Section-II

Chapter-4

RESULTS

4. RESULTS

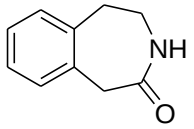
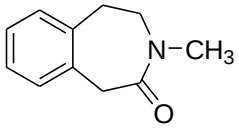
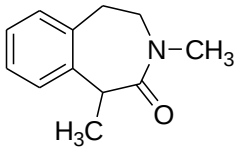
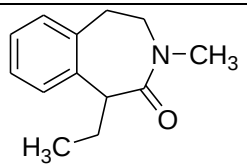
4.1. Biological evaluation

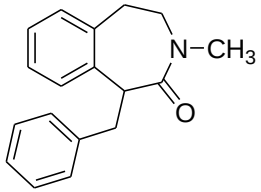
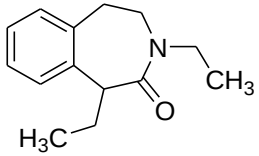
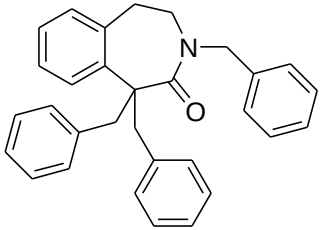
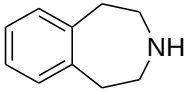
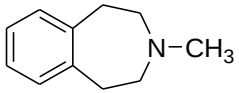
5-HT_{2A} and 5-HT_{2B} receptors are homologous to 5-HT_{2C} receptor due to their amino acid sequence similarities. 5-HT_{2A} and 5-HT_{2B} receptors are present in rat thoracic aorta and fundus respectively [141]. It was planned to screen the synthesized compounds by using classical pharmacological methods on isolated rat fundus and rat thoracic aorta preparations. The basic idea was to weed out those compounds which showed response to 5-HT_{2A} and/or 5-HT_{2B} receptors on these two isolated tissues and to select the inert compounds for additional studies for $5HT_{2C}$ sensibility.

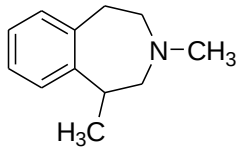
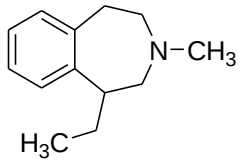
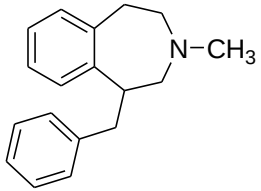
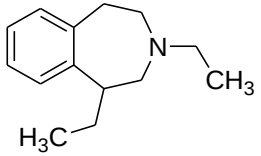
4.1.1. *In vitro* isolated rat fundus and isolated rat thoracic aorta experiments

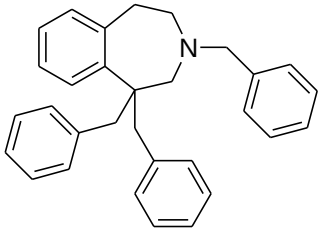
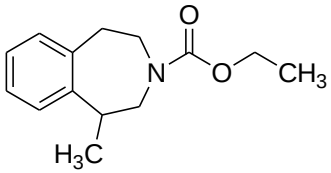
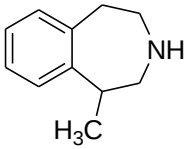
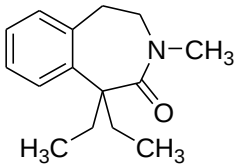
Compounds (**1-6, 15-17, 21, 22, 24, 25, 30, 31, 42-44, 47, 49, 50, 52-56** and **59**) were found to be active on 5-HT_{2B} or 5-HT_{2A} receptors and were eliminated from the study as those compounds that were selectively active on $5HT_{2C}$ receptors only were desired (**Table 1**). Compounds (**6, 15, 16, 17, 22, 42, 43, 44, 49** and **54**) have shown agonistic property on both 5-HT_{2B} and 5-HT_{2A} receptors on rat fundus and rat aorta preparations at 35 µM concentration. Compounds (**3, 5** and **56**) have shown agonistic property on 5-HT_{2B} receptor on rat fundus preparation at 35 µM concentration though they were found to be inactive at this concentration on rat thoracic aorta showing their inactivity on 5-HT_{2A} receptor. Compounds (**21, 24** and **47**) have shown antagonistic activity on rat thoracic aorta and no activity on rat fundus preparation. Compounds (**1, 2, 4, 8, 30** and **50**) have shown antagonistic activity on rat fundus and no activity on rat thoracic aorta preparation. Compounds (**7, 10-14, 18-20, 23, 26-29, 32-41, 45, 46, 48, 51, 57** and **58**) were found to be inactive on both the tissues, rat fundus as well as rat thoracic aorta at 35 µM concentration via 5-HT_{2A} & 5-HT_{2B} receptors.

