5-[1′-(2′-*N*-Arylsulfonyl-1′,2′,3′,4′-tetrahydroisoquinolyl)]-4,5-dihydro-2(3*H*)-furanones: Positive Allosteric Modulators of the $GABA_A$ Receptor with a New Mode of Action

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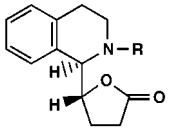
Introduction. γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter of the central nervous system. It acts at the $GABA_A$ receptor, a pentameric supramolecular complex which forms a ligand-gated ion channel controlling chloride ion flux in the neuron. Binding of GABA to its recognition site on the receptor has as principal effect the opening of the channel, allowing chloride ions to flow, leading to inhibition of neuronal activity. The subunits composing the $GABA_A$ receptor have been classified as α, β, γ, δ, ϵ, π, and, more recently, θ based on sequence homology.[1−6] These subunits are also generally present in the form of several isoforms (e.g. α1, α2, etc.), leading to a potentially high level of receptor heterogeneity, though the most common receptor composition has been found to consist of α1, β2, and γ2 subunits.[7,8]

In addition to the heterogeneity of the $GABA_A$ receptor system, each receptor also has a variety of allosteric sites, which, upon binding of their specific ligands, leads to modulation of GABA activity.[9] Thus, compounds which stimulate GABA-induced chloride ion currents are referred to as positive allosteric modulators. These include the therapeutically useful benzodiazepines which bind to the benzodiazepine recognition site. The latter in fact also binds a wide variety of compounds structurally unrelated to benzodiazepines and which have the same potentiating effect on GABA.[10] Other positive allosteric binding sites of the $GABA_A$ receptor include the barbiturate, neurosteroid, and loreclezole sites. On the other hand, negative allosteric modulation of the $GABA_A$ receptor (that is, inhibition of the neuroinhibitory action of GABA) is also possible with certain ligands as, for example, certain β-carbolines which bind to the benzodiazepine recognition site.[10,11]

Positive allosteric modulators of the GABA receptor generally display, to varying degrees, anticonvulsant, anxiolytic, sedative-hypnotic, anesthetic, and muscle-relaxant activities. Certain undesirable side effects are also associated with their use. These include amnesia, tolerance, dependence, alcohol potentiation, and oversedation (e.g. when only an anxiolytic effect is desired). To obtain drugs showing a more restrained pharmacological profile and/or decreased levels of side effects, a generally admitted strategy is to develop receptor subtype-selective ligands for one of the types of allosteric binding sites. A convincing example of this is zolpidem which binds to the benzodiazepine recognition site but which shows high selectivity for the α1β2γ2 $GABA_A$ receptor subtype and, consequently, predominantly sedative effects in vivo.[12] The search for subtype-selective ligands can also be approached from the point of view of new allosteric modulatory sites. Indeed, the large number of such sites on the $GABA_A$ receptor suggests that there may in fact be many others. Ligands for these sites could perhaps modulate the activity of GABA in more subtle ways than those presently known (e.g. as benzodiazepines do compared to barbiturates)[9] or they may be restricted to only certain receptor subunit combinations, thereby giving rise to the sought-after receptor subtype selectivity. The discovery of such novel allosteric modulatory sites can be accomplished using classical radioactive ligand displacement studies in combination with a functional assay, for example, the electrophysiological measurement of the potentiation of GABA-elicited currents by a compound.

We have recently reported the synthesis and preliminary pharmacological evaluation of (±)-ROD185, the *N*-benzyloxycarbonyl (N-Cbz) derivative of 5-(1-isoquinolyl)furan-2-one (**1**).[13] This novel compound was shown by in vitro radioactive ligand displacement studies using rat brain tissue to be a good ligand of the benzodiazepine recognition site of the $GABA_A$ receptor (IC_{50} = 60 nM in cerebellum). In electrophysiological studies, ROD185 strongly potentiated GABA-elicited currents in cells expressing the α1β2γ2 receptor subtype (351% at 100 μM). We now show that replacement of the N-Cbz group of ROD185 by an arylsulfonyl group (e.g. **2**) leads to compounds which are still strong positive allosteric modulators of the $GABA_A$ receptor but which do so mainly by acting in a novel fashion with this receptor.



