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Influence of the Terminal Amide Fragment Geometry in Some 3-Arylideneindolin-2(1*H*)-ones on Their 5-HT_{1A}/5-HT_{2A} Receptor Activity[1]

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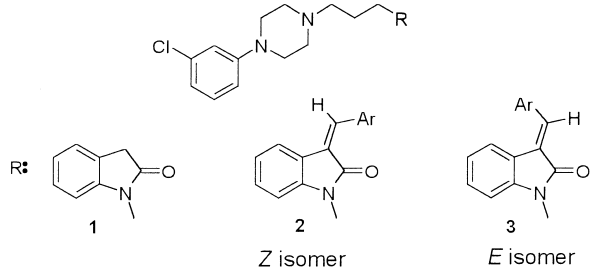
Abstract—Several 1,4-disubstituted arylpiperazine derivatives of 3-arylideneindolin-2(1*H*)-one (*Z* and *E* isomers) were tested for their 5-HT_{1A} and 5-HT_{2A} receptor activity in vitro and in vivo. It was shown that introduction of 3-arylidene substituents to indolin-2(1*H*)-one moiety allowed to change the mixed 5-HT_{1A}/5-HT_{2A} receptor ligands to 5-HT_{2A} ones with antagonistic in vivo activity.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) has been implicated in a variety of physiological and pathophysiological processes. Many therapeutically useful drugs have 5-HT receptors as targets.[2,3] Within the seven main classes of 5-HT receptors, 5-HT_{1A} and 5-HT_{2A} subtypes have been generally accepted as those involved in regulation of such mental disorders as depression, anxiety or schizophrenia.[3–5] For the past several years we have focused our attention on these receptor ligands in a search for structural parameters which determine their affinity and selectivity at 5-HT_{1A} and/or 5-HT_{2A} binding sites.[6,7] In the absence of detailed structural information about receptors we have had to adopt a structure–activity approach to study these problems.



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Within a group of 4-substituted 1-arylpiperazines possessing a terminal indolin-2(1*H*)-one fragment, we have already shown how 4-, 5-, and 6-substituents influence the activity, by not only promoting the binding to particular receptor subtypes but also by enhancing the pharmacological activity of the ligands.[8] Our present study is concerned with the indolin-2(1*H*)-one core by introducing 3-substituents which significantly elongate this fragment. The influence of geometry of the entire amide moiety on in vitro and in vivo properties of the newly synthesized compounds is the subject of this paper.

Materials and Methods

Chemistry

The starting 3-arylideneindolin-2(1*H*)-ones were obtained from indolin-2(1*H*)-one and the appropriate aromatic aldehydes in boiling EtOH in the presence of a few drops of piperidine.[9] Those 3-substituted indolin-2(1*H*)-ones (*E*, *Z* mixture) were alkylated with 1-(3-chloropropyl)-4-(3-chlorophenyl)piperazine in the presence of alumina-supported potassium fluoride (KF/Al_2O_3), in boiling acetonitrile. The obtained compounds were purified and separated into *E* and *Z* isomer derivatives by a column chromatography (SiO_2/$CHCl_3$–MeOH; 95:5), and their structure was determined on the basis of ^{1}H NMR spectra. Free bases were converted

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into hydrochloride or fumarate salts for biological assays.

Pharmacology

Radioligand binding studies were carried out in the hippocampus of rats for 5-HT_{1A} receptors, and in the cortex for 5-HT_{2A} receptors according to the previously published procedures.[10] The radioligands used were [^{3}H]-8-OH-DPAT (190 Ci/mmol, Amersham) and [^{3}H]-ketanserin (60 Ci/mmol, NEN Chemicals) for 5-HT_{1A} and 5-HT_{2A} receptors, respectively. K_i values were determined on the basis of at least three competition binding experiments in which the tested compounds were used at concentrations of 10^{-10}–10^{-3} M, run in triplicate.

To determine the postsynaptic 5-HT_{1A} agonistic effect of **2a** and **2e**, their ability to induce a lower lip retraction (LLR) in rats was tested. The ability of those compounds to inhibit that symptom produced by 8-OH-DPAT, a 5-HT_{1A} receptor agonist, was regarded as a postsynaptic 5-HT_{1A} antagonistic activity.[11,12] The blocking effect of the tested compounds on the head twitches induced by the 5-HT_{2A} agonist (±)DOI in mice[13] was a measure of their antagonistic activity at those receptors.

