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Imidazo[1,2-*a*]pyrazin-8-ones, imidazo[1,2-*d*][1,2,4]triazin-8-ones and imidazo[2,1-*f*][1,2,4]triazin-8-ones as α2/α3 subtype selective $GABA_A$ agonists for the treatment of anxiety

Simon C. Goodacre,* David J. Hallett, Robert W. Carling, José L. Castro, David S. Reynolds, Andrew Pike, Keith A. Wafford, Robert Newman, John R. Atack and Leslie J. Street

Neuroscience Research Centre, Merck, Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, UK

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Abstract—Imidazo[1,2-*a*]pyrazin-8-ones, imidazo[1,2-*d*][1,2,4]triazin-8-ones and imidazo[2,1-*f*][1,2,4]triazin-8-ones are high affinity $GABA_A$ agonists. Compound **16d** has good oral bioavailability in rat, functional selectivity for the $GABA_A$α2 and α3-subtypes and is anxiolytic in a conditioned animal model of anxiety with minimal sedation observed at full BZ binding site occupancy.

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain.[1,2] $GABA_A$ receptors are ligand-gated chloride ion channels composed of five transmembrane-spanning subunits.[3] These five subunits come from a family of 16 (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, π, and θ)[4] most frequently in a combination of two α, two β and one γ subunits.[5] These ion channels are also the site of action of the benzodiazepine (BZ) class of anxiolytic agents.[6] The BZ binding site occurs at the interface of a γ2 and either an α1, α2, α3 or α5 subunit, with the α subunit being a key determinant of the pharmacology of this site.[7] Clinically used BZ anxiolytics are high efficacy agonists that do not discriminate between α1, α2, α3 and α5 subtypes. These compounds, such as diazepam, also show sedative and muscle-relaxant properties. Studies using transgenic mice with point mutations in the α subunit have shown that $GABA_A$ receptors containing the α1 subunit mediate the sedative/muscle relaxant effects of BZs, whereas those containing an α2 and/or an α3 subunit mediate anxiolytic and anticonvulsant effects.[8–10]

The imidazo[1,2-*a*]pyrimidine **1**[11] and the imidazo[1,2-*a*][1,2,4]triazine **2**[12] have previously been reported as high affinity, $GABA_A$α2/α3 agonists with functional selectivity for the $GABA_A$α2/α3 receptors over $GABA_A$α1. Here we report our investigations on manipulation of the aza-bicyclic scaffold to afford compounds with excellent functional selectivity and pharmacokinetic properties (Fig. 1).

The nitrogen at the 8-position in the imidazo[1,2-*a*]pyrimidine **1** results in a tenfold increase in BZ affinity compared with the analogous imidazo[1,2-*a*]pyridine.[11] Work towards a new series of $GABA_A$α2/α3 agonists focused on exploiting this interaction with the $GABA_A$ receptor. Electron density mapping[13] of the imidazopyrimidine **3** suggested that a carbonyl group at the 8-posi-

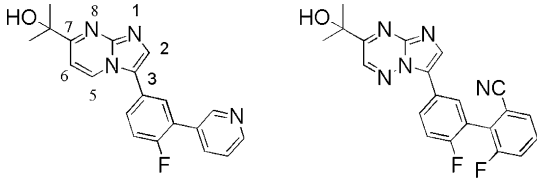

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Figure 1. Previously described clinical candidates, the imidazo[1,2-*a*]pyrimidine and imidazo[1,2-*a*][1,2,4]triazine.

Keywords: Imidazo[1,2-*a*]pyrazin-8-ones; Imidazo[1,2-*d*][1,2,4]triazin-8-ones; Imidazo[2,1-*f*][1,2,4]triazin-8-ones; $GABA_A$ agonists; Functionally selective for $GABA_A$α2 and α3; Anxiolytic.
* Corresponding author. Tel.: +44 (0) 1279440000; fax: +44 (0) 1279 440390; e-mail: simon_goodacre@merck.com

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tion, as in compound **4**, would have a very similar electron density to the nitrogen, Figure 2, and so may have similar affinity-enhancing properties (Table 1).

To test the hypothesis, the compounds targeted were the imidazo[1,2-*d*][1,2,4]triazin-8-one **10**, the imidazo[2,1-*f*][1,2,4]triazin-8-ones **13a**–**b** and the imidazo[1,2-*a*]pyrazin-8-ones **16a**–**d** and these compounds were prepared as shown in Schemes 1 and 2.[11,14,17] The aryl bromides and boronates required for the Heck and Suzuki couplings were prepared according to Scheme 1. Bromide **5**[15] was converted to the boronate ester under Miyaura conditions and then coupled with the desired aryl bromides using a Fu protocol,[16] before reduction with stannous chloride and conversion to the bromides **6a**–**c** by a Sandmeyer reaction. A Miyaura reaction with these bromides gave the boronate esters **7a**–**c**.

