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Potent anti-prostate cancer agents derived from a novel androgen receptor down-regulating agent

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Abstract—The search for novel androgen receptor (AR) down-regulating agents by catalyst HipHop pharmacophore modeling led to the discovery of some lead molecules. Unexpectedly, the effect of these leads on human prostate cancer LNCaP cell viability did not correlate with the ability of the compounds to cause down-regulation of AR protein expression. Through rational synthetic optimization of the lead compound (BTB01434), we have discovered a series of novel substituted diaryl molecules as potent anti-prostate cancer agents. Some compounds (**1**–**6**) were shown to be extremely potent inhibitors of LNCaP cell viability with GI_{50} values in the nanomolar range (1.45–83 nM). The most potent compound (4-methylphenyl)[(4-methylphenyl)sulfonyl]amine (**5**) with a GI_{50} value of 1.45 nM is *27,000 times more potent* than our lead compound BTB01434 (GI_{50} = 39.8 μM). In addition, some of the compounds exhibited modest anti-androgenic activities and one was also a potent inhibitor (GI_{50} = 850 nM) of PC-3 (AR-null) cell growth. A clear structure–activity relationship (SAR) has been established for activity against LNCaP cells, where potent molecules possess two substituted/unsubstituted aromatic rings connected through a sulfonamide linker. These novel compounds are strong candidates for development for the treatment of hormone-sensitive and importantly hormone-refractory prostate cancers in humans.

1. Introduction

Prostate cancer (PC) is the most common cancer among males of Western countries[1] and is a complex heterogeneous disease that acts differently in different men. The underlying cause of prostate cancer is still unknown. However, androgen and the androgen receptor (AR) are postulated to play crucial roles in the development of prostate cancer.[2] The current treatment for prostate cancer is a combination of surgery, radiation, and chemotherapy. The therapeutic agents used clinically include steroidal antiandrogens, such as cyproterone acetate, and nonsteroidal antiandrogens, such as flutamide and bicalutamide. The steroidal antiandrogens possess partial agonistic activity and overlapping effects with other hormonal systems, leading to many complications including severe cardiovascular problems, gynecomastia, loss of libido, and erectile dysfunction.[3–5] The nonsteroidal antiandrogens show fewer side effects and have improved oral bioavailability; therefore, they are favored over the steroidal antiandrogens. However, antiandrogen withdrawal syndrome has been discovered in patients receiving nonsteroidal antiandrogens for several months.[6,7] Long-term drug usage probably leads to mutation of the AR, and the nonsteroidal antiandrogens now exhibit agonistic activity to the mutant AR.[8] In addition, the clinically available antiandrogens are unable to kill prostate cancer cells, and within one to three years of drug administration, the cancer usually progresses into an androgen-refractory phenotype which is not curable. The poor clinical outcome of advanced metastatic PC highlights the urgent need to develop effective novel agents for prevention and treatment of this disease.

The addition of novel and potent drug candidates to the existing therapy would certainly improve the outcome of PC therapy. Androgen receptor down-regulating agents (ARDAs) have emerged as an attractive target for the development anti-prostate cancer drugs.[9–13] Until recently most of the agents known as ARDAs were natu-

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ral chemicals.[14–18] Recently, we discovered six synthetic small molecules, including BTB01434 (see Fig. 1a and b) as ARDAs (EC_{50} values 17.5–212 μM range) through the development of a qualitative 3D pharmacophore modeling strategy.[19] These compounds were also shown to be modest inhibitors (GI_{50} values 4.5–39.8 μM) of the growth of LNCaP cells.[19] However, the anti-proliferative effects on LNCaP cells by these molecules did not correlate to their ARDA activities.

Since it is well known that sulfonamides have a variety of biological activities, there appeared to be the possibility that new anti-cancer sulfonamides could be discovered from our new lead compounds. Because of the relatively simple chemical structure of BTB01434 and also potential ease of its chemical modifications, this compound was selected for lead optimization and subsequent evaluations of the new analogs for anti-prostate cancer activities. We have now prepared three series of

