SYNTHESIS AND POTENTIAL ANTIMICROBIAL ACTIVITY AND EVALUATION OF NAPHTHA [2, 3-D] IMIDAZOLES DERIVATIVES

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ABSTRACT

A new series of some naphtho[2,3-d]imidazoles derivatives have been synthesized. The structures of the synthesized compounds were confirmed by IR, 1H NMR and Mass spectral analysis and they were evaluated for their antibacterial activities by disc diffusion method. All of the synthesized compounds showed good antibacterial activity. However, the antibacterial activity was observed for all the compounds using four strains of Gram (+ve) and Gram (-ve) bacteria. The strains used were Staphylococcus aureus, Bacillus pumilis, Proteus mirabilis (+ve) and Escherchia coli (-ve) activity of the synthesized compounds against the tested organisms was found to be less than that of respective standard drug attested dose level.

Keywords: Napthalenediamine, Antibacterial activity, NMR
INTRODUCTION

The structural and therapeutic diversity coupled with commercial capability of small molecules has enthralled organic and medicinal chemists. There has been significant interest in the chemistry of heterocyclic systems which is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activity. In the past few years, research for new antibacterial, antifungal inflammatory agents has been reported that many compounds having an imidazole ring with substituents like 1,3,4-oxadiazole and azetidinederivatives are known for their antifungal and antibacterial activity. Therefore, it was enabled that compounds containing naphtho[2,3-d]imidazoles would result in interesting biological activities. In the present study, substituted naphtho[2,3-d]imidazoles were synthesized by treating 2,3 Naphthalene diamine with 4 amino carboxylic acids to get 5-amino 2-substituted naphtho[2,3-d]imidazoles derivatives. Then this derivatives were treated with different types of substituted aromatic benzaldehydes gives (E)-N-(4-(1H-naphtho[2,3-d]imidazol-2-yl)3-4 substituted phenyl)enamine then finally their thiazolidiones and azetidiones derivatives were synthesized.

MATERIALS AND METHODS

All used chemicals were purchased from Spectrochem and Alfa acer Company. Melting points were determined on open capillary tube and are uncorrected. The NMR spectra were recorded on a Bruker (300 MHz) spectrometer. Chemical shifts (ppm) were referred to the internal standard tetramethylsilane (TMS). The mass spectra were recorded on a JEOL JMS-D300 spectrometer operating at 70 eV. Reactions were monitored by thin layer chromatography using silica gel F254 aluminum sheets (ethyl acetate/ n-xene, 3:1).

Experimental

Synthesis of Comp 2 (4-(1H-naphtho[2,3-d]imidazol-2-yl)aniline)

The synthetic strategy leading to the target compounds 1 are illustrated in scheme 1. Synthesized by equimolar quantities (0.01 mol) of 2,3 naphthalenediamine, p-aminobenzoic acid (0.01 mol) in polyphospheric acid (PPA) (20 ml) was refluxed at 170°C for 2 hrs. The mixture is cooled and diluted with water and quenched by 10% NaOH, aqueous layer
was extracted by EtOAc, organic layer was separated dried over Na₂SO₄ and concentrated to afford desired compound as brown colored free flow solid. Obtained compound was purified by column chromatography as 4-(1H-naphtho[2,3-d]imidazol-2-yl)aniline Yielde59%; mp209-212⁰C DM-259+1

**General method of Synthesis of Compound (3a-3e)**

(E)-N-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)enamine

A compound 24-(1H-naphtho[2,3-d]imidazol-2-yl)aniline(0.01mol) and ofand aromatic benzaldehyde(a –e) was refluxed for16hrs In20 ml of ethan. Thereaction mixture was cooled and concentrated obtained solid was recrystallized from ether: pentane. The crystals found was filtered and obtained as desired comp (3a-3e).

