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Synthesis and carbonic anhydrase inhibitory properties of sulfamides structurally related to dopamine

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ABSTRACT

A series of novel sulfamides incorporating the dopamine scaffold were synthesized. Reaction of amines and *tert*-butyl-alcohol/benzyl alcohol in the presence of chlorosulfonyl isocyanate (CSI) afforded sulfamoyl carbamates, which were converted to the title compounds by treatment with trifluoroacetic acid or by palladium-catalyzed hydrogenolysis. Inhibition of six α-carbonic anhydrases (CAs, EC 4.2.1.1), that is, CA I, CA II, CA VA, CA IX, CA XII and CA XIV, and two β-CAs from *Candida glabrata* (CgCA) and *Mycobacterium tuberculosis* (Rv3588) with these sulfamides was investigated. All CA isozymes were inhibited in the low micromolar to nanomolar range by the dopamine sulfamide analogues. K_Is were in the range of 0.061–1.822 μM for CA I, 1.47–2.94 nM for CA II, 2.25–3.34 μM for CA VA, 0.041–0.37 μM for CA IX, 0.021–1.52 μM for CA XII, 0.007–0.219 μM for CA XIV, 0.35–5.31 μM for CgCA and 0.465–4.29 μM for Rv3588. The synthesized sulfamides may lead to inhibitors targeting medicinally relevant CA isoforms with potential applications as antiepileptic, antiobesity antitumor agents or anti-infective.

1. Introduction

Sulfamides have interesting biological activities,[1] this functional group being present in several drugs. The dopaminergic drug quinagolide (**1**), also known as norprolac, is used in the treatment of hyperprolactinemia.[2] Doripenem (**2**), (tradename doribax) also has a sulfamide moiety in its scaffold, being a broad-spectrum injectable antibiotic.[3] The recently developed drug candidate **3** (JNJ-26990990) is a powerful anticonvulsant.[4] Compound **3** has the potential to be used in the treatment of depression, neuropathic pain, and inflammatory pain.[5] Sulfamide **4** and its derivatives possess protein tyrosine kinase inhibitory activity,[6] therapeutic actions against diabetes, HCV replication inhibition,[7] and as endothelin receptor antagonists.[8] The autacoid dopamine (**5**) is a neurotransmitter and plays an important role in the central nervous system-related disorders such as schizophrenia and Parkinson's disease.[9] The carbonic anhydrase (CA, EC 4.2.1.1) activatory properties of dopamine (**5**) related compounds have also been reported[10,11] (Fig. 1).

CA is a well-suited protein to serve as a model in many types of biophysical, bioanalytical, and physical-organic chemical studies as well as for inhibitor drug design studies. This enzyme catalyzes the hydration of carbon dioxide (CO_2) and the dehydration of bicarbonate ion (HCO_3^-):[12]

$$CO_2 + H_2O \overset{CA}{\Longleftrightarrow} HCO_3^- + H^+$$

The CA enzyme family is particularly attractive for biophysical studies of protein–ligand binding and inhibition studies for the following reasons: (i) CA, a monomeric enzyme, is a single-chain protein of medium-sized molecular weight (around 30 kDa). Although, the most widespread CA II have no disulfide bonds, pendant sugar or phosphate groups, all the other isoforms have disulfide bonds, pendant sugar or phosphate groups, (ii) CA II is an widely available

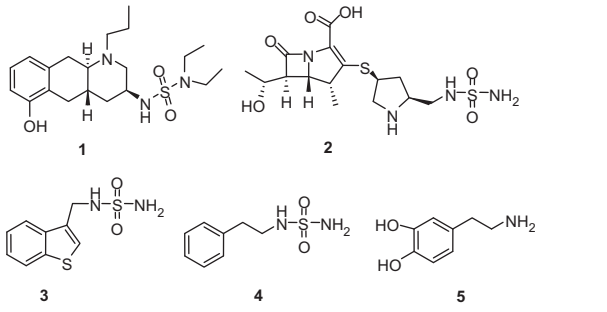

Fig. 1. Chemical structures of selected sulfamides (**1**–**4**) and of dopamine (**5**).

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inexpensive enzyme, (iii) CA II is relatively easy to handle and purify, due in large part to its excellent stability under standard laboratory conditions, (iv) The amino acid sequences are available for most isozymes, (v) The structure and active site geometries have been defined in detail by X-ray diffraction analysis for most of the 13 catalytically active mammalian isoforms,[12] and their catalytic mechanisms are well understood, (vi) It has not only a CO_2 hydrase/dehydratese activity with a high turnover number, but also possesses an esterase activity toward carboxylic acid esters, phosphoric acid esters, and even sulfate esters,[13–15] (vii) its inhibition mechanism with ligands that can bind to the Zn^{2+} ion is fairly simple and well characterized.[12,16]

CA catalyzes the reversible hydration of CO_2 to bicarbonate (HCO_3^-) (Scheme 1) in a two-step 'ping-pong' mechanism.[17] The first step involves the direct nucleophilic attack of a Zn^{2+}-bound hydroxyl group on CO_2 to form a metal-bound HCO_3^-, followed by displacement of HCO_3^- by a water molecule. In the second step, the Zn^{2+}-bound water molecule transfers a proton to molecules of buffer in the solvent and regenerates the zinc-hydroxide form of CA.[18]

So far, 16 different α-CA isoforms have been identified in mammals.[13] These isoenzymes are inhibited by three main inhibition mechanisms: (i) coordination of the inhibitor to the Zn^{2+} from the enzyme active site by replacing the zinc-bound water/hydroxide ion and leading to a tetrahedral geometry of Zn^{2+}, or by addition of the inhibitor to the metal coordination sphere, when the Zn^{2+} ion is in a trigonal bipyramidal geometry;[16,18–20] (ii) by anchoring the inhibitor to the Zn^{2+}-bound solvent molecule, that is, a water or a hydroxide ion, phenols[13,21–23] and polyamines;[24] or (iii) by occlusion of the entrance to the active site cavity,[25] when the inhibitors bind in the activator binding region from the CA active site (Scheme 2).[26–31]

