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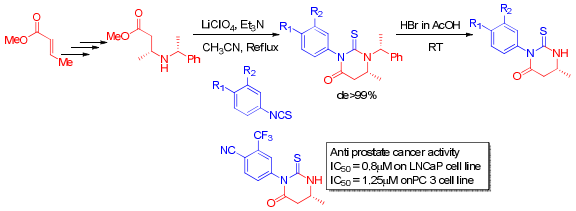
Table of contents



3-Aryl-6-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-ones were designed and synthesized as new class of anti-prostate cancer agents.

RSC Advances

Design And Synthesis of Optically Pure 3-Aryl-6-methyl-2-thioxotetrahydropyrimidin-4(1H)-ones as Anti-prostate Cancer Agents

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3-Aryl-6-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-ones were proposed as a new class of anti-prostate cancer agents on the basis of molecular modeling studies. Stereoselective synthesis of 3-aryl-6-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one derivatives was achieved by chiral induction employing (*R*)/(*S*)-*α*-methyl benzylamine and subsequent debenzylation with HBr in AcOH, afforded the desired enantiomers in good yields. The compounds were screened *in vitro* against prostate cancer cell lines, PC-3 and LNCaP and the most potent derivatives were identified.

Introduction

Prostate cancer is a major concern because of high prevalence, and is a leading cause of cancer related death in men.[1] The development and progression of the prostate cancer is directly related to the nuclear steroidal androgen receptor.[2] It regulates the binding of androgens like testesterone and its active metabolite dihydrotestesterone. Hence steroidal agents (cypreterone acetate, spironolactone) that block the androgens (antiandrogens) were employed for the treatment of prostate cancer in hormone therapy (Figure 1).[3] But the clinical applications of steroidal antiandrogens were limited because of cross reactivity, poor bioavailability, lack of tissue selectivity and other side effects.[3b] Hence the focus shifted to non-steroidal antiandrogens that not only overcome the side effects of the steroidal agents, but also provide sufficient scope for structural modifications to afford more potent scaffolds.[3,4]

Flutamide, nilutamide and bicalutamide are the available non-steroidal drugs for the treatment of prostate cancer,[3,4] but with elapse of time the therapy fails because of alterations in cell signaling pathways and mutations leading to antiandrogen withdrawal syndrome. Moreover, other adverse effects such as hepatotoxicity, gynaecomastia, loss of libido etc. necessitate the search for novel ligands for the treatment of prostate cancer.

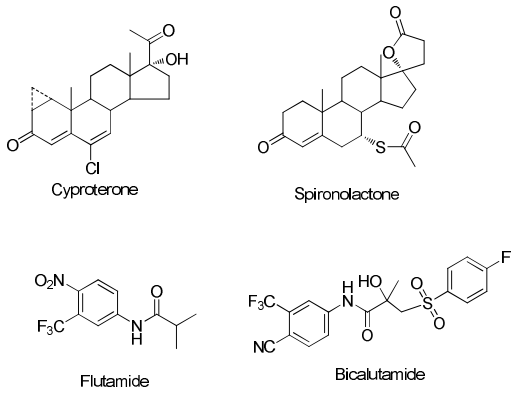

Figure 1. Steroidal and non-steroidal antiandrogens

Results and discussion

The X-ray crystal structure of bicalutmide in WL AR LBD complex revealed a bent conformation for bicalutamide, due to the hydrogen bonding interactions.[5] Therefore we assumed that a conformationally restricted model with similar interactions will be of high relevance to investigate the anti-prostate activity. With our current interests on heterocyclic scaffolds and anti-prostate cancer agents,[6] we designed conformationally restricted 2-thioxotetrahydropyrimidin-4(1*H*)-ones as pharmacophore of choice satisfying the criteria (Figure 2). The rationale is supported by recent literature reports where heterocycles with thioamide moiety, especially 3-aryl thiohydantoins, are found to be highly potent non-steroidal antiandrogens.[7] Further to understand the ligand binding interactions, docking of the proposed compounds was

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performed on the active site of the receptor. The results obtained from molecular docking of designed ligands are summarized in (Table 1), where the binding affinity of ligand towards mutant androgen receptor is represented in terms of docking score.[8]

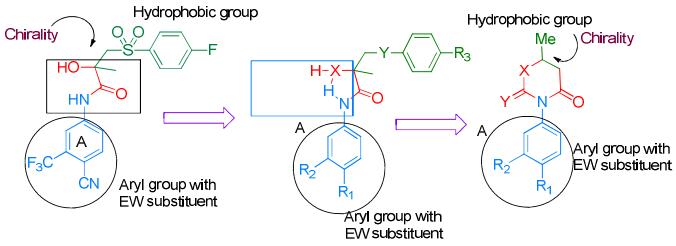


Figure 2. Design of proposed pharmacophore

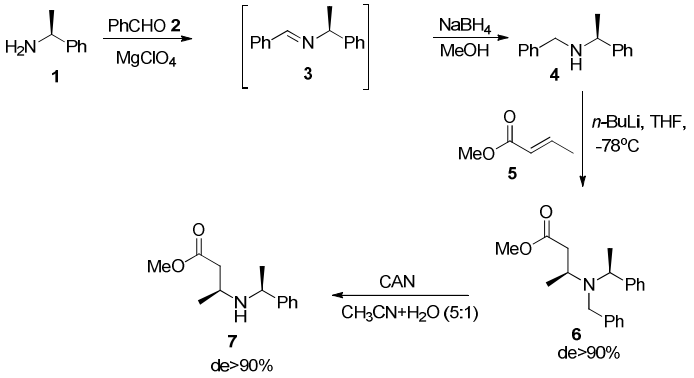
Table 1. Docking scores for the proposed ligands

Entry	Compound	Stereo-chemistry	Docking score[a]	Docking score[b]	Glide emodel
1	**Bicalutamide**	(*R*)	-10.74	-10.99	-94.62
2	**10a**	(*S*)	-7.41	-5.19	-55.65
3	**10b**	(*S*)	-7.76	-5.57	-59.13
4	**10c**	(*S*)	-8.28	-6.67	-62.36
5	**10d**	(*S*)	-7.85	-6.73	-57.80
6	**10e**	(*S*)	-8.07	-7.15	-63.20
7	**10f**	(*S*)	-8.01	-6.46	-60.20
8	**10'a**	(*R*)	-7.46	-5.63	-56.76
9	**10'b**	(*R*)	-7.99	-5.27	-58.51
10	**10'c**	(*R*)	-8.52	-7.07	-65.23
11	**10'd**	(*R*)	-8.05	-7.28	-55.30
12	**10'e**	(*R*)	-8.35	-6.94	-60.78
13	**10'f**	(*R*)	-7.59	-5.71	-43.47

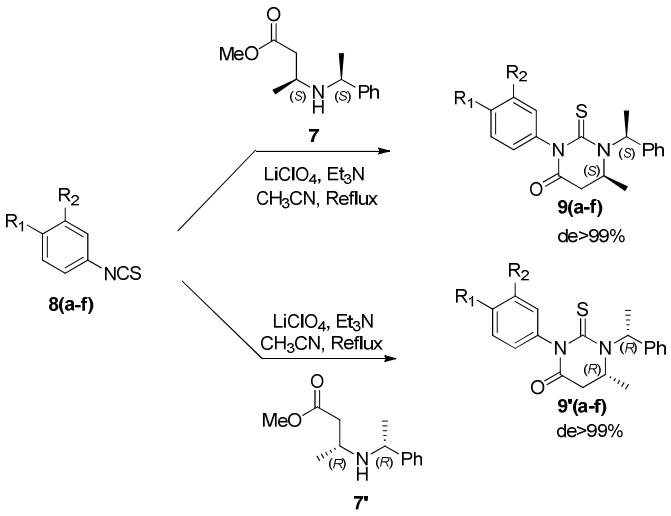
[a]Docking scores were calculated in standard precision mode
[b]Docking scores were calculated in extra precision mode

The results obtained from molecular docking of designed and synthesized analogues are summarized in Table 1. The molecular docking analysis showed that all ligands were docked in same ligand binding site as that of bicalutamide. The results also indicated the high binding affinity of the compounds towards the androgen receptor.

