MEDICINAL RESEARCH REVIEWS

Therapeutic Applications of Glycosidic Carbonic Anhydrase Inhibitors

Jean-Yves WINUM1*, Sally-Ann POULSEN2, Claudiu T. SUPURAN3*

1 Institut des Biomolécules Max Mousseron (IBMM) UMR 5247 CNRS-UM1-UM2 Bâtiment de Recherche Max Mousseron, Ecole Nationale Supérieure de Chimie de Montpellier, 8 rue de l’École Normale, 34296 Montpellier Cedex, France.
2 Griffith University, Eskitis Institute for Cell and Molecular Therapies, Don Young Road, Nathan, Qld 4111, Australia
3 Università degli Studi di Firenze, Polo Scientifico, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, 50019 Sesto Fiorentino (Florence), Italy.

*Correspondence authors. Tel: +33-467147234; Fax: +33-467144344;
E-mail: jean-yves.winum@univ-montp2.fr (JYW); claudiu.supuran@unifi.it (CTS)
Abstract:

The zinc enzymes carbonic anhydrases (CAs, EC 4.2.1.1) are very efficient catalysts for the reversible hydration of carbon dioxide to bicarbonate and hence play an important physiological role. In humans, 16 different isozymes have been described, some of them being involved in various pathological disorders. Several of these isozymes are considered as drug targets, and the design of selective inhibitors is a long-standing goal that has captured the attention of researchers for 40 years and has led to clinical applications against different pathologies such as glaucoma, epilepsy, and cancer. Among the different strategies developed for designing selective CA inhibitors (CAIs), the “sugar approach” has recently emerged as a new attractive and versatile tool. Incorporation of glycosyl moieties in different aromatic/heterocyclic sulfonamide/sulfamides/sulfamates scaffolds has led to the development of numerous and very effective inhibitors of potential clinical value. The clinical use of a highly active carbohydrate-based CA inhibitor, i.e., topiramate, constitutes an interesting demonstration of the validity of this approach. Other carbohydrate-based compounds also demonstrate promising potential for the treatment of ophthalmologic diseases. This review will focus on the development of this emerging sugar-based approach for the development of CAIs.

Keywords: carbonic anhydrase, enzyme inhibitors, carbohydrate, glycoconjugate, drug design.
1. Introduction:

Carbohydrates constitute an important class of biomolecules. They are involved in various biological/biochemical processes such as cell recognition, cell-cell interaction and adhesion, immune system regulation and fertilization among others.[1] Development of glycobiology has opened a promising area in carbohydrate-based drug design and drug delivery systems. This “glyco” strategy is currently a major focus in medicinal chemistry and has already contributed to the discovery of lead candidates with anti-infectious, anti-inflammatory or anti-cancer activity.[2-4]

In development of efficient enzyme inhibitors, the use of carbohydrate scaffolds in the design of novel compounds has proven to be a successful approach especially in the case of glycosidase inhibitors.[3] The incorporation of carbohydrate scaffolds within the design of CA inhibitors (CAIs) has been recently proposed by our groups, and now constitutes one of the most attractive ways to develop new generations of effective and selective inhibitors.

α-Carbonic anhydrases (CAs, EC 4.2.1.1) are zinc metalloproteins of which 16 different CA isozymes or CA-related proteins (CARP) are presently described in higher vertebrates including humans each differing by their relative hydrase activity, their subcellular localization and their susceptibility to inhibition. CAs catalyze a very simple physiological reaction — the conversion of carbon dioxide to the bicarbonate ion and protons, but considering the relevance of these three chemical entities (CO₂, HCO₃⁻ and H⁺) for physiological processes, this is a critical reaction in all life kingdoms. As a consequence, there are a large number of gene families encoding CAs all over the phylogenetic tree, whereas in vertebrates, including humans, a rather large number of isoforms are present and involved in a host of physiological processes. Indeed, there are five cytosolic forms (CA I-III, CA VII and CA XIII), five membrane-bound isozymes (CA IV, CA IX, CA XII, CA XIV and CA XV), two mitochondrial forms (CA VA and CA VB), as well as a secreted CA isozyme (CA VI)
described so far. In addition to the established role of carbonic anhydrase inhibitors (CAIs) as diuretics and antiglaucoma drugs, it has recently emerged that CAIs could have potential as novel anti-obesity, anticancer and anti-infective drugs. Physiological and pathological processes in which some CA isoforms are involved include among others gluconeogenesis, lipogenesis, ureagenesis, tumorigenicity and the growth and virulence of various pathogens. The modulation of the activity of these enzymes by inhibition with small molecules has gathered considerable momentum in the last few years. Much progress has been achieved in the design and synthesis of CAIs by means of rational drug design and many derivatives belonging to different classes of compounds (sulfonamide, sulfamide and sulfamate) are now known [4-7]. However, a hurdle related to this class of drugs is represented by the lack of selectivity of most clinically used sulfonamides/sulfamates (25 such compounds are known up until now [4]) for the inhibition of any of the 13 catalytically active isoforms involved in these physiologic/pathologic processes.

