Eur J Med Chem (1996) 31, 951–956

Isosteric replacement of the indole nucleus by benzothiophene and benzofuran in a series of indolylglyoxylylamine derivatives with partial agonist activity at the benzodiazepine receptor

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(Received 26 February 1996; accepted 31 May 1996)

Summary — A number of benzothienyl- and benzofurylglyoxylylamine derivatives, which are analogues of previously described indolylglyoxylylamines with a partial agonist activity, are reported in this paper. They were synthesized and tested to verify the importance of the presence of the indole NH group in the interaction of this class of compounds with the benzodiazepine agonist receptor site, since it was reported in literature that a hydrogen bond donor group such as NH was not necessary to elicit an agonist response. Several thienylglyoxylylamine derivatives were also prepared and tested. None of the compounds showed a high affinity at the BzR, demonstrating that the indole NH plays a decisive role in the interaction of the agonist glyoxylylamine ligands with the receptor site.

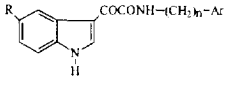
benzothienylglyoxylylamine / benzofurylglyoxylylamine / thienylglyoxylylamine / benzodiazepine receptor

Introduction

Benzodiazepines bind to a region on the $GABA_A$ (GABA = γ-aminobutyric acid) receptor/chloride ion channel complex, facilitating the inhibitory action of GABA [1, 2]. This benzodiazepine receptor (BzR) can accommodate not only classical benzodiazepines but also chemical agents with different structures [3–9], which can mediate a continuum of intrinsic activity ranging from full agonists (sedative-hypnotic, anxiolytic, anticonvulsant and myorelaxant agents) to antagonists (devoid of pharmacological efficacy) to inverse agonists (with proconvulsant, convulsant and anxiogenic properties). Considerable efforts have been focused on discovering features of various ligands (Bz) that are essential for their interaction with BzR. One widely employed method has been the superpositioning of the X-ray crystallographic or computational three-dimensional structures of a number of structurally different BzR ligands, to obtain maximum structural similarity among the agonists, antagonists, and inverse agonists, assuming their largely overlapping recognition sites [10–13]. Another working hypothesis used in approaching the problem consists of assuming that inverse agonists and/or antagonists bind to sites that are independent of the agonist site. Thus, an inverse agonist/antagonist pharmacophore model has been proposed, whose essential features include a hydrogen bond acceptor group designated as A_2, a donor site H_1, and a specific lipophilic area L_1. Ligand interaction with all these groups on the receptor is required to elicit potent inverse agonist activity [14–16].

The agonist model contains one additional hydrogen binding site of interaction, H_2, as well as two additional lipophilic areas, L_2 and L_3. Ligand interactions with the two receptor hydrogen bond donating sites H_1 and H_2, and with the lipophilic sites L_1, L_2 and/or L_3 determine an agonist activity [16–19]. The hydrogen binding site H_1, and the lipophilic region L_2 are points of interaction common to the agonist, antagonist and inverse agonist ligands in an inclusive pharmacophore/receptor model [20].

We recently reported the synthesis, the affinity data at the BzR and the pharmacological profile, as defined by the GABA ratio, of some *N*-(indol-3-ylglyoxylyl)-amine derivatives with a general formula **I** [21–22].



I

The pharmacological profile of this series of compounds varies from inverse agonist to partial agonist depending on the length of the alkyl chain which separates the aryl group from the amide NH (n = 1 or 2), and on the nature of the substituents on the aryl group. Interaction with the above-described inverse agonist and agonist receptor models has also been hypothesized. Indeed, in all cases, methylation of the indole NH causes a dramatic lowering of the affinity at the BzR. This is easily understandable for those derivatives with an inverse agonist activity, for which the anchoring at the receptor site should occur through formation of a hydrogen bond between the indole NH and the CO(2) of the oxalyl bridge with the A_2 and H_1 groups on the receptor, respectively.

