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Syntheses, pharmacological evaluation and molecular modelling of substituted 6-alkoxyimidazo[1,2-*b*]pyridazines as new ligands for the benzodiazepine receptor

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Summary — A series of 2,3-disubstituted-6-alkoxyimidazo[1,2-*b*]pyridazines has been synthesized and evaluated for in vitro affinity for the benzodiazepine receptor (BZR). 3-(Benzamidomethyl or substituted benzamidomethyl)-6-methoxy-2-(3,4-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazines were found to be the most potent BZR ligands (eg, **4a**, IC_{50} 7 nM; **4e**, IC_{50} 14 nM; **4v**, IC_{50} 8 nM). Imidazo[1,2-*b*]pyridazines unsubstituted in the 3-position, or containing bulkier alkoxy groups in the 6-position, were found to bind less strongly to the BZR. Selected compounds from the series were identified from in vitro GABA-shift experiments as BZR agonists. Molecular modelling has been employed to identify the common pharmacophoric points of lipophilic and hydrogen bonding, ligand–receptor interaction and areas of steric hindrance for these substituted imidazo[1,2-*b*]pyridazines at the BZR.

imidazo[1,2-*b*]pyridazine / benzodiazepine receptor / structure–activity relationship / molecular modelling

Introduction

The benzodiazepines are widely used in the treatment of central nervous system (CNS) disorders [1]. The pharmacological effects of the benzodiazepines result from their affinity for a specific binding domain on the $GABA_A$ receptor, known as the benzodiazepine receptor (BZR) [2, 3]. The $GABA_A$ receptor is a ligand-gated ion channel consisting of a combination of α, β, γ, δ and ρ polypeptide subunits [4]. The $GABA_A$ receptor is associated with a trans-membrane chloride ion channel, and this macromolecular complex has several major binding domains including sites for GABA, benzodiazepines, barbiturates, picrotoxin and the anaesthetic steroids [5–7]. Benzodiazepine agonists bind to the BZR and increase the frequency of chloride ion channel opening in response to GABA, causing anxiolytic, sedative and muscle-relaxant effects [8, 9]. Conversely, BZR inverse agonists produce anxiogenic and pro-convulsant effects by decreasing the frequency of chloride ion channel opening in response to GABA, and BZR antagonists block the pharmacological effects of agonists or inverse agonists [8, 9].

The major untoward side effects of the benzodiazepines used as anxiolytics include sedation, psychological and physical dependence, withdrawal symptoms and possible potentiation of the effects of alcohol [10, 11]. In the search for novel therapeutically useful agents without the adverse side effects of the benzodiazepines, other classes of compounds have been synthesized and found to bind with high affinity to the BZR, including β-carbolines, triazolopyridazines, imidazo[1,2-*a*]pyridines and cyclopyrrolones [12]. An examination of the structure–activity relationships (SAR) of affinity and efficacy of these and other compounds [13–20] at the BZR has assisted in the development of several pharmacophoric models for ligand–receptor interaction at the BZR. These models are characterized by a number of points of lipophilic and hydrogen-bonding ligand–receptor interaction [9, 21–23], and in some cases [24, 25] areas of steric hindrance have also been defined.

We have previously reported the syntheses and BZR affinities of many substituted imidazo[1,2-*b*]-

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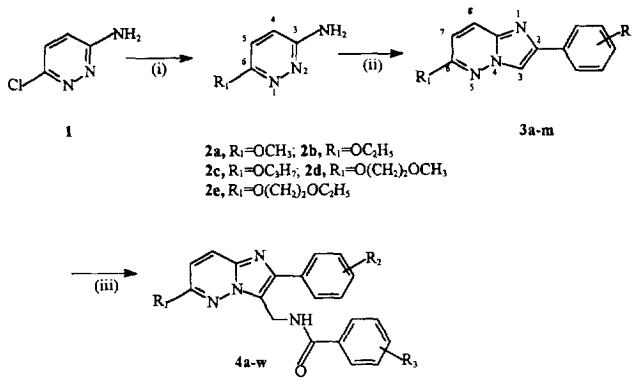
pyridazines, including the discovery of a number of high affinity BZR ligands [26, 27]. We now report the syntheses and BZR affinities of a series of substituted 6-alkoxyimidazo[1,2-*b*]pyridazines. Substituents of varying steric bulk and electronic properties have been placed in the 2-, 3- and 6-positions of the imidazo[1,2-*b*]pyridazine nucleus to further probe the steric and electronic requirements of the binding site of these ligands at the BZR. Molecular modelling studies have been carried out with selected compounds to determine the pharmacophoric points of ligand–receptor interaction for the imidazo[1,2-*b*]pyridazines at the BZR. The results of these studies have been compared with existing pharmacophore models.

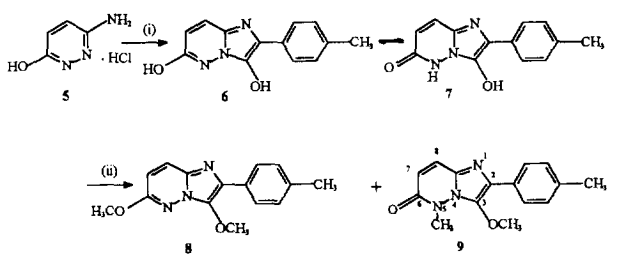
Chemistry

The substituted 6-alkoxyimidazo[1,2-*b*]pyridazines were synthesized using modified literature procedures [28–31]. 6-Chloropyridazin-3-amine **1** with the appropriate sodium alkoxide gave the 6-alkoxypyridazin-3-amines **2a–e**. Compounds **2a–e** when refluxed in ethanol with a substituted α-bromoacetophenone generated the 3-unsubstituted imidazo[1,2-*b*]pyridazines **3a–m**. Treatment of the compounds **3a–m** with *N*-(hydroxymethyl)benzamide or substituted *N*-(hydroxymethyl)benzamides in acetic acid and in the presence of a catalytic amount of concentrated sulphuric acid gave the required 3-(benzamidomethyl or substituted benzamidomethyl)imidazo[1,2-*b*]pyridazines **4a–w** (scheme 1).

The 3-methoxy compounds **8** and **9** were prepared from the condensation of 6-aminopyridazin-3-ol **5** with 4-methylphenylglyoxal hydrate in ethanolic hydrochloric acid to give 3,6-dihydroxyimidazo[1,2-*b*]-



Scheme 1. (i) NaR_1, 130–150 °C; (ii) $BrCH_2COC_6H_4R_2$, $NaHCO_3$, EtOH, 100 °C; (iii) $R_3C_6H_4CONHCH_2OH$, conc H_2SO_4, CH_3CO_2H, 100–120 °C. The substituents in compounds **3a–m** and **4a–w** are specified in tables I and II, respectively.



Scheme 2. (i) $4\text{-}CH_3C_6H_4COCHO\text{-}H_2O$, conc HCl, EtOH, 100 °C; (ii) CH_2N_2, $(C_2H_5)_2O$, 0 °C.

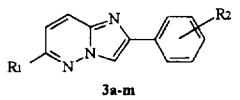
pyridazine **6** (or tautomer **7**), which when methylated with ethereal diazomethane gave both the *O,O*- and *O,N*-dimethylated **8** and **9** in a ratio of 7:6 (scheme 2). This situation is similar to one previously observed in which the methylation of 2-phenyl-3-unsubstituted imidazo[1,2-*b*]pyridazin-6(5*H*)-one with methyl iodide led to the formation of both 6-methoxy-2-phenylimidazo[1,2-*b*]pyridazine and 5-methyl-2-phenylimidazo-[1,2-*b*]pyridazin-6(5*H*)-one [32].

Compound **8** was found to have identical ^{1}H-NMR and analytical properties to those previously reported for 3,6-dimethoxy-2-(4-methylphenyl)imidazo[1,2-*b*]-pyridazine prepared from 6-methoxypyridazin-3-amine with 4-methylphenylglyoxal hydrate [33]. To confirm the structure of **9** and determine that methylation had taken place at N-5 and not N-1, NOE ^{1}H-NMR spectroscopy was employed. Irradiation of the singlet at δ 4.03 (3-OMe) gave a corresponding increase in the intensity of the singlet at δ 3.89 (NMe), with no effect on the signals at H-7 and H-8. Irradiation of the doublet at δ 7.49 (H-8) resulted in an increase in the intensity of the doublet at δ 6.51 (H-7) only. It was therefore concluded that the methylation had indeed occurred at N-5, and not at N-1.

Results and discussion

All the imidazo[1,2-*b*]pyridazines reported in this paper were evaluated for in vitro BZR affinity by their ability to displace [^{3}H]-diazepam binding from rat brain cortical membranes, using procedures described previously [34]. Table I shows the results for the 3-unsubstituted imidazo[1,2-*b*]pyridazines **3a–m**. None of these compounds demonstrated high affinity for the BZR, and it was difficult to identify consistent SAR trends. For example, an increase in the size of the 6-alkoxy group in the series of 2-(4-chlorophenyl)-imidazo[1,2-*b*]pyridazines **3b,f,j** led to a consistent decrease in BZR affinity, whereas a similar comparison with the series of 2-phenyl compounds **3c,g,k,m** showed variable results. The low overall BZR affinities of the 3-unsubstituted compounds reported here

Table I. In vitro displacement of [^{3}H]diazepam binding to the BZR by some 3-unsubstituted imidazo[1,2-*b*]pyridazines.



Compound	R_1	R_2	*Displacement (%)*[a]
3a	OCH_3	3,4-OCH_2O	58 ± 7
3b	OCH_3	4-Cl	46 ± 1
3c	OC_2H_5	H	19 ± 1
3d	OC_2H_5	4-CH_3	25 ± 7
3e	OC_2H_5	3,4-OCH_2O	54 ± 1
3f	OC_2H_5	4-Cl	35 ± 1
3g	OC_3H_7	H	26 ± 2
3h	OC_3H_7	4-CH_3	41 ± 10
3i	OC_3H_7	3,4-OCH_2O	22 ± 1
3j	OC_3H_7	4-Cl	22 ± 2
3k	$O(CH_2)_2OCH_3$	H	5 ± 1
3l	$O(CH_2)_2OCH_3$	4-CH_3	16 ± 1
3m	$O(CH_2)_2OC_2H_5$	H	23 ± 1

[a]Values are at a ligand concentration of 1000 nM and are the mean ± SEM of determinations in triplicate.

and their lack of consistent SAR suggests that they do not possess the structural or electronic features required for binding to the BZR.

