Acta Poloniae Pharmaceutica – Drug Research, Vol. 58 No. 1 pp. 43–52, 2001

ISSN 0001-6837
Polish Pharmaceutical Society

A STRUCTURE–ACTIVITY RELATIONSHIP STUDY OF THE AFFINITY OF SELECTED IMIDAZO[1,2–*a*]PYRIDINE DERIVATIVES, CONGENERS OF ZOLPIDEM, FOR THE ω_1–SUBTYPE OF THE BENZODIAZEPINE RECEPTOR

JERZY LANGE[1,*], JANINA KAROLAK–WOJCIECHOWSKA[2], KRYSTYNA WEJROCH[1] and SŁAWOMIR RUMP[3]

[1] Chemistry Department, University of Technology, 3 Noakowskiego Str., 00–664 Warsaw, Poland
[2] Institute of General and Ecological Chemistry, Technical University, 36 Żwirki Str., 90–924 Łódź, Poland
[3] Military Institute of Hygiene and Epidemiology, 4 Kozielska Str., 01–163 Warsaw, Poland

Abstract: A series of 6–substituted 2–aryl–*N,N*–dimethylimidazo[1,2–*a*]pyridine–3–acetamides, congeners of zolpidem and alpidem, was synthesized and tested *in vitro* for binding with the benzodiazepine receptor in the competition with ^{3}H–zolpidem as an ω_1–selective radioligand. Molecular electrostatic potential (MEP) and the HOMO and LUMO energies were calculated for the compounds by semi–empirical quantum chemistry methods. The lipophilicity parameter of the compounds, expressed as the logarithm of the octanol–water partition coefficient (log *P*), was calculated; alternatively, standard values of the Hansch hydrophobic substituent constants π were used. In agreement with earlier investigations on the benzodiazepine receptor ligands with a high preference for the ω_1–subtype, a quantitative correlation of the biological data with molecular parameters has revealed a significant dependence (r=0.954) of the binding affinity (IC_{50}) on the deepest MEP minimum, in this case associated with the amide carbonyl oxygen atom. The lipophilicity parameters were found to be of lower significance.

Keywords: imidazopyridines; benzodiazepine receptor ω_1–subtype; QSAR.

The benzodiazepine receptor (BZR) is located on the γ–aminobutyric acid type A ($GABA_A$) receptor channel, the main inhibitory neurotransmitter system of the brain, which also carries other receptor sites able to modulate the channel functions (1–3). A large body of knowledge regarding the molecular composition of $GABA_A$ receptors has been acquired to data. Four types of subunit families have been found in the structure of $GABA_A$, receptor in rodents: six α–subunits (α_1–α_6), three β–subunits (β_1–β_3), three γ–subunits (γ_1–γ_3) and one δ–subunit (4). Different combinations of the subunits induce quite distinct pharmacological responses.

The structure of BZR is not uniform throughout. The results of early radioligand (5–7) and radiohistochemical (8) experiments with 3–methyl–6–(3–trifluoromethylphenyl)triazolo[4,3–*b*]pyridazine (CL 218,872) made it possible to recognize heterogeneity of BZR and to delineate the existence and biological specifity of two receptor subtypes: ω_1 and ω_2, formerly denoted as the type–1 and type–2 BZ receptors, respectively (9). It was supposed that the ω_1 BZR mediated the anxiolytic and anticonvulsant activities, whereas the ω_2 BZR was responsible for the muscle–relaxant and sedative effects of the respective ligands (6). The actual phamacological situation is, however, more complicated, since the data obtained in later investigations demonstrates some sedative properties of typical ω_1 ligands, including CL 218,872, a variety of β–carbolines, and zolpidem (6, 10, 11). Receptor α_1–subunit in combination with β_2– and γ_2–subunits has been found to be the most distinctive element in the isoform construction of the ω_1–subtype (12–14). Other α–subunits in combinations with β_2– and γ_2–subunits are characteristic of the ω_2–subtype (13, 15–18).

Among the known ligands of BZR, two imidazopyridine derivatives, namely zolpidem and alpidem (*N,N*,6–trimethyl–2–(4–methylphenyl)– and 6–chloro–2–(4–chlorophenyl)–*N,N*–dipropylimidazo[1,2–*a*]pyridine–3 –acetamide, respectively), and a triazolopyridazine (3–methyl–6–(3–trifluoromethylphenyl)triazolo[4,3–*b*]pyridazine, CL 218,872) reveal a high affinity and selectivity for the ω_1–subtype of the receptor (19, 20). A considerable number of congeneric ligands have been synthesized and tested for the affinity for BZR to date, but the routine *in vitro* tests were usually performed with the use of non–selective radioligands, such as ^{3}H–diazepam or ^{3}H–flunitrazepam (20, 21).

In our recent *in vitro* investigations with a series of the congeners of CL 218,872, ^{3}H–zolpidem

