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Characterization, synthesis and biological evaluation of naphthalene based piperazines as anti bacterial agents

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ABSTRACT

The present research paper has been focused on synthesis of naphthalene based piperazine molecules by adopting appropriate synthetic steps. Purification of intermediates and final titled compounds has been done by recrystallization and also by chromatographic techniques. Characterization of all the synthesized naphthalene derivatives including intermediates by physical and spectral data like IR, Proton NMR and Mass. Biological evaluation of the newly synthesized compounds for their pharmacological activity has been studied and found that the synthesized compounds were active against Gram positive and Gram negative bacteria like Satphylococcus aureus, Escherichia coli, Bacillus subtilis and Klebsiella pneumonia by adopting standard protocols.

Key words: Naphthalene derivatives, piperazines, pharmacological activity, characterization and Evaluation.

INTRODUTION

Naphthalene containing piperazine heretocycles are medicinally important and molecules possessing them are associated with potent antimicrobial activity, anti inflammatory and antoxidants etc, which may suggest for design and development of new antimicrobial agents, anti inflammatory and antoxidants'. In view of the importance of the above hetrocyclics the author attempted and prepared some novel naphthalene based piperazine derivatives. The author in the present work characterized the titled compounds and studied the biological activities for the synthesized compounds.

Literature survey revealed that the Naphthalene based piperazines designed and synthesized both the quinoline and naphthalene containing molecules influenced by the unique structural makeup of Mefloquine and TMC207 respectively [1]. These compounds were evaluated for their anti-mycobacterial activity against drug sensitive *Mycobacterium tuberculosis* $H_{37}Rv$. Another series of N -[2-(2-naphthyl)ethyl]piperazinyl quinolones containing a carbonyl related functional groups (oxo- or oxyimino-) on the ethyl spacer and evaluated for antibacterial activity[2].Antifungal activity and synthesis of 1-keto,3-carboxy,6,7-methyene,1,2,3,4-tetrahydro naphthalene derivatives was reported[3].Antimicrobial activity of naphthalene derivatives against wide range of pathogens was also mentioned[4].Novel quinoline and naphthalene derivatives as anti mycobacterial agents by Ramshankar Upadhayaya et al[5] exists. Synthesis of antifungal, antibacterial and antitubercular activities of some piperazine derivatives was studied [6-8].Electrochemical properties of naphthalene derivatives were studied by

Banukoz[9].Antidepressant activity of phenothaizolyl-1H,2H,3H,4H-naphthalene derivatives reported[10].In view of the above the author find the importance of Naphthalene based piperazines as potent biologically important compounds and succeeded in synthesis of the said compounds.

MATERIALS AND METHODS

The following chemical were used in the present study of synthesis of Naphthalene containing piperzines such as Naphthalene, Benzoyl chloride, Acetone, Sodium chloride Sodium sulphate, Absolute alcohol, Dimethyl formamide, Isopropyl alcohol, Hexane and Ethyl acetate from SD Fine chemicals(LR grade).Anhydrous Aluminium chloride(AR grade), Carbon disulphide LR grade, Sodium borohydride, (±)*epi*-chlorohydrin AR from Merck chemicals. Molecular sieves (4A) LR from Wilson laboratories.4-MethylPiperazine, 4-EthylPiperazine,1-(o-olyl) piperazine, 4-phenyl piperazine and 1-(4-nitrophenyl) piperazine were from Alfa Aesar chemicals(AR grade) were used in the present work.

