


## Anti-obesity carbonic anhydrase inhibitors: challenges and opportunities

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### ABSTRACT

The mitochondrial isoforms VA/VB of metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) are involved in metabolic processes, such as *de novo* lipogenesis and fatty acid biosynthesis. We review the drug design landscape for obtaining CA VA/VB-selective/effective inhibitors, starting from the clinical observations that CA inhibitory drugs, such as the antiepileptics topiramate and zonisamide, or the diuretic acetazolamide induce a significant weight loss. The main approaches for designing such compounds consisted in drug repurposing of already known CA inhibitors (CAIs); screening of synthetic/natural products libraries both in the classical and virtual modes, and *de novo* drug design using the tail approach. A number of such studies allowed the identification of lead compounds diverse from sulphonamides, such as tropolones, phenols, polyphenols, flavones, glycosides, fludarabine, lenvatinib, rufinamide, etc., for which the binding mode to the enzyme is not always well understood. Classical drug design studies of sulphonamides, sulfamates and sulfamides afforded low nanomolar mitochondrial CA-selective inhibitors, but detailed antiobesity studies were poorly performed with most of them. A breakthrough in the field may be constituted by the design of hybrids incorporating CAIs and other antiobesity chemotypes.

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### 1. Introduction

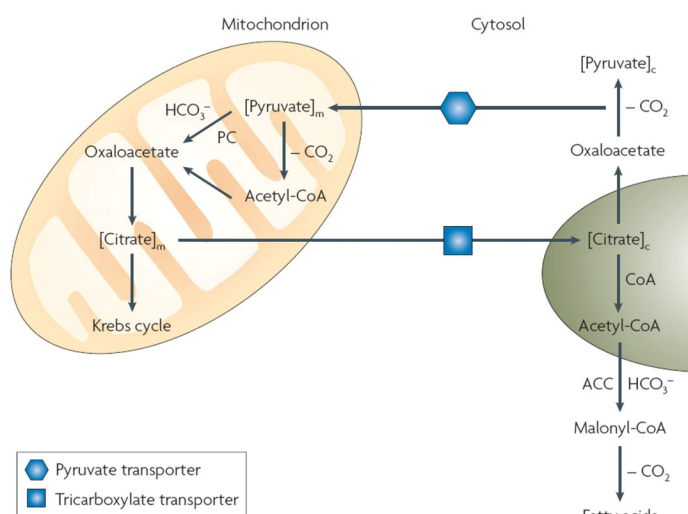
Obesity, a condition characterised by excessive fat accumulation, with body mass indexes (BMIs)  $\geq 30 \text{ kg/m}^2$  constitutes nowadays a challenging medical problem worldwide, with a large number of affected people both in the developed and developing countries. Only in USA it is estimated that 2/3 of the population has body weight problems, with 1 each three adults and 20% of the adolescents being obese<sup>1,2</sup>. Obesity is a multifactorial, complex medical problem, being considered nowadays as a chronic, degenerative disease<sup>3</sup> which however, has a large number of metabolic and psychological comorbidities, making it difficult to manage/treat, both medically and socially<sup>1–3</sup>. Some of these comorbidities include metabolic dysfunctions (type 2 diabetes, fatty liver diseases, dyslipidemia, gallstones, gout)<sup>1,2</sup>; cardiovascular diseases (atherosclerosis, hypertension, atrial fibrillation, heart failure); enhanced possibility of getting cancer (among which colon, breast and pancreas tumours); pulmonary problems (sleep apnoea, asthma); mental problems (cognition deficit, depression, anxiety and panic disorders)<sup>1–3</sup> and many other inconveniences which are not mentioned in detail here, as the field was recently reviewed in at least two excellent papers<sup>1,2</sup>. Apart a proper and balanced (hypocaloric) diet<sup>4</sup>, and bariatric surgery<sup>5</sup>, the pharmacological armamentarium for the treatment of obesity is rather scarce, with few available efficient drugs, which in addition possess a large number of side effects<sup>1,2,6</sup>. Furthermore, there is a rather relevant number of antiobesity drugs which have been developed, approved and withdrawn after a short period (few months – one year) due to serious side effects that emerged after their use in a relatively consistent number of patients, immediately after their approval<sup>1,2</sup>.

As already mentioned, the field of antiobesity drugs and the relevant drug design strategies to obtain them were recently reviewed<sup>1,2</sup>, and for this reason the physio-pathological processes involved in obesity as well as the various drug targets which have been explored in the last decades and which led to the few clinically used agents will be not dealt with here. The scope of this article is to review recent developments in the drug design of inhibitors of carbonic anhydrases (CAs), rather orphan anti-obesity drug targets<sup>7</sup>, which however may lead to interesting developments, as already shown in previous reviews on this argument, the latest of which was published in 2013<sup>8–12</sup>.

### 2. Carbonic anhydrases role(s) in obesity

CAs (EC 4.2.1.1) are enzymes which catalyse the interconversion between  $\text{CO}_2$  and bicarbonate, thus also generating a proton, and thus deeply involved in pH regulation<sup>13,14</sup>. Widespread in organisms all over the phylogenetic tree, from Archaea, Bacteria to Eukaryotes, at least 8 genetic families encoding CAs are known to date, of which only  $\alpha$ -CAs are present in vertebrates, including humans<sup>13–15</sup>. Fifteen human (h) CA isoforms, hCA I-hCA XIV (with two V-type ones, VA and VB) were described and characterised in detail, with many of them being consolidated drug targets for obtaining diuretics, antiglaucoma, antiepileptic and antitumor agents, among others<sup>13–15</sup>.

