ChemMedChem

Research Article
doi.org/10.1002/cmdc.202400345

Chemistry Europe
European Chemical Societies Publishing

www.chemmedchem.org

Thia- and Seleno-Michael Reactions for the Synthesis of Carbonic Anhydrases Inhibitors

Andrea Angeli,[a] Alessio Occhini,[b] Gioele Renzi,[a] Antonella Capperucci,[b] Marta Ferraroni,[b] Damiano Tanini,*[b] and Claudiu T. Supuran[a]

Novel chalcogen-containing amides and esters bearing the benzenesulfonamide moiety have been synthesised upon nucleophilic conjugate addition of thiols and selenols to suitable electron-deficient alkenes. The activity of the synthesised compounds as Carbonic Anhydrases inhibitors has been investigated *in vitro* and the inhibition mechanism has been elucidated by X-rays studies.

Introduction

Carbonic anhydrases (CAs, E.C. 4.2.1.1) are metalloenzymes present in all life kingdom, catalysing the reversible hydration reaction of carbon dioxide to bicarbonate and protons.[1] This reaction plays a pivotal role in a wide array of physiological and pathological processes associated with pH regulation, ion transport and fluid secretion. Furthermore, CAs are also involved in a number of biosynthetic pathways.[1,2]

Although seven CA enzymatic families are known to date, only α-CAs have been reported in vertebrates. In humans, 12 of the 15 discovered α-CA isoforms have been reported to be catalytically active. The remaining three isoforms (CA VIII, X, and XI) lack in catalytic activity but still play important functions in pathological processes.[1,2] In this context, CA inhibition represents an attractive strategy to develop therapeutic tools. CA inhibitors (CAIs) belonging to the sulfonamide or sulfamate classes are clinically used since 1954 as diuretics,[3] antiglaucoma agents,[4], antiepileptics[5] in different pathologies. More recently, inhibition of the tumor-associated isoforms CA IX/XII has also been validated as a novel therapeutic approach for cancer treatment.[6] Additionally, CAI-based strategies for the treatment of idiopathic intracranial hypertension[7] and for the management of cerebral ischemia,[8] arthritis[9] and neuropathic pain[10,11] have been studied.

The important role of chalcogens (*i.e.*, sulfur and selenium) in biological systems has been well demonstrated. Indeed, while selenium and sulfur occur in the active site of a variety of proteins,[12,13] chalcogen-containing organic derivatives can be employed as antioxidants,[15–17] antimicrobials,[18–20] and antitumor agents.[21–24] The activity of a number of organochalcogenides as modulators of enzymes, including nitric oxide synthase (NOS),[25] inosine monophosphate dehydrogenase (IMPDH),[26] lipoxygenases (LOX)[27] and carbonic anhydrases[28–31] has been unveiled. Very recently, selenium-containing SARS-CoV-2 cysteine proteases inhibitors have been described.[32–38] Selenium-based fluorescent probes and particles have also been reported.[39]

Particularly, selenium is an essential microelement for the human physiology. At least 25 human selenoproteins – containing the seleno-amino acid selenocysteine – have been discovered and demonstrated to be crucially involved in a wide variety of essential biological functions, ranging from the regulation of reactive oxygen species (ROS) concentration to the biosynthesis of hormones. These processes also play an important role in the onset and progress of several diseases, including cancer, diabetes, Alzheimer's disease, mental disorders, cardiovascular disorders, fertility impairments, inflammation, and infections (including SARS-CoV-2). Notably, selenium has been reported to possess both antioxidant and pro-oxidant properties which, potentially, could be exploited to develop different selenium-based therapeutic strategies. In this scenario, confusing effects of selenium have been documented in cancer biology and virus-associated diseases.[40–43] Similarly, selenium has been demonstrated to possess both protective and toxic effects on the nervous system and on the heart.

Although research on selenium-containing drugs is still in its early phase, significant evidences about the impact of selenium on the pharmacological activity, toxicity and biotransformation pathways of organoselenium compounds have been provided. In some cases, the introduction of selenium into small organic molecules has emerged as a rewarding strategy to modulate and increase their biological activity.[40–53] For example, the isosteric replacement of an oxygen or sulfur atom with selenium is widely employed in medicinal chemistry and may represent an attractive tool to explore new directions in drug discovery.[54,55] A variety of structurally diverse organoselenium compounds with chemopreventive and antioxidant activity have been synthesised and studied over the last few decades.

