SECTION-II

CHAPTER 3.
RESULTS AND DISCUSSION

3. RESULTS AND DISCUSSION

As envisaged it was planned to identify potential new 5-HT_{2C} agonist candidates considering lorcaserin as a lead molecule. A strategy of incorporating multiple chemical templates containing 3-benzazepine ring in common, was adopted, in order to enhance success rate of the compounds for advanced clinical trials. Since $5HT_{2A}$ and $5HT_{2B}$ receptors are homologous to $5HT_{2C}$ receptor due to similarity in their amino acid sequence and the activation of these receptors ($5HT_{2A}$ and $5HT_{2B}$) may lead to unwanted effects, it was planned to design and synthesize 5-HT_{2C} selective agonists.

The following section has been divided into two parts- one for chemical studies and the other for biological studies.

3.1. Chemical studies

C-1 substituted 3-benzazepin-2-one derivatives have been prepared by cyclizing the pre-substituted acyclic derivatives into the desired azepines using Heck reaction and other cyclization methods.[54] These methods use drastic conditions with or without metallic catalysts providing poor yields. To improve upon the yields using mild reaction conditions and avoiding costly reactants it was planned to construct the benzazepine ring system first and then to substitute the desired positions with appropriate substituents. For this purpose, 3-benzazepin-2-one (**1**) as the starting material was prepared from N-phenethyl-2-chloroacetamide by its cyclization in anhydrous aluminium chloride.[55,56]

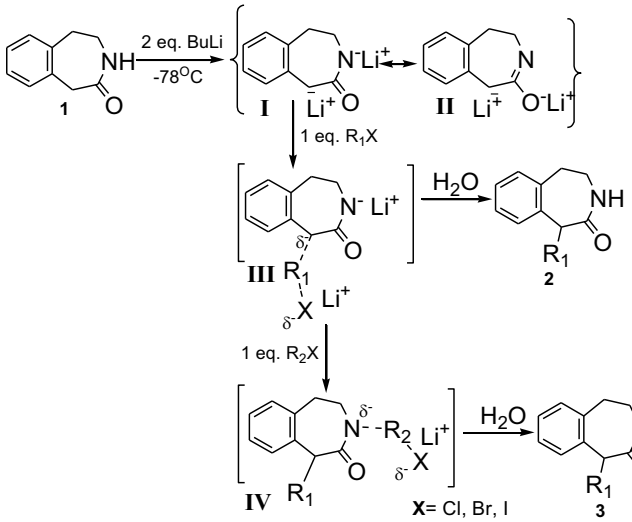
The synthetic protocols described here can be used in several different ways to obtain the desired products. Treatment of compound (**1**) with base can generate two

different anions depending on the reaction conditions and the strength of the base. It is possible to perform selective monoalkylation of either of the anions, double (symmetrical) alkylation, one-pot selective dialkylation of both of the anions with different substituents or general dialkylation of polyanions generated in one pot procedure. Furthermore, the reported methods offer the possibility to carry out reactions under milder conditions, more safely and involving less cumbersome separation of products with excellent yields. Selective mono/polyalkylation at C-1 and/or N-3 positions of 3-benzazepin-2-one was achieved using different synthetic protocols as discussed below.

Two equivalents of *n*-BuLi were used to generate the dianion (**I**, **Scheme 1**). Addition of one equivalent of alkyl halide offered the C-1 monoalkylated product (**2**) on the expected lines. For N-alkylation, a choice was available to select either the same alkyl halide as used earlier for C-alkylation or a different one to offer similarly or differently substituted alkylated products (**3**) respectively. Both types of products (**2** and **3**) were characterized by using ^{1}H-NMR spectra. PMR spectrum of **2** displayed the presence of a proton associated with N-3 at δ 5.88-5.95 ppm (confirmed by D_2O exchange studies) while spectrum of **3** showed the absence of protons at N-3. Selectivity at C-1 over N-3 could be achieved due to higher nucleophilicity of C-1 carbanion than the N-3 anion.

