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The α_1-adrenolytic and structural evaluation of new pyridoindole derivatives

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Abstract: α_1 – adrenolytic activities of pyridoindole derivatives recently synthesized in the Institute of Experimental Pharmacology, Slovak Academy of Sciences, were measured. A characteristic set of derivatives (five active, one with a threshold activity and two inactive) was chosen and an elementary structure-activity study was performed. The structure and energy properties were estimated by quantum-chemical semiempirical AM1 and molecular mechanics methods. The ionization constants pK_a of the individual derivatives were calculated by program Pallas or estimated by potenciometric titration. The α_1 blocking activities were measured by rat thoracic aorta model. The experimental model used was not α_1 – adrenoreceptor subtypes specific, however, the α_{1D} subtype could be considered to be a predominant type in a rat aorta. The obtained physico-chemical parameters were then compared with the blocking activities of the derivatives and following determining characters for α_1 – adrenolytic activities were determined: 1) the polarity of the substituted phenol ring represented by a map of molecular electrostatic potential and 2) the hexahydro-pyridine nitrogen pK_a constant, which represents the ability of the compound to be protonated by physiologic pH.

Keywords: α_1*–adrenolytic activity, pyridoindole, structure-activity, AM1 method*

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1 Introduction

The α_1-adrenergic receptors (α_1-AR) fall into the family of G-protein coupled receptors (GCPR) and participate in the regulation of the cardiovascular system and smooth muscle contraction [1]. To date, there exists no known high-resolution 3D structure for any adrenergic receptor, however, an α_1-pharmacophore proposed nineteen years ago has been successfully used for various sets of active substances [2]. Bremner et al. [3, 4] specified structural requirements for individual subtypes of α_1 adrenolytic pharmacophores ($\alpha_{1A,}\alpha_{1B}$ and α_{1D}). In addition to the necessary presence of protonated amine in molecules, the geometric constraints for mutual orientation of a positive charge center (P), the nearest aromatic ring center (A), and the hydrogen bond acceptor (HBA) existing in the antagonist structure were determined. Evers and Klaubunde [5, 6] refined this model for α_{1A} receptor and found two different α_{1A} adrenolytic pharmacophore models, the second one lacking the HBA.

Recently, new derivatives [7] of the pyridoindole stobadine [8, 9] were synthesized and tested in several biological experiments in Institute of Experimental Pharmacology of Slovak Academy of Sciences.. The main reason for synthesizing new derivatives was to enlarge the antioxidative (AO) and antiradical (AR) capacity of the lead substance. In biosciences, the AO (or AR) capacity is commonly interpreted as the capability of a given substance to protect concrete biological objects from the attack of defined oxidants (radicals) measured per unit amount of the substance. The most important part of the AR capacity is the radical scavenger property of the compound. However, other biological activities were also measured. The lead structure for this group – stobadine – was named the bellwether of a broader view of drug actions [10] due to its potential as a model drug for various pre-clinical studies. Having studied properties of newly synthesized compounds, we chose the methods already established stobadine pharmacological profile monitoring. The α_1– adrenolytic activities of 80 compounds were tested on the rat thoracic aorta and evaluated for their ability to shift the phenylephrine concentration – response curve to the right. Of the compounds tested, six structures **I** – **VI** (Fig. 1) proved to possess a competitive activity (**VI** on the margin); other structures (together more than 60 derivatives) were either inactive (e.g., **VII** and **VIII**) or uncompetitively active. In light of this knowledge we performed a simple structure-activity study for a chosen set of derivatives: active structures **I** – **V,** one poorly active structure, structure **VI**, and two inactive derivatives, **VII** – **VIII** (Fig. 1). In this comparative study, we tried to find possible structure-activity relationships which could explain the measured α_1– adrenolytic activities of the compounds.

