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Il Farmaco 56 (2001) 771–778

IL FARMACO

Synthesis of 11-aryl-5*H*-imidazo[2,1-*c*][1,4]benzodiazepines and their benzodiazepine and A_1 adenosine binding activity

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Received 16 June 2000; accepted 30 May 2001

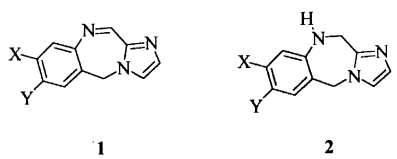
Abstract

In the context of a research program aimed at elucidating the properties of the 5*H*-imidazo[2,1-*c*][1,4]benzodiazepine system, a series of 11-aryl-5*H*-imidazo[2,1-*c*][1,4]benzodiazepines (**3a**–**i**) and their 10,11-dihydro-derivatives (**4a**–**i**) has been synthesized. The synthetic strategy includes the preparation of the aryl-[1-(2-nitrobenzyl)-1*H*-imidazol-2-yl]methanones (**5a**–**i**) followed by their reduction and subsequent cyclization. Affinities of compounds **3a**–**i** and **4a**–**i** for central benzodiazepine as well as for adenosine A_1-receptors were determined by radioligand binding assays. Among the unsaturated analogues, the highest activity at both receptors is displayed by 11-(2-thienyl) derivative **3e**. The hydrogenated analogues **4a**–**i** do not exhibit considerable binding affinity either for central benzodiazepine or for adenosine A_1-receptors. © 2001 Elsevier Science S.A. All rights reserved.

Keywords: Imidazobenzodiazepines; Benzodiazepine receptors; Adenosine receptors; Radioligand binding

1. Introduction

Classic benzodiazepine derivatives have attracted the attention of researchers owing to their interesting pharmacological activities and their low toxicity. They are widely used as anxiolytics, antidepressants, hypnotics, anticonvulsants and muscle relaxants. Annelation of the 1,4-benzodiazepine nucleus, formally obtained by fusion of a five-membered heterocycle on the diazepine skeleton, led to a number of compounds sharing attractive pharmacological properties [1].



1 **2**

Fig. 1.

As a further development on this area, we previously reported the synthesis of the novel 5*H*-imidazo[2,1-*c*][1,4]benzodiazepine tricyclic ring system [2].

Derivatives of type **1** and **2** (Fig. 1) were subjected to investigations to evaluate their pharmacological properties. Some of the tested compounds were comparable to chlordiazepoxide in sedative and muscle-relaxant activities, but did not show considerable binding affinity for central benzodiazepine receptors (BzR) [3].

The effects exerted in vivo by these compounds led us to proceed with further investigations in this field. In order to evaluate the effect of an additional aromatic or heteroaromatic group, a series of 11-aryl-5*H*-imidazo[2,1-*c*][1,4]benzodiazepines (**3a**–**i**) and their 10,11-dihydro-derivatives (**4a**–**i**) have been designed and synthesized (Fig. 2).

As preliminary in vivo assays (data not shown) indicated that these compounds were also able to induce sedative and muscle relaxant effects on mice, the title compounds were first studied for their interaction with BzR.

Furthermore, we chose to evaluate the affinity of the title compounds also towards the adenosine A_1-receptor. In fact, besides the GABA receptor system, the

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PII: S0014-827X(01)01133-8

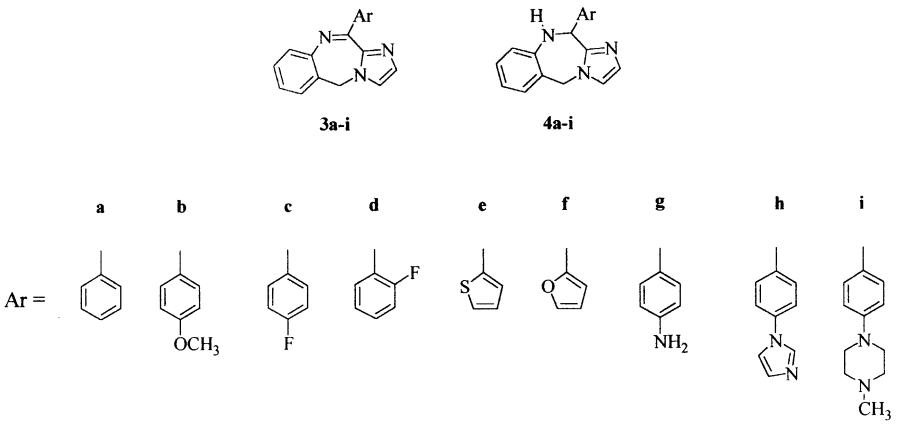


Fig. 2. Structures of 11-aryl-5*H*-imidazo[2,1-*c*][1,4]benzodiazepines (**3a**–**i**) and 10,11-dihydro-derivatives (**4a**–**i**).

purinergic system has also been involved in the central mechanism of action of benzodiazepines [4].

