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Pyrazolopyridinones as functionally selective $GABA_A$ ligands

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Abstract—2,5-Dihydro-3*H*-pyrazolo[4,3-*c*]pyridin-3-ones are $GABA_A$ receptor benzodiazepine binding site ligands, which can exhibit functional selectivity for the α_3 subtype over the α_1 subtype. SAR studies to optimize this functional selectivity are described.

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. It acts at the $GABA_A$ receptor on a pentameric supramolecular complex that forms a ligand-gated chloride ion channel. A functional receptor is formed by the co-assembly of subunits selected from the family of 16 gene products (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, π and θ), which are differentially expressed throughout the brain.[1,2] The most abundant $GABA_A$ receptor subtypes contain at least one each of the α, β and γ subunits in a 2:2:1 stoichiometry. The benzodiazepine class of drugs,[3,4] widely used as a therapy for anxiety and panic disorders, are generally unselective agonists that allosterically modulate the GABA-mediated chloride ion flux through the channel of $GABA_A$ receptors containing β, γ_2 and either α_1, α_2, α_3, or α_5 subunits.[1,2] However, the side-effect profile associated with the classical benzodiazepines is far from ideal.[5] Studies with transgenic mice and with subtype selective compounds suggest that α_1-containing receptors are responsible for mediating the sedative/muscle relaxant properties of benzodiazepines and that α_3 and/or α_2-containing receptors are important for anxiety.[6] The goal of our research was to identify subtype selective ligands, be they functionally selective or binding selective, with the expectation of gaining an improved side-effect profile over currently used benzodiazepines.

Tetrahydropyrazoloquinoline **1** (CGS-17867A)[7] (Fig. 1) was chosen as a starting lead, exhibiting high affinity for the benzodiazepine binding site, anxiolytic activity in several animal behavioral models,[7] and with an improved side-effect profile. We attributed this profile to the functional selectivity of this compound, which, in our hands, showed significant efficacy at α_3- and α_2-containing receptors and a much reduced efficacy at the α_1 subtype.[8] This paper describes modification of the tetrahydro ring by substituting the pyrazolopyridine core at the 6- and 7-positions to give a novel series (**2**) of high affinity compounds. This modification allows access to a similar region of space accessed by the 2,5-dihydropyrazolo[4,3-*c*]pyridin-3-one scaffold described recently by our laboratory.[8]

The 7-phenyl substituted pyrazolopyridinones were synthesised by condensation of phenylacetones with enamine **3**, followed by thermal cyclisation of the intermediate in Dowtherm® A to give the pyridones **5**.

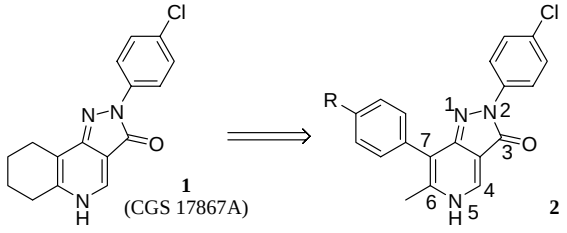

Figure 1.

Keywords: Pyrazolopyridinones; $GABA_A$.
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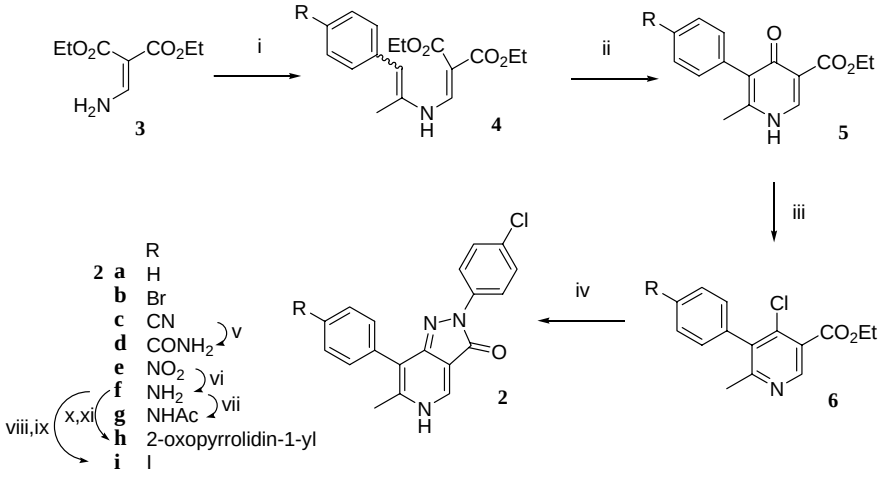