**General method of synthesis of azetidinones(4a-4e)**

Compound 3 (3a-3e) (0.001mol) and triethylamine(0.003mol)was dissolved in1,4 – Dioxan(25ml), stirred and cooled to 0⁰C this solution of chloroacetyl chloride (0.0012mol) in dioxane was added drop wise and reaction was stirred at rate for 16 hrs. Reaction was monitored by TLC after consumption of starting material reaction was concentrated to half of solvent separated and yield comp 1-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)-3-chloro-4-(4-substituted phenyl) azetidin-2-one, recrystallized from chloroform.

**General method of synthesis of thiazolidinones(5a-5e)**

A Compound 3 (3a-3e)(0.001mol) and thioglycollic acid(0.001mol) dissolved in1,4dioxane (20 ml), anhydrous zinc chloride (0.5mg)was added and refluxed at 900C for16h. The reaction was then cooled and the resulting solid was washed with sodiumbicarbonate solution and final compound as 3-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)-2-(4-substitutedphenylthiazolidin-4-one(5a-5e) recrystallized from absoluteethanol.
Scheme 1

Analytical Data

4-(1H-naphtho[2,3-d]imidazol-2-yl)aniline(2)

Brown Solid : Yield 59%; m.p.: 209-212; $^1$H NMR data (DMSO) $\delta$ (ppm) aromatic protons -7.70-7.75 (s, 2H), 8.11-8.15 (d, 2H), 7.69-7.70 (d, 2H), 7.90-7.92 (d, 2H), 6.70-6.71 (dd, 2H), NH 12.56 (bs, 1H), NH$_2$ 5.24 (s, 2H); MS: m/z 259 (M$^+$)
3a-3e

(E)-N-(4-(1H-naphtho[2,3-d]imidazol-2-y1)phenyl)-1-(4-methoxyphenyl)methanimine (3a)

Reddish colour  Solid Yield49%; m.p.: 221-223; $^1$H NMR data (DMSO) $\delta$ (ppm) aromatic protons 7.69-7.70 (d, 2H), 7.70-7.75 (s, 2H), 8.11-8.15 (d, 2H), 7.90-7.92 (dd, 2H), 6.70-6.71 (dd, 2H), 7.85-7.92 (dd, 2H), 7.05-7.06 (dd, 2H), NH 12.56 (bs, 1H), CH 8.65 (s, 1H), OCH$_3$ 3.81 (s, 3H); MS: m/z 377 (M$^+$)

(E)-N-(4-(1H-naphtho[2,3-d]imidazol-2-y1)phenyl)-1-(4-methoxy-3-nitrophenyl)methanimine (3b)

White Solid Yield39%; m.p.: 222-223; $^1$H NMR data (DMSO) $\delta$ (ppm) aromatic protons 7.69-7.70 (d, 2H), 7.70-7.75 (s, 2H), 8.11-8.15 (d, 2H), 7.90-7.92 (dd, 2H), 6.70-6.71 (dd, 2H), 8.55-8.52 (d, 1H), 8.20-8.25 (dd, 1H), 7.58-7.60 (dd, 1H), NH 12.56 (bs, 1H), CH 8.54 (s, 1H), OCH$_3$ 4.02 (s, 3H); MS: m/z 422 (M$^{+1}$)

(E)-N-(4-(1H-naphtho[2,3-d]imidazol-2-y1)phenyl)-1-(3-nitrophenyl)methanimine (3c)

OFF White Solid Yield62%; m.p.: 221-223; $^1$H NMR data (DMSO) $\delta$ (ppm) aromatic protons 7.69-7.70 (d, 2H), 7.70-7.75 (s, 2H), 8.11-8.15 (d, 2H), 7.76-7.81 (dd, 2H), 6.75-7.71 (dd, 2H), 8.15-8.19 (dd, 2H), 8.34-8.39 (dd, 2H), NH 12.56 (bs, 1H), CH 8.58 (s, 1H); MS: m/z 393 (M$^+$)

