Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1381–1384

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

N-(Indol-3-ylglyoxylyl)piperidines: High Affinity Agonists of Human GABA-A Receptors Containing the α_1 Subunit

Ian Collins,[a,*] William B. Davey,[a] Michael Rowley,[a] Kathleen Quirk,[b] Frances A. Bromidge,[b] Ruth M. McKernan,[b] Sally-Anne Thompson[c] and Keith A. Wafford[c]

[a]*Department of Medicinal Chemistry, Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex CM20 2QR, UK*

[b]*Department of Biochemistry, Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex CM20 2QR, UK*

[c]*Department of Pharmacology, Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex CM20 2QR, UK*

Received 5 January 2000; accepted 25 April 2000

Abstract—A new class of *N*-(indol-3-ylglyoxylyl)piperidines are high affinity agonists at the benzodiazepine binding site of human GABA-A receptor ion-channels, with modest selectivity for receptors containing the α_1 subunit over α_2 and α_3. All three receptor subtypes discriminate substantially between the two enantiomers of the chiral ligand **10**.

Compounds that act as agonists at the benzodiazepine binding site of the GABA-A receptor-chloride ion channel have proved a rich source of clinically effective drugs, particularly anxiolytics, hypnotics and anticonvulsants. Unfortunately the use of these classical benzodiazepine receptor (BZR) ligands can be compromised by the difficulty of separating their many pharmacological effects in man: for example, the treatment of anxiety with the agonist diazepam is accompanied by unwanted side-effects, including sedation, ataxia and the risk of dependence.[1,2] Mammalian GABA-A receptors have been shown to be heteropentameric assemblies of protein subunits ($\alpha,\beta,\gamma,\delta,\varepsilon,\theta,\pi,\rho$), each of which is expressed as a number of subtypes, where the most abundant receptor-ion channels have the composition $(\alpha_n)_2(\beta_n)_2(\gamma)$.[3–5] The apparent differential localisation of these subtypes within the brain[4,6] offers the exciting possibility that subtype selective ligands will allow separation of the many pharmacological effects of BZR agonists. Recent pharmacological and behavioural studies on transgenic mice in which the α_1 subunit was rendered benzodiazepine insensitive support this hypothesis, and imply that α_1 BZRs are important for sedation but not for anxiolytic effects.[7] Further study in this area requires the identification of more selective compounds, of which few are known.[8]

Although *N*-(indol-3-ylglyoxylyl)amines (Fig. 1) are a well established class of BZR ligands,[9] there is currently no reported data on their affinities or efficacies at subtypes of human GABA-A receptors. In this communication we describe the evolution of new *N*-(indol-3-ylglyoxylyl) piperidine agonists with high affinity and modest binding selectivity for $\alpha_1\beta_2\gamma_2$ containing human GABA-A receptors.

The pyrrolidine amide **1** was identified as a weak, non-selective BZR ligand from screening of the corporate sample collection. Compounds related to **1** were prepared by literature procedures[10–12] (Schemes 1, 2 and 3) and their binding affinities were measured by displacement of

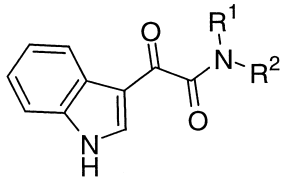


Figure 1. Representative structure.

*Corresponding author. Tel.: +1-279-440606; fax: +1-279-440187; e-mail: ian_collins@merck.com