2. Chemistry

The 11-aryl-imidazobenzodiazepines **3a**–**i** and **4a**–**i** (Table 2) were obtained by reduction and subsequent cyclization of the corresponding aryl-[1-(2-nitrobenzyl)-1*H*-imidazol-2-yl]methanones (**5a**–**i**) (Table 1) obtained as reported below. In particular, compounds **3a**–**f**,**h**,**i** were obtained by reduction of **5a**–**f**,**h**,**i** with iron(II) sulfate and ammonium hydroxide [2]. The 10,11-dihydroderivatives **4a**–**f**,**h**,**i** were prepared by reducing the nitro-methanones **5a**–**f**,**h**,**i** with iron powder in boiling glacial acetic acid [5] (Scheme 1).

When this method was applied to **5g**, the 11-(4-acetylaminophenyl)-5*H*-imidazo[2,1-*c*][1,4]benzodiazepine (**6**) was obtained, which, by hydrolysis with 20% NaOH, furnished **3g**. Compound **4g** was prepared through the reductive cyclization of **5g** with titanium trichloride [6] (Scheme 2).

The aryl-[1-(2-nitrobenzyl)-1*H*-imidazol-2-yl]methanones **5a**–**g** were obtained by reacting the 1-(2-nitrobenzyl)-1*H*-imidazole (**7**) [2] with the suitable aroyl chlorides in the presence of triethylamine (Scheme 3). Synthesis of **5h** was carried out reacting **5c** with imidazole in the presence of sodium hydride. Compound **5i** was obtained from **5c** by using an excess of methylpiperazine (Scheme 4).

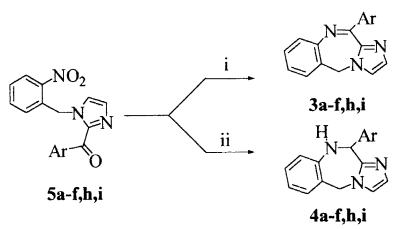
3. Results

*3.1. Biological evaluation of compounds **3a–i** and **4a–i** as ligands for benzodiazepine binding sites*

All compounds were screened at four concentrations (1, 10, 30, 100 μM) in radioligand binding assay for their potency to displace [^{3}H]flumazenil (at 0.5 nM) from its binding site at rat brain cortical membranes. Screening was repeated at six concentrations (1, 3, 10, 30, 100, 300 μM) for compounds **3a**,**d**–**f**, which presented at 100 μM a percentage of displacement higher than 50% and the K_i values were calculated. The affinity for the benzodiazepine recognition site was then investigated in the presence of GABA and the GABA shift (K_i compound/K_i compound + GABA) was determined (Table 3).

*3.2. Biological evaluation of compounds **3a–i** and **4a–i** as ligands for A_1-adenosine binding sites*

The title compounds were further investigated at three concentrations (1, 10, 100 μM) in radioligand binding assay for affinity at A_1-adenosine receptors in rat brain cortical membrane preparations. The A_1-selective agonist [^{3}H]2-chloro-N6-cyclopentyladenosine (CCPA) was used at 1 nM as ligand. Screening was repeated at seven concentrations (10, 30, 50, 100, 300, 500, 750 μM) for compounds **3a**,**e**, which presented at 100 μM a percentage of displacement higher than 40% and the K_i values were calculated (Table 4).



Scheme 1. Synthesis of compounds **3a**–**f**,**h**,**i** and **4a**–**f**,**h**,**i**. (i) $FeSO_4 \cdot 7H_2O$, NH_4OH, H_2O, EtOH; (ii) Fe, AcOH.

Table 1
Yields, physical, analytical and spectroscopic data of compounds **5a–i**