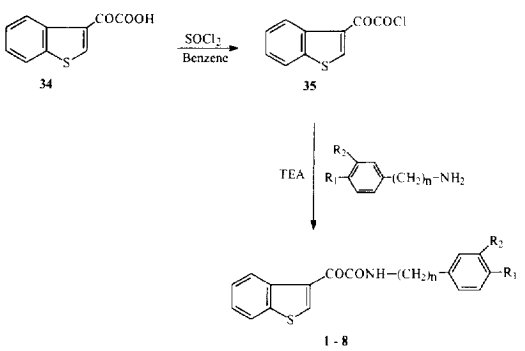
On the other hand, it is not easily understandable for those derivatives with a partial agonist efficacy, for which it was proposed that interaction with the two hydrogen bond donor groups H_1 and H_2 on the receptor is mediated by the two oxygen atoms of the oxalyl bridge. In this case, the interaction of the indole NH group with the hydrogen acceptor site A_2 of the inclusive model of the receptor could be only accessory, contributing to a higher affinity, but not strictly necessary. It has already been reported that for agonist β-carboline derivatives methylation of the indole NH determines a drastic reduction in the affinity [17–19]. This result was explained by hypothesizing the interaction of the *N*-methyl group with a zone of steric repulsion S_1 on the receptor.

Moreover, Cecchi et al [23] recently suggested that the hydrogen donor group, which they called d, is not essential for the anchoring of a ligand to the BzR recognition site, and that the lack of activity of *N*-methylated 6,6,5-tricyclic derivatives is not due to the lack of the hydrogen donor d, but to the steric hindrance of the alkyl substituent in the receptor–ligand interaction.

On the basis of this hypothesis, and taking into consideration some of the derivatives **I** with a partial agonist activity, we substituted the indole NH with an isoster group such as -S- or -O-, that is with moieties which did not possess the steric hindrance of the *N*-methyl analogues.

In this work we report the synthesis and the affinity data at the BzR of a series of benzothienyl **1–8** and benzofurylglyoxylylamine **9–13** derivatives, closely related to **I**.

We also describe several benzofuran derivatives **14–17** in which the glyoxylylamine chain was shifted from the 3- to the 2-position, and some simple (thien-2-yl)glyoxylylamine derivatives **18–33**.



Scheme 1.

Chemistry

The synthesis of (benzothien-3-yl)glyoxylylamine derivatives **1–8** involved the condensation of (benzothien-3-yl)glyoxylyl chloride **35** with the appropriate amines in benzene or THF solution and in the presence of triethylamine (scheme 1). The acid chloride **35** was prepared by treating the (benzothien-3-yl)-glyoxylic acid **34** [24], at reflux in a benzene solution with an excess of thionyl chloride, and it was used in the following reactions without further purification. The acid **34** was obtained by oxidation of 3-acetylbenzothiophene [25, 26] with selenium dioxide in a pyridine solution, introducing minor modifications into the literature procedure [24].

Also acids **36** and **37**, unlike reports in literature [27, 28], were prepared from the corresponding acetyl derivatives [29–32] by oxidation with selenium dioxide, following the same procedure employed to obtain compound **34**. Acids **39** and **40** were synthesized in accordance with known procedures [33, 34].

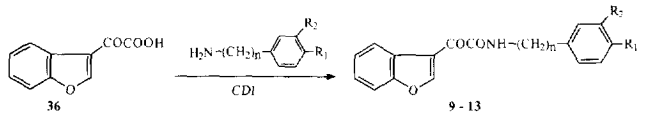
The (benzofur-3-yl)glyoxylylamine derivatives **9–13** were prepared from (benzofur-3-yl)glyoxylic acid **36** [27] and the appropriate amine, in an anhydrous THF solution, under a nitrogen atmosphere, using *N,N'*-carbonyldiimidazole [35] as the condensing agent (scheme 2).