[a] *A. Angeli, G. Renzi, C. T. Supuran*
NEUROFARBA Deptartment, Sezione di Scienze Farmaceutiche, University of Florence, Via Ugo Schiff 6, Sesto Fiorentino, 50019 Florence, Italy

[b] *A. Occhini, A. Capperucci, M. Ferraroni, D. Tanini*
Department of Chemistry "Ugo Schiff" (DICUS), University of Florence, Via della Lastruccia 3–13, Sesto Fiorentino, I-50019 Florence, Italy
E-mail: damiano.tanini@unifi.it

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cmdc.202400345

The incorporation of selenium into small molecules is often associated with additional benefits, closely related to the modulation of the oxidative stress status of cells.[40–43]

Owing to the possibility to conjugate the properties of organochalcogenides with those of sulfonamides, the development of new sulfur- and selenium-containing benzenesolfonamide-based CAIs represents an attractive research area, which may enable to enlarge the available structural diversity and to explore new chemical space in drug discovery.

During our studies towards the development of novel chalcogen-containing carbonic anhydrase inhibitors, we reported the synthesis and the investigation of the CA inhibition properties of variously functionalised sulfur- and selenium-containing benzenesulfonamide-based inhibitors. The general structures of these compounds, achieved via nucleophilic ring opening- and/or nucleophilic substitution-based methodologies, are reported in the Figure 1.[29–31]

In order to further enlarge the molecular diversity of the available inhibitors, and especially to investigate the activity of superior homologous of previously prepared α-chalcogeno-substituted amides (Figure 1, first structure on the left),[30] we focused our attention on compounds with the general structure reported in the Figure 2 (left).

Reasonably undergoing thia-Michael and seleno-Michael addition reactions, we envisaged α,β-unsaturated amide **1** and ester **2** (Figure 2, right) as possible precursors of the chalcogen-containing derivatives object of this study. The results of our investigations on the synthesis and the evaluation of the activity and of the inhibition mechanism of novel benzenesulfonamide-based sulfur- and selenium-containing CAIs are reported herein.

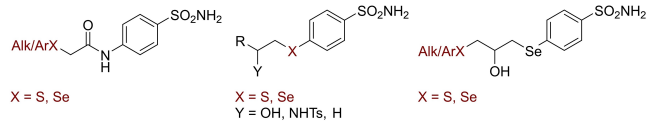


Figure 1. General structures of previously synthesised sulfur- and selenium-containing benzenesulfonamide-based CA inhibitors.

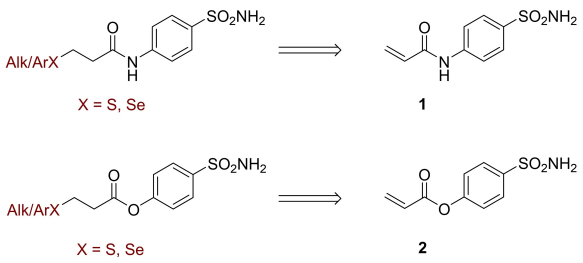


Figure 2. Sulfur- and selenium-containing CA inhibitors studied in this work (left) and their precursors (right).

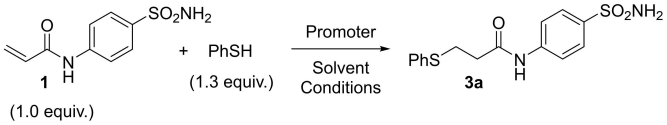
Results and Discussion

Synthesis of CA Inhibitors

We commenced our studies by establishing the optimal conditions required to synthesise the β-phenylthio-substituted amide **3a** through the conjugate addition of benzenethiol to the α,β-unsaturated amide **1**. On the basis of literature[56] and of our previously reported studies,[57] we considered the possibility of using both bases and Lewis acids to promote the desired conjugate addition. The results of this investigation are summarised in Scheme 1.

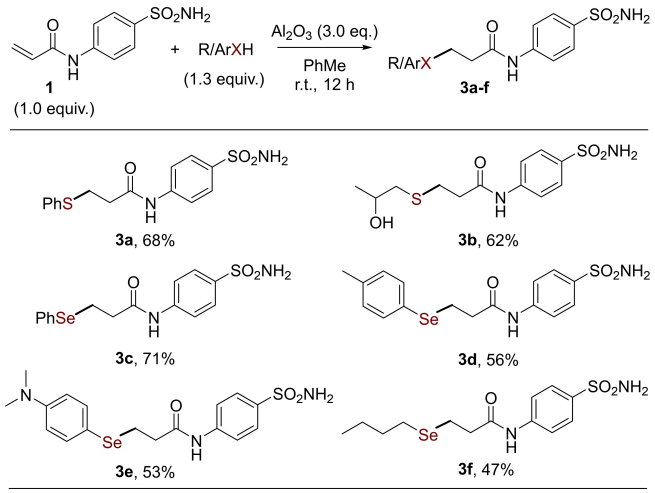
As previously observed for the reaction of alkyl selenols with electron-poor alkenes,[57] both aluminium oxide and triethylamine were effective in promoting the conjugate addition of benzenethiol to the α,β-unsaturated amide **1** (Scheme 1, entries 1 and 3). In both cases, higher yields were achieved upon performing the reaction at ambient temperature. Indeed, raising the temperature (Scheme 1, entries 2 and 4) proved to be detrimental to the reaction and led to the target β-phenylthio-substituted amide **3a** in lower yield. Inorganic bases such as KOH and Cs_2CO_3 were also evaluated and proved to be scarcely effective. Particularly, only very low yield or moderate yield of the thia-Michael addition product **3a** were achieved upon using the strong base KOH (Scheme 1, entry 5) or the Cs_2CO_3/TBAI system (Scheme 1, entry 6), respectively.

