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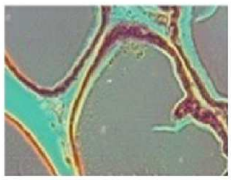
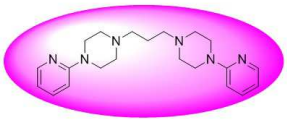
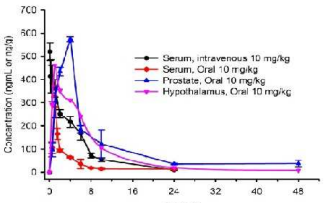
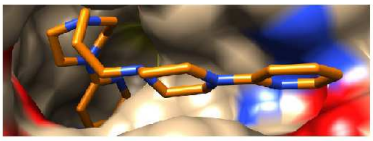
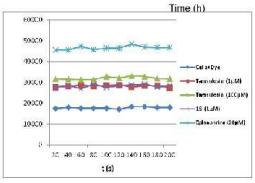




1, 3-Bis(4-(pyridin-2-yl)piperazin-1-yl)propane
Compound **19**
EC_{50} 15.6 μM
54% prostatic weight reduction







Novel Aryl Piperazines for alleviation of 'Andropause' associated Prostatic Disorders and Depression[#]

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ABSTRACT

A series of seventeen piperazine derivatives have been synthesized and biologically evaluated for the management of andropause-associated prostatic disorders and depression. Five compounds **16**, **19**, **20**, **21** and **22** significantly inhibited proliferation of androgen-sensitive LNCaP prostatic cell line with EC_{50} values of 12.4 µM, 15.6 µM, 11.8 µM, 10.4 µM, 12.2µM respectively and decreased Ca^{2+} entry through adrenergic-receptor α_{1A} blocking activity. Anti-androgenic behaviour of compound **19** and **22** was evident by decreased luciferase activity. The high EC_{50} value in AR-negative cells PC3 and DU145 suggested that the cytotoxicity of compounds was due to AR down regulation. Compound **19** reduced the prostate weight of rats by 53.8%. Further, forced-swimming and tail-suspension tests revealed antidepressant-like activity of compound **19,** lacking effects on neuromuscular co-ordination. *In silico* ADMET predictions revealed that the compound **19** had good oral absorption, aqueous solubility, non-hepatotoxic and good affinity for plasma protein binding. Pharmacokinetic and tissue uptake of **19** at 10 mg/kg demonstrated an oral bioavailability of 35.4%. *In silico* docking studies predicted similar binding pattern of compound 1**9** on androgen receptor as hydroxyflutamide. Compound **19** appears to be a unique scaffold with promising activities against androgen associated prostatic disorders in males like prostate cancer and BPH and associated depression.

Keywords: Piperazine derivatives; MTT assay; α_{1A} blocking activity, Pharmacokinetics

INTRODUCTION

Testosterone (T) and dihydrotestosterone (DHT) are the two androgenic hormones governing the normal tissue growth and differentiation of the prostate gland. These two male hormones are potent activators of androgen receptor (AR), the member of nuclear hormone receptor superfamily, which operates as a transcription factor and regulates downstream gene expressions [1]. AR signaling shows an array of pharmacological functions such as development and maintenance of male sexual characteristics and musculoskeletal tissues [2]. Androgen antagonists bind with the androgen receptors and display antagonist actions on prostate. Due to a range of side effects of steroidal androgen antagonists such as hepatotoxicity [3], loss of libido [4] etc. the non-steroidal template came into existence such as flutamide [5], nilutamide [6], and bicalutamide [7] (Figure 1) which are used for the treatment of proliferative androgen dependent prostatic disorders such as benign prostatic hyperplasia (BPH) and prostate cancer. Further, the tissue selective action of these androgen antagonists is yet ambiguous and needs clarification [8]. BPH and prostate cancer are age associated disorders and the risk of the incidence increases with age [9,10]. Globally, prostate cancer is the second most frequently diagnosed cancer in men (899,000 new cases, 13.6 % of the total) and the fifth most common cancer overall, leading to 258,000 deaths in 2008 [11]. On the other hand, BPH is increasingly becoming a major old-age disorder in human males causing morbidity due to troublesome Lower Urinary Tract Symptoms (LUTS). According to the global data it has been anticipated that the market value for treatment of BPH may rise from $2 billion in 2014 to approximately $4.9 billion by 2024 [12]. Similarly, the total estimated expenditure on prostate cancer in US was ~12 billion dollars ($) in 2010, and based on the growing aged population it is project to go up by 27%, by year 2020 [13].

ABBREVIATIONS

BPH, Benign prostatic hyperplasia; T, testosterone; DHT, dihydrotestosterone; SARM, selective androgen receptor modulator; PSA, prostate specific antigen; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetra-zolium bromide; DMF, dimethylsulphoxide; RFU, relative fluorescence unit; FST, forced swim test; TST, tail suspension test; AUC_{last}, area under the serum/tissue concentration-time curve up to last observation; C_{max}, peak serum/tissue concentration; t_{max}, time to C_{max}; V_d/F, volume of distribution; Cl/F, clearance; F, fraction of dose absorbed; HF, hydroxyflutamide; AR, androgen receptor; ET_3N, triethylamine; DCM, dichloromethane; ETOAc, ethyl acetate; DMSO, dimethyl sulfoxide; OD, optical density; SDS, sodium dodecyl sulphate; TBS, tri buffered saline; FBS, fetal bovine serum; UFLC, ultra fast liquid chromatography.

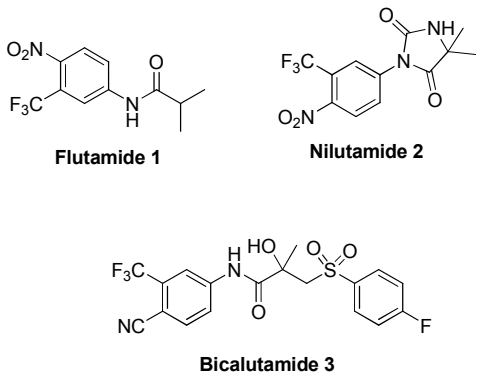


Figure 1. Structure of SARMs

It is well known that the progression of static component of the BPH [14] and early stages of prostate cancer [15] are androgen dependent. Besides being androgen sensitive, prostate cancer [16] and BPH [17] display increased adrenergic-α_{1A} receptor activity, hence sensitive to α_1-adrenergic blockers (e.g. prazosin, tamsulosin). Currently androgen antagonists are the potential option for management of androgen sensitive prostatic disorders in ageing males. Thus the discovery of novel androgen antagonists with α_{1A}-adrenergic blocking activity would be highly desirable to complement the activity of scaffolds. Different androgen antagonists have been reported in literature [18,19] based on different scaffolds.

It has been reported that prostatic disorders [20,21] and depression are closely associated with each other [10,22]. Depressive symptoms are generally diagnosed while experiencing complications due to prostatic problems and for a longer duration in elderly males [21,23]. Further, the presence of LUTS also increases the occurrence of depression in males [24,25]. BPH derived depression may be attributed to the fact that androgen imbalance (andropause, a hormone imbalance caused by lower levels of male hormone testosterone) [26] and depression are interlinked [27,28]. Hence it would be worthwhile to develop a scaffold with inbuilt anti-depressant activity.

Therapeutic potential and versatility of piperazines are well documented in literature as anticancer [29], anticonvulsant [30], antimalarial [31], antidepressant [32], etc. making it a privileged moiety in the field of medicinal chemistry. Additionally, piperazine bearing scaffolds hold a significant position in the area of non-steroidal androgen antagonists [19], ~~as well as~~ α_1 adrenoceptor antagonists [33] as well as dual activity towards α_1 adrenoceptor and 5-HT_{1A} receptors [34,35,36]. Our previous lab work explains that the piperazine containing scaffolds display an antagonistic profile against prostate, acting as androgen antagonists [19]. Piperazine

provides the molecular backbone to the molecule and the basicity of nitrogen atoms help in improving the potency of the scaffolds. Our endeavours of further exploring with piperazine motif containing scaffolds with both androgen antagonists and α_{1A}-adrenergic blocking activity led to the designing and development of new compounds containing two piperazines joined with variable number of methylene linkers. This report explains the synthesis, biological evaluation, pharmacokinetic studies and docking studies of synthesized piperazine based motifs (Figure 2).

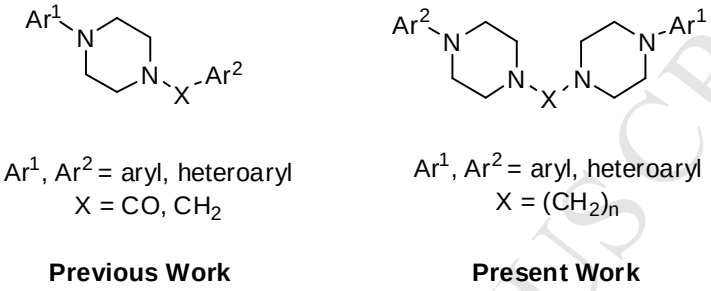


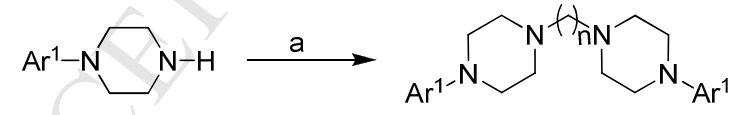
Figure 2. General structure of synthesized compounds

RESULT AND DISCUSSION

Chemistry

Aryl/hetroaryl piperazine derivatives may be synthesized by the following Scheme 1. In a single step reaction the substituted aryl/heteroaryl piperazine (**4-10**) were allowed to react with dihaloalkanes in basic conditions under heating in acetonitrile to furnish the respective final differently substituted aryl/heteroaryl piperazines derivatives (**11-23**).

Scheme 1. Synthesis of compounds **11-23**

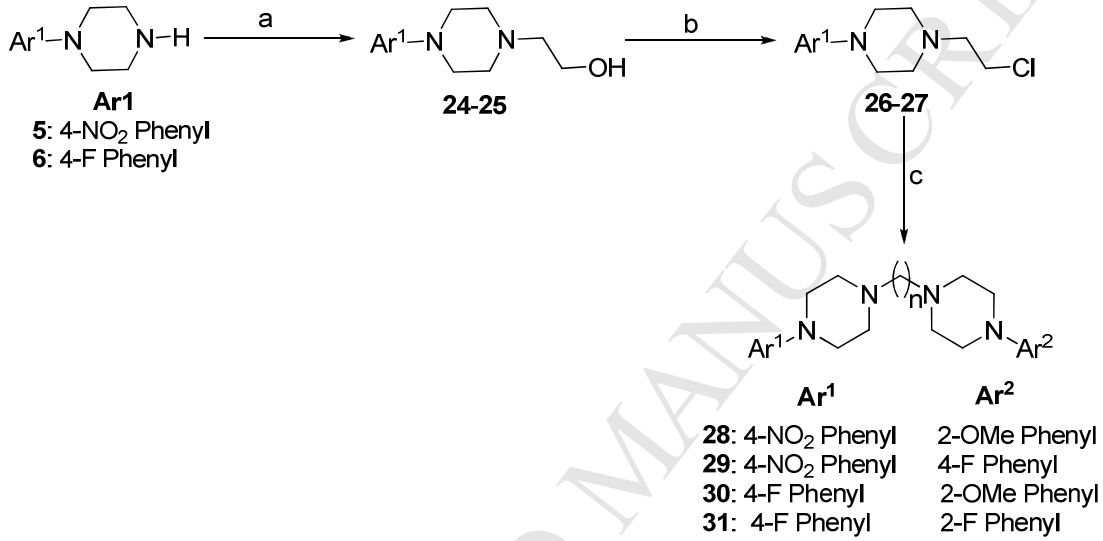


Ar1
4: 2-OMe Phenyl
5: 4-NO_2 Phenyl
6: 4-F Phenyl
7: 2-F Phenyl
8: 4-NO_2, 2-CF_3 Phenyl
9: 2-pyridyl
10: 4-CF_3, 2-NO_2 Phenyl

Reagents and condition: (a) dihaloalkanes (n = 2, 3), K_2CO_3, ACN, 50-55 ^oC, 7-9 h.

Scheme 2 further defines the synthesis of more derivatives (**28-31**). Substituted piperazine (**5-6**) on reaction with bromoethanol and subsequently with thionyl chloride gives the respective intermediates (**26-27**). Further **26-27** on refluxing with substituted piperazines in basic conditions gives final compounds (**28-31**).

