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Imidazo[1,2-*b*][1,2,4]triazines as α2/α3 subtype selective $GABA_A$ agonists for the treatment of anxiety

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Abstract—Imidazo[1,2-*a*]pyrimidines and imidazo[1,2-*b*][1,2,4]triazines are ligands for the benzodiazepine binding site of $GABA_A$ receptors that are functionally selective for the α2/α3 subtypes over the α1 subtype. SAR studies to optimise this functional selectivity, pharmacokinetic and behavioural data are described.

Inhibition of neurotransmission in the central nervous system is mediated predominantly by chloride ion flux into nerve cells through $GABA_A$ receptors. These receptors are ligand-gated ion channels which open in response to the binding of the neurotransmitter γ-aminobutyric acid (GABA). They are composed of five transmembrane subunits that come from a family of 19 ($\alpha_{1\text{-}6}$, $\beta_{1\text{–}3}$, $\gamma_{1\text{-}3}$, δ, ε, π, θ and $\rho_{1\text{-}3}$), most frequently consisting of α, β and γ subunits in a 2:2:1 ratio.[1,2] The $GABA_A$ receptors are the site of action of the benzodiazepine (BZ) class of molecules which allosterically modulate the GABA-mediated chloride ion flux.[3] The major BZ-sensitive receptors contain β, γ2 and either α1, α2, α3 or α5 subunits. Studies with transgenic mice and subtype selective compounds indicate that receptors containing different α subtypes mediate the various pharmacological effects of the non-subtype selective BZ agonists, such as diazepam, i.e., the α1-containing receptors are responsible for the sedative/muscle relaxant properties, while the α2 and/or the α3-containing receptors mediate the anxiolytic properties.[4,5] The goal of our research has been to identify ligands for the $GABA_A$ receptor that are selective α2/α3 agonists which could be potential anxiolytics without the concurrent sedation/ataxia observed with unselective BZs.

The imidazo[1,2-*a*]pyrimidine **1a** (Table 1) was identified as a high affinity ligand for the $GABA_A$ receptor BZ binding site, which also had the desired functional selectivity between α subtypes, i.e., antagonism at the α1 subtype and partial agonism at the α2 and α3 subtypes. The compound, as had been hypothesised for such a profile, proved to function as an anxiolytic in several animal behavioural models without showing the pronounced sedation and ataxia observed with unselective BZs.[6]

However, compound **1a** has modest pharmacokinetics (PK) in rat and dog (half-lives of 1.7 and 1.0 h, respectively—Table 3) and the desire was to find a compound with a longer half-life whilst maintaining the beneficial efficacy profile.

The pyridyl *N*-oxide of **1a** was observed as a major metabolite in vivo, therefore initial work focused on modification of the 3-pyridyl moiety. This was achieved using the route shown in Scheme 1[7] by forming the biaryl ring system **5**, which was then coupled to the imidazopyrimidine core **3**. Chemistry to introduce the diversity as the final step by Suzuki coupling to a late stage intermediate (**1** where R = Br or $B(OH)_2$) was explored, but proved to be problematic due to competing Dimroth rearrangement.[8]

Keywords: Imidazo[1,2-*a*]pyrimidines; Imidazo[1,2-*b*][1,2,4]triazines; GABAA receptors; α2/α3 subtype selective; Anxiolytic.
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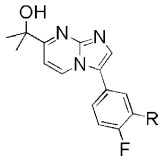
Table 1. Affinity and efficacy at α1 and α3 subtype $GABA_A$ receptors for imidazo[1,2-*a*]pyrimidines varying the terminal pyridyl ring