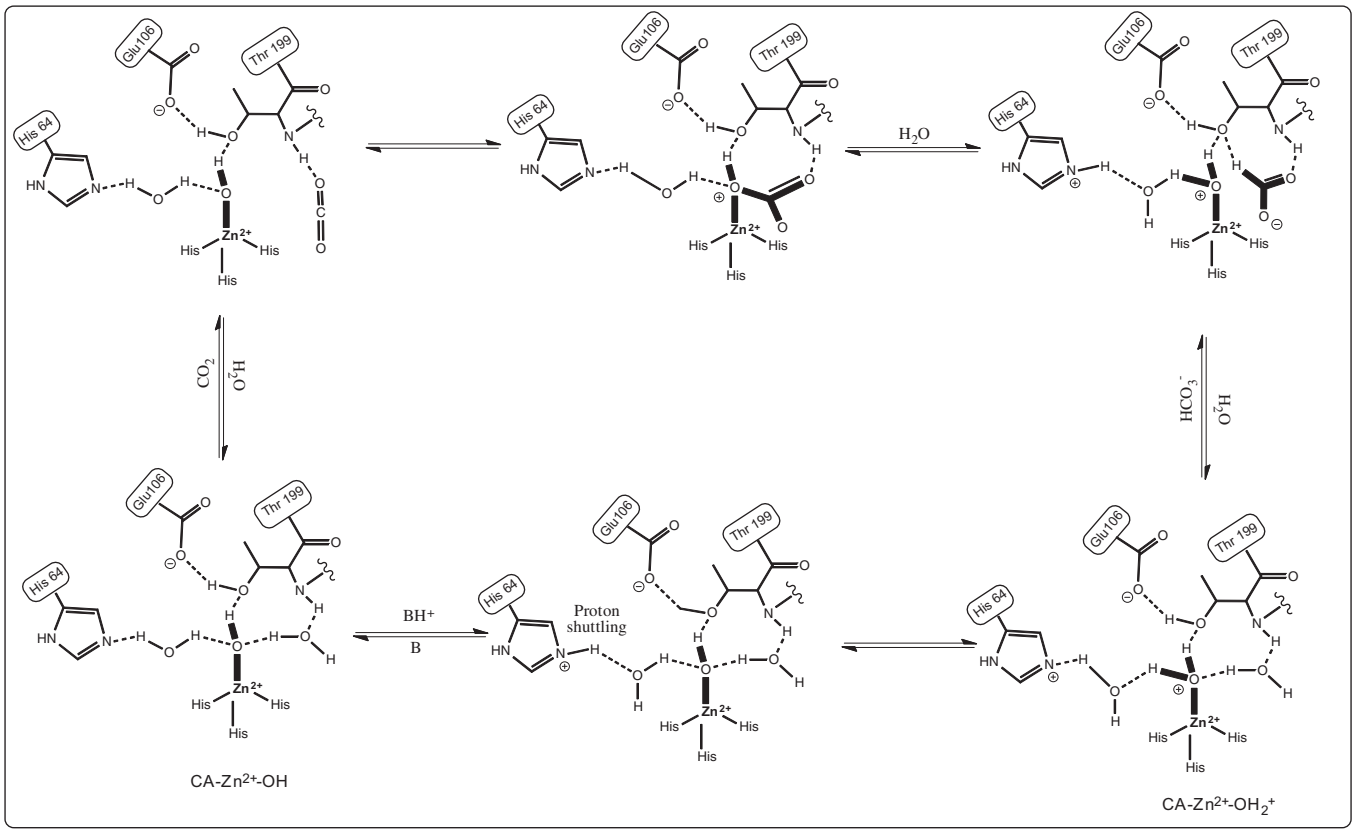
As sulfamides and dopamine related compounds show important biological activities, the synthesis and CA inhibitory properties of sulfamides derived from dopamine derivatives will be useful for further synthetic and biological purposes. In this context, here we report on the synthesis of sulfamides **19–23**. We also evaluate eight CA isoenzymes (CA I, CA II, CA VA, CA IX, CA XII, CA XIV, CgCA and Rv3588) inhibitory effects of the synthesized compounds **19–23**.

2. Results and discussion

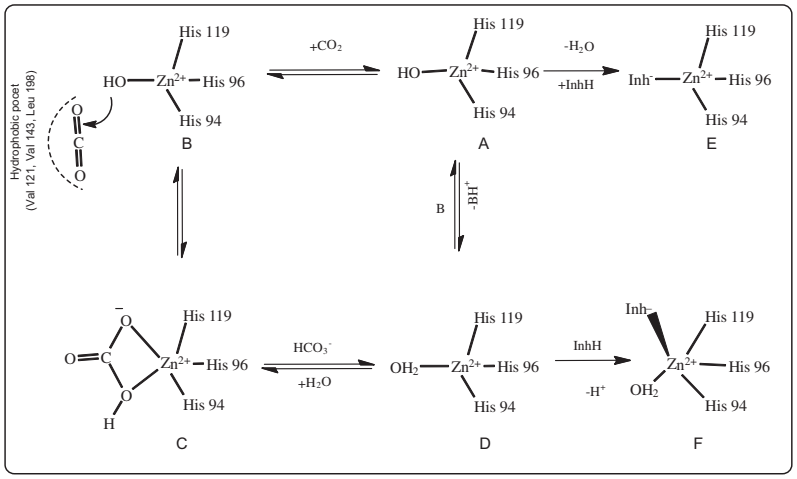
2.1. Chemistry

Curtius reaction of carboxylic acids with diphenylphosphoryl azide has been described clearly in the literature.[32,33] By applying this literature procedure, the reaction of acid **6** with diphenyl phosphoryl azide (DPPA) in the presence Et_3N at 80 °C for 6 h followed by addition of $PhCH_2OH$ and heating at 80 °C for 30 h afforded a new carbamate **7** in excellent yield. Hydrogenolysis of benzyloxycarbamates in the presence of $CHCl_3$ is a convenient method for the synthesis of amine hydrochloride salts.[34] Pd–C catalyzed hydrogenolysis of **7** in MeOH–$CHCl_3$ ($CHCl_3$ is used for producing HCl)[35] afforded dopamine analogue **8** as the hydrochloride salt which was neutralized to its free amine **9** with 10% NaOH (Scheme 3).