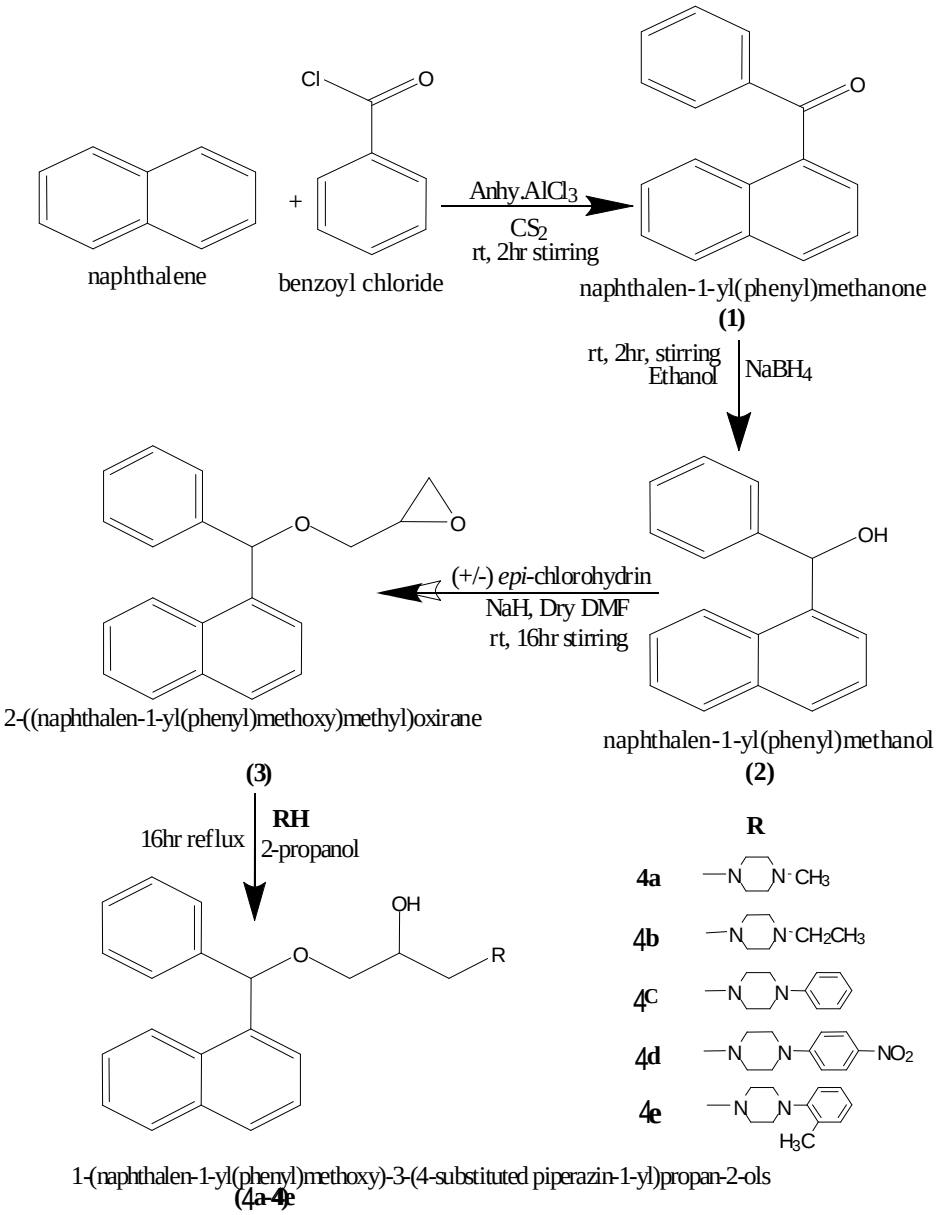
For biological activity: (antimicrobial): Cup-plate agar diffusion method. For the Nutrient Broth medium, Nutrient Agar medium, Dimethyl sulfoxide (DMSO), Ciprofloxacin and Distilled water. Bacterial organisms used were Gram-positive bacteria and Gram-negative bacteria such as Satphylococcus aureus, Escherichia coli, Bacillus subtilis and Klebsiella pneumonia respectively were used.

Instruments used:
The following are the instruments used for the characterization of synthesized compounds such as Melting points were recorded on Melter Fp-51 instrument and are uncorrected. Infrared spectra were recorded on Bruker Model 283B and Nicolet-740 FT-IR instruments and values are given in cm^{-1}.Proton magnetic resonance spectra were recorded on Varian Gemini-200,Varian unit-500 and Avance 300 MHz, BrukerUx-NMR instrument and the samples were made in $CDCl_3$ using tetra methyl silane (Me_4Si) as the internal standard. A mass spectrum is recorded on VG Micromass 7070H (ESI and EI) and was given in mass units (m/z).Each reaction was monitored by TLC by using appropriate solvent system, which was selected by trial and error method. Pre-coated TLC plates (0.25mm silica gel) were obtained from E. Merck. All solvents extracts were washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated at reduced pressures on Buchi-R-3000 rotary evaporator below 50^0C.For biological activity study of synthesized compounds Laminar air flow cabin, Incubator, Refrigerator, Micropipettes,Culture plate,Boiling tubes, Eppendorf tubes and Aerosol resistant tips were used.