The (benzofur-2-yl)glyoxylylamine derivatives **14–17**, the (thien-2-yl)- **18–25** and the (5-methylthien-2-yl)glyoxylylamine derivatives **26–33** were obtained by condensation of the appropriate amine via the corresponding acid chlorides **38**, **41** and **42** (schemes 3 and 4).

All products **1–33** were purified by recrystallization and their structure was confirmed by IR, ^{1}H NMR and elemental analyses (tables I and II).

Biochemistry

Compounds **1–33** were tested for their ability to displace [^{3}H]flunitrazepam from bovine brain mem-



Scheme 2.

branes. The inhibition of [^{3}H]flunitrazepam binding was first examined at a single concentration (10 μM) of the displacing agent, and then, for the most active compounds, the IC_{50} values were determined from log-probit plots, using four to six concentrations, and the K_i values were also derived in accordance with the equation of Cheng and Prusoff [36]. The ligand affinity (K_d) of [^{3}H]flunitrazepam was 1.8 nM.

Furthermore, using an exhaustively washed membrane preparation, the GABA ratio values of active compounds were evaluated as an in vitro indicator of the agonist, inverse agonist, or antagonist properties, in accordance with the suggestions of various authors [37, 38].

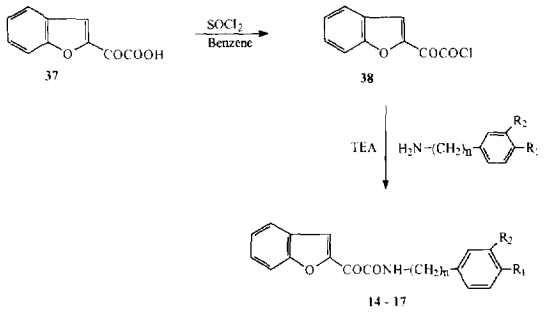
The binding data for compounds **1–33** are reported in tables I and II.

Results and discussion

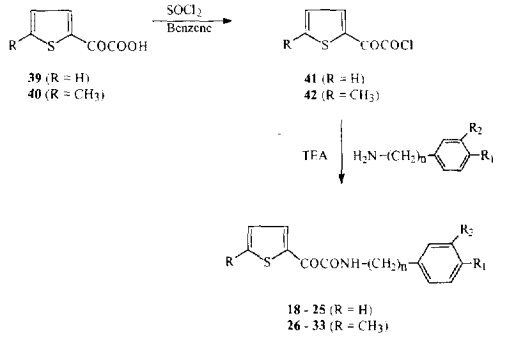
All the compounds synthesized **1–33** showed from moderate to scarce affinity at the BzR as reported in tables I and II. The benzothiophene **1–8** and the benzofuran derivatives **9–13** demonstrated a potency lower than that of the corresponding partial agonist indole derivatives **I**, which present K_i values ranging from 0.048 to 0.820 μM [21, 22]

The shift of the glyoxylylamine side chain from the 3- to the 2-position of benzofuran (compounds **14–17**) left the situation unchanged. Also the simple thiophene derivatives **18–33** showed a low affinity at the BzR, even if in this series, product **20** appeared to be the most potent, with a K_i value of 0.495 μM.

In conclusion, the substitution of the indole NH, either with a methyl, as previously reported [21], or with non-sterically hindered isoster groups (S, O) prevents these compounds from interacting adequately with the receptor site. It would therefore seem, at least for our indolylglyoxylylamine derivatives **I** which possess a high conformational freedom, that even if the indole NH group is not directly involved in the receptor interaction it contributes in an essential way in orientating the molecule in an appropriate alignment for interaction of the oxygen atoms of the glyoxylyl bridge with the two hydrogen bond donor groups H_1 and H_2, in the agonist site.



Scheme 3.



Scheme 4.

