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Bioisosterism of Fluorine and Cyano as Indole Substituents. Theoretical, *in vitro* and *in vivo* Examination

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Abstract: Fluorine substitution modifies structural attributes and often induces unexpected effects. When substituting fluorine with cyano in position 5 of the indole in indole-butyl-amines, the properties of the compounds proved to be very comparable. *In vitro* target-profile, metabolism, and *in vivo* activity in the ultrasonic vocalisation test indicate bioisosterism between the two substituents.

Keywords: Antidepressant · Bioisosterism · Metabolism · Pseudohalogen · Ultrasonic vocalisation

Introduction

The transformation of an organic substance into an active agent, suitable for development as pharmaceutical drug, necessitates the optimisation of selective target-activity as well as parameters like solubility or metabolic stability. Bioisosterism has been discussed since the middle of the last century [1] and it is often applied by medicinal chemists to fulfill the above-mentioned need for optimisation [2]. The directed exchange of individual substituents or even whole molecular fragments of a hit or lead structure should generate a broadly similar biological response and, more important, should result in optimised pharmacodynamic- and/or kinetic parameters [3].

In fundamental chemistry books cyanide is described as a pseudo-halogen, an expression which was introduced in 1925 for covalently bound radicals like CN, OCN, SCN and N_3 [4]. These groups can form anions X^-, hydrogen acids XH and neutral species like X_2, XY and so on. Comparability on a physico-chemical level between cyanide and the halogens has been described in the literature for biological systems only in certain rare cases and tends to refer to SAR approaches rather than bioisosterism.

Kelly and co-workers found that the exchange of chlorine by fluorine or cyano in 5-benzyluracil results in less potent liver uridine phosphorylase inhibitors (Fig. 1) [5a].

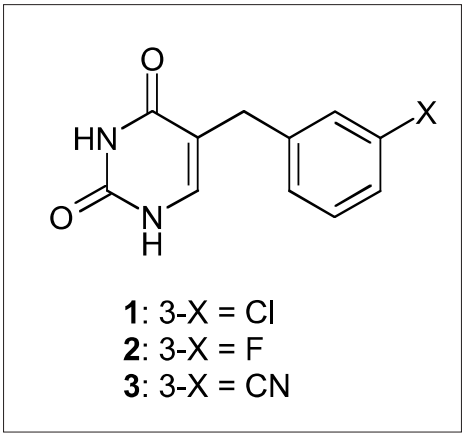

Fig. 1. Uridine phosphorylase inhibitors

The inhibition of the phosphorylase with the chloro derivative **1** is possible with an IC_{50} of 2.5 μM. The corresponding fluoro derivative **2** is weaker by a factor of 3 (IC_{50} = 7.6 μM) and the cyano compound **3** by a factor 5 (IC_{50} = 13.2 μM) [5b].

The replacement of the Cl-substituent by F or CN in benzodiazepine **3** results in retention of antagonistic activity at the cholecystokinin-A receptor as described by Evans *et al.* (Fig. 2) [6].

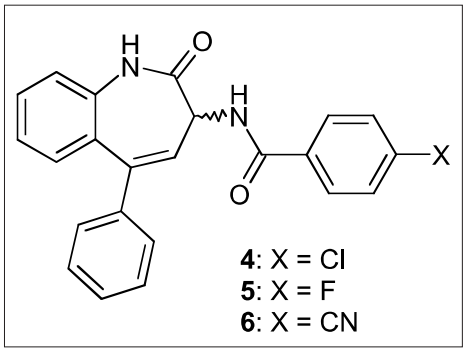

Fig. 2. CCK-A receptor antagonists

Though conserving functionality the activity decreases by about a factor of 12 (Cl → F) and ~18 (Cl → CN) with this exchange of electron-withdrawing groups [(IC_{50} nM) **4** = 41; **5** = 480; **6** = 730].

The incorporation of fluorine as either a bioisosteric replacement for hydrogen or an isoelectronic replacement for the hydroxyl group has been reported [7].

Bioisosterism between fluorine and the cyano-group has not been described in the literature explicitly to the best of our knowledge. In this paper we describe bioisosterism between fluorine and the cyano-group at position 5 of the indole using the serotonin (5-HT) receptor subtype 1A and the 5-HT transporter as biological targets. High

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potency at these targets is essential for compounds thought to increase 5-HT levels in the central nervous system and has a high potential to induce antidepressive effects in humans [8].

