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A new way of synthesizing heterocyclic primary sulfonamide probes for carbonic anhydrase

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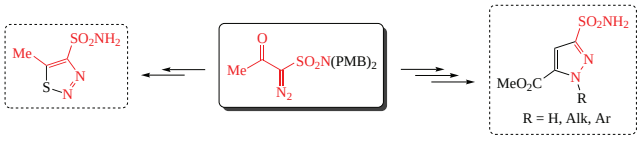
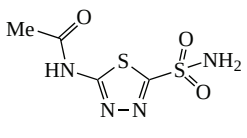
An *N*,*N*-bis(*p*-methoxybenzyl)-protected α-acetyl-α-diazomethane sulfonamide proved to be a useful building block for accessing new 5-methyl-1,2,3-thiadiazole-4-sulfonamide as well as methyl 3-sulfamoyl-1*H*-pyrazole-5-carboxylate. The latter was further subjected to *N*-alkylation and *N*-arylation reactions. All resulting compounds showed potent inhibition of I, II and particularly of cancer-related IX and XII isoforms of human carbonic anhydrase.

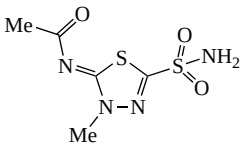


Keywords: α-acetyl-α-diazomethane sulfonamide, methyl propiolate, [3+2] dipolar cycloaddition, Lawesson' reagent, Chan–Evans–Lam arylation, pyrazoles, 1,2,3-thiadiazoles.

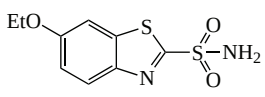
Carbonic anhydrases (CAs, EC 4.2.1.1), represented by 15 different isoforms in the human genome,[1] are ubiquitous zinc metalloenzymes that catalyze reversible hydration of carbon dioxide to bicarbonate anion. This reaction is central to the regulation of many physiological processes including the maintenance of intra- and extracellular pH.[2] Suppression of CA catalytic activity with small molecule inhibitors is a validated therapeutic approach in such disease areas as glaucoma, oedema, obesity, cancer, epilepsy and osteoporosis.[3] Many known CA inhibitors are derivatives of the basic benzenesulfonamide pharmacophore.[4,5] Despite the fact that four clinically used CA inhibitors such as Acetazolamide, Methazolamide, Ethoxzolamide and Dorzolamide contain heterocyclic cores, attempts to explore the chemical space of heterocyclic sulfonamides in search for CA inhibitors have been rather sporadic. The azole sulfonamide chemistry space, specifically, pyrazole and 1,2,3-thiadiazole[6] sulfonamides which are subject of the present study, have not been investigated in the context of CA inhibition.



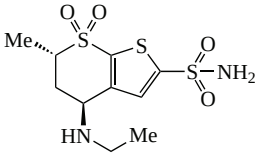
Acetazolamide



Methazolamide

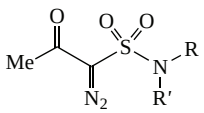


Ethoxzolamide



Dorzolamide

Recently, we introduced a new class of diazo compounds, namely, *N*,*N*-disubstituted acetyldiazomethane sulfonamides **1** which proved to be useful building blocks in the synthesis of 1,2,3-triazolines[7] and 1,2,3-triazoles.[8] We reasoned that if we prepared a special version of this diazomethane sulfonamide **2** where the two substituents on the sulfonamide nitrogen atom were easily removable protecting groups, this would open an entry into the chemical space of primary azole sulfonamides. As a candidate protecting group we chose *p*-methoxybenzyl (PMB) which proved to provide a convenient protection of sulfonamide moieties.[9,10]



1 R, R′ = Alk, Ar; or NRR′ = cyclic substituent

2 R = R′ = MeO– (PMB)

The synthesis of acetyldiazomethane sulfonamide **2** (Scheme 1) commenced with the high-yielding mesylation of bis(*p*-methoxybenzyl)amine **3** followed by LHMDS-promoted Claisen-type acetylation of sulfonamide **4** to afford CH acidic substrate **5**. The latter without purification was subjected to the recently developed 'sulfonyl-azide-free' (SAFE) protocol[11] for the Regitz-type diazo transfer in aqueous medium to give acetyl-substituted compound **2** in excellent yield over two steps.

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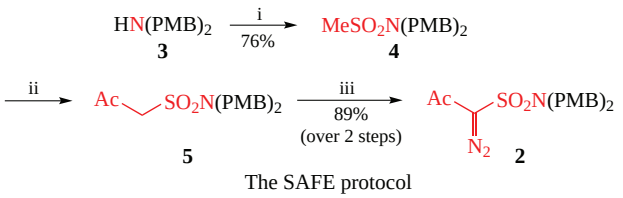
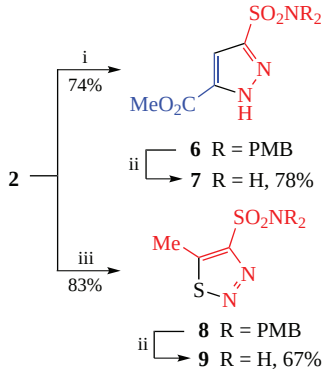


Scheme 1 *Reagents and conditions*: i, $MeSO_2Cl$, Et_3N, THF, 0 °C; ii, LiHMDS, –78 °C, THF, then EtOAc; iii, NaN_3 (2.0 equiv.), $3\text{-}HO_2CC_6H_4SO_2Cl$ (1.3 equiv.), K_2CO_3 (2.6 equiv.), $MeCN–H_2O$, room temperature, 1 h.



