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N-Hydroxysulfamides as Analog of *N*-Hydroxyurea: Synthesis and Biological Evaluation

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ABSTRACT: *A series of sulfa-analog of hydroxyurea, derived from N-hydroxysulfamide H_2NSO_2NHOH, were synthesized starting from chlorosulfonyl isocyanate (CSI) and O-substituted hydroxylamines. Their antiproliferative, antiviral (in synergy with ddI), and antifungal activities have been evaluated.* © 2001 John Wiley & Sons, Inc. Heteroatom Chem 12:1–5, 2001

INTRODUCTION

Structural similarities between the sulfonyl group and a tetrahedral carbonyl opening transition-state were often turned to account for the synthesis of analog usable as antagonists of carbonyl-containing biomolecules [1]. Surprisingly only few references were reported concerning the sulfonyl analogs of an old drug: Hydroxyurea (HU) **1** $H_2NCONHOH$, for which new uses have been found and more precisely in the treatment of HIV infection through the inhibition of ribonucleotide reductase. The *N*-hydroxy derivative of urea was originally synthesized in Germany in 1869 by Dressler and Stein [2], but its biological activity was recognized only in 1928, when Rosenthal et al. [3] observed leucopenia, macrocythemia, and death in animals treated with this compound; in 1963, Stearns et al. [4] found hydroxyurea to be active against L1210 mouse leukemia and other malignancies. Furthermore, HU was found to be an effective systemic agent for control of severe psoriasis [5] and was revealed as an efficient antisicklemic agent [6]. For instance, this compound is frequently used in the treatment of chronic myelogenous leukemia and polycythemia vera [7]; moreover in the treatment of HIV infection, HU significantly potentiates the action of didanosine [8]. It is well established that hydroxyurea is an inhibitor (through formation of a free radical) of the ribonucleotide reductase (EC 1.17.4.1), the enzyme involved in the reduction of ribonucleotides into deoxyribonucleotides, the crucial step of the de novo synthesis of DNA [9]. More generally, hydroxamic acids have been associated with diverse biological activities including antiinflammatory, antibacterial, antifungal, and antitumor profiles have become increasingly important in medicinal chemistry [10]. As shown by X-ray crystallographic studies, these compounds are known to inhibit various metalloproteins by their ability to form chelates with an active site metal atom [11].

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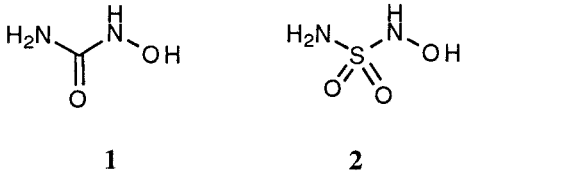
CHEMISTRY

Our purpose is the synthesis of *N*-hydroxysulfamide **2** derivatives, analogs of hydroxyurea, in order to modulate its chemical and pharmacological properties (Figure 1).

The first synthesis of *N*-hydroxysulfamides was described by Bliefert et al. [12], starting from substituted sulfamoyl chloride and hydroxylamine, with disheartening yields in the range 2–15%; improvements were then provided by Dusemund and Schurreit [13] using *O*-alkylated derivatives of hydroxylamine.

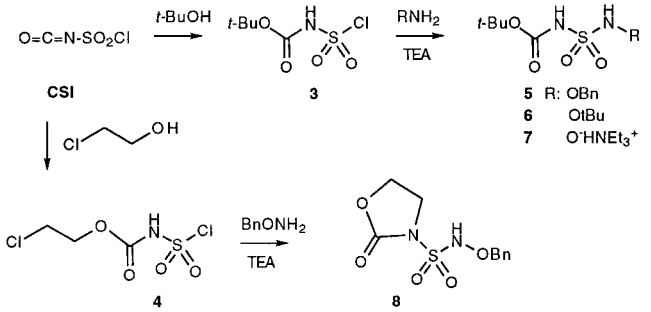
In our previous works, we have established that chlorosulfonyl isocyanate (CSI) is a suitable reagent allowing the introduction of a sulfamoyl group into biomolecules [14]. In the present case, by addition of *tert*-butyl alcohol or 2-chloroethanol, this isocyanate gave the corresponding *N*-chlorosulfonylcarbamates **3** and **4**, which present a better reactivity than sulfamoyl chloride itself. In reaction with nucleophiles, such as hydroxylamine hydrochloride and its *O*-benzyl and *O*-*tert*-butyl derivatives, **3** gave the *N*-carboxylsulfamides **5–7** in 60–90% yields (Scheme 1, top). Starting from 2-chloroethylcarbamate **4**, the 5-*exo-tet* heterocyclic closure was spontaneously carried out under the alkaline conditions of sulfamoylation [15] (the use of triethylamine in dichloromethane at 0°C), and the resulting compound **8** was obtained in 75% yield (Scheme 1, bottom).