Having established the adequate conditions for the synthesis of **3a**, we next applied this simple protocol towards differently substituted systems by varying the nature of the nucleophilic partner (Scheme 2). The amide **3b**, bearing the (2-hydroxypropyl)thio group on the β-carbon, was thus obtained upon selective functionalization of the SH group of 1-mercapto-2-propanol. Remarkably, the reaction was also extended to selenol partners, enabling the synthesis of amides **3c–f**, bearing arylseleno or alkylseleno moieties at the β-position. Both aromatic and aliphatic selenols efficiently reacted with **1** under the standard reaction conditions to provide the corresponding seleno–Michael addition products **3c–f** in good yield.



Entry	Promoter (eq.)	Solvent	Temperature, time	Yield (%)[a]
1	Al_2O_3 (3.0 eq.)[b]	PhMe	r.t., 12 h	72
2	Al_2O_3 (3.0 eq.)[b]	PhMe	90 °C, 3 h	53
3	Et_3N (1.1 eq.)	CH_2Cl_2	r.t., 2 h	68
4	Et_3N (1.1 eq.)	CH_2Cl_2	40 °C, 2 h	46
5	KOH (1.1 eq.)	DMF	0 °C to r.t., 3 h	19
6	Cs_2CO_3/TBAI (1.1 eq.)	DMF	0 °C to r.t., 3 h	34

Scheme 1. Optimisation of the reaction conditions for the synthesis of the β-phenylthio-substituted amide **3a** bearing the benzenesulfonamide moiety. [a]Isolated yield is given. [b]Neutral Al_2O_3 was used.



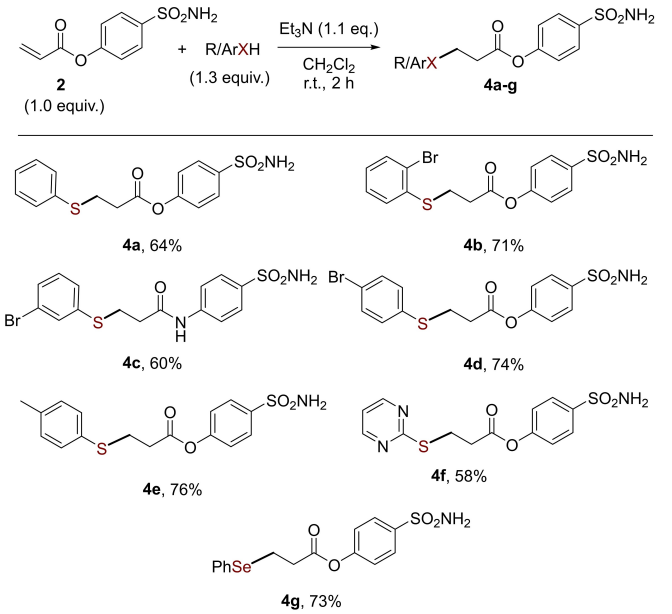
Scheme 2. Synthesis of β-chalcogenyl amides **3** bearing the benzenesulfonamide moiety.

Having synthesised a series of β-substituted amides **3**, we next turned our attention to evaluating the reactivity of the α,β-unsaturated ester **2** as a Michael acceptor in conjugate additions with thiols and selenols (Scheme 3). An initial set of comparative experiments with thiols led us to find that using Et_3N in dichloromethane led to higher yields with respect to those achieved conducting the reaction under the Al_2O_3/toluene conditions. Additionally, while the amide bond of **1** and **3** proved to be – as expected – stable under the conditions described in Scheme 1, a partial decomposition of the ester moiety of **2** and **4** was observed. The presence of the electron-withdrawing sulfonamide moiety at the *para* position of the aromatic ring significantly enhances the leaving group ability of the substituted phenoxide; thus, a nucleophilic acyl substitution competing with the conjugate addition, and leading to by-products, also occurred. Particularly, the use of Al_2O_3 (conditions of entry 1, Scheme 1) led to the formation of the desired functionalised esters in lower yield with respect to the same reactions performed in the presence of triethylamine (conditions of entry 3, Scheme 1). On the basis of these results, conjugated additions of thiols and selenols to the ester **2** were performed using Et_3N (Scheme 3).

