RIGA TECHNICAL UNIVERSITY

Anastasija Balašova

BENZOKSAFOSFEPĪNA ATVASINĀJUMI KĀ SELEKTĪVI OGĻSKĀBES ANHIDRĀZES INHIBITORI

Promocijas darbs

DEVELOPMENT OF BENZOXAPHOSPHEPINE DERIVATIVES AS SELECTIVE CARBONIC ANHYDRASE INHIBITORS

Doctoral Thesis

RTU Izdevniecība
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RIGA TECHNICAL UNIVERSITY
Faculty of Natural Sciences and Technology
Institute of Chemistry and Chemical Technology

Anastasija Balašova
Doktora studiju programmas "Ķīmija, materiālzinātne un tehnoloģijas" doktorante
Doctoral Student of the Study Programme "Chemistry, Materials Science and Engineering"

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Zinātniskais vadītājs
profesors *Ph. D.*
RAIVIS ŽALUBOVSKIS

Scientific supervisor
Professor PhD
RAIVIS ŽALUBOVSKIS

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OFICIĀLIE RECENZENTI

Vadošā pētniece *Dr. chem*. Aiva Plotniece,
Latvijas Organiskās sintēzes institūts, Latvija

Profesors *Ph. D. Jean-Yves Winum,*
Monpeljē Universitāte, Francija

Profesore *Ph. D. Eglė Arbačiauskienė,*
Kauņas Tehnoloģiju universitāte, Lietuva

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Apstiprinu, ka esmu izstrādājusi šo promocijas darbu, kas iesniegts izskatīšanai Rīgas Tehniskajā universitātē zinātnes doktora (*Ph. D.*) grāda iegūšanai. Promocijas darbs zinātniskā grāda iegūšanai nav iesniegts nevienā citā universitātē.

Anastasija Balašova …… (paraksts)

Datums ……

Promocijas darbs sagatavots kā tematiski vienota zinātnisko publikāciju kopa. Tas ietver kopsavilkumu latviešu un angļu valodā, četras publikācijas un nepublicētus rezultātus. Publikācijas uzrakstītas angļu valodā, to kopējais apjoms, ieskaitot elektroniski pieejamo informāciju, ir 81 lpp.

SATURS / CONTENTS

SAĪSINĀJUMI / ABBREVIATIONS

AAZ	acetazolamīds / acetazolamide
Ac	acetil- / acetyl
AEŠH	augstas efektivitātes šķidruma hromatogrāfija
Boc	*terc*-butiloksikarbonil- / *tert*-butyloxycarbonyl
CA	ogļskābes anhidrāze / carbonic anhydrase
CARP	ogļskābes anhidrāzei radniecīgs proteīns / carbonic anhydrase-related protein
Cys	cisteīns / cysteine
CSI	hlorsulfonilizocianāts / chlorosulfonyl isocyanate
DCE	dihloretāns / dichloroethane
DMF	*N*,*N*-dimetilformamīds / *N*,*N*-dimethylformamide
DNs	2,4-dinitrobenzolsulfonil- / 2,4-dinitrobenzenesulfonyl
dppf	1,1’-bis(difenilfosfīno)ferrocēns / 1,1’-bis(diphenylphosphino)ferrocene
EDC	1-etil-3-(3-(*N*,*N*-dimetilamino)propil)karbodiimīds / 1-ethyl-3-(3-(*N*,*N*-dimethylamino)propyl)carbodiimide
Et	etil- / ethyl
Gly	glicīns / glycine
h	stunda / hour
HMDS	bis(trimetilsilil)amīns / bis(trimethylsilyl)amine
HPLC	high-performance liquid chromatography
*i*Pr	izopropil- / isopropyl
i.t.	istabas temperatūra
K_i	inhibēšanas konstante / inhibition constant
LED	gaismu izstarojošā diode / light-emitting diode
Me	metil- / methyl
Naph	naftil- / naphthyl
NBS	*N*-bromsukcinimīds / *N*-bromosuccinimide
ND	nav noteikts / not determined
NMP	*N*-metil-2-pirolidons / *N*-methyl-2-pyrrolidone
PBS	fosfāta fizioloģiskais buferšķīdums / phosphate-buffered saline
Ph	fenil- / phenyl
Py	piridīns / pyridine
r.t.	room temperature
SAR	struktūras–aktivitātes likumsakarība / structure–activity relationship
*t*Bu	*terc*-butil- / *tert*-butyl
TFA	trifluoretiķskābe / trifluoroacetic acid
THF	tetrahidrofurāns / tetrahydrofuran
TMS	trimetilsilil- / trimethylsilyl
Tol	tolil- / tolyl
UV	ultravioletā gaisma / ultraviolet

PROMOCIJAS DARBA VISPĀRĒJS RAKSTUROJUMS

Tēmas būtība un aktualitāte

Ogļskābes anhidrāzes (CA, EC 4.2.1.1), atklātas 20. gadsimta 30. gadu sākumā, ir ļoti plaša un nozīmīga enzīmu saime, kuras pārstāvji atrodami visos dzīvajos organismos un katalizē apgriezenisku oglekļa dioksīda hidratēšanu, veidojot bikarbonāta anjonu.[1] Tās tiek iedalītas astoņās evolucionāri atšķirīgās gēnu ģimenēs: α, β, γ, δ, ε, ζ, η, θ un ι.[2] Lai arī pēc struktūras šie enzīmi ir daudzveidīgi, visām CA klasēm aktīvajā centrā ir metāla jons kā kofaktors. Piemēram, α-CA satur Zn^{2+}, un tam ir izšķiroša loma CA katalītiskajā ciklā.[3] Šī ir visplašāk pētītā CA klase, jo tā ir sastopama mugurkauliniekos; tās aktivitāte ir tieši saistīta ar dažādu fizioloģisko un patoloģisko procesu norisi, padarot α-CA par svarīgu farmakoloģisko mērķi.[3,4] Cilvēkos līdz šim ir zināmi 15 α-CA izoenzīmi, kas atšķiras pēc to struktūras, lokalizācijas un katalītiskās aktivitātes.[4]

Cilvēka CA izoformas tiek klasificētas atbilstoši to subšūnu lokalizācijai: citosoliskās izoformas (CA I, II, III, VII, XIII), mitohondriju izoformas (CA VA, VB), sekretētā izoforma (CA VI), kā arī ar membrānu saistītās izoformas (CA IV, IX, XII, XIV).[4] Pastāv arī trīs katalītiski neaktīvi CA radniecīgi proteīni (CARP VIII, X, XI), kuru bioloģiskās funkcijas joprojām ir nepietiekami aprakstītas.[4,5] CA katalītiski aktīvo formu regulācijas traucējumus un darbību nereti saista ar tādām slimībām kā vēzis, glaukoma, vielmaiņas un neiroloģiskās saslimšanas.[4] Tiek uzskatīts, ka divas transmembrānās izoformas — CA IX un CA XII —, kas ir paaugstināti ekspresētas cieto vēžu šūnās, nodrošina optimālu šūnas iekšējo un ārējo pH, tādējādi veicinot audzēju attīstību un metastazēšanos.[6–9] CA IX un CA XII izoformu ekspresija var kalpot kā diagnostikas un prognozēšanas biomarķieris noteiktiem vēža veidiem, kā arī korelē ar terapijas rezistenci hipoksijas un acidozes dēļ.[6,10,11] Līdz ar to selektīvu CA IX/XII inhibitoru izveide ir perspektīva stratēģija vēža ārstēšanai ar plašām lietošanas iespējām klīnikā.

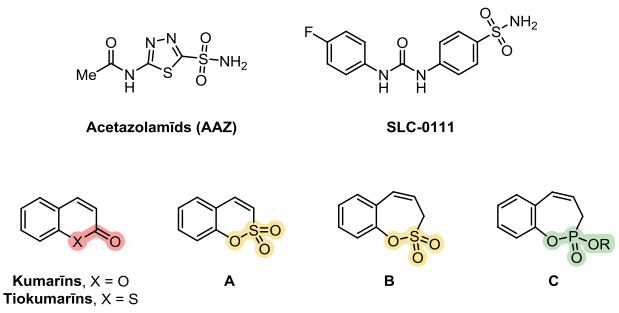
Ar rentgendifraktometrijas palīdzību ir izdevies noteikt trīsdimensionālo struktūru vairumam cilvēka CA izoformu, kas veicināja CA inhibitoru attīstību.[2–4] Katalītiski aktīvo izoformu aktīvais centrs ir novietots koniskā dobumā, ko veido divi atšķirīgi reģioni — hidrofobais un hidrofilais.[2–4] Šīs dažādās aktīvā centra daļas ir būtiskas enzīma darbībai un skaidrojamas ar substrāta un enzimātiskās reakcijas produkta ķīmisko dabu. Dobuma dziļumā atrodas Zn^{2+}, kas veido tetraedrisku kompleksu ar trim histidīna atlikumiem un ar ūdens molekulu vai hidroksīdjonu.[2–4] CA izoformu strukturālā līdzība un augsta aminoskābju secības identitāte katalītiskajā domēnā apgrūtina selektīvu inhibitoru konstruēšanu. Ņemot vērā CA IX un CA XII izoformu pārmērīgo ekspresiju vēža audos, bet zemu — normālos audos, šī promocijas darba pētījums ir mērķēts uz selektīvu CA IX/XII inhibitoru izstrādi, saglabājot organismā plaši sastopamo CA I un CA II izoformu aktivitāti.

Daudzi pētījumi tika vērsti uz CA un to inhibitoru mijiedarbības izpratni, kas sniegtu vērtīgas norādes racionālam zāļu dizainam. Patlaban ir zināmi četri dažādi inhibēšanas mehānismi: 1) kompleksēšanās ar enzīma aktīvajā centrā esošo cinka jonu (cinku saistošos inhibitorus sauc par klasiskajiem CA inhibitoriem, tostarp pirmējie sulfonamīdi, neorganiskie un organiskie anjoni, karbamāti, tioli, selenoli, benzoksaboroli); 2) koordinēšanās ar ūdens molekulu vai hidroksīda jonu, kas saistīts pie cinka (novērots fenolu, poliamīnu, sulfokumarīnu **A** (1. att.) gadījumā); 3) aktīvā centra ieejas aizsegšana (kumarīni, tiokumarīni); 4) perifēra saistīšanās (šim saistības veidam gan

zināms tikai viens piemērs — 2-(benzilsulfinil)-benzoskābe, tā saistās ārpus aktīvā centra, hidrofobajā kabatā blakus aktīvā centra ieejai),[2–4] kā arī ir vairāki CA inhibējoši savienojumi, kuriem darbības mehānisms nav noteikts.

Daži klasiskie CA inhibitori, piemēram, acetazolamīds (AAZ), metazolamīds, sultiāms un dorzolamīds tiek lietoti klīnikā kā diurētiķi, pretglaukomas vai pretepilepsijas līdzekļi.[2] Savienojumi, kas satur cinku saistošo $–SO_2NH_2$ grupu, ir vieni no visefektīvākajiem CA inhibitoriem, taču to neselektīvā CA izoformu inhibēšana ir saistīta ar nevēlamiem blakusefektiem. Tādēļ tiek konstruēti jauni inhibitori ar uzlabotu selektivitāti, to skaitā zāļu kandidāts SLC-0111, kas tika izstrādāts, lai inhibētu tieši ar vēzi saistītās CA IX un CA XII izoformas (1. att.).[12,13] SLC-0111 ir sasniedzis klīniskos pētījumus, kuros iesaistīti pacienti ar metastātiskiem cietiem audzējiem.[14] Tas atspoguļo CA inhibitoru terapeitisko potenciālu onkoloģisko slimību ārstēšanā.

Promocijas darba autores un viņas kolēģu pētniecības grupa fokusējas uz neklasisko kumarīna tipa CA inhibitoru izpēti un attīstīšanu. Enzīma aktīvajā centrā notiek kumarīnu hidrolīze, veidojot attiecīgās kanēļskābes, kas bloķē aktīvā centra ieeju.[15,16] Kumarīnu CA inhibēšanas spēja tika atklāta dabasvielu ekstraktu augstas caurlaidības skrīninga rezultātā, un tie izraisīja lielu interesi, pateicoties bioloģiskajai aktivitātei uz visām aktīvajām α-CA izoformām.[15,17] Grupas iepriekšējie pētījumi kumarīna tipa inhibitoru pilnveidošanā rezultējās ar sulfokumarīniem **A** (1,2-benzoksatiīn-2,2-dioksīdi) un to analogiem — homosulfokumarīniem **B** (3*H*-1,2-benzoksatiepīn-2,2-dioksīdi), kas uzrādīja augstu selektivitāti attiecībā pret CA IX un XII (1. att.).[18–24] Promocijas darba ietvaros tiek pētīta jauna savienojumu klase — 3*H*-1,2-benzoksafosfepīn-2-oksīdi **C** kā nākamās paaudzes CA inhibitori.



1. att. CA inhibitoru struktūras.

Pētījuma mērķis un uzdevumi

Promocijas darba mērķis ir, balstoties uz fosfepīna oksīda **C** struktūru, izstrādāt efektīvus un selektīvus ar audzēju saistīto CA IX un CA XII izoformu inhibitorus ar potenciālu terapeitisku lietojumu. Lai sasniegtu darba mērķi, tika definēti šādi uzdevumi:

1. izstrādāt 3*H*-1,2-benzoksafosfepīn-2-oksīda un tā atvasinājumu iegūšanas metodoloģiju;
2. noteikt sintezēto savienojumu inhibējošo aktivitāti uz izvēlētām cilvēka CA izoformām;
3. izpētīt savienojumu ietekmi uz šūnu dzīvotspēju, kā arī noteikt šķīdību ūdenī;
4. veikt struktūras–aktivitātes likumsakarību analīzi un sniegt ieskatu saistīšanās mehānismā.

Zinātniskā novitāte un galvenie rezultāti

Promocijas darba gaitā ir izstrādāti jauni 3*H*-1,2-benzoksafosfepīna 2-oksīda atvasinājumi, kas sniedz ieguldījumu selektīvo ogļskābes anhidrāzes inhibitoru jomā. Sintēzes stratēģija ļāva iegūt plašu benzoksafosfepīna atvasinājumu klāstu. Tika noteikta iegūto savienojumu spēja inhibēt cilvēka CA izoformas. Savienojumi selektīvi inhibēja ar audzēju saistītās izoformas IX un XII. Pētījuma rezultātā iegūta virkne inhibitoru, kam piemīt zema nanomolāra CA IX/XII inhibēšanas aktivitāte. Atsevišķiem pārstāvjiem papildus tika izvērtēta citotoksicitāte un noteikta šķīdība ūdenī. Noskaidrotās struktūras–aktivitātes likumsakarības deva ieskatu par iespējamo saistīšanās veidu ar mērķenzīmu. Kopumā rezultāti liecina, ka šie savienojumi ir potenciāli zāļvielu kandidāti un tos var izmantot turpmākai izpētei.

Darba struktūra un apjoms

Promocijas darbs sagatavots kā tematiski vienota zinātnisko publikāciju kopa par 3*H*-1,2-benzoksafosfepīna 2-oksīda atvasinājumu kā potenciālu pretvēža līdzekļu izstrādi, mērķējot tos uz ogļskābes anhidrāzes IX un XII izoformām.

Katra promocijas darba rezultātu apakšnodaļa ir balstīta vienas publikācijas saturā, ievērojot hronoloģisku secību.

Darba aprobācija un publikācijas

Promocijas darba galvenie rezultāti apkopoti trijās zinātniskajās oriģinālpublikācijās un vienā apskatrakstā. Pētījuma rezultāti prezentēti deviņās konferencēs.

Zinātniskās publikācijas

1. **Balašova, A.**; Žalubovskis, R. Synthetic methods toward phosphacoumarins (microreview). *Chem. Heterocycl. Comp.* **2022**, *58*, 310–312.
2. Pustenko, A.; **Balašova, A.**; Nocentini, A.; Supuran, C. T.; Žalubovskis, R. 3*H*-1,2-Benzoxaphosphepine 2-oxides as selective inhibitors of carbonic anhydrase IX and XII. *J. Enzyme Inhib. Med. Chem.* **2023**, *38* (1), 216–224.
3. **Balašova, A.**; Pustenko, A.; Nocentini, A.; Vullo, D.; Supuran, C. T.; Žalubovskis, R. Aryl derivatives of 3*H*-1,2-benzoxaphosphepine 2-oxides as inhibitors of cancer-related carbonic anhydrase isoforms IX and XII. *J. Enzyme Inhib. Med. Chem.* **2023**, *38* (1), 2249267.
4. **Balašova, A.**; Pustenko, A.; Angeli, A.; Andreucci, E.; Biagioni, A.; Nocentini, A.; Carta, F.; Supuran, C. T.; Žalubovskis, R. Unraveling the potential of amino-, acylamino-, and ureido-substituted 3*H*-1,2-benzoxaphosphepine 2-oxides toward nanomolar inhibitors of tumor-associated carbonic anhydrases IX and XII. *ACS Med. Chem. Lett.* **2025**, *16* (6), 1031–1037.
5. Manuskripts par pēdējo apakšnodaļu tiek gatavots publicēšanai.

Zinātniskās konferences

1. **Balašova, A.**; Žalubovskis, R. Benzoxaphosphepine 2-oxides as potential carbonic anhydrase inhibitors. *56th International Conference on Medicinal Chemistry (RICT 2021)*, virtual event, July 7–9, **2021**.

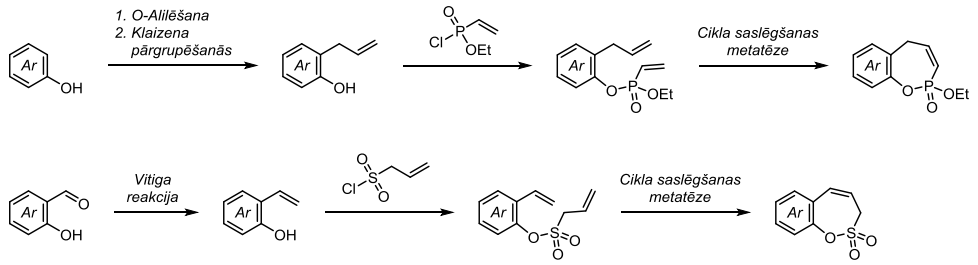
2. **Balašova, A.** Benzoxaphosphepine 2-oxides as potential carbonic anhydrase inhibitors. *12th Paul Walden Symposium on Organic Chemistry*, virtual event, October 28–29, **2021**.
3. **Balašova, A.**; Žalubovskis, R. Benzoxaphosphepine 2-oxides as potential carbonic anhydrase inhibitors. *Balticum Organicum Syntheticum (BOS 2022)*, Vilnius, Lithuania, July 3–6, **2022**.
4. **Balašova, A.**; Žalubovskis, R. Benzoxaphosphepine 2-oxides as potential carbonic anhydrase inhibitors. *Drug Discovery Conference 2022*, Riga, Latvia, September 22–24, **2022**.
5. **Balašova, A.**; Žalubovskis, R. Development of benzoxaphosphepine 2-oxides as carbonic anhydrase inhibitors. *81st International Scientific Conference of the University of Latvia*, Riga, Latvia, March 17, **2023**.
6. **Balašova, A.**; Žalubovskis, R. Design and synthesis of benzoxaphosphepine 2-oxides as carbonic anhydrase inhibitors. *12th International conference on Carbonic Anhydrases*, Naples, Italy, July 5–7, **2023**.
7. **Balašova, A.**; Pustenko, A.; Žalubovskis, R. Development of benzoxaphosphepine 2-oxides as carbonic anhydrase inhibitors. *Autoimmune diseases: main problems and solutions*, Riga, Latvia, November 9–10, **2023**.
8. **Balašova, A.**; Pustenko, A.; Žalubovskis, R. Benzoxaphosphepine 2-oxides — a novel class of carbonic anhydrase inhibitors. *Conference "Achievements of the SPRINGBOARD project"*, Riga, Latvia, May 2–3, **2024**.
9. **Balašova, A.**; Pustenko, A.; Žalubovskis, R. Benzoxaphosphepine 2-oxides — a novel class of carbonic anhydrase inhibitors. *Balticum Organicum Syntheticum (BOS 2024)*, Riga, Latvia, July 7–10, **2024**.

PROMOCIJAS DARBA GALVENIE REZULTĀTI

Prologs

Fosforu saturošiem savienojumiem piemīt plaša spektra bioloģiskās aktivitātes, tostarp pretvēža, pretmikrobu un pretiekaisuma īpašības, kā arī inhibējoša iedarbība uz dažādiem enzīmiem.[25,26] Fosfora grupu ievadīšana bieži vien uzlabo zāļvielu farmakokinētiku un farmakodinamiku, liecinot par augstu izmantošanas potenciālu medicīnas ķīmijā,[25–28] turklāt šīs grupas var būt vairāku citu funkcionālo grupu bioizostēri, kuru priekšrocība ir paaugstināta biopieejamība.[25,29] Līdz ar to tika paredzēts, ka benzoksafosfepīna oksīdi **C** ir iespējami sulfokumarīna analogu bioizostēri (1. att.).

Fosforu saturošu kondensētu heterociklu sintēzes metodoloģija ir salīdzinoši ierobežota. No literatūrā zināmajām pārejas metālu katalizētām pārvērtībām šādu *P*-heterociklu veidošanai cikla saslēgšanas metatēze ir izceļama kā universāla metode benzanelētu septiņlocekļu ciklu konstruēšanai. Šī metode tika veiksmīgi lietota gan sēru, gan fosforu saturošu analogu sintēzei (2. att.).[22,30] 3*H*-1,2-Benzoksafosfepīna 2-oksīda **C** atvasinājumus tika plānots iegūt, izmantojot līdzīgu pieeju. Nākamajās apakšnodaļās aprakstīta šo jauno fosfororganisko savienojumu sintēze un izvērtēta to bioloģiskā aktivitāte.

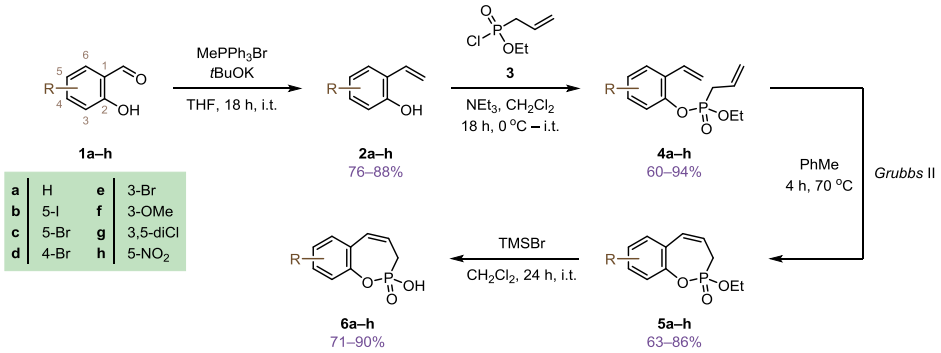


2. att. Benzanelētu *P*- vai *S*-saturošu septiņlocekļu heterociklu iegūšanas iespējas.

Informācija par fosfakumarīnu un to homologu sintēzes metodēm ir apkopota apskatrakstā *Chem. Heterocycl. Comp.* **2022**, *58*, 310–312.

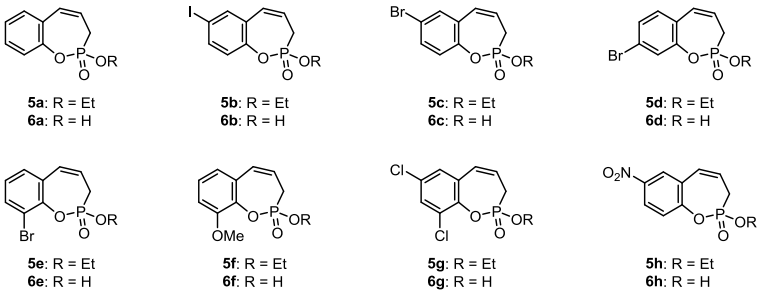
1. Benzoksafosfepīna oksīdu sintēze un inhibēšanas potenciāls

3*H*-1,2-Benzoksafosfepīn-2-oksīdu vispārīgais sintēzes ceļš tika izstrādāts, balstoties uz iepriekš aprakstīto strukturāli līdzīgu savienojumu iegūšanas metodoloģiju, kuras pamatā ir olefīnu metatēzes reakcija cikla saslēgšanai.[22,30] Sākumā komerciāli pieejamie salicilaldehīdi **1a–h** tika pārvērsti par vinilfenoliem **2a–h**, veicot *Wittig* olefinēšanas reakciju (1. shēma). Tālākā fosforilēšana ar hlorīdu **3** deva diolefīnus **4a–h**, kas tika ciklizēti par vēlamajiem benzoksafosfepīniem **5a–h**, izmantojot otrās paaudzes *Grubbs* katalizatoru. Apstrādājot etoksiatvasinājumus **5a–h** ar TMSBr, notika etilgrupas nošķelšana, veidojot hidrogēnfosfonātus **6a–h**. Produktu iznākumi reakcijās bija vidēji augsti līdz augsti.



1. shēma. Benzoksafosfepīna oksīdu **5** un **6** sintēze.

Lai novērtētu sintezēto ciklisko fosfonātu **5** un **6** (3. att.) inhibitoro aktivitāti un selektivitāti, tika noteikta šo savienojumu spēja inhibēt četras farmakoloģiski nozīmīgas cilvēka ogļskābes anhidrāzes izoformas — CA I, II, IX un XII.[31] Citosoliskās CA izoformas I un II ir sastopamas daudzos veselos audos, tāpēc to inhibēšana ir nevēlama, savukārt transmembrānās izoformas IX un XII paaugstināti ekspresējas audzēju šūnās un tiek uzskatītas par potenciāliem zāļvielu mērķiem. Iegūto inhibitoru aktivitātes uz CA ir izteiktas kā K_i (inhibēšanas konstantes) vērtības.



3. att. Potenciālo CA inhibitoru **5** un **6** struktūras.

In vitro analīzes dati liecina, ka testētie savienojumi **5**, **6** ir selektīvi inhibitori pret ar audzēju saistīto CA IX un CA XII izoformām un nav aktīvi pret CA I un CA II. Visi atvasinājumi uzrādīja K_i vērtības mikromolārā diapazonā, atsevišķos gadījumos sasniedzot pat submikromolāru līmeni

(1. tabula). Benzoksafosfepīna oksīdu **5**, **6** aizvietotāji pie aromātiskā fragmenta vai pie fosfora atoma (OH *vs* OEt) būtiski neietekmēja inhibitoro aktivitāti. Iegūtie rezultāti liecina arī par to, ka benzoksafosfepīna oksīdi ir sulfokumarīnu **A**, **B** bioizostēri analogi mijiedarbībā ar CA izoformām. Salīdzinot ar acetazolamīdu (AAZ), redzams, ka fosforu saturošie savienojumi **5**, **6** ir vājāki mērķenzīmu inhibitori, tomēr, pateicoties to augstajai selektivitātei un fizikālķīmiskajām īpašībām, benzoksafosfepīna pamatstruktūra ir piemērota tālākai optimizēšanai.

1. tabula

CA inhibēšanas dati savienojumu sērijām **5**, **6** un standarta inhibitoram AAZ

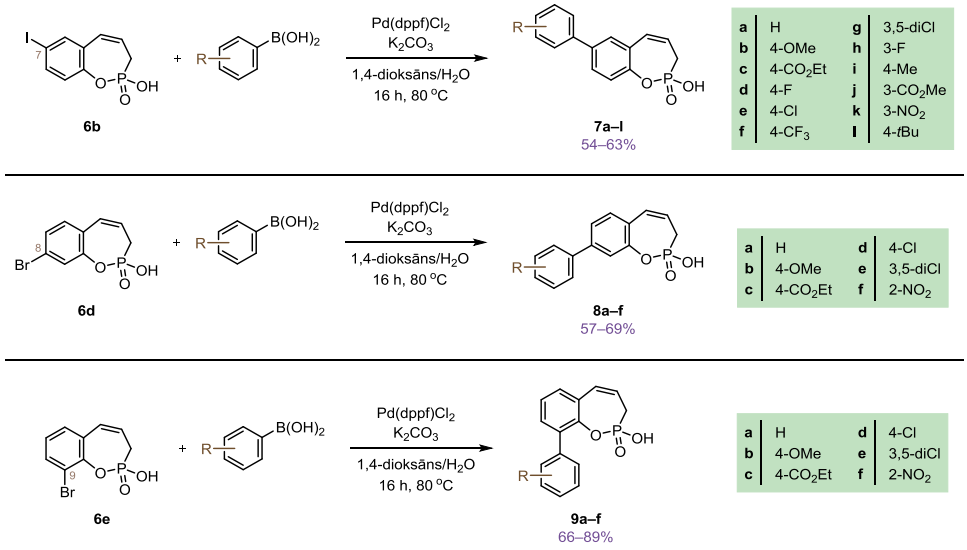
Savienojums	K_i **(µM)**[a,b,c]			
	CA I	CA II	CA IX	CA XII
5a	>100	>100	0,82	0,82
6a	>100	>100	1,3	0,51
5b	>100	>100	4,7	2,4
6b	>100	>100	0,88	0,68
5c	>100	>100	0,76	1,6
6c	>100	>100	1,0	0,96
5d	ND	ND	ND	ND
6d	>100	>100	3,4	1,5
5e	>100	>100	11,3	3,3
6e	>100	>100	2,5	1,8
5f	>100	>100	9,0	7,4
6f	>100	>100	1,8	1,2
5g	>100	>100	6,1	3,4
6g	>100	>100	0,80	1,7
5h	>100	>100	3,9	0,95
6h	>100	>100	0,67	1,0
AAZ*	0,25	0,012	0,025	0,006

[a] Noteikts sadarbībā ar prof. *C. T. Supuran* grupu Florences Universitātē. [b] Rezultāti ir aprēķināti kā vidējais no trīs neatkarīgiem mērījumiem (kļūdas ir ±5–10% robežās no norādītajām vērtībām). [c] Inhibitoru un enzīmu saturošie šķīdumi tika inkubēti 6 h i.t. * References standarts.

Šīs apakšnodaļas pētījumi ir aprakstīti oriģinālpublikācijā *J. Enzyme Inhib. Med. Chem.* **2023**, *38* (1), 216–224.

2. Benzoksafosfepīna pamatstruktūras ķīmiskās daudzveidības paplašināšana

Lai iegūtu plašāku informāciju par struktūras–aktivitātes likumsakarībām, tika veikta benzoksafosfepīna oksīda modificēšana, ievadot dažādus arilaizvietotājus 7-, 8- un 9-pozīcijā. Arilatvasinājumi **7–9** tika sintezēti palādija katalizētā *Suzuki–Miyaura* šķērssametināšanas reakcijā starp komerciāli pieejamajām arilborskābēm un halogenīdiem **6b,d,e** (2. shēma). No iepriekšējiem rezultātiem zināms, ka 6-pozīcijā aizvietotus atvasinājumus iegūt nav iespējams, jo attiecīgais diolefīns stērisko traucējumu dēļ neciklizējas metatēzes stadijā.[23] Iegūto produktu **7–9** bioloģiskās aktivitātes ļautu noskaidrot piemērotāko aizvietotāju novietojumu jaunu šāda tipa CA inhibitoru dizainā.



2. shēma. Benzoksafosfepīna oksīda 7-, 8-, 9-arilatvasinājumu **7–9** sintēze.

Arilatvasinājumi **7–9** uzrādīja samērā līdzīgas K_i vērtības (mikromolārā līmenī) uz CA IX un CA XII izoformām kā sākotnējie inhibitori **6b,d,e**, vienlaikus saglabājot selektivitāti (2. tabula). Būtiski, ka 9-pozīcijā aizvietotu atvasinājumu **9** gadījumā tika novērota aktivitātes samazināšanās, savukārt 7- un 8-aizvietotu analogu **7**, **8** CA IX/XII inhibēšanas spēja bija ievērojami augstāka. Kopumā savienojumu sēriju **7** un **8** pārstāvji uzrādīja līdzīgus rezultātus, tomēr **7a**, **7d** un **7h** ir aktīvākie šo sēriju inhibitori. Šī tendence ir novērojama arī sulfokumarīna **A** un homosulfokumarīna **B** analogu rindās.[20,23] Līdz ar to tālākā funkcionalizēšana, lai uzlabotu aktivitāti, tika veikta benzoksafosfepīna 7-pozīcijā.

2. tabula

CA inhibēšanas dati savienojumu sērijām **7–9** un standarta inhibitoram AAZ

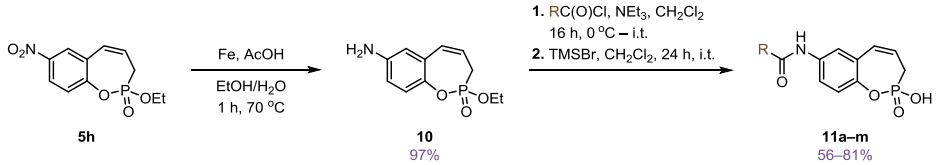
Savienojums	K_i **(µM)**[a,b,c]			
	CA I	CA II	CA IX	CA XII
7a	>100	>100	0,77	0,95
7b	>100	>100	4,6	1,7
7c	>100	>100	6,0	6,7
7d	>100	>100	0,86	0,25
7e	>100	>100	8,6	1,1
7f	>100	>100	3,7	0,59
7g	>100	>100	7,3	4,2
7h	>100	>100	0,63	0,56
7i	>100	>100	1,5	0,94
7j	>100	>100	9,5	1,5
7k	>100	>100	7,6	0,64
7l	>100	>100	4,9	0,97
8a	>100	>100	1,8	2,7
8b	>100	>100	2,1	5,6
8c	>100	>100	10,2	3,8
8d	>100	>100	5,0	7,1
8e	>100	>100	0,98	0,84
8f	>100	>100	12,9	0,67
9a	>100	>100	16,5	25,5
9b	>100	>100	39,4	52,4
9c	>100	>100	55,3	>100
9d	>100	>100	48,9	65,3
9e	>100	>100	22,4	28,2
9f	>100	>100	38,4	35,1
AAZ*	0,25	0,012	0,025	0,006

[a] Noteikts sadarbībā ar prof. *C. T. Supuran* grupu Florences Universitātē. [b] Rezultāti ir aprēķināti kā vidējais no trīs neatkarīgiem mērījumiem (kļūdas ir ±5–10% robežās no norādītajām vērtībām). [c] Inhibitoru un enzīmu saturošie šķīdumi tika inkubēti 6 h i.t. * References standarts.

Šīs apakšnodaļas pētījumi ir aprakstīti oriģinālpublikācijā *J. Enzyme Inhib. Med. Chem.* **2023**, *38* (1), 2249267.

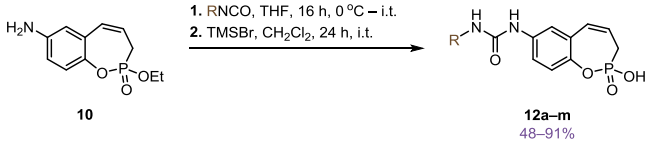
3. Benzoksafosfepīnu optimizēšana līdz nanomolāriem mērķenzīmu inhibitoriem

Iepriekšējos pētījumos par benzoksatiīna un benzoksatiepīna dioksīdiem **A** un **B**, kas ir benzoksafosfepīna oksīdu **C** tuvākie analogi, tika atklāts, ka acilaminofunkcijas ievadīšana pamatstruktūrā nozīmīgi ietekmēja savienojumu inhibitoro aktivitāti pret CA IX un CA XII.[21,24] Tādēļ nākamā darba posmā, lai sasniegtu labāku aktivitāti, tika nolemts sintezēt atvasinājumus ar amīda funkcionālajām grupām. Vispirms iepriekš iegūtais nitrobenzoksafosfepīns **5h** tika reducēts līdz amīnam **10**, kas tālāk tika acilēts ar virkni karbonskābju hlorīdu. Pēc starpproduktu etilgrupas nošķelšanas ar TMSBr tika iegūti attiecīgie amīdi **11a–m** (3. shēma).



3. shēma. Benzoksafosfepīna oksīda amino- un acilaminoatvasinājumu **10** un **11** sintēze.

Šī pētījuma ietvaros jaunu un selektīvu ogļskābes anhidrāzes inhibitoru klāsts tika papildināts arī ar urīnvielas atvasinājumiem **12**. Urīnvielas funkciju saturoši savienojumi tiek plaši izmantoti zāļvielu konstruēšanā un attīstīšanā, lai uzlabotu līdersavienojumu bioloģisko aktivitāti, kā arī fizikālķīmiskos parametrus.[32] Turklāt zāļvielas kandidāts SLC-0111, kas uzrādīja spēcīgu CA IX/XII inhibēšanu un kam ir pabeigti I fāzes klīniskie pētījumi (NCT02215850), satur urīnvielas fragmentu. Benzoksafosfepīna oksīda urīnvielas atvasinājumi tika sintezēti, amīnam **10** reaģējot ar dažādiem arilizocianātiem, kam seko *O*-deetilēšana, izmantojot TMSBr (4. shēma). Vēlamās *N,N'*-diarilurīnvielas **12a–m** tika iegūtas ar vidējiem līdz augstiem iznākumiem.



4. shēma. Benzoksafosfepīna oksīda urīnvielas atvasinājumu **12** sintēze.

Jaunsintezēto amino-, acilamino- un urīnvielas atvasinājumu **10–12** enzimātiskie testi uz CA I, II, IX, XII parādīja interesantus rezultātus. Tāpat kā iepriekš pētītie analogi **5–9**, savienojumi **10–12** neinhibē normālos audos plaši sastopamās CA I un CA II izoformas. Zīmīgi, ka amīns **10** un tā atvasinājumi **11**, **12** sasniedza nanomolāru līmeni — daži savienojumi uzrādīja labāku inhibēšanas aktivitāti uz CA IX vai CA XII nekā SLC-0111 un AAZ (3. tabula). Jāuzsver, ka spēja inhibēt abas

izoformas ir novērojama tikai amīna **10** un amīdu **11** gadījumā, savukārt urīnvielas **12** izrādījās selektīvi CA IX inhibitori.

3. tabula

CA inhibēšanas dati savienojumu sērijām **10–12** un inhibitoriem SLC-0111 un AAZ

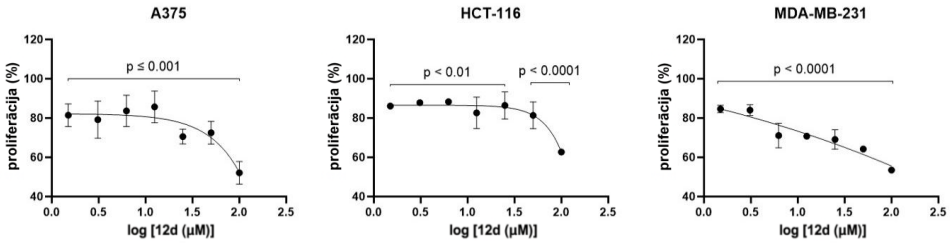
Savienojums	K_i **(nM)**[a,b,c]			
	CA I	CA II	CA IX	CA XII
10	>10000	>10000	3,5	16,6
11a	>10000	>10000	10,8	7,3
11b	>10000	>10000	20,5	7,8
11c	>10000	>10000	10,1	8,1
11d	>10000	>10000	29,4	6,7
11e	>10000	>10000	158,4	64,1
11f	>10000	>10000	25,8	84,4
11g	>10000	>10000	23,2	8,9
11h	>10000	>10000	18,7	57,5
11i	>10000	>10000	14,6	6,9
11j	>10000	>10000	27,4	4,0
11k	>10000	>10000	20,1	27,2
11l	>10000	>10000	22,3	9,7
11m	>10000	>10000	32,8	74,6
12a	>10000	>10000	34,9	>10000
12b	>10000	>10000	46,9	>10000
12c	>10000	>10000	53,3	>10000
12d	>10000	>10000	5,3	>10000
12e	>10000	>10000	52,9	>10000
12f	>10000	>10000	54,8	>10000
12g	>10000	>10000	46,9	>10000
12h	>10000	>10000	36,8	>10000
12i	>10000	>10000	36,0	>10000
12j	>10000	>10000	51,8	>10000
12k	>10000	>10000	4,4	>10000
12l	>10000	>10000	31,9	>10000
12m	>10000	>10000	42,7	>10000
SLC-0111[12]	5080	960	45,1	4,5
AAZ[*]	250	12,1	25,8	5,7

[a] Noteikts sadarbībā ar prof. *C. T. Supuran* grupu Florences Universitātē. [b] Rezultāti ir aprēķināti kā vidējais no trīs neatkarīgiem mērījumiem (kļūdas ir ±5–10% robežās no norādītajām vērtībām). [c] Inhibitoru un enzīmu saturošie šķīdumi tika inkubēti 6 h i.t. * References standarts.