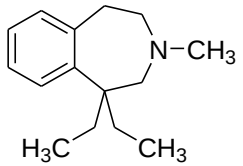
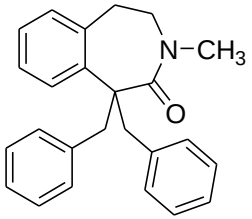
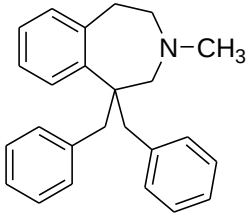
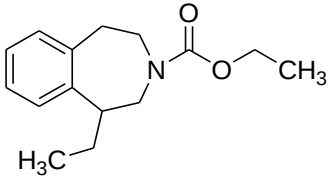
Table1: Compounds showing effect on isolated rat fundus and rat thoracic aorta preparations at a concentration of 35 µM

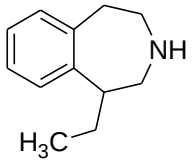
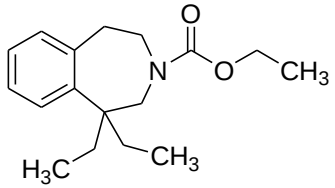
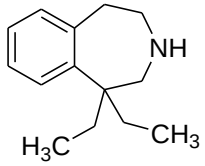
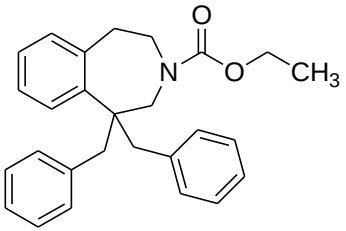
Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	Antagonist	Inactive	Agonist	Antagonist	Inactive
1			√				x
2			√				x
3		√					√
4			√				√

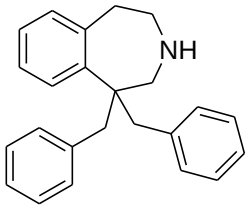
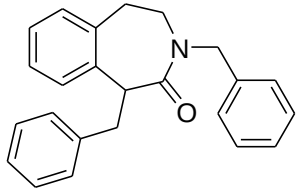
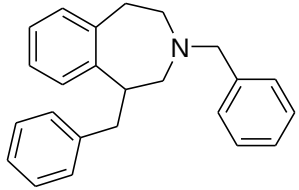
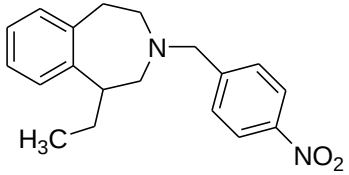
Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	**Antagonist**	**Inactive**	**Agonist**	**Antagonist**	**Inactive**
5		√					√
6		√			√		
7				x			x
8			√				x
9			√				x

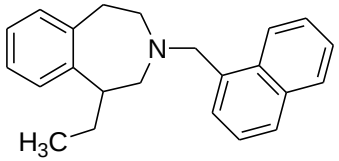
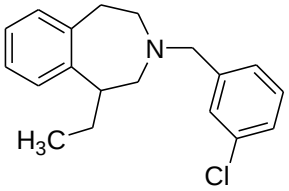
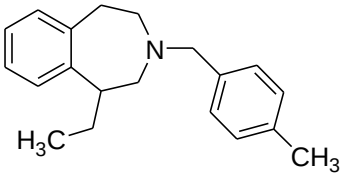
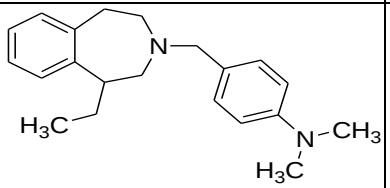
Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	**Antagonist**	**Inactive**	**Agonist**	**Antagonist**	**Inactive**
10				x			x
11				x			x
12				x			x
13				x			x

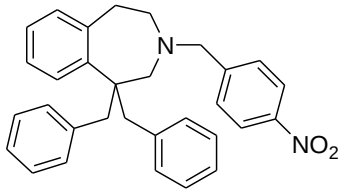
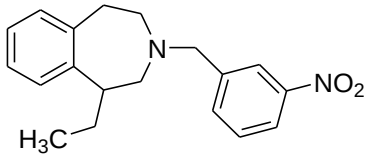
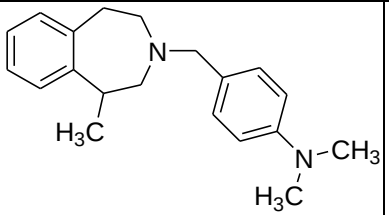
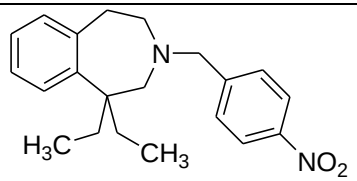
Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	**Antagonist**	**Inactive**	**Agonist**	**Antagonist**	**Inactive**
14				x			x
15		√			√		
16		√			√		
17		√			√		

