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Bioorganic & Medicinal Chemistry Letters 16 (2006) 1518–1522
Bioorganic & Medicinal Chemistry Letters

8-Fluoroimidazo[1,2-*a*]pyridine: Synthesis, physicochemical properties and evaluation as a bioisosteric replacement for imidazo[1,2-*a*]pyrimidine in an allosteric modulator ligand of the $GABA_A$ receptor

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Received 1 November 2005; revised 8 December 2005; accepted 11 December 2005
Available online 4 January 2006

Abstract—8-Fluoroimidazo[1,2-*a*]pyridine has been established as a physicochemical mimic of imidazo[1,2-*a*]pyrimidine, using both in silico and traditional techniques. Furthermore, a novel synthesis of a 3,7-disubstituted-8-fluoroimidazopyridine **3** has been developed and the utility of the physicochemical mimicry has been demonstrated in an in vitro system. Here, the 8-fluoroimidazopyridine ring contained in ligand **3** acts as a bioisosteric replacement for imidazopyrimidine in the $GABA_A$ receptor modulator **2**.

Nitrogen-bridgehead fused heterocycles containing an imidazole ring are a common structural motif in pharmacologically important molecules, with activities spanning a diverse range of targets. Probably the most widely used heterocyclic system from this group is imidazo[1,2-*a*]pyridine, which is contained in marketed drugs such as the benzodiazepine agonist Zolpidem®[1] and the PDE 3 inhibitor Olprinone®,[2] as well as other experimental molecules.[3] However, alternative derivatives, such as the closely related imidazo[1,2-*a*]pyrimidine Divaplon®,[4] are also prevalent (Fig. 1).

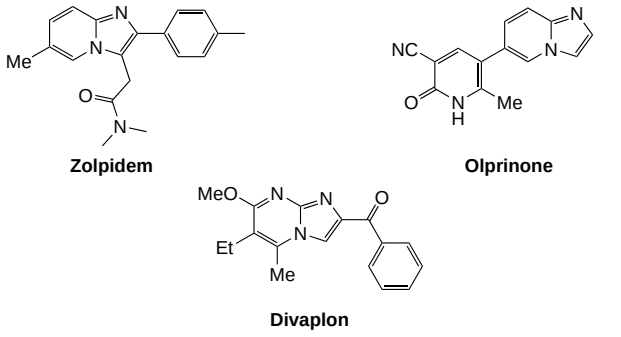

Figure 1. Some literature examples of imidazo[1,2-*a*]pyridine-type drugs.

During the course of a medicinal chemistry programme aimed at discovering novel allosteric modulators of the $GABA_A$ receptor, it was established that the imidazopyridine compound **1** (Fig. 2) has good affinity at multiple subtypes of the receptor (Table 1), with a degree of functional selectivity in vitro.[5] Subsequent work demonstrated that a 10-fold increase in affinity is achieved by replacement of the 8-position C–H with an azine nitrogen atom, giving the analogous imidazo[1,2-*a*]pyrimidine **2**. However, the strategy adopted in the Merck programme has been to minimise the functional effects at $GABA_A$ α_1 (seek an α_1 antagonist),[6] whilst maximising positive modulation at $GABA_A$ α_3 (agonist activity).[7] Since compound **2** still possesses significant functional activity at the α_1 subtype, there remained a desire to pursue further structural modifications of the heterocyclic core, with the aim of producing a second generation lead. Such a compound should retain the attractive sub-nanomolar activity of **2**, but possess lower functional activity at $GABA_A$ α_1 and increased functional selectivity. This paper details one of the strategies adopted.

Keywords: Imidazo[1,2-*a*]pyridine; Imidazo[1,2-*a*]pyrimidine; Bioisosterism; Physicochemical; $GABA_A$; Basicity; Lipophilicity.
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doi:10.1016/j.bmcl.2005.12.037

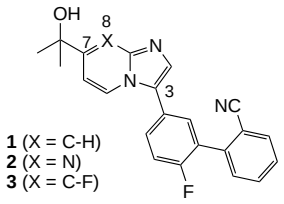


Figure 2. Allosteric modulators of the $GABA_A$ receptor.

