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Synthesis of Novel Bicaultamide Related Derivatives and their Evaluation of Biological Activity

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Abstract

A novel approach for the synthesis of novel Bicaultamide related derivatives (11-16) from (1S)-1-(oxiran-2-yl)-2-phenylethan-1-amine. The epoxide on treatment with Alkyl amines in presence of a potassium tert-butoxide followed by synthesis of Alkyl/Aryl sulphonamides on reaction with substituted sulphonyl chloride. All the synthesized compounds were screened in vitro for their antibacterial activity against two gram positive bacteria *Staphylococcus aureus* (MTCC – 96), *Bacillus subtilis* and two gram negative bacteria *Escherichia coli* (MTCC – 443), *Pseudomonas aeruginosa* (MTCC – 424) by the cup-plate agar diffusion methodat different concentrations (1 mg /mL).

Keywords

(1S)-1-(oxiran-2-yl)-2-phenylethan-1-amine., Green chemistry, N-alkyl 1,2-amino alcohol, substituted sulfonamides Antimicrobial activity.

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INTRODUCTION

Prostate cancer (PC) is one of the major causes of male death worldwide, representing the second most common cancer in males.1 Prostate cancer is a major cause of male death worldwide and the identification of new efficient treatments is constantly needed. An estimated 1.1 million men worldwide were diagnosed with prostate cancer in 2012, accounting for 15% of the cancers diagnosed in men, with almost 70% of the cases (759,000) occurring in more developed regions.

Prostate cancer is the development of cancer in the prostate, a gland in the male reproductive system. Most prostate cancers are slow growing; however, some grow relatively quickly2. The cancer cells may spread from the prostate to other area of the body, particularly the bones and lymph nodes. PC cell growth is strongly dependent on androgens, therefore blocking their effect can be beneficial to the patient's health. Such outcomes can be achieved by antagonism of the androgen receptor (AR) using anti-androgen drugs, which have been extensively explored either alone or in combination with castration 3(1), Flutamide (2) hydroxyflutamide (3), bicalutamide (4), Enzalutamide (5), Curcumin (6), nilutamide (7) and RU56279 (8) are all non-steroidal

androgen receptor antagonists (AR) approved for the treatment of PC. Among the drugs used for the treatment of PC, bicalutamide and enzalutamide selectively block the action of androgens while presenting fewer side effects in comparison with other AR antagonists4-6. Non-steroidal ligands are more favorable for clinical applications because of the lack of cross reactivity with other steroid receptors and improved oral bioavailability. Among them, Bicalutamide is the most potent and tolerated drug of choice administered either as monotherapy o8ikl\r with adjuvant castration or luteinizing hormone-releasing hormone. Structurally these are comprised of two differently substituted aromatic rings, named ring A and ring B, connected by a linker, either linear (Bicalutamide-like compounds) or cyclic Enzalutamide (like compounds), recently, a novel 4-(4-benzoylaminophenoxy) phenol anti-androgen scaffold, derived from the natural pigment Curcumin, has been reported, in which a central phenyl group is acting as linker connecting two different aromatic rings.

In the present chapter we reported the design and synthesis of novel molecular scaffolds of Bicaultamide as illustrated in Scheme 2, wherein our group has choosed the Bicaultamide as a lead compound. Due to their extensive range of applications, these compounds have usually a great deal of attention in linking with their synthesis.

RESULTS AND DISCUSSION

CHEMISTRY:

Synthesis of N-((2R,3S)-3-amino-2-hydroxy-4-phenylbutyl)-N-Alkyl substituted sulfonamides started from (1S)-1-(oxiran-2-yl)-2-phenylethan-1-amine.The opening of epoxide 8 in base condition epoxide on treatment with Alkyl amines in presence of a potassium tert-butoxide in reflux for 3 h, gave the N-alkyl 1,2-amino alcohol 9 in good % yields. The N-alkyl 1,2-amino alcohol was further converted to the Alkyl/Aryl sulphonamides 10 on reaction with substituted sulphonyl chloride in CH_2Cl_2 at 0 °C for 4-6 h efford in good yileds and all the compounds characterized by H-NMR and ESI-MS reports.