Scheme 2. Synthesis of compounds **28-31**



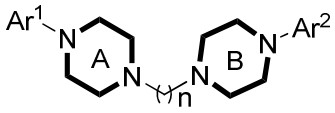
Reagents and condition: (a) Bromoethanol, Et_3N, $CHCl_3$, 50-55 ^oC, 20-22 h; (b) Thionylchloride, Et_3N, dry DCM, 0-5 ^oC, 5-6 h; (c) Substituted piperazine, Et_3N, $CHCl_3$, 80-85 ^oC, 12-14 h

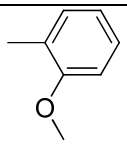
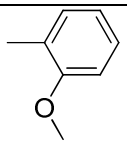
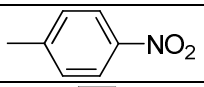
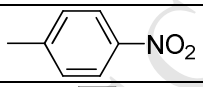
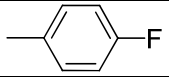
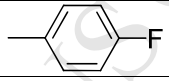
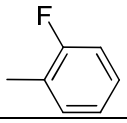
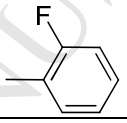
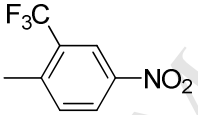
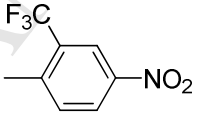
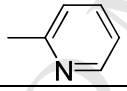
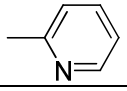
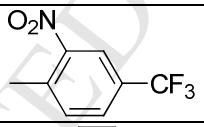
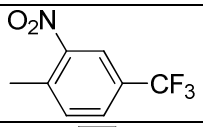
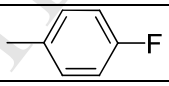
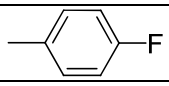
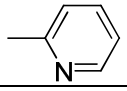
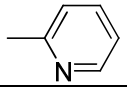
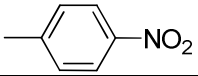
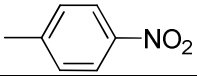
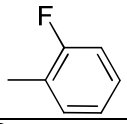
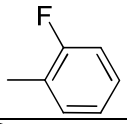
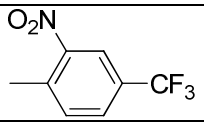
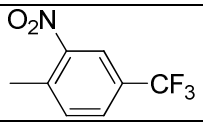
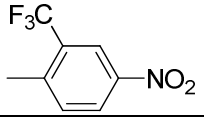
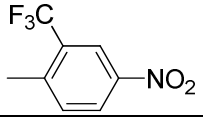
Biology

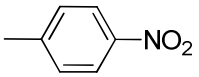
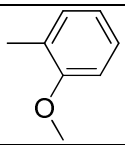
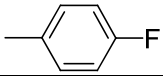
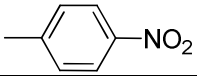
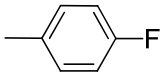
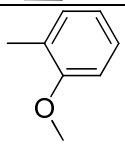
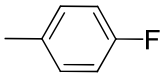
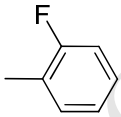
In vitro evaluation

Studies have shown that LNCaP cell are androgen dependent and also expresses α_{1A} [37]. Accordingly, the initial screening of seventeen synthesized compounds (**11-23**, **28-31**) has been done against the androgen dependent LNCaP cells via MTT assay taking flutamide (EC_{50} = 7.0 µM) as standard. The motive of this experiment was to obtain preliminary data on the inhibitory potential of compounds through a convenient screening method. The EC_{50} value of new compounds varied between 10.4-78.7 µM. Based on the results of MTT assay, compounds **16**, **19**, **20**, **21** and **22** exhibited good EC_{50} (µM) values in the range of 10 to 15 µM, which was comparable to the standard drug. Therefore, these five compounds were selected for further biological profiling. The structure of synthesized scaffolds and their EC_{50} (µM) values have been given in table 1.

Table 1. *In vitro* activity in LNCaP cell line (EC_{50} µM) of **11-23** and **28-31**



Compound number	n	Ar^1	Ar^2	EC_{50} (µM) in LNCaP
11	2			40
12	2			25.2
13	2			71.2
14	2			40
15	2			72.2
16	2			**12.4**
17	2			63.2
18	3			49.2
19	3			**15.6**
20	3			**11.8**
21	3			**10.4**
22	3			**12.2**
23	3			55.5

28	2			46.5
29	2			77.9
30	2			78.7
31	2			68

Detailed study of the data revealed the importance of methoxy, nitro, fluoro, trifluoromethyl substituent at the phenyl ring of piperazine and the heterocyclic pyridyl nucleus over the piperazine core in the synthesized scaffolds. The compounds **11-23** were symmetric having similar substituent on both the piperazines with 2 and 3 methylene spacer unit i.e. ethylene and propylene spacer while **28-31** were asymmetric with different substitution over both piperazines separated with 2 methylene spacers. It was found that in compounds **11-17** containing 2 methylene spacer units, compound **11** (EC_{50} = 40 µM) with 2-OCH_3 phenyl substituent on the N^4-position of both the piperazine showed moderate inhibition in the androgen dependent cell lines. Replacement of 2-OCH_3 with the 4-NO_2 group **12** (EC_{50} = 25.2 µM) increased the activity by 1.6 folds suggesting that the 4-NO_2 phenyl substituent was more favorable over 2-OCH_3 phenyl group. Further in scaffold **15** (EC_{50} = 72.2 µM), when 4-NO_2 group is accompanied by 2-CF_3 on the phenyl ring of both the piperazines, showed a considerable decrease in potency exhibiting that both the groups were not complementary to each other. On the other hand when the 4-NO_2 and 2-CF_3 were interchanged as in **17** (EC_{50} = 63.2 µM) having the 2-NO_2 and 4-CF_3 groups over the phenyl ring of both piperazine displayed a slight improvement in activity. Replacement of 4-F by 4-NO_2 group as in compound **13** (EC_{50} = 71.2 µM) resulted in decreased activity. The EC_{50} values of **13** and **15** and **17** confirm that the presence of 4-F, 2-CF_3 and 4-CF_3 respectively over the phenyl rings decrease the antagonistic behavior of the scaffolds. While the reshuffling of the fluoro substituent from the position-4 to position-2 of phenyl ring (compound **14**, EC_{50} = 40 µM) leads to the improvement of the activity of scaffold, showing that 2-F is more favourable than 4-F group. The switch over of the phenyl ring by the heterocyclic 2-pyridyl

nucleus (compound **16**, EC_{50} = 12.4 µM) causes a remarkable increase in the effectiveness of the scaffold as an antagonist.

Scaffolds **18-23,** had propylene as a linker between the differently substituted piperazine fragments. Compound **18** (EC_{50} = 49.2 µM) with 4-F phenyl core exhibited moderate inhibition. Moving this fluoro substituent from position-4 to position-2 (compound **21**, EC_{50} = 10.4 µM) caused five-fold enhancement of activity in comparison to **18**. Presence of 4-NO_2 phenyl (compound **20**, EC_{50} = 11.8 µM) led to a significant increase in the antagonistic behavior of the motif and additional 2-CF_3 group (compound **23**, EC_{50} = 55.5 µM) showed a diminished activity. However, interchanging the positions of these two groups (compound **22**, EC_{50} = 12.2 µM) enhanced the activity by about 4.5 folds. Adding the heterocyclic 2-pyridyl component to the nucleus (compound **19**, EC_{50} = 15.6 µM) also retained the good activity. The piperazine scaffolds **28-31** with dissimilar aryl substituent showed moderate activity (EC_{50} = 46.6-77.9 µM).

SAR of the scaffolds showed that with ethylene linker with the 2-pyridyl substitution (**16**) was the most privileged showing maximum inhibition followed by 4-NO_2 phenyl (**12**), 2-OCH_3 phenyl (**11**) = 2-F phenyl (**14**), 2-NO_2-4-CF_3 phenyl (**17**), 4-F phenyl (**13**) and 4-NO_2-2-CF_3 phenyl (**15**). While with propylene linker the order of activity was found to be 2-F phenyl (**21) >** 4-NO_2 phenyl (**20**) > 2-NO_2-4-CF_3 phenyl (**22**) > 2-pyridyl (**19**) > 4-F phenyl (**18**) > 4-NO_2-2-CF_3 phenyl (**23**).

The five scaffolds **16**, **19**, **20**, **21** and **22** with EC_{50} values of 12.4 µM, 15.6 µM, 11.8 µM, 10.4 µM, 12.2 µM respectively, and comparable with flutamide, were subjected to time dependent analysis of Ca^{2+} mobilization in α_{1A} expressing Chem-1 cells and *in vivo* evaluation in rats.

AR-antagonist nature of test compounds was exhibited by luciferase reporter gene assay. An AR negative Cos7 cell line was transiently transfected with AR and ARE- LUC plasmids and treated with compounds **19** and **22** at a concentration of 7.5 µM. Bicalutamide (3µM), was used as positive control. Test compounds (**19** and **22**, $P<0.01$) showed a significant decrease in luciferase activity as compared to control at 24 h, which displayed their anti-androgenic nature (Figure 3).

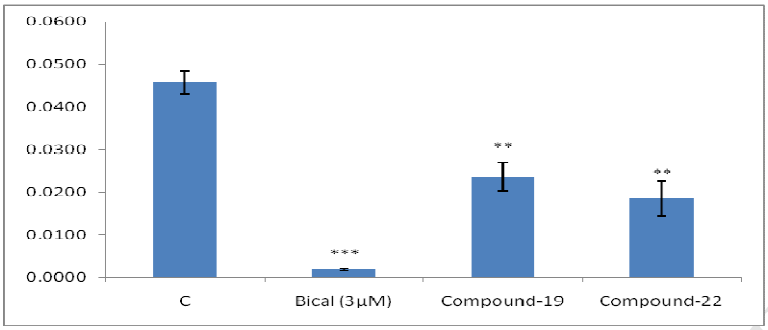


Figure 3. Transactivation of ARE-LUC by AR in presence of test compounds was measured by dual-luciferase reporter assay in terms of RLU (Control=100%). **19** (7.5 µM), **22** (7.5 µM) and bicalutamide (3 µM). Significant difference from control is indicated as **P<0.01 and ***P<0.001).

Cell viability test was performed in AR-negative PC3 and DU145 cells lines, which exhibited that the anti proliferative activity of test compounds **16**, **19**, **20**, **21** and **22** was due to AR down regulation. EC_{50} values of the compounds in AR-negative cells PC3, DU145 and AR-positive cells LNCaP cells was shown in table 2. The high EC_{50} value in AR-negative cells PC3 and DU145 suggested that the cytotoxicity of compounds was specifically mediated through AR.

Table 2. Cell Viability Test in PC3 and DU145 of compounds **16**, **19**, **20**, **21** and **22**

Compound	**EC_{50} for PC3 (µM)**	**EC_{50} for DU145 (µM)**	**EC_{50} for LNCaP (µM)**
16	>80	>80	12.4
19	>80	>80	15.6
20	>80	>80	11.8
21	>80	>80	10.4
22	>80	76.5	12.2

Alpha blocker (α_{1A}-adrenoceptor antagonist) alleviates the symptom score of the disease by binding with the α_{1A}-adrenoceptor present in the smooth muscle of the prostate. The five synthesized piperazine derivatives **16**, **19**, **20**, **21** and **22** were analyzed for the alpha blocking activity via time dependent analysis of Ca^{2+} mobilization in α_{1a} expressing Chem-1 cells. Assay was done with dose responses for compounds **16**, **19**, **20**, **21** and **22** at 1 µM, agonist epinephrine

at 20 μM and standard antagonist tamsulosin at 100 nM and 1 μM. The values of relative fluorescence unit (RFU) correspond to the Ca^{2+} released in the cells. It was elaborated from the results that the epinephrine, an agonist, increased the Ca^{2+} level significantly about 2.5 times in comparison to the untreated cell. Contrary to this the standard tamsulosin (1 μM), an antagonist causes the diminution of the Ca^{2+} level about 1.6 times of the epinephrine treated cells. The five tested scaffold exhibited the alpha blocking potency at concentration of 1 μM. Relative fluorescence unit (RFU) data reveals that the cells treated with compound **19** and **22** experience Ca^{2+} level reduction 1.6 times of epinephrine treated cells which is approximately same as that of tamsulosin. On the other side the next three scaffolds **16**, **20** and **21** showed a drop of 1.5 times which very close to standard drug tamsulosin (Figure 4). Hence, these scaffolds displayed good potency as α_{1A}-adrenoceptor antagonist.