Metallation of 3-benzazepin-2-one (**1**) was done at -78 ^{0}C in THF with 2 equiv. of *n*-BuLi. Alkyl halides (1 equiv) were then added at 0 ^{0}C. It took 2-18 h for the reaction to complete depending on the bulkiness and reactivity of the employed alkyl halides. Bulkier alkyl halides exhibited lower rates of reaction. As the first alkylation of the dianion proceeded much faster, selective monoalkylation at the more reactive

carbon could be achieved under conditions conducive for dialkylation by using simply only 1 equiv. of alkyl halide. Monoalkyalation reactions normally got completed within 2 h. For the reactive electrophiles (methyl, allyl etc.) traces of unselective alkylations were also observed along with the desired C-1 alkylated products. This problem was overcome by addition of the alkylating agent at a lower temperature (-20 ^{0}C) rather than at 0 ^{0}C. Addition of another equivalent of alkyl halide to the reaction mixture offered the disubstituted products (**3**) (**Scheme 1**). Addition of two equivalents of alkyl halide in the beginning itself provided the similarly substituted



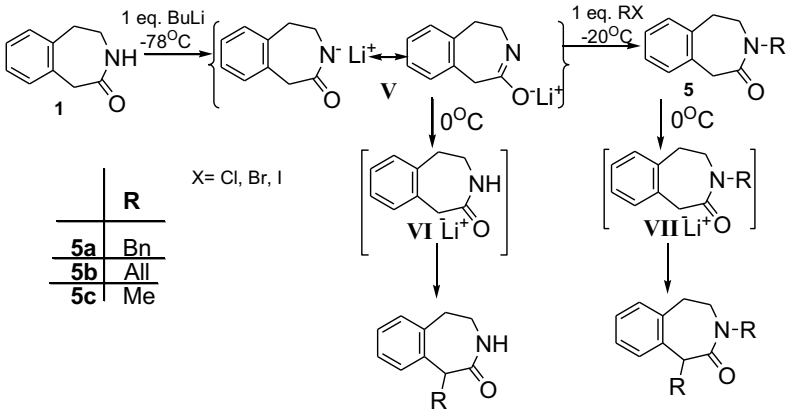
	R_1	R_2
2a	All	H
3a	Et	Me
3b	Et	Et
3c	Bn	Bn
3d	All	All
4a	Et	Me
4b	Et	Et
4c	Bn	Bn

Scheme 1

dialkylated product. Reactions were monitored by TLC and the stepwise character of the reactions could be easily observed running TLC of reaction mixtures at different time intervals; monoalkylated product (**2**) was formed quickly while the dialkylated products (**3**) took much longer time (8-18 h). Both of these reactions proceeded without the formation of byproducts. Dialkylated products from sterically more hindered electrophiles (*n*-Pr, *i*-Pr etc.) could not be obtained even on using large excess of the alkylating agents. It was also tried to perform 1,1,3-trialkylation using

excess of *n*-BuLi (5 equiv.) and excess of alkyl halides but without a success. The 1,3-disubstituted products (**3**) only could be isolated in these reactions even after stirring the reaction mixture for 18 h at RT.

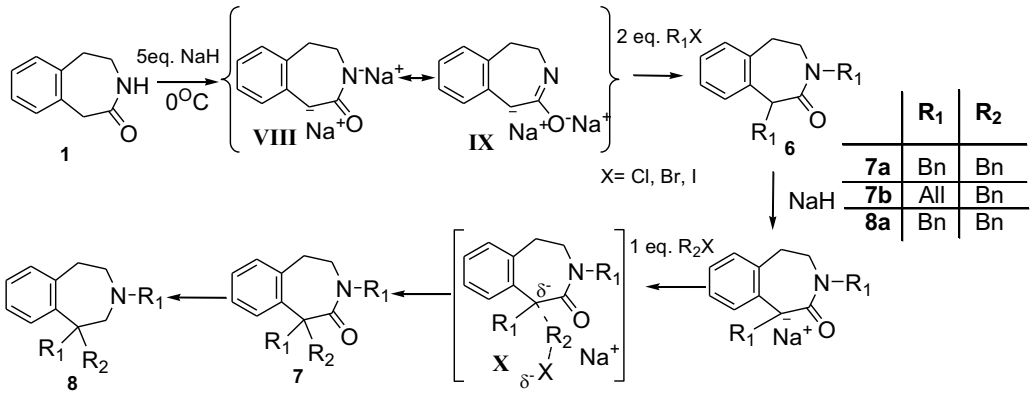
Selective monoalkylation of the N-3 position proved to be much trickier. It was achieved by using 1 equiv. each of *n*-BuLi and alkyl halide (**5**). The reaction took much longer a time (24-28 h) for completion. The reaction was carried out at below -20 ^{0}C, as an increase in reaction temperature up to 0 ^{0}C yielded impure products due



Scheme 2

to unselective alkylation of both C-1 and N-3 positions; probably due to formation of some amounts of anions (**VI** and **VII**) at temperatures higher than -20 ^{0}C (**Scheme 2**).