In the last period, CAs were not only considered as being involved in pH regulation/buffering in many cells and tissues, but also as metabolic enzymes<sup>16</sup>, due to their demonstrated role in several metabolic processes in tumours<sup>17,18</sup> and normal cells, including fatty acid biosynthesis and *de novo* lipogenesis (DNL) –



**Figure 1.** Role of mitochondrial and cytosolic CA isoforms in fatty acid biosynthesis: the transfer of acetyl groups from the mitochondrion to the cytosol (in the form of citrate) is required for the provision of substrate for *de novo* lipogenesis via malonyl-coenzyme A as key intermediate. Steps involving bicarbonate, both for the reaction catalysed by pyruvate carboxylase (PC) and acetyl-coenzyme A carboxylase (ACC) require the presence of CA isozymes: CA VA/VB in the mitochondrion and CA II in the cytosol.

**Figure 1**<sup>2,8-12</sup>. It has been known for decades that fatty acid biosynthesis and DNL involve both mitochondrial and cytosolic steps, in which several enzymes implicated both in the Krebs cycle as well as DNL, among which pyruvate carboxylase (PC) and acetyl-coenzyme A carboxylase (ACC) use bicarbonate and not CO<sub>2</sub> as one of their substrate<sup>8-12</sup>. In order to achieve the very rapid inter-conversion between these two species, highly catalytically active CA isoforms (among which CA II in the cytosol<sup>13-15</sup> and CA VA/VB in the mitochondria<sup>19,20</sup>) are necessary to participate<sup>21-25</sup>. It has been demonstrated already in the 90s that this is indeed the case, and that inhibition of mitochondrial/cytosolic CAs interferes with fatty acid biosynthesis and DNL in various cells, tissues and animal models<sup>21-25</sup>.

The most detailed study regarding the role of mitochondrial CAs in metabolism was reported by Minter's group in 2013<sup>26</sup> by using mitochondria wired onto electrodes and selective mitochondrial CA inhibitors (CAIs) – see discussion later in the text. The metabolism of pyruvate, acetate, and succinate were examined, in the presence of specific CA VA/B inhibitors, by measuring metabolic energy conversion, and comparing the resulting metabolic differences after treatment with structurally diverse, but effective CAIs of the primary sulphonamide type. It has been thus observed that some CA VA/B inhibitors showed a broad spectrum inhibition of metabolism, where others only had significant effects on some metabolic pathways, with pyruvate metabolism being the most dramatically affected by CA inhibition, followed by fatty acid metabolism, and finally by succinate metabolism<sup>26</sup>. These data conclusively demonstrated a clear role of mitochondrial CAs in metabolism and fatty acid biosynthesis, but the idea to use inhibition of such enzymes for obtaining antiobesity drugs started to be considered only in 2000s, when a drug company now no longer existing (Solvay Pharmaceuticals) and some academic research groups started a program for obtaining CAIs with antiobesity activity<sup>27</sup>. It should be noted, that as with other CA-related fields, such as CAIs as antitumor agents or anti-infectives, there is a serious "resistance" from members of the scientific community to accept a role of CAs in metabolism and obesity, with many

detractors trying to interfere with these findings by obstructing their publication, performing dishonest reviewing of manuscripts dealing with this topic and similar activities, which will however not stop a field which revealed to be an innovative one, with potential significant benefits for many patients suffering from this disease.

It should also be mentioned that there were many reports on the possible role of another CA isoform, the cytosolic CA III, in lipogenesis and obesity<sup>28-30</sup>. However, it was recently demonstrated that CA III is not involved in lipogenesis<sup>31</sup>, and we will not discuss this isoform as a possible antiobesity drug target here, also considering its very low catalytic activity for the CO<sub>2</sub> conversion reaction to bicarbonate as well as low affinity for sulphonamide/sulfamate inhibitors<sup>32</sup>.

### 3. Drug design of CA inhibitors as antiobesity agents

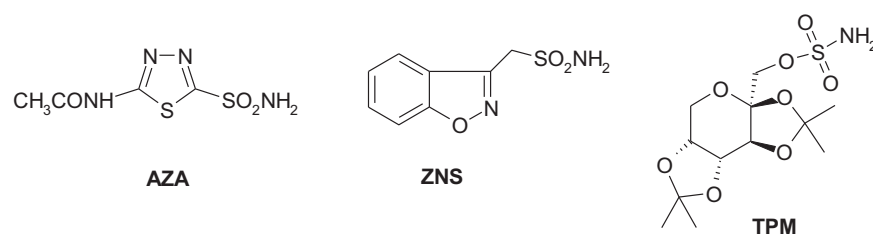
Although the CAI drug design panorama is quite rich, with many strategies and studies reported for applications as different as antiglaucoma, anticonvulsant, antitumor, anti-neuropathic pain and anti-infective agents<sup>13,33-35</sup>, the field of antiobesity CAIs was less investigated. However, three main approaches may be envisaged at this moment, which produced several interesting developments over the last two decades: (i) repurposing of drugs originally discovered for other pharmacologic applications than obesity; (ii) screening of natural products/synthetic libraries by using either virtual screening procedures or more classical enzyme inhibition assays; and (iii) *de novo* drug design studies based on already identified leads or on structural biology data of enzyme-inhibitor adducts characterised in detail, mainly by X-ray crystallographic techniques<sup>13,14,33-35</sup>.

#### 3.1. Drug repurposing

Sulphonamides and their isosteres (sulfamates, sulfamides) are the most investigated CAIs<sup>13</sup>, with many representatives in clinical use for decades as diuretics, antiglaucoma, antiepileptic, and even anti-infective agents, whereas many other such derivatives are in clinical trials for novel applications<sup>13,14,33-35</sup>. Sulphonamides such as acetazolamide (**AAZ**), launched in 1954 as the first non-mercurial diuretic<sup>13</sup>, zonisamide (**ZNS**) or the sugar sulfamate derivative topiramate (**TPM**) – **Figure 2**, are well known CAIs, with the last two compounds originally reported as antiepileptic agents<sup>13,36,37</sup> but later shown to induce a significant weight loss, both in animal models<sup>38</sup>, clinically controlled trials<sup>39-42</sup> and anecdotal reports<sup>43-48</sup>.

