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Bioorganic & Medicinal Chemistry Letters 14 (2004) 1679–1682
Bioorganic & Medicinal Chemistry Letters

Tricyclic pyridones as functionally selective human $GABA_A\alpha_{2/3}$ receptor-ion channel ligands

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Received 30 June 2003; revised 16 January 2004; accepted 21 January 2004

Abstract—A series of tricyclic pyridones has been evaluated as benzodiazepine site ligands with functional selectivity for the α_3 over the α_1 containing subtype of the human $GABA_A$ receptor ion channel. This investigation led to the identification of a high affinity, functionally selective, orally bioavailable benzodiazepine site ligand that demonstrated activity in rodent anxiolysis models and reduced sedation relative to diazepam.
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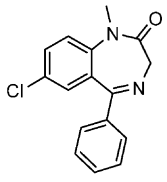
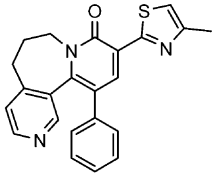
Neurotransmission by γ-aminobutyric acid (GABA) is the major inhibitory mechanism in the central nervous system (CNS). GABA exerts its effects via the ligand gated $GABA_A$ and $GABA_C$ receptor ion channels and the metabotropic $GABA_B$ receptors. However, the $GABA_A$ receptor has received most attention since modulation of its function by a number of classes of compounds such as barbituates, neurosteroids, ethanol and most notably, the benzodiazepines (BZs), results in a diverse range of responses, including changes in motor activity, seizures, sedation, modulation of cognition and mood, particularly anxiety states. $GABA_A$ receptors are oligomeric assemblies of a large range of subunits (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, π, and θ).[1] Most $GABA_A$ receptors in the brain contain α, β and γ subunits, and most specifically those with an α_1, α_2, α_3, or α_5, in conjunction with β and γ subunits, possess a specific binding site for benzodiazepines. Ligands at the BZ site are categorised on the basis of their ability to modulate GABA-activated currents. BZ agonists, inverse agonists or antagonists increase, decrease or have no effect, respectively on GABA induced currents. These different efficacies are mirrored in opposing behavioural effects. Generally agonists are anxiolytic and inverse agonists are anxiogenic, whereas antagonists have no effect. Classical BZ's such as Diazepam 1, have equivalent affinity and full agonist efficacy at α_1, α_2, α_3, and α_5 containing $GABA_A$ receptors. Although Diazepam **1** is efficacious in the treatment of anxiety, it causes side effects[2] such as sedation, ataxia, potentiation with alcohol and risk of tolerance and dependance with chronic use.



1 Diazepam



2a

The need for improved treatments which retain the anxiolytic efficacy but have reduced side effect liability is clear. It has been shown[3,4] that the α_1 subtype of the $GABA_A$ receptor is responsible for the sedative/motor effects of diazepam while the α_2/α_3 subtypes are primarily responsible for the anxiolytic activity. In the light of this, a subtype selective ligand (i.e., α_1 antagonist and α_2/α_3 agonist) should discriminate between sedation

Keywords: Tricyclic pyridones; $GABA_A$.
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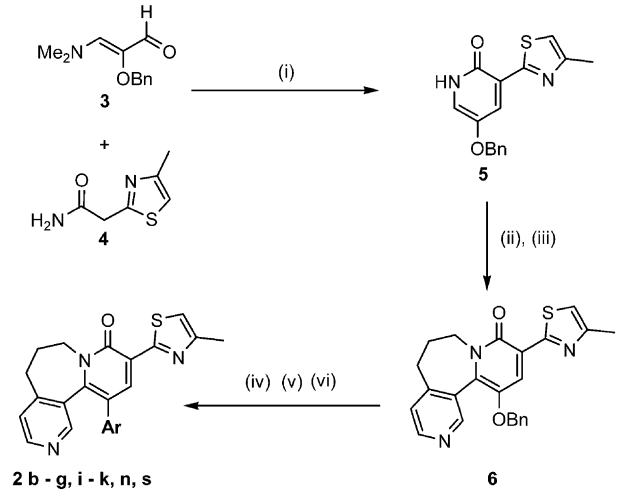
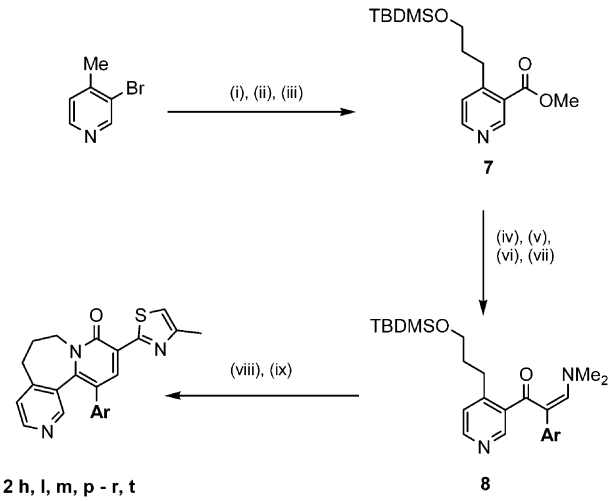
doi:10.1016/j.bmcl.2004.01.057