2 Statistical methods and Experimental Procedures

2.1 Experimental procedures

2.1.1 Estimation of pK_a constants

The ionization constants (pK_a) of new substances were determined potentiometrically, as pH values of the solutions of the compounds studied titrated to 50% with an alkali hydroxide. Because of poor water solubility of the bases liberated in the course of titration the medium used was a mixture of water – methanol 2:3 (v/v). The course of titration was followed on a pH meter (precision digital pH-meter OP 208, Radelkis, Hungary, glass electrode OP 0718 P and saturated calomel electrode OP 830 P, automatic burette type OP 930, Radelkis, Hungary connected to a personal computer). The pK_a values of the compounds studied were then calculated from the Henderson-Hasselbach equation after correction for the volume of methanol (-0.14 ml). With each compound two parallel assays were performed, and the presented results are the average values from these two assays. Compound stobadine dihydrochloride (**I**.2HCl) was titrated as dibasic acid; compound **VIII**.HCl was titrated as monobasic acid. Other pK_a's were not measured because of deficiency of the required amount of the remaining compounds.

2.1.2 Procedure

0.100 g of the compound studied was dissolved in a 200-ml conical flask in a 40=ml mixture of water and methanol (4:6 v/v). This was titrated with 0.1 M sodium hydroxide solution, determining the end point potentiometrically.

2.2 Estimation of α-adrenolytic activity of the compounds

The experiments were performed on male Wistar rats (Breeding Facility of IEPh SAS Dobrá Voda, Slovak Republic), weighing 250–270 g. The investigation conforms to the Guide for the Care and Use of Laboratory Animals. The animals were housed in cages (T4 Velaz, Prague, Czech Republic) with bedding composed of wood shaving (exchanged daily). Tap water and pelleted standard diet KKZ-P-M (Institute of Experimental Pharmacology, Slovak Academy of Sciences, Dobrá Voda, Slovak Republic) were available *ad libitum.* The animal room was kept under standard conditions. It was air-conditioned with 10 air changes per hour and the environment was continuously monitored to maintain a temperature of 23 ± 1^{o}C and relative humidity of 40-70 %.

The rats were sacrificed by cervical dislocation. After opening the thorax, the thoracic aorta was removed, cleaned of adherent tissue in physiological salt solution (PSS), and cut into 8 rings, each approximately 2 to 3 mm long. Special care was taken not to damage the endothelium. The rings were mounted between two L-shaped hooks in water-jacketed ($37°\pm$ 0.5°C) chambers containing PSS bubbled with a mixture of 95% O_2 and 5% CO_2 at pH 7.4. The composition of PSS was (in mmol/l): NaCl (118.0), KCl (4.7), KH_2PO_4 (1.2), $MgSO_4$(1.2), $CaCl_2$ (2.5), $NaHCO_3$ (25.0) and glucose (11.0). The