Table 1. Binding affinities and in vitro efficacy data for **1**, **2**, and **3** at $GABA_A$ α_1 and α_3 receptors

Compound	Affinity, K_i[a] (nM)		Efficacy (% CDZ)[b]	
	α_1	α_3	α_1	α_3
1	4.35	5.16	35[c]	61[c]
2	0.71	0.47	60[d]	79[c]
3	0.20	0.32	34[d]	104[c]

[a] K_i values for benzodiazepine sites on stably expressed human $GABA_A$ $\alpha_x\beta_3\gamma_2$ receptors ($x = 1$ or 3) in LTK mouse fibroblast cells. Inhibition curves were carried out using receptors labelled with [^{3}H]Ro15-1788 at a concentration of twice the K_d. K_i values were calculated according to the Cheng-Prussof equation. Data shown are means of three determinations (see Ref. 6).

[b] In vitro efficacy expressed relative to the maximal response of the full agonist chlordiazepoxide (CDZ) in stably expressed human $GABA_A$ $\alpha_x\beta_3\gamma_2$ receptors ($x = 1$ or 3) in LTK mouse fibroblast cells (see Ref. 6).

[c] Measured using a $^{36}Cl^-$ flux assay at 1000× K_i (drug) and GABA EC_{20}. Data shown are means of at least 7 determinations.

[d] Maximum modulation of the GABA EC_{20} measured by electrophysiology. Data shown are means of at least 5 determinations.

The increased affinity resulting from replacement of the 8-position C–H group (in **1**) to an azine nitrogen (in **2**) represents a relatively modest difference in binding energy (~6 kJ mol^{-1}).[8] Hence, we speculated that it most likely resulted from subtle changes to properties such as the electrostatic surface, the dipole moment and pK_a, which relate to the heterocycle as a whole, rather than a significantly altered receptor–ligand interaction in the locality of the 8-position, for example, a hydrogen bond.[9] We surmised that replacement of the same C–H unit by C–F would achieve a similar perturbation of electrostatic surface, dipole moment and pK_a, based on some of the known effects of fluorine in bioactive molecules.[10] However, incorporation of a C–F unit should be complementary to using an azine N, as other properties, including the lipophilicity and localised steric and electronic interactions, should be altered in quite different ways. Although a small number of compounds containing an 8-fluoroimidazopyridine are known in the literature,[11,12] no study has been carried out to compare the heterocycle's physicochemical properties with those of either the parent des-F imidazopyridine or the analogous imidazopyrimidine. To the best of our knowledge, there are also no examples of its use as a bioisostere for imidazopyrimidine in any biological system.[13] Therefore, we set out to make this comparison within the context of our known $GABA_A$ receptor modulators (Fig. 2).

Our first approach was to model and compare a series of simplified 3,7-disubstituted compounds (**4**–**6**) in silico using semi-empirical calculations on MMFF-minimised structures.[14] The results are displayed visually in Figure 3, with the magnitude of the dipoles being 3.37 D (**4**), 5.10 D (**5**) and 4.52 D (**6**). Consistent with our hypothesis, it is clear that the direction and strength of the dipole in the 8-fluoroimidazopyridine **6**, together with its overall electrostatic surface, show a much closer resemblance to the imidazopyrimidine **5** than to the des-F compound **4**.

Encouraged by the molecular modelling results, we set out to synthesise the 8-fluoroimidazopyridines **11** and **12**, in order to compare with the known[5] compounds **7**–**10** (Fig. 4).

In common with the general synthesis of imidazo[1,2-*a*]pyridines, 8-fluoroimidazopyridines can be prepared by the reaction of a 2-aminopyridine with bromoacetaldehyde, used as the diethyl or dimethyl acetal (Scheme 1).[11] However, we wished to incorporate an isopropanol substituent at the 7-position of the final ring system **11** (corresponding to the 4-position of the starting pyridine **13**)—giving a substitution pattern which had not been previously exemplified. To achieve our aim, we envisaged using Queguiner's procedure,[15] whereby lithiation followed by an electrophilic quench can be used to