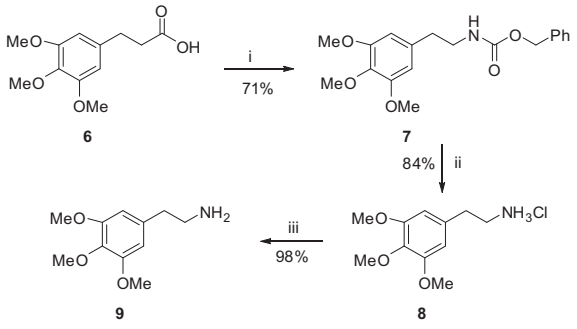
Dopamine related compounds **10–13** are commercially available. The synthesis of sulfamides from the reaction of amines with chlorosulfonyl isocyanate (CSI) has been reported.[36,37] Following these synthetic methodologies, the reactions of 1 equiv of amines **9–11** and 1.5 equiv of *t*-BuOH and the reactions of 1 equiv of amines **12**, **13** and 1.5 equiv of $PhCH_2OH$ with CSI at 0–25 °C for 4 h afforded novel sulfamoyl carbamates **14–18** in high yields. The cleavage of *N*-*tert*-butylamides with trifluoroacetic acid has been reported by Catt et al.[38] By a similar approach, the reaction



Scheme 1. Mechanism of catalysis of the hydration of CO_2 by hCA II.[17] The formal charge was shown only on the Zn^{2+}-bound water (and not the histidine residues) to emphasize that this water ligand is acidic.

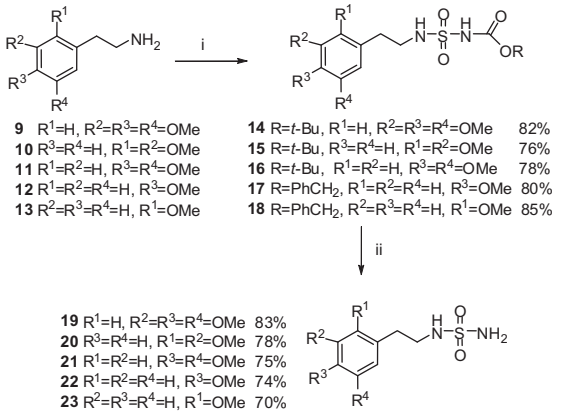


Scheme 2. General catalytic and inhibition mechanisms of carbonic anhydrases.



Scheme 3. Synthesis of mescaline **9**. Reagents and conditions: (i) DPPA/Et_3N, benzene, 80 °C, 6 h, then $PhCH_2OH$, 80 °C, 30 h; (ii) H_2/Pd–C, MeOH–$CHCl_3$, rt, 24 h; (iii) 10% solution of NaOH.

of sulfamide carbamates **14**–**16** with trifluoroacetic acid at room temperature afforded novel sulfamides **19**, **20**, and a known sulfamide **21**[39,40] in excellent yields. On the other hand, palladium catalyzed hydrogenolysis of carbamates **17** and **18** gave sulfamides **22**[7] and **23**[7] in good yields (Scheme 4). The structures of all synthesized compounds were characterized by ^1H and ^{13}C NMR spectroscopic techniques. The functional groups were characterized by IR.



Scheme 4. Synthesis of sulfamides. Reagents and conditions: (i) for the synthesis of **14**–**16**: CSI/NEt_3/*t*-BuOH, CH_2Cl_2, 0–25 °C, 4 h; for the synthesis of **17** and **18**: CSI/NEt_3/$PhCH_2OH$, CH_2Cl_2, 0–25 °C, 4 h; (ii) for the synthesis of **19**–**21**: CF_3CO_2H, 25 °C, 12 h; for the synthesis of **22** and **23**; H_2/Pd–C, MeOH, rt, 4 h.

2.2. CA isoenzymes inhibition studies

2.2.1. CA isoenzymes inhibition

Methods for isolation and purification of CA are well established and widely accessible. Commercial sources usually isolate isozymes hCA I and hCA II from fresh human red blood cells, in which CA is the second most abundant protein after hemoglobin. Research laboratories typically produce these isozymes by recombinant technology in *Escherichia coli*.[41,42] This method provides the structural flexibility and specificity of site-directed mutagenesis, which is useful for biophysical and biochemical studies.[18] The blood protein and the wild-type recombinant construct are identical except for the N-terminus, which is a post-translationally acetylated Ser in the native CA and exists as Met-Ala or Ala in the recombinant version.[43] The research laboratory procedure for the purification of hCA from red blood cells involves lysis of the cells and removal of the cellular remnants by centrifugation, followed by separation of hemoglobin from hCA on a sulfonamide-modified agarose affinity column.[44,45] Divalent anions like sulfate do not bind appreciably in the active site of CA. Therefore, they are used to remove nonspecifically bound protein by screening ionic interactions. Also, it was shown that CA isozymes from other species could be purified in a similar way with only minor modifications. Commercial suppliers do not use the chromatographic methods, although their exact procedures are proprietary.[18,44]

Inhibition of the CA isoenzymes has pharmacologic applications in the field of anti-glaucoma, anti-convulsant and anti-cancer agents. It is well known that sulfonamides constitute the main class of CA inhibitors, which can bind to the metal ion from the enzyme active site.[46] CA inhibitors block the function of the CA enzyme. The myriad inhibitors of CA can be divided into four main groups: (i) sulfonamides/sulfamides, (ii) other sulfonic acid derivatives, (iii) small monoanions such as halides, azides, and thiocyanate and, (iv) inhibitors with aromatic rings/phenolic compounds. The sulfonamides, sulfamides and sulfonic acid derivatives have values of K_i in the picomolar to micromolar range, whereas the inorganic monoanions bind CA with values of K_i in the micromolar to millimolar range.[18] Similarly, phenolic inhibitors with aromatic rings had K_i values in the micromolar to millimolar range.[13,23,47–51]