All the synthesized compounds were screened in vitro for their antibacterial activity against two gram positive bacteria *Staphylococcus aureus*(MTCC – 96), *Bacillus subtilis* and two gram negative bacteria *Escherichia coli* (MTCC- 443), *Pseudomonas aeruginosa* (MTCC-424) by the cup-plate agar diffusion methodat different concentrations (1 mg /mL).

EVALUATION OF BIOLOGICAL ACTIVITY OF THE SYNTHESISED SCAFFOLDS:

In view of varied biological and pharmacological importance of different series of novel N-Substituted analogues of bicalutamide derivatives, it is felt worthwhile to evaluate them for possible activities. These compounds therefore were screened for antimicrobial activity. The details of each of the methods are presented in the experimental section along with the observations recorded in tables.

All the synthesized compounds were screened *in vitro* for their antibacterial activity against two gram positive bacteria *Staphylococcus aureus* (MTCC- 96), *Bacillus subtilis* and two gram negative bacteria *Escherichia coli* (MTCC – 443), *Pseudomonas aeruginosa* (MTCC- 424) by the cup-plate agar diffusion method at different concentrations (1 mg /mL).

Antibacterial activity by Paper Disc method :[7,8]

Nutrient broth (pH –7.2) was used for the preparation of inoculum of bacteria. Nutrient agar medium was used for the antibacterial screening, contained 20.0 g of agar in addition to the composition of nutrient broth

Nutrient Broth: 23 gr of Nutrient Broth is added in one-liter water (23 gr /1Lit), autoclave at 121 ^{0}c and 15 lb pressure, inoculate bacterial cultures for growth in 250 ml beaker by using nutrient broth media.

Nutrient agar: 28 gr of Nutrient agar is added in one-liter water, (28 gr /1Lit)

For antibacterial screening, the agar medium was sterilized by autoclaving at 120ºC for 15 min. The Petri plates and pipettes were sterilized by dry heat in a hot- air oven at 150^{0}C for 1 hr. About 20 mL of the molten agar medium was poured in each of sterilized petri plates.

Bacterial Strains and Inoculum's preparation[9]

The microorganisms employed in this study were two-gram positive bacteria such as *Staphylococcus aureus* (MTCC- 96), *Bacillus subtilis* (MTCC-121) and two-gram negative bacteria *Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-424). The inoculum was standardized at 1* 106 CFU/ml comparing with turbidity standard (0.5 MacFarland tube).

Swabs preparation:

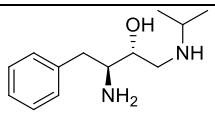
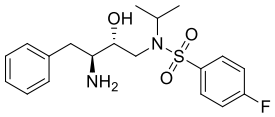
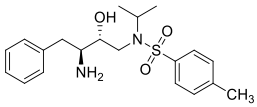
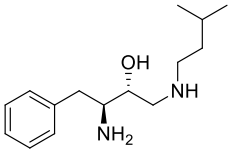
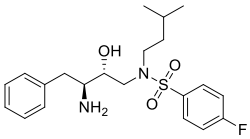
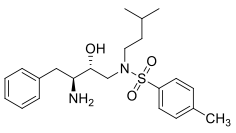
A supply of cotton wool swabs on wooden applicator sticks was prepared. They were sterilized in tins, culture tubes, or on paper, either in the autoclave or by dry heat.5 mm disks were prepared by using wt men filter paper and autoclaved.[10-12]

Methodology:

All the synthesized compounds were screened *in vitro* for their antibacterial activity against two gram positive bacteria *Staphylococcus aureus*(MTCC – 96),*Bacillus subtilis*and two gram negative bacteria *Escherichia coli* (MTCC – 443), *Pseudomonas aeruginosa* (MTCC – 424) by the cup-plate agar diffusion method[1]at different concentrations (1 mg /mL).