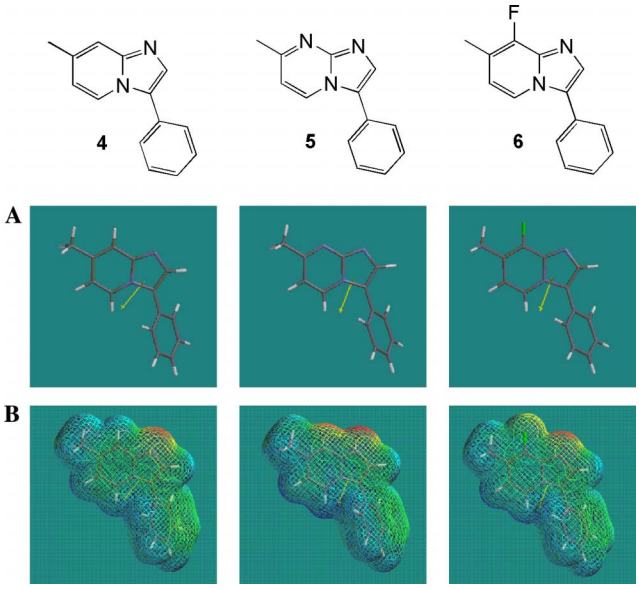


Figure 3. Dipole moments (A) and electron density surfaces (B) for the simplified $GABA_A$ ligands **4**, **5** and **6**. Red and blue colourations indicate high and low electron density, respectively.[14]

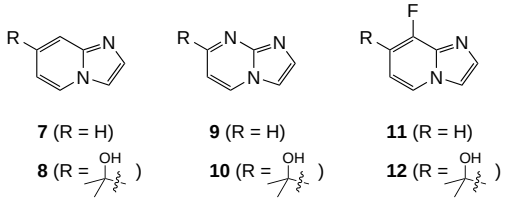
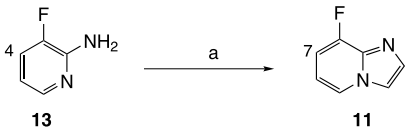
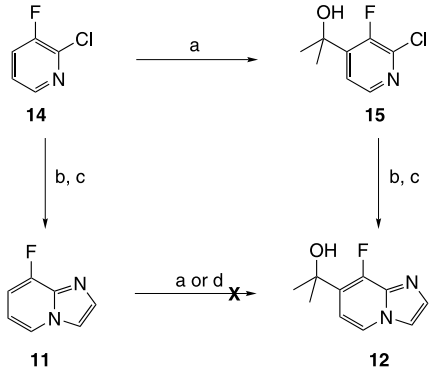


Figure 4. Structures **7**–**12** for physicochemical parameter comparison.

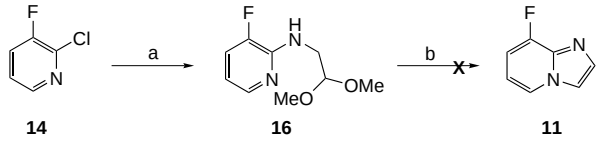


Scheme 1. Reagents and conditions:[11] (a) 2-bromoacetaldehyde diethylacetal, 24% hydrobromic acid, 100 °C, then room temperature, EtOH, $NaHCO_3$, then filter, then **13**, reflux, 52%.

install a substituent ortho to a fluorine atom in a pyridyl system. However, a 2-amino substituent (as in **13**) is incompatible with C-lithiation chemistry and hence, we adopted an alternative building block, that is, 2-chloro-3-fluoropyridine **14** (Scheme 2). As expected, **14** undergoes facile *ortho*-lithiation and an acetone quench afforded the required derivative **15** in high yield. This compound as well as the unfunctionalised pyridine **14** were then converted to the corresponding imidazopyridines **12** and **11**, respectively, using a novel two-step sequence commencing with replacement of the 2-chloro substituent by benzophenone imine under Buchwald conditions.[16] Rather than deprotecting the 'masked' aminopyridine in a stepwise fashion, imine hydrolysis was carried out concomitantly with deprotection of the bromoacetaldehyde diethyl acetal fragment, using hot hydrobromic acid.[5] On cooling, the solution was neutralised with sodium carbonate and heated again to afford **11** and **12** in satisfactory yield. We also examined whether it would be possible to functionalise the 7-position of the fused heterocycle **11** directly, using chemistry analogous to functionalising the pyridine 4-position. However, this strategy proved unsuccessful using any of Queguiner's conditions (i.e., lithium diisopropylamide, butyllithium/TMEDA or butyllithium/DABCO complexes),[15] with 3-substituted material being produced in each case. To try and overcome this, strategies involving dilithiation or site-blocking of the 3-position using trimethylsilane were also attempted, but again without success.