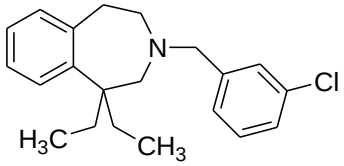
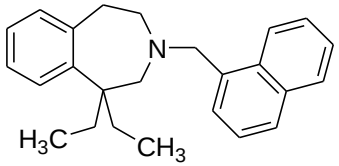
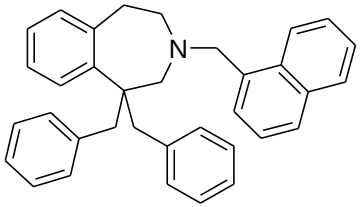
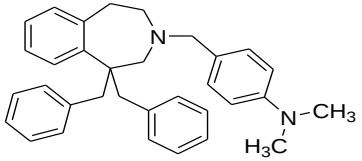
Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	**Antagonist**	**Inactive**	**Agonist**	**Antagonist**	**Inactive**
18				x			x
19				x			x
20				x			x
21				x		√	

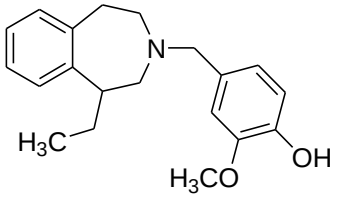
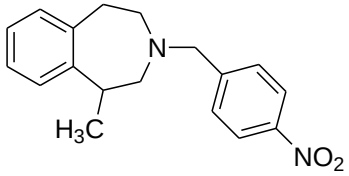
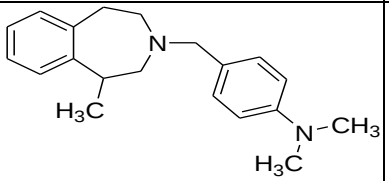
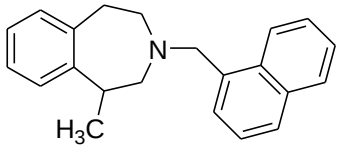
Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	**Antagonist**	**Inactive**	**Agonist**	**Antagonist**	**Inactive**
22		√			√		
23				x			x
24				x		√	
25				x		√	

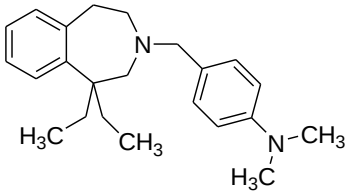
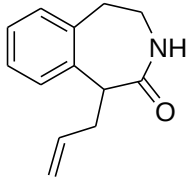
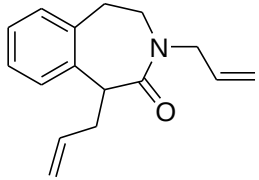
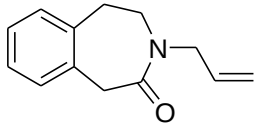
Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	**Antagonist**	**Inactive**	**Agonist**	**Antagonist**	**Inactive**
26				x			x
27				x			x
28				x			x
29				x			x

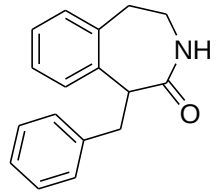
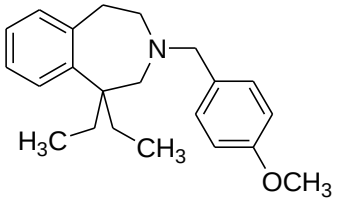
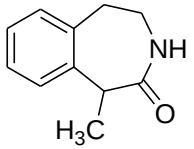
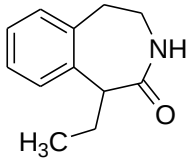
Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	**Antagonist**	**Inactive**	**Agonist**	**Antagonist**	**Inactive**
30			√				x
31			√				x
32				x			x
33				x			x