In auto calved nutrient broth media inoculation of *Pseudomonas aeruginosa* (gram-negative), *Escherichia coli* (*E. coli*) (gram-negative), *Bacillus subtilis* (Gram-positive), *Staphylococcus aureus* (gram-positive) and incubate over night at 37º C in shaker for bacterial growth. From that, 0.3ml of bacterial culture was taken and inoculated by using spreader on freshly prepared auto calved agar plates. After drying of plate 5 mm sample disc were kept on microbial plate along with positive controls NX (Norfloxacin) for *Staphylococcus, Pseudomonas* and OF (ofloxacin) for *Bacillus* and *E.coli*. Incubation over night at 37º C in BOD incubator. After overnight incubation zone of inhibition is measured by measuring scale. The zone of inhibition (in mm) was compared with standard drug NX (Norfloxacin) for *Staphylococcus, Pseudomonas* and OF (ofloxacin) for *Bacillus* and *E.coli*. The results are tabulated in **Table 1 and Figure 1.** The presence of definite zone of inhibition surrounding the disc indicated antimicrobial activity. The diameter of the zone of inhibition was recorded. The experiments were performed, at least in triplicate.

Table 1: Antibacterial activity of N-Substituted analogues of bicalutamide derivatives:

Compound	Structure	Zone of inhibition in (mm)			
		P.aeruginosa	***E. coli***	***S. aureus***	*B.subtilis*
		Gram-negative		***Gram-positive***	
1	 11	6	8	8	7
2	 12	2	4	2	5
3	 13	2	5	2	2
4	 14	2	11	7	10
5	 15	2	7	7	5
6	 16	2	6	4	3
Control (1µg/mL)		**9**	**10**	**9**	**10**

Norfloxacin: *Staphylococcus, Pseudomonas*
Ofloxacin: *Bacillus* and *E.coli*.

Table 1: Antibacterial activity of N-Substituted analogues of bicalutamide:

All the novel N-Substituted analogues of bicalutamide derivatives have been screened in vitro for their antibacterial activity against two gram positive bacteria *Staphylococcus aureus* (MTCC – 96), *Bacillus subtilis*and two gram negative bacteria *Escherichia coli* (MTCC – 443), *Pseudomonas aeruginosa* (MTCC – 424) by the cup-plate agar diffusion method at different concentrations (1 mg /mL).

Thus we observed that the amino alcohols compound **10**, **14** showed good activity against grampositive bacteria than their corresponding sulphonamides, whereas the sulphonamides with fluoro group in the *para* position compounds like **12**, showed good activity against grampositive bacteria than the corresponding sulphonamides with methyl substitution **13**, **16**. Inspired by these results the evaluation of the other derivatives for biological activity is in progress.