The biological results for the 3-substituted imidazo[1,2-*b*]pyridazines **4a–w**, **8** and **9** are given in table II. In contrast to the 3-unsubstituted compounds **3a–m**, a number of high affinity BZR ligands have been identified among this series. For compounds **4a–u**, the size of the 6-alkoxy group had a direct effect on BZR affinity. Maintaining constant 2- and 3-position substitutent groups, an increase in the size of the 6-alkoxy group led to a decrease in BZR affinity. This suggests that the bulkier 6-(propoxy, 2-methoxyethoxy and 2-ethoxyethoxy) groups are interacting with areas of steric hindrance on the BZR. In contrast, an alteration of the 3-position group from benzamidomethyl to substituted benzamidomethyl (as in **4e,v,w**) did not have a major effect on BZR affinity. The limited in vitro biological effects resulting from the introduction of the *o*-fluoro, and *m*- and *p*-nitro groups into the phenyl ring of the 3-benzamidomethyl substituent implies that the electronic and steric effects of these groups do not substantially alter the affinities of the compounds for the BZR. The functional group present at the 2-position of the molecules, however, influenced the BZR affinity considerably. With the 6- and 3-position groups remaining constant, the order of BZR affinity was 2-(3,4-methylenedioxyphenyl) > 2-(4-methylphenyl) > 2-(4-chlorophenyl) > 2-phenyl.

A comparison of the BZR affinities of **8** and **9** shows that the imidazo[1,2-*b*]pyridazin-6(5*H*)-one **9** binds dramatically more strongly in vitro than the 6-methoxy substituted imidazo[1,2-*b*]pyridazine isomer **8**. The BZR affinity of **9** is of a similar order of magnitude to that of 3-benzamidomethyl-6-methoxy-2-(4-methylphenyl)imidazo[1,2-*b*]pyridazine [35].

Compounds **4a,h,o** and **9** were evaluated for BZR efficacy by the measurement of in vitro IC_{50} values in the presence and absence of GABA and determination of GABA-shift ratios (table III). In general, inverse agonists have a GABA-shift ratio of less than 1, antagonists approximately 1, partial agonists 1–1.5 and full agonists greater than 1.5 [36, 37]. The compounds examined here all recorded GABA-shift ratios of over 1.5, consistent with full agonist properties at the BZR.

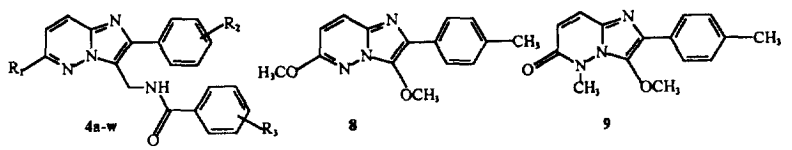
Molecular modelling was used to examine and attempt to rationalize the observed SAR of the series of imidazo[1,2-*b*]pyridazines. The structures of **4a,h,o** and **10** were generated and energy-minimized using the Sybyl molecular modelling software (Version 6.1, Tripos Inc, St Louis, MO) and Tripos force field [38] as described in the *Experimental protocols*. Compound **10** was not actually synthesized due to the low BZR affinities of related compounds **4s** and **4t**.

A system of conformational searches followed by a second energy minimization for **4a,h,o** and **10** allowed the most likely candidates for the biologically active conformers of these compounds to be determined. It was assumed that the proposed biologically active conformers of **4a,h,o** and **10** would all have similar geometrics as they would be required to bind to the same site on the BZR. The torsion angles determined are shown in table IV.

The pharmacophoric points of the prototypical 1,4-benzodiazepine, diazepam, were selected with reference to previously published BZR pharmacophore models [21–25]. Two areas of lipophilic interaction L1 and L2 (the centres of the fused phenyl and 5-phenyl rings) and two areas able to act as hydrogen bond accepting groups H1 and H2 (the carbonyl oxygen and the N-4 atoms) were chosen.

For the imidazo[1,2-*b*]pyridazines **4a,h,o** and **10**, L1 and L2 were defined as the centres of the phenyl rings of the 2-aryl and 3-benzamidomethyl groups respectively. Previous studies with analogous compounds have shown that replacement of a 2-aryl substituent with a 2-alkyl group results in a dramatic reduction in BZR affinity [39], whereas replacement

Table II. In vitro BZR affinities of some 3-substituted imidazo[1,2-*b*]pyridazines.



Compound	R_1	R_2	R_3	IC_{50} *(nM)*[a]	*Displacement (%)*[b]
4a[c]	OCH_3	3,4-OCH_2O	H	7 ± 1	–
4b	OCH_3	4-Cl	H	29 ± 1	–
4c	OCH_3	H	2-F	139 ± 17	–
4d	OCH_3	4-CH_3	2-F	21 ± 3	–
4e	OCH_3	3,4-OCH_2O	2-F	14 ± 1	–
4f	OC_2H_5	H	H	185 ± 19	–
4g	OC_2H_5	4-CH_3	H	35 ± 7	–
4h	OC_2H_5	3,4-OCH_2O	H	25 ± 1	–
4i	OC_2H_5	4-Cl	H	64 ± 7	–
4j	OC_2H_5	H	2-F	208 ± 16	–
4k	OC_2H_5	4-CH_3	2-F	51 ± 8	–
4l	OC_2H_5	3,4-OCH_2O	2-F	31 ± 1	–
4m	OC_3H_7	H	H	–	73 ± 3
4n	OC_3H_7	4-CH_3	H	181 ± 17	–
4o	OC_3H_7	3,4-OCH_2O	H	116 ± 16	–
4p	OC_3H_7	4-Cl	H	–	58 ± 8
4q	OC_3H_7	H	2-F	238 ± 1	–
4r	OC_3H_7	4-CH_3	2-F	143 ± 38	–
4s	$O(CH_2)_2OCH_3$	H	H	–	44 ± 4
4t	$O(CH_2)_2OCH_3$	4-CH_3	H	318 ± 40	–
4u	$O(CH_2)_2OC_2H_5$	H	H	–	47 ± 1
4v	OCH_3	3,4-OCH_2O	3-NO_2	8 ± 2	–
4w	OCH_3	3,4-OCH_2O	4-NO_2	23 ± 5	–
8				191 ± 13	–
9				26 ± 1	–

[a]Mean IC_{50} values determined ± SEM of three separate experiments. [b]Mean percentage displacement of [^{3}H]diazepam binding at a ligand concentration of 1000 nM determined ± SEM of determinations in triplicate. [c]The 2-phenyl and 2-(4-methylphenyl) analogues of **4a** have IC_{50} values of 79 and 23 nM respectively [35].

of a 3-benzamidomethyl with a 3-acetamidomethyl group generally causes a modest reduction in BZR affinity [35]. This is consistent with the L1 region of the imidazo[1,2-*b*]pyridazines being essential, and the L2 region being desirable, for high affinity binding to the BZR.

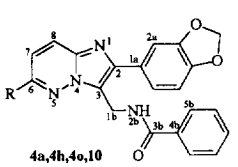
Among the ring nitrogens on the imidazo[1,2-*b*]-pyridazine nucleus, evidence suggesting that N-1 is the important pharmacophoric point H1 can be summarized as follows. First, theoretical calculations of the atomic charges of unsubstituted [40] and substituted [41] imidazo[1,2-*b*]pyridazines have identified N-1 as a strongly electronegative atom, whereas N-5 is less electronegative and N-4 is an area of moderate positive charge. The results given in figure 1 show that this is also the case for compounds **4a**, **8** and **9**. The N-1 atom would therefore be favoured to form hydrogen bonds with surrounding proton-donating groups

Table III. In vitro GABA-shift ratios for compounds **4a,h,o** and **9**.

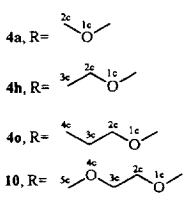
Compound	*IC_{50} (GABA) (nM)*[a]	*IC_{50} (no GABA) (nM)*[b]	*GABA-shift ratio*
4a	9.8	18.9	1.93
4h	28.9	68.1	2.36
4o	95.5	243.1	2.55
9	35.9	58.0	1.62

[a]IC_{50} values determined for the in vitro displacement of [^{3}H]flumazenil binding from rat brain in the presence of 100 μM γ-aminobutyric acid (GABA). [b]IC_{50} values determined for the in vitro displacement of [^{3}H]flumazenil binding from rat brain in the absence of GABA.

Table IV. Torsion angles τ (°)[a] of conformers selected for superimposition with diazepam.



4a,4h,4o,10



Compound	τ_1 (°)	τ_2 (°)	τ_3 (°)	τ_4 (°)	τ_5 (°)	τ_6 (°)	τ_7 (°)
4a	0.0	53.0	–164.7	61.6	–20.6	–	–
4h	–0.5	131.2	168.5	–62.0	21.9	–	–
4o	0.5	140.7	148.6	136.6	17.6	–179.8	–
10	0.0	–38.6	145.2	137.6	–156.9	179.9	179.8

[a]The torsion angles were measured using Sybyl and defined as follows: $\tau_1 = \tau$ (N5, C6, O1c, C2c); $\tau_2 = \tau$ (C2a, C1a, C2, N1); $\tau_3 = \tau$ (N2b, C1b, C3, N4); $\tau_4 = \tau$ (C3b, N2b, C1b, C3); $\tau_5 = \tau$ (C5b, C4b, C3b, N2b); $\tau_6 = \tau$ (O1c, C2c, C3c, X4c); $\tau_7 = \tau$ (C2c, C3c, X4c, C5c).

on the receptor protein. Second, identically substituted imidazo[1,2-*b*]pyridazines and imidazo[1,2-*a*]pyridines have previously been shown to have similar BZR affinities [39]. This implies that the N-5 atom of the imidazo[1,2-*b*]pyridazines (replaced by a CH group in the imidazo[1,2-*a*]pyridines) is not involved in stabilizing binding to the BZR.

The carbonyl oxygen atom of the 3-benzamidomethyl group was defined as H2, as it is another electronegative atom able to form hydrogen bonds by donation of electron density to surrounding proton donor groups on the receptor protein. The absence of the areas defined as L2 and H2, as in the 3-unsubstituted compounds **3a–m**, resulted in a considerable decrease in BZR affinity.