From earlier studies, three major basic structural elements have emerged as crucially important to the CA recognition pharmacophore [7]. The first one is the zinc binding function (ZBF) which interacts with the active site metal ion, and the residues Thr199 and Glu106, conserved in all CAs belonging to the $\alpha$-class. The second one is the organic scaffold, usually an aromatic or heteroaromatic moiety, which interacts both with the hydrophobic as well as the hydrophilic halves of the active site. The third one is a “tail” attached to the organic scaffold. Variations in the nature of the ZBF have been thoroughly investigated in the design of CAIs, and have been the subject of several interesting, recent reports.[8-13] Many studies have also focussed on the organic scaffold and tail moiety modifications of inhibitors to obtain tighter binding and eventually isozyme specific CAIs. Two general but complementary approaches have been developed during the last decade for the design of effective CAIs [6-7]:

(i) the “ring” approach, which consists of exploring a great variety of ring systems (aromatic
or heteroaromatic) to which a sulfonamide ZBF is attached, and used for the discovery of the
topical antiglaucoma agents dorzolamide and brinzolamide. (ii) the “tail” approach which
consists of attaching different tails to the scaffolds of well known aromatic/heterocyclic
sulfonamides in order to modulate the physicochemical properties such as for example water
solubility and enzyme binding capacity of these pharmacological agents. By applying the
latter approach, a large number of novel CAIs incorporating many types of new tails have
been generated. In this way, several categories can be distinguished within the tail approach
(i) substituted amino derivatives, in which tails introduced in the structure of the inhibitors
contains protonable amino groups belonging to amino acids (such as glycine, β-alanine,
GABA, sarcosine, creatine, etc.) (ii) cationic derivatives, which lead to the development of a
new class of positively charged membrane impermeant sulfonamides. This category, which is
based on the attachment of substituted-pyridinium moieties to the scaffold of
aromatic/heterocyclic sulfonamides, was used for the selective in vivo inhibition of membrane
bound versus cytosolic CA isozymes. A large variety of efficient inhibitors have been
generated by applying these approaches and lead compounds have been described with
potential biomedical applications. This particularly active research field has been subject to
various reviews [4, 7, 14-16].

A new class of CAIs has recently emerged – the glycoconjugate CAIs. While these
compounds can be incorporated into the general approaches described above and they
represent a new approach that we name the “sugar-approach”. This approach consists of the
use of a carbohydrate moiety, either directly attached to the ZBF of the inhibitors, or as a tail
group for inhibitors. The present review will discuss this sugar approach and summarize the
potential development of glycoconjugate CAIs in therapy.
2- Carbonic anhydrase inhibitors with carbohydrate scaffold: topiramate and their derivatives.

Topiramate 1 (TPM) is a marketed broad-spectrum antiepileptic drug (AED) with a well established efficacy as monotherapy or adjunctive therapy in the treatment of epilepsy in adult and paediatric patients. [17] Its potent anticonvulsant effects is due to multiple and complex mechanisms of action. Its pharmacological actions are diverse and include: (i) reduction of epileptiform discharges through a voltage-dependent block of Na(+) channels, (ii) enhancement of the activity of GABAergic transmission at some subtypes of gamma-aminobutyrate receptors, (iii) antagonism of non- N-methyl-D-aspartate (NMDA) glutamate receptors and (iv) inhibition of carbonic anhydrase. The effect of TPM on GABAergic transmission is supposed to be directly related to a decreased of the intracellular bicarbonate concentration, which may be caused by an inhibition of neuronal CA. [18-19]

Topiramate, designated chemically as 2,3:4,5-di-O-isopropylidene-β-D-fructopyranose sulfamate, is particularly interesting from a structural point of view because it is derived from a monosaccharide and bears a sulfamate functional group that is considered to be responsible for its anticonvulsant properties. The CA inhibitory effect of TPM may play an important role in the anticonvulsant activity as well as in the reported clinical side effects which might be encountered in patient treated with this drug (weight loss in human and animals [20] and nephrolithiasis risk [21] among others).