F and CN Most Comparable Electron-withdrawing Groups

The 5-hydroxy indole derivative **7h** shows only poor bio-availability because of metabolic issues [9]. To improve this feature we substituted the 5-OH functionality with different electron-withdrawing groups (**7a**-**7g**) to circumvent direct glucuronidation of the hydroxy-function and increase oxidative stability of the indole in general (Table 1).

The pharmaceutically less acceptable aldehyde **7d**, the oxime **7e** but also the cyano- **7b** and the fluoro derivative **7a** show nanomolar or even sub-nanomolar inhibition of the serotonin re-uptake transporter (SSRI) and binding to the 5-HT_{1A} receptor. The comparability of the *in vitro* results of **7a** and **7b** could be extended to *ex vivo* experiments. After *p*-chloramphetamine (PCA) induced 5-HT depletion in brains of living rats the 5-HT re-uptake could be inhibited with both compounds (**7a**/**7b**) to an equal amount [11].

These preliminary findings of F/CN-bioisosterism were followed by extended observations. Table 2 shows the scope of fluctuation of *in vitro* data when regarding piperidines (**8a**/**8b**), shortening chain length of **7a**/**7b** to **9a**/**9b** and **10a**/**10b**, and modifying rigidity of the linking area between the two aromatic ring systems (**11a**/**11b** and **12a**/**12b**).

The results differ by about a factor of 1 to 22 for the SSRI and from 1 to 11 for 5-HT_{1A} binding, which is in the range for bioisosters as indicated above. The modification of the aryl substituent on the piperazine gives a comparable variance of the results as shown in Table 3.

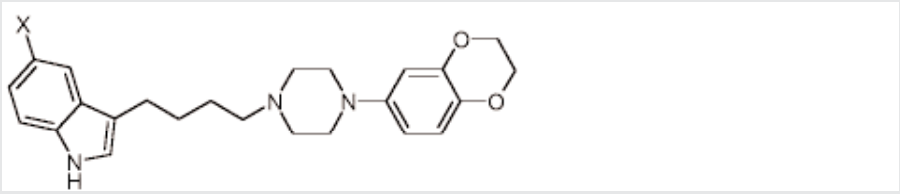
Bioisosterism is confirmed from the factor of 40 in 5-HT_{1A} binding for the monocylic derivatives **13a** and **13b** to nearly identical values for the chromenone derivatives **17a** and **17b** against both targets.

The pharmacodynamic activity (*in vivo* efficacy) of the compounds was tested in the ultrasonic vocalization (USV) test in rats, an animal model of anxiety and particularly sensitive to 5-HT_{1A} agonists [12]. Rats receiving a series of brief foot-shocks in a defined environment emit ultrasounds in the 22 kHz range, a frequency highly specific of intraspecies alarming function in anxiety-prone situations and environments [13]. When confronted again with the environment the next day, the duration of USV is measured, and a suppression of USV is regarded to be an anxiolytic effect. The method is described in detail elsewhere [14].

In the USV test, all compounds demonstrated a delayed onset of activity, although not as prominent with **13b**, **14b** and **17b**, and independent from the substitution pattern with F- or CN. However, CN-substitution seems to be associated with a higher potency with respect to the pharmacodynamic effects as determined in the USV test (*vide infra*) compared to F-substitution.

So far the compounds were analysed with respect to two targets, namely the 5-HT_{1A} receptor and the 5-HT re-uptake transporter. For the 'antidepressive' response and a 'clean drug', not only high potency at these biological objectives is essential but also weak binding at related

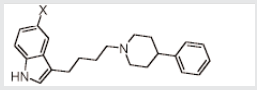
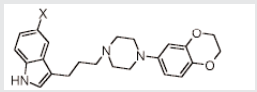
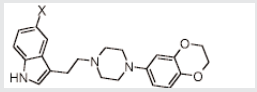
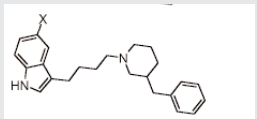
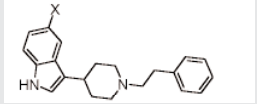
Table 1. Different EWGs in position 5 of the indole [IC_{50} nM]