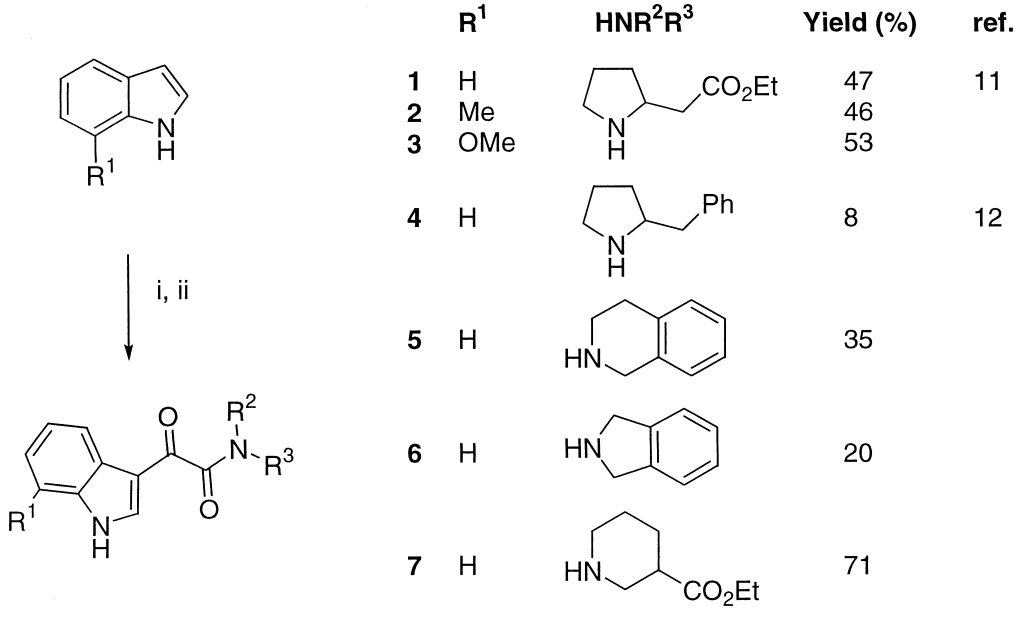
0960-894X/00/$ - see front matter
PII: S0960-894X(00)00245-6

[^{3}H]Ro15-1788 from recombinant human GABA-A receptors ($\alpha_1\beta_2\gamma_2$; $\alpha_2\beta_3\gamma_2$; $\alpha_3\beta_3\gamma_2$) stably expressed in L(tk$^-$) cells[13] (Table 1). These subtypes were chosen because of their major contribution to benzodiazepine sensitive GABA-A receptor populations in brain tissue.[4] Classical benzodiazepines such as diazepam do not bind to α_4- and α_6-containing receptors.[3,4] Although α_5-containing receptors are sensitive to diazepam, the distribution of this subtype within the brain is limited.[4,6b]

Investigation of substitution around the indole nucleus was largely fruitless; only groups in the 7-position were tolerated (compounds **2** and **3**) and these gave no significant improvement in affinity, although a trend towards higher affinities was noted with electron-rich 7-substituents. Notably, substitution patterns that were optimal in previously reported series of indole glyoxylamides,[9a] e.g., 5-NO_2, 5-Cl, gave much lower affinity (K_i >10 μM at all subtypes) than the unsubstituted lead **1**. Deletion of the pendant ester of the pyrrolidine lead **1** was not tolerated, but it could be replaced by a phenyl group, as in **4**. In addition to this the conformationally constrained benzylamine analogues **5** and **6** also retained affinity. The isoindoline amide **6**, although of moderate affinity, showed a good selectivity between receptors containing α_1-subunits and those comprised of α_2 (ca. 7-fold) and α_3 (ca. 100-fold). Comparison of **4**, **5** and **6** suggested that the α_3 subtype of the BZR was more sensitive to small changes in ligand structure than the α_1 or α_2 assemblies.

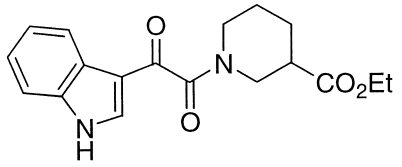
Replacement of the pyrrolidin-2-yl acetate with nipecotate (**7**) was tolerated and, for synthetic convenience, optimisation of this structure was pursued (Scheme 2). Increasing the size of the ester substituent was generally beneficial for affinity at all receptor subtypes in both the pyrrolidinyl and piperidinyl amides, illustrated by the increased affinity of the benzyl ester **8** relative to **7**. The affinity at the α_1 subtype was maintained in the secondary amide analogue **9** whilst an increase in selectivity against the α_2 and α_3 BZRs was seen. A further substantial increase in affinities was achieved on substitution of the amide nitrogen to give the racemic *N*-methyl-*N*-benzyl amide **10**, with poor selectivity for the α_1 BZR subtype.