The reported CA inhibition data of TPM appear rather controversial. Initially published as a millimolar inhibitor by Maryannoff et al. [22], the inhibition constant permanently decreased in the next 20 years in each of the publications from this group, with a submicromolar activity against CA II being reached in the last published works [23,24]. In the meantime our group demonstrated that TPM is a very potent (low nanomolar) inhibitor of several CA isozymes such as CA II, VA, VB, VI, VII, IX, XII and XIII, and a medium potency (micromolar)
inhibitor of CA I, CA IV and XIV [25] (See Table 1). TPM is also a very weak CA III inhibitor, as most sulfonamides and sulfamates [25]. Differences observed in inhibition data between our and Maryannoff’s reports may be attributed to limitations in their enzyme assays used to test inhibitors, as rather high amounts of reducing agents were present in their buffer [26]. The carbon dioxide hydration method as used by ourselves [27] consists of monitoring the physiologic reaction of CAs using a stopped-flow apparatus or similar equipment, and is the most appropriate and precise assay for evaluating CA inhibitors possessing a wide inhibition range, from milli- to subnanomolar ones [7]. By contrast the 4-nitrophenyl acetate esterase method [28] (using 4-NPA as CA substrate), whilst technically the most simple assay method, is not the method of choice as CAs are not a very efficient esterases. The thermofluor method employed more recently by Maryannoff et al. is also not yet validated for assaying CA inhibitors since it has been tested only on a very restricted number of compounds, and the rank of inhibition obtained for example with dichlorophenamide by Matulis et al. [29] is completely different as compared to all others method reported so far.

**Table 1 here**

The kinetic binding assay measurements reported in Table 1 have been validated by the resolution of X-ray crystal structures of TPM 1 [29], RWJ-37947 2 (the cyclic sulfate analogue of TPM) [30] and of the topiramate sulfamide analogue 3 [25] in adducts with hCA II (Fig. 1).

**Figure 1 here**

Both sugar sulfamate derivatives 1 and 2 bind with their deprotonated sulfamate moiety to zinc(II) ion, leading to a tetrahedral geometry of the metal ion (which is also coordinated by three imidazole residues of His94, 96 and 119, conserved in all α-CAs) [4]. Both compounds make two hydrogen bonds with the side chain oxygen atom of Thr199 and the backbone NH
nitrogen atom of the same residue. The hydroxyl group of Thr199 is involved in an additional hydrogen bond with Glu106. In this way, the hydroxyl moiety of Thr199 acts as a hydrogen-bond acceptor for inhibitor bound to the metal ion present in the active site (but also increase the nucleophilicity of the zinc hydroxide present in the uninhibited enzyme [4]). In the case of Topiramate, the X-ray crystal structure revealed a very tight association between bound inhibitor and the enzyme active site, and a network of eight strong hydrogen bonds with critical amino acids side chains from the cavity fixing the inhibitor within the active site in addition to the zinc coordination, consistent with TPMs very potent inhibitory activity against CA II (Ki of 5nM) and similar isozymes – see Table 1. [30] Compound 1 also participates in several hydrogen bonds with amino acid side chains in a hydrophilic binding pocket (Asn67, Gln92) and to a water molecule that donates a hydrogen bond to Thr200 (Fig. 2A). Contrary to topiramate 1, the structurally related cyclic sulfate RWJ-37947 2, adopts a totally different conformation when bound to the CA II active site. As compared to topiramate 1, the ring systems of 2 are rotated by approximately 180°. Surprisingly, the cyclic sulfate moiety points toward the hydrophobic pocket of the enzyme, interacting with residue Phe131, Pro202 and Leu198, whereas the isopropylidene moiety is oriented towards the amide groups side chains of Asn67, Asn62 and His94. RWJ-37497 was reported to have an IC50 of 36 nM against CA II by Maryanoff’s group [31]. Detailed modelling studies of the binding mode of compounds 1 and 2 to CA II have been performed by Klebe et al. [32].