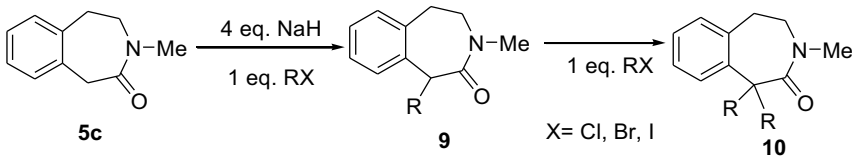
Since obtaining a tri-substituted (1,1,3-trialkylated) product was an overriding



Scheme 3

factor in our mind, experiments were repeated using NaH as the base in place of *n*-BuLi. Using one or two equivalents of NaH and one equivalent of alkyl halide gave a mixture of C-1 and N-3 monoalkylated products. Five equivalents of NaH and two equivalents of alkyl halide offered the symmetrical disubstituted product. In the same reaction mixture, if an additional equivalent quantity of a different alkyl halide was added then the tri-substituted product (**7**) could be obtained (**Scheme 3**).

For the preparation of tri-substituted product with same type of substituents at C-1 position, N-methyl-3-benzazepin-2-one derivative was chosen as the starting mat-

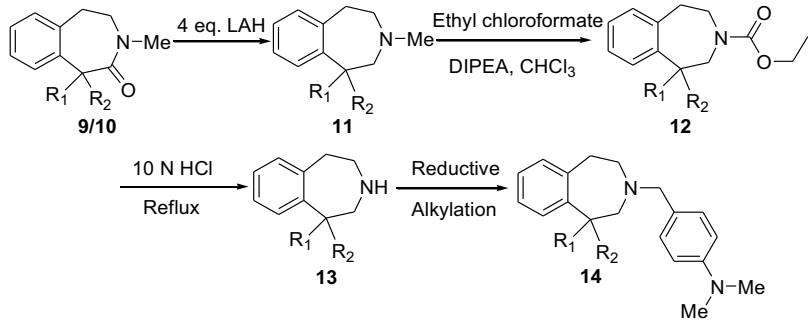


	R
9a	All
10a	Et
10b	Bn

Scheme 4

-erial and anions were generated on C-1 atom by using excess (3 eq) of NaH. Addition of 2 equiv of alkyl halide in the reaction mixture afforded the tri-substituted product (**10**) in satisfactory yields (**Scheme 4**).

Further, **9** or **10** with mono- or di-substituents at C-1 carbon were reacted with LAH to obtain **11**. For de-methylation, **11** was reacted with ethyl chloroformate in



	R_1	R_2
11a	Et	Et
11b	Bn	Bn
12a	Me	H
12b	Et	H
12c	Et	Et
12d	Bn	Bn
13a	Bn	Bn
14a	Bn	H

Scheme 5

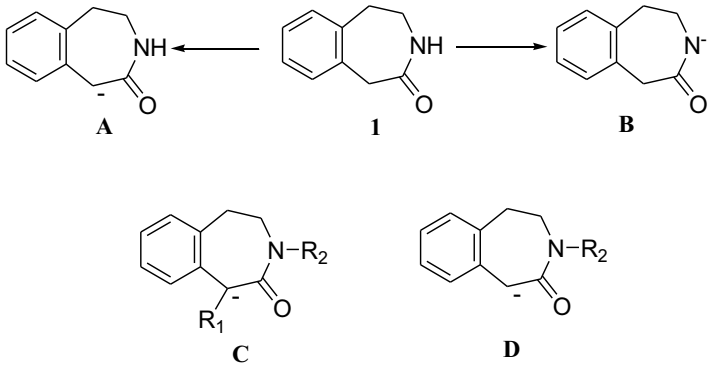
DIPEA and $CHCl_3$ yielding **12**. Compound (**12**) was then hydrolysed in conc. HCl and MeOH to obtain **13**, which was finally subjected to reductive alkylation for the synthesis of N-alkyl substituted 3-benzazepine derivative (**14**) (**Scheme 5**)