The choice of the BOC, *t*-Bu and Bn groups was a priori dictated by the possibility of the selective *N*- or *O*-deprotection of the resulting compounds **5,6**.



1 **2**

FIGURE 1



SCHEME 1

In addition, for compounds such as **8**, the oxazolidinone moiety can play the role of a leaving group and allows a transsulfamoylation reaction toward other amines [16].

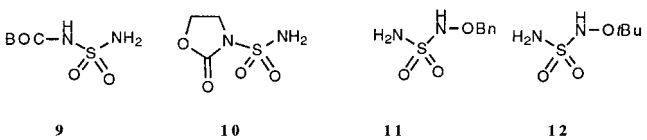
However, attempts to effect hydrogenolysis (Pd–C in the presence of cyclohexadiene or ammonium formate) on compounds **5** and **8** afforded the sulfamides **9** and **10**, respectively, resulting from a *N*-*O* cleavage. The treatment of **5** and **6** by trifluoroacetic acid (TFA) affected only the BOC group and led to the *O*-benzyl and *O*-*t*-butyl protected forms **11** and **12** of hydroxysulfamide. The free form **2** was synthesized in quantitative yield starting from the salt **7** in presence of TFA and characterized by MS, FT-IR, and NMR spectroscopy. The *N*-sulfamoylation (vs. the *O*-substitution) was clearly demonstrated by both signals of N*H*O*H*, at δ = 8.40 and 9.10 ppm, with ^3J = 3.5 Hz.

In comparison with the sulfamoyl chloride, chlororosulfonylcarbamates derived from CSI were thus revealed as convenient reagents for the synthesis of *O*-substituted sulfhydroxamates. Due to difficulties of the selective *NO*-alkyl cleavage, the only appropriate approach to prepare *N*-hydroxysulfamide in good yield requires the use of hydroxylamine hydrochloride.

BIOLOGICAL EVALUATION

Cytotoxicity and results concerning the evaluation of fungicidal, antiviral, and antiproliferative activities for the sulfonylhydroxamates **2** and **7** are reported in the following tables. Values are expressed in μM. Neither of the two sulfonylhydroxamates, were able to show any cytotoxic activity at the highest doses used against three kind of cells tested: CEM, Vero, and peripheral blood mononuclear cells from healthy donors. The fungal strain *Cryptococcus neoformans* is also insensitive to **2** and **7** at 120 and 60 μM, respectively. Antiviral evaluations of these compounds were made on HIV-1, HSV-1, and HSV-2. Except for **7**, which exhibits a relative anti HSV-1 activity with a IC_{50} of 70 μM, no activity was detected up to 100 and 10 or 500 μM on HSV and HIV, respectively (Table 1).

Moreover, compounds **2** and **7** did not show any antiproliferative effect, up to 100 μM, either on normal human fibroblasts (MRC5) or on four cancer cell



9 **10** **11** **12**

FIGURE 2

lines (Table 2). The effects of hydroxyurea and **2** in combination with 2′,3′-dideoxyinosine (ddI) were compared on the replication of HIV-1. In this experiment, a concentration of 25 μM HU was chosen. It was the highest dose of HU tested that did not have a proper toxic effect on PBMC (data not shown). For the nontoxic compound **2**, a dose of 100 μM was used. In the presence of HU, the 50% inhibitory concentration of ddI was reduced by 4.5-fold (from 1.8 to 0.4 μM) in activated PBMC. This result is compatible with previous data obtained by Gao et al. [17] With the same protocol, no potentiating effect on ddI was revealed by a preincubation of 100 μM of **2**.