Scheme 1. Reagents and conditions: (i) $ArCH_2COMe$, P_2O_5, THF, rt; (ii) Dowtherm A, reflux; (iii) $POCl_3$, reflux; (iv) *p*-chlorophenylhydrazine·HCl, BuOH, reflux; (v) H_2SO_4 (85% v/v); (vi) $Cu(acac)_2$, $NaBH_4$, EtOH; (vii) AcCl, pyridine, DCM; (viii) isoamyl nitrite, tetrafluoroboric acid, DMF; (ix) I_2, KI, DMSO; (x) 4-chlorobutanoyl chloride, pyridine, DCM; (xi) NaH, DMF.

A range of dehydration conditions for the formation of the enamine cyclisation precursor were examined; treatment with phosphorus pentoxide[9] in dichloromethane or THF gave the best results. Treatment of **5** with phosphorus oxychloride gave the chloropyridines **6**, which were reacted with 4-chlorophenylhydrazine to give the 6-methyl-7-phenyl substituted pyrazolopyridinones **2a**–**c**, **e** (see Scheme 1).

Copper-mediated borohydride reduction of the readily available 4-nitro compound **2e** gave aniline from which iodide **2i** was accessed by decomposition of the diazonium tetrafluoroborate salt in the presence of iodine and potassium iodide.[10] Pyrrolidinone **2h** was prepared by acylation of the aniline with 4-chlorobutanoyl chloride, followed by a base-promoted ring closure.[11]

Binding and efficacy data for the pyrazolopyridinones **2** are shown in Table 1. Introduction of small lipophilic substituents led to a loss in binding affinity (e.g., **2b**) compared to **1**; however, polar substituents, such as amides **2d** and **2g**, led to an increase in affinity. In our high-throughput functional efficacy assay, based on chloride ion flux,[13] **2d** and **2g** gave good efficacy at α_3-containing receptors. This was confirmed by patch-clamp efficacy on L(tk$^-$) cells containing $\alpha_1\beta_3\gamma_2$ and $\alpha_3\beta_3\gamma_2$GABA$_A$ receptors, with both **2d** and **2g** imparting functional selectivity for α_3 over α_1 similar to that of **1**. Interestingly, 2-oxopyrrolidin-1-yl **2h**, lacking the amide hydrogen bond donor (HBD), proved to be an unselective partial agonist. In a preliminary rat PK study, compound **2a** exhibited excellent oral bioavailability in rat ($F = 95\%$), a property not shared by **2g**. It was hypothesized that N-deacetylation of **2g** could be responsible for this lack of oral exposure and therefore we focused on a series of heterocyclic replacements for the amide, including heterocycles with a free NH group to probe if this were critical for functional selectivity.