Inhibition data against all the catalytically active CA isozymes, that is, CA I, CA II, CA VA, CA IX, CA XII, CA XIV, CgCA and Rv3588 with sulfamides **19**–**23** are presented in Table 1 and they were obtained at pH 7.4 in 10 mM Hepes buffer, at 25 °C, by a stopped-flow assay monitoring the CO_2 hydration reaction by Khalifah et al.[52] as described previously.[13,23] The reference compound

Table 1
CA I, CA II, CA VA, CA IX, CA XII, CA XIV and two β-CAs from *Candida glabrata* (CgCA) and Rv3588 from *Mycobacterium tuberculosis* inhibition data with some sulfamides **19**–**23** by an hydratase method.

Sulfamides	K_i (μM)							
	CA-I (μM)	CA II (nM)	CA VA (μM)	CA IX (μM)	CA XII (μM)	CA XIV (μM)	CgCA (μM)	Rv3588 (μM)
19	1.82	2.03	>50	0.221	1.52	0.22	3.34	2.20
20	0.25	2.95	>50	>50	0.047	0.18	4.59	>50
21	0.28	2.40	2.28	0.092	0.044	0.031	5.31	3.61
22	0.061	1.47	3.25	0.376	0.021	0.007	0.35	4.23
23	0.25	2.04	3.34	0.041	0.416	0.16	2.71	0.46
AAZ[a]	0.250	12.0	0.063	0.025	0.006	0.041	1.97	0.010

[a] Acetazolamide (AAZ) was used as a standard inhibitor for all CAs investigated here

acetazolamide (AAZ) has been tested for enzyme inhibition against all eight CA isoforms (Table 1). All tested sulfamides **19**–**23** showed K_i values in the lower μM to the nanomolar range (K_i <5 μM, see Table 1).

The following should be noted regarding the inhibition of these CA isozymes with sulfamides **19**–**23**:

(i) The hCA I enzyme was effectively inhibited well by all tested sulfamides **19**–**23**. Against isozyme hCA I, sulfamides **19**–**23** exhibited excellent inhibitory activity, with inhibition constants in the range of 61 nM–1.82 μM. The best inhibitor was sulfamide **22** (with a K_i of 61 nM), whereas the worst inhibition was observed for sulfamides **19** having three methoxy groups (–OCH_3) (with a K_i of 1.82 μM). Compound **22**, which has a methoxy group on the *para*-position (Scheme 4), showed a better K_i value compared to the other compounds. Thus, *para*-position of sulfamide and methoxy group (–OMe) lead to effective hCA I inhibitors. Despite all this, the differences in K_i value between sulfamides **19**–**23** were rather small.

(ii) The physiologically dominant[16,20] cytosolic human isoenzyme hCA II was very effectively inhibited by sulfamides **19**–**23**, with K_is in the range of 1.47–2.95 nM. The K_i values of sulfamides **19**–**23** were similar to each other. The differences between highest and lowest K_i values of sulfamides **19**–**23** were only twofold. The structure–activity relationship is rather similar to what outlined above for hCA I, because the two enzymes (hCA I and hCA II) have a high sequence homology of amino acid present within the active site), but these sulfamides were much more effective as hCA II inhibitors than as hCA I inhibitors.[16,20] The K_i values of all sulfamides **19**–**23** were lower than that of acetazolamide (K_i of 12.0 nM).

(iii) Isoform CA VA was poorly inhibited by sulfamides **19** and **20** (K_is >50 μM) The other sulfamides **21**–**23** showed K_is in the range of 2.28–3.34 μM. Sulfamides **21** was the best hCA VA inhibitor.

(iv) The two transmembrane, tumor-associated isozymes hCA IX and XII were also effectively inhibited by sulfamides **19**–**23** with K_is in the range of 41 nM–0.376 μM for hCA IX, and of 21 nM–1.52 μM for hCA XII, respectively. Sulfamides **20** was a, inefficient CA IX inhibitor. As these isozymes are overexpressed in hypoxic tumors where there are also reactive oxygen species damaging the tissue,[13,23] the inhibition of these isoforms with sulfamides also possessing antioxidant activity might be of interest to be studied in more details. CA XIV, another transmembrane isoform, was also effectively inhibited by sulfamides **19**–**23**, with inhibition constants in the range of 7 nM–0.22 μM.

(v) Another studied CA isoform was the β-CA (CgCA) purified from the pathogenic yeast *Candida glabrata*. CgCA exhibited a particular inhibition profile with sulfamides **19**–**23**. All investigated sulfamides **19**–**23** were effective inhibitors, with K_is in the range of 0.35–5.31 μM. The most potent CgCA inhibitor was sulfamide **22** with an inhibitor constant of 0.35 μM.

(vi) Finally another β-class CA (Rv3588) encoded in the genome of *Mycobacterium tuberculosis* was investigated here. Rv3588 was effectively inhibited by all investigated sulfamides (**19**–**23**), with inhibition constants in the range of 0.46–4.23 μM. As well as CA IX, Rv3588 is not regularly inhibited by sulfamide **20**. Hence, the inhibition profile of compound **20** was not determined on Rv3588.

Sulfamides are among the possible isoesteres of sulfonamides, in which an additional electron-withdrawing atom such as oxygen or grup such as NH is directly attached to the sulfamoyl function, generating compounds with general formula of R–NH–SO_2NH_2. These groups were considered as interesting candidates for CA inhibitors. First, Supuran's group showed that a simple sulfamide binds to the metal ion in the Co^{2+}-substituted CA II,[53] whereas the X-ray structures of the compound in adduct with CA II was reported several years later.[54] Nowadays, there are a large number of aromatic, heterocyclic, aliphatic, and sugar-based sulfamides, which were shown to possess highly effective inhibitory properties against all known mammalian isoforms.[16] Therefore, sulfamides constitute a highly important class of CA inhibitors, with some derivatives clinically used for the treatment of epilepsy and obesity.