Hydroxyurea is an inhibitor of ribonucleotide reductase (RR), but not urea and urethane. This fact immediately suggests that the active moiety is the hydroxylated nitrogen atom adjacent to the carbonyl group. HU allows the formation of a free radical, which is presumed to be the active form of the drug and selectively scavenges the tyrosyl free radical of the RR [18].

In our studies with the replacement of carbonyl by sulfonyl we wanted to investigate the influence of this modification on the chemical and pharmacological properties of HU. Unfortunately **2** or **7**/ddI vs. HU/ddi combinations didn't potentiate the anti-HIV effect of ddI monotherapy. This lack of activity and the absence of cytotoxic effect, even at high doses, suggest that these kind of compounds may not interact with cellular and viral RR perhaps by an inability to destroy the catalytically essential tyrosyl radical on the R2 subunit. As describe by Hendricks and Mathews [19] a physical method that monitors the decay (or not) of the radical could be necessary. Furthermore, since hydroxylamine derivatives produce aminoxy radicals, it should be interesting to study the abilities of such sulfonylhydroxamates to generate aminoxy radicals. On the other hand, some biochemistry assays (e.g., determination of the dNTP pools) should help us to design new potential inhibitors.

EXPERIMENTAL

Chemistry

Melting points were determined in open capillary tubes on a Büchi apparatus and are uncorrected. FT-IR spectra were recorded on a JASCO 410 spectrophotometer, with microporous polyethylene disposable supports (3M). Proton nuclear magnetic resonance was determined with a AC 250 Brüker spectrometer. Chemical shifts are expressed in parts per million, with TMS as a reference. The multiplicity is indicated as: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet). Fast-atom bombardment mass spectra (FAB-MS) were recorded in positive or negative mode on a JEOL DX 300 spectrometer with the GT matrix. Thin layer chromatography (TLC) was performed on precoated aluminium sheets of silicagel 60-F_{254} (Merck). Column chromatography was performed with silica gel 60.

General Procedures

Procedure 1: Carbamoylation-sulfamoylation. To a stirred solution of 1 mL of chlorosulfonyl isocyanate (1.63 g, 11.5 mmol) in 20 mL of anhydrous dichloromethane were added 11.5 mmol of absolute *tert*-butyl alcohol or 2-chloroethanol at 0°C. After having been stirred for 30 minutes, the resulting so-

TABLE 1 Cell Cytotoxicity, Antiviral, and Antifungal Activities of Compounds **2** and **7**

	Cell Cytotoxicity			*Antiviral Activity*			*Fungicidal Activity*
Compound	*CEM* $IC_{50}(\mu M)$	*Vero* $IC_{50}(\mu M)$	*PBMC* $IC_{50}(\mu M)$	*HIV-1* $IC_{50}(\mu M)$	*HSV-1* $IC_{50}(\mu M)$	*HSV-2* $IC_{50}(\mu M)$	*Cryptococcus neo.* $IC_{50}(\mu M)$
7	>50	>100	>500	>10	70	>100	>60
2	>50	>100	>500	>500	>100	>100	>120

TABLE 2 Antiproliferative Activity of Compounds **2** and **7**

	Antiproliferative Activity					
Compound	*MRC5-tox* $IC_{50}(\mu M)$	*MRC5-prol* $IC_{50}(\mu M)$	*NCI-H460* $IC_{50}(\mu M)$	*HT 29* $IC_{50}(\mu M)$	*Caki-1* $IC_{50}(\mu M)$	*HL-60* $IC_{50}(\mu M)$
7	>100	>100	>100	>100	>100	>100
2	>100	>100	>100	>100	>100	>100

lution of carbamatesulfamoyl chloride and 5 mL of triethylamine in 20 mL of dichloromethane were added dropwise to 11.5 mmol of a suspension of hydroxylamine derivative (hydrochloride salt) in 25 mL of dichloromethane in the presence of triethylamine (3 mL). The reaction temperature did not rise above 5°C. The resulting mixture was stirred at r.t. over 2 hours. Then, the reaction mixture was diluted with 100 mL of dichloromethane and washed with 0.1N HCl solution and brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The resulting compounds were recrystallized before analysis.