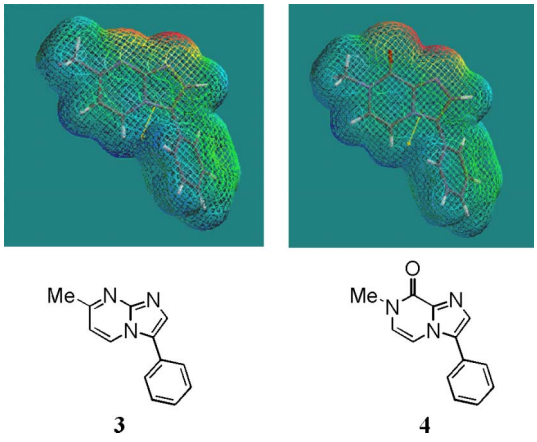
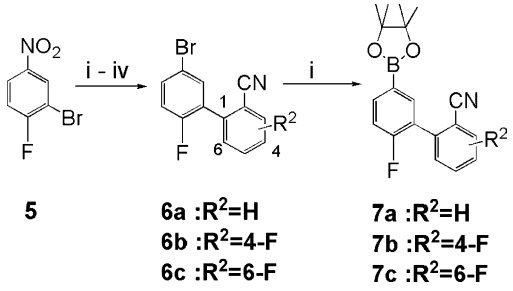


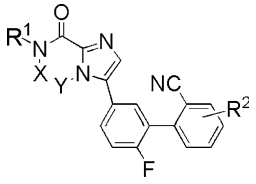
Figure 2. Electrostatic potential mapping of the imidazo[1,2-*a*]pyrimidine and imidazo[1,2-*a*]pyrazin-8-one. Red and blue colourations indicate high and low electron densities, respectively.



Scheme 1. Reagents and conditions: (i) Bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, 1,4-dioxane, 90 °C, 95%; (ii) Ar-Br, $Pd_2(dba)_3$, $PtBu_3$, KF, THF, rt to 50 °C, 90–95%; (iii) $SnCl_2{\cdot}2H_2O$, EtOH, 75–95%; (iv) $NaNO_2$, 48% HBr(aq), CuBr, 0–50 °C, 64–92%.

The imidazotriazinone **9** was prepared by cyclisation of 6-amino-4-methyl-3-(methylthio)-1,2,4-triazin-5(4*H*)-one **8**[17] with bromoacetaldehyde followed by removal of the thiomethyl group with Raney nickel. The imidazotriazinones **12a**–**b** were synthesized by cyclisation of imidazole **11**[18] with DMF-DMA at elevated temperatures followed by alkylation using the Curran protocol.[19] A Heck reaction[20] was used to couple the imidazotriazinones **9** and **12a**–**b** with bromide **6b** to give **10** and **13a**–**b**, respectively. The imidazopyrazinone analogues **16a**–**d** were prepared from 2-amino-3-chloropyrazine via cyclisation with bromoacetaldehyde to give chloroimidazopyrazine **15**. Bromination of **15**, followed by selective Suzuki coupling with **7a**–**c**, gave the biarylchloroimidazopyrazine. Methoxide displacement of the

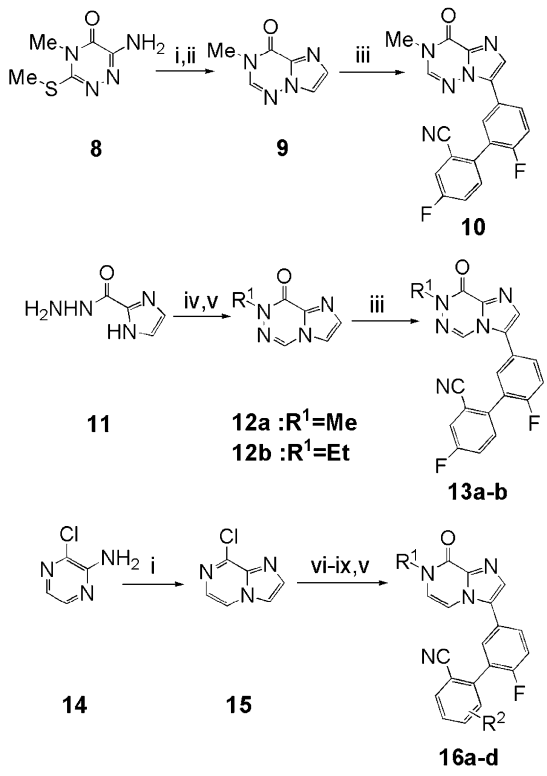
Table 1. Binding affinity and efficacy for imidazo[1,2-*a*]pyrazin-8-ones, imidazo[1,2-*d*][1,2,4]triazin-8-ones and Imidazo[2,1-*f*][1,2,4]triazin-8-ones at $GABA_A$ receptor subtypes