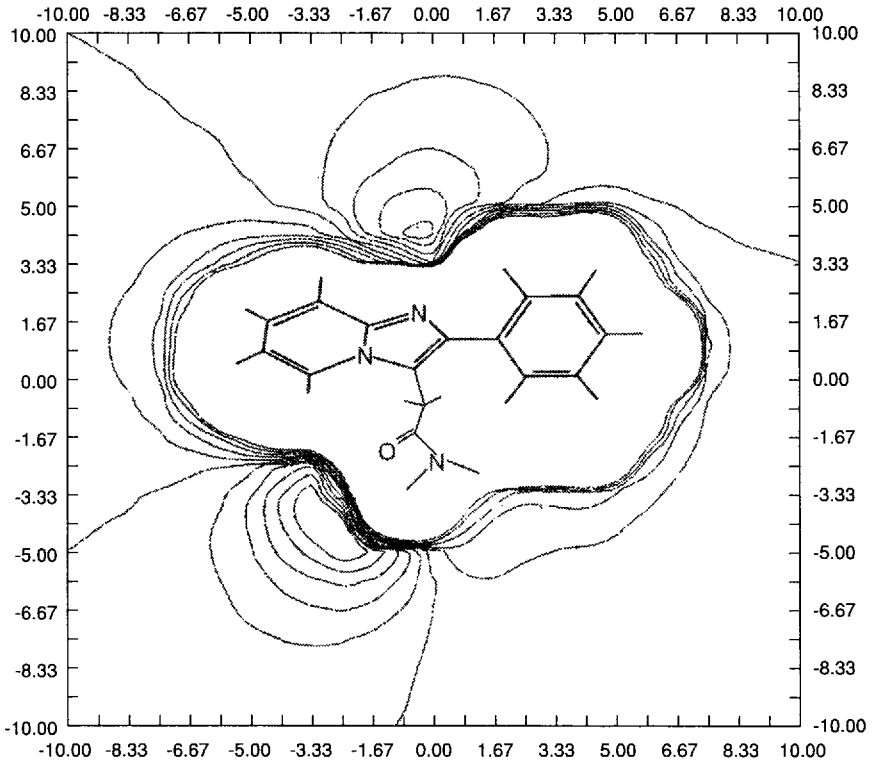


Figure 1. Molecular electrostatic potential (MEP) distribution in 2-phenylimidazo[1,2-*a*]pyridine-3-acetamide calculated with the VMNDO program (25) from the semi empirical quantum methods base. Equipotential lines are shown at intervals of 2.

was used as the displaceable radioligand because of its similar ω_1–selectivity (22). The results were mostly inconsistent with those reported for the same compounds in experiments with ^{3}H–diazepam (20, 23). In some cases, the compounds characterized by a high activity in displacing ^{3}H–diazepam were practically inactive in experiments with ^{3}H–zolpidem and *vice versa*. A QSAR analysis of the results obtained with ^{3}H–zolpidem also gave a different correlation equation (22). That means that the use of a non–selective radioligand distorts, or at least may distort, the results of experiments aimed at *in vitro* determination of the binding affinity of the investigated compounds for a given receptor or receptor subtype. In consequence, the effects of substituents distinguishing the congeneric compounds from one another may be ranked incorrectly.

With reference to the most advanced comprehensive model of the BZR pharmacophore (24), CL 218,872 and its analogs seem to be closely related to the zolpidem– like imidazopyridines in respect of the geometric distribution of the electronegative H_1 and H_2 (denotating according to (24)) sites which are generally considered to act as proton acceptors in anchoring the compounds to the receptor protein. A VMNDO (25) calculation of the molecular electrostatic potential (MEP) distribution in zolpidem has revealed now that the two MEP minima, which are to be identified with H_1 and H_2, are associated with the amide carbonyl group (minimum M_1) and the imidazole nitrogen atom (minimum M_2), respectively (Figure 1). Superimposition of the zolpidem base to CL 218,872 and to diazepam as the classic reference BZR ligand shows an almost perfect alignment of these anchoring sites (Figure 2).

In continuation of the research on BZR ligands with a presumed selective and high affinity for the ω_1–subtype, a series of imidazopyridines, congeners of zolpidem and alpidem, was now synthesized and tested *in vitro* for binding with the

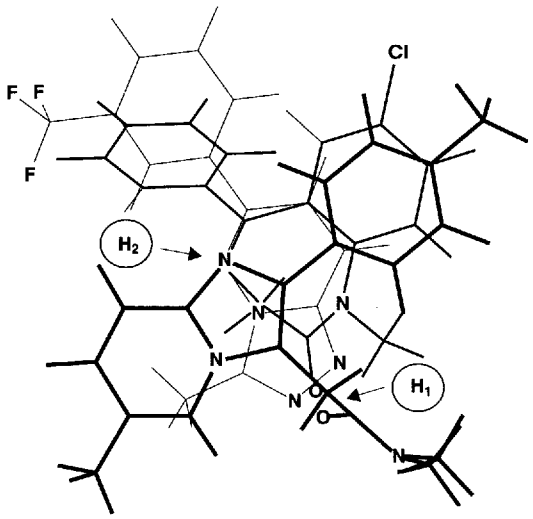


Figure 2. Superposition of the AMI–optimized molecules of zolpidem (thick line), CL 218,872 (thin line) and diazepam (medium line) with alignment of the imide nitrogen atom of all three compounds (H_2) and of the carbonyl oxygen atom of zolpidem and diazepam and the triazole N-N bond of CL 218,872 (H_1).

receptor in experiments using ^{3}H–zolpidem as the ω_1–selective displaceable radioligand. It was expected that the present results, possibly linked up with the conclusions drawn earlier from a similar study of triazolopyridazines (22, 23), do allow a more precise identification and evaluation of the molecular parameters which determine the binding ability.

EXPERIMENTAL

Melting points determined in a Büchi apparatus were uncorrected. The ^{1}H and ^{13}C NMR spectra were taken with a Varian 200 MHz spectrometer with TMS as internal standard. Microanalytical determinations were carried out by Mrs. E. Godzisz, Warsaw University of Technology, on a Perkin Elmer C–H–N analyzer; the results were within ±0.4% of the calculated values. Merck DC–Plastikfolien with Kieselgel 60 were used in the purity checking; the $CHCl_3$/MeOH/AcOH/satd. aq. NH_3 (3:2:1:0.1) developing system was applied. The reported melting points and elemental analyses refer to recrystallized, chromatographically homogeneous compounds.

1. 6–substituted 2–arylimidazo[1,2–*a*]pyridines [**I**]

The general procedure was as follows. An appropriate 4–substituted phenacyl bromide (0.1 mol) and 5–substituted 2–aminopyridine (0.125 mol) were dissolved in 200 ml of ethanol containing 13 g (0.155 mol) of sodium hydrogen carbonate. The mixture was stirred overnight at room temperature and next 5 h at reflux. Upon dilution with a liberal amount of water, the separated solid was collected by filtration, repeatedly washed with water, dried in a vacuum desiccator, and finally recrystallized from ethanol.