1 R = CO_2CH_2Ph (ROD185)

2 R = SO_2Ar

Results and Discussion. The *N*-sulfonyl derivatives **2** were prepared by exploiting the vinylogous Mannich reaction[14] previously used for the synthesis of ROD185. Thus, treatment of 3,4-dihydroisoquinoline **3** with benzyl chloroformate in acetonitrile for 15 min gave the iminium salt **4** which was reacted in situ with 2-(*tert*-butyldimethylsiloxy)furan (**5**)[15] to afford a mixture of the *threo* and *erythro* diastereomers **6** and **7**, respectively (Scheme 1). Overall yield was 66%, with a *threo*:

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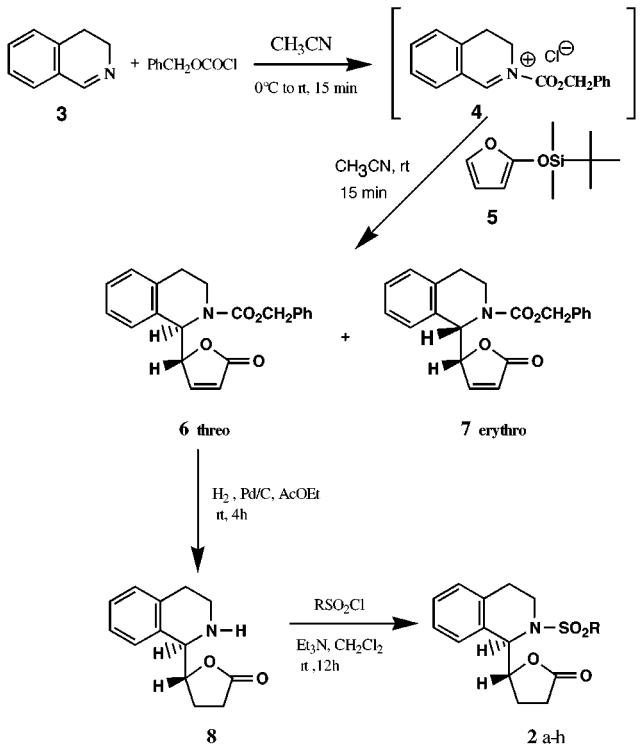
Scheme 1



erythro ratio of 3:2 based on the ^{1}H NMR spectrum of the mixture. Purification of the mixture on silica gel (heptane−ethyl acetate, 1:1) allowed isolation of pure isomers **6** and **7**. The relative configurations of the two asymmetric centers of **6** and **7** were established by comparison of their ^{13}C NMR spectra[16] and by X-ray crystallography of a derivative of **6** (see below). Because our previous studies showed that the *threo* derivatives of isoquinolylfuranones were generally more potent than the *erythro* isomers in stimulating GABA-elicited currents,[13] compound **6** was chosen for further transformations. Hydrogenolysis of the N-Cbz group of **6** using palladium-on-carbon in ethyl acetate led to simultaneous reduction of the lactone double bond to afford compound **8** in quantitative yield. The latter was efficiently N-sulfonylated by reaction with a variety of sulfonyl chlorides in the presence of triethylamine providing the target compounds **2a**−**h** (Table 1).

Compounds **2a**−**h** were first evaluated in vitro for their capacity to bind to the GABA, benzodiazepine, and picrotoxin (a chloride channel blocker)[9] recognition sites of the $GABA_A$ receptor by measuring their potency in displacing the appropriate specific radioligand (respectively [^{3}H]muscimol, [^{3}H]flunitrazepam, and [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS)).[17,18] Results (Table 1) are given as percent displacement of the radioactive ligand by 100 μM compound in whole rat brain tissue ([^{3}H])muscimol, [^{35}S]TBPS) or in cerebellum and forebrain tissue ([^{3}H]flunitrazepam). Compounds **2a**−**h** were also evaluated electrophysiologically for their capacity to stimulate GABA-elicited currents using *Xenopus laevis* oocytes expressing rat brain recombinant $\alpha1\beta2\gamma2$ $GABA_A$ receptors.[19,20]

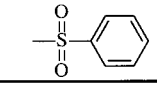
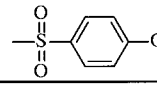
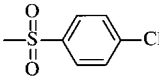
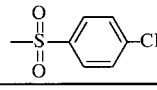
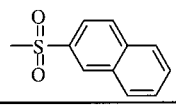
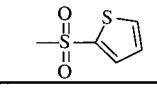
As in the case of ROD185, the *N*-sulfonyl analogues showed at best only weak displacement of [^{35}S]TBPS. Only the benzenesulfonyl and *p*-chlorobenzenesulfonyl derivatives (**2a**,**b**) showed significant displacement of this ligand at 100 μM (50% and 60%, respectively). In the case of [^{3}H]muscimol, either no displacement (**2d**,**f**,**h**) or a 5−15% stimulation of binding was observed. Moreover, binding to the benzodiazepine recognition site was considerably diminished by replacement of the benzylcarbamate group of ROD185 by an arylsulfonyl group. Thus, 100 μM benzenesulfonyl derivative **2a** was able to displace only 74% and 66% of [^{3}H]flunitrazepam in the cerebellum and forebrain, respectively, whereas ROD185 had IC_{50} values of 60 and 160 nM in the same tissues. All the other *N*-sulfonyl compounds synthesized were even weaker displacers of [^{3}H]flunitrazepam than **2a**.