Jāatzīmē, ka dažos vēža apakštipos CA XII ekspresijai, salīdzinot ar CA IX izoformu, ir minimāla ietekme uz audzēja šūnu proliferāciju.[33] Turklāt CA XII ir sastopama vairākos normālos audos, kas liecina par nepieciešamību noteiktos gadījumos izstrādāt selektīvākus CA IX inhibitorus

attiecībā pret CA XII.[33,34] Savienojumi **12**, kam piemīt CA IX inhibēšanas selektivitāte, varētu būt īpaši nozīmīgi farmakoloģijas jomā, pateicoties samazinātām blaknēm, saistītām ar neselektīvu CA izoformu aktivitātes nomākšanu.

No sintezētajiem benzoksafosfepīna oksīda **C** atvasinājumiem amīns **10**, amīdi **11** un urīnvielas **12** uzrādīja visaugstāko mērķenzīmu inhibēšanas spēju (ar K_i vērtībām nanomolārā diapazonā). No tiem **10**, **11c** un **12d** tika izvēlēti turpmākajiem *in vitro* eksperimentiem citotoksicitātes pārbaudei. Analizējamo inhibitoru citotoksicitāte tika pētīta uz A375 melanomas šūnu, HCT-116 kolorektālā vēža šūnu un MDA-MB-231 trīskārši negatīvā krūts vēža šūnu līnijām, kas kultivētas klasiskajos apstākļos. Šūnu dzīvotspējas noteikšanai tika izmantota MTT (3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolija bromīds) kolorimetriskā metode.[35,36] Rezultāti liecināja, ka amīnam **10** un amīdam **11c** ir zema citotoksiskā aktivitāte visās pētījumā analizētajās šūnu līnijās, savukārt urīnvielas atvasinājums **12d** uzrādīja izteiktāku citotoksisko efektu — inkubācija ar **12d** samazināja HCT-116 šūnu proliferāciju par 40% un A375, MDA-MB-231 šūnu proliferāciju par 50% 100 μM koncentrācijā (4. att.).



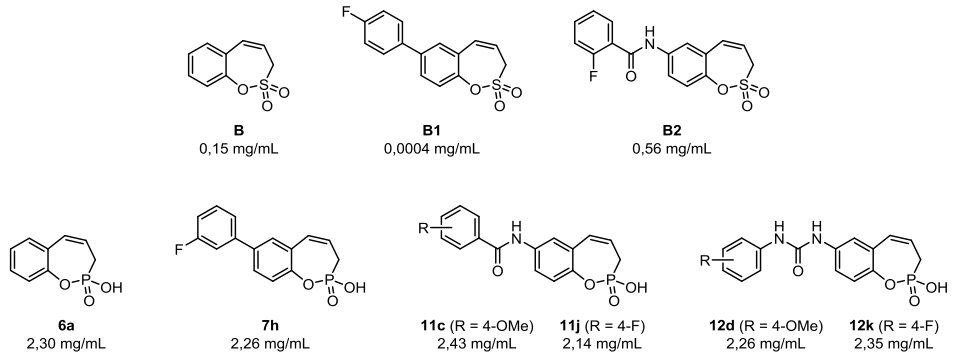
4. att. Savienojuma **12d** citotoksiskā iedarbība uz A375, HCT-116 un MDA-MB-231 šūnu līnijām pēc 72 h inkubācijas (N = 4; dati analizēti, izmantojot *GraphPad Prism 10.3.1*; noteikts Florences Universitātē sadarbībā ar *Dr. E. Andreucci* un *Dr. A. Biagioni*).

Dažiem literatūrā zināmajiem CA inhibitoriem piemīt augsta citotoksicitāte,[37,38] tomēr tiek uzskatīts, ka CA IX/XII inhibitoru pretvēža potenciāls galvenokārt ir saistīts ar to spēju modulēt audzēja mikrovidi, samazinot acidozi, kā arī kavējot ļaundabīgo šūnu augšanu un izplatīšanos.[9] Turklāt kombinācijā ar citām zāļvielām CA IX/XII inhibitori var darboties sinerģiski, sensibilizējot vēža šūnas un tādējādi palielinot ķīmijterapijas efektivitāti.[39,40] Interesanti, ka preklīniskajos *in vitro* pētījumos zāļvielas kandidāts SLC-0111 neietekmēja vēža šūnu dzīvotspēju normoksijas un neitrālā pH (7,4) apstākļu gadījumā, taču uzrādīja citotoksisko aktivitāti vēža šūnu līnijās, kultivētās skābā vidē (pH 6,7) vai hipoksijas apstākļos.[41] Izteiktu antiproliferatīvu un antimetastātisku iedarbību SLC-0111 uzrādīja *in vivo* krūts vēža modeļos.[12,13]

Citotoksicitātes testi plašākā šūnu līniju panelī, iekļaujot vēža šūnu līnijas ar paaugstinātu CA IX/XII ekspresiju un to inkubāciju hipoksijas apstākļos, sniegtu papildu informāciju par benzoksafosfepīna oksīdu terapeitisko potenciālu. Ņemot vērā novēroto selektivitāti CA izoformu inhibēšanā (3. tabula), pētījumi par šo savienojumu ietekmi uz vēža šūnām, kurām ir raksturīga pozitīva CA IX ekspresija un negatīva/vāja CA XII ekspresija (vai *vice versa*), ļautu izvērtēt saistību starp mērķproteīnu inhibēšanu un vēža šūnu proliferācijas spēju.

Darba turpinājumā 3*H*-1,2-benzoksafosfepīn-2-oksīdu klases, kā arī 3*H*-1,2-benzoksatiepīn-2,2-dioksīdu klases pārstāvjiem tika noteikta šķīdība ūdenī. Analīze tika veikta fosfāta fizioloģiskajā

buferšķīdumā (PBS; pH ~ 7,4) pie 25 °C, izmantojot AEŠH-UV metodi. No iegūtajiem rezultātiem redzams, ka pāreja no sultoniem uz cikliskajām fosfonskābēm uzlabo šķīdību ūdenī — fosfonātiem **6a**, **7h**, **11c**, **11j**, **12d**, **12k** tā ir izteikti augstāka nekā sultonu analogiem **B**, **B1**, **B2** (5. att.). Jāpiebilst, ka benzoksafosfepīna oksīdu šķīdības vērtības ir līdzvērtīgas neatkarīgi no aizvietotāja. Turklāt no fosfonskābēm ir iespējams iegūt sāļus, lai papildus paaugstinātu šķīdību ūdenī. Potenciālu zāļvielu šķīdība ir būtisks parametrs, kas ietekmē to absorbciju, biopieejamību un administrēšanu. Neapmierinošas farmakokinētikās īpašības nereti ir saistītas ar savienojumu zemu šķīdību un šūnu caurlaidību.[32]



5. att. Izvēlēto inhibitoru šķīdība PBS buferī (noteikts sadarbībā ar *Dr.* H. Kažokas grupu LOSI Hromatogrāfijas laboratorijā).

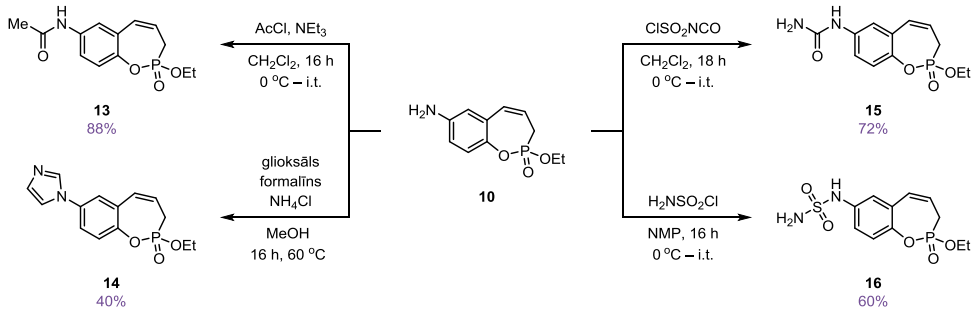
Šīs apakšnodaļas pētījumi ir aprakstīti oriģinālpublikācijā *ACS Med. Chem. Lett.* **2025**, *16* (6), 1031–1037.

4. Inhibitoru klāsta papildināšana un saistīšanās mehānisma izpēte

(Nepublicēti rezultāti)

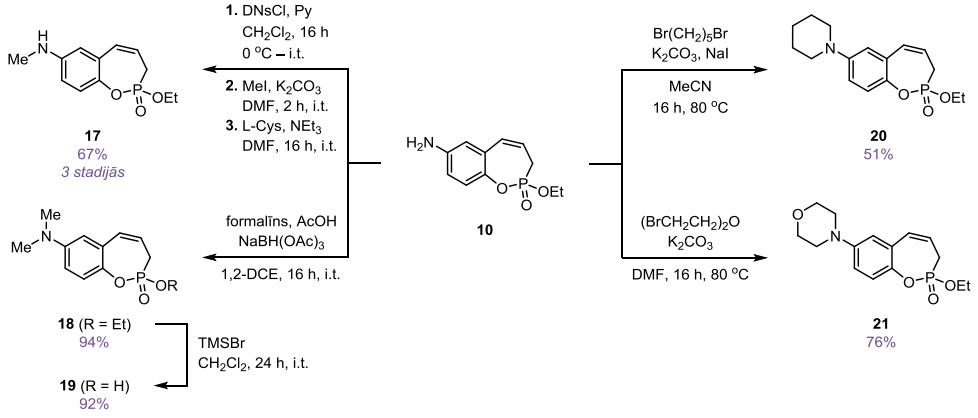
Izvērtējot slāpekli saturošu benzoksafosfepīna oksīdu **10–12** CA IX/XII inhibēšanas potenciālu, tika nolemts paplašināt šo atvasinājumu klāstu ar dažādiem aminofunkcijas aizvietotājiem. Amīns **10**, kas uzrādīja augstu inhibitoro aktivitāti un ligandu efektivitāti, kalpoja par atbilstošu references savienojumu, veicot tālāku struktūras modificēšanu.

Jauni mazmolekulāri CA inhibitori tika sintezēti, izmantojot benzoksafosfepīna oksīda aminoatvasinājumu **10** kā galveno būvbloku. Savienojuma **10** acetilēšana ļāva iegūt acetamīdu **13**. Reakcijā ar CSI amīns **10** tika pārvērsts par *N'*-neaizvietotu urīnvielu **15**, savukārt, amīnam **10** reaģējot ar sulfamoilhlorīdu, veidojās sulfamīds **16**. Veicot *Debus–Radziszewski* reakciju, no būvbloka **10** tika iegūts arī imidazols **14**, lai pārbaudītu, vai imidazolilgrupa varētu darboties kā arilgrupas bioizostērs (5. shēma).



5. shēma. *N*-Saturošu benzoksafosfepīna oksīda atvasinājumu **13–16** sintēze.

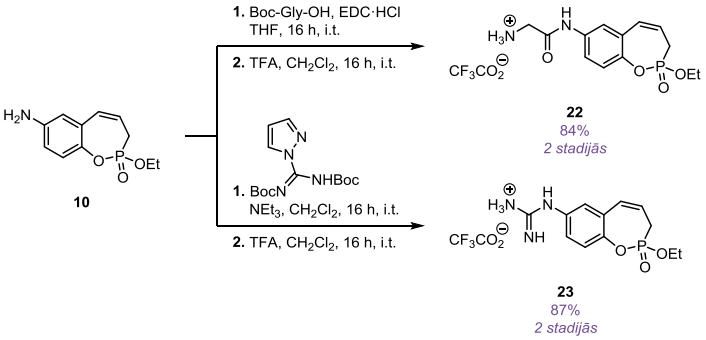
N-Metilatvasinājuma **17** iegūšanai tika izmantota *Fukuyama* metode,[42] kuras pamatā ir aminogrupas aizsargāšana ar 2,4-dinitrobenzolsulfonilgrupu. *N*-Aizsargāta amīna alkilēšana ar MeI un tai sekojoša DNs grupas nošķelšana ar L-cisteīnu NEt_3 klātienē deva vajadzīgo otrējo amīnu **17** (6. shēma).



6. shēma. *N*-Saturošu benzoksafosfepīna oksīda atvasinājumu **17–21** sintēze.

Būvbloka **10** pārvēršana par *N*,*N*-dimetilatvasinājumu **18** tika veikta ar formaldehīdu reducējošās aminēšanas ceļā. Pakļaujot savienojumu **18** iepriekš aprakstītajiem *McKenna* reakcijas apstākļiem, ar augstu iznākumu tika iegūts arī analogs **19**. Savukārt piperidīna un morfolīna atvasinājumi **20** un **21** tika sintezēti, attiecīgi alkilējot amīnu **10** ar 1,5-dibrompentānu vai bis(2-brometil)ēteri (6. shēma).

Slāpekli saturošu atvasinājumu rindas paplašināšanai amīns **10** tika transformēts par glicīnamīda **22** un guanidīna **23** analogiem, izmantojot reakciju ar *N*-Boc-aizsargātu glicīnu vai 1*H*-pirazol-1-karboksamidīnu. Pēc aizsarggrupu nošķelšanas skābā vidē mērķsavienojumi tika iegūti trifluoracetātu sāļu veidā (7. shēma).



7. shēma. *N*-Saturošu benzoksafosfepīna oksīda atvasinājumu **22** un **23** sintēze.

Jauno CA inhibitoru **13–23** K_i vērtības bija robežās no 23 nM līdz 3,44 μM (4. tabula).

4. tabula

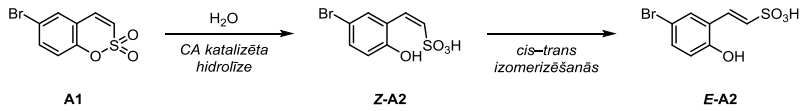
CA inhibēšanas dati savienojumiem **13–23** un standarta inhibitoram AAZ

Savienojums	**K_i (nM)**[a,b,c]			
	CA I	CA II	CA IX	CA XII
13	>10000	>10000	620	870
14	ND	ND	ND	ND
15	>10000	>10000	51	3440
16	370	94	37	23
17	>10000	>10000	840	710
18	>10000	>10000	950	760
19	>10000	>10000	920	810
20	>10000	>10000	1130	1070
21	>10000	>10000	990	1280
22	>10000	>10000	920	650
23	>10000	>10000	1230	1150
AAZ*	250	12,1	25,8	5,7

[a] Noteikts sadarbībā ar prof. *C. T. Supuran* grupu Florences Universitātē. [b] Rezultāti ir aprēķināti kā vidējais no trīs neatkarīgiem mērījumiem (kļūdas ir ±5–10% robežās no norādītajām vērtībām). [c] Inhibitoru un enzīmu saturošie šķīdumi tika inkubēti 6 h i.t. * References standarts.

Veicot benzoksafosfepīna oksīda atvasinājuma **10** aminogrupas modifikācijas, SAR pētījumos noskaidrots, ka alifātisko fragmentu ievadīšana kopumā samazināja inhibitoro aktivitāti (salīdzinot **13** ar **10** un **11a–m**; kā arī **17–22** ar **10**). Sintezētie acikliskie un cikliskie amīni **17–21**, kā arī amīdi **13**, **22** ir vājāki CA IX/XII inhibitori nekā attiecīgie aromātiskie atvasinājumi **11**, **12** un neaizvietots amīns **10** (3. un 4. tabula). Rezultāti liecina par nepieciešamību precīzi regulēt stēriskos un elektroniskos efektus konstruējamo ligandu aizvietotājiem *N*-saturošajā sānu ķēdē. Interesanti, ka *N'*-neaizvietotai urīnvielai **15** tika novērota līdzīga CA IX selektivitāte attiecībā pret CA XII inhibēšanu kā *N'*-arilurīnvielām **12a–m**. No visiem testētajiem savienojumiem tikai sulfamīds **16** inhibēja CA I un II izoformas, kas bija sagaidāms, ņemot vērā $–SO_2NH_2$ grupas koordinēšanos ar cinka jonu.[4] Toties savienojums **16** uzrādīja augstāku inhibēšanas aktivitāti uz mērķenzīmiem CA IX/XII nekā uz CA I/II, parādot benzoksafosfepīna struktūras ietekmi uz vēlamās selektivitātes nodrošināšanu.

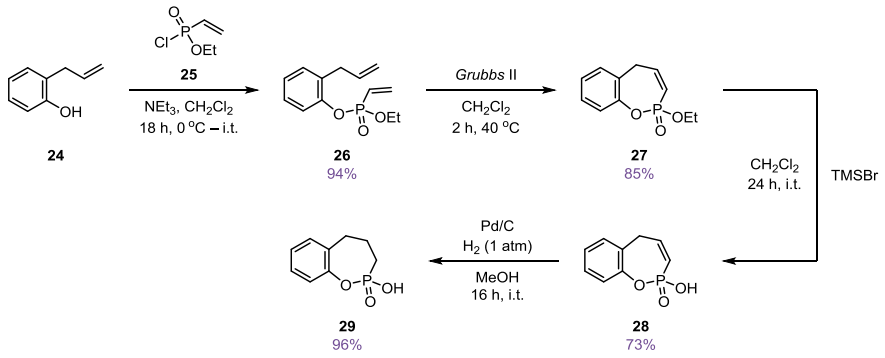
CA enzīma kristalizācijas mēģinājumi kompleksā ar dažādiem 3*H*-1,2-benzoksafosfepīna 2-oksīda atvasinājumiem nebija veiksmīgi, tāpēc joprojām paliek aktuāls jautājums par šo savienojumu konkrētu inhibēšanas mehānismu. Pētījuma gaitā tika izvirzītas divas galvenās hipotēzes: 1) 3*H*-1,2-benzoksafosfepīna 2-oksīdi iedarbojas uz CA neizmainītā veidā; vai 2) 3*H*-1,2-benzoksafosfepīna 2-oksīdi ir zāļvielu priekšteči, kas tiek pārveidoti par aktīvajiem savienojumiem hidrolīzes ceļā. Jāpiebilst, ka enzimātiskajos testos šo inhibitoru aktivitāte ir atkarīga no inkubācijas perioda, kas varētu liecināt par to, ka tie darbojas kā zāļvielu priekšteči. Piemēram, klasisko CA inhibitoru gadījumā enzīmu un inhibitoru šķīdumiem inkubācijas periods ir 15 minūtes, taču benzoksafosfepīna oksīdu aktivitātes noteikšanai bija nepieciešama sešu stundu inkubācija. Analoģisks efekts ir raksturīgs kumarīniem un sulfokumarīniem, kuru darbības mehānisms tika noskaidrots kokristalizācijas pētījumos un ietver heterocikliskā fragmenta hidrolītisko šķelšanos; rezultātā izveidojušies skābes atvasinājumi saistās ar enzīma aktīvo centru (6. att.).[16,19]



6. att. Sulfokumarīna **A1** CA katalizēta hidrolīze.[19]

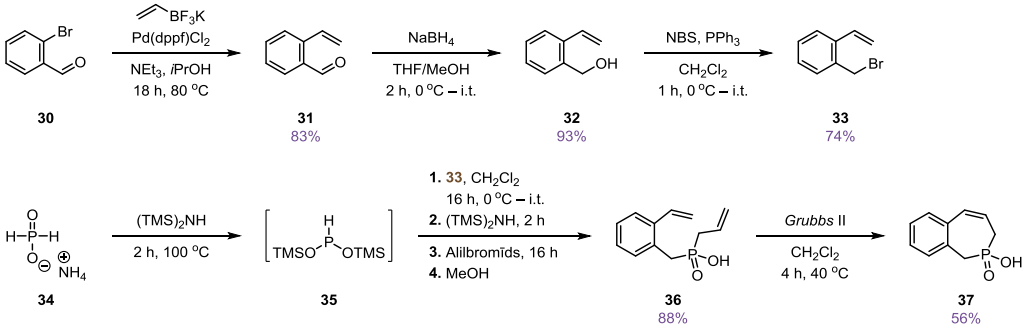
Lai izprastu benzoksafosfepīna oksīdu mijiedarbību ar CA, nepieciešama kompleksas pieejas lietošana, kas paredz tradicionālo SAR pētījumu un datormodelēšanas apvienošanu. Tādēļ tika nolemts iegūt vairākus papildu modeļsubstrātus, kas atklātu oksafosfepīna fragmentā esošās dubultsaites un skābekļa tiltiņa lomu CA inhibēšanā.

5*H*-1,2-Benzoksafosfepīn- un 3,4,5-trihidrobenzoksafosfepīn-2-oksīdi (**28** un **29**) tika sintezēti līdzīgā veidā kā iepriekšējās sērijas inhibitori **6**, sākot no 2-alilfenola (**24**). Fosforilēšanas reakcijā ar hlorīdu **25** no savienojuma **24** tika iegūts diolefīns **26**, kas veidoja ciklizēto produktu **27**, lietojot cikla saslēgšanas metatēzi (8. shēma). Jāmin, ka reakciju veicām sausā CH_2Cl_2 istabas temperatūrā, kas palīdzēja novērst olefīna **27** dubultsaites migrēšanu. Tālāka TMSBr veicināta etilgrupas noškelšana deva mērķsavienojumu **28**, no kura tika iegūts trihidroanalogs **29**, hidrogenējot heterocikliskajā fragmentā esošo dubultsaiti.



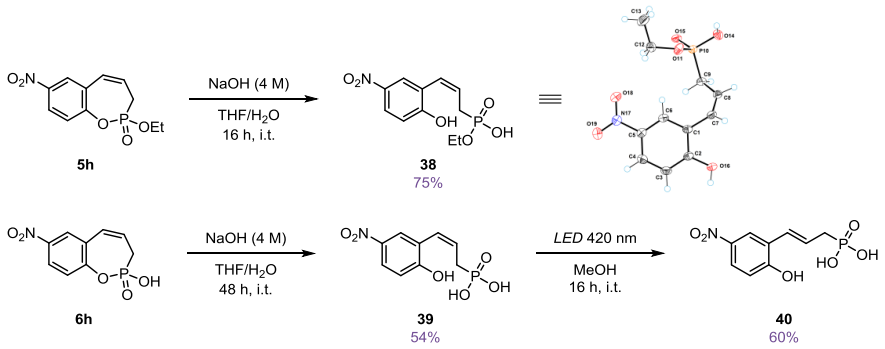
8. shēma. Benzoksafosfepīna oksīdu analogu **28** un **29** sintēze.

Lai pagatavotu ciklisko fosfīnskābi **37**, sākotnēji bija nepieciešams iegūt 2-vinilbenzilbromīdu (**33**). Savienojums **33** tika sintezēts no 2-brombenzaldehīda (**30**) saskaņā ar literatūras metodi,[43] kas paredzēja trīs soļu sekvenci — *Suzuki–Miyaura* sametināšanu starp **30** un kālija viniltrifluorborātu, aldehīda **21** reducēšanu un iegūtā spirta **32** *Appel* tipa bromēšanu (9. shēma). Fosforu saturoša fragmenta ievadīšana tika iesākta ar amonija hipofosfīta (**34**) pārvēršanu par bis(trimetilsilil)-fosfonītu (**35**), kas tika izmantots reakcijā ar bromīdu **33**.[44] Pēc HMDS pievienošanas un alilēšanas sekoja apstrāde ar MeOH, iegūstot dialkilfosfīnskābi **36**.[44] Pēdējā stadijā, veicot cikla saslēgšanas metatēzi, tika iegūts mērķsavienojums **37**.



9. shēma. 1,3-Dihidrobenzfosfepīna 2-oksīda **37** sintēze.

Ņemot vērā iespējamo inhibēšanas mehānismu, kas ietver oksafosfepīna hidrolīzi, iepriekš sintezētie benzoksafosfepīna oksīdi **5h** un **6h** tika pakļauti hidrolīzes reakcijai ar NaOH ūdens šķīdumu. Reakcijas rezultātā veidojās fosfonāts **38** un fosfonskābe **39** ar *Z*-konfigurācijas dubultsaiti. Savienojuma **38** struktūra tika pierādīta ar monokristāla rentgendifraktometriju. Salīdzināšanās nolūkā hidrolizētais analogs **39** tika pārvērsts par *E*-izomēru **40**, apstarojot to ar violetās gaismas *LED* (10. shēma).



10. shēma. Fosfonāta **38** un fosfonskābju **39**, **40** sintēze (rentgendifraktometrijas pētījumi tika veikti sadarbībā ar *Dr.* S. Beļakovu LOSI Fizikāli organiskās ķīmijas laboratorijā).

Modeļsavienojumu CA izoformu inhibēšanas testu dati daļēji apstiprināja izvirzīto hipotēzi par 3*H*-1,2-benzoksafosfepīna 2-oksīdu hidrolīzi enzīma vidē. Atbilstoši šai hipotēzei cikla atvēršanas produkti **38** un **39** saglabāja selektivitāti un uzrādīja inhibitoro aktivitāti pret CA IX/XII, kas bija salīdzināma ar ciklisko analogu **5h** un **6h** aktivitāti. Savienojuma **6a** dubultsaites reģioizomēra **28** un hidrogenētā atvasinājuma **29** rezultāti liecina, ka dubultsaitei benzoksafosfepīna bāzes struktūrā ir liela nozīme CA izoformu inhibēšanā, jo tās reducēšana izraisa aktivitātes zudumu. Iespējams, dubultsaite ierobežo struktūru kustīgumu hidrolīzes gadījumā un/vai veido nozīmīgas hidrofobās mijiedarbības ar enzīma aktīvajā centrā esošajām aminoskābēm. Savukārt benzofosfepīna **37** aktivitāti varētu skaidrot ar šī mazmolekulāra liganda spēju ieiet CA IX un CA XII izoformu katalītiskajā kabatā (5. tabula).

5. tabula

CA inhibēšanas dati savienojumiem **28**, **29**, **36–40** un standarta inhibitoram AAZ

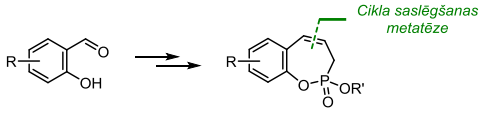
Savienojums	K_i (μM)[a,b,c]			
	CA I	CA II	CA IX	CA XII
28	>100	>100	1,6	3,5
29	>100	>100	>100	>100
36	ND	ND	ND	ND
37	>100	>100	0,86	0,78
38	>100	>100	2,14	1,98
39	>100	>100	1,80	1,67
40	ND	ND	ND	ND
AAZ*	0,25	0,012	0,025	0,006

[a] Noteikts sadarbībā ar prof. *C. T. Supuran* grupu Florences Universitātē. [b] Rezultāti ir aprēķināti kā vidējais no trīs neatkarīgiem mērījumiem (kļūdas ir ±5–10% robežās no norādītajām vērtībām). [c] Inhibitoru un enzīmu saturošie šķīdumi tika inkubēti 6 h i.t. * References standarts.

Savienojumu molekulārā modelēšana CA struktūrā ļautu noskaidrot izdevīgāko saistības veidu un sniegtu noderīgu informāciju par attīstīšanas iespējām turpmākajos pētījumos.

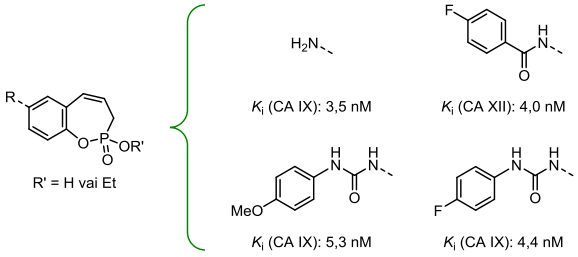
SECINĀJUMI

1. Izstrādātā sintēzes pieeja, kas ietver olefīnu veidošanos no 2-hidroksibenzaldehīdiem un cikla saslēgšanas metatēzi kā galveno stadiju, ļauj iegūt jaunus fosforu saturošus heterocikliskos savienojumus — 3*H*-1,2-benzoksafosfepīna 2-oksīdus. Šos savienojumus ir iespējams tālāk funkcionalizēt, iegūstot plašu atvasinājumu klāstu.

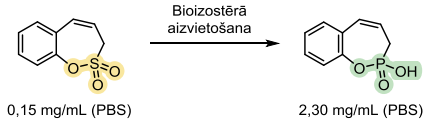


2. 3*H*-1,2-Benzoksafosfepīna 2-oksīdi ir jauna cilvēka CA inhibitoru klase, kuras pārstāvji uzrādīja augstu inhibēšanas spēju un selektivitāti uz vēža šūnās ekspresētajām CA IX un XII izoformām, salīdzinot ar organismā plaši sastopamajām CA I un II.

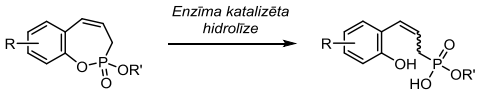
3. 7-Aminoatvasinājums, kā arī 7-acilamino- un 7-urīnvielas atvasinājumi ar dažām arilgrupām uzrādīja visaugstāko inhibitoro aktivitāti pret CA IX un/vai CA XII. Tie ir efektīvāki ar audzēju saistīto izoformu inhibitori nekā AAZ vai zāļvielas kandidāts SLC-0111. Iegūtie rezultāti liecina, ka savienojumi ir piemēroti turpmākai izpētei un attīstīšanai kā potenciāli pretvēža līdzekļi.



4. Benzoksafosfepīna oksīdi ir izmantojami kā kumarīna un tā sēru saturošu analogu bioizostēri attiecībā uz CA izoformu inhibēšanu. Sintezēto fosfororganisko mērķsavienojumu šķīdība ūdenī ir ievērojami augstāka nekā iepriekšējās paaudzes inhibitoriem, kas liecina par potenciāli uzlabotu biopieejamību.



5. CA inhibitoriem ar benzoksafosfepīna pamatstruktūru var būt līdzīgs darbības mehānisms kā radniecīgajiem kumarīniem un sulfokumarīniem. Piedāvātais savienojumu saistības veids CA IX vai CA XII aktīvajā centrā ietver oksafosfepīna hidrolīzi, kam seko saistīšanās pie aktīvā centra ieejas vai ar cinka neproteīna ligandu. Strukturālās atšķirības CA izoformu I, II, IX un XII aktīvā centra apkārtnē, kā arī 1,2-benzoksafosfepīn-2-oksīda aizvietotāju daba nosaka inhibēšanas selektivitāti.



DOCTORAL THESIS PROPOSED TO RIGA TECHNICAL UNIVERSITY FOR PROMOTION TO THE SCIENTIFIC DEGREE OF DOCTOR OF SCIENCE

To be granted the scientific degree of Doctor of Science (PhD), the present Doctoral Thesis has been submitted for defence at the open meeting of RTU Promotion Council on 11 December 2025 at 13.00 at the Faculty of Natural Sciences and Technology of Riga Technical University, Paula Valdena iela 3, Room 272.

OFFICIAL REVIEWERS

Senior researcher Dr. chem. Aiva Plotniece
Latvian Institute of Organic Synthesis, Latvia

Professor PhD Jean-Yves Winum
University of Montpellier, France

Professor PhD Eglė Arbačiauskienė
Kaunas University of Technology, Lithuania

DECLARATION OF ACADEMIC INTEGRITY

I hereby declare that the Doctoral Thesis submitted for review to Riga Technical University for promotion to the scientific degree of Doctor of Science (PhD) is my own. I confirm that this Doctoral Thesis has not been submitted to any other university for promotion to a scientific degree.

Anastasija Balašova …… (signature)

Date ……

The Doctoral Thesis has been prepared as a collection of thematically related scientific publications complemented by summaries in both Latvian and English. The Doctoral Thesis unites four scientific publications and unpublished results. The scientific publications have been written in English, with a total volume of 81 pages, including electronic supplementary data.

GENERAL OVERVIEW OF THE THESIS

Introduction

Discovered in the early 1930s, carbonic anhydrases (CA, EC 4.2.1.1) are a well-known enzyme superfamily recognised for catalysing the reversible hydration of carbon dioxide to bicarbonate anion in all living organisms.[1] They are divided into eight evolutionarily distinct gene families, namely α, β, γ, δ, ε, ζ, η, θ and ι.[2] While being structurally diverse, all CA enzymes possess a metal ion as a cofactor at their active site. The α-CA gene family contains Zn^{2+}, which plays a crucial role in the catalytic mechanism.[3] This is the most widely studied class of CAs, as it is present in vertebrates; its activity is involved in manifold physiological and pathological processes, making α-CA a target of therapeutic interest.[3,4] In humans, 15 different α-CA isozymes have been identified to date, according to their structure, cellular localisation and catalytic activity.[4]

Generally, human CA isoforms are grouped based on subcellular localisation: cytosolic (CA I, II, III, VII, XIII) isoforms, mitochondrial (CA VA, VB), secreted (CA VI), as well as membrane-bound (CA IV, IX, XII, XIV) isoforms.[4] Moreover, there are three catalytically inactive CA-related proteins (CARP VIII, X, XI), whose biological functions remain poorly defined.[4,5] The dysregulated expression and activity of catalytic forms of CA are often associated with a variety of medical conditions, including cancer, glaucoma, metabolic and neurological disorders.[4] In particular, two transmembrane isoforms, CA IX and CA XII, were shown to be overexpressed in many types of solid tumours, contributing to tumour progression and metastasis through the regulation of intra- and extracellular pH.[6–9] Their expression can also serve as a diagnostic and prognostic biomarker in certain cancer histotypes, and is correlated with therapeutic resistance due to involvement in hypoxia and acidosis.[6,10,11] Therefore, designing and developing selective CA IX/XII inhibitors represents a potential anticancer strategy with promising clinical applications in both conventional and combination therapies.

To date, the three-dimensional structures of the majority of the human CA isoforms have been elucidated by X-ray crystallography, facilitating the development of CA inhibitors.[2–4] The active site of catalytically active isoforms is located in a conical cavity comprising hydrophobic and hydrophilic regions, which are essential for enzymatic reactions.[2–4] At the deepest part of this cavity, Zn^{2+} is embedded and tetrahedrally coordinated by three histidine residues and a water molecule or hydroxide ion.[2–4] High amino acid sequence identity within the catalytic domain and overall structural similarity between human CAs pose a challenge for isoform-specific inhibition. In the framework of the Doctoral Thesis, the author decided to specifically target the inhibition of CA IX and CA XII, given their overexpression in cancerous tissues and limited expression in normal tissues, while preserving the activity of the housekeeping isoforms CA I and CA II.

Over the past few decades, numerous studies have focused on the interactions between CAs and their inhibitors, which can provide valuable clues for rational drug design. So far, four different inhibition mechanisms have been identified: 1) direct coordination to the catalytic zinc ion (zinc-binders are classical CA inhibitors, which include primary sulphonamides, (in)organic anions, carbamates, thiols, selenols, benzoxaboroles); 2) anchoring to the zinc-coordinated water/hydroxide ion (observed with phenols, polyamines and sulphocoumarins **A** (Fig. 1)); 3) occlusion of the active site entrance (coumarins, thiocoumarins); and 4) peripheral site binding (one carboxylic acid

derivative, 2-(benzylsulphinyl)benzoic acid, was found bound outside the active site — in a hydrophobic pocket adjacent to the entrance of the active site cavity).[2–4] Additionally, there are many CA-inhibiting compounds with unknown mechanisms of action.

Several classical CA inhibitors, such as acetazolamide (AAZ), methazolamide, sulthiame and dorzolamide, are clinically used as diuretics, antiglaucoma agents or antiepileptic drugs.[2] Compounds containing the $–SO_2NH_2$ group, which binds to Zn^{2+}, are among the most effective CA inhibitors; however, their non-selective inhibition profile is linked to severe side effects. For this reason, various novel CA inhibitors with improved selectivity have been introduced, one of which is the drug candidate SLC-0111, designed to inhibit the tumour-associated isoforms (Fig. 1).[12,13] SLC-0111 has advanced to clinical trials in patients with metastatic solid tumours.[14] This reflects the promising therapeutic niche that CA inhibitors may occupy in the treatment of oncological maladies.

Our research group's main priority is the exploration of non-classical coumarin-type CA inhibitors, whose binding mechanism does not involve coordination to Zn^{2+}.[15] Instead, coumarins bind within the active site of the enzyme in their hydrolysed form, thereby occluding substrate entry.[15,16] They were initially detected as CA inhibitors during high-throughput screening of natural products and attracted broad interest owing to their ability to inhibit multiple α-CA isoforms.[15,17] Efforts in advancing the potential of coumarin-based inhibitors have led to the development of sulphocoumarins **A** (1,2-benzoxathiine 2,2-dioxides) and their congeners — homosulphocoumarins **B** (3*H*-1,2-benzoxathiepine 2,2-dioxides) as selective CA IX and XII inhibitors (Fig. 1).[18–24] In this Doctoral Thesis, a novel chemotype — 3*H*-1,2-benzoxaphosphepine 2-oxide **C** — is studied as a platform for next-generation CA inhibitors.

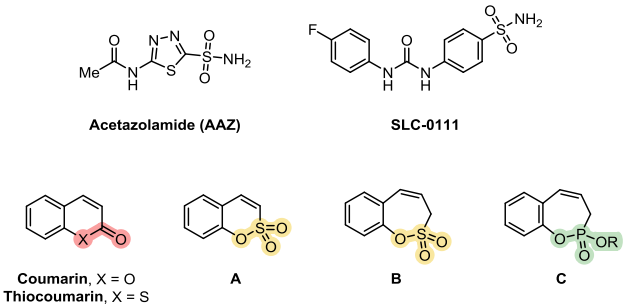


Fig. 1. Structures of CA inhibitors.

Aims and objectives

The Doctoral Thesis aims to develop effective and selective CA inhibitors on the basis of the phosphepine oxide **C** scaffold, targeting the tumour-associated isoforms IX and XII, with potential therapeutic applications. The following tasks were set to achieve this aim:

1. develop a methodology for the synthesis and derivatisation of 3*H*-1,2-benzoxaphosphepine 2-oxide;
2. determine the inhibitory activity of the synthesised analogues against a set of human CA isoforms;

3. explore the effects of the compounds on cell viability and evaluate their aqueous solubility;
4. investigate the structure–activity relationship (SAR) and provide insights into the binding mechanism.

Scientific novelty and main results

The research presented in the Thesis contributes to the field of selective carbonic anhydrase inhibitors by developing novel benzoxaphosphepine-based compounds. The established general synthetic pathway enabled access to a diverse array of substituted 3*H*-1,2-benzoxaphosphepine 2-oxides. The obtained compounds were investigated for their ability to inhibit various CA isoforms, showing the preferential activity towards the tumour-associated isoforms IX and XII. A series of inhibitors with low nanomolar potency against CA IX/XII, without off-target effects, was discovered. In addition, several representatives of the benzoxaphosphepine oxide class were evaluated for cytotoxicity and aqueous solubility. The core inferences from SAR studies, as well as mechanistic insights into the binding mode, were drawn. The results highlight the potential of these inhibitors as drug candidates that deserve further exploration.

Structure of the Thesis

The Thesis is a collection of thematically related scientific publications focused on the design and development of 3*H*-1,2-benzoxaphosphepine 2-oxide derivatives as potential anticancer agents targeting carbonic anhydrase IX and XII isoforms.

Each section of the results chapter of the Thesis presents a summary of a single publication in chronological order.

Publications and approbation of the Thesis

The main results of the Thesis were summarised in three scientific publications and one review article. Additionally, the research findings were presented at nine conferences.

Scientific publications

1. **Balašova, A.**; Žalubovskis, R. Synthetic methods toward phosphacoumarins (microreview). *Chem. Heterocycl. Comp.* **2022**, *58*, 310–312.
2. Pustenko, A.; **Balašova, A.**; Nocentini, A.; Supuran, C. T.; Žalubovskis, R. 3*H*-1,2-Benzoxaphosphepine 2-oxides as selective inhibitors of carbonic anhydrase IX and XII. *J. Enzyme Inhib. Med. Chem.* **2023**, *38* (1), 216–224.
3. **Balašova, A.**; Pustenko, A.; Nocentini, A.; Vullo, D.; Supuran, C. T.; Žalubovskis, R. Aryl derivatives of 3*H*-1,2-benzoxaphosphepine 2-oxides as inhibitors of cancer-related carbonic anhydrase isoforms IX and XII. *J. Enzyme Inhib. Med. Chem.* **2023**, *38* (1), 2249267.
4. **Balašova, A.**; Pustenko, A.; Angeli, A.; Andreucci, E.; Biagioni, A.; Nocentini, A.; Carta, F.; Supuran, C. T.; Žalubovskis, R. Unraveling the potential of amino-, acylamino-, and ureido-substituted 3*H*-1,2-benzoxaphosphepine 2-oxides toward nanomolar inhibitors of tumor-associated carbonic anhydrases IX and XII. *ACS Med. Chem. Lett.* **2025**, *16* (6), 1031–1037.

