Original Article

Synthesis and antibacterial evaluation of naphthalene based 1,5-benzodiazepine derivatives

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ABSTRACT

Various substituted 1,5-benzodiazepines were synthesized by condensing different naphthalene-based chalcones with o-phenylenediamine in methanol using piperidine as a base under refluxing conditions and were obtained in good yield after purification. The final compounds **(5a-e)** were structurally elucidated and evaluated in vitro for antibacterial activity against three human pathogenic bacterial strains using the disc diffusion method and MIC was determined by serial dilution method. The antibacterial evaluation led to the identification of compounds **5d** and **5e** as the most potent molecule of the series. The promising compounds identified in the study can be exploited in near future for the design of more potent candidates with better efficacy.

Keywords: 1,5-Benzodiazepines, antibacterial activity, chalcones, naphthalene

INTRODUCTION

Bacteria are a large domain of prokaryotic microorganisms. Typically a few micrometers in length, bacteria have a wide range of shapes, ranging from spheres to rods and spirals.[1] There are nearly 40 million of bacteria which are present in most habitats on Earth, growing in soil, acidic hot springs, radioactive waste, water, and deep in the Earth's crust, as well as in organic matter and the live bodies of plants and animals, providing outstanding examples of mutualism in the digestive tracts of humans, termites, and cockroaches.[2-4] They share a basic structure which is similar enough that antimicrobial drugs act in a fairly uniform fashion against them, regardless of the species. The permeability of bacteria to foreign substances such as antimicrobials is broadly determined, however, by the basic structure of the organism's cell wall, that is, by whether the organism is Gram-negative or Gram-positive.[5,6]

The antimicrobial agents which are used for the treatment of act by four different modes of action are: (1) Interference with cell wall synthesis, (2) inhibition of protein synthesis, (3) interference with nucleic acid synthesis, and (4) inhibition of a metabolic pathway.[7,8] Bacterial cells acquire drug resistance in a variety of ways which includes (a) acquiring the genes encoding enzymes which will destroy the antimicrobial agents such as β-lactamase, (b) acquiring an efflux pump which extrude the drug from the bacterial cells, or (c) by altering gene producing cell wall, which, in turn, produce altered proteins to which drug no longer can bind.[9,10] According to the World Health Organization, the human race soon enters into the "post-antibiotic" era, wherein bacteria become powerful enough to cause mass deaths. Some of the important examples of microbial agents becoming resistant toward first- and second-line antimicrobial therapies are vancomycin-resistant enterococci and multidrug-resistant *Staphylococcus aureus* which is posing life-threatening challenges especially in immune-compromised patients.[11] The mechanism of antimicrobial resistance is depicted in Figure 1.

1,5-benzodiazepines have presented a ubiquitous class of seven-membered heterocyclic molecules which represent a variety of biological activities. They are regarded as one of the most privileged classes of heterocyclic molecules in the recent past. They are valuable synthons for a variety of drug development processes.[12] They are the most widely used and prescribed class of drug molecules for their