In view of this loss of binding affinity for the benzodiazepine recognition site, it was very surprising to observe in the electrophysiological studies that the arylsulfonyl derivatives were in most cases *more* potent stimulators of GABA-elicited currents in *Xenopus* oocytes than ROD185. In particular, the *N*-*p*-toluenesulfonyl analogue **2c** stimulated currents by 579 ± 61% at 100 μM (mean of three experiments ± SEM unless otherwise stated), compared to 351 ± 10% for ROD185 at the same concentration. While the *N*-benzenesulfonyl and *N*-*p*-chlorobenzenesulfonyl derivatives **2a**,**b** were slightly less active in this assay (423 ± 32% and 479 ± 12% stimulation, respectively), both were still significantly more active than ROD185. Replacement of the *p*-methyl group of **2c** by a *p*-trifluoromethyl group (**2d**) led to considerable loss of stimulatory activity (90 ± 8% stimulation), as did suppression of the aromatic ring as in the *N*,*N*-dimethylaminosulfonamide **2e** (62 ± 7% stimulation) or the methylsulfonamide **2f** (32 ± 4% stimulation). Aryl groups other than phenyl were also active. Thus, though the naphthylsulfonyl derivative **2g** could not be assayed at 100 μM because of its insolubility, it demonstrated significant current stimulation even at 10 μM concentration (118 ± 24%). The thiophene derivative **2h** was in turn as active as ROD185 in stimulating GABA currents, though again it displayed a much weaker interaction with the benzodiazepine recognition site. None of the *N*-sulfonyl compounds produced significant currents in the absence of GABA, indicating that they are not acting as GABA agonists.

The question thus remained at this point as to which of the various allosteric modulatory sites of the $GABA_A$ receptor is involved in the stimulation of GABA currents by these arylsulfonyl derivatives. Further pharmacological profiling was thus undertaken on the most active of these compounds, the *N*-tosyl derivative **2c** (code named ROD188). However, because ROD188 (as well as all the compounds prepared via Scheme 1) is a racemic mixture, it was decided at this point to isolate each enantiomer of ROD188 and to study only the most active one. Separation was accomplished by subjecting ROD188 to HPLC on a chiral OD column. The pure enantiomers were obtained as solids and (−)-ROD188 was crystallized in a form suitable for X-ray analysis. As shown in Figure 1, (−)-ROD188 corresponds to the *SS* configuration of the two chiral centers.[21] That of (+)-ROD188 must therefore correspond to the *RR* configuration.

Comparison of the current stimulations produced by each enantiomer showed that (+)-ROD188 was considerably more potent than (−)-ROD188 (859 ± 43% and

Table 1. Displacement of Radioactive Ligands from Rat Brain Membranes and Effect on GABA-Elicited Currents in *Xenopus* Oocytes for Compounds **2a**−**h**

Compound n°	R	% Displacement of radioactive ligand at 100 μM [a] [^{3}H]- flunitrazepam	[^{35}S]- TBPS	[^{3}H]- Muscimol	% GABA current stimulation at 100 μM [c]
(±)-**1** (±)-ROD185	CO_2CH_2Ph	IC_{50} = 0.060 μM (ce) [b] = 0.160 μM (fo)	0%	0%	351 ± 10%
(±)-**2a**		74% (ce) 66% (fo)	50%	slight stimulation	423 ± 32%
(±)-**2b**		43% (ce) 30% (fo)	60%	slight stimulation	479 ± 12%
(±)-**2c**		66% (ce) 49% (fo)	15%	5-10% stimulation	579 ± 61%
(+)-**2c** *((+)-ROD188)*		67% (ce) 49% (fo)	-	-	859 ± 43%
(-)-**2c**		61% (ce) 35% (fo)	-	-	184 ± 25%
(±)-**2d**		42% (ce) 30% (fo)	0%	0 %	90 ± 8%
(±)-**2e**	$SO_2N(CH_3)_2$	20% (ce) 20% (fo)	0%	slight stimulation	62 ± 7%
(±)-**2f**	SO_2CH_3	26% (ce) 20% (fo)	0%	0%	32 ± 4%
(±)-**2g**		14% (ce) 9% (fo)	0%	~ 15% stimulation	118 ± 24% (at 10 μM) [d]
(±)-**2h**		66% (ce) 47% (fo)	25%	0%	353 ± 30% (48% at 10 μM)

[a] Determined in rat brain tissue as previously described.[17,18] Values are given as percent displacement of the radioactive ligand by each substance. Experiments were performed at least three times in triplicate. [b] ce refers to rat cerebellum; fo refers to rat forebrain tissue (whole brain minus cerebellum). [c] Determined electrophysiologically in *X. laevis* oocytes expressing $\alpha1\beta2\gamma2$ $GABA_A$ receptors as previously described.[19,20] Average of three assays ± SEM. [d] Values in parentheses refer to assays performed at a concentration other than 100 μM, as indicated.

