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Synthesis of 3-aryl-1-[(4-phenyl-1-piperazinyl)butyl]indazole derivatives and their affinity to 5-HT_{1A} serotonin and dopamine D_1 receptors

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Eight 3-arylindazole derivatives have been synthesized and their affinity to 5-HT_{1A} serotonin and D_1 dopamine receptors was investigated by radioligand analysis. Quantitative structure-activity relationships were studied using the Free-Wilson model. An increase in affinity to dopamine D_1 receptors within substituents $Br > Cl > CH_3$ at the 5-position of the 3-arylindazole molecule has been observed. Addition of a chlorine atom to the ortho-position the of phenyl ring let to even highes activity. Replacement of the hydrogen atom at the first position of the 3-arylindazole on the (phenylpiperazine)butyl substituent caused an increase of affinity and did not change the trends of affinity dependence on structure. An inverse dependence on the structure of the studied compounds was observed for the serotonin 5-HT_{1A} receptors. Compounds containing a methyl group at the 5-position of molecule were more active than compounds containing halogens. A chlorine atom at the ortho-position of the phenyl ring decreased affinity. Replacement of the hydrogen atom at the first position of the molecule on the phenylpiperazine)butyl substituent led to an increase in affinity. Selectivity of the studied compounds varied within a wide range. Generally, the presence of the 3-aryl-indazole fragment in the new buspirone analogues increased their affinity to dopamine receptors and reduced their affinity to serotonin receptors. Compounds containing a bromine atom in the 3-arylindazole moiety may be promising ligands for D_1 receptors.

1. Introduction

The arylpiperazinyl fragment is a common element of structure of new generation anxiolytics, such as buspirone, which binds with high affinity at both serotonin and dopamine receptors. Structure modifications of the arylpiperazinyl fragment cause significant changes in affinity and selectivity [1]. Study of relationships between structure and affinity of arylpiperazines showed that the features of the arylpiperazinyl fragment play an important role for recognition of the specific binding sites. As for the ligands of the 5-HT_{1A} receptors, the affinity is determined by the presence of a strongly basic nitrogen atom at a distance of 5.2–5.6 Å from the centre of the aromatic ring [2]. The hydrocarbon chain also plays an important role here, acting as a spacer or providing the hydrophobic interaction [3]. Signification of the N-terminal fragment has been studied unsufficiently. The influence of azaspirodecane, isoquinoline, phthalimide, imidazole and some other terminal fragments of buspirone analogues on their affinity is well known [1–4], whereas the influence of 3-arylindazole fragments on the affinity of the mentioned analogues has not been investigated yet.

It is also known, that some 3-arylindazoles have been proposed for clinical practice as CNS stimulating agents [5, 6]. The most perspective drug candidates are indazoles with alkylpiperazinyl substituents. Such compounds have sedative and anti-depressive properties that, probably, are due to their action on the serotonin- and dopaminergic systems [7].

This work has been carried out in order to study the influence of the indazole fragment on the affinity of 3-aryl-[(4-phenyl-1-piperazinyl)butyl]indazole derivatives to both 5-HT_{1A} serotonin and D_1 dopamine receptors and to reveal the perspective biologically active compounds of this series.

2. Investigations, results and discussion

2.1. Chemistry

The 3-arylindazoles **I–IV** were obtained according to Scheme 1.

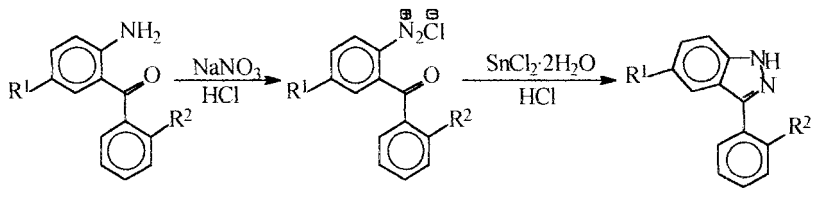
The starting 2-aminobenzophenones were previously described [8]. On the first stage these compounds were turned into the corresponding diazonium salts by sodium nitrite in acidic medium. The latter were reduced by stannum chloride to the 3-arylindazoles **I–IV** which were used for the synthesis of the phenylpiperazine derivatives **Ia–IVa** (Table 1) according to Scheme 2 [9].

