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Imidazo[1,2-*a*]pyrimidines as functionally selective $GABA_A$ ligands

Wesley P. Blackaby,* John R. Atack, Frances Bromidge, José L. Castro, Simon C. Goodacre, David J. Hallett, Richard T. Lewis, George R. Marshall, Andrew Pike, Alison J. Smith, Leslie J. Street, David F. D. Tattersall and Keith A. Wafford

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*]pyrimidines 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, a pentameric supramolecular complex that acts as a ligand-gated chloride ion channel. A functional receptor is formed by the co-assembly of subunits selected from the family of 19 gene products (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, π, θ, and ρ_{1-3}), which are differentially expressed throughout the brain.[1,2] The most abundant $GABA_A$ receptor subtypes contain α, β, and γ subunits in a 2:2:1 stoichiometry. The benzodiazepine class of drugs,[3,4] widely used as therapy for anxiety and panic disorders, are generally non-selective agonists in that they allosterically modulate the GABA-mediated chloride ion flux through the channel of $GABA_A$ receptors containing β, γ2, and either α1, α2, α3 or α5 subunits.[1,2] However, the side effect profile associated with the classical benzodiazepines, such as their sedative properties, 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 that *functionally selective* or *binding selective*, with the expectation of gaining an improved side effect profile over currently used benzodiazepines.[7]

Benzimidazole **1** (NS-2710)[8] (Fig. 1) exhibits anxiolytic activity in several animal behavioral models with an improved side effect profile. We attributed this profile to the functional selectivity of this compound, which in our hands showed full efficacy at α_3 and α_2-containing receptors (comparable with the full agonist chlorodiazepoxide [CDZ]) and somewhat reduced efficacy at the α_1 subtype. To test our hypothesis the ideal compound would be an antagonist at α_1 with significant efficacy at α_3- and/or α_2-containing receptors. We have recently disclosed the details of SAR studies around **1** which led to the discovery of imidazopyrimidine **2**.[9] This paper describes the SAR of a related series of compounds in which we have examined heterocyclic replacements for the biaryl moiety (rings A and B).

Attempts to prepare an imidazopyrimidine 3-zincate species (via lithium halogen exchange and transmetallation with zinc chloride) or 3-boronate ester (via palladium catalysis[10]) from bromide **4**[11] proved unsuccessful. However, as shown in Scheme 1 bromide **4** cleanly underwent magnesium bromine exchange[12] using *i*-PrMgCl and the resulting Grignard quenched with tributyltin chloride to give the key stannane intermediate **5**. Although the stannane could be chromatographed on silica gel (using aprotic solvents as eluent) it was more conveniently kept as a THF stock solution which was stable for extended periods when stored in the refrigerator. The stannane solution was subsequently used to couple to a variety of heterocyclic halides under standard Stille conditions to give the imidazopyrimidines **3a**–**h**. Best yields were obtained with heteroaryl bromides or 2-chloropyridines. 4-Chloropyridines were

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* Corresponding author. Tel.: +44 1279 440404; fax: +44 1279 440187; e-mail: Wesley_Blackaby@Merck.com

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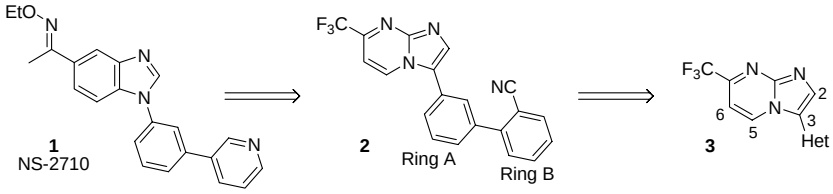
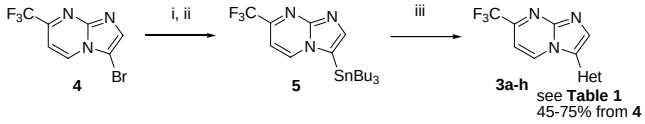


Figure 1.

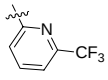
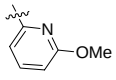
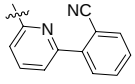


Scheme 1. Reagents and conditions: (i) *i*-PrMgCl, THF, −78 °C; (ii) Bu_3SnCl, −78 °C to rt; (iii) aryl halide, $Pd(PPh_3)_4$, reflux.

poorer coupling partners requiring longer reaction times. The mild Stille conditions negated the formation of products arising from Dimroth rearrangement[13] of the imidazopyrimidine core which has been observed during the Suzuki–Miyaura coupling reaction of bromide **4** with boronic acids.