Novel CA inhibitors have been designed for pharmacological and medicinal approaches, and many inhibitors have been synthesized recently.[55] There are many studies in the literature on the interactions of different compounds and CA isoenzymes. Recently, the inhibitory effects of dantrolene,[56] melatonin,[57] vitamin E,[58] morphine,[59] ethanol,[60] antioxidant phenols,[50] phenolic acids,[23,48] distinguished natural polyphenols products, prominent phenolic compounds,[49] bromophenols[61] with hCA I and hCA II has been investigated by our group. Indeed, the inhibition profiles of hCA I, and hCA II with compounds **19**–**23** are very variable, with inhibition constants ranging from the subnanomolar to the submicromolar levels. For this purpose, hCA I and hCA II inhibitory effects of the sulfamide compounds **19**–**23** were tested under in vitro conditions and their K_i values were calculated and presented in Table 1.

We report here the initial study on the inhibitory effects of synthesized sulfamides **19**–**23** and on CA I, CA II, CA VA, CA IX, CA XII, CA XIV, CgCA and Rv3588. Our data show that sulfamides **19**–**23** possess effective inhibitory properties against the various CA isoenzymes, the strongest inhibitory effect being observed with against the rapid cytosolic isoenzymes CA II (K_is: 1.47–2.95 nM).

It has been reported that sulfamide and sulfamic acid act as moderate hCA II inhibitors, with inhibition constants of 1130 μM for sulfamide and 390 μM for sulfamic acid at the physiological pH (7.4), respectively.[46] In the current study, we observed strong inhibitory effects of the newly synthesized sulfamides **19**–**23** on eight CA enzymes (Table 1).

3. Conclusions

In summary, dopamine analogue **8**, mescaline hydrochloride, was synthesized starting from 3-(3,4,5-trimethoxyphenyl)propanoic acid (**6**) via Curtius reaction by an alternative method. By this study, a series of sulfamides **19**–**23** were synthesized starting from the corresponding dopamine analogues. The synthesized sulfamoyl carbamates **14**–**18** and novel sulfamides **19** and **20** may be important for further synthetic and biological purposes. In addition, several sulfamide compounds **19**–**23** have been assayed for the inhibition of the physiologically relevant human carbonic anhydrase isoforms like CA I, CA II, CA VA, tumour-associated isozymes CA IX and CA XII, CA XIV, two β-carbonic anhydrase isoforms like CgCA from *Candida glabrata* and Rv3588 from *Mycobacterium tuberculosis*. The sulfamides investigated here showed effective CA inhibitory effects, in the low-micromolar to nanomolar range. In general, the compounds **19**–**23** had comparable inhibitory activity with the clinically used sulfonamide AAZ. These findings clearly showed that the synthesized sulfamides **19**–**23** may pave the way for generating potent CA inhibitors eventually targeting isoforms that have not been investigated in detail for their inhibition profile by sulfamides.

4. Experimental

All chemicals and solvents are commercially available and were used after distillation or treatment with drying agents. Melting points were determined on a capillary melting apparatus (BUCHI 530) and are uncorrected. IR spectra were obtained from solutions in 0.1 mm cells with a Perkin-Elmer spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a 400 (100)-MHz Varian and 400 (100)-MHz Bruker spectrometer; δ in ppm, Me_4Si as the internal standard. Elemental analyses were performed on a Leco CHNS-932 apparatus. All column chromatography was performed on silica gel (60-mesh, Merck). PLC is preparative thick-layer chromatography: 1 mm of silica gel 60 PF (Merck) on glass plates.

4.1. Synthesis

4.1.1. Synthesis of benzyl 3,4,5-trimethoxyphenethylcarbamate (7)

A solution of DPPA (2.75 g, 9.99 mmol) and NEt_3 (1.01 g, 9.99 mmol) in benzene (10 mL) were added to a solution of 3-(3,4,5-trimethoxyphenyl)propanoic acid (**6**) (2.00 g, 8.32 mmol) in benzene (30 mL). After the reaction mixture was stirred at 80 °C for 6 h, $PhCH_2OH$ (2.70 g, 24.97 mmol) was added to the reaction mixture and heated at 80 °C for 30 h. The solvent was evaporated. The column chromatography of the residue on silica gel (100 g) with 15% EtOAc–hexane yielded carbamate **7** (2.05 g, 71%). White solid. Mp 78–80 °C; IR (CH_2Cl_2, cm^{-1}) 3368, 2929, 1720, 1591, 1547, 1462, 1345, 1264, 1127. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.38 (m, 5H, Ph-H), 6.41 (s, 2H, Ar-H), 5.13 (s, 2H, CH_2), 4.82 (br s, 1H, NH), 3.87 (s, 3H, OCH_3), 3.85 (s, 6H, OCH_3), 3.50 (m, 2H, CH_2), 2.79 (m, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3 (CO), 153.3 (2C), 136.5 (2C), 134.4 (C), 128.6 (2CH), 128.19 (CH), 128.15 (2CH), 105.6 (2CH), 66.7 (OCH_2), 60.9 (OCH_3), 56.1 ($2OCH_3$), 42.2 (CH_2NH), 36.5 (CH_2). Anal. Calcd for ($C_{19}H_{23}NO_5$): C, 66.07; H, 6.71; N, 4.06. Found: C, 66.03; H, 6.74; N, 4.07.