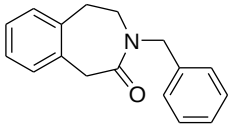
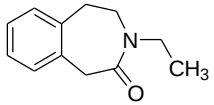
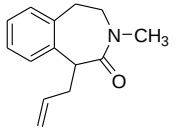
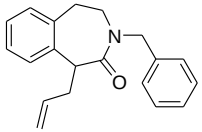
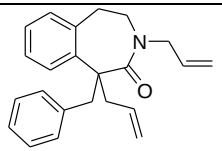
Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	**Antagonist**	**Inactive**	**Agonist**	**Antagonist**	**Inactive**
34				x			x
35				x			x
36				x			x
37				x			x

Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	**Antagonist**	**Inactive**	**Agonist**	**Antagonist**	**Inactive**
38				x			x
39				x			x
40				x			x
41				x			x

Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	**Antagonist**	**Inactive**	**Agonist**	**Antagonist**	**Inactive**
42		√			√		
43		√			√		
44		√			√		
45				x			x

Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	Antagonist	Inactive	Agonist	Antagonist	Inactive
46				x			x
47				√		√	
48				x			x
49		√			√		

Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	**Antagonist**	**Inactive**	**Agonist**	**Antagonist**	**Inactive**
50			√				x
51				x			x
52			√				x
53			√				x

Compound	Structure	Effect on Isolated rat fundus preparation ($5HT_{2B}$)			Effect on Isolated rat thoracic aorta preparation ($5HT_{2A}$)		
		Agonist	**Antagonist**	**Inactive**	**Agonist**	**Antagonist**	**Inactive**
54		√			√		
55			√				x
56		√					√
57				x			x
58				x			x

√- Compounds showing activity on a particular tissue.

x - Compounds showing no activity.

4.2. Tail suspension test (TST)

In mice, TST is used to evaluate anti-depressants. Fluoxetine, an anti-depressant of the selective serotonin reuptake inhibitor (SSRI) class, significantly reduces immobility time in the mice TST at 10 mg/kg, so it was used as a standard drug. All of the compounds found to be inactive on 5-TH_{2B} and 5-HT_{2A} receptors were screened initially for TST in mice and only those compounds that showed potent activity in TST were proceeded further for other *in vivo* evaluations. Compounds (**7, 19** and **28**) at 10 mg/kg i.p. reduced immobility time in the mice tail suspension test (p values<0.05 and <0.001; **Figure 4, 5 and 6**). This gives an indication of compounds (**7, 19** and **28**) having affinity for $5HT_{2C}$ receptor, as previous reports [142] showed that $5HT_{2C}$ receptor agonists were having antidepressant property. The remaining compounds (**10-14, 18, 20, 23, 26, 27, 29, 32-41, 45, 46, 48, 51, 57** and **58**) were not evaluated further as they did not show significant activity in this test (**Figure 4, 5 and 6**). The three active compounds (**7, 19** and **28**) were proceeded further for evaluation on anxiety, hypophagia and penile erection models.

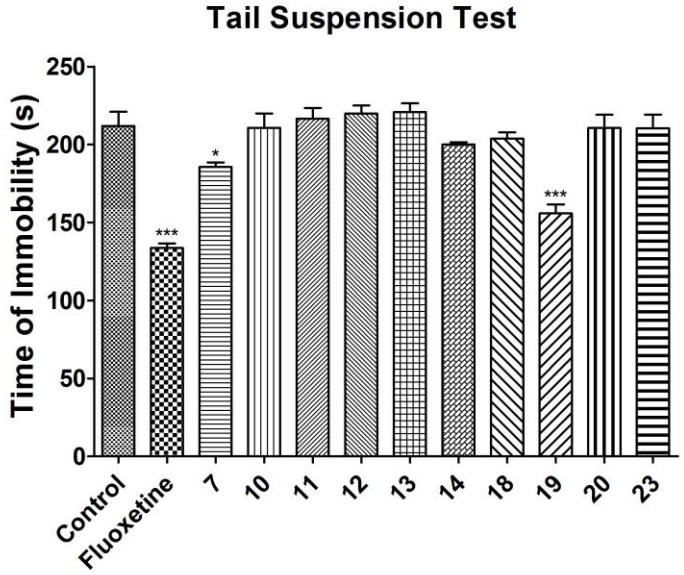


Figure 4: Effect of **Fluoxetine** and compounds (**7, 10-14, 18-20 and 23**) on immobility produced in the Tail suspension test (TST) in Swiss albino mice. Values represent mean immobility time ± SEM. Asterisks indicate values differ from vehicle treatment (*** P<0.001; *P<0.05) (n=6 per treatment group).

