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Use of Bicuculline, a GABA Antagonist, as a Template for the Development of a New Class of Ligands Showing Positive Allosteric Modulation of the $GABA_A$ Receptor

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Abstract—Analogues of bicuculline devoid of the benzo ring fused to the lactone moiety were prepared by reacting 2-(*tert*-butyldimethylsiloxy)furans with 3,4-dihydroisoquinolinium salts. Some of these compounds (e.g., ROD185, **8**) acted as modulators of the $GABA_A$ receptor, displacing ligands of the benzodiazepine binding site. They also strongly stimulated GABA currents mediated by recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes.

γ-Aminobutyric acid (GABA) is the major neuroinhibitory neurotransmitter of the central nervous system. One of its actions is binding to a receptor, the $GABA_A$ receptor, which forms a ligand-gated ion channel controlling neuronal chloride ion flux. Structurally, the $GABA_A$ receptor is a supramolecular complex formed by five protein subunits classified as α, β, γ, δ, ε, π and θ, and some of these subunits in turn occur generally in several isoforms.[1–6] While many distinct $GABA_A$ receptors have been identified in the CNS differing in their subunit compositions, the majority of these receptors consist of the α1, β2 and γ2 subunits.[7,8] In addition to the binding site for GABA itself, the $GABA_A$ receptor possesses several allosteric modulatory sites, the best known of which are the benzodiazepine, barbiturate, picrotoxin and neurosteroid binding sites.[9] Other less well characterized binding sites are those for the γ-butyrolactones[10] and loreclezole.[11] Such ligands can either stimulate currents produced by GABA (positive allosteric modulators, e.g., benzodiazepines)[12] or inhibit them (negative allosteric modulators, e.g., β-carbolines).[13]

(+)-Bicuculline (**1**) is a phthalide isoquinoline isolated by Manske[14] from *Dicentra cucullaria* in 1932 and shown by Curtis and co-workers[15] in 1970 to be a specific competitive antagonist of GABA. Surprisingly, since that time, very few structure–activity studies have been conducted on bicuculline, perhaps because the convulsant nature of this ligand obscured the possibility of developing therapeutically useful drugs. Simonyi and co-workers[16] determined the $GABA_A$ receptor activities of 45 phthalide isoquinoline alkaloids related to (+)-bicuculline. No compound was found to be more active than bicuculline itself but in all cases, the *erythro* stereoisomers (i.e. as in bicuculline **1**) were more active that the *threo* isomers. This conclusion was confirmed by the same authors in a more recent study.[17]

The importance for $GABA_A$ receptor activity of the benzo ring fused to the butyrolactone moiety of bicuculline has never been demonstrated. Removal of this aromatic ring would give rise to novel isoquinoline furanones of general structure **2**. In this communication, we wish to report the synthesis of molecules of type **2** and demonstrate that, unexpectedly, such molecules are potent positive allosteric modulators of the $GABA_A$ receptor, apparently acting via the benzodiazepine binding site.

Two different series of compounds of type **2** were prepared, one closely resembling bicuculline in which the R^2 group is methyl and another series having

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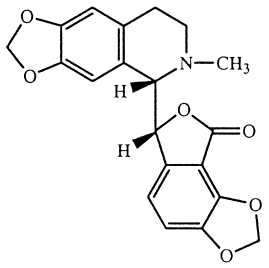
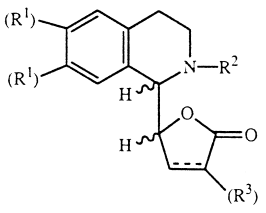
benzyloxycarbonyl (Cbz) as the R^2 group. The Cbz group was chosen as a convenient way of modifying the substituents on the isoquinoline nitrogen since, in principle, it can be easily removed by hydrogenolysis and the freed nitrogen atom alkylated or acylated as desired. Moreover, such molecules might be pharmacologically interesting in their own right.

The *N*-methyl derivatives were synthesized by quaternization of 3,4-dihydro-6,7-methylenedioxyisoquinoline (**3a**)[18,19] or 6,7-dimethoxy-3,4-dihydroisoquinoline (**3b**)[20] with methyl iodide in acetonitrile followed by reaction in situ of the resulting isoquinolinium salts (**4a**)[21] with the known 2-(*tert*-butyldimethylsiloxy)-3-methylfuran **5a** in the presence of 1.2 equiv of cesium fluoride[22–26] (Scheme 1). Coupling occurred at the C-5 position of the furan to give mixtures of the *threo* and *erythro* diastereomers **6a**,**b** and **7a**,**b**,[27] respectively. The diastereomers could be separated either by fractional crystallization or by chromatography on silica gel. Total yields were of the order of 40%, with the *threo* isomer predominating over the *erythro* isomer. The relative stereochemistry of compounds **6** and **7** was deduced principally by comparison of their ^{13}C NMR spectra since it has been shown in related systems that the ^{13}C chemical shifts of C-5 and C-1′ in the *threo* isomer are, respectively, further downfield and higher upfield than those of the corresponding carbons of the *erythro* isomer.[28,29] Unexpectedly, reaction of silyl enol ether **5b** ($R^3 = H$)[30] with isoquinolinium salts **4a** under identical reaction conditions used in the case of **5a** gave the products of C-3 rather than C-5 coupling with the furan. This explains our use of the 2-methyl derivative **5a** in this part of the study.



