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DIHYDRO ISOXAZOLE SCAFFOLDS: DESIGN, SYNTHESIS, AND BIOLOGICAL EVOLUTION

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ABSTRACT
In the present study, 6-methoxy naphthalene 2-ethanol **(1)** and substituted benzaldehyde **(2)** are dehydrated using Claisen-Schmidt to make chalcone derivative **(3a–l)**. This on cyclization with hydroxylamine hydrochloride gives the corresponding isoxazole scaffold **(4a-l)**. The formation of series was confirmed by spectrometric techniques such as IR, ^{1}H NMR, ^{13}C NMR and mass spectra. These compounds were evaluated for antimicrobial screening. Compounds **(4b, 4c &4g)** showed promising antibacterial activity, while compound **4e** shows good antifungal activity when compared against standard drugs.

KEYWORDS: Claisen-Schmidt, chalcone, isoxazole, spectrometric, antibacterial, antifungal.

INTRODUCTION
The discovery of the first antibiotic, penicillin, by Alexander Fleming in 1928 and the first commercially available antibacterial drug, Prontosil, in 1930 are some of the milestones in the human health system. From 1940 to 1960, most of the antibiotics were discovered and introduced to the market, considered the 'Golden Age' of antibiotics [i-iii]. During antimicrobial resistance (AMR) microorganisms evolved a mechanism over time that resists the medicines we consume to cure. AMR happens naturally over time, usually through genetic changes. Overuse or misuse of medicines is also a cause for AMR [iv-vii]. So it is important to develop new, potent, effective, less toxic drugs that will act against microorganisms. In synthetic chemistry, the formation of carbon-nitrogen bonds always plays an important role and is worthy of attention. Heterocyclic compounds have much important as they act as a bridge between chemical and life sciences. Isoxazole is an aromatic five-membered electron-rich heterocyclic compound (HC) containing two electronegative heteroatoms, nitrogen and oxygen, in a 1, 2-relationship, and three regular sp2 carbon atoms. Isoxazole and their scaffolds are privileged structures in pharmaceuticals, alkaloids, and organic materials. From

the first oxazole came into existence in 1840 [viii], several synthetic methodologies had been explored for the preparation of isoxazole derivatives with their merits and demerits. [ix-xii].

The presence of isoxazole can be found in a number of natural plants, such as Amanita muscaria and legume seeds. When currently marketed drugs are observed, the isoxazole ring ranks 33rd in frequency among the 351 ring systems found [xiii]. The structural characteristics of isoxazole make it possible for a variety of non-covalent interactions, especially hydrogen bonds, π-π stacks, and hydrophilic interactions [xiv]. Under certain situations, such as basic or reduction, there is a possibility of fragmentation of the O-N bond. This heteroaromatic system undergoes various reactions such as electrophilic-nucleophilic substitution, oxidation, reduction, thermolysis, photolysis, protonation, carbogenic condensations, quaterization, complexation, and transformation into other heterocyclic ring systems. The belongings of weak N-O bonds make isoxazole a prominent intermediate [xv].

Isoxazole derivatives have demonstrated their high-affinity binding ability to exhibit interactions with many targets at multiple distinct receptors, a variety of enzymes, and receptors[xvi]. Functionalized isoxazole derivatives possess biological activities such as anticancer, anti-inflammatory, antibacterial, anti-Alzheimer, antioxidant, antifungal, anti-viral, inhibitors, immune suppressants, anti-parasitic, anti-diabetic, anticonvulsant, herbicides, and insecticides [xvii-xxii]. In organic materials, these compounds had been applied as scaffolds for peptidomimetics, as chiral ligands, high-temperature lubricants, semiconductors, single-walled nanotubes, and in dyes [xii]. In material science, isoxazole derivatives are used in dye-sensitized solar cells, in optical properties, as an electrochemical probe, as a photochromic, as a liquid crystal, and as a high-energy material[xiii].