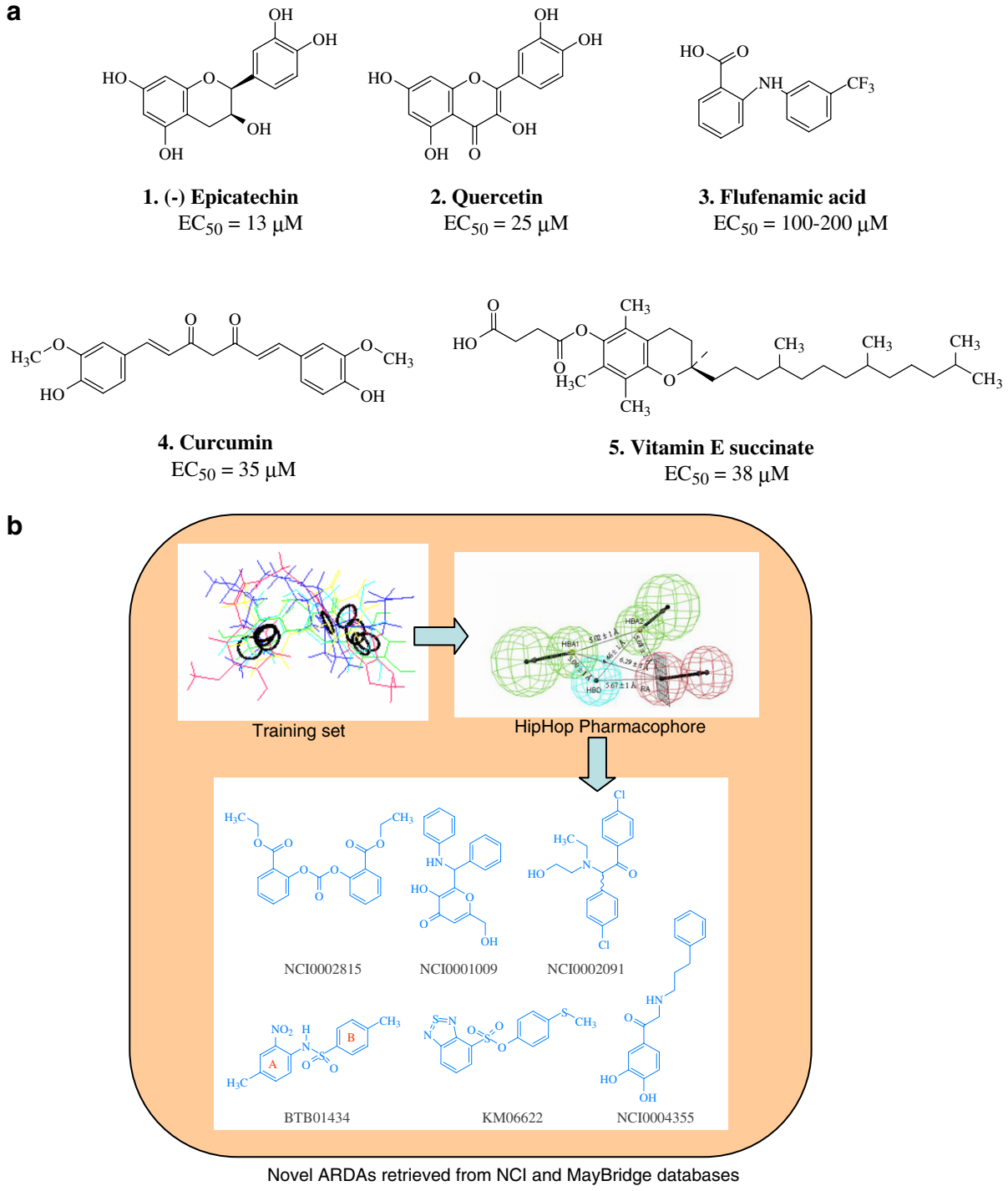
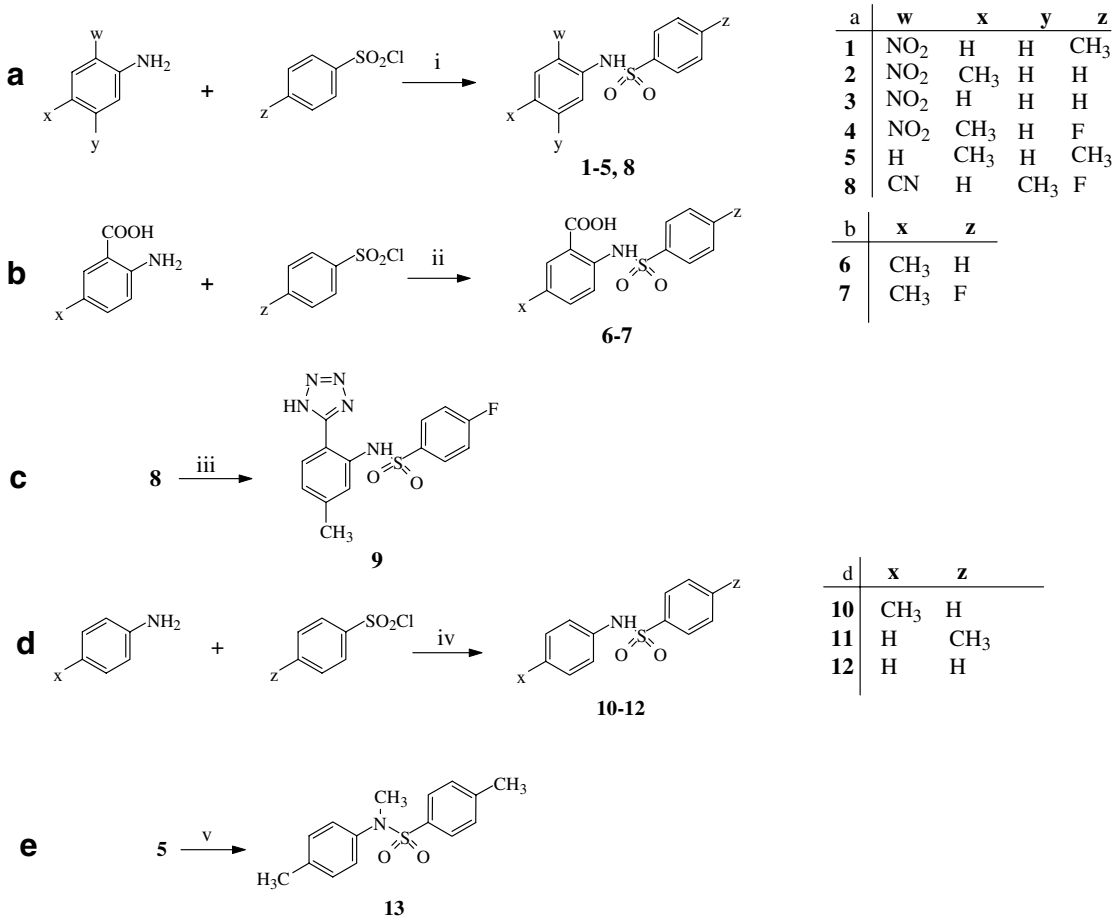


Figure 1. (a) Chemical structures of five known androgen receptor down-regulating agents (ARDAs) used to generate the pharmacophore model.[19] (b) Synthetic ARDAs discovered through generation of HipHop 3D pharmacophore modeling.[19]

new BTB01434 analogs, including diaryl sulfonamide analogs bearing various substituents on the phenyl rings (series A), aryl amide analogs (series B), and amine analogs (series C). These new compounds were evaluated for cell growth inhibition in two human prostate cancer cell lines, (LNCaP and PC-3) by the MTT assay. Based on the structures and anti-prostate cancer activities of the new compounds, a structure–activity relationship (SAR) was formulated. This SAR clearly reveals the structural features of the new compounds responsible for the growth inhibitory activities in human PC cells. This paper describes the discovery of novel anti- PC sulfonamide agents, including design, synthesis, and structure–activity relationship.[20]

2. Chemistry

A total of 16 compounds described in this study were prepared following straightforward chemistry as outlined in Scheme 1a–e (series A), Scheme 2 (series B), and Scheme 3 (series C). Compounds **1**–**5** and **8** were synthesized by refluxing the appropriate substituted aryl amines with the corresponding aryl sulfonyl chlorides in pyridine (Scheme 1a).[21] The carboxylic acid containing compounds **6** and **7** were synthesized by treatment of 2-amino-5-methylbenzoic acid in aqueous Na_2CO_3 solution at 60 °C followed by reaction with appropriate benzene sulfonyl chloride at 80 °C (Scheme 1b).[22] Treatment of the cyano compound **8** with sodium azide and ammonium chloride in DMF at 120 °C afforded the corresponding tetrazole **9** (Scheme 1c).[23] Compounds **10**–**12** were synthesized by simply triturating the appropriate substituted anilines with the corresponding aromatic sulfonyl chlorides (Scheme 1d).[24] The tertiary sulfonamide compound **13** was obtained by treatment of **5** with methyl iodide in the presence of 1-butyl-3-methyl imidazolium hexafluro phosphate at room temperature (Scheme 1e).[25] Reaction of the appropriate aryl amine with aryl aldehyde in the presence of triethyl amine afforded benzamide **14** (Scheme 2).[26] Finally, the aryl amines **16** and **18** were obtained by reducing intermediate imines **15** and **17**, respectively, with sodium borohydride. The intermediate imines (**15** and **17**) were prepared by condensation of appropriate aryl amines and benzaldehydes (Scheme 3).[27] All compounds were properly characterized by physical and spectroscopic analyses. However, with regard to compound **6**, we observed a significant difference in the melting point reported for this compound in the literature (130 °C) from that obtained in this study (197–198 °C). The spectral data (^{1}H NMR, ^{13}C NMR, and HRMS) confirm with certainty the integrity of this compound. Except compounds **4**, **7**, **8**, and **9**, all other compounds described in the study have previously been reported.[21,25,28–35]