Experimental methodology:
The various derivatives with naphthalene based piperazine compounds have got various activities like anti-inflammatory, antioxidant, anticancer, hypoglycemic, antibacterial, antifungal, antitubercular, antimalarial etc. In continuation of such investigations and in a search for less toxic and pharmacologically more potential derivatives, The author has been taken up the synthesis and pharmacological evaluation of some new naphthalene based piperazine derivatives. In view of the biological importance of the naphthalene and piperazines it was planned to synthesize **1-(naphthalen-1-yl (phenyl)methoxy)-3-(4-substituted pipera-zin-1-yl) propan-2-ols (4a-4e)** compounds and to screen them for antimicrobial activity. The compounds have been synthesized and were mentioned in the **Scheme 1.**

Experimental procedure: The preparation of Naphthalene based piperazines consists of four steps and was as follows.

Preparation of naphthalen-1-yl (phenyl) methanone(1):A solution of naphthalene(5.0g,0.031moles) in 13.5ml of carbon disulphide and a solution of Anhy.$AlCl_3$ (3.2g,0.023moles) and benzoyl chloride(3.2g,0.023moles) in 5.6ml of carbon disulphide were added and stirred at rt for 2hr and the stirring was stopped based on the TLC progress. Then, the reaction mixture was hydrolyzed in water, later organic phase was evaporated under reduced pressure and the resultant residue was extracted with ethyl acetate. The combined extract was washed with water (2times,10 ml each) followed by brine (10 ml), dried over anhydrous sodium sulfate, filtered and concentrated to obtain crude residue of napthalen-1-yl-phenyl-methanone.The crude residue was purified over silica gel using solvent mixture hexane and ethyl acetate(9:1) as eluent to getnapthalen-1-yl-phenyl-methanone as crystalline powder. Yield:5gm (75%); Melting point:112-114^0C, R_f:0.42 (hexane: ethyl acetate 9:1) IR (KBr) cm^{-1}: 3067(C-H str; aromatic),1681(C=O str; ketone), MASS (ESI) : 255$[M+Na]^+$,105 (100% base peak, benzoyl ion).



SCHEME-1

Preparation of Napthalen-1-yl-phenyl-methanol (2): Napthalen-1-yl-phenyl-methanone **(1)** (1 g, 4.2mmol) was taken in ethanol (20 ml) and the mixture was cooled to 0°C. (ice bath). Sodium borohydride (0.2g, 5.8mmol) was added to this solution, the cooling bath was removed and the reaction was stirred at rt for 2 h. Progress of the reaction was monitored by TLC, and after completion of the reaction the mixture was quenched by addition of ice pieces; the volatiles were removed under reduced pressure and extracted with ethyl acetate (2times, 10 ml). The combined extract was washed with water (2times,10 ml each) followed by brine (10 ml), dried over anhydrous sodium sulfate, filtered and concentrated to obtain pure light yellow colored residue of napthalen-1-yl-phenyl-methanol (0.76g, 76%). Yield: 0.76gm (76%); Melting Point: 120-121^{0}C,R_f:0.32 (hexane: ethyl acetate 8:2) IR (KBr) cm^{-1}:3400(O-H str; alcoholic), 3080(C-Hstr; aromatic), 2851(C-H str; aliphatic), 1672(C=Cstr; aromatic) ^{1}H-NMR [$CDCl_3$, 300 MHz]: δ=1.9 (s, 1H,-C*H*-OH), δ= 5.9(s,1H,-O*H*), δ=7.15(t,1H,Ar-*H*),δ=7.3 (t, 2H, Ar-*H*),δ=7.5(d,2H, Ar-*H*), δ=7.52(t,1H,Ar-*H*), δ=7.54(t,1H,Ar-*H*), δ=7.59(t,1H,Ar-*H*),δ=7.70(d,1H,Ar-*H*), δ=7.9 (d,1H,Ar-), δ=7.85(d,1H,Ar-*H*),δ=8.0(d,1H,Ar-*H*).