**Figure 2 here**

Previous studies demonstrated that the sulfamide moiety is also a suitable ZBF for the design of CAIs. [9-12] Our group reported recently the X-ray crystal structure of the topiramate sulfamide analogue 3 in complex with hCA II. Compound 3 was revealed to be roughly 210
times a weaker hCA II inhibitor as compared to topiramate 1. It is also a rather weak hCA I, IX and XII inhibitor too (affinity in the micromolar range, see also Table 1) but shows nanomolar inhibitory activity against other CA isoforms such as CA VA, VB, VII and XIII. The high resolution X-ray structure of the hCA II – 3 adduct, reveals tight binding within the active site, experienced by Zn(II) ion coordination through the deprotonated sulfamide moiety. Moreover the organic scaffold participates in an extended network of hydrogen bonds with Thr199, Gln92, His94, Asn62 and Thr200. Its binding to hCA II is also similar to that of topiramate 1 except that an important clash between the 8-methyl moiety of 3 and the methyl group of Ala65 has been evidenced (see Fig. 3).

Figure 3 here

This result supports our assay results and provides rationale for the possibility to obtain CAIs with diminished affinity for hCA II while still maintaining tight binding for other isoforms. We propose that newly designed CAIs that exploit the unfavourable interaction between the methyl group of Ala65 (which is unique to CA II among many mammalian CA isoforms described up to now) could lead to compounds possessing fewer side effects owing to improved selectivity over this physiologically dominant isoform.

In another recent study [33], we report the synthesis of a series of derivatives incorporating the protected fructopyranose moiety present in topiramate 1. This tail has been attached to the scaffold of aromatic/heterocyclic sulfonamides by means of the thioureido functionality, shown earlier to lead to potent CAIs belonging to several classes. The new derivatives (compounds 4-10) were active against hCA II (Kᵦs ranging from 6 to 750 nM) and hCA VII (Kᵦs ranging from 10 to 79 nM). Compounds 5 and 9 showed anticonvulsant properties in the MES test performed in mice. Intraperitoneal injected 2 hours prior to induce convulsions, the
fluorosulfanilamide derivative (5, 50 mg/kg) showed a more efficient anticonvulsant activity than topiramate 1 (87.5% and 69.7% respectively). Compound 5 is also characterized by the best inhibitory potency against the hCA VII ($K_I = 10$ nM) but lower than topiramate 1 ($K_I = 0.9$ nM) and acetazolamide ($K_I = 2.5$ nM).

**Figure 4 here**

These derivatives were also efficient inhibitors of the transmembrane isoforms hCA IX ($K_I$s of 10–4500 nM), especially compounds 7 ($K_I = 10$ nM) and 9 ($K_I = 11$ nM) (Fig. 4).

3- Glycoconjugated carbonic anhydrase inhibitors.

The design of CA-selective and isozyme-specific CAIs constitutes a field extensively investigated by several research groups and much progress which has been made though modification of the inhibitors scaffold structure. The recent alternative approach for the design of topically-acting antiglaucoma CA inhibitors consists of attaching carbohydrate tail moieties, that should induce the desired physicochemical properties such as for example water solubility, good penetrability through the cornea etc., to scaffolds of aromatic/heterocyclic sulfonamides.

In 2004 a small series of 4-sulfamoylphenylglycopyranosylamines were synthesized and reported as effective hCA II inhibitors [34]. These compounds showed inhibition constants in the range of 510–1200 nM against CA I and 10–25 nM against CA II, similarly to clinically used sulfonamides, such as acetazolamide, methazolamide, dichlorophenamide, dorzolamide and brinzolamide.

$N$-(4-sulfamoylphenyl)-$\alpha$-D-glucopyranosylamine 11 (Fig. 5) was shown to be a promising anti-glaucoma agent with topical activity in an animal model of the disease with
maximal intraocular pressure (IOP) lowering in the range of 10–16 mm Hg after administration directly into the eye, a high water solubility at neutral pH and a much longer duration of action compared to standard drug such as dorzolamide or brinzolamide.