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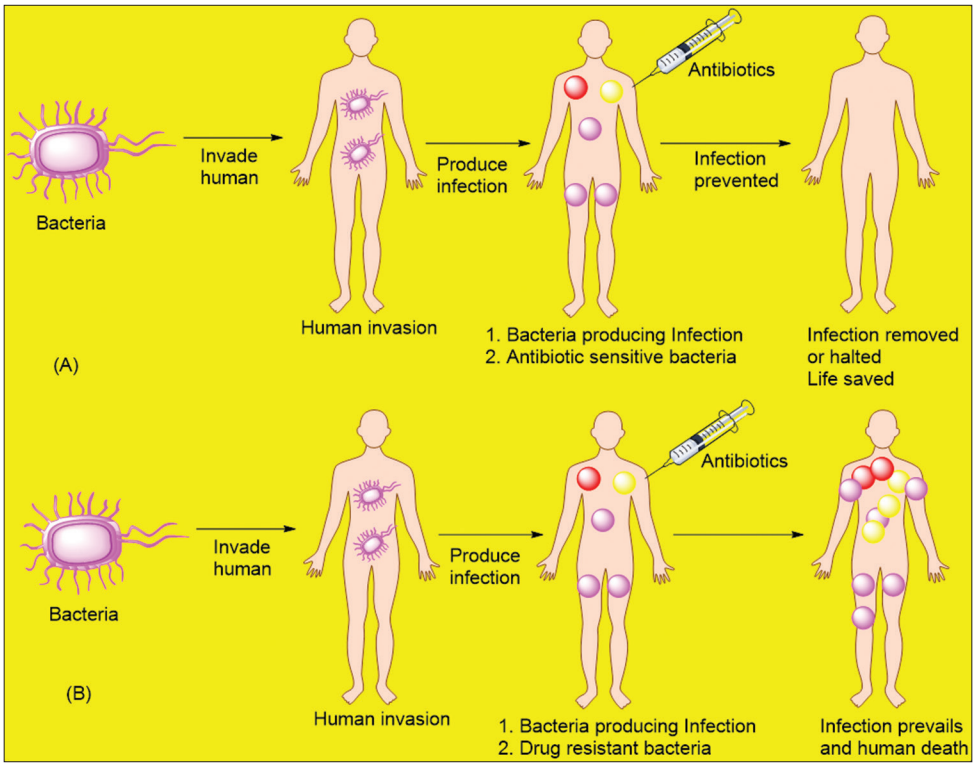


Figure 1: (a) Bacterial infection and its treatment using antibiotic (b) drug resistance preventing the drug to cure the infection

anxiolytic, antidepressant, and muscle relaxant, etc., properties. In the recent past, benzodiazepine derivatives were reported to possess various pharmacological activities such as antimicrobial, anticancer, anti-anxiolytic, antidepressant, anticonvulsant, anti-tubercular, anti-inflammatory, analgesic, antihistaminic, and anti-anxiety activities.[13-19]

Considering our previous work in the area[20] and the development of benzodiazepine as a potential antimicrobial agent, our group decided to synthesis some new 1,5-benzodiazepine derivatives and evaluated them as promising antimicrobial agents. In this work, various naphthyl-based chalcones were prepared and condensed with o-phenylenediamine to obtain the final 1,5-benzodiazepine derivative. The newly synthesized analogs were evaluated against three different microbial strains and their minimum inhibitory concentration (MIC) values were determined.