The reaction worked well with a series of aromatic thiols such as benzenethiol, 2-bromobenzenethiol, 3-bromobenzenethiol, 4-bromobenzenethiol, and 4-methylbenzenethiol, providing access to the corresponding β-functionalised esters **4a–e**. 2-Mercaptopyrimidine also reacted efficiently with **2** to afford the corresponding β-heteroarylthio-substituted derivative **4f** in good yield. To demonstrate the possibility to extend this methodology to the synthesis of selenium containing esters, the derivative **4g** was prepared upon treatment of **2** with benzeneselenol in the presence of Et_3N.

Carbonic Anhydrase Inhibition

Having in hands a series of sulfur- and selenium-containing amides and esters bearing the benzenesulfonamide moiety, we next studied their activity as hCA inhibitors. Compounds **3a–f**, **4a–g** were profiled *in vitro* against the physiologically most-relevant hCA isoforms such as hCAs I, II, IX and XII by applying the stopped-flow technique and results are reported in Table 1, along with data for the standard sulfonamide inhibitor acetazolamide (AAZ).



Scheme 3. Synthesis of β-chalcogenoaesters **4** bearing the benzenesulfonamide moiety.

Table 1. Inhibition data of human CA isoforms I, II, IX and XII with compounds **3a–f**, **4a–g** and AAZ by a stopped-flow CO_2 hydrase assay.[58]

K_I (nM)[a]				
Cmp	**hCA I**	**hCAII**	**hCA IX**	**hCA XII**
3a	54.9	20.7	27.1	23.1
3b	73.9	22.1	241.7	8.4
3c	36.5	3.8	26.7	6.5
3d	62.5	6.1	50.5	8.2
3e	407.9	48.5	90.5	5.5
3f	75.9	6.9	28.1	7.0
4a	688.5	7.0	147.6	7.1
4b	3194	125.3	1912	7.0
4c	358.7	53.9	820.6	50.3
4d	215.1	25.2	292.4	9.5
4e	7.8	3.9	757.0	23.1
4f	68.2	6.2	307.1	9.0
4g	72.3	5.3	87.3	8.3
AAZ	250.0	12.1	25.8	5.7

[a] Mean from 3 different assays, by a stopped flow technique (errors were in the range of ±5–10% of the reported values).

Based on the data presented in Table 1, the structure-activity relationship (SAR) of the tested compounds can be delineated as follows:

- The cytosolic isoform, hCA I, was effectively inhibited by almost all reported compounds. The inhibition ranged from the low nanomolar, with compound **4e** (K_I = 7.8 nM) to the micromolar range with **4d** (K_I = 3286 nM). Among the amide series (**3a–f**), the *N*-dimethyl substituent (**3e**) was identified as the least favourable moiety, and this observation was more pronounced in the ester series (**4a–g**). Notably, ester **4a** was 10-fold less potent than its amide analogue **3a**. The introduction of a bromine atom at the *ortho* position of the phenyl ring adversely affected potency, while its presence at the *meta* or *para* position increased potency. The replacement of bromine with a methyl group at the *para* position resulted in the most potent inhibition against hCA I, maintaining the inhibition in the low nanomolar range (K_I = 7.8 nM).
- Regarding the second cytosolic isoform, hCA II, isosteric replacement of sulfur (**3a**) with selenium (**3c**) led to a 5-fold increase in inhibition potency for the amide series (K_I of 20.7 nM and 3.9 nM, respectively). However, in the ester derivatives, there was no significant difference in inhibition potency between **4a** and **4g** (K_I of 7.0 nM and 5.3 nM, respectively). Similar to hCA I, the presence of a bromine group at the *ortho* position was detrimental to activity, with a K_I of 127.0 nM.
- The membrane-bound isoform, hCA IX, was effectively inhibited by the amide series, except for compound **3b**, which exhibited a K_I of 241.7 nM due to its aliphatic thio tail. Additionally, substituents at the *para* position of selenium derivatives were found to reduce potency, resulting in 2- (**3d**) and 3-fold (**3e**) lower activity than **3c** (K_I = 50.5 nM, 90.5 nM, and 28.1 nM, respectively). Similar to the cytosolic isoforms, the ester series generally displayed weaker inhibition than the amide series, and the position of the bromine atom on the phenyl ring resulted in the same inhibition pattern as mentioned above.
- The second membrane-bound isoform, hCA XII, exhibited a distinct inhibition profile. Among the amide series, compound **3a** with a thiophenyl moiety displayed the least activity with a K_I of 23.0 nM. The replacement of sulfur with selenium (**3c**) increased inhibition potency by 4.2 times (K_I = 6.6 nM). An interesting observation was made with the ester series regarding the position of the bromine atom; while the *ortho* location resulted in the most potent inhibition, the *meta* location was less active.