No	X	SSRI	5-HT_{1A}	PCA	USV[a] 30 min	USV[a] 120 min	USV[a] 210 min
7a	F	1	0.2	13%[b]	7%	8%	11%
7b	CN	0.4	1	10%[b]	n.d.	n.d.	n.d.
7c	Cl	20	40	n.d.	n.d.	n.d.	n.d.
7d	CHO	1	0.7	n.d.	n.d.	n.d.	n.d.
7e	C=N-OH	5	0.4	n.d.	0%	0%	0%
7f	CO_2Me	50	0.6	n.d.	100%	79%	71%
7g	$CONH_2$	60	0.2	n.d.	40%	100%	100%
7h	OH	0.2	0.9	n.d.	57%	36%	19%
7i	H	10	8	n.d.	n.d.	n.d.	n.d.

[a] Inhibition after p.o. application of 30 mg/kg when tested at various times after application [10]; [b] Antagonism after 210 min. and p.o. application of 0.3 mg/kg. n.d.: not determined

Table 2. F/CN comparison I [IC_{50} nM]

No	structure	X	SSRI	5-HT_{1A}	USV[a]
8a		F	4	60	n.d.
8b		CN	0.4	40	n.d.
9a		F	4	500	38%[a]
9b		CN	3	50	31%
10a		F	2	880	86%[a]
10b		CN	4	80	n.d.
11a		F	0.4	200	n.d.
11b		CN	9	200	n.d.
12a		F	60	30	n.d.
12b		CN	10	6	n.d.

[a] Inhibition after p.o. application of 30 mg/kg; given are the maximal effects observed when tested at various times [a]210 min; [b]120 min after application. n.d.: not determined

GPCRs. The following table shows the selectivity profile of the fluorine/cyano pair of the 2-carboxamido benzofuranes **16a**/**16b** (Table 4).

Binding at the 5-HT_{1A} receptor is nearly the same for both substances (factor 1.5), *in vitro* SSRI is within a narrow range (factor 6), and *ex vivo* examination of 5-HT reuptake inhibition (PCA) delivers identical results. Potency at the 5-HT receptor subtypes 1D and 2C is negligible as well as binding to the dopaminergic receptor subtype 2 (D_2) and the adrenergic receptors α_1 and α_2.

The compounds **16a** and **16b** were examined in more detail to select a clinical candidate. Metabolism has a high impact on the compound's bioavailability and both derivatives were analysed in this direction. Because of the difference in their absorption maxima the compounds were analysed at different wavelength (240 nm, 270 nm) during their HPLC runs (Fig. 3).

Fig. 3 shows the chromatograms after 120 min exposure of human liver microsomes on the compounds. Concerning the 5-F derivative **16a** there is no main metabolite detectable within generally weak metabolic activity. For the 5-CN compound **16b** it is obvious that there are a number of metabolites, as can be seen in the relevant region between 15 and 35 min. But the total amount of them is very low. The most prominent metabolite is the 6-hydroxy derivative (30–43% of all metabolites *in vivo*) [15a] which was validated after synthesis [15b]. Table 5 summarizes important data from the metabolism examination.

Particularly the poor solubility of the fluoro compound at pH 8.5 and the abovementioned pharmacodynamic effects influenced the decision to select the cyano derivative for clinical evaluation.

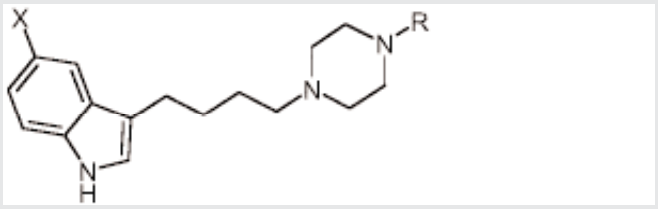
Prediction of Bioisosterism

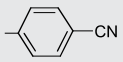
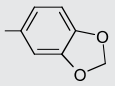
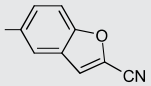
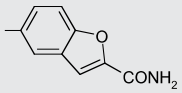
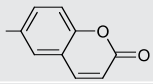
After this quite broad retrospective discussion of bioisosterism we want to focus on predictability. Could this bioisosterism have been expected? Regarding the abovementioned pseudo-halogen concept it suggests that the substituents behave the same. But for biological questions it should always be kept in mind that it is the whole molecule that induces the biological effect and not just one substituent.