RESULTS AND DISCUSSION

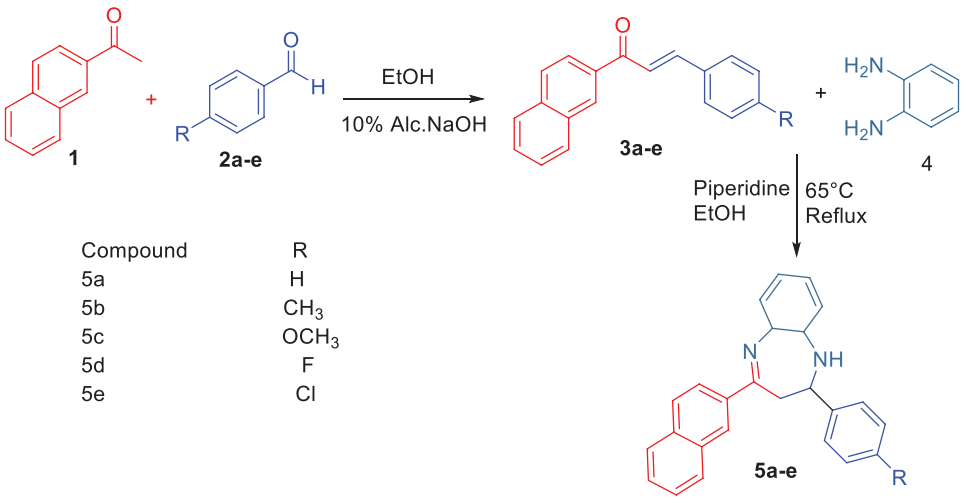
Chemistry

A series of substituted naphthalene-based benzodiazepine derivatives were prepared using solution-phase chemistry. The intermediate chalcones (**3a–e**) were synthesized through Claisen-Schmidt condensation of naphthyl ketone (**1**) with substituted aryl aldehydes (**2**). The intermediated obtained was treated with o-phenylenediamine (**4**) under reflux conditions to obtain the required pyrimidine derivative [**5a-e**, Scheme 1]. The IR spectra displayed two characteristic peaks primary amine group at a value of 3340–3356 cm^{-1} (N-H asymmetrical stretching) and peak at 1602–1610 cm^{-1} characterized as symmetrical stretching of the C-N group. The other peaks resembling aromatic region was also observed at the expected frequencies. The ^{1}H nuclear magnetic resonance (NMR) spectra showed a characteristic at 3.9–4.15 ppm which resembles the N-H proton of the naphthalene nucleus. The classical ABX pattern due to vicinal coupling of protons at C_3 and C_4 positions of benzodiazepine ring was also observed. The Hx proton was observed in the range of 5.40–5.50 ppm, H_B was observed in the range of 3.14–3.32ppm while the proton H_A was observed at 3.08–3.15 ppm in the synthesized compounds. All the protons of the aromatic region were observed at their expected chemical shifts. Further, the mass and elemental analysis supported their structures. The physical parameters of synthesized 1,5-benzodiazepines are summarized in Table 1.

Antibacterial activity

Antibacterial activity of compounds was assessed against three human pathogenic bacterial strains using the disc diffusion method on Mueller-Hinton agar plates. Ciprofloxacin was used as a standard drug. Two Gram-positive bacterial strains (*Bacillus subtilis* MTCC 2451 and *S. aureus* MTCC 96) and one Gram-negative bacterial strain (*Escherichia coli* MTCC 443) were used for assessing the antibacterial potential of title compounds. The potency of test compounds was described on the basis of measurement of the diameter of the zone of inhibition observed in the Petri plates with zone reader and by MIC values. Zone of inhibition data of various synthesized compounds is summarized in Table 2 whereas MIC values are given in Table 3.

The result of in vitro antibacterial assay revealed that compounds **5d** (4-fluorophenyl derivative) and **5e** (4-chloro derivative) exhibited the most potent inhibition of tested bacterial strains. Both the compounds represented a broad spectrum of activity. Compound **5a**



Scheme 1: General procedure of synthesis of benzodiazepines **(5a-e)**

having unsubstituted phenyl stood next to them while compounds **5b** and **5c** displayed poor activities against the tested strains of bacteria. In general, compounds were more active toward Gram-positive bacteria and less active toward Gram-negative bacteria. Moreover, the compounds having electron-withdrawing groups at phenyl represented the best inhibition of tested strains while those with electron-donating groups were less active. However, no compounds were found to be equal or more potent than the standard drug ciprofloxacin. The compounds were further evaluated for MIC value using serial dilution method wherein compound **5d** exhibited most significant activity against *S. aureus* (MIC = 7.8 μg/mL), followed by *B. subtilis* (MIC = 15.6 μg/mL). Compound **5e** displayed MIC in the range of 62.5–125 μg/mL against the tested strains. The other tested compounds either displayed poor inhibitory activity or found inactive.

Table 1: Physical data of the synthesized compounds (5a-e)