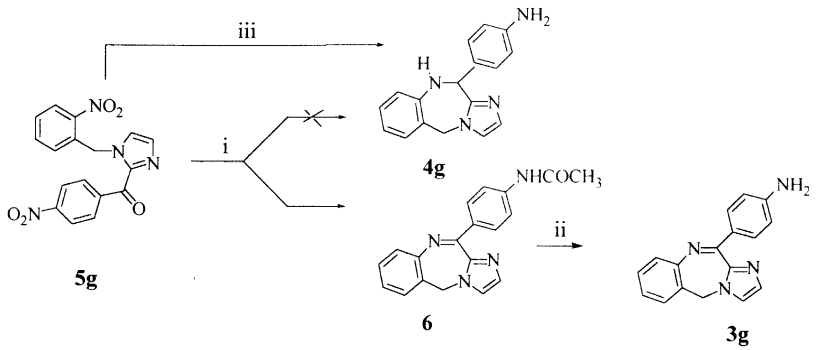
No.	Formula (MW)	Yield (%)	M.p. (°C) crystallization solvent	MS m/z [$M+$]	IR (cm^{-1})ν(C=O)	^{1}H NMR: δ (ppm), (J in Hz)
5a	$C_{17}H_{13}N_3O_3$ (307.30)	52	90 benzene: petroleum ether	307	1641	6.06 (s, 2H, CH_2), 6.74–7.78 (m, 1H, ArH), 7.22–7.63 (m, 7H, ArH), 8.14–8.28 (m, 3H, ArH).
5b	$C_{18}H_{15}N_3O_4$ (337.33)	68	80–82 ethanol	337	1625	3.83 (s, 3H, CH_3), 6.08 (s, 2H, CH_2), 6.73–6.81 (m, 1H, ArH), 7.29–7.62 (m, 4H, ArH), 8.19 (dd, $J = 8.0$ and 1.7, 1H, ArH), 8.30 (d, $J = 8.9$, 2H, ArH), 8.45 (d, $J = 8.9$, 2H, ArH).
5c	$C_{17}H_{12}N_3FO_3$ (325.29)	65	95 ethanol	325	1638	6.05 (s, 2H, CH_2), 6.72–6.79 (m, 1H, ArH), 7.09–7.60 (m, 6H, ArH), 8.19 (dd, $J = 8.1$ and $J = 1.5$, 1H, ArH), 8.31–8.42 (m, 2H, ArH).
5d	$C_{17}H_{12}N_3FO_3$ (325.29)	80	92–93 benzene: petroleum ether	325	1648	6.10 (s, 2H, CH_2), 6.74–6.81 (m, 1H, ArH), 7.08–7.73 (m, 8H, ArH), 8.18 (dd, $J = 7.9$ and $J = 1.5$, 1H, ArH).
5e	$C_{15}H_{11}N_3O_3S$ (313.33)	70	98–99 benzene: petroleum ether	313	1608	6.09 (s, 2H, CH_2), 6.67–6.75 (m, 1H, H benzene), 7.11–7.22 (m, 2H, H thiophene and H imidazole), 7.35–7.58 (m, 3H, H thiophene and H benzene), 7.71 (dd, $J = 4.8$ and $J = 1.0$, 1H, H thiophene), 8.19 (dd, $J = 7.6$ and $J = 1.6$, 1H, H benzene), 8.46 (dd, $J = 3.0$ and $J = 1.0$, 1H, H thiophene).
5f	$C_{15}H_{11}N_3O_4$ (297.27)	90	103–104 ethanol	297	1631	6.07 (s, 2H, CH_2), 6.58–6.62 (m, 1H, H furane), 6.68–6.76 (m, 1H, H benzene), 7.22 (s, 1H, H imidazole), 7.35 (s, 1H, H imidazole), 7.39–7.58 (m, 2H, H benzene), 7.64–7.68 (m, 1H, H furane), 8.08–8.20 (m, 2H, H benzene and H furane).
5g	$C_{17}H_{12}N_4O_5$ (352.30)	44	143–144 benzene: petroleum ether	352	1641	6.08 (s, 2H, CH_2), 6.73–6.82 (m, 1H, ArH), 7.28–7.62 (m, 4H, ArH), 8.21 (dd, $J = 8.0$ and $J = 1.7$, 1H, ArH), 8.30 (d, $J = 8.9$, 2H, ArH), 8.44 (d, $J = 8.9$, 2H, ArH).
5h	$C_{20}H_{15}N_5O_3$ (373.37)	28 [a]	120–123 ethanol: water	373	1637	6.05 (s, 2H, CH_2), 6.72–6.78 (m, 1H, ArH), 7.25–7.60 (m, 8H, ArH), 7.94 (s, 1H, ArH), 8.17 (dd, $J = 7.9$ and $J = 1.5$, 1H, ArH), 8.42 (d, $J = 8.5$, 2H, ArH).
5i	$C_{22}H_{23}N_5O_3$ (405.45)	64 [a]	123–125 benzene: petroleum ether	405	1622	2.35 (s, 3H, CH_3), 2.51–2.58 (m, 4H, H piperazine), 3.35–3.42 (m, 4H, H piperazine), 6.03 (s, 2H, CH_2), 6.72–6.79 (m, 1H, ArH), 6.88 (d, $J = 9.0$, 2H, ArH), 7.12–7.54 (m, 4H, ArH), 8.17 (dd, $J = 7.8$ and 1.5, 1H, ArH), 8.28 (d, $J = 9.0$, 2H, ArH).

[a] From **5c**.

