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Synthesis and Antidepressant activity of pyrazoline based MAO-inhibitors

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Abstract: A series of nine 3-(2-hydroxyphenyl)-5-aryl-*N*-phenyl-4,5-dihydropyrazole-1-carbothioamide derivatives (**3a-3i**) that were earlier reported as potent rMAO-A inhibitors were evaluated for their antidepressant activity in Porsolt's behavioral despair test (forced swim test) and Tail Suspension test activity, among them, compounds (**3e** and **3h**) were found to have potent antidepressant activity. Reduction in duration of immobility was significant for all the compounds in Porsolt's swim test compared with tail suspension test. 3h was further evaluated for anxiolytic activity in Elevated plus maze and was found to be devoid of it.

Keywords: pyrazolines; antidepressant activity; anxiolytic activity; porsolt's swim test; tail suspension test; elevated plus maze

1. Introduction

Pyrazolines are well known and important nitrogen containing 5-membered heterocyclic compounds and synthesis of which can be achieved through various methods.[1–3] They were also known for their diverse pharmacological activity[4,5] and well documented for their MAO inhibitory activity[6,7]. MAO inhibitors (Iproniazid[8], imiperamine, phenelzine, isocarboxazid and tranylcypromine) were the first drugs to be used clinically for the treatment of depression during 1950s,[9] but their use was restricted due to hypertensive crisis since 1960[10,11]. The side-effects was mainly attributed to their non-selective and irreversible inhibition of MAO and they were quickly replaced by drugs that were safer and effective (Selective Serotonin Reuptake Inhibitors).[12] Therapeutic utility of selective and reversible inhibitors of MAO isoforms in the treatment of depression and neurodegenerative disorders has renewed the intrest on design, synthesis and studies of newer compounds.[13] Presented study explores the antidepressant activity of nine potent rMAO-A inhibitors reported by our group[14] in Porsolt's behavioural despair test and Tail suspension test models. Potent compound was then screened for possible anxiolytic activity by elevated plus maze model.

2. Results and Discussion

2.1. Chemistry

Compounds (**3a-3i**) were synthesized according to the scheme and procedure reported by our group.[14]

2.2. *In vivo* Antidepressant & Anxiolytic activiy:

The LD_{50} for all the compounds were predicted through web based toxicity prediction program TOXBOX (http://www.pharma-algorithms.com/webboxes). One twentieth of the predicted LD_{50} value was selected for *in vivo* pharmacological studies (20 mg/kg body weight). Experimentation on animals were approved by Institutional Animal Ethics Committee wide Letter No. BIT/PH/IAEC/07/2009, dated 19/01/2009, All the synthesized compounds (**3a-3i**) were subjected to *in vivo* antidepressant activity investigated by Porsolt's behavioral despair test (forced swimming)[15] and Tail Suspension Test[16] model on swiss albino mice, at 20 mg/kg body weight dose level. Results are expressed as mean ± S.E.M. Data obtained from pharmacological experiments were analyzed with one-way analysis of variance (ANOVA) followed by Dunnet's post hoc test, using Microsoft Excel and Graphpad Instat Demo version. A p-value of less than 0.05 was considered statistically significant.

Except **3a**, all the other compounds have shown to reduce duration of immobility compared to the control. Compounds 3h and 3e were found to be potent amongst them showing significant reduction compared with control at $p< 0.05$ (**Table 1, Figure 1 & 2**). They were also found to be better than the standard Chlorgylin. Compound 3g having potent rMAO-A inhibitory activity (IC_{50}: 2.84±0.19 μM, Table 1) found to perform poorly in comparison with 3e and 3h. This may be due to its physicochemical properties influencing its pharmacokinetic behavior of compound that determines the concentration of drug at the site of action. In case of Tail suspension test reduction is immobility time compared with control was minimal for almost all the compounds tested. In order to rule out the possible anxiolytic activity, compound **3h** was tested on elevated plus maze model[17] and was found to

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Table 1. Antidepressant activity of compounds **3a-3i** in Porsolt's Swim Test Model and Tail Suspension Test Model