Compound	Molecular formula	Molecular weight	Melting point (°C)	R_f [a]	CHN
5a	$C_{25}H_{22}N_2$	350	145–147	0.62	Calculated: C, 85.68; H, 6.33; N, 7.99, Observed: C, 85.62; H, 6.28; N, 8.01
5b	$C_{26}H_{24}N_2$	364	150–152	0.54	Calculated: C, 85.68; H, 6.64; N, 7.69 Observed: C, 85.70; H, 6.70; N, 7.59
5c	$C_{26}H_{24}N_2O$	380	156–158	0.58	Calculated: C, 82.07; H, 6.36; N, 7.36 Observed: C, 81.99; H, 6.28; N, 7.28
5d	$C_{25}H_{21}FN_2$	368	148–150	0.65	Calculated: C, 81.50; H, 5.75; N, 7.60 Observed: C, 81.56; H, 5.75; N, 7.60
5e	$C_{25}H_{21}ClN_2$	384	160–162	0.60	Calculated: C, 78.01; H, 5.50; N, 7.28 Observed: C, 78.00; H, 5.55; N, 7.36

a = EtOAc : Hexane (1:9)

Table 2: Zone of inhibition of synthesized compounds (mm)

Compound	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*
5a	10	12	8
5b	NA	NA	10
5c	12	NA	NA
5d	22	24	18
5e	15	15	16
Ciprofloxacin*	24	26	30
Dimethyl sulfoxide	-	-	-

NA: No activity, *-standard drug

EXPERIMENTAL

Solvents and organic reagents were purchased from Sigma-Aldrich, Hi-media, and Loba-Chemie (India) and were used without further purification. Thin-layer chromatography was performed using commercially available pre-coated plates (Merck Kieselgel 60 F silica). Spots were visualized under ultraviolet light and iodine chamber. Mass spectra were recorded on gas chromatography-mass spectrometry (electrospray ionization [ESI]). Infrared (IR) spectra (KBr pellets) were recorded on a Thermo Fourier transform IR spectrophotometer. ^1H and ^{13}C NMR of the compounds were recorded on the JEOL or Bruker Advance II instrument at 300 MHz frequency, in CDCl3 and tetramethylsilane (TMS) $\delta = 0$) are used as an internal standard. The chemical shifts are reported in parts per million (δ) downfield from the signal of TMS added to the deuterated solvent. Spin multiplicities are given as s (singlet), b (broad), d (doublet), dd (double doublet), t (triplet), q (quartet), or m (multiplet). Microanalyses were performed on a Perkin-Elmer 240 CHN elemental analyzer. Melting points were recorded with the Stuart SMP30 melting point apparatus and are uncorrected.

General method for synthesis of chalcones (3a-e)

Required intermediate chalcones were synthesized by condensing equimolar portions of substituted aryl aldehydes (10 mmol, 1equiv) and acetophenones (10 mmol, 1equiv) using the synthetic methodology described in the literature.

General procedure for the synthesis of 1,

Table 3: Minimum inhibitory concentration of synthesized compounds

Compound (µg/ml)	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*
5a	125	62.5	125
5b	>125	>125	125
5c	62.5	>125	NA
5d	15.6	7.8	62.5
5e	62.5	125	62.5
Ciprofloxacin	0.25	0.5	0.15

5- benzodiazepines (5a-e)

Various chalcones (**3a-h**, 0.01 mole) and *o*-phenylenediamine (**4**, 0.01 mole) were dissolved in 25 mL of previously warm ethanol. When a clear solution was obtained, 2–3 drops of piperidine were added and the mixture was refluxed for 4–6 h. Progress of the reaction was determined by thin-layer chromatography using ethyl acetate: hexane (1:9) as solvent system. After completion, the reaction was allowed to stand overnight at room temperature. A yellow or pale yellow or white colored solid was separated. The precipitates were filtered using suction and the mother liquor was discarded. The precipitates were washed with water to remove excess of base and then with hexane and diethyl ether to remove impurities and dried to get pure compounds in satisfactory yield (55–70%).