Table 2
Yields, physical, analytical and spectroscopic data of compounds **3a–i** and **4a–i**

No.	Formula (MW)	Yield %	M.p. (°C) crystallization solvent	MS m/z $[M+]$	^{1}H NMR: δ (ppm), (J in Hz)
3a	$C_{17}H_{13}N_3$ (259.31)	95 [a]	180 ethanol:water	259	5.00 (s, 2H, CH_2), 7.10–7.67 (m, 9H, ArH), 8.15–8.40 (m, 2H, ArH).
3b	$C_{18}H_{15}N_3O$ (289.33)	79 [b]	158 ethanol:water	289	3.85 (s, 3H, CH_3), 4.98 (s, 2H, CH_2), 6.98 (d, $J = 8.9$, 2H, ArH), 7.07 (s, 1H, H imidazole), 7.15–7.51 (m, 5H ArH), 8.12 (d, $J = 8.9$, 2H, ArH).
3c	$C_{17}H_{12}N_3F$ (277.30)	81 [b]	144–145 ethanol:water	277	5.02 (s, 2H, CH_2), 7.08–7.52 (m, 8H, ArH), 8.10–8.22 (m, 2H, ArH).
3d	$C_{17}H_{12}N_3F$ (277.30)	81 [b]	206–208 ethanol:water	277	5.06 (s, 2H, CH_2), 7.04–7.58 (m, 9H, ArH), 7.86–7.98 (m, 1H, ArH).
3e	$C_{15}H_{11}N_3S$ (265.34)	98 [a]	164–165 ethanol	265	5.01 (s, 2H, CH_2), 7.08–7.56 (m, 8H, ArH), 7.98–8.02 (m, 1H, ArH).
3f	$C_{15}H_{11}N_3O$ (249.27)	98 [a]	151–152 ethanol	249	5.00 (s, 2H, CH_2), 6.58 (m, 1H, H furane), 7.10 (s, 1H, H imidazole), 7.20–7.58 (m, 6H, H benzene, H imidazole and H furane), 7.71 (m, 1H, H furane).
3g	$C_{17}H_{14}N_4$ (274.33)	99 [a,c]	220–222 benzene	274	3.97 (s, 2H, disappears with D_2O, NH_2), 5.00 (s, 2H, CH_2), 6.73 (d, $J = 8.7$, 2H, ArH), 7.07–7.5 (m, 6H, ArH), 7.99 (d, $J = 8.7$, 2H, ArH).
3h	$C_{20}H_{15}N_5$- (325.38)	52 [b]	231–233 benzene: petroleum ether	325	5.08 (s, 2H, CH_2), 7.12–7.57 (m, 10H, ArH), 7.94 (s, 1H, H imidazole), 8.28 (d, $J = 8.8$, 2H, ArH).
3i	$C_{22}H_{23}N_5$ (357.47)	80 [b]	208–210 benzene: ligroin	357	2.36 (s, 3H, CH_3), 2.54–2.62 (m, 4H, H piperazine), 3.33–3.39 (m, 4H, H piperazine), 5.00 (s, 2H, CH_2), 6.96 (d, $J = 8.9$, 2H, ArH), 7.06–7.50 (m, 6H, ArH), 8.07 (d, $J = 8.9$, 2H, ArH).
4a	$C_{17}H_{15}N_3$ (261.33)	98 [a]	225 benzene	261	4.37 (d, $J = 4.8$, 1H, disappears with D_2O, NH), 4.77 (d, $J = 14.8$, 1H, C*H*H), 5.03 (d, $J = 14.8$, 1H, C*H*H), 5.80 (d, $J = 4.8$, 1H, CH), 6.74–7.37 (m, 11H, ArH).
4b	$C_{18}H_{17}N_3O$ (291.35)	98 [a]	223–224 ethanol	291	3.77 (s, 3H, OCH_3), 4.30 (d, $J = 4.1$, 1H, disappears with D_2O, NH), 4.80 (d, $J = 14.8$, 1H, C*H*H), 5.03 (d, $J = 14.8$, 1H, C*H*H), 5.72 (d, $J = 4.1$, 1H, CH), 6.72–7.29 (m, 10H, ArH).
4c	$C_{17}H_{14}N_3F$ (279.31)	98 [a]	141–142 benzene	279	4.31 (d, $J = 4.1$, 1H, disappears with D_2O, NH), 4.82 (d, $J = 14.7$, 1H, C*H*H), 5.04 (d, $J = 14.7$, 1H, C*H*H), 5.76 (d, $J = 4.1$, 1H, CH), 6.74–7.36 (m, 10H, ArH).
4d	$C_{17}H_{14}N_3F$ (279.31)	98 [a]	173–174 ethanol	279	4.04 (d, 1H, $J = 3.6$, disappears with D_2O, NH), 5.08–5.24 (m, 2H CH_2), 6.12 (d, $J = 3.6$, 1H, CH), 6.65–7.29 (m, 10H, ArH).
4e	$C_{15}H_{13}N_3S$ (267.34)	66 [b]	184–185 ethyl acetate:petroleum ether	267	4.50 (d, $J = 4.6$, 1H, disappears with D_2O, NH), 4.82 (d, $J = 14.6$, 1H, C*H*H), 5.14 (d, $J = 14.6$, 1H, C*H*H), 6.05 (d, $J = 4.6$, 1H, CH), 6.75–7.20 (m, 9H, ArH).
4f	$C_{15}H_{13}N_3O$ (251.28)	60 [b]	167–171 benzene:ligroin	251	4.35 (d, $J = 4.3$, 1H, disappears with D_2O, NH), 5.00–5.12 (m, 2H, CH_2), 5.86 (d, $J = 4.3$, 1H, CH), 6.06 (m, 1H, H furane), 6.25 (m, 1H, H furane), 6.74–7.20 (m, 6H, H imidazole and H benzene), 7.31 (m, 1H, H furane).
4g	$C_{17}H_{16}N_4$ (276.34)	96 [a]	168–171 toluene: petroleum ether	276	3.70 (br s, 2H, disappears with D_2O, NH_2), 4.30 (br s, 1H disappears with D_2O, NH), 4.79 (d, $J = 14.7$, 1H, C*H*H), 5.05 (d, $J = 14.7$, 1H, C*H*H), 5.66 (br s, 1H, CH), 6.58–7.30 (m, 10H, ArH).
4h	$C_{20}H_{17}N_5$ (327.38)	60 [b]	220–222 toluene: petroleum ether	327	4.32 (d, $J = 3.8$, 1H disappears with D_2O, NH), 4.86 (d, $J = 14.8$, 1H, C*H*H), 5.08 (d, $J = 14.8$, 1H, C*H*H), 5.81 (d, $J = 3.8$, 1H, CH), 6.65–7.25 (m, 12H, ArH), 7.81 (s, 1H, H imidazole).
4i	$C_{22}H_{25}N_5$ (359.47)	83 [b]	148–150 ethyl acetate: petroleum ether	359	2.34 (s, 3H, CH_3), 2.54–2.60 (m, 4H, H piperazine), 3.18–3.25 (m, 4H, H piperazine), 4.33 (d, $J = 4.0$, 1H, disappears with D_2O, NH), 4.80 (d, $J = 14.7$, 1H, C*H*H), 5.06 (d, $J = 14.7$, 1H, C*H*H), 5.70 (d, $J = 4.0$, 1H, CH), 6.70–7.61 (m, 10H, ArH).