Binding and efficacy data for the imidazopyrimidines **3** are shown in Table 1. Although the parent pyridin-2-yl compound **3a** shows similar affinity to **1** at α_3-containing receptors, additional substitution at the 6-position as in compounds **3b–e** gives a marked increase in affinity. The pyrimidine analogs were more sensitive to substitution effects, with the 6-chloro compound **3g** losing affinity, whereas the 2-chloropyrimidine **3h** gave high affinity. In our high-throughput functional efficacy assay based

Table 1. Binding affinity and efficacy for imidazo[1,2-*a*]pyrimidines at $GABA_A$ receptor subtypes

Compound	Het	K_i[a] (nM)		Efficacy[b]	
		α1	α3	α1	α3
1	—	1.9	13	[0.4]	[0.99]
3a		9.1	11	−0.4	−0.07
b		0.31	0.68	0.11	0.02
c		0.31	0.88	−0.24	−0.4
d		1.2	1.7	−0.22 [−41.4%]	−0.09 [−51.6%]
e		0.36	0.46	−0.08	0.1
f		>33	>33	—	—
g		>33	>33	—	—
h		0.93	2.0	−0.22	−0.19 [−24.9%]

[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, α_3 or α_5 stably expressed in L(tk$^-$) cells. Values are means of 3–10 separate determinations.[15]

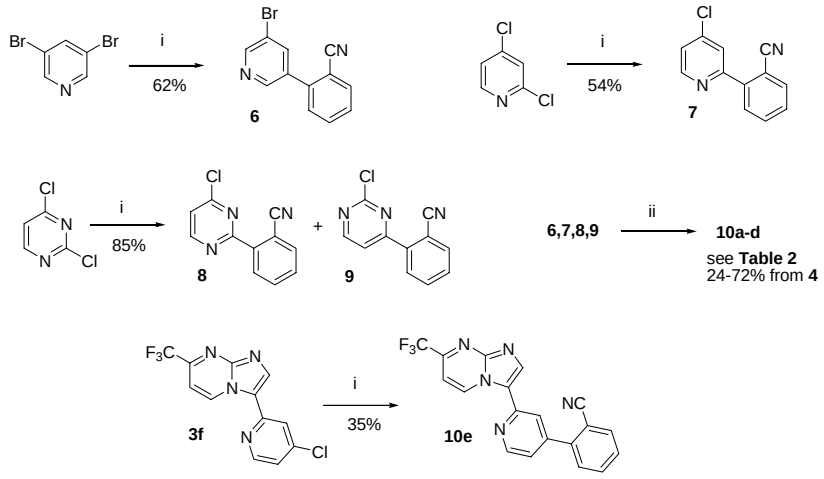
[b] Modulation of chloride ion flux in cells expressing $\beta_3\gamma_2$ plus either α_1 or α_3 produced by an EC_{20} equivalent concentration of GABA in the presence of an approximate $1000 \times K_i$ concentration of test compound. Efficacy is expressed relative to the full agonist chlorodiazepoxide (relative efficacy = 1.0). Values are means of at least seven independent experiments.[16] Negative values reflect a reduction in the GABA EC_{20} were induced chloride flux and reflect inverse agonist responses.

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 EC_{20}-equivalent of GABA relative to the full agonist chlorodiazepoxide (relative efficacy = 1.0).[17] Values shown as negative percentages reflect the fact that the GABA EC_{20} currents were attenuated rather than potentiated (i.e., responses were inverse agonist rather than agonist responses).

on chloride ion flux,[13] all the 2-aza heterocycles showed low efficacy at cells containing $\alpha_3\beta_3\gamma_2$ receptors. For compounds **3d** and **3h** patch-clamp measurements on L(tk$^-$) cells expressing α_1- and α_3-containing $GABA_A$ receptors confirm them to be unselective inverse agonists compared with the highly efficacious benzimidazole **1**. In the pentylenetetrazole (PTZ) assay carried out in Swiss Webster (SW) mice, compound **3h** was proconvulsant when dosed at 10 mg/kg i.p. (which is indicative of inverse agonists at the benzodiazepine binding site[14]).

