

RĪGAS TEHNISKĀ UNIVERSITĀTE

Materiālzinātnes un lietišķās ķīmijas fakultāte
Organiskās ķīmijas tehnoloģijas institūts

RIGA TECHNICAL UNIVERSITY

Faculty of Materials Science and Applied Chemistry
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**JAUNA VEIDA OĢĻSKĀBES ANHIDRĀŽU
INHIBITORU SINTĒZE**

Promocijas darba kopsavilkums

**SYNTHESIS OF A NEW TYPE OF CARBONIC
ANHYDRASES INHIBITORS**

Summary of the Doctoral Thesis

Zinātniskais vadītājs
asociētais profesors *Dr. chem.*
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RAIVIS ŽALUBOVSKIS

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PROMOCIJAS DARBS IZVIRZĪTS ZINĀTNES DOKTORA GRĀDA IEGŪŠANAI RĪGAS TEHNISKAJĀ UNIVERSITĀTĒ

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APSTIPRINĀJUMS

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

Aleksandrs Pustenko (paraksts)

Datums:

Promocijas darbs sagatavots kā tematiski vienota zinātnisko publikāciju kopa. Tajā ir kopsavilkums un piecas publikācijas. Publikācijas uzrakstītas angļu valodā, to kopējais apjoms ir 39 lpp.

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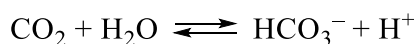
PROMOCIJAS DARBA VISPĀRĒJS RAKSTUROJUMS

Tēmas aktualitāte

Pēc Pasaules Veselības organizācijas datiem 2018. gadā pasaulē tika reģistrēti 9,6 miljoni onkoloģisko saslimšanu izraisīti nāves gadījumi un 18,1 miljoni jaunu onkoloģisko saslimšanas gadījumu. Katrs piektais vīrietis un katra sestā sieviete dzīves laikā saslimst ar vēzi [1]. Eiropā, kur dzīvo ~9 % no visiem pasaules iedzīvotājiem, 2018. gadā reģistrēti 23,4 % no visiem onkoloģisko saslimšanu gadījumiem, tai skaitā 20,3 % no visiem nāves gadījumiem, tāpēc ir ļoti svarīgi nepārtraukti attīstīt un pilnveidot onkoloģisko slimību ārstēšanas iespējas [1].

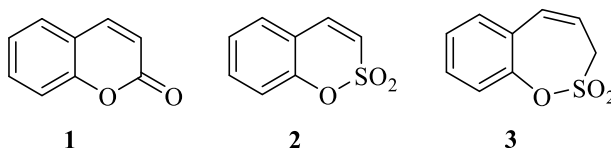
Mūsdienās ir zināmi vairāk nekā 300 dažādi enzīmi, kuriem cinks ir nozīmīgs kofaktors. Šie enzīmi organismā veic bioloģiski nozīmīgas funkcijas, to darbība ir tieši saistīta ar epiģenētiskās kontroles mehānismiem šūnās, kuru regulēšanas traucējumi ir viens no galvenajiem vēža rašanās cēloņiem [2].

Pēdējā desmitgadē pastiprināta uzmanība pievērsta cinku saturošiem metalloenzīmiem – ogļskābes anhidrāzēm (CA, EC 4.2.1.1), kas organismā katalizē apgriezenisku oglekļa dioksīda hidratāciju.



Tiek uzskatīts, ka no šobrīd zināmajām 15 cilvēka α -ogļskābes anhidrāžu izoformām, CA IX un CA XII tiek paaugstināti ekspresētas hipoksijai pakļautajās vēža šūnās, nodrošinot optimālu pH to izdzīvošanai un attīstībai. Lai apturētu vēža šūnu attīstību un izvairītos no nevēlamām blaknēm, jānodrošina selektīva CA IX un CA XII izoformu inhibēšana.

Literatūrā [3] zināms, ka kumarīna **1** atvasinājumiem piemīt selektīva CA IX un CA XII inhibēšanas spēja. Mūsu grupā tika sintezēti sulfokumarīna **2** atvasinājumi, kas izrādījās selektīvi CA IX un CA XII inhibitori [4]. Balstoties uz šīm zināšanām, tika sintezēti benzoksatiepīna-2,2-dioksīda **3** atvasinājumi, kas ir sulfokumarīna atvasinājumi ar paplašinātu ciklu.

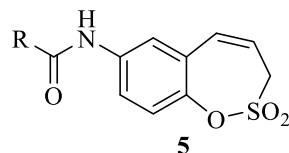
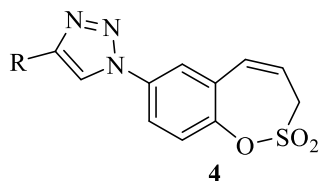


Promocijas darba mērķis

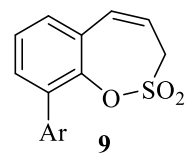
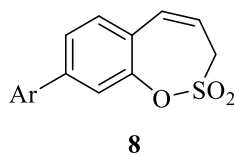
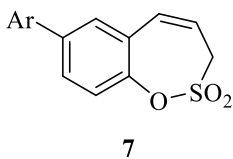
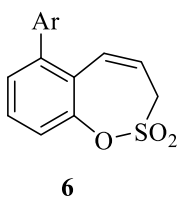
Izveidot jaunus, efektīvus un selektīvus CA IX un CA XII inhibitorus, no kuriem nākotnē, iespējams, varētu tikt izstrādāts jaunas paaudzes pretvēža līdzeklis.

Promocijas darba uzdevumi

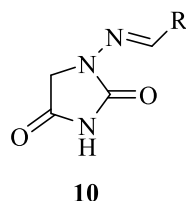
1. Izstrādāt 3*H*-1,2-benzoksatiepīna-2,2-dioksīda **3** atvasinājumu iegūšanas metodes.
2. Iegūt 3*H*-1,2-benzoksatiepīna-2,2-dioksīda 7-triazolil- **4** un 7-acilaminoatvasinājumus **5**.



3. Iegūt 6-, 7-, 8- un 9-aizvietotus 3*H*-1,2-benzoksatiepīna-2,2-dioksīda arilatvasinājumus **6–9**.



4. Iegūt 1-imidazolidīn-2,4-diona atvasinājumus **10**.



5. Izvērtēt sintezēto savienojumu CA inhibēšanas aktivitātes.

Zinātniskā novitāte un galvenie rezultāti

Ir atrasta jauna, selektīva CA IX un CA XII inhibitoru klase – 3*H*-1,2-benzoksatiepīna-2,2-dioksīdi. Sintezēta virkne 3*H*-1,2-benzoksatiepīna-2,2-dioksīda triazolil-, acilamino- un arilatvasinājumu.

Atklājām, ka furagīns, klīnikā lietots antibakteriālais līdzeklis, ir selektīvs CA IX un CA XII inhibitors. Attīstot šo virzienu, tika sintezēta virkne imidazolidīn-2,4-diona atvasinājumu. Visiem promocijas darbā iegūtajiem produktiem noteikta cilvēka CA (I, II, IX un XII) izoformu inhibēšanas aktivitāte.

Darba struktūra un apjoms

Promocijas darbs sagatavots kā tematiski vienota zinātnisko publikāciju kopa par ogļskābes anhidrāzes inhibitoru sintēzi.

Darba aprobācija un publikācijas

Promocijas darba rezultāti izklāstīti piecās zinātniskajās publikācijās, publikāciju kopējā ietekmes faktoru summa – 16,9. Darba rezultāti prezentēti sešās konferencēs.

Zinātniskās publikācijas

1. Pustenko, A., Nocentini, A., Gratteri, P., Bonardi, A., Vozny, I., Žalubovskis, R., Supuran, C. T. The antibiotic furagin and its derivatives are isoform-selective human carbonic anhydrase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2020**, *35*, 1011–1020.
2. Pustenko, A., Nocentini, A., Balašova, A., Krasavin, M., Žalubovskis, R., Supuran, C. T. 7-acylamino-3H-1,2-benzoxathiepine 2,2-dioxides as new isoform-selective carbonic anhydrase IX and XII inhibitors. *J. Enzyme Inhib. Med. Chem.* **2020**, *35*, 650–656.
3. Pustenko, A., Nocentini, A., Balašova, A., Alafeefy, A., Krasavin, M., Žalubovskis, R., Supuran, C. T. Aryl derivatives of 3H-1,2-benzoxathiepine 2,2-dioxide as carbonic anhydrase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2020**, *35*, 245–254.
4. Pustenko, A., Stepanovs, D., Žalubovskis, R., Vullo, D., Kazaks, A., Leitans, J., Tars, K., Supuran, C. 3H-1,2-benzoxathiepine 2,2-dioxides: a new class of isoform-selective carbonic anhydrase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2017**, *32*, 767–775.
5. Pustenko, A., Žalubovskis, R. Recent advances in sultone synthesis (microreview). *Chem. Heterocycl. Compd.* **2017**, *53*, 1283–1285.

Darba rezultāti prezentēti sešās zinātniskajās konferencēs

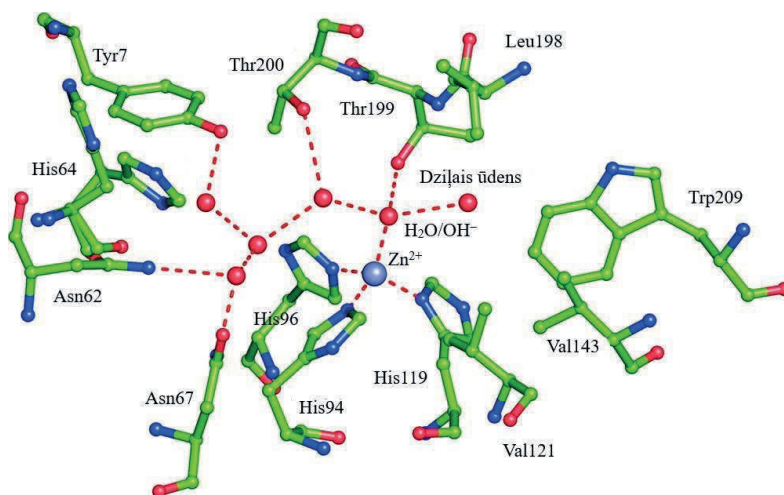
1. Žalubovskis, R., Grandāne, A., Ivanova, J., Balode, A., Pustenko, A., Domraceva, I., Tārs, K., Leitāns, J. Challenging design and synthesis of inhibitors of carbonic anhydrases. *International Conference on Organic Synthesis Balticum Organicum Syntheticum (BOS-2016)*. Riga, Latvia, July 3–6, **2016**.
2. Pustenko, A. Carbonic Anhydrases: Inhibitor Synthesis. *10th Paul Walden Symposium on Organic Chemistry*, Riga, Latvia, June 15–16, **2017**.
3. Žalubovskis, R., Ivanova, J., Pustenko, A., Grandane, A., Domraceva, I., Tars, K., Supuran, C., T. Inhibitors of Carbonic Anhydrases—challenges of design and synthesis. *3rd Satellite Meeting on Carbonic Anhydrase “New Trend in Carbonic Anhydrases Research”*, Montecatini Terme, Italy, May 24–27, **2017**.
4. Pustenko, A., Ivanova, J., Grandane, A., Vozny, I., Žalubovskis, R. Towards Novel Inhibitors of Cancer Associated Enzymes. *11th International Conference on Carbonic Anhydrases*, Bucharest, Romania, June 27–30, **2018**.
5. Pustenko, A., Balašova, A. Carbonic Anhydrases: Inhibitor Synthesis. *11th Paul Walden Symposium on Organic Chemistry*, Riga, Latvia, September 19–20, **2019**.
6. Pustenko, A., Balašova, A., Kapura, V., Žalubovskis, R. Inhibitors of cancer associated enzymes – design and synthesis. *4th Satellite Meeting on Carbonic Anhydrases*, Parma, Italy, November 14–17, **2019**.

PROMOCIJAS DARBA GALVENIE REZULTĀTI

Lai labāk izprastu promocijas darbā paveikto, sākotnēji jāaplūko mērķenzīmu ogļskābes anhidrāzes (CA). Ogļskābes anhidrāzes ir metalloenzīmi, kas katalizē apgriezenisku oglekļa dioksīda hidratāciju. Ogļskābes anhidrāzes tika atklātas 1933. gadā, kopš tā laika tās ir plaši pētītas. Mūsdienās ogļskābes anhidrāzes iedala astoņās dažādās klasēs: α , β , γ , δ , ξ , η , θ un ι [5], [6]. α -CA ir visplašāk pētītā klase, jo šīs klases ogļskābes anhidrāzes sastopamas zīdītājos. β -CA sastopamas augstākajos augos un dažos prokariotos. γ -CA sastopamas arheobaktērijās (*Archea*) un cianobaktērijās. δ - un ξ -CA sastopamas tikai aļģēs, savukārt η -CA – tikai vienšūņos [7]. α -, β - un δ -CA aktīvajā centrā satur Zn(II) jonus, γ -CA satur Fe(II) jonus, ξ -CA satur Co(II) jonus un ι -CA satur Mn(II) jonus [5, 6]. Daudzos organismos CA piedalās vitāli svarīgu fizioloģisko procesu norisē, kas saistīti ar pH regulēšanu un CO₂ homeostāzes nodrošināšanu [7].

Cilvēkos ir zināmas 15 α -CA izoformas. CA I, II, III, VII un XIII atrodas citosolā, CA IV, IX, XII un XIV ir piesaistītas membrānai, CA VA un VB atrodas mitohondrijā, savukārt CA VI atrodas siekalās un mātes pienā [8], [9]. Jāatzīmē, ka visām α -CA izoformām, izņemot CA VB, ir zināma 3D struktūra. Neatkarīgi no dažādā novietojuma šūnā visas α -CA izoformas ir strukturāli līdzīgas, tās ir monomēras, izņemot CA IX, CA XII un CA VI – tās ir dimēras [8].

α -CA aktīvais centrs ir novietots konusveida dobumā, kas ir aptuveni 12 Å plats un 13 Å dziļš. Cinka jons ir novietots dobuma apakšā, tas ir saistīts ar ligandiem – 3 histidīna atlikumiem (His119, His94 un His 96), ūdens molekulu / hidroksīdjonu (1. att.) [7], [8].



1. att. CAII aktīvā centra struktūra [8].

Zn²⁺ jons ar ūdenraža saitēm ir saistīts ar treonīna (Thr199) hidroksilgupu un divām pretēji novietotām ūdens molekulām. Ūdens molekulu, kas novietota hidrofobajā daļā, sauc par “dziļo ūdeni” (“*deep water*”), to ieskauj Val121, Val143, Leu198 un Trp207. Otra ūdens molekula novietota hidrofilajā daļā, aktīvā centra ieejā, un to ieskauj Asn62, His64 un Asn67.

Hidrofobais un hidrofilais apgabals skaidrojams ar substrāta (CO_2) un tā hidratācijas produktu (H^+ un HCO_3^-) dažādo ķīmisko dabu [8]. Makkenna (*McKenna*) ar līdzstrādniekiem parādīja, ka CO_2 molekula saistās enzīma hidrofobajā daļā, savukārt hidratācijas produkti – enzīma hidrofilajā daļā [10].

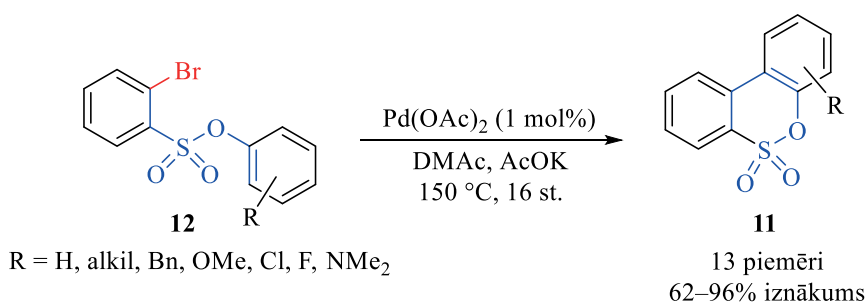
Jāatzīmē, ka visām līdz šim kristalizētajām cilvēka CA izoformām cinka jons ir saistīts ar trīs histidīna atlikumiem (His119, His94 un His 96) un tām visām ir novērota hidrofobā un hidrofilā daļa [7]. Balstoties uz šīm zināšanām par mērķenzīmu, tiek konstruēti un izstrādāti inhibitori.

Mūsdienās ir zināmi vairāki α -CA inhibīcijas mehānismi. Sulfonamīdi (RSO_2NH_2), sulfamāti (ROSO_2NH_2), sulfamīdi ($\text{RNHSO}_2\text{NH}_2$), karboksilāti (RCO_2^-), ureāti un fosfonāti ($\text{R}'\text{PO}(\text{OR})_2$) saistās ar enzīma aktīvajā centrā esošo cinka jonu un veido papildu H-saites ar Thr199. Fenoli un poliamīdi koordinējas ar ūdens molekulu / hidroksīdjonu, kas saistīts pie cinka. Kumarīni un to izostēri nosedz ieeju aktīvajā centrā, tādējādi CA aktivatori nevar piesaistīties pie enzīma [11], [12].

3*H*-1,2-Benzoksatiepīna-2,2-dioksīds ir uzskatāms par sultonu. Termins “sultoni” pirmo reizi tika lietots 1888. gadā. Mūsdienās sultoni tiek plaši izmantoti medicīnas ķīmijā kā enzīmu inhibitori, tiem piemīt pretvīrusu iedarbība. Labākās sultonu iegūšanas metodes ir pārejas metālu katalīze, ciklopievienošanās reakcijas un *Diels–Alder* tipa reakcijas [13].

Promocijas darba izstrādes laikā tika apkopota un vēlāk arī publicēta jaunākā informācija par pārejas metālu katalizētām sultonu sintēzes metodēm. Sultonus iespējams iegūt pallādija, rodija, vara, zelta un rutēnija katalizētās reakcijās, tuvāk tiks aplūkotas dažas no šīm metodēm.