Formation of some of the above discussed products under a particular set of conditions could be explained on well defined principles while the others were somewhat more difficult to explain. 1,1,3-Trialkylation of 3-benzazepin-2-one could not be achieved even on using excess of *n*-BuLi and alkyl halide as per **Scheme 1**. The reaction did not proceed beyond 1,3-dialkylation (**3**). While a change from *n*-BuLi to NaH yielded the desired 1,1,3-trialkylated products (**7**). Another interesting observation was that 2 equiv. of *n*-BuLi and 1 equiv. of alkyl halide yielded exclusively the C-1 substituted product in pure form; while use of just 1 equiv. of NaH and 1 equiv. of alkyl halide offered a mixture of C-1 and N-3 alkylated products along with some starting material. Using just 1 equiv. of NaH as a base could not yield selectively the N-3 alkylated product unlike the use of 1 or 2 equiv. of *n*-BuLi.

In the 3-benzazepin-2-one ring system, there are two reactive centres for the alkylation; one is C-1 carbon and the other N-3 nitrogen. Both of these centres have different chemical environments and negativity hence, the attached hydrogens have different acidities resulting in differential reactivities of these two centres. Abstraction of the proton from amidic nitrogen is much easier than abstraction of C-1 hydrogen due to the higher acidity of N-3 hydrogen, leading to easier formation of anion on N-3 than on C-1. The anion so generated on N-3 is stabilized by delocalisation of the negative charge on amide oxygen atom. Monoalkylation of the N-3 nitrogen is a straightforward reaction because only the mono anion (**V**) has been generated by using *n*-BuLi as a base (**Scheme 2**) at the reaction temperature of -20 ^{0}C. Although an

exclusive N-3 alkylated product was formed, the rate of formation of the product was slow due to delocalization of the negative charge on oxygen atom causing decreased nucleophilicity of the nitrogen. Moreover as per HSAB concept, nitrogen anion is a hard base while alkyl halides are soft acids, so their interaction is less favourable causing decreased rate of alkylation of amidic nitrogen.[57] But surprisingly, when monoalkylation of the 3-benzazepin-2-one was tried using 1 equiv. each of alkyl halide and NaH as base, a mixture of C-and N-alkylated products were obtained. It was puzzling because *n*-BuLi is a stronger base than NaH in a given set of conditions (although their basicity depends on factors like type of solvents used, concentration of the base and the temperature of the reaction). That means NaH was able to extract protons from the N-3 position as well as from C-1 position to generate both the anions resulting into formation of a mixture of C-1 as well as N-3 alkylated products at RT.

Another possible explanation is abstraction of C-1 proton by collision of the anion (**B**) with **1** at C-1 hydrogen leading to its abstraction and generating the carbani-



-on (**A**). Here, it is worthy to note that the reaction using *n*-BuLi was carried out at -20 ^{0}C while that of NaH was performed at room temp (about 25 ^{0}C); that means either *n*-BuLi at -20 ^{0}C, was unable to abstract C-1 proton unlike NaH when used in mono equivalent quantities or the collisions of anion (**B**) with **1** were not proving productive

at this temperature to generate the anion (**A**). The anion (**A**) once formed, will give the C-1 alkylated product faster than the anion (**B**) due to the fact that the anion (**A**) is a stronger nucleophile than anion (**B**). This can also be explained on HSAB concept as the alkyl halides and the anion (**A**) are soft acid and base respectively.

n-BuLi or NaH in excess quantities generated the same dianion (**I** and **VIII**) but monoalkylated product could be obtained by using *n*-BuLi only (using 1 equiv. of electrophile). It seems that both the anions at N-3 and C-1 are reactive enough at RT to offer C-1 and N-3-disubstituted products.

The most startling observation was the non-formation of 1,1,3-trialkylated product even on using excess of *n*-BuLi and alkyl halide (even with MeI, a powerful electrophile). It seems that the anion (**C**) never gets formed in the reaction medium with *n*-BuLi, because once formed it will surely offer the tri-substituted product, as it is a more powerful nucleophile than the anion (**D**). It was unexpected of a strong base like *n*-BuLi. *n*-BuLi in solid state has been known to exist in polymeric form.[58,59] In THF it has been reported to exist in dimeric and tetrameric forms.[60-62] It is assumed that under the above discussed reaction conditions *n*-BuLi has not been able to approach the sterically hindered tertiary C_1-H due to the bulky dimeric/tetrameric structures of *n*-BuLi. This assumption is further strengthened by the observation that the quality of monosubstituted product (**5**) (**Scheme 2**) further improved when the reaction temperature was lowered from 0^0C to -20^0C. To the best of our knowledge this is the first observation of this kind where *n*-BuLi has been reported not to abstract an acidic proton attached to a tertiary carbon.