Scheme 2. Reagents and conditions: (a) LDA, THF, −78 °C, then acetone (pre-dried over 4 Å molecular sieves), to room temperature, 95%; (b) benzophenone imine, Cs_2CO_3, BINAP (6 mol%), $Pd(OAc)_2$ (4 mol%), PhMe, 95 °C; (c) 2-bromoacetaldehyde diethylacetal, 24% hydrobromic acid, 90 °C, then room temperature, IPA, $NaHCO_3$, then filter, then 50 °C, 76% (**11**, 2 steps), 34% (**12**, 2 steps); (d) BuLi, TMEDA or DABCO, −78 °C, Et_2O or THF, then **11**, then acetone (pre-dried over 4 Å molecular sieves), to room temperature.

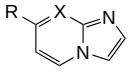


Scheme 3. Reagents and conditions: (a) 2-aminoacetaldehyde dimethylacetal, NaO^{t}Bu, BINAP, $Pd(OAc)_2$, THF, reflux, 79%; (b) 24% hydrobromic acid, 90 °C, then room temperature, IPA, $NaHCO_3$, then filter, then 50 °C.

Since this chemistry was first disclosed,[17] it has been established that Buchwald aminations of 3-fluoro-2-chloropyridine are general for a series of primary alkyl amines.[18] This led us to investigate an alternative 'reversed-synthon' approach to adding the two-carbon unit to the pyridine ring (Scheme 3).[19] In this case, **14** was reacted with aminoacetaldehyde dimethylacetal to form the aminopyridine **16**. However, this compound failed to cyclise to **11** under either acidic or neutral conditions after acid-catalysed aldehyde deprotection.

Physicochemical descriptors (pK_a and $\log D_{7.4}$) for the three unsubstituted cores (**7**, **9** and **11**), together with those for the three 7-substituted analogues (**8**, **10** and **12**) were measured and are presented in Table 2. The pK_a values were determined by analysing the UV chromophores during a pH titration (using Sirius SGA technology) and, for the compounds **7** and **9**, the values obtained are very close to those previously published in the literature.[20,21] Interestingly, in both the unsubstituted and 7-isopropanol series, it is clear that the C–F unit (in **11** and **12**) alters the pK_a in an identical manner to an azine nitrogen (in **9** and **10**), that is, by approximately two units. Log $D_{7.4}$ values were determined using the traditional 'shake-flask' method with partitioning between octanol and a $pH_{7.4}$ buffer. In this case, 8-fluoro derivative **11** shows closer similarity to the des-fluoro imidazopyridine **7**, rather than the imidazopyrimidine **9**. However, the effect of adding the isopropanol substituent is interesting, in that, for both **10** and **12**, the $\log D_{7.4}$ value has risen slightly (by 0.3 and 0.2, respectively), as a result of adding the lipophilic group while, on the other hand, the value for **8** is 0.2 lower than for the parent **7**. We believe this arises from the fact that

Table 2. Physicochemical data for compounds **7**–**12**



Compound	X	R	pK_a[a]	Log $D_{7.4}$[b]
7	C–H	–H	6.9 (6.8)[c]	0.8
8	C–H	–C(OH)Me_2	7.2	0.6
9	N	–H	4.9 (4.8)[d]	−0.2
10	N	–C(OH)Me_2	5.4	0.1
11	C–F	–H	4.9	0.9
12	C–F	–C(OH)Me_2	5.4	1.1

[a] Measured by analysis of the UV chromophore during a pH titration (Sirius SGA).

[b] Ratio of concentrations in octanol and $pH_{7.4}$ aqueous buffer after shaking for 20 min.

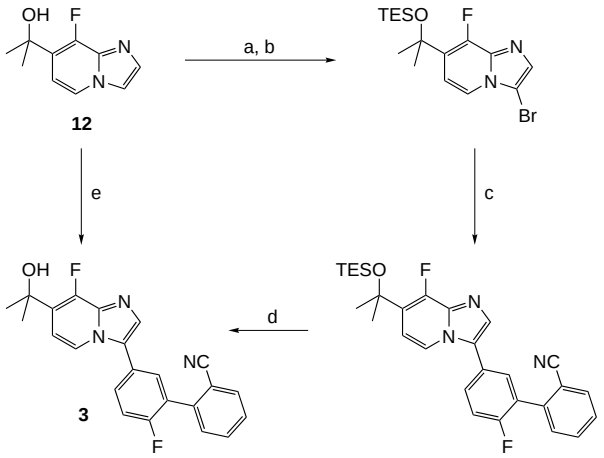
[c] Literature value (see Ref. 21).