To gain more insights of the binding site, an in-depth analysis was carried out, which showed that the active site is composed of mainly five sub pockets: (i) hydrophilic subpocket A that includes Arg752 and Gln711 and is responsible for hydrogen bonding interactions with electron withdrawing substituent at the *para* position of the *N*3 aryl group (ii) hydrophobic sub pocket B consists of Gly708, Met745, Val746, Met749, Phe764, Met787, Leu873, and Met895 which stabilizes the R_2 substituent as well as the thioamide moiety (iii) hydrophobic sub pocket C comprising of of Met895, Thr877, Phe891, Leu880 and Phe876, interacts with -CH_3 group of the *R*-stereoisomer (iv) hydrophobic sub pocket D composed of Leu704, Leu701, Met873, Met742 and Thr877 is responsible for interactions with the -CH_3 group of the *S*-stereoisomer (v) hydrophilic sub pocket E containing Asn705 is involved in hydrogen bonding with the -NH group of the ligands (**Figure 3**). The compounds having cyano group at the *para* position of the *N*3 aryl ring has higher docking scores in comparison with other substrates. This may be attributed to the simultaneous formation of hydrogen bonds with Arg752 and Gln711, and hence a stronger binding affinity. The higher binding affinity of compounds with *meta* substitution on *N*3 aryl group **10(b-e)** and **10'(b-e)** can be explained on the basis of hydrophobic interactions with amino acid residues Met745, Val746, Met749, Phe764, Met787 and Leu873 of the sub pocket B. A high docking score was observed for compound **10'e** due to the preferred geometry that allows hydrogen bonding interactions between the NH of the ligand and Asn705, in addition to the hydrogen bonding of the cyano group with Arg752 and Gln711.



Scheme 1. Diastereoselective synthesis of (*S*)-methyl 3-[(*S*)-1-phenylethylamino]butanoate



Scheme 2. Diastereoselective syntheses of 3-aryl-6(S)/(R)-methyl-1-[(S)/(R)-1-phenylethyl)]-2-thioxotetrahydropyrimidin-4(1*H*)-ones

Further, our efforts were to synthesize the (*R*) and (*S*) enantiomers of 3-aryl-6-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-ones to validate the conclusions drawn from the molecular modeling studies, and to identify the most active enantiomer as a potential androgen receptor antagonist. The key intermediate involved in the synthesis of 2-thioxotetrahydropyrimidin-4(1*H*)-ones are *β*-aminoesters which upon condensation with aryl isothiocyanates can afford the desired products. The diastereoselective synthesis of the chiral *β*-aminoesters[9] was attempted as depicted in Scheme 1. (*S*)-α-Methyl benzylamine **1** when treated with

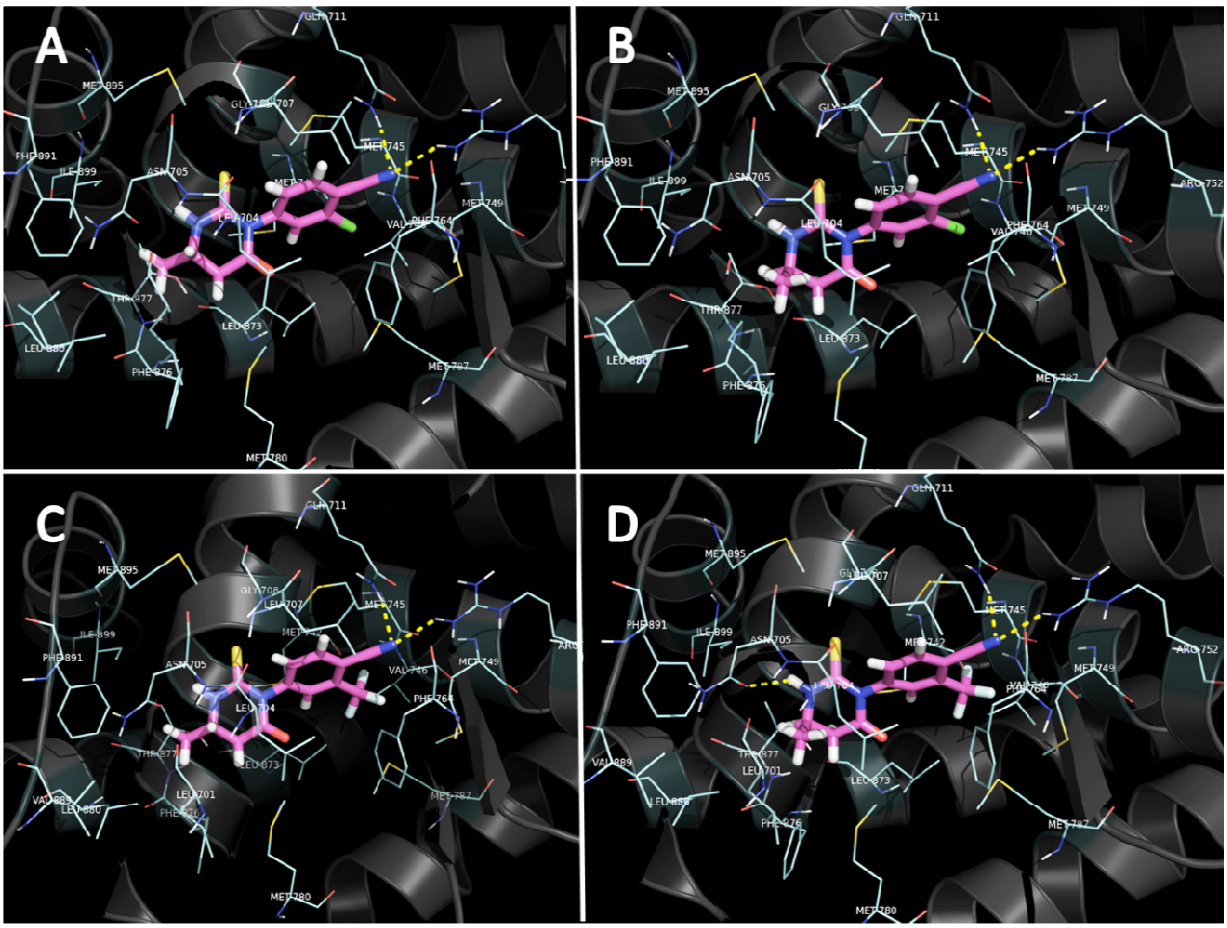


Figure 3. Binding orientations of compound **10c** (A), compound **10'c** (B), compound **10e** (C) and compound **10'e** (D) in 1z95 binding pocket. Hydrogen bonding interactions are highlighted in yellow color. Active site amino acid residues are in color lines model and ligands are in pink color stick model.

benzaldehyde **2** in presence of magnesium perchlorate as catalyst under neat condition afforded the imine **3**,which was subsequently reduced with $NaBH_4$ to obtain the chiral (*S*)-*N*-benzyl-*α*-methyl benzylamine **4** in excellent yields. The chiral

Table 2. Reactions of aryl isothiocyanates with (*S*)/(*R*)-methyl 3-[(*S*)/(*R*)-1-phenylethylamino]butanoates