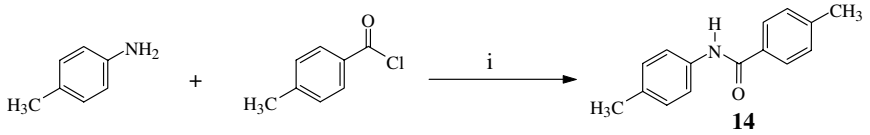


a	w	x	y	z
1	NO_2	H	H	CH_3
2	NO_2	CH_3	H	H
3	NO_2	H	H	H
4	NO_2	CH_3	H	F
5	H	CH_3	H	CH_3
8	CN	H	CH_3	F

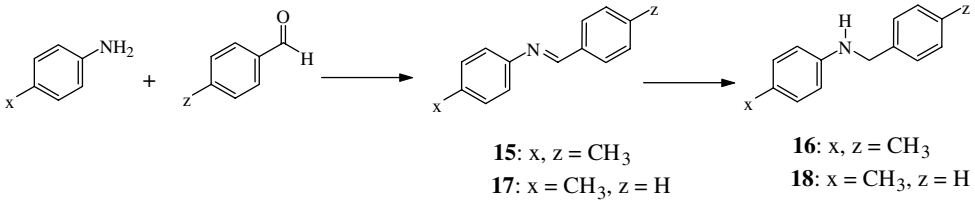
b	x	z
6	CH_3	H
7	CH_3	F

d	x	z
10	CH_3	H
11	H	CH_3
12	H	H

Scheme 1. Synthesis of sulfonamide compounds (series A) (**1**–**13**). Reagents and conditions: (i) pyridine, reflux, 125 °C, 6 h; (ii) Na_2CO_3, reflux, 80 °C, 6 h; (iii) NH_4Cl, NaN_3, DMF, 120 °C, 20 h; (iv) $NaHCO_3$, rt, 5–10 min; (v) CH_3I, 1-butyl-3-methyl imidazolium hexafluoro phosphate, rt, 2 h.



Scheme 2. Synthesis of arylamide derivative (series B) (**14**). Reagents and conditions: (i) TEA, EtOAc, rt, 12 h.



Scheme 3. Synthesis of aryl imines and amines (series C) (**15**–**18**). Reagents and conditions: (i) EtOH, reflux, 12 h; (ii) MeOH, $NaBH_4$, rt, 10 min.

3. Results and discussion

3.1. Inhibition of prostate cancer cell proliferation and SAR

All 16 compounds were tested for their ability to inhibit cell viability of both the LNCaP (mutant AR) and PC-3 (AR null) human prostate cancer cell lines using the MTT assay as we had previously described[19] and the GI_{50} values are presented in Table 1. Clearly, the LNCaP cell line was exquisitely more sensitive than the PC-3 cell line to the growth inhibitory effects of our compounds. The GI_{50} values for the LNCaP cell line ranged from 1.45 to >100,000 nM, while those for PC-3 cell line ranged from 850 to >100,000 nM. Figure 2 shows the dose–response curves for the effects of compounds **4** and **5** on inhibition of growth of both LNCaP and PC-3 human prostate cancer cell lines. The reason(s) underlying this difference in sensitivity between the two cell lines is unknown at this time. One possibility is that the mutant androgen receptor present in the LNCaP cell may be responsible for its sensitivity to our compounds. Therefore, the structure–activity relationship (SAR) discussed below is with respect to their activities against the LNCaP cell line.

The strategy for the design of new molecules was devised using lead compound BTB01434 (Fig. 1b, GI_{50} = 39,800 nM) on the basis of its simple structure and also envisioned ease of optimization. Thus, we planned to prepare a series of analogs by substituting and/or removing the functional groups on the two aromatic rings (A and B; see Fig. 1b) and also by introducing different linkers between the two aromatic rings.

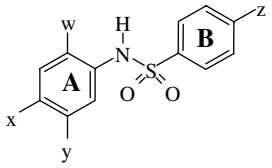
Firstly, examination of the growth inhibitory data reveals that substituting any of the three aryl substituents (i.e., CH_3 of ring B and CH_3 or NO_2 of ring A) with either H, F, or CO_2H led to interesting SAR information. Clearly, these substitutions led to excellent enhancement (480- to 27,000-fold) in growth inhibitory activities. Substitution of CH_3 of ring B with H resulted in the largest enhancement of activity (GI_{50} from 39,800 nM of BTB01434 → 2.95 nM of **2**), while replacement of the methyl group of ring A with H also yielded excellent enhancement of activity (GI_{50} from 39,800 nM of BTB01434 → 83.0 nM of **1**). Interestingly, removal of both CH_3 groups from rings A and B resulted in compound **3**, with GI_{50} of 14.8 nM, a value 5-fold less potent than **2** and 5.6-fold more potent than **1**. Introduction of the electron withdrawing fluorine in ring B of **2** yielded compound **4** (GI_{50} = 2.34 nM) with no significant change in activity. The strong electron withdrawing NO_2 group appears to cause a decrease in activity, because its replacement with H led to exquisite enhancement of activity (GI_{50} from 39,800 nM of BTB01434 → 1.45 nM for **5**). However, its replacement with the moderate electron withdrawing CO_2H in the absence of the CH_3 group of ring B resulted in a 6-fold decrease in activity (GI_{50} from 2.95 of **2** → 18.2 nM for **6**). Introduction of a *p*-fluoro group in ring B of compound **6** resulted in **7** with a drastic decrease in activity. In addition, as shown for compounds **8** and **9**, replacement of the nitro group with either CN or 1H-tetrazole also afforded deleterious effects on inhibitory activity.

We further investigated the effects of the methyl substituents on rings A and B of our most potent compound **5** (GI_{50} = 1.45 nM). As shown in compounds **10**, **11**, and **12**, removal of either or both methyl groups resulted in a drastic loss of activity. Clearly, the effects of the methyl groups in **5** are in contrast to their effects on the potency of compound **1** described above. To probe the contribution of the –NH-group, we synthesized compound **13**, an *N*-methyl derivative of **5**. Introduction of the *N*-methyl group resulted in a drastic decrease in activity (GI_{50} from 1.45 nM of **5** to 89, 150 nM of **13**). Furthermore, replacement of the sulfonamide linker ($–SO_2NH–$) with two other types of linkers, that is, amide (**14**), and amines (**16** and **18**) resulted in a significant loss of activity.

