Imidazo[1,2-*a*]pyrimidines as Functionally Selective and Orally Bioavailable $GABA_A\alpha2/\alpha3$ Binding Site Agonists for the Treatment of Anxiety Disorders

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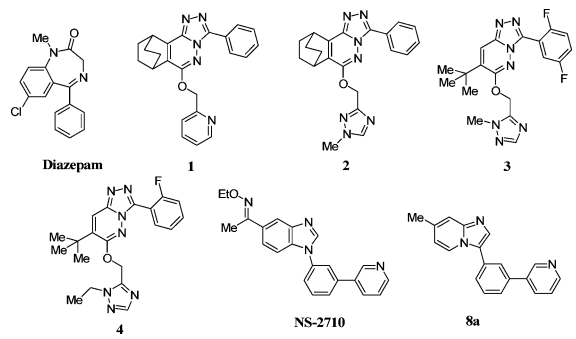
Abstract: A series of high-affinity $GABA_A$ agonists with good oral bioavailability in rat and dog and functional selectivity for the $GABA_A\alpha2$ and -$\alpha3$ subtypes is reported. The 7-trifluoromethylimidazopyrimidine **14g** and the 7-propan-2-olimidazopyrimidine **14k** are anxiolytic in both conditioned and unconditioned animal models of anxiety with minimal sedation observed at full BZ binding site occupancy.

Inhibitory neurotransmission in the central nervous system is mostly mediated through $GABA_A$ receptors by the binding of the agonist γ-aminobutyric acid (GABA).[1,2] These receptors are ligand-gated chloride ion channels that, in addition to binding GABA, are the site of action of a number of allosteric modulators including barbiturates, neurosteroids, loreclezole, anesthetics, ethanol, and benzodiazepines (BZs).[3] Purification, sequencing, and cloning of the $GABA_A$ receptor and its composite subunits has identified 16 subunits arranged within seven families ($\alpha1-\alpha6$, $\beta1-\beta3$, $\gamma1-\gamma3$, δ, ϵ, π, and θ).[4] Expression of recombinant $GABA_A$ receptors shows that at least one α, one β, and one γ (or δ or ϵ) subunit are required to form a pentameric, functional GABA-gated chloride ion channel.[4−7] The benzodiazepine binding site occurs at the interface of the α and γ subunits and the combination of α and γ subunits strongly influences both the affinity and efficacy (i.e., the ability to modulate GABA-induced chloride ion flux) of ligands for the benzodiazepine binding site.[8] Thus, it has been shown that the major benzodiazepine sensitive $GABA_A$ receptor subtypes in brain are those that contain β and $\gamma2$ subunits in conjunction with either an $\alpha1$, $\alpha2$, $\alpha3$, or $\alpha5$ subunit.[5] Since the $\gamma2$ is invariant, the benzodiazepine pharmacology of $GABA_A$ receptors is dictated primarily by the α subunit present.[5]

Currently used BZ anxiolytics such as diazepam are nonselective, high-efficacy agonists, and these compounds show sedative, muscle-relaxant, and amnesic properties. By use of transgenic mice with point mutations in the α subunit that render the corresponding receptor insensitive to diazepam (while retaining responsivity to GABA), it has been shown that $GABA_A$ receptors containing an $\alpha1$ subunit mediate the sedative/muscle relaxant effects of BZs, whereas those containing an $\alpha2$ or an $\alpha3$ subunit or both mediate anxiolytic and anticonvulsant effects.[9−11] There is also evidence that the $\alpha5$ subunit is associated with memory and learning and is not involved in anxiety.[12] We recently reported on the 1,2,4-triazolopyridazines **1**[13] and **2**[14] as moderately selective $GABA_A\alpha3$ agonists.



Optimization of these leads gave the $GABA_A\alpha2/3$ functionally selective agonists **3**[10,15,16] and **4**[17,18] as nonsedating anxiolytics in animal models, from which triazolopyridazine **4** was chosen as a clinical candidate. Second generation compounds were targeted that were structurally distinct with good oral bioavailability in rat and dog and with higher functional selectivity for the $GABA_A\alpha2$ and $\alpha3$ subtypes than **4**.