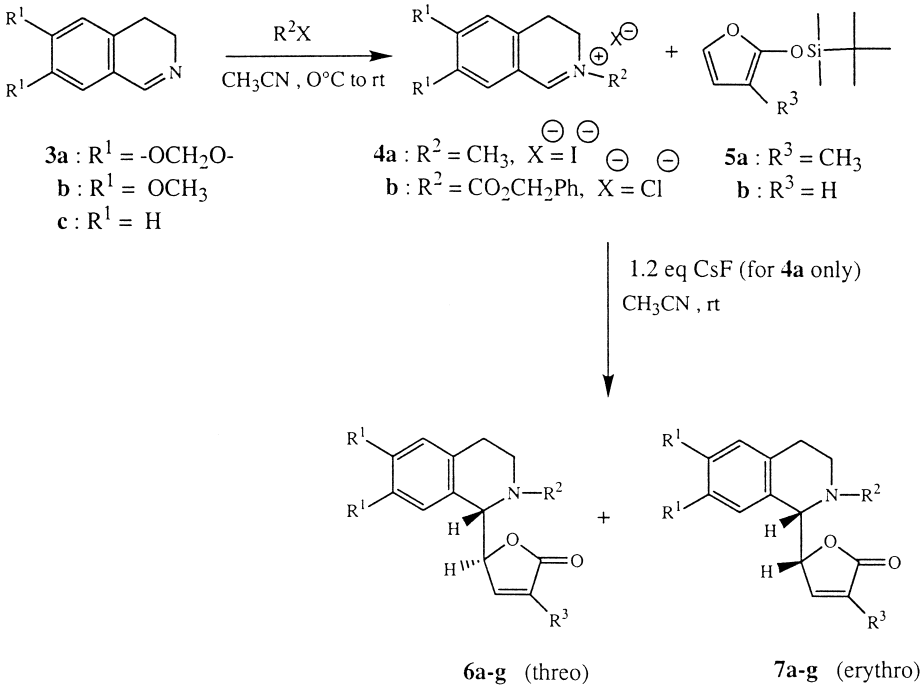
1: (+)-Bicuculline



2

The *N*-Cbz analogues of **6a**,**b** and **7a**,**b** (i.e. compounds **6c**–**g** and **7c**–**g**) were prepared in similar fashion from the *N*-Cbz isoquinolinium salts **4b** except that in this case, the addition of CsF was not necessary. Product yields (60–80%) were somewhat higher than in the case of the *N*-methyl analogues, no doubt due to the greater reactivity of the iminium having a strongly electron withdrawing *N*-substituent. In contrast to the *N*-methyl isoquinolinium salts **4a**, the *N*-Cbz derivatives **4b** reacted only at the C-5 position of enol ether **5b**. Finally, the double bond of the lactone ring of compound **6f** could be selectively reduced using sodium borohydride–nickel chloride complex[31] in methanol and THF to give compound **8** (code named ROD185) (Scheme 2).

The synthesized compounds were evaluated in vitro (Table 1) for their ability to displace appropriate radioactive ligands from rat brain membrane preparations. Thus, binding to the GABA site was determined by displacement of [^{3}H]-muscimol and interaction with the benzodiazepine binding site by displacement of [^{3}H]-flunitrazepam. Direct effects (competitive displacement)

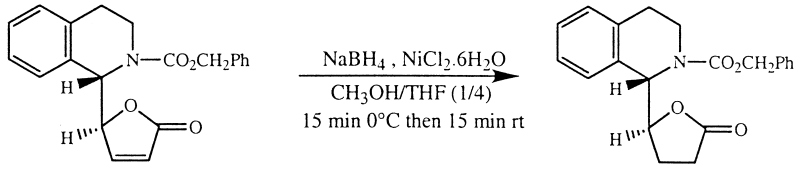


Scheme 1.