Results and Discussion

As seen in Table 1, the 3-arylidene substituted compounds have much diversified affinities at 5-HT_{1A} receptors. Of those compounds, only two *Z* isomers, which possess phenyl and 3-pyridyl substituents (**2a** and **2e**, respectively) have a relatively high affinity ($K_i \cong 50$ nM) at these sites; however, the values obtained in the present study were twice as low as those for the parent compound **1** ($K_i = 29$ nM).[14] The other tested compounds showed a low or a very low 5-HT_{1A} receptor affinity. A comparison of the 5-HT_{1A} binding data of *Z* isomers showed a strong influence of arylidene substituents, especially two of them with *p*-methoxyphenyl (**2c**) and 2-thienyl (**2d**) groups, in which the presence of additional heteroatoms rendered the respective derivatives practically inactive at 5-HT_{1A} binding sites. Among *Z* and *E* isomers, the former were generally better tolerated by those receptors.

On the other hand, the 5-HT_{2A} affinity remained at the same high level ($K_i = 200$–64 nM) for the majority of the tested compounds except for the (*Z*)-thiophene derivative **2d**, which is a less active compound ($K_i = 138$ nM), and the more active (*E*)-3-pyridyl one **3e** ($K_i = 7$ nM). Moreover, a clear difference between the 5-HT_{2A} binding constants of the two isomers was observed for those two compounds only, which suggests that the *E* isomer is more active than *Z*. For the rest of the compounds, K_i values seem to be independent of the structure and geometry of the 3-substituted indolinone core.

The next step of our investigation was concentrated on the in vivo effects induced by some selected compounds. As already mentioned, all of the presented compounds (except **2d**) demonstrated a high affinity for 5-HT_{2A} receptors and only two of them (**2a** and **2e**) for 5-HT_{1A} ones. For that reason those 5-HT_{1A} and/or 5-HT_{2A} ligands were studied in vivo in tests commonly used for evaluation of the 5-HT_{1A} or 5-HT_{2A} functional activity.[11–13] In those models, compounds **2a** and **2e**—like (*S*)-WAY-100135, a well-known 5-HT_{1A} antagonist—did not evoke LLR in rats, but, when used in a dose of 20 mg/kg, strongly inhibited the LLR induced by 8-OH-DPAT (Table 2). The above results show that derivatives **2a** and **2e** act as 5-HT_{1A} receptor antagonists. The ability of **2a–c**, **2e**, **3a–e** and ketanserin, a well-known 5-HT_{2A} receptor antagonist, to antagonize head twitches in mice, observed after administration of (±)DOI, a 5-HT_{2A} agonist,[13] was used to evaluate 5-HT_{2A} receptor antagonistic properties. The results presented in Table 1 indicate that all the investigated compounds, as well as the reference compounds ketanserin and **1** effectively inhibited the (±)DOI-induced head twitches in mice; ED_{50} values of **2a–3e** derivatives ranged between 9.5–28.5 mg/kg, hence they may be classified as 5-HT_{2A} antagonists. Interestingly, the tested derivatives—despite

Table 1. In vitro and in vivo activity of compounds **2** and **3**

No.	Ar	Isomer	K_i [nM]		ED_{50}[c](mg/kg), ip
			5-HT_{1A}	5-HT_{2A}	
1[a]	—	—	29±4	25±3	6.5 (4.5–9.4)
2a[b]	Phenyl	*Z*	54±13	56±3	27.5 (20.8–36.3)[d]
3a	Phenyl	*E*	399±11	38±6	28.5 (22.3–36.5)
2b[b]	*p*-Cl-C_6H_4	*Z*	204±13	36±4	15.0 (10.0–22.5)
3b	*p*-Cl-C_6H_4	*E*	1610±4	32±2	20.0 (14.3–28.0)
2c[b]	*p*-OCH_3-C_6H_4	*Z*	7930±18	36±1	16.0 (12.3–20.8)
3c	*p*-OCH_3-C_6H_4	*E*	538±3	20±4	28.5 (18.4–44.2)
2d	2-Thienyl	*Z*	20,500±100	138±21	—
3d	2-Thienyl	*E*	>50,000	64±15	16.0 (9.4–27.2)
2e	3-Pyridyl	*Z*	57±2	20±4	27.0 (21.6–33.8)
3e	3-Pyridyl	*E*	101±5	7±1	9.5 (7.0–12.8)
	Ketanserin		1933±219[e]	1.5±0.2[e]	0.12 (0.07–0.2)

[a]Ref 14.
[b]Ref 15.
[c]ED_{50} — the dose inhibiting the (±)DOI-induced (2.5 mg/kg, ip) head twitches in mice by 50%.
[d]Confidence limits (95%) given in parentheses.
[e]Data from ref 16.