The 7-methylimidazopyridine lead **8a**[19] was identified as a novel $GABA_A$ ligand and has some structural similarity to the high-efficacy $GABA_A\alpha3$ agonist NS-2710.[20] Compound **8a** had similar binding affinity for the $GABA_A\alpha3$ subtype as diazepam but was essentially a BZ antagonist (Table 1). With the aim of identifying compounds with higher efficacy for the $GABA_A\alpha2/3$ subtypes, alternative aryl groups for the pyridine ring of **8a** were targeted. Compounds were prepared by Suzuki coupling of the boronate ester **7** with a range of aryl bromides (Scheme 1). Compound **7** was prepared in four steps from 3-bromo-7-methylimidazopyridine **5**. [21] Binding affinities were measured by inhibition of [^{3}H]Ro15-1788 binding to human recombinant $GABA_A$ receptor subtypes stably expressed in L(tk$^-$) cells containing either $\alpha1$, $\alpha2$, $\alpha3$, or $\alpha5$ subunits in combination with $\beta3$ and $\gamma2$.[22] The in vitro efficacy for most compounds was measured on the same combination of $GABA_A$ receptor subtypes using whole cell patch clamp electrophysiological recordings in the presence of a submaximal (EC_{20}) concentration of GABA.[23] Alternatively, efficacies were determined in the same cell lines by measuring the modulation of ^{36}Cl ion flux produced by the EC_{20} concentration of GABA in the presence of a concentration of $1000K_i$ of the test compound.[24] The lead imidazopyridine identified from Suzuki reactions with **7**, the 2-cyanophenyl analogue **8b**, had 4-fold higher affinity at $GABA_A\alpha3$ compared with **8a** and, most importantly, was a $GABA_A$ agonist with functional selectivity for the $GABA_A\alpha3$ subtype over $\alpha1$- and $\alpha5$-containing $GABA_A$ receptors. Unfortunately, compound **8b** had low oral bioavailability ($F = 2\%$) and moderate plasma clearance in rat (Clp = 44 mL min^{-1} kg^{-1}), so structural changes were sought to improve pharmacokinetics, including modification of the imidazopyridine ring. A 10-fold increase in binding affinity for the $GABA_A\alpha3$ receptor was realized by replacing the imidazopyridine ring of **8b** with an imidazo[1,2-*a*]pyrimidine ring to give **14a** (Table 1). Functional selectivity for $GABA_A\alpha3$ was maintained, and in addition, **14a** had significantly improved oral bioavailability in rat ($F = 42\%$) and lower plasma clearance (13 mL min^{-1} kg^{-1}) compared with **8b**. Compound **14a**, however, had <5% oral bioavailability in dog with liver blood flow plasma clearance (Clp = 41 mL min^{-1} kg^{-1}). In vitro metabolism studies using liver microsomes identified the methyl group and central phenyl ring of **14a** as being major sites of oxidative metabolism in dog, so structural changes to these groups to improve metabolic stability were undertaken. The imidazo[1,2-*a*]pyrimidines **14a**−**k**

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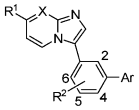
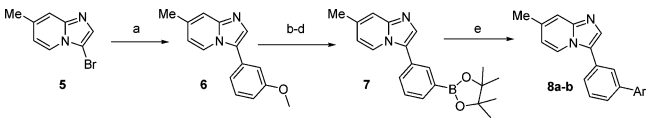
Table 1. Binding Affinity and Efficacy for Imidazo[1,2-*a*]pyridines and Imidazo[1,2-*a*]pyrimidines at $GABA_A$ Receptor Subtypes