and the anxiolytic properties, providing an improved anxiolytic therapy. Continuing from our work on 3-heteroaryl-2-pyridones[5] in this publication we describe work on a series of tricyclic pyridone $GABA_A$ BZ site ligands with functional selectivity for the α_2 and α_3 subtypes over the α_1 containing subtype. The compounds showed no selectivity between the α_2 and α_3 subtypes, binding affinities and efficacy being comparable. Our studies indicated that it was possible to obtain compounds that display good affinity at the α_1 and α_3 $GABA_A$ subtypes as exemplified by the 5-phenyl derivative **2a**[6] (Table 1). In general compounds in this series displayed no binding selectivity between α_1, α_2 and α_3 subtypes (α_1 0.7 nM; α_2 0.3 nM; α_3 0.4 nM). However, compound **2a**, demonstrated functional selectivity for α_3 over α_1 subtypes (efficacy α_1 0.10; α_3 0.32). **2a** suffered from a poor pharmacokinetic profile with high turnover in dog liver microsomes and low oral bioavailability in dogs. As a continuation of our studies we sought to investigate the 5-position on the pyridone core in order to improve the pharmacokinetic profile, whilst maintaining the functional selectivity. The derivatives **2b–t** were prepared as outlined in Schemes 1 and 2.[7,8]

Base-catalysed cyclisation of **3**[7] and the thiazole acetamide **4** gave the 2-pyridone **5**. *N*-alkylation of **5** with 3-bromo-4-(3-bromo-propyl)-pyridine using Curran's LiBr-mediated procedure[7] followed by radical cyclisation[6] gave the tricycle **6**. Subsequent deprotection of the alcohol with boron tribromide, formation of the triflate, and Pd(0)-catalysed arylation via either Suzuki coupling of arylboronic acids or Stille coupling of the corresponding arylstannane gave **2b–g**, **i–k**, **n**, **s**. An alternative synthetic sequence by Gibson et al.[9] could be employed as outlined in Scheme 2. Commercially available 3-bromo-4-methylpyridine was lithiated and alkylated with ethylene oxide.[10] Protection and carbonylation of the 3-bromo group gave the methyl ester **7**. The latter was hydrolysed to the nicotinic acid, activated as the imidazolide and condensed with the appropriate methyl



Scheme 1. Reagents: (i) NaH, MeOH, DMF, 70 °C, 73%; (ii) NaH, LiBr, 3-bromo-4-(3-bromopropyl)-pyridine, DME, DMF, 75 °C, 82%; (iii) AIBN, Bu_3SnH, benzene, 80 °C, 50%; (iv) BBr_3, DCM, 25 °C, 89%; (v) Tf_2O, pyr., DCM, −78–0 °C, 73%; (vi) $Pd(PPh_3)_4$, $ArB(OH)_2$, $CsCO_3$, or $ArSnBu_3$, LiCl, CuI, 24–76%.



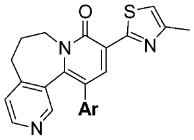
Scheme 2. Reagents: (i) LDA, ethylene oxide, −20 °C, 78%; (ii) TBDMSCl, imidazole, DCM, rt, 98%; (iii) $Pd(OAc)_2$, $Ph_2P(CH_2)_3PPh_2$, EtN^iPr_2, CO, MeOH, DMF, 95 °C, 88%; (iv) KOTMS, Et_2O, rt, 89%; (v) CDI, DMF, 50 °C, then $ArCH_2CO_2Me$, NaH, 0 °C–rt, 24–70%; (vi) NaCl, H_2O, DMSO, 150 °C, 50–76%; (vii) DMF.DMA, rt, 60–75%; (viii) **4**, NaH, DMF, 50 °C, 1N HCl, 45–75%; (ix) DEAD, PPh_3, THF, rt, 23–70%.