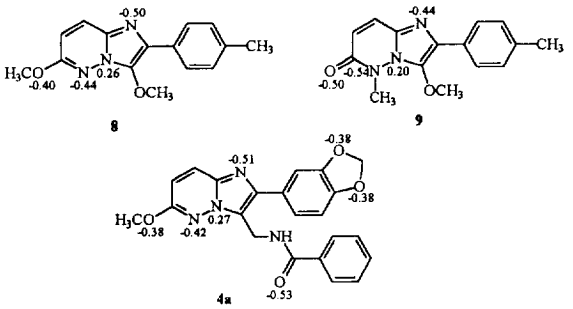


Fig 1. Atomic charges derived from electrostatic potential (see *Experimental protocols, Procedure c*).

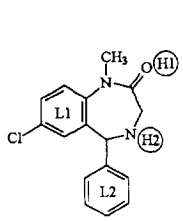
The distances between the proposed pharmacophoric areas of lipophilic (L1 and L2) and hydrogen bonding (H1 and H2) BZR–ligand interaction for diazepam and the selected biologically active conformation of **4a** were found to be similar for both of the compounds, suggesting that both the 1,4-benzodiazepines and the substituted 6-alkoxyimidazo[1,2-*b*]-pyridazines could be accommodated at the same area of the BZR protein (table V). In the case of the distances between L1 and H1, values for both compounds fall into the range stated by Fryer of approximately 4–5.5 Å for BZR agonist ligands [9]. The areas of lipophilic interaction L1 and L2 are closer together for the imidazo[1,2-*b*]pyridazines than for diazepam, but this does not appear to significantly reduce BZR affinity.

Close overall superimpositions of the imidazo[1,2-*b*]-pyridazines on to diazepam were achieved by superimposing the respective pairs of pharmacophoric points, L1–L1, H1–H1, etc, defined above. The root mean square (rms) distances (Å) between the four fitted atoms or ring centroids are given in table VI. The superimposition of **4o** with diazepam is shown in figure 2. Compounds **4a,h** and **10** also gave similar superimpositions.

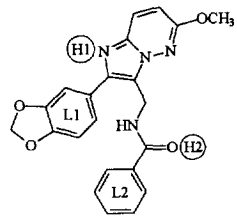
The 6-alkoxy groups of **4a,h,o** and **10** are orientated away from the seven-membered ring of diazepam, in the opposite direction to its fused phenyl ring. In this orientation, the 6-alkoxy groups interact with an area of the BZR not accessed by diazepam. Alkoxy groups larger in size than ethoxy appear to exceed the steric limits of this area of the BZR and result in decreased affinity for the receptor.

Previously published BZR pharmacophore models have defined areas of steric hindrance S1 in the region between the 7-chloro and 5-phenyl groups of diazepam [24, 25] and also S2 beyond the NMe group and fused phenyl ring of diazepam [25] (fig 3). The area of steric hindrance S3 occupied by the bulkier 6-alkoxy

Table V. Measurement of distances between the proposed points of ligand–receptor interaction for diazepam and **4a** at the BZR.



Diazepam



4a

Pharmacophoric points	*Distance*[a] *(Å)*	
	Diazepam	***4a***
L1–H1	4.95	3.95
L1–H2	3.80	3.89
L2–H1	6.76	6.49
L2–H2	3.69	3.65
H1–H2	3.46	4.13
L1–L2	4.86	3.46

[a]Distances measured using the MEASURE command within Sybyl. Distances including L1 or L2 were measured from the centre of the appropriate phenyl ring. Distances including H1 or H2 were measured to the centre of the appropriate atom for comparative purposes, though the area of actual hydrogen bonding interaction would be at a point approximately 1.5–2 Å from this.

groups of **4o** and **10**, however, is separate from these regions and is beyond the carbonyl groups of diazepam. A further area of negative ligand–receptor interaction at the BZR has therefore been defined.

The 3-methoxy compounds **8** and **9** lack the area of lipophilic interaction L2 provided by the phenyl ring of the 3-benzamidomethyl group of **4a**,**h**,**o** and **10**. Previous reports have demonstrated that, for a series of 2-aryl-3-methoxy-6-substituted imidazo[1,2-*b*]pyridazines, the BZR affinity varies depending on the 2-aryl groups present in the order 2-(3,4-methylenedioxyphenyl) > 2-(4-methylphenyl) > 2-phenyl [26, 33, 39]. This SAR is identical to that observed in this paper for compounds **4a–w**, and a possible explanation for this is that the 2-aryl groups of both series of compounds interact with the same area of the BZR. Other possible points of pharmacophoric interaction for **8** and **9** would be the oxygen atoms of the 6- and 3-position groups and the N-1 atom. The increased BZR affinity of **9** over **8** could be explained by the ability of the amide carbonyl moiety of **9** to interact with the same area of the receptor as the amide carbonyl moiety of diazepam. In this hypothesis, the 6-position oxygen atom would become H1, N-1 would be H2, and the 2-aryl group L1.

The atomic charges on the ring nitrogen and 6-position oxygen atoms for **8** and **9** were calculated (fig 1). These calculations show that the 6-position oxygen and 5-position nitrogen atoms of **9** have a greater negative charge than those of **8**. This may, at least in part, account for the increased BZR affinity of **9**, as the 6-position oxygen atom of **9** would be able to form a stronger hydrogen bond with a hydrogen bond donor group on the BZR protein than that of **8**. Other physicochemical factors not examined here, however, such as the overall lipophilicity of the compounds may also contribute towards the differences in the in vitro BZR affinity of **8** and **9**.

In conclusion, a number of the novel substituted 6-alkoxyimidazo[1,2-*b*]pyridazines reported in this paper have been found to be high-affinity BZR agonist ligands. One area of lipophilic interaction L1 and two hydrogen bond acceptor groups H1 and H2 have been found to be required for high affinity binding to the BZR, with the presence of a second area of lipophilic interaction L2 further stabilizing ligand binding to the BZR. 6-Alkoxy groups larger than ethoxy reduce BZR affinity, presumably by a negative ligand–receptor interaction. This area of steric hindrance is separate from similar areas previously defined at the BZR pharmacophore.

Table VI. Superimposition of the proposed biologically active conformers of **4a**,**h**,**o** and **10** with diazepam,.

Compound	E_{min}[a] *(kcal/mol)*	E_{cnf}[b] *(kcal/mol)*	*Fitted atoms*	*Rms*[c] *(Å)*
4a	42.61	42.61	L1-L1, L2-L2, H1-H1, H2-H2	0.96
4h	42.23	42.23	L1-L1, L2-L2, H1-H1, H2-H2	1.01
4o	43.30	43.30	L1-L1, L2-L2, H1-H1, H2-H2	1.65
10	44.70	45.16	L1-L1, L2-L2, H1-H1, H2-H2	1.70

[a]E_{min} is the global energy minimum of the molecule. [b]E_{cnf} is the energy of the proposed biologically active conformation of the molecule. [c]The rms value is the root mean square distance between the fitted atom points on the imidazo[1,2-*b*]pyridazines and diazepam.

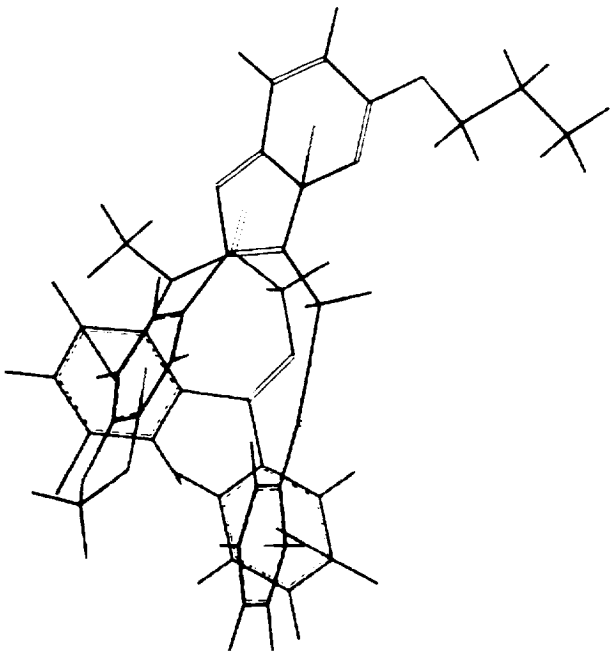


Fig 2. Superimposition of the proposed biologically active conformation of **4o** with diazepam.

Experimental protocols

In vitro binding assays. Procedure a

The abilities of the test compounds to displace [^{3}H]diazepam bound to BZR in rat brain cortex were determined using previously described techniques [34]. Male adult Wistar rats were decapitated, and their brains removed and placed on ice. The washed cortical membranes were prepared and stored frozen until use. On the day of assay the membrane preparations were thawed, washed once by centrifugation and resuspension in ice-cold water, then resuspended in 50 mM Tris-HCl buffer, pH 7.4, at 2 °C. For the receptor binding assays, the incubations contained ca 0.8 mg of protein, [^{3}H]diazepam (specific activity 86.6 Ci/mmol) at a final concentration of 0.70 ± 0.05 nM, various concentrations of the test ligands and 100 μM γ-aminobutyric acid (GABA) in a final volume of 2 mL 50 mM Tris-HCl buffer. Addition of the [^{3}H]diazepam initiated the incubations (at 0–4 °C) which were then terminated after 35 min by filtration under vacuum on glass fibre filters (Whatman GF/B, 2.5 cm) with two 6 mL washes of ice-cold buffer. Nonspecific binding was determined in separate tubes by the addition of a large excess (10 μM) of unlabelled diazepam. Initial assays were carried out at a test compound concentration of 1000 nM, and within each concentration determinations were at least in triplicate. IC_{50} values were determined over four concentrations of test compound and within each experiment all assays were performed in triplicate. IC_{50} values were determined using log-logit analysis (Mathcad 2.52, Mathsoft Inc) where the correlation coefficient of the lines of best fit to log-logit curves was not less than 0.95. Mean IC_{50} values were calculated ± SEM of three separate determinations.

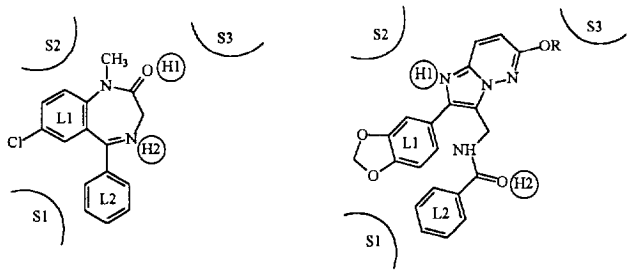


Fig 3. BZR pharmacophore models for diazepam and the substituted 6-alkoxy imidazo[1,2-*b*]pyridazines.