6–Chloro–2–(4–methoxyphenyl)imidazo[1,2–*a*] pyridine [**Id**], m.p. 228–230° (AcOEt), yield 65%. Anal.: Calcd. for $C_{14}H_{11}ClN_2O$: C, 65.00; H, 4.29; N, 10.83. Found: C, 64.82; H, 4.25; N, 10.82.

2–(4–Chlorophenyl)–6–methylimidazol[1,2–*a*] pyridine [**Ie**], m.p. 210–211° (MeOH–AcOEt), yield 59%. Anal.: Calcd. for $C_{14}H_{11}ClN_2$: C, 69.28; H, 4.57; N, 11.54. Found: C, 69.35; H, 4.41; N, 11.63.

2–(4–Methylphenyl)–6–methylimidazo[1,2–*a*] pyridine [**Ig**], m.p. 205–207° (MeOH–AcOEt), yield 75%. Anal.: Calcd. for $C_{15}H_{14}N_2$: C, 81.05; H, 6.35; N, 12.60. Found: C, 80.88; H, 6.41; N, 12.73.

2–(4–Methoxyphenyl)–6–methylimidazo[1,2–*a*] pyridine [**Ih**, m.p. 180–182° (dil. MeOH), yield 80%. Anal.: Calcd. for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.47; H, 5.98; N, 11.8.

Other compound **I** were prepared analogously. Their melting points were consistent with the literature data.

2. 6–substituted 2–aryl–*N,N*–dimethylimidazo[1,2–*a*] pyridine–3–methanamines [**II**]

The general procedure was as follows. To a stirred solution of 0.05 mol of an appropriate substituted imidazo[1,2–*a*]pyridine [**I**] in 60 ml of acetic acid was added dropwise at room temperature 8 ml (0.0625 mol) of a 40% aqueous solution of dimethylamine, followed by 4.5 ml (0.585 mol) of 37% formaldehyde. If necessary, moderate cooling was applied during the addition not to allow the temperature to exceed 50°C. The mixture was stirred 3–4 h at 50–55°C and left standing overnight. The evaporation of acetic acid (reduced pressure, temperature not exceeding 55°C) left a thick oil which was made slightly alkaline (pH 9–10) with aqueous ammonia and subsequently extracted with methylene chloride (5 × 30 ml). The extract was washed with water (2 × 25 ml), dried with magnesium sulfate, and finally evaporated under reduced pressure. Depending on the substituents in the starting compounds **I**, the product either crystallized on standing or remained oily.

Crude compounds **II** were used in the subsequent steps of the synthesis. For identification purposes, the solid compounds **II** which failed to crystallize spontaneously were dissolved in ethanol

(±5 ml/g) and the solutions were treated with a slight excess of a hydrogen chloride solution in ethanol and thoroughly cooled until separation of the hydrochloride salts commenced. Sodium hydrogen carbonate was used to convert the hydrochlorides back into the free Mannich bases.

6–Chloro–[2–(4–chlorophenyl)–*N,N*–dimethylimidazo[1,2–*a*]pyridine–3–methanamine [**IIa**], m.p. 140–142°, yield 77%. Anal.: Calcd. for $C_{16}H_{15}Cl_2N_3$: C, 60.01; H, 4.72; N, 13.12. Found: C, 59.84; H, 4.80; N, 13.02%.

6–Chloro–*N,N*–dimethyl–2–phenylimidazo[1,2–*a*]pyridine–3–methanamine [**IIb**], m.p. 125–127°, yield 62%. Anal.: Calcd. for $C_{16}H_{16}ClN_3$: C, 67.25; H, 5.64; N, 14.70. Found: C, 66.93; H, 5.55; N, 14.78%.

6–Chloro–*N,N*–dimethyl–2–(4–methylphenyl) imidazo[1,2–*a*]pyridine–3–methanamine [**IIc**], m.p. 127–128°, yield 64%. Anal.: Calcd. for $C_{17}H_{18}ClN_3$: C, 68.11; H, 6.05; N, 14.02. Found: C, 67.89; H, 5.96; N, 14.11%.

6–Chloro–*N,N*–dimethyl–2–(4–methoxyphenyl)imidazo[1,2–*a*]pyridine–3–methanamine [**IId**], m.p. 120–121°, yield 83%. Anal.: Calcd. for $C_{17}H_{18}ClN_3O$: C, 64.66; H, 5.75; N, 13.31. Found: C, 64.80; H, 5.59; N, 13.22.

N,N,6–Trimethyl–2–(4–methylphenyl)imidazo[1,2–*a*]pyridine–3–methanamine [**IIg**], m.p. 128–129°, yield 78%. Anal.: Calcd. for $C_{18}H_{21}N_3$: C, 77.38; H, 7.58; N, 15.04. Found: C, 77.11; H, 7.49; N, 15.18.

Other compounds **II** were obtained in 70–85% yields as oils which were neither characterized nor analyzed.

A representative ^{1}H NMR spectrum ($CDCl_3$, 200 MHz) was reported for **IIg**.: 2.23 (s, 6H, NCH_3), 2.34 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.83 (s, 2H, CH_2), 7.00–7.70 (m, 6H, arom. CH), 8.09 (m, 1H, arom. 5–CH).

3. 6–Substituted 2–aryl–*N,N,N*–trimethylimidazo[1,2–*a*]pyridine–3–methanaminium iodides [**III**]

The general procedure was as follows. An appropriate Mannich base (**II**, 0.02 mol) was dissolved in 40 ml of methylene chloride and 5 g (0.035 mol) of methyl iodide was added dropwise to this solution. In most cases, precipitation of the quaternary salt commenced soon. After 24 h at room temperature, the product was collected by filtration, washed with a small amount of methylene chloride, and dried. Compounds **III** were employed in subsequent steps of the synthesis without purification.