The reaction between the 3-arylindazoles **I–IV** and 8-phenyl-8-aza-5-azoniaspiro[4,5]decane bromide (**V**) was carried out in toluene in the presence of potassium hydroxide. The quaternary spiroammonium salt **V** was obtained by a known method [10] as a result of interaction of N-phenylpiperazine with 1,4-dibrombutane in isopropanol in the presence of sodium carbonate. UV, IR, NMR and mass-spectra confirm the proposed structures.

2.2. Binding studies

Addition of the tested compounds to the incubation medium has allowed to reveal significant differences in their

Scheme 1



I: R_1=CH_3, R_2=H **II**: R_1=Cl, R_2=H **III**: R_1=Br, R_2=H **IV**: R_1=Br, R_2=Cl

Table 1: 3-Arylindazoles derivatives

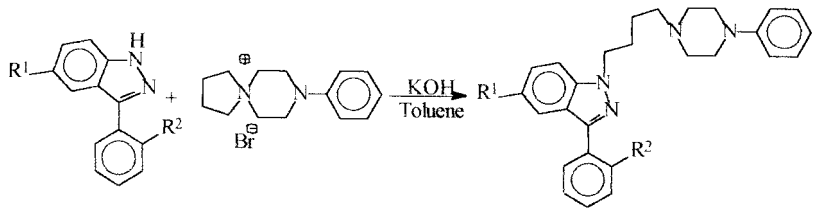
Compd.	Yield (%)	M.p. (°C)	Bruttoformula	Mass spectra $[M]^{+\cdot}$
I	70	133	$C_{14}H_{12}N_2$	208
II	67	123	$C_{13}H_9ClN_2$	228
III	66	148	$C_{13}H_9BrN_2$	272
IV	65	186	$C_{13}H_8BrClN_2$	306
Ia	55	160–165	$C_{28}H_{33}ClN_4$	424*
IIa	69	205–215	$C_{27}H_{30}Cl_2N_4$	444*
IIIa	71	210–220	$C_{27}H_{30}BrClN_4$	488*
IVa	64	205–215	$C_{27}H_{29}BrCl_2N_4$	522*

* Hydrochlorides of the compounds **Ia–IVa** have not a molecular ion peak in their MS but they have a $[M\text{-}HCl]^{+\cdot}$ free basis peak. ** Results of the elemental analyses use in

Table 2: Affinity of the 3-arylindazoles derivatives to 5-HT_{1A} and D_1 receptors of rat brain

Compd.	$-\log K_i$		Selectivity
	D_1	5-HT_{1A}	$K_i(5\text{-}HT_{1A})/K_i(D_1)$
I	4.41 ± 0.04	4.47 ± 0.03	0.85
II	4.58 ± 0.05	4.05 ± 0.04	3.37
III	4.79 ± 0.03	3.91 ± 0.03	7.54
IV	5.24 ± 0.06	3.82 ± 0.04	25.71
Ia	6.02 ± 0.04	6.34 ± 0.06	0.48
IIa	6.55 ± 0.06	5.56 ± 0.06	9.90
IIIa	6.83 ± 0.05	5.27 ± 0.05	36.41
IVa	6.96 ± 0.07	5.22 ± 0.04	54.92

Scheme 2



IV **V** **Ia - IVa**

affinity (Table 2). Compound **IVa** exhibited the highest affinity to dopamine D_1 receptors. A detailed study of the inhibitory effect depending on the concentration of these compounds showed that displacement of [^{3}H]SCH-23390 from its specific binding sites went according to the competition mode (similar data were also obtained for [^{3}H]8OH-DPAT displacement). An increase in affinity to dopamine D_1 receptors within substituents Br > Cl > CH_3 at 5-position of the 3-arylindazole molecule observed for compounds **I–III**. Addition of the chlorine atom to the orthoposition of the phenyl ring (**IV**) made its activity even higher. Thus, the value of $-\log K_i$ increased from 4.41 for compound **I** to 5.24 for compound **IV**. As it can be seen, replacement of the hydrogen atom at the first position of the 3-arylindazole on (phenylpiperazine)butyl substituent (**Ia–IVa**) caused an increase in affinity and did not change the trends of affinity dependence from the structure of 3-arylindazoles. The increase of $-\log K_i$ value within the substituents Br > Cl > CH_3 (**Ia–IIIa**) as well as the affinity increase in the presence of a chlorine atom in the ortho-position of the phenyl ring of 3-arylindazole (**IVa**) took place here, too. In this case, the value of $-\log K_i$ increased from 6.02 (**Ia**) to 6.96 (**IVa**).