As the BZR was known to discriminate between different enantiomers of some chiral indole glyoxylamides,[9b]



	R^1	HNR^2R^3	Yield (%)	ref.
1	H		47	11
2	Me		46	
3	OMe		53	
4	H		8	12
5	H		35	
6	H		20	
7	H		71	

Scheme 1. Reagents and conditions: (i) $(COCl)_2$, Et_2O, 0 °C; (ii) HNR^2R^3, Et_3N, CH_2Cl_2, rt.



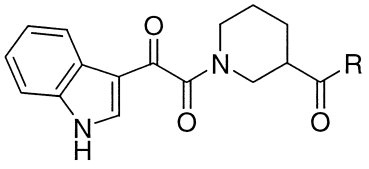
%ee

7

(*R*)-**7** 98

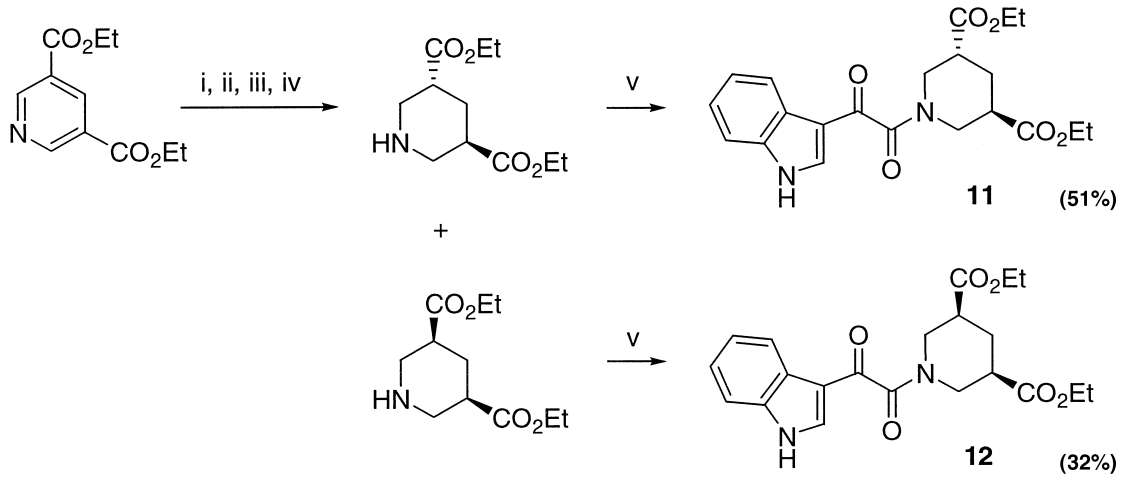
(*S*)-**7** 98

i, ii



		Yield (%)	%ee
8	R = OCH_2Ph	53	
9	R = $NHCH_2Ph$	20	
10	R = $N(Me)CH_2Ph$	29	
(*R*)-**10**	R = $N(Me)CH_2Ph$	12	91
(*S*)-**10**	R = $N(Me)CH_2Ph$	31	91

Scheme 2. Reagents and conditions: (i) LiOH, THF-H_2O, rt (76–93%); (ii) CDI, ROH, Et_3N, THF, rt or BOPCl, Et_3N, NHR^2R^3, CH_2Cl_2, rt.



Scheme 3. Reagents and conditions: (i) H_2, PtO_2, AcOH (58%); (ii) BOC_2O, Et_3N, CH_2Cl_2, rt (87%); (iii) silica chromatography; (iv) HCl, EtOAc, rt (100%); (v) indole-3-glyoxylyl chloride, Et_3N, MeCN, rt.