[a] Purified by recrystallization.
[b] Purified by column chromatography on alumina (eluent: chloroform).
[c] Yield of hydrolysis.



Scheme 2. Synthesis of compound **3g** and compound **4g** (i) Fe, AcOH; (ii) NaOH 20%; (iii) $TiCl_3$, HCl, H_2O, AcOH.

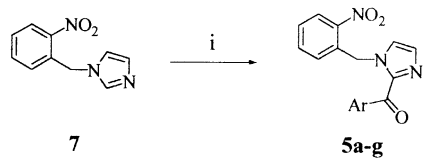
4. Discussion

The binding results show that the 10,11-dihydro-11-aryl-5*H*-imidazo[2,1-*c*][1,4]benzodiazepines **4a**–**i** do not exhibit noticeable binding affinity for central benzodiazepine receptors. Among the dehydrogenated analogues, derivatives with unsubstituted aryl moiety at position 11 (**3a**,**e**,**f**) exhibited a benzodiazepine affinity ranging from 14.0 to 47.7 μM (Table 3). The 11-(*o*-fluoro)phenyl derivative **4d** displayed a moderate increase in binding activity in comparison to the unsubstituted parent compound **4a**, whereas *para* substitution of the phenyl ring is endowed with a drastic decrease in affinity. In opposition, in compounds lacking the aryl moiety at position 11, only two hydrogenated derivatives of type **2** ($X = Y = H$ and $X = Y = OCH_3$) showed some affinity, with IC_{50} values of 22 and 14 μM, respectively (IC_{50} diazepam = 0.05 μM) [3].

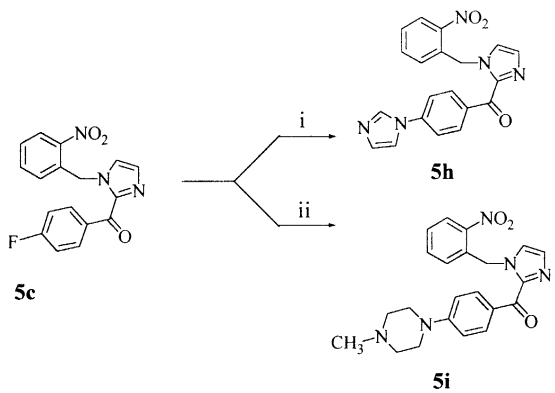
Since GABA shift values of the compounds **3a**,**d**–**f** are higher than 1, it can be assumed that they may act at benzodiazepine receptor as agonists. In fact the GABA shift indicates a different trend in the efficacy of a benzodiazepine receptor ligand: in this experiment the affinity for the benzodiazepine recognition site is determined in the presence and in the absence of GABA. The addition of GABA increases the affinity of various benzodiazepine receptor agonists while the potency of various antagonists remained unaltered [7,8].