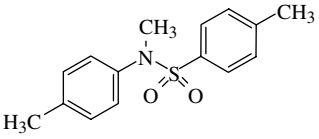
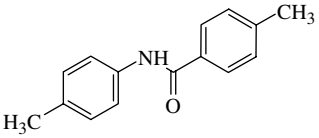
3.2. Other biological studies

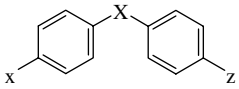
3.2.1. Effects of 1 and 5 on AR protein expression. As stated above, some compounds, such as **4** and **6** also

Table 1. In vitro growth inhibitory activities of LNCaP and PC-3 human prostate cancer cells by BTB01434 analogs



Compound	w	x	y	z	Cytotoxicity GI_{50} (nM)		ARDA
					LNCaP	PC-3	EC_{50} (μM)
A							
BTB01434	NO_2	CH_3	H	CH_3	39,800	43,670	76.0
1	NO_2	H	H	CH_3	83.00	50,110	>150
2	NO_2	CH_3	H	H	2.95	60,250	NT
3	NO_2	H	H	H	14.8	22,900	NT
4	NO_2	CH_3	H	F	2.34	850.0	NT
5	H	CH_3	H	CH_3	1.45	30,200	150
6	COOH	CH_3	H	H	18.20	1350	NT
7	COOH	CH_3	H	F	10,700	>100,000	NT
8	CN	H	CH_3	F	30,200	~100,000	NT
9	1H-tetrazole	H	CH_3	F	>100,000	>100,000	NT
10	H	CH_3	H	H	21,300	13,800	NT
11	H	H	H	CH_3	24,000	38,900	NT
12	H	H	H	H	53,500	>100,000	NT

	Structure	Cytotoxicity GI_{50} (nM)		ADRA (μM)
		LNCaP[a]	PC-3[a]	
13		89,150	44,660	NT
B				
14		54,950	26,920	NT



Compound	x	X	z	Cytotoxicity GI_{50} (nM)		ADRA (μM)
				LNCaP	**PC-3**	
C						
15	CH_3	N=CH	CH_3	NT	NT	NT
16	CH_3	HN–CH_2	CH_3	19,050	29,510	NT
17	CH_3	N=CH	H	NT	NT	NT
18	CH_3	HN–CH_2	H	>100,000	52,400	NT

[a] Values are means of three independent experiments, SEM = ±10%, NT, not tested. Compounds **15** and **17** were unstable under the assay conditions.

showed potent inhibition of PC-3 cell growth which suggests that they might have mechanisms of action other than interrupting the AR signaling pathway in human prostate cancer cells. Because these compounds are analogs of BTB01434, a modest ARDA (EC_{50} = 76 μM),[19] it was of interest to evaluate the effects of selected compounds on AR protein expression and also on their abilities to bind to and cause transactivation of the AR.

For comparison purposes, the most and least effective of the first series of compounds (i.e., **1**–**6**) in the LNCaP cells, **5** (GI_{50} = 1.45 nM), and **1** (GI_{50} = 83.0 nM), respectively, were assessed for their AR down-regulating activity as we have previously described.[19] Both compounds were not effective at decreasing AR protein expression since they each caused less than 50% AR protein expression at relatively high concentrations (150 μM). Although the other four compounds were not tested, it is probable that they may also behave in a similar fashion. These results demonstrates that the growth inhibitory effects of these compounds are due to properties not related to AR down-regulation.

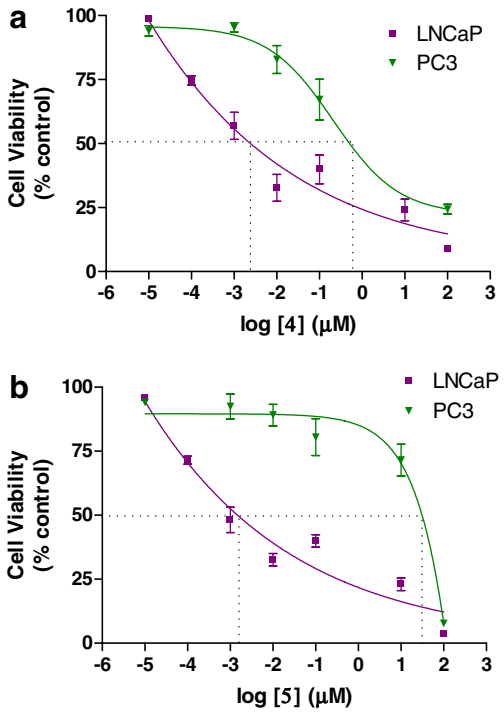


Figure 2. The effect of **4** (a) and **5** (b) on LNCaP and PC3 cell viability. Data plotted are mean values ±SEM for three independent experiments.

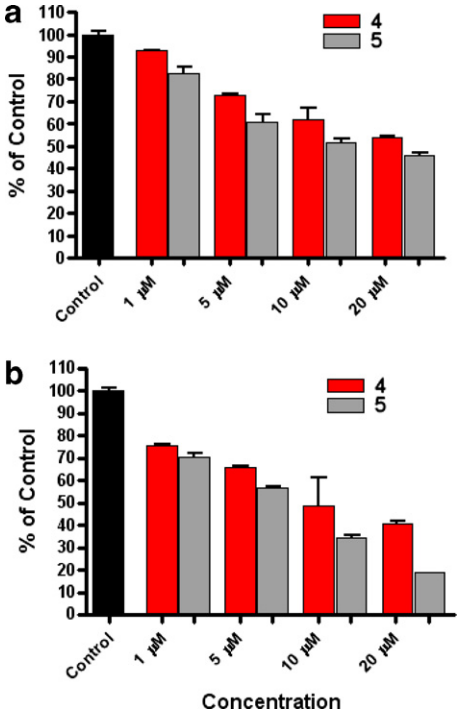


Figure 3. Dose–response for the competition of **4** and **5** for AR binding to [^{3}H]-R1881 in (a) LNCaP cells and in (b) PC-3-AR transfected cells. Data plotted are mean values ±SEM for three independent experiments.

3.2.2. LNCaP and PC-3AR androgen receptor binding and AR-mediated transcription studies. Competitive androgen binding studies were carried out utilizing the labeled synthetic androgen [^{3}H]-R1881 in androgen-dependent LNCaP cells that express a mutated AR, and androgen independent PC-3 cells stably transfected with the wild-type AR (PC-3-AR) as previously described by our group.[36] In this assay, we selected the most active compound **5** in LNCaP cells (GI_{50} = 1.45 nM) and **4** that was active in both LNCaP (GI_{50} = 2.34 nM) and PC-3 (GI_{50} = 850 nM) cell lines. In both cell lines, compounds **4** and **5** were able to effectively compete with labeled R1881 in a dose-dependent fashion, albeit at high concentrations (Fig. 3). In PC-3-AR cells **5** reduced [^{3}H]-R1881 binding by 65% and 80% at 10 and 20 μM, respectively. However, **5** favored binding to the wild-type AR over the mutated receptor, with an approximately 20% greater inhibition of [^{3}H]-R1881 binding to the wild-type AR at 10 μM and above. Compound **4** reduced [^{3}H]-R1881 binding by 50% and 65% at 10 and 20 μM, respectively, and was equipotent in both cell lines.