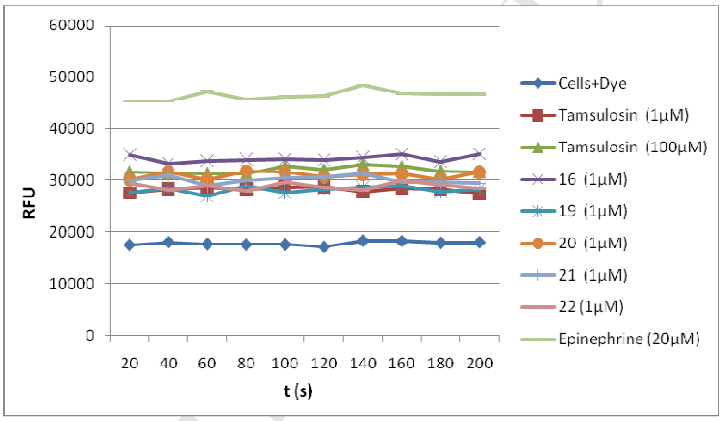


Figure 4. Time dependent analysis of Ca^{2+} mobilization in α1A expressing Chem-1 cells with different concentration of compounds **16** (1 μM), **19** (1 μM), **20** (1 μM), **21** (1 μM), **22** (1 μM), agonist epinephrine (20 μM) and antagonist tamsulosin (1 μM)

***In vivo* Experiment on *Sprague–Dawley* Rats**

Compounds **16**, **19**, **20**, **21** and **22** were subjected to *in vivo* experiment performed on the adult male *Sprague–Dawley* rats (weighing 250–280 g, n = 5). 29 days study included the induction of disease (prostatic hyperplasia) by initial 8 days oral dosing with citral (100 mg/kg) followed by giving compounds **16**, **19**, **20**, **21**, **22** and flutamide at 10 mg/kg for rest of 21 days parallel along with citral. Table 3 shows the results of experiment after dissection and weighing of the prostate 24 h of last treatment.

Table 3. Effect of compound **16**, **19**, **20**, **21** and **22** on prostate

Compd no.	Prostate wt. per 100 g of body weight	% Prostate wt.*	% Reduction of prostate weight
16	0.16	56.0	44.0
19	0.13	46.2	53.8
20	0.21	71.0	29.0
21	0.18	63.0	37.0
22	0.14	48.3	51.7
Flutamide	0.12	41.2	58.8
Citral	0.29	100	0
Control	0.11	37.9	-

* Prostate weight of citral treated rats was taken as 100%.

Citral being a disease inducer caused the increase in the prostatic weight up to 290 mg/100 g of body weight which is significantly higher than that of control 110 mg/100 g of body weight. Experiment displayed the prominent reduction of prostatic weight by treatment with all the five derivatives. Compound **19** and **22** exhibited reduction of 54% and 52% respectively which is comparable with flutamide. Interestingly, compounds did not affect the weights of seminal vesicle, testis and epididymis (Figure 5).

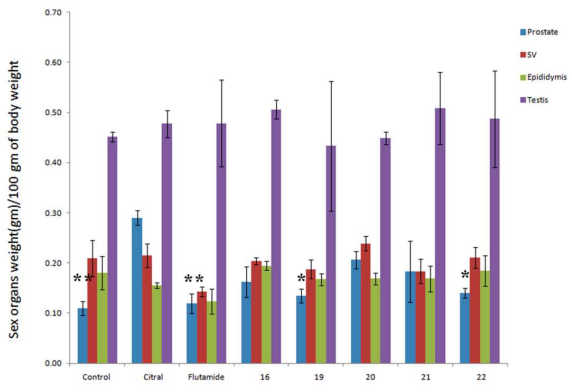


Figure 5. Effect of citral (10 mg/Kg) compound flutamide, **16**, **19**, **20**, **21** and **22** (10 mg/Kg) on prostate, testis, epididymis, seminal vesicle

Histological investigations of prostatic tissues were also carried out for all five compounds. Compound **19** was found to show the maximum (54%) reduction of the prostatic weight than other compounds so was also subjected for pharmacokinetic studies.

Histology

The histological investigations of periurethral prostatic tissues obtained from the *in vivo* experiments were done microscopically and presented in figure 6. Pictures clearly demonstrated that the BPH generated tissue have very little stromal differentiation as compared to control tissue having clear stroma. Treatment with compounds **16**, **19**, **20**, **21**, **22** and flutamide in parallel with citral increased the distinction between the stromal and acinar compartments. The results obtained from this study support the *in vitro* and *in vivo* results.

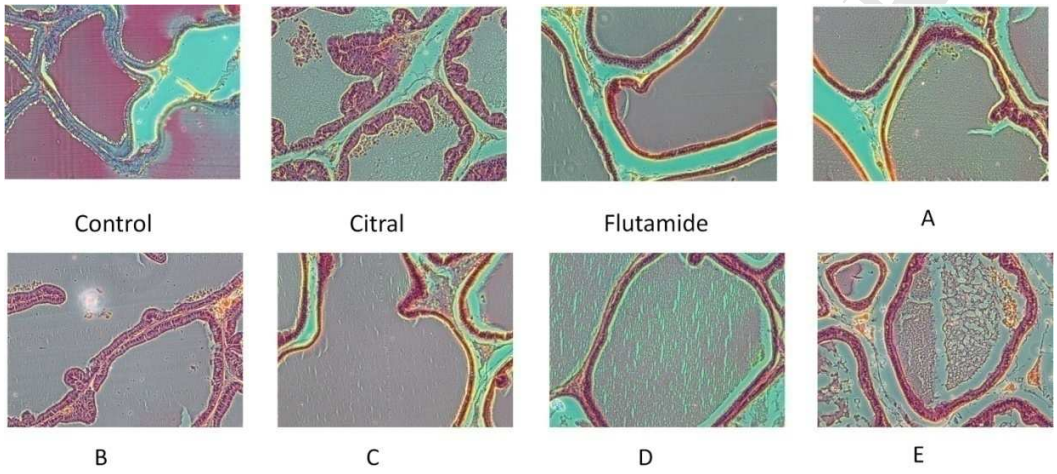


Figure 6. Representative histological architecture of the prostate of rats given vehicle control, citral (100 mg/Kg), citral + flutamide (10 mg/Kg), (A) citral + **16** (10 mg/Kg), (B) citral + **19** (10 mg/Kg), (C) citral + **20** (10 mg/Kg), (D) citral + **21** (10 mg/Kg) and (E) citral + **22** (10 mg/Kg) orally once daily for 21 days

Evaluation of antidepressant-like activity of BPH active compounds in different mice models

Forced Swim Test (FST)

Five compounds **16**, **19**, **20**, **21**, **22** active in *in vivo* studies for BPH were also studied for antidepressant like activity and tested in FST, a primary model of depression. Active compounds at dose of 20 mg/kg were administered orally to the mice and after 60 min of administration subjected to FST and immobility time was monitored. The reduction in the immobility time was found to be highly significant ($p<0.001$) after the administration of compound **19** (69.40%) and **20** (69.74%) as compared to control mice (Figure 7). The two compounds **19** and **20** also resulted

in significant reduction of immobility time as compared to Imipramine (IMI) (60 mg/kg) whereas was comparable with Flouxetine (FXT) (20mg/kg) (Table 4).

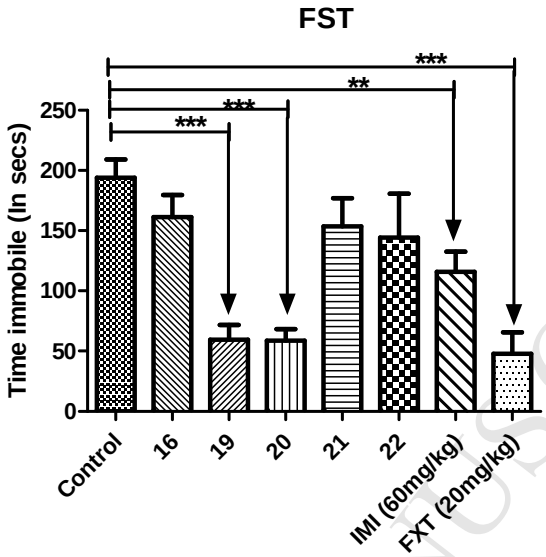


Figure 7. Force Swimming Test. Bar diagram shows immobility time duration (in seconds) during 5 minutes of test. The compounds were given orally at dose of 20 mg/kg.i.p. ***P<0.001; **P<0.01 vs control. Data was expressed as mean ± SEM; N=7-9 and analyzed by unpaired t-test

Table 4. Percentage decrease of immobility time (seconds) of compounds in FST. ***P < 0.001 vs control

Compound	Mean	SEM	% decrease of immobility	Significance level
Control	194.10	14.86	-	-
16 (29)	161.2	18.57	16.95	Ns
19 (32)	59.39	12.28	69.40	P < 0.001
20 (33)	58.72	9.55	69.74	P < 0.001
21 (34)	153.60	23.35	20.86	Ns
22 (35)	144.20	36.47	25.71	Ns
Imipramine (IMI)	115.70	17.02	40.39	P < 0.01
Fluoxetine (FXT)	47.84	17.66	75.35	P < 0.001

Tail Suspension Test (TST)

Further compounds **19** and **20** were also tested (at same dose) in tail suspension test, which is another model of antidepressant like activity to further confirm this activity. Treated mice were

suspended with tail in a horizontal rod and immobility time was recorded. Both the compounds showed significant reduction in immobility time and their effect was found comparable to Imipramine (60 mg/kg) in TST (Figure 8).

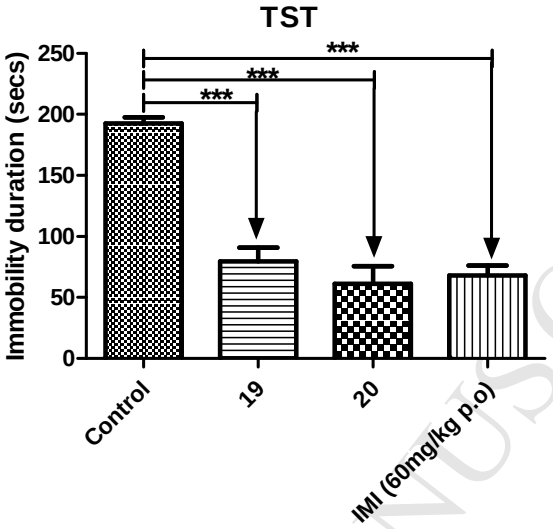


Figure 8. Tail Suspension Test. Diagram represents immobility time duration in TST when suspended in rod. ***P<0.001 as compared to control. Data was expressed as mean ± SEM; N=5

FST and TST experiments confirmed the antidepressant like activity of compounds **19** and **20**. Thereafter, both these compounds were further evaluated for their effect on motor activity.

Locomotor Activity and Rotarod Test

Locomotor activity and rotarod test (n = 6) were also performed for compounds **19** and **20,** which were found active in both FST and TST. There was no significant difference in locomotor activity when compared to control animals. This indicated that the compounds were not causing any alteration in locomotor activity (Figure 9). Similarly, effect of compounds on rotarod test for motor co-ordination showed that the latency to fall was not affected after the administration of compounds and stayed for 120 sec on the rotating rod. This suggests that the neuromuscular co-ordination of the animals was not impaired by treatment with the compounds **19** and **20**.