Entry	R_1	R_2	Time (h)	Product	Yield (%)	de
1	Cl	H	2.5	**9a**	78	>99
2	F	Cl	2.5	**9b**	80	>99
3	CN	Cl	3.0	**9c**	74	>99
4	Cl	CF_3	3.0	**9d**	76	>99
5	CN	CF_3	4.0	**9e**	78	>99
6	NO_2	CF_3	4.0	**9f**	72	>99
7	Cl	H	2.5	**9'a**	80	>99
8	F	Cl	2.5	**9'b**	82	>99
9	CN	Cl	3.0	**9'c**	72	>99
10	Cl	CF_3	3.0	**9'd**	76	>99
11	CN	CF_3	4.0	**9'e**	74	>99
12	NO_2	CF_3	4.0	**9'f**	72	>99

*Reactions were performed with 2.57 mmoles of aryl isothiocyante, 2.57mmoles of *β*-aminoester and 0.257 mmoles of catalyst

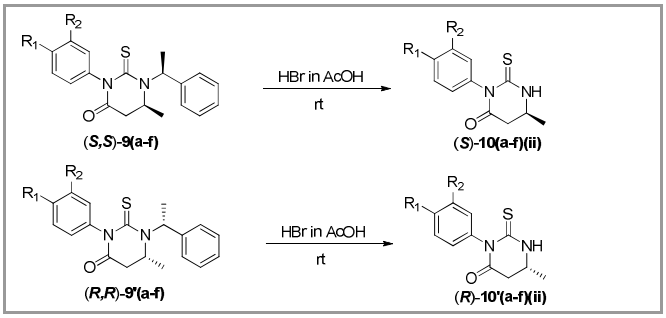
amine **4** when reacted with *n*-BuLi gave the corresponding lithium amide derivative which underwent Michael addition diastereoselectively with methyl crotonate **5** affording the *β*-aminoesters, methyl 3-(benzyl[(*S*)-1-phenylethyl]amino)butanoate **6** with (*S*,*S*) isomer as the major. Finally the oxidative cleavage of the *N*-benzyl group using cericammonium nitrate in acetonitrile-water mixture (5:1) afforded the (*S*)-methyl 3-[(*S*)-1-phenylethylamino]butanoate **7** with good yields and high diastereoselectivity. A similar reaction sequence with (*R*)-*α*-methyl benzylamine afforded the other enantiomer (*R*)-methyl 3-[(*R*)-1-phenylethylamino]butanoate (*R,R*)-**7'**. Synthesis of 3-aryl-6(*S*)-methyl-1-[(*S*)-1-phenylethyl)]-2-thioxotetrahydropyrimidin-4(1*H*)-ones **9(a-f)** were then achieved by the condensation of (*S*)-methyl 3-[(*S*)-1-phenylethylamino]butanoate 7 with aryl isothiocyanates **8(a-f)** using lithium perchlorate as catalyst and triethylamine as a base in acetonitrile medium under reflux conditions (Scheme 2). The reaction afforded good yields of the products in all the cases and recrystalization in MeOH afforded the (*S,S*) diastereomers as the sole product (de>99%) (Table 2). The corresponding (*R,R*) enantiomers **9'(a-f)** were obtained from (*R*)-methyl 3-[(*R*)-1-phenylethylamino]butanoate **(*R,R*)-7'**. After achieving chiral induction, our aim was to cleave the chiral handle in order to obtain the desired enantiomers of 3-aryl-6-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-ones stereospecifically. The cleavage of the handle, which involves the debenzylation at N1, was attempted by conventional methods (Table 3). Accordingly a solution 3-(4-chlorophenyl)-6(*S*)-methyl-1-[(*S*)-1-phenylethyl)]-2-thioxotetrahydropyrimidin -4(1*H*)-one **9a** in MeOH was treated with Pd catalyst under hydrogen atmosphere for 12 h. The reaction was unsuccessful and the starting material was recovered. A variation in the solvents or the oxidation state of the catalyst did not alter the outcome of the reaction. Oxidative *N*-debenzylation using CAN, DEAD and

DDQ also failed. Further, acid catalyzed cleavage of the chiral handle was investigated with HBr. Interestingly, the substrate in 33% HBr-AcOH at room temperature afforded the desired product 3-(4-chlorophenyl)-6(*S*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one **(*S*)-10a** in good yield (Scheme 3). The reaction conditions were then generalized (Table 4) with other substrates **9(a-f)** and **9'(a-f)** affording the corresponding products **(*S*)-10(a-f)** and **(*R*)-10'(a-f)** respectively in good yields.

Table 3. Reaction conditions employed for debenzylation at *N*1

Entry	Catalyst	Reagent	Solvent	Time (h)	Yield (%)
1	Pd	H_2	MeOH	12	-
2	Pd	H_2	AcOH	24	-
3	$Pd(OH)_2$	H_2	MeOH	18	-
4	$Pd(OH)_2$	H_2	AcOH	24	-
5	-	CAN	CH_3CN	5	-
6	-	DDQ	DCM	10	-
7	-	DEAD	Toluene	72	-
8	-	HBr	AcOH	2	55
9	-	HBr	AcOH	4	72

*Reactions were performed with 1.2 mmole of substrate and 0.12 mmoles of catalyst



Scheme 3. Debenzylation of 3-aryl-6(*S*)/(*R*)-methyl-1-(*S*)/(*R*)-1-phenyl ethyl)-2-thioxotetrahydro pyrimidin-4(1*H*)-ones

Table 4. Syntheses of enantiopure 3-aryl-6(*S*)/(*R*)-methyl-2-thioxo tetrahydropyrimidin-4(1*H*)-ones

Entry	Substrate	R_1	R_2	Product	Time (h)	Yield (%)
1	**9a**	Cl	H	**(*S*)-10a**	3.0	72
2	**9b**	F	Cl	**(*S*)-10b**	3.5	68
3	**9c**	CN	Cl	**(*S*)-10c**	3.5	75
4	**9d**	Cl	CF_3	**(*S*)-10d**	4.0	65
5	**9e**	CN	CF_3	**(*S*)-10e**	4.0	69
6	**9f**	NO_2	CF_3	**(*S*)-10f**	4.0	64
7	**9'a**	Cl	H	**(*R*)- 10'a**	3.0	74
8	**9'b**	F	Cl	**(*R*)- 10'b**	3.5	70
9	**9'c**	CN	Cl	**(*R*)- 10'b**	3.5	67
10	**9'd**	Cl	CF_3	**(*R*)- 10'd**	4.0	62
11	**9'e**	CN	CF_3	**(*R*)- 10'e**	4.0	66
12	**9'f**	NO_2	CF_3	**(*R*)- 10'f**	4.0	68

*Reactions were carried out with 1.2 mmole of substrate and 5ml of HBr at rt

To determine the cytotoxicity of the compounds, *in vitro* experiments were carried out at varying concentrations (0.01 nM to 100 μM) on prostate cancer cell lines, PC-3 and LNCaP. Bicalutamide was used as the positive control for the androgen dependent LNCaP cells, whereas as doxorubicin was preferred for PC-3 cell lines.[10] The cell viability was determined by colorimetric MTT assay.[11] It was observed that all the compounds exhibited antagonistic activity with IC_{50} values in mM to nM range. However none of the compounds showed agonistic activity. Though most of the compounds showed the antagonistic activity a total of four compounds (compound **10a**, **10e** and their enantiomers **10'a**, **10'e**) showed IC_{50} values below 50 μM that were further examined for the selectivity studies (**Table 5**). As expected on the basis of the docking studies the most potent compound turned out to be **10'e** that has the aryl ring with the CN group at the para position and CF_3 group at the meta position. A comparison of **10'e** with the standard drugs for prostate cancer showed an IC_{50} value of 1.25 μM, comparable to doxorubicin (1.05 μM) on PC-3 cell lines (**Figure 4**) and a better antagonistic activity of 782 nM on LNCap cell lines compared to flutamide (1 μM) and bicalutamide (1.7 μM) as shown in **Figure 5**. However the corresponding enantiomer **10e** was found to be less potent with IC_{50} value of 34 μM and 13 μM on PC-3 and LNCaP cell lines respectively which is indicative of the fact that chirality is also an important factor governing the binding affinity. Thus the cytotoxicity of the compounds not only depend on the substituent on the aryl ring but also on the orientation of the methyl group, with the *S* isomer being more active.