Thus, the use of **AAZ**, **ZNS** and **TPM** as anti-obesity drugs may be considered an interesting, successful but also problematic example of drug repurposing, since the use of these CAIs alone or in combination with other agents (phentermine, bupropion, metformin)<sup>49,50</sup> was demonstrated to induce weight loss in many obese patients, also improving their blood glucose levels<sup>39-50</sup>. How do these agents exert their antiobesity beneficial effects? Although the pharmacology of **TPM** and **ZNS** is rather complex, as these compounds bind to a multitude of targets<sup>27,36</sup>, both of them and obviously **AAZ**, are effective CAIs against human (h) CA (hCA) isoforms involved in fatty acid biosynthesis/DNL<sup>13,51,52</sup> – **Table 1**.

From data of **Table 1** it may be seen that all three drugs are effective hCA II, hCA VA and hCA VB inhibitors (inhibition constants of 10–63 nM) except **ZNS** against hCA VB, case in which the inhibition constant was in the micromolar range<sup>52</sup>. Furthermore, the binding of the two antiepileptics has also been investigated



**Figure 2.** Sulphonamide/sulfamate CAIs in clinical use: acetazolamide (AAZ), zonisamide (ZNS) and topiramate (TPM).

**Table 1.** CA inhibition data with selected drugs/investigational compounds against human (h) isoforms hCA I, II, VA and VB<sup>13,51,52</sup>.

Drug/Cmpnd	K <sub>i</sub> (nM)				Ref
	hCA I	hCA II	hCA VA	hCA VB	
AAZ	250	12	63	54	13
ZNS	56	35	20	6033	52
TPM	250	10	63	30	51

by X-ray crystallography on isoform hCA II<sup>51,52</sup> (as the X-ray crystal structure of hCA VA is not known at this moment<sup>53</sup>) and as seen in Figure 3, interesting findings emerged. **TPM** binds towards the hydrophilic half of the active site whereas **ZNS** scaffold is orientated more towards the hydrophobic half (Figure 3(A)), but both compounds coordinate through their zinc binding group (ZBG) in deprotonated form as sulfamate and sulfonamidate, respectively, to the catalytic metal ion, substituting the water molecule/hydroxide ion which acts as nucleophile in the catalytic cycle<sup>54</sup> – Figure 3(B,C). In the case of topiramate, the sugar scaffold makes a rather large number of H-bonds which involve the oxygen atoms from the drug and hydrophilic amino acid residues from the active site, among which Thr199, Thr200, Asn62, Asn67 and Gln92 (Figure 3(B)), which strongly stabilise the enzyme-inhibitor complex (K<sub>i</sub> of 10 nM). In the case of **ZNS** adduct, except the ZBG which participates in H-bonds with Thr199, the scaffold of the inhibitor participates only in van der Waals interactions with hydrophobic residues, among which Val121, Phe131 and Leu198 (Figure 3(C)). Although the two compounds show such a diverse binding mode to the enzyme, their inhibitory power towards hCA II is comparable (and also similar to that of **AAZ**, which as all sulphonamides, binds deeply within the active site, coordinating to the zinc ion though the SO<sub>2</sub>NH<sup>-</sup> moiety. The binding of **AAZ** was discussed in detail in many previous article, see refs.<sup>33,35,54</sup>) Although the detailed binding of **TPM** and **ZNS** to hCA II (and in the case of **TPM** also to hCA I<sup>55</sup>) are well understood, the two compounds were not used as leads for obtaining more efficient antiobesity CAIs, probably due to the difficulty to derivatize. Indeed, the only study in which the sulfamate ZBG of **TPM** was changed to sulfamide, led to compounds with decreased efficiency as CAIs<sup>56</sup>. It should be however mentioned that the binding of **ZNS** and **TPM** to hCA VA has been investigated by computational techniques, which explained the efficient binding to the enzyme and the differences in inhibitory power towards the mitochondrial and cytosolic isoforms<sup>57</sup>.

### 3.2. Screening of natural products/synthetic libraries

The first screening study based on a library of natural-based phenols for the identification of hCA VA and VB inhibitors was reported by Davis et al.<sup>58</sup>. Phenol is indeed a weak CAI, which binds differently from the sulphonamides, as its OH moiety is anchored to the zinc-coordinated water molecule<sup>54</sup>. Using phenol as lead, a library of simple and more complex natural product (NP) phenols

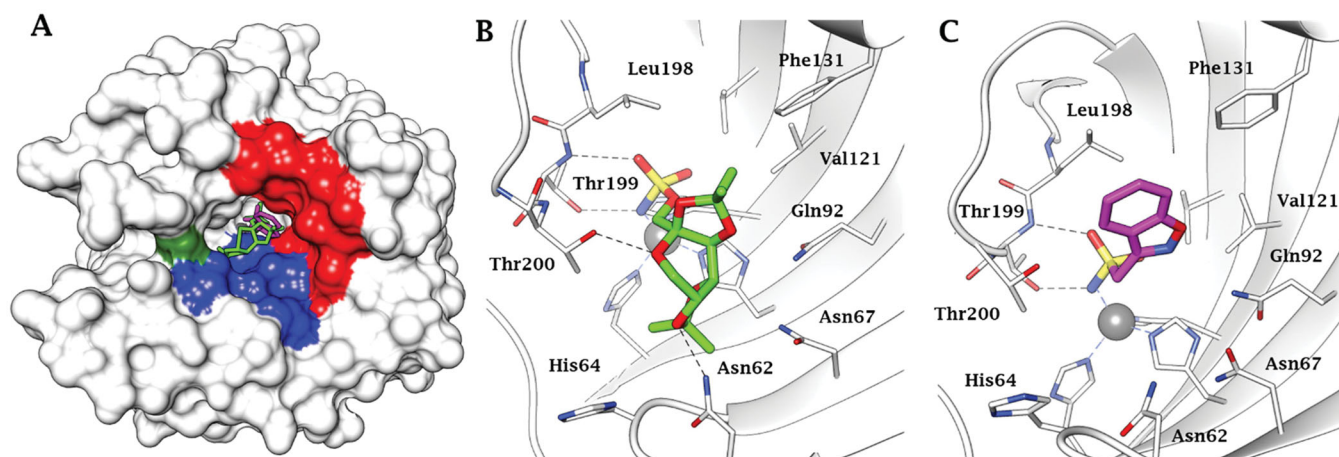
of types **1–13** have been screened (Figure 4) as inhibitors of hCA I, II (as offtargets) and hCA VA and VB (as target enzymes). Many of these derivatives were micromolar hCA I and II inhibitors, whereas acting more effectively as inhibitors of hCA VA/VB, with K<sub>s</sub> in the range of 90–105 nM<sup>58</sup>.