As mentioned above, the sulfur- and selenium-containing amides **3** synthesised in this work are superior homologous of previously prepared α-chalcogeno-substituted amides.[30] Particularly, compounds **3a**,**c**,**d**,**e** are structurally related to derivatives **5a–d** depicted in the Figure 3. For comparison purposes, the inhibition data of α-chalcogeno amides **5a–d** against hCAs I, II, IX and XII are reported in Table 2. Notably, the novel amides **3** displayed significantly higher activity against hCA I with respect to the inferior homologues **5**; the inhibition potency of phenylthio- and phenylseleno-substituted derivatives **3a** and **3c** against hCA I proved to be two order of magnitude higher than related α-chalcogeno amides **5a** and **5b**. Additionally, when compared to inferior homologues **5a** and **5d**, compounds **3a** and **3e** exhibited improved activity against tumor-associated hCA IX and XII, respectively.

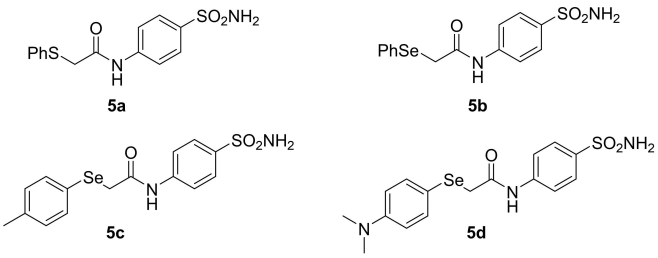


Figure 3. Structures of α-chalcogeno amides **5a–d**.

Table 2. Inhibition data of human CA isoforms I, II, IX and XII with compounds **5a–d** compared with those of homologous compounds **3a,c–e**. Inibition data have been determined by a stopped-flow CO_2 hydrase assay.[57] The ratio between K_I values of homologous compounds (K_I**5**/K_I**3**) is also reported for comparison purposes.

K_I (nM)[a]				
Cmp	**hCA I**	**hCAII**	**hCA IX**	**hCA XII**
5a	6049	6.1	272.1	29.2
3a	54.9	20.7	27.1	23.1
K_I5a/K_I3a	110.18	0.29	10.04	1.26
5b	2724	2.9	3.5	1.8
3c	36.5	3.8	26.7	6.5
K_I5b/K_I3c	73.82	0.74	0.12	0.27
5c	181.3	3.4	32.9	8.2
3d	62.5	6.1	50.5	8.2
K_I5c/K_I3d	2.90	0.56	0.65	1.00
5d	6979	48.9	46.9	35.8
3e	407.9	48.5	90.5	5.5
K_I5d/K_I3e	17.11	1.01	0.52	6.51

[a] Mean from 3 different assays, by a stopped flow technique (errors were in the range of ±5–10% of the reported values).

X-Ray Crystallography

To unravel the molecular mechanisms underlying the inhibition of CAs by the reported compounds, the X-ray structure of the complex formed with hCA I and derivative **4e**, which exhibited the best constant of inhibition value against this isoform, was studied. Following an initial round of refinement, a well-defined electron density map emerged within the active site, entirely consistent with the inhibitor **4e** (Figure S1). Notably, the benzenesulfonamide directly binds to the zinc ion with the deprotonated sulfonamide group, a characteristic feature typical of this compound class (Figure 4a).[59]

Furthermore, in the complex with **4e**, one of the oxygen atoms of the sulfonamide group forms a hydrogen bond with

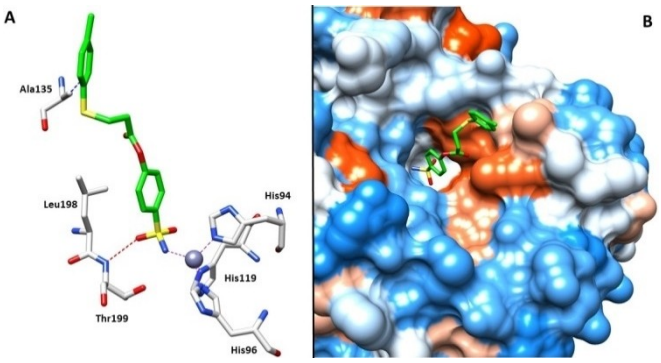


Figure 4. a) X-ray crystal structure of hCA I with **4e** bound within the active site (PDB: 9EP2). Residues involved in the binding of the inhibitor are also shown; the grey sphere represents the zinc ion in the active site of the proteins. Van der Waals interactions are shown in blue, hydrogen bonds and water bridges are shown in red. b) Compound **4e** inside the active site of hCA I. Hydrophobic (red) and hydrophilic (blue) residues are labelled.

the backbone amide of Thr199 (Figure 4a). In contrast, the benzenesulfonamide does not participate in the usual van der Waals interactions observed with the side chains of Ala121, Val143, and Leu198. The sole hydrophobic interaction identified was between the phenyl ring at the end of the tail of **4e** and Ala135, placing the inhibitor within a hydrophobic pocket (Figure 4b).[60]

Conclusions

A series of novel benzenesulfonamide-based chalcogen-containing CA inhibitors have been developed through a simple protocol relying on the reactivity of electron-poor functionalised alkenes with suitable thiol or selenol nucleophilic partners. The activity of the synthesised compounds has been investigated *in vitro* against the physiologically most-relevant hCA isoforms (hCAs I, II, IX and XII) and compared with previously reported data for structurally related inferior homologues. The inhibition mechanism of the synthesised compounds has been elucidated by X-rays studies. Overall, this work provides a valuable synthetic platform and additional structural information (*i.e.*, number of methylene groups inserted between the chalcogen atom and the carbonyl group) for the design and synthesis of chalcogen-containing benzenesulfonamide-based CA inhibitors.