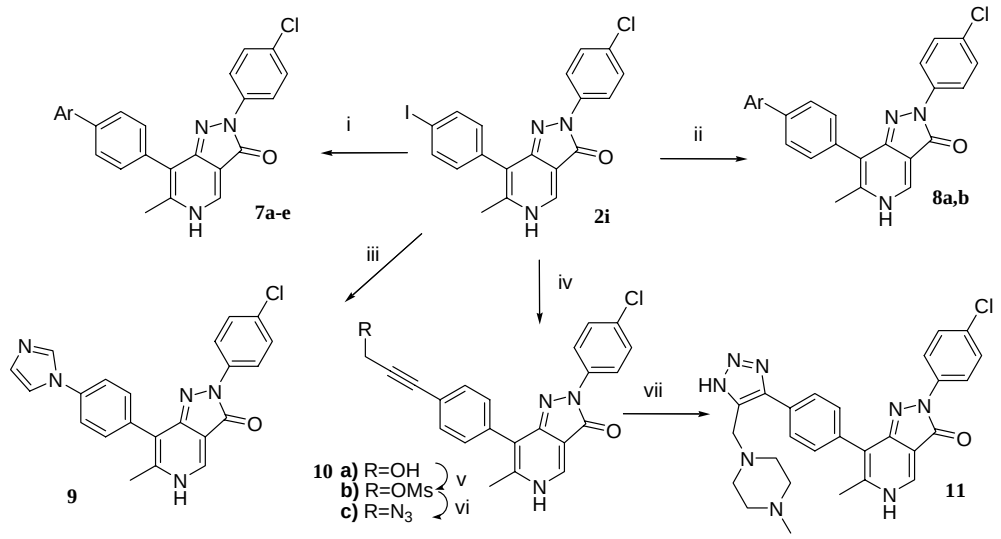
Table 1.

Entry	R	K_i (nM)[a]		Efficacy[b]	
		α_1	α_3	α_1	α_3
1	—	0.29 ± 0.04	1.5 ± 0.2	0.14 ± 0.06 [0.21 ± 0.03]	0.38 ± 0.05 [0.45 ± 0.05]
2a	H	6.8 ± 2.2	9.9 ± 3.0	—	—
b	Br	—	57 ± 7	—	—
c	CN	—	2.7 ± 0.3	—	0.13 ± 0.03
d	$CONH_2$	0.30 ± 0.07	0.67 ± 0.11	0.22 ± 0.08 [0.19 ± 0.02]	0.38 ± 0.06 [0.41 ± 0.06]
g	NHC(O)Me	0.25 ± 0.04	0.42 ± 0.03	— [0.23 ± 0.03]	0.48 ± 0.03 [0.43 ± 0.05]
h	2-Oxopyrrolidin-1-yl	—	2.5 ± 1.2	0.29 ± 0.04 [0.41 ± 0.04]	0.32 ± 0.05 [0.46 ± 0.06]

[a] Affinity was determined by the inhibition of [^{3}H]Ro 15-1788 (flumazenil) binding to 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 (means ± SD) of 2–10 separate determinations.[12]

[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 the test compound. Efficacy is expressed, relative to the full agonist chlorodiazepoxide (relative efficacy = 1.0), from at least seven independent experiments.[13]

Efficacy data, given in square brackets [] were measured at GABA$_A$ receptors stably expressed in L(tk$^-$) cells using whole cell patch-clamp recording and represent the effect of the test compound on the current produced by an EC20-equivalent of GABA, relative to the full agonist chlorodiazepoxide (relative efficacy = 1.0).[14]



Scheme 2. Reagents and conditions: (i) ArZnCl, $Pd(PPh_3)_4$, THF, reflux; (ii) $ArB(OH)_2$, K_2CO_3 (aq), $Pd(PPh_3)_4$, DME, reflux or $ArSnBu_3$, $Pd(PPh_3)_4$, DME, reflux; (iii) imidazole, copper bronze, K_2CO_3, NMP, reflux; (iv) propargyl alcohol, $Pd(PPh_3)_4$, CuI, piperidine 80 °C; (v) MsCl, LiCl, 2,6-lutidine, DMF; (vi) NaN_3, DMSO; (vii) *N*-methylpiperazine, DMSO, 100 °C.