[d] Literature value (see Ref. 22).

in going from **7** to **8**, the pK_a changes from 6.9 to 7.2, which is almost identical to the test pH. As a result, the ratio of protonated to non-protonated material rises by almost 50%, accounting for the net reduction in the log $D_{7.4}$. On the other hand, the pK_a changes for the other two cores (4.9–5.4) represent a very minor change in the degree of protonation at pH 7.4.

In order to determine the significance of the gathered physicochemical information within the biological system of interest ($GABA_A$), we needed to functionalise the 3-position to afford compound **3**. It is known that imidazopyridine-type structures can be readily arylated at the 3-position via electrophilic bromination, allowing Suzuki or Stille couplings,[5] or by direct Heck coupling of the unbrominated heterocycle.[22] Unsurprisingly, both of these methods worked well for the preparation of **3** (Scheme 4), although the direct Heck coupling proved to be considerably more efficient as there was no need for temporary alcohol protection.

8-Fluoroimidazopyridine **3** exhibits high affinity at both $GABA_A$ α_1 and α_3 receptors with K_i values of 0.20 and 0.32 nM, respectively (Table 1).[23] Comparison of this data with those previously established for the imidazopyridine **1** and imidazopyrimidine **2** demonstrates that replacement of the C–H unit by a C–F yields the same 6 kJ mol^{-1} increase in binding energy as replacement of the C–H with an azine N.[24] Hence, the replacements can be correctly termed bioisosteric, at least within this biological system.[11] However, the functional effects of the C–F and N replacements are not identical, with compound **3** giving a significantly lower in vitro efficacy at $GABA_A$ α_1-receptors (maximal response = 34% of CDZ, cf. 60% for **2**).[23] The compound also shows reasonable functional selectivity for the $GABA_A$ α_3 subtype (maximal response = 104% of CDZ, cf. 79% for **2**). These data makes **3** a promising starting point for further optimisation, for example, by small variations of the 3- and 7-substituents, which are known to influence functional efficacy.[5]



Scheme 4. Reagents and conditions: (a) Br_2, KBr, NaOAc, MeOH, 69%; (b) TESOTf, EtN*i*-Pr_2, CH_2Cl_2, −78 °C, to room temperature, 83%; (c) 2′-fluoro-5′-(pinacolataborolan-2-yl)biphenyl-2-carbonitrile,[5] $Pd(PPh_3)_4$ (5 mol%), 2 M Na_2CO_3, THF, 75 °C, 60%; (d) catalytic concd HCl (aq), EtOH, >95%; (e) 5′-bromo-2′-fluorobiphenyl-2-carbonitrile, [5] Cs_2CO_3, $Pd(PPh_3)_4$ (6 mol%), 1,4-dioxane, 35%.

In summary, we have demonstrated that 8-fluoroimidazo[1,2-*a*]pyridine closely resembles imidazo[1,2-*a*]pyrimidine in respect of a number of physicochemical parameters, such as electrostatic surface, dipole moment and pK_a, while, at the same time, it possesses a significantly higher log $D_{7.4}$, making it a complementary replacement for a C–H unit. Furthermore, the significance of the resemblance has been established in a $GABA_A$ system, where 8-fluoroimidazopyridine has been shown to be a bioisostere of imidazopyrimidine, with the ligands **3** and **2** both affording ~6 kJ mol^{-1} greater binding energy than the parent imidazopyridine **1**. However, the effects of **2** and **3** on in vitro efficacy at the $GABA_A$ α_1 and α_3 receptor subtypes have been shown to be different, with **3** producing a smaller functional effect at receptors containing the α_1-subtype, accompanied by a degree of functional selectivity for α_3-containing receptors. We therefore propose **3** as a lead for optimisation in the search for a pure 'α_3-agonist'. The results of optimisation studies to achieve this goal will be published in due course.

Acknowledgments

We thank Remya Muralikuttan for assistance in obtaining log $D_{7.4}$ values, Alison Smith for K_i determinations and George Marshall for determining the in vitro efficacy of compound **3**.

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