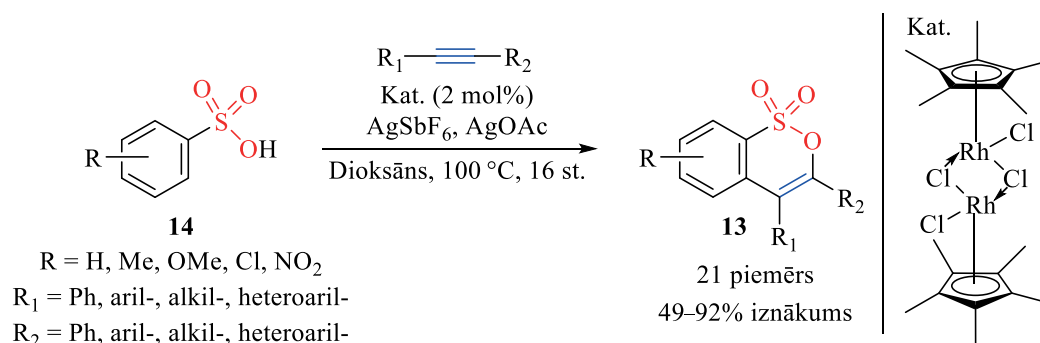
Doucets (*Doucet*) un līdzstrādnieki publicēja pallādija katalizētu sultonu **11** sintēzes metodi (2. att.), kā izejvielu izmantojot 2-brombenzosulfonskābes fenilesteri **12** [14].



2. att. Pallādija katalizēta sultonu **11** sintēze.

Jāatzīmē, ka reakcija ir atkarīga no aizvietotāja R dabas. Izmantojot elektrononus aizvietotājus, reakcijas produktu iznākums palielinās, savukārt, izmantojot elektronakceptorus aizvietotājus (NO_2 , CO_2Bu , CF_3), attiecīgie sultoni neveidojas. Visos gadījumos sultoni **11** tika iegūti ar augstu reģioselektivitāti.

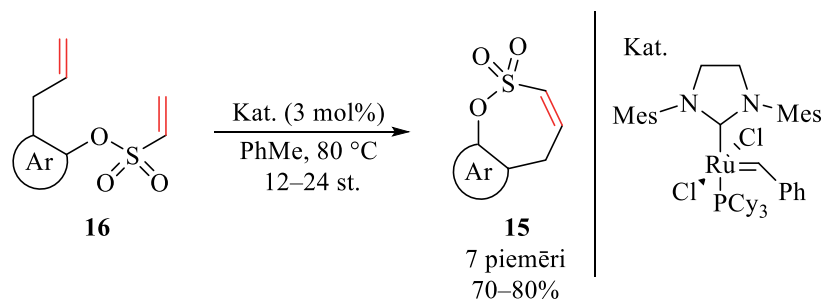
Li (*Li*) un līdzstrādnieki izstrādāja efektīvu Rh(III) katalizētu sultonu **13** sintēzes metodi no arilsulfonskābēm **14** un alkīniem (3. att.) [15].



3. att. Rodija katalizēta sultonu **13** sintēze.

Šajā metodē iespējams izmantot alkīnus gan ar elektrondonoriem, gan ar elektronakceptoriem aizvietotājiem. Jāatzīmē, ka, izmantojot alkīnu ar elektrondonoriem aizvietotājiem, reakcijas produkta iznākums ir augstāks. Izmantojot nesimetriskus alkīnus, attiecīgie sultoni tika iegūti ar ļoti labu reģioselektivitāti.

Mondals (*Mondal*) un līdzstrādnieki publicēja efektīvu sultonu **15** sintēzes metodi no attiecīgajiem diolefiem **16**, tos ciklizējot (4. att.) [16]. Diolefiem **16** tika ciklizēti, izmantojot rutēnija katalizētu olefīnu cikla saslēgšanas metatēzes reakciju. Jāatzīmē, ka, izmantojot Grabsa (*Grubbs*) pirmās paaudzes rutēnija katalizatoru, sultoni **15** neveidojās. Izmantojot Grabsa otrās paaudzes rutēnija katalizatoru, sultoni **15** tika iegūti ar labiem iznākumiem.



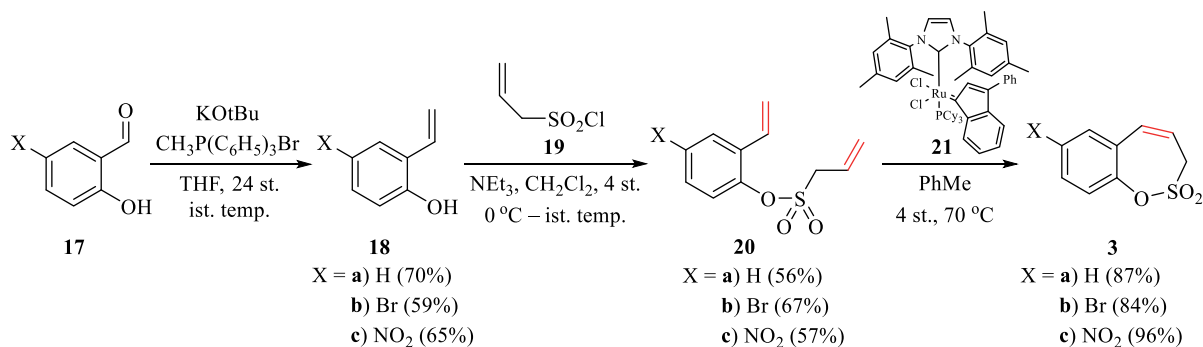
4. att. Rutēnija katalizēta sultonu **15** sintēze.

Izanalizējot literatūrā pieejamo informāciju, benzoksatiepīna-2,2-dioksīda **3** atvasinājumus nolēmām iegūt rutēnija katalizētā olefīnu cikla saslēgšanas metatēzes reakcijā. Izmantojot cikla saslēgšanas metatēzes reakciju, iespējams iegūt sultonus ar elektrondonoriem un elektronakceptoriem aizvietotājiem. Reakcijas produktu iznākumi parasti ir augsti.

1. 3*H*-1,2-Benzoksatiepīna-2,2-dioksīda atvasinājumu sintēze

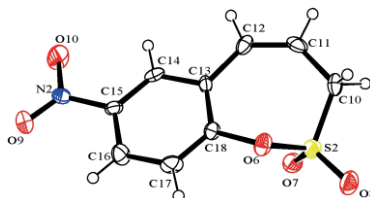
Pētījuma sākumā tika izstrādāta sintēzes metode. Sintēzi sākām no 5-aizvietota 2-hidroksibenzaldehīda **17**, tam veicot Vitiga reakciju, ieguvām olefīnus **18** (5. att.). Olefīnus **18** apstrādājām ar sulfonilhlorīdu **19**, iegūstot diolefīnus **20** ar pieņemamiem iznākumiem (56–67 %). Sulfonilhlorīds **19** ir komerciāli pieejams, taču dārgs reaģents. To veiksmīgi ieguvām, Na_2SO_3 vārot ar alilbromīdu, pēc tam iegūto nātrija sāli apstrādājām ar $POCl_3$. Jāatzīmē, ka sulfonilhlorīds **19** gaisā nav stabils, tāpēc to ieguvām lielākā daudzumā, lai attīrīšanu veiktu vakuumdestilācijas ceļā. Izmantojot nedestilētu sulfonilhlorīdu **19**, reakcijas produktu

iznākums būtiski samazinās. Kā galvenā stadija benzoksatiepīna-2,2-dioksīda iegūšanā tika izvēlēta diolefīna **20** ciklizācija, izmantojot olefīnu cikla saslēgšanas metatēzes reakciju. Ciklizāciju veiksmīgi veicām, izmantojot komerciāli pieejamo Grabsa otrās paaudzes katalizatora atvasinājumu **21**. Veiksmīgi ieguvām attiecīgos 7- aizvietotus 3*H*-1,2-benzoksatiepīna-2,2-dioksīdus **3** ar augstiem iznākumiem (84–96 %).



5. att. Benzoksatiepīna-2,2-dioksīda atvasinājumu **3a–3c** iegūšana.

Darba gaitā veiksmīgi izdevās iegūt 7-nitro-3*H*-1,2-benzoksatiepīna-2,2-dioksīda **3c** monokristālu, kas bija pietiekami kvalitatīvs struktūras noteikšanai, izmantojot monokristāla rentgendifraktometriju. Latvijas Organiskās sintēzes institūta Fizikāli organiskās ķīmijas laboratorijā tika iegūta rentgendifrakcijas aina, kas ir neapšaubāms savienojuma **3c** struktūras pierādījums (6. att.).

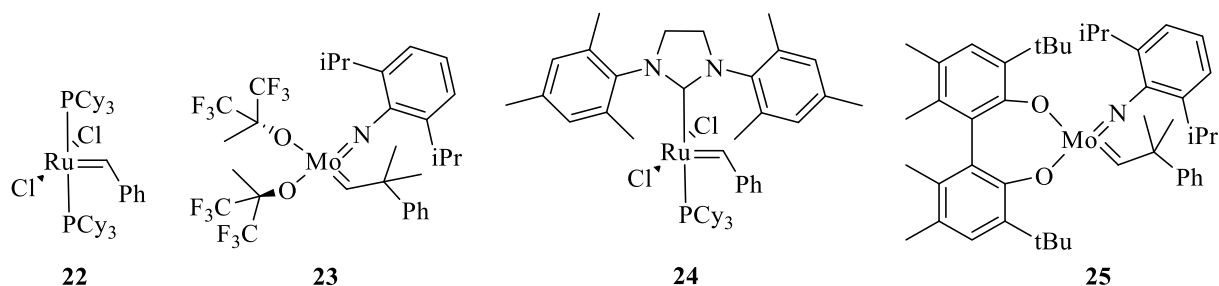


6. att. 7-Nitro-3*H*-1,2-benzoksatiepīna-2,2-dioksīda **3c** rentgenstruktūra.

Jāatzīmē, ka mūsdienās ir zināmi daudzi un dažādi cikla saslēgšanas metatēzes reakcijas katalizatori. Galvenokārt izmanto rutēnija un molibdēna katalizatorus. Pagājušā gadsimta 90. gados tika izstrādāti un komercializēti pirmās paaudzes katalizatori. Zināmākie no tiem ir Grabsa pirmās paaudzes katalizators **22** un Šroka (*Schrock*) katalizators **23** (7. att.). Diemžēl pirmās paaudzes katalizatoriem nepiemīt augsta funkcionālo grupu tolerance un selektivitāte, tie ir gaisa un mitruma jutīgi [17]. 1999. gada augustā Grabs publicēja rakstu, kurā ir apskatīti jauni, efektīvāki olefīnu cikla saslēgšanas metatēzes rutēnija katalizatori [18]. Mūsdienās tos pazīst kā Grabsa otrās paaudzes katalizatorus. Otrās paaudzes rutēnija katalizatori ir efektīvāki, tiem piemīt paaugstināta termiskā stabilitāte, katalītiskā aktivitāte, gaisa un mitrumizturība. Tas tika panākts, tricikloheksilfosfīna ligandu aizstājot ar *N*-heterociklisku karbēna ligandu [17], [19]. Zināmākais no šiem katalizatoriem ir Grabsa otrās paaudzes katalizators **24** (7. att.). Attīstot molibdēna katalizatorus, izstrādāja Šroka–Hoveidas (*Schrock–Hoveyda*) katalizatoru **25** (7. att.), kam piemīt augstāka funkcionālo grupu tolerance un selektivitāte nekā Šroka katalizatoram **23**. Tiek uzskatīts, ka molibdēna katalizatori tolerē

amīnus un fosfīnus, bet netolerē substrātus ar karboksil-, hidroksi-, un aldehīdgrupām. Savukārt rutēnija katalizatori netolerē amīnus un fosfīnus, bet tolerē substrātus ar karboksil-, hidroksi-, un aldehīdgrupām [17].

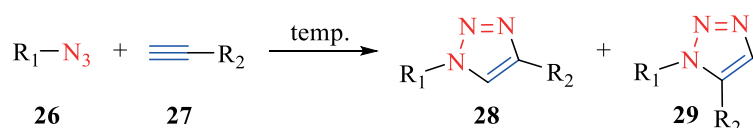
Otrās paaudzes rutēnija katalizatori ir termiski stabili, ar labu funkcionālo grupu toleranci, gaisa un mitrumizturību, tāpēc nolēmām izmantot komerciāli pieejamo otrās paaudzes katalizatora atvasinājumu **21**.



7. att. Olefīnu metatēzes reakcijas katalizatoru piemēri.

1.1. 3*H*-1,2-Benzoksatiepīna-2,2-dioksīda 1,2,3-triazolilatvasinājumu sintēze

Lai labāk izprastu struktūras–aktivitātes likumsakarības, nolēmām sintezēt 1,4-diaizvietotus benzoksatiepīna-2,2-dioksīda 1,2,3-triazolilatvasinājumus **4**. Mihaels (*Michael*) 1893. gadā publicēja pirmo 1,2,3-triazolu sintēzi no dietilacetilēndikarboksilāta un fenilazīda [20]. Neskatoties uz to, 1,2,3-triazolu sintēzi vairāk saista ar Huisgēna (*Huisgen*) vārdu. 20. gadsimta 60. gados viņš strādāja pie 1,3-dipolārajām ciklopievienošanās reakcijām, tai skaitā 1,2,3-triazolu sintēzes. Nodarbojās ar reakcijas mehānisma un kinētikas pētījumiem [21]. Kopš tā laika zināms, ka azīdu **26** reakcijās ar alkīniem **27** paaugstinātā temperatūrā veidojas reģioizomēru – 1,4- **28** un 1,5-diaizvietotu **29** 1,2,3-triazolu maisījums (8. att.).

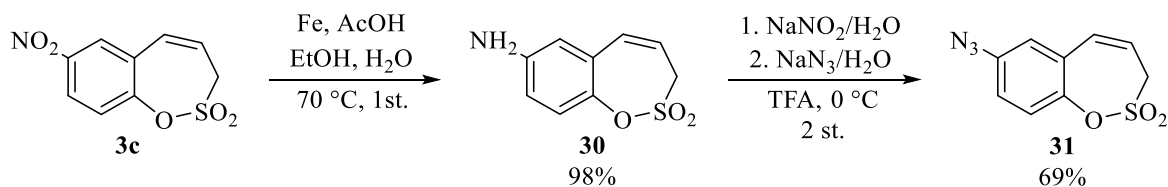


8. att. 1,2,3-Triazolilatvasinājumu veidošanās.

Mūsdienās ir izstrādātas metodes selektīvai 1,4- vai 1,5-diaizvietotu 1,2,3-triazolilatvasinājumu sintēzei. 2002. gadā Mendals (*Mendal*) un līdzstrādnieki [22] un Šarpless (*Sharpless*) un līdzstrādnieki [23] neatkarīgi viens no otra publicēja rakstus, kur aprakstīta Cu(I) katalizēta azīda-alkīna ciklopievienošanās, selektīvi veidojot 1,4-diaizvietotus 1,2,3-triazolilatvasinājumus. Jāatzīmē, ka Šarplesa izstrādātajā protokolā [23] tika izmantots CuSO₄, ko *in situ* reducēja ar nātrija askorbātu, veidojot Cu(I) nevis Cu(0). Selektīvu 1,5-diaizvietotu 1,2,3-triazolilatvasinājumu veidošanos iespējams panākt, izmantojot dažādus rutēnija katalizatorus [21], [24].

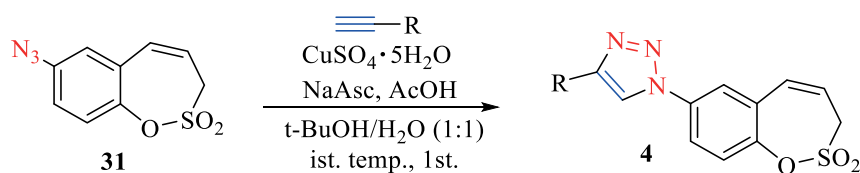
Turpinot darbu, nitroatvasinājumu **3c** veiksmīgi reducējām ar Fe(0), ar labu iznākumu iegūstot aminoatvasinājumu **30** (9. att.). No aminoatvasinājuma **30** ar pieņemamu iznākumu (69 %) pagatavoja azidoatvasinājumu **31** (9. att.), ko tālāk izmantoja kā izejvielu 1,4-

diaizvietotu 1,2,3-triazolilatvasinājumu sintēzē. Apstrādājot aminoatvasinājumu **30** ar NaNO₂ skābā vidē, *in situ* veidojas diazonija sāls, kuram reaģējot ar NaN₃, veidojas azīds **31**. Jāatzīmē, ka no NaN₃ skābā vidē veidojas HN₃ (slāpekļūdeņražskābe), kas ir viegli gaistošs, toksisks savienojums. Tāpēc reakcija jāveic 0 °C temperatūrā.



9. att. Benzoksatiepīna-2,2-dioksīda azidoatvasinājuma **31** iegūšana.

Selektīvai 1,4-diaizvietotu 1,2,3-triazolilatvasinājumu **4** iegūšanai izvēlējamies izmantot Cu(I) katalizētu reakciju starp azīdu **31** un dažādiem alkīniem (10. att.). Cu(I) ieguvām no CuSO₄, to *in situ* reducējot ar nātrija askorbātu, līdzīgi kā Šarpleša rakstā [23]. Kā šķīdinātāju lietojām *t*-BuOH/H₂O maisījumu attiecībā 1 : 1. Ar labiem iznākumiem tika iegūta rinda 1,4-diaizvietotu 1,2,3-triazolilatvasinājumu **4a–4j** (1. tabula). Jāatzīmē, ka tika izmantota etiķskābes piedeva. Ir pierādīts, ka vājas organiskas skābes (etiķskābe, benzoskābe) atvieglo vara eliminēšanos pēc 1,3-dipolārās ciklopievienošanās, tādējādi paaugstinot reakcijas ātrumu [25].



10. att. 1,4-Diaizvietotu 1,2,3-triazolu **4a–4j** sintēze.