Table 6 shows physico-chemical parameters of some indoles substituted with electron-withdrawing groups.

The value-variance for these descriptors indicates that they are not suitable for bioisosteric predictions. As mentioned in the literature it is the molecular hardness as defined by Parr and Pearson which, to some extent, allows comparison and prediction with regard to bioisosterism [16]. Valance orbital

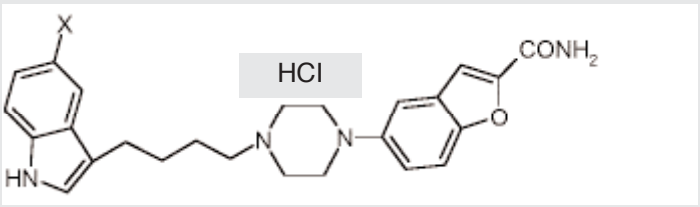
Table 3. F/CN comparison II [IC_{50} nM]



No	R	X	SSRI	5-HT_{1A}	USV[a] 30 min	USV[a] 120 min	USV[a] 210 min
13a		F	2	40	n.d.	n.d.	n.d.
13b		CN	1.3	1	25	14	15
14a		F	1	4	>30	7	6
14b		CN	0.6	0.7	14	9	10
15a		F	3	5	n.d.	n.d.	n.d.
15b		CN	1	0.4	n.d.	n.d.	n.d.
16a		F	0.3	3	>30	>30	>30
16b		CN	0.5	0.2	>30	13	12
17a		F	0.9	0.7	>30	20	18
17b		CN	0.7	0.1	9	4	4

[a] ID_{50} in mg/kg after p.o. application when tested at various times after application. n.d.: not determined

Table 4. F/CN comparison III [IC_{50} nM]



target	16a X = F	16b X = CN
SSRI[a]	0.3	0.2
5-HT_{1A}[a]	3.0	0.5
PCA[b]	3.8	3.8
USV[b]	> 30	12
5-HT_{1D}[a]	>1000	4000
5-HT_{2C}[a]	>1000	2000
D2[a]	> 100	666
α_1[a]	3000	1980
α_2[a]	9000	6000