Experimental protocols

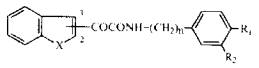
Chemistry

Melting points were determined on a Köfler hot-stage apparatus and are uncorrected. IR spectra were recorded with a Pye Unicam Infracord Model PU 9516 in Nujol mulls. Routine ^{1}H NMR spectra were determined on a Varian CFT 20 spectrometer operating at 80 MHz, using tetramethylsilane (TMS) as the internal standard. Magnesium sulfate was always used as the drying agent. Evaporations were made in vacuo (rotating evaporator). Analytical TLC was carried out on Merck 0.2 mm precoated silica-gel (60 F-254) aluminium sheets, with visualization by irradiation with a UV lamp. Silica gel 60 (70–230 mesh) was used for column chromatography and silica gel 60 (230–400 mesh) was used for flash chromatography. Elemental analyses were performed by our analytical laboratory and agreed with theoretical values to within ± 0.4%.

*General procedure for the synthesis of the (benzothien-3-yl)-glyoxylylamine derivatives **1–8**, (benzofur-2-yl)glyoxylylamine derivatives **14–17**, (thien-2-yl)glyoxylylamine derivatives **18–25** and (5-methylthien-2-yl)glyoxylylamine derivatives **26–33***

An excess of thionyl chloride (0.0032 mol) was added at 0 °C to a suspension of the acid **36**, **41**, **45** or **46** (0.0016 mol) in anhydrous benzene. The mixture was refluxed for 3 h, and then excess thionyl chloride was distilled off under reduced pressure

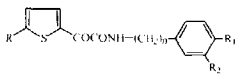
Table I. Physical properties and inhibition of [^{3}H]flunitrazepam specific binding from bovine brain membranes of benzothienyl- **1–8** and benzofuryl-glyoxylylamine derivatives **9–17**.



Compound	*X*	*Position*	R_1	R_2	*n*	*Yield (%)*	*Crystallization solvent*	*Mp (°C)*	*Formula*[a]	*Inhibition*[b] *(%) (10 μM)*	K_i[c] *(μM)*	*GABA ratio*[d]
1	S	3	H	H	2	42	Petroleum ether 60–80°	53–55	$C_{18}H_{15}NO_2S$	50		
2	S	3	Cl	H	2	46	Petroleum ether 60–80°	88–89	$C_{18}H_{14}ClNO_2S$	59		
3	S	3	F	H	2	37	Petroleum ether 60–80°	81–83	$C_{18}H_{14}FNO_2S$	53		
4	S	3	OCH_3	H	2	40	Petroleum ether 60–80°	88–89	$C_{19}H_{17}NO_3S$	84	1.62	1.15
5	S	3	H	OCH_3	2	47	Petroleum ether 100–140°	72–74	$C_{19}H_{17}NO_3S$	74	3.37	1.20
6	S	3	OCH_3	OCH_3	2	49	Petroleum ether 100–140°	95–97	$C_{20}H_{19}NO_4S$	67		
7	S	3	H	OH	1	50	Benzene	144–145	$C_{17}H_{13}NO_3S$	43		
8	S	3	OH	OH	1	62	Benzene	110–112	$C_{17}H_{13}NO_4S$	26		
9	O	3	H	H	2	45	Petroleum ether 60–80°	115–117	$C_{18}H_{15}NO_3$	11		
10	O	3	Cl	H	2	36	Petroleum ether 60–80°	137–138	$C_{18}H_{14}ClNO_3$	8		
11	O	3	F	H	2	32	Petroleum ether 60–80°	120–122	$C_{18}H_{14}FNO_3$	9		
12	O	3	OCH_3	H	2	52	Ethyl acetate	139–141	$C_{19}H_{17}NO_4$	20		
13	O	3	OCH_3	OCH_3	2	55	Ethyl acetate	114–115	$C_{20}H_{19}NO_5$	37		
14	O	2	H	H	2	42	Petroleum ether 60–80°	127–128	$C_{18}H_{15}NO_3$	11		
15	O	2	Cl	H	2	55	Petroleum ether 100–140°	149–151	$C_{18}H_{14}ClNO_3$	28		
16	O	2	F	H	2	52	Petroleum ether 100–140°	139–141	$C_{18}H_{14}FNO_3$	22		
17	O	2	OCH_3	H	2	65	Petroleum ether 100–140°	131–132	$C_{19}H_{17}NO_4$	64		