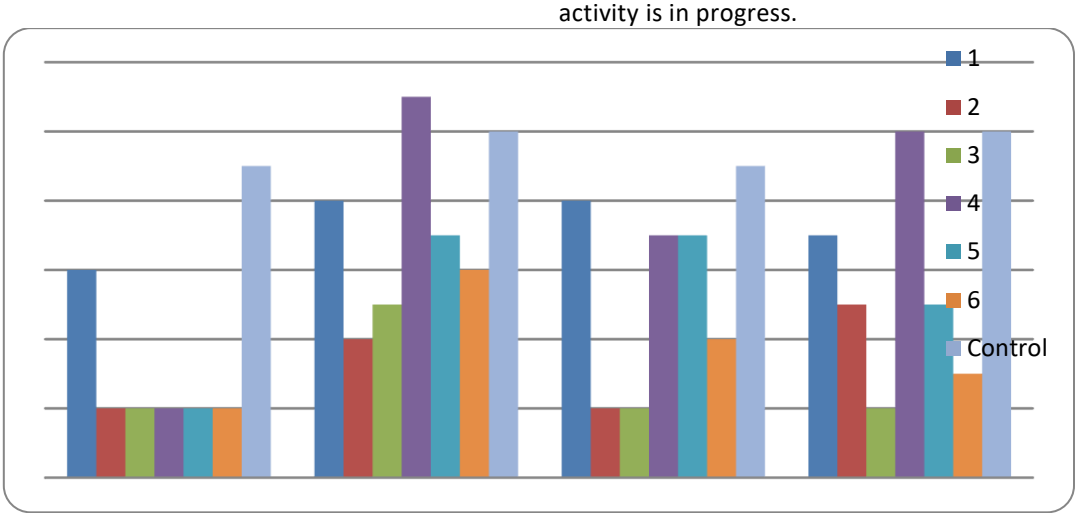
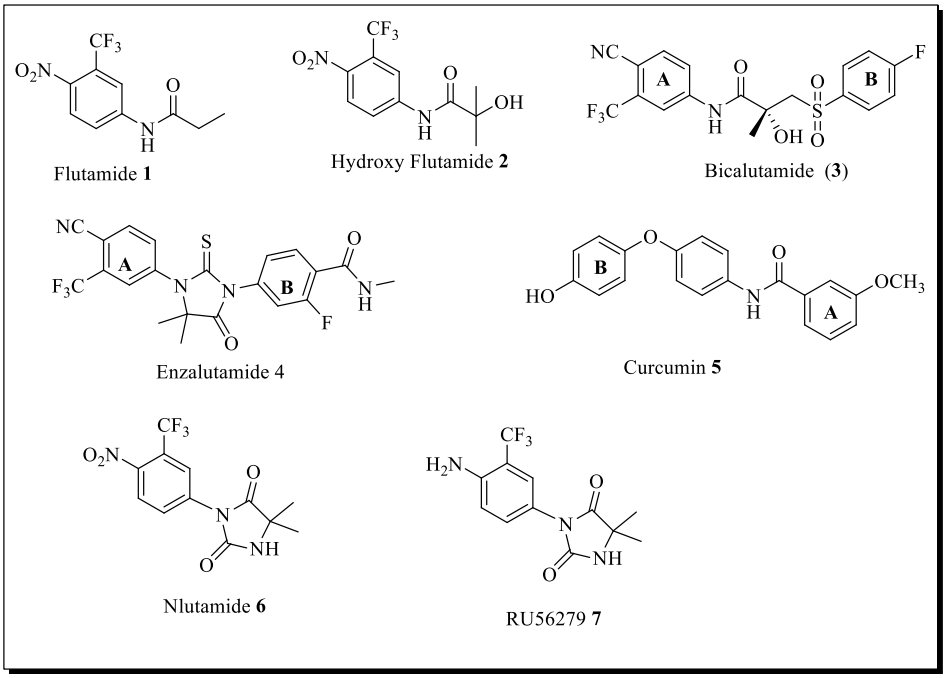
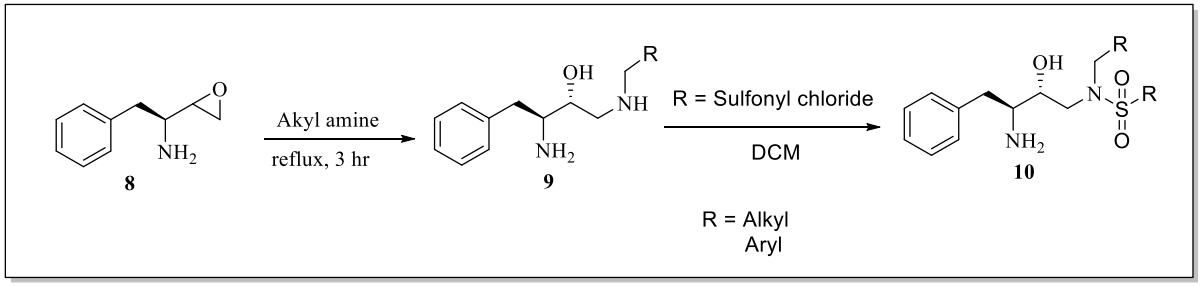


Figure 1: Antibacterial activity of compounds 1-6 against Staphylococcus *aureus,* Bacillus *subtilis* and, *Escherichia coli* and *Pseudomonas aeruginosa* (MTCC – 424).