(E)-N-(4-(1H-naphtho[2,3-d]imidazol-2-y1)phenyl)-1-(4chlorophenyl)methanimine (3d)

OFF white Yield52%; m.p.: 221-223; $^1$H NMR data (DMSO) $\delta$ (ppm) aromatic protons -7.69-7.70 (d, 2H), 7.70-7.75 (s, 2H), 8.11-8.15 (d, 2H), 7.76-7.81 (dd, 2H), 6.75-7.71 (dd, 2H), 7.59-7.61 (dd, 2H), 7.94-7.99 (dd, 2H), NH 12.56 (bs, 1H), CH 8.68 (s, 1H); MS: m/z 382 (M$^{+1}$)
(E)-4-(((4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)imino)methyl)-2-methoxyphenol (3e)

Brown colour Yield 50%; m.p.: 200-203; $^1$H NMR (DMSO) $\delta$ (ppm) aromatic protons -7.69-7.70 (d, 2H), 7.70-7.75 (s, 2H), 8.11-8.15 (d, 2H), 7.76-7.81 (dd, 2H), 7.65-7.71 (dd, 2H), 7.36 (s, 1H), 7.34 (d, 1H), 6.88 (d, 1H), NH 12.56 (bs, 1H), CH 8.63 (s, 1H), OH 9.95 (s, 1H)

OCH$_3$ 3.83 (s, 3H); MS: m/z 394 (M$^+$)

4a-4e

1-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)-3-chloro-4-(4-methoxyphenyl)azetidin-2-one (4a)

White solid Yield 79%; m.p.: 230-233; $^1$H NMR (DMSO) $\delta$ (ppm) aromatic protons -7.69-7.70 (d, 2H), 7.70-7.75 (s, 2H), 8.11-8.15 (d, 2H), 8.10 (dd, 2H), 7.45 (dd, 2H), 7.22 (dd, 2H), 6.88 (dd, 2H), aliphatic CH -5.16 (d, 1H), 5.23 (d, 1H), NH 12.56 (bs, 1H), OCH$_3$ 3.81 (s, 3H); MS: m/z 454 (M$^+$)

1-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)-3-chloro-4-(3-methoxy-4-nitrophenyl)azetidin-2-one (4b)

Off white yellowish Yield 39%; m.p.: 222-223; $^1$H NMR (DMSO) $\delta$ (ppm) aromatic protons -7.69-7.70 (d, 2H), 7.70-7.75 (s, 2H), 8.11-8.15 (d, 2H), 8.10 (dd, 2H), 7.45 (dd, 2H), 8.22 (d, 1H), 7.28 (d, 1H), 7.24 (d, 1H), aliphatic CH -5.16 (d, 1H), 5.23 (d, 1H), NH 12.56 (bs, 1H), OCH$_3$ 3.87 (s, 3H); MS: m/z 499 (M$^+$).

1-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)-3-chloro-4-(4-nitrophenyl)azetidin-2-one (4c)

Yellow Yield 65%; m.p.: 240-243; $^1$H NMR (DMSO) $\delta$ (ppm) aromatic protons -7.69-7.70 (d, 2H), 7.70-7.75 (s, 2H), 8.11-8.15 (d, 2H), 8.10 (dd, 2H), 7.45 (dd, 2H), 7.65 (dd, 2H), 8.18 (dd, 2H), aliphatic CH -5.26 (d, 1H), 5.43 (d, 1H), NH 12.56 (bs, 1H), MS: m/z 469 (M$^+$)
1-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)-3-chloro-4-(4-chlorophenyl)azetidin-2-one (4d)

White solid Yield65%; m.p.: 240-243; $^1$HNMR data (DMSO) $\delta$(ppm) aromatic protons -7.69-7.70(d, 2H), 7.70-7.75(s, 2H), 8.11-8.15(d, 2H), 8.10(dd, 2H), 7.45(dd, 2H), 7.48(dd, 2H), 7.50(dd, 2H), aliphatic CH -5.26 (d, 1H), 5.43 (d, 1H), NH 12.56 (bs, 1H), MS: m/z 458(M$^+$)