For the determination of the GABA-shift ratios, IC_{50} values were determined as described above in the presence and absence of 100 μM GABA. The radioligand used in these assays was [^{3}H]flumazenil rather than [^{3}H]diazepam as diazepam is a BZR agonist and would therefore bind more strongly to the BZR in the presence of GABA. The binding of flumazenil, a BZR antagonist, to the BZR is unaffected by the presence or absence of GABA.

Molecular modelling

Conformational analyses and superimpositions. Procedure b

The molecular modelling was carried out on a Silicon Graphics Personal Iris workstation. The molecular models of the compounds were constructed with standard bond lengths and bond angles as defined using the Sybyl 6.1 molecular modelling software. The structure of diazepam was constructed in the boat conformation found in crystalline diazepam and was thought to represent the most probable active conformation of the molecule [21]. The structures were energy-minimized using the Tripos force field [38] with Gasteiger–Hückel charges. These minimizations were carried out using the MINIMIZE command within Sybyl, using the Powell method with Simplex initial optimization and a termination gradient of 0.05 kcal/mol.

The systematic conformational searches were undertaken on the energy-minimized structures of the selected imidazo[1,2-*b*]-pyridazines. The SEARCH command within Sybyl was used for this purpose. Rotatable bonds were selected and varied from 0–359° with 30° increments. In the case of phenyl or symmetrically-substituted phenyl ring functional groups, the bonds were varied from 0–179° in 30° increments. The conformational searches were then filtered using the FILTER command within Sybyl to within 5 kcal/mol of the global energy minimum for each molecule. From these filtered systematic searches, the 20 lowest energy conformers were taken and energy minimized.

The energy-minimized conformers within 2 kcal/mol of the lowest energy conformer were then selected for superimposition with diazepam. This was to allow for the possibility of the biologically active conformation of the molecules not neccessarily being the lowest energy conformation due to the electronic and steric requirements of the BZR binding site. The molecular superimpositions were performed using the FIT ATOMS command within Sybyl.

Electrostatic potential derived atomic charges. Procedure c
The structures of the compounds were generated using standard bond lengths and angles using the Spartan software package (Version 3.1, Wavefunction Inc, Irvine CA) installed on an Onyx Silicon Graphics (model CMN AO11) workstation. The electrostatic potential derived charges (which are more suitable for studying receptor–ligand interactions than the Mulliken charges) were calculated following full AM1 optimization of the compounds with default values (convergence for the heat of formation 1.0 x 10^{-3} kcal/mol). Comparison of the AM1 charges with the corresponding ab initio (HF/6-31G*) data for a number of imidazo[1,2-*b*]pyridazines revealed a good agreement (P Mátyus, PW Harrison, GB Barlin, unpublished results).

Chemistry

All compounds were examined for the presence of impurities by thin-layer chromatography on alumina plates pre-coated with Merck Kieselgel 60 F_{254} of 0.25 mm thickness and by ^{1}H-NMR spectroscopy. Preparative thin layer chromotography was performed on glass plates pre-coated with Merck aluminium oxide 60 F_{254} (type E) of 1.5 mm thickness. The light petroleum used had bp 60–80 °C. ^{1}H-NMR spectra (δ values) were recorded from $CDCl_3$ solution with tetramethylsilane as internal standard, and at 90 MHz and 30 °C with a Jeol FX90Q Fourier transform spectrometer possessing digital resolution of 0.12 Hz. The ^{1}H-NMR NOE difference spectra were recorded from $CDCl_3$ solution under a nitrogen atmosphere at 500 MHz and 30 °C on a Varian VXR500S spectrometer. Melting points are uncorrected and were taken in open Pyrex capillaries using an Electrothermal melting point apparatus. Samples for analysis were dried at 100–120 °C/710 mmHg for 6–24 h unless otherwise specified. Microanalyses were performed by the Australian National University Analytical Services Unit and were within ±0.4% of calculated values for carbon, hydrogen and nitrogen except for compounds **4o,t,v** and **4w**. Low-resolution mass spectra were recorded on an Incos data system attached to a VG-Micromass 7070 double focusing mass spectrometer using electron ionization (EI) at 70 eV.

6-Chloropyridazin-3-amine **1** [28], 6-methoxypyridazin-3-amine **2a** [29], 6-ethoxypyridazin-3-amine **2b** [29], 6-hydroxypyridazin-3-amine hydrochloride **5** [29], α–bromoacetophenone, α-bromo-4-methylacetophenone [42], α-bromo-3,4-methylenedioxyacetophenone [43], α-bromo-4-chloroacetophenone [42], 4-methylphenylglyoxal hydrate [44], 6-methoxy-2-phenylimidazo[1,2-*b*]pyridazine [35], 6-methoxy-2-(4-methylphenyl)imidazo[1,2-*b*]pyridazine [35], *N*-(hydroxymethyl)benzamide [45], 2-fluoro-*N*-(hydroxymethyl)benzamide [46], and *N*-(hydroxymethyl)-(3 and 4) nitrobenzamide [46] were prepared according to the relevant literature procedures and characterized using ^{1}H-NMR spectroscopy.

6-Propoxypyridazin-3-amine ***2c****. Procedure d*
A mixture of 6-chloropyridazin-3-amine **1** (3.25 g, 0.025 mol) and sodium propoxide solution [from sodium (0.6 g, 0.026 mol) and propanol (25 mL, 0.33 mol)] was heated in a screw-top reaction vessel at 145 °C for 14 h. The reaction mixture was diluted with water, adjusted to pH 7, extracted with chloroform, the extract dried (Na_2SO_4) and the solvent evaporated to leave an oil (3.69 g, 96%). This oil was subjected to tlc (alumina; chloroform twice, then alumina; ether) and gave the title compound as an oil: ^{1}H-NMR δ 1.01 (t, 3 H, J = 7 Hz, CH_3), 1.80 (m, 2H, CH_2), 4.32 (t, 2H, J = 7 Hz, CH_2O), 4.40 (br s, NH_2), 6.79 (s, 2H, H 4,5). MS (EI) *m/z* 154 (M + 1, 33%), 124 (23), 111 (100), 54 (49); anal $C_7H_{11}N_3O$.

6-(2-Methoxyethoxy)pyridazin-3-amine ***2d****.* A mixture of 6-chloropyridazin-3-amine 1 (0.5 g, 0.0039 mol) and a solution of sodium 2-methoxyethanolate [prepared from sodium (0.089 g, 0.0039 mol) and 2-methoxyethanol (4.0 mL, 0.051 mol)] was treated as described in *Procedure d* to leave an oil (0.539 g, 83%). A portion was then subjected to tlc (alumina; chloroform) to give the title compound: mp 82–84 °C (from benzene); ^{1}H-NMR δ 3.43 (s, 3H, CH_3), 3.76 (m, 2H, CH_2), 4.50 (br s, NH_2), 4.55 (m, 2H, CH_2O), 6.83 (m, 2H, H 4,5). MS (EI) *m/z* 170 (M + 1, 3%), 111 (100), 54 (29); anal $C_7H_{11}N_3O_2$.

6-(2-Ethoxyethoxy)pyridazin-3-amine ***2e****.* A mixture of 6-chloropyridazin-3-amine **1** (1.0 g, 0.0077 mol) and a solution of sodium 2-ethoxyethanolate [prepared from sodium (0.18 g, 0.0078 mol) and 2-ethoxyethanol (8.0 mL, 0.082 mol)] was treated as described in *Procedure d* (but at 165 °C) to leave an oil (1.22 g, 86%). This compound was used without further purification. ^{1}H-NMR δ 1.22 (t, 3 H, J = 7 Hz, CH_3), 3.57 (q, 2 H, J = 7 Hz, CH_3CH_2), 3.75–3.85 (m, 2 H, $CH_3CH_2OCH_2$), 4.45–4.55 (m, 2H, CH_2O), 5.07 (br, 2H, NH_2), 6.82 (m, 2H, H 4,5).

6-Propoxy-2-(4-methylphenyl)imidazo[1,2-b]pyridazine ***3h****. Procedure e*
A mixture of 6-propoxypyridazin-3-amine **2c** (0.15 g, 0.001 mol), α-bromo-4-methylacetophenone (0.21 g, 0.001 mol) and ethanol (10 mL) was refluxed for 3 h. Sodium hydrogen carbonate (0.084 g, 0.001 mol) was then added and the refluxing continued for 3 h. The solvent was evaporated and the residue extracted with chloroform, the extract washed with water and dried (Na_2SO_4), and the chloroform evaporated to give an oil. The crude product was subjected to tlc (alumina; chloroform, light petroleum, 3:1) to provide **3h** (0.15 g, 56%), as yellow crystals: mp 86–88 °C (from light petroleum); ^{1}H-NMR δ 1.06 (t, 3H, J = 7 Hz, CH_3CH_2), 1.81 (m, 2H, CH_3CH_2), 2.39 (s, 3H, CH_3C), 4.27 (t, 2H, J = 7 Hz, CH_2O), 6.66 (d, 1H, J = 9.5 Hz, H 7), 7.24 (d, 2H, J = 8 Hz, H 2', 6', (3',5')), 7.79 (d, 1H, J = 9.5 Hz, H 8), 7.81 (d, 2H, J = 8 Hz, H 3',5'(2',6')), 7.99 (s, 1H, H 3). MS (EI) *m/z* 267 (M, 100%), 225 (100), 130 (13), 115 (22), 80 (14); anal $C_{16}H_{17}N_3O$.

The following compounds were prepared similarly by *Procedure e*.

2-(3,4-Methylenedioxyphenyl)-6-methoxyimidazo[1,2-b]pyridazine ***3a****.* 6-Methoxypyridazin-3-amine **2a** (0.19 g, 0.0015 mol) and α-bromo-3,4-methylenedioxyacetophenone (0.36 g, 0.0015 mol) gave **3a** as a brown solid (0.11 g, 28%): mp 193–194 °C (from toluene); ^{1}H-NMR δ 3.99 (s, 3H, CH_3O), 6.00 (s, 2H, OCH_2O), 6.67 (d, 1H, J = 9.5 Hz, H 7), 6.87 (d, 1H, J = 8.5 Hz) and 7.39–7.49 (m, 2H) (H 2',5',6'), 7.79 (d, 1H, J = 9.5 Hz, H 8), 7.93 (s, 1H, H 3). MS (EI) *m/z* 269 (M, 100%), 211 (10), 80 (14); anal $C_{14}H_{11}N_3O_3$.