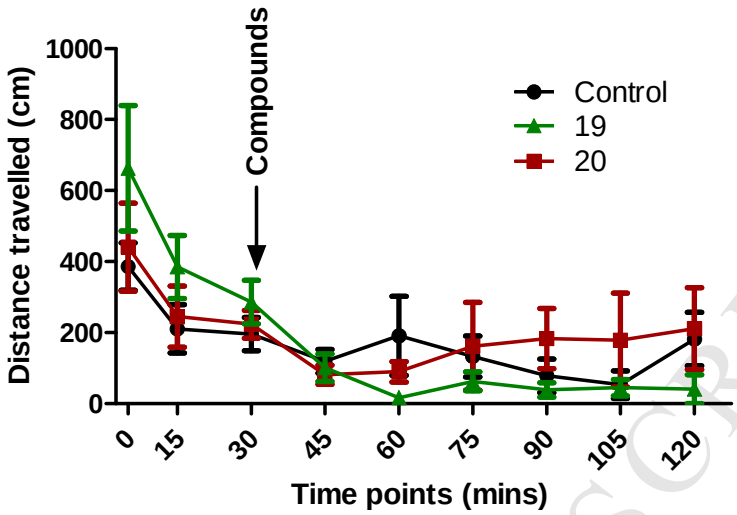


Figure 9. Locomotor activity of compound **19** and **20** treated and control mice (n=5)

In silico ADMET **Prediction of Selected Compounds**

The *in silico* ADMET predictions using BIOVIA Discovery Studio 4.1, for the compounds **16**, **19**, **20**, **21** and **22** are summarized in the table 5. The ADMET absorption ratio of 0 predicted for most of the compounds indicated good absorption. The ADMET solubility level of 2 indicated that although the compound is having some aqueous solubility but it is low, whereas ADMET solubility level of 3 indicated good aqueous solubility. The ADMET BBB level of 0, 1 and 4 indicated very high, high and undefined respective blood brain barrier penetration levels of compounds. The compounds with ADMET EXT CYP2D6 PRED as true indicated CYP2D6 inhibition potential and false indicated no inhibition potential. The compounds with false predicted ADMET hepatotoxicity are categorized into nontoxic. The ADMET EXT PPB PRED indicated good affinity of all the compounds for plasma protein binding [38]. Overall, the compound **19** was predicted to have good oral absorption, good aqueous solubility, non-hepatotoxic with CYP2D6 inhibition potential and good affinity for plasma protein binding. The confirmation of these predictions is provided by the *in vivo* pharmacokinetic studies of the compound **19** in *Sprague Dawley* rats.

Table 5. *In silico* ADMET Prediction of Selected Compounds

Comp.	ADMET solubility level	ADMET BBB_level	ADMET EXT CYP2D6 PRED	ADMET EXT hepatotoxic pred	ADMET EXT PPB PRED	ADMET absorption
16	3	1	true	false	true	0

19	3	1	true	false	true	0
20	2	4	false	true	true	0
21	2	0	true	true	true	0
22	2	4	false	false	true	2

Pharmacokinetic Studies

The animals tolerated the treatment as no peculiarities in the animals' behaviour were observed. Serum levels of the compound **19** were monitored up to 24 h. Following 10 mg/kg oral dose, it showed a peak serum concentration (C_{max}, 328.6 ± 45.9 ng/mL) at 1 h indicating rapid absorption (Figure 9). Moreover, it was monitored up to 48 h in hypothalamus and prostate with C_{max} at 1 and 4 h post dose, respectively. The concentration-time profile was analyzed using both non-compartmental and compartmental approach. The pharmacokinetic models were compared according to maximal correlation between observed and predicted concentration, minimal sum of squared residuals, Akaike's Information Criterion (AIC) and Schwarz Bayesian Criterion (SBC) [39,40]. The concentration-time data was best described by a two-compartmental open model and the calculated pharmacokinetic parameters are shown in table 6. The volume of distribution (28.7 ± 3.8 L/kg) is larger than the total blood volume of rat (0.054 L/kg [41]) and systemic clearance (4.1 ± 0.3 L/h/kg) is higher than the total hepatic blood flow in rats (2.9 L/h/kg [41]) indicating extravascular distribution along with the extrahepatic elimination of compound. The oral bioavailability was found to be 35.4%. Compound **19** displayed higher tissue to serum concentration ratio in prostate. However, hypothalamus showed lower tissue to serum ratio (Figure 10, table 6).

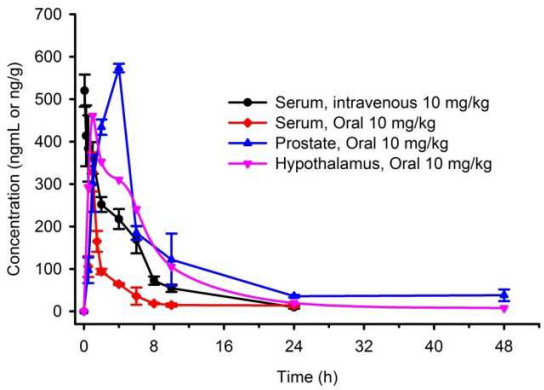


Figure 10. Mean concentration-time profile of **19** in serum, prostate and hypothalamus after single 10 mg/kg oral and intravenous administration in male *Sprague Dawley* rats. Bar represents SEM (n = 3).

Table 6. Pharmacokinetic parameters of **19** after single 10 mg/kg oral and intravenous administration in male *Sprague Dawley* rats[a]

Parameters	**Intravenous**	**Oral**		
	Serum	**Serum**	**Prostate**	**Hypothalamus**
C_{max} (ng/mL or ng/g)	520.0 ± 37.7	328.6 ± 45.9	573.3 ± 10.2	461.0
t_{max} (h)	-	1.0	4.0	1.0
AUC_{last} (µg h/mL or µg h/g)	2.5 ± 0.2	0.9	10.7	4.2
$T_{1/2}$ (h)	5.2 ± 0.7	5.4	155.5	128.8
Cl (L/h/kg or g/h/kg)	4.1 ± 0.3	3.9	0.3	0.8
V_{ss} (L/kg or g/kg)	28.3 ± 3.8	23.7	3.6	6.6
Tissue to serum ratio	-	1.0	11.8	4.6
Bioavailability (%)	-	35.4	-	-

[a]Each value represent the average of three rats. Serum and prostate concentration are mean ± SEM from three rats. Hypothalamus concentrations are the mean of the pooled tissue of three rats.

Docking Studies

Previous lab work showed that the piperazine containing scaffolds possibly act via androgen receptor modulation [19] therefore the present compounds were docked with androgen receptor. The binding mode of compounds **16**, **19**, **20**, **21** and **22** has been compared to Hydroxyflutamide (HF) which is known antagonist of androgen receptor (AR). The crystal structure of androgen receptor in complex with HF has been reported (PDB accession code: 2AX6). It has been shown that T877A mutation converts the HF to agonist [42]. In another study it has been proposed that repositioning of helix-12 of AR causes the change in activity of HF [43]. Therefore, docking studies of HF in the wild type AR was done and the reproduced binding pose was used as reference for the reported active compounds (Figure 11, table 7). The predicted binding pattern of compounds **16**, **19**, **20**, **21** and **22** was similar to binding mode of HF. The protein-ligand interactions were dominated by strong pi-pi stacking interactions with residues Phe 764 and Trp 741. The residues Met 749, Met 745 and Leu 707 are facilitating hydrophobic contacts. The backbone of residue Met 742 is involved in hydrogen bond formation in case of compound **16, 19** and **22** while residues Gln 711 and Arg 752 showed to form hydrogen bond in case of

compound **20**. Compound **19** with propylene linker showed strong pi-pi stacking in comparison to compound **16** with ethylene linker. Compound **21** with 2-fluoro phenyl substituent over piperazine showed good pi-pi stacking but devoid of hydrogen bonding character. However, compound **20** and **22** showed similar docking properties.

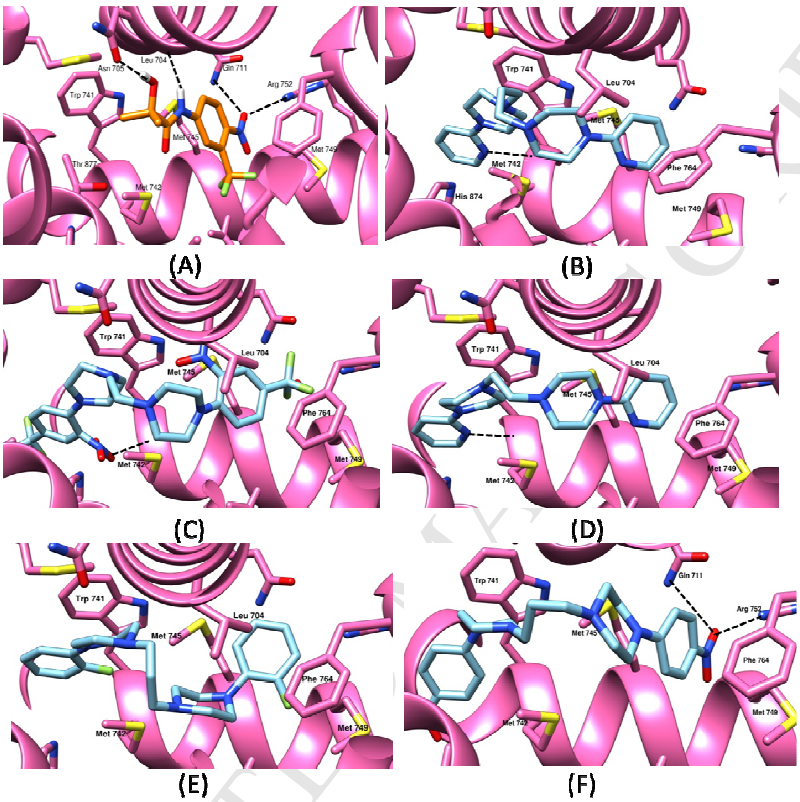


Figure 11. (A) Hydroxyflutamide binding mode to WT AR (B) Predicted binding mode of compound **19** (C) compound **22** (D) compound **16** (E) compound **21** (F) compound **20** in AR binding site. Protein is shown in mixed representation (ribbon and stick). Hydrogen bonds are shown as black line

Table 7. Docking studies and the residues involved in interaction

Compound No.	Hydrogen bond	Pi-pi stacking interactions
16	Met 742	Phe 764, Trp 741
19	Met 742	Phe 764, Trp 741, His 874
20	Arg 752/Gln711	Phe 764, Trp 741
21	-	Phe 764, Trp 741, His 874

22	Met 742	Phe 764, Trp 741

CONCLUSION

A series of seventeen alkylene bis(4-aryl/heteroarylpiperazine) derivatives (**11-23**, **28-31**) were synthesized and evaluated for proliferation inhibitory activity against androgen dependent LNCaP cell lines via MTT assay using flutamide as standard. Compounds **16**, **19**, **20**, **21** and **22,** which exhibited activity comparable to flutamide and were supplemented with α_{1A} blocking activity, were chosen for *in vivo* experiments, histology and docking studies. Anti-androgenic behavior of the compounds was assessed by luciferase reporter gene assay. Further the anti proliferative activity of the test compounds was due to AR down regulation. *In vivo* experiments revealed that all the five compounds **16**, **19**, **20**, **21** and **22** displayed a significant decrease in the prostatic weight. Compound **19** showed 54% reduction of prostatic weight, which is similar to flutamide (58%). Histological experiment also supported the results of *in vivo* studies. Further compound **19** and **20** were also found to possess significant antidepressant like behavior without affecting the neuromuscular co-ordination. *In silico* ADMET predictions revealed that the compound **19** had good oral absorption, good aqueous solubility, non-hepatotoxic with CYP2D6 inhibition potential and good affinity for plasma protein binding. The compound **19** exhibited a higher tissue to serum concentration ratio in prostate showing an oral bioavailability of 35.4%. In docking studies it displayed similar binding mode as that of hydroxyflutamide. Conclusively, 1, 2-bis(4-(pyridin-2-yl)piperazin-1-yl)propane (**19**) might act as a potent lead candidate for management of androgen sensitive prostatic disorders and associated depression in aged men.

EXPERIMENTAL

Chemistry

General Information

All reagents and solvents were commercial available and were used without further purification. Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel plates 60 GF_{254} (Aldrich) to monitor the progress of the reaction. Electrospray ionization mass spectra (ESI-MS) were recorded on Ion Ttrap LCQ Advantage Max-IT (Thermo Electron Corporation). High-resolution mass spectra (HRMS) were recorded on a 6520 Agilent Q Tof LC MS/MS (accurate mass).^{1}H and ^{13}C NMR spectra were recorded on Bruker Supercon Magnet Avance

DPX-200/DRX-300 spectrometers operating at 400 and 100 MHz respectively in deuterated solvents with TMS as internal reference (chemical shifts δ in ppm, *J* in Hz.). IR spectra (ν_{max} in cm^{-1}) of the compounds were recorded on Perkin Elmer's FT-IR RX1 PC spectrophotometer. Melting points were determined in open capillary tubes on the melting point apparatus. Elemental analyses were performed on Carlo Erba EA-1108 micro analyzer/Vario ELIII C H N S analyzer. All compounds were analyzed for C, H, N and the results obtained were within ±0.4% of calculated values. All compounds were characterized by TLC, 1H and 13C NMR, MS, and HRMS. Elemental analyses data meet the criteria of >95% purity.