or indirect effects on the picrotoxin binding site (shifting the channel to the open state and thus causing dissociation of the ligand)[32] were studied by displacement of [^{35}S]-TBPS (*t*-butylbicyclophosphorothionate) from its binding site.[33,34] Compounds were first screened at 100 μM concentrations and when displacement exceeded 50% or solubility was limited, 10 μM concentrations (or lower) were assayed. Compounds were also tested electrophysiologically for their aptitude to stimulate or inhibit GABA-elicited currents in *Xenopus laevis* oocytes expressing rat brain recombinant α1β2γ2 $GABA_A$ receptors.[35,36]

The compound having the most structural resemblance to bicuculline, i.e. *erythro* derivative **7a** (100 μM), was found to have very weak activity in inhibiting flunitrazepam binding (30±2%) while TBPS and muscimol binding were essentially unaffected. Its *threo* diastereomer **6a** was almost completely inactive in all three radioligand assays and, moreover, both compounds were inactive in stimulating or inhibiting GABA-elicited currents in oocytes. By comparison, bicuculline produced complete inhibition of GABA currents (−100% at 100 μM). Substituting the methylenedioxy group of **7a** by dimethoxy groups (**7b**) resulted in even weaker inhibition of [^{3}H]-flunitrazepam binding (12±4% at 100 μM). Diastereomer **6b** was similarly inactive, effects on GABA currents being again negligible. It thus appears that the replacement of the fused benzo ring of bicuculline by a methyl group results in significant loss of GABA antagonist activity.

We next turned our attention to the *N*-Cbz derivatives **6c–g** and **7c–g**. Interestingly, the methylenedioxy analogues **6c** and **7c** both showed, at 50 μM, good stimulation of GABA-elicited currents (+215 and +128%, respectively), in complete contrast to the inhibitory bicuculline. Both **6c** and **7c** had similar radioligand displacement profiles, with 10 μM concentrations of these compounds being able to displace 20–25% of flunitrazepam and TBPS but not muscimol. The dimethoxy analogues **6d** and **7d** demonstrated considerably reduced current stimulations (+20 and +43%, respectively) compared to the methylenedioxy derivatives **6c** and **7c**, though the radioligand inhibition profiles were similar. The 3-methyl-6′,7′-dimethoxy derivative **6e** was practically inactive, though significant current stimulation (+60%) was observed with its isomer **7e** which



6f **8 (ROD185)**

Scheme 2.

Table 1. Displacement of radioactive ligands from rat membranes and effect on GABA-elicited currents in *Xenopus* oocytes for compounds **6a–g**, **7a–g** and ROD185 (**8**)

				% Displacement of radioactive ligand[a]						% GABA current stimulation (+) or inhibition[b](−) at 100 μM
				[^{3}H]-flunitrazepam		[^{35}S]-TBPS		[^{3}H]-muscimol[g]		
Compounds	R^1	R^2	R^3	10 μM	100 μM	10 μM	100 μM	10 μM	100 μM	
6a	-OCH_2O-	CH_3	CH_3		21 ± 3		3 ± 3		3 ± 4	−3 ± 4
7a				6 ± 3	30 ± 2	1 ± 5	3 ± 3		5 ± 6	−7 ± 2
6b	OCH_3	CH_3	CH_3		8 ± 2		2 ± 3		4 ± 4	−8 ± 2
7b					12 ± 4		4 ± 4		−1 ± 4	−10 ± 3
6c	-OCH_2O-	Cbz	H	20 ± 5	ls[c]	19 ± 4	ls	−5 ± 4[g]	ls	+215 ± 16[d]
7c				27 ± 3	ls	18 ± 3	ls	−7 ± 5	ls	+128 ± 36[d]
6d	OCH_3	Cbz	H	5 ± 3	15 ± 5	5 ± 2	47 ± 4		−1 ± 5	+20 ± 7
7d				15 ± 4	51 ± 4	5 ± 2	50 ± 6	−7 ± 4	−10 ± 2	+43 ± 8
6e	OCH_3	Cbz	CH_3		4 ± 7		11 ± 6		−1 ± 4	+24 ± 5
7e					31 ± 4		63 ± 5		−23 ± 3	+60 ± 26
6f	H	Cbz	H	93 ± 1		14 ± 4	82 ± 2	−12 ± 4	−13 ± 3	+281 ± 35
7f				51 ± 2		18 ± 2	85 ± 1	−9 ± 3	−2 ± 6	+225 ± 68
6g	H	Cbz	CH_3	43 ± 2	ls	16 ± 3		−7 ± 4	ls	+130 ± 4
7g				27 ± 3	ls	20 ± 2		−4 ± 3	ls	+208 ± 4
8 (ROD185)	H	Cbz	H	96 ± 4		11 ± 3	82 ± 4	−18 ± 4	−2 ± 3	+351 ± 17 +111 ± 27[e]
Bicuculline					7 ± 3		−1 ± 2	45 ± 2		−100 ± 0
Diazepam				10 nM: 33 ± 2			25 ± 8		−13 ± 3	+150 to +300[f]