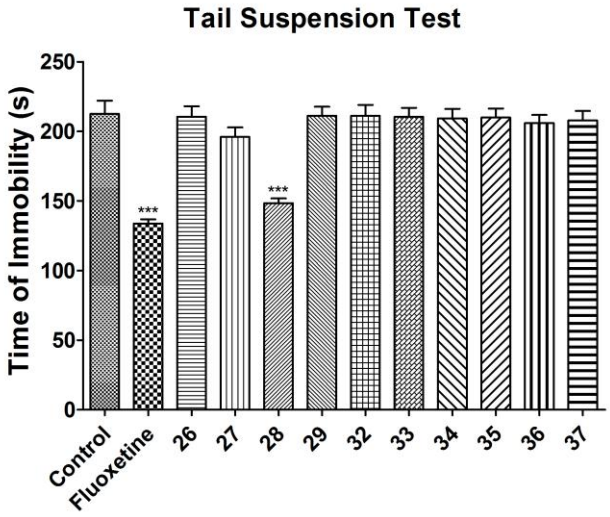


Figure 5: Effect of **Fluoxetine** and compounds (**26-29** and **32-37**) on immobility produced in the Tail suspension test (TST) in Swiss albino mice. Values represent mean immobility time ± SEM. Asterisks indicate values differ from vehicle treatment (*** P<0.001) (n=6 per treatment group).

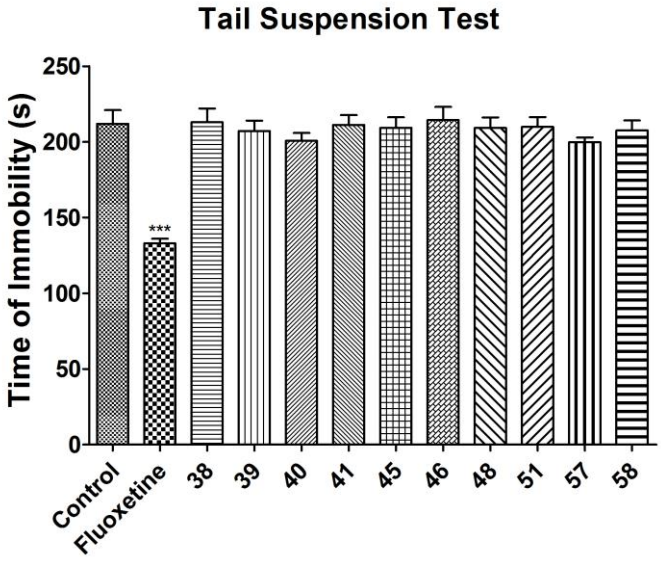


Figure 6: Effect of **Fluoxetine** and compounds (**38-41, 45, 46, 48, 51, 57 and 58**) on immobility produced in the Tail suspension test (TST) in Swiss albino mice. Values represent mean immobility time ± SEM. Asterisks indicate values differ from vehicle treatment (*** P<0.001) (n=6 per treatment group).

4.3. Elevated plus maze test

Elevated plus maze test is used to evaluate anxiolytics, using ***m*CPP** (1mg/kg i.p) as the positive control. As expected for a positive control, ***m*CPP** induced a selective anxiogenic-like effect in mice characterized by a significant decrease in the number of open arm entries, without changing the number of closed arm entries, compared to the negative control normal saline (**Figure 7a** and **7b**). Treatment with the compounds (**7, 19** and **28**) at 10 mg/kg significantly decreased the number of open arm entries and time spent in open arm of mice in the EPM.

