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Arylpiperazines for Management of Benign Prostatic Hyperplasia: Design, Synthesis, Quantitative Structure−Activity Relationships, and Pharmacokinetic Studies†

Amit Sarswat,‡ Rajeev Kumar,§ Lalit Kumar,‡ Nand Lal,‡ Smriti Sharma,‡ Yenamandra S. Prabhakar,‡ Shailendra K. Pandey,∥ Jawahar Lal,∥ Vikas Verma,§ Ashish Jain,§ Jagdamba P. Maikhuri,§ Diwakar Dalela,⊥ Kirti,⊥ Gopal Gupta,§ and Vishnu L. Sharma*,‡

‡*Medicinal & Process Chemistry Division,* §*Endocrinology Division, and* ∥ *Pharmacokinetics & Metabolism Division, Central Drug Research Institute (CSIR), Lucknow-226001, India, and* ⊥*Department of Urology, CSM Medical University, Lucknow-226001, India*

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A series of 27 aryl/heteroaryl/aralkyl/aroyl piperazines were synthesized, and most of these compounds reduced prostate weight of mature rats by 15−47%. Three compounds, **10**, **12**, and **18**, had better activity profile (reduced prostate weight by 47%, 43%, and 39%, respectively) than the standard drug flutamide (24% reduction). QSAR suggested structures with more cyclic and branched moieties, increased topological separation of O and N therein, and reduced solvation connectivity index for better activity. Pharmacokinetic study with compound **10** at an oral dose of 10.0 mg/kg indicated good absorption, negligible extrahepatic elimination, and rapid distribution to the target organ (prostate) but restricted entry through the blood−brain barrier. A 10-fold decrease in PSA and 15-fold increase in ER-β gene expressions of human prostate cancer cells (LNCaP) by compound **10** *in vitro* indicated AR and ER-β mediated actions. The findings may stimulate further explorations of identified lead for the management of benign prostatic hyperplasia.

Introduction

Benign prostatic hyperplasia (BPH)[a] is an ubiquitous[1] condition in aging males, such that the incidence of BPH detected at autopsy increases from approximately 30% at age 50 to >80% at age 80. Unfortunately, the urinary symptoms attributed to BPH lead to significant erosion in the quality of life for affected men.[2] The available treatments for BPH are either highly invasive (surgical) or partially effective with unwanted side effects and put a significant burden on employees and their employers through direct medical costs and lost work time. Direct and indirect private sector costs related to BPH treatment are an estimated $3.9 billion.[3] It is well established that androgens, such as testosterone and 5α-dihydrotestosterone (DHT), play an essential role in stimulating hyperplasia and carcinoma of hormone-sensitive tissue such as that of the prostate.[4] The two medical treatments currently used for BPH are α-adrenergic blockers (e.g., terazosin, tamsulosin) and 5α-reductase inhibitors (e.g., finasteride). While the former are more effective in relieving BPH symptoms,[5] only the latter reduce prostate volume and hence the risk of acute urinary retention.[6] Potential cardiovascular consequences and sexual dysfunction are the major issues associated with therapies involving α-adrenergic blockers and 5α-reductase inhibitors, respectively. Tamsulosin has fewer cardiovascular side effects due to its selectivity for α_{1a}-adrenergic receptors which are concentrated mainly in the prostate but may cause floppy iris syndrome and sulfa drug-type adverse effects.[7] Steroidal AR antagonists, such as cyproterone acetate[8] (Figure 1), block androgen action and also have progestational and glucocorticoid activities. Their overlapping effects with other hormonal systems cause a range of unpleasant side effects including thrombosis, fluid retention, and loss of libido, which hinder their use as agents for management of BPH.[9] A number of nonsteroidal AR antagonists, including flutamide[10−12] (Figure 1), nilutamide[13] (Figure 1), and bicalutamide[14−18] (Figure 1), have been reported in the literature.[19−29]

Nonsteroidal AR antagonists selectively block androgen action without affecting other hormonal systems, and side effects such as loss of libido and impotence are therefore less profound. However, they do inhibit the binding of testosterone to ARs in the central nervous system (CNS) which, in turn, interrupts the negative feedback of testosterone on gonadotropin secretion, causing an increased serum testosterone level.[30] This increase in serum testosterone impairs the antiandrogenic activity of AR antagonists and also causes side effects including gynecomastia and breast tenderness. Therefore, potent AR antagonists with fewer adverse effects are highly desirable.

Recently, arylpiperazine derivatives have been reported as potent nonsteroidal AR antagonists,[31,32] and some of them

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*To whom correspondence should be addressed. Phone: 91-522-2612411 ext 4320. Fax: 91-522-2623405. E-mail: vlscdri1@rediffmail.com; vlscdri@gmail.com.

[a] Abbreviations: BPH, benign prostatic hyperplasia; QSAR, quantitative structure−activity relationship; PSA, prostate-specific antigen; ER, estrogen receptor; AR, androgen receptor; DHT, 5α-dihydrotestosterone; SARMs, selective androgen receptor modulators; PCR, polymerase chain reaction; MRT, mean residence time; BR, biological response; CP-MLR, combinatorial protocol in multiple linear regression; LOO, leave-one-out; SPI, superpendentic index; TEA, triethylamine; DCM, dichloromethane; TURP, trans-urethral resection of prostate; FCS, fetal calf serum; dNTP, deoxyribonucleoside triphosphate; HPLC, high-performance liquid chromatography.

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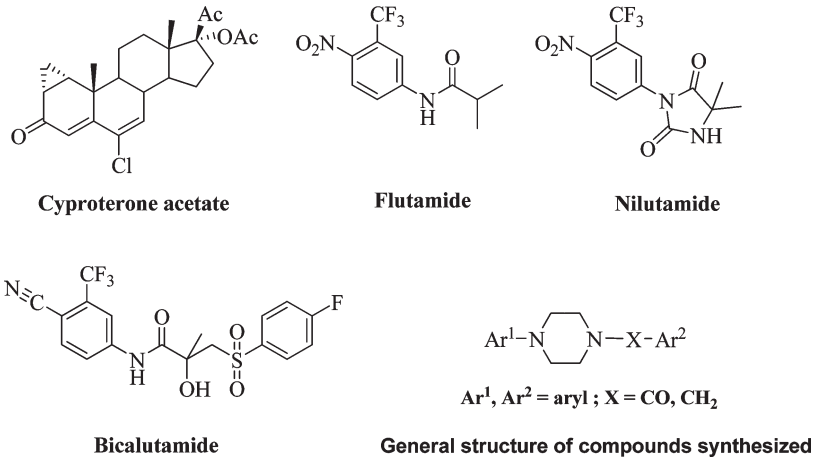


Figure 1. Structure of SARMs and general structure of compounds synthesized.

have exhibited better activity profile than bicalutamide (Figure 1) both *in vitro* and in the testosterone propionate treated castrated rat model.[33] However, they exhibited lower activity in the mature intact rat model which is a more appropriate model for BPH. Thus, it was thought worthwhile to synthesize aryl/heteroaryl/aralkyl/aroyl piperazine derivatives (Figure 1) as selective androgen receptor modulators (SARMs) that can achieve AR blockade without causing increased testosterone levels and to evaluate their *in vivo* effect on the prostate of mature intact rat model that simulates the clinical situation more appropriately.

The synthesis, quantitative structure−activity relationship (QSAR), and *in vivo* activity in mature rats of these piperazine derivatives (Figure 1) have been described in this report. The pharmacokinetics in rats and *in vitro* effect on prostate-specific antigen (PSA) and steroid hormone receptor expressions in human prostate cancer cells of the most promising structure are also presented.

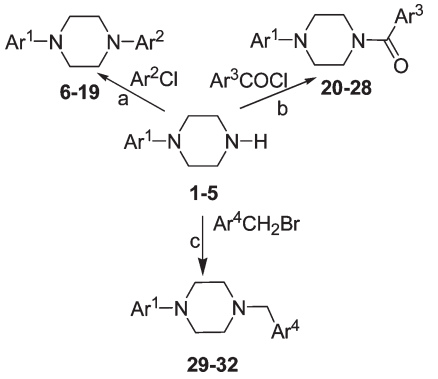
Chemistry

The aryl/heteroaryl/aralkyl/aroyl piperazines (**6**−**32**) were synthesized according to Scheme 1. Aryl/heteroaryl piperazines (**1**−**5**) were treated with appropriate aryl halide to provide diaryl piperazines (**6**−**19**) whereas the reaction of **1**−**5** with suitable aroyl chloride furnished 1-aryl-4-aroylpiperazines (**20**−**28**). Similarly, appropriate benzyl chlorides gave 1-aryl-4-arylmethylpiperazines (**29**−**32**). The attempts have been made to synthesize piperazines with trifluoromethyl (CF_3) and nitro (NO_2) group in aryl ring keeping an analogy with the structures of flutamide (Figure 1), nilutamide (Figure 1), and bicalutamide (Figure 1).