5. Manuscript regarding the last chapter of the Thesis is in preparation.

Conference participation

1. **Balašova, A.**; Žalubovskis, R. Benzoxaphosphepine 2-oxides as potential carbonic anhydrase inhibitors. *56th International Conference on Medicinal Chemistry (RICT 2021)*, virtual event, July 7–9, **2021**.
2. **Balašova, A.** Benzoxaphosphepine 2-oxides as potential carbonic anhydrase inhibitors. *12th Paul Walden Symposium on Organic Chemistry*, virtual event, October 28–29, **2021**.
3. **Balašova, A.**; Žalubovskis, R. Benzoxaphosphepine 2-oxides as potential carbonic anhydrase inhibitors. *Balticum Organicum Syntheticum (BOS 2022)*, Vilnius, Lithuania, July 3–6, **2022**.
4. **Balašova, A.**; Žalubovskis, R. Benzoxaphosphepine 2-oxides as potential carbonic anhydrase inhibitors. *Drug Discovery Conference 2022*, Riga, Latvia, September 22–24, **2022**.
5. **Balašova, A.**; Žalubovskis, R. Development of benzoxaphosphepine 2-oxides as carbonic anhydrase inhibitors. *81st International Scientific Conference of the University of Latvia*, Riga, Latvia, March 17, **2023**.
6. **Balašova, A.**; Žalubovskis, R. Design and synthesis of benzoxaphosphepine 2-oxides as carbonic anhydrase inhibitors. *12th International conference on Carbonic Anhydrases*, Naples, Italy, July 5–7, **2023**.
7. **Balašova, A.**; Pustenko, A.; Žalubovskis, R. Development of benzoxaphosphepine 2-oxides as carbonic anhydrase inhibitors. *Autoimmune diseases: main problems and solutions*, Riga, Latvia, November 9–10, **2023**.
8. **Balašova, A.**; Pustenko, A.; Žalubovskis, R. Benzoxaphosphepine 2-oxides — a novel class of carbonic anhydrase inhibitors. *Conference "Achievements of the SPRINGBOARD project"*, Riga, Latvia, May 2–3, **2024**.
9. **Balašova, A.**; Pustenko, A.; Žalubovskis, R. Benzoxaphosphepine 2-oxides — a novel class of carbonic anhydrase inhibitors. *Balticum Organicum Syntheticum (BOS 2024)*, Riga, Latvia, July 7–10, **2024**.

MAIN RESULTS OF THE THESIS

Prologue

Phosphorus-containing compounds have demonstrated a wide spectrum of biological activities, including antitumour, antimicrobial and anti-inflammatory properties as well as inhibitory effects against various enzymes.[25,26] The introduction of phosphorus moieties influences both the pharmacokinetics and the pharmacodynamics of drugs, which renders them attractive for application in medicinal chemistry.[25–28] Moreover, phosphorus functionalities can serve as bioisosteres of different functional groups, frequently providing enhanced bioavailability characteristics.[25,29] In this regard, we envisaged that benzoxaphosphepine oxides **C** might have potential as bioisosteres for (sulpho)coumarin analogues (Fig. 1).

The methodology for the synthesis of fused phosphorus-containing heterocycles is relatively limited. Among several transition-metal-catalysed approaches to form benzofused *P*-heterocycles, the use of ring-closing metathesis proved to be a versatile tool for the preparation of 7-membered benzannulated compounds. This method has been successfully applied to the synthesis of both sulphur- and phosphorus-containing analogues (Fig. 2).[22,30] Consequently, a similar strategy would allow to access 3*H*-1,2-benzoxaphosphepine 2-oxides **C**. The following sections outline the synthesis and biological evaluation of these novel organophosphorus derivatives.

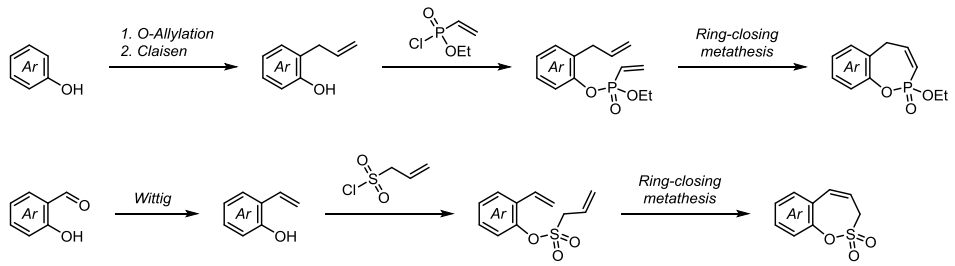
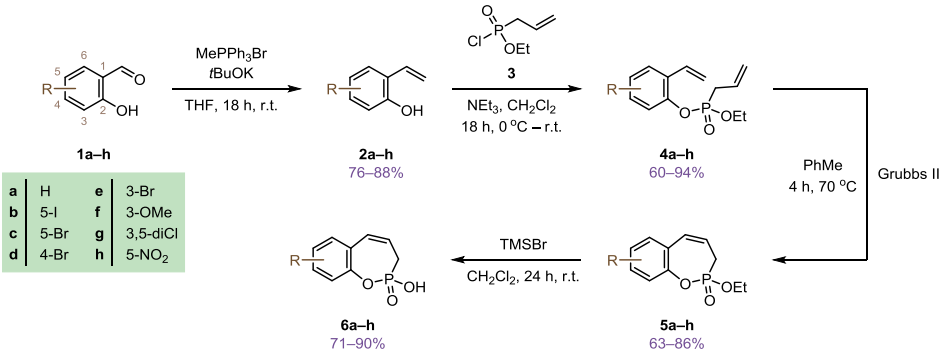


Fig. 2. Routes towards benzofused *P*- or *S*-containing 7-membered heterocycles.

The synthetic methods of phosphacoumarins and their homologues are summarised in the review article in *Chem. Heterocycl. Comp.* **2022**, *58*, 310–312.

1. Synthesis and inhibitory potential of benzoxaphosphepine chemotype

The general synthetic route leading to 3*H*-1,2-benzoxaphosphepine 2-oxides was established based on previously described methodology involving ring-closing metathesis reaction for the synthesis of structurally related compounds.[22,30] At first, commercially available salicylaldehydes **1a–h** were converted to vinylphenols **2a–h** using Wittig olefination (Scheme 1). Subsequent phosphorylation with chloride **3** afforded diolefins **4a–h**, which underwent ring-closing metathesis in the presence of Ru-based catalyst to generate benzoxaphosphepine scaffold. The final cleavage of the ethyl group in **5a–h** was achieved with TMSBr. This robust protocol provided compounds **6a–h** in overall good to excellent yields.



Scheme 1. Synthetic procedure towards benzoxaphosphepine oxides **5** and **6**.

In order to evaluate the inhibitory activity and selectivity of the synthesised cyclic phosphonates **5** and **6** (Fig. 3), compounds were screened against four pharmacologically relevant CA isoforms by means of the stopped-flow CO_2 hydration assay.[31] In this study, the ubiquitous CA I and II are regarded as off-target isoforms, whose inhibition is undesirable in contrast to the inhibition of the cancer-associated isoforms CA IX and XII. The activities of inhibitors, calculated as K_i (inhibition constants) values, were compared to the standard drug AAZ.

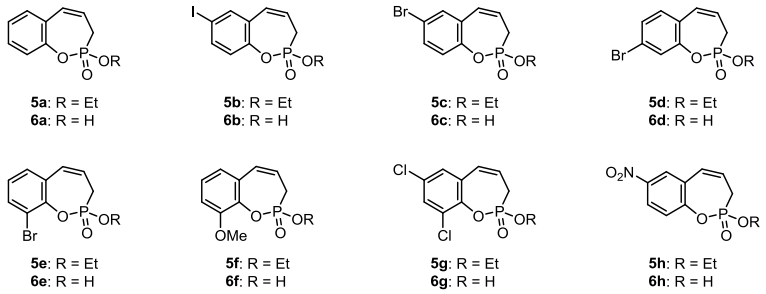


Fig. 3. The scope of potential CA inhibitors **5** and **6**.

The *in vitro* assay results revealed that all the tested compounds **5** and **6** possess the desired activity and isoform selectivity by inhibiting only CA IX and CA XII isoforms. Regardless of the substitution pattern on the aromatic fragment or phosphorus atom (OH *vs* OEt), the activity of these

derivatives lies in the micromolar range, reaching even the submicromolar level, as in compound **6b** (Table 1). These findings also indicate the bioisosteric relationship between sulphocoumarins **A**, **B** and benzoxaphosphepine oxides acting as CA IX/XII inhibitors. Although the inhibitory potential of cyclic phosphonates **5** and **6** was determined to be weaker than that of AAZ, this novel phosphorus-containing chemotype laid the groundwork for further structural optimisation in developing more potent inhibitors.

Table 1

Inhibition data of compound series **5**, **6** and the standard inhibitor AAZ against CA I, II, IX and XII

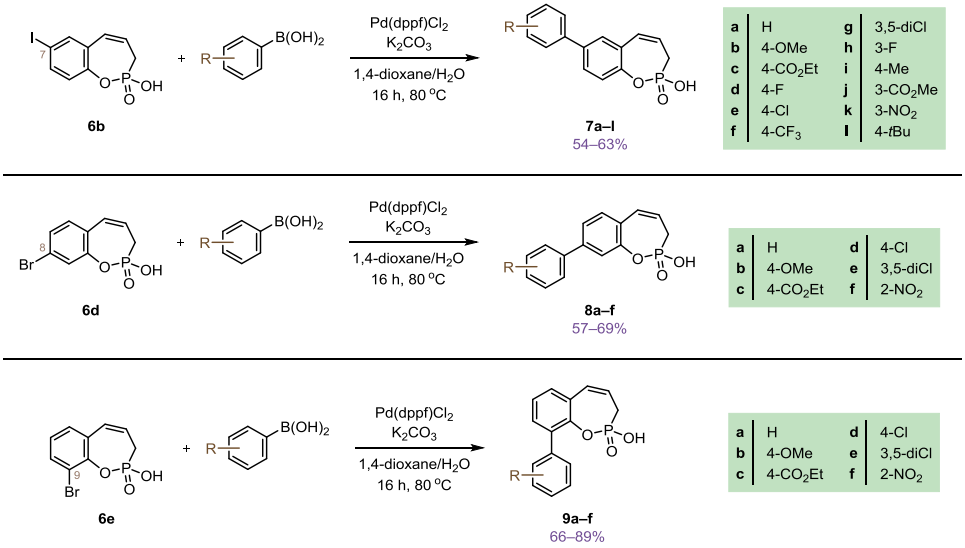
Compound	K_i (μM)[a,b,c]			
	CA I	CA II	CA IX	CA XII
5a	>100	>100	0.82	0.82
6a	>100	>100	1.3	0.51
5b	>100	>100	4.7	2.4
6b	>100	>100	0.88	0.68
5c	>100	>100	0.76	1.6
6c	>100	>100	1.0	0.96
5d	ND	ND	ND	ND
6d	>100	>100	3.4	1.5
5e	>100	>100	11.3	3.3
6e	>100	>100	2.5	1.8
5f	>100	>100	9.0	7.4
6f	>100	>100	1.8	1.2
5g	>100	>100	6.1	3.4
6g	>100	>100	0.80	1.7
5h	>100	>100	3.9	0.95
6h	>100	>100	0.67	1.0
AAZ*	0.25	0.012	0.025	0.006

[a] Determined in collaboration with Prof. C. T. Supuran's group from the University of Florence. [b] Values are the mean from three different assays using the stopped-flow technique (errors were in the range of ±5–10% of the reported values). [c] Inhibitor and enzyme solutions were preincubated together for 6 h at r.t. * Reference standard.

The results presented in this section are described in the scientific publication in *J. Enzyme Inhib. Med. Chem.* **2023**, *38* (1), 216–224.

2. Exploration of chemical space around benzoxaphosphepine scaffold

Going forward, the research was directed towards the detailed examination of the substitution effect on carbonic anhydrase inhibition by introducing various aryl groups in the benzene fragment of the benzoxaphosphepine core. A series of aryl derivatives **7–9** was conveniently obtained in the Suzuki–Miyaura cross-coupling reaction between halides **6b,d,e** and commercial arylboronic acids (Scheme 2). The resultant 7-, 8- and 9-aryl-substituted products would allow us to gain additional insight into the positional preference of different tails in inhibitors of such type for enhanced suppression of CA IX/XII activity. Alas, 6-aryl counterparts were not obtained due to increased steric hindrance during the ring-closing metathesis step in the synthesis of the corresponding halide.[23]



Scheme 2. Synthesis of benzoxaphosphepine oxide 7-, 8-, 9-aryl derivatives **7–9**.

The aryl analogues **7–9** were subjected to the same inhibition assay against CA I, II, IX and XII with AAZ serving as the reference inhibitor. The data indicated that these compounds exhibited similar activity (μM range) as their precursors **6b,d,e**, as well as retained selectivity for the target CA isoforms. Among the synthesised biaryls **7–9**, 9-substituted derivatives **9** showed the poorest inhibition of CA IX/XII, whereas the potencies of 7- and 8-substituted derivatives **7** and **8** were substantially higher (Table 2). Generally, representatives of compound series **7** and **8** displayed comparable inhibitory activity; however, a slightly better inhibition profile was observed in the case of **7**. This trend was also evident in aryl-substituted sulphocoumarins **A** and their congeners **B**.[20,23] Therefore, further functionalisation was performed at position 7 of the benzoxaphosphepine scaffold.

Table 2

Inhibition data of compound series **7–9** and the standard inhibitor AAZ against CA I, II, IX and XII

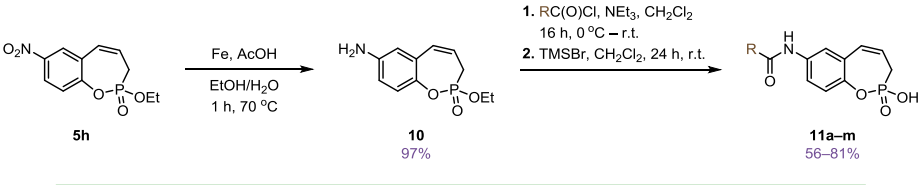
Compound	K_i (μM)[a,b,c]			
	CA I	CA II	CA IX	CA XII
7a	>100	>100	0.77	0.95
7b	>100	>100	4.6	1.7
7c	>100	>100	6.0	6.7
7d	>100	>100	0.86	0.25
7e	>100	>100	8.6	1.1
7f	>100	>100	3.7	0.59
7g	>100	>100	7.3	4.2
7h	>100	>100	0.63	0.56
7i	>100	>100	1.5	0.94
7j	>100	>100	9.5	1.5
7k	>100	>100	7.6	0.64
7l	>100	>100	4.9	0.97
8a	>100	>100	1.8	2.7
8b	>100	>100	2.1	5.6
8c	>100	>100	10.2	3.8
8d	>100	>100	5.0	7.1
8e	>100	>100	0.98	0.84
8f	>100	>100	12.9	0.67
9a	>100	>100	16.5	25.5
9b	>100	>100	39.4	52.4
9c	>100	>100	55.3	>100
9d	>100	>100	48.9	65.3
9e	>100	>100	22.4	28.2
9f	>100	>100	38.4	35.1
AAZ*	0.25	0.012	0.025	0.006

[a] Determined in collaboration with Prof. C. T. Supuran's group from the University of Florence. [b] Values are the mean from three different assays using the stopped-flow technique (errors were in the range of ±5–10% of the reported values). [c] Inhibitor and enzyme solutions were preincubated together for 6 h at r.t. * Reference standard.

The results presented in this section are described in the scientific publication in *J. Enzyme Inhib. Med. Chem.* **2023**, *38* (1), 2249267.

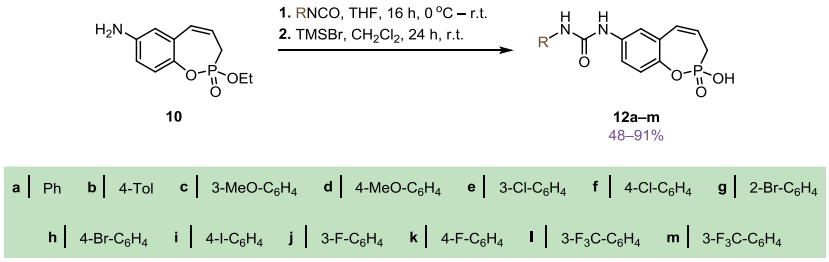
3. Paving the way to nanomolar inhibition of the target isozymes

In the following investigation, the incorporation of an amide functional group into the scaffold of benzoxaphosphepine oxide was considered. Previous findings on benzoxathiine and benzoxathiepine dioxides **A** and **B** uncovered that the presence of the acylamino functionality contributes to enhanced inhibitory activity towards CA IX and CA XII isoforms.[21,24] In the first step, nitro derivative **5h**, prepared in the earlier part of this research, was reduced to aniline **10**, which was then acylated by aroyl or heteroaroyl chlorides. The intermediate phosphonates underwent the ethyl group cleavage, furnishing the corresponding amides **11a–m** (Scheme 3).



Scheme 3. Synthesis of benzoxaphosphepine oxide (acyl)amino derivatives **10** and **11**.

Aside from acylamino derivatives **11**, ureido analogues **12** were also synthesised, as this structural motif is present in SLC-0111 — a potent CA IX/XII inhibitor that completed Phase I clinical trials (NCT02215850). Moreover, urea-containing compounds are increasingly utilised in drug design and development in order to improve pharmacological properties.[32] The preparation of benzoxaphosphepine oxide ureido derivatives was achieved by reaction of aniline **10** with various commercial isocyanates (Scheme 4). Successive TMSBr-mediated *O*-deethylation provided the corresponding *N,N'*-diarylureas **12a–m** in moderate to excellent yields.



Scheme 4. Synthesis of benzoxaphosphepine oxide ureido derivatives **12**.

The inhibition profiles of amino-, acylamino- and ureido-substituted benzoxaphosphepine oxides **10–12** against CA isoforms I, II, IX and XII showed intriguing results (Table 3). Consistent with previously studied analogues **5–9**, no inhibitory activity against off-target isoforms CA I and II was detected. Remarkably, all the newly synthesised compounds displayed inhibition of the tumour-associated CA IX in the nanomolar range. However, the activity of CA XII isoform was suppressed only by aniline **10** and acylamino derivatives **11**, while ureas **12** exclusively inhibited CA IX.

Table 3

Inhibition data of compound series **10–12** and the reference compounds SLC-0111, AAZ against CA I, II, IX and XII

Compound	K_i (nM)[a,b,c]			
	CA I	CA II	CA IX	CA XII
10	>10000	>10000	3.5	16.6
11a	>10000	>10000	10.8	7.3
11b	>10000	>10000	20.5	7.8
11c	>10000	>10000	10.1	8.1
11d	>10000	>10000	29.4	6.7
11e	>10000	>10000	158.4	64.1
11f	>10000	>10000	25.8	84.4
11g	>10000	>10000	23.2	8.9
11h	>10000	>10000	18.7	57.5
11i	>10000	>10000	14.6	6.9
11j	>10000	>10000	27.4	4.0
11k	>10000	>10000	20.1	27.2
11l	>10000	>10000	22.3	9.7
11m	>10000	>10000	32.8	74.6
12a	>10000	>10000	34.9	>10000
12b	>10000	>10000	46.9	>10000
12c	>10000	>10000	53.3	>10000
12d	>10000	>10000	5.3	>10000
12e	>10000	>10000	52.9	>10000
12f	>10000	>10000	54.8	>10000
12g	>10000	>10000	46.9	>10000
12h	>10000	>10000	36.8	>10000
12i	>10000	>10000	36.0	>10000
12j	>10000	>10000	51.8	>10000
12k	>10000	>10000	4.4	>10000
12l	>10000	>10000	31.9	>10000
12m	>10000	>10000	42.7	>10000
SLC-0111[12]	5080	960	45.1	4.5
AAZ*	250	12.1	25.8	5.7

[a] Determined in collaboration with Prof. C. T. Supuran's group from the University of Florence. [b] Values are the mean from three different assays using the stopped-flow technique (errors were in the range of ±5–10% of the reported values). [c] Inhibitor and enzyme solutions were preincubated together for 6 h at r.t. * Reference standard.

Of note, it has been reported that the expression of CA XII has minimal impact on tumour cell proliferation in some cancer subtypes, compared to CA IX.[33] Furthermore, the presence of CA XII in multiple normal tissues highlights the necessity of selective CA IX inhibitors over CA XII in certain cases.[33,34] From this perspective, urea-containing derivatives **12** hold particular importance

in the pharmacological field and may offer potential benefits due to reduced side effects arising from non-specific inhibition.

Aniline **10**, amides **11a,c** and ureas **12d,k** emerged as the most effective inhibitors of CA IX and/or CA XII, with K_i values equivalent to, or even exceeding, those of SLC-0111 and AAZ. These promising compounds warranted further investigation. Thus, the cytotoxicity of **10**, **11c**, **12d** was assessed *in vitro* on A375 melanoma cells, HCT-116 colorectal cancer cells and MDA-MB-231 triple-negative breast cancer cells cultivated under standard conditions. Cell viability was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay.[35,36] The results showed that compounds **10** and **11c** exhibited low cytotoxic activities across the tested cell lines. Conversely, compound **12d** exerted more pronounced cancer cell cytotoxicity — treatment with a 100 μM solution of **12d** reduced proliferation by 40% in HCT-116 cells and by 50% in A375 and MDA-MB-231 cells (Fig. 4).

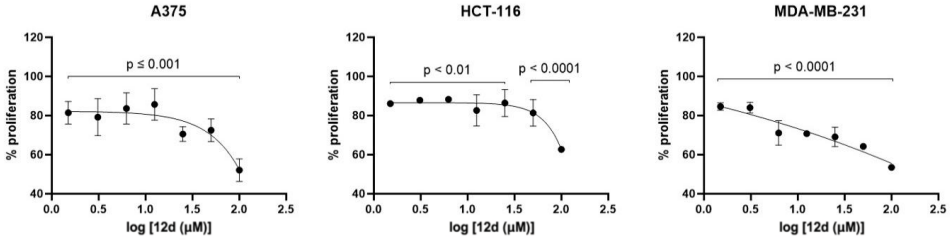


Fig. 4. Cytotoxic effect of **12d** on A375, HCT-116 and MDA-MB-231 cancer cell lines after 72 hours of incubation (N = 4; data was analysed using GraphPad Prism 10.3.1; determined in collaboration with Dr. E. Andreucci and Dr. A. Biagioni from the University of Florence).

While some known CA inhibitors possess high cytotoxic potential,[37,38] their primary anticancer value often lies in the ability to modulate the tumour microenvironment, reducing acidosis as well as impairing tumour growth and metastasis.[9] Moreover, CA IX/XII inhibitors can be synergistic with other drugs by chemosensitising tumour cells.[39,40] In preclinical *in vitro* studies, SLC-0111 did not affect cancer cell viability under normoxic and standard pH (7.4) conditions; however, a notable cytotoxic effect was observed under acidic pH (6.7) or hypoxic conditions.[41] Importantly, SLC-0111 featured antiproliferative and antimetastatic properties in *in vivo* breast cancer models.[12,13]

Additional information regarding the therapeutic potential of benzoxaphosphepine oxides can be gained by examining the toxicity and antiproliferative efficacy across a broader panel of cell lines, especially cancer cell lines with higher expression of CA IX/XII and under hypoxic conditions. Given the observed isozyme selectivity and potent activity in the inhibition of CA, the study on cancer cell lines that exhibit high expression of CA IX and do not express CA XII (or *vice versa* — CA IX-negative and CA XII-positive) would allow us to draw the correlation between the inhibition of these proteins and the antiproliferation of cancer cells.

Complementing the aforementioned findings, the aqueous solubility of members of the 3*H*-1,2-benzoxaphosphepine 2-oxide class was determined and compared with representatives of the 3*H*-1,2-benzoxathiepine 2,2-dioxide class. The analysis was performed in phosphate-buffered saline solution (PBS; pH ~ 7.4) at 25 °C using the HPLC-UV method. From the collected data, it is

discernible that the transition from sultones to cyclic phosphonic acids has a significant effect on aqueous solubility. The phosphono derivatives **6a**, **7h**, **11c**, **11j**, **12d**, **12k** have markedly higher solubility than the corresponding sultone derivatives **B**, **B1**, **B2** (Fig. 5). Interestingly, no influence on the solubility of benzoxaphosphepine oxides was observed upon varying the side chain. In addition, the presence of a free phosphonic acid group allows the formation of salts, which are expected to increase water solubility. In drug development, solubility in water is a critical factor for drug absorption, bioavailability and administration. Poor pharmacokinetic properties are often linked to low solubility and limited permeability.[32]

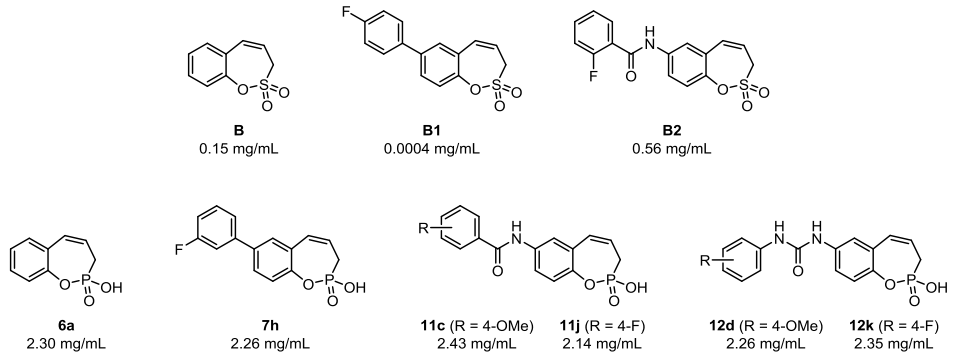


Fig. 5. Solubility of the selected inhibitors in PBS buffer (measurements were done in collaboration with Dr. H. Kažoka's group at the LIOS Laboratory of Chromatography).

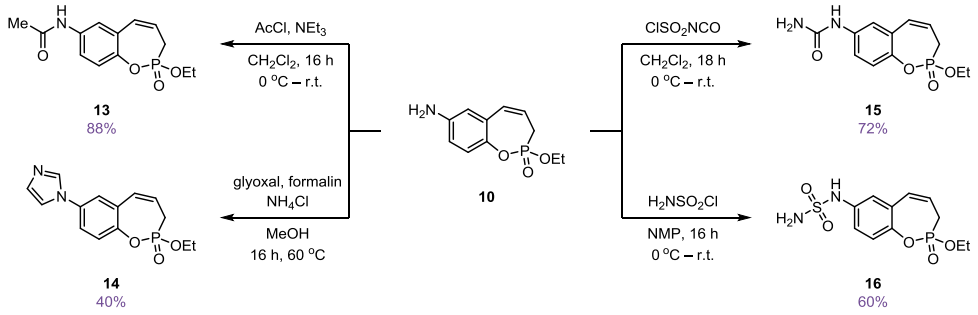
The results presented in this section are described in the scientific publication in *ACS Med. Chem. Lett.* **2025**, *16* (6), 1031–1037.

4. Expanding the library of inhibitors and probing the binding mechanism

(Unpublished results)

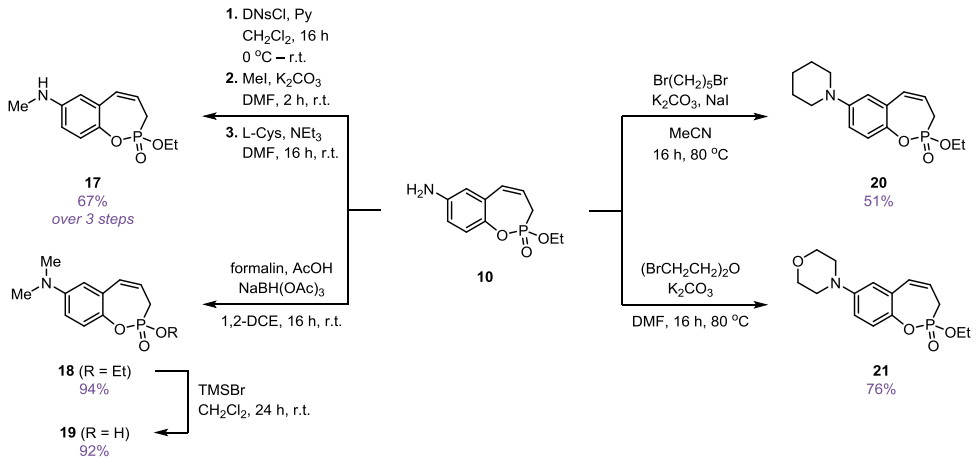
Building on the potential of nitrogen-containing benzoxaphosphepine oxides **10–12**, the synthesis of amino derivative **10** analogues as CA inhibitors was carried out. Aniline **10** showcased exceptional inhibitory activity against CA IX/XII, exhibiting high ligand efficiency, and thus served as the benchmark for further structural diversification of the benzoxaphosphepine scaffold.

A wide array of novel benzoxaphosphepine oxide derivatives were synthesised from aniline **10** as a starting material. Acetylation of **10** furnished acetamide **13**, whereas reaction with CSI provided *N'*-unsubstituted urea **15**. In turn, sulphamide **16** was prepared using sulphamoyl chloride. The Debus–Radziszewski reaction yielded imidazole **14** to verify the possible bioisosterism between imidazolyl and aryl groups (Scheme 5).



Scheme 5. Synthesis of *N*-containing benzoxaphosphepine oxide derivatives **13–16**.

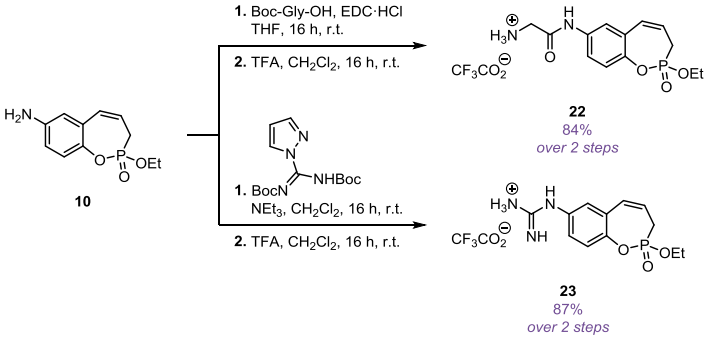
The preparation of *N*-methyl derivative **17** was accomplished by using the Fukuyama method,[42] in which the starting aniline **10** was converted to 2,4-dinitrobenzenesulphonamide. Alkylation with MeI followed by deprotection of the DNs group in the presence of L-cysteine and NEt_3 afforded the desired secondary amine **17** (Scheme 6).



Scheme 6. Synthesis of *N*-containing benzoxaphosphepine oxide derivatives **17–21**.

The employment of reductive amination of formaldehyde produced *N*,*N*-dimethyl derivative **18**, which was also *O*-deethylated under standard McKenna conditions to give **19** in excellent yield. The construction of piperidine and morpholine cycles in **20** and **21** was readily done utilising alkylation of **10** with 1,5-dibromopentane or bis(2-bromoethyl) ether, respectively (Scheme 6).

Adding to the structural diversity, aniline **10** was transformed into glycinamide derivative **22** and guanidine derivative **23** by treatment with *N*-Boc-protected glycine or 1*H*-pyrazole-1-carboxamidine. Removal of protecting groups under acidic conditions facilitated the isolation of the target compounds as trifluoroacetate salts (Scheme 7).



Scheme 7. Synthesis of *N*-containing benzoxaphosphepine oxide derivatives **22** and **23**.

These *N*-containing analogues **13–23** were tested for their inhibitory activity against CA I, II, IX and XII. Compounds showed varied activity, ranging from 23 nM to 3.44 μM (Table 4).

Table 4

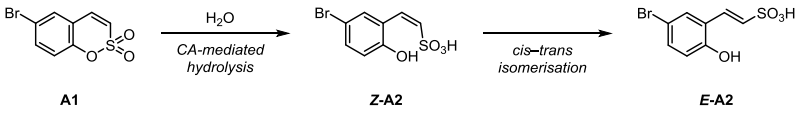
Inhibition data of compounds **13–23** and the standard inhibitor AAZ against CA I, II, IX and XII

Compound	K_i **(nM)**[a,b,c]			
	CA I	CA II	CA IX	CA XII
13	>10000	>10000	620	870
14	ND	ND	ND	ND
15	>10000	>10000	51	3440
16	370	94	37	23
17	>10000	>10000	840	710
18	>10000	>10000	950	760
19	>10000	>10000	920	810
20	>10000	>10000	1130	1070
21	>10000	>10000	990	1280
22	>10000	>10000	920	650
23	>10000	>10000	1230	1150
AAZ*	250	12.1	25.8	5.7

[a] Determined in collaboration with Prof. C. T. Supuran's group from the University of Florence. [b] Values are the mean from three different assays using the stopped-flow technique (errors were in the range of ±5–10% of the reported values). [c] Inhibitor and enzyme solutions were preincubated together for 6 h at r.t. * Reference standard.

The salient SAR findings indicated that tailored nitrogen-atom modifications to aniline **10**, particularly the introduction of aliphatic motifs, led to diminished inhibitory potency (as evidenced in **13** *vs* **10**, **11a–m**; and **17–22** *vs* **10**). All acyclic and cyclic aliphatic amines **17–21**, as well as amides **13** and **22**, were less potent against CA IX/XII when compared to the corresponding aromatic derivatives **11** and **12** and the unsubstituted amine **10** (Tables 3 and 4). These aliphatic substitutions in the *N*-containing side chain of the benzoxaphosphepine oxide scaffold had detrimental effects on the inhibitory activity, emphasising the importance of the delicate balance of electronic and steric effects required for optimal interactions with the target isozymes. Interestingly, the *N'*-unsubstituted urea derivative **15** demonstrated a similar selectivity trend, exhibiting high selectivity towards the CA IX isoform over CA XII, as observed in the previously analysed *N'*-aryl-substituted ureido derivatives **12a–m**. Furthermore, among the assessed compounds, only sulphamide **16** inhibited the off-target CA I and II, which was anticipated, given the pivotal role that the $–SO_2NH_2$ group has in the inhibition of active CA isoforms by coordinating with the catalytic zinc ion.[4] Compound **16**, in fact, was a more effective inhibitor of the target isoforms CA IX/XII than the off-target CA I/II, thereby showing the efficacy of the benzoxaphosphepine oxide moiety to induce the desired selectivity (Table 4).

Since cocrystallisation trials between CA and different 3*H*-1,2-benzoxaphosphepine 2-oxides were unsuccessful, the respective binding mechanism of benzoxaphosphepine-based CA inhibitors remains unclear and is a matter of ongoing discussion. Currently, two main hypotheses are under consideration: 1) 3*H*-1,2-benzoxaphosphepine 2-oxides are genuine inhibitors that directly interact with the active site in their intact form; 2) 3*H*-1,2-benzoxaphosphepine 2-oxides are prodrugs that undergo hydrolysis, with the resulting products then acting as active compounds. The observation that the inhibition of CAs was time-dependent, requiring a 6-hour incubation to detect the optimal effect, led us to assume that benzoxaphosphepine oxides can be considered as prodrugs. Moreover, structurally related coumarins and sulphocoumarins were shown to be hydrolysed by the esterase activity of CA to yield the corresponding acid derivatives prior to binding (Fig. 6); the mechanism of action of such compounds was corroborated by crystallographic studies.[16,19]



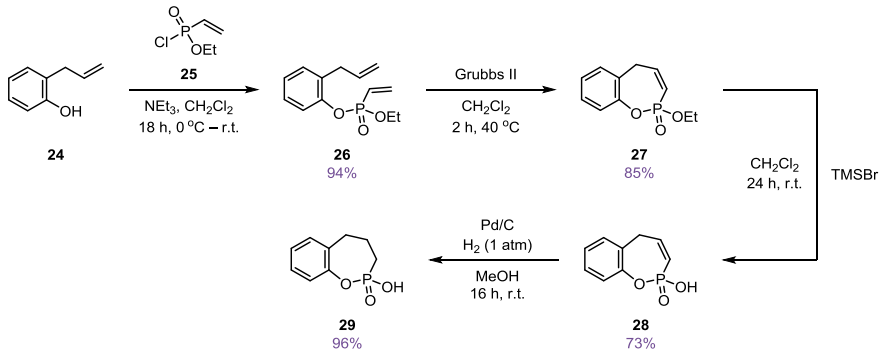
A1 **Z-A2** ***E*-A2**

Fig. 6. CA-mediated hydrolysis of sulphocoumarin **A1** to ***E*-A2**.[19]

The combination of *in silico* analysis with *in vitro* studies of various model substrates would provide a tool for understanding the binding mode in lieu of an X-ray cocrystal structure. To determine the contributions of the double bond and the *O*-tether in the oxaphosphepine moiety, several additional compounds were synthesised.

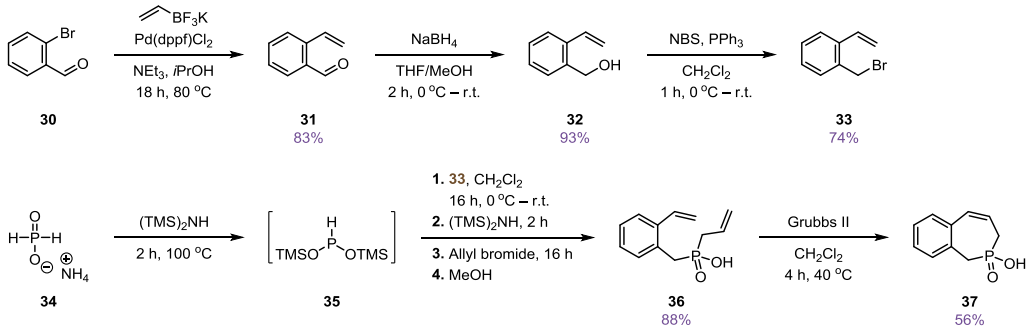
The synthesis of 5*H*-1,2-benzoxaphosphepine and 3,4,5-trihydrobenzoxaphosphepine 2-oxides (**28** and **29**) was completed in a similar manner to **6**, starting from 2-allylphenol (**24**) (Scheme 8). The resultant diolefin **26**, which was formed by phosphorylation of **24** with chloride **25**, gave the cyclisation product **27** *via* ring-closing metathesis. Notably, changing the solvent from PhMe to CH_2Cl_2, as well as decreasing the reaction temperature, prevented migration of the double bond in

27. The further removal of the ethyl group by means of TMSBr afforded product **28**, which was subjected to hydrogenation to yield trihydro analogue **29**.



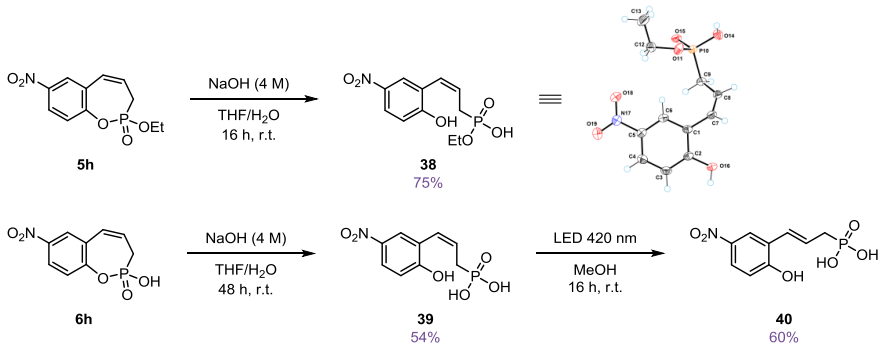
Scheme 8. Synthesis of benzoxaphosphepine oxide analogues **28** and **29**.

