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A STUDY OF 3-AMINO-N-HYDROXYPROPANESULFONAMIDE DERIVATIVES AS POTENTIAL $GABA_B$ AGONISTS AND THEIR FRAGMENTATION TO 3-AMINOPROPANESULFINIC ACID

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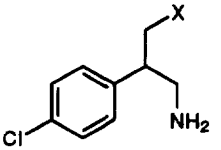
Abstract: A series of 3-amino-N-hydroxypropanesulfonamide analogs was prepared. Several compounds showed potent binding at the $GABA_B$ receptor and were active in an in vitro functional assay. The $GABA_B$ activity of these compounds appeared to be due to the fragmented product, 3-aminopropanesulfinic acid.

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian central nervous system. GABA receptors are subdivided into $GABA_A$ and $GABA_B$ subtypes.[1] The $GABA_B$ receptor is associated with the regulation of a variety of physiological mechanisms,[2] such as modulation of both the cholinergic system in lung,[3] anaphylactic responses in the airways,[4] as well as affecting the cough reflex in cats and guinea pigs.[5] $GABA_B$ agonists have potential therapeutic use in asthma by modulating neural control of airway function. A $GABA_B$ agonist would be expected to inhibit the release of acetylcholine and neurokinins from lung tissue, potentially reducing airway hyperreactivity, reflex bronchospasm, pulmonary inflammation, and cough.

During the past two decades, many $GABA_A$ agonists, antagonists, and uptake inhibitors have been described in the literature.[6a] However, only a limited number of $GABA_B$ agonists and antagonists have been reported.[6b,c] Several $GABA_B$ agonists and antagonists contain bioisosteric replacements for the carboxyl function of GABA or baclofen. These include phosphinic acid agonists,[7a-d] the antagonists phaclofen,[8] and saclofen,[9,10] as well as the agonist siclofen (a sulfinic acid derivative of baclofen).[11]

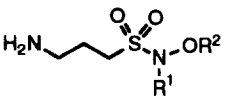


X = $-CO_2H$	X = $-P(O)(OH)_2$	X = $-SO_3H$	X = $-SO_2H$
baclofen	phaclofen	saclofen	siclofen

In a search for novel, selective, orally active $GABA_B$ agonists, we have synthesized a series of 3-amino-N-hydroxypropanesulfonamides in which the N-hydroxysulfonamide is a replacement for the carboxylic acid of GABA. Here we report the synthesis of 3-amino-N-hydroxypropanesulfonamide analogs and their biological activity.

The analogs of 3-amino-N-hydroxypropanesulfonamide **1a–f** (Figure 1) were synthesized according to Schemes 1 and 2.

Figure 1

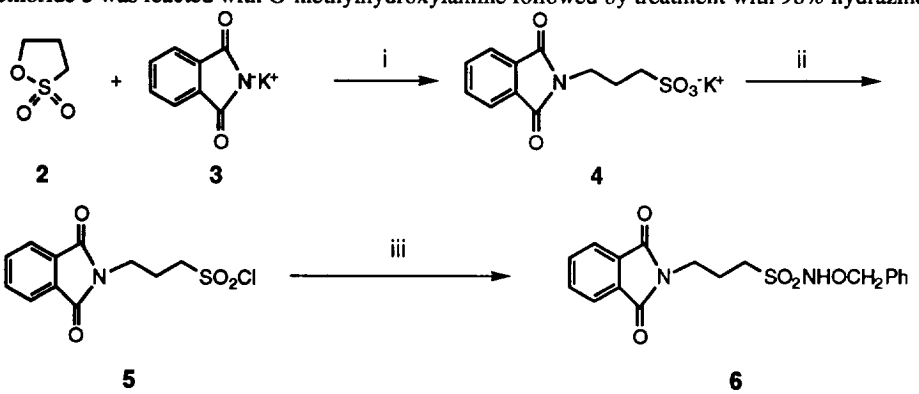


1

a. $R^1 = H$ $R^2 = CH_2Ph$
b. $R^1 = H$ $R^2 = CH_3$
c. $R^1 = H$ $R^2 = H$
d. $R^1 = CH_3$ $R^2 = H$
e. $R^1 = CH_2Ph$ $R^2 = H$
f. $R^1 = CH_2COOH$ $R^2 = H$

Chemistry: The key intermediate **6** was prepared according to the method shown in Scheme 1. Ring opening of 3-propanesultone (**2**) using potassium phthalimide (**3**) gave **4**, which was converted to the sulfonyl chloride derivative **5**. Reaction of **5** with O-benzylhydroxylamine yielded the key intermediate **6**, which was obtained in 67% overall yield from **2**. Treatment of **6** (Scheme 2) with 98% hydrazine, followed by debenzylation of **1a** with boron tribromide produced compound **1c** in good yield. However, debenzylation of compound **1a** with 5% Pd/C in 0.5M HCl or with 10% $Pd(OH)_2$ in anhydrous HCl/EtOH gave variable amounts of the over-reduced product, 3-aminopropanesulfonamide, which was very difficult to separate from the desired compound **1c**.