Result and Discussion

***In Vivo* Effect on Prostate Weight.** Adult mature rats (Sprague-Dawley strain, $n = 5$) were given test compounds **6**−**32** as water/gum acacia suspension at an oral dose of 10.0 mg/kg, daily for 21 days. Parallel control animals received vehicle while positive control animals received flutamide (10.0 mg/kg) for similar duration. At autopsy (24 h after the last treatment), the prostates were dissected out and weighed (Table 1). Twenty-one of these piperazine derivatives (**8**−**19**, **21**, **24**−**27**, **29**−**32**) decreased the size of prostate appreciably by 15−47% whereas four compounds (**20**, **22**, **23**, **28**) decreased the prostatic size moderately by up to 10%

Scheme 1[a]

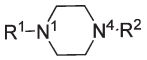


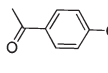
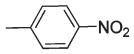
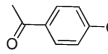
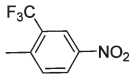
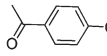
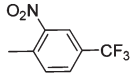
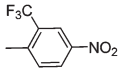
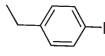
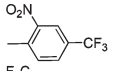
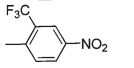
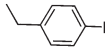
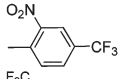
[a] Reagents and conditions: (a) $(Et)_3N$, neat, 80−100 °C, 5−10 h; (b) $(Et)_3N$, dry DCM, −5 to 0 °C, 0.5−2 h; (c) $(Et)_3N$, neat, 80−100 °C, 2−4 h.

and two compounds (**6** and **7**) had no effect at all. Nine compounds (**9**−**12**, **14**−**16**, **18**, **32**) were more active than flutamide (nonsteroidal SARM, 24% reduction at 10.0 mg/kg) while five compounds (**17**, **25**, **29**−**31**) were comparable to flutamide in activity. Three compounds (**10**, **12**, **18**) were almost twice as active as flutamide. The data have been presented in Table 1.

This study included two types of aryl piperazines, compounds having an aryl group directly attached to N^4-position (**6**−**19**) and compounds in which the N^4-aryl substituent was separated by a one-carbon linker (a methylene or a carbonyl group, **20**−**32**). The aryl substituents at N^1-position were 3-chlorophenyl, 3-(trifluoromethyl)phenyl, 4-fluorophenyl, 2-pyridyl, and 2-pyrimidyl, while the aryl group at N^4-position was either nitro substituted or nitro and trifluoromethyl disubstituted. A broad analysis of the structure−activity relationship indicated that a 4-nitrophenyl (**9**, **10**, **12**, **14**, **16**, **18**) or a 4-nitrobenzyl (**29**−**32**) group at N^4-position of piperazines generally resulted in potent activity. Addition of a carbonyl linker (**20**−**28**) imparted better activity, and addition of a methylene linker (**29**−**32**) reduced activity. For example, introducing the carbonyl linker in compounds **7** and **8** augmented the activity (**24**, **25**), while placing the methylene linker in compound **9** decreased the activity (**31**). Nevertheless, the active compound (**32**) with linker had a 4-nitrophenylmethyl group at N^4-position. On the other hand, structure−activity comparisons of compounds without

Table 1. Effect of Compounds **6**−**32** on Prostate of Rat[a]



Compd	R¹	R²	Prostate Weight*	LogBR obs	LogBR pred	Compd	R¹	R²	Prostate Weight*	LogBR obs	LogBR pred
6			99.36	1.99	1.97	**24**			86.60	1.94	1.93
7			100	2.00	1.95	**25**[a]			75.60	1.88	1.88
8			84.80	1.93	1.91	**26**			82.40	1.92	1.94
9[a]			65.15	1.81	1.89	**27**			84.10	1.92	1.91
10			52.30	1.72	1.78	**28**			92.30	1.96	1.97
11			68.08	1.83	1.75	**29**			77.60	1.89	1.82
12			56.60	1.75	1.82	**30**			77.80	1.89	1.95
13			81.40	1.91	1.85	**31**[a]			76.70	1.88	1.89
14			67.70	1.83	1.75	**32**			66.20	1.82	1.89
15[a]			68.28	1.83	1.83						
16			64.39	1.81	1.87	**Control**			100		
17			72.70	1.86	1.92	**Flutamide**			75.80		
18			60.50	1.78	1.84						
19			84.10	1.92	1.86						
20			91.20	1.96	1.95						
21			82.40	1.92	1.95						
22[a]			92.20	1.96	1.98						
23			91.40	1.96	1.94						

[a] * = prostate weight (per 100 mg of control); a = test set compounds.

linker (**10**−**19**) indicated that molecules having nitro group at position 4 and trifloromethyl group at position 2 (**10**, **12**, **14**, **16**, and **18**) in the aryl ring at N^4-position of piperazine were more potent than those having nitro group at position 2 and trifloromethyl group at position 4 (**11**, **13**, **15**, **17**, and **19**). Among the former (**10**, **12**, **14**, **16**, and **18**), the activity profile with respect to the substituent at N^1-position (R^1) of piperazine was of the following order: 3-(trifluoromethyl)phenyl (**10**, 47.70%) > pyridyl (**12**, 43.40%) > 4-fluorophenyl (**18**, 39.50%) > 3-chlorophenyl (**16**, 35.61%) > 2-pyrimidyl (**14**, 32.30%). However, in the entire study, the most active compound (**10**) had a 3-(trifluoromethyl)phenyl group at N^1-position and a (4-nitro-2-trifloromethyl)phenyl group directly attached at N^4-position of piperazine.

With a view to assess the potential of the most active compound **10** as a candidate for BPH management, it was evaluated for the expression of prostate-specific antigen (PSA) gene expression (Table 2) and estrogen receptors (Figure 2) in human BPH-derived prostatic stromal cells and prostate cancer derived epithelial cell

line, as well as the pharmacokinetic parameters (Table 3, Figure 3).

Expression of Prostate-Specific Antigen (PSA) in Human Prostate Cancer Epithelial Cells (LNCaP). Prostatic cells secrete a serine protease called PSA which is used as an early marker for prostatic hyperplasia, especially cancer. The regulation of PSA gene is strictly androgen dependent and regulated by AR at transcriptional level.[34] Consequently, PSA mRNA expression has been used for antiandrogenic assays.[35] We established the SARM nature of new compounds by establishing the effect of the most promising compound **10** on expression of PSA gene in androgen-dependent human prostate cancer epithelial cells (LNCaP) by real time PCR. Flutamide was used as positive control. Compound **10** (10.0 μmol) reduced PSA secretion of LNCaP cells by ~90%, which established its antagonistic activity against AR. Flutamide (10.0 μmol) reduced PSA secretion of LNCaP cells by 99% (Table 2).

Compound **10** stimulated the expressions of ER-α and ER-β by approximately 3- and 6-fold in human BPH stromal cells and 11- and 16-fold in LNCaP cells, respectively. Flutamide stimulated the expressions of ER-α and ER-β by approximately 11- and 14-fold in LNCaP cells. Both flutamide and compound **10** shifted ER-β/ER-α ratio in favor of ER-β, though compound **10** was more effective (1.37) than flutamide (1.32). Hence, compound **10** is likely to promote prostatic regression through elevated ER-β mediated signaling in stromal and epithelial cells. Human prostatic stromal cells are highly responsive to androgens due to the high expression of AR and the highly favorable AR:ER-β ratio, which further increases 3-fold in BPH stromal cells.[36] Hence, upregulation of ER-β expression is highly desirable to control aberrant growth.