1, 2-Bis(4-(2-methoxyphenyl)piperazin-1-yl)ethane (11)

A mixture of 1-bromo-2-chloroethane (0.43 mL, 5.2 mmol), K_2CO_3 (0.71 g, 5.2 mmol) in ACN (10mL) was stirred at 50 °C for 15 min and then 1-(2-methoxyphenyl)piperazine (**4**, 0.5 g, 2.6 mmol) in ACN (5 mL), was added dropwise within 1 h duration. Reaction mixture was further stirred for 4 h. Then the mixture was concentrated under reduced pressure and residue was dissolved in EtOAc (15 mL) and the EtOAc layer was then washed with water (10 mL × 3). The combined organic layer was dried (anhyd. Na_2SO_4) and concentrated under reduced pressure. The resultant was washed with distilled hexane and again dried under high vaccum. The pure offwhite crystals were obtained by recrystallization using EtOAc/Hexane (yield: 69%); mp: 137-140 °C; IR (KBr) ν (cm^{-1}): 3019, 2834, 2400, 1594, 1499, 1451, 1411; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.04-6.87 (8H, m), 3.88 (6H, s), 3.15 (8H, bs), 2.78-2.72 (12H, m); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 152.3, 141.3, 122.9, 121.0, 118.2, 111.2, 55.9, 55.3, 53.9, 50.6; HRMS (ESI positive) m/z calcd. for $C_{24}H_{34}N_4O_2$ $[M+H]^+$: 411.2760, found: 411.2761; Anal calcd. for $C_{24}H_{34}N_4O_2$: C, 70.21; H, 8.35; N, 13.65, found: C, 70.09; H, 8.21; N, 13.43.

Further compounds **12-23** was synthesized by using the procedure similar to compound **11.**

1, 2-Bis(4-(4-nitrophenyl)piperazin-1-yl)ethane (12)

The title compound was synthesized using 1-(4-nitrophenyl)piperazine (**5**) and 1-bromo-2-chloroethane as a starting material and yellow colour crystals were obtained as a product (yield 77%); mp: 189-193 °C; IR (KBr) ν (cm^{-1}); 3021, 2955, 2401, 1595, 1502, 1397, 1327; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06-8.04 (4H, m), 6.76-6.74 (4H, m), 3.36 (8H, t, *J* = 5.0 Hz), 2.59 (8H, t, *J* = 5.1 Hz), 2.5 (4H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.8, 138.5, 125.9, 112.6, 55.7, 53.1, 47.0; HRMS (ESI positive) m/z calcd. for $C_{22}H_{28}N_6O_4$ $[M+H]^+$: 441.2250, found: 441.2247; Anal calcd. for $C_{22}H_{28}N_6O_4$: C, 59.99; H, 6.41; N, 19.08, found: C, 59.72; H, 6.31; N, 19.01.

1, 2-Bis(4-(4-fluorophenyl)piperazin-1-yl)ethane (13)

The title compound was synthesized from 1-(4-fluorophenyl)piperazine (**6**) and 1-bromo-2-chloroethane as white solid (yield 70%); mp: 183-185 °C; IR (KBr) ν (cm^{-1}): 3019, 2954, 2827, 2400, 1600, 1509, 1452, 1420, 1386; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.97-6.93 (4H, m), 6.88-6.85 (4H, m), 3.12 (8H, t, *J* = 4.8 Hz), 2.67 (8H, t, *J* = 5.0 Hz), 2.62 (4H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.3, 156.0, 147.9, 117.8, 117.7, 115.6, 115.4, 55.8, 53.6, 50.1; HRMS (ESI positive) m/z calcd. for $C_{22}H_{28}F_2N_4$ $[M+H]^+$: 387.2360, found: 387.2349; Anal calcd. for $C_{22}H_{28}F_2N_4$: C, 68.37; H, 7.30; N, 14.50, found: C, 68.12; H, 7.21; N, 14.35.

1, 2-Bis(4-(2-fluorophenyl)piperazin-1-yl)ethane (14)

The title compound was synthesized from 1-(2-fluorophenyl)piperazine (**7**) and 1-bromo-2-chloroethane to give pure compound as white solid (yield 59%); mp: 149-153 °C; IR (KBr) ν (cm^{-1}) 3020, 2951, 2829, 2401, 1613, 1501, 1450; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00-6.82 (8H, m), 3.05 (8H, t, *J* = 4.6 Hz), 2.63 (8H, t, *J* = 4.7 Hz), 2.56 (4H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.9, 154.5, 140.1, 140.0, 124.4, 124.4, 122.4, 122.4, 118.9, 118.9, 116.2, 115.9, 55.9, 53.7, 50.5, 50.4; HRMS (ESI positive) m/z calcd. for $C_{22}H_{28}F_2N_4$ $[M+H]^+$: 387.2360, found: 387.2359; Anal calcd. for $C_{22}H_{28}F_2N_4$: C, 68.37; H, 7.30; N, 14.50; found: C, 68.24; H, 7.01; N, 14.29.

1, 2-Bis(4-nitro-2-(trifluoromethyl)phenyl)piperazin-1-yl)ethane (15)

The title compound was synthesized from 1-(4-nitro-2-(trifluoromethyl)phenyl)piperazine (**8**) and 1-bromo-2-chloroethane to give pure compound as yellow solid (yield 70%); mp: 138-143 °C; IR (KBr) ν (cm^{-1}): 1638, 1386; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (2H, d, *J* = 2.6 Hz), 8.25 (2H, dd, *J* = 2.4, 8.9 Hz), 7.20-7.19 (2H, m), 3.10 (8H, t, *J* = 4.5 Hz), 2.63-2.62 (8H, m) 2.57 (4H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.9, 142.1, 127.8, 124.6, 124.6, 122.2, 122.0, 55.7, 53.4, 52.7, 51.2; HRMS (ESI positive) m/z calcd. for $C_{24}H_{26}F_6N_6O_4$ $[M+H]^+$: 577.1998, found: 577.1995; Anal calcd. for $C_{24}H_{26}F_6N_6O_4$: C, 50.00; H, 4.55; N, 14.58, found: C, 49.85; H, 4.41; N, 14.46.

1, 2-Bis(4-(pyridin-2-yl)piperazin-1-yl)ethane (16)

The title compound was synthesized from 1-(pyridine-2-yl)piperazine (**9**) and 1-bromo-2-chloroethane as yellow solid (yield 74%); mp: 129-131 °C; IR (KBr) ν (cm^{-1}): 3399, 2953, 1602, 1482, 1386; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (2H, dd, *J* = 1.4, 4.8 Hz), 7.50-7.46 (2H, m),

6.66-6.61 (4H, m), 3.56 (8H, t, *J* = 4.9 Hz), 2.63 (12H, t, *J* = 4.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.5, 147.9, 137.4, 113.2, 107.7, 56.0, 53.5, 45.1; HRMS (ESI positive) m/z calcd. for $C_{20}H_{28}N_6$ $[M+H]^+$: 353.2454, found: 353.2449; Anal calcd. for $C_{20}H_{28}N_6$: C, 68.15; H, 8.01; N, 23.84, found: C, 68.04; H, 7.90; N, 23.62.

1, 2-Bis(2-nitro-4-(trifluoromethyl)phenyl)piperazin-1-yl)ethane (17)

The title compound was synthesized from 1-(2-nitro-4-(trifluoromethyl)phenyl)piperazine (**10**) and 1-bromo-2-chloroethane as yellow crystals (yield 78%); mp: 150-153 °C; IR (KBr) ν (cm^{-1}): 3019, 2400, 1625, 1534, 1385, 1326; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (2H, d, *J* = 1.6 Hz), 7.57 (2H, dd, *J* = 1.9, 8.8 Hz), 7.07 (2H, d, *J* = 8.7 Hz), 3.09 (8H, t, *J* = 4.7 Hz), 2.58 (8H, t, *J* = 4.8 Hz), 2.53 (4H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 146.9, 139.5, 129.1, 129.1, 123.2, 123.1, 119.5, 54.6, 52.0, 49.8; HRMS (ESI positive) m/z calcd. for $C_{24}H_{26}F_6N_6O_4$ $[M+H]^+$: 577.1998, found: 577.2023; Anal calcd. for $C_{24}H_{26}F_6N_6O_4$: C, 50.00; H, 4.55; N, 14.58, found: C, 49.82; H, 4.33; N, 14.36.

1, 2-Bis(4-(4-fluorophenyl)piperazin-1-yl)propane (18)

The title compound was synthesized from 1-(4-fluorophenyl)piperazine (**6**) and 1-bromo-3-chloropropane when pure compound was obtained as white solid (yield 55%); mp: 176-179 °C; IR (KBr) ν (cm^{-1}): 3019, 1654, 1627, 1509, 1404; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.97-6.93 (4H, m), 6.89-6.85 (4H, m), 3.13 (8H, t, *J* = 4.8 Hz), 2.61 (8H, t, *J* = 5.0 Hz), 2.45 (4H, t, *J* = 7.5 Hz), 1.80-1.75 (2H, m) ; ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.3, 155.9, 147.9, 147.9, 117.8, 117.7, 115.6, 115.3, 56.6, 53.2, 50.1, 24.3; HRMS (ESI positive) m/z calcd. for $C_{23}H_{30}F_2N_4$ $[M+H]^+$: 401.2517, found: 401.2512; Anal calcd. for $C_{23}H_{30}F_2N_4$: C, 68.97; H, 7.55; N, 13.99, found: C, 68.75; H, 7.36; N, 13.74.

1, 2-Bis(4-(pyridin-2-yl)piperazin-1-yl)propane (19)

The title compound was synthesized from 1-(pyridine-2-yl)piperazine (**9**) and 1-bromo-3-chloropropane as offwhite crystals (yield 66%); mp: 166-168 °C; IR (KBr) ν (cm^{-1}): 3019, 2823, 1636, 1595, 1481, 1437, 1402, 1385, 1313; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19-8.18 (2H, m), 7.49-7.45 (2H, m), 6.65-6.60 (4H, m), 3.55 (8H, t, *J* = 5.0 Hz), 2.57 (8H, t, *J* = 5.1 Hz), 2.44 (4H, t, *J* = 7.4 Hz), 1.82-1.75 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.5, 147.9, 137.4, 113.2, 107.0, 56.7, 53.0, 45.1, 24.2; HRMS (ESI positive) m/z calcd. for $C_{21}H_{30}N_6$ $[M+H]^+$: 367.2610, found, 367.2606; Anal calcd. for $C_{21}H_{30}N_6$: C, 68.82; H, 8.25; N, 22.93, found: C, 68.90; H, 8.14; N, 22.72.

1, 2-Bis(4-(4-nitrophenyl)piperazin-1-yl)propane (20)

The title compound was synthesized from 1-(4-nitrophenyl)piperazine (**5**) and 1-bromo-2-chloropropane as pure yellow colour crystals (yield 80%); mp: 144-149 °C; IR (KBr) ν (cm^{-1}): 3019, 1626, 1598, 1458, 1403, 1385, 1328; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (4H, d, *J* = 9.4 Hz), 6.75 (4H, d, *J* = 9.4 Hz), 3.36 (8H, t, *J* = 5.0 Hz), 2.53 (8H, t, *J* = 5.1 Hz), 2.39 (4H, t, *J* = 7.4 Hz), 1.71-1.63 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.8, 138.4, 125.9, 112.6, 56.4, 52.7, 47.0, 24.3; HRMS (ESI positive) m/z calcd. for $C_{23}H_{30}N_6O_4$ $[M+H]^+$: 455.2407, found, 455.2400; Anal calcd. for $C_{23}H_{30}N_6O_4$: C, 60.78; H, 6.65; N, 18.49, found: C, 60.61; H, 6.42; N, 18.25.

1, 2-Bis(4-(2-fluorophenyl)piperazin-1-yl)propane (21)

The title compound was synthesized from 1-(2-fluorophenyl)piperazine (**7**) and 1-bromo-2-chloropropane to give pure compound as white solid (yield 68%); mp: 136-138 °C; IR (KBr) ν (cm^{-1}): 3016, 2915, 1654, 1457, 1403, 1385; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.00-6.84 (8H, m), 3.05 (8H, t, *J* = 4.7 Hz), 2.58 (8H, t, *J* = 4.7 Hz), 2.40 (4H, t, *J* = 7.5 Hz), 1.74-1.69 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.9, 154.5, 140.1, 140.0, 124.4, 124.4, 122.4, 122.3, 118.9, 118.9, 116.1, 115.9, 56.6, 53.3, 50.5, 50.4, 24.2; HRMS (ESI positive) m/z calcd. for $C_{23}H_{30}F_2N_4$ $[M+H]^+$: 401.2517, found, 401.2511; Anal calcd. for $C_{23}H_{30}F_2N_4$: C, 68.97; H, 7.55; N, 13.99, found: C, 68.79; H, 7.34; N, 13.72.