Comp	R	rMAO-A (IC_{50} in μM)#	Porsolt's Swim Test Model		Tail Suspension Test Model	
			Duration of immobility (sec.)	% Change from control	Duration of immobility (sec.)	% Change from control
3a	H	49.16±3.50	192.75±24.036	20.28	128±11.590	-28.19
3b	2-Cl	20.05±3.56	129.00±41.354	-19.50	96.66±4.631**	-46.14
3c	4-Cl	23.18±1.58	53.25±18.145*	-66.77	91.33±21.927**	-48.48
3d	2-OH	58.10±3.63	43.25±4.137**	-73.01	125.50±20.209	-29.59
3e	4-OH	67.22±5.80	32.75±29.511**	-79.56	150.75±14.879	-15.54
3f	2-OCH_3	74.20±6.76	106.00±5.730	-33.85	111.33±49.166*	-37.54
3g	4-OCH_3	2.84±0.19	70.67±7.52**	-64.25	149.5±12.23**	-27.37
3h	a	5.56±0.45	27.17±4.84**	-86.26	130.83±12.88**	-36.44
3i	b	33.41±2.85	65.5±5.694	-59.10	101.33±10.269*	-43.15
CTRL			160.25±24.955	-	178.25±7.052	-
MOC		3.90±0.19	13.75±8.509**	-91.41	62.67±8.511**	-64.04
CLOR		2.05±0.19	39.5±7.79**	-80.02	62.5±14.54**	-69.64

Values are mean ± SEM (n=6), **P<0.01 vs control (Anova, Dunnett test), *P<0.05 vs Control (Anova, Dunnet test), MOC=Moclobemide, CLOR-Clorgyline, CTRL-Control [a]Thiophene-2-yl replaces R-C_6H_4- ring.[b]Furan-2-yl replaces R-C_6H_4- ring; # values are from ref 14

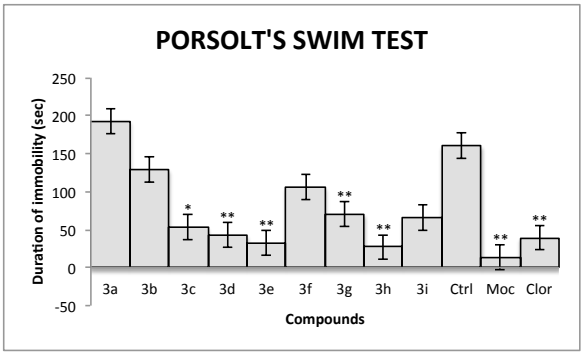

Figure 1. Antidepressant activity of compounds **3a-3i**: Porsolt's swim test

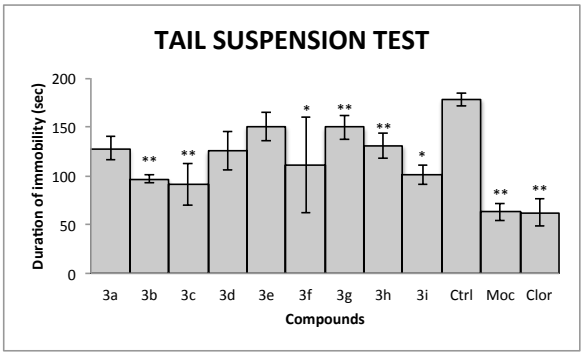

Figure 2. Antidepressant activity of compounds **3a-3i**: Tail suspension test

Table 2. Anxiolytic activity of compounds **3h** in Elevated plus maze test model

Comp	Time spent (s)			Frequency of entry		
	Open arm	Close arm	Centre	Open arm	Close arm	Centre
3h	3.17± 2.10*	228.1± 14.32	67± 12.39	0.5± 0.22	3.33± 0.80	2.83± 0.87
ALP	205± 9.99**	48± 13.57	46.67± 9.35	5.83± 1.22**	2.33± 0.56	7.17± 1.64
CTRL	23± 5.05	252.7± 7.80	24.33± 4.38	2.17± 0.48	8.83± 1.58	10± 2.05

Values are mean±SEM (n=6), **P<0.01 vs control, *P<0.05 vs control (Anova, Dunnett test); ALP-Alprazolam; CTRL-Control

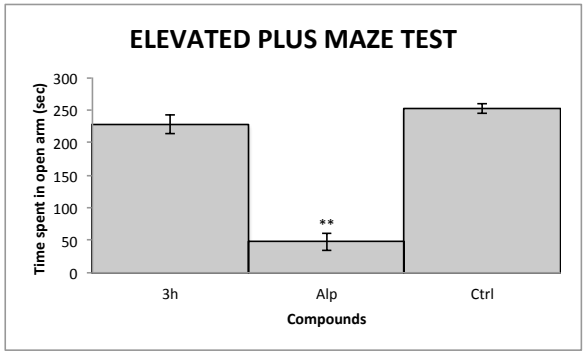

Figure 3. Anxiolytic activity of compounds **3h**: Elevated plus maze test

have insignificant activity compared with control and standard Alprazolam (**Table 2, Figure 3**).