Another order of affinity dependence on the structure of the studied compounds was observed for serotonin 5-HT_{1A} receptors. Displacement of [^{3}H]8OH-DPAT by two different groups of 3-arylindazoles (**I–IV, Ia–IVa**) showed that compounds containing the methyl group at the 5-position (**I** and **Ia** are more active than compounds containing a halogen. Inclusion of the chlorine atom to the ortho-position of the phenyl ring (**IV, IVa**) caused a much more decrease in affinity. Replacement of the hydrogen atom at the first position of the 3-arylindazole on the (phenylpiperazine)butyl substituent (**Ia–IVa**) led to an increase in affinity (Table 2). Such an influence of the substituents on the molecule resulted in a sharp increase of $-\log K_i$ for the 3-arylindazoles turning into the corresponding piperazinyl derivatives (compounds **Ia–IVa**). The quantitative influence of structure modifications on the receptor affinity of compounds **I–IV** and **Ia–IVa** was analysed by the Free-Wilson method [12]. In this nonparametric QSAR model we have used the logarithmic scale of affinity. For each compound of the series the value of affinity A_i was expressed as the sum of the average affinity A_0 and individual affinity contributions a_{ij} of the substituents R_i in the position j:

$$A_i = A_0 + \sum a_{ij}X_{ij} \quad (1)$$

where, X_{ij} has a value of "one" when the substituent R_i is present at the position j, otherwise its value is "zero".

For the 5-HT_{1A} binding experiments:

$$\log K_i = -4.83 - 2.93 \cdot (R^1_{CH_3}) - 2.57 \cdot (R^1_{Cl}) - 2.21 \cdot (R^1_{Br}) + 0.66 \cdot (R^2_H) + 1.02 \cdot (R^2_{Cl}) + 2.47 \cdot (R^3_H) + 1.02 \cdot (R^3_{Piperazine})$$
$$n = 8 \qquad r = 0.981 \qquad s = 0.04 \quad (2)$$

For the D_1 binding experiments:

$$\log K_i = -5.67 - 2.7 \cdot (R^1_{CH_3}) - 3.12 \cdot (R^1_{Cl}) - 3.54 \cdot (R^1_{Br}) + 1.54 \cdot (R^2_H) + 1.12 \cdot (R^2_{Cl}) + 2.63 \cdot (R^3_H) + 0.95 \cdot (R^3_{Piperazine})$$
$$n = 8 \qquad r = 0.973 \qquad s = 0.04 \quad (3)$$

The correlations given in eqs. **2** and **3** are highly significant. The influence of substituents R_i on receptor affinity of the compounds **I–IV** and **Ia–IVa** are independent of each other and fully additive.

The selectivity of the studied compounds determined by inhibitory constants dividing (5-HT_{1A}/D_1) varied within a wide range. Compounds having the highest activity to D_1 dopamine receptors were also the most selective. Thus, addition of the 3-arylindazole fragment in new buspirone analogues increased their affinity to dopamine receptors and reduced their affinity to serotonin receptors. In general, the selectivity of the studied compounds testified that they could be considered as dopaminomimetics rather than serotoninomimetics. Particularly interesting from this point of view are compounds **IIIa** and **IVa** which possess high selectivity and high affinity to D_1 receptors. The inhibitory constants of the mentioned compounds ($K_i \approx 100$ nM)

practically are not significantly different ($P > 0.05$). Evidently, they might be interesting for further pharmacological investigation. Compound **IVa** appears to be the most promising because its selectivity is the highest.