**Figure 5 here**

The high resolution X-ray crystal structure of the \(N\)-(4-sulfamoylphenyl)-\(\alpha\)-D-glucopyranosylamine 11 with the target isoform involved in glaucoma, i.e., CA II, was also reported by this group [35]. The crystal structure demonstrated that the sulfonamide zinc binding anchor and the phenyl ring of the inhibitor bound in the expected way: coordinated to the metal ion, and filling the channel of the enzyme cavity, respectively. The glycosyl moiety, responsible for the high water solubility of the compound, was oriented towards the hydrophilic region of the active site, where other inhibitors were never observed to be bound up to now. A relay of seven hydrogen bonds with four water molecules and the amino acid residues Pro201, Pro202 and Gln92 further stabilized this enzyme–inhibitor adduct. [36]

Recently we reported the synthesis of a series of glycosyl-thioureido sulfonamides and their inhibitory activity against four physiologically relevant CA isoforms, the cytosolic, ubiquitous hCA I and II as well as the transmembrane, tumor associated hCA IX and hCA XII. [36] Many of the new compound showed micromolar-submicromolar affinity for the inhibition of CA I and II, but low nanomolar binding to CA IX and XII. The most selective inhibitors against CAIX and CA XII were compounds 12 and 13 with selectivity ratios in the range of 955 to 1227-fold. (Fig.6)

**Figure 6 here**

Multiple libraries of benzene sulfonamides conjugated to monosaccharide and disaccharide “tails” have been prepared as a dual isozyme-differentiating and drug solubilizing strategy by Poulsen and co-workers [37-40]. This work has shown that carbohydrates, through the combination of a high degree of polyfunctionality and hydrophilicity incorporated onto the
aromatic sulfonamide CA pharmacophore, impart unique properties to this family of CA inhibitors. The stereochemical diversity across the carbohydrate tails proved very effective at discriminating subtle differences in active site topology of CA isozymes, while the physicochemical properties of the complete inhibitor set provided the potential to impair membrane diffusion to selectively target transmembrane hCA isozymes wherein the CA active site is located extracellularly.

The Cu(I) catalyzed ligation of a terminal acetylene to an organic azide to form regiospecifically a 1,4-disubstituted-1,2,3-triazole has emerged as a valued synthetic tool in the field of medicinal chemistry (Scheme 1) [41,42]. This transformation, now known as ‘click chemistry’, occurs under ambient conditions and has a high degree of biocompatibility.

Scheme 1 here

Poulsen and co-workers applied click chemistry to generate libraries of glycosyltriazole CA inhibitors using a “click-tailing” strategy. Initial studies encompassed a detailed synthetic analysis of the utility of the click chemistry reaction using carbohydrate as substrates, as then an assessment of the stability of the formed glycosyl triazole linkage towards typical carbohydrate reaction sequences [42]. These investigations showed that click chemistry was orthogonal to many pre-existing synthetic methodologies involving carbohydrates [43]. Specifically, the robustness of the triazole moiety was demonstrated on a model glucosyl triazole 14 was interrogated under reaction conditions for alcohol group protection/deprotection (with Tr, TBDMS, Ac, Bz or Bn as the protecting group), nucleophilic displacement and O-glycosylation, in all situations the triazole moiety was retained intact (Scheme 2).

Scheme 2 here
To facilitate the generation of glycosyltriazole CA inhibitors the aromatic sulfonamide pharmacophore was endowed with either a terminal alkyne or an azide functionality to act as the reaction partner for click chemistry with a larger panel of sugar building blocks possessing complementary functionality. The library format thus was a combination of either: (i) an acetylene containing sulfonamide fragment with a panel of azido sugars, or ii) an azide containing benzene sulfonamide fragment with a panel of sugar acetylenes (Scheme 3).

Scheme 3 here

The glycoconjugate CA inhibitor libraries were readily prepared in a parallel fashion using this “click-tailing” strategy (Scheme 4). In the first reported glycoconjugate library the 1,2,3-triazole linker was in combination with either an ester or amide functionality, while in the second library it was in combination with an O-glycoside functionality. A third library installed the 1,2,3-triazole directly on the benzene sulfonamide fragment. The latter library should have enhanced stability towards endogenous esterase, protease or glycosidase activity as found in the *in vivo* environment [44].