2-(4-Chlorophenyl)-6-methoxyimidazo[1,2-b]pyridazine ***3b****.* 6-Methoxypyridazin-3-amine **2a** (0.25 g, 0.002 mol) and α-bromo-4-chloroacetophenone (0.47 g, 0.002 mol) gave **3b** as a cream-coloured solid (0.36 g, 69%): mp 177–179 °C (from light petroleum); ^{1}H-NMR δ 4.00 (s, 3H, CH_3), 6.68 (d, 1H, J = 9.5 Hz, H 7), 7.39 (d, 2H, J = 9 Hz, H 2',6'(3',5')), 7.77 (d, 1H, J = 9.5 Hz, H 8), 7.85 (d, 2H, J = 9 Hz, H 3',5'(2',6')), 8.02 (s, 1H, H 3). MS (EI) *m/z* 259 (M, 100%), 216 (12), 80 (26); anal $C_{13}H_{10}N_3OCl$.

6-Ethoxy-2-phenylimidazo[1,2-b]pyridazine ***3c***. 6-Ethoxypyridazin-3-amine **2b** (0.28 g, 0.002 mol) and α-bromoacetophenone (0.40 g, 0.002 mol) gave **3c** as yellow crystals (0.10 g, 42%): mp 126–128 °C (from cyclohexane); ^{1}H-NMR δ 1.45 (t, 3 H, J = 7 Hz, CH_3), 4.39 (q, 2 H, J = 7 Hz, CH_2), 6.69 (d, 1 H, J = 9.5 Hz, H 7), 7.29–8.00 (m, 6 H, H 8 and Ph), 8.02 (s, 1 H, H 3). MS (EI) *m/z* 239 (M, 100%), 211 (75), 102 (34), 80 (16); anal $C_{14}H_{13}N_3O$.

6-Ethoxy-2-(4-methylphenyl)imidazo[1,2-b]pyridazine ***3d***. 6-Ethoxypyridazin-3-amine **2b** (0.14 g, 0.001 mol) and α-bromo-4-methylacetophenone (0.22 g, 0.001 mol) provided **3d** as a cream-coloured solid (0.06 g, 24%): mp 139–140 °C (from cyclohexane); ^{1}H-NMR δ 1.44 (t, 3H, J = 7 Hz, CH_3CH_2), 2.38 (s, 3H, CH_3C), 4.38 (q, 2H, J = 7 Hz, CH_3CH_2), 6.65 (d, 1H, J = 9.5 Hz, H 7), 7.39 (d, 2H, J = 9 Hz, H 2',6'(3',5')), 7.80 (d, 1H, J = 9.5 Hz, H 8), 7.85 (d, 2H, J = 9 Hz, H 3',5' (2',6')), 7.98 (s, 1H, H 3). MS (EI) *m/z* 253 (M, 100%), 225 (62), 115 (20), 80 (14); anal $C_{15}H_{13}N_3O$.

6-Ethoxy-2-(3,4-methylenedioxyphenyl)imidazo[1,2-b]pyridazine ***3e***. 6-Ethoxypyridazin-3-amine **2b** (0.22 g, 0.0016 mol) and α-bromo-3,4-methylenedioxyacetophenone (0.39 g, 0.0016 mol) gave **3e** as a white solid (0.27 g, 59%): mp 169–171 °C (from cyclohexane); ^{1}H-NMR δ 1.44 (t, 3 H, J = 7 Hz, CH_3), 4.37 (q, 2H, J = 7 Hz, CH_3CH_2), 5.99 (s, 2H, OCH_2O), 6.64 (d, 1H, J = 9.5 Hz, H 7), 6.87 (d, 1H, J = 8.5 Hz) and 7.38–7.48 (m, 2H) (H 2',5',6'), 7.76 (d, 1H, J = 9.5 Hz, H 8), 7.90 (s, 1H, H 3). MS (EI) *m/z* 283 (M, 100%), 255 (60), 197 (11), 128 (11), 80 (10); anal $C_{15}H_{13}N_3O_3$.

2-(4-Chlorophenyl)-6-ethoxyimidazo[1,2-b]pyridazine ***3f***. 6-Ethoxypyridazin-3-amine **2b** (0.28 g, 0.002 mol) and α-bromo-4-chloroacetophenone (0.47 g, 0.002 mol) furnished **3f** as a yellow solid (0.19 g, 35%): mp 157–159 °C (from light petroleum); ^{1}H-NMR δ 1.45 (t, 3H, J = 7 Hz, CH_3), 4.38 (q, 2H, J = 7 Hz, CH_2), 6.67 (d, 1H, J = 9.5 Hz, H 7), 7.39 (d, 2H, J = 9 Hz, H 2',6'(3',5')), 7.77 (d, 1H, J = 9.5 Hz, H 8), 7.85 (d, 2H, J = 9 Hz, H 3',5'(2',6')), 7.99 (s, 1H, H 3). MS (EI) *m/z* 273 (M, 100%), 245 (60), 136 (28), 80 (23); anal $C_{14}H_{12}N_3OCl$.

2-Phenyl-6-propoxyimidazo[1,2-b]pyridazine ***3g***. 6-Propoxypyridazin-3-amine **2c** (0.15 g, 0.001 mol) and α-bromoacetophenone (0.20 g, 0.001 mol) gave **3g** as yellow crystals (0.08 g, 32%): mp 102–104 °C (from light petroleum); ^{1}H-NMR δ 1.06 (t, 3H, J = 7 Hz, CH_3), 1.81 (m, 2H, CH_3CH_2), 4.27 (t, 2H, J = 7 Hz, CH_2O), 6.66 (d, 1H, J = 9.5 Hz, H 7), 7.29–7.97 (m, 6H, H 8 and Ph), 8.02 (s, 1H, H 3). MS (EI) *m/z* 253 (M, 82%), 211 (100), 102 (34); anal $C_{15}H_{15}N_3O$.

2-(3,4-Methylenedioxyphenyl)-6-propoxyimidazo[1,2-b]pyridazine ***3i***. 6-Propoxypyridazin-3-amine **2c** (0.15 g, 0.001 mol) and α-bromo-3,4-methylenedioxyacetophenone (0.24 g, 0.001 mol) afforded **3i** as a yellow solid (0.15 g, 50%): mp 125–126 °C from light petroleum, twice); ^{1}H-NMR δ 1.05 (t, 3 H, J = 7 Hz, CH_3), 1.89 (m, 2H, CH_3CH_2), 4.27 (t, 2H, J = 7 Hz, CH_2O), 5.99 (s, 2H, OCH_2O), 6.65 (d, 1 H, J = 9.5 Hz, H 7), 6.87 (d, 1H, J = 9 Hz) and 7.38–7.47 (m, 2H) (H 2',5',6'), 7.76 (d, 1H, J = 9.5 Hz, H 8), 7.91 (s, 1H, H 3). MS (EI) *m/z* 297 (M, 100%), 255 (81), 225 (12), 80 (11); anal $C_{16}H_{15}N_3O_3$.

2-(4-Chlorophenyl)-6-propoxyimidazo[1,2-b]pyridazine ***3j***. 6-Propoxypyridazin-3-amine **2c** (0.23 g, 0.0015 mol) and α-bromo-4-chloroacetophenone (0.35 g, 0.0015 mol) gave **3j** as a yellow solid (0.20 g, 47%): mp 149–151 °C (from light petroleum, twice); ^{1}H-NMR δ 1.06 (t, 3H, J = 7 Hz, CH_3), 1.85 (m, 2 H, CH_3CH_2), 4.27 (t, 2H, J = 7 Hz, CH_2O), 6.67 (d, 1H, J = 9.5 Hz, H 7), 7.39 (d, 2H, J = 8 Hz, H 2',6'(3',5')), 7.76 (d, 1H, J = 9.5 Hz, H 8), 7.84 (d, 2H, J = 8 Hz, H 3',5'(2',6')), 7.89 (s, 1H, H 3). MS (EI) *m/z* 287 (M, 85%), 245 (100), 136 (25), 80 (13); anal $C_{15}H_{14}N_3OCl$.

6-(2-Methoxyethoxy)-2-phenylimidazo[1,2-b]pyridazine ***3k***. 6-(2-Methoxyethoxy)pyridazin-3-amine **2d** (0.25 g, 0.0015 mol) and α-bromoacetophenone (0.30 g, 0.0015 mol) gave **3k** as a cream-coloured solid (0.18 g, 45%): mp 91–92 °C (from light petroleum, twice); ^{1}H-NMR δ 3.46 (s, 3H, CH_3), 3.73–3.84 (m, 2H, CH_3OCH_2), 4.43–4.54 (m, 2H, $CH_3OCH_2CH_2O$), 6.74 (d, 1H, J = 9.5 Hz, H 7), 7.36–7.97 (m, 6H, H 8 and Ph), 8.02 (s, 1H, H 3). MS (EI) *m/z* 269 (M, 92%), 211 (100), 102 (28), 59 (48); anal $C_{15}H_{15}N_3O_2$.

6-(2-Methoxyethoxy)-2-(4-methylphenyl)imidazo[1,2-b]pyridazine ***3l***. 6-(2-Methoxyethoxy)pyridazin-3-amine **2d** (0.25 g, 0.0015 mol) and α-bromo-4-methylacetophenone (0.32 g, 0.0015 mol) afforded **3l** as a yellow solid (0.22 g, 52%): mp 118–120 °C (from light petroleum, twice); ^{1}H-NMR δ 2.38 (s, 3H, CH_3C), 3.46 (s, 3H, CH_3O), 3.73–3.84 (m, 2H, CH_3OCH_2), 4.43–4.54 (m, 2H, $CH_3OCH_2CH_2O$), 6.72 (d, 1H, J = 9.5 Hz, H 7), 7.24 (d, 2H, J = 8 Hz, H 2',6'(3',5')), 7.78 (d, 1H, J = 9.5 Hz, H 8), 7.81 (d, 2H, J = 8 Hz, H 3',5'(2',6')), 7.99 (s, 1H, H 3). MS (EI) *m/z* 283 (M, 100%), 225 (94), 115 (16), 59 (42); anal $C_{16}H_{17}N_3O_2$.