Scheme 2 *Reagents and conditions*: i, K_2CO_3 (for deacetylation), then $HC{\equiv}CCO_2Me$ (neat); ii, CF_3CO_2H, 80 °C, 1 h; iii, Lawesson's reagent, PhMe, reflux, 18 h.

Compound **2** was deacylated followed by reaction with methyl propiolate to achieve the formation of pyrazole bis-PMB-protected sulfonamide **6** (Scheme 2). Its treatment with trifluoroacetic acid afforded primary sulfonamide **7**. On the other hand, without deacetylation, compound **2** was treated with the Lawesson's reagent in refluxing toluene to give 1,2,3-thiadiazole bis-PMB-protected sulfonamide **8** which was deprotected into product **9**. The structure of compound **9** was confirmed by single-crystal X-ray analysis (Figure 1).†

Pyrazole derivative **7** is potentially amenable to further selective modifications at the N^1 atom. Both modifications comprised the use of protected precursor **6** and were aimed at increasing the lipophilic bulk at the pyrazole ring to provide

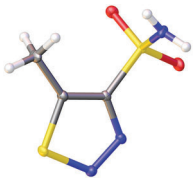
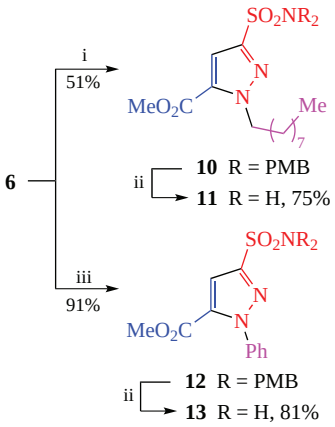


Figure 1 Single-crystal X-ray data for compound **9**.



Scheme 3 *Reagents and conditions*: i, $n\text{-}C_9H_{19}Br$, K_2CO_3, Bu_4NBr; ii, CF_3CO_2H, 80 °C, iii, $PhB(OH)_2$, $Cu(OAc)_2$, 3 Å MS, Py, CH_2Cl_2, room temperature, contact with air, 9 days.

hydrophobic contacts with the lipophilic side[12] of the CA active site (Scheme 3). Alkylation with *n*-nonyl bromide under phase transfer conditions (neat) gave, after deprotection of **10**, compound **11**. The Chan–Evans–Lam arylation[13] with phenylboronic acid afforded, after deprotection of **12**, compound **13**. Importantly, both transformations proceeded with exclusive N^1 regioselectivity.[14] The latter aspect was judged (in case of compound **10**) by analogous alkylation whose outcome was confirmed by X-ray analysis[15] and (in case of compound **12**) by the single-crystal X-ray analysis (Figure 2).‡

Primary amides **7**, **9**, **11** and **13** were tested towards human CA (*h*CA) I, II, IX and XII isoforms to reveal their inhibitory properties in the low nanomolar to submicromolar range (Table 1). Particularly encouraging was the potent and selective inhibition profile exhibited by these compounds (particularly by 1,2,3-thiadiazole **9** and *N*-phenyl pyrazole **13**) towards tumor-associate isoforms *h*CA IX and XII. However, two striking structure–activity relationship facts are notable. Firstly, *N*-arylation of pyrazole ring (**7**→**13**) significantly improves the selectivity towards these two isoforms compared to the off-target *h*CA II (the inhibition of the latter

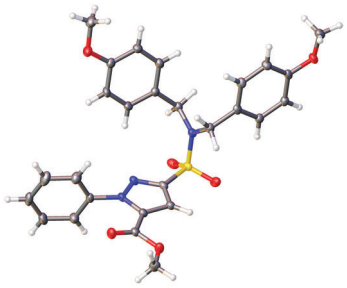


Figure 2 Single-crystal X-ray data for compound **12**.

† Crystal of compound **9** was grown from acetonitrile.

Crystal data for **9**. $C_3H_5N_3O_2S_2$, M = 179.22, triclinic, space group $P\bar{1}1$, 100(2) K, a = 6.5322(5), b = 7.4854(4) and c = 7.8022(7) Å, α = 69.663(7)°, β = 70.575(7)°, γ = 87.832(5)°, Z = 2, V = 336.05(5) Å^3, d_{calc} = 1.771 g cm^{-3}, $F(000)$ = 184.0. Clear colorless prism single crystal with dimensions 0.26 × 0.2 × 0.06 mm was selected and intensities of 2227 reflections were measured using an Agilent Technologies SuperNova Atlas and an Agilent Technologies Xcalibur Eos diffractometers (λ[CuKα] = 1.54184 Å, μ = 6.749 mm^{-1}, $2\theta_{max}$ = 134.958°). After merging of equivalents and absorption and absorption correction, 1201 independent reflections (R_{int} = 0.0245) were used for the structure solution and refinement. The final R_1 was 0.0375 [$I > 2\sigma(I)$] and wR_2 was 0.0998 (all data), GOF = 1.070.