					Ki (nM)[a]				Efficacy		
Compd.	X	R^1	R^2	Ar	α1	α2	α3	α5	α1	α3	α5
Diazepam	-	-	-	-	13.0	6.6	33.0	11.0	+103%[b]	+118%[b]	+106%[b]
1	-	-	-	-	56	21.7	10.0	1.0	+32%[b]	+45.6[b]	ND
2	-	-	-	-	31.0	16.3	8.0	2.1	+34%[b]	+55%[b]	ND
3	-	-	-	-	0.80	0.70	0.70	2.3	+2%[b]	+43%[b]	+39%[b]
4	-	-	-	-	0.26	0.3	0.3	0.2	+1%[b]	+33%[b]	+6%[b]
NS-2710	-	-	-	-	1.5	13.1	9.2	1.6	+42%[b]	+127%[b]	+26%[b]
8a	C	Me	H		ND	ND	16.0	ND	-15%[c]	+9%[c]	ND
8b	C	Me	H		4.5	ND	3.6	1.2	-10%[b]	+57%[b]	-14.6%[b]
14a	N	Me	H		0.39	0.78	0.37	0.12	+7%[b]	+81%[b]	1%[b]
14b	N	CF_3	H		0.66	ND	0.33	0.21	+20%[b]	+69%[b]	ND
14c	N	CF_3	2F		2.7	ND	2.6	0.94	+2%[b]	+43%[b]	+18%[b]
14d	N	CF_3	4F		0.5	0.52	0.34	0.2	+12.4%[b]	+43%[b]	+14%[b]
14e	N	CF_3	5F		5.3	ND	14	1.6	+4%[c]	+18%[c]	ND
14f	N	CF_3	4F		4.6	ND	7.6	2.8	+31%[c]	+56%[c]	ND
14g	N	CF_3	4F		1.2	1.0	0.73	0.50	-2%[b]	+25%[b]	+2%[b]
14h	N	CF_3	4F		0.65	ND	0.62	0.26	+34%[b]	ND	ND
14i	N	CF_3	4F		0.87	ND	0.38	0.50	+8%[b]	+33%[b]	ND
14j	N		4F		2.7	3.3	1.3	0.10	+45%[c]	+45%[c]	ND
14k	N		4F		0.85	3.7	4.0	0.53	+6%[b]	+57%[b]	+20%[b]

[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−Prussof equation. Data shown are mean values for three to six determinations. [b] Efficacy was 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 submaximal concentration of GABA (EC_{20}). [c] Modulation of ^{36}Cl chloride ion flux in cells expressing $\beta 3\gamma 2$ and either α1 or α3 produced by a submaximal concentration of GABA (EC_{20}) in the presence of an approximate 1000K_i concentration of test compound. Values are the mean of at least seven independent experiments. ND = not determined.

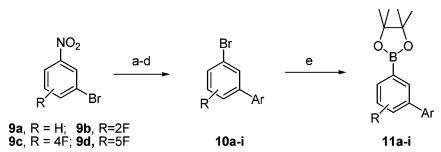
Scheme 1[a]



[a] Reagents and conditions: (a) 3-methoxybenzeneboronic acid, $Pd(PPh_3)_4$, DME, 2 N Na_2CO_3, reflux; (b) HBr, AcOH, reflux; (c) Tf_2O, pyridine, DCM, 0 °C to rt; (d) bis(pinacolato)diboron, $PdCl_2$(dppf), dppf, KOAc, 1,4-dioxane, 80 °C; (e) Ar−Br, Cs_2CO_3, $Pd(PPh_3)_4$, DME, reflux.

were prepared as shown in Schemes 2 and 3.[25−29] The aryl bromides **10a**−**i** and boronate esters **11a**−**i** required for Heck reaction with imidazopyrimidines **13a**−**c** or Suzuki couplings with the 3-bromoimidazopyrimidine derivatives were prepared according to Scheme 2. Bromides **9a**−**d** were converted to the

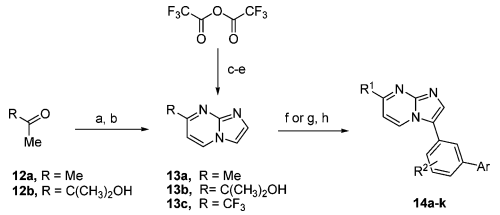
Scheme 2[a]



[a] Reagents and conditions: (a) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, 1,4-dioxane, 90 °C; (b) Ar−Br, $Pd_2(dba)_3$, $PtBu_3$, KF, THF, rt to 50 °C; (c) $SnCl_2\cdot 2H_2O$, EtOH; (d) $NaNO_2$, 48% HBr, CuBr, 0−50 °C; (e) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, 1,4-dioxane, 90 °C.

boronate esters under Miyaura conditions and then coupled with the desired aryl bromides using a Fu protocol, before reduction with stannous chloride and conversion to the bromides **10a**−**i** by Sandmeyer reaction. A Miyaura reaction with bromides

Scheme 3[a]



[a] Reagents and conditions: (a) $HC(OEt)_3$, $BF_3{\cdot}Et_2O$, CH_2Cl_2, −40 to 0 °C, then $RCOCH_3$, $EtiPr_2N$, −78 °C to rt; (b) 2-aminoimidazole hemisulfate, NaOMe, EtOH, reflux; (c) ethyl vinyl ether, pyridine, CH_2Cl_2; (d) guanidine hydrochloride, NaOH, EtOH; (e) $BrCH_2CH(OEt)_2$, 48% HBr, EtOH, reflux; (f) **10**, Cs_2CO_3, $Pd(PPh_3)_4$, 1,4-dioxane, reflux; (g) Br_2, KBr, NaOAc, MeOH, 0 °C; (h) **11**, K_3PO_4, $Pd(PPh_3)_4$, DMA, 65 °C.