3.2. Biological studies

Since $5HT_{2A}$ and $5HT_{2B}$ receptors are present on rat thoracic aorta and fundus respectively, all of the synthesized compounds were screened on isolated rat fundus & isolated rat thoracic aorta preparations; the compounds which were inactive on $5HT_{2B}$ & $5HT_{2A}$ receptors even at 35 μM concentration only were selected for $5HT_{2C}$ activity as the aim was to choose the compounds selective for $5HT_{2C}$ receptors. The selected compounds were evaluated on anxiety, depression, obesity and penile erection models mediated via $5HT_{2C}$ receptor. The action of the newly synthesized compounds on $5HT_{2C}$ receptor was further confirmed by using $5HT_{2C/2B}$ antagonist SB-206553 (Sigma Aldrich). In absence of *in vitro* receptor binding studies it was planned to screen the synthesized compounds on isolated rat fundus and rat thoracic aorta preparations. The basic idea was to weed out those compounds which were found to be active on these two preparations for further experimentation as that would show their $5HT_{2A}$ and $5HT_{2B}$ responsiveness, and inactive compounds only would be proceeded for additional studies for $5HT_{2C}$ sensibility.

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

After performing activity on the two isolated preparations, compounds (**2a, 3a, 3c, 3d, 5a, 5b, 9a** and **10a**) were found to be active on $5HT_{2B}$ or $5HT_{2A}$ and were eliminated from the study as compounds with selectivity for $5HT_{2C}$ receptors only were required (**Table 1**). Compounds (**3a, 3c, 3d, 5a, 5b, 9a** & **10a**) have shown agonistic property on $5HT_{2B}$ receptor at 35 μM concentration on rat fundus preparation but were found to be inactive at this concentration on rat thoracic aorta showing their inactivity on $5HT_{2A}$ receptor whereas compound (**2a**) has shown

antagonistic activity on rat thoracic aorta and no activity on rat fundus preparation, which shows the activity of compound (**2a**) on $5HT_{2A}$ receptor. Compounds (**3b, 4a,**

Table1. Compounds showing effect on isolated rat fundus and rat thoracic aorta.

Comp. Entry	Effect of compounds on Isolated rat fundus preparation ($5HT_{2B}$) at 35 µM conc.			Effect of compounds on Isolated rat thoracic aorta preparation ($5HT_{2A}$) at 35 µM conc.		
	Agonist	Antagonist	Inactive	Agonist	Antagonist	Inactive
2a			+		+	
3a	+			+		
3b			+			+
3c	+			+		
3d	+			+		
4a			+			+
4b			+			+
4c			+			+
5a	+			+		
5b	+			+		
7a			+			+
7b			+			+
8a			+			+
9a	+			+		
10a	+			+		
10b			+			+
11a			+			+
12a			+			+
12b			+			+
12c			+			+
13a			+			+
14a			+			+

4b, 4c, 7a, 7b, 8a, 10b, 11a, 12a, 12b, 12c, 13a and **14a**) were found to be inactive on both rat fundus as well as rat thoracic aorta at 35 µM concentration showing their inability to interact with $5\text{-}HT_{2B}$ and $5\text{-}HT_{2A}$ receptors.

3.2.2. Tail Suspension Test (TST) in mice

In mice, TST is used to evaluate anti-depressants. Fluoxetine, an anti-depressant of the selective serotonin reuptake inhibitor (SSRI) class, at 10 mg/kg significantly

reduces immobility time in the mice TST, so it was used as a standard. All the inactive compounds on $5TH_{2B}$ and $5HT_{2A}$ were screened initially for TST in mice and compounds showing potent activity in TST were screened further for other *in vivo* evaluations. Compounds (**4c, 7a, 10b, 12c and 13a**) at 10mg/kg i.p. reduced immobility time in the mice tail suspension test (p values <0.001; **Fig. 1**). This gives an indication of compounds (**4c, 7a, 10b, 12c and 13a**) having affinity for $5HT_{2C}$ receptor as previous reports showed that $5HT_{2C}$ receptor agonists are having antidepressant property. Compounds (**3b, 4a, 4b, 7b, 8a, 11a, 12a, 12b** and **14a**) did not show significant results (**Figure 1**). The remaining five active compounds (**4c, 7a, 10b, 12c** and **13a**) only were further evaluated on anxiety, hypophagia and penile erection models.