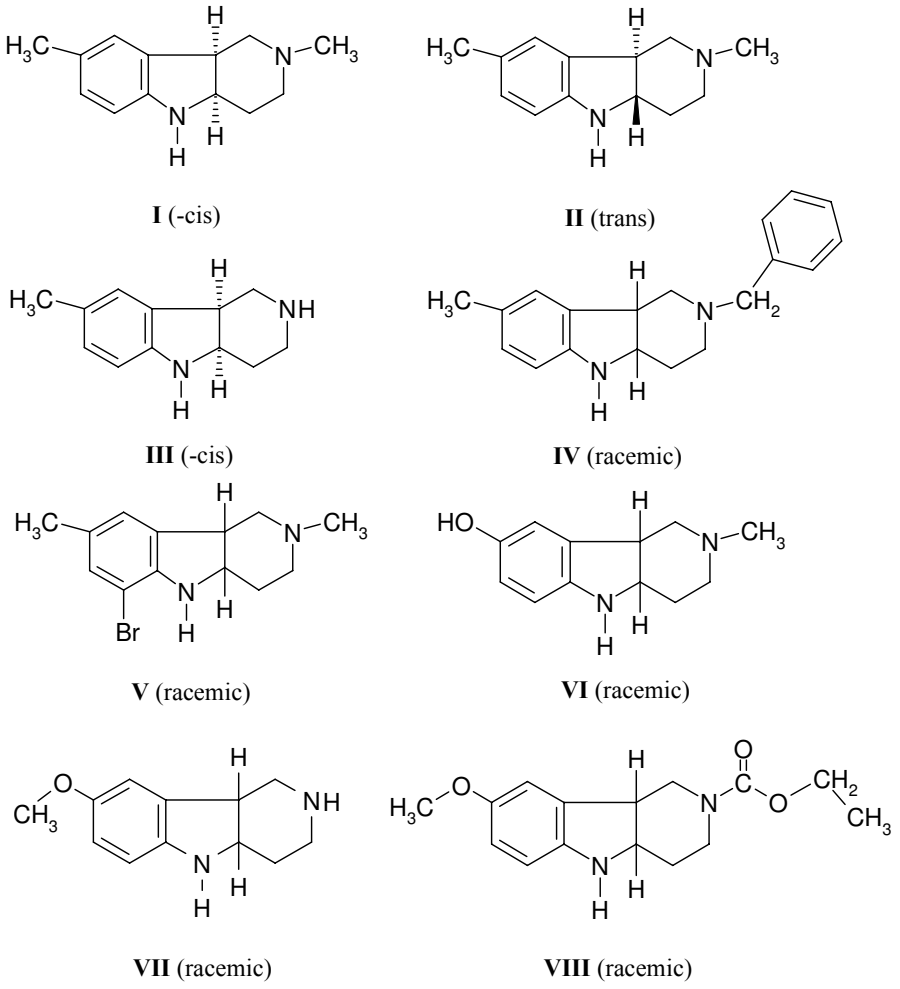

Fig. 1 Identification of the studied pyridoindole derivatives.

preparations were connected to an isometric transducer (M 1101, Czech Republic) and stretched passively to optimal length by imposing an optimal initial tension of 20 mN, as tested in preliminary experiments. After application of the initial tension, the arterial preparations were equilibrated for 60 minutes. Isometric contractions were recorded on a Kutesz 185 line-recorder (Hungary). The experimental protocol was as follows: Preparations were contracted by phenylephrine in concentrations increasing cumulatively from 10^{-9} to 10^{-6} mol/l. Subsequently, the preparations were washed several times with PSS and then relaxed to initial tension values. After 60 minutes, the concentration-response curve of phenylephrine was performed again, and the second curve was considered to be the control. Then the preparations were washed several times during the 30-minute period. The drug was then tested, in the concentration of 10^{-7} mol/l, added to the PSS for 30 minutes, and the concentration-response curve of phenylephrine was done. This protocol was repeated with the compound at concentrations of 10^{-6}, and 10^{-5} mol/l. Four compounds were tested in parallel, each in a separate preparation vessel.

Sensitivity of the preparations to phenylephrine was expressed from the concentration-response curve, the maximal response in control conditions was considered as 100%. The

EC_{50} was evaluated as the concentration of phenylephrine when the responses reached 50% of the maximal value. α-adrenolytic activity of the compounds was expressed in pA_2 (negative logarithms of the antagonist concentration inducing depression of the effect of the agonist by 50%).

2.3 Computational methods

All quantum-chemical calculations were performed by means of the HyperChem 7.1 software package [11]. Conformational analysis was executed by *Conformational Search* module using the molecular mechanics MM+ approximation, to maximally retain the flexibility of the basic structure. We used the usage directed method with the acceptance energy criterion 6 kcal/mol. After finishing the conformational search, further optimization of individual conformers in AM1 by semiempirical method was performed. First, we chose the first 20 samples from molecular mechanics calculations. When the number of total conformers found was greater than 20, we chose further conformers with a frequency greater than the maximum from the first twenty samples. The conformer with minimum energy in AM1 optimization (with the optimization criterion RMS<0.01 kcal/mol) was considered to be the optimal structure, taking into account the optical isomerism of the given compound.

The electrostatic potential maps were calculated by the *Molecular Graphs* module in HyperChem and they ranged as multiples of e/a_0. The pKa constants were calculated by the program Pallas [12].