184 ± 25%, respectively). Threshold of stimulation was about 1 μM for (+)-ROD188. Both compounds showed approximately the same weak affinities for the benzodiazepine recognition sites (<70% displacement of [^{3}H]-flunitrazepam at 100 μM), consistent with the hypothesis that current stimulation is not principally due to interaction with these sites. This was furthermore corroborated by the observation that at a concentration that would normally completely inhibit current stimulations by diazepam, co-administration of the benzodiazepine antagonist Ro15-1788 (1 μM) resulted in inhibition of only about 34% of the current stimulation produced by (+)-ROD188 (20 μM). Thus, stimulation was reduced from 207 ± 42% to 136 ± 20% (n = 5).

The possibility that (+)-ROD188 interacts with other known allosteric binding sites on the $GABA_A$ receptor was then investigated. Both barbiturates (e.g. pentobarbital)[22] and steroids (e.g. 5α-pregnan-3α-ol-20-one)[23] modulate $GABA_A$ receptor currents and, moreover, activate the chloride ion channel in the absence of GABA. In the oocyte preparation expressing $\alpha1\beta2\gamma2$ receptors, (+)-ROD188 (30 μM) was found to strongly stimulate currents elicited by pentobarbital (100 μM) and 5α-pregnan-3α-ol-20-one (3 μM) (588 ± 218% and 644 ± 47%, respectively) suggesting that the binding sites of these two ligands are different from that of (+)-ROD188. Furthermore, while an $\alpha1\beta2$ receptor combination responds to current stimulation by loreclezole, the point-mutated form $\alpha1\beta2$N265S is only weakly responsive to loreclezole.[24,25] However, both the wild-type and mutated receptor respond almost equally well to (+)-ROD188, current stimulation being 859 ± 75% in the first case and 600 ± 46% (n = 4) in the second. The mode of action of (+)-ROD188 thus also appears to be distinct from that of loreclezole. Finally, the current stimulation produced by α-EMTBL, which acts via a postulated γ-butyrolactone binding site,[26] was also decreased by the same mutation (unpublished results) indicating a mode of action different from that of (+)-ROD188.

Summary. In conclusion, *N*-arylsulfonylisoquinolylfuranones of general structure **2** are a new class of

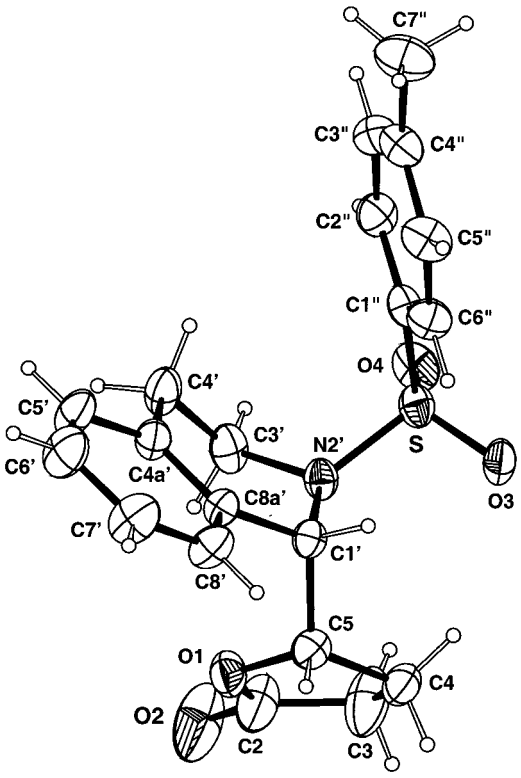


Figure 1. ORTEP drawing of (−)-ROD188 ((−)-**2c**) showing the 5*S*,1′*S* configuration.

positive allosteric modulators of the $GABA_A$ receptor. Preliminary experiments conducted on the most potent of the compounds synthesized, (+)-ROD188 ((+)-**2c**), showed that while this compound presents weak residual affinity for the benzodiazepine recognition site, binding to this site cannot entirely account for the strong current stimulation observed. Furthermore, it appears unlikely that (+)-ROD188 interacts with the barbiturate, steroid, picrotoxin, loreclezole, γ-butyrolactone, or GABA binding sites. It is thus concluded that (+)-ROD188 and its analogues interact with the $GABA_A$ receptor[27] by binding to a new allosteric modulatory site on this receptor in addition to binding to the benzodiazepine recognition site. Confirmation of this possibility will be the object of further study.

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Supporting Information Available: Full experimental details and spectroscopic data for compounds **2a**−**h** and **6**−**8**. This material is available free of charge on the Internet at http://pubs.acs.org.

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