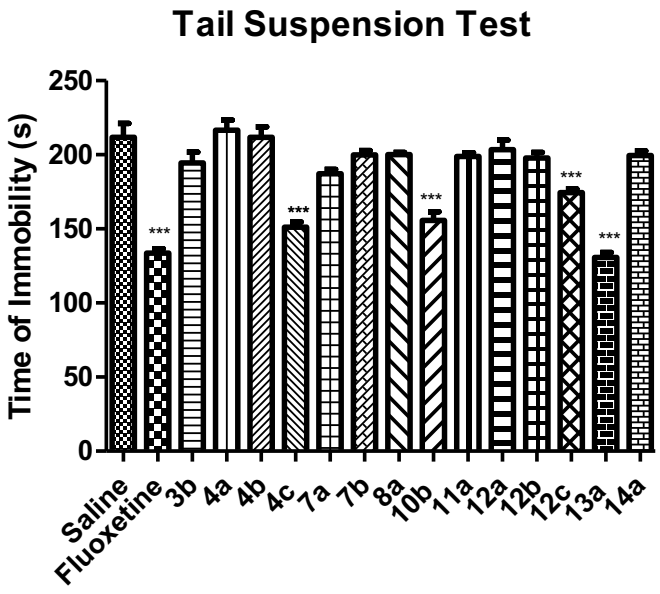


Figure 1. Effect of **Fluoxetine**, compounds (**3b, 4a, 4b, 4c, 7a, 7b, 8a, 10b, 11a, 12a, 12b, 12c, 13a** and **14a**) on immobility produced in the Tail suspension test (TST) in Swiss albino mice. Values represent mean immobility time±SEM. Asterisks indicate values differ from vehicle treatment (*** p<0.001) (n=6 per treatment group).

3.2.3. Elevated Plus-Maze (EPM) test

Elevated plus maze test is used to evaluate anxiolytics. As expected for a positive control, ***m*CPP** (1mg/kg i.p.) 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 enclosed arm entries, compared to the negative control, normal saline (**Fig. 2a** and **2b**). Treatment with the compounds (**4c, 7a, 10b, 12c** and **13a**) at 10 mg/kg significantly decreased the number of open arm entries and time spent in open arm of mice in the EPM. These parameters confirmed that **4c, 7a, 10b, 12c** and **13a** had anxiogenic like activity and prefiguration of $5HT_{2C}$ agonistic activity. Further **4c, 7a, 10b, 12c** and **13a** at 10mg/kg in presence of **SB206553**, a

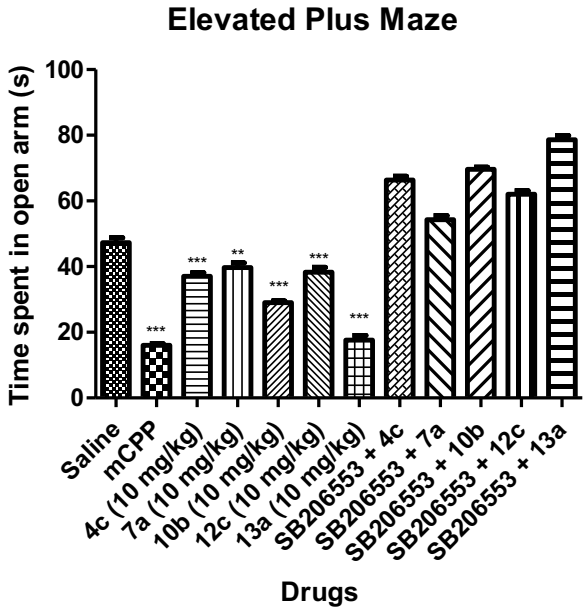


Figure 2a. Effect of ***m*CPP**, **4c, 7a, 10b, 12c** and **13a** and **SB-206553** in combination with **4c, 7a, 10b, 12c** and **13a** (i.p. 30 minutes pretest except **SB-206553** 45 minutes pre-test) on time spent in open arms in mice for 5 min Elevated Plus Maze (EPM) test. All data expressed as Mean ± SEM, n = 6 and are significantly different from vehicle-treated group: ***P<0.001 by Dunnett's test and 1-way ANOVA.