The aryl iodide **2i** proved to be an invaluable intermediate for the introduction of heterocycles via a range of metal-catalysed chemistries, examples of which are shown in Scheme 2. Compounds **7a**–**e** were accessed by Negishi cross couplings with the appropriate heterocycle, NH heterocycles protected as the SEM derivatives,[15] prior to metallation, and deprotected after the coupling reaction with HCl in ethanol. The *N*-linked imidazole **9** was prepared by copper catalysed amination of the aryl iodide. Suzuki and Stille couplings also proceeded smoothly to give **8a** and **8b**, respectively. Sonogashira coupling with propargyl alcohol gave acetylene **10a**, which could be transformed to the mesylate **10b**. Displacement by azide (**10c**), followed by base-catalysed rearrangement,[16] gave amino substituted 1,2,3-triazoles **11** via an allenyl azide, which was trapped with nucleophilic amine bases. This type of group has been shown to increase solubility in a series of NK1 antagonists.[17]

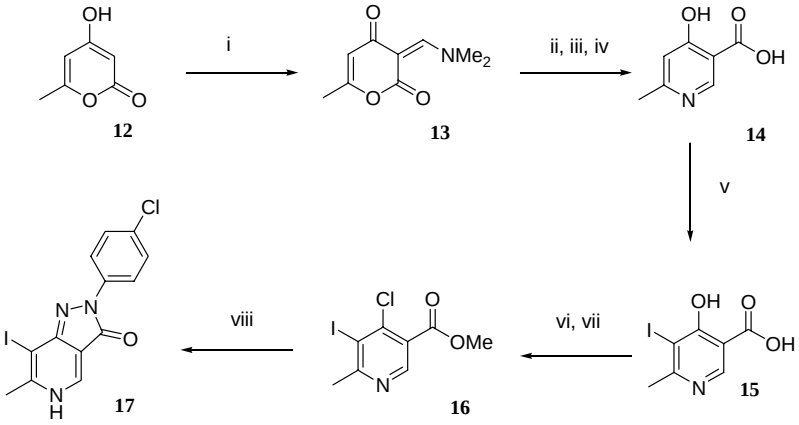
From the data shown in Table 2, it can be seen that a range of polar heterocyclic replacements for the amide show excellent binding affinity at α_3 containing receptors, although the more lipophilic heterocycles **7e** and **8b** led to a significant loss in affinity. None of the heterocycles lacking a HBD exhibited any functional selectivity (**7d**, **8a** and **8b**). However, although NH triazoles **7a**, **7b** also lack functional selectivity, imidazole **7c** and triazolopiperazine **11** do exhibit functional selectivity for α_3 containing receptors. Triazolopiperazine **11**, having a higher efficacy at α_3, was chosen for further profiling but gave no in vivo displacement of [^{3}H]Ro 15-1788 binding when dosed at 10 mg/kg po in 0.5% methocel to rats.[18] The poor physical properties (high crystallinity and poor solubility) of this series, which have also been observed in the related pyrimidin-5(6*H*)-one series,[19] precluded further in vivo evaluation of any of the other derivatives.

To improve the physical properties, replacements for the phenyl spacer were investigated. To facilitate this, the synthetic route was modified to give access to a pyrazolopyridine core with a halogen at the 7-position (Scheme 3). Treatment of 4-hydroxy-6-methyl-2-pyrone **12** with