1. tabula

Benzoksatiepīna-2,2-dioksīda 1,2,3-triazolilatvasinājumus **4** sintēze, CA inhibēšanas rezultāti

N. p. k.	R	4, iznākums, %	K _I [*] , μM			
			hCA I	hCA II	hCA IX	hCA XII
1.	C ₆ H ₅	4a , 95	>50	>50	1,71	>50
2.	4-ClC ₆ H ₄	4b , 74	>50	>50	3,59	>50
3.	3-OMeC ₆ H ₄	4c , 51	>50	>50	2,56	>50
4.	4-FC ₆ H ₄	4d , 66	>50	>50	1,75	>50
5.	4-OCF ₃ C ₆ H ₄	4e , 83	>50	5,77	0,34	1,72
6.	3-FC ₆ H ₄	4f , 74	>50	>50	1,15	>50
7.	2-NH ₂ C ₆ H ₄	4g , 57	>50	>50	0,46	2,32
8.	CH ₂ OH	4i , 81	>50	>50	0,87	>50
9.	4-CF ₃ C ₆ H ₄	4j , 85	>50	>50	0,43	>50
10.	AAZ*	–	0,25	0,012	0,025	0,006

* 10. rindā parādīta acetazolamīda (AAZ) dažādu CA izoformu inhibēšanas spēja.

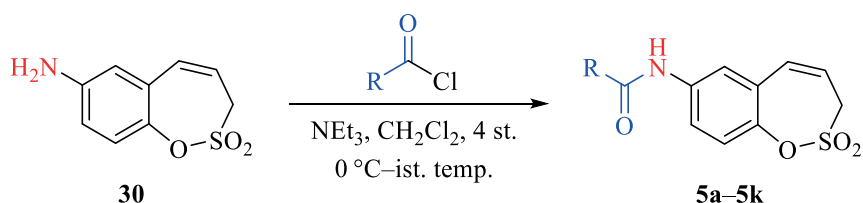
Promocijas darbā sintezētajiem savienojumiem Florences Universitātē profesora C. T. Supurāna (*Supuran*) grupā tika noteiktas cilvēka ogļskābes anhidrāžu (CA I, CA II, CA IX un CA XII) inhibēšanas aktivitātes. Visos gadījumos kā salīdzināšanas standarts izmantots 5-acetamido-1,3,4-tiadiazol-2-sulfonamīds (acetazolamīds, AAZ), kas ir izoformu neselektīvs inhibitors. Jāatzīmē, ka citosolisko CA izoformu CA I un CA II inhibēšana ir nevēlama, jo tās ir plaši sastopamas cilvēka organismā (nemērķenzīmi). Savukārt uz šūnu membrānas virsmas saistītās CA izoformas CA IX un CA XII ir mērķenzīmi, jo audzēju šūnās tās tiek pastiprināti ekspresētas.

Kā redzams 1. tabulā, neviens no triazolilatvasinājumiem neinhibē citosoliskās CA I un CA II, izņemot savienojumu **4e**, kam piemīt vāja CA II inhibitorā aktivitāte ($K_I = 5,77 \mu\text{M}$) (5. rinda). Triazolilatvasinājumi **4a–4j** inhibē CA IX, inhibitorā aktivitāte ir 0,43–3,59 μM , kas ir vājāka par acetazolamīda CA IX inhibēšanas aktivitāti. Vislabākos rezultātus uzrādīja triazolilatvasinājumi **4e**, **4g–4j**. Savienojums **4i** satur hidroksimetilgrupu triazolilgredzenā, tā CA IX inhibēšanas konstante ir $K_I = 0,87 \mu\text{M}$ (8. rinda). Arilgrupu saturošajiem triazolīem **4e**, **4g** un **4j**, kas satur 4-trifluormetoksi-, 2-amino- un 4-trifluoraizvietotājus fenilgredzenā K_I ir 0,34 μM ; 0,46 μM un 0,43 μM attiecīgi (5., 7. un 9. rinda).

Septiņi no deviņiem triazolilatvasinājumiem neinhibē CA XII, $K_I > 50 \mu\text{M}$ (1.–4. rinda, 6. rinda un 8.–9. rinda). Atlikušie divi savienojumi **4e** un **4g** ir vāji CA XII inhibitori – $K_I = 1,72 \mu\text{M}$ un 2,32 μM . No bioloģiskajiem rezultātiem izriet, ka 1,4-diazvietotie 1,2,3-triazolilatvasinājumi **4a–4j** ir selektīvi CA IX inhibitori.

1.2. 3H-1,2-Benzoksatiepīna-2,2-dioksīda 7-acilaminoatvasinājumu sintēze

Lai paplašinātu savienojumu klāstu bioloģisko aktivitāšu pētījumiem, tika nolemts iegūt benzoksatiepīna-2,2-dioksīda 7-acilaminoatvasinājumus **5**. Tos sekmīgi ieguva no aminoatvasinājuma **30** reakcijās ar dažādiem acilchlorīdiem (11. att., 2. tab.).



11. att. Benzoksatiepīna-2,2-dioksīda 7-acilaminoatvasinājumu **5** sintēze.

Neviens no produktiem **5a–5g** neinhibē citosoliskās CA I un CA II ($K_I > 100\,000 \text{ nM}$). Savukārt visi produkti **5a–5g** inhibē mērķenzīmus CA IX un CA XII nanomolārās koncentrācijās. Produkts **5i** uzrāda lielisku gan CA IX, gan arī CA XII inhibēšanas spēju – $K_I = 19,7 \text{ nM}$ un 8,7 nM (8. rinda). Tas ir aktīvāks CA IX inhibitors nekā medicīnā lietotais acetazolamīds, kam CA IX $K_I = 25 \text{ nM}$. Produktiem **5a–5g** un **5j–5k** piemīt vājāka bioloģiskā aktivitāte uz CA IX un CA XII nekā acetazolamīdam ($K_I = 25 \text{ nM}$ un 5,7 nM), to inhibēšanas konstantes ir 45,4–353,3 nM un 40,3–643,7 nM attiecīgi (1.–7., 9., 10. rinda).

Benzoksatiepīna-2,2-dioksīda 7-acilaminoatvasinājumu **5** sintēze, CA inhibēšanas rezultāti

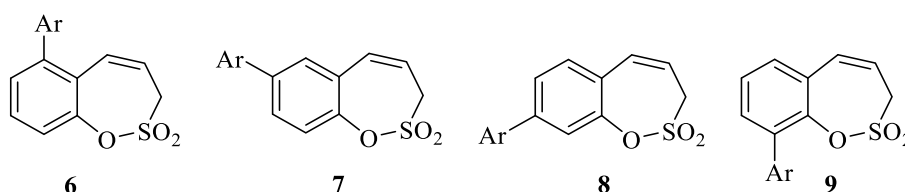
N. p. k.	R	5, iznākums, %	K_1^* , nM			
			hCA I	hCA II	hCA IX	hCA XII
1.	CH ₃	5a , 70	>100 000	>100 000	61,8	162,5
2.	C ₆ H ₅	5b , 72	>100 000	>100 000	208,6	370,1
3.	4-CH ₃ C ₆ H ₄	5c , 73	>100 000	>100 000	83	309,3
4.	4-BrC ₆ H ₄	5d , 59	>100 000	>100 000	353,3	140,7
5.	2-IC ₆ H ₄	5e , 88	>100 000	>100 000	45,4	643,7
6.	2-BrC ₆ H ₄	5f , 82	>100 000	>100 000	66,8	96,2
7.	2-FC ₆ H ₄	5g , 79	>100 000	>100 000	74,6	40,3
8.	2-CF ₃ C ₆ H ₄	5i , 87	>100 000	>100 000	19,7	8,7
9.	2-tienil	5j , 81	>100 000	>100 000	177,5	73,2
10.	2-furil	5k , 81	>100 000	>100 000	210,1	134,4
11.	*AAZ	–	250	12	25	5,7

* 10. rindā parādīta acetazolamīda (AAZ) dažādu CA izoformu inhibēšanas spēja.

Produkts **5d**, kas satur 4-bromfenilgrupu, ir mazāk aktīvs CA IX inhibitors (CA IX $K_1 = 353,3$ nM, 4. rinda) nekā savienojums **5f**, kas satur 2-bromfenilgrupu (CA IX $K_1 = 66,8$ nM, 6. rinda). Iespējams, fenilkarboksiamīdatvasinājumi, kas satur aizvietotāju fenilgrupas otrajā pozīcijā, ir aktīvāki nekā savienojumi, kas satur aizvietotāju fenilgrupas 4. pozīcijā. Ja neaizvietotu fenilgrupu (savienojums **5b**, 2. rinda) aizvieto ar metilgrupu (savienojums **5a**, 1. rinda), CA IX inhibitorā aktivitāte palielinās. Savukārt, ja neaizvietotu fenilgrupu (savienojums **5b**, 2. rinda) aizvieto ar pieclocekļu heterociklu (**5j** un **5g**), CA IX inhibitorā aktivitāte būtiski nemainās – K_1 vērtības 117,5 nM un 210,1 nM attiecīgi (9. un 10. rinda).

1.3. 3H-1,2-Benzoksatiepīna-2,2-dioksīda arilatvasinājumu sintēze

Lai gūtu priekšstatu par struktūras-aktivitātes likumsakarībām un paplašinātu savienojumu klāstu, tika nolemts sintezēt benzoksatiepīna-2,2-dioksīda arilatvasinājumus **6–9** (12. att.).



12. att. Benzoksatiepīna-2,2-dioksīda arilatvasinājumu **6–9** vispārējās struktūrformulas.

Arilatvasinājumus izvēlējamies iegūt Pd katalizētas Suzuki–Mijauras šķērssametnāšanas reakcijas ceļā attiecīgo halogēnatvasinājumu reakcijās ar arilborskābēm.

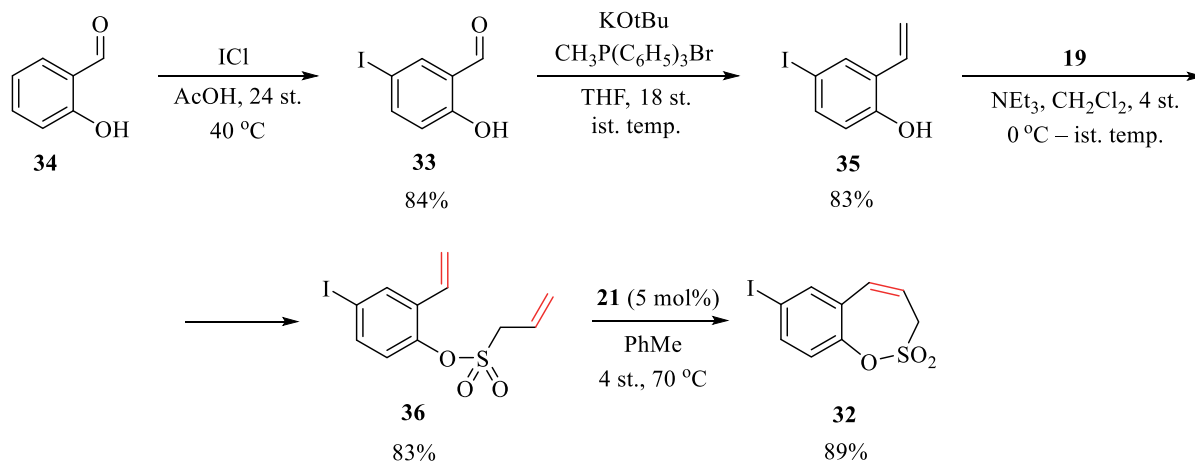
Suzuki–Mijauras šķērssametnāšanas reakcijā galvenokārt tiek izmantoti pallādijs un niķeļa katalizatori. Pallādijs katalizatoru gadījumā reaģētspējīgākie ir ariljodīdi, kam seko triflāti un bromīdi. Izmantojot arilhlorīdus, reakcijas produktu iznākums būtiski samazinās. Tas skaidrojams ar oksidējošās pievienošanās (pirmās katalītiskā cikla stadijas) ātruma palēnināšanos [26]. Visbiežāk izmantotie pallādijs katalizatori Suzuki–Mijauras šķērssametnāšanas reakcijā ir pallādijs katalizatori ar fosfina ligandiem: Pd(PPh₃)₄, Pd(dppf)Cl₂, Pd(PPh₃)₂Cl₂, jo tie ir termiski izturīgi un komerciāli pieejami [26], [27].

Niķeļa katalizatoru attīstība ir veicinājusi mazāk reaģētspējīgu elektrofilu, piemēram, arilhlorīdu, fluorīdu, esteru, nitrilu un arilamīdu, izmantošanu Suzuki–Mijauras šķērssametnāšanas reakcijā [28]. Tomēr, neskatoties uz šīm priekšrocībām, praksē vairāk izmanto tieši pallādijs katalīzi. Parasti niķeļa katalizētas Suzuki–Mijauras šķērssametnāšanas reakcijās nepieciešams liels katalizatora iesvars (3–10 mol %), un tās ir jutīgas pret reakcijas apstākļiem. Ļoti svarīga ir bāzes un šķīdinātāja izvēle. Lielākoties izmanto THF, dioksānu vai toluolu apvienojumā ar slikti šķīstošu neorganisko bāzi, piemēram, K₃PO₄ vai K₂CO₃ [28]. Hidroksīdu [29], kā arī ūdens izmantošana [30] deaktivē niķeļa katalizatorus, un reakcijas produktu iznākums samazinās.

Turpretī pallādijs katalizētā Suzuki–Mijauras šķērssametnāšanas reakcijā izmanto gan organiskās, gan neorganiskās bāzes, piemēram, Na/K₃PO₄, Na/Cs/K₂CO₃, Na/KOH, Na/KOt-Bu, NaOEt, NaOMe. Svarīgi atzīmēt, ka reakcija nenorisinās bez bāzes klātbūtnes. Kā šķīdinātāju parasti izmanto organisko šķīdinātāju maisījumā ar ūdeni. Visbiežāk lietotie organiskie šķīdinātāji ir dioksāns, THF, DMF un toluols [31].

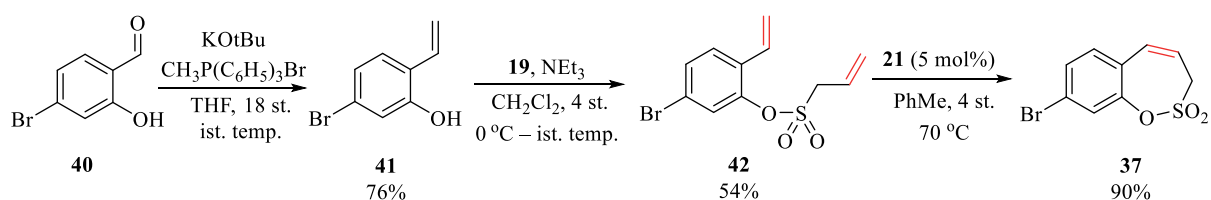
Šķērssametnāšanas reakcijas attīstību lielā mērā ir sekmējusi organobora atvasinājumu vājā nukleofilā daba un stabilitāte. Tie ir komerciāli pieejami, kā arī ir izstrādātas vairākas metodes to iegūšanai. Šķērssametnāšanu iespējams veikt ar labu ķīmisko, reģio- un stereoselektivitāti.

Mērķsavienojumu **6–9** sintēzi sākām ar izejvielu iegūšanu. Pallādijs katalizētā Suzuki–Mijauras šķērssametnāšanas reakcijā jodīdi reaģē aktīvāk nekā bromīdi, tāpēc nolēmām iegūt 7-jodatvasinājumu **32**. Jāatzīmē, ka jodsalicilaldehīds **33**, lai arī komerciāli pieejams, tomēr ir dārgs reaģents. To veiksmīgi ieguva no salicilaldehīda **34**, to apstrādājot ar joda monohlorīdu skābā vidē. Tālāk jodsalicilaldehīdam **33** veica Vitiga reakciju, iegūstot olefīnu **35**, ko veiksmīgi sulfonilēja ar sulfonilhlorīdu **19**, iegūstot diolefīnu **36**. Diolefīnu ciklizēja izmantojot rutēnija katalizatoru **21**, ar labu reakcijas produktu iznākumu iegūstot 7-jodatvasinājumu **32** (13.att.).

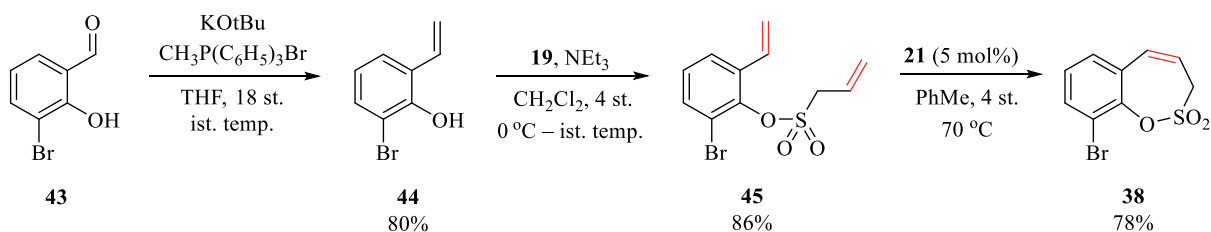


13.att. 7-Jod-3H-1,2-benzoksatiepīna-2,2-dioksīda (**32**) sintēze.

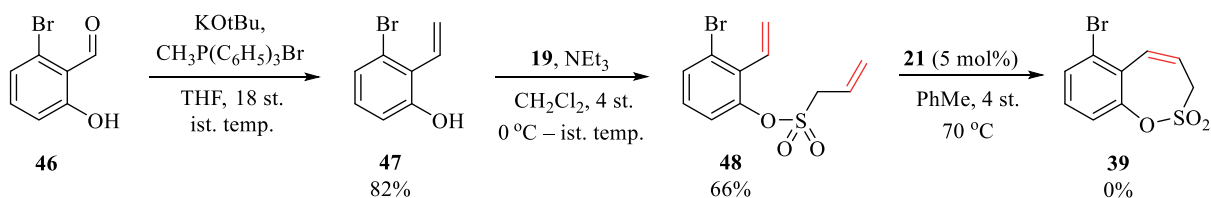
3-, 4- un 6-bromsalicilaldehīdi ir komerciāli pieejami, un nav ērtas sintēzes metodes attiecīgo jodsalicilaldehīdu iegūšanai, tāpēc nolēmām sintetēt bromatvasinājumus **37–39** (14.–16. att.).



