Synthesis and antimicrobial activity of naphtho-[1,2-e][1,3]oxazines linked benzimidazole

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A new series of 2-(1H-benzo[d]imidazol-2-yl)-2,3-dihydro-1-aryl-1H-naphtho[1,2-e][1,3]oxazines have been accomplished by a green protocol utilizing an efficient atom economic three component coupling reaction. The reaction of 2-amino benzimidazole, aromatic aldehydes with β-naphthol on oil bath has produced the corresponding 1-(1H-benzo[d]imidazol-2-yl amino) phenyl (methyl) naphthalene-2-ols, which have been then cyclized to the title products by treatment with formaldehyde. The title compounds have been screened for their antimicrobial activity. Some of the compounds show promising antimicrobial activity.

Keywords: 2-(1H-benzo[d]imidazol-2-yl)-2,3-dihydro-1-aryl-1H-naphtho[1,2-e][1,3]oxazines, one-pot synthesis, three-component reaction, antimicrobial activity

These has been a considerable interest in the synthesis of molecules having 1,3-oxazine moiety due to large spectrum of pharmacological activities such as antitumor1, antimicrobial2, anti HIV3, and antimalarial agents4. In particular, naphthoxazines exhibited therapeutic potential for the treatment of Parkinson’s disease5. Benzimidazole is an important nucleus that has been extensively used in medicinal chemistry, notable examples being the antihistaminic asterizole and the antiulcerative omeprazole6. Benzimidazoles are also known for their anti-inflammatory7, antibiotic8, antihelmintic9, anticancer10, and antiviral activities11.

Molecular hybridization is a relatively new terminology in the field of drug design and development involving the fusion of two or more pharmacophoric submits from the molecular structure of ligands previously reported to have an inhibitory effect against the target properties or disease. The newly designed architecture can lead to compounds having improved affinity and efficacies than the parent compounds with reduced side effects, while retaining the desired characteristics of original template. Various literature reports have explored this methodology in designing newer analogues as potential candidates for biological evaluation12.

Based on these findings, we are interested to construct oxazine-benzimidazole hybrids by utilizing a green protocol to evaluate the antimicrobial activity of the compounds. We, herein, report the synthesis and antimicrobial activity of 2-(1H-benzo[d]imidazol-2-yl)-2,3-dihydro-1-aryl-1H-naphtho[1,2-e][1,3]oxazines.

Results and Discussion
Initially, we attempted a three component reaction using benzaldehyde 2, β-naphthol 3 and 2-amino benzimidazole 1 as substrates to stabilize the reaction. The reaction was carried out at 110-120°C in an oil bath for 2 h. to afford the 1-(1H-benzo[d]imidazol-2-yl amino) phenyl (methyl)naphthalene-2-ol 4 in high yield under solvent free conditions by employing a green protocol.

To investigate the scope of the reaction, a number of differently substituted aromatic aldehydes are reacted with β-naphthol and 2-amino benzimidazole 1 as substrates to stabilize the reaction. The reaction was carried out at 110-120°C in an oil bath for 2 h. to afford the 1-(1H-benzo[d]imidazol-2-yl amino) phenyl (methyl)naphthalene-2-ols 4 in high yield under solvent free conditions by employing a green protocol.

The naphthalene-2-ols 4 were refluxed in CH3CN while stirring at 90°C for 2 h with formaldehyde (37%) to afford the corresponding 2-(1H-benzo[d]imidazol-2-yl)2,3-dihydro-1-aryl-1H-naphtho[1,2-e][1,3]oxazines 5 in good yields. In general, all the reactions were clean, and all the products were characterized by IR, 1H and 13C NMR, and mass spectra (Scheme I).

The newly synthesized imidozolyl naphthol 4a in its IR spectrum exhibited a strong absorption band at
3430 due to OH and two bands at 3360 and 3375 cm\(^{-1}\) due to NH functional group stretching vibrations respectively. \(^1\)H NMR spectrum of 4a showed two broad signals at \(\delta\) 5.24 and 10.25 due to NH protons and a broad singlet at \(\delta\) 5.50 due to OH proton, which are D\(_2\)O exchangeable. A singlet at \(\delta\) 5.19 is assignable to CH proton. The mass spectrum of 4a displayed the molecular ion \([M+H]^+\) peak at \(m/z\) 366 confirming the formation of a three-component product.