Table 5. Cytotoxicity studies of most active compounds on PC-3 and LNCaP cells[a]

Entry	Compound	R_1	R_2	IC_{50} (μM) on PC-3 cells	IC_{50} (μM) on LNCaP cells
1	**Doxorubicin**[b]	---	---	1.05	nd[c]
2	**Flutamide**[b]	---	---	nd[c]	1.0
3	**Bicalutamide**	---	---	nd[c]	1.7
4	**(*S*)-10a**	Cl	H	>100	>100
5	**(*R*)-10'a**	Cl	H	>100	>100
6	**(*S*)-10b**	F	Cl	50	>100
7	**(*R*)-10'b**	F	Cl	>100	>100
8	**(*S*)-10c**	CN	Cl	100	39
9	**(*R*)-10'c**	CN	Cl	90	14
10	**(*S*)-10d**	Cl	CF_3	>100	>100
11	**(*R*)-10'd**	Cl	CF_3	>100	>100
12	**(*S*)-10e**	CN	CF_3	34	13.0
13	**(*R*)-10'e**	CN	CF_3	1.25	0.8
14	**(*S*)-10f**	NO_2	CF_3	>100	>100
15	**(*R*)-10'f**	NO_2	CF_3	>100	>100

[a]The data represents the mean of three experiments in triplicate
[b]Used as a positive control. [c]nd = not determined.

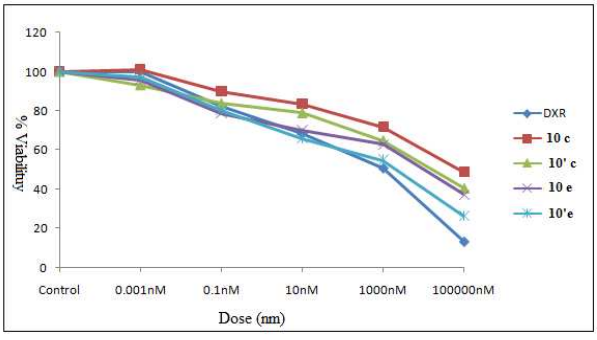


Figure 4. Dose response curve on PC-3cells

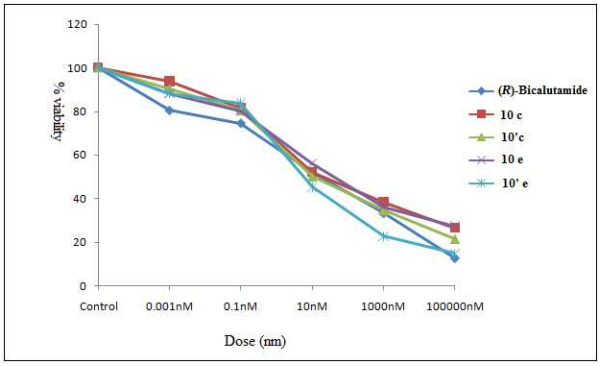


Figure 5. Dose response curve on LNCaP cells

Further to study the selectivity of the compounds for prostate cancer, cytotoxicity of the most potent compounds were examined on non cancerous cell line MCF 10A and breast cancer cell line MCF-7. The results obtained were impressive as no compound exhibited toxicity on the other cell lines at conc. of 1 μM. Hence 3-(4-cyano-3-(trifluoromethyl)phenyl)-6(*R*)-methyl-2-thioxotetrahyro pyrimidn-4(1*H*)-one was identified as the lead compound for further studies in this direction.

Experimental Section

Chemical Synthesis

The ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively on a Bruker Avance 400 (400 MHz) spectrometer in $CDCl_3$ using TMS as an internal standard. The chemical shifts (δ) for ^{1}H and ^{13}C are given in *ppm* relative to residual signals of the solvent. Coupling constants are given in Hz. The following abbreviations are used to indicate the multiplicity: s, singlet; d, doublet; m, multiplet. Mass spectra were recorded on Finnigan Mat LCQ LCMS. The reactions were monitored by TLC (Merck). Evaporation of solvents was performed under reduced pressure using a Buchi rotary evaporator. Commercial grade reagents and solvents were used without further purification. (s)-α-methyl benzyl amine, benzaldehyde, sodium borohydride ($NaBH_4$), lithium diisopropylamide (LDA), methyl acrylate, lithium hexamethyldisilazane (LHMDS), cerric ammonium nitrate, 4-chloro aniline, 4-chloro-3-trifluoromethylaniline, 4-amino-2-chlorobenzonitrile, 4-cyno-3-trifluoromethylaniline, 4-nitro-3-trifluoromethylaniline (Aldrich), thiophosgene, Methanol, THF (Spectrochem); dichloromethane (DCM) 4-chloroaniline (Merck) and sodium hydrogen carbonate (CDH).

Synthesis of (*S*)-methyl 3-[(*S*)-1-phenylethylamino] butanoate: In a typical experiment, (*S*)-α-methylbenzylamine (6.36 mL, 50.0 mmol) was taken in MeOH and benzaldehyde (5.50 mL, 55 mmol) followed by $MgClO_4$ (0.24 g, 2.0 mmol) was added to the reaction mixture. It was stirred at room temperature for 2h to obtain the corresponding imine. After completion of the reaction as determined from TLC, $NaBH_4$ (2.22 g, 60.0 mmol) was slowly added to it and stirred for 2.5h. The reaction mixture was then concentrated, diluted with water and extracted into ethyl acetate. The organic layer was dried and the solvent was evaporated to obtain the crude product. It was purified by column chromatography on silica gel (60–120 mesh) using EtOAc-hexane mixture (1:9) as the eluent to afford the (*S*)-*N*-benzyl-1-phenylethanamine (8.89 g, 85%). The amine (8.36 g, 40.0 mmol) dissolved in THF (100 mL) was cooled to 0 ^{o}C and *n*-butyl lithium (27.47 mL, 1.6 M in hexanes, 44.0 mmol) was added to it. The solution was stirred at 0 ^{o}C for 15 min and further cooled to -78 ^{o}C. Methyl crotonate (4.04 mL, 40.0 mmol) in THF (50 mL) was added dropwise and the reaction mixture was stirred for another 15 min at -78 ^{o}C before quenching with saturated ammonium chloride (2 mL). The reaction was allowed to warm, poured into saturated aqueous sodium chloride solution, extracted with ether (2 x 100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product as a pale yellow oil. The product (*S*)-methyl 3-(benzyl[(*S*)-1-phenylethyl]amino) butanoate, 8 (7.70 mL, 62%) was purified by column chromatography on silica gel (60–120 mesh) using EtOAc-hexane mixture (1:9) as the eluent. To a solution of (*S*)-methyl 3-(benzyl[(*S*)-1-phenylethyl]amino)butanoate (6.2 g, 20.0 mmol) in CH_3CN:H_2O (5:1), at room temperature, was added ceric ammonium nitrate (23.01 g, 42.0 mmol). The reaction mixture was stirred for 2h, neutralized with aqueous $NaHCO_3$, extracted with Et_2O, dried over Na_2SO_4, and concentrated in vacuum to afford a crude oil which was purified by column chromatography on silica gel (60-120 mesh) using hexane-ethyl acetate mixture (70:30) as the eluent to obtain the desired β-amino ester **9**, (3.3 g, 77%). (*R*)-methyl 3-(*R*)-1-phenylethylamino butanoate was synthesized from (*R*)-α-methylbenzylamine by an analogous procedure.