[a] [IC50 nM]; [b] [ED_{50} mg/kg] 210 min after p.o. administration

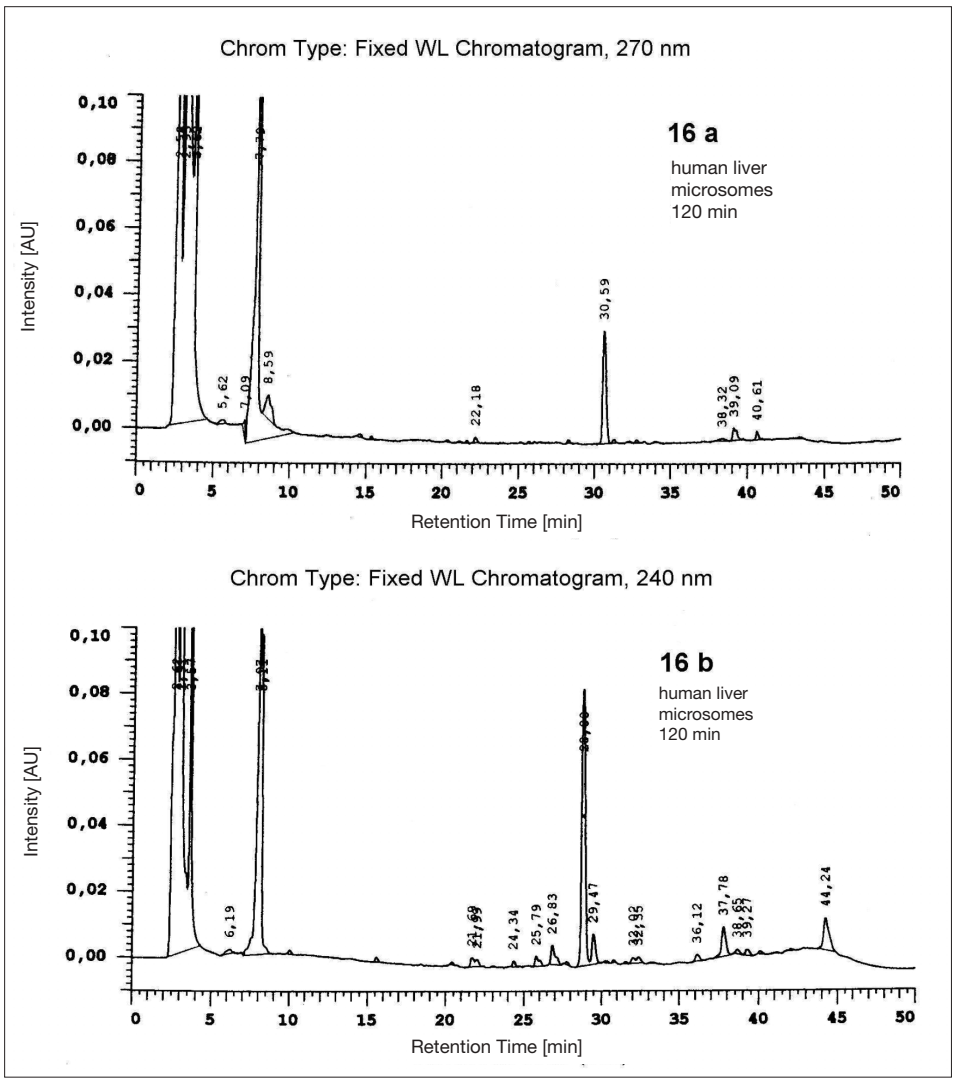


Fig. 3. HPLC analysis of *in vitro* metabolism studies of the fluorine analogue **16a** and *vilazodone* **16b**

Table 5. Metabolism examination summary

	16a	**16b**
artificial gastric juice	stable	stable
pH 8.5	stable	poor solubility
	logD pH 7.4: 2.96	logD pH 7.4: 3.61
human plasma	stable	stable
$t_{1/2}$ rat microsomes	40 min	18 min
	(23 metabolites)	(11 metabolites)
$t_{1/2}$ human microsomes	> 60 min	> 60 min
	(13 metabolites)	(5 metabolites)

calculations for larger molecules are quite extensive, of course, or even impossible, and that is why we thought of an alternative or compromise. Ultimately hardness quantifies valence electron binding to the core structure and indicates polarizability. Even if quantification is difficult, visualisation in terms of qualification should be helpful. Some considerations are shown in Table 7.

Here molecular electrostatic potential and dipole vector are shown for the unsubstituted indole and substituted derivatives. It is interesting to see how the unsubstituted 'mother' structure and the 5-chloro analogue have one negative area of charge in contrast to the fluorine and cyano derivatives which show comparability with regard to two centres of negative charge and the dipole vector in length and direction. The 5-ethynyl-indole has an equal charge distribution around the core but a positively charged endpoint at the ethynyl moiety. Therefore the dipole vector is shorter for this carbon-chain indole than for the fluoro- and cyano derivatives. Nevertheless it would be interesting to synthesize the ethynyl *vilazodone* analogue and to compare the biological features. The same applies for the tri-fluoro methyl substituent, too. Charge distribution and dipole vector of this indole scaffold are very comparable to the fluoro and cyano framework and equal biological results can be expected from the tri-fluoromethyl *vilazodone* derivative.

Conclusions

This graphical presentation of the calculated potential surfaces allows visualisation and thus might contribute to a better understanding of the bioisosterism approach. Perhaps it would make sense to treat all practically synthesised and virtually constructed molecules within a certain project in this illustrating way to search purposively for bioisosters. There are hints in the literature that this approach might work [17].

In this context we have observed that fluorine and cyano are exchangeable and behave as bioisosters at the 5-HT_{1A} receptor subtype and the 5-HT re-uptake transporter.