Subtype	Maximum potentiation (%)[a]	Efficacy relative to chlordiazepoxide[b]
α1β3γ2	6.2 ± 3.0	0.05 ± 0.03
α2β3γ2	48 ± 6	0.39 ± 0.06
α3β3γ2	57 ± 10	0.54 ± 0.08
α5β3γ2	20 ± 3	0.18 ± 0.02

Figure 1. Efficacy of **14k** at human $GABA_A$ receptor subtypes expressed in L(tk⁻) cells measured by whole cell patch-clamp electrophysiology: *a* indicates that data shown are mean ± SEM of four to six separate concentration−response curves for each subtype as shown; *b* indicates efficacy of **14k** relative to the nonselective full agonist chlordiazepoxide (relative efficacy = 1).

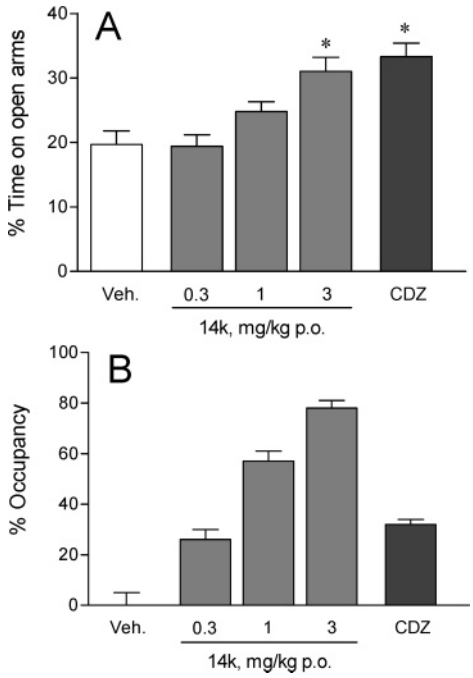


Figure 2. (A) The anxiolytic effects of **14k** in the rat elevated plus maze test. The mean time (± SEM, $n = 18$/group) spent on the open arms (expressed as a percentage of the total time, 5 min, on the maze) after 30 min pretreatment with vehicle (0.5% methyl cellulose, p.o.), **14k** (0.3, 1, or 3 mg kg^{-1} p.o.), or chlordiazepoxide (CDZ, 5 mg kg^{-1} i.p.). The statistically significant differences between vehicle and drug-treated groups were determined using ANOVA followed by Dunnett's post hoc tests: ∗ indicates $p < 0.05$. (B) Occupancy of brain $GABA_A$ receptor benzodiazepine binding sites by **14k** in a subset of rats taken after completion of the elevated plus maze trial as measured by inhibition of the in vivo binding of [^{3}H]-Ro15-1788. Occupancy was dose-dependent with an ID_{50} of 0.8 mg kg^{-1}.

10a−**i** gave the boronate esters **11a**−**i**. The imidazopyrimidines **13a** and **13b** were prepared by reaction of the ketones **12a** and **12b**, respectively, with diethoxycarbenium fluoroborate followed by treatment of the resultant keto-acetals with 2-aminoimidazole under basic conditions. 7-Trifluoromethyl imidazo[1,2-*a*]pyrimidine **13c** was prepared in three steps from trifluoroacetic anhydride and ethyl vinyl ether (Scheme 3).