1, 2-Bis(2-nitro-4-(trifluoromethyl)phenyl)piperazin-1-yl)propane (22)

The title compound was synthesized from 1-(2-nitro-4-(trifluoromethyl)phenyl)piperazine (**10**) and 1-bromo-2-chloropropane to give pure compound as yellow solid (yield 80%); mp: 131-135 °C; IR (KBr) ν (cm^{-1}): 3020, 1626, 1403, 1326; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (2H, d, *J* = 1.5 Hz), 7.66 (2H, dd, *J* = 2.0, 8.7 Hz), 7.17 (2H, d, *J* = 8.7 Hz), 3.18 (8H, t, *J* = 4.7 Hz), 2.61 (8H, t, *J* = 4.8 Hz), 2.47 (4H, t, *J* = 7.3 Hz), 1.79-1.70 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.9, 140.6, 130.1, 130.0, 124.1 124.1, 120.5, 56.3, 52.7, 50.8, 24.1; HRMS (ESI positive) m/z calcd. for $C_{25}H_{28}F_6N_6O_4$ $[M+H]^+$: 591.2154, found, 591.2151; Anal calcd. for $C_{25}H_{28}F_6N_6O_4$: C, 50.85; H, 4.78; N, 14.23, found: C, 50.62; H, 4.52; N, 14.02.

1, 2-Bis(4-nitro-2-(trifluoromethyl)phenyl)piperazin-1-yl)propane (23)

The title compound was synthesized from 1-(4-nitro-2-(trifluoromethyl)phenyl)piperazine (**8**) and 1-bromo-2-chloropropane as yellow solid (yield 63%); mp: 169-172 °C; IR (KBr) ν (cm^{-1}) 3429, 1626, 1459, 1402, 1385, 1333; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (2H, d, *J* = 2.5 Hz),

8.24 (2H, dd, *J* = 2.4, 8.9 Hz), 7.21-7.18 (2H, m), 3.11 (8H, bs), 2.63-2.57 (8H, m) 2.42 (4H, t, *J* = 7.4 Hz), 1.73-1.66 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ156.9, 142.0, 127.8, 124.6, 124.6, 122.2, 56.4, 53.0, 52.8, 52.7, 51.2, 24.1; ESI-MS *m/z* calcd for $C_{25}H_{28}F_6N_6O_4$ $[M+H]^+$: 591; Anal calcd. for $C_{25}H_{28}F_6N_6O_4$: C, 50.85; H, 4.78; N, 14.23; found: C, 50.71; H, 4.65; N, 14.12.

2-(4-(4-Nitrophenyl)piperazin-1-yl)ethanol (24)

A mixture of 1-(4-nitrophenyl)piperazine (**5**, 2.5 g, 12 mmol), 2-bromoethanol (1.28 mL, 18 mmol) and Et_3N (1.6 mL, 18 mmol) were heated at 50 °C in $CHCl_3$ (15 mL) for 30-35 h. Reaction mixture was then concentrated under reduced pressure and residue was dissolved in EtOAc (15 mL). The organic layer was washed with water (10 mL × 3). The combined organic layer was then dried (anhyd. Na_2SO_4) and was concentrated under reduced pressure. The solid separated out was washed with distilled hexane and again dried under high vacuum. The pure yellow crystals were recrystallized using EtOAc/Hexane in excellent yield (yield 97%); mp: 180-184 °C; IR (KBr) ν (cm^{-1}): 3019, 2399, 2344, 1597, 1535, 1507, 1476, 1350, 1330; ^{1}H NMR (400 MHz, $CDCl_3$): δ8.12-8.09 (2H, m), 6.83-6.81 (2H, m), 3.69 (2H, t, *J* = 5.2 Hz), 3.47-3.43 (4H, m), 2.67 (4H, t, *J* = 5.1 Hz), 2.62 (2H, t, *J* = 5.4 Hz), 2.33 (1H, bs); ^{13}C NMR (100 MHz, $CDCl_3$): δ154.7, 138.5, 125.9, 112.7, 59.3, 57.9, 52.4, 47.0; HRMS (ESI positive) m/z calcd. for $C_{12}H_{17}N_3O_3$ $[M+H]^+$: 252.1348, found, 252.1347; Anal calcd. for $C_{12}H_{17}N_3O_3$; C, 57.36; H, 6.82; N, 16.72, found: C, 57.13; H, 6.64; N, 16.60.

Similar procedure was used to synthesize compound **25.**

2-(4-(4-Fluorophenyl)piperazin-1-yl)ethanol (25)

The title compound was synthesized from 1-(4-fluorophenyl)piperazine (**6**) and 2-bromoethanol as offwhite crystals (yield 89%); mp: 136-139 °C; IR (KBr) ν (cm^{-1}) 3019, 2400, 1638, 1510, 1405; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.97-6.93 (2H, m), 6.88-6.85 (2H, m), 3.69 (2H, t, *J* = 5.2 Hz), 3.31 (1H, bs), 3.15 (4H, t, *J* = 4.8 Hz), 2.72 (4H, t, *J* = 5.0 Hz), 2.64 (2H, t, *J* = 5.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ158.4, 147.7, 118.0, 117.9, 115.6, 155.4, 59.4, 57.7, 52.9, 50.0; HRMS (ESI positive) m/z calcd. for $C_{12}H_{17}FN_2O$ $[M+H]^+$: 225.1403, found, 225.1400; Anal calcd. for $C_{12}H_{17}FN_2O$: C, 64.26; H, 7.64; N, 12.49, found: C, 64.01; H, 7.40; N, 12.23.

1-(2-Chloroethyl)-4-(4-nitrophenyl)piperazine (26)

The mixture of **24** (1 g, 0.39 mmol), Et_3N (1 mL, 0.78 mmol) in dry DMF (5mL) was stirred at room temperature for 15 min under dry condition and then thionyl chloride (0.28 mL, 0.39 mmol) in dry DMF (2 mL) was added dropwise at 0 to 5 °C within 1 h duration. Reaction

mixture was further stirred at room temperature for 3 h. The resultant mixture was concentrated under reduced pressure, dissolved in $CHCl_3$ (10mL) and washed with cold distilled water (10mL x 5). The combined organic layer was dried (anhyd. Na_2SO_4) and concentrated under reduced pressure and recrystallization was done using EtOAc/Hexane. The title compound was obtained as yellow solid (yield 62%); mp: 140-143 °C; IR (KBr) ν (cm^{-1}): 3019, 2963, 2400, 1637, 1599, 1509, 1475, 1419, 1331, 1261; ^{1}H NMR (400 MHz, $CDCl_3$): δ8.14-8.12 (2H, m), 6.84-6.82 (2H, m), 3.69-3.63 (2H, m), 3.49-3.45 (4H, m), 2.84-2.80 (2H, m), 2.71-2.68 (4H, m); ESI-MS *m/z* calcd. for $C_{12}H_{16}ClN_3O_2$ $[M+H]^+$: 270; Anal calcd. for $C_{12}H_{16}ClN_3O_2$; C, 53.43; H, 5.98; N, 15.58, found: C, 53.59; H, 6.12; N, 15.69.

Compound **27** was also synthesized using the procedure similar to compound **26**.

1-(2-Chloroethyl)-4-(4-fluorophenyl)piperazine (27)

The title compound was synthesized from **25** and thionyl chloride as pure white crystals (yield 60%); mp: 152-155 °C; IR (KBr) ν (cm^{-1}): 3019, 2400, 1638, 1510, 1403; ^{1}H NMR (400 MHz, $CDCl_3$): δ7.01-6.96 (2H, m), 6.92-6.89 (2H, m), 4.14-4.03 (4H, m), 3.55 (1H, bs), 3.51-3.46 (3H, m), 3.38-3.29 (4H, m); ESI-MS *m/z* calcd. for $C_{12}H_{16}ClFN_2$ $[M+H]^+$: 243; Anal calcd. for $C_{12}H_{16}ClFN_2$: C, 59.38; H, 6.64; N, 11.54, found: C, 59.52; H, 6.82; N, 11.73.

Further compounds **28-31** was synthesized by using the procedure similar to compound **11.**

1-(2-Methoxyphenyl)-4-(2-(4-(4-nitrophenyl)piperazin-1-yl)ethyl)piperazine (28)

The title compound was synthesized from **26** and 1-(2-methoxyphenyl)piperazine (**4**) to give pure yellow crystals (yield 72%); mp: 181-183 °C; IR (KBr) ν (cm^{-1}) 3019, 2400, 1637, 1599, 1419, 1330; ^{1}H NMR (400 MHz, $CDCl_3$): δ8.20-8.13 (2H, m), 7.04-6.83 (6H, m), 3.88 (2H, bs), 3.69 (3H, bs), 3.47-3.44 (2H, m), 3.16-3.13 (5H, m), 2.76-2.67 (4H, m), 2.35 (6H, bs), 2.03 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ154.8, 152.2, 141.1, 138.4, 125.9, 123.4, 123.0, 121.0, 118.4, 118.2, 112.3, 111.3, 55.4, 55.3, 53.8, 53.1, 50.4, 50.2, 46.9, 46.1, 45.0; HRMS (ESI positive) m/z calcd. for $C_{23}H_{31}N_5O_3$ $[M+H]^+$: 426.2505, found, 426.2505; Anal calcd. for $C_{23}H_{31}N_5O_3$: C, 64.92; H, 7.34; N, 16.46, found: C, 64.81; H, 7.20; N, 16.32.

1-(4-Fluorophenyl)-4-(2-(4-(4-nitrophenyl)piperazin-1-yl)ethyl)piperazine (29)

The title compound was synthesized from **26** and 1-(4-fluorophenyl)piperazine (**6**) as pure yellow crystals (yield 57%); mp: 142-144 °C; IR (KBr) ν (cm^{-1}): 3019, 2400, 2346, 1636, 1599, 1510, 1476, 1420, 1330; ^{1}H NMR (400 MHz, $CDCl_3$): δ8.15-8.12 (4H, m), 6.85-6.82 (4H, m), 3.46-3.39 (8H, m), 3.16-3.13 (1H, m), 3.05-3.03 (7H, m), 2.69-2.64 (4H, m); ^{13}C NMR (100

MHz, $CDCl_3$): δ 155.2, 154.8, 147.9, 138.3, 126.0, 125.9, 117.8, 117.7, 115.6, 115.4, 112.5, 112.2, 55.8, 53.6, 53.1, 50.1, 48.1, 47.0, 45.7; HRMS (ESI positive) m/z calcd. for $C_{22}H_{28}FN_5O_2$ $[M+H]^+$: 414.2305, found, 414.2301; Anal calcd. for $C_{22}H_{28}FN_5O_2$; C, 63.90; H, 6.83; N, 16.94, found: C, 63.75; H, 6.61; N, 16.73.

1-(4-Fluorophenyl)-4-(2-(4-(4-methoxyphenyl)piperazin-1-yl)ethyl)piperazine (30)

The title compound was synthesized from **27** and 1-(2-methoxyphenyl)piperazine (**4**) as pure yellow crystals (yield 82%); mp: 133-136 °C; IR (KBr) ν (cm^{-1}) 3019, 2399, 1638, 1533, 1500, 1419, 1350, 1301; ^1H NMR (400 MHz, $CDCl_3$): δ 6.93-6.76 (8H, m), 3.77 (4H, s), 3.05-2.95 (12H, m), 2.60-2.55 (4H, m), 2.39 (3H, bs); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.3, 155.9, 152.2, 147.9, 141.7, 122.9, 120.9, 118.2, 117.7, 115.6, 115.3, 111.2, 59.6, 57.9, 55.9, 55.3, 53.9, 53.6, 53.0, 51.9, 50.6, 50.1, 46.1; HRMS (ESI positive) m/z calcd. for $C_{23}H_{31}FN_4O$ $[M+H]^+$: 399.2560, found, 399.2550; Anal calcd. for $C_{23}H_{31}FN_4O$; C, 69.32; H, 7.84; N, 14.06, found: C, 69.49; H, 7.96; N, 14.35.