Gidaro et al.<sup>59</sup> screened a library of NPs including polyphenols, flavones and some of their glycosides, for the inhibition of several CA isoforms, including hCA VA (Figure 5). Many of these derivatives acted as low micromolar hCA VA inhibitors (but they also inhibited isoforms hCA I, IX and XII), and the most effective mitochondrial CA inhibitors were detected to be apigenin **14** (K<sub>i</sub> of 0.30 μM) and eriocitrin **16** (K<sub>i</sub> of 0.15 μM). In the same work a computational study has been performed for the binding of **16** to murine (m) mCA VA (for which a truncated X-ray crystal structure is available<sup>54</sup>) which led to the proposal that this flavone coordinates with its catecholic system to the zinc ion from the enzyme active site<sup>59</sup>. This binding mode is however not much plausible, considering that catechols were recently shown by X-ray crystallography to anchor to the zinc-coordinated water and to the deep water molecule within the active site of hCA II<sup>60a</sup>. Some natural product polyphenols isolated from blueberries and their glycosides were also investigated as mitochondrial CA inhibitors and showed micromolar inhibitory action<sup>60b</sup>.

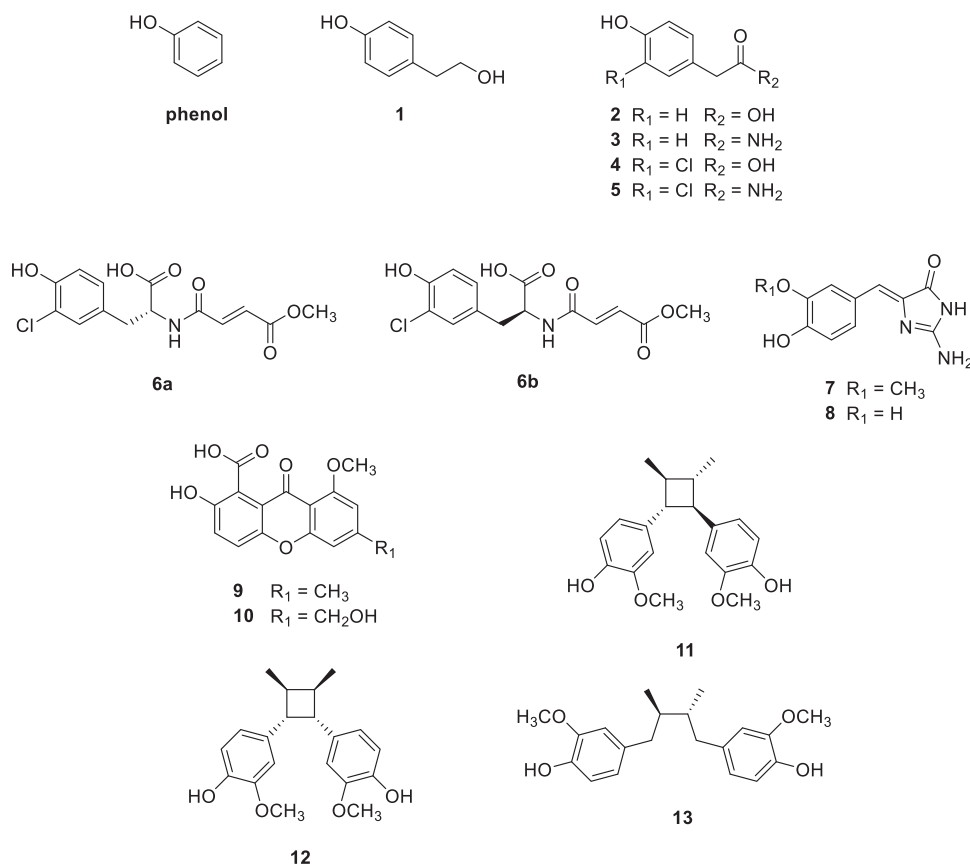
In another study, Costa et al.<sup>61</sup> examined NP present in essential oils isolated from natural sources, of types **24–33** for the inhibition of hCA I, II and VA (Table 2). The tropolone **24** was thus reported as a new chemotype with CA inhibitory properties, being proposed (by using computational data) that it coordinates bidentately with its CO-CHOH moiety to the zinc ion from the enzyme active site. In this case, X-ray crystallography of a simple tropolone confirmed the proposed binding mode<sup>62</sup>. 2-Hydroxyisobutyric acid **25** and 3Z-nonenic acid **29** were the most effective hCA VA inhibitors detected in that study, with K<sub>s</sub> < 5 μM and also showing selectivity for inhibiting hCA VA over the dominant and widespread offtarget isoform hCA II<sup>61</sup>.

Virtual screening (VS) procedures were also applied by Alcaro's group<sup>63,64</sup> in order to detect antiobesity compounds based on the inhibition of hCA VA. A library of 93522 compounds was used in a VS procedure, which led to 12 hits, which were thereafter tested experimentally for inhibition of hCA I, II and VA<sup>63</sup>. Among the obtained hits were the anticancer drugs fludarabine **34**, lenvatinib **35**, the antiepileptic rufinamide **36** (micromolar inhibitors, K<sub>s</sub> of 130–344 nM), as well as the homovanillic acid sulphate **37** and the desacetyl metabolite of the antibacterial cephalosporin **38**, which were active in the nanomolar range against hCA VA, with no inhibition at all of hCA I and II (Figure 6).