To determine if the compounds act as AR agonists or antagonists, androgen regulated transcriptional activation studies were performed in LNCaP cells transiently transfected with the probasin luciferase reported construct ARR2-Luc.[36] The results are presented in Figure 4. 5 nM DHT stimulated luciferase activity approximately 200-fold. Compound **5** alone had no effect on luciferase activity at 0.1 and 10 μM concentrations. Compound **4** did not increase luciferase expression at 10 μM, however, there was a slight increase at 0.1 μM. In the presence of DHT, compounds **4** and **5** reduced luciferase expression by 50% and 80% at 10 μM, respectively. Taken together, these results suggest that both compounds do not possess AR agonistic activity and may be considered as modest, pure androgen receptor antagonists. It is relevant to point out that the potency of compound **5** is similar to potencies of our steroidal CYP17/antiandrogens and casodex, a clinically used anti-androgen that were previously tested by us in a similar assay[36] as that used in this study. Although we did not test the compounds with PC-3AR/LU cells,

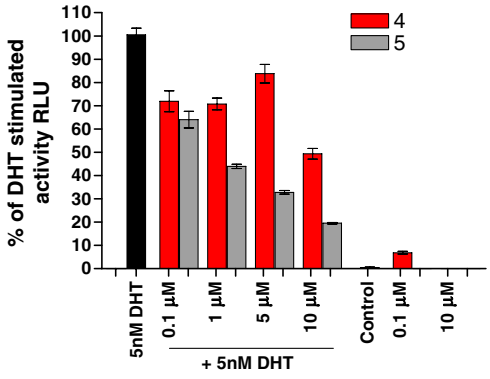


Figure 4. The effects of **4** and **5** on transcriptional activity of luciferase mediated through LNCaP-AR in LNCaP-ARR2-lu prostate cancer cells. Cells in steroid-free medium were treated with vehicle, or increasing concentrations of either **4** or **5** with and without 5 nM DHT for 18 h. Cells were then assayed for luciferase activity as described previously.[36] Data plotted are mean values ±SEM for three independent experiments.

which express wild-type AR, it is likely that they may also behave in a similar fashion. In general, these agents interacted modestly with both AR types, an indication that the compounds may be useful for the treatment of patients with tumors expressing either wild-type or mutated AR.

The biological studies with these lead-modified compounds (**1**–**14**, **16**, and **18**) suggest that their PC growth inhibitory activities are not due to effects on AR expression, but in some cases may involve AR antagonistic properties. However, either with or without anti-androgenic activities, these compounds have the potential to treat both androgen-dependent and androgen-refractory PC. The anti-proliferative properties of some of these diaryl sulfonamides in both LNCaP and PC-3 cell lines suggest that the compounds are capable of interacting with multiple cellular targets. There are extensive literature precedents that support the potential of sulfonamide compounds as a useful class of drugs, with a variety of biological activities.[37–40]

4. Conclusions

Lead optimization of BTB01434 previously identified from a qualitative 3D pharmacophore modeling study afforded sulfonamide compounds that are extremely potent inhibitors of LNCaP cell growth and some are strong inhibitors of PC-3 cell growth as well. Interestingly, the compounds generated in this study possess new scaffolds that are different from those of molecules used for generation of pharmacophore model (see Ref. 19). Because of their exceptional prostate cancer growth inhibitory potencies and high synthetic feasibility, these compounds are strong candidates for development for treatment of prostate cancer. On the basis of these impressive results, our next focus will be towards large-scale syntheses of compounds **4** and **5** and their molecular and pharmacological characterization and anti-prostate tumor (LNCaP and PC-3) evaluations in suitable model systems.

5. Experimental

5.1. Cell culture

Androgen-dependent LNCaP cells were obtained from American Type Culture Collection (Rockville, MD, USA). Cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA, USA) and 1% penicillin/streptomycin. Cells were grown as a monolayer in T75 or T150 tissue culture flasks in a humidified incubator (5% CO_2, 95% air) at 37 °C.

The lead compound (BTB01434) was from Maybridge (Ryan Scientific, Inc., Isle of Palms, SC, USA) databases.

5.2. Western blot analysis

For immunoblot detection of the AR, LNCaP cells were cultured as described above in T25 flasks. Cells were treated with various concentrations of compounds and whole cell lysates were prepared using lysis buffer containing 0.1 M Tris, 0.5% Triton X-100, and protease inhibitor. Protein content was determined using the Bradford Assay (Bio-Rad, Hercules, CA, USA). Protein was subjected to SDS–PAGE (10% acrylamide) and transferred onto nitrocellulose membrane. The blots were blocked overnight in 5% nonfat milk in PBS-T buffer at 4 °C. Monoclonal antibody was used against the AR (AR441; sc-7305; Santa Cruz Biotechnology, Santa Cruz, CA; 1:500 dilution) at room temperature for 1 h. Membranes were then incubated with a goat anti-mouse IgG secondary antibody conjugated to horseradish peroxidase (Bio-Rad cat # 170-6516; 1:2000 dilution) at room temperature for 1 h. Blots were rinsed with PBS-T between each step and specific bands were visualized by enhanced chemiluminescence (ECL; Amersham Biosciences, Arlington Heights, IL, USA). Equivalent loading of samples was determined by reprobing membranes with β-actin (Calbiochem, USA). Protein expression was normalized to β-actin.

5.3. Cell growth inhibition (MTT colorimetric assay)

LNCaP cells were seeded in 24-well plates (Corning Costar) at a density of 2×10^4 cells per well per 1 mL of medium. Cell were allowed to adhere to the plate for 24 h and then treated with different concentrations of compounds dissolved in DMSO. Cells were treated for five days with renewal of compounds and media on day 3. On the fifth day, the medium was renewed and 100 μL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide from Sigma] solution (0.5 mg MTT/mL of media) was added to the medium such that the ratio of MTT:medium was 1:10. The cells were incubated with MTT for 2 h. The medium was then aspirated and 500 μL of DMSO was added to solubilize the violet MTT-formazan product. The absorbance at 560 nm was measured by spectrophotometer (Victor 1420 multilabel counted, Wallac). For each concentration of compounds there were triplicate wells in each independent experiment. GI_{50} values (concentrations that cause 50% cell growth inhibition) were calculated by nonlinear regression analysis using GraphPad Prism software.

5.4. Competitive androgen receptor (AR) binding and luciferase transactivation assays

These assays were performed as previously described by our group.[36]

5.4.1. Chemistry. General procedures and techniques were identical with those previously reported.[21–27] ^1H NMR spectra were recorded in $CDCl_3$ (unless mentioned) with Me_4Si as an internal standard and ^{13}C NMR were recorded in DMSO-d_6 using Varian Inova 500 MHz spectrometer operating at 150 MHz. High-resolution mass spectra (HRMS) were determined on a 12T Bruker FTICR–MS with an Apollo II ion source, by positive ion electrospray. Low-resolution mass spectra (LRMS) were determined on a Finnegan LCR–MS. Infrared spectra were recorded on a Perkin-Elmer 1600

FTIR spectrometer using solutions in $CHCl_3$. Melting points (mp) were determined with fisher Johns melting point apparatus and are uncorrected. As a criterion of purity for key target compounds, we provided high-resolution mass spectral data with HPLC chromatographic data indicating compound homogeneity. All the precursors required for synthesis were purchased from Aldrich–Sigma.