(*S*)-*N*-benzyl-1-phenylethanamine (4): Colourless oil; Yield 85%;; R_f 0.3 (1:10 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.40 (d, 3H, J = 6.80 Hz), 3.68 (dd, 2H, J = 13.20 and J = 14.40 Hz), 3.85 (q, 1H, J = 6.80 Hz), 7.24-7.39 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.5, 51.6, 57.6, 126.6, 126.8, 126.9, 128.1, 128.3, 128.4, 140.6, 145.6 ; MS (APCI): $[M+1]^+$ = 212.40; $[\alpha]_{20}^{D}$ -39.6 (neat)

(*S*)-methyl 3-(benzyl[(*S*)-1-phenylethyl]amino) butanoate (6): Light yellow oil; Yield 62%; R_f 0.6 (1:10 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17 (d, 3H, J = 6.9 Hz), 1.38 (d, 3H, J = 6.9 Hz), 2.15 (dd, 1H, J = 7.8 and 7.8 Hz), 2.40 (dd, 1H, J = 7.8 and 7.8 Hz), 3.48 (q, 1H, J = 6.6 Hz), 3.53 (s,3H), 3.69-3.74 (m, 2H), 3.92 (q, 1H, J = 6.9 Hz), 7.20-7.45 (m, 10H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.3, 18.3, 39.5, 49.5, 49.9, 51.3, 57.5, 126.6, 127.7, 128.0, 128.1, 128.2, 128.3, 141.6, 144.2, 172.6; MS (APCI): $[M+1]^+$ = 312.48; $[\alpha]_{20}^{D}$ -1.1 (c=1.0, $CHCl_3$).

(*S*)-methyl 3-(*S*)-1-phenylethylamino butanoate (7): (*S*,3*S*)-**5a**: Light brown oil; R_f 0.2 (1:10 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (d, J = 6.4 Hz, 3H), 1.31 (d, J = 6.4 Hz, 3H), 1.66 (br s, 1H), 2.33-2.47 (m, 2H), 3.65 (s, 3H), 3.87 (q, J = 6.4 Hz, 1H), 7.19-7.36(m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.4, 24.6, 40.6, 47.7, 51.3, 55.2, 126.5, 126.8, 128.4, 146.0, 172.7; MS (APCI): $[M+1]^+$ = 222.34; $[\alpha]_{20}^{D}$ -38.0 (c=1.0, $CHCl_3$).

General procedure for the syntheses of 3-aryl-6(*S*)/(*R*)-methyl-1-[(*S*)/(*R*)-1-phenylethyl)-2-thioxotetrahydro pyrimidin-4(1*H*)-ones (9, 9')(a-f): To a solution of aryl isothiocyanate (2.57 mmol) in acetonitrile (30 mL) was added ethyl 3-(1-phenylethylamino)butanoate (0.6 g, 2.57 mmol) followed by triethylamine (0.4 mL, 3.08 mmol) and $LiClO_4$ (10 mol%, 0.03 g, 0.26 mmol). The reaction mixture was refluxed for 1.2h and then concentrated under reduced pressure. The residue was diluted with DCM, washed with water (2 x 25 mL), then with brine (1 x 25 mL) and dried over anhydrous Na_2SO_4. From the concentrated reaction mixture the purified product was obtained by column chromatography on silica gel (60–120) using EtOAc-hexane mixture (15:85) as the eluent. The products were recrystalized from MeOH.

3-(4-Chlorophenyl)-6(*S*)-methyl-1-[(*S*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one 9(a): White solid; Yield 78%; mp 177-179 ^{o}C; R_f 0.66 (3:7 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (d, 3H, J = 6.80 Hz), 1.73 (d, 3H, J = 7.2 Hz), 2.41-2.58 (m, 2H), 3.67-3.70 (m, 1H), 6.97 (q, 1H, J = 7.2 Hz), 7.13 (s, 1H), 7.25-7.46 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3, 19.4, 38.7, 46.3, 59.7, 127.1, 128.3, 128.4, 129.0, 129.3, 134.2, 138.1, 139.3, 165.9, 180.7; MS (APCI): $[M+1]^+$ = 359.03; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{19}H_{19}ClN_2OS$: 381.0805, found: 381.0808; $[\alpha]_{20}^{D}$ -173.62 (c=1.0, $CHCl_3$)

3-(4-Chlorophenyl)-6(*R*)-methyl-1-[(*R*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one 9'(a): White solid; Yield 80%; mp 177-179 ^{o}C; R_f 0.66 (3:7 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.48 (d, 3H, J = 6.6 Hz), 1.75 (d, 3H, J = 7.0 Hz), 2.44-2.60 (m, 2H), 3.69-3.72 (m, 1H), 6.99 (q, 1H, J = 7.20 Hz), 7.15

(s, 1H), 7.37-7.56 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.4, 19.4, 38.7, 46.3, 59.7, 127.1, 128.3, 128.4, 129.0, 129.3, 134.2, 138.1, 139.3, 165.9, 180.6; MS (APCI): $[M+1]^+$ = 359.07; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for for $C_{19}H_{19}ClN_2OS$: 381.0805, found: 381.0800; $[\alpha]_{20}^D$ +173.70 (c=1.000, $CHCl_3$).

3-(3-Chloro-4-fluorophenyl)-6(*S*)-methyl-1-[(*S*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one 9(b): White solid; Yield 80%; mp 170-171 °C; R_f 0.66 (3:7 EtOAc-hexane); ^{1}H NMR; (400 MHz, $CDCl_3$): δ 1.47 (d, 3H, *J* = 6.8 Hz), 1.75 (d, 3H, *J* = 7.2 Hz), 2.44-2.59 (m, 2H), 3.70-3.73 (m, 1H), 6.97 (q, 1H, *J* = 7.20 Hz), 7.21-7.48 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3, 19.4, 38.7, 46.3, 59.8, 116.6, 121.3, 127.0, 128.5, 129.0, 131.6, 135.9, 139.2, 156.5, 159.0, 165.8, 180.5; MS (APCI): $[M+1]^+$ = 377.13; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{19}H_{18}ClFN_2OS$: 399.0710, found: 399.0714; $[\alpha]_{20}^D$ -168.20 (c=1.000, $CHCl_3$).

3-(3-Chloro-4-fluorophenyl)-6(*R*)-methyl-1-[(*R*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one 9'(b): White solid; Yield 82%; mp 170-171 °C; R_f 0.66 (3:7 EtOAc-hexane); ^{1}H NMR; (400 MHz, $CDCl_3$): δ 1.47 (d, 3H, *J* = 6.5 Hz), 1.75 (d, 3H, *J* = 6.9 Hz), 2.42-2.59 (m, 2H), 3.70-3.73 (m, 1H), 6.97 (q, 1H, *J* = 6.9 Hz), 7.11-7.60 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3, 19.4, 38.7, 46.3, 59.8, 116.6, 121.3, 127.1, 128.5, 129.0, 131.6, 135.8, 139.2, 156.5, 159.0, 165.9, 180.5; MS (APCI): $[M+1]^+$ = 376.95; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{19}H_{18}ClFN_2OS$: 399.0710, found: 399.0704; $[\alpha]_{20}^D$ +168.35 (c=1.000, $CHCl_3$).