Supporting Information Summary

Experimental details, synthetic procedures, and spectroscopic characterisation data are reported in the Supporting Information. The authors have cited additional references within the Supporting Information.[61–71]

Acknowledgements

The financial support provided by the MUR-Dipartimenti di Eccellenza 2023–2027 (DICUS 2.0) to the Department of Chemistry "Ugo Schiff" of the University of Florence is acknowledged. Open Access publishing facilitated by Università degli Studi di Firenze, as part of the Wiley - CRUI-CARE agreement.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Thiols · Selenols · Alkenes · Conjugate addition · Solfonamides · X-rays

[1] C. T. Supuran, *Biochem. J.* **2016**, *14*, 2023.
[2] C. T. Supuran, *J. Enzyme Inhib. Med. Chem.* **2016**, *3*, 345.
[3] F. Carta, C. T. Supuran, *Expert Opin. Ther. Pat.* **2013**, *6*, 681.
[4] A. Scozzafava, C. T. Supuran, *Subcell Biochem.* **2014**, *75*, 349.
[5] C. B. Mishra, S. Kumari, A. Angeli, S. M. Monti, M. Buonanno, M. Tiwari, C. T. Supuran, *J. Med. Chem.* **2017**, *6*, 2456.
[6] H. I. Gul, C. Yamali, H. Sakagami, A. Angeli, J. Leitans, A. Kazaks, K. Tars, D. O. Ozgun, C. T. Supuran, *Bioorg. Chem.* **2018**, *77*, 411.
[7] C. T. Supuran, *Expert Rev. Neurother.* **2015**, *15*, 851–856.
[8] L. Di Cesare Mannelli, L. Micheli, F. Carta, A. Cozzi, C. Ghelardini, C. T. Supuran, *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 894.
[9] F. Margheri, M. Ceruso, F. Carta, A. Laurenzana, L. Maggi, S. Lazzeri, G. Simonini, F. Annunziato, M. Del Rosso, C. T. Supuran, R. Cimaz, *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 60.
[10] F. Carta, L. Di Cesare Mannelli, M. Pinard, C. Ghelardini, A. Scozzafava, R. McKenna, C. T. Supuran, *Bioorg. Med. Chem.* **2015**, *23*, 1828.
[11] C. T. Supuran, *Expert Rev. Neurother.* **2016**, *16*, 961.
[12] H. S. Olcott, W. D. Brown, J. Van Derveen, *Nature* **1961**, *191*, 1201.
[13] R. Walter, I. L. Schwartz, J. Roy, *Ann. N Y Acad. Sci.* **1972**, *192*, 175.
[14] A. Capperucci, M. Coronnello, F. Salvini, D. Tanini, S. Dei, E. Teodori, L. Giovannelli, *Bioorg. Chem.* **2021**, *110*, 104812.
[15] A. J. Pacula, F. Mangiavacchi, L. Sancineto, E. J. Lenardao, J. Scianowski, C. Santi, *Curr. Chem. Biol.* **2015**, *9*, 97.
[16] S. Yoshida, F. Kumakura, I. Komatsu, K. Arai, Y. Onuma, H. Hojo, B. G. Singh, K. I. Priyadarsini, M. Iwaoka, *Angew. Chem. Int. Ed.* **2011**, *50*, 2125.
[17] C. W. Nogueira, G. Zeni, J. B. T. Rocha, *Chem. Rev.* **2004**, *104*, 6255.
[18] A. Angeli, M. Pinteala, S. S. Maier, B. C. Simionescu, A. Milaneschi, G. Abbas, S. del Prete, C. Capasso, A. Capperucci, D. Tanini, F. Carta, C. T. Supuran, *Int. J. Mol. Sci.* **2020**, *21*, 598.
[19] P. Tran, J. Kopel, B. Ristic, H. Marsh, J. Fralick, T. Reid, *Adv. Redox Res.* **2022**, *4*, 100031.
[20] P. Tran, A. Hamood, T. Mosley, T. Gray, C. Jarvis, D. Webster, B. Amaechi, T. Enos, T. Reid, *J. Dent. Res.* **2013**, *92*, 461.
[21] D. Tanini, L. Ricci, A. Capperucci, L. Di Cesare Mannelli, C. Ghelardini, T. S. Peat, F. Carta, A. Angeli, C. T. Supuran, *Eur. J. Med. Chem.* **2019**, *181*, 111586.
[22] S. Garbo, S. Di Giacomo, D. Łażewska, E. Honkisz-Orzechowska, A. Di Sotto, R. Fioravanti, C. Zwergel, C. Battistelli, *Pharmaceutics* **2023**, *15*, 104.
[23] D. Radomska, R. Czarnomysy, D. Radomski, K. Bielawski, *Int. J. Mol. Sci.* **2021**, *22*, 1009.
[24] V. Gandin, P. Khalkar, J. Braude, A. P. Fernandes, *Free Radic. Biol. Med.* **2018**, *127*, 80.
[25] K. S. Prabhu, F. Zamamiri-Davis, J. B. Stewart, J. T. Thompson, L. M. Sordillo, C. C. Reddy, *Biochem. J.* **2002**, *366*, 203.