5.4.2. [(4-Methylphenyl)sulfonyl](2-nitrophenyl)amine (1).[21] To a solution of 2-nitroaniline (1 g, 7.24 mmol) in dry pyridine (4 mL) was added 4-methyl-benzenesulfonyl chloride (1.38 g, 7.24 mmol) over a period of 3 min, and the reaction mixture was stirred for 6 h at 125 °C. The reaction mixture was concentrated under reduced pressure and the concentrate was poured into ice cold water (300 mL). The resulting reddish brown solid precipitate was filtered, washed with water and dried to give **1** (1.12 g, 52.9%). Crystallization from EtOH gave (0.82 g) yellow needle shaped crystals, mp: 112–113 °C (lit[21] 111–112 °C); IR ($CHCl_3$) 3289, 1530, 1349, 1170, 1147, 757 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 7.14-8.11 (m, 8H, aromatic), and 9.84 (s, 1H, NH). ^{13}C NMR (500 MHz, DMSO-d_6) δ 143.79, 142.99, 136.33, 134.20, 130.39, 129.78, 126.84, 126.14, 125.48, 125.23, 21.00. HRMS calcd 315.0409 ($C_{13}H_{12}N_2O_4S{\cdot}Na^+$), found 315.0407.

5.4.3. (4-Methyl-2nitrophenyl)(phenylsulfonyl)amine (2). The method followed that described for compound **1** but using 2-nitro-4-methylaniline (1 g, 6.57 mmol) and benzenesulfonyl chloride (1.17 g, 6.57 mmol) in dry pyridine (4 mL), to give **2** (1.22 g, 63.48%). Crystallization with EtOH gave (1.1 g) yellow needle shaped crystals, mp: 95–96 °C (lit[29] 101–102 °C); IR ($CHCl_3$) 3292, 1532, 1349, 1171, 1147, 686 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.34 (s, 3H, CH_3), 7.26–7.87 (m, 8H, aromatic) and 9.62 (s, 1H, NH). ^{13}C NMR (500 MHz, DMSO-d_6) δ 143.76, 139.29, 136.94, 134.55, 133.19, 129.31, 127.33, 126.71, 126.34, 125.31, 19.95. HRMS calcd 315.0409 ($C_{13}H_{12}N_2O_4S{\cdot}Na^+$), found 315.0412.

5.4.4. (2-Nitrophenyl)(phenylsulfonyl)amine (3). The method followed that described for compound **1** but using 2-nitroaniline (1 g, 7.24 mmol) and benzenesulfonyl chloride (1.28 g, 7.24 mmol) in dry pyridine (4 mL) to give **3** (1.54 g, 66.51%). Crystallization with EtOH gave (1.34 g) yellow crystals, mp: 102 °C (lit[21] 101–102 °C); IR ($CHCl_3$) 3286, 1530, 1350, 1172, 1147, 686 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.16–8.11 (m, 9H, aromatic), and 9.85 (s, 1H, NH). ^{13}C NMR (500 MHz, DMSO-d_6) δ 143.30, 139.20, 134.16, 133.32, 130.14, 129.36, 126.76, 126.38, 125.64, 125.47. HRMS calcd 301.0253 ($C_{12}H_{10}N_2O_4S{\cdot}Na^+$), found 301.0257.

5.4.5. [(4-Flurophenyl)sulfonyl](4-methyl-2-nitrophenyl) amine (4). The method followed that described for compound **1** but using 2-nitro-4-methylaniline (1 g, 6.57 mmol) and 4-fluoro-benzenesulfonyl chloride (1.4 g, 6.57 mmol)in dry pyridine (4 mL), to give **4** (1.7 g, 83.33%). Crystallization with EtOH gave (1.4 g) white crystals, mp: 109–112 °C; IR ($CHCl_3$) 3289, 1533, 1351, 1173, 1156, 661 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.35 (s, 3H, CH_3), 7.10–7.89 (m, 7H, aromatic) and 9.59 (s, 1H, NH). ^{13}C NMR (500 MHz, DMSO-d_6) δ 165.51, 163.50, 144.072, 137.23, 135.66, 134.58, 129.85, 127.01, 126.76, 125.36, 116.58, 20.01. HRMS calcd 333.0315($C_{13}H_{11}FN_2O_4S{\cdot}Na^+$), found 333.0314.

5.4.6. (4-Methylphenyl)[(4-methylphenyl)sulfonyl]amine (5). The method followed that described for compound **1** but using *p*-toluedine (0.5 g, 4.66 mmol) and 4-methyl-benzenesulfonyl chloride (0.89 g, 4.66 mmol)in dry pyridine (2 mL), to give **5** (1.0 g, 82%). Crystallization with EtOH gave (0.73 g) white crystals, mp: 114–115 °C (lit[30] 116 °C); IR ($CHCl_3$) 3256, 1510, 1332, 1160, 766^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.18 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 6.94–7.60 (m, 8H, aromatic) and 10.01 (s, 1H, NH). ^{13}C NMR (500 MHz, DMSO-d_6) δ 143.03, 136.74, 135.16, 133.22, 129.52, 126.71, 120.47, 20.91, 20.26. HRMS calcd 284.0715 ($C_{14}H_{15}NO_2S{\cdot}Na^+$), found 284.0716.

5.4.7. 5-Methyl-2-[(phenylsulfonyl)amino]benzoic acid (6).[22] To a solution of 2-amino-5-methyl-benzoic acid (0.2 g, 1.32 mmol), sodium carbonate (0.34 g, 3.17 mmol) in water (2 mL) at 60 °C was added benzenesulfonyl chloride (0.28 g, 1.58 mmol) over a period of 2 min, and the reaction mixture was stirred for 6 h at 80 °C. The reaction mixture was cooled to room temperature and acidified with 6 N HCl and the resulting cream colored solid precipitate was filtered, washed with water and dried to give **6** (0.39 g, 98.7%). Crystallization from EtOH gave (0.22 g) white needle shaped crystals, mp: 197–198 °C (lit[28] 130 °C); IR (nujol) 3202, 1669, 1341, 1167, 1149, cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.50 (s, 3H, CH_3), 7.35–7.77 (m, 8H, aromatic) and 1093 (br, 1H, COOH). ^{13}C NMR (500 MHz, DMSO-d_6) δ 169.74, 138.55, 137.23, 135.03, 133.45, 132.84, 131.50, 129.43, 126.79, 118.98, 116.98, 19.99. HRMS calcd 314.0457 ($C_{14}H_{13}NO_4S{\cdot}Na^+$), found 314.0460.

5.4.8. {[(4-Fluorophenyl)sulfonyl]amino}-5-methylbenzoicacid (7). The title compound was synthesized by reacting 2-amino-5-methylbenzoic acid (0.25 g, 1.65 mmol), 4-fluoro-benzenesulfonyl chloride (0.39 g, 1.98 mmol), and sodium carbonate (0.36 g, 3.39 mmol) in 3 mL of water, following the reaction procedure of **6**. The white solid obtained after acidification of reaction mixture was filtered, washed with water and dried to give **7** (0.44 g, 85.42%). Crystallization with EtOH gave (0.3 g) white amorphous solid, mp: 159–160 °C; IR ($CHCl_3$) 3177, 1662, 1342, 1148, 667 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.31 (s, 3H, CH_3), 7.08–7.86 (m, 7H, aromatic), 9.32 (br, 1H, NH), and 10.18 (s, 1H, COOH). ^{13}C NMR (500 MHz, DMSO-d_6)δ 169.61, 165.59, 163.59, 136.91, 135.00, 133.10, 131.54, 130.02, 129.95, 119.34, 117.42, 116.75, 116.58, 20.00. HRMS calcd 332.0363 ($C_{14}H_{12}FNO_4S{\cdot}Na^+$), found 332.0372.