Preparation of 2-((naphthalen-1-yl(phenyl) methoxy) methyl) oxirane(3): Napthalen-1-yl-phenyl-methanol **(2)** (1g, 4mmol) was dissolved in dry DMF (1mL), cooled to 0°C and sodium hydride (0.12g, 5mmol) was added portion wise. Then, cooling bath was removed and the reaction mixture was stirred at rt for 30 min. (±)*epi*-chlorohydrin (0.64mL, 8.4mmol) was added and stirring was continued for further 16 hr at rt. Reaction mixture was concentrated under reduced pressure; ice-cold water was added and extracted with ethyl acetate (2times, 10 ml each).The combined extract was washed with water (2times,10 ml each) and brine (10 ml). Organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to obtain light yellow colored residue (0.62, 62%).with the yield of 0.62gm (62%); Melting point:118-119^{0}C,R_f:0.72(hexane: ethyl acetate 7:3)^{1}H-NMR [$CDCl_3$, 300 MHz]: δ =2.84, 2.92 (d, 2H, -C*H*$_2$-CH- of oxirane), δ=2.5(p,1H,-CH_2-C*H*-CH_2-),δ=3.57, 3.7(d,2H,O-C*H*$_2$-CH),δ=5.9(s,1H,-C*H*-O), δ=7.7(d,1H,Ar-*H*),δ=7.5(t,1H,Ar-*H*),δ=7.85(d,1H,Ar-*H*),δ=7.9 (d,1H,Ar-*H*), δ=7.5(t,1H,Ar-*H*),δ=7.52 (t,1H,Ar-*H*), δ=8.0 (d,1H, Ar-*H*), δ =7.52(d,2H,Ar-*H*), δ=7.2(t, 1H,Ar-*H*),δ=7.1(t, 1H, Ar-*H*).

Prepartion of 1-(naphthalen-1-yl(phenyl)methoxy)-3-(4-substituted piperazin-1-yl) propan-2-ols (4a-4e): Oxirane**(3)** (0.3g, 1.02mmol) and **RH** (0.27g, 1.02mmol) were dissolved in 2-propanol (30 ml) and the mixture was refluxed for 16 hr and the reaction was monitored by TLC. After completion of reaction the mixture was concentrated under reduced pressure to get thick liquid as a crude product. Crude product was purified by column chromatography (eluent: ethyl acetate, hexane; gradient elution) to obtain pure1-(naphthaalen-1-yl(phenyl) methoxy)-3-(4-substituted piperazin-1-yl)propan-2-ols(4a-e).Yield:0.13g(45%),Melting point:122-124^{0}C, MASS(ESI): 391[M+1]$^+$, ^{1}H-NMR [$CDCl_3$, 300MHz]:δ=2.25(s,3H,-C*H*$_3$),δ=2.32(t,4H,-C*H*$_2$-$CH_2$of piperazine), δ=2.7(t,4H,-C*H*$_2$-$CH_2$of piperazine), δ=2.95, 2.8(d,2H, -CH-C*H*$_2$-N-),δ=4.1(p,1H,-CH_2-C*H*-CH_2),δ=3.75(s,1H,-CH-*H*), δ=3.35, 3.2 (d,2H,-C*H*$_2$-CH),δ=6.35(s,1H,-C*H*-O),δ=7.7(d,1H,Ar-), δ=7.5 (t,1H,Ar-*H*), δ=7.9(d,1H,Ar-*H*),δ=7.95(d,1H,Ar-*H*), δ=7.56 (t,1H, Ar-*H*),δ=7.51(t,1H, Ar-*H*), δ=8.1(d,1H, Ar-*H*),δ=7.52(d,2H, Ar-*H*), δ=7.2(t,2H, Ar-*H*), δ=7.15(t,1H, Ar-*H*).The detailed Mass and ^{1}H-NMR spectrum of compound (4a) was incorporated in figure-5 and 6 respectively.

Biological evaluation for anti-bacterial activity:
The antibacterial activity of the substituted **1-(naphthalen-1-yl (phenyl)methoxy)-3-(4-substituted piperazin-1-yl)propan-2-ol(4a-4e)**has been studied against four different strains of bacteria by cup-plate agar diffusion method by measuring the zone of inhibition in mm. Said Gram-positive bacteria and Gram-negative bacteria were selected for the said activity of synthesized compounds.

The bacterial growth inhibition can be measured by two methods namely cup plate method and serial dilution method. Nutrient broth has been used for the preparation of inoculums of the bacteria and nutrient agar is used for the evaluation of antibacterial activity as Bacterial culture medium. Composition of the nutrient agar medium such as Peptone 5gm, Sodium chloride5gm, Beef extract 1.5gm, Yeast extract 1.5gm, Agar1.5gm, Distilled water up to1000mLpH: 7.4±0.2 were used.

Procedure: (Cup plate method): The test organisms were sub cultured using nutrient broth medium. The tubes containing sterilized medium were inoculated with respective bacterial strains. After incubation at 37±1^{0}C for 24 hours, they were stored in refrigerator. The stock cultures were maintained. The flasks were incubated at 37±1^{0}C for 48 hours before the experimentation.