4-(naphthalen-2-yl)-2-phenyl-2,3,5a,9a-tetrahydro-1H-benzo[b][1,5]diazepine (5a)

White powder; 67% yield; IR (KBr, ν_{max}, cm^{-1}); 3342.13(N-H str), 1604.75(C=N str),1561.84 (C-N str), 1465.01 (C=C str), 1H NMR (400, Mhz, $CDCl_3$, δ (ppm),TMS = 0, J_{Hz}); 7.96–7.93 (2H, m), 7.81–7.80 (2H, dd, *J* = 8, 9.6), 7.75–7.70 (1H, m), 7.46–7.45 (2H, dd, *J* = 7.9, 9.4), 7.27–7.20 (7 H, m), 5.93–5.90 (2H, dd, *J* = 10, 8.8), 5.80–5.78 (2H, dd, *J* = 9.9, 8.7), 5.47-5.45 (1H, dd, *J* = 4.0, 9.8), 4.15 (1H, bs), 3.18–3.16 (1H, dd, *J* = 3.9, 12.6), 3.10–3.08 (1H, dd, *J* = 9.7, 12.5), mass (ESI) m/z [M+H] 351.

4-(naphthalen-2-yl)-2-(p-tolyl)-2,3,5a,9a-tetrahydro-1H-benzo[b][1,5]diazepine (5b)

Yellow crystalline powder; 70% yield; IR (KBr, ν_{max}, cm^{-1}); 3340.15 (N-Hstr), 1606.75(C=Nstr),1564.65 (C-Nstr), 1460.25 (C=Cstr), 1H NMR (400, Mhz, $CDCl_3$, δ (ppm), TMS = 0, J_{Hz}); 7.98–7.95 (2H, m), 7.79–7.77 (2H, dd, *J* = 7.8, 9.2), 7.73–7.69 (1H, m), 7.40–7.35 (2H, dd, *J* = 8.1, 9.1), 7.21–7.13 (6 H, m), 5.91–5.89 (2H, dd, *J* = 9.9, 8.6), 5.76–5.70 (2H, dd, *J* = 9.8, 8.6), 5.45–5.43 (1H, dd, *J* = 3.9, 10.8), 4.13 (1H, bs), 3.14-3.12 (1H, dd, *J* = 3.9, 13), 3.08–3.06 (1H, dd, *J* = 10.8, 12.9), 2.33 (3H, s); mass (ESI) m/z [M+H] 365.

2-(4-methoxyphenyl)-4-(naphthalen-2-yl)-2,3,5a,9a-tetrahydro-1H-benzo[b][1,5]diazepine (5c) Cream white powder; 55% yield; IR (KBr, ν_{max}, cm^{-1})

3350.13(N-Hstr), 1602.80 (C=N str), 1570.84 (C-N str), 1460.20 (C=C str), 1H NMR (400, Mhz, $CDCl_3$, δ (ppm), TMS = 0, J_{Hz}); 8.01 (1H,t, *J* = 9)), 7.97–7.94 (2H, m), 7.75–7.72 (2H, dd, *J* = 8.0, 9.0), 7.40–7.35 (2H, dd, *J* = 8.1, 9.1), 7.21–7.13 (6 H, m), 5.91-5.89 (2H, dd, *J* = 9.9, 8.6), 5.76–5.70 (2H, dd, *J* = 9.8, 8.6), 5.50–5.45 (1H, dd, *J* = 4.2, 11.2), 3.98 (1H, bs), 3.87 (3H,s), 3.14–3.12 (1H, dd, *J* = 4.1, 11.9), 3.08–3.06 (1H, dd, *J* = 11.2, 11.9); mass (ESI) m/z [M+H] 381.

2-(4-fluorophenyl)-4-(naphthalen-2-yl)-2,3,5a,9a-tetrahydro-1H-benzo[b][1,5]diazepine (5d)

Yellow crystal; 65% yield; IR (KBr, ν_{max}, cm^{-1}); 3355.10 (N-Hstr), 1608.75 (C=N str), 1565.08 (C-N str), 1458.12 (C=C str), 1H NMR (400, Mhz, $CDCl_3$, δ (ppm), TMS = 0, J_{Hz}); 8.10-7.99(t,1H, *J* = 9.2),7.92-7.90 (dd, 2H, *J* = 8,9.6),7.86–7.84 (d, 1H, *J*=7.2) 7.62–7.59 (t, 2H, *J* = 8.8), 7.39–7.35 (m, 4H), 7.01–6.91 (m,6H), 6.72–6.70 (d, 2H, *J* = 2.8), 5.46–5.43 (1H, dd, *J* = 4.2, 13.8) 3.9 (1H, bs), 3.36–3.32 (dd,1H, *J* = 4.2,8.1) 3.20-3.18 (dd, 1H, *J* = 7.9,13.7); mass (ESI) m/z [M+H] 381.