14. att. 8-Brom-3H-1,2-benzoksatiepīna-2,2-dioksīda (**37**) sintēze.

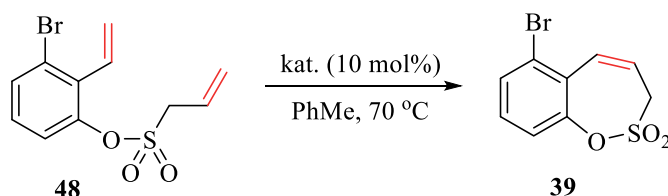


15. att. 9-Brom-3H-1,2-benzoksatiepīna-2,2-dioksīda (**38**) sintēze.



16. att. 6-Brom-3H-1,2-benzoksatiepīna-2,2-dioksīda (**39**) sintēze.

Bromatvasinājumus **37**, **38** ieguva līdzīgi kā jodatvasinājumu **32**. Attiecīgajam salicilaldehīdam veica Vitiga reakciju, iegūstot olefīnu. Olefīnu sulfonilēja ar sulfonilhlorīdu **19**, iegūstot attiecīgo diolefīnu, kuru veiksmīgi ciklizēja izmantojot iepriekš izstrādātos ciklizācijas apstākļus, savienojumu **39** iegūt neizdevās. Veicanieliu reakcijas apstākļu optimizāciju (17. att., 3. tab.).



17. att. Diolefīna **48** cikla saslēgšanas metatēzes reakcijas apstākļu optimizācija.

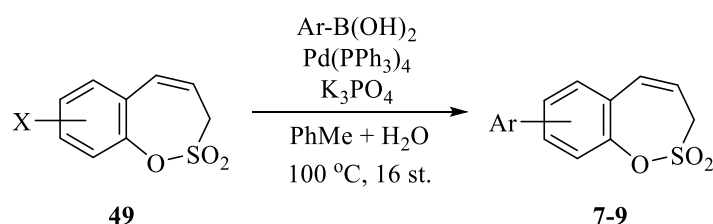
3. tabula

Diolefīna **48** cikla saslēgšanas metatēzes reakcijas apstākļu optimizācijas rezultāti

N. p. k.	Katalizators	Laiks, st.	Iznākums, %
1.	21	40	–
2.	23	16	–
3.	25	16	–

Izmantojot iepriekš lietoto katalizatoru **21**, palielinot reakcijas laiku un divreiz palielinot katalizatoru iesvaru, produkta veidošanos nenovēroja (1. rinda). Izvēlējāmies izmēģināt Šroka (*Schrock*) katalizatorus **23** un **25**, jo tiek uzskatīts, ka molibdēna katalizatori ir aktīvāki par rutēnija katalizatoriem. Diemžēl produktu **39** iegūt neizdevās, iespējams, tas neveidojās stērisko traucējumu dēļ.

Arilatvasinājumus **7–9** tika sekmīgi sintezēti pallādijs katalizētā Suzuki–Mijauras šķerssametināšanas reakcijā, kā katalizatoru izmantojot pallādijs tetrakis ($\text{Pd}(\text{PPh}_3)_4$) (18. att., 4.–6. tab.). Reakcijas veiksmīgai norisei bija nepieciešama paaugstināta temperatūra un ūdens piedeva. Bez ūdens piedevas reakcijas produktu iznākums būtiski samazinās.



18. att. Optimizētie Suzuki–Mijauras sametināšanas reakcijas apstākļi.

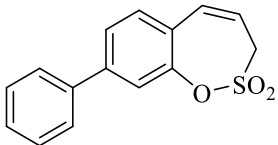
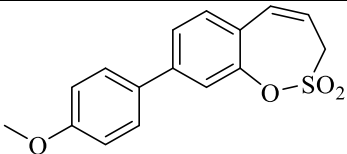
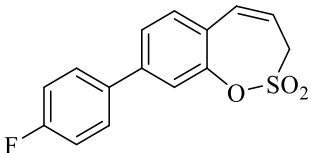
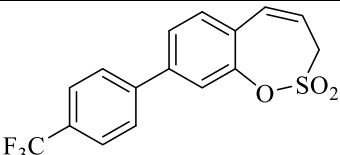
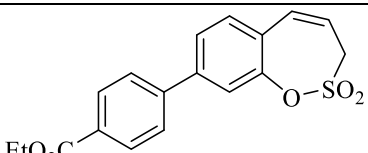
7-Aril-3H-1,2-benzoksatiēpīna-2,2-dioksīda **7** atvasinājumu sintēze, CA inhibēšanas rezultāti

N. p. k.	Produkta Nr., iznākums, %	Produkts	K_1^* , nM	
			hCA IX	hCA XII
1.	7a , 56		654,8	1376
2.	7b , 61		407,6	2934
3.	7c , 44		330,8	890,5
4.	7d , 66		221,4	4017
5.	7e , 44		620,8	2398

* CA I un CA II $K_1 > 100 \mu\text{M}$. Kā standarts izmantots AAZ, tā CA IX $K_1 = 25 \text{ nM}$ un CA XII $K_1 = 5,7 \text{ nM}$.

Kā redzams 4. tabulā, 7-arilaizvietoti benzoksatiēpīna-2,2-dioksīda atvasinājumi **7a–7e** iegūti ar labiem (1., 2., 4. rinda) un vidējiem (3., 5. rinda) iznākumiem. 7-Arilatvasinājumi **7a–7e** neinhibē citosoliskās CA I un CA II, savukārt inhibē mērķenzīmus CA IX un CA XII. Savienojumi **7a–7e** spēcīgāk inhibē CA IX ($K_1 = 221,4–654,8 \text{ nM}$) nekā CA XII ($K_1 = 890,5–4017 \text{ nM}$).

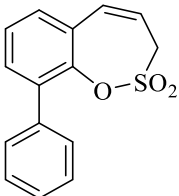
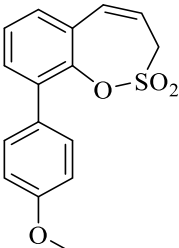
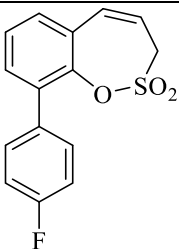
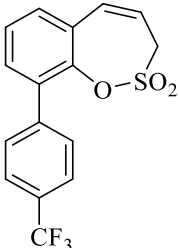
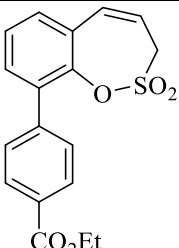
8-Aril-3H-1,2-benzoksatiepīna-2,2-dioksīda **8** atvasinājumu sintēze, CA inhibēšanas rezultāti

N. p. k.	Produkta Nr., iznākums, %	Produkts	K_1^* , nM	
			hCA IX	hCA XII
1.	8a , 44		104,8	473,2
2.	8b , 44		63,1	168,6
3.	8c , 41		95,2	77,9
4.	8d , 46		44,0	247,8
5.	8e , 38		79,8	289,3

* CA I un CA II $K_1 > 100 \mu\text{M}$. Kā standarts izmantots AAZ, tā CA IX $K_1 = 25 \text{ nM}$ un CA XII $K_1 = 5,7 \text{ nM}$.

Kā redzams 5. tabulā, 8-arilaizvietoti benzoksatiepīna-2,2-dioksīda atvasinājumi **8a–8e** iegūti ar vidējiem iznākumiem. 8-Arilatvasinājumi **8a–8e** neinhibē citosoliskās CA I un CA II, savukārt inhibē mērķenzīmus CA IX un CA XII. Savienojumi **8a–8e** spēcīgāk inhibē CA IX ($K_1 = 44,0\text{--}104,8 \text{ nM}$) nekā CA XII ($K_1 = 77,9\text{--}473,2 \text{ nM}$).

9-aril-3*H*-1,2-benzoksatiepīna-2,2-dioksīda **9** atvasinājumu sintēze, CA inhibēšanas rezultāti

N. p. k.	Produkta Nr., iznākums, %	Produkts	K_1^* , μM	
			hCA IX	hCA IX
1.	9a , 42		21,1	>100
2.	9b , 40		60,9	>100
3.	9c , 39		33,7	>100
4.	9d , 44		47,1	>100
5.	9e , 36		16,4	>100

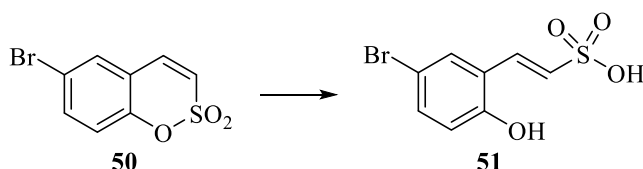
* CA I un CA II $K_1 > 100 \mu\text{M}$. Kā standarts izmantots AAZ, tā CA IX $K_1 = 25 \text{ nM}$ un CA XII $K_1 = 5,7 \text{ nM}$.

Kā redzams 6. tabulā, 9-arilaizvietoti benzoksatiepīna-2,2-dioksīda atvasinājumi **9a–9e** iegūti ar vidējiem iznākumiem. 9-Arilatvasinājumi **9a–9e** neinhibē citosoliskās CA I un CA II un mērķenzīmu CA XII. Tie vāji inhibē CA IX, $K_1 = 16,4\text{--}60,9 \mu\text{M}$.

Jāatzīmē, ka netika novērota arilborskābju aizvietotāju ietekme uz Suzuki–Mijauras sametināšanas reakcijas norisi. Visos gadījumos produkti **7–9** tika iegūti ar līdzīgiem iznākumiem.

Salīdzinot 7-aril- (4. tab.), 8-aril- (5. tab.) un 9-arilsavienojumu (6. tab.) bioloģiskās aktivitātes, tika secināts, ka visaktīvākie CA IX un CA XII inhibitori ir 8-arilatvasinājumi **8a–8e**, kam seko 7-arilatvasinājumi **7a–7e**. 9-Arilatvasinājumi **9a–9e** vāji inhibē CA IX un neinhē CA XII. Vislabākais CA IX inhibitors ir 8-(4-(trifluormetil)fenil)-benzoksatiepīna-2,2-dioksīds (**8d**) (5. tab., 4. rinda), savukārt vislabākais CA XII inhibitors ir 8-(4-fluorfenil)-3*H*-1,2-benzoksatiepīna-2,2-dioksīds (**8c**) (4. tab., 3. rinda). 7-Arilatvasinājumi **7a–7e** un 8-arilatvasinājumi **8a–8e** ir selektīvi CA IX inhibitori.

Lai izprastu benzoksatiepīna-2,2-dioksīda mijiedarbību ar CA, Latvijas Biomedicīnas pētījumu centrā K. Tāra grupā tika veikti benzoksatiepīna-2,2-dioksīdu un cilvēka CA IX kokristalizācijas mēģinājumi. Diemžēl līdz šim brīdim enzīma-inhibitora kokristālu iegūt nav izdevies. Jāatzīmē, ka iepriekš mūsu grupā izstrādātajam un sintezētajam sulfokumarīna atvasinājumam **50** Latvijas Biomedicīnas pētījumu centrā K. Tāra vadībā izdevās iegūt CA II / CA IX mimētiķa-sulfokumarīna kokristālu [4a]. Izpētot kokristāla struktūru, secinājām, ka aktīvajā centrā notikusi sulfokumarīna cikla atvēršanās, veidojot vinilsulfonskābi **51** (19. att.). Kumarīniem notiek analoga cikla atvēršanās, veidojot attiecīgos kanēļskābes atvasinājumus [3].

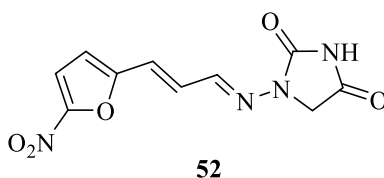


19. att. Sulfokumarīna cikla atvēršanās enzīma aktīvajā centrā.

Benzoksatiepīna-2,2-dioksīda-mērķenzīma kokristāla mums nav, tāpēc varam tikai izteikt minējumus par inhibēšanas mehānismu. Iespējams, ka oksatiepīna-2,2-dioksīda cikls enzīma aktīvajā centrā atveras līdzīgi kā sulfokumarīnu gadījumā.

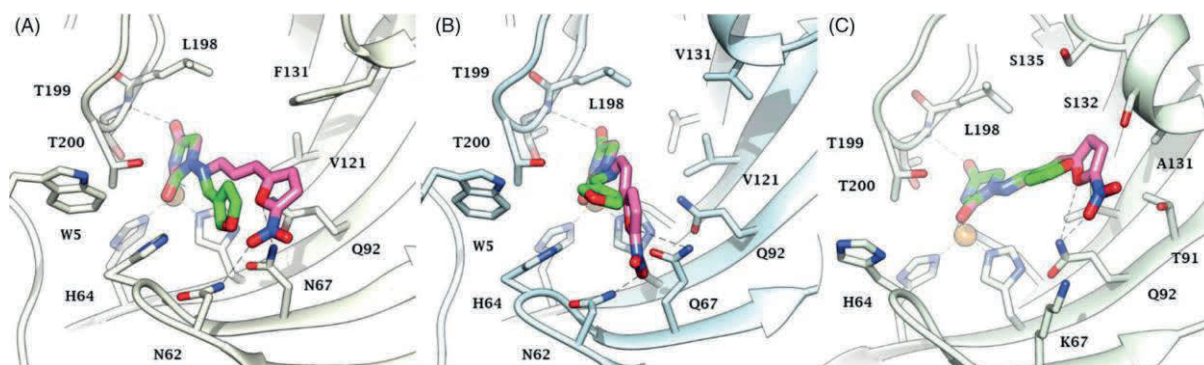
2. Imidazolidīn-2,4-diona atvasinājumu sintēze

Promocijas darba iztrādes beigu posmā atklājām, ka furagīns **52** (20. att.), klīnikā lietots antibakteriālais līdzeklis, ir selektīvs CA IX un CA XII inhibitors. Furagīns tiek pieskaitīts pie nitrofurāna preparātiem. Iekšķīgi to lieto pret urīnceļu infekcijām, ārīgi – ķirurģijā un ginekoloģijā [32].



20. att. Furagīna struktūrformula.

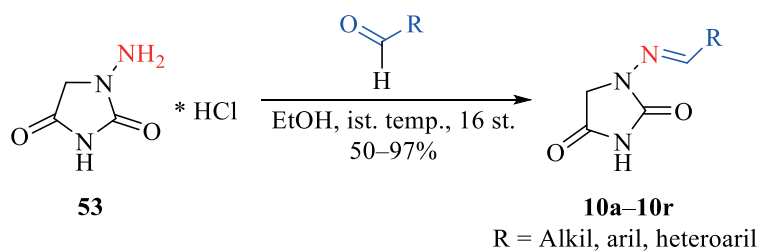
Lai gūtu priekšstatu par to, kā furagīns saistās ar dažādām cilvēka CA izoformām (CA II, CA IX un CA XII), mūsu sadarbības partneri no Florences Univesitātes veica molekulārās modelēšanas (21. att.) un molekulārās dinamikas simulāciju eksperimentus.



21. att. Paredzamās furagīna (rozā) un imidazolidīn-2,4-diona atvasinājuma **10f** (zaļā) orientācijas enzīmu (A) CA II, (B) CA IX un (C) CA XII aktīvajā centrā.

No molekulārās modelēšanas eksperimentiem var secināt, ka imidazolidīn-2,4-diona funkcija ir cinku saistošā grupa. Molekulārās dinamikas simulāciju eksperimenti 100 nanosekunžu robežās parāda, ka furagīns veido spēcīgu H-saites mijiedarbību ar mērķenzīmiem CA IX un CA XII. Savukārt ar CA II furagīns neveido spēcīgu H-saites mijiedarbību, tāpēc tas ir selektīvs CA IX un CA XII inhibitors.

Ņemot vērā iegūtos rezultātus, tika nolemts attīstīt šo virzienu, sintezējot virkni imidazolidīn-2,4-diona atvasinājumu **10a–10r** (22. att., 7. tab.). Savienojumus **10a–10r** veiksmīgi tika iegūti 1-aminoimidazolidīna-2,4-dinona hidrohlorīda **53** reakcijās ar izvēlētajiem aldehīdiem.



22. att. Imidazolidīn-2,4-diona atvasinājumu **10a–10r** sintēze.

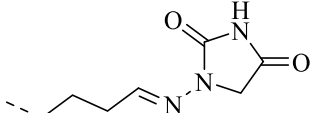
Kā redzams 7. tabulā, iegūta virkne savienojumu ar alkil- **10h** un **10i** (8.–9. rinda), aril- **10a–10c**, **10g** un **10j–10l** (1.–4., 7., 10.–12. rinda), heteroaril- **10f** un **10m–10p** (6. un 13.–16. rinda) aizvietotajiem. Neatkarīgi no aizvietotāja dabas reakcijas produkti tika iegūti ar labiem un ļoti labiem iznākumiem (50–97 %). Jāatzīmē, ka visus savienojumus **10a–10r** izdevās veiksmīgi attīrīt, izmantojot kristalizāciju no etanola, papildu attīrīšana ar kolonnu hromatogrāfiju nebija nepieciešama.

Visiem sintezētajiem imidazolidīn-2,4-diona atvasinājumiem **10a–10r** tika noteiktas bioloģiskās aktivitātes uz dažādām CA izoformām, iegūtie rezultāti apkopoti 7. tabulā. Visi imidazolidīn-2,4-diona atvasinājumi **10a–10r** ir vāji citosoliskās CA I inhibitori, $K_I = 16\ 800\text{--}100\ 000\ \text{nM}$. Tie labāk inhibēja CA II nekā CA I ($K_I = 620\text{--}59\ 000\ \text{nM}$). Savienojumi **10a**, **10f**, **10g** un **10n** (1., 6.–7. un 14. rinda), kas satur neaizvietotas fenil- vai heteroarilgrupas, izrādījās visspēcīgākie CA II inhibitori ($K_I = 540\text{--}900\ \text{nM}$). Atlikušie savienojumi uzrādīja zemu CA II inhibitoro aktivitāti – $K_I = 3100\text{--}59\ 000\ \text{nM}$. Jāatzīmē, ka savienojums **10k**

(11. rinda), kas satur dihidroksifenilgrupu, izrādījās gandrīz trīs reizes vājāks CA II inhibitors par otru vājāko inhibitoru **10h** (8. rinda).