In order to prepare the cyclic phosphinic acid **37**, 2-vinylbenzyl bromide (**33**) was obtained from 2-bromobenzaldehyde (**30**) according to the literature procedure (Scheme 9).[43] The synthetic sequence included the Suzuki–Miyaura coupling between **30** and potassium vinyltrifluoroborate, reduction of aldehyde **31** and Appel-type bromination of alcohol **32**. The inclusion of phosphorus-containing moiety began with the conversion of ammonium hypophosphite (**34**) to bis(trimethylsilyl) phosphonite (**35**), which was then treated with bromide **33**.[44] Subsequent addition of HMDS followed by allylation and methanolic work-up formed dialkylphosphinic acid **36**.[44] The final ring-closing metathesis step furnished the target compound **37**.



Scheme 9. Synthesis of 1,3-dihydrobenzophosphepine 2-oxide **37**.

Considering the plausible inhibition mechanism through hydrolysis of the oxaphosphepine moiety, hydrolysed forms of benzoxaphosphepine oxides **5h** and **6h** were generated upon treatment with aqueous alkali. The obtained phosphonate **38** and phosphonic acid **39** possess the *Z*-configuration of the double bond, which was unambiguously confirmed by single-crystal X-ray diffraction for **38**. To ensure proper SAR comparison, compound **39** was irradiated with a violet light LED, affording phosphonic acid **40** with the *E*-configuration of the double bond (Scheme 10).



Scheme 10. Synthesis of phosphonate **38** and phosphonic acids **39** and **40** (X-ray diffraction studies were performed by Dr. S. Belyakov at the LIOS Laboratory of Physical Organic Chemistry).

Inhibition assays of the model substrates against CA isoforms I, II, IX and XII partially supported the presumption of 3*H*-1,2-benzoxaphosphepine 2-oxides' hydrolysis in enzymatic media. In particular, the open *Z*-forms **38** and **39** preserved the selectivity and exhibited inhibitory activity against CA IX/XII similar to that of the cyclic forms **5h** and **6h**. Moreover, the results of the double bond positional isomer of **6a**, namely **28**, and hydrogenated derivative **29** imply that the double bond in benzoxaphosphepine-based CA inhibitors has to be retained for maintaining their potency. It is possible that the double bond restricts conformational mobility in the case of hydrolysis and/or contributes to the formation of key hydrophobic interactions. The activity of benzophosphepine **37** could be explained by this small-molecule ligand's ability to enter the binding pockets of CA IX and CA XII isoforms (Table 5).

Table 5

Inhibition data of compounds **28**, **29**, **36–40** and the standard inhibitor AAZ against CA I, II, IX and XII

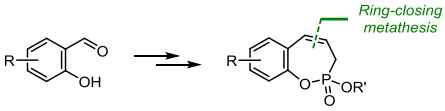
Compound	K_i (μM)[a,b,c]			
	CA I	CA II	CA IX	CA XII
28	>100	>100	1.6	3.5
29	>100	>100	>100	>100
36	ND	ND	ND	ND
37	>100	>100	0.86	0.78
38	>100	>100	2.14	1.98
39	>100	>100	1.80	1.67
40	ND	ND	ND	ND
AAZ*	0.25	0.012	0.025	0.006

[a] Determined in collaboration with Prof. C. T. Supuran's group from the University of Florence. [b] Values are the mean from three different assays using the stopped-flow technique (errors were in the range of ±5–10% of the reported values). [c] Inhibitor and enzyme solutions were preincubated together for 6 h at r.t. * Reference standard.

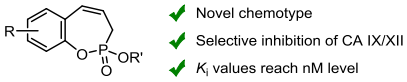
The utilisation of *in silico* docking could be beneficial for elucidating the binding mechanism of 3*H*-1,2-benzoxaphosphepine 2-oxides and will be applied in future studies.

CONCLUSIONS

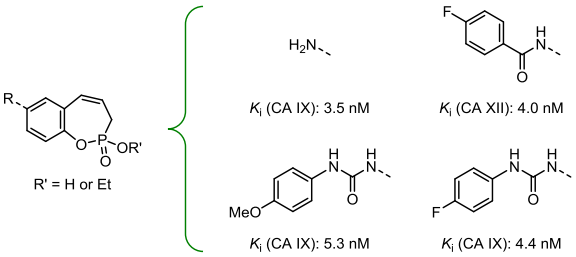
1. The developed synthesis strategy employing ring-closing metathesis has enabled access to novel phosphorus-containing heterocyclic compounds — 3*H*-1,2-benzoxaphosphepine 2-oxides. Their structural features, in combination with the established synthetic approach, allow further derivatisation of the scaffold and the preparation of diverse analogues.



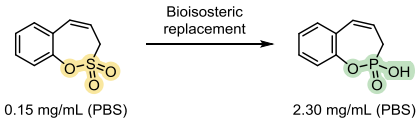
2. The structural framework of 3*H*-1,2-benzoxaphosphepine 2-oxides represents a promising new class of human CA inhibitors, exhibiting high affinity and exceptional isoform selectivity for the tumour-associated CA IX and XII over the ubiquitous CA I and II.



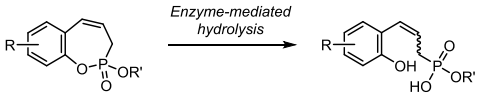
3. Among the synthesised series, 7-amino-substituted, as well as acylamino- and ureido-substituted derivatives bearing specific aryl groups, emerged as the superior inhibitors of CA IX and/or CA XII, outperforming the reference compounds such as AAZ and SLC-0111. The preliminary bioactivity profiles suggest that the compounds are suitable for future development and evaluation as anticancer agents.



4. The bioisosteric relationship between the benzoxaphosphepine and (sulpho)coumarin scaffolds was highlighted with respect to CA inhibition. The aqueous solubility of the phosphorus-containing compounds surpasses that of the previous-generation analogues, indicating a potentially improved bioavailability profile.



5. Benzoxaphosphepine-based CA inhibitors are expected to share a similar mechanism of action with coumarins and sulphocoumarins. The proposed binding mode within the active site of CA IX or CA XII involves putative hydrolysis of the oxaphosphepine motif, followed by binding at the entrance of the catalytic site or anchoring to the zinc-bound water molecule. The spatial differences in the vicinity of the active sites of CA isoforms I, II, IX and XII are a possible reason for the observed selectivity in inhibition.



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PIELIKUMI / APPENDICES

Balašova, A.; Žalubovskis, R. Synthetic methods toward phosphacoumarins (microreview).

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Химия гетероциклических соединений **2022**, *58*(6/7), 310–312

ГЕТЕРОЦИКЛЫ В ФОКУСЕ

Химия гетероциклических соединений

Synthetic methods toward phosphacoumarins (microreview)

Anastasija Balašova[1,2]*, Raivis Žalubovskis[1,2]

[1] *Latvian Institute of Organic Synthesis, 21 Aizkraukles St., Riga LV-1006, Latvia; e-mail: balasova@osi.lv*

[2] *Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, 3/7 Paula Valdena St., Riga LV-1048, Latvia*

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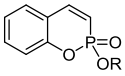


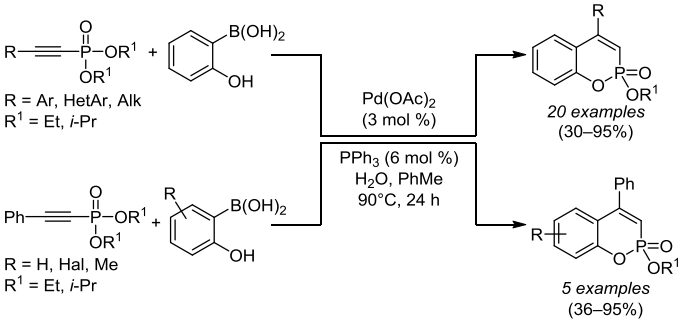
This microreview briefly summarizes recent synthetic approaches toward phosphacoumarins and their derivatives. The synthetic approaches include mainly transition metal-catalyzed reactions, as well as Kolbe oxidation.

Introduction

Phosphorus-containing heterocycles have attracted noticeable attention of synthetic chemists due to their broad applicability in medicinal chemistry,[1] organic synthesis,[2] agriculture,[3] and materials science.[4] In particular, phosphacoumarins, the phosphorus analogs of coumarin, exhibit interesting biological activities and could serve as novel pharmaceuticals.[5,6] Earlier works on the synthesis of phosphacoumarins include the Knoevenagel condensation reaction,[7,8] intermolecular Horner–Wadsworth–Emmons reaction,[9] intramolecular condensation of 2-methoxycarbonylbenzylphosphonates,[10] and reaction of oxaphospholes with terminal acetylenes.[11,12] Noteworthy, a review on the synthesis of related cyclic phosphonates was recently published.[13] This microreview focuses on the synthetic approaches toward phosphacoumarins, their derivatives and analogs covering the literature of the past decade with the emphasis on the recent literature sources.

Palladium catalysis

Pd-catalyzed addition/cyclization reaction of (2-hydroxyaryl)boronic acids with alkynyl phosphonates for the synthesis of a variety of phosphacoumarins has been developed by Zhao and coworkers.[14] Utilizing this procedure, phosphacoumarins were obtained in moderate to excellent yields (30–95%). In general, the reaction yields were lower when aliphatic and heterocyclic alkynyl phosphonates were used. Higher yields were achieved when aryl alkynyl phosphonates were used. In all cases, the regioselectivity was high.

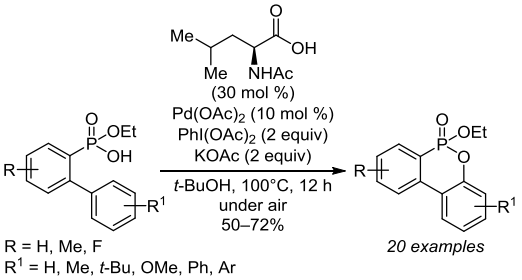


Anastasija Balašova was born in 1998 in Riga, Latvia. She received her MSc degree in chemistry at the University of Latvia in 2021. Currently she is working toward her PhD under the supervision of Dr. chem. Raivis Žalubovskis at the Latvian Institute of Organic Synthesis. Her research is devoted to the design and synthesis of novel carbonic anhydrase inhibitors.

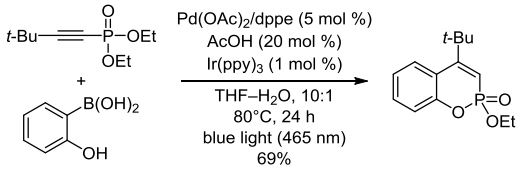
Raivis Žalubovskis was born in 1975 in Riga, Latvia. He received his PhD in organic chemistry under the supervision of professor Christina Moberg at the KTH (Royal Institute of Technology), Sweden in 2006. At present, he is a research group leader at the Latvian Institute of Organic Synthesis and an associated professor at the Riga Technical University. His scientific interests concern medicinal chemistry, heterocyclic compounds, and organic synthesis.

Palladium catalysis (continued)

Lee and coworkers presented a method to ethoxy dibenzo-oxaphosphorine oxide derivatives, which could be considered as phosphacoumarin derivatives, from phosphonic acid monoesters by intramolecular Pd-catalyzed oxidative cyclization.[15] The optimal reaction conditions included a combination of $Pd(OAc)_2$ and $PhI(OAc)_2$ with *N*-acetyl-L-leucine as a ligand. Under optimized reaction conditions, the products were obtained in good yields (50–72%).

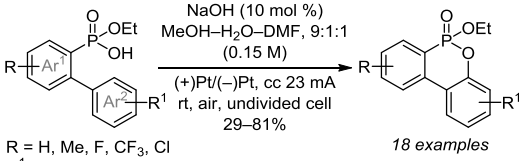


In 2020, Carretero and coworkers published their findings on the *anti*-hydroarylation of activated alkynes with aryl-boronic acids mediated by $Pd(OAc)_2$ and $Ir(ppy)_3$.[16] Reaction of the 2-hydroxyphenylboronic acid with alkynyl phosphonate smoothly yielded the phosphacoumarin derivative through a tandem sequence involving hydro-arylation, isomerization, and cyclization.



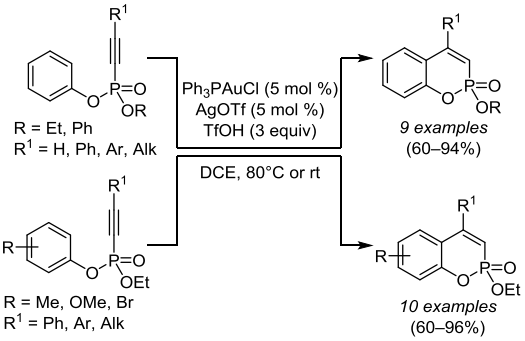
Phosphonic Kolbe oxidation

In 2018, Mo and coworkers reported another approach to benzoxaphosphinine oxides.[17] They have developed an anodic oxidation/cyclization method toward the synthesis of benzoxaphosphinine oxide derivatives starting from 2-(aryl)-arylphosphonic acid monoesters. The method is transition metal free, supporting electrolyte and oxidant free, as well as proceeds at room temperature. The desired cyclized products were obtained in moderate to good yields (29–81%). This method tolerates diverse functional groups. Electron-donating substituents on Ar^2 ring were favored, while electronic nature of substituents on Ar^1 ring did not have a prominent influence on this electrochemical oxidation reaction.



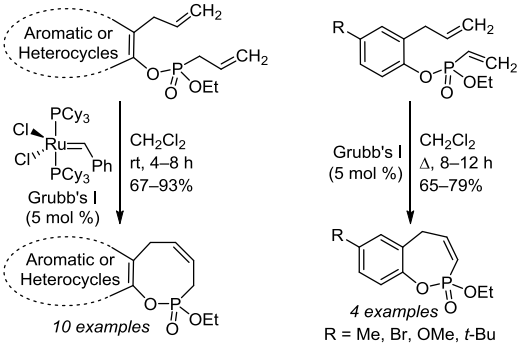
Gold catalysis

Lee and coworkers reported an efficient Au(I)-catalyzed intramolecular hydroarylation of aryl alkynyl phosphonates for the synthesis of phosphacoumarins.[18] The desired compounds were produced in good to excellent yields (60–96%) *via* a 6-*endo*-*dig* cyclization. The ethoxy and phenoxy groups attached to phosphorus did not affect the efficiency of this reaction. A variety of electron-withdrawing or electron-donating substituents on the phenyl ring attached to alkynyl group were tolerated as well.



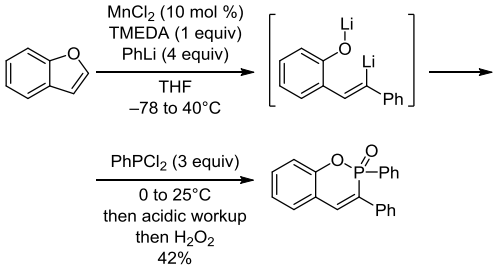
Ruthenium catalysis

Majumdar and coworkers reported a convenient synthetic route to ring-expanded analogs of phosphacoumarin by Ru-catalyzed ring-closing metathesis.[19] According to this procedure, various 7- and 8-membered P-heterocycles were synthesized and studied for biochemical properties.[20,21] Grubb's first generation catalyst was used in the metathesis for the formation of cyclized products. The 8-membered products were obtained at room temperature in good to excellent yields (67–93%), whereas the formation of the 7-membered products required an increase of the temperature. Under refluxing conditions, the 7-membered cyclized products were obtained in good yields (65–79%). The observed functional group tolerance was high – this type of reaction tolerated both electron-donating and electron-withdrawing groups.



Aromatic metamorphosis

The group of Yorimitsu published a novel class of aromatic metamorphosis in which benzofurans are converted into 6-membered oxaheterocycles.[22] This skeletal transformation is composed of two reactions in one pot: Mn-catalyzed arylative or alkylative ring opening and subsequent electrophilic trapping with multivalent heteroatom electrophiles. Using this methodology, various 6-membered heterocycles containing P, B, Si, Ge, and Ti atoms could be prepared. The phosphacoumarin derivative was obtained in moderate yield from benzofuran by ring opening with organolithium reagent and treatment with $PhPCl_2$, followed by oxidation with H_2O_2.



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Aleksandrs Pustenko, Anastasija Balašova, Alessio Nocentini, Claudiu T. Supuran & Raivis Žalubovskis

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3*H*-1,2-Benzoxaphosphepine 2-oxides as selective inhibitors of carbonic anhydrase IX and XII

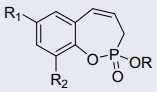
Aleksandrs Pustenko[a], Anastasija Balašova[a,b], Alessio Nocentini[c], Claudiu T. Supuran[c] and Raivis Žalubovskis[a,b]

[a]Latvian Institute of Organic Synthesis, Riga, Latvia; [b]Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia; [c]Department of Neurofarba, Università degli Studi di Firenze, Florence, Italy

ABSTRACT

The synthesis of 3*H*-1,2-benzoxaphosphepine 2-oxides and evaluation of their inhibitory activity against human carbonic anhydrase (hCA) isoforms I, II, IX, and XII are described. The target compounds were obtained via a concise synthesis from commercial salicylaldehydes and displayed low to sub-micromolar inhibition levels against the tumour-associated isoforms hCA IX and XII. All obtained benzoxaphosphepine 2-oxides possess remarkable selectivity for inhibition of hCA IX/XII over the off-target cytosolic hCA isoforms I and II, whose inhibition may lead to side effects.

GRAPHICAL ABSTRACT



Strong and selective human CA inhibitors

K_i (hCA IX) = 670 nM
K_i (h CA XII) = 510 nM

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Carbonic anhydrase; benzoxaphosphepine 2-oxide; isoform-selective inhibitors; anti-tumour

Introduction

Carbonic anhydrases (CA, EC 4.2.1.1) are a superfamily of metalloenzymes present across all kingdoms of life[1]. These enzymes catalyse a simple yet essential physiological reaction – the reversible hydration of carbon dioxide[2]. To date, 15 different human CA (hCA) isoforms have been identified, out of which hCA IX and XII isoforms are highly overexpressed in different tumour types and may contribute in the survival and progression of tumour cells by regulating intra- and extracellular pH[2–6]. Therefore, the development of selective hCA IX/XII inhibitors is a potential strategy for designing anti-tumour agents.

Due to the high degree of structural homology and sequence similarities within the active site of the hCA isoforms, the design and development of isoform-selective hCA inhibitors pose a challenge[7]. A variety of compounds have been reported as potent and selective inhibitors of tumour-associated isoforms hCA IX and XII including coumarins[8–11], thiocoumarins[8,11], sulphocoumarins[8,12–15], as well as their congeners, homosulphocoumarins (3*H*-1,2-benzoxathiepine 2,2-dioxides)[16]. In this work, attention was drawn to phosphorus, as phosphorus-containing molecules display a multitude of biological activities relevant in medicinal chemistry[17]. Additionally, several groups have shown the use of organophosphorus compounds as CA inhibitors[18].

Considering isosteric relationship between sulphonyl derivatives and phosphonates[19], our research group designed and synthesised a series of benzoxaphosphepine 2-oxides pursuing the development of new classes of selective CA inhibitors. These compounds showed interesting inhibitory activity against hCA IX and XII. Moreover, the results of current study demonstrate the bioisosteric utility of the cyclic phosphonate moiety in the design of novel CA inhibitors.

Materials and methods

Chemistry

The air- or moisture-sensitive reactions were performed under argon atmosphere using dry glassware. Toluene was freshly distilled from Na prior to use. DCM and NEt_3 were distilled from CaH_2. Other reagents, starting materials and solvents were purchased from commercial sources and used as received. TLC was performed on silica gel plates (60 F_{254}) and visualised under UV light (254 and 365 nm). Melting points were determined on an OptiMelt MPA100 apparatus. IR spectra were recorded on a Shimadzu FTIR IR Prestige-21 spectrophotometer. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker Avance Neo 400 MHz spectrometer. The chemical shifts (δ) were reported in parts per million (ppm) relative to the residual solvent peak as an internal reference (DMSO-d_6: ^{1}H 2.50, ^{13}C 39.52; $CDCl_3$: ^{1}H 7.26, ^{13}C 77.16; C_6D_6: ^{1}H 7.16, ^{13}C 128.06). ^{31}P shifts were referenced externally to H_3PO_4. The coupling constants (J) were expressed in Hertz (Hz). HRMS was performed on a Q-TOF Micro mass spectrometer.

CONTACT Claudiu T. Supuran claudiu.supuran@unifi.it Department of Neurofarba, Università degli Studi di Firenze, Florence, Italy; Raivis Žalubovskis raivis@osi.lv Latvian Institute of Organic Synthesis, 21 Aizkraukles St., Riga, LV-1006, Latvia

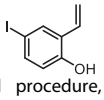
General procedure for the synthesis of vinylphenols 2

To a stirred solution of $MePPh_3Br$ (2.3 eq) in dry THF (3 ml/1 mmol of $MePPh_3Br$) was added *t*-BuOK (2.35 eq) in small portions over 20 min. The reaction mixture was stirred under inert atmosphere for 2 h at rt. The corresponding hydroxybenzaldehyde **1** (1.0 eq) was added, and the mixture was kept stirring at rt for another 18 h. The reaction mixture was treated with sat. aq NH_4Cl (25 ml) and then was diluted with Et_2O (3 ml/1 mmol of $MePPh_3Br$). The organic layer was washed with water (2 × 40 ml) and brine (2 × 40 ml), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (PE/EtOAc 4:1).

2-Vinylphenol (2a)

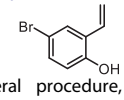
By following the general procedure, **2a** was prepared from $MePPh_3Br$ (13.46 g, 37.7 mmol), *t*-BuOK (4.32 g, 38.5 mmol), and 2-hydroxybenzaldehyde (**1a**) (2.00 g, 16.4 mmol) as a yellowish oil (1.71 g, 87%)[16a]. ^1H NMR (400 MHz, $CDCl_3$) $\delta = 5.34–5.38$ (m, 2H), 5.75 (dd, 1H, $J = 17.8$, 1.4 Hz), 6.80 (dd, 1H, $J = 8.1$, 1.1 Hz), 6.89–7.01 (m, 2H), 7.12–7.17 (m, 1H), 7.40 (dd, 1H, $J = 7.7$, 1.7 Hz) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) $\delta = 115.8$, 116.0, 121.0, 125.0, 127.4, 129.0, 131.6, 153.0 ppm.

4-Iodo-2-vinylphenol (2b)



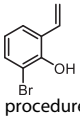
By following the general procedure, **2b** was prepared from $MePPh_3Br$ (16.56 g, 46.4 mmol), *t*-BuOK (5.32 g, 47.4 mmol), and 2-hydroxy-5-iodobenzaldehyde (**1b**) (5.00 g, 20.2 mmol) as a yellowish solid (4.17 g, 84%)[16b]. ^1H NMR (400 MHz, $CDCl_3$) $\delta = 5.23$ (dd, 1H, $J = 11.3$, 1.3 Hz), 5.80 (dd, 1H, $J = 17.6$, 1.3 Hz), 6.67 (d, 1H, $J = 8.6$ Hz), 6.77–6.87 (m, 1H), 7.37 (dd, 1H, $J = 8.6$, 2.4 Hz), 7.70 (d, 1H, $J = 2.4$ Hz), 9.94 (s, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) $\delta = 81.4$, 115.1, 118.4, 126.9, 130.4, 134.4, 137.0, 154.6 ppm.

4-Bromo-2-vinylphenol (2c)



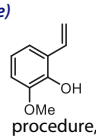
By following the general procedure, **2c** was prepared from $MePPh_3Br$ (8.17 g, 22.9 mmol), *t*-BuOK (2.62 g, 23.4 mmol), and 5-bromo-2-hydroxybenzaldehyde (**1c**) (2.00 g, 10 mmol) as a yellowish solid (1.74 g, 88%)[16a]. ^1H NMR (400 MHz, $CDCl_3$) $\delta = 4.98$ (s, 1H), 5.40 (dd, 1H, $J = 11.3$, 1.0 Hz), 5.74 (dd, 1H, $J = 17.8$, 1.0 Hz), 6.68 (d, 1H, $J = 8.6$ Hz), 6.85 (dd, 1H, $J = 17.8$, 11.3 Hz), 7.23 (dd, 1H, $J = 8.6$, 2.4 Hz), 7.49 (d, 1H, $J = 2.4$ Hz) ppm.

2-Bromo-6-vinylphenol (2d)



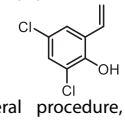
By following the general procedure, **2d** was prepared from $MePPh_3Br$ (16.35 g, 45.8 mmol), *t*-BuOK (5.25 g, 46.8 mmol), and 3-bromo-2-hydroxybenzaldehyde (**1d**) (4.00 g, 19.9 mmol) as a yellowish solid (3.05 g, 77%)[16b]. ^1H NMR (400 MHz, $CDCl_3$) $\delta = 5.34$ (dd, 1H, $J = 11.2$, 1.3 Hz), 5.72 (s, 1H), 5.79 (dd, 1H, $J = 17.7$, 1.3 Hz), 6.77–6.82 (m, 1H), 7.00 (dd, 1H, $J = 17.7$, 11.2 Hz), 7.35–7.41 (m, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) $\delta = 111.2$, 116.2, 121.6, 126.2, 126.5, 131.1, 131.3, 149.6 ppm.

2-Methoxy-6-vinylphenol (2e)



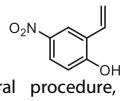
By following the general procedure, **2e** was prepared from $MePPh_3Br$ (2.70 g, 7.6 mmol), *t*-BuOK (0.87 g, 7.7 mmol), and 2-hydroxy-3-methoxybenzaldehyde (**1e**) (0.50 g, 3.3 mmol) as a yellowish solid (0.40 g, 81%)[20]. ^1H NMR (400 MHz, $CDCl_3$) $\delta = 3.89$ (s, 3H), 5.33 (dd, 1H, $J = 11.2$, 1.5 Hz), 5.83 (dd, 1H, $J = 17.8$, 1.5 Hz), 5.93–5.94 (m, 1H), 6.76–6.80 (m, 1H), 6.81–6.86 (m, 1H), 7.00–7.11 (m, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) $\delta = 56.2$, 109.7, 114.9, 118.9, 119.5, 124.1, 131.2, 143.4, 146.8 ppm.

2,4-Dichloro-6-vinylphenol (2f)



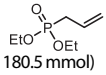
By following the general procedure, **2f** was prepared from $MePPh_3Br$ (2.15 g, 6.0 mmol), *t*-BuOK (0.69 g, 6.2 mmol), and 3,5-dichloro-2-hydroxybenzaldehyde (**1f**) (0.50 g, 2.6 mmol) as a yellowish solid (0.38 g, 76%)[21]. ^1H NMR (400 MHz, $CDCl_3$) $\delta = 5.39$ (dd, 1H, $J = 11.2$, 1.0 Hz), 5.70–5.72 (m, 1H), 5.80 (d, 1H, $J = 17.7$, 1.0 Hz), 6.92 (dd, 1H, $J = 17.7$, 11.2 Hz), 7.23 (d, 1H, $J = 2.5$ Hz), 7.32–7.34 (m, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) $\delta = 117.3$, 121.0, 125.5, 125.6, 127.2, 127.4, 130.1, 147.5 ppm.

4-Nitro-2-vinylphenol (2g)



By following the general procedure, **2g** was prepared from $MePPh_3Br$ (9.83 g, 27.5 mmol), *t*-BuOK (3.16 g, 28.1 mmol), and 2-hydroxy-5-nitrobenzaldehyde (**1g**) (2.00 g, 11 mmol). The solution of nitrobenzaldehyde **1g** in THF (20 ml) was added at −78 °C. The title product was obtained as a yellow solid (1.70 g, 86%)[22]. ^1H NMR (400 MHz, DMSO-d_6) $\delta = 5.39$ (dd, 1H, $J = 11.3$, 1.2 Hz), 5.98 (dd, 1H, $J = 17.8$, 1.2 Hz), 6.92 (dd, 1H, $J = 17.8$, 11.3 Hz), 7.01 (d, 1H, $J = 9.0$ Hz), 8.02 (dd, 1H, $J = 9.0$, 2.9 Hz), 8.28 (d, 1H, $J = 2.9$ Hz), 11.32 (s, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 116.1$, 116.9, 122.3, 124.7, 124.8, 130.1, 139.9, 161.0 ppm.

Diethyl allylphosphonate (S1)



Triethylphosphite (31.0 ml, 180.5 mmol) and allyl bromide (18.7 ml, 216.7 mmol) were stirred and heated for 24 h at 70 °C. After evaporation of the remaining allyl bromide, the residue was distilled *in vacuo* (~4 mbar) to afford product **S1** as a colourless liquid (29.00 g, 90%), b.p. 60–62 °C/4 mbar[23]. ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 26.81$ ppm. ^1H NMR (400 MHz, DMSO-d_6) $\delta = 1.22$ (t, 6H, $J = 7.1$ Hz), 2.62 (dt, 1H, $J = 7.3$, 1.3 Hz), 2.67 (dt, 1H, $J = 7.3$, 1.3 Hz), 3.93–4.06 (m, 4H), 5.10–5.26 (m, 2H), 5.63–5.73 (m, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 16.2$ (d, $J_{P,C} = 5.7$ Hz), 30.6 (d, $J_{P,C} = 136$ Hz), 61.2 (d, $J_{P,C} = 6.3$ Hz), 119.5 (d, $J_{P,C} = 14.2$ Hz), 128.3 (d, $J_{P,C} = 10.9$ Hz) ppm.

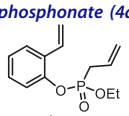
Ethyl allylphosphonochloridate (3)

Diethyl allylphosphonate(**S1**) (28.6 g, 160.5 mmol) was dissolved in dry DCM (200 ml). The solution was cooled down to 0 °C, and oxalyl chloride (49.0 ml, 0.562 mol) was added dropwise. The reaction mixture was stirred for 16 h at rt. After evaporation of the solvent and remaining oxalyl chloride, the residue was distilled *in vacuo* (~4 mbar) to afford product **3** as a colourless liquid (21.64 g, 80%), b.p. 88–90 °C/4 mbar[23]. ^{31}P NMR (162 MHz, DMSO-d_6) δ = 39.17 ppm. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.37 (t, 3H, J = 7.1 Hz), 2.93 (dt, 1H, J = 7.3, 1.2 Hz), 2.99 (dt, 1H, J = 7.3, 1.2 Hz), 4.16–4.37 (m, 2H), 5.26–5.36 (m, 2H), 5.72–5.86 (m, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 15.9 (d, $J_{P,C}$ = 7.0 Hz), 39.1 (d, $J_{P,C}$ = 123 Hz), 63.5 (d, $J_{P,C}$ = 8.4 Hz), 122.2 (d, $J_{P,C}$ = 16.8 Hz), 125.4 (d, $J_{P,C}$ = 12.8 Hz) ppm.

General procedure for the synthesis of diolefins 4

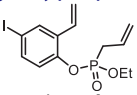
The corresponding vinylphenol **2** (1.0 eq) was dissolved in dry DCM (10 ml/1 mmol of **2**). After cooling down the solution to 0 °C, ethyl allylphosphonochloridate (**3**) (1.2 eq) and NEt_3 (1.25 eq) were added. The reaction mixture was stirred under inert atmosphere at rt for 18 h. Water (30 ml) was added, and the mixture was extracted with EtOAc (3 × 40 ml). The combined organic layers were washed with brine (2 × 40 ml), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (PE/EtOAc 1.5:1).

Ethyl (2-vinylphenyl) allylphosphonate (4a)



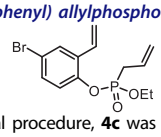
By following the general procedure, **4a** was prepared from 2-vinylphenol (**2a**) (0.38 g, 3.16 mmol), ethyl allylphosphonochloridate (**3**) (0.56 ml, 3.79 mmol), and NEt_3 (0.55 ml, 3.95 mmol) as a colourless oil (0.48 g, 60%). IR (thin film, cm^{-1}): 1260 (P = O), 1219 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) δ = 24.63 ppm. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.19 (t, 3H, J = 7.0 Hz), 2.84–2.97 (m, 2H), 4.00–4.16 (m, 2H), 5.19–5.32 (m, 2H), 5.36 (dd, 1H, J = 11.2, 0.9 Hz), 5.71–5.83 (m, 1H), 5.86 (dd, 1H, J = 17.7, 0.9 Hz), 6.96 (dd, 1H, J = 11.7, 11.2 Hz), 7.16–7.22 (m, 1H), 7.26–7.33 (m, 2H), 7.67 (d, 1H, J = 7.7 Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 16.1 (d, $J_{P,C}$ = 5.8 Hz), 31.0 (d, $J_{P,C}$ = 138 Hz), 62.6 (d, $J_{P,C}$ = 6.8 Hz), 116.3, 120.4 (d, $J_{P,C}$ = 14.6 Hz), 120.9 (d, $J_{P,C}$ = 2.8 Hz), 125.0, 126.3, 127.5 (d, $J_{P,C}$ = 11.4 Hz), 128.8 (d, $J_{P,C}$ = 5.0 Hz), 129.1, 130.2, 147.4 (d, $J_{P,C}$ = 8.9 Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{13}H_{18}O_3P$: 253.0994, found 253.1003.

Ethyl (4-iodo-2-vinylphenyl) allylphosphonate (4b)



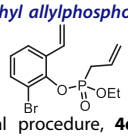
By following the general procedure, **4b** was prepared from 4-iodo-2-vinylphenol (**2b**) (2.50 g, 10.2 mmol), ethyl allylphosphonochloridate (**3**) (1.81 ml, 12.2 mmol), and NEt_3 (1.77 ml, 12.7 mmol) as a colourless oil (3.53 g, 92%). IR (thin film, cm^{-1}): 1265 (P = O), 1220 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) δ = 25.01 ppm. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.19 (t, 3H, J = 7.1 Hz), 2.90 (dt, 1H, J = 7.3, 1.2 Hz), 2.96 (dt, 1H, J = 7.3, 1.2 Hz), 4.00–4.16 (m, 2H), 5.19–5.32 (m, 2H), 5.40 (dd, 1H, J = 11.2, 0.7 Hz), 5.69–5.83 (m, 1H), 5.93 (dd, 1H, J = 17.7, 0.7 Hz), 6.84 (dd, 1H, J = 17.7, 11.2 Hz), 7.11 (dd, 1H, J = 8.6, 1.3 Hz), 7.63 (dd, 1H, J = 8.6, 2.2 Hz), 7.98 (d, 1H, J = 2.2 Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 16.1 (d, $J_{P,C}$ = 5.6 Hz), 30.9 (d, $J_{P,C}$ = 137 Hz), 62.7 (d, $J_{P,C}$ = 6.7 Hz), 89.6 (d, $J_{P,C}$ = 1.4 Hz), 117.9, 120.5 (d, $J_{P,C}$ = 15.0 Hz), 123.2 (d, $J_{P,C}$ = 2.7 Hz), 127.3 (d, $J_{P,C}$ = 11.6 Hz), 128.8, 131.3 (d, $J_{P,C}$ = 5.3 Hz), 132.2 (d, $J_{P,C}$ = 2.6 Hz), 134.7, 137.5, 147.3 (d, $J_{P,C}$ = 9.0 Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{13}H_{17}O_3PI$: 378.9960, found 378.9966.

Ethyl (4-bromo-2-vinylphenyl) allylphosphonate (4c)



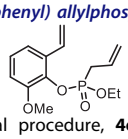
By following the general procedure, **4c** was prepared from 4-bromo-2-vinylphenol (**2c**) (1.63 g, 8.19 mmol), ethyl allylphosphonochloridate (**3**) (1.46 ml, 9.83 mmol), and NEt_3 (1.42 ml, 10.2 mmol) as a colourless oil (1.71 g, 63%). IR (thin film, cm^{-1}): 1266 (P = O), 1224 (P = O), 1174 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) δ = 25.10 ppm. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.16–1.22 (m, 3H), 2.91 (dt, 1H, J = 7.3, 1.2 Hz), 2.97 (dt, 1H, J = 7.3, 1.2 Hz), 3.99–4.17 (m, 2H), 5.19–5.32 (m, 2H), 5.43 (dd, 1H, J = 11.2, 0.8 Hz), 5.70–5.83 (m, 1H), 5.97 (dd, 1H, J = 17.7, 0.8 Hz), 6.87 (dd, 1H, J = 17.7, 11.2 Hz), 7.26 (dd, 1H, J = 8.7, 1.3 Hz), 7.48 (dd, 1H, J = 8.7, 2.5 Hz), 7.86 (d, 1H, J = 2.5 Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 16.1 (d, $J_{P,C}$ = 6.0 Hz), 30.9 (d, $J_{P,C}$ = 137 Hz), 62.8 (d, $J_{P,C}$ = 6.7 Hz), 117.3 (d, $J_{P,C}$ = 1.5 Hz), 118.2, 120.5 (d, $J_{P,C}$ = 15.0 Hz), 123.0 (d, $J_{P,C}$ = 2.8 Hz), 127.3 (d, $J_{P,C}$ = 11.8 Hz), 128.8 (d, $J_{P,C}$ = 9.7 Hz), 131.1 (d, $J_{P,C}$ = 5.3 Hz), 131.6, 146.6 (d, $J_{P,C}$ = 8.9 Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{13}H_{17}O_3PBr$: 331.0099, found 331.0103.

2-Bromo-6-vinylphenyl ethyl allylphosphonate (4d)



By following the general procedure, **4d** was prepared from 2-bromo-6-vinylphenol (**2d**) (1.00 g, 5.02 mmol), ethyl allylphosphonochloridate (**3**) (0.89 ml, 6.03 mmol), and NEt_3 (0.87 ml, 6.28 mmol) as a colourless oil (1.28 g, 77%). IR (thin film, cm^{-1}): 1262 (P = O), 1219 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) δ = 25.07 ppm. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.16 (dt, 3H, J = 7.0, 0.4 Hz), 3.00–3.10 (m, 2H), 3.96–4.15 (m, 2H), 5.22–5.37 (m, 2H), 5.42 (dd, 1H, J = 11.1, 0.9 Hz), 5.77–5.92 (m, 2H), 7.06 (dd, 1H, J = 17.7, 11.1 Hz), 7.13–7.19 (m, 1H), 7.61 (dd, 1H, J = 7.9, 1.5 Hz), 7.69 (dd, 1H, J = 7.9, 1.5 Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 16.0 (d, $J_{P,C}$ = 6.0 Hz), 32.1 (d, $J_{P,C}$ = 139 Hz), 63.1 (d, $J_{P,C}$ = 6.9 Hz), 116.5 (d, $J_{P,C}$ = 4.0 Hz), 117.6, 120.5 (d, $J_{P,C}$ = 15.2 Hz), 125.6 (d, $J_{P,C}$ = 1.5 Hz), 126.7, 127.4 (d, $J_{P,C}$ = 11.4 Hz), 130.7, 132.2 (d, $J_{P,C}$ = 2.8 Hz), 132.8 (d, $J_{P,C}$ = 1.5 Hz), 145.0 (d, $J_{P,C}$ = 10.7 Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{13}H_{17}O_3PBr$: 331.0099, found 331.0092.

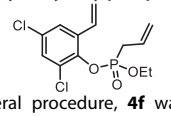
Ethyl (2-methoxy-6-vinylphenyl) allylphosphonate (4e)



By following the general procedure, **4e** was prepared from 2-methoxy-6-vinylphenol (**2e**) (0.32 g, 2.13 mmol), ethyl allylphosphonochloridate (**3**) (0.38 ml, 2.56 mmol), and NEt_3 (0.37 ml, 2.66 mmol) as a colourless oil (0.40 g, 66%). IR (thin film, cm^{-1}): 1274 (P = O), 1179 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) δ = 24.86 ppm. ^{1}H NMR

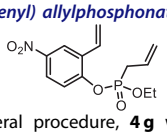
(400 MHz, DMSO-d_6) $\delta = 1.19–1.24$ (m, 3H), 2.86–2.96 (m, 2H), 3.82 (s, 3H), 3.96–4.18 (m, 2H), 5.18–5.31 (m, 2H), 5.37 (dd, 1H, $J = 11.1$, 1.1 Hz), 5.74–5.88 (m, 2H), 6.97 (dd, 1H, $J = 17.7$, 11.1 Hz), 7.04 (dd, 1H, $J = 8.1$, 1.5 Hz), 7.11–7.17 (m, 1H), 7.22 (dd, 1H, $J = 7.9$, 1.5 Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 16.2$ (d, $J_{P,C} = 6.1$ Hz), 31.8 (d, $J_{P,C} = 139$ Hz), 55.9, 62.1 (d, $J_{P,C} = 7.0$ Hz), 112.2, 116.6, 117.3, 119.9 (d, $J_{P,C} = 15.0$ Hz), 125.2, 128.1 (d, $J_{P,C} = 11.5$ Hz), 130.4, 130.5 (d, $J_{P,C} = 3.5$ Hz), 137.1 (d, $J_{P,C} = 9.6$ Hz), 151.2 (d, $J_{P,C} = 3.0$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{14}H_{20}O_4P$: 283.1099, found 283.1105.