Other analogs of 3-amino-N-hydroxypropanesulfonamide (**1d–f**), were prepared by treating intermediate **6** with various alkylating agents R^1X to give the alkylated products **7**, following the same sequence of reactions as described above. Compound **1b** was prepared via the same sequence as described in Scheme 1 in which sulfonyl chloride **5** was reacted with O-methylhydroxylamine followed by treatment with 98% hydrazine.

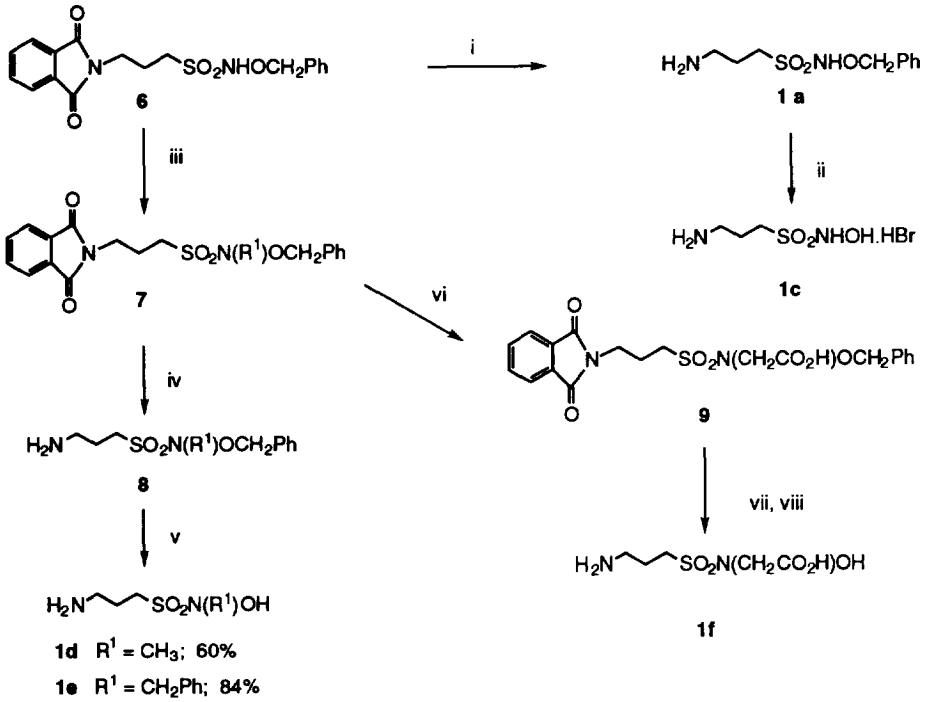


Scheme 1 *Reagents and conditions:* (i) EtOH, reflux, 2 h, 88%; (ii) PCl_5, benzene, reflux, 92%; (iii) $PhCH_2ONH_2 \cdot HCl$, Hünig's Base, CH_2Cl_2, 83%

When **7** ($R^1 = CH_2CO_2tBu$) was treated with hydrazine, decomposition occurred. However, when the t-butyl ester of **7** was hydrolyzed with TFA acid **9** was obtained cleanly. Treatment of **9** with 98% hydrazine, followed by removal of the benzyl protecting group readily afforded target **1f.**

Alkylation of key intermediate **6** with secondary halides such as isopropyl bromide or cyclopentyl bromide was unsuccessful. When R^1 was allyl, removal of the protecting phthalyl group of **7** with 98% hydrazine in ethanol gave a mixture of desired target and the reduced propyl derivative. This mixture was very difficult to purify.

Purification of the final products (**1c–f**) was initially problematic since the compounds were unstable to silica chromatography with acidic, basic, or alcoholic eluants. Although the products hydrolyzed to 3-amino-propanesulfonic acid (**11 in Table 1**) on attempted recrystallization from aqueous methanol, a satisfactory procedure was devised effecting recrystallization from anhydrous ethanol/dichloromethane mixtures.