3-(3-Chloro-4-cyanophenyl)-6(*S*)-methyl-1-[(*S*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one 9(c): White solid; Yield 74%; mp 218-220 °C; R_f 0.50 (3:7 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.46 (d, 3H, *J* = 6.8 Hz), 1.74 (d, 3H, *J* = 7.2 Hz), 2.44-2.59 (m, 2H), 3.69-3.76 (m, 1H), 6.88 (q, 1H, *J* = 7.2 Hz), 7.22-7.46 (m, 7H), 7.74 (d, 1H, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.4, 19.5, 38.6, 46.4, 59.7, 113.1, 115.6, 127.0, 128.6, 128.9, 129.1, 131.5, 134.0, 137.1, 138.9, 144.3, 165.5, 179.5; MS (APCI): $[M+1]^+$ = 384.20; HRMS(ESI): *m/z* $[M+Na]^+$ calcd for $C_{20}H_{18}ClN_3OS$: 406.0757, found: 406.0757; $[\alpha]_{20}^D$ -195.87 (c=1.000, $CHCl_3$).

3-(3-Chloro-4-cyanophenyl)-6(*R*)-methyl-1-[(*R*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one 9'(c): White solid; Yield = 72%; mp = 218-220 °C; R_f 0.50 (3:7 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.48 (d, 3H, *J* = 6.7 Hz), 1.75 (d, 3H, *J* = 7.2 Hz), 2.45-2.60 (m, 2H), 3.71-3.77 (m, 1H), 6.92 (q, 1H, *J* = 7.2 Hz), 7.22-7.47 (m, 7H), 7.74 (d, 1H, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3, 19.5, 38.7, 46.4, 59.7, 113.1, 115.5, 127.0, 128.6, 128.9, 129.0, 131.5, 133.9, 137.1, 139.0, 144.3, 165.4, 179.6; MS (APCI): $[M+1]^+$ = 384.00; HRMS(ESI):*m/z* $[M+Na]^+$ calcd for $C_{20}H_{18}ClN_3OS$: 406.0757, found: 406.0766; $[\alpha]_{20}^D$ +195.82 (c=1.000, $CHCl_3$).

3-(4-Chloro-3-(trifluoromethyl)phenyl)-6(*S*)-methyl-1-[(*S*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one 9(d): White solid; Yield 76%; mp 126-127 °C; R_f 0.50 (3:7 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.49 (d, 3H, *J* = 6.8 Hz); 1.76 (d, 3H, *J* = 7.2 Hz), 2.45-2.61 (m, 2H), 3.70-3.77 (m, 1H), 6.95 (q, *J* = 7.1 Hz, 1H), 7.27-7.50 (m, 7H), 7.60 (d, 1H, *J* = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3, 19.5, 38.7, 46.3, 59.8, 121.1, 123.8, 127.0, 128.5, 129.0, 132.0, 132.2, 138.2, 139.1, 165.8, 180.1; MS (APCI): $[M+1]^+$ = 426.93; HRMS(ESI):*m/z* $[M+Na]^+$ calcd for $C_{20}H_{18}ClF_3N_2OS$: 449.0678, found: 449.0677; $[\alpha]_{20}^D$ -146.99 (c=1.000, $CHCl_3$).

3-(4-Chloro-3-(trifluoromethyl)phenyl)-6(*R*)-methyl-1-[(*R*)-1-phenylethyl]-2-thioxotetra hydropyrimidin-4(1*H*)-one 9'(d): White solid; Yield 76%; mp 126-127 °C; R_f 0.50 (3:7 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.49 (d, 3H, *J* = 6.4 Hz), 1.76 (d, 3H, *J* = 6.8 Hz), 2.46-2.61 (m, 2H), 3.72-3.75 (m, 1H), 6.95 (q, *J* = 6.8 Hz, 1H), 7.27-7.48 (m, 7H), 7.60 (d, 1H, *J* = 8.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3, 19.5, 38.7, 46.3, 59.8, 121.1, 123.8, 127.0, 128.5, 129.0, 132.0, 132.2, 138.2, 139.1, 165.8, 180.1; MS (APCI): $[M+1]^+$ = 426.95; HRMS(ESI):*m/z* $[M+Na]^+$ calcd for $C_{20}H_{18}ClF_3N_2OS$: 449.0678, found: 449.0685; $[\alpha]_{20}^D$ +146.91 (c=1.000, $CHCl_3$).

3-(4-Cyano-3-(trifluoromethyl)phenyl)-6(*S*)-methyl-1-[(*S*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one 9(e): White solid; Yield 78%; mp 178-180 °C; R_f 0.50 (3:7 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.49 (d, 3H, *J* = 6.8 Hz), 1.75 (d, 3H, *J* = 7.2 Hz), 2.46-2.61 (m, 2H), 3.71-3.78 (m, 1H), 6.88 (q, *J* = 7.1 Hz, 1H), 7.25-7.52 (m, 6 H),7.62 (s, 1H), 7.91 (d, 1H, *J* = 8.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3, 19.6, 38.6, 46.4, 59.8, 109.7, 115.1, 117.9, 120.7, 123.4, 126.1, 127.0, 128.6, 129.1, 135.2, 138.9, 143.7, 165.6, 179.5; MS (APCI): $[M+1]^+$ = 417.96; HRMS(ESI):*m/z* $[M+Na]^+$ calcd for $C_{21}H_{18}F_3N_3OS$: 440.1021, found: 440.1021; $[\alpha]_D$ -169.38 (c=1.000, $CHCl_3$).

3-(4-Cyano-3-(trifluoromethyl)phenyl)-6(*R*)-methyl-1-[(*R*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one 9'(e): White solid; Yield 74%; mp 178-180 °C; R_f 0.50 (3:7 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.49-1.51 (d, 3H, *J* = 6.28 Hz), 1.77 (d, 3H, *J* = 6.7 Hz), 2.48-2.63 (m, 2H), 3.76 (m, 1H), 6.87 (q, *J* = 7.1 Hz, 1H), 7.27-7.54 (m, 6H), 7.63 (s, 1H), 7.91 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3, 19.6, 38.6, 46.4, 59.8, 109.7, 115.1, 117.9, 120.7, 123.4, 126.1, 127.0, 128.6, 129.1, 135.2, 138.9, 143.7, 165.6, 179.5; MS (APCI): $[M+1]^+$ = 418.02; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{21}H_{18}F_3N_3OS$: 440.1021, found: 440.1019; $[\alpha]_{20}^D$ +169.42 (c=1.000, $CHCl_3$).

3-(4-Nitro-3-(trifluoromethyl)phenyl)-6(*S*)-methyl-1-[(*S*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one 9(f): Yellow solid; Yield 72%; mp 134-136 °C; R_f 0.50 (3:7 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.48-1.50 (d, 3H, *J* = 6.40 Hz), 1.75-1.77 (d, 3H, *J* = 7.20 Hz), 2.46-2.62 (m, 2H), 3.72-3.79 (m, 1H), 6.86-6.91 (q, 1H, *J* = 7.20 Hz), 7.25-7.64 (m, 7H), 7.99-7.97 (d, 1H, *J* = 8.40 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3, 19.6, 38.6, 46.4, 59.8, 120.3, 123.3, 124.8, 125.9, 127.1, 128.4, 129.0, 138.9, 140.6, 143.4, 147.0, 165.6, 179.5; MS (APCI): $[M+1]^+$ = 437.93; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{20}H_{18}F_3N_3O_3S$: 460.0919, found: 460.0921; $[\alpha]_{20}^D$ -119.81 (c=1.000, $CHCl_3$).

3-(4-Nitro-3-(trifluoromethyl)phenyl)-6(*R*)-methyl-1-[(*R*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one 9'(f): Yellow solid; Yield 72%; mp 134-136 °C; R_f 0.50 (3:7 EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50 (d, 3H, *J* = 6.2 Hz), 1.76 (d, 3H, *J* = 6.8 Hz), 2.47-2.62 (m, 2H), 3.76 (m, 1H), 6.90 (q, 1H, *J* = 6.6 Hz), 7.25-7.64 (m, 7H), 7.99 (d, 1H, *J* = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.3, 19.6, 38.6, 46.5, 59.8, 120.3, 123.0, 124.8, 125.9, 127.0, 128.7, 129.1, 134.3, 138.9, 143.4, 147.0, 165.6, 179.5; MS (APCI): $[M+1]^+$ = 437.97; HRMS (ESI): *m/z* $[M+Na]^+$ Calcd for $C_{20}H_{18}F_3N_3O_3S$: 460.0919, found: 460.0919; $[\alpha]_{20}^D$ +119.86 (c=1.000, $CHCl_3$).