The 7-trifluoromethylpyrimidine **14b** had comparable binding affinity to the methyl analogue **14a** but was less functionally selective for the $GABA_A\alpha3$ subtype (Table 1). In the 7-trifluoromethylimidazopyrimidine series, the effect of fluorine substitution around the central phenyl ring on $GABA_A$ binding affinity, functional selectivity, and pharmacokinetics was explored. Substitution with fluorine was best tolerated at the 4-position, compound **14d**, the 2-fluoro isomer **14c** and 5-fluoro isomer **14e** losing 8-fold and 43-fold $GABA_A\alpha3$ binding affinity, respectively. Substitution on the 2-cyanophenyl ring of **14d** with fluorine was tolerated at all positions though the 2-cyano-3-fluorophenyl- (**14f**) and 2-cyano-5-fluorophenyl- (**14h**) analogues have significant efficacy for $GABA_A\alpha1$-containing receptors. The 2-cyano-4-fluorophenyl analogue **14g** was functionally selective for $GABA_A\alpha3$ and $GABA_A\alpha2$ (+20.3%) over α1- and α5-containing $GABA_A$ receptors and had a comparable efficacy profile to the lead 1,2,4-triazolopyridazine **4**. Compound **14g** had good pharmacokinetics in rat ($F = 53\%$; Clp = 10.3 mL min^{-1} kg^{-1}; $t_{1/2} = 7.3$ h), dog ($F = 29\%$; Clp = 2.9 mL min^{-1} kg^{-1}; $t_{1/2} = 32$ h), and rhesus monkey ($F = 49\%$; Clp = 3.0 mL min^{-1} kg^{-1}; $t_{1/2} = 13.4$ h) with improved plasma half-life compared to **4** (rat $F = 35\%$, $t_{1/2} = 1.4$ h; dog $F = 53\%$; $t_{1/2} = 1.5$ h)[17] and displaced [^{3}H]-Ro15-1788 in an in vivo binding assay used to measure central BZ binding site occupancy[30] with an ID_{50} of 0.27 mg kg^{-1} p.o. and an EC_{50} of 7.6 ng mL^{-1} (19nM). Compound **14g** was active in the rat elevated plus maze assay, an unconditioned model of anxiety,[31] at 1 mg kg^{-1} p.o. (91% BZ site occupancy) with no impairment shown in the rat chain pulling assay, a test of sedation, at 30 mg kg^{-1} p.o.[32]

An alternative, metabolically stable replacement for the methyl group of **8a** and **14a** was shown to be propan-2-ol as represented by compounds **14j** and **14k (**Table 1**)**. Compound **14k** had selective efficacy for $GABA_A\alpha3$ over the $GABA_A\alpha1$ subtype (Table 1) with higher efficacy for $GABA_A\alpha3$ subtype than the first generation compound **4** and **14g**. The efficacy of **14k** relative to the nonselective high-efficacy BZ agonist chlordiazepoxide (CDP) is shown in Figure 1. Compound **14k** is a $GABA_A\alpha1$ antagonist relative to CDP and a partial agonist on both the $GABA_A\alpha2$ (0.39 relative to CDP) and $GABA_A\alpha3$ (0.54 relative to CDP) subtypes. Compound **14k** had good pharmacokinetics in rat ($F = 77\%$; Clp = 9.1 mL min^{-1} kg^{-1}; $t_{1/2} = 1.7$ h) and dog ($F = 48\%$; Clp = 8.2 mL min^{-1} kg^{-1}; $t_{1/2} = 0.7$ h) with an ID_{50} of 0.84 mg kg^{-1} p.o. and an EC_{50} of 202 ng mL^{-1} (469 nM) in the rat in vivo binding assay. Compound **14k** was active in the rat elevated plus maze assay (Figure 2) at 3 mg kg^{-1} p.o. (78% BZ site occupancy) with no impairment seen in the rat chain pulling assay at 30 mg kg^{-1} p.o. The nonselective full BZ agonist CDP was effective at 5 mg kg^{-1} i.p. in the elevated plus maze, which equated to 30% BZ receptor occupancy. Both **14g** and **14k** were anxiolytic in the squirrel monkey conditioned emotional response test, a conditioned model of anxiety, at 0.3 and 3 mg kg^{-1} p.o., respectively.[15,33]

On the basis of the $GABA_A$ efficacy profiles and pharmacokinetics of **14g** and **14k**, these compounds were chosen as additional development compounds for the treatment of anxiety disorders, **14g** having a similar $GABA_A$ in vitro efficacy profile to the first development compound **4** but with a plasma half-life in preclinical species indicative of once a day dosing in man and **14k** having higher efficacy for the $GABA_A\alpha3$ subtype relative to **4**.

Supporting Information Available: Experimental procedures and characterization of intermediates and final compounds. This material is available free of charge via the Internet at http://pub.acs.org.

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