3 Results

As mentioned above, derivatives II – VIII were not designed especially to maximize their α_1 – adrenolytic activities, however, these properties were also monitored. This may be the main reason that the structures presented here are not typical representatives of α_1– adrenolytic compounds.

he experimental model used (rat aorta) was not α_1– adrenoreceptor subtypes specific. As it was demonstrated by Faber et al. [13], the predominant type of α_1– adrenoreceptor in a rat aorta is the α_{1D}, although the populations of α_{1A} and α_{1B} are also significant. The main structural difference of our substances with the α_{1D}pharmacophore elaborated by Bremner et al. [3, 4] is a missing hydrogen bond acceptor for our structures with the observed distinct adrenolytic activity (I-V substances). However, we attempted to compare the adrenergic activities of our substances and to find the potential relation, if exists, between their activities and physicochemical and structural properties.

As to the range of pA_2 values (Table 1), the active derivatives (**I-VI**) belong to the family of average and weakly active compounds (e.g., pA_2 of indoramin = 6.8, pA_2 of prazosin = 9.5, see Table 5 in Ref. [4] for α_{1D} selective activities). An average activity is connected with effective concentrations (EC) in approximately micromolar region, ($pA_2 \approx$ 6), weak is beyond it and inactive compounds are commonly accepted with EC in the

Table 1 Experimental α_{1A}activities (pA_2), ionization constants $pK_{a,t}$ (predicted) and pK_a(measured), distances of the protonated nitrogen from the middle of the aromatic ring P-A and RMS errors of geometric fit of the given substance with derivative **I** (see text).

	pA_2	$pK^a_{a,t}$	pK_a	P-A [Å]	RMS [Å]
I	7.3±0.1	9.03, 2.53	6.77, 2.66	5.0	-
II	6.3±0.4	b	c	5.4	0.36
III	6.6±0.3	10.23, 5.17	c	4.1	0.46
IV	6.6±0.5	8.34, 2.53	c	4.1	0.46
V	6.1±0.0	8.92, 0.49	c	4.1	0.63
VI	<5.0	8.74, 3.02	c	4.1	0.46
VII	inactive	9.87, 5.58	c	4.1	0.46
VIII	inactive	4.95, -3.58	4.07	5.1	0.01

a – the first value of pK_a corresponds to the piperidine nitrogen, the second one is related to the indole nitrogen;
b – software used [12] does not distinguish optical isomers;
c – not measured, see text in Methods.

milimolar region.

Structures **I** and **III** were optically pure substances in (*-cis*) configuration, structure **II** was a (*trans*) modification of substance **I**, and the substances **IV-VIII** were (*cis*) racemic mixtures.

The conformational analysis of all derivatives studied was performed as described in the Experimental section with the nitrogen N-R2 chosen as the protonation site at physiological pH (Fig. 2). The distance P-A (distance of the protonated nitrogen from the middle of the aromatic ring, denoted as *) was calculated for each optimal protonated conformer. Considering the optimal geometry of the derivative **I** to be the reference structure, we can calculate the RMS fit for the "basic backbone" of individual pairs of the molecules, as pointed out by thick lines in Fig. 2.

4 Discussion

Concentrating on the values of the geometric similarities, we can see no clear relation between the $\alpha_1$activities (pA_2) and the structural characterization of the compounds studied. All compounds have the values of P-A distance in the range of 4.1 – 5.4 Å, whereas the optimal value according to Bremner [4] for α_{1D}pharmacophore is 5.4 Å. Bremner et al. also used a data mining approach to search for the molecular substructures proper for the α_{1A}blocker design [14]. Their search was influenced by the choice of tryptamine as the initial query structure.

Klabunde and Evers [5, 6] presented pharmacophore models for two different groups of α_{1A} antagonists. According to the structural features, compounds **I-VIII** belongs to the second group they described, which lacked the hydrogen-bond acceptor group. However,

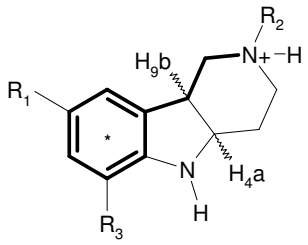

Fig. 2 Numbering scheme of the derivatives studied. Asterisk (*) denotes the middle of the aromatic ring.

the distance of the aromatic group from the positively charged nitrogen atom is much longer in both classes of α_{1A} ligands (9.5 or 7.2 Å) than any of those given in Table 1. This difference points out that our experiments evaluated the α_{1D} rather than α_{1A} activity.