3. Experimental

3.1. Chemistry

3.1.1. 5-Brom-3-(ortho-chlorphenyl)indazole (**IV**)

2-Amino-5-brom-2′-chlorobenzophenone (2.5 g, 0.04 mol), 100 ml of acetic acid and 100 ml of concentrated HCl were placed into a four-neck flask (0.5 l) equipped with stirrer, thermometer and dropping funnel. Benzophenone was dissolved at stirring and heating. The reaction mixture was cooled to 3–6 °C with an external ice-salt bath. A solution of $NaNO_2$ (3.3 g of $NaNO_2$ in 8 ml of H_2O) was added dropwise and stirred at a rate to keep temperature at +1 °C. The reaction mixture was stirred for 30 min, then poured at a small rate and with stirring into solution of $SnCl_2$ (25 g $SnCl_2 \cdot 2\,H_2O$ in 130 ml of concentrated HCl), and cooled with an ice-salt bath. After settling for 30 min the reaction mass was washed with H_2O (400 ml). The precipitated residue was filtered on the Buhner's funnel and then placed into a beaker with concentrated NaOH solution in H_2O (40 ml). The obtained suspension was extracted with boiling toluene (3×50 ml). The combined toluene solution was dried over Na_2SO_4, filtered off, and concentrated under reduced pressure with a rotary evaporator. The precipitated crystals were recrystallized from a $CHCl_3/CH_3OH$ (2 : 1) mixture. Yield 65.3% (8.1 g); m.p. 186 °C; ^{1}H NMR: AM-250 "Bruker" (250.13 MHz, TMS, $CDCl_3$, δ, ppm): 6.9–7.9 (m, 7 H, aromatics), 12.38 (s, 1 H, NH); UV [CH_3OH, nm (lg ε)]: $\lambda_{max} = 218(4.57)$, 307 (3.84)
Compounds **I–III** were obtained in the same manner.

3.1.2. 8-Phenyl-8-aza-5-azoniaspiro[4,5] decane bromide (**V**)

N-Phenylpiperazine (10 g, 0.062 mol), 6.6 g (0.062 mol) of finely ground Na_2CO_3, 120 ml of isopropanol and 18.6 ml (0.155 mol) of 1,4-dibrombutane are placed in a two-neck flask with stirrer and reflux condenser. The reaction mixture is refluxed for 8 h with stirring, then cooled to room temperature and filtered through a folding filter. The isopropanol solution was concentrated in a rotary evaporator. The final product was recrystallized from an isopropanol-ethylacetate (1 : 2) mixture. The crystals were collected on Shott's filter and dried for 1 h in drying box at 100 °C. Yield 92.7% (17 g), m.p. 212 °C.

3.1.3. 5-Brom-3-(ortho-chlorphenyl)-1-[4-(4-phenyl-1-piperazinyl)butyl] indazole (**IVa**)

5-Brom-3-(ortho-chlorphenyl)indazole (3.12 g, 0.01 mol), 3 g (0.01 mol) of **V** and 0.85 g (0.015 mol) of carefully ground KOH were placed into a two-neck round-bottom flask with stirrer and reflux condenser. Dry toluene (60 ml) was added to the reaction mixture and then it was heated under reflux with stirring for 6 h. Afterwards the mixture was cooled and filtered through a folding filter. The toluene solution was concentrated in a rotary evaporator. The obtained oil-like basis was transformed into the monohydrochloride by an equimolar amount of HCl in C_2H_5OH. The hydrochloride was recrystallized from an C_2H_5OH diethyl ether (1 : 2) mixture.
Yield 64% (3.64 g); m.r. 205–213 °C; ^{1}H NMR: AM-250 "Bruker" (250.13 MHz, TMS, $CDCl_3$, δ, ppm): 6.9–8.0 (m, 12 H, aromatics), 4.55 (t, 2 H, NCH_2); 2.12 (quint, 2 H, $CH_2\underline{CH_2}CH_2CH_2$), 1.66 (quint, 2 H, $CH_2CH_2\underline{CH_2}CH_2$), 2.49 (t, 2 H, CH_2N), 2.62 (t, 4 H, piperazine), 3.25 (t, 4 H, piperazine); UV [CH_3OH, nm (lg ε)]: $\lambda_{max} = 209(4.63)$, 223 (4.65), 314 (3.87).
Compounds **Ia–IIIa** were obtained in the same manner.