[26] G. Gebeyehu, V. E. Marquez, A. Van Cott, D. A. Cooney, J. A. Kelley, H. N. Jayaram, G. S. Ahluwalia, R. L. Dion, Y. A. Wilson, D. G. Johns, *J. Med. Chem.* **1985**, *1*, 99.
[27] J. Ghosh, *Biochem. Biophys. Res. Commun.* **2004**, *3*, 624.
[28] A. Angeli, F. Carta, S. Donnini, A. Capperucci, M. Ferraroni, D. Tanini, C. T. Supuran, *Chem. Commun.* **2020**, *56*, 4444.
[29] A. Angeli, D. Tanini, A. Capperucci, C. T. Supuran, *ACS Med. Chem. Lett.* **2017**, *8*, 1213.
[30] D. Tanini, A. Capperucci, M. Scopelliti, A. Milaneschi, A. Angeli, C. T. Supuran, *Bioorg. Chem.* **2019**, *89*, 102984.
[31] A. Angeli, D. Tanini, A. Capperucci, G. Malevolti, F. Turco, M. Ferraroni, C. T. Supuran, *Bioorg. Chem.* **2018**, *81*, 642.
[32] Han K, Jia Y. Application of mono-selenoether compound in preparation of anti-coronavirus drugs and drugs, CN2022/114246867.
[33] F. B. Omage, A. Madabeni, A. R. Tucci, P. A. Nogara, M. Bortoli, A. Dos Santos Rosa, V. N. Dos Santos Ferreira, J. B. T. Rocha, M. D. Miranda, L. Orian, *J. Chem. Inf. Model.* **2023**, *63*, 2226.
[34] H. Sies, M. J. Parnham, *Free Radic. Biol. Med.* **2020**, *156*, 107.
[35] E. Weglarz-Tomczak, J. M. Tomczak, M. Talma, M. Burda-Grabowska, M. Giurg, S. Brul, *Sci. Rep.* **2021**, *11*, 3640.
[36] N. Suzuki, Y. Ogra, Y. Fukumoto, K. Ichikawa, Therapeutic or prophylactic agent for covid19 which comprises selenoneine. WO2022/177029 A1.
[37] G. Barchielli, A. Capperucci, D. Tanini, *Expert Opin. Ther. Pat.* **2024**, *34*, 17.
[38] G. Wildner, A. R. Tucci, A. D. S. Prestes, T. Muller, A. D. S. Rosa, N. R. R. Borba, V. N. Ferreira, J. B. T. Rocha, M. D. Miranda, N. V. Barbosa, *Vaccines* **2023**, *11*, 1222.
[39] A. Abdillah, P. M. Sonawane, D. Kim, D. Mametov, S. Shimodaira, Y. Park, D. G. Churchill, *Molecules* **2021**, *26*, 692.
[40] G. Barchielli, A. Capperucci, D. Tanini, *Antioxidants* **2022**, *11*, 251.
[41] S. Debnath, A. Agarwal, N. R. Kumar, A. Bedi, *Future Pharmacol.* **2022**, *2*, 595–607.
[42] W. Ali, R. Benedetti, J. Handzlik, C. Zwergel, C. Battistelli, *Drug Discov. Today* **2021**, *26*, 256.
[43] W. Hou, H. Dong, X. Zhang, Y. Wang, L. Su, H. Xu, *Drug Discov. Today* **2022**, *27*, 2268.
[44] A. Angeli, M. Ferraroni, A. Capperucci, D. Tanini, G. Costantino, C. T. Supuran, *ChemMedChem.* **2022**, *17*, e202200085.
[45] D. Tanini, S. Carradori, A. Capperucci, L. Lupori, S. Zara, M. Ferraroni, C. Ghelardini, L. Di Cesare Mannelli, L. Micheli, E. Lucarini, F. Carta, A. Angeli, C. T. Supuran, *Eur. J. Med. Chem.* **2021**, *225*, 113793.
[46] C. W. Nogueira, J. B. T. Rocha, *Arch. Toxicol.* **2011**, *85*, 1313.
[47] B. J. Bhuyan, G. Mugesh, *Organoselenium Chemistry: Synthesis and Reactions* (Ed. T. Wirth), Wiley-VCH Verlag & Co, Weinheim, Germany **2012**, 361–379.
[48] J. B. T. Rocha, C. S. Oliveir, P. A. Nogara, *Organoselenium Compounds in Biology and Medicine: Synthesis, Biological and Therapeutic Treatments* (Eds. V. K. Jain, K. I. Priyadarsini) Royal Society of Chemistry, London **2018**, 342–376.