Table 2. Fold Change in PSA Expression of LNCaP (Androgen-Dependent Human Prostate Cancer) Cells after 48 h of Treatment with Compound **10** and Flutamide

treatment	fold change
control	1
flutamide (10 μmol)	0.01 ± 0.23
compound **10** (10 μmol)	0.106 ± 0.16

Pharmacokinetic Studies. The pharmacokinetic studies revealed that the animals tolerated the treatment as no peculiarities in the animals' behavior were observed. In a comparative study of tissue distribution and pharmacokinetics, the absorption of the compound was rapid with a peak concentration ($C_{max\,1}$) at 0.5 h and could be monitored up to 24 h (Figure 3, Table 3). The compound exhibited a double-peak phenomenon, and the $C_{max\,2}$ (139.31 ± 56.64 ng/mL) occurred after 3.0 h postdose. Because of the occurrence of a double-peak

Table 3. Pharmacokinetic Parameters of Compound **10** after Single Administration at 10 mg/kg (per Oral) in Male Sprague-Dawley Rats [a]

parameter		serum	prostate	hypothalamus
C_{max} (ng/mL or μg/g)	1	106.01 ± 2.47	442.19 ± 18.82	99.47
	2	139.31 ± 2.47	364.73 ± 39.94	123.82
t_{max} (h)	1	0.5	1.5	1.0
	2	2.0	6.0	2.0
AUC_{0-t} ((ng·h)/mL or (μg·h)/g)		712	5653	1999
ratio to prostate		0.13	1.00	0.35
MRT (h)		6.97	10.98	11.57
CL_{oral} (L h^{-1} kg^{-1})		0.010	0.001	0.001

[a] Each value represents the average of three rats dosed at 10 mg/kg po. Serum and prostate concentrations are mean ± SEM from three rats. Hypothalamus concentrations were the mean of replicates from the pooled tissue of three rats.

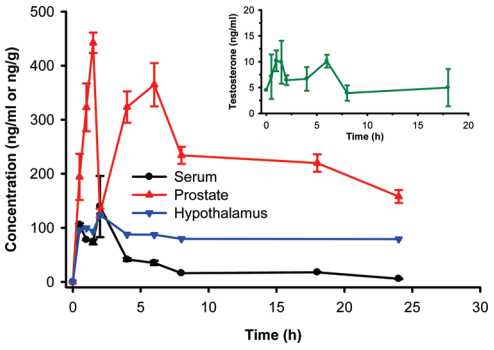


Figure 3. Concentration−time profile of compound **10** and testosterone after a single 10 mg/kg oral dose in male Sprague-Dawley rats. Bar represents SEM.

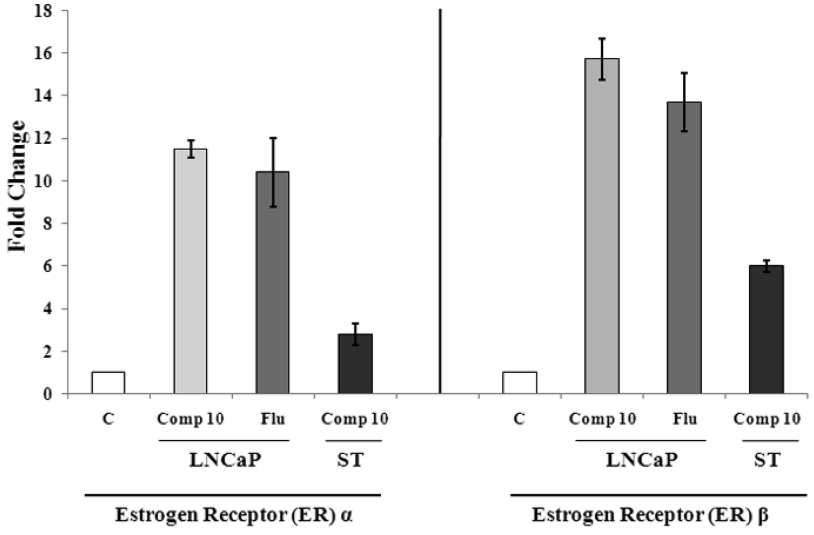


Figure 2. Changes in ER-α and ER-β gene expressions by compound **10** (10.0 μmol) and flutamide (10.0 μmol) in human prostate cancer epithelial cells (LNCaP) and BPH-derived stromal cells (ST) *in vitro* (mean ± SE of three values).

phenomenon, the data sets could not be fitted to an appropriate compartmental pharmacokinetic model. Hence, the pharmacokinetic parameters were determined by a noncompartmental analysis. The mean residence time (MRT) and AUC_{0-t} were 6.97 h and 712 (ng·h)/mL, respectively. The clearance (0.01 L h^{-1} kg^{-1}) was smaller than the hepatic blood flow (2.9 L h^{-1} kg^{-1})[37] of the rat, suggesting an insignificant amount of extrahepatic elimination of this compound. However, the prostate levels achieved the C_{max} ($C_{max\,1}$, 442.19 ± 18.82 ng/g, and $C_{max\,2}$, 364.73 ± 39.94 ng/g) occurred after 1.5 and 6.0 h postdose. Whereas the hypothalamus showed the C_{max} ($C_{max\,1}$, 99.47 ng/g, and $C_{max\,2}$, 123.82 ng/g) which occurred after 1.0 and 2.0 h post dose. The compound levels were higher in prostate (target tissues for main effects) than in hypothalamus (target tissues for side effects such as increased serum testosterone concentrations), probably because of poor penetration across the blood−brain barrier (Figure 3, Table 3). However, since some amount of compound crosses the blood−brain barrier, its interference with the negative feedback mechanism resulted in increased serum testosterone levels during 0.5−6.0 h postdose. Similar rise in serum testosterone levels has been reported for bicalutamide.[33] Moreover, the flip-flop serum testosterone levels correspond to the levels of compound **10** in serum, prostate, and hypothalamus (Figure 3). Nevertheless, significantly higher levels of compound **10** in prostate than in brain (hypothalamus) kept the prostate weight suppressed.

QSAR Study. A QSAR study has been carried out on the 27 piperazine derivatives shown in the Table 1 to offer rationale for the activity. Adopting the standard procedure, the structures were generated in the ChemDraw.[38] These structures were used in Dragon software to compute the topological features of the compounds.[39] This has resulted in 222 descriptors as explanatory variables of the molecular structures (Table 1). The biological response (BR) of these compounds (effect on prostate) was considered as dependent variable after logarithmic transformation and expressed as log BR. For the purpose of QSAR model validation, these 27 compounds are randomly divided into training (22 compounds) and test sets (5 compounds). Only the training set compounds are used for the model development, and test set compounds are used for the external validation of the developed model. The QSAR analysis has been carried out using combinatorial protocol in multiple linear regression (CP-MLR) procedure.[40] Among several models the following equation has optimally explained the activity:

$$\begin{aligned}\log BR &= 1.918 - 0.000016(0.0000043)SPI \\ &+ 0.045(0.029)X5sol - 0.025(0.008)VRA2 \\ &- 0.001(0.0005)T(N\cdots O)\end{aligned}$$

$$\begin{aligned} &n = 22 && r = 0.861 \\ &r^2_t = 0.544 && Q^2 = 0.516 \\ &Q^2_{G3} = 0.498 && s = 0.044 \\ &F = 12.242 \\ &r^2_{Yrand}(max) = 0.290(0.376)\end{aligned} \quad (1)$$

$$\begin{aligned}\log BR &= 1.969 - 0.139SPI_S + 0.046X5sol_S \\ &- 0.092VRA2_S - 0.127T(N\cdots O)_S\end{aligned} \quad (2)$$

In this regression equation, n is the number of compounds, r is the multiple correlation coefficient of regression, r^2_t is the test set r^2 value, Q^2 and Q^2_{G3} are cross-validated r^2 from leave-one-out (LOO) and leave group of three out, respectively, s is the standard error of the estimate, F is the F-ratio between the variances of calculated and observed activities, and r^2_{Yrand} is the average squared correlation coefficient from randomization study involving 100 simulations with its maximum (max). In the equation, the values given in the parentheses are the standard errors of the regression coefficients. The model is validated with an external test set of five compounds. The predictions of the test set compounds are found to be satisfactory as reflected in the test set r^2. Equation 2 is a derivative of eq 1, derived by using the scaled X (X_S) in place of X as shown.

$$X_S = \frac{X - X_{min}}{X_{max} - X_{min}} \quad (3)$$

where X_{min} and X_{max} are the training set feature X's minimum and maximum values. This transforms the descriptor values between "0" and "1" and provides an opportunity for direct comparison of the regression coefficients within the equation. The scaled descriptors are identified with subscript "S" suffixed to the abbreviated names.