General procedure for the syntheses of 3-aryl-6-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-ones [10/10' (a-f): In a typical experiment 3-aryl-6(*S*)-methyl-1-[(*S*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one (1.2 mmol) was taken in a RB flask and HBr in AcOH (5 mL) was added to it. The solution was stirred at room temperature until the reaction was complete as observed by TLC. It was concentrated and the residue was diluted with DCM, washed with $NaHCO_3$ (2 x 15 mL), then with brine (1 x 15 mL) and dried over anhydrous Na_2SO_4. The reaction mixture was concentrated and purified by column chromatography on silica gel (60–100) using EtOAc-hexane mixture (35:65) as the eluent to afford 3-aryl-6(*S*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one. By an analogous procedure 3-aryl-6(*R*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one was obtained from 3-aryl-

6(*R*)-methyl-1-[(*R*)-1-phenylethyl]-2-thioxotetrahydropyrimidin-4(1*H*)-one.

3-(4-Chlorophenyl)-6(*S*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one, 10(a): White solid; Yield 72%; mp 249-251 °C; R_f 0.44 (5:5 EtOAc-hexane); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25 (d, 3H, J = 5.6 Hz), 2.66–2.72 (m, 1H), 2.84-2.88 (m, 1H), 3.87 (m, 1H), 7.16 (d, 2H, J = 7.5 Hz), 7.43 (d, 2H, J = 7.6 Hz), 10.06 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.3, 37.6, 44.9, 128.3, 131.6, 132.2, 138.2, 167.1, 180.4; MS (APCI): $[M+1]^+$ = 255.20; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{11}H_{11}ClN_2OS$: 277.0179; found: 277.0168; $[\alpha]_{20}^D$ –20.18 (c=1.00, acetone).

3-(4-Chlorophenyl)-6(*R*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one, 10'a: White solid; Yield 74%; mp 249-251 °C; R_f 0.44 (5:5 EtOAc-hexane); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.26 (d, 3H, J = 6.4 Hz), 2.66–2.73 (m, 1H), 2.84-2.89 (m, 1H), 3.87 (m, 1H), 7.16 (d, 2H, J = 7.6 Hz), 7.44 (d, 2H, J = 7.6 Hz), 10.06 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.8, 38.1, 45.4, 128.8, 132.1, 132.7, 138.7, 167.6, 180.9; MS (APCI): $[M+1]^+$ = 255.27; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{11}H_{11}ClN_2OS$: 277.0179; found: 277.0169; $[\alpha]_{20}^D$ +20.15 (c=1.00, acetone).

3-(3-Chloro-4-fluorophenyl)-6(*S*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one, 10b: White solid; Yield 68%; mp 230-233 °C; R_f 0.48 (5:5 EtOAc-hexane); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25 (d, 3H, J = 5.4 Hz), 2.66–2.71 (m, 1H), 2.84-2.89 (m, 1H), 3.87 (m, 1H), 7.20 (s, 1H), 7.41-7.49 (m, 2H), 10.10 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.3, 37.6, 44.9, 116.3, 118.7, 118.9, 131.9, 136.2, 155.3, 157.7, 167.1, 180.3; MS (APCI): [M+1]+ = 273.29; HRMS (ESI*)*: *m/z* [M+H]+ calcd for $C_{11}H_{10}ClFN_2OS$: 273.0265; found: 273.0812; $[\alpha]_{20}^D$ –19.31 (c=1.00, acetone).

3-(3-Chloro-4-fluorophenyl)-6(*R*)-methyl-2-thioxotetrahydropyrimidin-4(1H)-one, 10'b: White solid; Yield 70%; mp 230-233 °C; R_f 0.48 (5:5 EtOAc-hexane); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.26 (d, 3H, J = 6.6 Hz), 2.65–2.72 (m, 1H), 2.84-2.89 (m, 1H), 3.85-3.90 (m, 1H), 7.18-7.21 (s, 1H), 7.41-7.50 (m, 2H), 10.10 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.3, 37.6, 44.9, 116.3, 118.7, 118.9, 131.9, 136.2, 155.3, 157.7, 167.1, 180.3; MS (APCI): [M+1]+ = 273.32; HRMS (ESI): *m/z* [M+Na]+ calcd for $C_{11}H_{10}ClFN_2OS$: 295.0084; found: 295.0093; $[\alpha]_{20}^D$ +19.37 (c=1.00, acetone).

3-(3-Chloro-4-cyanophenyl)-6(*S*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one, 10c: White solid; Yield 75%; mp 236-238 °C; R_f 0.35 (5:5 EtOAc-hexane); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.26 (m, 3H, J = 5.6 Hz), 2.67–2.73 (m, 1H), 2.85-2.90 (m, 1H), 3.89 (m, 1H), 7.42 (d, 1H, J= 8.0 Hz), 7.73(s, 1H), 8.02 (d, 1H, J = 8.0 Hz), 10.21 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.2, 37.4, 45.1, 111.2, 115.7, 130.1, 131.7, 134.3, 134.9, 144.8, 167.0, 179.5; MS (APCI): $[M+1]^+$ = 280.28; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{12}H_{10}ClN_3OS$: 302.0131; found: 302.0136; $[\alpha]_{20}^D$ –30.72 (c=1.00, acetone).

3-(3-Chloro-4-cyanophenyl)-6(*R*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one, 10'c: White solid; Yield 67%; mp 236-238 °C; R_f 0.35 (5:5 EtOAc-hexane); ^{1}H NMR (400 MHz, DMSO-*d*6): δ 1.26 (m, 3H, J = 5.6 Hz), 2.67–2.73 (m, 1H), 2.85-2.90 (m, 1H), 3.89 (m, 1H), 7.42 (d, 1H, J= 8.0 Hz), 7.73 (s, 1H), 8.02 (d, 1H, J = 8.0 Hz), 10.21 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.2, 37.4, 45.1, 111.2, 115.7, 130.1, 131.7, 134.3, 134.9, 144.8, 167.0, 179.5; [M+1]$^+$ = 280.28; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{12}H_{10}ClN_3OS$: 302.0131; found: 302.0123; $[\alpha]_{20}^D$ +30.78 (c=1.00, acetone).

3-(4-Chloro-3-(trifluoromethyl)phenyl)-6(*S*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one, 10d: White solid; Yield 65%; mp 239-241 °C; R_f 0.51 (5:5 EtOAc-hexane); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.26 (d, 3H, J = 6.5 Hz), 2.67–2.74 (m, 1H), 2.84-2.89 (m, 1H), 3.88-3.93 (m, 1H), 7.52 (d, 1H, J= 8.5 Hz), 7.76 7.76 (s, 2H), 10.16 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.3, 37.5, 45.0, 121.2, 123.9, 126.7, 129.6, 131.7, 135.7, 138.8, 167.2, 180.0; MS (APCI): $[M+1]^+$ = 323.24; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{12}H_{10}ClF_3N_2OS$ 345.0052; found: 345.0044; $[\alpha]_{20}^D$ –38.29 (c=1.00, acetone).