2,4-Dichloro-6-vinylphenyl ethyl allylphosphonate (4f)



By following the general procedure, **4f** was prepared from 2,4-dichloro-6-vinylphenol (**2f**) (0.80 g, 4.23 mmol), ethyl allylphosphonochloridate (**3**) (0.75 ml, 5.08 mmol), and NEt_3 (0.74 ml, 5.29 mmol) as a colourless oil (1.17 g, 86%). IR (thin film, cm^{-1}): 1262 (P = O), 1217 (P = O). ^{31}P NMR (162 MHz, C_6D_6) $\delta = 24.33$ ppm. ^{1}H NMR (400 MHz, C_6D_6) $\delta = 0.88$ (dt, 3H, $J = 7.1$, 0.4 Hz), 2.63–2.73 (m, 2H), 3.76–3.98 (m, 2H), 5.00–5.11 (m, 3H), 5.27 (d, 1H, $J = 17.6$ Hz), 5.76–5.89 (m, 1H), 7.03 (d, 1H, $J = 2.5$ Hz), 7.19–7.21 (m, 1H), 7.25 (dd, 1H, $J = 17.6$, 11.0 Hz) ppm. ^{13}C NMR (101 MHz, C_6D_6) $\delta = 16.3$ (d, $J_{P,C} = 5.7$ Hz), 33.0 (d, $J_{P,C} = 141$ Hz), 63.4 (d, $J_{P,C} = 7.0$ Hz), 118.0, 120.4 (d, $J_{P,C} = 15.0$ Hz), 125.0 (d, $J_{P,C} = 1.9$ Hz), 127.5 (d, $J_{P,C} = 11.5$ Hz), 128.9 (d, $J_{P,C} = 3.7$ Hz), 129.4, 130.8, 130.9 (d, $J_{P,C} = 2.1$ Hz), 134.3 (d, $J_{P,C} = 3.0$ Hz), 143.8 (d, $J_{P,C} = 10.7$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{13}H_{16}O_3PCl_2$: 321.0214, found 321.0233.

Ethyl (4-nitro-2-vinylphenyl) allylphosphonate (4 g)

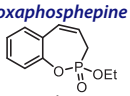


By following the general procedure, **4 g** was prepared from 4-nitro-2-vinylphenol (**2 g**) (1.00 g, 6.06 mmol), ethyl allylphosphonochloridate (**3**) (1.08 ml, 7.27 mmol), and NEt_3 (1.05 ml, 7.57 mmol) as a yellowish oil (1.70 g, 94%). IR (thin film, cm^{-1}): 1273 (P = O), 1232 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 25.61$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.22$ (dt, 3H, $J = 7.1$, 0.3 Hz), 3.00 (dt, 1H, $J = 7.3$, 1.2 Hz), 3.06 (dt, 1H, $J = 7.3$, 1.2 Hz), 4.06–4.22 (m, 2H), 5.21–5.35 (m, 2H), 5.54–5.58 (m, 1H), 5.71–5.85 (m, 1H), 6.11 (dd, 1H, $J = 17.7$, 0.6 Hz), 6.96 (dd, 1H, $J = 17.7$, 11.2 Hz), 7.59 (dd, 1H, $J = 9.1$, 1.2 Hz), 8.19 (dd, 1H, $J = 9.1$, 2.9 Hz), 8.45–8.48 (m, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 16.1$ (d, $J_{P,C} = 5.8$ Hz), 30.9 (d, $J_{P,C} = 137$ Hz), 63.1 (d, $J_{P,C} = 6.9$ Hz), 119.6, 120.8 (d, $J_{P,C} = 15.0$ Hz), 121.7 (d, $J_{P,C} = 3.0$ Hz), 121.8, 124.2, 127.0 (d, $J_{P,C} = 11.7$ Hz), 128.6, 129.9 (d, $J_{P,C} = 5.5$ Hz), 144.3, 152.0 (d, $J_{P,C} = 8.6$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{13}H_{17}NO_5P$: 298.0844, found 298.0858.

General procedure for the synthesis of 2-ethoxy-3H-1,2-benzoxaphosphepine 2-oxides 6

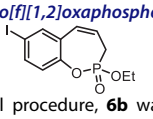
The corresponding diolefin **4** (1.0 eq) was dissolved in dry, degassed PhMe (18 ml/1 mmol of **4**). The solution was sparged with argon followed by addition of ruthenium catalyst **5** (CAS: 254972–49-1) (5 mol%). The reaction mixture was stirred at 70 °C for 4 h, then cooled down to rt, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc 100%).

2-Ethoxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (6a)



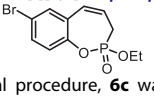
By following the general procedure, **6a** was prepared from ethyl (2-vinylphenyl) allylphosphonate (**4a**) (0.43 g, 1.70 mmol), and ruthenium catalyst **5** (81 mg, 0.085 mmol) as a greenish dense oil (0.28 g, 74%). IR (thin film, cm^{-1}): 1265 (P = O), 1203 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 40.00$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.28$ (t, 3H, $J = 7.1$ Hz), 2.62–2.88 (m, 2H), 4.14–4.23 (m, 2H), 5.92–6.04 (m, 1H), 6.71 (dd, 1H, $J = 10.8$, 5.3 Hz), 7.17–7.27 (m, 2H), 7.31–7.41 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 16.3$ (d, $J_{P,C} = 5.9$ Hz), 25.5 (d, $J_{P,C} = 125$ Hz), 62.2 (d, $J_{P,C} = 6.9$ Hz), 121.6 (d, $J_{P,C} = 3.4$ Hz), 122.7 (d, $J_{P,C} = 12.2$ Hz), 125.0, 127.6 (d, $J_{P,C} = 1.1$ Hz), 129.4, 129.5, 129.6 130.6, 147.5 (d, $J_{P,C} = 8.3$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{11}H_{14}O_3P$: 225.0681, found 225.0692.

2-Ethoxy-7-iodo-3H-benzo[f][1,2]oxaphosphepine 2-oxide (6b)



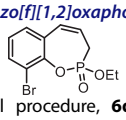
By following the general procedure, **6b** was prepared from ethyl (4-iodo-2-vinylphenyl) allylphosphonate (**4b**) (3.50 g, 9.26 mmol), and ruthenium catalyst **5** (438 mg, 0.46 mmol) as a greenish dense oil (2.46 g, 76%). IR (thin film, cm^{-1}): 1265 (P = O), 1173 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 39.83$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.27$ (t, 3H, $J = 7.1$ Hz), 2.66–2.92 (m, 2H), 4.13–4.22 (m, 2H), 5.95–6.07 (m, 1H), 6.63–6.70 (m, 1H), 6.99–7.04 (m, 1H), 7.68 (dd, 1H, $J = 8.5$, 2.2 Hz), 7.71 (d, 1H, $J = 2.2$ Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 16.2$ (d, $J_{P,C} = 5.6$ Hz), 25.5 (d, $J_{P,C} = 125$ Hz), 62.4 (d, $J_{P,C} = 6.7$ Hz), 89.3 (d, $J_{P,C} = 1.4$ Hz), 123.8, 123.9, 124.0, 128.3 (d, $J_{P,C} = 8.7$ Hz), 130.2, 137.9, 138.8, 147.4 (d, $J_{P,C} = 8.0$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{11}H_{13}O_3PI$: 350.9647, found 350.9659.

7-Bromo-2-ethoxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (6c)



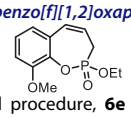
By following the general procedure, **6c** was prepared from ethyl (4-bromo-2-vinylphenyl) allylphosphonate (**4c**) (0.92 g, 2.78 mmol), and ruthenium catalyst **5** (132 mg, 0.14 mmol) as a greenish dense oil (0.53 g, 63%). IR (thin film, cm^{-1}): 1274 (P = O), 1220 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 44.68$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.27$ (t, 3H, $J = 7.1$ Hz), 2.68–2.93 (m, 2H), 4.13–4.23 (m, 2H), 5.97–6.10 (m, 1H), 6.65–6.71 (m, 1H), 7.15–7.19 (m, 1H), 7.54 (dd, 1H, $J = 8.6$, 2.5 Hz), 7.58 (d, 1H, $J = 2.5$ Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 16.2$ (d, $J_{P,C} = 5.4$ Hz), 25.4 (d, $J_{P,C} = 125$ Hz), 62.5 (d, $J_{P,C} = 7.0$ Hz), 116.9 (d, $J_{P,C} = 1.5$ Hz), 123.8 (d, $J_{P,C} = 3.5$ Hz), 124.1 (d, $J_{P,C} = 12.3$ Hz), 128.4 (d, $J_{P,C} = 9.0$ Hz), 130.0, 132.1, 132.9, 146.7 (d, $J_{P,C} = 7.8$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{11}H_{13}O_3PBr$: 302.9786, found 302.9791.

9-Bromo-2-ethoxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (6d)



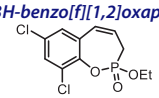
By following the general procedure, **6d** was prepared from 2-bromo-6-vinylphenyl ethyl allylphosphonate (**4d**) (2.00 g, 6.04 mmol), and ruthenium catalyst **5** (286 mg, 0.30 mmol) as a greenish dense oil (1.58 g, 86%). IR (thin film, cm^{-1}): 1268 (P = O), 1232 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 39.08$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.29$ (t, 3H, $J = 7.1$ Hz), 2.70–2.83 (m, 1H), 2.87–3.00 (m, 1H), 4.18–4.32 (m, 2H), 5.95–6.08 (m, 1H), 6.67–6.74 (m, 1H), 7.17 (td, 1H, $J = 7.8$, 0.6 Hz), 7.34 (dd, 1H, $J = 7.8$, 1.6 Hz), 7.68 (dd, 1H, $J = 7.8$, 1.6 Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 16.2$ (d, $J_{P,C}$ = 6.1 Hz), 25.8 (d, $J_{P,C}$ = 126 Hz), 62.8 (d, $J_{P,C}$ = 7.0 Hz), 115.4 (d, $J_{P,C}$ = 3.8 Hz), 123.4 (d, $J_{P,C}$ = 12.2 Hz), 126.1, 129.1 (d, $J_{P,C}$ = 9.0 Hz), 129.3, 130.4, 132.7, 144.2 (d, $J_{P,C}$ = 7.8 Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{11}H_{13}O_3PBr$: 302.9786, found 302.9795.

2-Ethoxy-9-methoxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (6e)



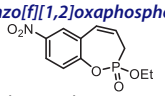
By following the general procedure, **6e** was prepared from ethyl (2-methoxy-6-vinylphenyl) allylphosphonate (**4e**) (315 mg, 1.12 mmol), and ruthenium catalyst **5** (53 mg, 0.056 mmol) as a greenish dense oil (0.23 g, 81%). IR (thin film, cm^{-1}): 1270 (P = O), 1244 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 41.74$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.26$ (t, 3H, $J = 7.1$ Hz), 2.50–2.63 (m, 1H), 2.79–2.91 (m, 1H), 3.84 (s, 3H), 4.13–4.22 (m, 2H), 5.92–6.04 (m, 1H), 6.65–6.72 (m, 1H), 6.86 (dd, 1H, $J = 7.8$, 1.4 Hz), 7.08 (dd, 1H, $J = 8.2$, 1.4 Hz), 7.14–7.20 (m, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 16.1$ (d, $J_{P,C}$ = 6.1 Hz), 25.4 (d, $J_{P,C}$ = 127 Hz), 55.9, 62.0 (d, $J_{P,C}$ = 6.9 Hz), 112.0, 121.3, 122.9 (d, $J_{P,C}$ = 12.2 Hz), 125.0, 128.7, 129.6 (d, $J_{P,C}$ = 8.8 Hz), 136.6 (d, $J_{P,C}$ = 8.4 Hz), 151.2 (d, $J_{P,C}$ = 3.1 Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{12}H_{16}O_4P$: 255.0786, found 255.0800.

7,9-Dichloro-2-ethoxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (6f)



By following the general procedure, **6f** was prepared from 2,4-dichloro-6-vinylphenyl ethyl allylphosphonate (**4f**) (0.70 g, 2.18 mmol), and ruthenium catalyst **5** (103 mg, 0.109 mmol) as a greenish dense oil (0.46 g, 72%). IR (thin film, cm^{-1}): 1276 (P = O), 1242 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 40.02$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.29$ (t, 3H, $J = 7.1$ Hz), 2.76–3.05 (m, 2H), 4.15–4.31 (m, 2H), 6.02–6.15 (m, 1H), 6.66–6.72 (m, 1H), 7.46 (d, 1H, $J = 2.6$ Hz), 7.73 (d, 1H, $J = 2.6$ Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 16.2$ (d, $J_{P,C}$ = 6.0 Hz), 25.7 (d, $J_{P,C}$ = 126 Hz), 62.9 (d, $J_{P,C}$ = 6.9 Hz), 125.0 (d, $J_{P,C}$ = 12.2 Hz), 126.8 (d, $J_{P,C}$ = 3.6 Hz), 128.0 (d, $J_{P,C}$ = 9.2 Hz), 128.8, 128.9, 129.1, 130.8, 142.3 (d, $J_{P,C}$ = 7.6 Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{11}H_{12}O_3PCl_2$: 292.9901, found 292.9908.

2-Ethoxy-7-nitro-3H-benzo[f][1,2]oxaphosphepine 2-oxide (6 g)

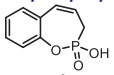


By following the general procedure, **6 g** was prepared from ethyl (4-nitro-2-vinylphenyl) allylphosphonate (**4 g**) (1.85 g, 6.22 mmol), and ruthenium catalyst **5** (295 mg, 0.31 mmol) as a brown dense oil (1.12 g, 67%). IR (thin film, cm^{-1}): 1278 (P = O), 1233 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 38.90$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.29$ (t, 3H, $J = 7.1$ Hz), 2.78–3.03 (m, 2H), 4.18–4.28 (m, 2H), 6.06–6.19 (m, 1H), 6.81–6.88 (m, 1H), 7.44–7.48 (m, 1H), 8.22 (dd, 1H, $J = 8.9$, 2.8 Hz), 8.30 (d, 1H, $J = 2.8$ Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 16.2$ (d, $J_{P,C}$ = 5.8 Hz), 25.7 (d, $J_{P,C}$ = 124 Hz), 62.8 (d, $J_{P,C}$ = 6.8 Hz), 123.2 (d, $J_{P,C}$ = 3.8 Hz), 124.6, 125.0 (d, $J_{P,C}$ = 12.4 Hz), 126.4, 128.2 (d, $J_{P,C}$ = 9.2 Hz), 128.8, 144.1, 151.9 (d, $J_{P,C}$ = 8.0 Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{11}H_{13}NO_5P$: 270.0531, found 270.0539.

General procedure for the synthesis of 2-hydroxy-3H-1,2-benzoxaphosphepine 2-oxides 7

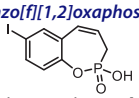
The corresponding ethoxy derivative **6** (1.0 eq) was dissolved in dry DCM (20 ml/1 mmol of **6**), then TMSBr (6.0 eq) was added dropwise. The reaction mixture was stirred under inert atmosphere at rt for 24 h. The volatiles were removed *in vacuo*, and the residue was treated with MeOH (15 ml/1 mmol of **6**), concentrated, purified by column chromatography on silica gel (EtOAc 100%). Products were recrystallised from EtOAc.

2-Hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide(7a)



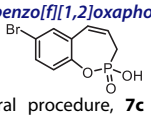
By following the general procedure, **7a** was prepared from 2-ethoxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**6a**) (0.32 g, 1.43 mmol) and TMSBr (1.12 ml, 8.56 mmol) as a white solid (0.25 g, 88%). Mp: 128–129 °C. IR (KBr, cm^{-1}): 2487 (O = P-OH), 2203 (O = P-OH), 1665 (O = P-OH), 1258 (P = O), 1223 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 36.64$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.57$ (dd, 1H, $J = 6.7$, 1.0 Hz), 2.62 (dd, 1H, $J = 6.7$, 1.0 Hz), 5.88–6.01 (m, 1H), 6.64 (dd, 1H, $J = 10.8$, 5.0 Hz), 7.09–7.14 (m, 1H), 7.17–7.23 (m, 1H), 7.27–7.37 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.1$ (d, $J_{P,C}$ = 125 Hz), 121.8 (d, $J_{P,C}$ = 3.4 Hz), 123.6 (d, $J_{P,C}$ = 12.2 Hz), 124.5, 127.9, 129.1 (d, $J_{P,C}$ = 8.4 Hz), 129.2, 130.6, 147.9 (d, $J_{P,C}$ = 7.6 Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_9H_{10}O_3P$: 197.0368, found 197.0371.

2-Hydroxy-7-iodo-3H-benzo[f][1,2]oxaphosphepine 2-oxide (7b)



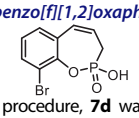
By following the general procedure, **7b** was prepared from 2-ethoxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**6b**) (2.22 g, 6.34 mmol) and TMSBr (4.98 ml, 38.0 mmol) as a white solid (1.66 g, 81%). Mp: 193–194 °C.IR (KBr, cm^{-1}): 2490 (O = P-OH), 2198 (O = P-OH), 1652 (O = P-OH), 1259 (P = O), 1217 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 36.14$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.59$ (dd, 1H, $J = 6.7$, 1.0 Hz), 2.65 (dd, 1H, $J = 6.7$, 1.0 Hz), 5.91–6.03 (m, 1H), 6.56–6.62 (m, 1H), 6.92 (dd, 1H, $J = 8.4$, 1.1 Hz), 7.64 (dd, 1H, $J = 8.4$, 2.2 Hz), 7.68 (d, 1H, $J = 2.2$ Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.1$ (d, $J_{P,C}$ = 125 Hz), 88.6 (d, $J_{P,C}$ = 1.5 Hz), 124.2 (d, $J_{P,C}$ = 3.2 Hz), 124.9 (d, $J_{P,C}$ = 12.2 Hz), 127.8 (d, $J_{P,C}$ = 8.4 Hz), 130.6, 137.6, 138.7, 147.9 (d, $J_{P,C}$ = 7.4 Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_9H_9O_3PI$: 322.9334, found 322.9345.

7-Bromo-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (7c)



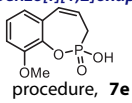
By following the general procedure, **7c** was prepared from 7-bromo-2-ethoxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**6c**) (0.31 g, 1.02 mmol) and TMSBr (0.80 ml, 6.14 mmol) as a white solid (0.23 g, 82%). Mp: 163–164 °C. IR (KBr, cm^{-1}): 1652 (O = P-OH), 1224 (P = O), 1206 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 36.27$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.61$ (dd, 1H, $J = 6.7$, 0.9 Hz), 2.66 (dd, 1H, $J = 6.7$, 0.9 Hz), 5.93–6.06 (m, 1H), 6.58–6.64 (m, 1H), 7.07 (dd, 1H, $J = 8.6$, 0.9 Hz), 7.50 (dd, 1H, $J = 8.6$, 2.5 Hz), 7.54 (d, 1H, $J = 2.5$ Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.1$ (d, $J_{P,C} = 125$ Hz), 116.3 (d, $J_{P,C} = 1.5$ Hz), 124.0 (d, $J_{P,C} = 3.4$ Hz), 125.1 (d, $J_{P,C} = 12.0$ Hz), 127.9 (d, $J_{P,C} = 8.6$ Hz), 130.3, 131.8, 132.8, 147.2 (d, $J_{P,C} = 7.6$ Hz) ppm. HRMS (ESI) $[M + H]^+$: *m/z* calcd for $C_9H_9O_3PBr$: 274.9473, found 274.9470.

9-Bromo-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (7d)



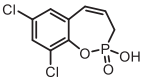
By following the general procedure, **7d** was prepared from 9-bromo-2-ethoxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**6d**) (0.60 g, 1.98 mmol) and TMSBr (1.55 ml, 11.9 mmol) as a white solid (0.49 g, 90%). Mp: 180–181 °C. IR (KBr, cm^{-1}): 2545 (O = P-OH), 2125 (O = P-OH), 1214 (P = O), 1210 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 36.44$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.63$ (dd, 1H, $J = 6.6$, 0.9 Hz), 2.68 (dd, 1H, $J = 6.6$, 0.9 Hz), 5.92–6.05 (m, 1H), 6.61–6.67 (m, 1H), 7.09–7.14 (m, 1H), 7.30 (dd, 1H, $J = 7.8$, 1.3 Hz), 7.64 (dd, 1H, $J = 7.9$, 1.5 Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.3$ (d, $J_{P,C} = 125$ Hz), 115.9 (d, $J_{P,C} = 3.8$ Hz), 124.6 (d, $J_{P,C} = 12.1$ Hz), 125.5, 128.6 (d, $J_{P,C} = 8.6$ Hz), 129.7, 130.2, 132.4, 144.9 (d, $J_{P,C} = 7.4$ Hz) ppm. HRMS (ESI) $[M + H]^+$: *m/z* calcd for $C_9H_9O_3PBr$: 274.9473, found 274.9473.

2-Hydroxy-9-methoxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (7e)



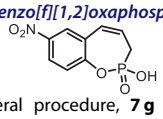
By following the general procedure, **7e** was prepared from 2-ethoxy-9-methoxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**6e**) (185 mg, 0.73 mmol) and TMSBr (0.57 ml, 4.37 mmol) as a white solid (120 mg, 73%). Mp: 200–201 °C. IR (KBr, cm^{-1}): 2527 (O = P-OH), 2224 (O=P-OH), 1275 (P = O), 1256 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 37.38$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.56$ (dd, 1H, $J = 6.6$, 0.9 Hz), 2.61 (dd, 1H, $J = 6.6$, 0.9 Hz), 3.80 (s, 3H), 5.87–5.99 (m, 1H), 6.59 (dd, 1H, $J = 10.9$, 4.8 Hz), 6.82 (dd, 1H, $J = 7.7$, 1.3 Hz), 7.01–7.05 (m, 1H), 7.08–7.14 (m, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.3$ (d, $J_{P,C} = 126$ Hz), 55.9, 112.0, 121.6, 123.7 (d, $J_{P,C} = 12.2$ Hz), 124.4, 128.9, 129.0, 129.1, 137.2 (d, $J_{P,C} = 7.6$ Hz), 151.5 (d, $J_{P,C} = 3.3$ Hz) ppm. HRMS (ESI) $[M + H]^+$: *m/z* calcd for $C_{10}H_{12}O_4P$: 227.0473, found 227.0477.

7,9-Dichloro-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (7f)



By following the general procedure, **7f** was prepared from 7,9-dichloro-2-ethoxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**6f**) (0.37 g, 1.26 mmol) and TMSBr (1.00 ml, 7.57 mmol) as a white solid (0.27 g, 81%). Mp: 192–193 °C. IR (KBr, cm^{-1}): 2522 (O = P-OH), 2219 (O = P-OH), 1230 (P = O), 1155 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 36.50$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.68$ (dd, 1H, $J = 6.7$, 0.9 Hz), 2.73 (dd, 1H, $J = 6.7$, 0.9 Hz), 5.99–6.11 (m, 1H), 6.62 (dd, 1H, $J = 11.1$, 4.9 Hz), 7.41 (d, 1H, $J = 2.6$ Hz), 7.66 (d, 1H, $J = 2.6$ Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.3$ (d, $J_{P,C} = 125$ Hz), 126.1 (d, $J_{P,C} = 12.2$ Hz), 127.2 (d, $J_{P,C} = 3.8$ Hz), 127.5 (d, $J_{P,C} = 8.6$ Hz), 128.2, 128.5, 128.9, 131.2, 143.0 (d, $J_{P,C} = 7.4$ Hz) ppm. HRMS (ESI) $[M + H]^+$: *m/z* calcd for $C_9H_8O_3PCl_2$: 264.9588, found 264.9595.

2-Hydroxy-7-nitro-3H-benzo[f][1,2]oxaphosphepine 2-oxide (7 g)



By following the general procedure, **7 g** was prepared from 2-ethoxy-7-nitro-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**6 g**) (0.33 g, 1.23 mmol) and TMSBr (0.96 ml, 7.36 mmol) as a white solid (0.21 g, 71%). Mp: 183–184 °C. IR (KBr, cm^{-1}): 2558 (O = P-OH), 2263 (O = P-OH), 1262 (P = O), 1221 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 39.26$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.69$ (dd, 1H, $J = 6.6$, 0.9 Hz), 2.74 (dd, 1H, $J = 6.6$, 0.9 Hz), 6.02–6.15 (m, 1H), 6.73–6.80 (m, 1H), 7.32–7.36 (m, 1H), 8.18 (dd, 1H, $J = 8.9$, 2.9 Hz), 8.25 (d, 1H, $J = 2.9$ Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.4$ (d, $J_{P,C} = 125$ Hz), 123.2 (d, $J_{P,C} = 3.6$ Hz), 124.3, 126.0 (d, $J_{P,C} = 12.0$ Hz), 126.3, 127.7 (d, $J_{P,C} = 9.0$ Hz), 129.1, 143.7, 152.7 (d, $J_{P,C} = 7.6$ Hz) ppm. HRMS (ESI) $[M + H]^+$: *m/z* calcd for $C_9H_9NO_5P$: 242.0218, found 242.0226.

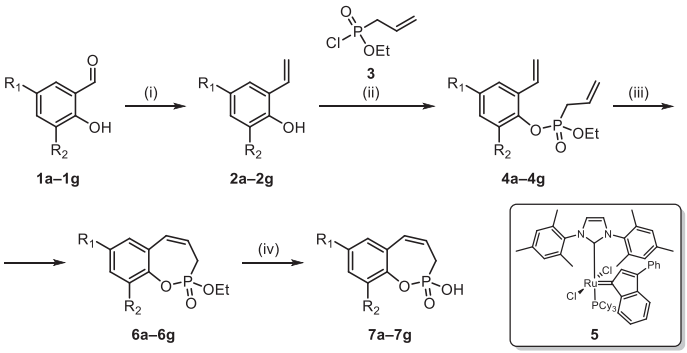
Carbonic anhydrase inhibition assay

The CA-catalysed CO_2 hydration activity was assayed by using an applied photophysics stopped-flow apparatus[24]. Phenol red (0.2 mM) was used as indicator following the initial rates of the CA-catalysed CO_2 hydration reaction for a period of 10–100 s. The indicator worked at the absorbance maximum of 557 nm, with 20 mM HEPES buffer (pH 7.4) and 20 mM $NaClO_4$ for maintaining constant ionic strength. For the determination of the kinetic parameters and inhibition constants, the CO_2 concentrations were varied from 1.7 to 17 mM. For each inhibitor, at least six traces of the initial 5–10% of the reaction were used for determining the initial velocity. The uncatalysed rates were determined in the same fashion and subtracted from the total observed rates. The stock solutions of inhibitor were prepared as 1 mM solutions in distilled, deionised water. Afterwards, dilutions down to 0.01 nM were prepared in distilled and deionised water. Inhibitor and enzyme were preincubated together for 6 h at room temperature in order to allow for the formation of the enzyme–inhibitor complex. The inhibition constants were acquired by the non-linear least squares method using PRISM 3 and the Cheng–Prusoff equation, whereas the kinetic parameters of uninhibited enzymes were obtained from Lineweaver–Burk plots and represent the mean from at least three different determinations. All CA isoforms were recombinant, obtained in-house as reported earlier[25–27].

Results and discussion

Chemistry

The synthetic strategy for the synthesis of 3*H*-1,2-benzoxaphosphepine 2-oxides is outlined in Scheme 1. The synthesis



1, 2, 4, 6, 7	a	b	c	d	e	f	g
R_1	H	I	Br	H	H	Cl	NO_2
R_2	H	H	H	Br	OMe	Cl	H

Scheme 1. Reagents and conditions: (i) $MePPh_3Br$, *t*-BuOK, THF, rt, 18 h, 76–88%; (ii) NEt_3, DCM, 0 °C to rt, 18 h, 60–94%; (iii) **5**, PhMe, 70 °C, 4 h, 63–86%; (iv) TMSBr, DCM, rt, 24 h, 71–90%.

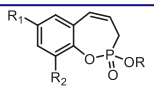
commenced with the Wittig reaction of commercially available 2-hydroxybenzaldehydes **1**, which provided olefins **2** in high yields. In the following step, compounds **2** were treated with ethyl allylphosphonochloridate (**3**, the reagent was prepared according to the literature procedure[23]) to give diolefins **4** in good to excellent yields. These key intermediates **4** were successfully cyclised by ring-closing metathesis, utilising commercially accessible Ru-based catalyst **5**. The reaction furnished corresponding cyclic ethyl phosphonates **6** in good yields. Finally, compounds **6** were treated with TMSBr to afford hydroxy derivatives **7** in very good yields.

Carbonic anhydrase inhibition

The newly synthesised compounds **6** and **7** were evaluated for their CA inhibition activity by using the stopped-flow CO_2 hydrase assay[24]. The study was carried out against four human CA isoforms – the ubiquitous cytosolic CA I and II as well as *trans*-membrane tumour-associated CA IX and XII[1–7]. The clinically used acetazolamide (AAZ) was used as the reference drug. The results of this study are shown in Table 1, and the following inferences could be drawn:

i. All synthesised benzoxaphosphepine2-oxide derivatives **6–7** have no inhibitory activity towards cytosolic isoforms hCA I and hCA II (K_I > 100 μM), whose inhibition in most cases is undesirable, as hCA I and II isoforms are found in many tissues of the organism[1,2,7]. It should be mentioned that AAZ is a highly effective inhibitor of all the four hCA isoforms considered here, which explains the many side effects of that drug[28,29].
ii. The tumour-associated hCA IX isoform was inhibited by all synthesised compounds **6–7** with inhibition constants in the sub-micromolar to low micromolar range (K_I: 0.67–11.3 μM). The compound **7g** was found to be the most potent hCA IX inhibitor among tested compounds with K_I = 0.67 μM.
iii. The other tumour-associated isoform hCA XII was also notably inhibited by all the synthesised derivatives **6–7** with K_I values in the low micromolar and sub-micromolar range (K_I: 0.51–7.4 μM). Among all tested compounds, compound **7a** was the most effective inhibitor against hCA XII with K_I = 0.51 μM.
iv. Overall, hydroxy derivatives **7** showed slightly higher inhibition potency against tumour-associated hCA IX and XII than the corresponding ethoxy derivatives **6**. In the case of hydroxy derivatives **7**, the range of K_I values was found to be from 0.67 to 2.5 μM for hCA IX and from 0.51 to 1.8 μM for hCA XII. Regarding ethoxy derivatives **6**, the range of K_I values was from 0.76 to 11.3 μM for hCA IX and from 0.95 to 7.4 μM for hCA XII.

Table 1. Inhibition data of compounds **6–7** and the standard inhibitor acetazolamide (AAZ) against human CA isoforms I, II, IX and XII.



6, 7

				K_I (μM)[a,b]			
Cmpd	R	R_1	R_2	hCA I	hCA II	hCA IX	hCA XII
6a	Et	H	H	>100	>100	0.82	0.82
7a	H	H	H	>100	>100	1.3	0.51
6b	Et	I	H	>100	>100	4.7	2.4
7b	H	I	H	>100	>100	0.88	0.68
6c	Et	Br	H	>100	>100	0.76	1.6
7c	H	Br	H	>100	>100	1.0	0.96
6d	Et	H	Br	>100	>100	11.3	3.3
7d	H	H	Br	>100	>100	2.5	1.8
6e	Et	H	OMe	>100	>100	9.0	7.4
7e	H	H	OMe	>100	>100	1.8	1.2
6f	Et	Cl	Cl	>100	>100	6.1	3.4
7f	H	Cl	Cl	>100	>100	0.80	1.7
6g	Et	NO_2	H	>100	>100	3.9	0.95
7g	H	NO_2	H	>100	>100	0.67	1.0
AAZ	–			0.25	0.012	0.025	0.006

[a]Values are mean from three different assays using the stopped-flow technique (errors were in the range of ± 5–10% of the reported values).
[b]6 h incubation.

Albeit the efficacy of the synthesised compounds **6–7** was lower in comparison to the reference drug AAZ, these compounds displayed desirable isoform-selective inhibition activity for tumour-associated isoforms hCA IX and hCA XII. The establishing of the selectivity is necessary to prevent possible side effects from inhibition of cytosolic hCA I and II isoforms[7,28,29].

Conclusions

Herein we report the synthesis of novel benzoxaphosphepine 2-oxide derivatives as a new class of tumour-associated CA IX and XII inhibitors. These compounds were investigated against four human CA isoforms with pharmacological applications (hCA I, hCA II, hCA IX, and hCA XII). All tested compounds exhibited selective inhibition of the tumour-associated hCA isoforms IX and XII with activities in the sub-micromolar or low micromolar range, whereas the off-target cytosolic isoforms hCA I and II were not significantly inhibited by these compounds. Considering that hCA IX and XII are implicated in processes connected to tumourigenesis[2–6], present findings give an insight towards development of new selective anti-tumour agents.

Disclosure statement

No potential competing interest was reported by all authors except CTS. CT Supuran is Editor-in-Chief of the Journal of Enzyme Inhibition and Medicinal Chemistry. He was not involved in the assessment, peer review, or decision-making process of this paper. The authors have no relevant affiliations of financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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ORCID

Aleksandrs Pustenko http://orcid.org/0000-0003-4930-7372
Anastasija Balašova http://orcid.org/0000-0002-1185-1922
Alessio Nocentini http://orcid.org/0000-0003-3342-702X
Claudiu T. Supuran http://orcid.org/0000-0003-4262-0323
Raivis Žalubovskis http://orcid.org/0000-0002-9471-1342

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Anastasija Balašova, Aleksandrs Pustenko, Alessio Nocentini, Daniela Vullo, Claudiu T. Supuran & Raivis Žalubovskis

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RESEARCH ARTICLE

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Aryl derivatives of 3*H*-1,2-benzoxaphosphepine 2-oxides as inhibitors of cancer-related carbonic anhydrase isoforms IX and XII

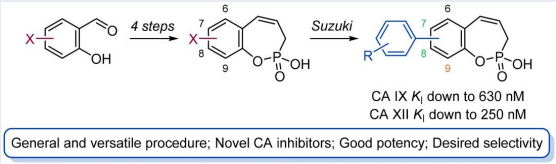
Anastasija Balašova[a,b], Aleksandrs Pustenko[a], Alessio Nocentini[c], Daniela Vullo[c], Claudiu T. Supuran[c] and Raivis Žalubovskis[a,b]

[a]Latvian Institute of Organic Synthesis, Riga, Latvia; [b]Institute of Technology of Organic Chemistry, Riga Technical University, Riga, Latvia; [c]Department of Neurofarba, Section of Pharmaceutical and Nutraceutical Sciences, Florence, Italy

ABSTRACT

A range of 3*H*-1,2-benzoxaphosphepine 2-oxide aryl derivatives with various substitution patterns at positions 7, 8, or 9 of the scaffold was synthesised in five steps from the commercially available salicylaldehydes. All of the newly obtained compounds were studied for their inhibition potency against carbonic anhydrase (CA) isoforms I, II, IX, and XII. Delightfully, these compounds showed a striking selectivity for the cancer-associated CA IX and XII over the cytosolic CA I and II, whose inhibition may lead to side-effects. Overall, a structure–activity relationship (SAR) revealed that 7- and 8-substituted aryl derivatives were more effective inhibitors of CA IX and XII than 9-substituted derivatives. In addition, the fluorine-containing analogues emerged as the most potent CA IX/XII inhibitors in this series.

GRAPHICAL ABSTRACT



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Introduction

Cancer is a devastating group of diseases which is one of the major causes of death worldwide, accounting for nearly 10 million deaths in 2020[1]. By approaching to the middle of the 21st century, the cancer incidence and mortality rates are expected to increase to 29.5 million and 16.4 million per year, respectively[2]. The development of novel and improved therapies to combat cancer is therefore of a paramount priority. Carbonic anhydrase (CA, EC 4.2.1.1) isoforms CA IX and CA XII are presently serving as biomarkers and anticancer drug targets[3]. Both of these isozymes are highly overexpressed in various cancer types and may contribute to the growth of cancer, subsequent metastasis, as well as impaired therapeutic response[4].

CA IX and CA XII are transmembrane zinc metalloenzymes that belong to the α-CA family[5]. Humans have 15 α-CA isoforms with different expression patterns, molecular features and kinetic properties[6]. These enzymes are involved in many important physiological processes (e.g. respiration, homeostasis, and metabolism), as they catalyse the reversible hydration of CO_2[6]. Hence, the development of selective CA IX and XII inhibitors is highly desired to prevent possible side effects.

At present, none of the clinically used CA inhibitors displays selectivity for a specific isoform[7]. Due to the high level of structural homology between the CA isoforms and sequence similarities within the active site, the design and development of isoform-specific CA inhibitors remain challenging[8]. However, to our knowledge, to date, several molecules have been reported as potent and selective CA IX and XII inhibitors, including coumarins[9–12], isocoumarins[13], thiocoumarins[9,12], sulfocoumarins[9,14–17], and their congeners—homosulfocoumarins[18] (3*H*-1,2-benzoxathiepine 2,2-dioxides).

In the course of our research, devoted to discovering novel chemotypes acting as selective CA IX/XII inhibitors that could be employed in cancer chemotherapy, we previously designed and synthesised a series of 3*H*-1,2-benzoxaphosphepine 2-oxides as bioisosteres of homosulfocoumarins[19]. These new compounds showed excellent selectivity and good inhibitory activity against both CA IX and XII. Our interest in phosphorus heterocycles stems from the fact that phosphorus functionalities can improve the pharmacokinetics profile, bioavailability and water solubility of drugs[20]. Moreover, several groups reported the use of organophosphorus compounds as CA inhibitors[21].

CONTACT Raivis Žalubovskis raivis@osi.lv Latvian Institute of Organic Synthesis, 21 Aizkraukles St., Riga, LV-1006, Latvia.
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In continuation of our previous work on the development of benzoxaphosphepine 2-oxides as CA inhibitors[19], the current study is aimed to investigate the chemical space around this novel chemotype. In this paper, we present the synthesis and biological evaluation of 7-, 8- and 9-aryl-substituted benzoxaphosphepine 2-oxides.

Materials and methods

Chemistry

The air- or moisture-sensitive reactions were performed under an argon atmosphere using dry glassware. All reagents, starting materials and solvents were purchased from commercial sources and used as received. TLC was performed on silica gel plates (60 F_{254}) and visualised under UV light (254 and 365 nm). Reversed-phase chromatography was done on a Biotage Isolera One system using Biotage SNAP KP-C18-HS cartridges. Melting points were determined on an OptiMelt MPA100 apparatus. IR spectra were recorded on a Shimadzu FTIR IR Prestige-21 spectrophotometer. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker Avance Neo 400 MHz spectrometer. The chemical shifts (δ) were reported in parts per million (ppm) relative to the residual solvent peak as an internal reference (DMSO-d_6: ^{1}H 2.50, ^{13}C 39.52; CD_3OD: ^{1}H 3.31, ^{13}C 49.00). ^{31}P shifts were referenced externally to H_3PO_4. The coupling constants (J) were expressed in Hertz (Hz). HRMS was performed on a Q-TOF Micro mass spectrometer.

The synthesis and characterisation of 4-iodo-2-vinylphenol (**2a**), 2-bromo-6-vinylphenol (**2c**), ethyl allylphosphonochloridate (**3**), ethyl (4-iodo-2-vinylphenyl) allylphosphonate (**4a**), 2-bromo-6-vinylphenyl ethyl allylphosphonate (**4c**) together with corresponding 3*H*-1,2-benzoxaphosphepine 2-oxides **6a,c** and **7a,c** are reported by our group in the previous paper[19].