4. Conclusions

Compounds (**3e** and **3h**) have shown significant antidepressant activity at a dose of 20 mg/kg body weight in Porsolt's swim test model. Further compound 3h was studied using Elevated plus maze model for possible anxiolytic activity but was found to be devoid of any anxiolytic activity. Analogues of these compounds having variation in aryl ring at 1st and 3rd position of Pyrazolines may lead to potent and promising antidepressant molecule.

5. Experimental

Materials and methods: *Animals:* Swiss Albino mice of either sex weighing 24±2 gms were selected for studying antidepressant activity. The animals were housed in group of ten/cage in a controlled temperature and humidity (22±3°C and 60±5%, respectively) on a 12 h light/dark cycle and with standard lab chow and tap water *ad libitum*. Food and water intake of the subjects was not restricted during the study. *Chemicals and Standard drugs:* Tween-80 was purchased from Merck and other standard drugs (Clorgyline, Moclobemide and Alprazolam)used in the study were purchased from the local market. *Apparatus:* Fabricated Plexiglass cylinder of standard dimension (for Porsolt's swim test) and Fabricated Frame of standard dimension (for Tail suspension test) were used for the study. *Software:* Microsoft Excel and Graphpad Instat Demo version

were employed for statistical interpretation of the data generated. Also a web based toxicity prediction program TOXBOX (http://www.pharma-algorithms.com/webboxes/) was used to predict the LD_{50} of the selected molecules.

5.1 *In-vivo* pharmacological screening:

5.1.1 Porsolt's swim test: On the day of experiment, mice were assigned into different groups (n=6 for each group). The test compounds (**3a-3i)** and standard drugs (Clorgyline, Moclobemide) were suspended in aqueous Tween-80 (2% w/v) at the concentration of 1 mg/mL. Suspension of test compounds (10 mg/kg body weight) and Clorgyline, Moclobemide (10 mg/kg body weight) were injectedintraperitoneally to mice at a volume of 1 mL per 100g body weight. After 30 min. mice were droppedone at a time into a Plexiglass cylinder (25 cm height, 30 cm diameter, containing 20 cm height of water at 21-23 ℃) and observed for 6min. At the end of 2 min, the animals having vigorous struggling tendency were immobile. The immobility time for each mousewas then recorded during last 4 min. A control group was also maintained.[15] The percentage change from control has been calculated using the following formula,

$$\% \text{ change from control} = \left[\frac{T_D}{T_C} - 1\right] \text{x } 100$$

T_D = Immobility time for drug treated group

T_C = Immobility time for control group

5.1.2 Tail suspension test: On the day of experiment, mice were assigned into different groups (n=6 foreach group). The test compounds (**3a-3i)** and standard drug (Clorgyline, Moclobemide) were suspended in aqueous Tween-80 (2% w/v) at the concentration of 1 mg/mL. Suspension of test compounds (10 mg/kg body weight) and Clorgyline, Moclobemide (10 mg/kg body weight) were injected intraperitoneally to mice at a volume of 1 mL per 100 g body weight. After 30 min, the mice were hung one at a time using a paper adhesive tape from 65 cm above the table top. Tape was placedapproximately 1cm from the tip of the tail. Animals wereallowed to hang for 6min and the duration of immobility was then recorded during last 5 min. A control group was also maintained.[16] The results have been presented in **Table 1.** The percentage change from control has been calculated using the same formula as discussed in Porsolt's swim test.

Elevated plus maze test: On the day of experiment, mice were assigned into different groups (n=6 for each group). The test compounds (**3h)** was suspended in aqueous Tween-80 (2% w/v) at the concentration of 1 mg/mL, while standard drug Alprazolam at the concentration of 0.05 mg/mL. Suspension of test compound (10 mg/kg body weight) and Alprazolam (0.5 mg/kg body weight) were injected intraperitoneally to mice at a volume of 1 mL per 100g body weight. Half an hour later, the mice were placed in the middle of the X-maze facing a corner of the centre platform (equal choice of entering an open or closed arm) and observed for a period of 5 min. Number of entries and time spent in open arm, closed arm and center platform were recorded.[17]

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