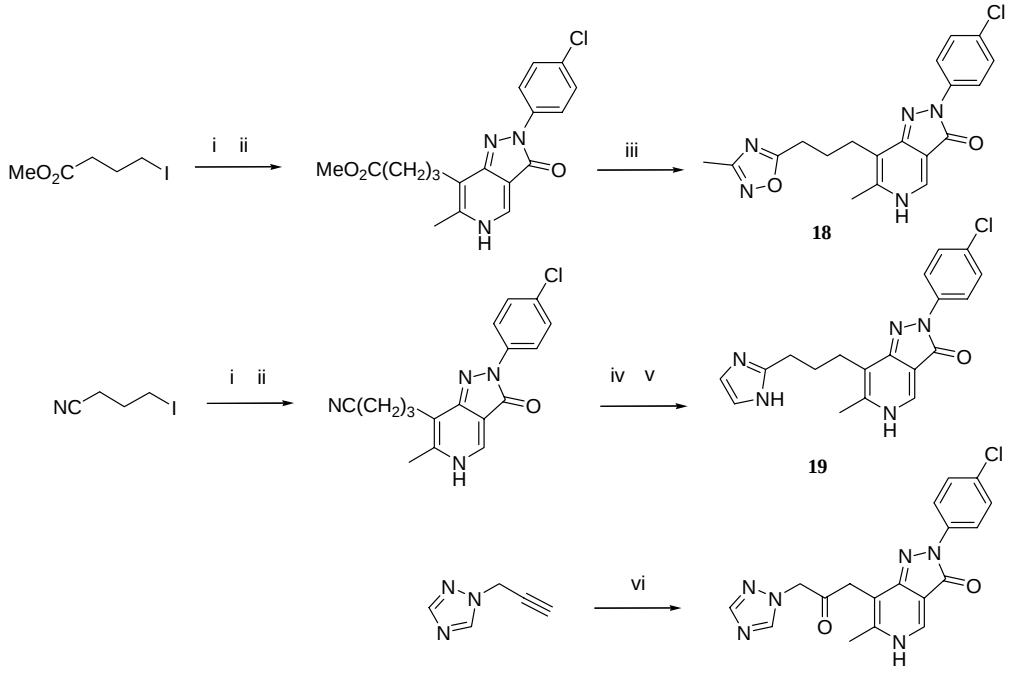
Table 2.

Entry	R	K_i (nM)[a]		Efficacy[b]	
		α_1	α_3	α_1	α_3
2d	$CONH_2$	0.30 ± 0.07	0.67 ± 0.11	0.22 ± 0.08	0.38 ± 0.06
7a	1,2,4-Triazol-3-yl	—	0.85 ± 0.22	0.01 ± 0.04	0.03 ± 0.04
b	1,2,3-Triazol-5-yl	—	0.79 ± 0.18	0.23 ± 0.06	0.26 ± 0.04
c	Imidazol-2-yl	—	1.2 ± 0.2	0.03 ± 0.04	0.22 ± 0.04
d	1-Methylimidazol-2-yl	—	1.5 ± 0.1	0.34 ± 0.03	0.4 ± 0.04
e	Thiazol-2-yl	—	51 ± 4	—	—
8a	Pyridin-3-yl	—	2.4 ± 0.3	0.15 ± 0.03	0.18 ± 0.03
b	Oxazol-2-yl	—	14 ± 1	0.22 ± 0.02	0.25 ± 0.02
9	Imidazol-1-yl	—	1.8 ± 0.7	0.1 ± 0.05	0.08 ± 0.04
11	4-[(4-Methylpiperazin-1-yl) methyl]-1,2,3-triazol-5-yl	—	0.44 ± 0.02	0.23 ± 0.04	0.43 ± 0.04

[a,b]As for Table 1.



Scheme 3. Reagents and conditions: (i) DMF/DMA, dioxan; (ii) NH_4OH; (iii) $NHMe_2$, 50 °C; (iv) H_2SO_4; (v) ICl, $CaCO_3$, DMF, 50 °C; (vi) $POCl_3$, 95 °C; (vii) MeOH, DCM, 0 °C, (viii) *p*-chlorophenylhydrazine·HCl, BuOH, reflux.



Scheme 4. Reagents and conditions: (i) activated zinc, THF, 50 °C; (ii) **17**, $Pd_2(dba)_3$, tris(2-furylphosphine), DMF, 50 °C; (iii) acetamide oxime, NaOEt, EtOH, 50 °C; (iv) aminoacetaldehyde dimethylacetal, CuCl, DMSO, 80 °C; (v) formic acid, 70 °C; (vi) **17**, $Pd_2(dba)_3$, tris(2-furylphosphine), CuI, piperidine, 80 °C.