4.1.2. Synthesis of 2-(3,4,5-trimethoxyphenyl)ethanamine hydrochloride (8)

Pd–C (100 mg) and benzyl 3,4,5 trimethoxyphenethylcarbamate (**7**) (1.75 g 5.07 mmol) in MeOH–$CHCl_3$ (4:1, 100 mL) were placed into a 250-mL flask. A balloon filled with H_2 gas (3 L) was fitted to the flask. The mixture was deoxygenated by flushing with H_2. The reaction mixture was hydrogenated at rt for 24 h. The catalyst was removed by filtration. Recrystallization of the residue from MeOH–Et_2O afforded hydrochloride salt **8** (1.05 g, 84% yield). White solid. Mp 184–186 °C. (lit.[62] Mp: 183–184) ^{1}H NMR (400 MHz, CD_3OD) δ 6.59 (s, 2H, Ar-H), 4.91 (br s, H of NH_3 and MeOH), 3.84 (s, 6H, OCH_3), 3.73 (s, 3H, OCH_3), 3.18 (t, 2H, J = 7.6 Hz, CH_2), 2.90 (t, 2H, J = 7.6 Hz, CH_2). ^{13}C NMR (100 MHz, CD_3OD) δ 153.7 (2C), 140.3 (C), 132.8 (C), 105.8 (2CH), 59.9 (OCH_3), 55.4 ($2OCH_3$), 40.8 (CH_2N), 33.7 (CH_2).

4.1.3. The synthesis of 3,4,5-trimethoxyphenylethanamine (9)

Amine hydrochloride salt **8** (0.95 g, 3.83 mmol) was dissolved in MeOH (30 mL) and cooled to 0 °C. To this solution was added a solution of 10% NaOH (15 mL). The reaction mixture was stirred at room temperature for 3 h. After most of the MeOH was evaporated, CH_2Cl_2 (50 mL) and H_2O (20 mL) were added to the residue. Organic layer was separated and H_2O layer was extracted with CH_2Cl_2 (2 × 30 mL). Combined organic layers were dried over Na_2SO_4 and CH_2Cl_2 was evaporated to give oily mescaline **9** (0.79 g, 98%).

^{1}H and ^{13}C NMR data is in agreement with data given in the literature.[63]

4.1.4. Standard procedure for the synthesis of *tert*-butyl sulfamoylcarbamates: *tert*-butyl *N*-(3,4,5-trimethoxyphenethyl)sulfamoylcarbamate (14)

t-BuOH (0.49 g, 6.63 mmol) was added to a solution of CSI (0.67 g, 4.73 mmol) in CH_2Cl_2 (10 mL) at 0 °C. A solution of amine (**9**) (1.00 g, 4.73 mmol) in CH_2Cl_2 (30 mL) and NEt_3 (0.53 g, 5.21 mmol) were added to the solution of CSI–*t*-BuOH drop wise and it was stirred at 0 °C for 1 h. then at room temperature for 3 h. The reaction mixture was cooled to 0 °C and to this mixture was added a solution of 0.1 N HCl (50 mL). Organic phase was separated and H_2O phase was extracted with CH_2Cl_2 (2 × 30 mL). Combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. Column chromatography of the residue on silica gel (50 g) with 25% EtOAc–hexane gave carbamate **14** (1.52 g, 82%). Yellow oil. IR (CH_2Cl_2, cm^{-1}) 3361, 3274, 2977, 2939, 1702, 1593, 1460, 1367, 1349, 1244, 1146, 1127. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (br s, 1H, NH), 6.36 (s, 2H, Ar-H), 5.14 (t, 1H, NH, J = 6.1 Hz), 3.79 (s, 6H, OCH_3), 3.76 (s, 3H, OCH_3), 3.26 (q, 2H, CH_2NH, J = 6.7 Hz), 2.76 (t, 2H, CH_2; J = 7.0 Hz), 1.38 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.5 (2C), 150.16 (CO), 136.9 (C), 133.3 (C), 105.6 (2CH), 83.9 (OC), 60.9 (OCH_3), 56.1 ($2OCH_3$), 44.9 (CH_2NH), 35.8 (CH_2), 27.9 ($3CH_3$). Anal. Calcd for ($C_{16}H_{26}N_2O_7S$): C, 49.22; H, 6.71; N, 7.17; S, 8.21. Found: C, 49.20; H, 6.74; N, 7.12; S, 8.23.

t-Butyl sulfamoylcarbamates **15** and **16** were also synthesized by this procedure with yields of 76% and 78%, respectively.

4.1.5. *tert*-Butyl *N*-(2,3-dimethoxyphenethyl)sulfamoylcarbamate (15)

White solid. Mp 113–115 °C. IR (CH_2Cl_2, cm^{-1}) 3274, 2974, 1720, 1655, 1586, 1480, 1351, 1268, 1144. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (br s, 1H, NH), 6.98–7.02 (m, 1H, Ar-H), 6.83 (dd, 1H, Ar-H, J = 8.2, J = 1.4 Hz), 6.77 (dd, 1H, Ar-H, J = 7.6, J = 1.3 Hz), 5.40 (t, 1H, NH, J = 5.8 Hz), 3.85 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.30 (m, 2H, CH_2), 2.90 (t, 2H, CH_2, J = 7.0 Hz), 1.43 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.8 (CO), 150.2 (C), 147.3 (C), 131.5 (C), 124.3 (CH), 122.3 (CH), 111.5 (CH), 83.6 (OC), 60.7 (OCH_3), 55.7 (OCH_3), 44.4 (CH_2NH), 30.1 (CH_2), 27.9 ($3CH_3$). Anal. Calcd for ($C_{15}H_{24}N_2O_6S$): C, 49.99; H, 6.71; N, 7.77; S, 8.90. Found: C, 50.01; H, 6.73; N, 7.78; S, 8.88.