These compounds were docked within the murine (m) enzyme mCA VA active site in the same study, but the binding poses obtained were not yet validated by X-ray crystallography. However, they may provide interesting hints for the drug design of novel CA VA inhibitors belonging to new chemotypes compared to the well known sulphonamides, sulfamates or phenol derivatives. Acipimox **39**, a nicotinic acid derivative in clinical use



**Figure 3.** (A) hCA II complexed with superimposed topiramate (PDB 3HKU) in green, and zonisamide (PDB not deposited, available from the authors<sup>52</sup>) in magenta. The Zn(II) is shown as a grey sphere that is bound to the protein ligands His94, His96 and His119. The hydrophobic half of the active site is coloured in red, the hydrophilic one in blue. His64, the proton shuttle residue, in green. Active site ribbon view of hCA II in adduct with B) TPM and C) ZNS. H-bonds are represented as black dashed lines. Active site ribbon view of hCA II in adduct with B) topiramate and C) zonisamide. H-bonds are represented as black dashed lines.



**Figure 4.** Phenol and NP based phenols screened as hCA VA/VB inhibitors.

for the treatment of hyperlipidaemia (Figure 6) was also reported recently<sup>65</sup> to act as low micromolar hCA VA/VB inhibitor. By using computational methods, the same study suggested that acipimox coordinates through its carboxylate moiety to the zinc ion from the CA active site<sup>65</sup>.

### 3.3. De novo drug design

Several studies explored the *de novo* drug design of hCA VA/VB inhibitors, mainly belonging to zinc binders (sulphonamides and

their isosteres<sup>54</sup> by using the tail approach, which consists in attaching moieties (tails) on the scaffold of the CALs in such a way as to permit the contact with the more external parts (entrance to the cavity and its rim) of the CA active site, where the amino acid composition between the various isoforms has the highest variability<sup>66,67</sup>.

One of the first such studies<sup>68</sup> examined a series of 46 aromatic and heterocyclic sulphonamides, many of which were simple derivatives of benzenesulfonamide and 1,3,4-thiadiazole-5-sulfonamide for the inhibition of mCA VA and hCA I, II and IV (as offtargets). Some of the best inhibitors (in the nanomolar



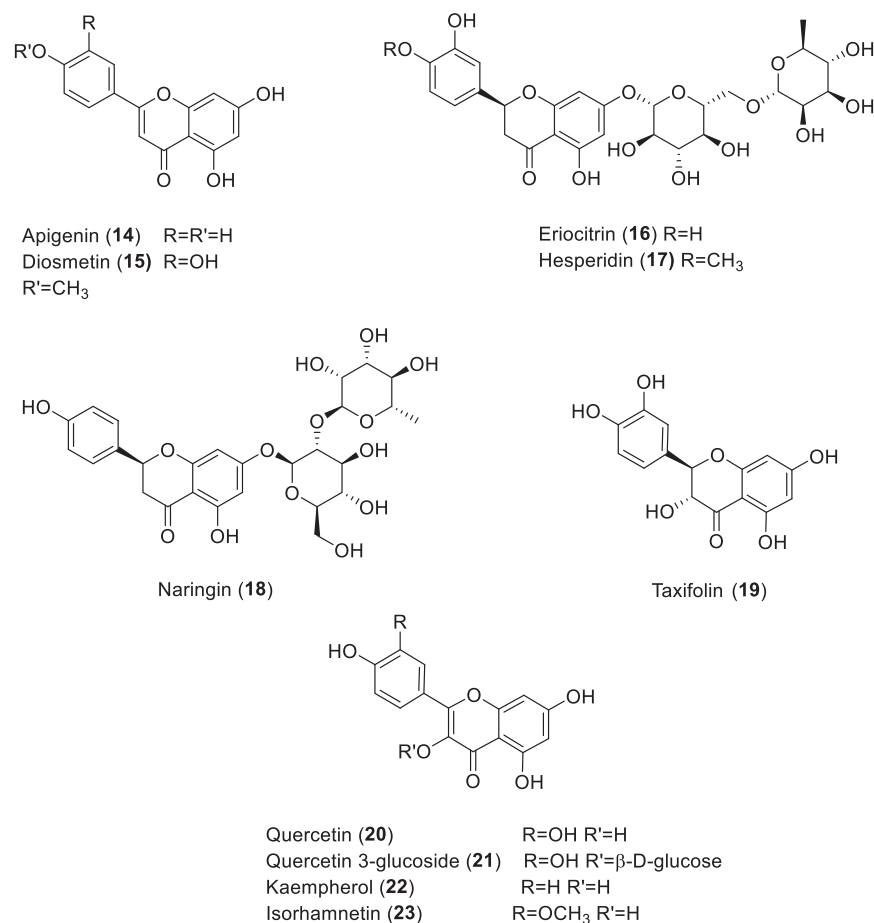


Figure 5. NPs screened as hCA VA/VB inhibitors.

range, typically  $K_i$ s of 6–95 nM) incorporated the benzenesulfonamide head and acylamido or ureido tails on the sulphanilamide scaffold, which in turn possessed alkyl or aryl moieties. Although this series was not tested for the inhibition of hCA VA/VB (but only the murine isoform VA), the leads detected were useful for the next generation hCA VA/VB inhibitors that were reported thereafter. Indeed, Guzel et al.<sup>69</sup> reported a large series of aromatic/heterocyclic sulphonamides incorporating phenacetyl, pyridylacetyl and thienylacetyl tails of types 40–44 (Table 3), some of which were among the most effective and isoform V-selective sulphonamides discovered to date.

It can be seen that these sulphonamides showed low nM inhibitory action against both mitochondrial isoforms ( $K_i$ s of 6.1–10.2 nM) and they were also employed by Arechederra et al.<sup>26</sup> to investigate the metabolic fluxes in the presence of hCA VA/B inhibitors, as mentioned earlier in this review. Although these sulphonamides also inhibit the cytosolic hCA I and II (data not shown), their  $K_i$ s against these isoforms are at least an order of magnitude higher compared to the same parameters measured for hCA VA/B<sup>69</sup>.