7. tabula

Imidazolidīn-2,4-diona atvasinājumu **10a–10r** iznākumi, CA inhibēšanas rezultāti

N. p. k.	Produkta Nr., iznākums, %	R	K_I^* , nM			
			CA I	CA II	CA IX	CA XII
1.	10a , 90	C ₆ H ₅	39 600	900	3500	5600
2.	10b , 80	4-OCH ₃ -C ₆ H ₄	57 600	6400	1200	4700
3.	10c , 82	4-NO ₂ -C ₆ H ₄	>100 000	11 100	7400	2800
4.	10d , 95	4-(CO ₂ CH ₃)-C ₆ H ₄	>100 000	8300	4900	930
5.	10e , 50		19 100	4000	1100	160
6.	10f , 89	3-furil	16 800	710	850	1700
7.	10g , 90	4-(OCH ₂ C ₆ H ₅)-C ₆ H ₄	>100 000	540	350	910
8.	10h , 81	CHCH(CO ₂ C ₂ H ₅)	45 900	23 600	810	440
9.	10i , 72	CHC(CH ₃) ₂	28 800	16 500	2900	880
10.	10j , 71	CHCH(4-OCH ₃ -C ₆ H ₄)	>100 000	3100	400	360
11.	10k , 93	2,4-(OH) ₂ -C ₆ H ₃	>100 000	59 900	5800	150
12.	10l , 88	4-(B(OH) ₂)-C ₆ H ₄	90 700	14 200	7300	230
13.	10m , 95	2-piridil	51 800	4200	4500	1300
14.	10n , 90	3-piridil	45 600	620	2300	3200
15.	10o , 91	4-piridil	26 600	3300	1600	810
16.	10p , 97	5-imidazolil	9600	12 400	560	350
17.	Furagīns (52)*	–	>100 000	9600	260	57
18.	AAZ*	–	250	12	25	6

* 17. rindā parādīta furagīna **52** dažādu CA izoformu inhibēšanas spēja, 18. rindā parādīta acetazolamīda (AAZ) dažādu CA izoformu inhibēšanas spēja.

Savienojumi **10f–10h** (6.–8. rinda), **10j** (10. rinda), **10p** (16. rinda) un furagīns **50** (17. rinda) mērķenzīmu CA IX inhibēja nanomolārās koncentrācijās – $K_I = 260–850$ nM. Efektīvākais CA IX inhibitors no šiem savienojumiem izrādījās furagīns **50**. Atlikušie savienojumi uzrādīja par kārtu zemāku CA IX inhibitoro aktivitāti ($K_I = 1100–7300$ nM). Vērojama zināma likumsakarība, ka savienojumi, kas satur vinilgrupu **10h** (8. rinda), **10j** (10. rinda) vai mazu heteroarilaizvietotāju **10f** (6. rinda) un **10p** (16. rinda) ir labāki CA IX inhibitori nekā pārējie atvasinājumi. Izņēmums ir savienojums **10g** (7. rinda), kas fenilgredzenā satur ētera grupu.

Vislabāk no visām pārbaudītajām izoformām tika inhibēta CA XII, vislabākais inhibitors bija furagīns **52** (17. rinda), $K_I = 57$ nM. Par kārtu mazāka inhibitorā aktivitāte tika novērota savienojumiem **10d** un **10e** (4.–5. rinda), **10g–10l** (7.–12. rinda), **10o** un **10p** (14.–15. rinda), CA XII $K_I = 150–930$ nM.

SECINĀJUMI

1. Rutēnija katalizēta olefīnu cikla saslēgšanas metatēzes reakcija ir piemērota *3H*-1,2-benzoksatiepīna-2,2-dioksīda un tā atvasinājumu iegūšanai.
2. Olefīnu cikla saslēgšanas metatēzes reakcija, izmantojot gan rutēnija, gan molibdēna katalizatoru, nav piemērota 6-brom-*3H*-1,2-benzoksatiepīna-2,2-dioksīda sintēzei.
3. *3H*-1,2-Benzoksatiepīna-2,2-dioksīdi, kas 7. vietā satur triazolil-, acilamino- vai arilatvasinājumus, ir selektīvi un efektīvi hipoksijai pakļautajās šūnās ekspresēto CA IX un CA XII izoformu inhibitori.
4. 8-Aril *3H*-1,2-benzoksatiepīna-2,2-dioksīdi CA IX un CA XII izoformas inhibē visefektīvāk, salīdzinot ar atbilstošajiem 7- un 9-aril *3H*-1,2-benzoksatiepīna-2,2-dioksīdiem.
5. Furagīns un sintezētie imidazolidīn-2,4-diona atvasinājumi ir selektīvi un efektīvi hipoksijai pakļautajās šūnās ekspresēto CA IX un CA XII izoformu inhibitori.

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Paldies zinātniskajam vadītājam Raivim Žalubovskim par sniegtajām zinātniskajām konsultācijām visu šo gadu laikā!

Pateicība Florences Universitātes (Itālija) profesoram Klaudiu T. Supurānam (*Claudiu T. Supuran*) un viņa grupai par ogļskābes anhidrāžu izoformu inhibēšanas noteikšanu un *In Silico* pētījumiem.

Paldies Latvijas Organiskās sintēzes institūta Fizikāli organiskās ķīmijas laboratorijas kolektīvam par KMR, IS spektru uzņemšanu un AIMS veikšanu!

Aleksandrs Pustenko

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

To be granted the scientific degree of Doctor of Science (Ph. D.), the present Doctoral Thesis has been submitted for the defence at the open meeting of RTU Promotion Council on 30 September 2020 at the Faculty of Materials Science and Applied Chemistry of Riga Technical University, Riga, 3 Paula Valdena Street, Room 272.

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DECLARATION OF ACADEMIC INTEGRITY

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

Aleksandrs Pustenko (signature)

Date:

The Doctoral Thesis has been prepared as a thematically united collection of scientific publications. It consists of a summary and five scientific publications. Publications have been written in English, their total volume is 39 pages.

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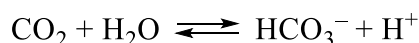
GENERAL OVERVIEW OF THE THESIS

Introduction

According to the World Health Organization, in 2018, 9.6 million deaths due to oncological diseases and 18.1 million new oncological cases were registered worldwide. One in 5 men and one in 6 women develop cancer in their lifetime [1]. In Europe, where ~9 % of the world's population lived in 2018, 23.4 % of all oncological diseases were registered, including 20.3 % of all deaths [1]. Therefore, it is very important to continuously develop and improve cancer treatment.

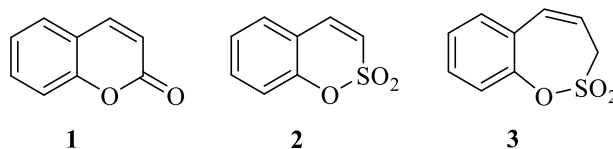
Today, more than 300 different enzymes are known for which zinc is an important cofactor. These enzymes perform various biologically important functions in human organism. Their activity is directly related to epigenetic control mechanisms in cells, the deregulation of which is one of the main causes of cancer [2].

In the last decade, increased attention has been paid to zinc-containing metalloenzymes carbonic anhydrases (CA, EC 4.2.1.1), which catalyze the reversible hydration of carbon dioxide in the living organisms.



From currently known 15 human α -carbonic anhydrase isoforms, CA IX and CA XII are thought to be over expressed in hypoxic cancer cells providing an optimal pH for their survival and development. To stop the development of cancer cells and avoid unwanted side effects, selective inhibition of CA IX and CA XII isoforms must be developed.

In the literature [3] it has been shown that coumarin **1** derivatives are selective CA IX and CA XII inhibitors. In our group, sulfocoumarin **2** derivatives were synthesized, which proved to be selective inhibitors of CA IX and CA XII [4]. Therefore, we decided to synthesize benzoxathiepine-2,2-dioxide **3** derivatives, which are sulfocoumarin derivatives with an extended cycle.

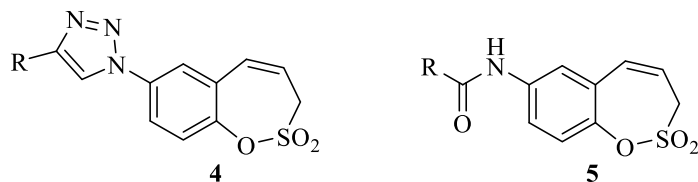


The aim of the dissertation

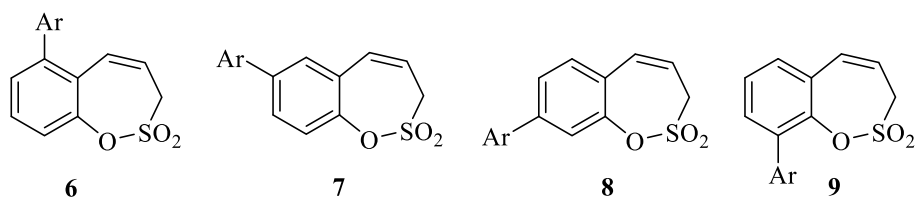
To develop new, effective and selective inhibitors of CA IX and CA XII, from which a new generation of anticancer agent could be developed in the future.

Objectives

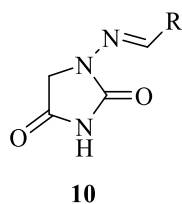
1. To develop synthesis method of 3*H*-1,2-benzoxathiepine 2,2-dioxide **3** derivative.
2. To synthesize 3*H*-1,2-benzoxathiepine 2,2-dioxide 7-triazolyl **4** and 7-acylamino **5** derivatives.



3. To synthesize 6-, 7-, 8- and 9-substituted 3*H*-1,2-benzoxathiepine 2,2-dioxide aryl derivatives **6–9**.



4. To synthesize 1-imidazolidine-2,4-dione derivatives **10**.



5. To evaluate inhibitory activities of synthesized compounds against hCA isoforms.

Scientific novelty and main results

A new, selective class of CA IX and CA XII inhibitors – 3*H*-1,2-benzoxathiepine 2,2-dioxides, has been found. A series of 3*H*-1,2-benzoxathiepine 2,2-dioxide triazolyl, acylamino and aryl derivatives was synthesized.

We discovered that furagin, a clinically used antibacterial agent, is a selective inhibitor of CA IX and CA XII. Developing this concept, we synthesized a series of imidazolidine-2,4-dione derivatives.

Inhibitory activity on relevant human CA isoforms (I, II, IX, and XII) was determined for all products synthesized within the scope of the Doctoral Thesis.

Structure of the Thesis

The Thesis is a summary of scientific publications focused on carbonic anhydrase inhibitor synthesis.

Publications and approbation of the Thesis

Main results of Thesis were summarized in four scientific publications and a review article. Total IF sum is 16.9. Results of the research were presented at six conferences.

Scientific publications

1. Pustenko, A., Nocentini, A., Gratteri, P., Bonardi, A., Vozny, I., Žalubovskis, R., Supuran, C. T. The antibiotic furagin and its derivatives are isoform-selective human carbonic anhydrase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2020**, *35*, 1011–1020.
2. Pustenko, A., Nocentini, A., Balašova, A., Krasavin, M., Žalubovskis, R., Supuran, C. T. 7-acylamino-3H-1,2-benzoxathiepine 2,2-dioxides as new isoform-selective carbonic anhydrase IX and XII inhibitors. *J. Enzyme Inhib. Med. Chem.* **2020**, *35*, 650–656.
3. Pustenko, A., Nocentini, A., Balašova, A., Alafeefy, A., Krasavin, M., Žalubovskis, R., Supuran, C. T. Aryl derivatives of 3H-1,2-benzoxathiepine 2,2-dioxide as carbonic anhydrase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2020**, *35*, 245–254.
4. Pustenko, A., Stepanovs, D., Žalubovskis, R., Vullo, D., Kazaks, A., Leitans, J., Tars, K., Supuran, C. T. 3H-1,2-benzoxathiepine 2,2-dioxides: a new class of isoform-selective carbonic anhydrase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2017**, *32*, 767–775.
5. Pustenko, A., Žalubovskis, R. Recent advances in sultone synthesis (microreview). *Chem. Heterocycl. Compd.* **2017**, *53*, 1283–1285.

Results of the research were presented at the following conferences

1. Žalubovskis, R., Grandāne, A., Ivanova, J., Balode, A., Pustenko, A., Domraceva, I., Tārs, K., Leitāns, J. Challenging design and synthesis of inhibitors of carbonic anhydrases. *International Conference on Organic Synthesis Balticum Organicum Syntheticum (BOS-2016)*. Riga, Latvia, July 3–6, **2016**.
2. Pustenko, A. Carbonic Anhydrases: Inhibitor Synthesis. *10th Paul Walden Symposium on Organic Chemistry*, Riga, Latvia, June 15–16, **2017**.
3. Žalubovskis, R., Ivanova, J., Pustenko, A., Grandane, A., Domraceva, I., Tars, K., Supuran, C. T. Inhibitors of Carbonic Anhydrases—challenges of design and synthesis. *3rd Satellite Meeting on Carbonic Anhydrase “New Trend in Carbonic Anhydrases Research”*, Montecatini Terme, Italy, May 24–27, **2017**.
4. Pustenko, A., Ivanova, J., Grandane, A., Vozny, I., Žalubovskis, R. Towards Novel Inhibitors of Cancer Associated Enzymes. *11th International Conference on Carbonic Anhydrases*, Bucharest, Romania, June 27–30, **2018**.
5. Pustenko, A., Balašova, A. Carbonic Anhydrases: Inhibitor Synthesis. *11th Paul Walden Symposium on Organic Chemistry*, Riga, Latvia, September 19–20, **2019**.
6. Pustenko, A., Balašova, A., Kapura, V., Žalubovskis, R. Inhibitors of cancer associated enzymes – design and synthesis. *4th Satellite Meeting on Carbonic Anhydrases*, Parma, Italy, November 14–17, **2019**.

MAIN RESULTS OF THE DOCTORAL THESIS

To better understand the results of this Doctoral Thesis, first, we will take a look at the target enzyme – carbonic anhydrases (CA). CAs are metalloenzymes which catalyze reversible carbon dioxide hydration. CAs were discovered in 1933 and since then they have been extensively studied. Today at least 8 genetic families of CA are known: α , β , γ , δ , ξ , η , θ , and ι [5], [6]. The α -CAs are the most widely studied class because it is found in mammals. The β -CAs are found in higher plants and in some prokaryotes. The γ -CAs are found in cyanobacteria and *Archaea*. The δ and ξ -CA are found only in marine diatoms, whereas the η -CAs in protozoa [7]. The α -, β -, and δ -CAs contain Zn(II) in the active site, the γ -CAs contain Fe(II) ions, ξ contain Co(II) ions and ι contain Mn(II) ions [5], [6]. In many organisms, the CAs are involved in vital physiological processes – pH regulation and providing of CO₂ homeostasis [7].

In humans, 15 α -CA isoforms have been described. CA I, II, III, VII, and XIII are found in cytosol, CA IV, IX, XII, and XIV are membrane bound, CA VA and VB are found in mitochondria, CA VI is found in saliva and breast milk [8], [9]. It should be noted, that all α -CA isoforms, except CA VB, have a known 3D structure. Regardless of the different subcellular localization, all α -CA isoforms are structurally similar, they are monomers, except for CA IX, CA XII, and CA VI – which are dimers [8].

The active site of α -CAs is located in a conical cavity that is approximately 12 Å wide and 13 Å deep. Zinc ion is placed at the bottom of the cavity and it is bound to ligands – 3 histidine residues (His119, His94, and His96), water molecule / hydroxide ion (Fig. 1) [7], [8].

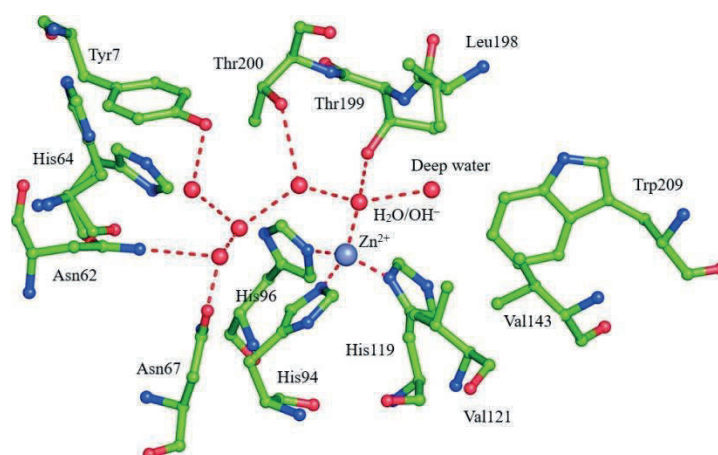


Fig. 1. Active site structure of human CAII [8].

Zn²⁺ ion with hydrogen bonds is bonded with threonine (Thr199) hydroxyl group and two opposite water molecules. The water molecule located in hydrophobic part is called “deep water” and is surrounded by Val121, Val143, Leu198, and Trp207. The second water molecule is located in hydrophilic part, in the entrance of active site, and is surrounded by Asn62, His64, and Asn67.

The hydrophobic and hydrophilic regions can be explained by the different chemical nature of the substrate (CO₂) and its hydration products (H⁺ and HCO₃⁻) [8]. McKenna with co-workers showed that the CO₂ molecule binds in the hydrophobic part of the enzyme, while the hydration products bind in the hydrophilic part of the enzyme [10].

It should be noted, that for all human CA isoforms crystallized so far, the zinc ion is bound to three histidine residues (His119, His94, and His96), and all of them have a hydrophobic and hydrophilic parts [7]. Inhibitors are designed and developed based on this knowledge about target enzyme.

Nowadays several α -CAs inhibition mechanisms are known. Sulphonamides (RSO₂NH₂), sulfamates (ROSO₂NH₂), sulfamides (RNHSO₂NH₂), carboxylates (RCO₂⁻), urates, and phosphonates (R'PO(OR)₂) bind to the zinc ion located in the active site and form additional H-bonds with Thr199. Phenols and polyamides coordinate with water molecule / hydroxide ion, which is bound to zinc. Coumarins and their isosteres block the active site entrance, so CA activators cannot bind to the enzyme [11], [12].