Table 2. Induction of a lower lip retraction (LLR) by **2a** and **2e** and the effect of the investigated compounds on the 8-OH-DPAT-induced LLR in rats (1 mg/kg, sc)

Treatment	Dose (mg/kg)	Induction[a]	Inhibition[b] Mean±SEM
Vehicle	—	0.1±0.1	2.8±0.2
2a	10	0.1±0.1	1.9±0.4
	20	0.2±0.1	0.8±0.1[c]
Vehicle	—	0.1±0.1	2.7±0.1
2e	10	0.1±0.2	2.3±0.3
	20	0.1±0.1	0.9±0.3[c]
(*S*)-WAY-100135	10	0.0±0.0	0.8±0.3[c]

[a]The investigated compounds were administered ip 15 min before the test.
[b]The investigated compounds were administered ip 45 min before 8-OH-DPAT.
[c]$P < 0.01$ versus vehicle + 8-OH-DPAT.

high 5-HT_{2A} affinity—were significantly less potent than ketanserin at inhibition of (±)DOI-induced effect in mice.

The major finding of this study is that introduction of a 3-arylidene substituent to the indolin-2(1*H*)-one moiety allowed to shift off mixed 5-HT_{1A}/5-HT_{2A} receptor ligands to 5-HT_{2A} receptor ones. Additionally, the study has shown that 5-HT_{2A} binding sites tolerate equally readily both *Z* and *E* isomers of the 3-substituted indolinone core, and that they are capable of accommodating a variety of 3-arylidene substituents, while the 5-HT_{1A} binding pocket is much more sensitive to both the type of arylidene substituents and the structure of the entire amide fragment.

References and Notes

1. Part 42 of the series: Structure–Activity Relationship Studies of CNS Agents.
2. Roth, B. L. *Ann. Clin. Psychol.* **1994**, *6*, 67.
3. Fletcher, A.; Cliffe, I. A.; Dourish, C. T. *Trends Pharmacol. Sci.* **1993**, *14*, 41.
4. Glennon, R. A.; Dukat, M. *ID Res. Alert* **1997**, *2*, 107.
5. Albinsson, A.; Björk, A.; Svantengren, J.; Klint, T.; Andersson, G. *Eur. J. Pharmacol.* **1994**, *262*, 285.
6. Mokrosz, M. J.; Kowalski, P.; Kowalska, T.; Majka, Z.; Duszyńska, B.; Charakchieva-Minol, S.; Szaro, A.; Tatarczyńska, E.; Kłodzińska, A.; Chojnacka-Wójcik, E. *Pol. J. Pharmacol.* **1998**, *50*, 333.
7. Mokrosz, M. J.; Kowalski, P.; Kowalska, T.; Majka, Z.; Duszyńska, B.; Bojarski, A.; Fruziński, A.; Karolak-Wojciechowska, J.; Wesołowska, A.; Kłodzińska, A.; Tatarczyńska, E.; Chojnacka-Wójcik, E. *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 373.
8. Mokrosz, M. J.; Duszyńska, B.; Misztal, S.; Kłodzińska, A.; Tatarczyńska, E.; Chojnacka-Wójcik, E.; Dziedzicka-Wasylewska, M. *Arch. Pharm. Pharm. Med. Chem.* **1998**, *331*, 325.
9. Sun, L.; Tran, N.; Tang, F.; App, H.; Horth, P.; Mc Mahon, G.; Tang, C. *J. Med. Chem.* **1998**, *41*, 2599.
10. Bojarski, A. J.; Cegła, M. T.; Charakchieva-Minol, S.; Mokrosz, M. J.; Maćkowiak, M.; Misztal, S.; Mokrosz, J. L. *Pharmazie* **1993**, *48*, 289.
11. Mokrosz, J. L.; Paluchowska, M. H.; Chojnacka-Wójcik, E.; Filip, M.; Charakchieva-Minol, S.; Dereń-Wesołek, A.; Mokrosz, M. J. *J. Med. Chem.* **1994**, *37*, 2754.
12. Moore, N. A.; Rees, G.; Sander, G.; Perrett, L. *Pharmacol. Biochem. Behav.* **1993**, *46*, 141.
13. Darmani, N. A.; Martin, B. R.; Pandey, V.; Glennon, R. A. *Pharmacol. Biochem. Behav.* **1990**, *36*, 901.
14. Mokrosz, M. J.; Mokrosz, J. L.; Duszyńska, B.; Dereń-Wesołek, A.; Kłodzińska, A.; Kowalski, P.; Charakchieva-Minol, S.; Tatarczyńska, E.; Kowalska, T.; Majka, Z.; Chojnacka-Wójcik, E.; Misztal, S. *Pharmazie* **1997**, *52*, 423.
15. Mokrosz, M. J.; Charakchieva-Minol, S.; Kozioł, A.; Kłodzińska, A.; Chojnacka-Wójcik, E. *Pol. J. Pharmacol.* **2000**, *52*, 47.
16. Titeler, M.; Jyon, R. A.; Davis, K. H.; Glennon, R. A. *Biochem. Pharmacol.* **1987**, *36*, 3265.