Benzimidazolyl naphthoxazine 5a in its IR spectrum did not show the absorption bands at 3430 and 3360 cm\(^{-1}\) due to OH and NH functional groups which are present in its precursor 4 confirming the cyclization. The \(^1\)H NMR spectrum of 5a exhibited a singlet at \(\delta\) 5.79 due to the methylene protons confirming the formation of 1,3-oxazine ring. The disappearance of NH and OH protons signals at \(\delta\) 5.24 and 5.50, which are present in its precursor confirming the cyclization. The mass spectrum of 5a showed the molecular ion \([M+H]^+\) peak at 378, which is in agreement with the proposed structure. Data from the elemental analyses further confirmed the assigned structures of 4 & 5.

**Scheme I**

### Antimicrobial activity

**Antibacterial activity**

The newly synthesized 2-(1H-benzo[d]imidazol-2-yl)2,3-dihydro-1-aryl-1H-naphtho[1,2-e][1,3]oxazines 5a-j were evaluated for their *in vitro* antibacterial activity against Gram-positive bacteria *viz.*, *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 511) and *Staphylococcus aureus* (MTCC 96) and Gram-negative bacteria *viz.*, *Pseudomonas aeruginosa* (MTCC 741), *Klohsinella aerogenes* (MTCC 39) and *Chromobacterium violaceum* (MTCC 2656) at 100 µg/mL concentration. The *in vitro* antibacterial activity of the tested compounds was assessed by minimum inhibitory concentration (MIC) using broth dilution method\(^{13}\). Ciprofloxacin was used as standard drug for comparison.

The antibacterial activity results showed that compounds 5a-j displayed a better activity and were more active than standard drug Ciprofloxacin (Table I). The activity was expressed in terms of minimum inhibitory concentration (MIC). The compounds 5b, 5c and 5d are highly active, because the activity is considerably affected by the presence of methyl and methoxy as substituents on benzene ring. Compounds
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The antibacterial activity of compounds 5b and 5c is promising compared to standard Ciprofloxacin, and they can be exploited for formulation of bacteriocides after further study.

The antibacterial activity was done by broth dilution method and expressed as minimum inhibitory concentration. The ready made nutrient broth medium (Himedia, 24 g) was suspended in distilled water (100 mL) and heated to boiling until it dissolved completely. The medium and test tubes were autoclaved at pressure of 15 lb/in² for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compound 5 is dissolved in suitable solvent (acetone) and concentration of 100 µg/mL of test compound is added in the first test tube, which is serially diluted. A fixed volume of 0.5 mL overnight culture is added in all test tubes and are incubated at 37°C for 24 h. After 24 h, these tubes were measured for turbidity. Bacterial strains used for the present investigation, *Bacillus subtilis* (MTCC 441), *Bacillus"sphaericus* (MTCC 511), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 741), *Klobsiella aerogenes* (MTCC 39) and *Chromobacterium violaceum* (MTCC 2656), were obtained from the Institute of Microbial Technology, Chandigarh.

**Antifungal activity**

The newly synthesized 2-[(1H-benzo[d]imidazol-2-yl)2,3-dihydro-1-aryl-1H-naphtho[1,2-e][1,3]oxazines 5a-j were also evaluated for their antifungal activity against *Fusarium oxysporum*, *Verticillium dahliae*, *Alternaria solani*, *Rhizoctonia solani*, *Colletotrichum capsici* and *Pythium aphanidermatum* in acetone by agar cup bioassay method using Fluconazole as the standard drug.

Antifungal activity data (Table II) revealed that compounds 5a-g are highly toxic towards all the fungi under investigation. Compounds 5b, 5c and 5d exhibited high antifungal activity by inhibiting the growth of fungi to a remarkable extent, when compared to standard drug Fluconazole, which may be due to...
the presence of methyl and methoxy substituents on the benzene ring. Compound 5a showed good activity. Compounds 5e, 5f and 5g are moderately active. However, the degree of spore germination inhibition varied with the test compound as well as with the fungi under investigation. It is noteworthy that compounds 5b and 5c showed better activity, when compared with the standard drug Fluconazole, hence, they may be exploited for control of wilt diseases of different crops as fungicides after further studies.

In conclusion, the results revealed that compounds 5b and 5c are highly toxic towards the fungi under investigation and they are lethal even at 100 μg/mL concentration in comparison with standard Fluconazole at the same concentration, and may be exploited for control of wilt diseases of different crops as fungicides after detailed study.

The antifungal activity was done by using agar cup bioassay method. The readymade potato dextrose agar (PDA) medium (Himedia, 39g) was suspended in distilled water (100 mL) and heated to boiling until it dissolved completely. The medium and petri-dishes were autoclaved at pressure of 15 lb/ inc2 for 20 min. When the medium in the plates solidified, 0.5 mL of (week old) culture of test organism was inoculated under aseptic conditions in a laminar flow chamber. The medium was poured into sterile petri-dishes with acetone and Fluconazole. The concentrations of test solutions were added. Controls were maintained with acetone and Fluconazole. The reaction was allowed to cool. The solid obtained after completion of the reaction as indicated by TLC, was washed with ethanol (3 × 10 mL) thrice to obtain the corresponding pure products.