[49] L. Wang, Z. Yang, J. Fu, H. Yin, K. Xiong, Q. Tan, H. Jin, J. Li, T. Wang, W. Tang, J. Yin, G. Cai, M. Liu, S. Kehr, K. Becker, H. Zeng, *Free Radic. Biol. Med.* **2012**, *52*, 898.
[50] C. K. Chu, L. Ma, S. Olgen, C. Pierra, J. Du, G. Gumina, E. Gullen, Y–C. Cheng, R. F. Schinazi, *J. Med. Chem.* **2000**, *43*, 3906.
[51] R. Zhao, A. Holmgren, *J. Biol. Chem.* **2002**, *277*, 39456.
[52] C. W. Nogueira, F. C. Meotti, E. Curte, C. Pilissão, G. Zeni, J. B. T. Rocha, *Toxicology* **2003**, *183*, 29.
[53] E. J. Lenardão, C. Santi, L. Sancineto, *New Frontiers in Organoselenium Compounds*, Springer, Switzerland **2018**.
[54] A. K. Sharma, Sk. U. Hossain, J. M. Peters, S. Amin, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4050.
[55] D. Moreno, D. Plano, Y. Baquedano, A. Jiménez-Ruiz, J. A. Palop, C. Sanmartín, *Parasitol. Res.* **2011**, *108*, 233.
[56] R. N. Salvatore, R. A. Smith, A. K. Nischwitz, T. Gavin, *Tetrahedron Lett.* **2005**, *46*, 8931.
[57] D. Tanini, S. Scarpelli, E. Ermini, A. Capperucci, *Adv. Synth. Catal.* **2019**, *361*, 2337.
[58] R. G. Khalifah, *J. Biol. Chem.* **1971**, *246*, 2561.
[59] M. Ferraroni, A. Angeli, M. Pinteala, C. T. Supuran, *J. Mol. Struct.* **2022**, *1268*, 133672.
[60] Atomic coordinates were deposited in the Protein Data Bank (PDB accession code: 9EP2).
[61] A. Angeli, D. Tanini, A. Nocentini, A. Capperucci, M. Ferraroni, P. Gratteri, C. T. Supuran, *Chem. Commun.* **2019**, *55*, 648.
[62] C. T. Supuran, *Nat. Rev. Drug Discov.* **2008**, *7*, 168.
[63] A. Angeli, E. Trallori, F. Carta, L. Di Cesare Mannelli, C. Ghelardini, C. T. Supuran, *ACS Med. Chem. Lett.* **2018**, *9*, 947.
[64] E. Berrino, A. Angeli, D. D. Zhdanov, A. P. Kiryukhina, A. Milaneschi, A. De Luca, M. Bozdag, S. Carradori, S. Selleri, G. Bartolucci, T. S. Peat, M. Ferraroni, C. T. Supuran, F. Carta, *J. Med. Chem.* **2020**, *63*, 7392.
[65] A. Angeli, I. Chelli, L. Lucarini, S. Sgambellone, S. Marri, S. Villano, M. Ferraroni, V. De Luca, C. Capasso, F. Carta, C. T. Supuran, *J. Med. Chem.* **2024**, *67*, 3066.
[66] S. G. Narella, M. G. Shaik, A. Mohammed, M. Alvala, A. Angeli, C. T. Supuran, *Bioorg. Chem.* **2019**, *87*, 765.
[67] A. G. W. Leslie, H. R. Powell, *Evolving methods for macromolecular crystallography*, vol. 245 (Eds. R. J. Read, J. L. Sussman) NATO Science series, Springer, Dordrecht **2007**, 41–51.
[68] G. N. Murshudov, A. A. Vagin, E. J. Dodson, *Acta Crystallogr. D* **1997**, *53*, 240.
[69] P. Emsley, B. Lohkamp, W. Scott, K. Cowtan, *Acta Crystallogr. D* **2010**, *66*, 486.
[70] S. C. Lovell, I. W. Davis, W. B. Arendall III, P. I. W. de Bakker, J. M. Word, M. G. Prisant, J. S. Richardson, D. C. Richardson, *Proteins* **2003**, *50*, 437.
[71] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* **2004**, *25*, 1605–1612.

Manuscript received: May 6, 2024
Revised manuscript received: June 20, 2024
Version of record online: August 8, 2024