As to the (-*cis*) and (*trans*) isomers (substances **I** and **II**, respectively), the P-A distance is longer for **II**, in agreement with their activities order. The AM1 method (like other semiempirical quantum-chemical methods) provides energetic equivalence for optical antipodes, with inverse geometrical structures. In short, the P-A distance for (+cis) isomers will be the same as for the (-cis) isomers in Table 1. The same could be said about the structural similarity measured by RMS error, which provided a narrow range of values, with the minimum for substance **VIII**, which is inactive.

To consider the effect of the real protonation of the given compound in solution, we calculated the ionization constants [12]. According to these theoretical values, protonation at physiological pH is enabled for all active substances and one inactive substance.

Molecular electrostatic maps provide data that may explain the differences in activities rather than the differences in geometries alone (Fig. 3). The substituted aromatic ring for all derivatives with distinct activities (**I**-**V**) showed only positive molecular potential, while both inactive derivatives (**VII** and **VIII**) and the derivative with a weak activity (**VI**) obtained minimum values near the aromatic rings due to the hydroxyl- or methoxy-substitution. This suggests that a possible improvement of the parent structure through the introduction of hydrogen bond acceptor group should be planned in the residual part of the molecule, not at the aromatic ring itself. The value of this study could be enhanced by the type-selective method, which exceeded our present methodological capabilities.

The structurally similar pyridoindole derivatives **I** – **VIII** were studied from the point of view of their α_1 - adrenolytic activity and compounds with potential affinity towards the α_{1D} adrenergic receptor. The main properties that could determine their activities are the values of their pK_{a2} constant (i.e., occurrence of their protonated form at physiological pH), as well as the shape of the molecular electrostatic potential around the substituted aromatic ring. The possible improvement of the given α_1 - adrenolytic activity should be connected with preserving the protonation ability of the compound and introducing the hydrogen bond acceptor group at places other than the aromatic ring.

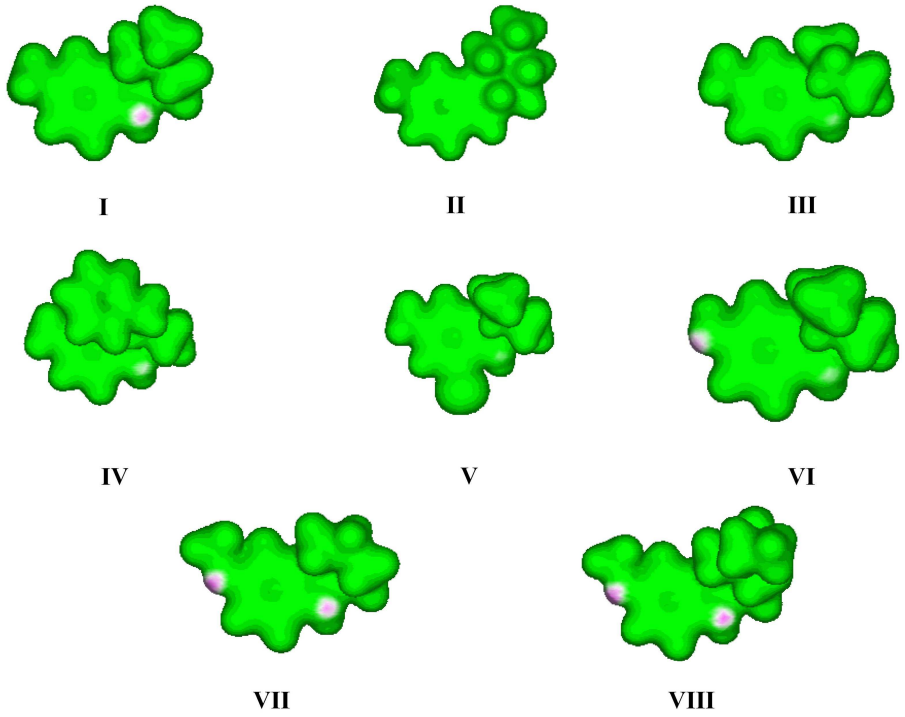

Fig. 3 Molecular electrostatic potential maps of the compounds studied.

Acknowledgment

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