In the equation the coefficient of solvation connectivity index of order 5 (X5sol) suggested in favor of smaller connectivity values for better activity. Its regression coefficient is marginally less significant when compared to coefficients of other descriptors. However, this and the other three descriptors are statistically significant in the regression equation of full data set. The regression coefficients of SPI (superpendentic index), VAR2 (average Randic-type eigenvector-based index from adjacency matrix), and T(N···O) (sum of topological distances between nitrogen and oxygen) suggested that a larger value of these features is favorable for the activity. The index SPI accounts for the branches and/or loops present in the molecules. The equation suggests in favor of increased cyclic and branched analogues for the activity. Also, this regression equation suggests that an increased separation of O and N is favorable for the activity. This in turn suggests in favor of increased and/or distantly placed amino, nitro, and hydroxyl groups on the template molecule for better activity.

Conclusion

The new series of designed SARMs consisting of aryl/heteroaryl/aralkyl/aroyl piperazines have yielded some very interesting structures that reduced prostatic weight in mature rats to the extent of 47%, which was twice more than that reduced by flutamide (24%). The QSAR study revealed that cyclic and branched moieties with topological separation of O and N and reduced solvation connectivity index showed better activity profile. The most promising compound **10** was well absorbed and rapidly distributed to the target organ (prostate) but restricted by blood−brain barrier from reaching hypothalamus (the target organ for major side effect). Compound **10** inhibited PSA expression in prostatic cells by 90%, which is a marker for both prostatic hyperplasia and AR activation. On the other hand, upregulation of ER-β in prostatic (especially BPH) cells indicated an ER-β mediated mechanism of action. Some of these structures are worth further optimization for activity against prostatic hyperplasia.

Experimental Section

Chemistry. In general, all reagents and solvents were commercial quality and were used without further purification. Melting points

were determined in open capillary tubes on an electrically heated block and are uncorrected. IR spectra (ν_{max} in cm^{-1}) of the compounds were recorded on Perkin-Elmer's FT-IR RX1 PC spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Supercon Magnet Avance DPX-200/DRX-300 spectrometers (operating at 200 and 300 MHz, respectively, for ^1H and 50 and 75 MHz, respectively, for ^{13}C) in deuterated solvents with TMS as internal reference (chemical shifts δ in ppm, J in Hz). Electrospray ionization mass spectra (ESI-MS) were recorded on Thermo Lcq Advantage Max-IT, and HR-DART MS were recorded on JEOL, JMS T100LC Accu TOF. Elemental analyses were performed on Carlo Erba EA-1108 micro analyzer/Vario EL-III C H N S analyzer. All compounds were analyzed for C, H, and N, and the results obtained were within ±0.4% of calculated values. The reaction progress was routinely monitored by thin-layer chromatography (TLC) on precoated alumina/silica gel plates (Aldrich). Column chromatography was performed over Merck silica gel (60−120 mesh). All compounds were characterized by TLC, ^1H and ^{13}C NMR, MS, and HRMS. Elemental analyses data meet the criteria of ≥95% purity. All chemicals and solvents were procured from Sigma-Aldrich/Merck India Ltd.

1-(3-Chlorophenyl)-4-(2-nitrophenyl)piperazine (6). To the mixture of 1-(3-chlorophenyl)piperazine (**1**, 1.7 g, 9.0 mmol) and triethylamine (TEA, 2.5 mL, 18.1 mmol) was added 1-chloro-2-nitrobenzene (2.1 g, 13.5 mmol) and stirred at 100 °C for 10 h. EtOAc (15.0 mL) was added in reaction mixture, and solid triethylamine hydrochloride salt was filtered off. Filtrate was washed with water (2 × 5.0 mL), and organic layer was separated. Combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. Solid product was washed with hexane (10.0 mL) and recrystallized from EtOAc/hexane to give the title compound (2.4 g, 85%) as yellow solid: mp 91−93 °C; IR (KBr) ν (cm^{-1}) 2941, 2928, 1596, 1483, 1231; ^1H NMR (200 MHz, $CDCl_3 + CCl_4$) δ 7.76 (1H, d, J = 7.5 Hz), 7.45−7.53 (1H, m), 7.04 −7.19 (3H, m), 6.75 −6.87 (3H, m), 3.29 −3.31 (4H, m), 3.20 (4H, br s); ^{13}C NMR (75 MHz, $CDCl_3 + CCl_4$) δ 152.3 (s), 145.9 (s), 144.1 (s), 135.2 (s), 133.5 (d), 130.3 (d), 126.0 (d), 122.5 (d), 121.3 (d), 119.8 (d), 116.2 (d), 114.2 (d), 51.8 (t), 49.0 (t); ESI-MS m/z 318 (MH^+ with ^{35}Cl), 320 (MH^+ with ^{37}Cl). Anal. Calcd for $C_{16}H_{16}ClN_3O_2$: C, 60.47; H, 5.08; N, 13.22. Found: C, 60.51; H, 5.25; N, 13.29.

The following compounds **7**−**19** were prepared using a procedure similar to that described for compound **6** from the corresponding substituted piperazines and aryl halides.

1-(2-Nitrophenyl)-4-(3-(trifluoromethyl)phenyl)piperazine (7). The title compound was synthesized from 1-(3-(trifluoromethyl)-phenyl)piperazine (**2**) and 1-chloro-2-nitrobenzene in 88% yield as yellow solid: mp 66−68 °C; IR (KBr) ν (cm^{-1}) 2982, 2857, 1597, 1540, 1280; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 7.77 (1H, dd, J = 8.1, 1.4 Hz), 7.47−7.52 (1H, m), 7.32−7.37 (1H, m), 7.19 (1H, d, J = 8.2 Hz), 7.08−7.13 (4H, m), 3.38−3.41 (4H, m), 3.21−3.25 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.4 (s), 145.9 (s), 143.8 (s), 133.8 (d), 130.9−132.1 (CF_3), 129.8 (d), 126.0 (d), 122.7 (s), 122.6 (d), 121.3 (d), 119.12−119.13 (d), 116.2−116.4 (d), 112.4−112.6 (d), 51.8 (t), 48.9 (t); HRMS m/z calcd for $C_{17}H_{17}F_3N_3O_2$ (MH^+) 352.1273, found 352.1258. Anal. Calcd for $C_{17}H_{16}F_3N_3O_2$: C, 58.12; H, 4.59; N, 11.96. Found: C, 57.91; H, 4.58; N, 11.79.

1-(2-Nitrophenyl)-4-(pyridin-2-yl)piperazine (8). The title compound was synthesized from 1-(pyridin-2-yl)piperazine (**3**) and 1-chloro-2-nitrobenzene in 80% yield as yellow solid: mp 118−120 °C; IR (KBr) ν (cm^{-1}) 2888, 2831, 1598, 1498, 1229; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.17 (1H, d, J = 3.9 Hz), 7.77 (1H, dd, J = 8.1, 1.1 Hz), 7.44−7.50 (2H, m), 7.17 (1H, d, J = 8.20 Hz), 7.04−7.09 (1H, m), 6.60−6.65 (2H, m), 3.69−3.72 (4H, m), 3.17−3.20 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3 + CCl_4$) δ 159.5 (s), 148.3 (d), 146.2 (s), 144.1 (s), 137.6 (d), 133.5 (d), 126.1 (d), 122.3 (d), 121.3 (d), 113.7 (d), 107.2 (d), 51.8 (t), 45.5 (t); ESI-MS m/z 285 (MH^+). Anal. Calcd for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.51; H, 5.73; N, 19.53.

1-(4-Nitrophenyl)-4-(pyridin-2-yl)piperazine (9). The title compound was synthesized from **3** and 1-chloro-4-nitrobenzene in 82% yield as yellow solid: mp 165−167 °C; IR (KBr) ν (cm^{-1}) 2926, 2848, 1594, 1535, 1280; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.20−8.23 (1H, m), 8.14 (2H, d, J = 9.4 Hz), 7.50−7.56 (1H, m), 6.84 (2H, d, J = 9.4 Hz), 6.66−6.70 (2H, m), 3.73−3.77 (4H, m), 3.57−3.60 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3 + CCl_4$) δ 159.0 (s), 154.8 (s), 148.2 (d), 138.7 (s), 137.9 (d), 126.2 (d), 114.1 (d), 112.6 (d), 107.2 (d), 46.8 (t), 44.7 (t); ESI-MS m/z 285 (MH^+). Anal. Calcd for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.52; H, 5.71; N, 19.97.