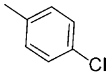
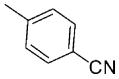
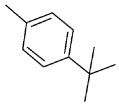
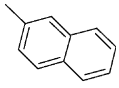
arylacetate, and decarboxylated under Krapcho conditions.[11] Reaction of the ketone with dimethylformamide-dimethylacetal yielded the dimethylaminopropen-2-ones **8**. Base-catalysed condensation of the thiazole acetamide **4** and **8**, deprotection of the alcohol, and closure of the seven membered ring under Mitsunobu conditions gave the tricyclic 2-pyridones **2h**, **l**, **m**, **p–r**, **t**.

Table 1 summarises the affinities and efficacies at both α_1 and α_3 containing $GABA_A$ receptors for substituted phenyl groups at the 5-position of the pyridone core. Generally, a wide variety of small substituents are well tolerated in terms of binding affinity. There is, however, a size limitation in this part of the molecule as illustrated by the 4-*tert*-butylphenyl **2f** and 2-napthyl **2g** derivatives, which both lose affinity relative to **2a**. Only the 4-chlorophenyl **2b** retains the binding affinity of the parent **2a**, but exhibits a poor functional selectivity profile. It is also clear from Table 1, that while substitution is broadly tolerated for binding affinities, a wide spread of functional efficacy is observed at both the α_1 and α_3 subtypes, indicating that this could be an effective way of moderating the efficacy to give the desired subtype selective profile.

If we only consider the compounds that display close to the desired subtype selectivity, (i.e., an antagonist at α_1 and agonist at α_3), then from Table 1 only the 2-methylphenyl **2c** and 4-cyanophenyl **2e** derivatives approach this profile. Unfortunately the 2-methylphenyl analogue **2c**, despite good binding affinity and functional selectivity, showed high turnover when exposed to dog liver microsomes (all test compound incubated at 1 μM at a protein concentration of 0.4 mg/mL for 15 min at 37 °C). However, for the 4-cyanophenyl **2e** the turnover in dog liver microsomes was only 28% as compared to 67% for **2a**, suggesting that plasma clearance might also be reduced by modifications in this

Table 1. Substituted phenyl derivatives

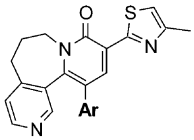


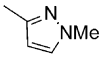
No.	Ar	K_i (nM)[a]		Efficacy[b]	
		α_1	α_3	α_1	α_3
2a		0.7 (±0.2)	0.4 (±0.1)	0.10 (±0.05)	0.32 (±0.03)
2b		0.2 (±0.0)	0.4 (±0.1)	0.17 (±0.2)	0.26 (±0.01)
2c		1.5 (±0.1)	1.2 (±0.1)	0.08 (±0.0)	0.36 (±0.1)
2d		5.0 (±0.2)	3.0 (±0.6)	0.56 (±0.07)	0.48 (±0.1)
2e		5.4 (±0.8)	3.1 (±0.3)	−0.04 (±0.03)	0.39 (±0.07)
2f		>33	>33	—	—
2g		>33	>33	—	—

[a] Affinity was determined by measuring the displacement of [^{3}H]Ro15-1788 from human recombinant $GABA_A$ receptors containing $\beta_3\gamma_2$ plus either α_1 or α_3 stably expressed in L(tk$^-$) cells. Values are the (mean±SD) of 2–10 separate determinations.

[b] Modulation of chloride ion flux in cells expressing $\beta_3\gamma_2$ plus either α_1 or α_3 produced by an EC_{20} equivalent concentration of GABA in the presence of an approximate $1000\times K_i$ concentration of test compound. Efficacy is expressed relative to the full agonist chlorodiazepoxide (relative efficacy = 1.0), from at least seven independent experiments.[12]