Figure 2: Structures of various anti androgens for the treatment of prostate cancer



Scheme 2: N-((2R,3S)-3-amino-2-hydroxy-4-phenylbutyl)-N-Alkyl substituted sulfonamides



CONCLUSION:

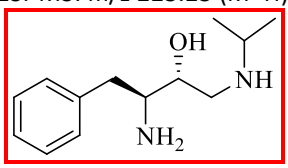
In conclusion, we have developed a new and efficient method for the synthesis of novel Bicaultamide analogues in excellent yields. It is interesting to note from the results, that compounds of 11 and 14 have been more effective against gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and two-gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* containing alkyl groups like isopropyl and isopentyl than other groups. whereas the sulphonamides with fluoro group in the para position compounds like 12 and 15 showed good activity against grampositive bacteria than the corresponding sulphonamides with methyl substitution analogues like 13 and 16.

EXPERIMENTAL SECTION:

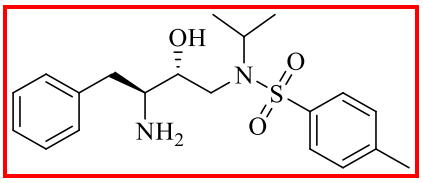
All reactions were monitored by thin-layer chromatography (TLC) using silica-coated plates and visualization under UV light. Light petroleum of the distillation range 60–80°C was used. Melting points were determined using a Buchi R-535 apparatus and are uncorrected. Mass spectra were recorded under electron impact at 70 eV on an LC-MSD (Agilent Technologies). ^{1}H NMR spectra were recorded on Varian FT 200-MHz (Gemini) and Bruker UXNMR FT 300-MHz (Avance) instruments in $CDCl_3$. Chemical shift values were reported in parts per million (d) relative to tetramethylsilane (TMS) (δ 0.0) as an internal standard. EtOH 95% was used for recrystallization. Yields refer to pure products isolated by crystallization and spectroscopically (^{1}H, IR) homogeneous material.

GENERAL PROCEDURE FOR THE SYNTHESIS OF COMPOUNDS (11-16):

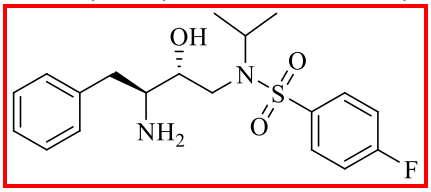
(2R,3S)-3-amino-1-(isopropylamino)-4-phenylbutan-2-ol (11): To a stirred solution of **10** (5 g, 0.0306 mol) in 50 ml of IPA and to this added Isopropyl amine (10 mL) drop wise for 10 min. And the reaction mixture was heated to reflux for 3 h, then Reaction mass was monitored by TLC. After completion of reaction mass was concentrated under reduced pressure then the crude was diluted with 100 ml of Ethyl acetate and washed with water followed by Brine solution, Organic layer was dried under sodium sulphate, then concentrated under reduced pressure, obtained crude compound was purified by flash silica column chromatography using 100-200 silica gel, Product **11** was eluted at 40% Ethyl acetate in Hexane, then the fractions were collected to concentrated. Yield: 4.1 g (60.29%) HPLC: 95.76%; 1HNMR (300 MHz, DMSO-d6): ppm 7.28-7.13 (m, 5H), 6.41-6.38 (d, lH), 4.64 (bs, 1H), 3.69-3.64 (m, 1H), 3.45 (bs, 1H), 2.82-2.76 (m, 1H), 2.68-2.59 (m, 2H), 2.43-2.36 (m, 1H), 1.44-1.19 (m, 3H), 0.94-0.91(m, 6H); ESI-MS: m/z 223.23 (M+H)+.