Procedure 2: BOC Cleavage. A solution of TFA (50% in dried dichloromethane; 3 equiv.) was added dropwise into a stirred solution of *t*-butyl-substituted compound (5 mmol) in dried dichloromethane (15 mL) at 0°C. The reaction medium was stirred for 2–5 hours concentrated under reduced pressure, and coevaporated with diethyl ether.

Procedure 3: Hydrogenolysis. To an ethanolic solution of benzylated compound (0.3 mmol in 10 mL) was added 15 mg of palladium on charcoal (5%) and ammonium formate (3 equiv.). The reaction mixture was heated 30–45 minutes at 50°C. The reaction mixture was then filtered through celite and concentrated under reduced pressure. The resulting debenzylated compound was purified by recrystallization.

N-Hydroxysulfamide **2** (*Procedures 1 and 2*)

Yield: 95%, m.p. 83–84°C (lit. 86–87°C [12]): ^{1}H NMR (DMSOd_6, δ): 6.85 (s, 2H, NH_2, exch.); 8.40 (d, J = 3.5 Hz, 1H, OH, exch.); 9.10 (d, J = 3.5 Hz, 1H, NH, exch.). MS (FAB pos.): 113 ($M+H]^+$). IR (ν cm^{-1}): 3350, 3205, 1375, 1170.

Benzyl N-(tert-butyloxycarbonyl)sulfamoylhydroxamate **5** (*Procedure 1*)

Yield: 81%; m.p. 130°C. Rf: 0.70 (CH_2Cl_2:MeOH 95:5). ^{1}H NMR (DMSOd_6, δ): 1.50 (s, 9H, *t*-Bu); 5.03 (s, 2H, CH_2); 7.40 (m, 5H, ArH); 7.85 (sb, 2H, 2 NH, exch.). MS (FAB pos.): 303 ($M+H]^+$).

tert-Butyl N-(tert-butyloxycarbonyl)sulfamoylhydroxamate **6** (*Procedure 1*)

Yield: 90%; m.p. 147°C. Rf: 0.75 (CH_2Cl_2:MeOH 95:5) ^{1}H NMR (DMSOd_6, δ): 1.30 (s, 9H, *t*-Bu); 1.50 (s, 9H, BOC); 7.30, 7.40 (2 s, 2H, NH, exch.). MS (FAB pos.): 269 ($M + H]^+$).

Triethylammonium N-(tert-butyloxycarbonyl) sulfamoylhydroxamate **7** (*Procedure 1*)

Yield: 66%; m.p. 122°C; ^{1}H NMR (DMSOd_6, δ): 1.22 (t, 9H, 3 CH_3–CH_2); 1.38 (s, 9H, *t*-Bu); 3.04 (q, 6H, 3 CH_3–CH_2); 7.95, 8.80 (2 sb, 2H, 2 NH, exch.). MS (FAB neg.): 211 (hydroxamate anion).

N-(N-benzyloxysulfamoyl) 2-Oxazolidinone **8** (*Procedure 1*)

Yield: 76%. m.p. 87°C. Rf: 0.50 (CH_2Cl_2:MeOH 95:5); ^{1}H NMR (DMSOd_6, δ): 3.65 (t, 2H, J = 8.25 Hz, CH_2CH_2–O); 4.40 (t, 2H, CH_2CH_2–N); 4.95 (s, 2H, CH_2Ar); 7.33 (m, 5H, ArH); 7.75 (s, 1H, NH, exch.). MS (FAB pos.): 273 ($M + H]^+$).

N-(tert-butyloxycarbonyl) Sulfamide **9** (*Procedures 1 and 3*)

Yield: 86%; m.p. 128°C; ^{1}H NMR (DMSOd_6, δ): 1.45 (s, 9H, *t*-Bu); 7.10–7.35 (m, 3H, NH + NH_2, exch.). MS (FAB pos.): 197 ($M + H]^+$).