2-(4-chlorophenyl)-4-(naphthalen-2-yl)-2,3,5a,9a-tetrahydro-1H-benzo[b][1,5]diazepine (5e)

Yellow crystal; 56% yield; IR (KBr, ν_{max}, cm^{-1}); 3356.10 (N-Hstr), 1610.75 (C=N str), 1560.08 (C-N str), 1460.12 (C=C str), 1H NMR (400, Mhz, $CDCl_3$, δ (ppm),TMS = 0, J_{Hz}); 8.15-8.10 (t,1H, *J* = 9.5),7.90–7.86 (dd, 2H, *J* = 7.8, 9.4),7.83-7.80 (d, 1H, *J* = 7.1) 7.6 (t, 2H, *J* = 8.5), 7.35-7.30 (m, 4H), 7.01–6.91 (m, 6H), 6.72–6.70 (d, 2H, *J* = 2.8), 5.46-5.43 (1H, dd, *J* = 4.2, 13.8), 4.15 (1H, bs), 3.36–3.32 (dd, 1H, *J* = 4.2,8.1) 3.22–3.20 (dd, 1H, *J* =7.9,13.7); mass (ESI) m/z [M+H] 386.

BIOLOGICAL EVALUATION

Antibacterial assay of synthesized compounds

Antibacterial activity of newly synthesized compounds was established against three human pathogenic bacterial strains. Two Gram-positive bacterial strains (*B. subtilis* MTCC 2451 and *S. aureus* MTCC 96) and one Gram-negative bacterial strain (*E. coli* MTCC 443) were used for assessing the antimicrobial potential of test compounds. Stock solution (1 mg/mL) of all test compounds was made in dimethyl sulfoxide (DMSO) and antibacterial activity of compounds was determined in comparison with standard discs of ciprofloxacin (10 µg). All bacterial strains were grown in LB Broth until their O.D. reached 0.5–0.6. For determining antibacterial activity pre-warmed Mueller-Hinton agar plates were inoculated with 200 µL of bacterial suspensions and kept aside for 30 min. Previously marked sterile filter paper discs (5 mm diameter) were placed on the surface of inoculated agar plates and 30 µL of each compound was pipette onto the discs. After 1 h plates were incubated at 37°C for 24 h. Antimicrobial activity was determined in triplicates. DMSO was used as a negative control. The data of the zone of inhibition are shown in Table 2.

MIC of synthesized compounds

MIC of compounds was calculated using the serial dilution method. Different dilutions (125, 62.5, 31.25, 15.6, 7.8, and 3.9 µg/mL) of all selected compounds were prepared in DMSO. Five milliliters of nutrient broth were taken in previously marked test tubes and 100 µL of microbial suspension was added to these test tubes. One milliliter of different concentrations of compounds was added in test tubes and tubes were kept in an incubator at 37°C for 24 h and were viewed

for assessing MIC of compounds against different test organisms. The concentration showing no growth was considered to be MIC of the respective compound against that strain.

CONCLUSION

The increasing drug resistance in bacteria soon led the human race into a post-antibiotic era where microbes will be powerful enough to cause mass deaths as in the pre-antibiotic era. The alarming situations prompt the scientific community to discover new antimicrobial agents with novel mechanisms to treat bacterial resistance. In an attempt to identify new leads, we designed and synthesized naphthalene-based 1,5-benzodiazepines and evaluated their antibacterial potential. Compounds 5d and 5e were identified as the most potent antibacterial compound possessing MIC values in the range of 7.8–62.5 μg/mL. The compounds were more active toward Gram-positive bacteria than the Gram-negative bacteria.

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