Table 1. Affinities of selected compounds at subtypes of human GABA-A receptors[a]

Compound	K_i (nM)[b]		
	$\alpha_1\beta_2\gamma_2$	$\alpha_2\beta_3\gamma_2$	$\alpha_3\beta_3\gamma_2$
1	890 (±60)	430 (±63)	570 (±70)
2	510 (±70)	440 (±82)	500 (±73)
3	460 (±43)	270 (±12)	320 (±34)
4	440 (±30)	830 (±200)	960 (±160)
5	380 (±33)	680 (±120)	720 (±50)
6	200 (±28)	1300 (±310)	19,000[c] (13,600, 26,400)
7	880[c] (710, 1100)	390[c] (330, 450)	990[c] (890, 1100)
8	77 (±11)	180 (±25)	91 (±14)
9	300 (±22)	970 (±55)	1,500 (±70)
10	14 (±2.5)	40 (±8.4)	96 (±15)
(*S*)-**10**	140 (±8.9)	250 (±42)	670 (±13)
(*R*)-**10**	7.3 (±1.5)	19 (±3.3)	50 (±6.5)
11	400[c] (270, 598)	200[c] (178, 215)	250[c] (245, 247)
12	14 (±3.0)	22 (±1.9)	23 (±2.2)
Diazepam[d]	12 (±2.2)	6.6 (±0.9)	33 (±5.3)
Flunitrazepam[d]	3.9 (±0.9)	1.1 (±0.5)	5.9 (±1.5)

[a]Displacement of [^{3}H]Ro15-1788 from hGABA-A subtypes stably expressed in L(tk$^-$) cells.
[b]Mean (±SEM) for $n=4$–6, data expressed to 2 significant figures.
[c]Mean of $n=2$ expressed to 2 significant figures, individual determinations in parentheses.
[d]Mean (±SD) for $n>6$.

compound **10** was prepared as separate enantiomers starting from resolved ethyl nipecotate[14] (Scheme 2). Slight degradation of the enantiopurity of the materials occurred during the synthesis, presumably in the basic hydrolysis step, and *R*-**10** and *S*-**10** were each isolated with 91% enantiomeric excess as determined by chiral analytical HPLC. Nevertheless, the *R* enantiomer was clearly some 10- to 20-fold more active in the binding assay than the *S* enantiomer, providing a compound with excellent binding affinity at the α_1 BZR subtype, although with modest selectivity over α_2- and α_3-containing receptors. The high affinity compounds **8**, **10** and (*R*)-**10** from this study were tested for their ability to displace [^{3}H]Ro15-1788 from human α_5-containing receptors ($\alpha_5\beta_3\gamma_2$) but showed no appreciable affinity (K_i >350 nM; < 50% inhibition of radiolabel binding at test concentration of 2 μM in two independent determinations).

The evolution of the high affinity ligand *R*-**10** could be viewed as mainly the optimisation of lipophilic binding of the substituent distal to the indole nucleus, but the conformational effects of the amide rotamers present in this class of compound were also considered. With the exception of the symmetrically substituted isoindoline amide **6**, compounds **1–10** existed as mixtures of rotational isomers about the glyoxylyl amide bond at room temperature. The compounds **10** showed an additional barrier to rotation about the pendant amide. This was seen clearly in the NMR spectra of the compounds and variable temperature NMR experiments, (e.g., of **1**) showed no coalescence of signals at temperatures up to 353K, indicating a substantial barrier to interconversion. To assess the degree to which the population of lower affinity conformations could compromise the binding of these compounds, symmetrical disubstituted analogues of the nipecotate **7** were prepared (Scheme 3).

Diethyl 3,5-pyridinedicarboxylate was exhaustively hydrogenated to give a mixture of the *cis* and *trans* piperidines, which were *N*-protected and separated by chromatography. The individual piperidine isomers were deprotected and coupled to indole-3-glyoxylyl chloride as before to give **11** and **12**. Both disubstituted analogues were of higher affinity at all BZR subtypes than the parent **7**, but the *cis* compound **12** showed a particularly dramatic (20- to 60-fold) increase in affinity. This increase in affinity could be due to additional specific binding of the second ester substituent to the BZR, coupled with the effect of the partial symmetry of the piperidine moieties, which would render either amide rotamer of **11** and **12** equally able to occupy the binding site effectively. The especially high affinity of the *cis* isomer **12** may result from the relatively flat overall conformation of the diequatorially substituted 6-membered ring, compared to the *trans* isomer **11** where one ester is axially oriented and may interact less favourably with

the receptor. Models of the binding of indole glyoxylyl amides to the BZR emphasize the apparent planarity of the binding site.[9] Nonetheless, compound **11** still showed an increase in affinity relative to the parent **7** as a result of both rotamers being able to present at least one substituent in the optimal orientation for binding.