Smaine et al.<sup>70</sup> reported a small series of 2-substituted-1,3,4-thiadiazole-5-sulfamides 45 which showed inhibitory activity against hCA I, II and IV ( $K_i$ s in the high nanomolar to the micromolar range) but were low nanomolar inhibitors of hCA VA/VB, with inhibition constants in the range of 4.2–32 nM and 1.3–74 nM, respectively. The most effective derivatives incorporated as R the groups CF<sub>3</sub> ( $K_i$  of 7.3 nM for hCA VA and of 3.9 nM against hCA VB) and 4-bromophenyl ( $K_i$  of 4.2 nM for hCA VA and of 4.5 nM against hCA VB)<sup>70</sup> – see Figure 7.

Poli et al.<sup>71</sup> reported a series of *N*-aryl-*N'*-ureido-*O*-sulfamates of types 46–48 (Figure 7) and tested them against the two mitochondrial CA isoforms hCA VA and VB. The results revealed an interesting selectivity profile, especially against hCA VB over the VA, observed for all the analysed compounds. For one derivative 46o ( $K_i$  against hCA VA >10 μM,  $K_i$  against hCA VB of 515 nM), molecular modelling studies highlighted the importance of amino acid residues which are diverse at the entrance of the cavity between the two mitochondrial isoforms, which substantially influenced the tail orientation of the inhibitor, its interaction with the enzymes and were reflected on the potency of the inhibitor against the mitochondrial CAs<sup>71</sup>.

Maresca and Supuran<sup>72</sup> reported a series of (R)- and (S)-10-camphorsulfonyl-substituted aromatic/heterocyclic sulphonamides of types 49 and 50 which showed effective (usually low nanomolar efficacy) for inhibiting hCA VA/VB and having less affinity for hCA I and II. The (R) enantiomers were generally more effective as mitochondrial CA inhibitors over the corresponding (S) enantiomers<sup>72</sup>. Poulsen et al.<sup>73</sup> reported triazole-sulphonamides 51 obtained by click chemistry and incorporating various groups on the second aromatic ring (Figure 8), which showed effective, nanomolar inhibition of hCA VA and VB, but also potently inhibited hCA II, being thus non-selective inhibitors.

#### 4. Conclusions and future developments

Starting from the observations that clinically used antiepileptics (topiramate, zonisamide) or diuretics (acetazolamide) induce weight loss in obese patients, as well as reduction of blood

**Table 2.** Structures of compounds 24–33 screened for the inhibition of hCA I, II and VA.

Compound	$K_i$ ( $\mu\text{M}$ )		
	hCA I	hCA II	hCA VA
24	4.98	90.60	7.50
25	5.18	>100	3.80
26	3.44	85.40	7.38
27	8.27	>100	8.36
28	3.75	>100	7.83
29	6.09	>100	4.52
30	8.81	18.90	9.07
31	5.06	3.71	43.10
32	6.82	>100	9.69
33	4.89	>100	44.00

The traditional names of the compounds are: **24:**  $\beta$ -thujaplicin; **25:** 2-hydroxyisobutyric acid; **26:** 4-isopropylbenzoic acid; **27:** methyl geranate; **28:** 3-phenylpropyl benzoate; **29:** 3Z-nonenic acid; **30:** Z-geranic acid; **31:** 2-methylhexanoic acid; **32:** ferulic acid; **33:** 5-methylfuran-2-carboxylic acid.

glucose levels, a rationale was searched for explaining this effect, which concentrated on the fact that these compounds are effective inhibitors of several CA isoforms, including the mitochondrial ones, hCA VA and VB, known to be involved in DNL and fatty acids biosynthesis. It should be stressed here that these drugs do not induce mitochondrial toxicity as erroneously stated

by some reviewers of this manuscript when it has been submitted in another journal to which I refuse to continue to contribute due to conceptual errors and misunderstanding during the reviewing process. Although the two antiepileptic drugs mentioned here possess a complicated polypharmacology, topiramate in combination with phentermine was approved by FDA in 2012 for a second use in the management of obesity, whereas there are clinical trials on the similar use of zonisamide, alone or in combination with other agents (bupropion, metformin)<sup>49,50</sup>. However, the side effects due to the non-selective inhibition of the target CA isoforms and the polypharmacology of these drugs, makes them effective but scarcely used antiobesity agents.

As a consequence, drug design studies have emerged in the last two decades for the design of potent and isoform-selective hCA VA/VB inhibitors. The main strategies which have been used consisted (in addition to the drug repurposing just mentioned) in the screening (experimentally or by using VS) of large libraries of synthetic and natural products, which afforded interesting and somehow unexpected hints, such as for example the tropolones (a new chemotype with CA inhibitory properties)<sup>61</sup>, several flavones, polyphenols and their glycosides<sup>59</sup>, but also clinically used drugs, such as fludarabine, lenvatinib, rufinamide, etc.<sup>63</sup>. The classical drug design studies for obtaining effective and isoform-selective hCA VA/VB inhibitors concentrated on the other hand on the classical sulphonamide/sulfamate/sulfamide chemotypes<sup>66–73</sup>. However, a rather limited number of such studies has been reported to date, and although several highly effective compounds were detected, detailed pharmacological studies which prove their efficacy in the inhibition of DNL and in the promotion of weight loss in experimental models of obesity, are unfortunately lacking.