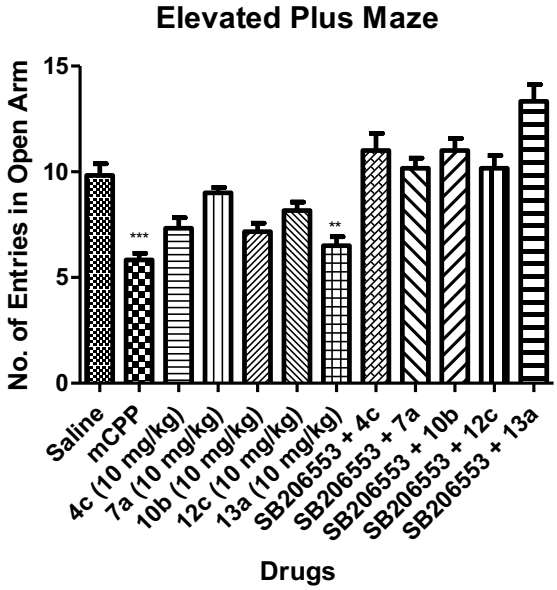


Figure 2b. Effect of ***m*CPP, 4c, 7a, 10b, 12c** and **13a** and **SB-206553** in combination with **4c, 7a, 10b, 12c** and **13a** (i.p. 30 minutes pretest except **SB-206553** 45 minutes pre-test) on number of entries in open arms in mice for 5 min Elevated Plus Maze (EPM) test. All data expressed as Mean ± SEM, n = 6 and are significantly different from vehicle-treated group: ***P<0.001 by Dunnett's test and 1-way ANOVA.

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. Out of these five compounds **13a** showed anxiogenic property equivalent to ***m*CPP**, while **4c** and **10b** showed lesser anxiogenic property than **13a**. **7a** and **12c** were found to be least anxiogenic. These findings suggested that **4c, 10b** and **13a** are having higher affinity for $5HT_{2C}$ receptor than **7a** and **12c** (**Figure 2a, 2b**).

3.2.4. Hypophagic response in rats

$5HT_{2C}$ agonist ***m*CPP** at 5 mg/kg i.p. showed hypophagic response and as compared to the control treated animals with normal saline at 10 ml/kg. **4c, 7a, 10b,**

12c and **13a** also showed hypophagic responses. However, the hypophagic responses of **4c, 7a, 10b, 12c** and **13a** were reversed by **SB206553** (**Fig 3**). Responses were

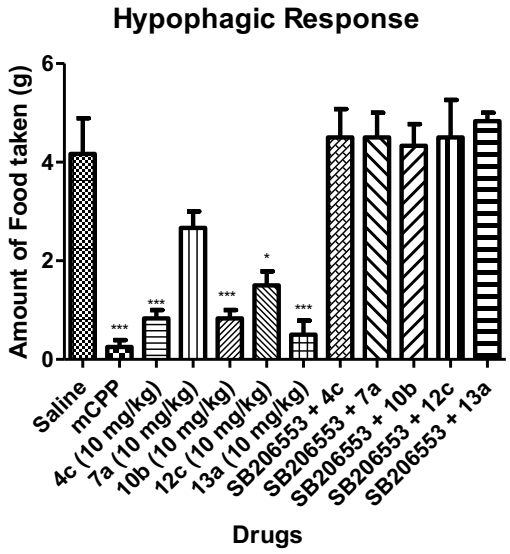


Figure 3. Effect of **SB-206553** on the **4c, 7a, 10b, 12c** and **13a** mediated reduction in 2 h food intake in 24 h fasted normal Sprague–Dawley rats. Antagonist (**SB-206553**, 2 mg/kg) was administered i.p. 15 min prior to administration of **4c, 7a, 10b, 12c** and **13a** (10 mg/kg). ***m*CPP** (5 mg/kg) i.p.was given as standard. Mean ± SEM (n = 3 per group) is significantly different from vehicle-treated group: ***P<0.001 by Dunnett's test and 1-way ANOVA.

recorded as the amount of food taken in 2 hr. Similar to EPM test, compounds **4c, 10b** and **13a** showed better hypophagic responses than **7a** and **12c** which validated the higher selectivity of compounds **4c, 10b** and **13a** towards $5HT_{2C}$ receptor than **7a** and **12c**.