Table 4 shows that, among all the tested compounds the highest affinity towards adenosine A_1-receptor is displayed by compound **3e**, with a K_i value of 7.07 μM. The same compound was the most active at the benzodiazepine receptor. Compound **3a**, bearing a 11-unsubstituted phenyl ring, exhibits a K_i value of 15.17 whereas all the other compounds, both saturated and unsaturated, do not display notable binding activity.

Taken together, the results of the binding studies do not appear to support the hypothesis of a direct involvement of benzodiazepine- or adenosine A_1-receptors in mediating the behavioral changes induced by administration of the tested compounds. However, an involvement of the purinergic system in the mechanism of action of these compounds can still be put forward



Scheme 3. Synthesis of aryl-[1-(2-nitrobenzyl)-1*H*-imidazol-2-yl]methanones **5a**–**g** (i) ArCOCl, $N(C_2H_5)_3$, acetonitrile.



Scheme 4. Synthesis of aryl-[1-(2-nitrobenzyl)-1*H*-imidazol-2-yl]methanones **5h**,**i** (i) NaH, dimethylacetamide (ii) methylpiperazine, 120 °C.

Table 3
Binding constants at benzodiazepine receptor for compounds **3a**,**d**–**f**

Compound	$K_i \pm$ SEM (μM) [a]	GS [b]
3a	47.7 ± 9.33	1.58 ± 0.15
3d	24.7 ± 3.42	1.7 ± 0.2
3e	14.0 ± 0.93	1.43 ± 0.5
3f	33.4 ± 1.02	1.44 ± 0.18
Diazepam	0.0185 ± 0.0025	1.55 ± 0.09

[a] K_i values are means of four determinations.
[b] GABA-shift = K_i compound/K_i (compound + 100 μM GABA) performed in four independent experiments.

Table 4
Binding constants at A_1-adenosine receptor for compounds **3a**,**e**

Compound	K_i (μM) [a]
Theophylline	2.53
3a	15.17
3e	7.07

[a] K_i values are means of two determinations.

on the basis of the ability of benzodiazepine derivatives to bind to the adenosine equilibrative transport system [9–11]. As a consequence of the blockade of adenosine uptake, the extracellular levels of the purine rise, thus allowing activation of the purinergic system-mediated responses. Since this mechanism could at least in part mediate the sedative and muscle relaxant effects, the title compounds are presently under evaluation for their putative ability to interfere with the uptake of adenosine.

5. Experimental

5.1. Chemistry

Melting points were determined on a Büchi 530 apparatus and are uncorrected. Mass spectra data were determined on a V6-Micromass 7070H mass spectrometer. The infrared spectra were recorded on a Jasco (FT/IR-200) spectrophotometer. The ^{1}H NMR spectra were obtained at 200 MHz using a Varian instrument, using $CDCl_3$ as solvent unless indicated, with TMS as internal standard. Alumina chromatography was performed using Merck aluminium oxide 90 (0.063–0.200 mm). Elemental analysis were performed by Dr. Emilio Cebulec at the Chemistry Department of the University of Trieste and the results of the C, H, N determinations were within $\pm 0.4\%$ of those calculated for the corresponding formulae.

5.1.1. General procedure for preparation of the aryl-[1-(2-nitrobenzyl)-1H-imidazol-2-yl]methanones (**5a**–**g**)

A solution of the appropriate aroyl chloride (33.0 mmol) in dry acetonitrile (30 ml) was added to a solution of 1-(2-nitrobenzyl)-1*H*-imidazole (**7**) (6.1 g, 30.0 mmol) and triethylamine (3.34 g, 33.0 mmol) in the same solvent (50 ml). The mixture was stirred at 80 °C overnight and, after cooling at room temperature, the solid was filtered off. The solution was evaporated to give an oil which was treated with water (100 ml) and extracted with chloroform (3 × 50 ml). The combined organic layers were washed with a saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent gave an oil which was purified by chromatography (alumina) with chloroform as eluent. The first eluates were collected and the solvent was evaporated to afford compounds **5a**–**g**. Yields, melting points, analytical and spectroscopic data are reported in Table 1.

5.1.2. [4-(1H-Imidazol-1-yl)phenyl]-[1-(2-nitrobenzyl)-1H-imidazol-2-yl]methanone (**5h**)

To a suspension of sodium hydride (1.08 g, 45.0 mmol) in dry dimethylacetamide (50 ml) a solution of **5c** (9.75 g, 30.0 mmol) and imidazole (3.06 g, 45.0 mmol) in the same solvent (50 ml) was added dropwise; the mixture was stirred at 100 °C for 18 h. The reaction mixture was cooled, poured into water (300 ml), acidified to pH 2.0 with concentrated hydrochloric acid and then extracted with chloroform (3 × 50 ml). The combined organic layers were discarded while the aqueous phase was made alkaline by adding sodium hydrogen carbonate and extracted with chloroform (3 × 50 ml), washed with a saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent gave an oil which was purified by chromatography (alumina) with chloroform as eluent. The first eluates were collected and solvent evaporated to give compound **5h**. Yield, melting point, analytical and spectroscopic data are reported in Table 1.