4.1.6. *tert*-Butyl *N*-(3,4-dimethoxyphenethyl)sulfamoylcarbamate (16)

White solid. Mp 163–164 °C. IR (CH_2Cl_2, cm^{-1}) 3442, 2926, 1726, 1656, 1518, 1459, 1346, 1262, 1143. ^1H NMR (400 MHz, $CDCl_3$) δ 7.20 (br s, 1H, NH), 6.82 (d, 1H, Ar-H, J = 8.1 Hz), 6.76–6.72 (m, 2H, Ar-H), 5.12–5.10 (m, 1H, NH), 3.88 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.33–3.30 (m, 2H, CH_2), 2.84 (t, 2H, CH_2, J = 7.0 Hz), 1.45 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.0 (CO), 149.3 (C), 148.1 (C), 130.0 (C), 120.7 (CH), 111.9 (CH), 111.6 (CH), 83.91 (OC), 56.0 (OCH_3), 55.9 (OCH_3), 45.1 (CH_2NH), 35.0 (CH_2), 27.9 ($3CH_3$). Anal. Calcd for ($C_{15}H_{24}N_2O_6S$): C, 49.99; H, 6.71; N, 7.77; S, 8.90. Found: C, 49.94; H, 6.69; N, 7.80; S, 8.93.

4.1.7. Standard procedure for the synthesis of benzyl sulfamoylcarbamates: benzyl *N*-(4-methoxyphenethyl) sulfamoylcarbamate (17)

$PhCH_2OH$ (0.27 g, 2.48 mmol) was added to a solution of CSI (0.24 g, 1.65 mmol) in CH_2Cl_2 (10 mL) at 0 °C. A solution of amine (**12**) (0.25 g, 1.65 mmol) in CH_2Cl_2 (15 mL) and NEt_3 (0.19 g, 1.82 mmol) were added to the solution of CSI–$PhCH_2OH$ drop wise and it was stirred at 0 °C for 1 h. then at room temperature for 3 h. The reaction mixture was cooled to 0 °C. A solution of 0.1 N HCl (50 mL) was added to the mixture. Organic phase was separated and H_2O phase was extracted with CH_2Cl_2 (2 × 30 mL). Combined organic layers were dried over Na_2SO_4. The solvent was evaporated. Carbamate **17** was purified by column chromatography of the residue on silica gel (30 g) with 20% EtOAc–Hexane (0.48 g, 80%). White solid. Mp 137–139 °C; IR (CH_2Cl_2, cm^{-1}) 3422, 3288, 2926, 1731, 1449, 1341, 1265, 1149; ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (br s, 1H, NH), 7.38–7.32 (m, 5H, Ar-H), 7.08 (A part of AB, d, 2H, J = 8.6 Hz, Ar-H), 6.85 (B part of AB, d, 2H, J = 8.6 Hz, Ar-H), 5.32 (t, 1H, J = 6.0 Hz, NH), 5.14 (s, 2H, OCH_2), 3.80 (s, 3H, OCH_3), 3.32–3.27 (m, 2H, CH_2NH), 2.79 (t, 2H, CH_2, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5 (CO), 151.2 (C), 134.5 (C), 129.7 (2CH), 129.3 (C), 128.9 (CH), 128.8 (2CH), 128.5 (2CH), 114.2 (2CH), 68.6 (OCH_2), 55.3 (OCH_3), 45.1 (CH_2NH), 34.5 (CH_2); Anal. Calcd for ($C_{17}H_{20}N_2O_5S$): C, 56.03; H, 5.53; N, 7.69; S, 8.80. Found: C, 56.07; H, 5.56; N, 7.66; S, 8.79.

Benzyl-sulfamoylcarbamate **18** was also synthesized by this procedure with a yield of 85%.

4.1.8. Benzyl *N*-(2-methoxyphenethyl)sulfamoylcarbamate (18)

White solid. Mp: 105–108 °C; IR (CH_2Cl_2, cm^{-1}) 3494, 3279, 2937, 1722, 1495, 1455, 1351, 1244, 1158. ^1H NMR (400 MHz, $CDCl_3$) δ 7.64 (br s, 1H, NH), 7.39–7.32 (m, 5H, Ar-H), 7.26 (dt, 1H, Ar-H, J = 8.0, J = 1.7 Hz), 7.12 (dd, 1H, Ar-H, J = 7.4, J = 1.5 Hz), 6.93–6.87 (m, 2H, Ar-H), 5.46 (t, 1H, NH, J = 6.1 Hz), 5.13 (s, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 3.36–3.31 (m, 2H, CH_2NH), 2.87 (t, 2H, CH_2, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.4 (CO), 151.2 (C), 134.6 (C), 130.7 (CH), 128.8 (2CH), 128.7 (2CH), 128.6 (C), 128.4 (CH), 128.3 (CH), 126.0 (C), 120.8 (CH), 110.5 (CH), 68.4 (OCH_2), 55.3 (OCH_3), 44.0 (CH_2NH), 30.3 (CH_2). Anal. Calcd for ($C_{17}H_{20}N_2O_5S$): C, 56.03; H, 5.53; N, 7.69; S, 8.80. Found: C, 56.02; H, 5.57; N, 7.71; S, 8.79.

4.1.9. Standard procedure for the synthesis of sulfamides (hydrolysis of *tert*-butyl sulfamoylcarbamates): *N*-(3,4,5-trimethoxyphenethyl)sulfamide (19)

t-Butyl sulfamoylcarbamate (**14**) (0.73 g, 1.87 mmol) was added to a solution of TFA–CH_2Cl_2 (1:1 10 mL). The mixture was stirred at room temperature for 12 h. Then, the solvent was evaporated. Recrystallization of the residue from CH_2Cl_2–hexane gave **19** (0.45 g, 83% yield). White solid. 109–111 °C. IR (CH_2Cl_2, cm^{-1}) 3269, 2936, 1737, 1593, 1509, 1460, 1423, 1332, 1153, 1125. ^1H NMR (400 MHz, $CDCl_3$) δ 6.42 (s, 2H, Ar-H), 4.80 (br s, 3H, NH and NH_2), 3.82 (s, 6H, OCH_3), 3.79 (s, 3H, OCH_3), 3.32 (t, 2H, CH_2, J = 7.1 Hz), 2.79 (t, 2H, CH_2, J = 7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.5 (2C), 136.7 (C), 134.2 (C), 106.0 (2CH), 61.1 (OCH_3), 56.3 ($2OCH_3$), 44.8 (CH_2NH), 36.2 (CH_2). Anal. Calcd for ($C_{11}H_{18}N_2O_5S$): C, 45.51; H, 6.25; N, 9.65; S, 11.04. Found: C, 45.49; H, 6.28; N, 9.63; S, 11.05.