1-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)-3-chloro-4-(4-hydroxy-3-methoxyphenyl)azetidin-2-one (4e)

Pinkish white – Yield39%; m.p.: 232-223; $^1$HNMR data (DMSO) $\delta$(ppm) aromatic protons --7.69-7.70(d, 2H), 7.70-7.75(s, 2H), 8.11-8.15(d, 2H), 8.10(dd, 2H), 7.45(dd, 2H), 6.81(d, 1H), 6.73(d, 1H), 6.95(d, 1H), aliphatic CH -5.16 (d, 1H), 5.43 (d, 1H), NH 12.56 (bs, 1H), OCH$_3$ 3.77(s, 3H), OH 9.96(s, 1H); MS: m/z 499(M$^+$).

5a-5e

3-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)-2-(4-methoxyphenyl)thiazolidin-4-one (5a)

Off white – Yield35%; m.p.: 210-212; $^1$HNMR data (DMSO) $\delta$(ppm) aromatic protons -7.69-7.70(d, 2H), 7.70-7.75(s, 2H), 8.11-8.15(d, 2H), 8.0(dd, 2H), 7.43(dd, 2H), 7.83(dd, 2H), 6.83(dd, 2H), NH 12.56 (bs, 1H), CH 6.44(s, 1H), CH$_2$ 4.02(s, 2H), OCH$_3$ 3.81(s, 3H); MS: m/z 452(M$^+$)

3-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)-2-(4-methoxy-3-nitrophenyl)thiazolidin-4-one (5b)

Yellowish solid Yield35%; m.p.: 210-212; $^1$HNMR data (DMSO) $\delta$(ppm) aromatic protons -7.69-7.70(d, 2H), 7.70-7.75(s, 2H), 8.11-8.15(d, 2H), 8.00-8.12(dd, 2H), 7.43(dd, 2H), 8.23(d, 1H), 7.25(s, 1H), 7.20(d, 1H), NH 12.56 (bs, 1H), CH 6.44(s, 1H), CH$_2$ 4.02(s, 2H), OCH$_3$ 3.87(s, 3H);
previously sterilized petri dishes (9 cm in diameter). After solidification, petri plates were prepared and sterilized by an autoclave (121°C) for 20 min. Nutrient agar (antibacterial activity) and sabouraud’s dextrose agar medium (antifungal activity) was prepared and sterilized by an autoclave (121°C and 15 lbs for 20 min) and transferred to previously sterilized petri dishes (9 cm in diameter). After solidification, petri plates were

MS:m/z 497(M⁺)

3-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)-2-(4-nitrophenyl)thiazolidin-4-one (5c)

Yellow Solid – Yield 40%: m.p.: 205-207; ¹H NMR (DMSO) δ (ppm) aromatic protons: 7.69-7.70 (d, 2H), 7.70-7.75 (s, 2H), 8.11-8.15 (d, 2H), 8.0 (dd, 2H), 7.43 (dd, 2H), 7.54 (dd, 2H), 8.21 (dd, 2H), NH 12.56 (bs, 1H), CH 6.44 (s, 1H), CH₂ 4.02 (s, 2H); MS:m/z 467(M⁺)

3-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)-2-(4-chlorophenyl)thiazolidin-4-one (5d)

White Solid: Yield 40%: m.p.: 205-207; ¹H NMR (DMSO) δ (ppm) aromatic protons: 7.69-7.70 (d, 2H), 7.70-7.75 (s, 2H), 8.11-8.15 (d, 2H), 8.0 (dd, 2H), 7.43 (dd, 2H), 7.20 (dd, 2H), 7.38 (dd, 2H), NH 12.56 (bs, 1H), CH 6.44 (s, 1H), CH₂ 4.02 (s, 2H); MS:m/z 456(M⁺)