5-Bromo-2-vinylphenol (2b)

The titled compound **2b** was obtained according to the general procedure previously reported[19] using $MePPh_3Br$ (16.35 g, 45.77 mmol), *t*BuOK (5.25 g, 46.8 mmol), and 4-bromo-2-hydroxybenzaldehyde (4.00 g, 19.9 mmol) as a yellowish solid (3.21 g, 81%). The NMR spectra are consistent with the literature[19]. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 5.24$ (dd, 1H, $J = 11.3$, 1.6 Hz), 5.79 (dd, 1H, $J = 17.6$, 1.6 Hz), 6.87 (dd, 1H, $J = 17.6$, 11.3 Hz), 6.95 (dd, 1H, $J = 8.3$, 2.0 Hz), 7.00 (d, 1H, $J = 2.0$ Hz), 7.37 (d, 1H, $J = 8.3$ Hz), and 10.13 (s, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 114.6$, 118.3, 120.8, 122.0, 123.5, 128.0, 130.8, and 155.7 ppm.

5-Bromo-2-vinylphenyl ethyl allylphosphonate (4b)

The titled compound **4b** was obtained according to the general procedure previously reported[19] using 5-bromo-2-vinylphenol (**2b**) (3.87 g, 19.4 mmol), ethyl allylphosphonochloridate (**3**) (3.46 ml, 23.3 mmol) and NEt_3 (3.38 ml, 24.3 mmol) as a colourless oil (4.23 g, 66%). IR (thin film, cm^{-1}): 1265 (P = O), 1218 (P = O), 1181 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 25.50$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.20$ (t, 3H, $J = 7.0$ Hz), 2.94 (dt, 1H, $J = 7.3$, 1.2 Hz), 2.99 (dt, 1H, $J = 7.3$, 1.2 Hz), 4.02–4.18 (m, 2H), 5.20–5.33 (m, 2H), 5.42 (dd, 1H, $J = 11.2$, 1.0 Hz), 5.70–5.83 (m, 1H), 5.91 (dd, 1H, $J = 16.6$, 1.0 Hz), 6.84–6.93 (m, 1H), 7.38–7.43 (m, 1H), 7.48–7.50 (m, 1H), and 7.62–7.66 (m, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 16.1$ (d, $J_{P,C} = 5.6$ Hz), 30.9 (d, $J_{P,C} = 137$ Hz), 62.8 (d, $J_{P,C} = 6.8$ Hz), 117.3, 120.5 (d, $J_{P,C} = 1.4$ Hz), 120.6 (d, $J_{P,C} = 15.0$ Hz), 123.7 (d, $J_{P,C} = 2.6$ Hz), 127.3 (d, $J_{P,C} = 11.6$ Hz), 128.0, 128.2 (d, $J_{P,C} = 5.0$ Hz), 129.2, and 147.8 (d, $J_{P,C} = 9.1$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{13}H_{17}O_3PBr$: 331.0099, found 331.0114.

8-Bromo-2-ethoxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (6b)

The titled compound **6b** was obtained according to the general procedure previously reported[19] using 5-bromo-2-vinylphenyl ethyl allylphosphonate (**4b**) (2.00 g, 6.04 mmol) and ruthenium catalyst **5** (CAS: 250220–36-1) (286 mg, 0.30 mmol) as a greenish dense oil (1.51 g, 83%). IR (thin film, cm^{-1}): 1270 (P = O), 1237 (P = O), 1202 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 39.41$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.28$ (t, 3H, $J = 7.0$ Hz), 2.68–2.93 (m, 2H), 4.15–4.25 (m, 2H), 5.94–6.04 (m, 1H), 6.66–6.70 (m, 1H), 7.28–7.32 (m, 1H), 7.43–7.48 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 16.2$ (d, $J_{P,C} = 5.7$ Hz), 25.6 (d, $J_{P,C} = 125$ Hz), 62.5 (d, $J_{P,C} = 6.8$ Hz), 121.3, 123.3 (d, $J_{P,C} = 12.2$ Hz), 124.4 (d, $J_{P,C} = 3.5$ Hz), 127.1, 128.0, 128.7 (d, $J_{P,C} = 8.8$ Hz), 132.3, and 147.9 (d, $J_{P,C} = 7.8$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{11}H_{13}O_3PBr$: 302.9786, found 302.9781.

8-Bromo-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (7b)

The titled compound **7b** was obtained according to the general procedure previously reported[19] using 8-bromo-2-ethoxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**6b**) (1.13 g, 3.73 mmol) and TMSBr (2.93 ml, 22.4 mmol) as a white solid (0.88 g, 86%). Mp: 182–184 °C. IR (KBr, cm^{-1}): 2519 (O = P-OH), 2245 (O = P-OH), 1250 (P = O), and 1205 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 35.97$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.58$–2.68 (m, 2H), 5.91–6.04 (m, 1H), 6.57–6.63 (m, 1H), 7.24–7.29 (m, 1H), 7.31–7.34 (m, 1H), 7.38–7.42 (m, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.3$ (d, $J_{P,C} = 125$ Hz), 120.9, 124.4 (d, $J_{P,C} = 12.0$ Hz), 124.6 (d, $J_{P,C} = 3.2$ Hz), 127.5, 128.2 (d, $J_{P,C} = 8.4$ Hz), 132.2, and 148.5 (d, $J_{P,C} = 7.4$ Hz) ppm. HRMS (ESI) $[M-H]^-$: *m/z* calcd for $C_9H_7O_3PBr$: 272.9316, found 272.9320.

General procedure for the synthesis of 3H-1,2-benzoxaphosphepine 2-oxide aryl derivatives 8–10

The corresponding 3*H*-1,2-benzoxaphosphepine 2-oxide halogen derivative **7** (200 mg, 1.0 eq) was placed in a pressure tube and dissolved in 1,4-dioxane (5 ml) followed by the addition of degassed water (1 ml). The corresponding boronic acid (1.5 eq), K_2CO_3 (2.0 eq) and Pd(dppf)Cl_2 (10 mol% for iodo derivative **7a**; 20 mol% in case of bromo derivatives **7b** and **7c**) were added to the solution. The reaction mixture was purged with argon for 5 min, the tube was sealed and heated for 16 h at 80 °C. Upon cooling to rt, the reaction mixture was filtered through a pad of celite, which was washed with MeCN. The pH of the filtrate was adjusted to 2 by addition of TFA. After that, the filtrate was concentrated *in vacuo*. The crude product was purified by reversed-phase flash chromatography (MeCN/water = 10 to 95%) and recrystallised from EtOAc.

2-Hydroxy-7-phenyl-3H-benzo[f][1,2]oxaphosphepine 2-oxide (8a)

By following the general procedure, **8a** was prepared from 2-hydroxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7a**) (200 mg, 0.62 mmol), phenylboronic acid (114 mg, 0.93 mmol), K_2CO_3 (172 mg, 1.24 mmol), and Pd(dppf)Cl_2 (45 mg, 0.062 mmol) as a white solid (101 mg, 60%). Decomp. > 205 °C. IR (KBr, cm^{-1}): 2611 (O = P-OH), 2161 (O = P-OH), 1619 (O = P-OH), 1215 (P = O), and 1196 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 35.77$ ppm. ^{1}H

NMR (400 MHz, DMSO-d_6) $\delta = 2.62$ (d, 1H, $J = 6.6$ Hz), 2.67 (d, 1H, $J = 6.6$ Hz), 5.92–6.05 (m, 1H), 6.69–6.76 (m, 1H), 7.15–7.22 (m, 1H), 7.32–7.40 (m, 1H), 7.42–7.50 (m, 2H), and 7.56–7.70 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.3$ (d, $J_{P,C} = 125$ Hz), 122.3 (d, $J_{P,C} = 3.2$ Hz), 124.0 (d, $J_{P,C} = 12.2$ Hz), 126.7, 127.5 (d, $J_{P,C} = 2.4$ Hz), 128.3, 128.8, 129.0, 129.1 (d, $J_{P,C} = 8.6$ Hz), 136.4, 139.2, and 147.6 (d, $J_{P,C} = 7.5$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{15}H_{14}O_3P$: 273.0681, found 273.0685.

2-Hydroxy-7–(4-methoxyphenyl)-3H-benzo[f][1,2]oxaphosphepine 2-oxide (8b)

By following the general procedure, **8b** was prepared from 2-hydroxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7a**) (200 mg, 0.62 mmol), (4-methoxyphenyl)boronic acid (142 mg, 0.93 mmol), K_2CO_3 (172 mg, 1.24 mmol), and Pd(dppf)Cl_2 (45 mg, 0.062 mmol) as a white solid (115 mg, 61%). Mp: 214–216 °C. IR (KBr, cm^{-1}): 2522 (O = P-OH), 2207 (O = P-OH), 1265 (P = O), and 1219 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 36.14$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.61$ (d, 1H, $J = 6.1$ Hz), 2.66 (d, 1H, $J = 6.1$ Hz), 3.79 (s, 3H), 5.91–6.04 (m, 1H), 6.67–6.74 (m, 1H), 6.98–7.05 (m, 2H), 7.13–7.19 (m, 1H), and 7.50–7.64 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.2$ (d, $J_{P,C} = 125$ Hz), 55.2, 114.4, 122.2 (d, $J_{P,C} = 3.2$ Hz), 123.8 (d, $J_{P,C} = 12.2$ Hz), 126.9, 127.7, 128.1, 128.2, 129.2 (d, $J_{P,C} = 8.5$ Hz), 131.5, 136.1, 147.0 (d, $J_{P,C} = 7.5$ Hz), and 158.9 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{16}H_{16}O_4P$: 303.0786, found 303.0798.

Ethyl 4–(2-hydroxy-2-oxido-3H-benzo[f][1,2]oxaphosphepin-7-yl) benzoate (8c)

By following the general procedure, **8c** was prepared from 2-hydroxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7a**) (200 mg, 0.62 mmol), (4-(ethoxycarbonyl)phenyl)boronic acid (182 mg, 0.93 mmol), K_2CO_3 (172 mg, 1.24 mmol), and Pd(dppf)Cl_2 (45 mg, 0.062 mmol) as a white solid (126 mg, 59%). Mp: 250–252 °C. IR (KBr, cm^{-1}): 2512 (O = P-OH), 2187 (O = P-OH), 1710 (C = O), 1286 (P = O), and 1218 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 35.17$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.34$ (t, 3H, $J = 7.0$ Hz), 2.61 (d, 1H, $J = 6.0$ Hz), 2.66 (d, 1H, $J = 6.0$ Hz), 5.93–6.06 (m, 1H), 6.67–6.76 (m, 1H), 7.17–7.25 (m, 1H), 7.63–7.74 (m, 2H), 7.77–7.86 (m, 2H), and 7.97–8.07 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 14.2$, 27.4 (d, $J_{P,C} = 125$ Hz), 60.8, 122.6 (d, $J_{P,C} = 3.2$ Hz), 124.3 (d, $J_{P,C} = 12.2$ Hz), 126.8, 127.7, 128.6, 128.7, 128.8 (d, $J_{P,C} = 8.4$ Hz), 129.2, 129.8, 134.9, 143.6, 148.4 (d, $J_{P,C} = 7.6$ Hz), and 165.5 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{18}H_{18}O_5P$: 345.0892, found 345.0901.

7–(4-Fluorophenyl)-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (8d)

By following the general procedure, **8d** was prepared from 2-hydroxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7a**) (200 mg, 0.62 mmol), (4-fluorophenyl)boronic acid (130 mg, 0.93 mmol), K_2CO_3 (172 mg, 1.24 mmol), and Pd(dppf)Cl_2 (45 mg, 0.062 mmol) as a white solid (114 mg, 63%). Decomp. > 232 °C. IR (KBr, cm^{-1}): 2514 (O = P-OH), 2177 (O = P-OH), 1648 (O = P-OH), 1214 (P = O), and 1200 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 35.88$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.59$–2.70 (m, 2H), 5.91–6.06 (m, 1H), 6.67–6.75 (m, 1H), 7.15–7.22 (m, 1H), 7.24–7.33 (m, 2H), 7.55–7.63 (m, 2H), and 7.66–7.75 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.3$ (d, $J_{P,C} = 125$ Hz), 115.7 (d, $J_{F,C} = 21.0$ Hz), 122.3 (d, $J_{P,C} = 3.2$ Hz), 124.0 (d, $J_{P,C} = 12.2$ Hz), 127.4, 128.3, 128.6 (d, $J_{F,C} = 8.2$ Hz), 128.8, 129.1 (d, $J_{P,C} = 8.6$ Hz), 135.4, 135.6 (d, $J_{F,C} = 2.4$ Hz), 147.5 (d, $J_{P,C} = 7.4$ Hz), and 162.0 (d, $J_{F,C} = 244$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{15}H_{13}O_3PF$: 291.0586, found 291.0586.

7–(4-Chlorophenyl)-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (8e)

By following the general procedure, **8e** was prepared from 2-hydroxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7a**) (200 mg, 0.62 mmol), (4-chlorophenyl)boronic acid (146 mg, 0.93 mmol), K_2CO_3 (172 mg, 1.24 mmol), and Pd(dppf)Cl_2 (45 mg, 0.062 mmol) as a white solid (112 mg, 59%). Decomp. > 212 °C. IR (KBr, cm^{-1}): 2089 (O = P-OH), 1637 (O = P-OH), 1201 (P = O), and 1117 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 35.74$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.62$ (d, 1H, $J = 6.0$ Hz), 2.67 (d, 1H, $J = 6.0$ Hz), 5.92–6.06 (m, 1H), 6.67–6.75 (m, 1H), 7.16–7.23 (m, 1H), 7.47–7.54 (m, 2H), and 7.58–7.73 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.3$ (d, $J_{P,C} = 125$ Hz), 122.4 (d, $J_{P,C} = 3.2$ Hz), 124.1 (d, $J_{P,C} = 12.2$ Hz), 127.4, 128.4, 128.8, 128.9, 129.0 (d, $J_{P,C} = 8.6$ Hz), 132.4, 135.0, 137.9, and 147.8 (d, $J_{P,C} = 7.5$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{15}H_{13}O_3PCl$: 307.0291, found 307.0290.

2-Hydroxy-7–(4-(trifluoromethyl)phenyl)-3H-benzo[f][1,2]oxaphosphepine 2-oxide (8f)

By following the general procedure, **8f** was prepared from 2-hydroxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7a**) (200 mg, 0.62 mmol), (4-(trifluoromethyl)phenyl)boronic acid (177 mg, 0.93 mmol), K_2CO_3 (172 mg, 1.24 mmol), and Pd(dppf)Cl_2 (45 mg, 0.062 mmol) as an off-white solid (133 mg, 63%). Decomp. > 280 °C. IR (KBr, cm^{-1}): 1669 (O = P-OH), 1222 (P = O), and 1197 (P = O). ^{31}P NMR (162 MHz, CD_3OD) $\delta = 40.27$ ppm. ^{1}H NMR (400 MHz, CD_3OD) $\delta = 2.52$ (d, 1H, $J = 6.7$ Hz), 2.57 (d, 1H, $J = 6.7$ Hz), 5.98–6.11 (m, 1H), 6.63–6.69 (m, 1H), 7.23–7.27 (m, 1H), 7.47–7.50 (m, 1H), 7.52–7.57 (m, 1H), 7.60–7.63 (m, 2H), and 7.83–7.87 (m, 2H) ppm. ^{13}C NMR (101 MHz, CD_3OD) $\delta = 29.2$ (d, $J_{P,C} = 125$ Hz), 121.7, 124.1 (d, $J_{P,C} = 3.0$ Hz), 124.3 (q, $J_{F,C} = 3.6$ Hz), 124.7 (q, $J_{F,C} = 3.5$ Hz), 126.5 (d, $J_{P,C} = 12.2$ Hz), 127.1, 128.4, 129.7 (d, $J_{P,C} = 8.3$ Hz), 129.9, 130.7, 131.6, 132.2 (q, $J_{F,C} = 32.0$ Hz), 136.3, 142.7, and 151.1 (d, $J_{P,C} = 7.5$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{16}H_{13}O_3PF_3$: 341.0554, found 341.0566.

7–(3,5-Dichlorophenyl)-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (8 g)

By following the general procedure, **8 g** was prepared from 2-hydroxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7a**) (200 mg, 0.62 mmol), (3,5-dichlorophenyl)boronic acid (178 mg, 0.93 mmol), K_2CO_3 (172 mg, 1.24 mmol), and Pd(dppf)Cl_2 (45 mg, 0.062 mmol) as a white solid (119 mg, 56%). Decomp. > 180 °C. IR (KBr, cm^{-1}): 2508 (O = P-OH), 2255 (O = P-OH), 1230 (P = O), and 1206 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 34.87$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.61$ (d, 1H, $J = 5.8$ Hz), 2.66 (d, 1H, $J = 5.8$ Hz), 5.92–6.05 (m, 1H), 6.66–6.73 (m, 1H), 7.15–7.21 (m, 1H), 7.55–7.60 (m, 1H), and 7.66–7.78 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.5$ (d, $J_{P,C} = 125$ Hz), 122.5 (d, $J_{P,C} = 3.2$ Hz), 124.3 (d, $J_{P,C} = 12.2$ Hz), 125.3, 126.8, 127.7, 128.6, 128.8 (d, $J_{P,C} = 8.5$ Hz), 129.4, 133.2, 134.7, 142.6, and 148.5 (d, $J_{P,C} = 7.5$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{15}H_{12}O_3PCl_2$: 340.9901, found 340.9906.

7–(3-Fluorophenyl)-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (8h)

By following the general procedure, **8h** was prepared from 2-hydroxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7a**) (200 mg, 0.62 mmol), (3-fluorophenyl)boronic acid (130 mg, 0.93 mmol), K_2CO_3 (172 mg, 1.24 mmol), and $Pd(dppf)Cl_2$ (45 mg, 0.062 mmol) as a white solid (180 mg, 60%). Mp: 171–173 °C. IR (KBr, cm^{-1}): 2579 (O = P-OH), 2308 (O = P-OH), and 1190 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 35.68$ ppm. ^1H NMR (400 MHz, DMSO-d_6) $\delta = 2.60–2.72$ (m, 2H), 5.90–6.08 (m, 1H), 6.67–6.77 (m, 1H), 7.14–7.24 (m, 2H), 7.46–7.60 (m, 3H), and 7.62–7.70 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.3$ (d, $J_{P,C} = 125$ Hz), 113.4 (d, $J_{F,C} = 22.2$ Hz), 114.2 (d, $J_{F,C} = 21.0$ Hz), 122.4 (d, $J_{P,C} = 3.2$ Hz), 122.7, 124.0 (d, $J_{P,C} = 12.2$ Hz), 127.6, 128.4, 129.0, 129.1, 130.9 (d, $J_{P,C} = 8.6$ Hz), 135.0, 141.6 (d, $J_{F,C} = 7.8$ Hz), 148.0 (d, $J_{P,C} = 7.6$ Hz), and 162.7 (d, $J_{F,C} = 243$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{15}H_{13}O_3PF$: 291.0586, found 291.0588.

2-Hydroxy-7-(o-tolyl)-3H-benzo[f][1,2]oxaphosphepine 2-oxide (8i)

By following the general procedure, **8i** was prepared from 2-hydroxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7a**) (200 mg, 0.62 mmol), *o*-tolylboronic acid (127 mg, 0.93 mmol), K_2CO_3 (172 mg, 1.24 mmol), and $Pd(dppf)Cl_2$ (45 mg, 0.062 mmol) as a white solid (101 mg, 57%). Mp: 156–158 °C. IR (KBr, cm^{-1}): 2585 (O = P-OH), 2303 (O = P-OH), 1623 (O = P-OH), and 1193 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 35.70$ ppm. ^1H NMR (400 MHz, DMSO-d_6) $\delta = 2.23$ (s, 3H), 2.63 (d, 1H, $J = 6.6$ Hz), 2.68 (d, 1H, $J = 6.6$ Hz), 5.90–6.03 (m, 1H), 6.64–6.74 (m, 1H), and 7.14–7.32 (m, 7H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 20.2$, 27.3 (d, $J_{P,C} = 125$ Hz), 121.6 (d, $J_{P,C} = 3.2$ Hz), 123.8 (d, $J_{P,C} = 12.2$ Hz), 126.0, 127.5, 127.7, 129.1 (d, $J_{P,C} = 8.6$ Hz), 129.6, 129.8, 130.4, 131.0, 134.8, 137.4, 140.2, and 147.0 (d, $J_{P,C} = 7.5$ Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{16}H_{16}O_3P$: 287.0837, found 287.0841.

Methyl 3–(2-hydroxy-2-oxido-3H-benzo[f][1,2]oxaphosphepin-7-yl) benzoate (8j)

By following the general procedure, **8j** was prepared from 2-hydroxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7a**) (200 mg, 0.62 mmol), (3-(methoxycarbonyl)phenyl)boronic acid (168 mg, 0.93 mmol), K_2CO_3 (172 mg, 1.24 mmol), and $Pd(dppf)Cl_2$ (45 mg, 0.062 mmol) as a white solid (117 mg, 57%). Mp: 145–147 °C. IR (KBr, cm^{-1}): 2303 (O = P-OH), 1718 (C = O), 1252 (P = O), and 1220 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 35.62$ ppm. ^1H NMR (400 MHz, DMSO-d_6) $\delta = 2.62$ (d, 1H, $J = 6.6$ Hz), 2.67 (d, 1H, $J = 6.6$ Hz), 3.89 (s, 3H), 5.93–6.06 (m, 1H), 6.71–6.76 (m, 1H), 7.19–7.24 (m, 1H), 7.59–7.68 (m, 3H), 7.93–7.97 (m, 2H), and 8.17–8.19 (m, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.4$ (d, $J_{P,C} = 125$ Hz), 52.3, 122.5 (d, $J_{P,C} = 3.0$ Hz), 124.2 (d, $J_{P,C} = 12.2$ Hz), 127.1, 127.6, 128.1, 128.6, 128.9, 129.0, 129.5, 130.4, 131.5, 135.2, 139.7, 148.0 (d, $J_{P,C} = 7.6$ Hz), and 166.1 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{17}H_{16}O_5P$: 331.0735, found 331.0734.

2-Hydroxy-7–(3-nitrophenyl)-3H-benzo[f][1,2]oxaphosphepine 2-oxide (8k)

By following the general procedure, **8k** was prepared from 2-hydroxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7a**) (200 mg, 0.62 mmol), (3-nitrophenyl)boronic acid (156 mg, 0.93 mmol), K_2CO_3 (172 mg, 1.24 mmol), and $Pd(dppf)Cl_2$ (45 mg, 0.062 mmol) as a yellow solid (120 mg, 61%). Mp: 231–233 °C. IR (KBr, cm^{-1}): 2580 (O = P-OH), 1646 (O = P-OH), and 1214 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 35.39$ ppm. ^1H NMR (400 MHz, DMSO-d_6) $\delta = 2.63$ (d, 1H, $J = 6.6$ Hz), 2.68 (d, 1H, $J = 6.6$ Hz), 5.94–6.07 (m, 1H), 6.71–6.77 (m, 1H), 7.21–7.26 (m, 1H), 7.72–7.78 (m, 3H), 8.12–8.23 (m, 2H), and 8.43–8.46 (m, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.3$ (d, $J_{P,C} = 125$ Hz), 121.0, 122.1, 122.6 (d, $J_{P,C} = 3.2$ Hz), 124.3 (d, $J_{P,C} = 12.2$ Hz), 127.8, 128.6, 128.8 (d, $J_{P,C} = 8.5$ Hz), 129.3, 130.5, 133.2, 133.9, 140.7, and 148.3, 148.4 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{15}H_{13}NO_5P$: 318.0531, found 318.0537.

7–(4-(tert-Butyl)phenyl)-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (8 l)

By following the general procedure, **8 l** was prepared from 2-hydroxy-7-iodo-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7a**) (200 mg, 0.62 mmol), (4-(*tert*-butyl)phenyl)boronic acid (166 mg, 0.93 mmol), K_2CO_3 (172 mg, 1.24 mmol), and $Pd(dppf)Cl_2$ (45 mg, 0.062 mmol) as a white solid (110 mg, 54%). Decomp. > 210 °C. IR (KBr, cm^{-1}): 2162 (O = P-OH), 1654 (O = P-OH), 1220 (P = O), and 1195 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 31.16$ ppm. ^1H NMR (400 MHz, DMSO-d_6) $\delta = 1.31$ (s, 9H), 2.41–2.47 (m, 1H), 5.83–5.98 (m, 1H), 6.53–6.63 (m, 1H), 7.01–7.11 (m, 1H), 7.41–7.51 (m, 4H), and 7.52–7.60 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 28.7$ (d, $J_{P,C} = 125$ Hz), 31.1, 34.2, 122.5 (d, $J_{P,C} = 2.8$ Hz), 125.3 (d, $J_{P,C} = 11.4$ Hz), 125.6, 126.2, 126.7, 128.3, 128.4 (d, $J_{P,C} = 8.4$ Hz), 128.7, 135.3, 136.6, 148.6 (d, $J_{P,C} = 7.4$ Hz), and 149.6 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{19}H_{22}O_3P$: 329.1307, found 329.1307.

2-Hydroxy-8-phenyl-3H-benzo[f][1,2]oxaphosphepine 2-oxide (9a)

By following the general procedure, **9a** was prepared from 8-bromo-2-hydroxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7b**) (200 mg, 0.73 mmol), phenylboronic acid (133 mg, 1.09 mmol), K_2CO_3 (201 mg, 1.45 mmol), and $Pd(dppf)Cl_2$ (106 mg, 0.15 mmol) as a white solid (121 mg, 61%). Mp: 155–157 °C. IR (KBr, cm^{-1}): 2582 (O = P-OH), 2162 (O = P-OH), 1255 (P = O), and 1184 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 34.14$ ppm. ^1H NMR (400 MHz, DMSO-d_6) $\delta = 2.60$ (d, 1H, $J = 6.4$ Hz), 2.65 (d, 1H, $J = 6.4$ Hz), 5.87–6.01 (m, 1H), 6.60–6.67 (m, 1H), 7.34–7.41 (m, 3H), 7.44–7.52 (m, 3H), and 7.67–7.72 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.7$ (d, $J_{P,C} = 125$ Hz), 119.6 (d, $J_{P,C} = 2.8$ Hz), 122.4, 124.0 (d, $J_{P,C} = 11.6$ Hz), 126.6, 127.1, 128.0, 128.6 (d, $J_{P,C} = 8.0$ Hz), 129.0, 131.3, 138.7, 141.0, and 148.7 (d, $J_{P,C} = 7.1$ Hz) ppm. HRMS (ESI) $[M-H]^-$: *m/z* calcd for $C_{15}H_{12}O_3P$: 271.0524, found 271.0536.

2-Hydroxy-8–(4-methoxyphenyl)-3H-benzo[f][1,2]oxaphosphepine 2-oxide (9b)

By following the general procedure, **9b** was prepared from 8-bromo-2-hydroxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7b**) (200 mg, 0.73 mmol), (4-methoxyphenyl)boronic acid (166 mg, 1.09 mmol), K_2CO_3 (201 mg, 1.45 mmol), and $Pd(dppf)Cl_2$ (106 mg, 0.15 mmol) as a white solid (147 mg, 67%). Mp: 205–207 °C. IR (KBr, cm^{-1}): 2556 (O = P-OH), 2305 (O = P-OH), 1248 (P = O), and 1183 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 34.96$ ppm. ^1H NMR (400 MHz, DMSO-d_6) $\delta = 2.63$ (d, 1H, $J = 6.4$ Hz), 2.68 (d, 1H, $J = 6.4$ Hz), 3.80 (s, 3H), 5.85–6.00 (m, 1H), 6.61–6.67 (m, 1H), 7.00–7.06 (m, 2H), 7.32–7.36 (m, 2H), 7.45–7.49 (m, 1H), and 7.63–7.69 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.4$ (d, $J_{P,C} = 125$ Hz), 55.2, 114.5, 118.9 (d, $J_{P,C} = 3.2$ Hz), 122.1, 123.3 (d, $J_{P,C} = 11.8$ Hz), 126.2, 127.8, 128.8 (d, $J_{P,C} = 8.2$ Hz), 130.9, 131.3, 140.8, 148.4 (d, $J_{P,C} = 7.2$ Hz), and 159.3 ppm. HRMS (ESI) $[M-H]^-$: *m/z* calcd for $C_{16}H_{14}O_4P$: 301.0630, found 301.0641.

Ethyl 4–(2-hydroxy-2-oxido-3H-benzo[f][1,2]oxaphosphepin-8-yl) benzoate (9c)

By following the general procedure, **9c** was prepared from 8-bromo-2-hydroxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7b**) (200 mg, 0.73 mmol), (4-(ethoxycarbonyl)phenyl)boronic acid (212 mg, 1.09 mmol), K_2CO_3 (201 mg, 1.45 mmol), and Pd(dppf)Cl_2 (106 mg, 0.15 mmol) as a white solid (145 mg, 58%). Mp: 189–191 °C. IR (KBr, cm^{-1}): 2577 (O = P-OH), 2287 (O = P-OH), 1708 (C = O), 1283 (P = O), and 1192 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 35.08$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.34$ (t, 3H, $J = 7.1$ Hz), 2.65 (d, 1H, $J = 6.2$ Hz), 2.70 (d, 1H, $J = 6.2$ Hz), 4.34 (q, 2H, $J = 7.1$ Hz), 5.92–6.06 (m, 1H), 6.64–6.72 (m, 1H), 7.39–7.51 (m, 2H), 7.57–7.65 (m, 1H), 7.85–7.91 (m, 2H), and 8.01–8.06 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 14.2$, 27.4 (d, $J_{P,C}$ = 125 Hz), 60.8, 119.9 (d, $J_{P,C}$ = 3.2 Hz), 122.9, 124.2 (d, $J_{P,C}$ = 12.0 Hz), 126.9, 127.9, 128.6 (d, $J_{P,C}$ = 8.2 Hz), 129.1, 129.9, 131.5, 139.6, 143.0, 148.5 (d, $J_{P,C}$ = 7.2 Hz), and 165.5 ppm. HRMS (ESI) $[M–H]^-$: *m/z* calcd for $C_{18}H_{16}O_5P$: 343.0735, found 343.0750.

8–(4-Chlorophenyl)-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (9d)

By following the general procedure, **9d** was prepared from 8-bromo-2-hydroxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7b**) (200 mg, 0.73 mmol), (4-chlorophenyl)boronic acid (171 mg, 1.09 mmol), K_2CO_3 (201 mg, 1.45 mmol), and Pd(dppf)Cl_2 (106 mg, 0.15 mmol) as a white solid (134 mg, 60%). Mp: 203–205 °C. IR (KBr, cm^{-1}): 2583 (O = P-OH), 2292 (O = P-OH), 1218 (P = O), and 1187 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 35.06$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.64$ (d, 1H, $J = 6.4$ Hz), 2.69 (d, 1H, $J = 6.4$ Hz), 5.90–6.03 (m, 1H), 6.63–6.70 (m, 1H), 7.36–7.42 (m, 2H), 7.50–7.55 (m, 3H), and 7.72–7.77 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.4$ (d, $J_{P,C}$ = 125 Hz), 119.6 (d, $J_{P,C}$ = 3.2 Hz), 122.6, 123.9 (d, $J_{P,C}$ = 12.0 Hz), 127.3, 128.4, 128.7 (d, $J_{P,C}$ = 8.4 Hz), 129.0, 131.4, 132.9, 137.4, 139.7, and 148.4 (d, $J_{P,C}$ = 7.2 Hz) ppm. HRMS (ESI) $[M–H]^-$: *m/z* calcd for $C_{15}H_{11}O_3PCl$: 305.0134, found 305.0143.

8–(3,5-Dichlorophenyl)-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (9e)

By following the general procedure, **9e** was prepared from 8-bromo-2-hydroxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7b**) (200 mg, 0.73 mmol), (3,5-dichlorophenyl)boronic acid (208 mg, 1.09 mmol), K_2CO_3 (201 mg, 1.45 mmol), and Pd(dppf)Cl_2 (106 mg, 0.15 mmol) as a white solid (141 mg, 57%). Mp: 136–138 °C. IR (KBr, cm^{-1}): 2533 (O = P-OH), 2262 (O = P-OH), 1221 (P = O), and 1196 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 33.47$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.59$ (d, 1H, $J = 6.4$ Hz), 2.64 (d, 1H, $J = 6.4$ Hz), 5.90–6.03 (m, 1H), 6.60–6.66 (m, 1H), 7.35–7.39 (m, 1H), 7.46–7.49 (m, 1H), 7.54–7.61 (m, 2H), and 7.74–7.78 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.8$ (d, $J_{P,C}$ = 125 Hz), 120.2, 122.6, 124.7, 125.3, 127.2, 128.3, 128.4, 131.4, 134.7, 137.8, 142.2, and 148.8 ppm. HRMS (ESI) $[M–H]^-$: *m/z* calcd for $C_{15}H_{10}O_3PCl_2$: 338.9745, found 338.9760.

2-Hydroxy-8–(2-nitrophenyl)-3H-benzo[f][1,2]oxaphosphepine 2-oxide (9f)

By following the general procedure, **9f** was prepared from 8-bromo-2-hydroxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7b**) (200 mg, 0.73 mmol), (2-nitrophenyl)boronic acid (182 mg, 1.09 mmol), K_2CO_3 (201 mg, 1.45 mmol), and Pd(dppf)Cl_2 (106 mg, 0.15 mmol) as a yellowish solid (159 mg, 69%). Mp: 218–220 °C. IR (KBr, cm^{-1}): 2604 (O = P-OH), 2240 (O = P-OH), 1185 (P = O), and 1126 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 34.84$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.65$ (d, 1H, $J = 6.4$ Hz), 2.70 (d, 1H, $J = 6.4$ Hz), 5.92–6.05 (m, 1H), 6.64–6.70 (m, 1H), 7.07–7.10 (m, 1H), 7.16 (dd, 1H, $J = 8.0$, 1.6 Hz), 7.38 (d, 1H, $J = 8.0$ Hz), 7.57–7.61 (m, 1H), 7.62–7.68 (m, 1H), 7.75–7.81 (m, 1H), and 8.01 (dd, 1H, $J = 8.0$, 1.0 Hz) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.4$ (d, $J_{P,C}$ = 125 Hz), 120.9 (d, $J_{P,C}$ = 3.3 Hz), 123.9, 124.2 (d, $J_{P,C}$ = 11.6 Hz), 124.3, 127.7, 128.6 (d, $J_{P,C}$ = 8.6 Hz), 129.3, 131.3, 131.8, 133.1, 133.7, 137.8, 148.0 (d, $J_{P,C}$ = 7.2 Hz), and 148.7 ppm. HRMS (ESI) $[M–H]^-$: *m/z* calcd for $C_{15}H_{11}NO_5P$: 316.0375, found 316.0384.

2-Hydroxy-9-phenyl-3H-benzo[f][1,2]oxaphosphepine 2-oxide (10a)

By following the general procedure, **10a** was prepared from 9-bromo-2-hydroxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7c**) (200 mg, 0.73 mmol), phenylboronic acid (133 mg, 1.09 mmol), K_2CO_3 (201 mg, 1.45 mmol), and Pd(dppf)Cl_2 (106 mg, 0.15 mmol) as a white solid (166 mg, 84%). Mp: 184–186 °C. IR (KBr, cm^{-1}): 2592 (O = P-OH), 2261 (O = P-OH), 1255 (P = O), and 1204 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 33.69$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.67$ (dd, 1H, $J = 6.7$, 1.0 Hz), 2.72 (dd, 1H, $J = 6.7$, 1.0 Hz), 5.86–5.99 (m, 1H), 6.62–6.68 (m, 1H), 7.23–7.39 (m, 4H), 7.40–7.46 (m, 2H), and 7.60–7.64 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.8$ (d, $J_{P,C}$ = 125 Hz), 123.3 (d, $J_{P,C}$ = 11.6 Hz), 124.4, 127.2, 128.0, 128.5, 129.3 (d, $J_{P,C}$ = 8.6 Hz), 129.6, 130.5 (d, $J_{P,C}$ = 4.6 Hz), 134.3 (d, $J_{P,C}$ = 3.4 Hz), 137.4, and 144.8 (d, $J_{P,C}$ = 7.4 Hz) ppm. HRMS (ESI) $[M–H]^-$: *m/z* calcd for $C_{15}H_{12}O_3P$: 271.0524, found 271.0527.

2-Hydroxy-9–(4-methoxyphenyl)-3H-benzo[f][1,2]oxaphosphepine 2-oxide (10b)

By following the general procedure, **10b** was prepared from 9-bromo-2-hydroxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7c**) (200 mg, 0.73 mmol), (4-methoxyphenyl)boronic acid (166 mg, 1.09 mmol), K_2CO_3 (201 mg, 1.45 mmol), and Pd(dppf)Cl_2 (106 mg, 0.15 mmol) as a white solid (196 mg, 89%). Mp: 199–201 °C. IR (KBr, cm^{-1}): 2573 (O = P-OH), 2257 (O = P-OH), 1248 (P = O), and 1205 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 34.24$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.66$ (d, 1H, $J = 6.4$ Hz), 2.71 (d, 1H, $J = 6.4$ Hz), 3.80 (s, 3H), 5.85–6.00 (m, 1H), 6.61–6.67 (m, 1H), 6.95–7.02 (m, 2H), 7.20–7.34 (m, 3H), and 7.54–7.60 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.6$ (d, $J_{P,C}$ = 125 Hz), 55.1, 113.5, 123.3 (d, $J_{P,C}$ = 11.3 Hz), 124.4, 128.4, 129.4 (d, $J_{P,C}$ = 7.8 Hz), 129.7, 130.0, 130.4, 130.8, 134.0, 144.8 (d, $J_{P,C}$ = 7.4 Hz), and 158.6 ppm. HRMS (ESI) $[M–H]^-$: *m/z* calcd for $C_{16}H_{14}O_4P$: 301.0630, found 301.0636.

Ethyl 4–(2-hydroxy-2-oxido-3H-benzo[f][1,2]oxaphosphepin-9-yl) benzoate (10c)

By following the general procedure, **10c** was prepared from 9-bromo-2-hydroxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7c**) (200 mg, 0.73 mmol), (4-(ethoxycarbonyl)phenyl)boronic acid (212 mg, 1.09 mmol), K_2CO_3 (201 mg, 1.45 mmol), and Pd(dppf)Cl_2 (106 mg, 0.15 mmol) as a white solid (208 mg, 83%). Mp: 194–196 °C. IR (KBr, cm^{-1}): 2566 (O = P-OH), 2272 (O = P-OH), 1710 (C = O), 1285 (P = O), and 1190 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 34.39$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 1.34$ (t, 3H, $J = 7.0$ Hz), 2.66 (d, 1H, $J = 6.1$ Hz), 2.71 (d, 1H, $J = 6.1$ Hz), 4.35 (q, 2H, $J = 7.0$ Hz), 5.88–6.02 (m, 1H), 6.64–6.70 (m, 1H), 7.25–7.42 (m,

3H), 7.72–7.78 (m, 2H), and 7.98–8.03 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 14.2$, 27.6 (d, $J_{P,C} = 125$ Hz), 60.8, 123.6 (d, $J_{P,C} = 11.6$ Hz), 124.6, 128.6, 128.8, 129.1 (d, $J_{P,C} = 8.4$ Hz), 130.0, 130.3, 131.2, 133.2 (d, $J_{P,C} = 3.4$ Hz), 142.2, 144.8 (d, $J_{P,C} = 7.4$ Hz), and 165.7 ppm. HRMS (ESI) $[M–H]^-$: *m/z* calcd for $C_{18}H_{16}O_5P$: 343.0735, found 343.0740.

9–(4-Chlorophenyl)-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (10d)

By following the general procedure, **10d** was prepared from 9-bromo-2-hydroxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7c**) (200 mg, 0.73 mmol), (4-chlorophenyl)boronic acid (171 mg, 1.09 mmol), K_2CO_3 (201 mg, 1.45 mmol), and Pd(dppf)Cl_2 (106 mg, 0.15 mmol) as a white solid (161 mg, 72%). Mp: 217–219 °C. IR (KBr, cm^{-1}): 2568 (O = P-OH), 2261 (O = P-OH), 1256 (P = O), 1205 (P = O), and 1190 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 37.90$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.61$ (d, 1H, $J = 6.2$ Hz), 2.66 (d, $J = 6.2$ Hz), 5.84–5.97 (m, 1H), 6.58–6.65 (m, 1H), 7.21–7.27 (m, 1H), 7.28–7.35 (m, 2H), 7.44–7.50 (m, 2H), and 7.62–7.68 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 28.0$ (d, $J_{P,C} = 125$ Hz), 123.9 (d, $J_{P,C} = 10.4$ Hz), 124.3, 127.5, 128.0, 128.7, 129.0 (d, $J_{P,C} = 8.2$ Hz), 130.2, 130.8, 131.5, 133.0 (d, $J_{P,C} = 2.4$ Hz), 136.0, and 145.1 (d, $J_{P,C} = 7.4$ Hz) ppm. HRMS (ESI) $[M–H]^-$: *m/z* calcd for $C_{15}H_{11}O_3PCl$: 305.0134, found 305.0143.