1-(2-Fluorophenyl)-4-(2-(4-(4-fluorophenyl)piperazin-1-yl)ethyl)piperazine (31)

The title compound was synthesized from **27** and 1-(2-fluorophenyl)piperazine (**7**) to obtain offwhite crystals (yield 71%); mp: 171-174 °C; IR (KBr) ν (cm^{-1}) 3019, 2952, 2829, 2400, 1613, 1535, 1509, 1453, 1406, 1385, 1331, 1302; ^1H NMR (400 MHz, $CDCl_3$): δ 7.00-6.78 (8H, m), 3.07-3.04 (4H, m), 2.99 (6H, s), 2.64-2.59 (5H, m), 2.56-2.52 (2H, m), 2.29 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.4, 157.0, 154.5, 147.9, 140.5, 124.4, 124.4, 122.4, 117.8, 117.8, 117.7, 115.6, 59.3, 57.8, 53.7, 53.6, 52.9, 51.6, 50.1, 46.0; HRMS (ESI positive) m/z calcd. for $C_{22}H_{28}F_2N_4$ $[M+H]^+$: 387.2360, found, 387.2357; Anal calcd. for $C_{22}H_{28}F_2N_4$; C, 68.37; H, 7.30; N, 14.50, found: C, 68.63; H, 7.50; N, 14.68.

Biology

Cell Cultures

The PC3 and DU145, monkey kidney Cos-7 and LNCaP prostate cancer cells were procured from National Centre for Cell sciences (NCCS, Pune). Human prostate cancer cells, PC3 and DU145, were maintained in DMEM/ HAM'S F-12 medium (Sigma–Aldrich, St. Louis, MO), and Cos-7 cells were grown in DMEM medium (Sigma–Aldrich, St. Louis, MO) supplemented with 10% FBS and 0.01% antibiotic/antimycotic solutions. These cells were grown in Roswell Park Memorial Institute (RPMI) medium supplemented with 12.5% fetal bovine serum (charcoal

stripped, Life Technologies Inc), 100 units/mL penicillin G sodium and 100 µg/mL streptomycin sulfate (Sigma-Aldrich, USA) in an atmosphere of 5% CO_2/95% air at 37 °C.

Ready-to-Assay α_{1a} Adrenergic Receptor GPCR frozen cells were used for rapid calcium assays and procured from Millipore (cat no. **HTS087RTA**).

Cell Proliferation Assay

Prostate cancer cells and normal cells were seeded in 96-well plates at a density of 2×10^4 cells/well and allowed 24 h for attachment. The treatments were proceed in triplicate with flutamide **(**Stock 10mM; Sigma-Aldrich, USA) and with compounds (Stock 10mM) at concentrations ranging from 2.5 µM to 80 µM in 2% charcoal stripped serum for 24 h at 37 °C in 5% CO_2 atmosphere. The stock solution of flutamide (10mM) was dissolved in molecular grade dimethyl sulfoxide (DMSO) and diluted with culture medium to different concentrations before adding to the cells. Final concentration of DMSO was not more than 0.05%. Controls were treated with DMSO (0.05% in culture medium). After 24 h of incubation in CO_2 incubator, 5 µl of 5mg/ml MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetra-zolium bromide)] was added to the cells. After a further incubation of 3 h, the formazan crystals formed in viable cells were dissolved in DMSO with gentle shaking on a plate shaker for 5 min. The absorbance was measured at 540 nm using a microplate reader (Microquant, Bio-Tek, USA). The percent viability was calculated according to formula given below:

% viability of cells = {(OD of treatment)/OD of Vehicle control} x 100

Reporter Gene Assay by Luciferase Expression

AR-negative Cos7 cells were seeded at a density of $5x10^4$ cells/well into 48-well plates on the day prior to transfection with 500ng of luciferase reporter gene construct pARE-eElB-LUC along with the expression vector for AR, pCR 3.1.AR, using DharmaFECT transfection reagent according to manufacturer's protocol. At the end of 8 h transfection period, cells were switched to complete medium and thereafter, treated with test compounds and Bicalutamide (AR-antagonist, 3µM) was used as positive control for 24 h. Luciferase activity was determined with luciferase assay systems (Promega, Madison, WI) following the manufacturer's protocol to detect the AR mediated transcriptional activity. Luciferase activity was normalized for transfection efficiency using pRL-SV40-luc as an internal control.

Calcium Mobilization Assay

All the analyses were performed with α_{1A}-adrenergic receptor GPCR cells (Millipore). The cells were cultured immediately by immersing in 37ºC water bath in a vial pre-sterilized with 70% ethanol. Pre-warmed media component (1 mL) was added to the vial of cells and the volume was raised up to 10 mL with media component. The cell suspension was centrifuged at 190 x g for 4 min and then the supernatant was removed. 5 mL of pre-warmed media was added to re-suspend the cell pellet. 4 x 10^4 cells per well were seeded overnight in 100 µL using 96-well plates and growth medium. After incubation overnight, the growth medium was removed from the cells and 100 µL/well fluoforte™ dye-loading solution (**Enzo life sciences FluoForte Calcium Assay Kit cat. No. ENZ-51017)** was added to each well. Thereafter, the culture plates were incubated for 45 min at 37°C and 15 min at room temperature. α_1-agonist (epinephrine, 20 µM), and antagonists (tamsulosin 1 µM and 100 nM) and the test compounds **16**, **19**, **20**, **21** and **22** (1 µM) were prepared and the calcium flux assay was run by monitoring the fluorescence at excitation wavelength at 490 nm and emission wavelength of 525 nm, using a fluorescence plate reader (Biotek Flx 800), for 180 sec with agonist addition at 10 sec. Calcium assay was optimized for agonist at one fifth of the final volume and relative fluorescence unit (RFU) values were determined for Ca^{2+} released in the cells.

***In vivo* Experiment**

Adult mature *Sprague Dawley* rats (weighing 250–280 g), for *in vivo* study, were procured from the Animal Division of the CSIR-Central Drug Research Institute, Lucknow, India. Prostatic hyperplasia was induced in rats by a known agent citral [44]. Animals were randomly divided into eight groups, viz. Group I (vehicle control), Group II (Citral 100 mg/kg); Group III (Citral 100 mg/kg + Flutamide 10 mg/kg), Group IV (Citral 100 mg/kg + **16**), Group V (Citral 100 mg/kg + **19**), Group VI (Citral 100 mg/kg + **20**), Group VII (Citral 100 mg/kg + **21**), Group VIII (Citral 100 mg/kg + **22**). Groups II to VIII were administered with citral at a dose of 100 mg/kg once daily for 28 days and further animals in Group III to VIII were coadministered with Flutamide, **16**, **19**, **20**, **21** and **22** respectively at 10 mg/kg, treatment beginning from the 8th day of citral treatment and continuing once daily for remaining 21 days of treatment. All treatments were oral. At the end of experiment, animals were sacrificed according to the guidelines of Institutional Animal Ethics Committee, and the weights of testis, epididymis, seminal vesicles and prostate were recorded. Prostatic tissues were fixed in 10% formalin solution for histology.

Histology

Prostate tissues set overnight in 10% formalin solution and then were processed for histology. The standard protocol was followed and 5.0 µ sections were stained with hematoxyline & eosin and then examined under a light microscope (Eclipse 80i, Nikon Corporation, Japan).

Animals for antidepressant activities

In this study, adult male Swiss albino mice (20–25 g) were used to screen antidepressant-like activities. The mice were housed in polycarbonate cages six in each groups with free access to food and water and maintained under the controlled temperature (22±2 °C) and 12 h light/dark cycle (8:00 AM to 8:00 PM). Experimental protocol used in the study was approved by Institutional Animal Ethical Committee (IAEC) following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) India.

Drugs and treatment schedule:

Flouxetine (FXT) and imipramine (IMI), were purchased from Sigma Aldrich, UK. Compounds were prepared in 1% gum acacia at a dose of 20 mg/kg and administered to the mice orally at rate of 10 mL/kg of body weight. IMI (60 mg/kg) and FXT (20 mg/kg) were also given to the mice in the same fashion.

Behavior Studies

Forced Swimming Test

Mice were administered orally either with vehicle (equal volume of 1% gum-acacia; control group), test compounds (20 mg/kg) or FXT (20 mg/kg) and IMI (60 mg/kg) as standard drugs. After 60 min of drug administration, mice were subjected to forced swimming test (FST), a behavior despair test to model depression [45]. Briefly, mice were placed individually in plexi-glass cylindrical vessel (20 cm height, 10 cm diameter) filled with water at depth of 10 cm at 25 ±1 ºC. Mice were placed in water and removed after 15 min, dried, and returned back to their home cages. Mice were again placed in the cylinder after 24 h and Immobility was recorded for 5 min after the initial 1 min acclimatization period with the camera equipped with computer and analyzed by software ANY MAZE. Mice were considered to be ‘‘immobile’’ when they were floating motionless.

Tail Suspension Test

The tail suspension test (TST) was performed as described by Steruet *et. al*. [46]. In brief, the mice were individually suspended 60 cm above the surface of table by tail to the horizontal bar using adhesive tape affixed about 2 cm from tip of tail. The immobility time was recorded for 5 minutes after acclimatization of 1 minute. Immobility is defined as the absence of any limb or body movements, except those caused by respiration. TST was performed for compounds found active in forced swim test at the same dose. IMI was used as a standard drug.

Open-field Test

Animals were assessed for locomotor activity using actometers, OptoVarimex activity monitors [47] (Columbus Inst., USA). Each cage (43*44*25 cm) perimeter was lined with an array of 15*15 photocell beams located 3 cm from the floor surface. Interruptions of the photocell beams were counted as a measure of locomotor activity and defined as the distance traveled (in cm). Animals were placed in activity chamber and locomotor activity was recorded using Multidevice software (Columbus Instruments Device Interface Version 1.4). The test was carried out for 24 intervals (120 minutes) each of 5 minute duration. Activity of animal is observed when it interrupts the infrared beam. The drug was administered orally after 6^{th} interval when activity was recorded. Each group comprised of six animals.

Rotarod Test

Rotarod test is commonly used for evaluation of neuromuscular coordination in rodents [48]. The test was performed using Rotamex 4/8 apparatus (M/s Columbus Instruments, USA). Rotarod consists of a rod coated with polypropylene foam which provides friction to the animals. The rod is driven by a motor which can regulate rotational speed. The test was started at speed 5 rpm and end at 10 rpm in our study. Animals were trained on the rotarod for duration of 2 min per trial, with 3 trials per day for two days. On third day the trained animals were tested before the drug administration (0 min) then drug was given orally, further tested at 1 hr, 2hr, 3hr and 4 hr, time interval and latency to fall was recorded.

Statistical Analysis

The comparisons among different groups were made using unpaired t-test. The values are represented as mean ± SEM. A $p<0.05$ was considered as statistically significant.

Pharmacokinetic Studies

The pharmacokinetic studies of **19** were carried out in young and healthy male *Sprague Dawley* rats weighing 250 ± 25 g obtained from Laboratory Animal Division, CDRI, Lucknow. The

animals were housed in plastic cages in standard laboratory conditions with a regular 12 h day-night cycle. Standard pelleted laboratory chow (Goldmohar Laboratory Animal Feed, Lipton India Ltd, Chandigarh, India) and water were allowed *ad libitum.* The rats were acclimatized to this environment for at least five days before conducting the experiment. The study was conducted in overnight fasted (12-16 h) rats (n = 3 per time point). All experiments, euthanasia and disposal of carcasses were carried out as per the guidelines of Local Ethics Committee for animal experimentation.

Suspension formulation containing 2.5 mg/mL of **19** was prepared by triturating **19**, gum acacia (1% w/v) and water (drop wise addition) in a mortar and pestle. A single 10 mg/kg oral dose was given to conscious rats using rat feeding needle. For intravenous administration, the drug solution was prepared by dissolving 15 mg of compound in 150 µL dimethyl sulfoxide and a single 10 mg/kg dose was administered and blood samples were withdrawn at various predefined times. The animals were sacrificed at various predefined times up to 48 h post oral dose and blood, prostate and hypothalamus were collected. Serum samples were harvested. The hypothalamus of three rats were pooled and homogenized in order to ensure a measurable quantity. All samples were stored at -80 °C until analysis.