5.1.3. [4-(4-Methyl-piperazin-1-yl)phenyl]-[1-(2-nitrobenzyl)-1H-imidazol-2-yl]methanone (**5i**)

A mixture of **5c** (3.25 g, 10.0 mmol) and methylpiperazine (5.01 g, 50.0 mmol) was heated at 120 °C for 6 h. After cooling, the reaction mixture was taken up in chloroform (300 ml), washed with a saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent gave an oil which was purified by chromatography (alumina) with chloroform as eluent. The first eluates were collected and solvent evaporated to give compound **5h**. Yield, melting point, analytical and spectroscopic data are reported in Table 1.

5.1.4. General procedure for preparation of the 5H-imidazo [2,1-c][1,4]benzodiazepines (**3a**–**f**,**h**,**i**)

A boiling solution of the suitable aryl-[1-(2-nitrobenzyl)-1*H*-imidazol-2-yl]methanone (**5a**–**f**,**h**,**i**) (4.31 mmol) in ethanol (20 ml) was added to a suspension of iron(II) sulfate heptahydrate (12.0 g, 43.1 mmol) in water (20 ml) and 32% ammonium hydroxide (2 ml). The reaction mixture was heated under reflux for 4 h while 32% ammonium hydroxide was dropped (20 ml). The hot mixture was filtered and the inorganic material was washed with boiling ethanol. The solution was partially evaporated and then extracted with chloroform (3 × 100 ml). The combined organic layers were washed with water followed by a saturated sodium chloride solution, dried over anhydrous sodium sulfate

and evaporated to give compounds **3b**–**d**,**f**,**h**,**i**. For compounds **3a**,**e** the crude residue was dissolved in toluene (100 ml) and refluxed overnight in the presence of *p*-toluensulfonic acid with azeotropic removal of water by a Dean–Stark trap. After cooling the solution was washed with a saturated sodium chloride solution, dried over anhydrous sodium sulfate and evaporated to give compounds **3a**,**e**. Purification methods, yields, melting points, analytical and spectroscopic data are reported in Table 2.

*5.1.5. 11-(4-Aminophenyl)-5H-imidazo[2,1-c]-[1,4]benzodiazepine (**3g**)*

Iron powder (3.40 g, 60.0 mg-atom) was added slowly to a solution of **5g** (1.76 g, 5.00 mmol) in glacial acetic acid (100 ml) and the mixture was refluxed overnight while stirring. After concentration of the solvent under reduced pressure, the residue was taken up with ethyl acetate (100 ml) and water (50 ml). The organic phase was washed twice with water followed by a saturated sodium chloride solution, dried over anhydrous sodium sulfate and evaporated to give the 11-(4-acetylaminophenyl) - 5*H* - imidazo[2,1 - *c*][1,4]benzodiazepine (**6**) (1.17 g, 3.70 mmol, 74% yield), m.p. 242 °C (ethanol); IR (potassium bromide): ν(C=O) 1687, N–H 3067 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.10 (s, 3H, CH_3), 5.22 (s, 2H, CH_2), 7.15–7.80 (m, 10H, ArH), 10.23 (s, 1H, 1H, disappears with D_2O, NH), ms: m/z 316 (M^+). A solution of the acetylderivative **6** (0.50 g, 1.58 mmol) in ethanol (50 ml) was treated with an aqueous solution of 20% sodium hydroxide (3 ml) and the reaction mixture was heated under reflux overnight. After cooling the solution was concentrated under reduced pressure, diluted with water (50 ml) and extracted with chloroform (3 × 50 ml). The combined extracts were washed with water, dried over anhydrous sodium sulfate and evaporated to give **3g**. Purification method, yield, melting point, analytical and spectroscopic data are reported in Table 2.

*5.1.6. General procedure for preparation of the 10,11-dihydro-5H-imidazo [2,1-c][1,4]benzodiazepines (**4a**–**f**,**h**,**i**)*

Iron powder (1.70 g, 30.0 mg-atom) was added slowly to a solution of the suitable aryl-[1-(2-nitrobenzyl)-1*H*-imidazol-2-yl]methanone (**5**) (5.00 mmol) in glacial acetic acid (100 ml) and the mixture was refluxed overnight while stirring. After concentration of the solvent under reduced pressure, the residue was taken up with ethyl acetate (100 ml) and water (50 ml). The organic phase was washed twice with water followed by a saturated sodium chloride solution, dried over anhydrous sodium sulfate and evaporated to give **4a**–**f**,**h**,**i**. Purification methods, yields, melting points, analytical and spectroscopic data are reported in Table 2.