3-(4-(1H-naphtho[2,3-d]imidazol-2-yl)phenyl)-2-(4-hydroxy-3-methoxyphenyl)thiazolidin-4-one (5e)

Brown sticky solid: Yield 35%: m.p.: 210-212; ¹H NMR (DMSO) δ (ppm) aromatic protons: 7.69-7.70 (d, 2H), 7.70-7.75 (s, 2H), 8.11-8.15 (d, 2H), 8.00-8.12 (dd, 2H), 7.43 (dd, 2H), 7.34 (d, 1H), 7.45 (s, 1H), 6.94 (d, 1H), NH 12.56 (bs, 1H), CH 6.44 (s, 1H), CH₂ 4.02 (s, 2H), OCH₃ 3.87 (s, 3H) OH 9.96 (s, 1H); MS:m/z 468(M⁺)

Biological Evaluation:

Antimicrobial Activity

The synthesized compounds were tested for antimicrobial activity by disc diffusion method. They were dissolved in and sterilized by filtering through 0.45μm Millipore filter. Final into culums of 100μl suspension containing 108 CFU/ml of each bacterium used. Nutrient agar (antibacterial activity) and sabouraud’s dextrose agar medium (antifungal activity) was prepared and sterilized by an autoclave (121°C and 15 Lbs for 20 min) and transferred to previously sterilized petri dishes (9 cm in diameter). After solidification, petri plates were
inoculated with bacterial organisms in sterile nutrient agar medium at 45°C, organisms in sterile sabouraud’s dextrose agar medium at 45°C in aseptic condition. Sterile whatmann filter paper discs (previously sterilized in U.V. lamp) were impregnated with synthesized compounds at a concentration of 300µg/ml and 300µg/ml were placed in the organism-impregnated petri plate under sterile condition. The plates were left for 30 min to allow the diffusion of compounds at room temperature. Antibiotic discs of streptomycin (300µg /ml) and 600µg /ml) as standard were used as positive control, while DMSO used as negative control. Then the plates were incubated for 24 hrs at 37 ± 1°C for antibacterial activity and 48 h at 37±1°C for antifungal activity. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no microbial growth around the each disc.
The synthesized compounds were screened for antimicrobial activity by zone of inhibition method. Antibacterial activity was observed for all the compounds using four strains of Gram (+ve) and Gram (-ve) bacteria. The strains used were Staphylococcus aureus, Bacillus pumilis, Proteus mirabilis (+ve) and Escherchia coli (-ve). The concentrations taken were 300 µg/mL, 600 µg/ml. By the analysis of antimicrobial data found that, compounds 4b and 4c were found to be more active against Escherchia coli (-ve) organism and 5b is more active against Bacillus pumilis used. 4d and 5d also showing good activity against microorganism at both strengths. The remaining compounds showed moderate and low activity against organisms.
Table 1 Antimicrobial activity of synthesized compound Standard as streptomycin

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<th>Proteus mirabilis</th>
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RESULTS AND DISCUSSION

All the synthesized compounds exhibited significant antibacterial activity. Azetidinone and thiazolidinone derivatives were found to exhibit most potent antimicrobial activity against all the microbial stains tested. All the compounds were active against all tested microorganism with a range of MIC values for *Staphylococcus aureus*, *Bacillus pumilis*, *Proteus mirabilis* (+ve) and *Escherchia coli* (-ve) Compounds, exhibited potent antimicrobial activity against *E. coli* and compounds were found to be active. The data revealed that electron-withdrawing groups like -NO₂, -Cl, and electron donating group like -OCH₃, -OH were found to increase the antimicrobial properties, whereas electron donating group like -CH₃ group found to have moderate activity. The most of the synthesized compounds exhibited significant antibacterial activity.

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