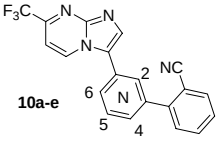
To examine the effect upon efficacy in biaryl-containing system **2** (Fig. 1) more closely, ring B was kept constant as 2-cyanophenyl whilst carbon was switched for nitrogen around ring A. The required halides were readily prepared by Suzuki coupling of the pyridine or pyrimidine dihalide with 2-cyanophenyl boronic acid as shown in Scheme 2. Coupling with 2,4-dichloropyridine took place predominantly at the 2-position as expected based on literature precedent[18] to give **7**. Under similar conditions 2,4-dichloropyrimidine gave a 1:2 mixture of the 2-coupled isomer (**9**) and 4-isomer (**8**) which, fortuitously, were readily separable by flash chromatography on silica. The heterocyclic halides were then coupled to stannane **5** as previously described. Pyridine **10e** was available via a Suzuki coupling of **3f** in refluxing THF, use of higher temperatures or microwave irradiation led to significant amounts of Dimroth products.

The data shown in Table 2 show that a nitrogen for carbon switch is well tolerated at the 2 and 4 positions of ring A; pyridines **3e**, **10b** and pyrimidine **10d** show excellent binding affinity at α_1-, α_3-, and α_5-containing subtypes. Nitrogen at position 6 was not tolerated in either the pyridine (**10e**) or pyrimidine (**10c**) case. The position of the nitrogen also has a profound effect in terms of efficacy. Whereas compound **3e** shows low efficacy at both α_1 and α_3 receptors, compound **10b** shows excellent functional selectivity with low efficacy at α1-containing receptors and moderate efficacy at the



Scheme 2. Reagents and conditions: (i) 2-Cyanophenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3 (aq), THF, reflux; (ii) **5**, $Pd(PPh_3)_4$, THF reflux.

Table 2. Binding affinity, efficacy and DLM turnover data for imidazo[1,2-*a*]pyrimidines



Compound	*N* position	K_i[a] (nM)		Efficacy[b]		Turnover[c] % DLM
		α1	α3	α1	α3	
2	—	0.66	0.33	0.10 [0.19]	0.53 [0.59]	100
3e	2	0.36	0.46	−0.08	0.1 [−22.0%]	15
10a	5	18	>33	−0.55	−0.39	—
10b	4	2.5	2.9	0.04 [0.15]	0.43 [0.45]	4
10c	2.6	>33	>33	—	—	—
10d	2.4	1.0	1.4	−0.29	−0.15	56
10e	6	>33	>33	—	—	—

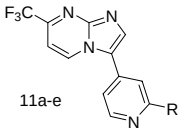
[a] As for Table 1.
[b] As for Table 1.
[c] Figure represents % of test compound metabolized when incubated with dog liver miocrosomes [DLM] at a drug concentration of 1 μM and a protein concentration of 0.4 mg/ml for 15 min at 37 °C.

α_3 subtype. The stability of the compounds was also examined in dog liver microsome [DLM] preparations. Gratifyingly the turnover for the N-4 nitrogen compound **10b** was dramatically reduced compared with the parent biphenyl compound **2**, suggesting that plasma clearance might also be reduced by this modification.

To achieve the required antagonism at α_1-containing receptors, our attention shifted to ring B modification whilst keeping the functionally selective pyridin-4-yl (ring A) of compound **10b** constant. As shown in Scheme 3, chloropyridine **13** was prepared as a late stage intermediate. Coupling of 4-bromo-2-methoxypyridine with stannane **5** proceeded smoothly to give **11a**. Hydrolysis with TMS iodide followed by chlorination with thionyl chloride gave **13**. Suzuki couplings with compound **13** were surprisingly sluggish and the more forcing conditions and lengthy reaction times required led to substantial amounts of Dimroth by-products, which were difficult to separate. Although fluorophenyl compound **11b** was accessed via this route, in general it was easier to prepare the biaryl coupling precursor. Suzuki coupling of 2-chloro-4-methoxypyridine with 3-fluorophenylboronic acid under Suzuki conditions gave **14**, which on treatment with PBr_3 gave the coupling precursor bromide **15**. Suzuki coupling of Boc-protected 2-chloro-4-aminopyridine followed by TFA treatment gave aminopyridine **16**. Diazotization in concentrated HBr then gave bromide **17**, the coupling precursor. Chloropyridine **18** was accessed from 2,4-dichloropyridine as described for compound **10b** above. The biaryl halide precursors were then coupled to stannane **5**.