Despite optimisation of the binding of these compounds for the α_1 BZR subtype, the selectivities observed between the α_1-, α_2- and α_3-containing receptors were at best modest. There is high sequence homology between the BZR subtypes,[3] and this may account for the difficulty in achieving high selectivities. The benzodiazepine binding site is probably located between the α- and γ-subunits in the receptor complex.[3] Although specific residues on the α-subunits have been show to be critical for benzodiazepine binding,[15] it is also known that amino acids in the γ-subunits contribute to the binding pocket.[16]

The benzodiazepine binding site is an allosteric modulatory site on the GABA-A receptor, and BZR ligands can be classified according to their efficacy; i.e., the ability to enhance (agonists), diminish (inverse agonists) or leave unchanged (antagonists) the response of the chloride ion channel to the endogenous neurotransmitter γ-aminobutyric acid (GABA). Selected compounds were therefore tested for their ability to affect the GABA-induced chloride current in *Xenopus* oocytes transiently expressing human GABA-A $\alpha_1\beta_2\gamma_2$ receptors using two-electrode voltage-clamp electrophysiology[17] (Table 2).

The lead pyrrolidine **1** was a low efficacy partial agonist at the α_1-containing BZR. Both the α_1-selective isoindolinamide **6** and the high affinity diester **12** were also partial agonists, with somewhat higher efficacy. In contrast, the high affinity, selective *N*-methyl-*N*-benzylamide (*R*)-**10** was a full agonist with similar efficacy to the benzodiazepines diazepam and flunitrazepam. The association of higher efficacy with appropriately placed aromatic or other lipophilic groups is a common feature of many BZR pharmacophore models.[18]

In summary, a new class of *N*-(indol-3-ylglyoxylyl)piperidines are high affinity agonists at the benzodiazepine binding site of human GABA-A receptor ion-channels, with modest selectivity for receptors containing the α_1 subunit over α_2 and α_3. All three receptor subtypes discriminate substantially between the two enantiomers of the chiral ligand **10**, and it is possible to overcome the effect of low affinity rotational conformers in this series by appropriate symmetrical or pseudosymmetrical substitution of the piperidine ring.

Table 2. Efficacy of selected compounds at human $\alpha_1\beta_2\gamma_2$ GABA-A receptors

Compound	Test concentration (μM)	Modulation of GABA EC_{20} current at $\alpha_1\beta_2\gamma_2$ receptors (%)[a,b]
Diazepam	1	+157 (±10)
Flunitrazepam	1	+121 (±9)
1	30	+28 (±4)
6	3	+64 (±10)
(*R*)-**10**	1	+110 (±2)
12	1	+56 (±5)

[a]Mean (± SEM) for $n = 3$–5.
[b]The GABA EC_{20} (concentration eliciting 20% of maximum GABA current) was determined for each oocyte (range 4–30 μM). The modulatory effects of each compound were then determined on coapplication with GABA at the EC_{20}.

Acknowledgement

We thank Sharon Penn for VT NMR experiments on **1** and analogues.