**General procedure for the synthesis of 1-(1H-benzo[d]-imidazol-2-yl amino) (aryl)(methyl)naphthalene-2-ols, 4a-j**

A mixture of 2-amino benzimidazole 1 (1 mmol), aromatic aldehyde 2 (1 mmol), β-naphthol 3 (1 mmol) was heated in preheated oil bath at 110-120°C for 2 h. Chemical shift values are given in δ (ppm) with tetramethyl silane as an internal standard. ESI Mass spectra were recorded on a Varian Gemini 300 MHz spectrometer. Chemical shift values are given in δ (ppm) with tetramethyl silane as an internal standard. ESI Mass spectra were recorded on a Agilent LC-MSD mass spectrometer. Elemental analyses were performed on a Carlo Erba 106 and Perkin-Elmer model 240 analyzers.

**Experimental Section**

All the melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Analytical TLC was performed on Merck precoated 60 F254 silica gel plates. Visualization was carried out by exposure to iodine vapour. IR spectra (KBr pellet) were recorded on a Perkin-Elmer BX series FT-IR spectrometer. 1H NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer. 13C NMR spectra were recorded on a Bruker 75 MHz spectrometer. Elemental analyses were performed on a Carlo Erba 106 and Perkin-Elmer model 240 analyzers.
13C NMR (75MHz, CDCl3): δ 50.16, 115.38, 115.61, 115.94, 118.68, 123.45, 123.69, 123.99, 126.32, 126.52, 128.12, 128.17, 128.48, 128.65, 128.60, 128.88, 129.51, 131.60, 133.48, 138.29, 138.58, 141.55, 142.88, 153.59; ESI-MS: m/z 444 [M+H]+. Anal. Calcd for C24H18N2OBr: C, 65.01; H, 4.06; N, 13.72%. Found: C, 65.86; H, 4.10; N, 13.75%.

1-(1H-Benzox[d]imidazol-2-ylamino)(4-methoxyphenyl)methyl)naphthalen-2-ol, 4d: Pale yellow solid. Yield 80%. IR (KBr): 3438 (OH), 3382 (NH), 3376 (NH) cm−1; 1H NMR (300MHz, CDCl3): δ 5.19 (s, 1H, CH), 5.26 (s, 1OH, D2O exchangeable), 7.00-7.83 (m, 14H), 10.89 (s, 1NH, D2O exchangeable); 13C NMR (75MHz, CDCl3): δ 50.16, 115.38, 115.61, 115.94, 118.68, 123.45, 123.69, 123.99, 126.32, 126.52, 128.12, 128.17, 128.48, 128.65, 128.60, 128.88, 129.51, 131.60, 133.48, 138.29, 138.58, 141.55, 142.88, 153.59; ESI-MS: m/z 444 [M+H]+. Anal. Calcd for C24H18N2OBr: C, 65.01; H, 4.06; N, 13.72%. Found: C, 65.86; H, 4.10; N, 13.75%.

1-(1H-Benzox[d]imidazol-2-ylamino)(4-nitrophenyl)methyl)naphthalen-2-ol, 4j: Pale yellow solid. Yield 80%. IR (KBr): 3438 (OH), 3382 (NH), 3376 (NH) cm−1; 1H NMR (300MHz, CDCl3): δ 5.19 (s, 1H, CH), 5.26 (s, 1OH, D2O exchangeable), 7.00-7.83 (m, 14H), 10.89 (s, 1NH, D2O exchangeable); 13C NMR (75MHz, CDCl3): δ 50.16, 115.38, 115.61, 115.94, 118.68, 123.45, 123.69, 123.99, 126.32, 126.52, 128.12, 128.17, 128.48, 128.65, 128.60, 128.88, 129.51, 131.60, 133.48, 138.29, 138.58, 141.55, 142.88, 153.59; ESI-MS: m/z 444 [M+H]+. Anal. Calcd for C24H18N2OBr: C, 65.01; H, 4.06; N, 13.72%. Found: C, 65.86; H, 4.10; N, 13.75%.