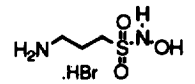
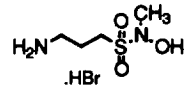
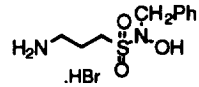
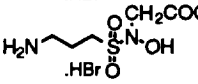
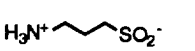
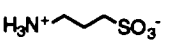
Scheme 2 *Reagents and conditions*: (i) 98% N_2H_4, EtOH, 50 °C, 83%; (ii) BBr_3, CH_2Cl_2, 0 °C, 88%; (iii) R^1X, Cs_2CO_3, n-$Bu_4N^+HSO_4^-$, DMF, 71–87%; (iv) (R^1 = CH_3 or CH_2Ph), 98% N_2H_4,EtOH, 50 °C, 90–97%; (v) BBr_3, CH_2Cl_2, 0 °C; (vi) (R^1 = CH_2CO_2t-Bu), CF_3CO_2H; (vii) 98% N_2H_4, EtOH, 50 °C; (viii) BBr_3, CH_2Cl_2, °C, 56%

Pharmacology: The apparent $GABA_B$ and $GABA_A$ data for the 3-amino-N-hydroxypropanesulfonamides are listed in Table 1. Compounds **1c–f** showed comparable $GABA_B$ activity to (±)-baclofen but they were not specific. They were about one-sixth to one-eighth as potent at the $GABA_A$ receptor. The N-benzyl and N-methyl derivatives (**1d–e**) showed higher apparent potency than baclofen at the $GABA_B$ receptor, similar to that of 3-amino-propanesulfinic acid (**10**). Later we will show that the $GABA_B$ binding activity of compounds (**1c–e**) is probably due to the fragmentation of these compounds to **10**. None of the compounds, including **10**, show much activity in an in vivo $GABA_B$ model. Baclofen inhibits electrical field stimulated (EFS) vagally-mediated broncho-constriction in guinea-pigs[3a] whereas **10** and its precursors are almost inactive. Table 1 shows that the sulfinic acid analog **10** possesses much more potent $GABA_B$ binding activity than the sulfonic acid analog **11**.

Among the four 3-amino-N-hydroxypropanesulfonamide analogs, the apparent $GABA_B$ binding activity was in the order: R = H < CH_2COOH < CH_3 = CH_2Ph (Table 1).

Table 1

Apparent Biological Activity of 3-Amino-N-hydroxypropanesulfonamide Derivatives

	Cpd.	$GABA_B$ Binding[a] IC_{50} μM 30 min	InVitro Trachea[b] IC_{30} μM 10 min	Vagal Bronchospasm[c] % Inh. 3 mg/Kg 10 min	$GABA_A$ Binding[a] IC_{50} μM 30 min
	1c	0.28 (n=1)	16 (8.4–26.4) (n=10)	0	2 (n=1)
	1d	0.04 ± 0.01 (n=3)	2.6 (1.6–6.6) (n=4)	17 ± 16 (n=4)	0.28 (n=1)
	1e	0.04 ± 0.01 (n=4)	4.2 (2.1–9.0) (n=4)	17 ± 16 (n=4)	0.23 (0.21,0.25)[e] (n=2)
	1f	0.08 (n=1)	NT	NT	0.62 (n=1)
	10	0.06 ± 0.01 (n=3)	1.7 (0.7–4.4) (n=4)	25 ± 21[d] (n=4)	0.47 (n=1)
	11	30 ± 4 (n=4)	NT	NT	0.3 ± 0.05 (n=3)
(±)-Baclofen		0.2 ± 0.01 (n=5)	6.4 (4.2–10.7) (n=8)	75 ± 5 (n=4)	inactive

NT = Not tested. [a]The preparation of rat brain synaptosomes and the assays for receptor binding were performed as described elsewhere.[12] [b]Effect of drug on EFS (electrical field stimulation) stimulated neuronal cholinergic contractions of guinea pig tracheal rings.[13] [c]Effect of drug on the bronchospasm caused by vagal nerve stimulation (EFS) in anesthetized, mechanically ventilated guinea-pigs.[3a] [d]56% at 30 min [e]where n=2 results are expressed as the average, and the individual values are shown in parentheses.