9–(3,5-Dichlorophenyl)-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (10e)

By following the general procedure, **10e** was prepared from 9-bromo-2-hydroxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7c**) (200 mg, 0.73 mmol), (3,5-dichlorophenyl)boronic acid (208 mg, 1.09 mmol), K_2CO_3 (201 mg, 1.45 mmol), and Pd(dppf)Cl_2 (106 mg, 0.15 mmol) as a white solid (164 mg, 66%). Mp: 210–212 °C. IR (KBr, cm^{-1}): 2542 (O = P-OH), 2228 (O = P-OH), 1259 (P = O), and 1192 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 36.19$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.64$ (d, 1H, $J = 6.4$ Hz), 2.70 (d, 1H, $J = 6.4$ Hz), 5.90–6.03 (m, 1H), 6.64–6.70 (m, 1H), 7.25–7.31 (m, 1H), 7.34–7.43 (m, 2H), 7.60–7.63 (m, 1H), and 7.66–7.68 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.5$ (d, $J_{P,C} = 125$ Hz), 123.9 (d, $J_{P,C} = 12.0$ Hz), 124.6, 126.9, 128.4, 129.0 (d, $J_{P,C} = 8.6$ Hz), 130.4, 131.3, 131.5, 133.7, 140.8, and 144.7 (d, $J_{P,C} = 7.4$ Hz) ppm. HRMS (ESI) $[M–H]^-$: *m/z* calcd for $C_{15}H_{10}O_3PCl_2$: 338.9745, found 338.9751.

2-Hydroxy-9–(2-nitrophenyl)-3H-benzo[f][1,2]oxaphosphepine 2-oxide (10f)

By following the general procedure, **10f** was prepared from 9-bromo-2-hydroxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**7c**) (200 mg, 0.73 mmol), (2-nitrophenyl)boronic acid (182 mg, 1.09 mmol), K_2CO_3 (201 mg, 1.45 mmol), and Pd(dppf)Cl_2 (106 mg, 0.15 mmol) as a yellowish solid (161 mg, 70%). Mp: 226–228 °C. IR (KBr, cm^{-1}): 2359 (O = P-OH), 1229 (P = O), and 1192 (P = O). ^{31}P NMR (162 MHz, DMSO-d_6) $\delta = 35.15$ ppm. ^{1}H NMR (400 MHz, DMSO-d_6) $\delta = 2.66$ (d, 1H, $J = 6.4$ Hz), 2.72 (d, 1H, $J = 6.4$ Hz), 5.90–6.03 (m, 1H), 6.65–6.72 (m, 1H), 7.29–7.35 (m, 1H), 7.38–7.45 (m, 2H), 7.86–7.91 (m, 2H), and 8.25–8.31 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) $\delta = 27.5$ (d, $J_{P,C} = 125$ Hz), 123.2, 123.9 (d, $J_{P,C} = 12.0$ Hz), 124.7, 128.8, 129.0 (d, $J_{P,C} = 8.6$ Hz), 130.4, 131.0, 131.7, 132.3 (d, $J_{P,C} = 3.4$ Hz), 144.4, 144.8 (d, $J_{P,C} = 7.4$ Hz), and 146.6 ppm. HRMS (ESI) $[M–H]^-$: *m/z* calcd for $C_{15}H_{11}NO_5P$: 316.0375, found 316.0385.

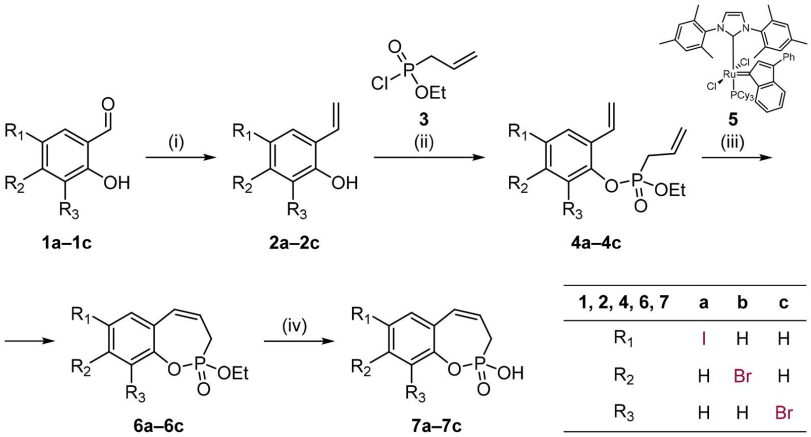
Carbonic anhydrase inhibition assay

The CA-catalysed CO_2 hydration activity was assayed by using an applied photophysics stopped-flow apparatus as reported in previous papers from our group[22]. All CA isoforms were recombinant proteins, obtained as reported earlier[23–25].

Results and discussion

Chemistry

Our group recently developed a strategy for the synthesis of simple derivatives of 3*H*-1,2-benzoxaphosphepine 2-oxide from the commercially available salicylaldehydes[19]. This methodology employs a ring-closing metathesis (RCM) reaction as a key step to construct benzo-fused oxaphosphepine ring. Following this synthetic route[19], we have prepared iodo- and bromo-substituted analogues **7a–c** (Scheme 1). First, halosalicylaldehydes **1a–c** were



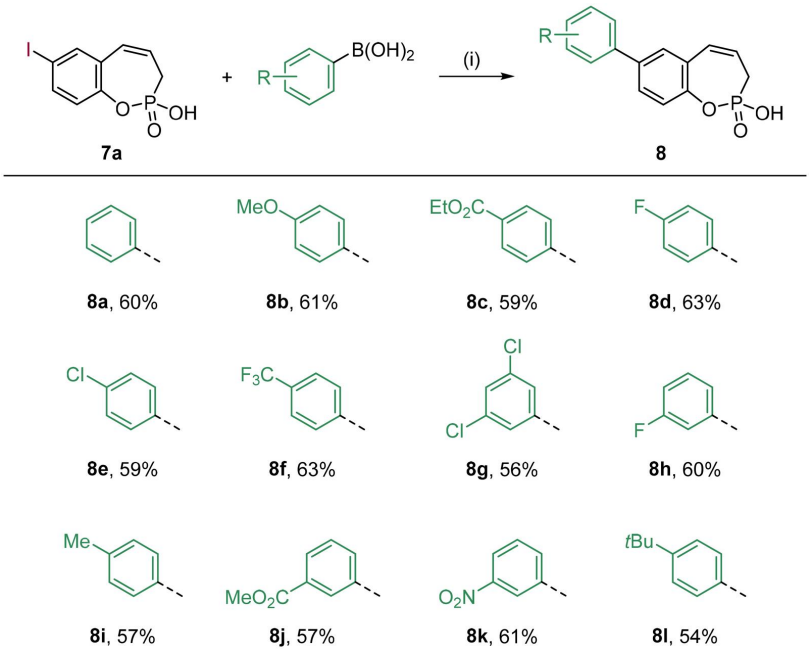
Scheme 1. Reagents and conditions: (1) $MePPh_3Br$, *t*BuOK, THF, rt, 18 h; (2) NEt_3, CH_2Cl_2, 0 °C to rt, 18 h; (3) **5** (CAS: 250220–36-1), PhMe, 70 °C, 4 h; (iv) TMSBr, CH_2Cl_2, rt, 24 h.

converted to styrenes **2a–c**. Subsequent phosphorylation and RCM gave the cyclised compounds **6a–c**. Lastly, deprotection was achieved using TMSBr and the target compounds **7a–c** were successfully obtained.

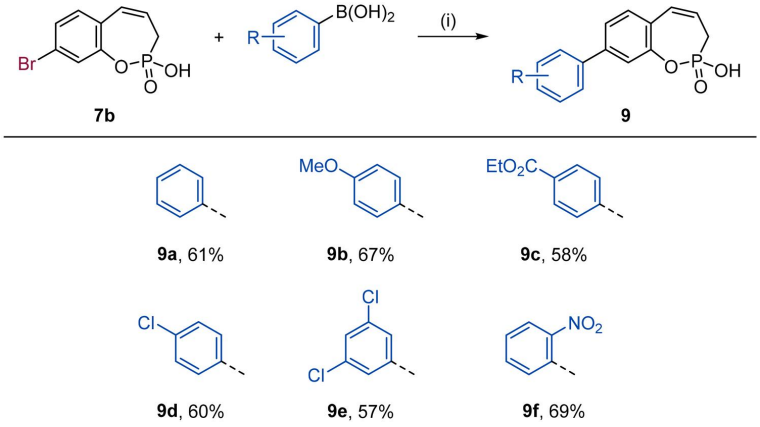
With the halo derivatives **7a–c** in hand, we next proceeded to the Suzuki–Miyaura cross-coupling reaction, employing commercial arylboronic acids. As a result, 7-, 8-, and 9-aryl-substituted benzoxaphosphepine 2-oxides **8–10** were furnished in good to excellent yields (Schemes 2–4). Noteworthy, the electronic nature and substitution patterns of arylboronic acids did not significantly affect isolated yields. However, the coupling reactions occurring at position 9 of benzoxaphosphepine 2-oxide core displayed higher efficacy in yields, compared to positions 7 and 8.

Carbonic anhydrase inhibition

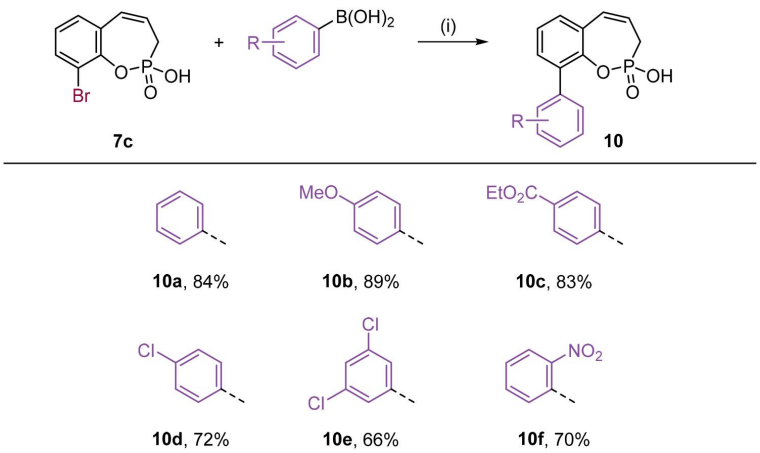
The newly synthesised compounds **7–10** were investigated for their CA inhibition activity against four pharmacologically relevant human CA isoforms—the ubiquitous cytosolic CA I and II as well as the cancer-associated CA IX and XII. In this study, CA I and CA II are considered off-target isoforms, that were tested in order to



Scheme 2. Reagents and conditions: (1) $Pd(dppf)Cl_2$, K_2CO_3, 1,4-dioxane/H_2O (5:1), 80 °C, 16 h, 54–63%.



Scheme 3. Reagents and conditions: (1) $Pd(dppf)Cl_2$, K_2CO_3, 1,4-dioxane/H_2O (5:1), 80 °C, 16 h, 57–69%.



Scheme 4. Reagents and conditions: (1) Pd(dppf)Cl_2, K_2CO_3, 1,4-dioxane/H_2O (5:1), 80 °C, 16 h, 66–89%.

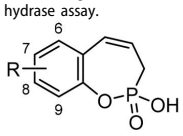
explore the selectivity of inhibitors towards the CA IX and CA XII isoforms. The clinically utilised acetazolamide (AAZ) was used as a reference drug. The following structure-activity relationship (SAR) can be deduced from the inhibition data reported in Table 1:

i. Equally, as previously reported simple derivatives of benzoxaphosphepine 2-oxide[19], all aryl derivatives **8–10** reported here, as well as **7b**, showed no inhibitory activity towards the off-target CA isoforms I and II (K_I > 100 μM). In the context of cancer treatment with CA inhibitors, this is a desirable feature to prevent possible side effects, since CA I and CA II isoforms are found in many tissues throughout the body[6]. The standard drug AAZ has a very good affinity for CA I and CA II.

ii. The cancer-associated CA IX isoform was inhibited by all of the tested compounds; however, 9-aryl-substituted derivatives **10** were weak inhibitors, with K_I values ranging from 16.5 to 55.3 μM. In general, 7- and 8-aryl-substituted compounds **8** and **9** were much more effective inhibitors with good or moderate activity. The 7-aryl derivative with the 3-fluoro substituent **8h** emerged as the most potent CA IX inhibitor with K_I = 0.63 μM. The inclusion of –CO_2R, –NO_2 or –Cl substituent typically resulted in decreased inhibitory activity against CA IX.

iii. Similarly, another cancer-associated isoform CA XII was inhibited by 7-aryl and 8-aryl derivatives **8** and **9**, whereas 9-aryl-substituted derivatives **10** displayed weak or no inhibitory activity (K_I: 25.5–65.3 μM for **10a,b,d–f** and K_I > 100 μM for **10c** that bears the 4-CO_2Et substituent). Only the 9-bromo derivative **7c** had a moderate inhibition potency against both CA IX and XII. The 7-aryl derivative with the 4-fluoro substituent **8d** was the most effective inhibitor against CA XII with K_I = 0.25 μM. Other fluorine-containing compounds also exhibited good activity against CA XII (K_I = 0.56 μM for **8h**; K_I = 0.59 μM for **8f**). In contrast to CA IX inhibition profile, 7- and 8-aryl derivatives with the nitro group showed good inhibition of CA XII (K_I = 0.64 μM for **8k**; K_I = 0.67 μM for **9f**).

Table 1. Inhibition data of compounds **7–10** and the standard inhibitor acetazolamide (AAZ) against human CA isoforms I, II, IX and XII by the stopped-flow CO_2 hydrase assay.



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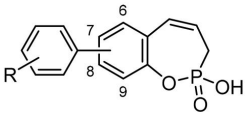

8–10

Cmpd	Substitution position (7 / 8 / 9)	R	K_I (μM)[a,b] CA I	CA II	CA IX	CA XII
7a[19]	7	I	>100	>100	0.88	0.68
8a	7	H	>100	>100	0.77	0.95
8b	7	4-OMe	>100	>100	4.6	1.7
8c	7	4-CO_2Et	>100	>100	6.0	6.7
8d	7	4-F	>100	>100	0.86	0.25
8e	7	4-Cl	>100	>100	8.6	1.1
8f	7	4-CF_3	>100	>100	3.7	0.59
8g	7	3,5-diCl	>100	>100	7.3	4.2
8h	7	3-F	>100	>100	0.63	0.56
8i	7	4-Me	>100	>100	1.5	0.94
8j	7	3-CO_2Me	>100	>100	9.5	1.5
8k	7	3-NO_2	>100	>100	7.6	0.64
8l	7	4-*t*Bu	>100	>100	4.9	0.97
7b	8	Br	>100	>100	3.4	1.5
9a	8	H	>100	>100	1.8	2.7
9b	8	4-OMe	>100	>100	2.1	5.6
9c	8	4-CO_2Et	>100	>100	10.2	3.8
9d	8	4-Cl	>100	>100	5.0	7.1
9e	8	3,5-diCl	>100	>100	0.98	0.84
9f	8	2-NO_2	>100	>100	12.9	0.67
7c[19]	9	Br	>100	>100	2.5	1.8
10a	9	H	>100	>100	16.5	25.5
10b	9	4-OMe	>100	>100	39.4	52.4
10c	9	4-CO_2Et	>100	>100	55.3	>100
10d	9	4-Cl	>100	>100	48.9	65.3
10e	9	3,5-diCl	>100	>100	22.4	28.2
10f	9	4-NO_2	>100	>100	38.4	35.1
AAZ	–		0.25	0.012	0.025	0.006

[a]Values are mean from three different assays using the stopped-flow technique (errors were in the range of ± 5–10% of the reported values).
[b]Incubation time: 6 h.

Collectedly, benzoxaphosphepine 2-oxide derivatives that are substituted with aryl groups in positions 7 or 8 displayed superior inhibition efficiency of CA IX and XII as compared to the 9-aryl-substituted derivatives. In comparison with the standard drug AAZ, which is a highly effective inhibitor of all the four CA isoforms considered in this study, the analogues **7–10** were less effective as CA IX and XII inhibitors. However, benzoxaphosphepine 2-oxide derivatives showed desirable selectivity as none of them inhibited the off-target CA I and CA II. The most potent inhibitors of both cancer-associated CA IX and CA XII were fluorine-containing compounds **8d** and **8h**.

It is worthwhile to underline here that a 6-h incubation of the enzyme and each compound **7–10** solutions is essential. When the incubation period of 15 min was used for assaying the inhibition, as generally done for the other types of CA inhibitors, only a weak inhibition was observed (data not shown). Furthermore, it appears to be the case with coumarins and related bioisosteres[9–18]. These compounds were shown to act as prodrug inhibitors, being hydrolysed within the CA active site to the corresponding acids; subsequently, the obtained hydrolysis products bind within the enzyme active site cavity[10,12,14a]. By considering aforementioned points, we assume that benzoxaphosphepine 2-oxides are likely to undergo the CA-mediated hydrolysis of oxaphosphepine ring with formation of phosphonic acid derivatives that act as CA inhibitors.

Conclusions

3*H*-1,2-Benzoxaphosphepine 2-oxides represent a novel chemotype acting as isoform-selective CA inhibitors. In this paper, we have expanded the chemical space around the benzoxaphosphepine scaffold by synthesising aryl derivatives. The latter were evaluated for their inhibitory activity against CA I, II, IX and XII. Most of the compounds tested manifested promising potency in inhibiting the cancer-associated CA isoforms IX and XII. Furthermore, none of the target compounds showed significant inhibition of the cytosolic CA I and II. The SAR studies indicated that 7- and 8-substituted aryl derivatives of 3*H*-1,2-benzoxaphosphepine 2-oxide were considerately more active CA IX/XII inhibitors than the 9-aryl derivatives. The introduction of aryl groups at the 9th position of the scaffold resulted in decreased potency. Among all the tested compounds, derivatives **8d,h** with the fluoro-substituted aryl groups demonstrated the highest inhibitory activity against CA IX/XII, with K_I values in the sub-micromolar range. Taking into account the efficiency and significant selectivity of these novel molecules, further development and evaluation will be pursued.

Disclosure statement

No potential competing interest was reported by all authors except CTS. CT Supuran is Editor-in-Chief of the Journal of Enzyme Inhibition and Medicinal Chemistry. He was not involved in the assessment, peer review, or decision-making process of this paper. The authors have no relevant affiliations or financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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ORCID

Anastasija Balašova http://orcid.org/0000-0002-1185-1922
Aleksandrs Pustenko http://orcid.org/0000-0003-4930-7372
Alessio Nocentini http://orcid.org/0000-0003-3342-702X
Daniela Vullo http://orcid.org/0000-0002-1352-1900
Claudiu T. Supuran http://orcid.org/0000-0003-4262-0323
Raivis Žalubovskis http://orcid.org/0000-0002-9471-1342

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Letter

Unraveling the Potential of Amino-, Acylamino-, and Ureido-Substituted 3*H*-1,2-Benzoxaphosphepine 2-Oxides toward Nanomolar Inhibitors of Tumor-Associated Carbonic Anhydrases IX and XII

Anastasija Balašova, Aleksandrs Pustenko, Andrea Angeli, Elena Andreucci, Alessio Biagioni, Alessio Nocentini, Fabrizio Carta, Claudiu T. Supuran, and Raivis Žalubovskis*

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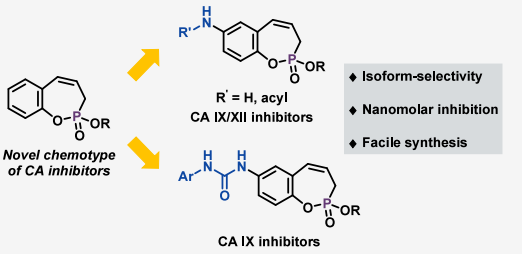
ABSTRACT: 3*H*-1,2-Benzoxaphosphepine 2-oxides were recently identified as a novel class of carbonic anhydrase (CA) inhibitors. In this study, which aims to broaden the chemical space around this scaffold and improve inhibition potency against cancer-related isoforms (CA IX and CA XII), we report the synthesis and biochemical evaluation of amino-, acylamino-, and ureido-substituted benzoxaphosphepine oxides **2**−**4**. All members of these series showed no off-target inhibition of cytosolic CA I and CA II activity, while the inhibition of the target isoforms was strongly dependent on the substitution pattern. To our delight, several compounds managed to inhibit tumor-associated CA isoforms at the nanomolar level, which is equal to or even surpasses that of the reference drugs. The results of the current study bolster and extend previous research, demonstrating the capability of the benzoxaphosphepine oxide chemotype to serve as a platform for the future development of new therapeutic agents.



KEYWORDS: *carbonic anhydrase, carbonic anhydrase inhibitors, benzoxaphosphepine oxide, anticancer, amidation*

Carbonic anhydrases (CA, EC 4.2.1.1) constitute an ample family of metalloenzymes that catalyze the reversible hydration of carbon dioxide to bicarbonate anion, thus participating in numerous physiological processes.[1] Their modulators have broad application prospects in the design of pharmacological agents for the treatment or prevention of a variety of maladies, including cancer, glaucoma, bacterial infections as well as cognitive disorders.[2,3] In particular, two transmembrane CA isoforms IX and XII have enhanced expression in solid tumors, which leads to increased tumor growth, survival, and metastasis due to maintaining favorable intra- and extracellular pH level.[1,4] Frequently, the overexpression of these isoforms is associated with drug resistance and poor prognosis in certain cancers.[5] The inhibition of CA IX and CA XII is a promising strategy for the development of novel anticancer drugs.

The main obstacle in the elaboration of CA inhibitors is connected to the isoform selectivity problem due to the structural similarity among the different human CA isoforms.[6,7] Achieving selectivity is crucial for minimizing side effects that arise from the inhibition of the ubiquitous CA I and CA II. To address this issue, extensive research efforts have been devoted to the discovery and development of chemotypes targeting specific isoforms of CA.[8−12] Several studies led to a number of inhibitors of cancer-associated CA isoforms, such as (iso)-coumarins,[13−17] thiocoumarins,[17,18] and other related compounds.[19−25] Moreover, one selective CA IX/XII inhibitor possessing a benzenesulfonamide moiety with a urea tether, SLC-0111, is currently undergoing clinical trials for the treatment of hypoxic malignancies (Phase I was completed successfully; Figure 1).[26,27] Considering the critical role of CA IX and CA XII in tumor development, it is necessary to introduce new chemotypes to efficiently combat cancer amid rising of the annual death toll from oncological diseases.[28]

Recent findings in our group revealed benzoxaphosphepine oxides **A** as selective CA IX and XII inhibitors.[29,30] Albeit displaying the desired isoform selectivity, compounds of previous studies suppressed the activity of both CA IX and CA XII at the micromolar level. Aiming to increase the potency of these inhibitors toward the target isoforms, herein we

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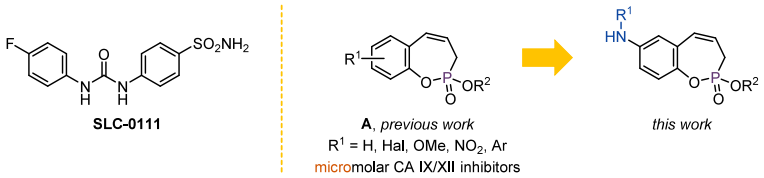
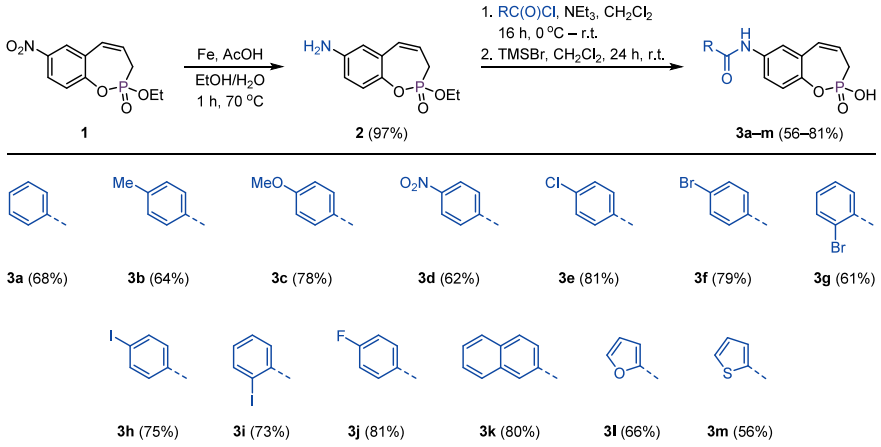
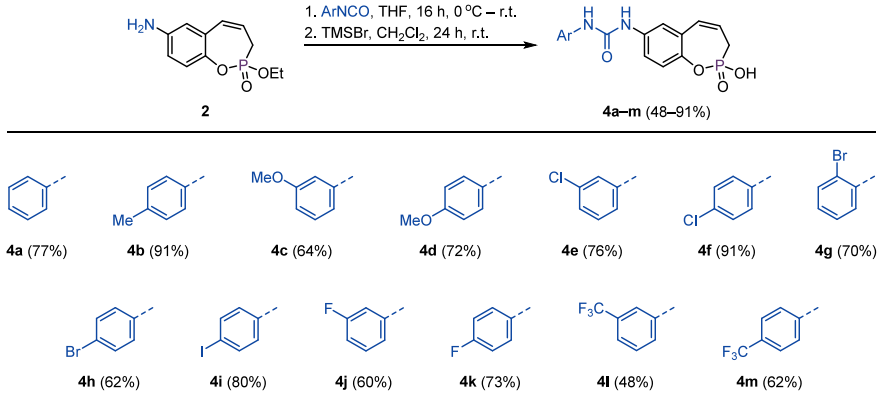


Figure 1. Structure of SLC-0111 and schematic representation of compounds studied.

Scheme 1. Synthesis and Substrate Scope of Benzoxaphosphepine Oxide Acylamino Derivatives 3a−m from Precursor 1



Scheme 2. Synthesis and Substrate Scope of Benzoxaphosphepine Oxide Ureido Derivatives 4a−m from Aniline 2



designed analogues of **A** with a primary amine, an acylamino or a ureido substituent at the 7-position on the basis of prior structure−activity relationship (SAR) studies (Figure 1). Exploration of the SAR of these novel series was also conducted in conjunction with cytotoxicity assays of the most promising representatives. Additionally, the solubility of the selected compounds was determined, showing the potential of benzoxaphosphepine oxide derivatives as drug candidates.

The synthesis of benzoxaphosphepine oxides bearing an amine moiety, an acylamino or a ureido substituent at the 7-position began with nitrobenzoxaphosphepine oxide **1**, which was prepared in 3 steps from commercially available starting material following our previously established experimental protocol (Scheme 1).[29] Compound **1** was then reduced using iron powder in acidic media to afford aniline **2** in a nearly quantitative yield. To obtain a series of acylamino derivatives **3**, aniline **2** was treated with the respective acyl chlorides in the presence of NEt_3 followed by *O*-dealkylation with TMSBr. This robust method provided target compounds **3** in good yields.

The introduction of the ureido group was achieved by reaction of aniline **2** with various aryl isocyanates (Scheme 2). Subsequent cleavage of phosphonate ethyl ester was performed

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by action of TMSBr giving the final compound **4a−m** in moderate to excellent yields. Notably, the purification of products **4a−m** did not require column chromatography.

All the prepared derivatives **2−4** were screened for inhibitory activity against four relevant CA isoforms (I, II, IX, and XII) using the stopped-flow CO_2 hydration assay (Table 1).[31] Their activities, expressed as K_I values, were compared with those of the reference compounds acetazolamide (AAZ) and SLC-0111. The obtained results allowed us to derive the following SAR:

Table 1. Inhibition Data of Compounds 2−4, SLC-0111, and the Standard Inhibitor AAZ against CA Isoforms I, II, IX, and XII

	K_I (nM)[a,b]			
Cmpd	CA I	CA II	CA IX	CA XII
2	>10000	>10000	3.5	16.6
3a	>10000	>10000	10.8	7.3
3b	>10000	>10000	20.5	7.8
3c	>10000	>10000	10.1	8.1
3d	>10000	>10000	29.4	6.7
3e	>10000	>10000	158.4	64.1
3f	>10000	>10000	25.8	84.4
3g	>10000	>10000	23.2	8.9
3h	>10000	>10000	18.7	57.5
3i	>10000	>10000	14.6	6.9
3j	>10000	>10000	27.4	4.0
3k	>10000	>10000	20.1	27.2
3l	>10000	>10000	22.3	9.7
3m	>10000	>10000	32.8	74.6
4a	>10000	>10000	34.9	>10000
4b	>10000	>10000	46.9	>10000
4c	>10000	>10000	53.3	>10000
4d	>10000	>10000	5.3	>10000
4e	>10000	>10000	52.9	>10000
4f	>10000	>10000	54.8	>10000
4g	>10000	>10000	46.9	>10000
4h	>10000	>10000	36.8	>10000
4i	>10000	>10000	36.0	>10000
4j	>10000	>10000	51.8	>10000
4k	>10000	>10000	4.4	>10000
4l	>10000	>10000	31.9	>10000
4m	>10000	>10000	42.7	>10000
SLC-0111	5080	960	45.1	4.5
AAZ	250	12.1	25.8	5.7

[a]Values are mean from 3 different assays using the stopped-flow technique (errors were in the range of ± 5−10% of the reported values). [b]Incubation time: 6 h.

- Analogously to the previous studies, current representatives of benzoxaphosphepine oxide class showed no inhibition against cytosolic and widely expressed off-target CA I and CA II. This feature distinguishes these compounds from AAZ, a potent clinically approved CA inhibitor, which can cause various side effects due to its nonselectivity.[2]
- All synthesized compounds **2−4** displayed remarkable inhibitory activity against tumor-associated CA IX isoform, with K_I in the medium to low nanomolar range. The best inhibition was observed for aniline **2** and urea derivatives **4d**, **4k**, bearing methoxy and fluoro substituent at the 4-position of the phenyl ring, respectively. In most cases, acylamino derivatives **3** and ureas **4** exhibited comparable potencies to the reference inhibitors AAZ and SLC-0111. However, 4-chlorobenzamide **3e** showed the poorest inhibition for CA IX relative to all the compounds reported. Of note, increasing lipophilicity by incorporating aromatic unit into aniline **2** generally led to decreased inhibitory activity. No firm correlation was observed between the electronic nature of the aryl substituent and inhibition potency.
- As for the other tumor-associated CA isoform XII, compounds **2−4** demonstrated a notably different inhibition profile when compared to that of CA IX. Specifically, the introduction of ureido group led to completely inactive compounds **4a−m**, suggesting that the conformational rigidity of *N*,*N*′-diarylureas may be responsible for the observed inactivity.[32] In turn, other derivatives **2−3** showed effective inhibition of CA XII at nanomolar concentrations, with 4-fluorobenzamide **3j** being the most potent inhibitor in this series. Changing the fluorine substituent to chlorine, bromine or iodine at the 4-position resulted in decrease of potency, as shown in compounds **3e, 3f, 3h**. Interestingly, the switch from the 4-position to the 2-position of the bromo and iodo substituent in compounds **3g** and **3i** gave more than an 8-fold increase in activity. Amides **3a−d**, **3i**, and **3j** were highly effective in inhibiting the target isoform and possessed activity on par with the reference drugs.
- The discrepancy in the inhibition profile between (acyl)amino and ureido compounds may be attributed to differences in amino acid residues within the "selective pocket" located in close proximity to the active site, which is more sterically hindered in case of CA XII.[33] Hence, conformationally locked compounds, such as arylureas **4**, may exhibit difficulty entering the active site.

In vitro cytotoxicity assays were conducted for the compounds with the highest inhibitory activity on CA IX (**2**, **3c**, and **4d**) on A375 melanoma cells, HCT-116 colorectal cancer cells, and MDA-MB-231 triple-negative breast cancer cells (Figure 2, also see Supporting Information). After 72 h of treatment, **2** exhibited minimal cytotoxic activity across all tumor types, achieving a maximal effect of approximately 10−15% at a concentration of 100 μM (Figure 2A). Similarly, treatment with 100 μM **3c** resulted in a maximal cytotoxic activity of about 10% in A375 and MDA-MB-231 cells, while HCT-116 cells showed around 30% inhibition of proliferation (Figure 2B). In contrast, compound **4d** demonstrated significantly higher tumor cell cytotoxicity across all of the tested cell types. Specifically, 100 μM **4d** reduced proliferation by 50% in A375 and MDA-MB-231 cells and by 40% in HCT-116 cells (Figure 2C).

Additional solubility studies for benzoxaphosphepine oxides **8**,[29] **9**,[30] **3c**, **3j**, **4d**, **4k** along with the previously reported by our group benzoxathiepine dioxides **5−7**[21−23] were conducted in phosphate buffered saline solution (PBS) at pH ∼ 7.4 (Table 2). The obtained data indicate a notable difference between these two chemotypes; as expected, members of the benzoxaphosphepine class exhibited good aqueous solubility in comparison to the benzoxathiepine analogues. Indeed, the switch from the sultone moiety to cyclic phosphonic acid resulted in a more than 15-fold increase of water solubility (**5**

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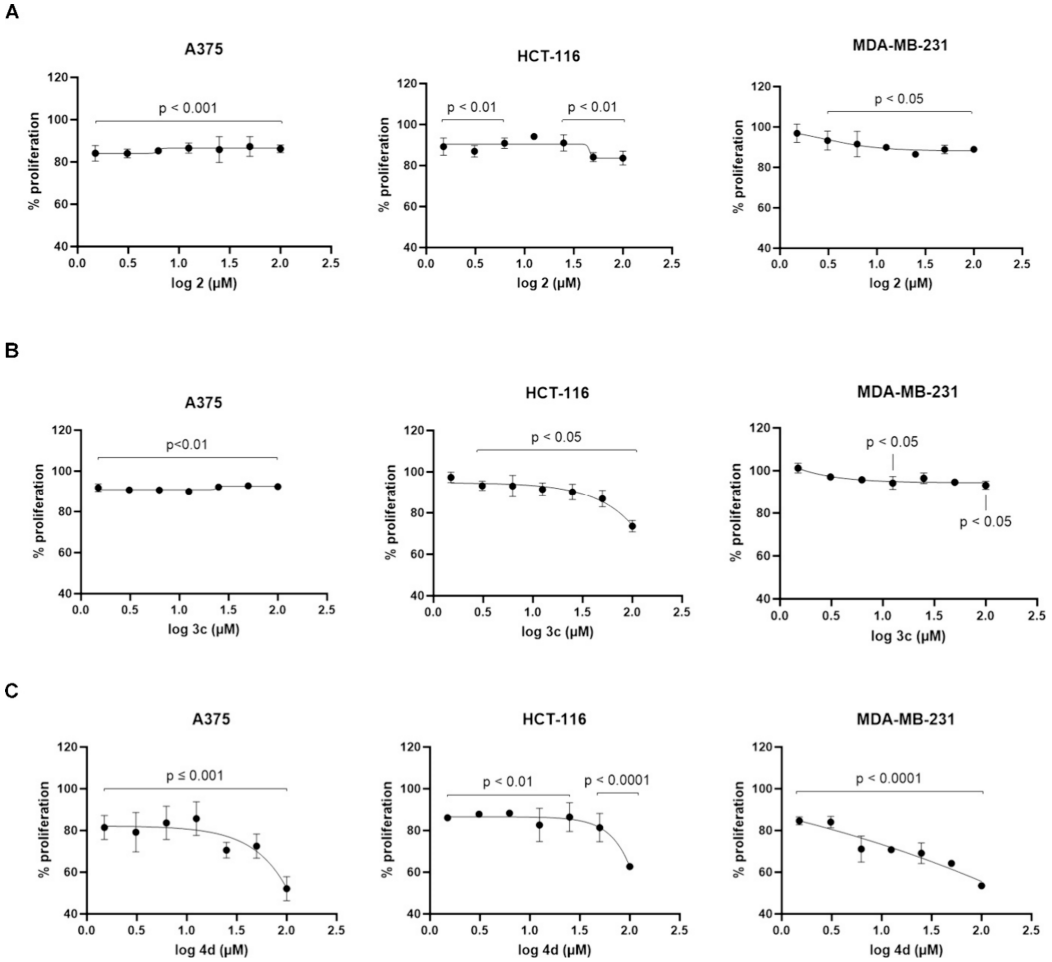

Figure 2. Cytotoxic activity of compounds **2** (A), **3c** (B), and **4d** (C) on A375 melanoma cells, HCT-116 colorectal cancer cells, and MDA-MB-231 breast cancer cells, $N = 4$, GraphPad Prism 10.3.1.

vs **8**). Furthermore, regardless of the substituents in the benzene ring of **8**, the aqueous solubility remains high (>2 mg/mL). This can potentially lead to improved bioavailability profile of benzoxaphosphepine-derived inhibitors of CA described herein.

In conclusion, the presented expansion of the 3*H*-1,2-benzoxaphosphepine 2-oxide library allowed for the identification of novel CA inhibitors with superior potency against cancer-associated CA isoforms IX and XII. All title compounds − aniline **2**, amides **3**, and ureas **4** − were biologically evaluated for the inhibition of several CA isoforms involved in essential physiological (CA I, II) and pathological (CA IX, XII) processes. Gratifyingly, the entire set of derivatives investigated here showed no inhibitory activity against the off-target cytosolic isoforms CA I and II, while demonstrating interesting inhibition profiles for the cancer-related isoforms CA IX and CA XII. All synthesized molecules indicated significant inhibitory effects against CA IX, with aniline **2** and ureas **4d**, **4k** being the best in this wide array of inhibitors (K_I: 3.5, 5.3, and 4.4 nM, respectively). However, in the case of CA XII, the SAR analysis disclosed that urea derivatives of benzoxaphosphepine oxide **4** were entirely inactive; benzamide **3j** was the most potent CA XII inhibitor with a K_I of 4.0 nM. Presumably, the inactivity of ureas **4** toward CA XII may be linked to the spatial features nearby the active site. The mechanism of action of these inhibitors will be investigated in our future studies. Since CA-inhibitor cocrystallization experiments with compounds reported in this paper have failed so far, the future goal of our research is to perform *in silico* docking studies to verify the activity and selectivity of the change to the amide/ureas' moiety.

Although the cytotoxicity of selected CA inhibitors was not prominent, overall presented findings, including solubility, provide prospects for further optimization in the development of antitumor agents. This supports the need for dedicated studies aimed at refining cytotoxicity assessment protocols to

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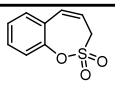
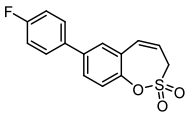
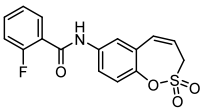
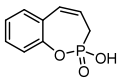
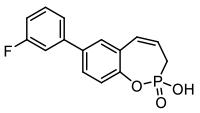
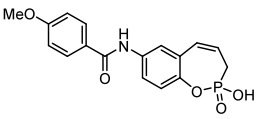
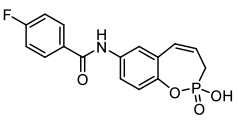
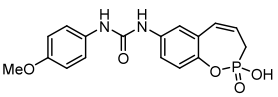
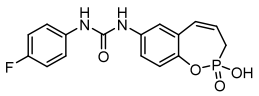
Table 2. Solubility of Inhibitors 5−9, 3c, 3j, 4d, and 4k in PBS Buffer

Cmpd	Structure	Solubility (mg/mL)[a]
5[21]		0.15
6[22]		0.0004
7[23]		0.56
8[29]		2.30
9[30]		2.26
3c		2.43
3j		2.14
4d		2.26
4k		2.35

[a]Solubility was determined by the HPLC-UV method at 25 °C.

be conducted *in vitro* across a broader panel of tumor cell lines and histotypes and under diverse microenvironmental conditions (e.g., normoxia vs. hypoxia). The inclusion of three-dimensional tumor models may further improve the translational relevance. Finally, *in vivo* studies in appropriate tumor models will be crucial to evaluate pharmacokinetics, biodistribution, and therapeutic efficacy under physiologically relevant contexts. Collectively, these strategies will guide further optimization of lead compounds toward clinical translation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.5c00099.