1-(1H-Benzox[d]imidazol-2-ylamino)benzo[d][1,3]dioxol-6-yl)methyl)naphthalen-2-ol, 4e: Pale yellow solid. Yield 82%. m.p. 245-57°C. IR (KBr): 3438 (OH), 3382 (NH), 3376 (NH) cm−1; 1H NMR (300MHz, CDCl3): δ 5.19 (s, 1H, CH), 5.26 (s, 1OH, D2O exchangeable), 7.00-7.83 (m, 14H), 10.89 (s, 1NH, D2O exchangeable); 13C NMR (75MHz, CDCl3): δ 50.16, 115.38, 115.61, 115.94, 118.68, 123.45, 123.69, 123.99, 126.32, 126.52, 128.12, 128.17, 128.48, 128.65, 128.60, 128.88, 129.51, 131.60, 133.48, 138.29, 138.58, 141.55, 142.88, 153.59; ESI-MS: m/z 444 [M+H]+. Anal. Calcd for C24H18N2OBr: C, 65.01; H, 4.06; N, 13.72%. Found: C, 65.86; H, 4.10; N, 13.75%.
brown solid. Yield 89%. m.p.249-51°C. IR (KBr): 3447 (OH), 3387 (NH), 3378 (NH) cm⁻¹; ¹H NMR (300MHz, CDCl₃): δ 5.17 (s, 1H, CH), 5.20 (s, 1NH, D₂O exchangeable ), 5.90 (s, 2H,CH) 5.50 (s, 1 OH, D₂O exchangeable), 7.00-7.83 (m, 13 H), 10.58 (s, 1 NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): δ 50.11, 101.01, 115.33, 115.81, 116.13, 118.91, 123.42, 126.43, 128.29, 128.37, 128.69, 128.80, 128.99, 129.14, 129.53, 129.91, 133.63, 135.65, 138.32, 138.84, 141.65, 142.95,146.45, 149.34, 153.69; ESI-MS: m/z 392 [M+H]⁺. Anal. Calcd for C₂₅H₁₈N₃O₃: C, 73.34; H, 5.05; N, 10.72%.  

**General procedure for the synthesis of 2-(1H-benzo[d]imidazol-2-yl)-2,3-dihydro-1-aryl-1H-naphtho[1,2-e][1,3]oxazines, 5a-j**

Benzimidazol naphthols 4 (1 mmol) and formaldehyde (37%, 1 mmol) were taken in acetonitrile (15 mL). The reaction mixture was refluxed while stirring for 2h. After the completion of the reaction (monitored by TLC) the solvent was removed under reduced pressure and 30 mL water was added. The contents were extracted with ethyl acetate, and solvent distilled out under reduced pressure. The residue obtained was recrystallized from ethanol to produce 5 in high yield.

2-(1H-Benzol[d]imidazol-2-yl)-2,3-dihydro-1-phenyl-1H-naphtho[1,2-e][1,3]oxazine, 5a: Pale yellow solid. Yield 89%. m.p.229-31°C. IR (KBr): 3376 (NH) cm⁻¹; ¹H NMR (300MHz, CDCl₃): δ 5.20 (s, 1H, CH), 5.79 (s, 2H, oxazine ring CH₂), 7.00-7.83 (m, 15H), 10.25 (s, Benzimidazol NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): δ 55.60, 87.62, 115.34, 115.57, 115.85, 118.73, 123.31, 123.89, 126.21, 126.58, 128.02, 128.11, 128.39, 128.50, 128.69, 128.83, 129.50, 129.87, 133.51, 138.20, 138.74, 141.59, 142.84, 152.58, 160.21; ESI-MS: m/z 378 [M+H⁺]. Anal. Calcd for C₂₅H₁₉N₃O: C, 79.79; H, 5.37; N, 10.74%. Found: C, 79.76; H, 5.34; N, 10.71%.

2-(1H-Benzol[d]imidazol-2-yl)-2,3-dihydro-1-(4-methoxyphenyl)-1H-naphtho[1,2-e][1,3]oxazine, 5d: Pale yellow solid. Yield 89%. m.p.251-53°C. IR (KBr): 3385 (NH) cm⁻¹; ¹H NMR (300MHz, CDCl₃): δ 3.90 (s, 3H,OH), 5.18 (s, 1H, CH), 5.65 (s, 2H, oxazine ring CH₂), 7.00-7.83 (m, 14 H), 10.39 (s,1 NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): δ 64.49, 76.93, 115.30, 115.51, 115.88, 118.75, 123.39, 123.99, 126.25, 126.63, 128.02, 128.13, 128.35, 128.49, 128.58, 128.89, 129.57, 129.93, 133.58, 138.25, 138.74, 141.69, 142.89, 152.62, 160.67; ESI-MS: m/z 408 [M+H⁺]. Anal. Calcd for C₂₆H₂₁N₃O: C, 76.62; H, 5.12; N, 10.26%.