DMF/DMA[20] gave the enamine **13** which on treatment with aqueous ammonia, followed by acidification with sulfuric acid,[21] gave the hydroxy acid **14**. The acid was halogenated with iodine monochloride and transformed to pyrazolopyridine **17**, as outlined in Scheme 3.

From iodide **17**, it was possible to gain access to compounds with a flexible alkyl spacer via palladium-catalysed coupling with an alkyl zinc reagent prepared by the method of Knochel et al.[22] Thus, methyl 4-iodobutanoate was converted to the alkyl zinc reagent, coupled to **17**, and reacted with acetamide oxime[23] to give oxadiazole **18**. Similarly, coupling with the organozinc derived from 4-iodobutyronitrile, followed by copper catalysed amidine formation[24] and acid-mediated ring closure, gave imidazole **19**. Attempted Sonogashira coupling of **17** using the propargylic triazole (Scheme 4) gave concomitant hydration of the acetylene to afford ketone **20** as the sole product. This facile hydrolysis (presumably via an allene intermediate) is probably due to enhanced acidity of the propargylic methylene.[†]

As shown in Table 3, replacement of the phenyl spacer with alkyl spacers gave compounds with high affinity at α_3 receptors. In addition, compounds **18**, **19** and **20** demonstrated a range of efficacy profiles. Compounds **18** and **19** have the efficacy profile of an antagonist and

[†] The hydrolysis may be catalysed by trace amounts of the hydroxide present in the triethylamine, which was dried over potassium hydroxide before use.

Table 3.

Entry	R	K_i (nM)[a]		Efficacy[b]	
		α_1	α_3	α_1	α_3
18	4-(3-Methyl-1,2,4-oxadiazol-5-yl)	0.30 ± 0.02	0.80 ± 0.14	−0.2 ± 0.05 [1.9%]	0.08 ± 0.03 [−6.3%]
19	3-(Imidazol-2-yl)-propyl	0.30 ± 0.04	0.75 ± 0.04	0.23 ± 0.04	0.28 ± 0.04
20	2-Oxo-3-(1,2,4-triazol-1-yl)-propyl	0.45 ± 0.04	0.75 ± 0.18	−0.06 ± 0.03	0.23 ± 0.05

[a,b]As for Table 1.

unselective partial agonist, respectively. However, compound **20** exhibited the desired efficacy profile, being a partial agonist at α_3 containing receptors and an antagonist at α_1 containing receptors. The occupancy of compound **20** was determined in vivo by displacement of [^{3}H]Ro 15-1788 binding in the mouse. When dosed at 3 mg/kg iv in 70% PEG, **20** gave a 44% receptor occupancy.[18]

In conclusion, replacement of the fused tetrahydro ring of CGS-17867A gave a novel series of high affinity $GABA_A$ ligands by pendantly substituting the pyrazolopyridine ring at the 6- and 7-positions. Although binding selectivity for $\alpha_3\beta_3\gamma_2$ over $\alpha_1\beta_3\gamma_2$ receptors was rarely greater than about 5-fold, modification of the 7-position of the pyrazolopyridinone ring enabled modulation of the efficacy profile to introduce functional selectivity. Compounds, such as **2d** and **11**, achieved functional selectivity comparable to that of the original lead. Replacement of the 7-phenyl with a ketotriazole moiety gave a compound (**20**) with subnanomolar affinity at $\alpha_3\beta_3\gamma_2$ receptors and an attractive efficacy profile, being an antagonist at $\alpha_1\beta_3\gamma_2$ receptors and a partial agonist at $\alpha_3\beta_3\gamma_2$ receptors.