5.4.9. 2-{[(4-Fluorophenyl)sulfonyl]amino}-4-methylbenzenecarbonitrile (8). The title compound was synthesized by reacting 2-amino-4-methylbenzonitrile (0.25 g, 1.89 mmol) and 4-fluoro-benzenesulfonyl chloride

(0.37 g, 1.89 mmol) in 2 mL of dry pyridine, following the reaction procedure of **1**.[21] The concentrated reaction mixture was poured into ice cold water (300 mL), and resulting pale white solid was filtered, washed with water, and dried to gave **8** (0.24 g, 42.8%). Crystallization with EtOH gave (0.15 g) white amorphous solid, mp: 130–132 °C; IR ($CHCl_3$) 3260, 2226, 1376, 1340, 1171, 1156 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.42 (s, 3H, CH_3) and 6.90–7.83 (m, 7H, aromatic). ^{13}C NMR (500 MHz, DMSO-d_6) δ 165.51, 163.51, 144.83, 138.27, 135.88, 133.69, 129.93, 129.86, 128.04, 127.31, 116.62, 116.58, 116.44, 106.96, 21.20. HRMS calcd 313.0417 ($C_{14}H_{11}FN_2O_2S{\cdot}Na^+$), found 313.0418.

5.4.10. (2-(1H-1,2,3,4-Tetrazole-5-yl)-5-methylphenyl)[(4-fluorophenyl)sulfonyl]amine (9). The title compound was synthesized by reacting 2-{[(4-fluorophenyl)sulfonyl]amino}-4-methylbenzenecarbonitrile (**8**) (0.1 g, 0.34 mmol), sodium azide (0.09 g, 1.37 mmol), and ammonium chloride (0.74 g, 1.37 mmol) in 3 mL of DMF at 120 °C for 20 h.[23] The reaction mixture was poured into ice cold water (30 mL) and acidified with dilute HCl solution. The white precipitate obtained on acidification was extracted with ethyl acetate. The organic layer was washed (H_2O), dried (Na_2SO_4), and concentrated to give crude product (0.1 g), which was purified by FCC (silica gel, CH_2Cl_2, MeOH, CH_3COOH, 10:2:0.1, v/v/v) to give **9** as a white solid (0.086 g, 74.9%), mp: 252–254 °C; IR ($CHCl_3$) 3136, 1457, 1376, 1348, 1165, 1153 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.33 (s, 3H, CH_3), 6.60–7.77 (m, 7H, aromatic) and 10.54 (br, 1H, NH). ^{13}C NMR (500 MHz, DMSO-d_6) δ 154.00, 152.79, 142.40, 136.53, 128.56, 128.50, 124.08, 122.88, 120.11, 110.70, 109.92, 21.31. HRMS calcd 356.0587 ($C_{14}H_{12}FN_5O_2S{\cdot}Na^+$), found 356.0587.

5.4.11. (4-Methylphenyl)(phenylsulfonyl)amine (10). The title compound was synthesized by triturating powdered mixture of *p*-toluedine (0.5 g, 4.66 mmol) and sodium bicarbonate (0.78 g, 9.33 mmol) with benzene sulfonyl chloride (0.82 g, 4.66 mmol) for 10 min at rt.[24] Then the reaction mixture was stirred with water, filtered, washed with water to remove sodium bicarbonate, and dried to give **10** (1.1 g, 95.14%). The product was purified by FCC using solvent gradient (silica gel, petroleum ether, ethyl acetate, triethyl amine, 9:1:0.2, v/v/v, and petroleum ether and ethyl acetate 8:2, v/v) to give **10** as white needles (0.89 g), mp: 118–119 °C (lit[31] 120–121 °C); IR ($CHCl_3$) 3254, 1510, 1389, 1330, 1163 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.27 (s, 3H, CH_3), 6.42 (br, 1H, NH), and 6.92–7.74 (m, 9H, aromatic). ^{13}C NMR (500 MHz, DMSO-d_6) δ 139.60, 135.03, 133.41, 132.68, 129.55, 129.10, 126.67, 120.66, 20.24. HRMS calcd 270.05592 ($C_{13}H_{13}NO_2S{\cdot}Na^+$), found 270.05568.

5.4.12. [(4-Methylphenyl)sulfonyl]phenylamine (11). The title compound was synthesized by triturating aniline (0.5 g, 5.36 mmol), powdered sodium bicarbonate (0.9 g, 1.07 mmol), and powdered *p*-toluene sulfonyl chloride (1.02 g, 5.36 mmol) for 5 min at rt. The reaction mixture was stirred with water, filtered, washed with water, and dried to give **11** (1.13 g, 84.9%). The product was purified by FCC by using eluent as in **10** to give **11** as a white powder (0.9 g), mp: 95–97 °C (lit[32] 96 °C); IR ($CHCl_3$) 3255, 1599, 1342, 1160 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.37 (s, 3H, CH_3), 6.53 (br, 1H, NH), and 7.04–7.65 (m, 9H, aromatic). ^{13}C NMR (500 MHz, DMSO-d_6) δ 143.18, 137.83, 136.72, 129.63, 129.10, 126.70, 123.92, 119.90, 20.90. HRMS calcd 270.05592 ($C_{13}H_{13}NO_2S{\cdot}Na^+$), found 270.05589.

5.4.13. Phenyl(phenylsulfonyl)amine (12). The title compound was synthesized by triturating aniline (0.5 g, 5.36 mmol), powdered sodium bicarbonate (0.9 g, 1.07 mmol), and benzene sulfonyl chloride (0.95 g, 5.36 mmol) for 10 min at rt. Then the reaction mixture was stirred with water at 60 °C for 30 min, filtered, washed with water, and dried to gave **12** (0.85 g, 68%). The product was purified by FCC by using eluent as in **10** to give **12** as white flakes (0.79 g), mp: 107–109 °C (lit[33] 110 °C); IR ($CHCl_3$) 3256, 1599, 1331, 1162 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.42 (br, 1H, NH), and 7.04–7.77 (m, 10H, aromatic). ^{13}C NMR (500 MHz, DMSO-d_6) δ 139.63, 137.75, 132.75, 129.13, 126.69, 124.09, 120.13. HRMS calcd 256.04027 ($C_{12}H_{11}NO_2S{\cdot}Na^+$), found 256.04016.