2-(1H-Benzol[d]imidazol-2-yl)-2,3-dihydro-1-(4-chlorophenyl)-2,3-dihydro-1H-naphtho[1,2-e][1,3]oxazine, 5e: Pale yellow solid. Yield 86%. m.p.264-66°C. IR (KBr): 3385 (NH) cm⁻¹; ¹H NMR (300MHz, CDCl₃): δ 5.20 (s, 1H, CH), 5.77 (s, 2H, oxazine ring CH₂), 7.00-7.83 (m, 14 H), 10.42 (s, 1H, CH₃, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): δ 55.71, 87.62, 115.44, 115.57, 115.89, 118.79, 123.30, 123.82, 126.51, 126.73, 128.14, 128.29, 128.39, 128.51, 128.74, 128.88, 129.56, 129.89, 133.54, 138.27, 138.79, 141.78, 142.88, 152.59, 160.29; ESI-MS: m/z 412 [M+H⁺]. Anal. Calcd for C₂₆H₂₁N₃OCl: C, 72.99; H, 4.39; N, 10.21%. Found: C, 72.96; H, 4.41; N, 10.19%.

2-(1H-Benzol[d]imidazol-2-yl)-1-(4-bromophenyl)-2,3-dihydro-1H-naphtho[1,2-e][1,3]oxazine, 5f: Pale brown solid. Yield 86%. m.p.260-62°C. IR (KBr): 3389 (NH) cm⁻¹; ¹H NMR (300MHz, CDCl₃): δ 5.19 (s, 1H, CH₃), 5.79 (s, 2H, oxazine ring CH₂), 7.00-7.83 (m, 14 H), 10.45 (s,1 NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): δ 55.66, 87.63, 115.34, 115.65, 115.87, 118.78, 123.39, 123.89, 126.27, 126.60, 128.12,
2-(1H-Benz[d]imidazol-2-yl)-1-(2,4-dichlorophenyl)-2,3-dihydro-1H-naphtho[1,2-e][1,3]oxazine, 5g: Pale yellow solid. Yield 88%. m.p. 268-70°C. IR (KBr): 3382 (NH) cm$^{-1}$; 1H NMR (300MHz, CDCl$_3$): $\delta$ 5.50 (s, 1H, CH), 5.79 (s, 2H, oxazine ring CH$_2$), 7.00-7.83 (m, 13H), 10.45 (s, 1NH, D$_2$O exchangeable); 13C NMR (75MHz, CDCl$_3$): $\delta$ 55.60, 87.62, 115.34, 115.71, 115.95, 118.75, 123.37, 123.89, 126.40, 126.91, 128.01, 128.12, 128.39, 128.49, 128.83, 129.50, 129.87, 133.51, 138.74, 141.59, 142.84, 152.58, 160.21; ESI-MS: m/z 446 [M+H$^+$]. Anal. Calcd for C$_{26}$H$_{19}$N$_3$O$_3$: C, 74.10; H, 4.51; N, 9.97%. Found: C, 74.07; H, 4.54; N, 9.95%.

4-(2-(1H-Benz[d]imidazol-2-yl)-2,3-dihydro-1H-naphtho[1,2-e][1,3]oxazine-1-yl)-N,N-dimethylbenzamine, 5i: Pale brown solid. Yield 80%. m.p. 246-48°C; IR (KBr): 3390 (NH) cm$^{-1}$; 1H NMR (300MHz, CDCl$_3$): $\delta$ 5.20 (s, 1H, CH), 5.81 (s, 2H, oxazine ring CH$_2$), 7.00-7.83 (m, 13H), 10.47 (s, 1NH, D$_2$O exchangeable); 13C NMR (75MHz, CDCl$_3$): $\delta$ 50.11, 87.90, 115.34, 115.71, 115.95, 118.75, 123.37, 123.93, 126.31, 126.60, 128.11, 128.19, 128.45, 128.54, 128.74, 128.89, 129.57, 129.85, 133.63, 138.28, 138.74, 141.63, 142.89, 152.78, 160.29; ESI-MS: m/z 423 [M+H$^+$]. Anal. Calcd for C$_{25}$H$_{17}$N$_3$OCl$_2$: C, 68.37; H, 3.78; N, 9.40%.

References

Conclusion
In conclusion, we developed an efficient atom economic three component coupling reaction under a green protocol procedure. The reactions are fairly clean, easy to handle and the products formed can be easily purified by simply washing the reaction mixture with ethanol without intervention of chromatography. The newly synthesized benzimidazolyl naphthoxazines exhibited good in vitro antimicrobial activity. It is significant to note that by effecting a simple modification in structure, a new potent analogue can be generated with the desired activity with good efficacy.

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