3-(4-Chloro-3-(trifluoromethyl)phenyl)-6(*R*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one, 10'd:White solid; Yield 62%; mp 239-241 °C; R_f 0.51 (5:5 EtOAc-hexane); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.26 (d, 3H, J = 6.0 Hz), 2.70–2.74 (m, 1H), 2.84-2.89 (m, 1H), 3.91 (m, 1H), 7.52 (d, 1H, J= 8.4 Hz), 7.76 (s, 2H), 10.16 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.8, 38.0, 45.5, 121.7, 124.4, 126.9, 130.1, 132.2, 136.2, 139.2, 167.7, 180.5; MS (APCI): $[M+1]^+$ = 323.20; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{12}H_{10}ClF_3N_2OS$ 345.0052; found: 345.0048; $[\alpha]_{20}^D$ +38.37 (c=1.00, acetone).

3-(4-Cyano-3-(trifluoromethyl)phenyl)-6(*S*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one, 10e: White solid; Yield 69%; mp 225-227 °C; R_f 0.35 (5:5 EtOAc-hexane); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.27 (d, 3H, J = 5.8 Hz), 2.69–2.76 (m, 1H), 2.86-2.91 (m, 1H), 3.93 (m, 1H), 7.77 (d, 1H, J = 7.9 Hz), 8.0 (s, 1H), 8.23 (d, 1H, J = 7.9 Hz), 10.24 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.2, 37.4, 45.1, 107.6, 115.2, 123.7, 129.2, 131.0, 135.1, 135.8, 144.3, 167.1, 179.5; MS (APCI): $[M+1]^+$ = 314.27; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{13}H_{10}F_3N_3OS$: 336.0395; found: 336.0383; $[\alpha]_{20}^D$ –28.20 (c=1.00, acetone).

3-(4-Cyano-3-(trifluoromethyl)phenyl)-6(*R*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one, 10'e: White solid; Yield 66%; mp 225-227 °C; R_f 0.35 (5:5 EtOAc-hexane); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.27 (d, 3H, J = 5.8 Hz), 2.69–2.76 (m, 1H), 2.86-2.91 (m, 1H), 3.93 (m, 1H), 7.77 (d, 1H, J = 7.9 Hz), 8.0 (s, 1H), 8.23 (d, 1H, J = 7.9 Hz), 10.26 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.7, 37.9, 45.6, 108.1, 115.7, 124.2, 129.6, 131.2, 135.6, 136.3, 144.8, 167.6, 180.0; MS (APCI): $[M+1]^+$ = 314.35; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{13}H_{10}F_3N_3OS$: 336.0395; found: 336.0388; $[\alpha]_{20}^D$ +28.26 (c=1.00, acetone).

3-(4-Nitro-3-(trifluoromethyl)phenyl)-6(*S*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one, 10f: Yellow solid;Yield 64%; mp 199-201 °C; R_f 0.52 (5:5 EtOAc-hexane); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.28 (d, 3H, J = 6.0 Hz), 2.70–2.76 (m, 1H), 2.86-2.91 (m, 1H), 3.94 (m, 1H), 7.82 (d, 1H, J = 8.4 Hz), 8.03 (s, 1H), 8.21 (d, 1H, J = 8.3 Hz), 10.26 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.2, 37.4, 45.1, 121.7, 123.2, 125.9, 130.0, 136.0, 143.6, 146.2, 167.2, 179.6; MS (APCI): $[M+1]^+$ = 334.19; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{12}H_{10}F_3N_3O_3S$: 356.0293; found: 356.0286; $[\alpha]_{20}^D$ –36.97 (c=1.00, acetone).

3-(4-Nitro-3-(trifluoromethyl)phenyl)-6(*R*)-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-one, 10'f: Yellow solid; Yield 68%; mp 199-201 °C; R_f 0.52 (5:5 EtOAc-hexane); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.29 (d, 3H, J = 6.6 Hz), 2.71–2.77 (m, 1H), 2.87-2.92 (m, 1H), 3.93 (m, 1H), 7.83 (d, 1H, J = 8.5 Hz), 8.04 (s, 1H), 8.22 (d, 1H, J = 8.5 Hz), 10.26 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.7, 37.9, 45.6, 122.2, 123.7, 126.4, 130.5, 136.5, 144.1, 146.7, 167.7, 180.1; MS (APCI): $[M+1]^+$ = 334.27; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{12}H_{10}F_3N_3O_3S$: 356.0293; found: 356.0287; $[\alpha]_{20}^D$ +36.91 (c=1.00, acetone).

Molecular Modeling Studies

The molecular docking studies were performed to predict the ligand binding affinity as well as correct binding poses in the protein active site.[12] Molecular docking of the designed analogues was performed using Glide software included in Schrodinger suite 9.0.211. The crystal structure of the androgen receptor ligand-binding domain, W741L mutant complex with *R*-bicalutamide (pdb id 1Z95) is used

for molecular docking.[13] Protein structure was prepared using protein preparation wizard for molecular docking as follows; hydrogen atoms were added, hydrogen bonding network was optimized, and protein was minimized to RMSD (Root Mean Square Deviation) 0.30Å using OPLS (Optimized Potential for Liquid Simulations) 2005 force field. The designed ligands were prepared in LigPrep and minimized using OPLS 2005 force field. The co-crystallized ligand bicalutamide was used to define binding site grid in protein structure. The ligand docking calculations were done in the standard precision mode of glide software. Ligands were passed through a scaling factor of 0.80 and partial charge cutoff of 0.15.1[14]

Pharmacological Studies

All *in-vitro* experiments for cell proliferation/inhibition were performed in triplicates. PC-3 cells were originally derived from advanced androgen independent bone metastasized prostate cancer. For the PC-3 cell growth inhibition assay, cells were cultured in DMEM supplement and trypsinized, further diluted to 2.0 x 10^4 cell/mL with DMEM supplemented with 10% fetal bovine serum. This cell suspension was transferred to 96-well microtiter plates, and incubated in the presence or absence of increasing concentration of positive control (doxorubicin) or the test compounds (10^{-12}M, 10^{-10}M, 10^{-8}M, 10^{-6}M and 10^{-4}M) at 37^{o}C and 5% CO_2. After 24h incubation, cells were treated with MTT solution for 4h in a cell culture incubator at 37 ^{o}C and 5% CO_2. Cell proliferation was determined by the MTT method. MTT which is a tetrazolium salt is converted into insoluble formazan by mitochondrial dehydrogenases in live cells. Formazan is dissolved in DMSO (Merck) and absorbance was measured at dual wavelength of 550 nm and 630 nm on an ELISA plate spectrophotometer (Biotrek instruments). Similar experiments were performed in LNCaP cells also. Since LNCaP cells have demonstrated androgen dependent cell growth; promotion and inhibition of cell growth was considered as agonistic and antagonistic respectively. These cells are known to possess an aberrant AR with a mutation of 877Thr to Ala. LNCaP cells were cultured as described above in RPMI1640 media and were transferred into a 96-well plate with a 2.0 x 10^4 cell/mL well density supplemented with testosterone (final concentration in each well was kept 10 nM). After 24h, the cells were treated with testosterone in the presence or absence of each concentration of positive control (Flutamide) or test compounds (10^{-10}M, 10^{-8}M, 10^{-6}M and 10^{-4}M) for another 24h. The total number of viable cells relative to viable cells in untreated control was plotted. For LNCaP cells, the number of cells on wells with testosterone alone was defined as 100% viability.

Conclusions

In conclusion, optically pure enantiomers of 3-aryl-6-methyl-2-thioxotetrahydropyrimidin-4(1*H*)-ones were obtained in good yields and high selectivities by asymmetric induction using chiral (*R*)/(*S*)-α-methyl benzylamine. *In vitro* cytotoxicity of these derivatives was evaluated on PC-3 and LNCaP cell lines for the anti-prostate cancer activity. Compound **10'e** was found to be highly potent with cytotoxicity comparable with doxorubicin on PC-3 cell lines and better than the standard drugs flutamide and bicalutamide on LNCap cell lines though its enantiomer was 20 fold less active.

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