Scheme 4 here

The triazole linked glycoconjugates were screened using the CO$_2$ hydration assay against the cytosolic hCA I and II isozymes, as well as a selection of the transmembrane hCA IX, XII and XIV isozymes, the latter possessing extracellular catalytic domains. The inhibition results have provided a comparative surveillance of the active sites of these clinically relevant CA isozymes, and have proven immensely informative in advancing the quest to discover novel CA-based therapeutics and/or diagnostics displaying improved potency and selectivity. The impressive potency and selectivity of several glycoconjugates confirms the effectiveness of the carbohydrate tail at differentiating amongst isozymes. Inhibition of these isozymes was in most cases non-clustered for each of the libraries. Conservation of active site structure and topology within the CA enzyme family has made it generally difficult to target subtle isozyme
differences by rational drug design, however these ‘click-tailed’ glycoconjugates were able to impart selectivity for the isozymes relative to the non-selective parent benzenesulfonamide scaffolds PhSO$_2$NH$_2$. The qualitative structure-activity demonstrated that the stereochemical diversity within the carbohydrate tails effectively interrogated the CA active site topology. One powerful example are the glucose (28, 29, 42, 43, 56, 61) and galactose (30, 31, 44, 45, 57, 62) epimer derivatives of Library 1 and Library 2. These compounds have drastically different inhibition and selectivity profiles yet the sole stereochemical difference is many bonds removed from the sulfonamide zinc binding moiety.

There were numerous glycosyltriazole candidates identified within these libraries with interesting $K_i$ profiles across the clinically relevant hCA isozymes, the interested reader is directed to the original research papers for more detail, here we will highlight a selection of the most noteworthy inhibitors. The methyl-β-D-glucuronate triazole 37 of Library 1 was found to be a potent inhibitor of the tumour-associated isozyme, hCA IX ($K_i$ 23 nM), comparable to the bis-sulfonamide indisulam, which is in phase II clinical trials for the treatment of solid tumours. Remarkably, compound 37 was shown to be 26-fold selective than indisulam for hCA IX over the physiologically dominant hCA II isozyme, indisulam is non-selective. This is an important step forward in the quest for new cancer chemotherapeutics. The galactose-OAc derivative of Library 2 (44) was also a potent, but this time non-selective, inhibitor of hCA IX ($K_i$ 9.7 nM). In Library 3 the methyl-D-glucuronate triazoles 57 and 65 were again proven to induce potent inhibition of hCA IX ($K_is$ 9.9 and 8.4 nM, respectively) – this affirms the glucuronate tail fragment as an important candidate for more intense SAR investigation when targeting the tumour-associated hCA IX isozyme. Library 3 identified two additional candidates (58 and 66) with low-nM potency at hCA IX ($K_is$ 5.0 and 5.4 nM, respectively) – the lowest yet at hCA IX. These compounds both have a mannose tail moiety,
this sugar fragment was not assessed in the first two libraries and therefore is worthy of further scrutiny.

From a synthetic viewpoint the reactions used to generate molecular diversity in carbohydrate-based libraries need to be facile, mild, high yielding and ideally give a predictable stereochemical outcome. We have shown that the click chemistry 1,3-DCR fulfils these demands. The facile conjugation of carbohydrate tails to a primary aryl sulfonamide fragment using click chemistry has provided an expedient and powerful means to generate several diverse libraries of glycoconjugate CA inhibitors. The stereochemical and structural variability of the carbohydrate ‘tail’ region allowed for exploration of active site architecture and has the potential to develop neutral, water soluble CA inhibitors for drug delivery applications, and as a means to specifically target extracellular and clinically relevant CA isozymes due to impaired permeation of the plasma membrane.