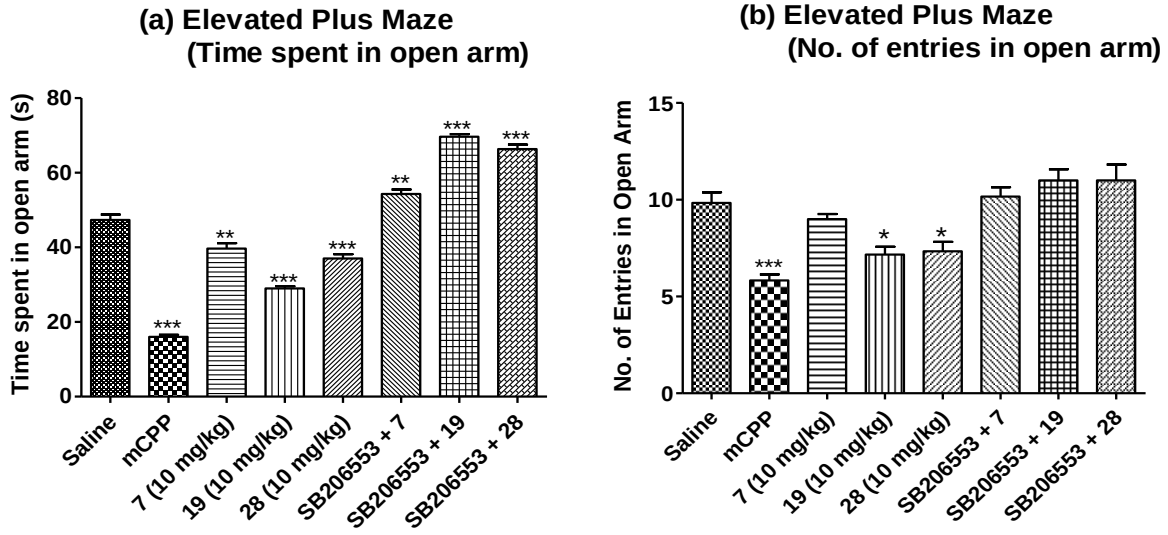


Figure 7: (a) Effect of ***m*CPP**, **7, 19** and **28,** and **SB-206553** in combination with **7, 19** and **28** (i.p. 30 minutes pretest except **SB-206553** 45 minutes pre-test) on time spent in open arms in mice for 5 min Elevated Plus Maze (EPM) test. All data expressed as Mean ± SEM, n = 6 and are significantly different from vehicle-treated group: ***P<0.001 and **P<0.01 by Dunnett's test and 1-way ANOVA. **(b)** Effect of ***m*CPP, 7, 19** and **28**, and **SB-206553** in combination with **7, 19** and **28** (i.p. 30 minutes pretest except **SB-206553** 45 minutes pre-test) on number of entries in open arms in mice for 5 min Elevated Plus Maze (EPM) test. All data expressed as Mean ± SEM, n = 6 and are significantly different from vehicle-treated group: ***P<0.001 and *P<0.05 by Dunnett's test and 1-way ANOVA.

These observations confirmed that compounds (**7, 19** and **28**) had anxiogenic-like activity and prefiguration of $5HT_{2C}$ agonistic activity. Further, compounds (**7, 19** and **28**) at 10 mg/kg in presence of **SB206553**, a selective $5HT_{2C/2B}$ antagonist at 2mg/kg i.p. increased the number of entries in open arm as well as time spent in open arm that showed their selectivity for $5HT_{2C}$ receptors. Compounds (**19** and **28**) showed higher anxiogenic property

than **7**. These findings suggested that **19** and **28** are having higher affinity for $5HT_{2C}$ receptor than **7** (**Figures 7a** and **7b**).

4.4. Hypophagic response

As expected of a $5HT_{2C}$ agonist, ***m*CPP** at 5 mg/kg i.p. showed hypophagic response in comparison to the control (normal saline at 10 ml/kg) treated animals. Compounds (**7, 19** and **28**) also showed hypophagic responses. The hypophagic responses of compounds (**7, 19** and **28**) were reversed by **SB206553** (**Figure 8**). Responses were recorded as the amount of food (g) taken in 2 h. Similar to elevated plus maze test, compounds (**19** and **28**) showed better hypophagic responses than **7** which validated the higher selectivity of compounds (**19** and **28**) towards $5HT_{2C}$ receptor than **7**.

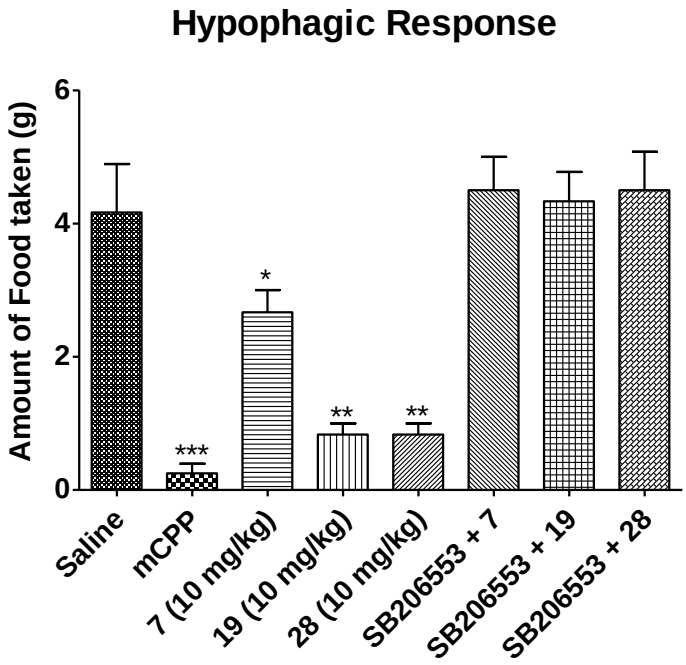


Figure 8: Effect of **SB-206553** on the test compound-mediated reduction in 2 h food intake in 24 h fasted normal Sprague–Dawley rats. Antagonist (**SB-206553**; 2 mg/kg) was administered i.p. 15 min prior to administration of test compounds (**7, 19** and **28**; 10 mg/kg each). ***m*CPP** (5 mg/kg) i.p. was given as a standard drug. Mean ± SEM (n = 3 per group) is significantly different from vehicle-treated group: ***P<0.001, **P<0.01 and *P<0.05 by Dunnett's test and 1-way ANOVA.