5.4.14. Methyl(4-methylphenyl)[(4-methylphenyl)sulfonyl]amine (13). The title compound was synthesized by adding methyl iodide (0.09 g, 0.63 mmol) to a stirring mixture of approximately 0.5 mL of 1-butyl-3-methylimidazolium-hexa-fluorophosphate, powdered KOH (0.04 g, 0.72 mmol), and **5** (0.15 g, 0.57 mmol) at rt.[25] The reaction mixture was stirred for 2 h and then diluted with water, extracted with ethyl acetate. The organic layer was treated with brine solution, dried with Na_2SO_4, and evaporated to get a thick liquid. The product was purified by FCC (silica gel, petroleum ether, ethyl acetate, 9:1, v/v) to give **13** as a colorless viscous liquid, which solidified after several days (0.14 g, 88.5%), mp: 52–54 °C (lit[25] 59–60 °C); IR (Neat) 1598, 1510, 1348, 1171, 1154, 679 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.32 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.13 (s, 3H, CH_3), and 6.95–7.44 (m, 8H, aromatic). ^{13}C NMR (500 MHz, DMSO-d_6) δ 143.51, 138.65, 136.56, 133.20, 129.58, 129.32, 127.47, 126.03, 37.89, 21.00, 20.51. HRMS calcd 298.08722 ($C_{15}H_{17}NO_2S{\cdot}Na^+$), found 298.08699.

5.4.15. (4-Methylphenyl)-N-(4-methylphenyl)carboxamide (14). The title compound was synthesized by adding (drop wise) a solution of *p*-toluoyl chloride (0.86 g, 5.59 mmol) in 11 mL ethyl acetate (2 mL/mmol) to a solution of TEA (0.66 g, 6.5 mmol), and *p*-toluidine (0.5 g, 4.6 mmol) in 25 mL ethyl acetate (5 mL/mmol) at rt.[26] The white precipitate obtained was stirred over-night. The next day, most of the solvent was removed in vacuo. Petroleum ether was added, and the solids were collected on a filter, washed twice with a mixture of petroleum ether/ethyl acetate (5/1), and three times with water. The residue was dried to obtain white crystalline needles (0.92 g, 85%). Crystallization from EtOH gave (0.75 g) white needle shaped shining crystals, mp: 163 °C (lit[34] 163–164 °C); IR ($CHCl_3$) 3437, 3323,

1665, 1516, 1217, 1212 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.33 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 7.16–7.77 (m, 8H, aromatic) and 7.72 (s, 1H, NH). ^{13}C NMR (500 MHz, DMSO-d_6) δ 165.14, 141.39, 136.75, 132.45, 132.19, 128.99, 128.85, 127.64, 120.39, 20.97, 20.47. HRMS calcd 248.10458 ($C_{15}H_{15}NO{\cdot}Na^+$), found 248.10446.

5.4.16. (1*E*)-1-Aza-1,2-bis(4-methylphenyl)ethane (15). The title compound was synthesized by reacting *p*-toluidine (0.05 g, 0.46 mmol) and *p*-tolualdehyde (0.056 g, 0.46 mmol) for 5 min, then to this reaction mixture 3 mL of ethanol was added and refluxed over-night.[27] The next day the solvent was evaporated and the crude product was purified by FCC (silica gel, petroleum ether, ethyl acetate, 9:1, v/v) to give **15** as a yellow fluffy solid (0.045 g, 46%), mp: 83–85 °C (lit[35] 107 °C); IR ($CHCl_3$) 3018, 2923, 2399, 1626, 1512, 1503, 1214, 751 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.36 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 7.11–7.79 (m, 8H, aromatic) and 8.42 (s, 1H). ^{13}C NMR (500 MHz, DMSO-d_6) δ 159.47, 148.93, 141.27, 135.14, 133.63, 129.67, 129.39, 128.57, 120.91, 21.14, 20.58. HRMS calcd 232.10967 ($C_{15}H_{15}N{\cdot}Na^+$), found 232.12772.

5.4.17. (4-Methylphenyl)[(4-methylphenyl)methyl]amine (16). The title compound was synthesized by reducing the crude (1*E*)-1-aza-1,2-bis(4-methyl phenyl)ethane (**15**) in 4 mL methanol with sodium borohydride (0.15 g, 3.96 mmol) at rt for 10–20 min.[27] Methanol was evaporated under vacuo and the residue treated with saturated sodium bicarbonate solution and extracted with MDC. The organic layer was dried with sodium sulfate and evaporated under vacuo to get crude **16**. The crude product was purified by FCC (silica gel, petroleum ether, ethyl acetate, 9:1, v/v) to give **16** as a pale yellow solid (0.079 g, 80%), mp: 50–51 °C; IR ($CHCl_3$) 3416, 3015, 2921, 2864, 1615, 1518, 1215, 810 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.23 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.84 (br, 1H, NH), 4.25 (s, 2H), and 6.55–7.25 (m, 8H, aromatic). ^{13}C NMR (500 MHz, DMSO-d_6) δ 146.45, 137.36, 135.46, 129.21, 128.77, 127.12, 123.89, 112.44, 46.51, 20.64, 20.06. HRMS calcd 234.12532 ($C_{15}H_{17}N{\cdot}Na^+$), found 234.12517.

5.4.18. (1*E*)-1-Aza-1-(4-methylphenyl)-2-phenylethene (17). The title compound was synthesized by reacting *p*-toluedine (0.2 g, 1.86 mmol) and benzaldehyde (0.198 g, 1.86 mmol) for 5 min, then to this reaction mixture 3 mL of ethanol added and refluxed for approx. 12 h.[27] Next day solvent evaporated and crude product was purified by FCC (silica gel, petroleum ether, ethyl acetate, 9:1, v/v) to give **17** as colorless liquid (0.155 g, 42%), IR ($CHCl_3$) 3023, 1626, 1512, 1504, 1190, 814, 691 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.36 (s, 3H, CH_3), 7.13–7.89 (m, 9H, aromatic) and 8.46 (s, 1H). ^{13}C NMR (500 MHz, DMSO-d_6) δ 159.64, 148.80, 136.16, 135.36, 131.27, 129.69, 128.77, 128.56, 120.95, 20.58. HRMS calcd 218.09402 ($C_{14}H_{13}N{\cdot}Na^+$), found 218.09387.

5.4.19. (4-Methylphenyl)benzylamine (18). The title compound was synthesized by reducing, the crude (1*E*)-1-aza-1-(4-methylphenyl)-2-phenylethene (**17**) in 6 mL methanol with sodium borohydride (0.6 g, 15.8 mmol) at rt for 10–20 min.[27] Methanol evaporated under vacuo and residue treated with saturated sodium bicarbonate solution and extracted with MDC. Organic layer dried with sodium sulfate and evaporated under vacuo to get crude **18**. Crude product was purified by FCC (silica gel, petroleum ether, ethyl acetate, 9:1, v/v) to give **18** as colorless low melting solid (0.32 g, 86%), mp: 20 °C; IR ($CHCl_3$) 3414, 3025, 2357, 1616, 1520, 806 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.22 (s, 3H, CH_3), 3.88 (s, 1H, NH), 4.29 (s, 2H), and 6.54–7.36 (m, 9H, aromatic). ^{13}C NMR (500 MHz, DMSO-d_6) δ 146.49, 140.52, 129.30, 128.25, 127.19, 126.53, 124.15, 112.50, 46.88, 20.12. HRMS calcd 220.10967 ($C_{14}H_{15}N{\cdot}Na^+$), found 220.10936.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.02.031.

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