Acknowledgments

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References and notes

1. Simon, J.; Wakimoto, H.; Fujita, N.; Lalande, M.; Barnard, E. A. *J. Biol. Chem.* **2004**, *279*, 41422.
2. Sieghart, W. *Pharmacol. Rev.* **1995**, *47*, 181.
3. *The GABA Receptors*; Bowery, G., Enna, S. J., Eds., 2nd ed., 1997, p 332.
4. Gupta, S. P. *Prog. Drug Res.* **1995**, *45*, 67.
5. Atack, J. R. *Curr. Drug Targets CNS Neurol. Disord.* **2003**, *2*, 213.
6. 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. *Nat. Neurosci.* **2000**, *3*, 587.
7. Bennett, D. A.; Amrick, C. L.; Wilson, D. E.; Boast, C. A.; Loo, P.; Bernard, P. S.; Schmutz, M.; Gerhardt, S. C.; Braunwalder, A.; Klebs, K.; Yokoyama, N.; Liebman, J. M. *Drug Dev. Res.* **1987**, *11*, 219.
8. Mitchinson, A.; Atack, J. R.; Blurton, P.; Carling, R. W.; Castro, J. L.; Curley, K. S.; Russell, M. G. N.; Marshall, G.; McKernan, R. M.; Moore, K. W.; Narquizian, R.; Smith, A.; Street, L. J.; Thompson, S.; Wafford, K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3441.
9. Ohtsuka, Y. *J. Org. Chem.* **1976**, *41*, 629.
10. Citterio, A.; Arnoldi, A. *Synth. Commun.* **1981**, *11*, 639.
11. Michael, J. P.; Cirillo, P. F.; Denner, L.; Hosken, G. D.; Howard, A. S.; Tinkler, O. S. *Tetrahedron* **1990**, *46*, 7923.
12. Hadingham, K. L.; Wingrove, P.; Le Bourdelles, B.; Palmer, K. J.; Ragan, C. I.; Whiting, P. J. *Mol. Pharmacol.* **1993**, *43*, 970.
13. 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.
14. Brown, N.; Kerby, J.; Bonnert, T. P.; Whiting, P. J.; Wafford, K. A. *Br. J. Pharmacol.* **2002**, *136*, 965.
15. Holzer, W.; Ruso, K. *J. Heterocycl. Chem.* **1992**, *29*, 1203.
16. Banert, K. *Chem. Ber.* **1989**, *122*, 1963.
17. Harrison, T.; Owens, A. P.; Williams, B. J.; Swain, C. J.; Williams, A.; Carlson, E. J.; Rycroft, W.; Tattersall, F. D.; Cascieri, M. A.; Chicchi, G. G.; Sadowski, S.; Rupniak, N. M. J.; Hargreaves, R. J. *J. Med. Chem.* **2001**, *44*, 4296.
18. Atack, J. R.; Smith, A. J.; Emms, F.; McKernan, R. M. *Neuropsychopharmacology* **1999**, *20*, 255.
19. Albaugh, P. A.; Marshall, L.; Gregory, J.; White, G.; Hutchison, A.; Ross, P. C.; Gallagher, D. W.; Tallman, J. F.; Crago, M.; Cassella, J. V. *J. Med. Chem.* **2002**, *45*, 5043.
20. Kilbourn, E. E.; Seidel, M. C. *J. Org. Chem.* **1972**, *37*, 1145.
21. Yamada, H.; Tobiki, H.; Jimpo, K.; Gooda, H.; Takeuchi, Y.; Ueda, S.; Komatsu, T.; Okuda, T.; Noguchi, H. *J. Antibiot.* **1983**, *36*, 532.
22. Knochel, P.; Perea, J. J. A.; Jones, P. *Tetrahedron* **1998**, *54*, 8275.
23. Street, L. J.; Baker, R.; Book, T.; Kneen, C. O.; MacLeod, A. M.; Merchant, K. J.; Showell, G. A.; Saunders, J.; Herbert, R. H. *J. Med. Chem* **1990**, *33*, 2690.
24. Rousselet, G.; Capdevielle, P.; Maumy, M. *Tetrahedron Lett.* **1993**, *34*, 6395.