*5.1.7. 10,11-Dihydro-11-(4-aminophenyl)-5H-imidazo-[2,1-c][1,4]benzodiazepine (**4g**)*

A solution of **5g** (1.00 g, 2.84 mmol) in acetic acid–water 1:1 (15 ml) was treated with a 15% titanium trichloride hydrochloric acid solution (39.0 ml, d = 1.20, 16 mol equiv.) and stirred for 10 min at room temperature. The reaction mixture was basified with 15% sodium hydroxide aqueous solution and extracted with dichloromethane–methanol 95:5 (3 × 70 ml). The combined organic layers were washed with water, dried over anhydrous sodium sulfate and evaporated to give **4g**. Purification method, yield, melting point, analytical and spectroscopic data are reported in Table 2.

5.2. Biochemistry

5.2.1. Benzodiazepine binding assay

Affinity for the benzodiazepine recognition site was determined in membranes prepared from rat cerebral cortex according to Lipartiti et al. [12]. In brief, cerebral cortices from adult, male Sprague–Dawley rats were dissected on ice and homogenized in 10 volumes of ice-cold 0.32 M sucrose in a glass homogenized. After centrifugation at 1000 × *g* for 20 min at 4 °C, the pellet was discarded and the supernatant was centrifuged at 20 000 × *g* for 20 min at 4 °C. The resulting pellet was resuspended in ice-cold distilled water and centrifuged at 8000 × *g* for 20 min at 4 °C. The supernatant was collected, centrifuged at 48 000 × *g* for 20 min at 4 °C and the final pellet was stored at −20 °C for at least 24 h. On the day of the assay, the pellet was thawed, suspended in 50 mM Tris–HCl, pH 7.4, centrifuged at 20 000 × *g* for 20 min at 4 °C and resuspended in Tris–HCl pH 7.4 containing 120 mM NaCl and 5 mM KCl.

For binding assay, 0.5 nM [^{3}H]flumazenil (87 Ci/mmol, New England Nuclear, Boston, MA) was incubated in 96-well MultiScreen-FB microtiter plates (Millipore) in 250 μl (total volume) of Tris–HCl pH 7.4 containing 120 mM NaCl and 5 mM KCl. Nonspecific binding was determined in the presence of 3 μM diazepam and was 4–7% of total binding. Test compounds were dissolved in dimethyl sulfoxide and diluted with incubation buffer immediately before use. Final vehicle concentration in incubation wells was maintained constant (1%) in each well. For GABA-shift measurement, 100 μM GABA was included in the incubation mixture. Assays were performed in duplicate. The incubation was started by the addition of 50 μg protein/well, carried out for 60 min at 25 °C and terminated by vacuum filtration on a MultiScreen vacuum manifold (Millipore). Wells were rinsed twice with 200 μl of ice-cold Tris–HCl, 50 mM, pH 7.4 and the flexible plate underdrain was removed. The plate was placed inside the Wallac cassette for Millipore MultiScreen Filtration Plate, SuperMix liquid scintillation

cocktail (25 μl/well) was added and the plate counted in a MicroBeta Trilux liquid scintillation counter (Wallac, Finland).

5.2.2. Adenosine binding assay

Affinity for the adenosine A_1 receptor was determined in membranes prepared from mouse cerebral cortex as previously described [13]. In brief, cerebral cortex from adult, male CBA mice were dissected on ice, homogenized in 10 volumes of ice-cold Tris–HCl 50 mM, pH 7.4 with an Ulta-Turrax and centrifuged at $40\,000 \times g$ for 20 min at 4 °C. The pellet was washed with 10 volumes of Tris–HCl and again centrifuged at $40\,000 \times g$ for 20 min at 4 °C. The final pellet was resuspended in 10 volumes of Tris–HCl and 1 ml aliquots were stored at −80 °C. On the day of the assay, membranes were thawed and incubated for 60 min at 25 °C with 2U/ml adenosine deaminase, diluted and used in the binding assay.

For binding assay, 1 nM [^{3}H]CCPA (42.8 Ci/mmol, New England Nuclear, Boston, MA) was incubated in 96-well MultiScreen-FB microtiter plates (Millipore) in 300 μl (total volume) of Tris–HCl pH 7.4. Nonspecific binding was determined in the presence of 1 mM theophylline and was 5% of total binding. Test compounds were dissolved in dimethyl sulfoxide and diluted with Tris–HCl buffer immediately before use. Final vehicle concentration in incubation wells was maintained constant (1%) in each well. Assays were performed in triplicate. The incubation was started by the addition of 50 μg protein/well, carried out for 180 min at 25 °C and terminated by vacuum filtration as described above.

Protein content was determined by the method of Lowry [14], using bovine serum albumine as a standard. Potencies for displacement (IC_{50} values) were determined with the aid of the computerized program PHARM/PCS using at least six different concentrations for each compound [15].

The binding affinity constants of the compounds (K_i) were calculated according to the following equation [16]: $K_i = IC_{50}/(1 + [L]/K_d$, where [L] is the concentration of the radioligand utilized in binding experiments.

Acknowledgements

Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST) is gratefully acknowledged for its financial support.

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