CCDC 2205764 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

‡ Crystal of compound **12** was grown from toluene.

Crystal data for **12**. $C_{27}H_{27}N_3O_6S$, M = 521.57, monoclinic, space group $P2_1$, 100(2) K, a = 11.59590(10), b = 5.14010(10) and c = 21.3426(3) Å, β = 103.3540(10)°, Z = 2, V = 1237.71(3) Å^3, d_{calc} = 1.400 g cm^{-3}, $F(000)$ = 548.0. Clear colorless needle single crystal with dimensions 0.4 × 0.04 × 0.03 mm was selected and intensities of 14438 reflections were measured using an Agilent Technologies SuperNova Atlas and an Agilent Technologies Xcalibur Eos diffractometers (λ[CuKα] = 1.54184 Å, μ = 6.749 mm^{-1}, $2\theta_{max}$ = = 134.94°). After merging of equivalents and absorption and absorption correction, 4451 independent reflections (R_{int} = 0.0385) were used for the structure solution and refinement. The final R_1 was 0.0288 [$I > 2\sigma(I)$] and wR_2 was 0.0761 (all data), GOF = 1.070.

CCDC 2205762 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

Table 1 Inhibition of *h*CA I, II, IX and XII by compounds **7**, **9**, **11** and **13**.

Compound	K_i/nM[a]			
	*h*CA I	*h*CA II	*h*CA IX	*h*CA XII
7	122.1	65.6	9.6	29.9
9	902.0	725.3	23.8	5.7
11	913.3	12.8	193.5	9.5
13	375.2	527.6	4.3	6.3

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

drops nearly tenfold while inhibition of the *h*CA IX/XII duo improves). On the other hand, *n*-nonylation of the pyrazole ring (**7**→**11**) dramatically lowers the *h*CA IX inhibition potency.

To account for the latter changes in the profile of compound **7** on introducing substitutions, we performed docking simulation of both pairs **7**/**13** and **7**/**11** in the active site of *h*CA II and *h*CA IX, respectively. Analysis of side-chain contacts with the target revealed that *N*-phenylpyrazole **13** displayed significantly more strained interactions (Figure 3, highlighted with red gradient) with *h*CA II compared to unsubstituted pyrazole **7** which likely accounts for the nearly tenfold affinity to that isoform. Likewise, compound **11** showed the same tendency with respect to *h*CA IX compared to its unsubstituted counterpart [see Figure 3(*c*),(*d*)].

In summary, we have shown that *N*,*N*-bis(*p*-methoxybenzyl)-protected α-acetyl-α-diazomethane sulfonamide, synthesized in three straightforward steps, is a useful reagent in constructing primary sulfonamides based on either a pyrazole (*via* dipolar [3+2] cycloaddition) and a 1,2,3-thiadiazole (*via* reaction with the Lawesson's reagent) cores. We have also demonstrated that both compounds are efficient inhibitors of various isoforms of human carbonic anhydrase and the inhibitory profile can be altered significantly by introducing substitutions at the pyrazole ring.

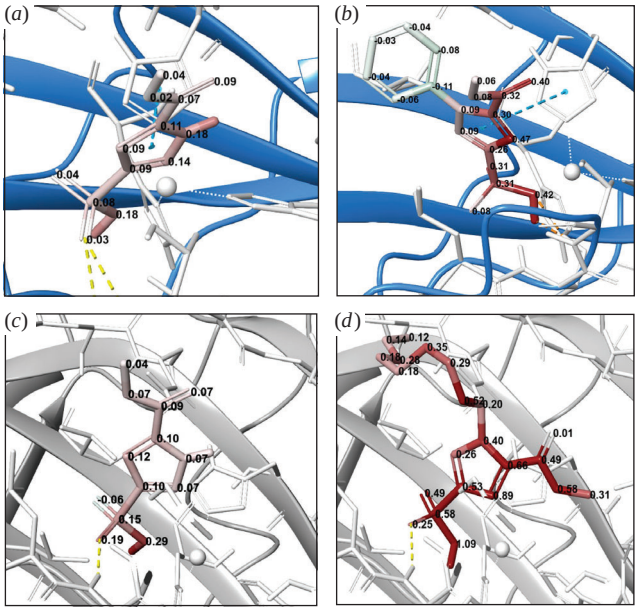


Figure 3 Docking poses of (*a*) compound **7** and (*b*) compound **13** in the active site of *h*CA II as well as (*c*) compound **7** and (*d*) compound **11** in the active site of *h*CA IX.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.04.009.

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