References

1. Ashton, H. A. *Drugs* **1994**, *48*, 25.
2. In *Martindale: The Complete Drug Reference*; Parfitt, K., Ed.; Pharmaceutical Press: 1999; pp 661–668.
3. Stephenson, F. A. *Biochem. J.* **1995**, *310*, 1 and references therein.
4. McKernan, R. M.; Whiting, P. J. *Trends Neurosci.* **1996**, *19*, 139 and references therein.
5. Farrar, S. J.; Whiting, P. J.; Bonnert, T. P.; McKernan, R. M. *J. Biol. Chem.* **1999**, *274*, 10100.
6. (a) Korpi, E. R.; Lüddens, H. *Br. J. Pharmacol.* **1997**, *120*, 741. (b) Sur, C.; Fresu, L.; Howell, O.; McKernan, R. M.; Atack, J. R. *Brain Res.* **1999**, *822*, 265.
7. Rudolph, U.; Crestani, F.; Benke, D.; Brünig, I.; Benson, J. A.; Fritschy, J.-M.; Martin, J. R.; Bluethmann, H.; Möhler, H. *Nature* **1999**, *401*, 796.
8. (a) Atack, J. R.; Smith, A. J.; Emms, F.; McKernan, R. M. *Neuropsychopharmacology* **1999**, *20*, 255. (b) Quirk, K.; Blurton, P.; Fletcher, S.; Leeson, P.; Tang, F.; Mellilo, D.; Ragan, C. I.; McKernan, R. M. *Neuropharmacol.* **1996**, *35*, 1331. (c) Huang, Q.; Cox, E. D.; Gan, T.; Ma, C.; Bennett, D. W.; McKernan, R. M.; Cook, J. M. *Drug Des. Discovery* **1999**, *16*, 55. (d) He, X.; Huang, Q.; Yu, S.; Ma, C.; McKernan, R.; Cook, J. M. *Drug Des. Discovery* **1999**, *16*, 77.
9. (a) Martini, C.; Gervasio, T.; Lucacchini, A.; Da Settimo, A.; Primofiore, G.; Marini, A. M. *J. Med. Chem.* **1985**, *28*, 506. (b) Primofiore, G.; Marini, A. M.; Da Settimo, F.; Martini, C.; Bardinelli, A.; Giannaccini, G.; Lucacchini, A. *J. Med. Chem.* **1989**, *32*, 2514. (c) Bianucci, A. M.; Da Settimo, A.; Da Settimo, F.; Primofiore, G.; Martini, G.; Martini, C.; Giannaccini, G.; Lucacchini, A. *J. Med. Chem.* **1992**, *35*, 2214. (d) Primofiore, G.; Da Settimo, F.; Marini, A. M.; La Motta, C.; Martini, C.; Senatore, G.; Lucacchini, A. *Il Farmaco* **1995**, *50*, 5. (e) Da Settimo, A.; Primofiore, G.; Da Settimo, F.; Marini, A. M.; Novellino, E.; Greco, G.; Martini, C.; Giannaccini, G.; Lucacchini, A. *J. Med. Chem.* **1996**, *39*, 5083.
10. Shaw, K. N. F.; McMillan, A.; Gudmundson, A. G.; Armstrong, M. D. *J. Org. Chem.* **1958**, *23*, 1171.
11. Mandell, L.; Roberts, E. C. *J. Het. Chem.* **1965**, *2*, 479.
12. Beak, P.; Lee, W. K. *J. Org. Chem.* **1993**, *58*, 1109.
13. Hadingham, K. L.; Wingrove, P.; Le Bourdelles, B.; Palmer, K. J.; Ragan, C. I.; Whiting, P. J. *Mol. Pharmacol.* **1993**, *43*, 970.
14. Magnus, P.; Thurston, L. S. *J. Org. Chem.* **1991**, *56*, 1166.
15. McKernan, R. M.; Farrar, S.; Collins, I.; Emms, F.; Asuni, A.; Quirk, K.; Broughton, H. B. *Mol. Pharmacol.* **1998**, *54*, 33.
16. Wingrove, P. B.; Thompson, S. A.; Wafford, K. A.; Whiting, P. J. *Mol. Pharmacol.* **1997**, *52*, 874.
17. Wafford, K. A.; Whiting, P. D.; Kemp, J. A. *Mol. Pharmacol.* **1992**, *43*, 240.
18. Zhang, W.; Koehler, K. F.; Zhang, P.; Cook, J. M. *Drug Des. Discovery* **1995**, *12*, 193 and references therein.