In the preparation of compound **IIIg**, methylene chloride was replaced with ethanol.

4. 6–Substituted 2–arylimidazo[1,2–*a*]pyridine–3–acetic acids [**V**]

The general procedure was as follows. An appropriate ammonium iodide (**III**, 0.03 mol) was added to the solution of 3.34 g (0.05 mol) of potassium cyanide in 300 ml of water (50% ethanol in the case of **IIIg**) and the mixture was refluxed for 4 h. Upon cooling, the solid product, which was identified as a mixture of the 6–substituted 2–arylimidazo[1,2–*a*]pyridine–3–acetamide [**IV**] and the corresponding nitrile, was collected by filtration, washed with cold water and dried. Depending on the substituents, the yields were in the 40–80% range. Since recrystallization from methanol only slightly increased the amide–to–nitrile ratio, the crude product was hydrolyzed without purification. Thus, a mixture prepared by suspending 0.01 mol of crude **IV** in 55 ml of ethanol and next by adding a solution of 4.9 g (0.085 mol) of potassium hydroxide in 10 ml of water was refluxed for 8 h. The ethanol was removed under reduced pressure and a minimum amount of water was added to the residue to dissolve completely any solid material. Acidification to pH=4 with an 18% aqueous hydrogen chloride (approximately 12 ml) precipitated the acid which was filtered, repeatedly washed with water and left to dry. The crude product was purified by recrystallization from methanol. The yields and melting points are produced in Table 1.

A representative ^{1}H NMR spectrum ($CDCl_3$–CF_3COOH, 200 MHz) was reported for **Vh**: 2.56 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 4.225 (s, 2H, CH_2), 7.14–7.83 (m, 6H, arom. CH), 8.24 (s, 1H, arom. 5–CH).

5. 6–Substituted 2–aryl–*N,N*–dimethylimidazo-[1,2–*a*]pyridine–3–acetamides [**VI**]

The general procedure was as follows. In a flask flushed with argon, 0.01 mol of the appropriate carboxylic acid **V** was suspended in 55 ml of dry THF and the mixture was stirred for 1 h at room temperature, next for 1 h at 50–52°C, cooled to room temperature, and bubbled through with gaseous dimethylamine for 1 h. THF was removed under reduced pressure, the residue was dissolved in 45 ml of methylene chloride, washed with two 15 ml portions of a sodium hydrogen carbonate solution, next with water, and finally dried with magnesium sulfate. Upon evaporation of the solvent, the solid product was recrystallized from 60% aq. methanol. If necessary, the recrystallization was repeated. The yields and melting points of the new compounds **VI** are produced in Table 1; the melting points of other compounds **VI** were consistent with the literature data (37).

A representative ^{1}H NMR spectrum ($CDCl_3$, 200 MHz) was reported for **VIh**: 2.315 (s, 3H, CH_3), 2.87 (s, 3H, NCH_3), 2.92 (s, 3H, NCH_3), 3.825 (s, 3H, OCH_3), 4.03 (s, 2H, CH_2), 6.94–7.58 (m, 6H, arom. CH), 7.95 (s, 1H, arom. 5–CH).

6. Receptor tests (2, 41)

Rat brains were homogenized at 0°C in 20 vols. of 0.32 M sucrose and the homogenate was incubated for 30 min at 37°C and centrifuged at 20,000 × *g*. The pellet obtained was homogenized in 60 vols. of Tris–HCl (0°C, pH=7.4). A 800–µl sample of the latter homogenate was incubated for 5 min at 37°C with 100 µl of the methanol solution of the investigated compound (10^{-5}–10^{-9} M concentrations), 100 µl of a ^{3}H–zolpidem solution (3 nM, specific activity 80 Ci/mM) was then added, and the incubation was continued for 30 min. The mixture was filtered through a sintered glass filter Whatman GF/C which had been soaked overnight at 4°C in a 0.3% solution of poly(ethyleneimine) in Tris–HCl. The filter was washed twice with 5 µl portions of Tris, dried, immersed in 10 ml of a scintillation liquid (POPOP 50 mg, PPO 4 g, methanol 20 ml, toluene 1000 ml). Radioactivity was measured in a Packard 2100TR β–scintillation counter and the results were expressed as % inhibition of binding of the labelled zolpidem.

RESULTS AND DISCUSSION

The synthesis of imidazo[1,2–*a*]pyridines was preceded by molecular modeling studies which involved 54 derivatives with R^1 = H, CH_3, OCH_3, and Cl; R^2 = H, Cl, F, CH_3, OCH_3, and CF_3 (see Scheme 1, formula **VI**). A few *N,N*–diethyl homologs were also covered by the study. The basic geometry of the rigid bicyclic core was taken from the published crystallographic reports on related compounds (26, 27).

Two computation methods were considered in optimizing the structure of the investigated compounds: *ab initio* calculations with the aid of the GAUSSIAN program (28) and calculations with the semi–empirical AM1 method from the MOPAC–93 program (29). In order to substantiate the choice of the most convenient and reasonably accurate method, optimization of the first four compounds was carried out with a parallel use of both methods. The semi–empirical calculations were applied to both, free and solvated molecules, the dielectric constant of water (ε = 78.4) being used in the calculations concerning the solvated species. Since the dipole moment values calculated by any semi–empirical method for molecules in an aqueous solvation medium are known to be overestimated, the dipole moment criterion could not be used in comparing the results of the two methods. The results were, therefore, compared by using the geometry criterion. As it may be seen in Figure 3a, which shows a superimposition of the structure of *N,N*–diethyl–6–chloro–2–(4–chlorophenyl)–imidazo[1,2–*a*]pyridine–3 –acetamide (R = C_2H_5, R^1 = R^2 = Cl) optimized with the AM1 method to that, obtained by *ab initio* calculations, the structures do not differ a lot. A still better fit, in particular in the