In general, 3*H*-1,2-benzoxathiepine 2,2-dioxide is a sultone. The term “sultone” was first used in 1888 by Endermann. Nowadays sultones are widely used in medicinal chemistry as enzyme inhibitors, they exhibit antiviral activity. The most powerful sultone synthesis methods include transition metal catalyzed reaction, cycloaddition reactions, and Diels-Alder type reactions [13].

In the process of development of the Doctoral Thesis, the latest information on transition metal-catalyzed sultone synthesis methods was summarized and published in a review article. Sultones can be synthesized using palladium, rhodium, copper, gold and ruthenium catalyzed reactions, we will take a closer look at some of these methods.

Doucet with co-workers reported palladium catalyzed, phosphine free sultone **11** synthesis method using 2-bromobenzenesulfonic acid phenyl esters **12** as a starting material (Fig. 2) [14].

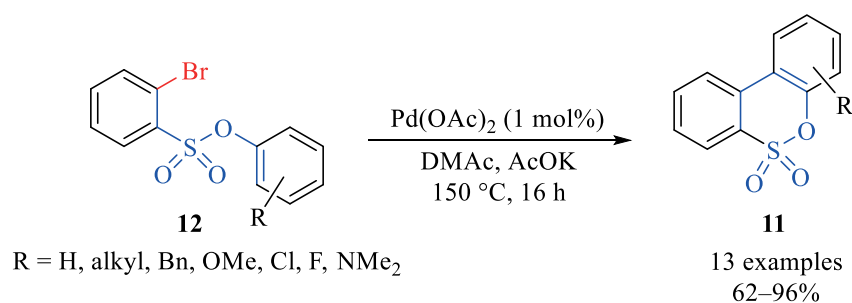


Fig. 2. Palladium catalyzed sultone **11** synthesis.

It should be noted, that substituent R has a strong influence on the reaction outcome. Using electron donor substituents, the yield of reaction products increases, while using electron acceptor substitutes (NO₂, CO₂Bu, CF₃) corresponding sultones do not form. In all cases when reaction occurred high product regioselectivity was observed.

Li with co-workers reported efficient Rh(III) catalyzed sultone **13** synthesis method by coupling aryl sulfonic acids **14** with internal alkynes (Fig. 3) [15].

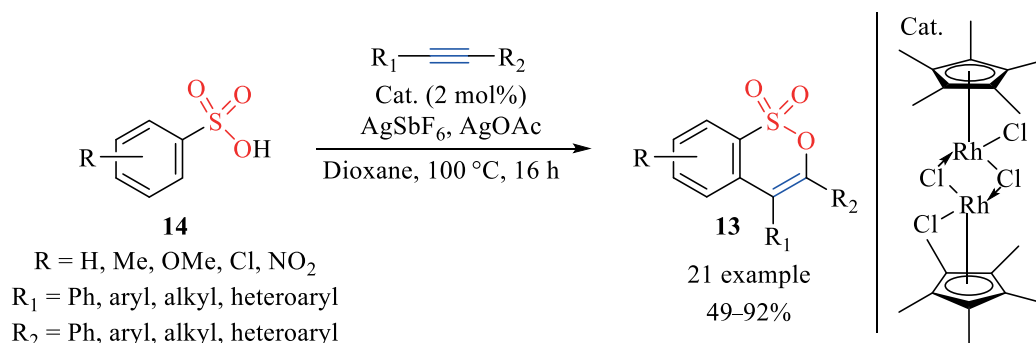


Fig. 3. Rhodium catalyzed sultone **13** synthesis.

Alkynes with both electron donor and electron acceptor substituents can be used in this method, but reaction product yield is higher using alkynes with electron donor substituents. In case of unsymmetrical alkynes, product regioselectivity is very high.

Mondal with co-workers reported convenient sultone **15** synthesis method by cyclization of corresponding diolefines **16** via Ru-catalyzed ring closing metathesis (RCM) (Fig. 4) [16]. Using Grubbs 1st generation catalyst, no sultone **15** formation was observed. Changing catalyst to Grubbs 2nd generation catalyst, sultones **15** were synthesized in good yields.

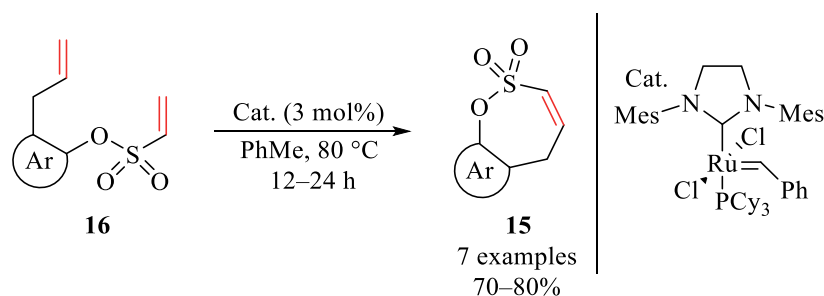


Fig. 4. Ruthenium catalyzed sultone **15** synthesis.

After summarizing the information available in the literature, we decided to synthesize benzoxathiepine 2,2-dioxide **3** derivatives in a ruthenium catalyzed olefin ring closing metathesis reaction.

1. Synthesis of 3*H*-1,2-benzoxathiepine 2,2-dioxide derivatives

We started our research with the development of synthesis method. At first, in Wittig reaction from 5-substitued 2-hydroxybenzaldehydes **17** we prepared corresponding olefins **18** (Fig. 5). Olefins **18a–18c** were sulfonylated with sulfonyl chloride **19** to give diolefins **20** in moderate yields (56–67 %). Sulfonyl chloride **19**, although commercially available, is an expensive reagent. Therefore it was successfully synthesized by boiling allyl bromide with Na₂SO₃, then obtained sodium salt was treated with POCl₃. It should be noted, that sulfonyl chloride **19** is air sensitive, therefore, we obtained it in larger quantities and purified by vacuum distillation. Using non-distilled sulfonyl chloride **19**, the yield of reaction products was significantly reduced. Cyclization of diolefin **20** using olefin ring closing metathesis reaction was chosen as the key step in synthesis of benzoxathiepine-2,2-dioxide. Cyclization

was successfully performed using a commercially available Grubbs 2nd generation catalyst derivative **21**. We obtained corresponding 7-substituted 3*H*-1,2-benzoxathiepine 2,2-dioxides **3** in good yields (84–96 %).

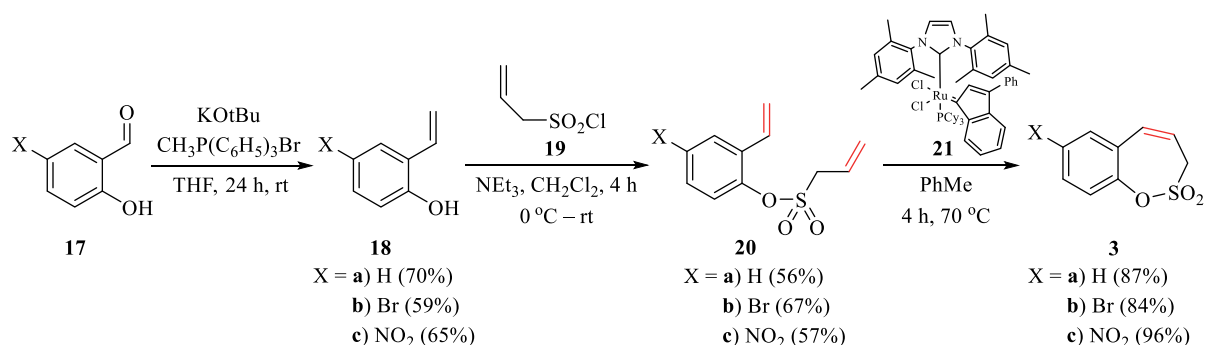


Fig. 5. Synthesis of 3*H*-1,2-benzoxathiepine 2,2-dioxide derivatives **3a–3c**.

7-Nitro-3*H*-1,2-benzoxathiepine 2,2-dioxide **3c** mono crystal, which was of sufficient quality for structure determination using single crystal X-ray diffraction was obtained. In the laboratory of Physical Organic Chemistry of the Latvian Institute of Organic Synthesis, an X-ray pattern was obtained. X-ray pattern is an unequivocal proof of the structure of compound **3c** (Fig. 6).

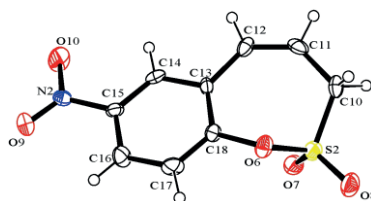


Fig. 6. 7-Nitro-3*H*-1,2-benzoxathiepine 2,2-dioxide **3c** X-ray structure.

It should be noted, that nowadays a lot of different olefin ring closing metathesis reaction catalysts are known. Mostly ruthenium and molybdenum catalysts are used. In 1990s, first generation catalysts were developed and commercialized. The best known ones are Grubbs 1st generation catalyst **22** and Schrock's catalyst **23** (Fig. 7). Unfortunately, 1st generation catalysts do not exceed high functional group tolerance and selectivity; they are air and moisture sensitive [17]. In August 1999, Grubbs published a paper in which he described new, more efficient ruthenium olefin ring closing metathesis reaction catalysts [18]. Today they are known as Grubbs 2nd generation catalysts. 2nd generation ruthenium catalysts are more efficient, they have increased thermal stability, catalytic activity, air and moisture resistance. That was achieved by replacing the tricyclohexylphosphine ligand with an N-heterocyclic carbene (NHCs) ligand [17], [19]. The best known of these catalysts is Grubbs 2nd generation catalyst **24** (Fig. 7). Continuing the development of molybdenum catalysts, Schrock–Hoveyda catalyst **25** (Fig. 7) was developed. It has a higher functional group tolerance and selectivity than the Schrock's catalyst **23**. In general molybdenum catalysts tolerate amines and phosphines, but do not tolerate substrates with carboxyl, hydroxy, and

aldehyde groups. Ruthenium catalysts, on the other hand, do not tolerate amines and phosphines, but tolerate substrates with carboxyl, hydroxy, and aldehyde groups [17].

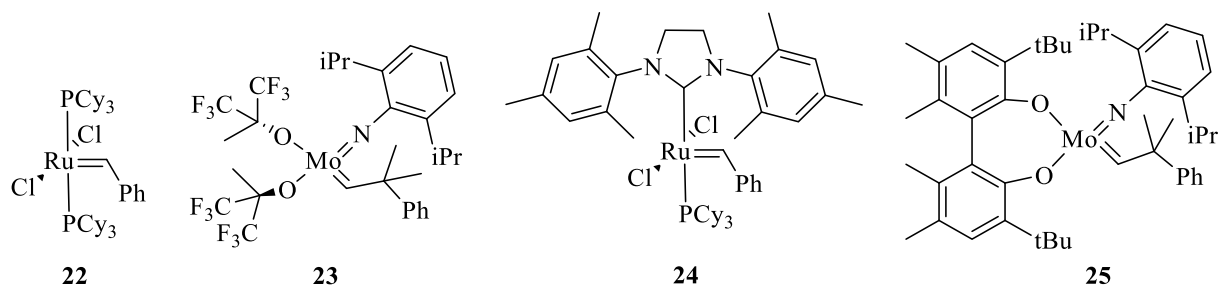


Fig. 7. Some olefin ring closing metathesis reaction catalysts.

Since 2nd generation ruthenium catalysts are thermally stable, with good functional group tolerance, air and moisture resistance, we decided to use a commercially available 2nd generation catalyst derivative **21**.

1.1. Synthesis of 3*H*-1,2-benzoxathiepine 2,2-dioxide 1,2,3-triazolyl derivatives

To better understand structure–activity relationship (SAR) we decided to synthesize 1,4-disubstituted benzoxathiepine-2,2-dioxide 1,2,3-triazolyl derivatives **4**. Michael, in 1893, published the first ever synthesis of 1,2,3-triazoles from diethyl acetylenedicarboxylate and phenyl azide [20]. Despite this, the synthesis of 1,2,3-triazoles is more related with Huisgen. In the 1960s, he worked on 1,3-dipolar cycloaddition reactions, including the synthesis of 1,2,3-triazoles, studied the reaction mechanism and kinetics [21]. Since then, it has been known that the reaction of azides **26** with alkynes **27** at high temperature forms a mixture of regioisomers – 1,4- **28** and 1,5-disubstituted **29** 1,2,3-triazoles (Fig. 8).

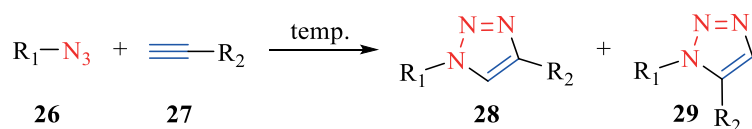


Fig. 8. Synthesis of 1,2,3-triazoles.

Nowadays, there are methods for selective synthesis of 1,4- or 1,5-disubstituted 1,2,3-triazolyl derivatives. In 2002, Mendal with co-workers [22] and Sharpless with co-workers [23] independently of each other published papers describing Cu (I) catalyzed azide-alkyne cycloaddition to selectively form 1,4-disubstituted 1,2,3-triazole derivatives. It should be noted, that in the protocol developed by Sharpless [23] CuSO₄ was used, which was reduced *in situ* with sodium ascorbate to selectively form Cu(I) instead of Cu(0). Selective formation of 1,5-disubstituted 1,2,3-triazolyl derivatives can be achieved using various ruthenium catalysts [21], [24].

Continuing the work, we successfully reduced nitro derivative **3c** with Fe(0), obtaining the amino derivative **30** (Fig. 9). From amino derivative **30** in decent yield (69 %) we synthesized azide derivative **31** (Fig. 9.), which was further used as a starting material in the synthesis of 1,4-disubstituted 1,2,3-triazolyl derivatives. Treatment of amino derivative **30** with NaNO₂ in

acid medium *in situ* produces diazonium salt, which then reacts with NaN₃ to form azide **31**. It should be noted, that from NaN₃ in acid medium HN₃ (hydrazoic acid) forms, which is a volatile, toxic compound. Therefore, the reaction must be carried out at 0 °C temperature.

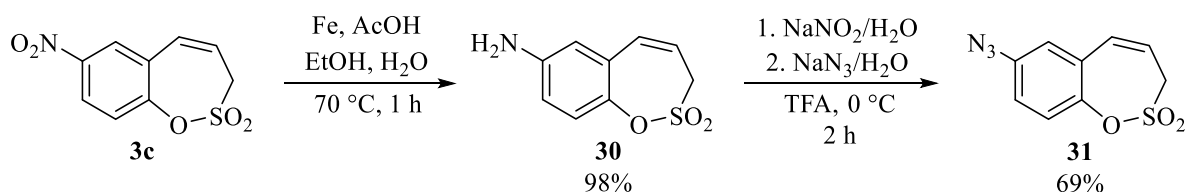


Fig. 9. Synthesis of 3*H*-1,2-benzoxathiepine 2,2-dioxide azido derivative **31**.

For selective synthesis of 1,4-disubstituted 1,2,3-triazolyl derivatives we chose to use a Cu(I) catalyzed reaction between azide **31** and various alkynes (Fig. 10). Cu(I) was obtained from CuSO₄ by *in situ* reduction with sodium ascorbate, similar like in Scharpless article [23]. As a solvent, we chose to use a 1 : 1 mixture of *t*-BuOH/H₂O. With good yields we synthesized a series of 1,4-disubstituted 1,2,3-triazole derivatives **4a–4j**. It should be noted, that we decided to use acetic acid additive. It has been proven, that weak organic acid additive (acetic, benzoic acid) facilitates the elimination of copper after 1,3-dipolar cycloaddition, thus increasing the reaction rate [25].

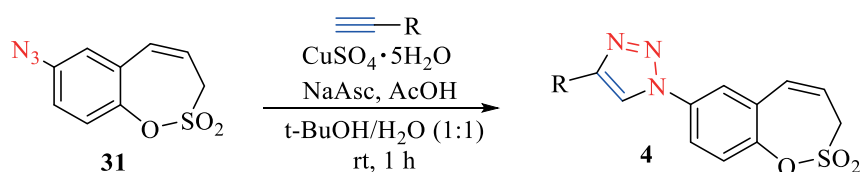


Fig. 10. Synthesis of 1,4-disubstituted 1,2,3-triazole derivatives **4**.

Table 1

Synthesis of benzoxathiepine-2,2-dioxide 1,2,3-triazolyl derivatives **4**, CA inhibition results

Entry	R	4 , yield, %	K_I^* , μM			
			hCA I	hCA II	hCA IX	hCA XII
1	C ₆ H ₅	4a , 95	>50	>50	1.71	>50
2	4-ClC ₆ H ₄	4b , 74	>50	>50	3.59	>50
3	3-OMeC ₆ H ₄	4c , 51	>50	>50	2.56	>50
4	4-FC ₆ H ₄	4d , 66	>50	>50	1.75	>50
5	4-OCF ₃ C ₆ H ₄	4e , 83	>50	5.77	0.34	1.72
6	3-FC ₆ H ₄	4f , 74	>50	>50	1.15	>50
7	2-NH ₂ C ₆ H ₄	4g , 57	>50	>50	0.46	2.32
8	CH ₂ OH	4i , 81	>50	>50	0.87	>50
9	4-CF ₃ C ₆ H ₄	4j , 85	>50	>50	0.43	>50
10	AAZ*	–	0.25	0.012	0.025	0.006

* Different CA isoform inhibition of acetazolamide (AAZ, Entry 10).

For the compounds synthesized within the scope of the Doctoral Thesis, inhibitory activities of human carbonic anhydrases (CA I, CA II, CA IX, and CA XII) were determined at University of Florence in Prof. C. T. Supuran's group. In all cases, 5-acetamido-1,3,4-thiadiazole-2-sulfonamide (acetazolamide, AAZ), a non-selective isoform inhibitor, was used as a reference standard.