6-(2-Ethoxyethoxy)-2-phenylimidazo[1,2-b]pyridazine ***3m***. 6-(2-Ethoxyethoxy)pyridazin-3-amine **2e** (0.27 g, 0.0015 mol) and α-bromo-acetophenone (0.30 g, 0.0015 mol) gave **3m** as a yellow solid (0.19 g, 44%): mp 76–78 °C (from light petroleum, twice); ^{1}H-NMR δ 1.26 (t, 3H, J = 7 Hz, CH_3), 3.61 (q, 2H, J = 7 Hz, CH_3CH_2), 3.77–3.88 (m, 2H, $CH_3CH_2OCH_2$), 4.43–4.59 (m, 2H, $CH_3CH_2OCH_2CH_2$), 6.73 (d, 1H, J = 9.5 Hz, H 7), 7.30–7.95 (m, 5H, Ph), 7.80 (d, 1H, J = 9.5 Hz, H 8), 8.02 (s, 1H, H 3). MS (EI) *m/z* 283 (M, 80%), 211 (100), 102 (23); anal $C_{16}H_{17}N_3O_2$.

3-(2-Fluorobenzamidomethyl)-6-methoxy-2-phenylimidazo[1,2-b]pyridazine ***4c***. *Procedure f*

A mixture of 2-fluoro-*N*-(hydroxymethyl)benzamide (0.12 g, 0.0007 mol) in glacial acetic acid (6.0 mL) with concentrated sulphuric acid (0.11 mL, 0.002 mol) was heated at 50 °C for 15 min, then 6-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (0.16 g, 0.0007 mol) was added and the mixture was heated under reflux in an oil bath at 120 °C for 24 h. The acetic acid was removed in vacuo and the residue diluted with water, adjusted with aqueous ammonia to pH 10, and the product extracted into chloroform. The extract was washed with water, dried (Na_2SO_4), solvent evaporated and the residue subjected to tlc (alumina; chloroform/light petroleum, 4:1) which gave **4c** (0.11 g, 42%) as a white solid: mp 200–201 °C (from methanol); ^{1}H-NMR δ 4.07 (s, 3H, CH_3O), 5.24 (dd, 2H, J = 5.5 Hz, 1.5 Hz, CH_2N), 6.75 (d, 1H, J = 9.5 Hz, H 7), 6.95–7.61 (m, 6H) and 7.92–8.25 (m, 3H) (H 3',4',5',6' and Ph), 7.84 (d, 1H, J = 9.5 Hz, H 8); MS (EI) *m/z* 376 (M, 11%), 253 (100), 123 (78), 95 (26); anal $C_{21}H_{17}N_4O_2F$.

The compounds listed below were also prepared using *Procedure f*.

3-Benzamidomethyl-6-methoxy-2-(3,4-methylenedioxyphenyl)-imidazo[1,2-b]pyridazine ***4a***. 6-Methoxy-2-(3,4-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazine **3a** (0.10 g, 0.00037 mol) and *N*-(hydroxymethyl)benzamide (0.06 g, 0.00040 mol) afforded **4a** as a cream-coloured solid (0.04 g, 25%): mp 247–

249 °C (from toluene); ^{1}H-NMR δ 4.01 (s, 3H, CH_3O), 5.17 (d, 2H, J = 5.5 Hz, CH_2N), 6.00 (s, 2H, OCH_2O), 6.72 (d, 1H, J = 9.5 Hz, H 7),6.94 (br s, NH) 6.85–7.82 (m, 8H, H 2',5',6' and Ph), 7.81 (d, 1H, J = 9.5 Hz, H 8); MS (EI) *m/z* 402 (M, 31%), 297 (81), 267 (100), 123 (50), 105 (35), 77 (24); anal $C_{22}H_{18}N_4O_4$.

3-Benzamidomethyl-2-(4-chlorophenyl)-6-methoxyimidazo[1,2-b]-pyridazine ***4b***. 2-(4-Chlorophenyl)-6-methoxyimidazo[1,2-*b*]-pyridazine **3b** (0.16 g, 0.0006 mol) and *N*-(hydroxymethyl)-benzamide (0.091 g, 0.0006 mol) gave **4b** as a white solid (0.07 g, 30%): mp 259–261 °C (from toluene); ^{1}H-NMR δ 4.03 (s, 3H, CH_3O), 5.18 (d, 2H, J = 5.5 Hz, CH_2N), 6.75 (d, 1H, J = 9.5 Hz, H 7), 6.90 (br, NH), 7.39–7.93 (m, 5H, Ph), 7.44 (d, 2H, J = 9 Hz, H 2',6'(3',5')), 7.82 (d, 1H, J = 9.5 Hz, H 8), 7.88 (d, 2H, J = 9 Hz, H 3',5'(2',6')); MS (EI) *m/z* 392 (M, 23%), 287 (100), 105 (47), 77 (36); anal $C_{21}H_{17}N_4O_2Cl$.

3-(2-Fluorobenzamidomethyl)-6-methoxy-2-(4-methylphenyl)-imidazo[1,2-b]pyridazine ***4d***. 6-Methoxy-2-(4-methylphenyl)-imidazo[1,2-*b*]pyridazine (0.14 g, 0.0006 mol) and 2-fluoro-*N*-(hydroxymethyl)benzamide (0.10 g, 0.0006 mol) furnished **4d** as white crystals (0.10 g, 43%): mp 197–199 °C (from methanol); ^{1}H-NMR δ 2.40 (s, 3H, CH_3C), 4.05 (s, 3H, CH_3O), 5.22 (dd, 2H, J = 5.5 Hz, 1.5 Hz, CH_2N), 6.72 (d, 1H, J = 9.5 Hz, H 7), 6.94–8.25 (m, 8 H, H 3',4',5',6', 2",3",5",6") 7.82 (d, 1H, J = 9.5 Hz, H 8); MS (EI) *m/z* 390 (M, 32%), 267 (100), 123 (47), 95 (17); anal $C_{22}H_{19}N_4O_2F$.

3-(2-Fluorobenzamidomethyl)-6-methoxy-2-(3,4-methylenedi-oxyphenyl)imidazo[1,2-b]pyridazine ***4e***. 6-Methoxy-2-(3,4-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazine **3a** (0.16 g, 0.0006 mol) and 2-fluoro-*N*-(hydroxymethyl)benzamide (0.10 g, 0.0006 mol) provided **4e** as a white solid (0.11 g, 44%): mp 226–227 °C (from methanol); ^{1}H-NMR δ 4.06 (s, 3H, CH_3O), 5.20 (dd, 2H, J = 5.5 Hz, 1.5 Hz, CH_2N), 6.01 (s, 2H, OCH_2O), 6.74 (d, 1H, J = 9.5 Hz, H 7), 6.94 (d, 1H, J = 9 Hz), 6.99–7.51 (m, 5H) and 8.06–8.26 (m, 1H, (H3',4',5',6',2",5",6")), 7.82 (d, 1H, J = 9.5 Hz, H 8); MS (EI) *m/z* 420 (M, 12%), 390 (21), 297 (31), 267 (70), 120 (42), 84 (83), 49 (100); anal $C_{22}H_{17}N_4O_4F$.

3-Benzamidomethyl-6-ethoxy-2-phenylimidazo[1,2-b]pyrida-zine ***4f***. 6-Ethoxy-2-phenylimidazo[1,2-*b*]pyridazine **3c** (0.17 g, 0.0007 mol) and *N*-(hydroxymethyl)-benzamide (0.11 g, 0.0007 mol) gave **4f** as cream-coloured crystals (0.12 g, 46%): mp 183–185 °C (from toluene); ^{1}H-NMR δ 1.42 (t, 3H, J = 7 Hz, CH_3), 4.35 (q, 2H, J = 7 Hz, CH_3CH_2), 5.15 (d, 2H, J = 5.5 Hz, CH_2N) 6.53 (d, 1H, J = 9.5 Hz, H 7), 7.12 (br NH), 7.30–7.86 (m, 10H, 2 x Ph), 7.68 (d, 1H, J = 9.5 Hz, H 8); MS (EI) *m/z* 372 (M, 8%), 267 (100), 105 (83), 77 (80); anal $C_{22}H_{20}N_4O_2$.

3-Benzamidomethyl-6-ethoxy-2-(4-methylphenyl)imidazo[1,2-b]-pyridazine ***4g***. 6-Ethoxy-2-(4-methylphenyl)imidazo[1,2-*b*]-pyridazine **3d** (0.15 g, 0.0006 mol) and *N*-(hydroxymethyl)-benzamide (0.09 g, 0.0006 mol) gave **4g** as a white solid (0.07 g, 30%): mp 216–218 °C (from toluene); ^{1}H-NMR δ 1.41 (t, 3H, J = 7 Hz, CH_3CH_2), 2.36 (s, 3H, CH_3C), 4.35 (q, 2 H, J = 7 Hz, CH_3CH_2), 5.13 (d, 2H, J = 5.5 Hz, CH_2N), 6.60 (d, 1H, J = 9.5 Hz, H 7), 7.15 (br s, NH), 7.33–7.46 (m, 6H) and 7.64–7.87 (m, 3H) (Ph and H 2',3',5',6'), 7.65 (d, 1H, J = 9.5 Hz, H 8); MS (EI) *m/z* 386 (M, 17%), 381 (100), 105 (41), 84 (31), 49 (40); anal $C_{23}H_{22}N_4O_2$.

3-Benzamidomethyl-6-ethoxy-2-(3,4-methylenedioxyphenyl)-imidazo[1,2-b]pyridazine ***4h***. 6-Ethoxy-2-(3,4-methylene-dioxyphenyl)imidazo[1,2-*b*]pyridazine **3e** (0.17 g, 0.0006 mol) and *N*-(hydroxymethyl)benzamide (0.09 g, 0.0006 mol) afforded **4h** as cream-coloured crystals (0.05 g, 20%): mp 252–254 °C (from toluene); ^{1}H-NMR δ 1.44 (t, 3H, J = 7 Hz, CH_3), 4.40 (q, 2H, J = 7 Hz, CH_3CH_2), 5.16 (d, 2H, J = 5.5 Hz, CH_2N), 5.99 (s, 2H, OCH_2O), 6.71 (d, 1H, J = 9.5 Hz, H 7), 6.91 (d, 1H, J = 8.5 Hz), 7.25–7.48 (m, 6H) and 7.72–7.83 (m, 1H) (Ph and H 2',5',6'), 7.80 (d, 1H, J = 9.5 Hz, H 8); MS (EI) *m/z* 416 (M, 43%), 311 (100), 283 (18), 105 (39), 77 (21); anal $C_{23}H_{20}N_4O_4$.