Solution of the test compound was prepared by dissolving the sample in DMSO.A reference standard for both Gram-positive and Gram-negative bacteria was made by dissolving accurately weighed quantity of Ciprofloxacin in sterile distilled water. The nutrient agar medium was sterilized by autoclaving at 121^{0}C for 15min. The petriplates, tubes and flasks plugged with cotton were sterilized in hot air oven at 160^{0}C for an hour. Into each sterilized petriplate, about 25 ml of molten nutrient agar medium inoculated with the respective strains of bacteria was transferred, aseptically. The plates were left at room temperature to allow solidification. In each plate, cups of 10mm diameter were made with sterile borer. Then 100µl of the test solution of each compound in concentration of 50, 100, 200 µg/ml was added to the respective cups aseptically. The plates were kept undisturbed for atleast 2hours in refrigerator to allow diffusion of the solution properly into the nutrient agar medium. After the incubation of the plates at 37±1^{0}c for 24 hours, the diameter of the zone of inhibition surrounding each of the cups was measured with the help of the scale and tabulated. All the experiments were carried out in triplicate. Simultaneously controls were maintained employing 0.1ml of DMSO to observe the solvent effects. Results of anti-bacterial activity of compounds are presented in **Table-1.**(Gram-negative bacteria), **Table-2.** (Gram-positive bacteria) and **Table-3** and

figure-1 give the antibacterial activity data of test compounds which are active against four organisms at one concentration i.e., 200µg/ml and labeled accordingly.

RESULTS AND DISCUSSION

Total of five compounds were prepared by adopting standard procedure. In which 1-(4-methylpiperazin-1-yl)-3-(naphthalen-1-yl (phenyl) methoxy) propan-2-ol**(4a)** was obtained as a brown solid,yield: 0.13g (45%) and melting point:122-124^{0}C.1-(4-ethylpiperazin-1-yl)-3-(naphthalen-1-yl(phenyl)methoxy)propan-2-ol**(4b)**was obtained as yellow solid with an yield of 0.2g (60%), melting point:135-138^{0}C was recorded.1-(naphthalen-1-yl (phenyl) methoxy)-3-(4-phenylpiperazin-1-yl)propan-2-ol **(4c)** was obtained as yellow solid have an yield of 0.16g (55%) with melting point: 160-161^{0}C.1-(naphthalen-1-yl(phenyl)methoxy)-3-(4-(4-nitro- phenyl) piperazin-1-yl)propan-2-ol**(4d)** was obtained as dark yellow solid, yield - 0.14g (48%) and melting point of 152-153^{0}C were recorded. 1-(naphthalen-1-yl(phenyl)methoxy)-3-(4-(o-tolyl)piperazin-1-yl)propan-2-ol **(4e)** was obtained as yellow solid, yield-0.15g (52%), melting point - 160-161^{0}C.1-(naphthalen-1yl(phenyl)methoxy)-3-(piperazin-1-yl)propan-2-ol(**4a-4e**) compounds were synthesized as per **scheme 1.**The details of IR, ^{1}H-NMR and Mass spectral data for the synthesised 1-(naphthalen-1-yl (phenyl) methoxy)-3-(4-substituted piperazin-1-yl) propan-2-ols compounds **(4a-4e)** and their precursors namely **1,2** and **3** were incorporated. The synthesized derivatives **(4a-4e)** has been evaluated for their antibacterial activity against Gram-positive (Staphylococcus aureus, Bacillus subtilis) and Gram-negative (Escherichia coli, Klebsilla pneumonia) bacteria by measuring the zone of inhibition. The results have been compared with a broad spectrum antibacterial agent Ciprofloxacin as standard drug. Among all the synthesized compounds, compound **4c**(R= 4-phenyl piperazin-1-yl), **4b**(R= 4-ethyl piperazine-1-yl), **4e** (R=4-(2-methyl phenyl) piperazin-1-yl) were found to be more effective than the other compounds. The compound**4b, 4c** showed maximum activity against E.coli, K.pneumonia at 200µg/ml with zone of inhibition 6mm.The compound **4c** showed maximum activity against S.aureus at 200µg/ml with zone of inhibition 6mm.The compound **4b** showed less activity against Gram-positive bacteria (B.subtilis) at 200µg/ml with zone of inhibition 4mm, whereas compounds **4a**(R= 4-methyl piperazine-1-yl), **4d** (R= 4-(4-nitro phenyl) piperazine-1-yl) did not show any zone of inhibition at all three concentrations tested. The compound **4e** showed maximum activity against Gram-negative (E.coli, K.pneumonia)at 200µg/ml with zone of inhibition 5mm but are least active against Gram-positive (S.aureus, B.subtilis) at 200µg/ml with zone of inhibition 4mm.The compound **4a** and **4d** were inactive at all concentrations tested, against both Gram-positive and Gram-negative organisms. Among all those compounds, piperazine ring substituted with ethyl **(4b)** and simple phenyl ring **(4c)** and with 2-methyl phenyl substitution **(4e)** showed good activity against all the four organisms tested.