1-(4-Nitro-2-(trifluoromethyl)phenyl)-4-(3-(trifluoromethyl)-phenyl)piperazine (10). The title compound was prepared from **2** and 1-chloro-4-nitro-2-(trifluoromethyl)benzene in 95% yield as dark brown solid: 110−112 °C; IR (KBr) ν (cm^{-1}) 2841, 1612, 1591, 1216; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.53 (1H, d, J = 2.4 Hz), 8.35 (1H, dd, J = 8.9, 2.4 Hz), 7.34−7.40 (2H, m), 7.10−7.16 (3H, m), 3.40−3.42 (4H, m), 3.29−3.32 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.1 (s), 151.4 (s), 142.9 (s), 131.1−132.4 (CF_3), 129.9 (d), 128.2 (d), 125.1 (s), 124.7−124.8 (CF_3, s), 122.9 (d), 119.3 (d), 116.6−116.7 (d), 112.6−112.7 (d), 52.9 (t), 49.1 (t); HRMS m/z calcd for $C_{18}H_{16}F_6N_3O_2$ (MH^+) 420.1147, found 420.1130. Anal. Calcd for $C_{18}H_{15}F_6N_3O_2$: C, 51.56; H, 3.55; N, 10.02. Found: C, 51.32; H, 3.66; N, 10.11.

1-(2-Nitro-4-(trifluoromethyl)phenyl)-4-(3-(trifluoromethyl)-phenyl)piperazine (11). The title compound was prepared from **2** and 1-chloro-2-nitro-4-(trifluoromethyl)benzene in 95% yield as dark yellow solid: mp 76−78 °C; IR (KBr) ν (cm^{-1}) 2926, 2848, 1622, 1526, 1275; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.06 (1H, d, J = 1.4 Hz), 7.70 (1H, dd, J = 8.7, 1.7 Hz), 7.33−7.39 (1H, m), 7.20 (1H, d, J = 8.7 Hz), 7.12−7.13 (2H, m), 7.06 (1H, d, J = 8.3 Hz), 3.39−3.43 (4H, m), 3.31−3.34 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.1 (s), 148.0 (s), 140.9 (s), 131.8 (d), 131.4 (d), 130.4−130.5 (CF_3), 129.9 (d), 126.3 (s), 124.3−124.4 (CF_3), 123.1 (s), 120.8 (d), 119.1 (d), 116.5−116.6 (d), 112.4−112.5 (d), 50.6 (t), 48.6 (t); ESI-MS m/z 420 (MH^+). Anal. Calcd for $C_{18}H_{15}F_6N_3O_2$: C, 51.56; H, 3.61; N, 10.02. Found: C, 51.70; H, 3.67; N, 10.10.

1-(4-Nitro-2-(trifluoromethyl)phenyl)-4-(pyridin-2-yl)piperazine (12). The title compound was prepared from **3** and 1-chloro-4-nitro-2-(trifluoromethyl)benzene in 82% yield as dark brown solid: mp 62−64 °C; IR (KBr) ν (cm^{-1}) 2964, 2847, 1593, 1570, 1283; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.52 (1H, d, J = 2.6 Hz), 8.33 (1H, dd, J = 8.9, 2.6 Hz), 8.17−8.20 (1H, m), 7.46−7.52 (1H, m), 7.32 (1H, d, J = 8.9 Hz), 6.63−6.67 (2H, m), 3.70−3.74 (4H, m), 3.22−3.25 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.2 (s), 148.2 (d), 148.2 (s), 140.6 (s), 137.9 (d), 130.3−130.5 (d), 124.5−124.6 (CF_3), 120.5 (d), 114.1 (d), 107.3 (d), 50.7 (t), 45.0 (t); ESI-MS m/z 353 (MH^+). Anal. Calcd for $C_{16}H_{15}F_3N_4O_2$: C, 54.55; H, 4.29; N, 15.90. Found: C, 54.63; H, 4.45; N, 15.87.

1-(2-Nitro-4-(trifluoromethyl)phenyl)-4-(pyridin-2-yl)piperazine (13). The title compound was prepared from **3** and 1-chloro-2-nitro-4-(trifluoromethyl)benzene in 87% yield as oil: IR (neat) ν (cm^{-1}) 2968, 2850, 1598, 1570, 1290; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.16−8.18 (1H, m), 8.05 (1H, d, J = 1.4 Hz), 7.66 (1H, dd, J = 8.7, 1.8 Hz), 7.45−7.51 (1H, m), 7.19 (1H, d, J = 8.7 Hz), 6.61−6.66 (2H, m), 3.71−3.74 (4H, m), 3.27−3.30 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5 (s), 157.2 (s), 148.2 (d), 142.6 (s), 137.9 (d), 128.1 (d), 125.2 (s), 124.3−124.9 (d, CF_3), 122.7 (d), 114.1 (d), 107.5 (d), 52.8 (t), 45.6 (t); HRMS m/z calcd for $C_{16}H_{16}F_3N_4O_2$ (MH^+) 353.1225, found 353.1216. Anal. Calcd for $C_{16}H_{15}F_3N_4O_2$: C, 54.55; H, 4.29; N, 15.90. Found: C, 54.78; H, 4.37; N, 15.87.

2-(4-(4-Nitro-2-(trifluoromethyl)phenyl)piperazin-1-yl)pyrimidine (14). The title compound was prepared from 2-(piperazin-1-yl)-pyrimidine (**4**) and 1-chloro-4-nitro-2-(trifluoromethyl)benzene in 75% yield as brown solid: mp 95−97 °C; IR (KBr) ν (cm^{-1}) 2922, 2854, 1587, 1496, 1226; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.53 (1H, d, J = 2.6 Hz), 8.30−8.36 (3H, m), 7.31 (1H, d, J = 8.9 Hz), 6.53 (1H, t, J = 4.7 Hz), 3.99−4.02 (4H, m), 3.16−3.19

(4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.8 (s), 157.9 (d), 157.3 (s), 142.7 (s), 128.1 (d), 125.3 (s), 124.6−124.9 (d, CF_3), 122.8 (d), 110.6 (d), 52.9 (t), 43.9 (t); HRMS m/z calcd for $C_{15}H_{15}F_3N_5O_2$ (MH^+) 354.1178, found 354.1172. Anal. Calcd for $C_{15}H_{14}F_3N_5O_2$: C, 50.99; H, 3.99; N, 19.82. Found: C, 51.02; H, 3.45; N, 19.88.

2-(4-(2-Nitro-4-(trifluoromethyl)phenyl)piperazin-1-yl)pyrimidine (15). The title compound was prepared from **4** and 1-chloro-2-nitro-4-(trifluoromethyl)benzene in 78% yield as yellow solid: mp 66−68 °C. IR (KBr) ν (cm^{-1}) 3020, 2856, 1624, 1586, 1501, 1218; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (2H, d, J = 4.8 Hz), 8.10 (1H, d, J = 1.4 Hz), 7.69 (1H, dd, J = 8.8, 1.8 Hz), 7.20 (1H, d, J = 8.7 Hz), 6.55 (1H, t, J = 4.7 Hz), 3.99−4.03 (4H, m), 3.24−3.28 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.7 (s), 157.9 (d), 148.3 (s), 141.0 (s), 130.1−130.2 (d), 124.3−124.5 (CF_3), 122.9 (s), 120.7 (d), 110.6 (d), 50.9 (t), 43.5 (t); HRMS m/z calcd for $C_{15}H_{15}F_3N_5O_2$ (MH^+) 354.1178, found 354.1158. Anal. Calcd for $C_{15}H_{14}F_3N_5O_2$: C, 50.99; H, 3.99; N, 19.82. Found: C, 50.86; H, 4.09; N, 19.98.

1-(3-Chlorophenyl)-4-(4-nitro-2-(trifluoromethyl)phenyl)piperazine (16). The title compound was prepared from **1** and 1-chloro-4-nitro-2-(trifluoromethyl)benzene in 93% yield as dark brown solid: mp 55−57 °C; IR (KBr) ν (cm^{-1}) 2857, 1625, 1586, 1270; ^{1}H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.52 (1H, d, J = 2.3 Hz), 8.34 (1H, dd, J = 8.9, 2.3 Hz), 7.34 (1H, d, J = 8.9 Hz), 7.16−7.22 (1H, m), 6.92 (1H, s), 6.82−6.85 (2H, m), 3.34−3.36 (4H, m), 3.27−3.29 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.0 (s), 152.2 (s), 142.8 (s), 135.2 (s), 130.3 (d) 128.1 (d), 125.4 (s), 124.7−125.1 (CF_3), 122.8 (d), 120.1 (d), 116.4 (d), 114.4 (d), 52.9 (t), 49.1 (t); HRMS m/z calcd for $C_{17}H_{16}ClF_3N_3O_2$ (MH^+) 386.0883, found 386.0910. Anal. Calcd for $C_{17}H_{15}ClF_3N_3O_2$: C, 52.93; H, 3.92; N, 10.89. Found: C, 52.79; H, 3.73; N, 10.97.