3.2.5. Rat model of penile erection

***m*CPP** at 0.75 mg/kg s.c. induced penile erection and engorged penis as compared to the control animals treated with normal saline at 10 ml/kg. Similarly, **4c, 7a, 10b, 12c** and **13a** had shown penile erections at dose of 20 mg/kg s.c. However,

penile erections were antagonized by **SB206553** at 2 mg/kg i.p. (**Fig. 4**). More number of lickings in animals treated with **4c, 10b** and **13a** further exhibited the higher affinity of these compounds for $5HT_{2C}$ receptor than **7a** and **12c**.

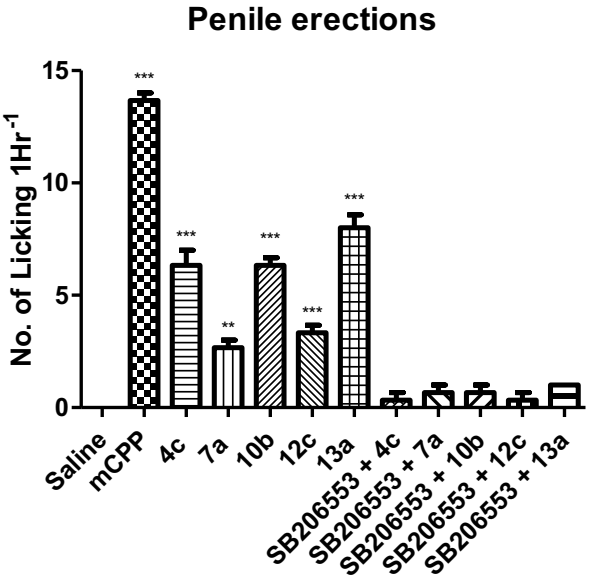


Figure 4. Interaction of **SB-206553** with **4c, 7a, 10b, 12c** and **13a** on penile erections in Sprague Dawley rats. **SB-206553** was injected i.p. 15 min before the s.c. injection of **4c, 7a, 10b, 12c** and **13a** (20 mg/kg) and the penile erections were counted over a period of 60-min, starting from the injection of **4c, 7a, 10b, 12c** and **13a**. Mean± SEM was calculated from n=3 rats per group and was significantly different from vehicle-treated group: ***P<0.001 by Dunnett's test and 1-way ANOVA.

The functional activity of the compounds for the 5-HT_{2C}, 5-HT_{2A} and 5-HT_{2B} receptors was found to be dependent on the substitution pattern on 3-benzazepine ring. Compound (**2a**), our first C-1 substituted compound in this series, demonstrated 5-HT_{2A} antagonistic activity and was eliminated from the study. Simultaneously compounds (**3a** and **3c**) have shown $5HT_{2B}$ agonistic activity and were also eliminated from the study but compound (**3b**) with more lipophilic and bulkier aryl substituents

at C-1 and N-3 has shown no activity for $5HT_{2A}$ and $5HT_{2B}$ and compounds (**4a**, **4b** and **4c**) also showed no interactions with $5HT_{2A}$ and $5HT_{2B}$. With these results in hand, a small set of compounds based on 3-benzazepine was designed to explore the effects of substitution at the C-1 and N-3 positions. At the C-1 position, di-substitutions of the bulkier benzyl group resulted into good $5HT_{2C}$ potency. Compound (**13a**) with no substitution at N-3 position and no oxo- at C-2 position demonstrated highest agonist potency for $5HT_{2C}$ receptor. Compounds (**2a, 3a, 3c, 9a** and **10a**) with oxo- at C-2 position and less lipophilic substituents or no substituents at C-1 and N-3 positions resulted in high potencies towards $5HT_{2A}$ and $5HT_{2B}$ receptors. Compounds (**4c** and **12c**) with no oxo- at C-2 and bulkier substituents at C-1 and N-3 positions exhibited good $5HT_{2C}$ potencies and selectivities. At the C-1 and N-3 positions, replacing the benzyl with methyl, ethyl or allyl groups showed a trend towards decreasing the potency at the $5HT_{2C}$ receptor with decreasing bulk size of the substituent. In summary bulkier substituents at C-1 and N-3 positions and no substituent at C-2 position showed good $5HT_{2C}$ potencies while oxo substitution at C-2 position and small alkyl groups at C-1 and N-3 positions showed decreased activity towards $5HT_{2C}$ and increased activity for $5HT_{2A}$ and $5HT_{2B}$ receptors.