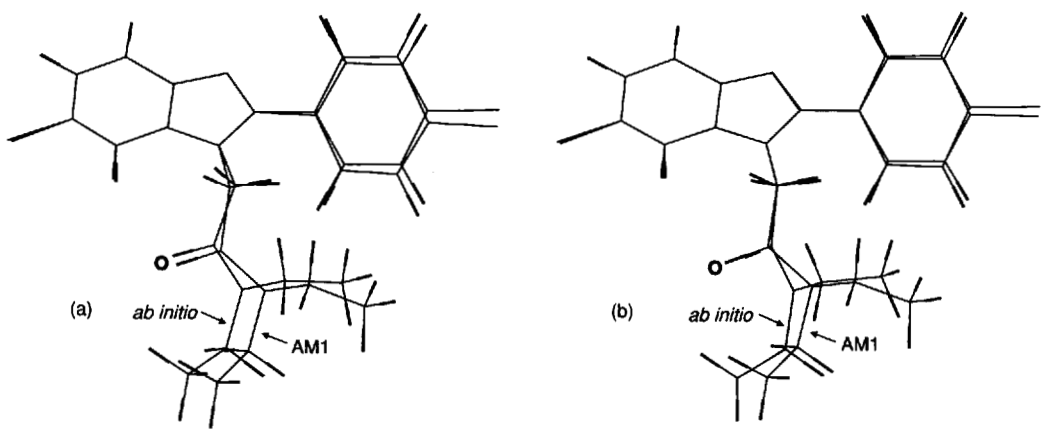
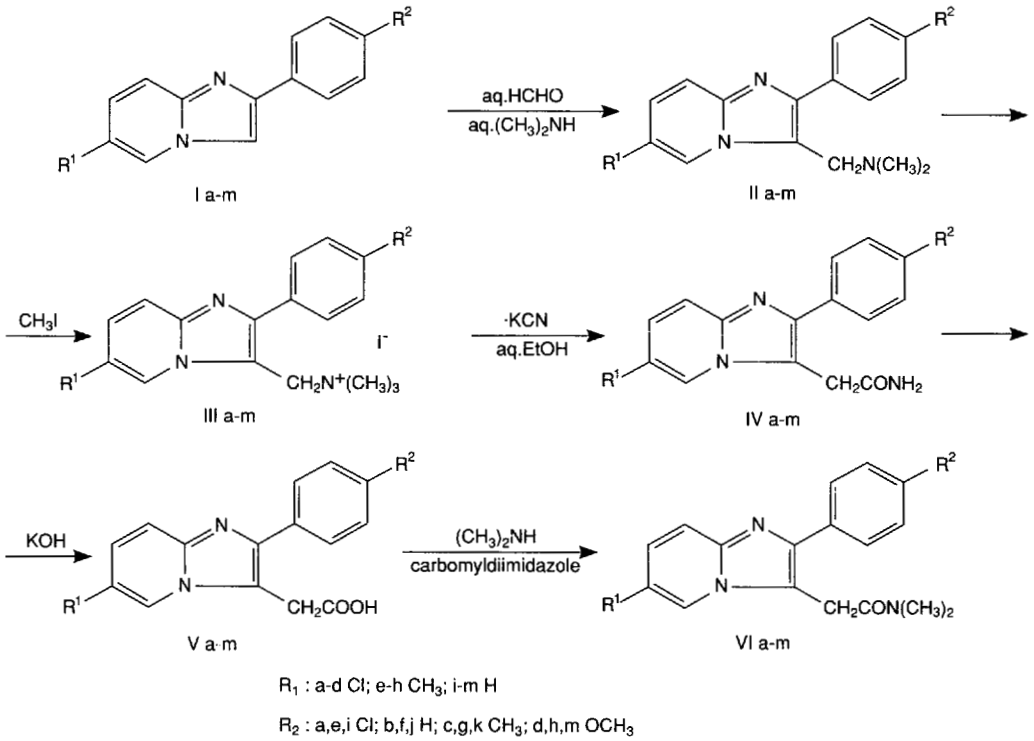


Figure 3. Superpositions of 6-chloro-2-(4-chlorophenyl)-N,N-diethylimidazo [1,2-a]pyridine-3-acetamide optimized with the *ab initio* program (28) and the AMI program (29): (a) free molecules and (b) solvated molecules.



Scheme 1.

position of the amide carbonyl group, which acts as a potential proton acceptor and is, therefore, a crucial factor determining the ability of binding with the receptor, may be seen in a similar superimposition of the solvated molecule (Figure 3b). Consequently, the AM1 method was considered accurate sufficiently to use for calculating the parameters of solvated molecules in all compounds of the series.

On the basis of the molecular modeling data, 12 compounds (R = CH_3; R^1–H, CH_3, and Cl; R^2 = H, CH_3, OCH_3, and Cl), all with the *N,N*–dimethyl substituent, were selected for the synthesis (Table 1). In order to verify in a direct [^{1}H]– *versus* ^{3}H–zolpidem test of the reliability of the binding determination method used, zolpidem (base) was included in the series. The series comprised also some known compounds for which no receptor binding data were found in the literature. The idea of maximal differentation of the substituent volume and electronic interactions was underlying the selection of R^1 and R^2. However, only such substituents were taken into consideration, which were known to appear most often in the compounds with a definite affinity, either as agonists or as antagonists, for the receptors controlling the functions of the central nervous system and which appeared in the zolpidem–related compounds synthesized earlier.

The synthetic route, which is shown in Scheme 1, followed, in general, the one used by George et al. (30) and recommended for the laboratory scale purpose.