Sulfamides **20** and **21** were also synthesized by this procedure with yields of 78% and 75% respectively.

4.1.10. *N*-(2,3-Dimethoxyphenethyl)sulfamide (20)

White solid. Mp 107–108 °C. IR (CH_2Cl_2, cm^{-1}) 3350, 3274, 2929, 1736, 1630, 1548, 1481, 1266, 1157. ^1H NMR (400 MHz, $CDCl_3$) δ 7.02–6.98 (m, 1H, Ar-H), 6.77–6.83 (m, 2H, Ar-H), 4.81 (t, 1H, NH, J = 5.8 Hz), 4.74 (br s, 2H, NH_2), 3.85 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.35 (q, 2H, CH_2, J = 6.7 Hz), 2.89 (t, 2H, J = 6.8 Hz, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.8 (C), 147.2 (C), 132.1 (C), 124.4 (CH), 122.3 (CH), 111.2 (CH), 60.7 (OCH_3), 55.7 (OCH_3), 44.2 (CH_2NH), 30.1 (CH_2). Anal. Calcd for ($C_{10}H_{16}N_2O_4S$): C, 46.14; H, 6.20; N, 10.76; S, 12.32. Found: C, 46.10; H, 6.23; N, 10.74; S, 12.34.

4.1.11. *N*-(3,4-Dimethoxyphenethyl)sulfamide (21)

White solid. Mp 96–98 °C. IR (CH_2Cl_2, cm^{-1}) 3391, 2925, 2845, 1636, 1517, 1465, 1330, 1262, 1156. ^1H NMR (400 MHz, $CDCl_3$) δ 6.81 (d, 1H, Ar-H, J = 8.3 Hz), 6.76–6.74 (m, 2H, Ar-H), 4.74 (br s, 3H, NH and NH_2), 3.87 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.35 (t, 2H, CH_2NH, J = 7.0 Hz), 2.82 (t, 2H, CH_2, J = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.3 (C), 148.1 (C), 130.0 (C), 120.7 (CH), 111.9 (CH), 111.6 (CH), 56.0 (OCH_3), 55.9 (OCH_3), 45.1 (CH_2NH), 35.0 (CH_2). Anal. Calcd for ($C_{10}H_{16}N_2O_4S$): C, 46.14; H, 6.20; N, 10.76; S, 12.32. Found: C, 46.17; H, 6.16; N, 10.73; S, 12.33.

4.1.12. Standard procedure for the synthesis of sulfamides (hydrogenolysis of benzyl sulfamoylcarbamates): *N*-(4-methoxyphenethyl)sulfamide (22)

Into a 100 mL flask were placed Pd–C (50 mg) and benzyl sulfamoylcarbamate **17** (0.45 g 1.23 mmol) in MeOH (50 mL). A balloon filled with H_2 gas (3 L) was fitted to the flask. The mixture was deoxygenated by flushing with H_2 and then hydrogenated at room temperature for 4 h. The catalyst was removed by filtration. Recrystallization of the residue from EtOAc–hexane gave **22** (0.21 g, 74%). White solid. Mp 95–98 °C. IR (CH_2Cl_2, cm^{-1}) 3283, 2927, 2851, 1735, 1612, 1547, 1514, 1247, 1157. ^1H NMR (400 MHz, $CDCl_3$) δ 7.13 (d, 2H, Ar-H, J = 8.5 Hz), 6.85 (d, 2H, J = 8.5 Hz, Ar-H), 4.63 (br s, 2H, NH_2), 4.49 (br s, 1H, NH), 3.78 (s, 3H, OCH_3), 3.34 (t, 2H, CH_2NH, J = 6.9 Hz), 2.82 (t, 2H, CH_2, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5 (C), 129.94 (C), 129.85 (2CH), 114.2 (CH), 55.3 (OCH_3), 44.8 (CH_2NH), 34.7 (CH_2). Anal. Calcd for ($C_9H_{14}N_2O_3S$): C, 46.94; H, 6.13; N, 12.16; S, 13.92. Found: C, 46.90; H, 6.12; N, 12.14; S, 13.94.

Sulfamide **23** was also synthesized by this procedure with a yield of 70%.

4.1.13. *N*-(2-Methoxyphenethyl)sulfamide (23)

Brown oil. IR (CH_2Cl_2, cm^{-1}) 3282, 3002, 2955, 1601, 1587, 1495, 1464, 1439, 1327, 1245, 1158. ^1H NMR (400 MHz, $CDCl_3$) δ 7.21–7.13 (m, 2H, Ar-H), 6.89–6.82 (m, 2H, Ar-H), 4.75 (br s, 3H, NH and NH_2), 3.79 (s, 3H, OCH_3), 3.31 (t, 2H, CH_2NH, J = 7.0 Hz), 2.86 (t, 2H, CH_2, J = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.6 (C), 130.8 (CH), 128.1 (CH), 126.7 (C), 120.7 (CH), 110.6 (CH), 55.4 (OCH_3), 43.5 (CH_2NH), 30.5 (CH_2). Anal. Calcd for ($C_9H_{14}N_2O_3S$): C, 46.94; H, 6.13; N, 12.16; S, 13.92. Found: C, 46.91; H, 6.11; N, 12.13; S, 13.94.

4.2. Inhibition studies

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity.[52] Phenol

red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.4) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s.[52] The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (10 mM) were prepared in distilled–deionized water and dilutions up to 0.001 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E–I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3, as reported earlier,[11,13] and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in house as reported earlier.[10–15] The concentration of the enzymes in the assays was in the range of 5.3–12.5 nM. All enzymes were recombinant ones prepared as described earlier by one of our groups.[23–25]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.03.077.

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