[a]Determined in rat whole brain membrane preparations as previously described;[33,34] data expressed as means ± SD; experiments were performed at least three times in triplicate.
[b]Determined electrophysiologically in *Xenopus laevis* oocytes expressing rat recombinant α1β2γ2 $GABA_A$ receptors as previously described;[35,36] average of three assays; data expressed as means ± SD.
[c]Limited solubility.
[d]Assay performed at 50 μM of test substance.
[e]Assay performed at 10 μM of test substance.
[f]Assay performed at 1 μM of test substance.
[g]A negative value indicates stimulation of radioligand binding.

Table 2. IC_{50} values of compounds **6g**, **7g**, ROD185 and diazepam in rat cerebellar and forebrain tissue

Compounds	IC_{50} (μM)[a] against [^{3}H]-flunitrazepam	
	ce[b]	fb[c]
6g	7	12
7g	17	25
ROD185	0.06±0.004	0.16±0.002
Diazepam	0.02±0.002	0.02±0.002

[a]Assays performed as previously described;[33,34] data expressed as means±SD; assays of three experiments in triplicate.
[b]ce: Rat cerebellar tissue.
[c]fb: Rat forebrain tissue (whole brain minus cerebellum).

may be correlated to its greater capacity to inhibit [^{3}H]-flunitrazepam binding (31% at 100 μM). Compound **7e** also showed appreciable inhibition of TBPS binding (63% at 100 μM) but stimulated muscimol binding (123% at 100 μM).

Removal of the methoxy groups from the isoquinoline ring, as in compounds **6f** and **7f**, led to increased current stimulation (+281 and +225%, respectively) as well as increased flunitrazepam and TBPS binding inhibition potencies. For **6f**, this corresponded to 93% inhibition of flunitrazepam binding at 10 μM and 82% of TBPS binding at 100 μM. Some stimulation of muscimol binding by **6f** (112% at 10 μM) was again observed. Significantly, the current stimulation produced by **6f** could be completely reversed by the presence of the benzodiazepine antagonist flumazenil. The addition of a methyl group at the C-2 position of the lactone ring (i.e., compounds **6g** and **7g**) had only minor effects on radioligand binding and current stimulation as far as the *erythro* isomer was concerned (compare **7g** and **7f**) but in the case of the *threo* isomer, both inhibition of flunitrazepam binding and current stimulation were diminished (compare **6g** and **6f**). The IC_{50}s of compounds **6g** and **7g** with respect to [^{3}H]-flunitrazepam were determined to correspond to 7 μM and 17 μM in cerebellar tissue, respectively (Table 2). Both compounds displayed higher IC_{50}s (12 and 25 μM, respectively) in cortical tissue. Finally, a potent and selective interaction with the benzodiazepine binding site was demonstrated by the reduced analogue of **6f**, ROD185 (**8**). This substance stimulated currents by +351% at 100 μM and by 111% at 10 μM concentrations. The latter stimulation could be essentially completely (80%) reversed by flumazenil (1 μM). Furthermore, ROD185, the most potent stimulator of GABA currents in this study, also demonstrated the highest capacity to displace [^{3}H]-flunitrazepam, with IC_{50}s of 60 nM in cerebellar tissue and 160 nM in cortical tissue (Table 2). ROD185 also inhibited TBPS binding to a lesser degree (11% at 10 μM) and displayed a weak stimulatory action on muscimol binding (118% at 10 μM).

In conclusion, initiated by a desire to study structure–activity relationships of the convulsant GABA antagonist bicuculline, we have discovered a new class of positive allosteric modulators of the $GABA_A$ receptor. Compared to bicuculline, the most active compound of this series, ROD185, has 1 — no fused benzo ring on the lactone moiety, 2 — no alkoxy groups on the isoquinoline ring, 3 — an electron-withdrawing aromatic substituent instead of a methyl group attached to the isoquinoline nitrogen, 4 — a *threo* configuration instead of an *erythro* configuration. In analogous fashion to diazepam (and other positive modulators of the $GABA_A$ receptor), but in distinct contrast to bicuculline, ROD185 strongly stimulates GABA currents as measured electrophysiologically in *Xenopus* oocytes. Compounds of this type should thus display a pharmacological profile similar to that of the therapeutically useful benzodiazepines. This possibility is currently under study.

Acknowledgements

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