Conclusion

CAs catalyze a very simple physiological reaction — the conversion of carbon dioxide to the bicarbonate ion and protons and underpin physiological and pathological processes including among others gluconeogenesis, lipogenesis, ureagenesis, tumorigenicity and the growth and virulence of various pathogens. In addition to the established role of CAIs as diuretics and antiglaucoma drugs, it has recently emerged that CAIs could have potential as novel anti-obesity, anti-cancer and anti-infective drugs. Recently, some progress has been registered in the design of compounds with favourable inhibition profiles and a certain degree of selectivity against several pharmacologically relevant isozymes (hCA II, VA, IX, XII, XIII and XIV among others), especially by using the tail approach [4-7]. The tail approach consists of introducing moieties with various physicochemical properties onto the scaffold of aromatic/heterocyclic sulfonamides and their bioisosteres (sulfamates, sulfamides, etc) in such
a way as to assure a larger number of interactions of the tail moieties with amino acid residues towards the entrance of the enzyme active site. Indeed, that is the region which contains the most variable composition of amino acids within the active site of all mammalian CA isoforms [4-7], and a positive or negative (clash) interaction with some of them may lead to different inhibition profiles of the corresponding compounds and as a consequence to CAIs showing a certain degree of isozyme-selectivity. The most interesting examples of this approach for the design of isozyme-selective CAIs was offered just by attaching sugar moieties to the scaffold of aromatic/heterocyclic sulfonamides, sulfamates or sulfamides, or by attaching zinc binding groups of the sulfonamide, sulfamate or sulfamide type to various sugar derivatives. As a consequence, this variant of the tail approach has been denominated the “sugar approach” for designing CAIs, and many such examples are now available in the literature, as shown throughout this review. Prior to this work tail groups covering only a relatively limited area of chemical space had been tethered to the aryl sulfonamide CA pharmacophore. The carbohydrate moieties, through the combination of a high degree of polyfunctionality and hydrophilicity incorporated onto the aromatic/heterocyclilc sulfonamide CA pharmacophore, were shown to impart unique properties to this family of CAIs. The stereochemical diversity across the carbohydrate tails proved very effective at discriminating subtle differences in active site topology of CA isozymes, while the physicochemical properties carbohydrate-based inhibitors is set to provide the potential to impair membrane diffusion and selectively target transmembrane isozymes wherein the CA active site is located extracellularly. Considering that this field is quite young, we can be optimistic to forecast further, rapid developments of the sugar approach, that may lead indeed to compounds with enhanced activity and selectivity for targeting many of the CA isozymes known to play fundamental physiological and pathological roles, which in the end may lead to better drugs belonging to this class of pharmacological agents.
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Biosketch:

**Jean-Yves Winum** received his Ph.D. in chemistry from the University of Montpellier II (France) in 1998. He then worked as post-doctoral fellow in the Department of Chemistry of Georgetown University (Washington, DC) and in the Department of Organic Chemistry of the University of Geneva (Switzerland). In 1999, he joined the Department of Chemistry of the University of Montpellier II, where he is now assistant professor. His research interests are focused on organic/medicinal chemistry of metallo-enzyme inhibitors.

**Sally-Ann Poulsen** received her PhD in medicinal chemistry from Griffith University, Australia in 1996 in the group of Professor Ron Quinn. After a period of postdoctoral research in the pharmaceutical industry (Astra Charnwood, UK, 1997) she was awarded a Royal Society/NHMRC Howard Florey Research Fellowship in 1998 (Department of Chemistry, University of Cambridge, UK). Following this she took up an Australian Research Council QEII Fellowship at Griffith University, Australia (2000-2007) and is now the Chemical Biology Program Leader within the Eskitis Institute for Cell and Molecular Therapies, Griffith University. Her research interests include medicinal chemistry, protein mass spectrometry and exploiting chemistry for bioimaging applications.

**Claudiu T. Supuran** received his BSc in chemistry from the Polytechnic University of Bucharest, Romania (1987) and PhD in chemistry at the same university in 1991. In 1990 he became assistant professor of chemistry at the University of Bucharest. After a short stage at the University of Florida in Gainesville, USA, he returned in Bucharest where he became associate professor in the Department of Organic Chemistry. In 1995 he moved at the University of Florence, Italy, where he is currently research fellow and contract professor of chemistry. His main research interests include medicinal chemistry, design of enzyme inhibitors and activators, heterocyclic chemistry, chemistry of sulfonamides, sulfamates and sulfamides, biologically active organo-element derivatives, QSAR studies, X-ray crystallography of metallo-enzymes, metal complexes with biologically active ligands (metal-based drugs), carbonic anhydrases, cyclooxygenases, serine proteases, matrix metalloproteinases, bacterial proteases, and amino acid derivatives among others. He has published more than 400 original research papers in these fields.