N-sulfamoyl-2-oxazolidinone **10** (*Procedures 1 and 3*)

Yield: 67%; m.p. 130°C. ^{1}H NMR (DMSOd_6, δ): 4.00 (t, 2H, J = 8.25 Hz, CH_2CH_2–O); 4.37 (t, 2H, CH_2CH_2–N); 7.75 (s, 2H, NH_2, exch.). MS (FAB pos.): 167 ($M + H]^+$).

N-benzyloxysulfamide **11** (*Procedures 1 and 2*)

Yield: 75%; m.p. 63°C. ^{1}H NMR ($CDCl_3$, δ): 4.80 (s, 2H, NH_2, exch.); 5.05 (s, 2H, CH_2Ar); 7.00 (sb, 1H, NH, exch.); 7.38 (m, 5H, ArH). MS (FAB pos.): 203 ($M + H]^+$).

N-tert-butyloxysulfamide **12** (*Procedures 1 and 2*)

Yield: 98%; m.p. 60°C. ^{1}H NMR (DMSOd_6, δ): 1.12 (s, 9H, *t*Bu); 6.85 (s, 2H, NH_2, exch.); 8.65 (s, 1H, NH, exch.). MS (FAB pos.): 169 ($M + H]^+$).

BIOLOGY

Material and Methods

CT50 (cytotoxic concentration) of compounds **2** and **7** were determined on the human T lymphoblastoid cell line CEM (ATCC # CC-119) after 4 days of culture at exponential rate. At the end of this period, cells were incubated with propidium iodide that stained only dead cells. The cytotoxic effect of compounds

was measured in duplicates on a flow cytometry cell analyzer (Facscalibun, Becton-Dickinson). The ratio between living and dead cells is representative of the cytotoxic properties of the compounds.

Antiproliferative activities of the compounds **2** and **7** were determined after 3 days using four cancer cell lines in exponential growth rate [NCI H460 (lung large cell carcinoma, ATCC #HTB-177), HT 29 (colon adenocarcinoma, ATCC # HTB-38), Caki-1 (kidney carcinoma ATCC # HTB-46), and HL60s (promyelotic leukemia, ATCC # CCL-240)]. A normal cell line [MRC5 (fS tal lung fibroblast, ATCC # CCL-171)] was used as control. With MRC5 cells, cytotoxic (MRC5-tox) and antiproliferative (MRC5-prol) effects of each compound were determined in stationary and exponential growth rate, respectively. After 3 days of culture, cell growth was determined using the MTT essay as previously described by Park *et al.* [20]. Briefly, the MTT test is based on the enzymatic reduction of the tetrazolium salt MTT in living, metabolically active cells but not in dead cells. The reaction product, a purple-colored formazan soluble in DMSO, was measured colorimetrically at 550 nm, using a multiwell plate reader. Measurements are currently duplicated. Antifungal activities were explored using the *Cryptococcus neoformans* strain (IP960-67). Fungi (105/mL) were seeded in a Sabouraud liquid medium with dilutions of the compounds **2** and **7** and were incubated for 18 hours at 37°C. Fungal development was measured spectrophotometrically at 620 nm.

Peripheral blood mononuclear cells from healthy donors were used to determine the activity of compound **2** on HIV-1 Lai. Cells activated by PHA and IL2 were treated by dilutions of *N*-hydroxysulfamide, or DMSO for control, 1 hour before infection by 100 TCID50. After 7 days, the reverse transcriptase activity in culture supernatant was measured using the RetroSys kit (Innovagen, Lund, Sweden). Activities against HSV-1 and HSV-2 viruses were determined on Vero cells (kidney, African green monkey, ATCC # CCL-81). Cells were infected by HSV-1 or -2. One hour after infection, dilutions of compounds **2** and **7** or DMSO for control were added. After 3 days, the cell layer was fixed by trichloroacetic acid and colored by sulforhodanine B. After dissolution, optical density, which was inversely proportional to the virus activity, was measured at 520 nm using a multiwell plate reader. Measurements were currently duplicated. The cytotoxicity of each compound was also determined in the same conditions but without virus on Vero cells using the MTT assay as described earlier.

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