As shown in Table 3 replacement of the cyanophenyl with methoxy (**11a**) (cf. 6-methoxypyridine **3d**) or 3-fluorophenyl (**11b**) is not well tolerated both modifications losing an order of magnitude in affinity. 2-Methoxyphenyl (**11c**) and 2-fluorophenyl (**11e**) were better tolerated in terms of affinity and also retained functional selectivity for α_3-containing receptors over α_1-containing receptors. However, the 2-cyano-4-fluorophenyl compound

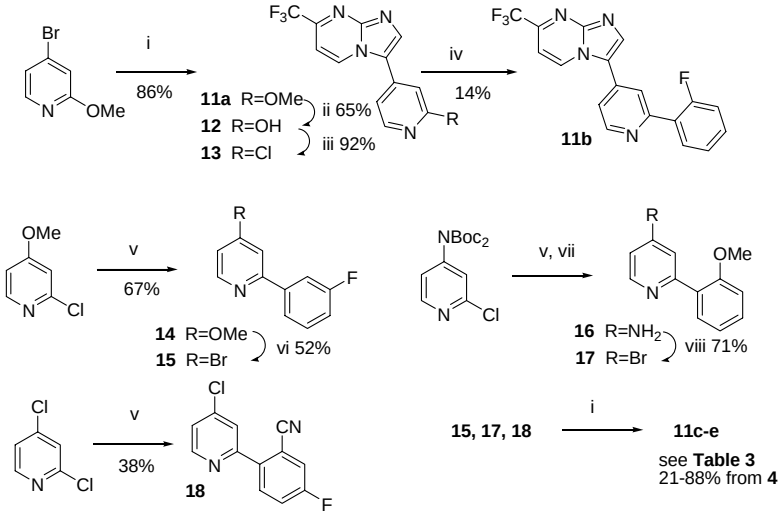
Table 3. Binding affinity and efficacy for imidazo[1,2-*a*]pyrimidines at $GABA_A$ receptor subtypes



Compound	R	K_i[a] (nM)		Efficacy[b]	
		α1	α3	α1	α3
10b		2.5	2.9	0.04 [0.15]	0.43 [0.45]
11a	OMe	29	>33	—	—
11b		>33	>33	—	—
11c		5.8	6.1	0.35	0.54
11d		0.99	2.2	0.09 [0.0]	0.21 [0.22]
11e		8.3	14	0.06	0.23

[a] As for Table 1.
[b] As for Table 1.

11d retained good affinity and the desired efficacy profile being an *antagonist* at α_1-containing receptors and a *partial agonist* at α_3 receptors. The stability of compound **11d** was also examined in dog and human liver microsome preparations; less than 5% turnover was observed in both species, suggesting that plasma clearance might be low for this compound. Compound **11d** was then progressed into our in vivo occupancy assay in rat which



Scheme 3. Reagents and conditions: (i) **5**, $Pd(PPh_3)_4$, THF, reflux; (ii) TMSI, DCM; (iii) thionyl chloride; (iv) $ArB(OH)_2$, $PdCl_2(dppf)$, Na_2CO_3 dioxane, 50 °C; (v) $ArB(OH)_2$, $PdCl_2(dppf)$, Na_2CO_3 dioxane, 80 °C; (vi) PBr_3; (vii) TFA, DCM; (viii) $NaNO_2$, Cu(I)Br, HBr.

uses displacement of [^{3}H]Ro 15-1788 to measure the extent to which the compound penetrates the CNS and occupies $GABA_A$ receptor benzodiazepine binding sites. When dosed at 1 mg/kg po as a suspension in 0.5% methocel, **11d** gave 50% receptor occupancy with a plasma Occ_{50} of 0.15 μM and brain to plasma ratio of 0.73.

In conclusion, the SAR of heterocyclic replacements for the biaryl moiety of **2** has been investigated. Introducing nitrogen into ring A of compound **2** has a profound effect on affinity, efficacy and metabolic stability. 6-Substituted pyridin-2-yls such as **3b**–**e** showed reduced efficacy at α_3 receptors. Pyridines with the nitrogen at the 4-position such as **10b**, **11c**, and **11e** showed higher efficacy at α_3 and functional selectivity over α_1 receptors. Modification of rings A and B in tandem enabled further modulation of the efficacy profile which led to compound **11d** with high affinity at $GABA_A$ 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 which exhibits good receptor occupancy in the rat on oral dosing.

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