Materials and methods; synthetic procedures for final compounds **2**−**4**; characterization of compounds **2**−**4** (^{1}H, ^{13}C, ^{31}P NMR, IR, HRMS); CA inhibition assay protocol; cytotoxicity assay protocol and bar graph depiction for **2**, **3c**, **4d**; solubility assay protocol; HPLC chromatograms (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Raivis Žalubovskis − *Latvian Institute of Organic Synthesis, LV-1006 Riga, Latvia; Institute of Chemistry and Chemical Technology, Faculty of Natural Sciences and Technology, Riga Technical University, LV-1048 Riga, Latvia;* orcid.org/0000-0002-9471-1342; Email: raivis@osi.lv

Authors

Anastasija Balašova − *Latvian Institute of Organic Synthesis, LV-1006 Riga, Latvia; Institute of Chemistry and Chemical Technology, Faculty of Natural Sciences and Technology, Riga Technical University, LV-1048 Riga, Latvia*

Aleksandrs Pustenko − *Latvian Institute of Organic Synthesis, LV-1006 Riga, Latvia*

Andrea Angeli − *Department of Neurofarba, Università degli Studi di Firenze, 50019 Florence, Italy;* orcid.org/0000-0002-1470-7192

Elena Andreucci − *Department of Experimental and Clinical Biomedical Sciences, Università degli Studi di Firenze, 50134 Florence, Italy*

Alessio Biagioni − *Department of Experimental and Clinical Biomedical Sciences, Università degli Studi di Firenze, 50134 Florence, Italy*

Alessio Nocentini − *Department of Neurofarba, Università degli Studi di Firenze, 50019 Florence, Italy;* orcid.org/0000-0003-3342-702X

Fabrizio Carta − *Department of Neurofarba, Università degli Studi di Firenze, 50019 Florence, Italy;* orcid.org/0000-0002-1141-6146

Claudiu T. Supuran − *Department of Neurofarba, Università degli Studi di Firenze, 50019 Florence, Italy;* orcid.org/0000-0003-4262-0323

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.5c00099

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

No unexpected or unusually high safety hazards were encountered.

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■ ABBREVIATIONS

CA, carbonic anhydrase; AAZ, acetazolamide

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Nepublicēti rezultāti / Unpublished results

Investigation on the benzoxaphosphepine-based carbonic anhydrase inhibitors: synthesis, biological evaluation and mechanistic studies

Anastasija Balašova[1,2], Aleksandrs Pustenko[1], Andrea Angeli[3], Alessio Nocentini[3], [...] Claudiu T. Supuran[3], Raivis Žalubovskis[1,2,*]

[1] Latvian Institute of Organic Synthesis, LV-1006 Riga, Latvia

[2] Institute of Chemistry and Chemical Technology, Faculty of Natural Sciences and Technology, Riga Technical University, LV-1048 Riga, Latvia

[3] Department of Neurofarba, Università degli Studi di Firenze, 50019 Florence, Italy

[*] Corresponding author: raivis@osi.lv (R. Žalubovskis)

Keywords

Carbonic anhydrase; inhibitors; benzoxaphosphepines; anticancer

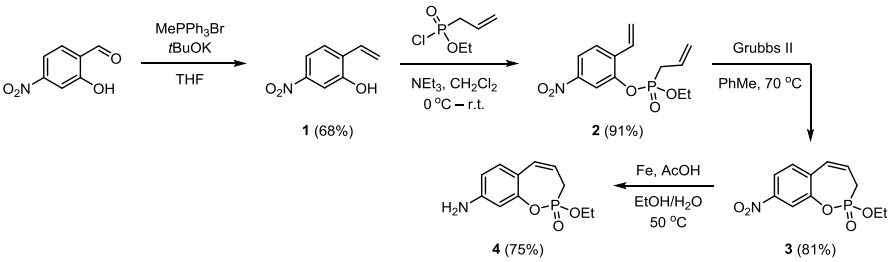
Introduction

Carbonic anhydrases (CAs) are a family of metalloenzymes that catalyze the reversible interconversion between carbon dioxide and bicarbonate anion.[#] These enzymes are critical for maintaining acid–base homeostasis and for facilitating various physiological processes such as respiration, renal function, and bone resorption.[#] The inhibition of carbonic anhydrases has emerged as a promising therapeutic strategy, particularly in the context of cancer. Cancer cells exhibit an altered metabolic state, characterized by an acidic tumor microenvironment. The dysregulation of pH homeostasis within tumor cells and their surroundings promotes tumor progression, invasion, and resistance to therapy. CA isoforms IX and XII are overexpressed in many solid tumors, making them attractive targets for anticancer therapy.[#]

Results and Discussion

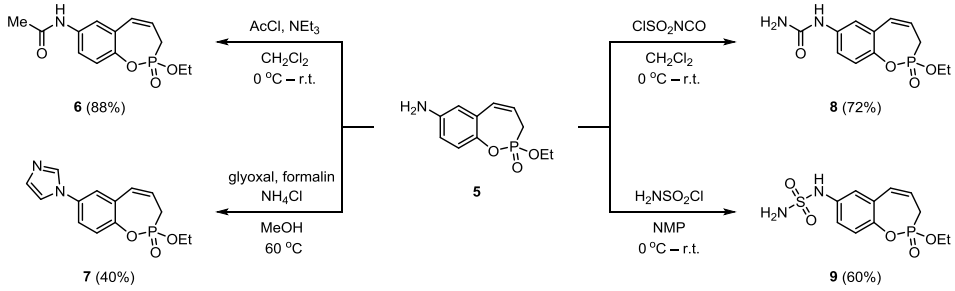
Chemistry

The synthesis of 8-aminobenzoxaphosphepine 2-oxide **4** was accomplished using a similar procedure, which was previously used by our group for the preparation of a 7-substituted analogue (Scheme 1).[#] Nitrosalicylaldehyde underwent the Wittig olefination to furnish 2-vinylphenol **1**, which was phosphorylated to give diolefin **2**. Ring-closing metathesis of **2** provided 8-nitro-substituted derivative **3**, which was subsequently reduced to aniline **4**.



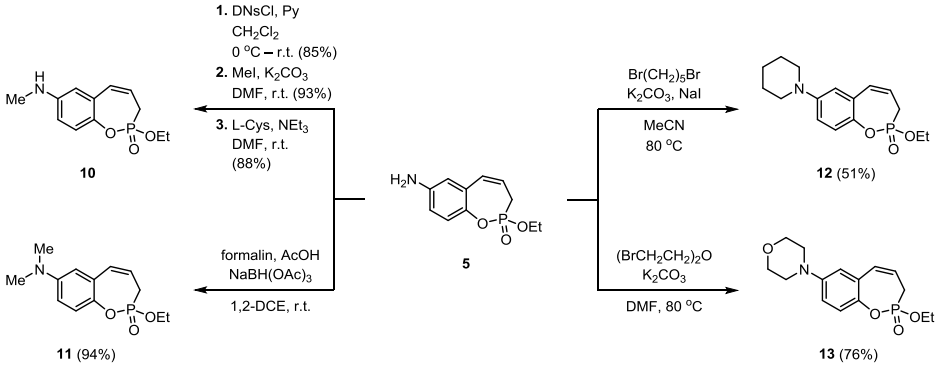
Scheme 1. Synthesis of aniline **4**.

Aniline **5**, reported in our earlier work,[#] was converted into acetamide **6** and urea **8** *via* reaction with AcCl or chlorosulfonyl isocyanate, respectively. The Debus–Radziszewski reaction with glyoxal, formaldehyde and NH_4Cl yielded imidazole **7**, whereas reaction with sulfamoyl chloride afforded sulfamide **9** (Scheme 2).



Scheme 2. Synthesis of compounds **6**–**9**.

The preparation of *N*-methyl derivative **10** involved Fukuyama method, in which the starting aniline **5** was transformed to 2,4-dinitrobenzenesulfonamide, which was then methylated with MeI. The mild cleavage of sulfonamide protecting group was performed in the presence of L-cysteine and NEt_3, yielding the target product **10**. The synthesis of *N*,*N*-dimethylaniline **11** was performed utilizing reductive amination of formaldehyde. The construction of piperidine and morpholine analogues **12**, **13** was readily done using alkylation of **5** with 1,5-dibromopentane and bis(2-bromoethyl)ether, respectively (Scheme 3).

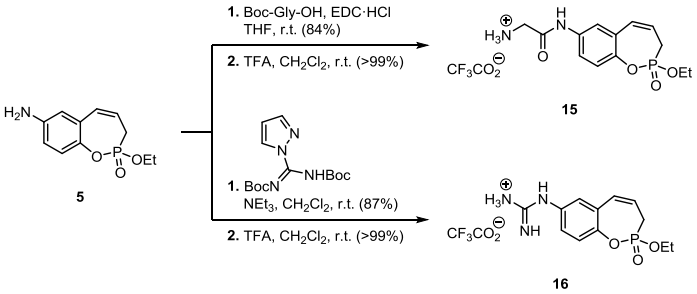


Scheme 3. Synthesis of compounds **10**–**13**.

O-Deethylation of compound **11** was done using standard McKenna conditions (Scheme 4).

Scheme 4. Deethylation of compound **11**.

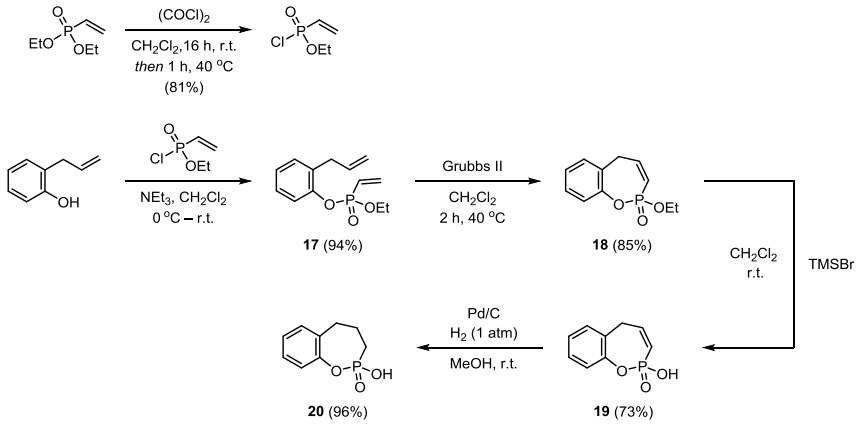
Treatment of aniline **5** with the respective *N*-Boc-protected glycine or di-Boc-1*H*-pyrazole-1-carboxamidine and subsequent removal of the Boc groups with TFA afforded the desired compounds **15** and **16** as trifluoroacetate salts (Scheme 5).



Scheme 5. Synthesis of compounds **15** and **16**.

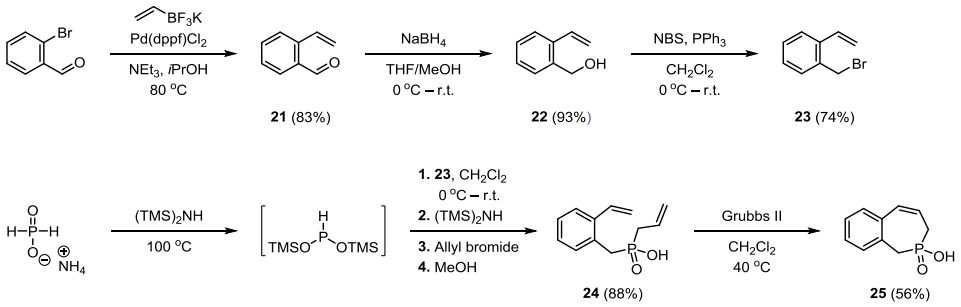
The synthesis of 2,5-dihydro- and 2,3,4,5-tetrahydrobenzoxaphosphepine 2-oxides **19** and **20** was completed as shown in Scheme 6. In the first step, commercially available 2-allylphenol was phosphorylated with ethyl vinylphosphonochloridate, which was prepared in one step by Arbuzov-type

reaction from diethyl vinylphosphonate. The resultant diolefin **17** underwent ring-closing metathesis furnishing 2-ethoxylated derivative **18**. Removal of the ethyl group by TMSBr facilitated the formation of 2-hydroxy analogue **19**, whose hydrogenation afforded **20**.



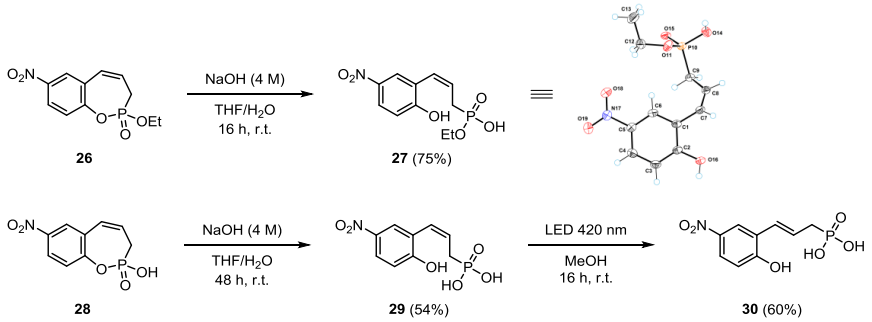
Scheme 6. Synthesis of compounds **19** and **20**.

In order to prepare the cyclic phosphinic acid **25**, 2-vinylbenzyl bromide (**23**) was obtained from 2-bromobenzaldehyde, which underwent vinylation under Suzuki–Miyaura conditions. The resultant 2-vinylbenzaldehyde (**21**) was reduced and brominated under Appel-type conditions (Scheme 7). The introduction of phosphorus atom was carried out by conversion of ammonium hypophosphite to bis(trimethylsilyl)phosphonite, which then reacted with bromide **23**. Subsequent reactions with hexamethyldisilazane, allyl bromide and MeOH yielded diolefin **24**, which furnished the target compound **25** after ring-closing metathesis.



Scheme 7. Synthesis of compound **25**.

In addition, 7-nitrobenzoxaphosphepine 2-oxides **26** and **28** prepared by previously established methodology[#] were hydrolyzed under alkaline conditions to give *Z*-phosphonate **27** and *Z*-phosphonic acid **29**, respectively (Scheme 6). The double bond geometry in compound **27** was unambiguously proven by single-crystal X-ray diffractometry. Compound **29** was irradiated with violet light LED to give the corresponding *E*-phosphonic acid analogue **30**.



Scheme 8. Synthesis of compounds **27**, **29**, and **30**.

Carbonic Anhydrase Inhibitory Activity

Table 1. Inhibition data of human CA I, II, IX, and XII with compounds of series **NB** and the standard sulfonamide inhibitor acetazolamide (AAZ).

Cmpd	K_I (μM)[a,b]			
	hCA I	hCA II	hCA IX	hCA XII
4	ND	ND	ND	ND
6	>100	>100	0.62	0.87
7	ND	ND	ND	ND
8	>100	>100	0.051	3.44
9	0.37	0.094	0.037	0.023
10	>100	>100	0.84	0.71
11	>100	>100	0.95	0.76
12	>100	>100	1.13	1.07
13	>100	>100	0.99	1.28
14	>100	>100	0.92	0.81
15	>100	>100	0.92	0.65
16	>100	>100	1.23	1.15
19	>100	>100	1.6	3.5
20	>100	>100	>100	>100
24	ND	ND	ND	ND

25	>100	>100	0.86	0.78
27	>100	>100	2.14	1.98
29	>100	>100	1.80	1.67
30	ND	ND	ND	ND
AAZ	0.25	0.012	0.025	0.006

[a]Values are mean from 3 different assays using the stopped-flow technique (errors were in the range of ±5–10% of the reported values). [b]Incubation time: 6 h.

Materials and Methods

General Information

All non-aqueous reactions were performed under argon atmosphere using dry glassware and dry solvents. Anilines **4** and **5** were prepared following the literature procedure described in our previous paper.[#] Other reagents were purchased from commercial sources and used as received. TLC was performed on Merck 60 F254 precoated silica gel plates and was visualized with UV light at 254 nm. Flash column chromatography was carried out using Kieselgel (0.035–0.070 mm) silica gel. Reversed-phase chromatography was done on a Biotage Isolera One system using Biotage SNAP KP-C18-HS cartridges. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker Avance Neo 400 MHz spectrometer. Exact molecular masses (HRMS) were determined on a Waters Synapt G2-Si hybrid quadrupole time-of-flight (TOF) mass spectrometer equipped with an electron spray ion source (ESI).

*Synthesis of 8-amino-2-ethoxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (**4**)*

8-Nitrobenzoxaphosphepine oxide **3** was synthesized from 4-nitrosalicylaldehyde according to a general literature procedure.[#] 8-Amino-2-ethoxy-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**4**) was prepared by applying previously established procedure.[#]

*5-Nitro-2-vinylphenol (**1**).* Compound **1** was prepared from $MePPh_3Br$ (9.83 g, 27.5 mmol, 2.3 equiv), *t*-BuOK (3.16 g, 28.1 mmol, 2.35 equiv) and 4-nitrosalicylaldehyde (2.00 g, 12.0 mmol, 1 equiv) as a yellow solid (1.35 g, 68%).[#] ^{1}H NMR (600 MHz, $CDCl_3$, δ): 7.53 (dd, J = 8.5, 2.1 Hz, 1H), 7.43 (d, J = 2.1 Hz, 1H), 7.27 (d, J = 8.5 Hz, 1H), 6.71 (dd, J = 17.7, 11.2 Hz, 1H), 5.66 (d, J = 17.7 Hz, 1H), 5.30 (t, J = 5.6 Hz, 2H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, δ): 153.1, 147.7, 131.8, 130.1, 127.8, 119.9, 116.3, 111.1 ppm.

*Ethyl (5-nitro-2-vinylphenyl) allylphosphonate (**2**).* Compound **2** was prepared from 5-nitro-2-vinylphenol (**1**) (1.34 g, 8.14 mmol, 1.0 equiv), ethyl allylphosphonochloridate[#] (1.45 mL, 9.76 mmol, 1.2 equiv), and NEt_3 (2.04 mL, 14.65 mmol, 1.8 equiv) as a yellowish oil (2.21 g, 91%). ^{1}H NMR

(400 MHz, $CDCl_3$, δ): 8.23 (dd, J = 2.3, 1.4 Hz, 1H), 8.01 (ddt, J = 8.7, 2.3, 0.5 Hz, 1H), 7.69 (d, J = 8.7 Hz, 1H), 7.02 (dd, J = 17.7, 11.1 Hz, 1H), 5.93 (dd, J = 17.7, 0.6 Hz, 1H), 5.92–5.79 (m, 1H), 5.57 (dd, J = 11.1, 0.6 Hz, 1H), 5.35–5.25 (m, 2H), 4.31–4.12 (m, 2H), 2.87 (dt, J = 7.3, 1.1 Hz, 1H), 2.82 (dt, J = 7.4, 1.3 Hz, 1H), 1.31 (td, J = 7.1, 0.4 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, δ): 147.7 (d, $J_{P,C}$ = 9.3 Hz), 136.2 (d, $J_{P,C}$ = 5.0 Hz), 129.5, 127.0, 126.2 (d, $J_{P,C}$ = 11.7 Hz), 121.5, 121.4, 120.2, 120.1, 116.9 (d, $J_{P,C}$ = 2.9 Hz), 63.7 (d, $J_{P,C}$ = 7.0 Hz), 32.2 (d, $J_{P,C}$ = 140 Hz), 16.5 (d, $J_{P,C}$ = 5.8 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$, δ): 25.02 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{13}H_{17}NO_5P$: 298.0844, found 298.0851.

*2-Ethoxy-8-nitro-3H-benzo[f][1,2]oxaphosphepine 2-oxide (**3**).* Compound **3** was prepared from ethyl (5-nitro-2-vinylphenyl) allylphosphonate (**2**) (1.00 g, 3.36 mmol, 1.0 equiv) and ruthenium catalyst (CAS: 246047-72-3) (200 mg, 0.235 mmol, 0.07 equiv) as a yellowish oil (0.73 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.08–8.03 (m, 2H), 7.40 (d, J = 8.3 Hz, 1H), 6.73 (dd, J = 10.9, 5.4 Hz, 1H), 6.15 (ddt, J = 17.9, 10.9, 6.8 Hz, 1H), 4.44–4.29 (m, 2H), 2.91–2.64 (m, 2H), 1.42 (t, J = 7.1, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, δ): 148.0 (d, $J_{P,C}$ = 7.9 Hz), 134.33, 134.31, 131.3, 129.0 (d, $J_{P,C}$ = 9.2 Hz), 125.5 (d, $J_{P,C}$ = 12.5 Hz), 119.9, 117.8 (d, $J_{P,C}$ = 3.8 Hz), 63.6 (d, $J_{P,C}$ = 7.2 Hz), 26.6 (d, $J_{P,C}$ = 128 Hz), 16.6 (d, $J_{P,C}$ = 5.7 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$, δ): 39.37 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{11}H_{13}NO_5P$: 270.0531, found 270.0535.

*8-Amino-2-ethoxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (**4**).* The target compound **4** was prepared from 2-ethoxy-8-nitro-3*H*-benzo[*f*][1,2]oxaphosphepine 2-oxide (**3**) (706 mg, 2.62 mmol, 1 equiv), Fe powder (732 mg, 13.1 mmol, 5 equiv), and glacial AcOH (1.35 mL, 23.6 mmol, 9 equiv) as a yellowish oil (627 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.97–6.93 (m, 1H), 6.54–6.49 (m, 1H), 6.48–6.45 (m, 2H), 5.71 (ddt, J = 18.6, 10.6, 6.6 Hz, 1H), 4.34–4.18 (m, 2H), 3.90 (br s, 2H), 2.78–2.59 (m, 2H), 1.35 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, δ): 149.0 (d, $J_{P,C}$ = 7.7 Hz), 148.1, 131.6, 130.4 (d, $J_{P,C}$ = 8.5 Hz), 118.2 (d, $J_{P,C}$ = 12.8 Hz), 111.9, 107.6 (d, $J_{P,C}$ = 3.8 Hz), 62.8 (d, $J_{P,C}$ = 7.1 Hz), 26.2 (d, $J_{P,C}$ = 128 Hz), 16.6 (d, $J_{P,C}$ = 5.7 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$, δ): 38.66 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{11}H_{15}NO_3P$: 240.0790, found 240.0789.

*N-(2-Ethoxy-2-oxido-3H-benzo[f][1,2]oxaphosphepin-7-yl)acetamide (**6**)*

Aniline **5** (250 mg, 1.05 mmol, 1.0 equiv) was dissolved in dry DCM (10 mL). After the solution was cooled to 0 °C, acetyl chloride (90 µL, 1.25 mmol, 1.2 equiv) and NEt_3 (0.44 mL, 3.14 mmol, 3.0 equiv) were added. The reaction mixture was stirred under inert atmosphere at r.t. for 16 h, then it was treated with water (10 mL). The organic layer was separated, and the aqueous layer was extracted with

EtOAc (2 × 30 mL). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give a crude product, which was purified by flash column chromatography on silica gel (EtOAc/MeOH 10:1) to provide 6 as a white foam (230 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 9.40 (s, 1H), 7.48–7.43 (m, 1H), 7.36 (dd, J = 8.8, 2.6 Hz, 1H), 6.91 (d, J = 8.8 Hz, 1H), 6.43 (dd, J = 10.7, 5.1 Hz, 1H), 5.86–5.72 (m, 1H), 4.25–4.10 (m, 2H), 2.69–2.45 (m, 2H), 2.02 (s, 3H), 1.30–1.23 (m, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, δ): 169.3, 143.4 (d, $J_{P,C}$ = 7.8 Hz), 135.7, 130.2 (d, $J_{P,C}$ = 8.9 Hz), 127.5, 121.5 (d, $J_{P,C}$ = 3.4 Hz), 121.3 (d, $J_{P,C}$ = 12.8 Hz), 121.2, 121.0, 62.9 (d, $J_{P,C}$ = 7.3 Hz), 25.5 (d, $J_{P,C}$ = 128 Hz), 23.9, 16.3 (d, $J_{P,C}$ = 5.8 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$, δ): 40.79 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{13}H_{17}NO_4P$: 282.0907, found 282.0895.

*1-(2-Ethoxy-2-oxido-3H-benzo[f][1,2]oxaphosphepin-7-yl)urea (**8**)*

Aniline **5** (255 mg, 1.07 mmol, 1.0 equiv) was dissolved in dry DCM (4 mL). After the solution was cooled to 0 °C, chlorosulfonyl isocyanate (0.10 mL, 1.17 mmol, 1.1 equiv) was added dropwise. The reaction mixture was stirred under inert atmosphere at r.t. for 2 h, then it was treated with water (10 mL) and stirred for an additional 16 h. The organic layer was separated and concentrated in vacuo to give a crude product, which was purified by flash column chromatography on silica gel (EtOAc/MeOH 5:1) to provide **8** as a white foam (156 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.59 (s, 1H), 7.36 (d, J = 2.6 Hz, 1H), 7.32 (dd, J = 8.7, 2.6 Hz, 1H), 7.05 (d, J = 8.7 Hz, 1H), 6.64 (dd, J = 10.7, 5.3 Hz, 1H), 5.95 (ddt, J = 17.4, 10.7, 6.8 Hz, 1H), 5.86 (s, 2H), 4.16 (dq, J = 8.5, 7.0 Hz 2H), 2.84–2.57 (m, 2H), 1.27 (t, J = 7.0 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, δ): 156.0, 141.7 (d, $J_{P,C}$ = 7.8 Hz), 137.4, 129.8 (d, $J_{P,C}$ = 8.7 Hz), 127.6, 122.7 (d, $J_{P,C}$ = 12.5 Hz), 121.7 (d, $J_{P,C}$ = 3.1 Hz), 119.0, 118.7, 62.2 (d, $J_{P,C}$ = 6.8 Hz), 25.3 (d, $J_{P,C}$ = 126 Hz), 16.3 (d, $J_{P,C}$ = 5.6 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$, δ): 40.91 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{12}H_{16}N_2O_4P$: 283.0848, found 283.0855.

*N-(2-Ethoxy-2-oxido-3H-benzo[f][1,2]oxaphosphepin-7-yl)sulfamide (**9**)*

Aniline **5** (200 mg, 0.84 mmol, 1.0 equiv) was dissolved in NMP (*N*-methyl-2-pyrrolidinone, 8 mL). After the solution was cooled to 0 °C, sulfamoyl chloride[#] (116 mg, 10.0 mmol, 1.2 equiv) was added. The reaction mixture was stirred at r.t. for 16 h, then it was treated with water (30 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give a crude product, which was

purified by flash column chromatography on silica gel (EtOAc/MeOH 10:1) to provide **9** as a white foam (160 mg, 60%). ^{1}H NMR (400 MHz, acetone-d_6, δ): 8.49 (s, 1H), 7.29 (dd, J = 8.7, 2.7 Hz, 1H), 7.23 (d, J = 2.7 Hz, 1H), 7.16–7.11 (m, 1H), 6.66 (dd, J = 10.7, 5.4 Hz, 1H), 6.41 (s, 2H), 6.04 (ddt, J = 17.3, 10.6, 6.8 Hz, 1H), 4.24 (dq, J = 8.6, 7.1 Hz, 2H), 2.80–2.59 (m, 2H), 1.34 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (101 MHz, acetone-d_6, δ): 145.0 (d, $J_{P,C}$ = 7.9 Hz), 137.1, 130.4 (d, $J_{P,C}$ = 8.7 Hz), 129.4, 124.0 (d, $J_{P,C}$ = 12.7 Hz), 123.2 (d, $J_{P,C}$ = 3.2 Hz), 121.74, 121.67, 63.1 (d, $J_{P,C}$ = 6.6 Hz), 26.4 (d, $J_{P,C}$ = 127 Hz), 16.8 (d, $J_{P,C}$ = 5.7 Hz) ppm. ^{31}P NMR (162 MHz, acetone-d_6, δ): 40.32 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{11}H_{16}N_2O_5PS$: 319.0518, found 319.0521.

*7-(Dimethylamino)-2-ethoxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (**11**)*

A mixture of aniline **5** (200 mg, 0.84 mmol, 1.0 equiv), formaldehyde (37% wt in H_2O, 0.16 mL, 2.09 mmol, 2.5 equiv), $NaBH(OAc)_3$ (1.7 g, 8.03 mmol, 9.6 equiv), and glacial AcOH (0.05 mL, 0.84 mmol, 1.0 equiv) in 1,2-DCE (5 mL) was stirred at r.t. for 16 h. Saturated aq. $NaHCO_3$ (10 mL) was added, and the aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give a crude product, which was purified by flash column chromatography on silica gel (EtOAc/MeOH 10:1) to provide **11** as a yellowish oil (210 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.06–7.02 (m, 1H), 6.68 (dd, J = 9.0, 3.1 Hz, 1H), 6.63 (dd, J = 10.6, 5.4 Hz, 1H), 6.46 (d, J = 3.1 Hz, 1H), 5.92 (ddt, J = 17.3, 10.6, 6.8 Hz, 1H), 4.34–4.21 (m, 2H), 2.92 (s, 6H), 2.78–2.55 (m, 2H), 1.37 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, δ): 148.0, 139.6 (d, $J_{P,C}$ = 7.7 Hz), 131.0 (d, $J_{P,C}$ = 8.7 Hz), 128.0, 122.3 (d, $J_{P,C}$ = 3.3 Hz), 121.6 (d, $J_{P,C}$ = 12.8 Hz), 114.0, 113.0, 62.3 (d, $J_{P,C}$ = 7.1 Hz), 41.1, 25.8 (d, $J_{P,C}$ = 128 Hz), 16.6 (d, $J_{P,C}$ = 5.7 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$, δ): 41.94 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{13}H_{19}NO_3P$: 268.1103, found 268.1107.

*7-(Dimethylamino)-2-hydroxy-3H-benzo[f][1,2]oxaphosphepine 2-oxide (**14**)*

^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.06–7.02 (m, 1H), 6.68 (dd, J = 9.0, 3.1 Hz, 1H), 6.63 (dd, J = 10.6, 5.4 Hz, 1H), 6.46 (d, J = 3.1 Hz, 1H), 5.92 (ddt, J = 17.3, 10.6, 6.8 Hz, 1H), 4.34–4.21 (m, 2H), 2.92 (s, 6H), 2.78–2.55 (m, 2H), 1.37 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, δ): 148.0, 139.6 (d, $J_{P,C}$ = 7.7 Hz), 131.0 (d, $J_{P,C}$ = 8.7 Hz), 128.0, 122.3 (d, $J_{P,C}$ = 3.3 Hz), 121.6 (d, $J_{P,C}$ = 12.8 Hz), 114.0, 113.0, 62.3 (d, $J_{P,C}$ = 7.1 Hz), 41.1, 25.8 (d, $J_{P,C}$ = 128 Hz), 16.6 (d, $J_{P,C}$ = 5.7 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$, δ): 41.94 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{11}H_{15}NO_3P$: 240.0790, found 240.0794.

*2-Ethoxy-7-(piperidin-1-yl)-3H-benzo[f][1,2]oxaphosphepine 2-oxide (**15**)*

To a stirred solution of aniline **5** (200 mg, 0.84 mmol, 1.0 equiv) in dry MeCN (10 mL) were added 1,5-dibromopentane (0.14 mL, 1.00 mmol, 1.2 equiv), K_2CO_3 (138 mg, 1.00 mmol, 1.2 equiv), and NaI (150 mg, 1.00 mmol, 1.2 equiv). The reaction mixture was stirred under inert atmosphere at 80 °C for 16 h. After complete conversion, the reaction mixture was cooled down to r.t. and diluted with Et_2O. The solids were filtered off, and the filtrate was then concentrated in vacuo to give a crude product, which was purified by flash column chromatography on silica gel (EtOAc/MeOH 10:1) to provide **15** as a white amorphous solid (131 mg, 51%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.04–6.99 (m, 1H), 6.93 (dd, J = 9.0, 3.0 Hz, 1H), 6.80 (d, J = 3.0 Hz, 1H), 6.64 (dd, J = 10.7, 5.3 Hz, 1H), 5.93 (ddt, J = 17.4, 10.6, 6.7 Hz, 1H), 4.21–4.09 (m, 2H), 3.13–3.04 (m, 4H), 2.82–2.55 (m, 2H), 1.65–1.56 (m, 4H), 1.55–1.47 (m, 2H), 1.27 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6, δ): 148.8, 140.1 (d, $J_{P,C}$ = 7.7 Hz), 130.2 (d, $J_{P,C}$ = 8.7 Hz), 127.8, 122.2 (d, $J_{P,C}$ = 12.4 Hz), 121.8 (d, $J_{P,C}$ = 3.2 Hz), 117.3, 116.6, 62.1 (d, $J_{P,C}$ = 6.7 Hz), 49.9, 25.32 (d, $J_{P,C}$ = 126 Hz), 25.26, 23.8, 16.3 (d, $J_{P,C}$ = 5.7 Hz) ppm. ^{31}P NMR (162 MHz, DMSO-d_6, δ): 40.81 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{16}H_{23}NO_3P$: 308.1416, found 308.1414.

*2-Ethoxy-7-morpholino-3H-benzo[f][1,2]oxaphosphepine 2-oxide (**16**)*

To a stirred solution of aniline **5** (357 mg, 1.49 mmol, 1.0 equiv) in dry DMF (6 mL) were added 2-bromoethyl ether (0.21 mL, 1.64 mmol, 1.1 equiv) and K_2CO_3 (227 mg, 1.64 mmol, 1.1 equiv). The reaction mixture was stirred under inert atmosphere at 80 °C for 16 h. After complete conversion, the reaction mixture was cooled down to r.t. and diluted with EtOAc. The solids were filtered off, and the filtrate was then concentrated in vacuo to give a crude product, which was purified by flash column chromatography on silica gel (EtOAc/MeOH 10:1) to provide **16** as a colorless oil (352 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.11–7.06 (m, 1H), 6.87 (dd, J = 8.9, 3.0 Hz, 1H), 6.68 (d, J = 3.0 Hz, 1H), 6.61 (dd, J = 10.5, 5.3 Hz, 1H), 5.94 (ddt, J = 17.3, 10.5, 6.8 Hz, 1H), 4.35–4.21 (m, 2H), 3.88–3.81 (m, 4H), 3.14–3.07 (m, 4H), 2.78–2.55 (m, 2H), 1.37 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, δ): 148.5, 141.8 (d, $J_{P,C}$ = 7.9 Hz), 130.6 (d, $J_{P,C}$ = 8.8 Hz), 128.2, 122.5 (d, $J_{P,C}$ = 3.4 Hz), 122.0 (d, $J_{P,C}$ = 13.0 Hz), 117.4, 116.6, 67.0, 62.9 (d, $J_{P,C}$ = 7.2 Hz), 49.8, 25.9 (d, $J_{P,C}$ = 128 Hz), 16.6 (d, $J_{P,C}$ = 5.7 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$, δ): 41.54 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{15}H_{21}NO_4P$: 310.1208, found 310.1215.

*N-(2-Ethoxy-2-oxido-3H-benzo[f][1,2]oxaphosphepin-7-yl)-2,4-dinitrobenzenesulfonamide (**NB-612**)*

^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.67 (d, J = 2.2 Hz, 1H), 8.42 (dd, J = 8.6, 2.2 Hz, 1H), 8.08 (d, J = 8.6 Hz, 1H), 7.55 (br s, 1H), 7.16–7.07 (m, 3H), 6.55 (dd, J = 10.8, 5.2 Hz, 1H), 5.99 (ddt, J = 18.3, 10.8, 6.7 Hz, 1H), 4.37–4.22 (m, 2H), 2.70 (ddt, J = 21.2, 6.7, 1.1 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, δ): 150.3, 148.7, 146.8 (d, $J_{P,C}$ = 7.6 Hz), 137.7, 133.6, 131.4, 129.24, 129.23 (d, $J_{P,C}$ = 8.6 Hz), 127.1, 125.6, 124.5, 123.5 (d, $J_{P,C}$ = 2.8 Hz), 123.4, 120.9, 63.4 (d, $J_{P,C}$ = 7.0 Hz), 26.3 (d, $J_{P,C}$ = 128 Hz), 16.6 (d, $J_{P,C}$ = 5.6 Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{17}H_{17}N_3O_9PS$: 470.0423, found 470.0430.

*N-(2-Ethoxy-2-oxido-3H-benzo[f][1,2]oxaphosphepin-7-yl)-N-methyl-2,4-dinitrobenzenesulfonamide (**NB-614**)*

^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.45 (d, J = 2.2 Hz, 1H), 8.36 (dd, J = 8.7, 2.2 Hz, 1H), 7.81 (d, J = 8.7 Hz, 1H), 7.19–7.08 (m, 3H), 6.58 (dd, J = 10.8, 5.3 Hz, 1H), 6.01 (ddt, J = 17.6, 10.8, 6.8 Hz, 1H), 4.39–4.24 (m, 2H), 3.41 (s, 3H), 2.78–2.68 (m, 2H), 1.40 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, δ): 150.0, 148.5, 147.8 (d, $J_{P,C}$ = 7.7 Hz), 136.7, 136.3, 133.5, 130.8, 129.3, 129.2 (d, $J_{P,C}$ = 8.9 Hz), 128.2, 125.7, 123.5 (d, $J_{P,C}$ = 7.1 Hz), 123.4 (d, $J_{P,C}$ = 2.1 Hz), 119.7, 63.4 (d, $J_{P,C}$ = 7.0 Hz), 40.2, 26.3 (d, $J_{P,C}$ = 128 Hz), 16.6 (d, $J_{P,C}$ = 5.6 Hz) ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{18}H_{19}N_3O_9PS$: 484.0580, found 484.0603.

*2-Ethoxy-7-(methylamino)-3H-benzo[f][1,2]oxaphosphepine 2-oxide (**10**)*

^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.03–6.96 (m, 1H), 6.60 (dd, J = 10.6, 5.4 Hz, 1H), 6.54 (dd, J = 8.7, 2.9 Hz, 1H), 6.36 (d, J = 2.9 Hz, 1H), 5.91 (ddt, J = 15.9, 10.6, 6.8 Hz, 1H), 4.35–4.20 (m, 2H), 3.73 (br s, 1H), 2.81 (s, 3H), 2.77–2.56 (m, 2H), 1.37 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, δ): 146.5, 139.9 (d, $J_{P,C}$ = 7.6 Hz), 130.8 (d, $J_{P,C}$ = 8.6 Hz), 128.3, 122.5 (d, $J_{P,C}$ = 3.1 Hz), 121.7 (d, $J_{P,C}$ = 12.9 Hz), 113.9, 112.1, 62.7 (d, $J_{P,C}$ = 7.1 Hz), 31.1, 25.8 (d, $J_{P,C}$ = 128 Hz), 16.6 (d, $J_{P,C}$ = 5.7 Hz) ppm. ^{31}P NMR (162 MHz, $CDCl_3$, δ): 42.03 ppm. HRMS (ESI) $[M+H]^+$: *m/z* calcd for $C_{12}H_{17}NO_3P$: 254.0946, found 254.0945.

Anastasija Balašova dzimusi 1998. gadā Rīgā. Latvijas Universitātē ieguvusi dabaszinātņu bakalaura (2019) un maģistra (2021, ar izcilību) grādu ķīmijā. Kopš 2018. gada strādā Latvijas Organiskās sintēzes institūtā. Patlaban ir profesora Raivja Žalubovska vadītās Lietderīgās ķīmijas grupas zinātniskā asistente. Pētījuma "Jauns virziens pretvēža preparātu izveidē", ko Latvijas Zinātņu akadēmija nosaukusi par vienu no 2020. gada Latvijas nozīmīgākajiem sasniegumiem zinātnē, līdzautore. Zinātniskās intereses saistītas ar medicīnas ķīmiju un bioloģiski aktīvu heterociklisku savienojumu sintēzi. Ir piecu oriģinālpublikāciju un viena apskatraksta līdzautore.

Anastasija Balašova was born in 1998 in Riga. She obtained a Bachelor's degree (2019) and a Master's degree with distinction (2021) in Natural Sciences in Chemistry from the University of Latvia. Since 2018, she has been working at the Latvian Institute of Organic Synthesis. Currently, she is a research assistant in the Applied Chemistry Group under the supervision of Prof. Raivis Žalubovskis. She is a co-author of the study "A new direction in the development of anticancer drugs", recognised by the Latvian Academy of Sciences as one of Latvia's most significant scientific achievements of 2020. Her research interests are related to medicinal chemistry and the synthesis of biologically active heterocyclic compounds. She is a co-author of five original publications and one review article.