3.2. Radioligand study

The affinity for both D_1 dopamine and 5-HT_{1A} serotonin receptors was tested by determination of the ability to inhibit the specific binding of [^{3}H]SCH-23390 and [^{3}H]8OH-DPAT, respectively, on the synaptic membranes of rat brains. White male rats (200 ± 20 g) used in all experiments were housed under standard conditions with free access to food and water.

3.2.1. Preparation of dopamine receptor membranes [13]

The rats were quickly decapitated and their brains were removed on cold. Striatum was dissected and homogenized in 50 volumes (wet weight/volume) of cold-ice 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) using a manual glass/Teflon homogenizer (20 strokes). The obtained suspension of crude membranes was centrifuged at 40,000 × g and at 4 °C for 20 min. The supernatant was removed, the pellet was rehomogenized in the same volume of cold Tris-HCl saline buffer (pH 7.4 at 21 °C), containing 120 mM of NaCl, 5 mM of KCl, 2 mM of $CaCl_2$, 1 mM of $MgCl_2$, and 0.1% of ascorbic acid, and the obtained suspension was centrifuged again. Rehomogenization and centrifugation were repeated at the same conditions. Then the supernatant was removed and the final pellet was weighted and rehomogenized in an assay cold buffer (pH 7.4 at 21 °C), containing 50 mM of Tris-HCl, 120 mM of NaCl, 5 mM of KCl, 2 mM of $CaCl_2$, 1 mM of $MgCl_2$ and 1 mM of EDTA. Membrane preparations containing 20–30 mg/ml protein were obtained using appropriate volumes of assay buffer.

3.2.2. Preparation of serotonin receptor membranes [14–15]

The frontal rat brain was homogenized immediately after decapitation in 20 volumes of ice-cold Tris-HCl 50 mM buffer (pH 7.5 at 20 °C) and it was centrifuged at 15,000 × g and at 4 °C for 20 min. The pellet was rehomogenized and recentrifuged twice at the same conditions. Then it was frozen to −30 °C overnight. After defrozing the pellet was homogenized in assay buffer (pH 7.6 at 20 °C), containing 50 mM of Tris-HCl, 5 mM of $CaCl_2$ and 0.1% of ascorbic acid. The obtained homogenate was preincubated at 25 °C for 30 min and centrifuged at the mentioned conditions once more. The final pellet was rehomogenized again in an appropriate volume of assay buffer to obtain 15–25 mg/ml tissue protein.

3.2.3. Binding assays

All experiments were performed using [^{3}H]SCH-23390 (75 Ci/mmol, Amersham) and [^{3}H]8OH-DPAT (211 Ci/mmol, Amersham). Dopamine D_1 receptor binding assays consisted of 8–12 mg of tissue suspension, 0.3 nM of [^{3}H]SCH-23390 and different concentrations of displacing drugs dissolved in 0.5 ml final buffer assay volume. The following incubation at 20 °C for 40 min was interrupted by addition of 6 ml buffer and the assays were rapidly filtered under vacuum through GF/B Whatman's filters with the 12-position harvester. Serotonin 5-HT_{1A} receptor binding assays consisted of 6–10 mg of tissue suspension, 0.5 nM of [^{3}H]8OH-DPAT and different concentrations of displacing compounds dissolved in 0.5 ml final buffer assay volume. All assays were incubated at 20 °C for 40 min. The binding reaction was interrupted by a 12-times dilution of the assays and a quick filtration under vacuum. Specific binding was defined using unlabeled SCH-23390 (100 nM) or 8OH-DPAT (10 nM), respectively. Specific binding was generally 80–85% of the total binding of both [^{3}H]SCH-23390 and [^{3}H]8OH-DPAT. The dried filters were placed into the vials with 10 ml of OptiPhase (LKB, Sweden) scintillation cocktail and radioactivity was measured on the liquid scintillation counter RackBeta (LKB, Sweden). To define 50% inhibitory concentration (IC_{50}) eight concentrations of the tested substances within a range of 0.1–100 nM were used. All experiments were repeated 6 times and the IC_{50} value was determined by processing of experimental data with the methods of regressive analysis. The Inhibition constant was calculated by the formula:

$$K_i = IC_{50}/(1 + L/K_d) \quad (4)$$

where L = radioligand concentration; K_d = dissociation constant of the ligand-receptor complex.

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