3-Benzamidomethyl-2-(4-chlorophenyl)-6-ethoxyimidazo[1,2-b]-pyridazine ***4i***. 2-(4-Chlorophenyl)-6-ethoxyimidazo[1,2-*b*]-pyridazine **3f** (0.16 g, 0.0006 mol) and *N*-(hydroxymethyl)-benzamide (0.091 g, 0.0006 g) afforded **4i** as a cream-coloured solid (0.12 g, 50%): mp 229–230 °C (from toluene); ^{1}H-NMR δ 1.45 (t, 3H, J = 7 Hz, CH_3), 4.39 (q, 2H, J = 7 Hz, CH_3CH_2), 5.14 (d, 2H, J = 5.5 Hz, CH_2N), 6.68 (d, 1H, J = 9.5 Hz, H 7), 7.00 (br, NH), 7.34–7.48 (m, 5H) and 7.65–7.86 (m, 5H) (H 8,2',3',5',6' and Ph); MS (EI) *m/z* 406 (M, 26%), 301 (100), 273 (23), 105 (63), 77 (33); anal $C_{22}H_{19}N_4O_2Cl$.

6-Ethoxy-3-(2-fluorobenzamidomethyl)-2-phenylimidazo[1,2-b]pyridazine ***4j***. 6-Ethoxy-2-phenylimidazo[1,2-*b*]pyridazine **3c** (0.17 g, 0.0007 mol) and 2-fluoro-*N*-(hydroxymethyl)benzamide (0.12 g, 0.0007 mol) gave **4j** as cream-coloured crystals (0.10 g, 37%): mp 184–186 °C (from toluene); ^{1}H-NMR δ 1.46 (t, 3 H, J = 7 Hz, CH_3), 4.44 (q, 2 H, J = 7 Hz, CH_3CH_2), 5.21 (dd, 2 H, J = 5.5 Hz, 1.5 Hz, CH_2N), 6.71 (d, 1 H, J = 9.5 Hz, H 7), 6.96–7.60 (m, 6 H) and 7.90–8.25 (m, 3 H) (H 3',4',5',6' and Ph), 7.81 (d, 1 H, J = 9.5 Hz, H 8); MS (EI) *m/z* 390 (M, 11%), 267 (96), 123 (100), 95 (33); anal $C_{22}H_{19}N_4O_2F$.

6-Ethoxy-3-(2-fluorobenzamidomethyl)-2-(4-methylphenyl)-imidazo[1,2-b]pyridazine ***4k***. 6-Ethoxy-2-(4-methylphenyl)-imidazo[1,2-*b*]pyridazine **3d** (0.16 g, 0.0006 mol) and 2-fluoro-*N*-(hydroxymethyl)benzamide (0.10 g, 0.0006 mol) provided **4k** as a white solid (0.10 g, 42%): mp 196–198 °C (from toluene); ^{1}H-NMR δ 1.47 (t, 3H, J = 7 Hz, CH_3CH_2), 2.40 (s, 3H, CH_3C), 4.44 (q, 2H, J = 7 Hz, CH_3CH_2), 5.20 (dd, 2H, J = 5.5 Hz, 1.5 Hz, CH_2N), 6.73 (d, 1H, J = 9.5 Hz, H 7), 6.95–7.60 (m, 5H), 7.85 (d, 2H, J = 8.5 Hz) and 8.05–8.25 (m, 1H) (H 3',4',5',6',2",3",5",6"), 7.84 (d, 1H, J = 9.5 Hz, H 8); MS (EI) *m/z* 404 (M, 17%), 281 (89), 123 (100), 95 (29); anal $C_{23}H_{21}N_4O_2F$.

6-Ethoxy-3-(2-fluorobenzamidomethyl)-2-(3,4-methylenedioxy-phenyl) imidazo[1,2-b]pyridazine ***4l***. 6-Ethoxy-2-(3,4-methyl-enedioxyphenyl)imidazo[1,2-*b*]pyridazine **3e** (0.17 g, 0.0006 mol) and 2-fluoro-*N*-(hydroxymethyl)benzamide (0.10 g, 0.0006 mol) gave **4l** as cream-coloured crystals (0.05 g, 19%): mp 202–203 °C (from methanol); ^{1}H-NMR δ 1.47 (t, 3H, J = 7 Hz, CH_3CH_2), 4.45 (q, 2H, J = 7 Hz, CH_3CH_2), 5.18 (dd, 2H, J = 5.5 Hz, 1.5 Hz, CH_2N), 6.00 (s, 2H, OCH_2O), 6.72, (d, 1H, J = 9.5 Hz, H 7), 7.02 (br, NH), 6.88–7.50 (m, 6 H) and 8.06–8.26 (m, 1H) (H 3',4',5',6',2",5",6"), 7.82 (d, 1H, J = 9.5 Hz, H8); MS (EI) *m/z* 434 (M, 28%), 311 (72), 123 (100), 95 (29); anal $C_{23}H_{19}N_4O4F$.

3-Benzamidomethyl-2-phenyl-6-propoxyimidazo[1,2-b]pyrida-zine ***4m***. 2-Phenyl-6-propoxyimidazo[1,2-*b*]pyridazine **3g** (0.15 g, 0.0006 mol) and *N*-(hydroxymethyl)benzamide (0.091 g, 0.0006 mol) afforded **4m** as cream-coloured crystals (0.07 g, 30%): mp 177–179 °C (from toluene); ^{1}H-NMR δ 1.02 (t, 3H,

J = 7 Hz, CH_3), 1.82 (m, 2H, CH_3CH_2), 4.26 (t, 2H, J = 7 Hz, CH_2O), 5.17 (d, 2H, J = 5.5 Hz, CH_2N), 6.66 (d, 1H, J = 9.5 Hz, H 7), 7.02 (br, NH), 7.35–7.50 (m, 6H) and 7.66–7.90 (m, 5H) (H 8 and 2 x Ph); MS (EI) *m/z* 386 (M, 23%), 281 (100), 257 (20), 239 (34), 105 (59), 77 (40); anal $C_{23}H_{22}N_4O_2$.

3-Benzamidomethyl-2-(4-methylphenyl)-6-propoxyimidazo-[1,2-b]pyridazine ***4n***. 2-(4-Methylphenyl)-6-propoxyimidazo[1,2-*b*]pyridazine **3h** (0.16 g, 0.0006 mol) and *N*-(hydroxymethyl)benzamide (0.091 g, 0.0006 mol) gave **4n** as yellow crystals (0.10 g, 42%): mp 195–197 °C (from toluene); ^{1}H-NMR δ 1.02 (t, 3H, J = 7 Hz, CH_3CH_2), 1.73 (m, 2H, CH_3CH_2), 2.38 (s, 3H, CH_3C), 4.26 (t, 2H, J = 7 Hz, CH_2O), 5.17 (d, 2H, J = 5.5 Hz, CH_2N), 6.65 (d, 1H, J = 9.5 Hz, H 7), 7.01 (br, NH), 7.18–7.48 (m, 5H) and 7.65–7.87 (m, 4H) (H 2',3',5',6' and Ph), 7.70 (d, 1 H, J = 9.5 Hz, H 8); MS (EI) *m/z* 400 (M, 22%), 295 (100), 253 (22), 105 (32); anal $C_{24}H_{24}N_4O_2$.

3-Benzamidomethyl-2-(3,4-methylenedioxyphenyl)-6-propoxy-imidazo[1,2-b]pyridazine ***4o***. 2-(3,4-Methylenedioxyphenyl)-6-propoxyimidazo[1,2-*b*]pyridazine **3i** (0.15 g, 0.0005 mol) and *N*-(hydroxymethyl)benzamide (0.76 g, 0.0005 mol) afforded **4o** as yellow crystals (0.08 g, 36%): mp 219–220 °C (from toluene); ^{1}H-NMR δ 1.02 (t, 3H, J = 7 Hz, CH_3CH_2), 1.83 (complex, 2H, CH_3CH_2), 4.27 (t, 2H, J = 7 Hz, CH_2O), 5.13 (d, 2H, J = 5.5 Hz, CH_2N), 5.98 (s, 2H, OCH_2O), 6.67 (d, 1H, J = 9.5 Hz, H 7), 6.98 (br, NH), 6.87 (d, 1H, J = 8.5 Hz), 6.95–7.47 (m, 5H) and 7.78–7.88 (m, 2H) (H 2',5',6' and Ph), 7.71 (d, 1H, J = 9.5 Hz, H 8); MS (EI) *m/z* 430 (M, 45%), 325 (100), 297 (39), 283 (27), 105 (59), 77 (30); anal $C_{24}H_{22}N_4O_4$.

3-Benzamidomethyl-2-(4-chlorophenyl)-6-propoxyimidazo[1,2-b]-pyridazine ***4p***. 2-(4-Chlorophenyl)-6-propoxyimidazo[1,2-*b*]-pyridazine **3j** (0.17 g, 0.0006 mol) and *N*-(hydroxymethyl)-benzamide (0.091 g, 0.0006 mol) gave **4p** as white crystals (0.13 g, 52%): mp 218–220 °C (from toluene); ^{1}H-NMR δ 1.04 (t, 3H, J = 7 Hz, CH_3), 1.81 (m, 2H, CH_3CH_2), 4.28 (t, 2H, J = 7 Hz, CH_2O), 5.15 (d, 2H, J = 5.5 Hz, CH_2N), 6.71 (d, 1H, J = 9.5 Hz, H 7), 6.99 (br, NH), 7.35–7.48 (m, 5H) and 7.68–7.89 (m, 5H) (H 8,2',3',5',6' and Ph); MS (EI) *m/z* 420 (M, 30%), 315 (100), 273 (30), 105 (53), 77 (22); anal $C_{23}H_{21}N_4O_2Cl$.

3-(2-Fluorobenzamidomethyl)-2-phenyl-6-propoxyimidazo[1,2-b]-pyridazine ***4q***. 2-Phenyl-6-propoxyimidazo[1,2-*b*]pyridazine **3g** (0.15g, 0.0006 mol) and 2-fluoro-*N*-(hydroxymethyl)benzamide (0.10 g, 0.0006 mol) afforded **4q** as yellow crystals (0.13 g, 54%): mp 185–187 °C (from toluene); ^{1}H-NMR δ 1.06 (t, 3H, J = 7 Hz, CH_3), 1.87 (m, 2H, CH_3CH_2), 4.34 (t, 2H, J = 7 Hz, CH_2O), 5.22 (dd, 2H, J = 5.5 Hz, 1.5 Hz, CH_2NH), 6.74 (d, 1H, J = 9.5 Hz, H 7), 6.95–7.60 (m, 6H) and 7.92–8.26 (m, 3H) (H 3',4',5',6' and Ph), 7.83 (d, 1H, J = 9.5 Hz, H 8); MS (EI) *m/z* 404 (M, 22%), 281 (100), 239 (24), 123 (48); anal $C_{23}H_{21}N_4O_2F$.