Table 2. 5-Membered heterocycles



No.	Ar	K_i (nM)[a]		Efficacy[b]	
		α_1	α_3	α_1	α_3
2a		0.7 (±0.2)	0.4 (±0.1)	0.10 (±0.05)	0.32 (±0.03)
2h		14.3 (±4.3)	16.8 (±8.6)	−0.33 (±0.05)	−0.01 (±0.06)
2i		3.9 (±1.0)	4.4 (±0.15)	0.04 (±0.05)	0.05 (±0.07)
2j		4.6 (±0.3)	3.1 (±0.6)	−0.11 (±0.07)	0.41 (±0.04)
2k		7.8 (±0.1)	4.2 (±0.5)	−0.21 (±0.06)	0.41 (±0.03)
2l		4.0 (±0.1)	3.0 (±0.0)	0.00 (±0.02)	0.46 (±0.03)
2m		10.0 (±2.9)	20.0 (±3.1)	0.29 (±0.04)	0.47 (±0.04)

[a] Affinity was determined by measuring the displacement of [^{3}H]Ro15-1788 from human recombinant $GABA_A$ receptors containing $\beta_3\gamma_2$ plus either α_1 or α_3 stably expressed in L(tk$^-$) cells. Values are the (mean±SD) of 2–10 separate determinations.

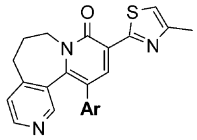
[b] Modulation of chloride ion flux in cells expressing $\beta_3\gamma_2$ plus either α_1 or α_3 produced by an EC_{20} equivalent concentration of GABA in the presence of an approximate $1000\times K_i$ concentration of test compound. Efficacy is expressed relative to the full agonist chlorodiazepoxide (relative efficacy = 1.0), from at least seven independent experiments.[12]

area. Table 2 summarises the effects of five membered heterocycles at the 5-position of the pyridone core. As with the substituted phenyl groups, this position proved tolerant in terms of binding affinity, of a wide variety of functional groups.

Although none of the five membered heterocycles retained the subnanomolar binding affinity of **2a**, a wide range of functional selectivities was observed, again indicating this strategy as a good way of modifying the functional efficacy of this class of compounds. The most active compounds were the isoxazole **2j** and 1-methylpyrrole **2l** which both maintained good affinity. Isoxazole **2j** displayed a good overall selective efficacy for α_3 over α_1 $GABA_A$ receptors. The 1-methylpyrrole **2l** also showed good binding to both α_1 and α_3 subtypes and encouragingly also displayed the desired functional selectivity profile (Table 2). Compound **2l** was again screened in dog liver microsomes as a quick method for predicting in vivo clearance. The turnover (53%), although lower than the lead compound **2a**, was still considered too high to be taken further.

Table 3 summarises data for the 6-ring heterocycles. The 4-pyrimidyl analogue **2p** displays good functional selectivity but has poor binding affinity. Pleasingly, the 4-pyridyl compound **2t**[14] retained the binding affinity at the α_3 subtype as compared to **2a**, and also exhibits excellent functional selectivity, with the desired antagonism at α_1, and an agonist profile at α_3. Binding selectivity of **2t** for α_3 over α_4 (27.9 nM), α_5 (>33.3 nM), and α_6 (65.9 nM), containing subtypes was achieved, but no binding or functional selectivity over α_2 (0.65 nM, efficacy 0.37) was observed. Compound **2t** also showed an improvement in turnover in dog liver microsomes (14%). This reduced turnover translated into improved oral bioavailability in dog of 20% as compared to 5% for the starting lead **2a**. Compound **2t** was shown to have 28% oral bioavailability in rat. In line with the programme hypothesis, compound **2t** was anxiolytic in

Table 3. 6-Membered heterocycles



No.	Ar	K_i (nM)[a]		Efficacy[b]	
		α_1	α_3	α_1	α_3
2a		0.7 (±0.2)	0.4 (±0.1)	0.10 (±0.05)	0.32 (±0.03)
2n		21.1 (±4.9)	9.0 (±3.0)	−0.03 (±0.04)	0.16 (±0.02)
2p		12.2 (±1.6)	8.5 (±1.0)	0.03 (±0.08)	0.32 (±0.08)
2q		8.3 (±0.7)	5.0 (±2.1)	−0.41 (±0.05)	0.20 (±0.03)
2r		26.1 (±2.1)	17.3 (±2.8)	−0.33 (±0.07)	−0.44 (±0.1)
2s		5.3 (±1.1)	2.4 (±0.5)	−0.16 (±0.07)	−0.02 (±0.08)
2t		1.3 (±0.2)	0.5 (±0.1)	0.00# (±0.02)	0.45# (±0.1)