[a]Elemental analyses for C, H, N were within ± 0.4% of the calculated values. [b]Percentages of inhibition of specific [^{3}H]flunitrazepam binding at 10 μM compound concentration are means ± SEM of five determinations. [c]K_i values are means ± SEM of three determinations. [d]GABA ratio = K_i without GABA/K_i with GABA.

Table II. Physical properties and inhibition of [^{3}H]flunitrazepam specific binding from bovine brain membranes of thienylglyoxylylamine derivatives **18–33**.



Compound	*R*	R_1	R_2	*n*	*Yield (%)*	*Crystallization solvent*	*Mp (°C)*	*Formula*[a]	*Inhibition*[b] *(%) (10 μM)*	K_i[c] *(μM)*	*GABA ratio*[d]
18	H	H	H	2	35	Petroleum ether 60–80°	59–61	$C_{14}H_{13}NO_2S$	43		
19	H	Cl	H	2	47	Petroleum ether 60–80°	83–85	$C_{14}H_{12}ClNO_2S$	82	1.80	2.10
20	H	F	H	2	39	Petroleum ether 60–80°	70–72	$C_{14}H_{12}FNO_2S$	94	0.495	1.75
21	H	OCH_3	H	2	52	Petroleum ether 60–80°	83–85	$C_{15}H_{15}NO_3S$	53		
22	H	H	OCH_3	2	38	Petroleum ether 60–80°	53–54	$C_{15}H_{15}NO_3S$	80	1.90	1.35
23	H	OCH_3	OCH_3	2	52	Petroleum ether 100–140°	105–107	$C_{16}H_{17}NO_4S$	50		
24	H	H	OH	1	31	Benzene	115–116	$C_{13}H_{11}NO_3S$	36		
25	H	OH	OH	1	46	Benzene	127–128	$C_{13}H_{11}NO_4S$	4		
26	CH_3	H	H	2	39	Petroleum ether 60–80°	51–53	$C_{15}H_{15}NO_2S$	50		
27	CH_3	Cl	H	2	65	Petroleum ether 60–80°	106–108	$C_{14}H_{14}ClNO_2S$	87	1.53	1.75
28	CH_3	F	H	2	40	Petroleum ether 60–80°	78–79	$C_{15}H_{14}FNO_2S$	66		
29	CH_3	OCH_3	H	2	36	Petroleum ether 60–80°	100–102	$C_{16}H_{17}NO_3S$	76	4.33	1.1
30	CH_3	H	OCH_3	2	47	Petroleum ether 60–80°	65–67	$C_{16}H_{17}NO_3S$	67		
31	CH_3	OCH_3	OCH_3	2	60	Petroleum ether 100–140°	103–105	$C_{17}H_{19}NO_4S$	58		
32	CH_3	H	OH	1	32	Petroleum ether 100–140°	144–145	$C_{14}H_{13}NO_3S$	43		
33	CH_3	OH	OH	1	41	Benzene	172–174	$C_{14}H_{13}NO_4S$	43		

[a]Elemental analyses for C, H, N were within ± 0.4% of the calculated values. [b]Percentages of inhibition of specific [^{3}H]flunitrazepam binding at 10 μM compound concentration are means ± SEM of five determinations. [c]K_i values are means ± SEM of three determinations. [d]GABA ratio = K_i without GABA/K_i with GABA.