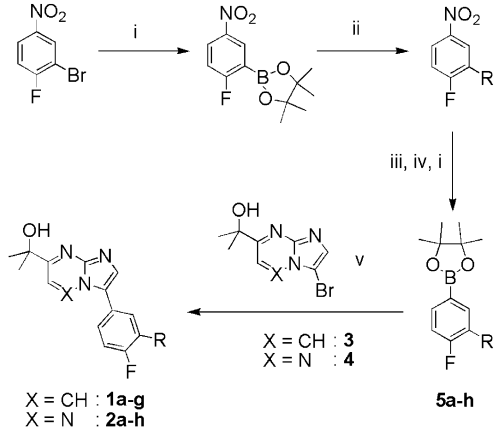


Compound	R	K_i (nM)[a]				Flux efficacy (vs CDZ)[b]		Patch–clamp efficacy (vs CDZ)[c]	
		α1	α2	α3	α5	α1	α3	α1	α3
1a		0.85	3.70	4.00	0.53	0.06	0.50	0.05	0.53
1b		0.68		2.25	0.31	0.18	0.47	0.42	
1c		1.60		1.94	0.42	0.38	0.57	0.40	
1d		0.80		0.42	1.24	0.44	0.68		
1e		1.79		3.76	1.13	0.18	0.21		
1f		1.43		3.64	0.67	0.26	0.52		
1g		0.60		0.28	0.14	0.30	0.59		

[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 of 3–10 separate determinations.[9]

[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 chlordiazepoxide (CDZ) (relative efficacy = 1.0), from at least seven independent experiments.[10]

[c] Measured with $GABA_A$ 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 an EC_{20}-equivalent of GABA relative to the full agonist chlordiazepoxide (CDZ) (relative efficacy = 1.0). Data show mean maximal efficacy from at least four individual cells.[4]



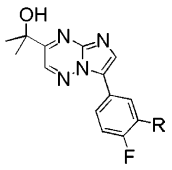
Scheme 1. Reagents and conditions: (i) Bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, 1,4-dioxane, 90 °C; (ii) R-Cl/Br, $Pd_2(dba)_3$, P^tBu_3, KF, THF, rt to 50 °C; (iii) $SnCl_2 \cdot 2H_2O$, EtOH; (iv) $NaNO_2$, 48% HBr (aq), CuBr, 0–50 °C; (v) $Pd(PPh_3)_4$, Na_2CO_3 (aq), DME, 80 °C.

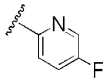
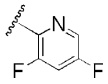
Some key compounds to come from this work are shown in Table 1. In terms of affinity, the pyridyl nitrogen was tolerated at all three positions (**1a**–**c**). Fluoro or cyano substituents could also be introduced to give promising compounds (**1c**–**g**) with high affinity. In our high throughput chloride ion flux efficacy assay,[10] all the compounds showed a window between the agonism produced at the α3 and α1 subtypes, however none acted as an antagonist at α1 (confirmed for compounds **1b** and **1c** by using a whole cell patch–clamp efficacy assay).[4]

It was noted, during concurrent work modifying the core heterocyclic-ring system, that replacing the imidazo[1,2-*a*]pyrimidine by an imidazo[1,2-*b*][1,2,4]triazine conferred a lowered agonism profile at both α3 and α1 subtypes whilst maintaining an equivalent level of binding affinity.[11] For example, the direct analogue of **1a**, compound **2a** (Table 2), has equivalent affinity to **1a** and exhibits lower efficacy in the patch–clamp assay: 0.24 relative to the standard full agonist chlordiazepoxide (CDZ) at the α3 subtype compared to 0.53 for **1a**.

With this in mind several of the pyridine compounds were made in the imidazotriazine series (conveniently by coupling the previously synthesised biaryls **5** to bromide **4** as shown in Scheme 1)[12] with the aim of

Table 2. Affinity and efficacy at α1 and α3 subtype $GABA_A$ receptors for imidazo[1,2-*b*][1,2,4]triazines varying the terminal pyridyl ring



Compound	R	K_i (nM)[a]				Patch–clamp efficacy (vs CDZ)[b]			
		α1	α2	α3	α5	α1	α2	α3	α5
2a		0.81		1.63	0.50	0.00		0.24	
2b		0.55	0.92	0.52	0.50	0.06			
2c		1.45	2.3	2.77	1.24	0.15		0.50	
2d		2.01		3.42	1.57	0.24		0.52	
2e		0.67	3.44	4.50	2.71	0.00	0.39	0.50	0.09
2f		1.28	8.43	9.53	9.34	0.01	0.37	0.69	0.20
2g		0.76	1.54	1.06	0.32	0.00	0.23	0.37	0.41
2h		0.67		3.30		0.01		0.34	