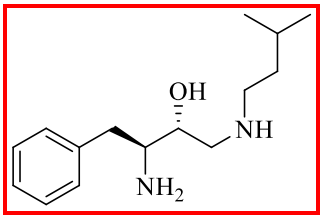
N-((2R,3S)-3-amino-2-hydroxy-4-phenylbutyl)-N-isopropyl-4-methylbenzenesulfonamide (13) : To a stirred solution of **11** (1.0 g, .0044 mol) in 10 ml CH_2Cl_2 of and cooled to 0°C, to this added p-toluene sulfonyl chloride (0.94 g, 0.0049mol) drop wise for 5 min. And allowed the reaction mixture to room temperature for 6 hour then Reaction mass was monitored by TLC. After completion of reaction mass was diluted with 50 mL of CH_2Cl_2 and washed with water followed by Brine solution, Organic layer was dried under sodium sulphate, then concentrated under reduced pressure, obtained crude compound was purified by flash silica column chromatography using 100-200 silica gel, Product **13** was eluted at 25% Ethyl acetate in Hexane, then the fractions were collected to concentrated. Yield: 0.8g (82%) HPLC: 95.76%; 1HNMR (300 MHz, DMSO-d6): ppm 7.66-7.63 (m, 2H), 7.38-7.36 (d, 2H), 7.27-7.17 (m, 5H), 6.53-6.50 (d, 1H), 3.88-3.68 (m, 3H), 3.20-3.16 (m, 1H), 2.94-2.78 (m, 3H), 2.69-2.62 (m, 1H), 2.38 (m, 3H), 1.31-1.22 (m, 2H), 0.97-0.95 (m, 3H), 0.84-0.82 (m, 3H); ESI-MS:m/z 399.02 (M+Na)+.



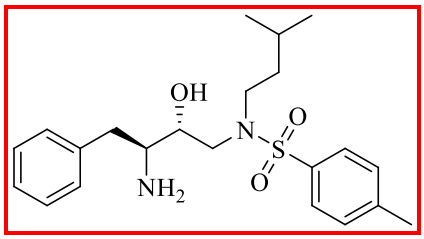
N-((2R,3S)-3-amino-2-hydroxy-4-phenylbutyl)-4-fluoro-N-isopropylbenzenesulfonamide (12): To a stirred solution of **11** (1.0 g, 0.0044 mol) in 10 mL CH_2Cl_2 of and cooled to 0°C, to this added 4-flouro benzene sulfonyl chloride (0.96 g, 0.0049 mol) drop wise for 5 min. And allowed the reaction mixture to room temperature for 6 hours then Reaction mass was monitored by TLC. After completion of reaction, reaction mass was diluted with 50 mL of CH_2Cl_2 and washed with water followed by Brine solution, Organic layer was dried under sodium sulphate, then concentrated under reduced pressure, obtained crude compound was purified by flash silica column chromatography using 100-200 silica gel, Product **12** was eluted at 20% Ethyl acetate in Hexane, then the fractions were collected to concentrated. Yield: 1.12 (65.49%) HPLC: 95.76%; ^{1}HNMR (300 MHz, DMSO-d6): ppm 7.87-7.82 (m, 2H),7.44-7.38 (m, 2H),7.27-7.17(m, 5H), 6.55-6.52 (d, lH), 4.93-4.91 (d, 1H), 3.89-3.67 (m, 3H), 3.23-3.19 (m, 1H), 2.98-2.90 (m, 1H), 2.85-2.79 (m, 2H), 2.68-2.61 (m, 1H), 1.00-0.98 (m, 3H), 0.87-0.85 (m, 3H); ESI-MS:m/z 403.35 (M+Na)+.