A Shimadzu UFLC pump (LC-20AD) with online degasser (DGU-20A3), an auto-sampler (SIL-HTc) with a temperature-controlled peltier-tray and a triple quadrupole API 4000 mass spectrometer (Applied Biosystems, Toronto, Canada) was used for analysis. Chromatographic separation was made on a Discovery HS C-18 column (5 µm, 100 x 4.6 mm id) preceded with a guard column (5 µm, 50 x 4.0 mm, id) packed with the same material with mobile phase [acetonitrile: methanol: aqueous ammonium acetate buffer (0.01M) (50:40:10, % v/v)] pumped at a flow rate of 0.6 mL/min under isocratic condition. The mobile phase was degassed by ultrasonication for 15 min before use. LC-MS/MS system was equilibrated for approximately 20 min before commencement of analysis. The column oven temperature was 40 °C. Total analysis time was 5 min per sample. The mass spectral analysis was performed in positive ionization mode at 5500 V using multiple reaction monitoring technique to monitor the transitions *m/z* 367.1 → *m/z* 204.0 for **19** and *m/z* 180.1 → *m/z* 138.1 for phenacetin (internal standard). Data acquisition and quantitation were performed using analyst software (version 1.4.2; AB Sciex, Toronto, Canada). The method utilizes 50 µL of serum or tissue homogenate. Liquid-liquid

extraction was used for sample clean up. The method showed linearity over the range of 5 – 400 ng/mL with recovery of >75% and acceptable accuracy and precision [49].

Docking Studies

In order to probe the binding mode of compounds **16**, **19**, **20**, **21** and **22** in the binding site of androgen receptor docking studies was done. The sketch module of the molecular modeling suite Sybyl 2.1 was used to draw and optimize the geometry of compounds [50]. Tripos force field and Gasteiger−Huckel charges was applied, followed by energy minimization using the Powell method with an energy convergence gradient of 0.001 kcalmol^{-1}. Surflex-Dock program was used for molecular docking studies [51]. It uses Hammerhead scoring function based search engine to dock the ligands. The consensus score is result of linear combination of non-linear functions of protein-ligand atomic surface distances including steric, polar, entropic, and solvation terms. The co-crystallised structure of wild type Androgen receptor with bound ligand designated as S-1 was retrieved from the protein databank (PDB accession code: 2AXA) and used as receptor. For receptor preparation, ligand was extracted and substructures were removed. The hydrogen atoms were added, bumps were relaxed, unknown atom types were assigned and water molecules were removed. The receptor was assigned Kollman-all atom charges. Ligand based protomol was generated and the residues Leu 704, Asn 705 and Arg 752 were considered as flexible. The Geom mode of Surflex-Dock program was selected for generating the docking poses. *In silico* ADMET prediction were done using BIOVIA Discovery Studio 4.1. (Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 4.1, San Diego: Dassault Systèmes, 2015).

SUPPORTING INFORMATION AVAILABLE

^{1}H NMR, ^{13}C NMR and HRMS of compounds **11-31** and rotarod activity of active compounds **19** and **20**. Representative chromatogram of compound **19**.

CONFLICT OF INTEREST

Authors confirm that this article has no conflict of interest

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REFERENCES

1. E. L. Piatnitski Chekler, R. Unwalla, T. A. Khan, R. S. Tangirala, M. Johnson, M. St Andre, J. T. Anderson, T. Kenney, S. Chiparri, C. McNally, E. Kilbourne, C. Thompson, S. Nagpal, G. Weber, S. Schelling, J. Owens, C. A. Morris, D. Powell, P. R. Verhoest, A. M. Gilbert, *J. Med. Chem*., 2014, **57**, 2462-71.
2. R. P. Trump, J. B. Blanc, E. L. Stewart, P. J. Brown, M. Caivano, D. W. Gray, W. J. Hoekstra, T. M. Willson, B. Han, P. Turnbull, *J. Comb. Chem.*, 2007, **9**, 107-14.
3. N. Schlienger, B. W. Lund, J. Pawlas, F. Badalassi, F. Bertozzi, R. Lewinsky, A. Fejzic, M. B. Thygesen, A. Tabatabaei, S. R. Bradley, L. R. Gardell, F. Piu, R. Olsson, *J. Med. Chem.*, 2009, **52**, 7186-91.
4. B. J. Furr, *Eur. Urol.*, 1996, **29**, 83-95.
5. D. K. Wysowski, . P. Freiman, J. B. JTourtelot, M. L. Horton, *Ann. Intern. Med.*, 1993, **118**, 860-4.
6. J. M. Kuhn, T. Billebaud, H. Navratil, A. Moulonguet, J. Fiet, P. Grise, J. F. Louis, P. Costa, J. M. Husson, R. Dahan, et al., *N. Engl. J. Med.*, 1989, **321**, 413-8.
7. E. E. Parent, C. S. Dence, C. Jenks, T. L. Sharp, M. J. Welch, J. A. Katzenellenbogen, *J. Med. Chem*,. 2007, **50**, 1028-40.
8. E. G. Vajda, F. J. Lopez, P. Rix, R. Hill, Y. Chen, K. J. Lee, Z. O'Brien, W. Y. Chang, M. D. Meglasson, Y. H. Lee, *J. Pharmacol. Exp. Ther.*, 2009, **328**, 663-70.
9. http://www.cdc.gov/cancer/prostate/statistics/age.htm (accessed on 17.02.2016).
10. B. Pietrzyk, M. Olszanecka-Glinianowicz, A. Owczarek, T. Gabryelewicz, A. Almgren-Rachtan, A. Prajsner, J. Chudek, *Int. Urol. Nephrol.*, 2015, ***47***, 431-40.
11. http://www.iarc.fr/en/media-centre/iarcnews/2010/globocan2008.php (accessed on 17.02.2016).

12. http://healthcare.globaldata.com/media-center/press-releases/pharmaceuticals/benign-prostatic-hyperplasia-treatment-market-value-to-reach-nearly-5-billion-by-2024-says-globaldata (accessed on 13.10.2016).
13. http://www.cancer.gov/news-events/press-releases/2011/CostCancer2020 (accessed on 17.02.2016).
14. T. M. Nicholson, W. A. Ricke, *Differentiation*, 2011, **82**, 184-99.
15. http://www.cancer.gov/types/prostate/prostate-hormone-therapy-fact-sheet (accessed on 18.02.2015).
16. S. Thebault, M. Roudbaraki, V. Sydorenko, Y. Shuba, L. Lemonnier, C. Slomianny, E. Dewailly, J. L. Bonnal, B. Mauroy, R. Skryma, N. Prevarskaya, *J. Clin. Invest.*, 2003, **111**, 1691-701.
17. F. C. Lowe, *Clin. Ther.*, 2004, **26**, 1701-13.
18. H. Nejishima, N. Yamamoto, M. Suzuki, K. Furuya, N. Nagata, S. Yamada, *Prostate*, 2012, **72**, 1580-7.
19. A. Sarswat, R. Kumar, L. Kumar, N. Lal, Sharma S., Y. S. Prabhakar, S. K. Pandey, J. Lal, V. Verma, A. Jain, J. P. Maikhuri, D. Dalela, Kirti; Gupta G., V. L. Sharma, *J. Med. Chem.*, 2011, **54**, 302-11.
20. I. J. Korfage, M. L. Essink-Bot, A. C. Janssens, F. H. Schroder, H. J. de Koning, *Br. J. Cancer*, 2006, **94**, 1093-8.
21. W. F. Pirl, G. I. Siegel, Goode M. J., M. R. Smith, *Psychooncology*, 2002, **11**, 518-23.
22. M. Rom, G. Schatzl, N. Swietek, E. Rucklinger, C. Kratzik, *BJU international*, 2012, **110**, E918-21.
23. L. Ma, X. Zhao, H. Liu, H. Zhu, Yang W., Y. Qian, J. Wang, M. Feng, Y. Li, *Int. J. Clin. Exp. Med.*, 2015, **8**, 4031-7.
24. R. Y. Chung, J. C. Leung, D. C. Chan, Woo J., C. K. Wong, S. Y. Wong, *PloS One*, 2013, **8**, e76017.
25. S. Y. Wong, A. Hong, J. Leung, T. Kwok, P. C. Leung, J. Woo, *J. Affect. Disord.*, 2006, **96**, 83-8.
26. http://www.testsymptomsathome.com/sym_male_hormones.asp (accessed on 07.01.2016)
27. http://www.livescience.com/51453-low-testosterone-men-depression-risk.html (accessed on 07.01.2016)

28. https://www.recoveryranch.com/articles/mental-health-articles/testosterone-therapy-shown-to-ease-depression-in-men/ (accessed on 07.01.2016)
29. H. S. Roth, R. C. Botham, S. C. Schmid, Fan T. M., L. Dirikolu, P. J. Hergenrother, *J. Med. Chem.*, 2015, **58**, 4046-65.
30. M. D. Aytemir, E. Septioglu, U. Calis, *Arzneimittelforschung*, 2010, **60**, 22-9.
31. A. Mendoza, S. Perez-Silanes, M. Quiliano, A. Pabon, S. Galiano, G. Gonzalez, G. Garavito, M. Zimic, A. Vaisberg, I. Aldana, A. Monge, E. Deharo, *Exp. Parasitol.*, 2011, **128**, 97-103.
32. A. M. Waszkielewicz, K. Pytka, A. Rapacz, E. Welna, M. Jarzyna, G. Satala, A. Bojarski, J. Sapa, P. Zmudzki, B. Filipek, H. Marona, *Chem. Boil. Drug. Des.*, 2015, **85**, 326-35.
33. L. A. Romeiro, M. da Silva Ferreira, L. L. da Silva, H. C. Castro, A. L. Miranda, C. L. Silva, F. Noel, J. B. Nascimento, C. V. Araujo, E. Tibirica, E. J. Barreiro, C. A. Fraga, *Eur. J. Med. Chem.*, 2011, **46**, 3000-12.
34. S. Franchini, U. M. Battisti, A. Baraldi, A. Prandi, P. Fossa, E. Cichero, A. Tait, C. Sorbi, G. Marucci, A. Cilia, L. Pirona, L. Brasili, *Eur. J. Med. Chem.*, 2014, **87**, 248-66.
35. A. Prandi, S. Franchini, L. I. Manasieva, P. Fossa, E. Cichero, G. Marucci, M. Buccioni, A. Cilia, L. Pirona, L. Brasili, *J. Med. Chem*., 2012, **55**, 23-36.
36. S. Franchini, A. Prandi, A. Baraldi, C. Sorbi, A. Tait, M. Buccioni, G. Marucci, A. Cilia, L. Pirona, P. Fossa, E. Cichero, L. Brasili, *Eur. J. Med. Chem*., 2010, **45**, 3740-51.
37. C. H. Song, S. H. Yang, E. Park, S. H. Cho, E. Y. Gong, D. B. Khadka, W. J. Cho, K. Lee, *J. Biol. Chem.*, 2012, **287**, 30769-80.
38. J. X. Qui, Z. W. Zhou, Z. X. He, X. Zhang, S. F. Zhou, S. Zhu, *Drug Des Devel Ther.*, 2015, **9**, 841-66.
39. K. Yamaoka, T. Nakagawa, T. Uno, *J. Pharmacokinet. Biopharm.*, 1978, **6**, 165-75.
40. G. Schwarz, *Ann Statist*, 1978, **6**, 461-468.
41. B. Davies, T. Morris, *Pharm. Res.*, 1993, **10**, 1093-5.
42. N. Poujol, J. M. Wurtz, B. Tahiri, S. Lumbroso, J. C. Nicolas, D. Moras, C. Sultan, *J Biol Chem*., 2000, **275**, 24022-31.
43. J. Zhou, B. Liu, G Geng., Wu J. H., *Proteins*, 2010, **78**, 623-37.

44. D. Engelstein, J. Shmueli, S. Bruhis, C. Servadio, A. Abramovici, *Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol.*, 1996, **115**, 169-77.
45. R. D. Porsolt, M. Le Pichon, M. Jalfre, *Nature*, 1977, **266**, 730-2.
46. L. Steru, R. Chermat, B. Thierry, P. Simon, *Psychopharmacology*, 1985, **85**, 367-70.
47. M. Filip, L. Antkiewicz-Michaluk, M. Zaniewska, M. Frankowska, Golda A., J. Vetulani, E. Przegalinski, *J. Physiol. Pharmacol.*, 2007, **58**, 625-39.
48. N. W. Dunham, T. S. Miya, *J. Am. Pharm. Assoc. Am. Pharm. Assoc.*, 1957, **46**, 208-9.
49. http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf, FDA, Guidance for Industry: Bioanalytical Method Validation.
50. SYBYL-X, version 2.1, Tripos, **1991-2012**, Certara.
51. A. N. Jain, *J. Med. Chem.*, 2003, **46**, 499-511.

Highlights

- Seventeen aryl/heteroaryl piperazines designed to manage prostate and depression.
- MTT assay on LNCaP cells and Ca^{++} mobilization in α_{1A} expressing cells done.
- Luciferase reporter gene assay and cell viability were also carried out.
- *In vivo*, PK and docking studies of the most promising compounds accomplished.
- Compound **19** may be a lead candidate for andropause associated disorders in males.