The starting 6–substituted 2–arylimidazo[1,2–*a*]pyridines [**I**] were prepared in >80% yields in the reaction of the appropriately substituted 2–aminopyridines with ω–bromoacetophenone and its 4–substituted analogs in an ethanol medium and in the presence of sodium hydrogen carbonate (31, 32). With the exception of compounds **Ic, Ie, Ig**, and **Ih**, they all were known compounds. Lower yields were obtained, when a 100% excess of the aminopyridine was used for neutralization of the formed hydrogen chloride (33).

Further steps of the synthesis followed, in general, the procedures used by Kaminski et al. (34), and Almirante et al. (35, 36), as well as those, outlined in the patent literature (37–40). Thus, the Mannich bases [**II**] were prepared by making compound **I** react with aqueous solutions of dimethylamine and formaldehyde. Crude compounds **II** were initially isolated as thick oils but, depending on the substituents R^1 and R^2, some of them solidified on standing at room temperature. In the subsequent reaction with methyl iodide, compounds **II** were used without purification, although samples of the solid compounds **II** were recrystallized for identification purposes. Substitution of ethanol for methylene chloride as the reaction solvent resulted in a higher purity of the quaternary iodides [**III**], the yields were, however, somewhat lower.

In accordance with some earlier observations (34), the reaction of the quaternary salts with potassium cyanide, which was carried out in an aqueous ethanol, gave mixtures of the corresponding nitriles and amides, in some cases containing also the corresponding carboxylic acids. The nitrile/amide ratio, roughly estimated by analyzing the IR spectra, notably varied depending on the nature of the substituents, but purification by a single recrystallization from ethanol or methanol resulted in a considerable increase in the amide [**IV**] content. Further purification proved its unnecessity since alkaline hydrolysis of such amide–enriched mixtures yielded the carboxylic acids [**V**] in satisfactory yields. In the last synthetic step, the reaction of compounds **V** with gaseous dimethylamine in the presence of carbonyldiimidazole afforded the final compounds [**VI**] in yields varying from 72 to 88%. The overall yield of the synthesis 12–28% was depended on the substituents. No attempts were made to improve the yields by optimizing the reaction conditions.

The physico–chemical characteristics of compounds **V** and of new compounds **VI** are presented in Table 1, while those of new compounds **I** and **II** are given in the experimental part.

Determination of binding with the ω_1 subtype of the benzodiazepine receptor was carried out by a standard procedure using ^{3}H–zolpidem as the labelled ligand (1, 2, 41). The results expressed as the concentration of the investigated compound displacing 50% of the radioligand (IC_{50}) are given in Table 2, which also includes all the calculated molecular parameters used in the correlation study.

Preliminary correlations of IC_{50} with the calculated molecular parameters revealed some dependence on the HOMO energy (correlation coefficient r = 0.643) and a very low dependence on the LUMO energy (r = 0.275). Moreover, IC_{50} satisfactorily correlated (r = 0.840) with the magnitude of the M_1 molecular electrostatic potential (MEP) minimum but not with that of M_2. The regression combining the HOMO and M_1 parameters gave the following correlation equation:

Eq.1

$$IC_{50} \times 10^{-3} = 11.26M_1 + 81.33HOMO + 962.18$$

$n = 12$; $r = 0.872$; $s = 15.16$; $p = 0.001$; Calcd.F = 14.26; Table$F$(2, 9, 0.01) = 8.02

in which, however, only the M_1 term was of statistical significance. Equation 1 indicates that a deep M_1 minimum characterizes the compounds with low IC_{50}, *i.e.*, those with a high affinity for the receptor.

The classical approach to QSAR always calls for the use of parameters which determine the affinity of the compound for the aqueous and the lipid phase. The partition coefficient (P), most often used in its logarithmic form (log P), meets these requirements. It may be either determined directly in the water–*n*–octanol system or computed with the aid of a specific progam (42), as it has been done in the present research. However, correlation of the determined values of IC_{50} with the computed values of log P was rather poor (r = 0.504). Another poor correlation (r = 0.520) was obtained when the Hansch substituent constants (π_R^1, π_R^2) were used to replace log P.

However, when log P was combined with the most significant electronic parameter M_1, a highly significant regression equation was obtained:

Eq.2

$$IC_{50} \times 10^{-3} = 13.36M_1 - 24.58\log P + 333.50$$

$n = 12$; $r = 0.932$; $s = 11.25$; Calcd.F = 29.52; Table$F$(2, 9, 0.01) = 8.02

Both terms of this equation were statistically significant, with the highest significance level of the M_1 term.

A similarly highly significant correlation (Equation 3) was obtained when the log P parameter was replaced with the respective hydrophobic constants π:

Eq.3

$$IC_{50} \times 10^{-3} = 12.88M_1 - 33.46\pi_R^1 - 16.95\pi_R^2 + 289.28$$

$n = 12$; $r = 0.945$; $s = 10.74$; $p = 0.01$; Calcd.F = 22.26; Table$F$(3, 8, 0.01) = 7.59

Table 1. 6-Substituted-2-arylimidazo[1,2-*a*]pyridine-3-acetic acids [**V**] and new 6-substituted-2-arylimidazo-*N*,*N*-dimethyl[1,2-*a*]pyridine-3-acetamides [**VI**].