Stability Studies: Because we had observed some degree of instability in the 3-amino-N-hydroxypropanesulfonamide analogs during isolation of these compounds, it was important to examine whether they have intrinsic activity or if the $GABA_B$ activity is due to the fragmented product. We performed a stability study of three compounds (**1c** (R=H), **1d** (R=CH_3), **1e** (R=CH_2Ph)) under various pH conditions at room temperature, and investigated the products by examining the NMR spectra after various time intervals. We found that all three compounds gradually hydrolyzed to 3-aminopropanesulfonic acid (**11**) in acidic media, but fragmented to 3-aminopropanesulfinic acid (**10**) in pH 7.4 buffer or at higher pH. The results are shown in Table 2(A). We investigated the stability of these compounds at pH 7.4 and found that only 11% of **1c** fragmented to 3-aminopropanesulfinic acid, yet most of **1d** and **1e** fragmented to the 3-aminopropanesulfinic acid (**10**) within 40 min. The results are shown in Table 2(B) and 2(C). It is interesting to note that **1c** has about one fifth of

both $GABA_B$ and $GABA_A$ activities of **10** yet **1d** and **1e** have comparable biological activities to those of **10** (see Table 1). These results somewhat correlate with their fragmentation rates to the sulfinic acid. Since the $GABA_B$ activity was measured 30 min after administration of drug, it suggests that 3-amino-N-hydroxypropanesulfonamides may possess little intrinsic activity.

Table 2

A. Stability Study of 3-Amino-N-hydroxypropanesulfonamide Derivatives

	Relative Reaction Rate	Reaction Product
pH 2 buffer	**1d** < **1c**	$H_2N(CH_2)_3SO_3H$; **11**
D_2O (HBr salts)	**1d** < **1c** < **1e**	$H_2N(CH_2)_3SO_3H$; **11**
pH 7.4 buffer	**1c** < **1d** < **1e**	$H_2N(CH_2)_3SO_2H$; **10**
pH 8.0 buffer	**1c** < **1d**	$H_2N(CH_2)_3SO_2H$; **10**

The amounts of these compounds present were measured by ^{1}H-NMR using the integral over the methylene protons next to $-SO_2$ (α-CH_2)
δ (ppm) in $CDCl_3$: **1c**: 3.28; **1d**: 3.20; **1e**: 3.20; **10**: 2.29; **11**: 3.18.

B. Fragmentation of 0.1M 1c to 10 in 0.53M Tris buffer (pH = 7.4)

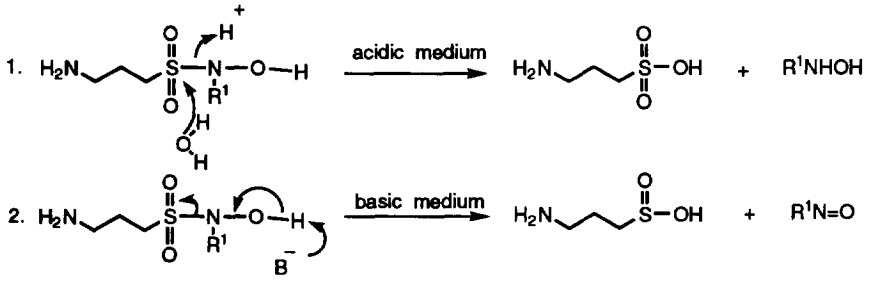
Elapsed time	5–15 min	20–30 min	45–60 min	2 h–3.5 h	4 h 20 min	68 h
Amount of **10**	9–10%	11–11.5%	13–15%	30–46%	61%	100%

C. Fragmentation of 1d (R = CH_3) and 1e (R = CH_2Ph) to 10 at various pH levels

Cpd.	Medium	5 min	15 min	40 min
1d	pH 7.4	15%	60%	71%
	pH 6.5	0%	12%	22–25% (1.5–4 h)
	pH 6.0	0%	5%	
1e	pH 7.4	33%	85%	100%
	pH 6.5	2%	15–20%	40% (1.5–4 h)
	pH 6.0	0%	10%	

We also prepared compounds **1a** and **1b** where R^2 = CH_2Ph and CH_3, respectively. The $GABA_B$ binding assay showed that neither of these compounds is active at 20 μg/mL. In addition, **1a** and **1b** are stable at pH 7.4 condition at room temperature for several days. This result suggests that the hydroxy proton of the 3-amino-N-hydroxypropanesulfonamides is important for the apparent $GABA_B$ binding activity because, once it is substituted, the compound can not be converted to the sulfinic acid derivative (**10**) under physiological conditions.

A similar fragmentation to sulfinic acid derivatives has been reported by Nagasawa et al.[14a] and by Penketh et al.[14b] The proposed mechanisms for decomposition of 3-amino-N-hydroxypropanesulfonamides are as follows.



In conclusion, we have prepared several 3-amino-N-hydroxypropanesulfonamide derivatives. They are not stable under physiological conditions but fragment to 3-aminopropanesulfinic acid **10** which shows potent $GABA_B$ and moderate $GABA_A$ receptor binding and in vitro $GABA_B$ activity comparable to baclofen.

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