It should be noted, that inhibition of the cytosolic CA isoforms CA I and CA II is undesirable because they are widespread in the human body (off-target enzymes). On the other hand, transmembrane isoforms (CA IX and CA XII) are drug targets, because they are overexpressed in tumour cells.

As shown in Table 1, all synthesized triazole derivatives do not inhibit cytosolic CA I and CA II, except compound **4e**, which showed a moderate inhibitory profile against CA II ($K_I = 5.77 \mu\text{M}$, Entry 5). Tumour associated isoform CA IX was inhibited by all triazole derivatives **4a–4j**, with K_{IS} ranging between $0.43 \mu\text{M}$ and $3.59 \mu\text{M}$. Four compounds **4e**, **4g–4j** showed submicromolar CA IX inhibitory activity. Compound **4i** contains a hydroxymethyl group in the triazolyl ring, its CA IX inhibition constant is $K_I = 0.87 \mu\text{M}$ (Entry 8). For phenyl group containing triazoles **4e**, **4g**, and **4j** substituted with 4-trifluoromethoxy-, 2-amino-, or 4-trifluoromethyl substituents on the aryl fragment K_{IS} are $0.34 \mu\text{M}$; $0.46 \mu\text{M}$ and $0.43 \mu\text{M}$ (Entry 5, 7, 9).

Seven out of nine triazole derivatives do not inhibit CA XII, $K_I > 50 \mu\text{M}$ (Entry 1–4, 6, 8–9). Remaining two compounds **4e** and **4g** are moderate CA XII inhibitors – $K_I = 1.72$ and $2.32 \mu\text{M}$ (Entry 5, 7). Based on biological results, we can conclude that 1,4-disubstituted 1,2,3-triazole derivatives **4a–4j** are selective CA IX inhibitors.

1.2. Synthesis of 7-acylamino-3H-1,2-benzoxathiepine 2,2-dioxides

Continuing development of the work, we decided to synthesize 7-acylamino-3H-1,2-benzoxathiepine 2,2-dioxide derivatives **5** to better understand SAR. 7-Acylamino derivatives were successfully synthesized from amino derivative **30** in reactions with various acyl chlorides (Fig. 11, Table 2).

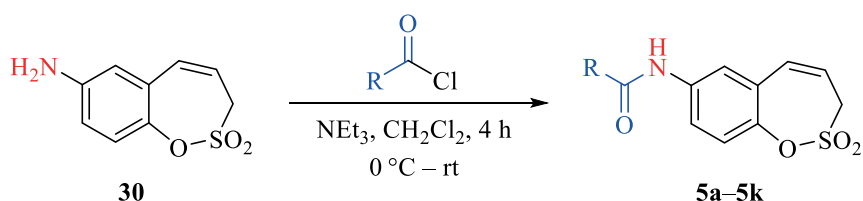


Fig. 11. 7-Acylamino-3H-1,2-benzoxathiepine 2,2-dioxide **5** synthesis.

As shown in Table 2, all synthesized products **5a–5g** do not inhibit cytosolic (off-target) CA I and CA II ($K_I > 100\,000 \text{ nM}$). On the other hand, they inhibit target enzymes CA IX and CA XII in nanomolar concentrations. Derivative **5i** is the most active CA IX and CA XII inhibitor – $K_I = 19.7 \text{ nM}$ and 8.7 nM (Entry 8), it is even more active than AAZ (Entry 11).

Table 2

Synthesis of 7-acylamino benzoxathiepine 2,2-dioxide derivatives **5**, CA inhibition results

Entry	R	Product No., yield, %	K_I^* , nM			
			hCA I	hCA II	hCA IX	hCA XII
1	CH ₃	5a , 70	>100 000	>100 000	61.8	162.5
2	C ₆ H ₅	5b , 72	>100 000	>100 000	208.6	370.1
3	4-CH ₃ C ₆ H ₄	5c , 73	>100 000	>100 000	83	309.3
4	4-BrC ₆ H ₄	5d , 59	>100 000	>100 000	353.3	140.7
5	2-IC ₆ H ₄	5e , 88	>100 000	>100 000	45.4	643.7
6	2-BrC ₆ H ₄	5f , 82	>100 000	>100 000	66.8	96.2
7	2-FC ₆ H ₄	5g , 79	>100 000	>100 000	74.6	40.3
8	2-CF ₃ C ₆ H ₄	5i , 87	>100 000	>100 000	19.7	8.7
9	2-thienyl	5j , 81	>100 000	>100 000	177.5	73.2
10	2-furyl	5k , 81	>100 000	>100 000	210.1	134.4
11	AAZ*	–	250	12	25	5.7

* Different CA isoform inhibition of acetazolamide (AAZ, Entry 10).

Compounds **5a–5g** and **5j–5k** exhibit weaker biological activity on CA IX and CA XII than AAZ (Entry 11), their inhibition constants are 45.4–353.3 and 40.3–643.7 nM (Entry 1–7, 9, 10). Compound **5d**, which contains 4-bromophenyl substituent, is less active CA IX inhibitor (CA IX K_I = 353.3 nM, Entry 4) than compound **5f**, which contains 2-bromophenyl substituent (CA IX K_I = 66.8 nM, Entry 6). In general, compounds containing substituents in second position of phenyl ring are more active than the compounds containing substituent in fourth position of phenyl ring. If unsubstituted phenyl group (**5b**, Entry 2) is replaced by methyl group (**5a**, Entry 1), increases inhibitory activity on target enzymes. If unsubstituted phenyl group (**5b**, Entry 2) is replaced by five membered heterocycle **5j** (Entry 9) and **5g** (Entry 10), the target enzyme inhibitory activity does not change significantly.

1.3. Synthesis of 3H-1,2-benzoxathiepine 2,2-dioxide aryl derivatives

To gain a better understanding about SAR and expand the range of compounds, we decided to synthesize benzoxathiepine-2,2-dioxide aryl derivatives **6–9** (Fig. 12).

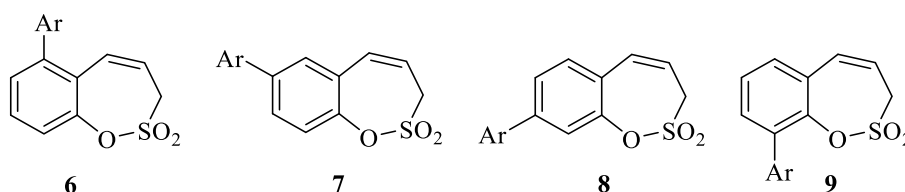


Fig. 12. General structures of benzoxathiepine-2,2-dioxide aryl derivatives **6–9**.

We decided to synthesize benzoxathiepine-2,2-dioxide aryl derivatives by palladium catalyzed Suzuki–Miyaura cross-coupling reaction, from corresponding benzoxathiepine-2,2-dioxide halogen derivatives and aryl boronic acids.

In general, palladium and nickel catalysts are mainly used in Suzuki–Miyaura cross-coupling reaction. In the case of palladium catalysts, the most reactive are aryl iodides, followed by triflates and bromides. The use of aryl chlorides significantly reduces the reaction product yield. Oxidative addition (the first stage of the catalytic cycle) in most cases is the limiting step, its speed decreases in order $I \gg OTf \approx Br \gg Cl$ [26]. The most commonly used palladium catalysts in the Suzuki–Miyaura cross-coupling reaction are palladium catalysts with phosphine ligands: $Pd(PPh_3)_4$, $Pd(dppf)Cl_2$, $Pd(PPh_3)_2Cl_2$, they are thermally stable and commercially available [26], [27].

The development of nickel catalysts has contributed to the use of less reactive electrophiles such as aryl chlorides, fluorides, esters, nitriles and aryl amides Suzuki–Miyaura cross-coupling reaction [28]. However, despite these advantages, palladium catalysis is more widely used in practical synthesis than nickel catalysis. Generally, nickel catalyzed Suzuki–Miyaura cross-coupling reactions require high catalyst loading (3–10 mol %), and they are sensitive to reaction conditions. The choice of base and solvent is very crucial. Mostly THF, dioxane or toluene is used in combination with a poorly soluble inorganic base such as K_3PO_4 or K_2CO_3 [28]. Alkali hydroxides [29], water addition [30] deactivates nickel catalysts and the reaction product yield decreases.

In palladium catalyzed Suzuki–Miyaura cross-coupling reaction both – inorganic and organic bases such as Na/K_3PO_4 , $Na/Cs/K_2CO_3$, Na/KOH , $Na/KOt-Bu$, $NaOEt$, $NaOMe$ can be used. It is important to note that the role of base is crucial, cross-coupling reaction will not proceed without the presence of a base. Mainly organic solvent is used in mixture with water. Commonly used organic solvents are dioxane, THF, DMF, and toluene [31].

Weak nucleophilic nature and stability of organoboron compounds contributed to the development of cross-coupling reaction. Organoboron compounds are commercially available, several methods have been developed to synthesize them. Cross-coupling can be done with good chemical, regio- and stereoselectivity.

We started target compound **6–9** synthesis with preparation of starting materials. In palladium catalyzed Suzuki–Miyaura cross-coupling reaction iodides react more actively than bromides, therefore we decided to synthesize 7-iodo derivative **32**. It should be noted, that iodosalicylaldehyde **33**, although commercially available, is an expensive reagent. It was successfully synthesized from salicylaldehyde **34** treating it with iodine monochloride in acid medium (Fig. 13). Next, we performed Wittig reaction on iodosalicylaldehyde **33** to yield olefin **35**. Olefin **35** was successfully sulfonylated with sulfonyl chloride **19** to give diolefin **36**. Diolefin **36** was successfully cyclised using ruthenium olefin ring-closing metathesis catalyst **21** to give 7-iodo derivative **32** in good yield.

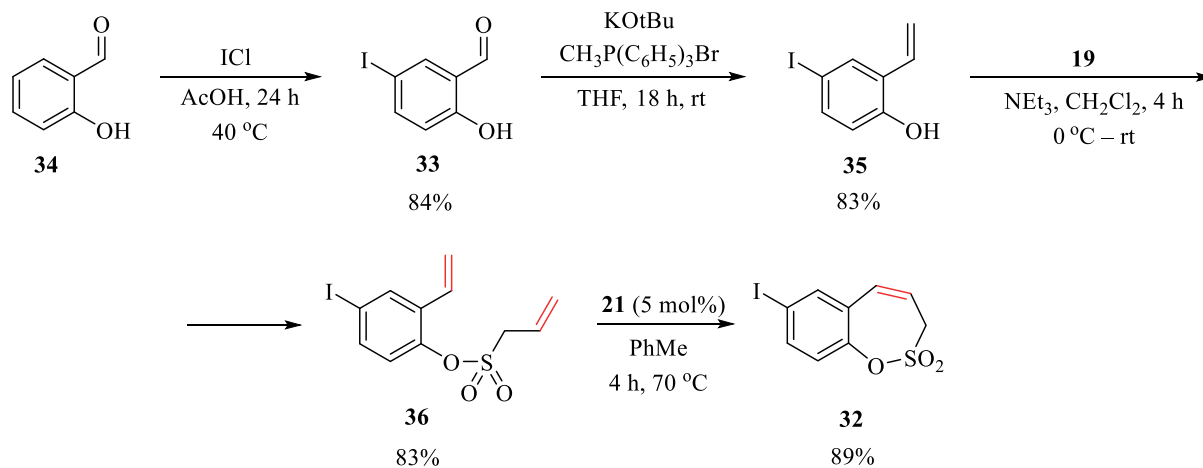


Fig. 13. Synthesis of 7-iodo-3*H*-1,2-benzoxathiepine 2,2-dioxide **32**.

As 3-, 4-, and 6-bromosalicylaldehydes are commercially available and there is no convenient synthesis method for preparation of corresponding iodosalicylaldehydes, we decided to synthesize bromo derivatives **37–39** (Fig. 14–16).

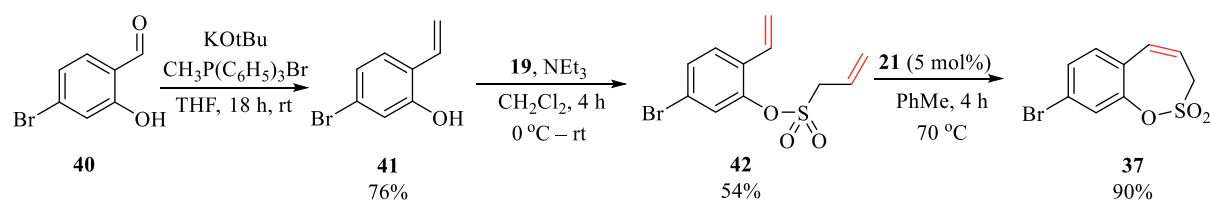


Fig. 14. Synthesis of 8-bromo-3*H*-1,2-benzoxathiepine 2,2-dioxide **37**.

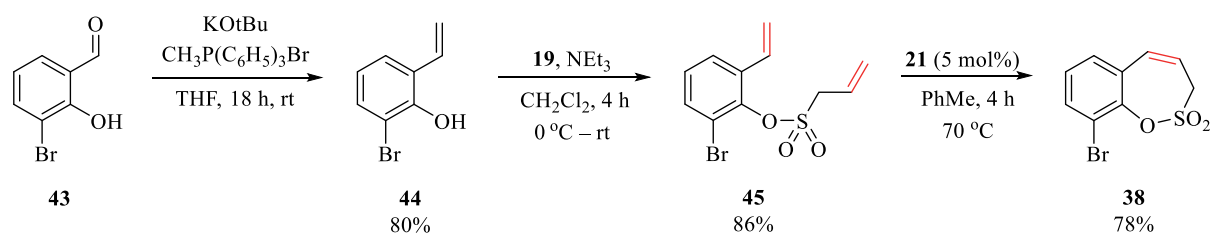


Fig. 15. Synthesis of 9-bromo-3*H*-1,2-benzoxathiepine 2,2-dioxide **38**.

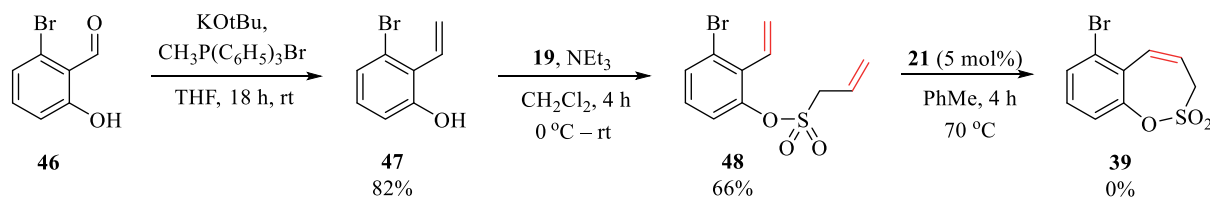


Fig. 16. Synthesis of 6-bromo-3*H*-1,2-benzoxathiepine 2,2-dioxide **39**.

Bromo derivatives **37** and **38** were obtained similarly to iodine derivative **32**. At first we performed Wittig reaction on the corresponding salicylaldehyde to obtain corresponding olefin. The olefin was sulfonylated with sulfonyl chloride **19** to give the corresponding diolefin, which was successfully cyclized using ruthenium ring-closing metathesis catalyst **21**. Unfortunately using the previously developed cyclization conditions, compound **39** was not obtained. We performed a small optimization of the reaction conditions (Fig. 17 and Table 3).

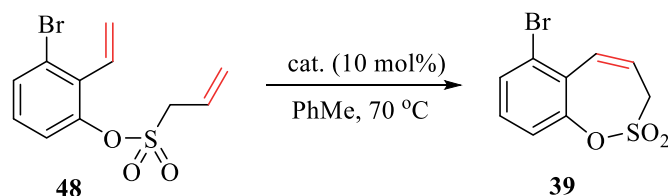


Fig. 17. Optimization of diolefin **48** ring-closing metathesis reaction conditions.

Table 3

Optimization Results of Diolefin **48** Ring-Closing Metathesis Reaction Conditions

Entry	Catalyst	Time, h	Yield, %
1	21	40	–
2	23	16	–
3	25	16	–

Using previously used catalyst **21**, increasing reaction time and twice increasing the amount of catalyst, no product formation was observed (*Entry 1*). We decided to try Schrock's molybdenum catalysts **23** (*Entry 2*) and **25** (*Entry 3*), because in general molybdenum catalysts are more active than ruthenium catalysts. Unfortunately, even by changing the catalyst the desired product **39** was not forming. Most likely it is due to steric factors.

Benzoxathiepine 2,2-dioxide aryl derivatives **7–9** were successfully synthesized in a palladium catalyzed Suzuki–Miyaura cross-coupling reaction using palladium tetrakis ($\text{Pd}(\text{PPh}_3)_4$) as a catalyst (Fig. 18, Tables 4–6). The reaction required heating and addition of water. Without water additive yield of reaction products decreased significantly.

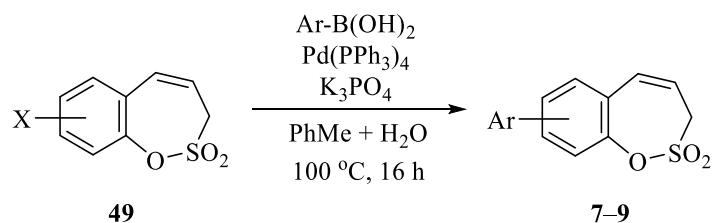
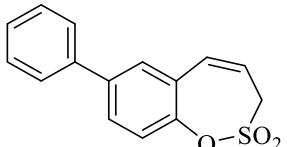
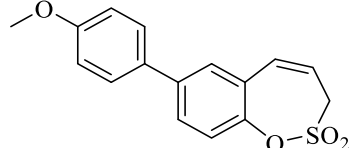
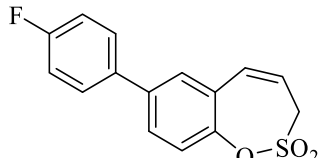
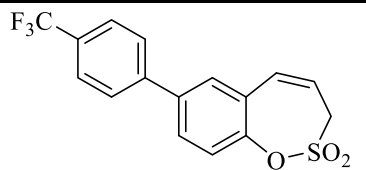
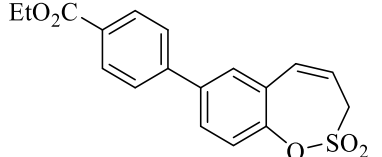


Fig. 18. Optimized Suzuki–Miyaura cross-coupling reaction conditions.