Compd.	R^1	R^2	Formula Mol. mass	M.p.[1] [°C]	Yield [%]	Analysis: calcd./found		
						C%	H%	N%
Va	Cl	Cl	$C_{15}H_{10}Cl_2N_2O_2$ 321.16	227-229	85	56.10 55.9	3.14 3.1	8.72 8.9
Vb	Cl	H	$C_{15}H_{11}ClN_2O_2$ 286.71	233-236	82	62.84 62.6	3.87 3.9	9.77 9.6
Vc	Cl	CH_3	$C_{16}H_{13}ClN_2O_2$ 300.74	231-233	87	63.90 63.8	4.36 4.4	9.31 9.4
Vd	Cl	OCH_3	$C_{16}H_{13}ClN_2O_3$ 316.74	229-231	80	60.67 60.5	4.14 4.2	8.84 8.9
Ve	CH_3	Cl	$C_{16}H_{13}ClN_2O_2$ 300.74	222-224	76	63.90 63.6	4.36 4.2	9.31 9.3
Vf	CH_3	H	$C_{16}H_{14}N_2O_2$ 266.29	223-224	82	72.17 72.0	5.30 5.3	10.52 10.4
Vg	CH_3	CH_3	$C_{17}H_{16}N_2O_2$ 280.32	227-229	87	72.84 72.6	5.75 5.6	9.99 10.1
Vh	CH_3	OCH_3	$C_{17}H_{16}N_2O_3$	214-216	72	68.91 68.7	5.44 5.4	9.45 9.3
Vi	H	Cl	$C_{15}H_{11}ClN_2O_2$ 296.32	223-225	82	62.84 62.7	3.87 3.9	9.77 9.5
Vj	H	H	$C_{15}H_{12}N_2O_2$ 252.27	231-233	86	71.42 71.5	4.79 4.6	11.10 10.9
Vk	H	CH_3	$C_{16}H_{14}N_2O_2$ 266.29	230-232	76	72.17 72.0	5.30 5.2	10.52 10.5
Vm	H	OCH_3	$C_{16}H_{14}N_2O_3$ 282.29	219-222	82	68.08 67.9	5.00 5.1	9.92 9.8
VIf	CH_3	H	$C_{18}H_{17}N_3O$ 291.35	220-223	86	74.20 74.0	5.88 5.8	14.42 14.3
VIh	CH_3	OCH_3	$C_{19}H_{19}N_3O_2$ 321.37	212-215	88	71.01 70.8	5.96 5.9	13.07 12.9
VIj	H	H	$C_{17}H_{15}N_3O$ 277.32	179-182	84	73.63 73.5	5.45 5.4	15.15 15.0
VIk	H	CH_3	$C_{18}H_{17}N_3O$ 291.35	154-156	74	74.20 74.0	5.88 5.9	14.42 14.3
VIm	H	OCH_3	$C_{18}H_{17}N_3O_2$ 307.35	143-146	72	70.34 70.2	5.58 5.7	13.67 13.6

[1] Melting points of all compounds **V** with decomposition.

Statistical analysis of this correlation revealed again a high significance of the M_1 term, a much lower significance of the π_{R^1} term, and a low significance of the π_{R^2} term. Nevertheless, elimination of the last listed term caused the correlation coefficient to drop to 0.92. The regression generating Equation 3 explained 89.3% of the variance in IC_{50}.

This quantitative relation between the affinity for the benzodiazepine receptor (IC_{50}) and the molecular electronic parameters of the molecules is fully consistent with the distribution of the molecu-

Table 2. Experimental receptor affinity data and calculated molecular parameters of 6 -substituted 2-aryl-*N,N*-dimethylimidazo[1,2-*a*]pyridine-3-acetamides [**VI**]

Compound			M_1MEP minimum [kcal/mol]	M_2MEP minimum [kcal/mol]	log P	IC_{50} [nM]	HOMO [eV]	LUMO [eV]
No.	R^1	R^2						
VIa	Cl	Cl	-18.177	-16.715	2.94	23351	-9.008	-0.892
VIb	Cl	H	-19.457	-19.005	2.20	26529	-8.995	-0.818
VIc	Cl	CH_3	-18.765	-19.188	2.72	283	-8.975	-0.800
VId	Cl	OCH_3	-19.160	-18.954	2.27	28851	-8.939	-0.795
VIe	CH_3	Cl	-19.211	-19.199	2.72	13222	-8.908	-0.765
VIf	CH_3	H	-20.265	-21.730	1.98	10634	-8.896	-0.646
VIg	CH_3	CH_3	-20.143	-21.722	2.50	5.15	-8.876	-0.646
VIh	CH_3	OCH_3	-19.716	-21.218	2.05	1280	-8.857	-0.627
VIi	H	Cl	-19.127	-19.256	2.20	43168	-8.898	-0.758
VIj	H	H	-21.091	-21.855	1.46	19567	-8.884	-0.636
VIk	H	CH_3	-21.008	-21.736	1.98	1496	-8.862	-0.649
VIm	H	OCH_3	-14.472	-19.188	1.53	>100000	-8.642	-0.640

lar electrostatic potential (MEP) around the molecules of imidazopyridines (Figure 1). The deepest minimum of MEP (M_1), which is associated with the carbonyl oxygen atom, acts as a proton acceptor anchoring *via* a hydrogen bond the ligand molecule to a specific site of the receptor protein. The depth of this minimum, which is modulated in a congeneric series by the electronic properties of the substituents, is certainly an important determinant of the binding ability and hence of the affinity for the receptor. As far as other effects of substituents are concerned, their higher or lower hydrophobicity affects the transport to and penetration into the receptor tissue, whereas their greater or lesser volume defines the ability to fit the receptor pocket(s).

It seems noteworthy that our earlier QSAR studies of the triazolo[4,3–*b*]pyridazine congeners of CL 218,872 (22) and the pyrazolo[4,3–*c*]quinoline congeners of CGS–9896 and CGS–8216 (43) have also revealed the importance of the electronic factors and in particular of the molecular electrostatic potential distribution.

Acknowledgment

The research was carried out under grant no. 4 505F 013 09 from the Committee of Scientific Research, Poland. This financial support is gratefully acknowledged.