3-(2-Fluorobenzamidomethyl)-2-(4-methylphenyl)-6-propoxy-imidazo[1,2-b]pyridazine ***4r***. 2-(4-Methylphenyl)-6-propoxy-imidazo[1,2-*b*]pyridazine **3h** (0.16 g, 0.0006 mol) and 2-fluoro-*N*-(hydroxymethyl)benzamide (0.10 g, 0.0006 mol) provided **4r** as a cream-coloured solid (0.21 g, 84%): mp 180–181 °C (from methanol); ^{1}H NMR δ 1.05 (t, 3H, J = 7 Hz, CH_3CH_2), 1.86 (m, 2H, CH_3CH_2), 2.40 (s, 3H, CH_3C), 4.33 (t, 2H, J = 7 Hz, CH_2O), 5.21 (dd, 2H, J = 5.5 Hz, 1.5 Hz, CH_2N), 6.72 (d, 1H, J = 9.5 Hz, H7), 6.94–7.50 (m, 5H), 7.75–7.89 (m, 3H) and 8.05–8.25 (m, 1H) (H 8,3',4',5',6' and 2",3",5",6"); MS (EI) *m/z* 418 (M, 36%), 295 (100), 253 (31), 123 (62); anal $C_{24}H_{23}N_4O_2F$.

3-Benzamidomethyl-6-(2-methoxyethoxy)-2-phenylimidazo-[1,2-b]pyridazine ***4s***. 6-(2-Methoxyethoxy)-2-phenylimidazo-[1,2-*b*]pyridazine **3k** (0.13 g, 0.0005 mol) and *N*-(hydroxymethyl)benzamide (0.076 g, 0.0005 mol) gave **4s** as cream-coloured needles (0.08 g, 40%): mp 188-189 °C (from toluene); ^{1}H-NMR δ 3.40 (s, 3 H, CH_3O), 3.68–3.79 (m, 2H, CH_3OCH_2), 4.45–4.52 (m, 2H, $CH_3OCH_2CH_2$), 5.17 (d, 2H, J = 5.5 Hz, CH_2N), 6.73 (d, 1H, J = 9.5 Hz, H7), 6.95 (br, NH), 7.34–7.50 (m, 6 H) and 7.75–7.89 (m, 4 H) (2 × Ph), 7.72 (d, 1H, J = 9.5 Hz, H8); MS (EI) *m/z* 402 (M, 21%), 297 (100), 239 (33), 105 (43), 77 (23); anal $C_{23}H_{22}N_4O_3$.

3-Benzamidomethyl-6-(2-methoxyethoxy)-2-(4-methyl-phenyl)imidazo[1,2-b]pyridazine ***4t***. 6-(2-Methoxyethoxy)-2-(4-methylphenyl)imidazo[1,2-*b*]pyridazine **3l** (0.17 g, 0.0006 mol) and *N*-(hydroxymethyl)benzamide (0.091 g, 0.0006 mol) afforded **4t** as a white solid (0.09 g, 36%): mp 187–189 °C (from toluene); ^{1}H-NMR δ 2.38 (s, 3 H, CH_3C), 3.40 (s, 3H, CH_3O), 3.68–3.79 (m, 2H, CH_3OCH_2), 4.41–4.52 (m, 2 H, $CH_3OCH_2CH_2$), 5.16 (d, 2 H, J = 5.5 Hz, CH_2N), 6.72 (d, 1H, J = 9.5 Hz, H7), 6.90 (br, NH), 7.18–7.47 (m, 5H) and 7.75–7.86 (m, 4H) (H 2',3',5',6' and Ph), 7.72 (d, 1H, J = 9.5 Hz, H8); MS (EI) *m/z* 416 (M, 17%), 311 (100), 253 (30), 105 (38), 77 (18); anal $C_{24}H_{24}N_4O_3$.

3-Benzamidomethyl-6-(2-ethoxyethoxy)-2-phenylimidazo[1,2-b]-pyridazine ***4u***. 6-(2-Ethoxyethoxy)-2-phenylimidazo[1,2-*b*]-pyridazine **3m** (0.14 g, 0.0005 mol) and *N*-(hydroxymethyl)-benzamide (0.076 g, 0.0005 mol) gave **4u** as cream-coloured crystals (0.10 g, 48%): mp 195–197 °C (from toluene); ^{1}H-NMR δ 1.22 (t, 3H, J = 7 Hz, CH_3), 3.55 (q, 2H, J = 7 Hz, CH_3CH_2), 3.73–3.83 (m, 2H, $CH_3CH_2OCH_2$), 4.41–4.51 (m, 2H, $CH_3CH_2OCH_2CH_2$), 5.16 (d, 2H, J = 5.5 Hz, CH_2N), 6.72 (d, 1H, J = 9.5 Hz, H7), 7.01 (br, NH), 7.35–7.47 (m, 6H) and 7.66–7.87 (m, 5H) (H 8 and 2 × Ph); MS (EI) *m/z* 416 (M, 21%), 325 (20), 311 (100), 239 (48), 105 (54), 77 (26); anal $C_{24}H_{24}N_4O_3$.

6-Methoxy-2-(3,4-methylenedioxyphenyl)-3-(3-nitrobenzami-domethyl)imidazo[1,2-b]pyridazine ***4v***. 6-Methoxy-2-(3,4-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazine **3a** (0.40 g, 0.0015 mol) and *N*-(hydroxymethyl)-3-nitrobenzamide (0.44 g, 0.00225 mol) afforded **4v** as brown crystals (0.13 g, 19%): mp 252–254 °C (from toluene); ^{1}H-NMR δ 4.04 (s, 3H, CH_3O), 5.20 (d, 2H, J = 5.5 Hz, CH_2N), 6.00 (s, 2H, OCH_2O), 6.74 (d, 1H, J = 9.5 Hz, H7), 6.89 (d, 1H, J = 9 Hz), 7.35–7.65 (m, 3H) 8.17–8.40 (m, 3H) (H 2',5',6',4",5",6"), 7.77 (d, 1H, J = 9.5 Hz, H8), 8.59 (br s, 1H, H2"). MS (EI) *m/z* 447 (M, 28%), 297 (100), 149 (31), 91 (32), 57 (43); anal $C_{22}H_{17}N_5O_6$.

6-Methoxy-2-(3,4-methylenedioxyphenyl)-3-(4-nitrobenzami-domethyl)imidazo[1,2-b]pyridazine ***4w***. 6-Methoxy-2-(3,4-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazine **3a** (0.40 g, 0.0015 mol) and *N*-(hydroxymethyl)-4-nitrobenzamide (0.44 g, 0.00225 mol) gave **4w** as a yellow solid (0.11 g, 16%): mp 280–282 °C (from toluene); ^{1}H NMR δ 4.01 (s, 3H, CH_3), 5.19 (d, 2H, J = 5.5 Hz, CH_2N), 6.00 (s, 2H, OCH_2O), 6.67–8.32 (complex, 9H, H7,8,2',5',6',2",3",5",6"). MS (EI) *m/z* 447 (M, 55%), 297 (100), 273 (52), 245 (42), 57 (34); anal $C_{22}H_{17}N_5O_6$.

3,6-Dimethoxy-2-(4-methylphenyl)imidazo[1,2-b]pyridazine ***8*** *and 3-methoxy-5-methyl-2-(4-methylphenyl)imidazo[1,2-b]-pyridazin-6(5H)-one* ***9***. *Procedure g*

Methylation with diazomethane. A mixture of 6-hydroxypyridazin-3-amine hydrochloride (0.10 g, 0.00068 mol) and 4-methylphenylglyoxal hydrate (0.11 g, 0.00066 mol) in ethanol

(10.0 mL) with concentrated hydrochloric acid (0.07 mL) was refluxed with stirring in an oil bath at 95 °C for 17 h. Excess diazomethane and solvents were then evaporated and the residue subjected to tlc (alumina; chloroform / light petroleum, 1:1). The product (0.054 g, 34%) in the light fluorescent band at higher R_f was recrystallized from light petroleum and gave 3,6-dimethoxy-2-(4-methylphenyl)imidazo[1,2-*b*]pyridazine **8** as a yellow solid: mp 93–95 °C, ^{1}H-NMR spectrum identical to that of the compound described from 6-methoxypyridazin-3-amine **2a** [33].

The product (0.047 g, 29%) in the fluorescent band at lower R_f was recrystallized from light petroleum to give 3-methoxy-5-methyl-2-(4-methylphenyl)imidazo[1,2-*b*]pyridazin-6(5*H*)-one **9** as a yellow solid: mp 140–142 °C; ^{1}H-NMR δ 2.39 (s, 3H, CH_3C), 3.89 (s, 3 H, NCH_3), 4.03 (s, 3H, CH_3O), 6.51 (d, 1H, J = 10 Hz, H7), 7.26 (d, 2H, J = 8.5 Hz, H2',6' (3',5')), 7.49 (d, 1H, J = 10 Hz, H8), 7.80 (d, 2H, J = 8.5 Hz, H3',5'(2',6')). MS (EI) *m/z* 269 (M, 57%), 226 (100), 198 (20), 119 (27), 80 (18); anal $C_{15}H_{15}N_3O_2$.

Methylation with methyl iodide. 6-Hydroxypyridazin-3-amine hydrochloride (0.1 g, 0.00068 mol) and 4-methylphenylglyoxal hydrate (0.11 g, 0.00066 mol) were condensed as in *Procedure g* and the product stirred in dimethylformamide (3.0 mL) with potassium carbonate (0.23 g, 0.0018 mol) and methyl iodide (0.48 g, 0.21 ml, 0.00338 mol) at 20 °C for 24 h. The mixture was diluted with water and extracted with ether. The product obtained was washed several times with water and then subjected to tlc (alumina; chloroform). It gave **8** (0.029 g, 18%) and **9** (0.008 g, 5%), identical (^{1}H-NMR) with the products isolated above *(Methylation with diazomethane).*

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