4.5. Penile erections in rats

***m*CPP** at 0.75 mg/kg s.c. induced penile erections and engorged penis as compared to the control animals treated with normal saline at 10 ml/kg. Similarly, compounds (**7, 19** and

28) had shown penile erections at doses of 20 mg/kg s.c. However, penile erections were antagonized by **SB206553** at 2 mg/kg i.p. (**Figure 9**). More number of lickings in animals treated with **19** and **28** further supplemented the higher affinity of these compounds for $5HT_{2C}$ receptor than **7**.

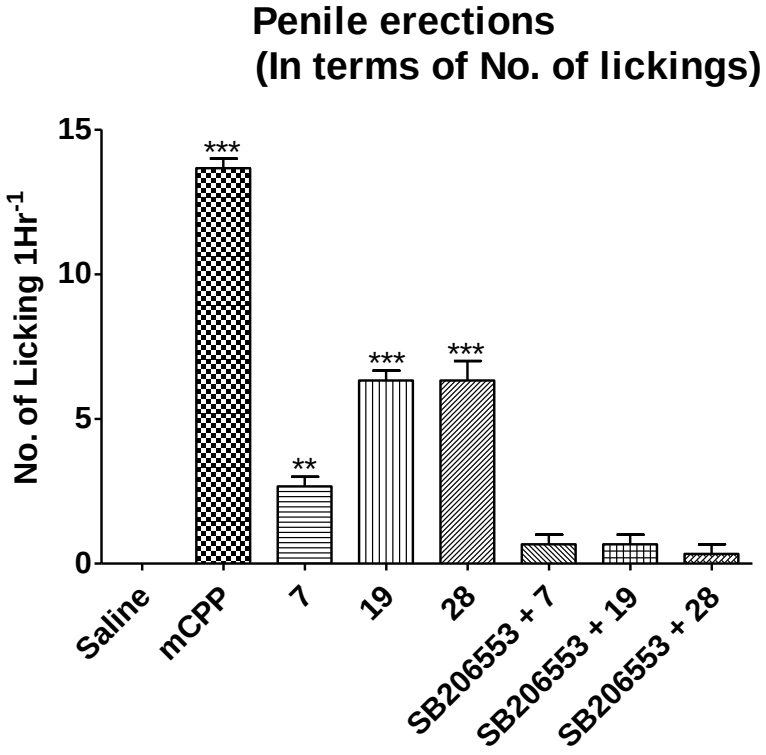


Figure 9: Interaction of **SB-206553** with compounds (**7, 19** and **28**) on penile erections in Sprague Dawley rats. **SB-206553** was injected i.p. 15 min before the s.c. injection of **7, 19** and **28** (20 mg/kg) and the penile erections were counted over a period of 60-min, starting from the injection of **7, 19** and **28**. Mean± SEM was calculated from n=3 rats per group and was significantly different from vehicle-treated group: ***P<0.001and **P<0.01 by Dunnett's test and 1-way ANOVA.

4.6. Estimation of monoamines

First, a standard chromatogram was developed to estimate the DA and 5-HT levels in the brains of the untreated animals (**Figure 10**). There was a significant decrease in the brain concentrations of DA and 5-HT in the animals exposed to the compounds (**19** and **28)** (p<0.001). Pre-treatment of **SB206553** significantly reversed the decreased level of DA and 5-HT which further confirmed the above finding (p<0.001) (**Figure 11a, Figure 11b**).