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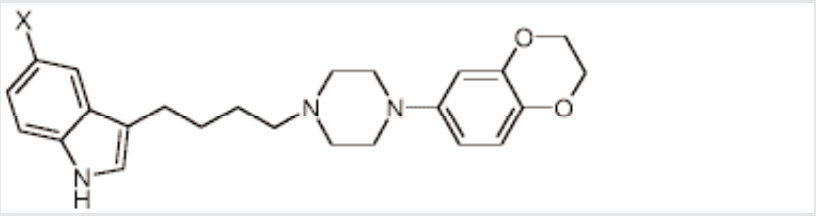
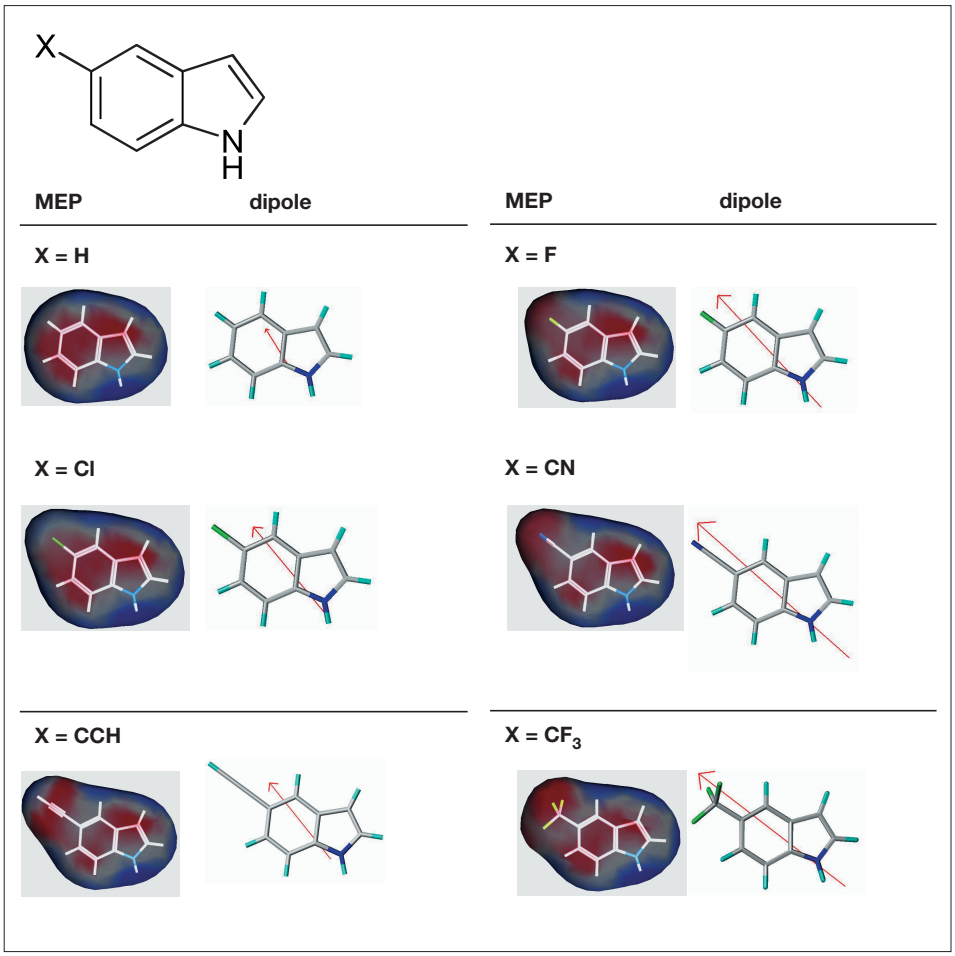
Table 6. Physico-chemical parameters



No	X	σ-p	mol.-refrac.	logD calc.	pKa exp.	S exp. [μg/ml]	S calc. [μg/ml]
7a	F	0.06	0.09	4.62	7.78	< 1	2.5
7b	CN	0.66	0.63	3.96	7.75	1.4	6.8
7c	Cl	0.23	0.60	5.06	7.82	< 1	1.3
7d	CHO	0.42	0.69	4.13	8.01	8.3	8.5
7e	C=N-OH	0.10	1.028	4.29	7.91 (calc.)	1	0.5
7f	CO_2Me	0.45	1.29	4.25	7.49	3.5	4.4
7g	$CONH_2$	0.36	0.98	3.16	8.09	74	3.0
7h	OH	-0.37	0.285	3.94	7.85	1	56.7
7i	H	0	0.10	4.42	7.91	1.2	7.3

Table 7. MEP and dipole moment for some 5-substituted indoles



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[10] Whereas compounds **7f** and **7h** exhibit maximal activity when tested 30 min after application, compound **7g** demonstrate a delayed onset of activity. For compound **7g,** with the most prominent separation between 5-HT1A *versus* SSRI, also the dose-dependency was determined giving ID50 values of 6 mg/kg when tested 120 min after application and 2.4 mg/kg p.o. when tested 210 min after application.

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