REFERENCES

1. Squires R.F., Braestrup C.: Nature 266, 732 (1977).
2. Möhler H., Okada T.: Science 198, 849 (1977).
3. Gavish M., Snyder S.H.: Life Sci. 26, 579 (1980).
4. Herb A., Wisden W., Lüddens H., Puia G., Vicini S., Seeburg P.H.: Proc. Natl. Acad. Sci. USA 89, 1433 (1992).
5. Squires R.F., Benson D.I., Braestrup C., Coupet J., Klepner C.A., Myers V., Beer B.: Pharmacol. Biochem. Behav. 10, 825 (1979).
6. Klepner C.A., Lippa A.S., Benson D.I., Sano M.C., Beer B.: Pharmacol. Biochem. Behav. 11, 457 (1979).
7. Sieghart W., Karobath M.: Nature 286, 285 (1980).
8. Young III W.S., Niehoff D., Kuhar M.J., Beer B., Lippa A.S.: J. Pharmacol. Exp. Ther. 216, 434 (1981).
9. Sieghart W.: Trends Pharmacol. Sci. 10, 407 (1989).
10. Doble A., Martin I.L.: Trends Pharmacol. Sci. 13, 76 (1992).
11. Gardner C.R.: Drug Dev. Res. 12, 1 (1988).
12. Lüddens H., Wisden W.: Trends Pharmacol. Sci. 12, 49 (1991).
13. Pritchett D.B., Seeburg P.H.: J. Neurochem. 54, 1802 (1990).

14. Zivkovic B.: Pol. J. Pharmacol. 45, 88 (1994).
15. Mohler H., Knoflach F., Paysan J., Motejlek K., Benke D., Luescher B., Fritschy J.M.: Neurochem. Res. 20, 631 (1995).
16. Korpi E.R., Mattila M.J., Wisden W., Lüddens H.: Ann. Med. 29, 275 (1997).
17. Huang Q., Liu R.Y., Zhang P.W., He X.H., McKernan R., Gan T., Bennett D.W., Cook J.M.: J. Med. Chem. 41, 4130 (1998).
18. Hadingham K.L., Wingrove P., Le-Bourdelles B., Palmer K.J., Ragan C.I., Whiting P.J.: Mol. Pharmacol. 43, 970 (1993).
19. Langer S.Z., Arbilla S., Benavides J., Scatton B.: Adv. Biochem. Psychopharmacol. 46, 61 (1990).
20. Albright J.D., Moran D.B., Wright W.B., Jr., Collins J.B., Beer B., Lippa A.S., Greenblatt E.N.: J. Med. Chem. 24, 592 (1981).
21. Trapani G., Franco M., Ricciardi L., Latrofa A., Genchi G., Sanna E., Tuveri F., Cagetti E., Biggio G., Liso G.: J. Med. Chem. 40, 3109 (1997).
22. Lange J., Karolak-Wojciechowska J., Wejroch K., Rump S.: Acta Pol. Pharm. – Drug Res. 56, 61 (1999).
23. Karolak-Wojciechowska J., Lange J., Kwiatkowski W., Gniewosz M., Plenkiewicz J.: Bioorg. Med. Chem. 2, 773 (1994).
24. Zhang W., Koehler K.F., Zhang P., Cook J.M.: Drug. Des. Disc. 12, 193 (1995).
25. Kwiatkowski W., Karolak-Wojciechowska J.: SAR QSAR Environ. Res. 1, 233 (1993).
26. Aslanov L.A., Tafeenko V.A., Paseshnichenko K.A., Bundel Yu.G., Gromov S.P., Gerasimov B.G.: Zh. Strukt. Khim. 24, 115 (1983).
27. Georges G.J., Vercauteren D.P., Evrard G.H., Durant F.V., George P.G., Wick A.E.: Eur. J. Med. Chem. 28, 323 (1993).
28. GAUSSIAN 94 computer program, Gaussian, Inc., Carnegie, PA, USA.
29. MOPAC-93 Programme, Indiana University, Bloomington, USA, 1993.
30. George P., Rossey G., Depoortere H., Mompon B., Allen J., Wick A.: Farmaco 46, 277 (1991).
31. Julian P.L., Meyer E.W., Magnani A., Cole W.: J. Am. Chem. Soc. 67, 1203 (1945).
32. I.A. Kaye, C.L. Parris, W.J. Burlant: J. Am. Chem. Soc. 75, 746 (1953).
33. Buu-Hoï Ng. Ph., Jacquignon P., Xuong Ng.D., Lavit D.: J. Org. Chem. 19, 1370 (1954).
34. Kaminski J.J., Bristol J.A., Puchalski C., Lovey R.G., Elliott A.J., Guzik H., Salomon D.M., Conn D.J., Domalski M.S., Wong S.-C., Gold E.H., Long J.F., Chiu P.J.S., Steinberg M., McPhail A.T.: J. Med. Chem. 28, 876 (1985).
35. Almirante L., Mugnaini A., Polo Friz L., Provincial E.: Boll. Chim. Farm. 105, 32 (1966).
36. Almirante L., Mugnaini A., Rugarli P., Gamba A., Zefelippo E., de Toma N., Murmann W.: J. Med. Chem. 12, 122 (1969).
37. Kaplan J.-P., George P.: US Patent 4,382,938 (1983), 14 pp.
38. Kaplan J.-P., George P.: US Patent 4,460,592 (1984), 12 pp.
39. Kaplan J.-P., George P., Bernardon J.-M.: European Patent 92,459 (1983), 18 pp.
40. Allen G., George P.: Fr. Demande FP 2, 599, 368 (1986), 9 pp.
41. Wu Y., Rosenberg H.C., Chiu T.H., Ramsey-Williams V.: J. Pharmacol. Exp. Ther. 268, 675 (1994).
42. Pallas for Windows 1.2, Compu-Drug Chemistry, Ltd., Budapest, Hung., 1995.
43. Karolak-Wojciechowska J., Lange J., Książek W., Gniewosz M., Rump S.: Farmaco 53, 579 (1998).

Received: 27.03.2000