The lack of X-ray crystal structures of isoforms hCA VA/VB<sup>53,54</sup> was detrimental to the structure-based drug design of inhibitors targeting these isoforms, although several homology models and other computational studies addressed this problem<sup>61,63,64,71</sup>. In some cases<sup>61</sup> the proposed binding modes of inhibitors such as the tropolones were confirmed by crystallography<sup>62</sup>, whereas in other cases, such as for some catechol natural products<sup>59</sup> the crystallography<sup>60</sup> did not confirm the binding poses obtained by computational techniques. One of the detailed such studies, by Tuccinardi's group<sup>71</sup> for sulfamate CAIs analysed in detail the selectivity profile of compounds which potently inhibited hCA VB and were weak or ineffective as hCA VA inhibitors, revealing the amino acid residues at the entrance of the active site cavity involved in binding. With all these limitations, the field of CA VA/B inhibitors with potential anti-obesity activity made relevant progress in the last decade since the last review in the field was published<sup>12</sup>. Nowadays there are many classes of such potent and also selective mitochondrial CA inhibitors, apart the sulphonamides and sulfamates reported earlier, which strengthen the rationale of using of topiramate and zonisamide as anti-obesity agents, alone or in combination with other drugs, with all their limitations mentioned above due to side effects correlated or no with offtarget CA inhibition<sup>40–42</sup>.

Although a highly innovative procedure for evaluating the metabolic fluxes in the presence of mitochondrial CA inhibitors has been reported already in 2013<sup>26</sup>, which made use of electrode wired mitochondria, this type of experiments were performed only with a limited number of sulphonamide CAIs, which however, conclusively showed that the pyruvate, succinate and overall cell metabolism undergoes significant changes when these two enzymes are inhibited (without mitochondrial toxicity,

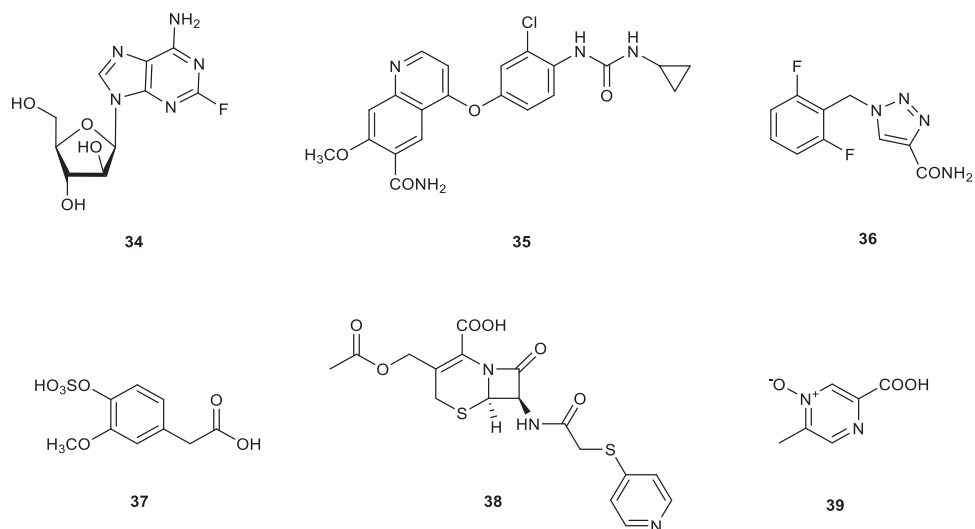


Figure 6. hCA VA inhibitors 34–38 identified by VS techniques, and acipimox 39, identified by classical screening procedures.

Table 3. Benzenesulfonamides and 1,3,4-thiadiazole-sulphonamides acting as low nanomolar hCA VA and VB inhibitors<sup>69</sup>.

Compound	n	X	K <sub>i</sub> (nM)	
			hCA VA	hCA VB
40	0	–	7.2	7.0
41	0	Cl	7.7	8.6
42	1	–	9.1	7.2
43	2	–	10.2	8.0
44	–	–	8.4	6.1

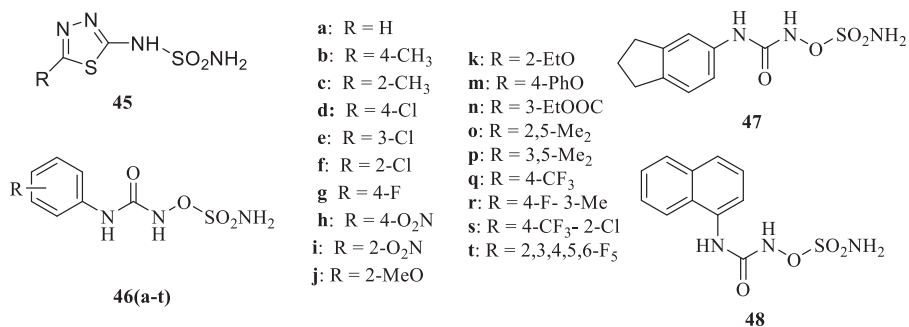


Figure 7. Sulfamides 45 and sulfamates 46–48 reported as hCA VA/VB inhibitors.