and the residue was washed three times with anhydrous benzene. The oily residue obtained was dissolved in 20 mL anhydrous benzene (THF for **8**, **25** and **33**) and cooled at 0 °C; triethylamine (0.0017 mol) and, subsequently, a solution of the appropriate amine (0.0017 mol) in 2 mL benzene (THF for **8**, **25** and **33**) was added. The reaction mixture was left under stirring at room temperature for 24–36 h (TLC analysis). After filtering off the triethylamine hydrochloride, the solution was concentrated to dryness. The residue was triturated with dilute hydrochloric acid and then with saturated sodium hydrogen carbonate aqueous solution, washed with water and collected to give a crude product, which was purified by recrystallization from the appropriate solvent, if necessary after silica-gel column filtration. Yields, recrystallization solvents and melting points are reported in tables I and II. The spectrometric data for **1** and **26**, which are representative of the title compounds, are listed below.

*N-[(Benzothien-3-yl)glyoxylyl]phenylethylamine **1**.* IR ν cm^{-1}: 3380, 1680, 1630, 1380, 1160. ^{1}H-NMR ($CDCl_3$): δ 2.85 (t, 2H, ArCH_2); 3.60 (q, 2H, NHCH_2); 7.1–9.8 (m, 11H, ArH, NH).

*N-[(5-Methylthien-2-yl)glyoxylyl]phenylethylamine **26**.* IR ν cm^{-1}: 3350, 1680, 1640, 1220, 780. ^{1}H-NMR ($CDCl_3$): δ 2.33 (s, 3H, CH_3); 2.70 (t, 2H, ArCH_2); 3.55 (q, 2H, NHCH_2); 6.70–8.35 (m, 8H, ArH, NH).

*(Benzofur-3-yl)glyoxylylamine derivatives **9–13**.* *N,N'*-Carbonyldiimidazole (0.26 g, 0.0016 mol) was added, under stirring in a nitrogen atmosphere, to a solution of (benzothien-3-yl)-glyoxylic acid **39** (0.30 g, 0.0016 mol) in anhydrous THF (10 mL). After carbon dioxide evolution had ceased, a solution of the appropriate amine (0.0016 mol) in 2 mL anhydrous THF was added to the reaction mixture, which was left to stir at room temperature for 2–3 h (TLC analysis). The solution obtained was concentrated to dryness, and the residue, dissolved in chloroform, was washed with saturated sodium hydrogen carbonate aqueous solution, and then with water. After drying, the chloroform solution was concentrated to dryness, and the crude product was purified by recrystallization, after filtration, when necessary, on a silica-gel column. Yields, recrystallization solvents and melting points are reported in table I. The spectrometric data for **9**, which is representative of the title compounds, are listed below.

*N-[(Benzofur-3-yl)glyoxylyl]phenylethylamine **9**.* IR ν cm^{-1}: 3180, 3080, 1690, 1640, 1510, 1130, 750. ^{1}H-NMR ($CDCl_3$): δ 2.91 (t, 2H, ArCH_2); 3.65 (q, 2H, NHCH_2); 7.10–9.35 (m, 11H, ArH, NH).

Biochemistry

Tritiated flunitrazepam was obtained from Du Pont de Nemours, New England Nuclear Division (Dreieichenhaim, Germany) and had a specific activity of 83.5 Ci/mol and a radiochemical purity > 99%. All other chemicals were of reagent grade and obtained from commercial suppliers.

Bovine cerebral cortex membranes were prepared in accordance with reference [39]. The membrane preparations were subjected to a freeze–thaw cycle, washed by suspension and centrifugation in 50 mM Tris-citrate buffer pH 7.4 and used in the binding assay. Protein concentration was assayed by the method of Lowry et al [40].

Binding studies were performed by using a filtration technique essentially as previously reported [21].

Acknowledgments

This work was supported by grants from the Ministry of University and Scientific and Technological Research (MURST) (Research fund 60%) and from the Italian National Research Council (Progetto di Ricerca CNR: Chimica Fine II).

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