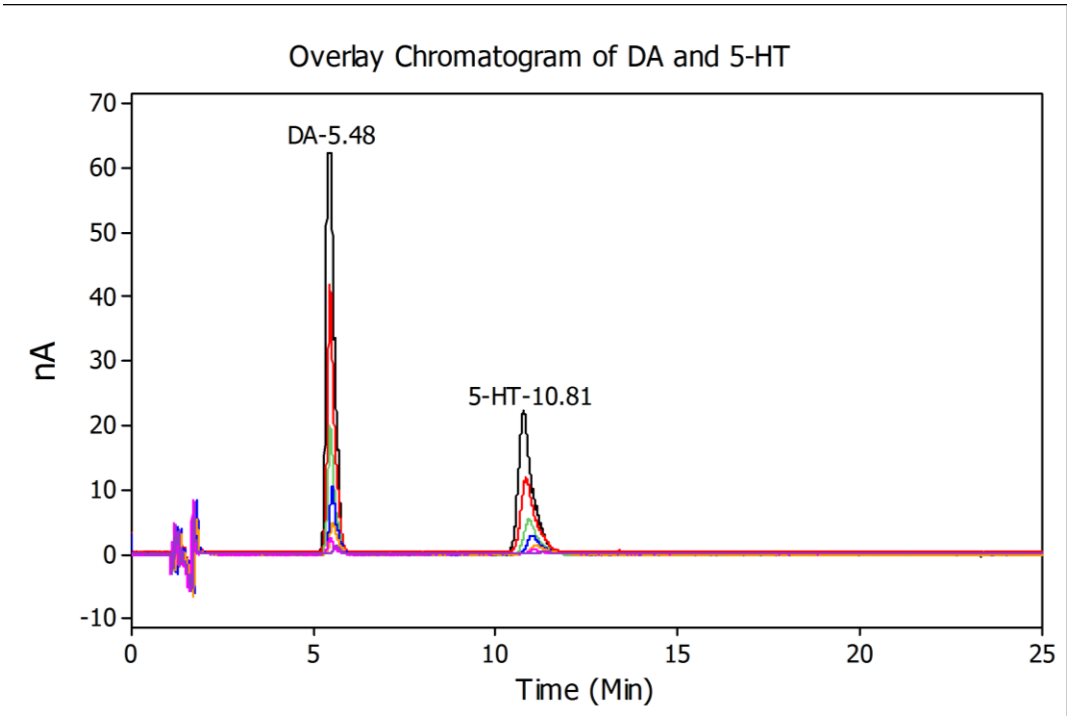


Figure 10: Standard chromatogram of DA (Dopamine) and 5-HT (Serotonin)

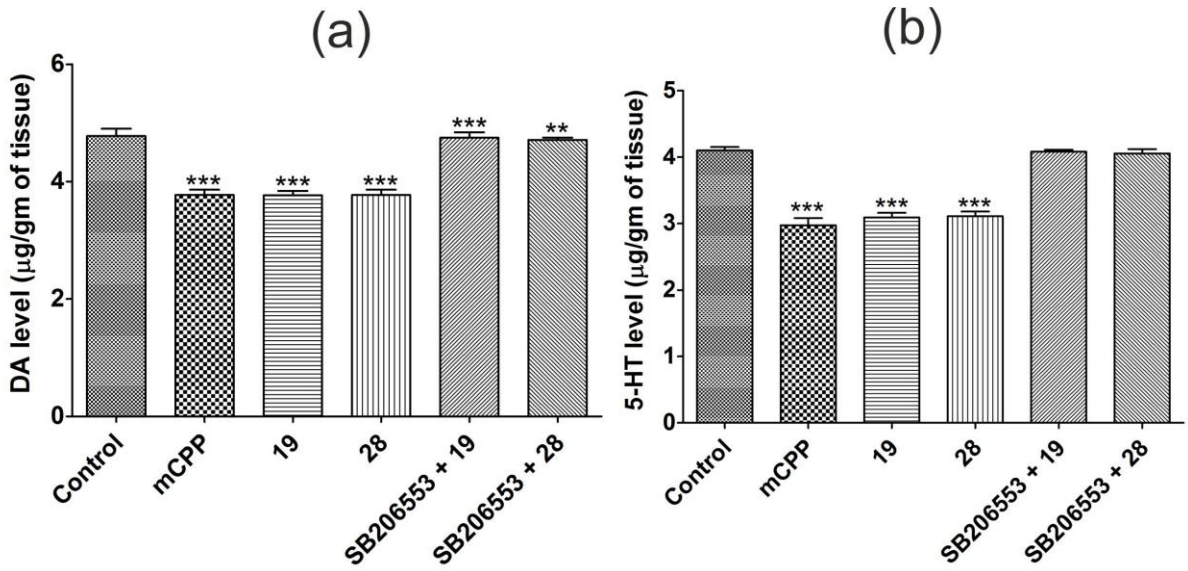


Figure 11: (a, b) Effect of ***m*-CPP** (2 mg/kg, p.o.) and the test compounds (**19** and **28**) (10 mg/kg, p.o.) on DA and 5-HT levels in the rat brain. Values represent the brain concentration of DA and 5-HT (µg of DA and 5-HT/gm of tissue). Data are expressed as mean ± SEM. ***p<0.001 and **p<0.01 vs vehicle control, (N=6).