[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 the mean of 3–10 separate determinations.[9]

[b] Measured with $GABA_A$ 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 an EC_{20}-equivalent of GABA relative to the full agonist chlordiazepoxide (CDZ) (relative efficacy = 1.0). Data shows mean maximal efficacy from at least four individual cells.[4]

reducing the efficacy at the α1 subtype to antagonism levels. The resulting compounds are shown in Table 2. Gratifyingly, the anticipated change in efficacy profile did indeed manifest itself; 4-pyridyl and 2-pyridyl-4-fluoro compounds **2b** and **2c** when compared with analogues **1b** and **1c** showed a reduction in their α1 efficacy of approximately 0.3 units in the patch–clamp assay. Utilising this discovery, several compounds with very interesting selective efficacy profiles (**2e**–**h**) were identified.

Having measured the affinity and efficacy for these compounds at the α2 and α5 subtypes (Table 2), all were shown to be partial agonists, but compounds **2e** and **2f** stood out: **2f** due to its high efficacy at the α3 subtype and **2e** due to its low efficacy at the α5 subtype (α5 efficacy has been implicated in memory/learning impairment).[13]

The pharmacokinetic profiles of **2e** and **2f** were examined in rat and dog (Table 3). Both were found to have

Table 3. Pharmacokinetic parameters in rat and dog

Compound	Rat[a]				Dog[b]			
	F (%)	Cl (ml/min/kg)	$T^{1/2}$ (h)	V_{dis} (l/kg)	*F* (%)	Cl (ml/min/kg)	$T^{1/2}$ (h)	V_{dis} (l/kg)
1a	77	9.1	1.7	1.0	46	8.1	1.0	0.6
2e	69 ± 8	1.2 ± 0.1	22	2.2 ± 0.1	64 ± 28	0.5 ± 0.1	9/65[c]	2.3 ± 0.3
2f	102 ± 20	2.4 ± 0.3	7.9	1.7 ± 0.4	99 ± 32	2.2 ± 0.7	9.7	1.5 ± 0.1

[a] Determined in six male Sprague–Dawley rats. Three dosed 1 mg/kg iv and three dosed 1 mg/kg po.

[b] Determined in six female beagle dogs. Three dosed 1 mg/kg iv and three dosed 1 mg/kg po.

[c] Two-phase elimination (2–10 h/10–48 h).

excellent parameters in the two species, with significantly increased half-lives when compared to **1e**.

Compound **2e** was an anxiolytic in the rat elevated plus maze assay[14] giving a statistically significant increase in time spent on the open arms at an oral dose of 1 mg/kg (which corresponded with an occupancy of 84% as measure by in vivo displacement of [^{3}H]Ro 15-1788)[15], and showed no ataxia in the mouse rotarod assay[4] either at oral doses up to 30 mg/kg or in the presence of a sub-threshold level of ethanol (1.5 g/kg ip) at an oral dose of 3 mg/kg (92% occupancy). Compound **2e** was also effective in the squirrel monkey conditioned emotional response (CER) paradigm, a more stringent test of anxiolytic potential, at an oral dose of 0.3 mg/kg.

In conclusion, we have shown that certain changes to the pyridyl moiety of imidazo[1,2-*a*]pyrimidine, **1a**, are tolerated in terms of affinity but lose the desired efficacy profile. This can be regained by modulating the efficacy profile with an imidazo[1,2-*b*][1,2,4]triazine ring system, which leads to compounds that are selective agonists at the α2/α3 subtypes of the $GABA_A$ receptor over α1. These compounds also exhibit improved pharmacokinetics in rat and dog over **1a** and **2e** has shown anxiolysis in two species at levels which show no effect in an animal model of ataxia.

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