1-(3-Chlorophenyl)-4-(2-nitro-4-(trifluoromethyl)phenyl)piperazine (17). The title compound was prepared from **1** and 1-chloro-2-nitro-4-(trifluoromethyl)benzene in 90% yield as light brown solid: mp 126−128 °C; IR (KBr) ν (cm^{-1}) 2965, 2841, 1625, 1593, 1282; ^{1}H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.06 (1H, br s), 7.69 (1H, dd, J = 8.7, 1.8 Hz), 7.15−7.21 (2H, m), 6.83−6.88 (2H, m), 6.78 (1H, dd, J = 8.3, 2.1 Hz), 3.35−3.37 (4H, m), 3.30−3.32 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.1 (s), 148.1 (s), 141.1 (s), 135.3 (s), 130.4 (d), 125.3 (d) 124.4−124.5 (CF_3), 122.8 (d), 121.7 (d), 120.8 (d), 120.2 (d), 116.3 (d), 114.3 (d), 51.0 (t), 48.8 (t); ESI-MS m/z 386 (MH^+). Anal. Calcd for $C_{17}H_{15}ClF_3N_3O_2$: C, 52.93; H, 3.92; N, 10.89. Found: C, 52.72; H, 3.60; N, 10.67.

1-(4-Fluorophenyl)-4-(4-nitro-2-(trifluoromethyl)phenyl)piperazine (18). The title compound was prepared from 1-(4-fluorophenyl)piperazine (**5**) and 1-chloro-4-nitro-2-(trifluoromethyl)benzene in 90% yield as brown solid: mp 80−82 °C; IR (KBr) ν (cm^{-1}) 2932, 2858, 1587, 1498, 1230; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (1H, d, J = 2.4 Hz), 8.34 (1H, dd, J = 8.9, 2.4 Hz), 7.35 (1H, d, J = 9.0 Hz), 6.87−6.99 (4H, m), 3.27 (8H, s); ^{13}C NMR (50 MHz, $CDCl_3 + CCl_4$) δ 162.20 (s), 157.1 (s), 155.4 (s), 147.8 (d), 147.8 (d), 143.0 (s), 128.1 (d), 124.6−124.9 (CF_3), 122.7 (d), 118.7 (d), 118.5 (d), 116.2 (d), 115.7 (d), 53.2 (t), 50.7 (t); ESI-MS m/z 369 (MH^+). Anal. Calcd for $C_{17}H_{15}F_4N_3O_2$: C, 55.29; H, 4.09; N, 11.38. Found: C, 55.42; H, 4.2; N, 11.58.

1-(4-Fluorophenyl)-4-(2-nitro-4-(trifluoromethyl)phenyl)piperazine (19). The title compound was prepared from **5** and 1-chloro-2-nitro-4-(trifluoromethyl)benzene in 90% yield as brown solid: mp 70−72 °C; IR (KBr) ν (cm^{-1}) 2935, 2858, 1580, 1496, 1237; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (1H, s), 7.69 (1H, dd, J = 8.6, 1.4 Hz), 7.20 (1H, d, J = 8.7 Hz), 6.86−6.99 (4H, m), 3.25−3.31 (8H, m); ^{13}C NMR δ (75 MHz, $CDCl_3$) δ 159.1 (s), 155.9 (s), 147.9 (s), 147.5 (d), 147.5 (d), 130.2−130.3 (s), 124.1−124.3 (CF_3), 120.7 (d), 118.3 (d), 118.2 (d), 115.8 (d), 115.5 (d), 51.0 (t), 50.0 (t); ESI-MS m/z 369 (MH^+). Anal. Calcd for $C_{17}H_{15}F_4N_3O_2$: C, 55.29; H, 4.09; N, 11.38. Found: C, 55.46; H, 4.22; N, 11.48.

Phenyl(4-(pyridin-2-yl)piperazin-1-yl)methanone (20). To a solution of **3** (0.6 mL, 3.9 mmol) and TEA (1.0 mL, 7.8 mmol) in dichloromethane (DCM, 15.0 mL) was added benzoyl chloride (0.5 mL, 4.7 mmol) and stirred at −5 to 0 °C for 2 h. Reaction mixture was treated with ice (2.0 g), and organic layer was separated, followed by water washing (2 × 5.0 mL). Combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. Solid product was recrystallized from EtOAc/hexane to give the title compound (0.97 g, 97%) as white solid: mp 68−70 °C; IR (KBr) ν (cm^{-1}) 2910, 2857, 1629, 1595; ^{1}H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.20 (1H, d, J = 3.6 Hz), 7.43−7.53 (6H, m), 6.65−6.68 (2H, m), 3.89 (2H, br s), 3.57 (6H, br s); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.7 (s), 159.3 (s), 148.2 (d), 137.9 (d), 135.8 (s), 130.1 (d), 128.7 (d), 127.3 (d), 114.2 (d), 107.5 (d), 45.6 (t); ESI-MS m/z 268 (MH^+). Anal. Calcd for $C_{16}H_{17}N_3O$: C, 71.89; H, 6.41; N, 15.72. Found: C, 71.79; H, 6.59; N, 15.56.

The following compounds **21**−**28** were prepared using a procedure similar to that described for compound **20** from the corresponding substituted piperazines and benzoyl chloride.

Phenyl(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)methanone (21). The title compound was prepared from **2** and benzoyl chloride in 95% yield as white solid: mp 75−77 °C; IR (KBr) ν (cm^{-1}) 2907, 2839, 1715, 1636, 1598; ^{1}H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 7.42 (5H, br s), 7.33−7.38 (1H, m), 7.05−7.14 (3H, m), 3.77 (4H, br s), 3.25 (4H, br s); ^{13}C NMR (75 MHz, $CDCl_3 + CCl_4$) δ 170.3 (s), 151.3 (s), 135.6 (s), 131.2−132.5 (CF_3), 130.1 (d), 129.8 (d), 128.7 (d), 127.4 (d), 122.5 (s), 119.6 (d), 116.9−117.0 (d), 112.9−113.1 (d), 49.4 (t); ESI-MS m/z 335 (MH^+). Anal. Calcd for $C_{18}H_{17}F_3N_2O$: C, 64.66; H, 5.13; N, 8.38. Found: C, 64.86; H, 5.25; N, 8.67.

(4-(3-Chlorophenyl)piperazin-1-yl)(phenyl)methanone (22). The title compound was prepared from **1** and benzoyl chloride in 91% yield as oil: IR (neat) ν (cm^{-1}) 2910, 2857, 1639, 1595, 1570; ^{1}H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 7.30 (5H, br s), 7.02−7.07 (1H, m), 6.71−6.75 (2H, m), 6.66 (1H, d, J = 8.3 Hz), 3.45−3.76 (4H, br m), 3.03 (4H, br s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3 (s), 152.0 (s), 135.5 (s), 135.0 (s), 130.2 (d), 129.9 (d), 128.6 (d), 127.1 (d), 120.1 (d), 116.4 (d), 114.5 (d), 49.1 (t); ESI-MS m/z 301 (MH^+). Anal. Calcd for $C_{17}H_{17}ClN_2O$: C, 67.88; H, 5.70; N, 9.31. Found: C, 67.94; H, 5.94; N, 9.21.

Phenyl(4-(pyrimidin-2-yl)piperazin-1-yl)methanone (23). The title compound was prepared from **4** and benzoyl chloride in 88% yield as white solid: mp 113−115 °C; IR (KBr) ν (cm^{-1}) 2924, 2855, 1628, 1585, 1550; ^{1}H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.29 (2H, d, J = 4.7 Hz), 7.42 (5H, s), 6.51 (1H, t, J = 4.7 Hz), 3.70−3.86 (8H, m); ^{13}C NMR (50 MHz, $CDCl_3 + CCl_4$) δ 170.5 (s), 161.7 (s), 157.9 (d), 135.9 (s), 130.0 (d), 128.7 (d), 127.4 (d), 110.6 (d), 44.1 (t); ESI-MS m/z 269 (MH^+). Anal. Calcd for $C_{15}H_{16}N_4O$: C, 67.15; H, 6.01; N, 20.88. Found: C, 67.29; H, 6.27; N, 20.98.