[a] As Tables 1 and 2. For compounds marked # efficacy measurement of effect of test compound on a current at GABA EC_{20} using whole-cell patch-clamp electrophysiological recording.[13]

the elevated plus maze[3,15] at a dose of 1 mg/kg po. In addition, **2t** demonstrated no sedative effects up to 30 mg/kg po and no potentiation of ethanol interaction up to 10 mg/kg as shown by the mouse rotarod performance test.[3]

In summary, replacement of the phenyl moiety in **2a** with a 4-pyridyl group gave **2t**; a high affinity benzodiazepine site ligand with α_3-subtype functional selectivity for the human $GABA_A$ receptor-ion channel. The compound was efficacious in animal models of anxiety, and showed no sedation or potentiation of ethanol effects.

Acknowledgements

We would like to thank Dr. Jason Elliott and Dr. Jose L. Castro for their help during the preparation of this manuscript.

References and notes

1. Sieghart, W.; Fuchs, K.; Tretter, V.; Ebert, V.; Jechlinger, M.; Höger, H.; Adamiker, D. *Neurochem. Int*. **1999**, *34*, 379.
2. Korpi, E. R.; Mattila, M. J.; Wisden, W.; Lüddens, H. *Ann. Med*. **1997**, *29*, 275.
3. McKernan, R. M.; Rosahl, T. W.; Reynolds, D. S.; Sur, C.; Wafford, K. A.; Atack, J. R.; Farrar, S.; Myers, J.; Cook, G.; Ferris, P.; Garrett, L.; Bristow, L.; Marshall, G.; Macaulay, A.; Brown, N.; Howell, O.; Moore, K. W.; Carling, R. W.; Street, L. J.; Castro, J. L.; Ragan, C. I.; Dawson, G. R.; Whiting, P. J. *Nature Neuroscience* **2000**, *3*, 587.
4. Löw, K.; Crestani, F.; Keist, R.; Benke, D.; Brünig, I.; Benson, J. A.; Fritschy, J.-M.; Rülicke, T.; Bluethmann, H.; Möhler, H.; Rudolph, U. *Science* **2000**, *290*, 131.
5. Collins, I.; Moyes, C. R.; Davey, W. B.; Rowley, M.; Bromidge, F. A.; Quirk, K.; Atack, J. R.; McKernan, R. M.; Thomson, S.; Wafford, K.; Dawson, G. R.; Pike, A.; Sohal, B.; Tsou, N. N.; Ball, R. G.; Castro, J. L. *J. Med. Chem*. **2002**, *45*, 1887.
6. Harrison, T.; Moyes, C. R.; Nadin, A.; Owens, A. P.; Lewis, R. T. PCT Int. Appl. (1998) WO 9850384.
7. Nadin, A.; Harrison, T. *Tetrahedron Lett*. **1999**, *40*, 4073.
8. Liu, H.; Ko, S. B.; Josien, H.; Curran, D. P. *Tetrahedron Lett*. **1995**, *36*, 8917.
9. Gibson, K. R.; Hitzel, L.; Mortishire-Smith, R. J.; Gerhard, U.; Jelley, R. A.; Reeve, A. J.; Rowley, M.; Nadin, A.; Owens, A. P. *J. Org. Chem*. **2002**, *67*, 9354.
10. Mink, K.; Bracher, F. *Liebigs Ann*. **1995**, 645.
11. Krapcho, P. A.; Lovey, A. J. *Tetrahedron Lett*. **1973**, *14*, 957.
12. Smith, A. J.; Alder, L.; Silk, J.; Adkins, C.; Fletcher, A. E.; Scales, T.; Kerby, J.; Marshall, G.; Wafford, K. A.; McKernan, R. M.; Atack, J. R. *Mol. Pharmacol*. **2001**, *59*, 1108.
13. Whiting, P. J.; McAllister, G.; Vassilatis, D.; Bonnet, T. P.; Heavens, R. P.; Smith, D. W.; Hewson, L.; O'Donnell, R.; Rigby, M. R.; Sirinathsinghji, D. J. S.; Marshall, G.; Thompson, S. A.; Wafford, K. A. *J. Neuroscience* **1997**, *17*, 5027.
14. Crawforth, J. M.; Gibson, K. R.; Rowley, M. U.S. Pat. Appl. Publ. (2001) U.S 20010053776.
15. Collinson, N.; Dawson, G. R. *Psycopharmacology* **1997**, *132*, 751.