Table 1. Antibacterial activity of 1-(naphthalen-1-yl (phenyl)methoxy)-3-(4-substituted piperazin-1-yl)propan-2-ol (4a-e) (Gram negavive bacteria)

Compound	Escherichia coli			Klebsilla pneumonia		
	Concentration(µg/ml)			Concentration(µg/ml)		
	50	100	200	50	100	200
4a	NA	NA	NA	NA	NA	NA
4b	NA	2mm	6mm	NA	2mm	4mm
4c	NA	2mm	6mm	NA	NA	NA
4d	NA	NA	NA	NA	NA	NA
4e	NA	2mm	5mm	NA	3mm	5mm
Ciprofloxacin	10mm	12mm	14mm	10mm	12mm	14mm

All values are expressed as zone of inhibition in mm.
NA =No activity, Standard = Ciprofloxacin.

Table 2. Antibacterial activity of 1-(naphthalen-1-yl(phenyl)methoxy)-3-(4-substituted piperazin-1-yl)propan-2-ol (4a-e) (Gram positive bacteria)

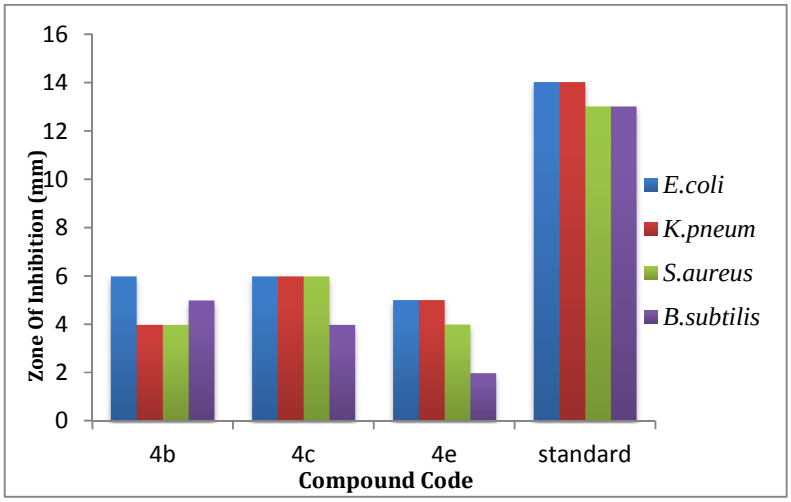
Compound	Steptocacus Aureus			Bacilus Subtilis		
	Concentration(µg/ml)			Concentration(µg/ml)		
	50	100	200	50	100	200
4a	NA	NA	NA	NA	NA	NA
4b	NA	2mm	4mm	NA	2mm	5mm
4c	NA	2mm	6mm	NA	2mm	4mm
4d	NA	NA	NA	NA	NA	NA
4e	NA	2mm	4mm	NA	2mm	2mm
Ciprofloxacin	10mm	11mm	13mm	10mm	11mm	13mm

All values are expressed as zone of inhibition in mm.
NA =No activity, Standard = Ciprofloxacin.

Table 3. Antibacterial activity of 1-(naphthalen-1-yl(phenyl)methoxy)-3-(4-substituted piperazin-1-yl)propan-2-ol (4b, 4c, 4e) at 200µg/ml concentration

Compound	Escherichia coli	Kebsiella pneumonia	Staphylococcus aureus	Bacius subtilis
4b	6mm	4mm	4mm	5mm
4c	6mm	6mm	6mm	4mm
4e	5mm	5mm	4mm	2mm
Ciprofloxacin	14mm	14mm	13mm	13mm

Figure-1. Antibacterial activity of 1-(naphthalen-1-yl(phenyl)methoxy)-3-(substituted piperazin-1-yl)propan-2-ol (4b, 4c, 4e) at 200µg/ml concentration(Bar diagram)



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