(2-Nitrophenyl)(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)-methanone (24). The title compound was prepared from **2** and 2-nitrobenzoyl chloride in 95% yield as pale yellow solid: mp 62−64 °C; IR (KBr) ν (cm^{-1}) 2936, 2858, 1700, 1630, 1594, 1278; ^{1}H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.21 (1H, d, J = 8.1 Hz), 7.72−7.77 (1H, m), 7.58−7.62 (1H, m), 7.43 (1H, d, J = 6.7 Hz), 7.34−7.40 (1H, m), 7.06−7.14 (3H, m), 3.95−4.05 (2H, m), 3.39−3.41 (4H, m), 3.17 (2H, br s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.8 (s), 151.3 (s), 145.7 (s), 134.8 (s), 131.5−132.7 (CF_3), 131.1 (d), 130.2 (d), 129.9 (d), 128.3 (d), 125.1 (d), 122.6 (s), 119.7−119.8 (d), 116.9−117.1 (d), 113.0−113.2 (d), 49.0 (t), 48.9 (t), 46.7 (t), 41.8 (t); HRMS m/z calcd for $C_{18}H_{17}F_3N_3O_3$ (MH^+) 380.1222, found 380.1195. Anal. Calcd for $C_{18}H_{16}F_3N_3O_3$: C, 56.99; H, 4.25; N, 11.08. Found: C, 56.69; H, 4.53; N, 11.04.

(2-Nitrophenyl)(4-(pyridin-2-yl)piperazin-1-yl)methanone (25). The title compound was prepared from **3** and 2-nitrobenzoyl chloride in 92% yield as yellow solid: mp 110−112 °C; IR (KBr) ν (cm^{-1}) 2923, 2853, 1718, 1635, 1592, 1235; ^{1}H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.19−8.24 (2H, m), 7.71−7.76 (1H, m) 7.58−7.63 (1H, m) 7.49−7.54 (1H, m), 7.43 (1H, d, J = 7.5 Hz), 6.66−6.68 (2H, m), 3.96 (2H, br s), 3.70 (2H, br s), 3.56 (2H, br s), 3.33−3.36 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7 (s), 159.1 (s), 148.1 (d), 145.5 (s), 137.8 (d), 134.7 (d), 132.8 (s), 130.1 (d), 128.2 (d), 124.9 (d), 114.1 (d), 107.5 (d), 46.6 (t), 45.1 (t), 45.0 (t), 41.7 (t); ESI-MS m/z 313 (MH^+). Anal. Calcd for $C_{16}H_{16}N_4O_3$: C, 61.53; H, 5.16; N, 17.94. Found: C, 61.39; H, 5.28; N, 17.74.

(4-(Pyridin-2-yl)piperazin-1-yl)(4-(trifluoromethyl)phenyl)-methanone (26). The title compound was prepared from **3** and 4-(trifluoromethyl)benzoyl chloride in 92% yield as white solid: mp 80−82 °C; IR (KBr) ν (cm^{-1}) 2923, 2851, 1638, 1596; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.18 (1H, d, J = 3.8 Hz), 7.70 (2H, d, J = 8.0 Hz), 7.46−7.56 (3H, m), 6.62−6.69 (2H, m), 3.59−3.89 (8H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.2 (s), 159.1 (s), 148.1 (s), 139.3 (s), 137.9 (d), 131.6 (s), 127.7 (d), 125.7−125.9 (CF_3), 114.3 (d), 107.6 (d), 45.6 (t); ESI-MS m/z 336 (MH^+). Anal. Calcd for $C_{17}H_{16}F_3N_3O$: C, 60.89; H, 4.81; N, 12.53. Found: C, 61.18; H, 4.98; N, 12.34.

(4-(Trifiuoromethyl)phenyl)(4-(3-(trifluoromethyl)phenyl)-piperazin-1-yl)methanone (27). The title compound was prepared from **2** and 4-(trifluoromethyl)benzoyl chloride in 90% yield as white solid: mp 65−66 °C; IR (KBr) ν (cm^{-1}) 2927, 2857, 1656, 1598; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 7.71 (2H, d, J = 8.1 Hz), 7.55 (2H, d, J = 8.0 Hz), 7.34−7.39 (1H, m), 7.04−7.15 (3H, m), 3.46−3.90 (4H, m), 3.25 (4H, br s); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.9 (s), 151.3 (s), 139.3 (s), 131.9−132.6 (CF_3), 130.0 (d), 127.9 (d), 126.2 (d), 125.9−126.0 (CF_3), 122.6 (s), 122.1 (s), 119.9 (d), 117.4−117.5 (d), 113.3−113.4 (d), 49.7 (t); HRMS m/z calcd for $C_{19}H_{17}F_6N_2O$ (MH^+) 403.1245, found 403.1228. Anal. Calcd for $C_{19}H_{16}F_6N_2O$: C, 56.72; H, 4.01; N, 6.96. Found: C, 56.81; H, 4.20; N, 7.04.

(4-(3-Chlorophenyl)piperazin-1-yl)(4-(trifluoromethyl)phenyl)-methanone (28). The title compound was prepared from **1** and 4-(trifluoromethyl)benzoyl chloride in 88% yield as oil; IR (neat) ν (cm^{-1}) 2925, 2846, 1689, 1594; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 7.70 (2H, d, J = 8.1 Hz), 7.54 (2H, d, J = 8.0 Hz), 7.14−7.20 (1H, m), 6.85−6.87 (2H, m), 6.77 (1H, dd, J = 8.2, 1.8 Hz), 3.56−3.90 (4H, m), 3.20 (4H, br s); ESI-MS m/z 369 (MH^+). Anal. Calcd for $C_{18}H_{16}ClF_3N_2O$: C, 58.62; H, 4.37; N, 7.60. Found: C, 58.92; H, 4.40; N, 7.51.

1-(4-Nitrobenzyl)-4-(3-(trifluoromethyl)phenyl)piperazine (29). To the mixture of **2** (0.7 g, 3.3 mmol) and TEA (0.9 mL, 6.7 mmol) was added 1-(bromomethyl)-4-nitrobenzene (1.0 g, 5.0 mmol) and heated at 100 °C for 4 h. EtOAc (15.0 mL) was added in reaction mixture and washed with water (2 × 5.0 mL), and organic layer was separated. Combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/5) to give the title compound in 83% yield as yellow oil: IR (neat) ν (cm^{-1}) 2925, 2838, 1582, 1544, 1255; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.19 (2H, d, J = 8.6 Hz), 7.54 (2H, d, J = 8.6 Hz), 7.29−7.35 (1H, m), 67.00−7.07 (3H, m), 3.66 (2H, s), 3.24−3.27 (4H, m), 2.61−2.64 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 151.5 (s), 147.3 (s), 146.2 (s), 130.4−132.3 (CF_3), 129.7 (d), 129.6 (d), 127.2 (s), 123.6 (d), 118.8 (d), 115.7−115.9 (d), 112.0−112.2 (d), 62.0 (t), 53.0 (t), 48.7 (t); ESI-MS m/z 366 (MH^+). Anal. Calcd for $C_{18}H_{18}F_3N_3O_2$: C, 59.17; H, 4.97; N, 11.50. Found: C, 59.28; H, 5.10; N, 11.48.

The following compounds **30**−**32** were prepared using a procedure similar to that described for compound **29** from the corresponding substituted piperazines and 1-(bromomethyl)-4-nitrobenzene.

1-(3-Chlorophenyl)-4-(4-nitrobenzyl)piperazine (30). The title compound was prepared from **1** and 1-(bromomethyl)-4-nitrobenzene in 85% yield as pale yellow solid: mp 108−110 °C; IR (KBr) ν (cm^{-1}) 2942, 2878, 1592, 1564, 1230; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.18 (2H, d, J = 8.7 Hz), 7.54 (2H, d, J = 8.7 Hz), 7.13−7.18 (1H, m), 6.85−6.87 (1H, m), 6.76−6.81 (2H, m), 3.64 (2H, s), 3.19−3.22 (4H, m), 2.58−2.61 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.4 (s), 147.3 (s), 146.2 (s), 135.0 (s), 130.2 (d), 129.6 (d), 123.7 (d), 119.4 (d), 115.8 (d), 114.0 (d), 62.1 (t), 53.1 (t), 48.7 (t); ESI-MS m/z 332 (MH^+ with ^{35}Cl), 334 (MH^+ with ^{37}Cl). Anal. Calcd for $C_{17}H_{18}ClN_3O_2$: C, 61.54; H, 5.47; N, 12.66. Found: C, 61.29; H, 5.61; N, 12.81.