(2R,3S)-3-amino-1-(isopentylamino)-4-phenylbutan-2-ol (14): To a stirred solution of **11** (5.0 g, 0.0199 mol) in 50 mL of IPA and to this added Isopentyl amine (10 mL) drop wise for 10 min. And the reaction mixture was heated to reflux for 3 h, then Reaction mass was monitored by TLC. After completion of reaction, reaction mass was concentrated under reduced pressure then the crude was diluted with 100 ml of Ethyl acetate and washed with water followed by Brine solution, Organic layer was dried under sodium sulphate, then concentrated under reduced pressure, obtained crude compound was purified by flash silica column chromatography using 100-200 silica gel, compound **14** was eluted at 40% Ethyl acetate in Hexane, then the fractions were collected to concentrated. HPLC: 91.35%; 1HNMR (300 MHz, DMSO-d6): ppm 7.26-7.14 (m, 5H), 3.79 (bs,2H), 3.65-3.64 (m, 1H), 3.55-3.51(m, 1H), 2.79-2.77(m, 1H), 2.74-2.73 (m, 1H), 2.66-2.38 (m, 5H), 1.56-1.47 (m, 1H), 1.26 (m, 4H), 0.81-0.79 (m, 6H),; ESI-MS: m/z 251.25 (M+H)+.

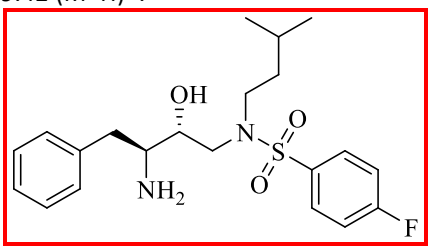


N-((2R,3S)-3-amino-2-hydroxy-4-phenylbutyl)-N-isopentyl-4-methylbenzenesulfonamide (16): To a stirred solution of **14** (1.0 g, 0.0039 mol) in 10 mL CH_2Cl_2 of and cooled to 0°C, to this added p-toluene sulfonyl chloride (0.83 g, 0.0043 mol) drop wise for 5 min. And allowed the reaction mixture to room temperature for 6 hours then Reaction mass was monitored by TLC. After completion of reaction mass was diluted with 50 mL of CH_2Cl_2 and washed with water followed by Brine solution, Organic layer was dried under sodium sulphate, then concentrated under reduced pressure, obtained crude compound was purified by flash silica column chromatography using 100-200 silica gel, Product **16** was eluted at 10% Ethyl acetate in Hexane, then the fractions were collected to concentrated. Yield: 1.01 g (62.73%) HPLC: 92.19%; ^{1}HNMR (300 MHz, DMSO-d6): ppm 7.64-7.61 (d, 2H), 7.39-7.36 (d, 2H), 7.28-7.14 (m, 5H), 6.51-6.48 (d, lH), 4.95-4.93 (d, 1H), 3.64-3.62 (m, 2H), 3.18-2.94 (m, 6H), 2.80-2.38 (m, 5H), 1.44-1.21 (m, 4H), 0.78-0.76 (m, 6H); ESI-MS: m/z 405.32 (M+H)+.



N-((2R,3S)-3-amino-2-hydroxy-4-phenylbutyl)-4-fluoro-N-isopentylbenzenesulfonamide (15): To a stirred solution of 14(1.0 g, 0.0039 mol) in 10 mL CH_2Cl_2 of and cooled to 0°C ,to this added 4-flouro benzene sulfonyl chloride (0.85 g, 0.0043 moles) drop wise for 5 min. And allowed the reaction mixture to room temperature for 6 h. then Reaction mass was monitored by TLC. After completion of reaction mass was diluted with 50 mL of CH_2Cl_2 and washed with water followed by Brine solution, Organic layer was dried under sodium sulphate, then concentrated under reduced pressure, obtained crude compound was purified by flash silica column chromatography using 100-200 silica gel, Product **15** was eluted at 10% Ethyl acetate in Hexane, then the fractions were collected to concentrated. Yield: 0.81g (49.69%) HPLC: 93.32%; ^{1}HNMR (300 MHz, DMSO-d6): 6 ppm 7.85-7.80 (m, 2H), 7.44-7.38 (m,

2H), 7.28-7.16 (m, 5H), 6.53-6.50 (d,lH), 4.98 (bs, 1H), 3.63-3.62(111, 2H), 3.23-2.99 (m, 4H), 2.81-2.57(m, 2H), 1.31-1.22 (m, 4H), 0.79-0.77 (m, 6H),; ESI-MS: m/z 409.42 (M+H)+.



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