Table 4

Synthesis of 7-aryl-3*H*-1,2-benzoxathiepine 2,2-dioxide derivatives **7**, CA inhibition results

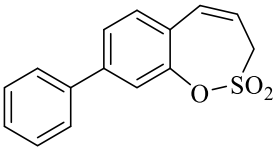
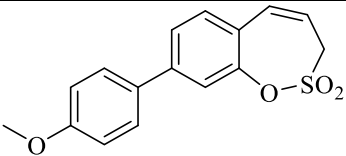
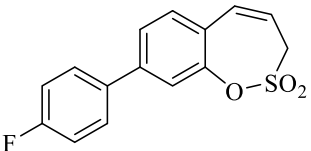
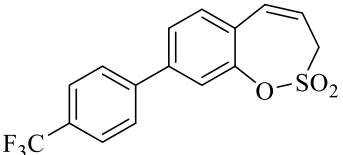
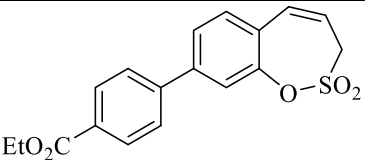
Entry	Product No., yield, %	Product	K_I^* , nM	
			hCA IX	hCA XII
1	7a , 56		654.8	1376
2	7b , 61		407.6	2934
3	7c , 44		330.8	890.5
4	7d , 66		221.4	4017
5	7e , 44		620.8	2398

* For compounds **7a–7e** hCA I and hCA II $K_I > 100 \mu\text{M}$. AAZ used as a standard CA inhibitor, its hCAI $K_I = 0.25 \text{ nM}$, hCAII $K_I = 0.012 \text{ nM}$, CA IX $K_I = 25 \text{ nM}$, and CA XII $K_I = 5.7 \text{ nM}$.

As shown in Table 4, 7-aryl substituted benzoxathiepine 2,2-dioxide derivatives **7a–7c** were obtained in good (*Entry* 1, 2 and 4) and moderate yields (*Entry* 3 and 5). 7-aryl derivatives **7a–7c** do not inhibit cytosolic (off-target) CA I and CA II. On the other hand, 7-aryl derivatives **7a–7c** inhibit target enzymes CA IX ($K_I = 221.4\text{--}654.8 \text{ nM}$) and CA XII ($K_I = 890.5\text{--}4017 \text{ nM}$), they are more effective CA IX than CA XII inhibitors.

Table 5

Synthesis of 8-aryl-3*H*-1,2- benzoxathiepine 2,2-dioxide derivatives **8**, CA inhibition results

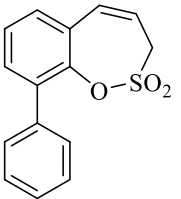
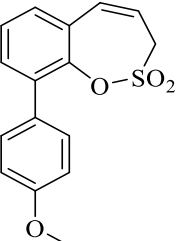
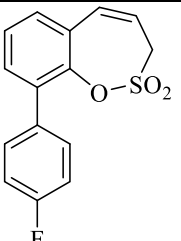
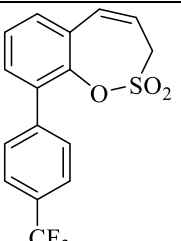
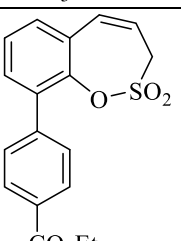
Entry	Product No., yield, %	Product	K_I^* , nM	
			hCA IX	hCA XII
1	8a , 44		104.8	473.2
2	8b , 44		63.1	168.6
3	8c , 41		95.2	77.9
4	8d , 46		44.0	247.8
5	8e , 38		79.8	289.3

* For compounds **8a–8e** hCA I and hCA II $K_I > 100 \mu\text{M}$. AAZ used as a standard CA inhibitor, its hCAI $K_I = 0.25 \text{ nM}$, hCAII $K_I = 0.012 \text{ nM}$, CA IX $K_I = 25 \text{ nM}$, and CA XII $K_I = 5.7 \text{ nM}$.

As shown in Table 5, 8-aryl substituted benzoxathiepine 2,2-dioxide derivatives **8a–8c** were obtained in moderate yields. 8-aryl derivatives **8a–8c** do not inhibit cytosolic (off-target) CA I and CA II. However, 8-aryl derivatives **8a–8c** inhibit target enzymes CA IX ($K_I = 44.0\text{--}104.8 \text{ nM}$) and CA XII ($K_I = 77.9\text{--}473.2 \text{ nM}$).

Table 6

Synthesis of 9-aryl-3*H*-1,2-benzoxathiepine 2,2-dioxide derivatives **9**, CA inhibition results

Entry	Product No., yield, %	Product	K_1^* , μM	
			hCA IX	hCA IX
1	9a , 42		21.1	>100
2	9b , 40		60.9	>100
3	9c , 39		33.7	>100
4	9d , 44		47.1	>100
5	9e , 36		16.4	>100

* For compounds **9a–9e** hCA I and hCA II $K_1 > 100 \mu\text{M}$. AAZ used as a standard CA inhibitor, its hCAI $K_1 = 0.25 \text{ nM}$, hCAII $K_1 = 0.012 \text{ nM}$, CA IX $K_1 = 25 \text{ nM}$, and CA XII $K_1 = 5.7 \text{ nM}$.

As shown in Table 6, 9-aryl substituted benzoxathiepine 2,2-dioxide derivatives **9a–9c** were obtained in moderate yields. 9-aryl derivatives **9a–9c** do not inhibit cytosolic (off-target) CA I, CA II, and cancer associated (target) isoform CA XII. They poorly inhibit other cancer associated isoform CA IX ($K_1 = 16.4\text{--}60.9 \mu\text{M}$).

It should be noted, that no effect of aryl boronic acid substituent was observed on Suzuki–Miyaura cross-coupling reaction. In all cases, products **7–9** were obtained in similar yields.

Comparing 7-aryl (Table 4), 8-aryl (Table 5), and 9-aryl (Table 6) benzoxathiepine 2,2-dioxide biological activities, we conclude that the most active cancer associated isoform (CA IX and CA XII) inhibitors are 8-aryl derivatives **8a–8e** (Table 5), followed by 7-aryl derivatives **7a–7e** (Table 4). 9-aryl derivatives **9a–9e** exhibit very weak inhibitory activity on CA IX and they do not inhibit CA XII.

7-aryl derivatives **7a–7e** (Table 4) are more selective CA IX inhibitors than 8-aryl derivatives **8a–8e** (Table 5), but best inhibitory activity on CA IX was observed for compound **8d** (Table 5, *Entry 4*), the best CA XII inhibitor was compound **8c** (Table 4, *Entry 3*).

In order to understand the interaction of benzoxathiepine 2,2-dioxide with CA, Professor K. Tars' group at Latvian Biomedical Research and Study Centre performed co-crystallization experiments of benzoxathiepine 2,2-dioxide and human CA IX. Unfortunately, the enzyme-inhibitor cocrystal has not been obtained so far. It should be noted, that the sulfocoumarin derivative **50** previously developed and synthesized in our group was successfully cocrystallized with CA II / CA IX mimetic under the supervision of K. Tars [4a].

Examining the structure of the cocrystal, we concluded that the sulfocoumarin ring has opened in the active site of enzyme forming vinylsulfonic acid **51** (Fig. 19). Coumarins undergo an analogous ring opening forming corresponding cinnamic acid derivatives [3].

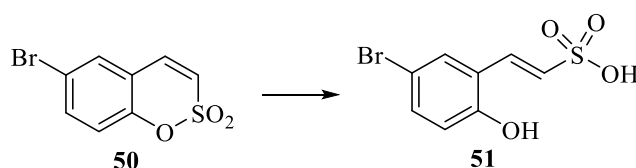


Fig. 19. Sulfocoumarin ring opening in CA II / CA IX mimic active site.

Since we do not have a benzoxathiepine 2,2-dioxide-target enzyme cocrystal, we can only guess the mechanism of inhibition. It is possible that oxathiepine 2,2-dioxide ring opens in the active site of the enzyme in a similar way to sulfocoumarins.

2. Synthesis of imidazolidine-2,4-dione derivative

In the final stage of the Doctoral Thesis, we discovered that furagin **52** (Fig. 20), an antibacterial drug used in clinics, is a selective inhibitor of tumour associated CA IX and CA XII. Furagin, nitrofurantoin analog, is used in the therapy of urinary tract infections [32].

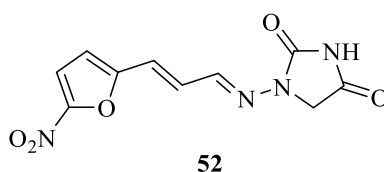


Fig. 20. Furagin structure.

To better understand how furagin binds to different CA isoforms (CA II, CA IX, and CA XII), our collaboration partners from the University of Florence performed molecular modelling (Fig. 21) and molecular dynamics simulation experiments.

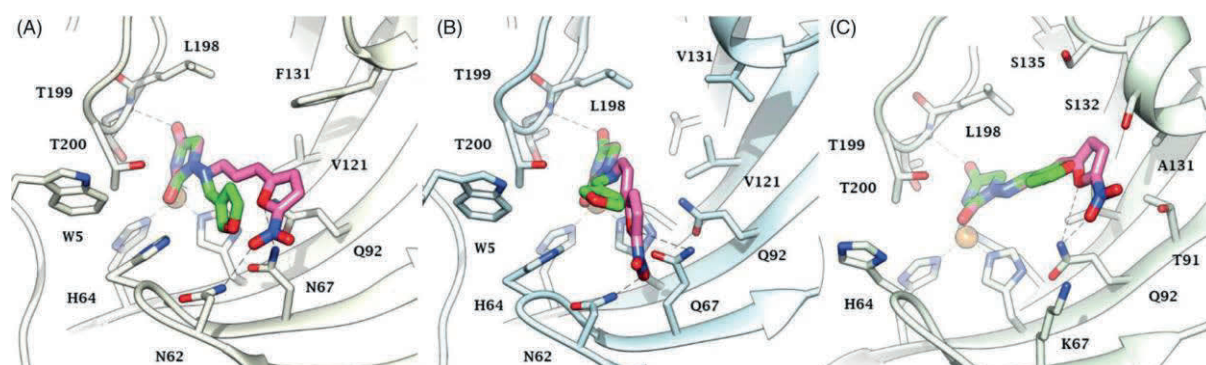


Fig. 21. Predicted docking orientations of furagin (pink) imidazolidine-2,4-dione (green) to (A) CA II, (B) CA IX, and (C) CA XII.

From the molecular modelling experiments we conclude that imidazolidine-2,4-dione function is a zinc binding group. Molecular dynamics simulation experiments in the range of 100 nanoseconds show that furagin forms a strong H-bond interaction with target enzymes CA IX and CA XII. In contrast, furagin does not form a strong H-bond interaction with CA II, that is why it is a selective inhibitor of CA IX and CA XII.

Based on docking and molecular simulation experiments, we decided to develop this direction by synthesizing a series of imidazolidine-2,4-dione derivatives **10a–10r** (Fig. 22 and Table 7). Imidazolidine-2,4-dione derivatives **10a–10r** were successfully synthesized by reaction of 1-aminoimidazolidine-2,4-dione hydrochloride **53** with various aldehydes.

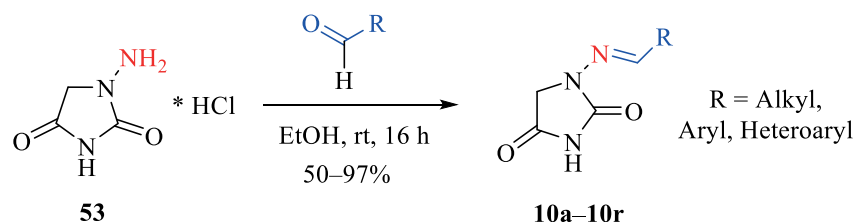
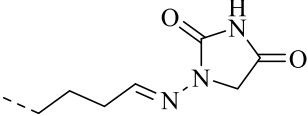


Fig. 22. Synthesis of imidazolidine-2,4-dione derivative **10a–10r**.

As shown in Table 7, a number of compounds with alkyl **10h** and **10i** (Entry 8–9), aryl **10a–10c**, **10g** and **10j–10l** (Entry 1–4, 7, 10–12), heteroaryl substituents **10f** and **10m–10p** (Entry 6 and 13–16) were obtained. Regardless of the type of substituent, all reaction products were obtained in good and very good yield 50–97 %. It should be noted, that all compounds **10a–10r** were successfully purified by crystallization from ethanol, no further purification by column chromatography was required.

Table 7

Imidazolidine-2,4-dione derivative **10a–10r** synthesis, CA inhibition results

Entry	Product No., yield, %	R	K_I^* , nM			
			CA I	CA II	CA IX	CA XII
1	10a , 90	C ₆ H ₅	39 600	900	3500	5600
2	10b , 80	4-OCH ₃ -C ₆ H ₄	57 600	6400	1200	4700
3	10c , 82	4-NO ₂ -C ₆ H ₄	>100 000	11 100	7400	2800
4	10d , 95	4-(CO ₂ CH ₃)-C ₆ H ₄	>100 000	8300	4900	930
5	10e , 50		19 100	4000	1100	160
6	10f , 89	3-furanyl	16 800	710	850	1700
7	10g , 90	4-(OCH ₂ C ₆ H ₅)-C ₆ H ₄	>100 000	540	350	910
8	10h , 81	CHCH(CO ₂ C ₂ H ₅)	45 900	23 600	810	440
9	10i , 72	CHC(CH ₃) ₂	28 800	16 500	2900	880
10	10j , 71	CHCH(4-OCH ₃ -C ₆ H ₄)	>100 000	3100	400	360
11	10k , 93	2,4-(OH) ₂ -C ₆ H ₃	>100 000	59 900	5800	150
12	10l , 88	4-(B(OH) ₂)-C ₆ H ₄	90 700	14 200	7300	230
13	10m , 95	2-pyridyl	51 800	4200	4500	1300
14	10n , 90	3-pyridyl	45 600	620	2300	3200
15	10o , 91	4-pyridyl	26 600	3300	1600	810
16	10p , 97	5-imidazolyl	9600	12 400	560	350
17	Furagin (52)*	–	>100 000	9600	260	57
18	AAZ*	–	250	12	25	6

* Row 17 shows the ability of furagin **52** to inhibit different CA isoforms; Row 18 shows the ability of acetazolamide (AAZ) to inhibit different CA isoforms.

Inhibition activities of various CA isoforms were determined for all synthesized imidazolidine-2,4-dione derivatives **10a–10r**, see Table 7 for the obtained results. All tested imidazolidine-2,4-dione derivatives **10a–10r** exhibited weak inhibitory effect on cytosolic CA I isoform, $K_I = 16\,800\text{--}100\,000$ nM. The physiologically relevant isoform, CA II, was better inhibited, $K_I = 620\text{--}59\,000$ nM. Compounds **10a**, **10f**, **10g**, and **10n** (Entry 1, 6–7, and 14) containing unsubstituted phenyl moiety, or heteroaryl group showed the best inhibitory activities, $K_I = 540\text{--}900$ nM. The rest of the compounds showed weaker inhibitory effect of CA II, $K_I = 3100\text{--}59\,000$ nM. It should be noted, that the compound **10k** (Entry 11), having dihydroxyphenyl substituent, proved to be almost a three times weaker CA II inhibitor, than the second weakest CA II inhibitor **10h** (Entry 8).

Compounds **10f–10h** (Entry 6–8), **10j** (Entry 10), **10p** (Entry 16), and furagin (Entry 17) inhibited cancer associated target enzyme CA IX at nanomolar concentrations ($K_I = 260\text{--}850$ nM), the strongest inhibition was observed for furagin. The rest of imidazolidine-2,4-dione derivatives showed one order weaker CA IX inhibition activities, $K_I = 1100\text{--}7300$ nM.

Certain pattern can be observed, compounds containing vinyl substituent **10h** (Entry 8), **10j** (Entry 10) and furagin (Entry 17) or small heteroaryl substituent **10f** (Entry 6) and **10p**

(Entry 16) are better CA IX inhibitors than the rest of compounds, except in the case of compound **10g** (Entry 7), containing ether moiety.

Of all the isoforms studied, the other cancer associated isoform CA XII was inhibited best. The best inhibitor was furagin (Entry 17), $K_I = 57$ nM. One order weaker CA XII inhibition compared to furagin was observed for compounds **10d** and **10e** (Entry 4–5), **10g–10l** (Entry 7–12), **10o** and **10p** (Entry 14–15), CA XII, $K_I = 150–930$ nM.

In summary, we have showed that furagin and imidazolidine-2,4-dione derivatives are potential CA inhibitors. Good selectivity against cancer associated isoforms (CA IX and CA IX) compared to cytosolic ones (CA I and CA II) was observed for furagin and compound **10h**.

CONCLUSIONS

1. The ruthenium-catalyzed olefin ring closing metathesis reaction is suitable for the preparation of 3*H*-1,2-benzoxathiepine 2,2-dioxide and its derivatives.
2. The olefin ring closing metathesis reaction using both ruthenium and molybdenum catalysts is not suitable for the synthesis of 6-bromo-3*H*-1,2-benzoxathiepine-2,2-dioxide.
3. 3*H*-1,2-Benzoxathiepine 2,2-dioxides containing triazolyl, acylamino, or aryl derivatives at 7th position are selective and effective inhibitors of tumour associated CA isoforms – CA IX and CA XII.
4. 8-Aryl 3*H*-1,2-benzoxathiepine 2,2-dioxides are the most effective CA IX and CA XII inhibitors compared to the corresponding 7- and 9-aryl 3*H*-1,2-benzoxathiepine 2,2-dioxides.
5. Furagin and the synthesized imidazolidine-2,4-dione derivatives are selective and effective inhibitors of tumour associated CA isoforms – CA IX and CA XII.

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