Compound	R^1	X	Y	R^2	K_i[a] (nM)			Efficacy[b]		
					α1	α3	α5	α1 (%)	α3 (%)	α5 (%)
1	—	—	—	—	1.4	4.0	0.52	+6	+57	+20
2	—	—	—	—	0.6	1.5	0.9	+4	+65	+44
10	Me	CH	N	4-F	2.5	4.6	4.8	−46	—	—
13a	Me	N	CH	4-F	6.5	4.7	2.3	−9	+1	—
13b	Et	N	CH	4-F	2.4	3.4	0.95	−19	−7	—
16a	Me	CH	CH	H	0.53	0.7	0.1	+27	+51	—
16b	Me	CH	CH	4-F	0.7	1.9	0.39	−27	+44	—
16c	Me	CH	CH	6-F	0.16	0.59	0.19	+3	+28	—
16d	Et	CH	CH	4-F	0.28	0.9	0.15	−8	+43	−4

[a] K_i values for binding to the benzodiazepine sites of stably expressed human recombinant $GABA_A$ receptors with the composition $\alpha x\beta 3\gamma 2$ (x = 1, 2, 3 or 5). Inhibition of the binding of 1.8 nM [^{3}H]Ro15-1788 was measured and the concentration required to inhibit binding by 50% (IC_{50}) was converted to a K_i value according to the Cheng–Prusoff equation. Data shown are mean values for 3–6 determinations.

[b] Efficacy measured at $GABA_A$ receptors stably expressed in L(tk$^-$) cells using whole cell patch clamp recording and represents the effect of the test compound on the current produced by a sub-maximal concentration of GABA (EC_{20}).[21] Values are means of at least seven independent experiments.



Scheme 2. Reagents and conditions: (i) $BrCH_2CH(OEt)_2$, 48% HBr(aq) then $NaHCO_3$, ^{i}PrOH, 47–65%; (ii) Ra-Ni, EtOH, 40 °C, 24%; (iii) **6a**–**c**, $Pd(OAc)_2$, PPh_3, KOAc, DMA, 120 °C, 66–77%; (iv) DMF-DMA, Dowtherm®, 210 °C, 83%; (v) NaH, LiBr, R^1I, 60 °C, 73–83%; (vi) Br_2, KBr, NaOAc, MeOH, 0 °C, 90-95%; (vii) **7a**–**c**, Cs_2CO_3, $Pd(PPh_3)_4$, THF, reflux, 64–74%; (viii) NaOMe, MeOH, CH_2Cl_2, 40 °C, 90–95%; (ix) HBr (30% w/w in AcOH), 95 °C, 74–85%.

8-chloro substituent and subsequent cleavage of the methoxy group under acidic conditions gave the 7*H*-imidazopyrid-8-ones which were then alkylated to give compounds **16a**–**d**.

As suggested by the electrostatic potential mapping of **3** and **4**, the carbonyl group afforded compounds with comparable affinity to **1** and **2**. The imidazotriazinone **10** retained good BZ affinity however it was a strong inverse agonist at $GABA_A\alpha1$ containing receptors. A second series of imidazotriazinones were investigated where the nitrogen at the 5-position was transposed to the 6-position. The two analogues **13a** and **13b** again retained nanomolar affinity at $GABA_A$ receptors but had significantly reduced selectivity between α3 and α1 containing receptors. Utilising the same concept in the imidazo[1,2-*a*]pyrimidine ring system gave a series of imidazopyrazinones **16a**–**d**. The unsubstituted benzonitrile analogue **16a** showed sub-nanomolar affinity at BZ receptors but had significant efficacy at α1 containing receptors. To improve functional selectivity in this series, the substituents R^1 and R^2 were modified. The 4-fluoro-2-cyanophenyl analogue **16b** had excellent functional selectivity but was an inverse agonist at α1. The 6-fluoro-2-cyanophenyl analogue **16c** was an antagonist at α1 containing receptors but with weak partial efficacy at the $GABA_A\alpha3$ subtype. To optimise the $GABA_A\alpha3$ efficacy profile of **16b**, replacements for the methyl at the 7-position were investigated. Increasing the lipophilicity of the 7-substituent by replacing the *N*-methyl with *N*-ethyl gave **16d**, a compound with the desired profile, similar to previously described development candidates **1** and **2**.[11,12]

Thus, **16d** is a $GABA_A\alpha1$ antagonist and a partial agonist on both the $GABA_A\alpha2$ (+27%) and the $GABA_A\alpha3$ subtypes. Compound **16d** had good pharmacokinetics in rat (F = 38%; Clp = 17 ml/min/kg; $t_{1/2}$ = 5.0 h), with an ID_{50} of 0.7 mg/kg po in a rat [^{3}H]Ro15-1788 in vivo binding assay.[22] Compound **16d** was active in the rat elevated plus maze assay,[23] a model of anxiety, at 3 mg/kg po (86% BZ site occupancy) with no impairment seen in the beam walking assay,[24] a model of sedation, at 100 mg/kg po.

In summary, we have described the synthesis and biological activity for a series of imidazopyrazinones and two series of imidazotriazinones as novel BZ receptor ligands. Compound **16d** was identified as a functionally selective $GABA_A\alpha2/\alpha3$ agonist which is an orally bioavailable, non-sedating anxiolytic in animal models.

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