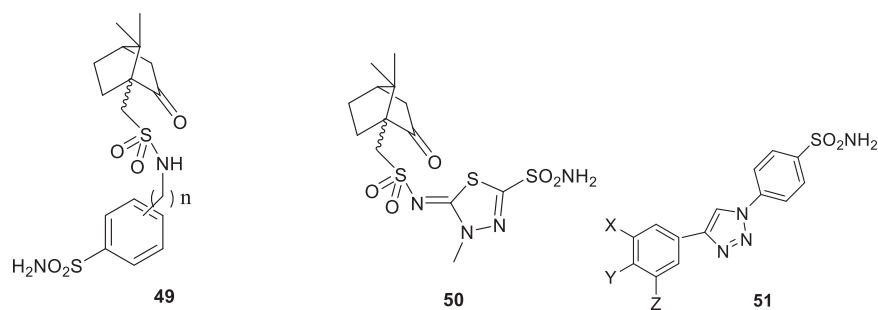
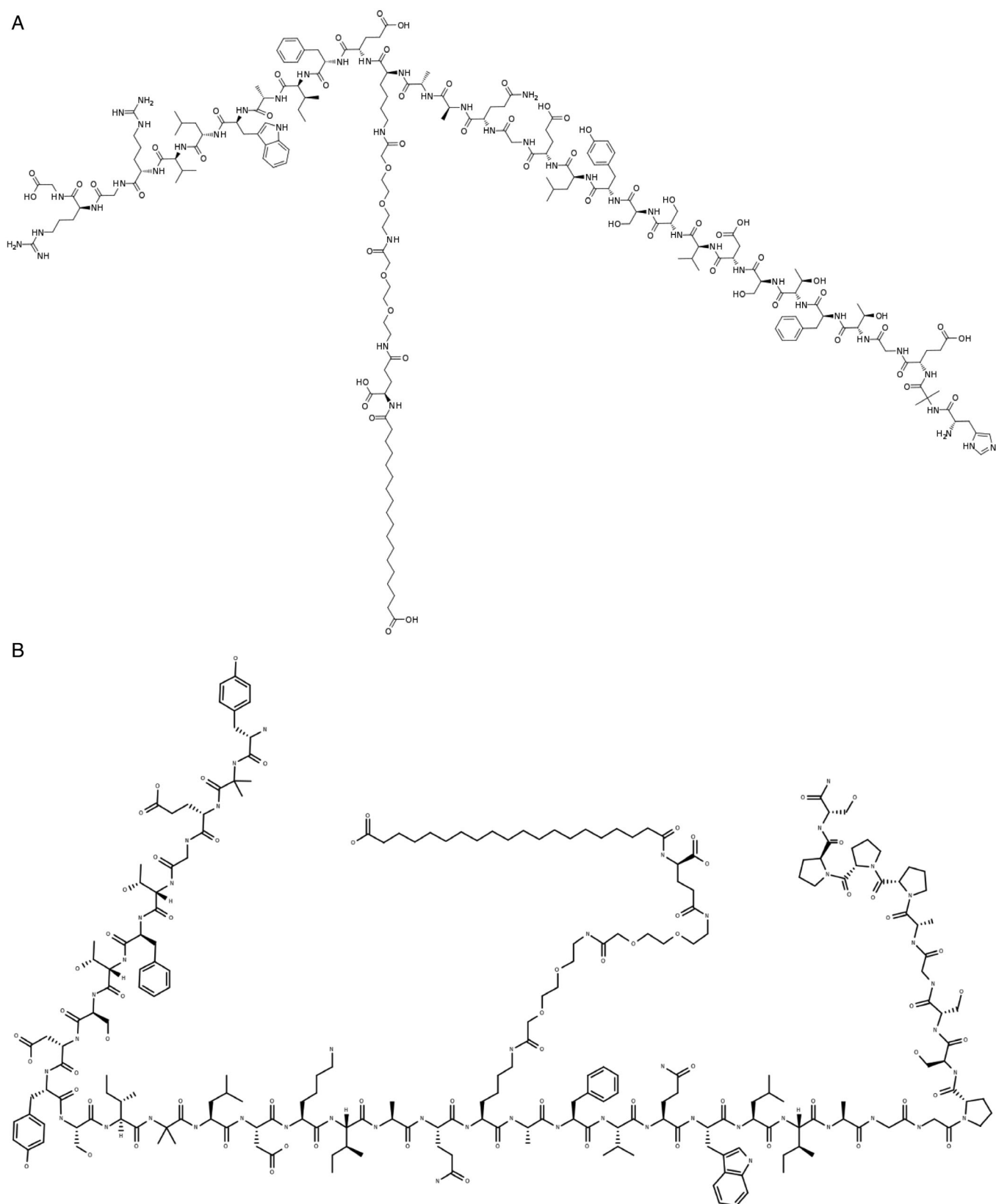


Figure 8. Aromatic/heterocyclic sulfonamides 49 and 50 incorporating 10-camphorsulfonyl tails. In derivatives 49,  $n = 0, 1$  and 2. Triazole-sulphonamides 51 incorporate X, Y Z groups of the type H, F, Me, OMe, CF<sub>3</sub>, SO<sub>2</sub>NH<sub>2</sub>.



**Figure 9.** Chemical structures of Semaglutide (A), and Tirzepatide (B), recently approved anti-obesity agents.

I stress this again). This type of measurements would be desirable for other classes of newly identified CA VA/B inhibitors, prior to *in vivo* anti-obesity experiments, in animal models of the disease, which are more expensive, as well as time- and resource consuming.

CA VA/B inhibition on the other hand might be beneficial for the management of other diseases for which few therapeutic options are available. For example, several groups<sup>74,75</sup> demonstrated that the elevated glucose-induced mitochondrial respiration and formation of reactive oxygen species (ROS), typical of



type II diabetes, has been reversed by pharmacological inhibition of mitochondrial CAs with topiramate, in a mouse cerebral pericytes model of the disease. The connection between type II diabetes and obesity is even closer, considering that two of the recently approved anti-obesity agents, semaglutide and tirzepatide<sup>76</sup> – Figure 9, were initially licenced for the management of type II diabetes.

Both drugs act like human glucagon-like peptide-1 (GLP-1) mimics, increasing insulin secretion and sugar metabolism. GLP-1 and the glucose-dependent insulinotropic polypeptide (GIP) are hormones involved in blood sugar control. A thought-provoking idea would be to combine this type of novel anti-obesity agents with CA VA/VB inhibitors, either as a combination therapy or by including CA inhibitory moieties in the oligopeptide structure, in order to obtain hybrids with dual action on GLP-1 as well as the two mitochondrial CA enzymes.

Overall, the field of antiobesity agents based on mitochondrial CA inhibitors achieved a certain level of maturity in the last decade, although many critical issues remain to be solved, among which more detailed pharmacological studies of the potent inhibitors already detected, a simpler method to screen compounds that interfere with the mitochondrial metabolic fluxes than the wired mitochondria one mentioned here, and last but not least, the conservatory and old fashioned idea of some scientists that CAls are “boring drugs” that have many side effects and should not be investigated for other applications than as diuretics. Probably in the 80s this could be even understood, as few sulphonamide pan-inhibitors CAls were known, but the progress over that last two decades in the field<sup>7</sup> demonstrated that there are many additional CA inhibition mechanisms and a wealth of new inhibitory chemotypes which are devoid of the many side effects of first and second generation such drugs.

## Disclosure statement

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