1-(4-Nitrobenzyl)-4-(pyridin-2-yl)piperazine (31). The title compound was prepared from **3** and 1-(bromomethyl)-4-nitrobenzene in 80% yield as yellow solid: mp 136−138 °C; IR (KBr) ν (cm^{-1}) 2941, 2847, 1685, 1594, 1560, 1248; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.19 (2H, d, J = 8.7 Hz), 8.14−8.16 (1H, m), 7.55 (2H, d, J = 8.6 Hz), 7.41−7.47 (1H, m), 6.58−6.62 (2H, m), 3.64 (2H, s), 3.54−3.57 (4H, m), 2.55−2.58 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 159.6 (s), 148.1 (d), 147.4 (s), 146.3 (s), 137.6 (d), 129.7 (d), 123.7 (d), 113.5 (d), 107.2 (d), 62.3 (t), 53.2 (t), 45.4 (t); ESI-MS m/z 299 (MH^+). Anal. Calcd for $C_{16}H_{18}N_4O_2$: C, 64.41; H, 6.08; N, 18.78. Found: C, 64.50; H, 6.27; N, 18.57.

2-(4-(4-Nitrobenzyl)piperazin-1-yl)pyrimidine (32). The title compound was prepared from **4** and 1-(bromomethyl)-4-nitrobenzene in 76% yield as pale yellow solid: mp 130−132 °C; IR (KBr) ν (cm^{-1}) 2925, 2838, 1594, 1544, 1255; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$) δ 8.20 (2H, d, J = 4.7 Hz), 8.13 (2H, d, J = 8.7 Hz), 7.47 (2H, d, J = 8.6 Hz), 6.39 (1H, t, J = 4.7 Hz), 3.75−3.78 (4H, m), 3.56 (2H, s), 2.42−2.45 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 161.8 (s), 157.8 (d), 147.3 (s), 146.3 (s), 129.6 (d), 123.7 (d), 110.1 (d), 62.3 (t), 53.2 (t), 43.8 (t), ESI-MS m/z 300 (MH^+). Anal. Calcd for $C_{15}H_{17}N_5O_2$: C, 60.19; H, 5.72; N, 23.40. Found: C, 60.23; H, 5.68; N, 23.20.

Biology

Expression of Estrogen Receptors in Human BPH Stromal and Cancer Epithelial (LNCaP) Cells. Estrogen receptors (ER-α, ER-β) are ligand-dependent transcription factors belonging to the superfamily of nuclear receptors. Recent studies have shown that estrogen signaling plays an important role in pathogenesis of prostatic hyperplasia. Compelling evidence is available in literature which supports that ER-β signaling mediates antiproliferative and proapoptotic effects while ER-α mediates proliferative effects (aberrant growth) in prostate.[41] ER-α is chiefly located in the stroma, which is the site of BPH initiation, and regulates epithelial growth through paracrine signaling. ER-β is present in both stromal and epithelial compartments, with higher prevalence in the latter. The mRNA expression of these receptors in human BPH-derived stromal cells and cancer epitheial cells (LNCaP) in response to treatment with compound **10** was studied by real time PCR. Human BPH-derived stromal cells [isolated from prostatic tissue of patients undergoing transurethral resection of prostate (TURP) for BPH at the CSM Medical University, Lucknow] were maintained in primary culture by following standard method.[42] Briefly, viable tissues were washed three times in medium A [a mixture of DMEM/Ham's F-12 and Waymouth MD 752/1 (1:1 v/v) supplemented with antibiotic−antimycotic solution (Sigma Aldrich)], minced, and digested with collagenase (5.0 mg/mL) in medium A enriched with 2% fetal calf serum (FCS) at 37 °C for 4 h. Subsequently, the tissues were passed 6−7 times through an 18G needle and centrifuged at 1200 rpm for 3 min. The pellets were washed and resuspended in medium A with 10% FCS. The resulting cells were vimentin positive and grown in DMEM/Ham's F-12 medium supplemented with 10% FCS. Cells from passages 4−6 were used in these studies. LNCaP cells were grown in RPMI-1640 medium supplemented with 10% FCS, 100 units/mL penicillin G sodium, and 100 μg/mL streptomycin sulfate using gelatin-coated plates. Prostatic cells grown to 80% confluence were treated with either 10.0 μmol of compound **10** or flutamide for 24 h. Later, total RNA of cells was extracted using the Trireagent (Invitrogen) by following the manufacturer's instructions. cDNA was prepared from 1.0−2.0 μg of total RNA using Revert Aid H-Minus first strand cDNA synthesis kit (Fermentas Life Sciences, USA). Synthesis was performed for 1 h at 42 °C (for reverse transcription), and the thermocycling for each reaction was done in a final volume of 25.0 μL containing 1.0 μL of cDNA sample, 0.5 μmol of

each primer, 2× ready-to-use reaction mixture (ABi SYBR green master mixture) including Taq DNA polymerase, reaction buffer, and deoxyribonucleoside triphosphate (dNTP) mixture. After 10 s of initial denaturation at 95 °C, the following cycling conditions (45 cycles) were used: denaturation at 95 °C for 20 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 30 s. The detection of the PCR reaction based on fluorescence monitoring (Light Cycler 480; Roche) was employed. Quantitative results were obtained by the cycle threshold (CT) value where a signal rose above background level. Expression of the investigated genes was compared to the steady expression of GAPDH. The primer sequences used were as follows. ER-α: forward, 5′-CCACCAACCAGTGCACCATT-3′; reverse, 5′-GGTCTTTTCGTATCCCACCTTTC-3′. ER-β: forward, 5′-AAGAAGATTCCCGGCTTTGT-3′; reverse, 5′-CTTCTACGCATTTCCCCTCA-3′.

Pharmacokinetic Evaluation. The pharmacokinetic studies of compound **10** were carried out in young and healthy male Sprague-Dawley rats weighing 250 ± 25 g, obtained from Laboratory Animal Division, CDRI, Lucknow, India. The animals were housed in plastic cages under 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 2 days before conducting the experiments. In all of the studies mentioned below the dose was administered after overnight fasting (12−16 h). The study was conducted in $n = 3$ per time point. In all experiments, euthanasia and disposal of carcasses were carried out as per the guidelines of Local Ethics Committee for animal experimentation.

Suspension formulation containing 12.5 mg of compound **10** was prepared by triturating compound **10** with gum acacia and water in a pestle with mortar, and a single oral dose of 10 mg/kg was given to conscious rats by oral gavage. The animals were sacrificed at various times up to 24 h after drug administration, and blood, prostate, and hypothalamus were collected. Serum samples were also harvested. The hypothalamus of three rats was pooled and homogenized in order to ensure a measurable quantity. All samples were stored at −20 °C until analysis.

Chromatographic separations and quantification of the candidate drug were achieved by a reverse-phase HPLC method on a Phenomenex C-18 column (5 μmol, 150 × 4.6 mm i.d.) preceded with a guard column (5 μmol, 30 × 4.6 mm i.d.) packed with the same material under isocratic condition at a flow rate of 1 mL/min. The HPLC system used in this study consisted of a pump (LC-10AT VP with FCV-10AL VP; Shimadzu), degasser (DGU-14A; Shimadzu), and autoinjector (SIL-HTc, fixed with a 100 μL loop). Eluents were monitored at 260 nm with UV−vis multiple wavelength detector (Shimadzu, Japan), and chromatograms were integrated using Class-VP (version 6.12 SP5) software. The mobile phase composition was aqueous sodium acetate (0.01 M, pH 6.0) and acetonitrile (30:70% v/v) at a flow rate of 1.0 mL/min, and it was degassed by ultrasonication for 15 min before use. The HPLC system was equilibrated for approximately 30 min before commencement of analysis, and chromatography was carried out at ambient temperature. The lower limit of quantification for the analytical method was 10 ng/mL of test article in serum and tissues. The mean and SEM of the serum concentrations of the candidate drug at each time point were calculated using Microsoft Excel for Windows. All pharmacokinetic parameters were calculated by noncompartmental models using WinNonlin program, version 1.5 (Scientific Consulting Inc.).

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectral data of compounds **6**−**32**, HRMS data of compounds **7**, **10**, **13**, **14**, **15**, **16**, **24**, and **27**, and representative chromatogram of compound **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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