EXPERIMENTAL

Material and Methods

Chemicals used during the experimental work were of commercial grade. Reaction progress and purity of developed spots were visualised using alumina thin-layer chromatography (TLC) plates coated with silica gel 60 F254, 0.25 mm thickness (Merck), under ultraviolet (254 nm) light and in iodine vapours. The open capillary method was employed to determine melting points using Mettler Toledo MP50 instruments. IR spectra in the range of 4000 to 650 cm^{-1} were recorded on a Bruker FTIR spectrometer using potassium bromide pellets. Mass spectra were recorded on Make: Agilent Technologies. The Bruker Avance (400 MHz) spectrometer (Bruker Scientific Ltd., Switzerland) was used to record ^{1}H NMR spectra with dimethyl sulfoxide (DMSO) as solvent and tetramethylsilane (TMS) as an internal standard. ^{13}C NMR was recorded at 100 MHz on the AvanceNeo NB instrument (100 MHz) using DMSO-d6 as solvent. HPLC purity was performed using Waters HPLC.

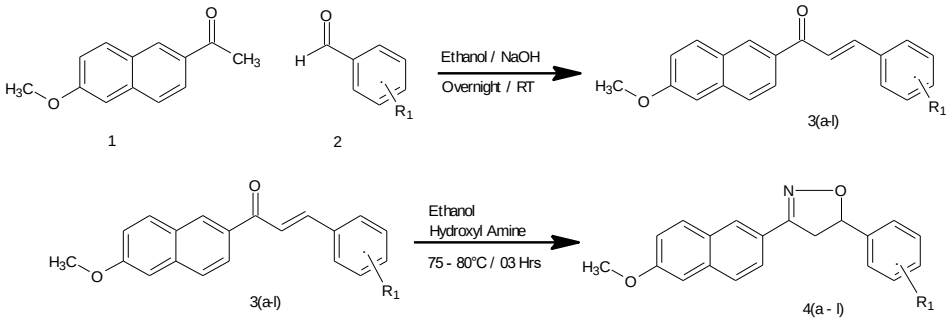
General procedure for the synthesis of E-1(6-methoxynaphthalene-2-yl)-3-(substituted phenyl) prop-2-en-1-one (3):

To clean the round bottom flask containing ethanol (30 ml), add (0.1 mol) of 1-(6-methoxynaphthyl-2-yl) ethoxy **(1)** and sodium hydroxide (0.15 mol) with constant stirring. The mixture was stirred for 15 min to get a clear solution. Add benzaldehyde derivative (0.15 mol) **(2)** under constant stirring. The reaction mixture was stirred overnight at room temperature. After confirmation of completion of the reaction by TLC, cool the reaction mixture and pour in cold distilled water. Filter the crude product and crystallise in ethanol to get the pure compound. Other compounds are prepared in the same procedure **(3a-l)**. Yield = 75 to 80%.

General procedure for the synthesis of 3-(6-methoxynaphthalen-2-yl)-5-(substituted phenyl)-4,5-dihydro-1,2-oxazole compounds 4(a-l):

To the mixture of E-1(6-methoxynaphthalene-2-yl)-3-(substituted phenyl) prop-2-en-1-one (0.1 mol) **(3a-l)** in ethanol (30 ml), add hydroxyl amine hydrochloride (0.12 mol). The whole mixture was refluxed for three hours. The progress of the reaction was monitored by TLC. On completion of the reaction, the reaction mass was cooled and neutralised with liquor ammonia solution. Pour the reaction mixture in cold distilled water and isolate the crude compound by filtration. Crude solid was crystallised in ethanol to get pure compound. This reaction was extended for the preparation of remaining compounds **(4a-l).**

Reaction Scheme:



Scheme: 1. Synthesis of isoxazole series (4a-l)

3-(6-methoxynaphthalen-2-yl)-5-(2-methoxyphenyl)-4,5-dihydro-1,2-oxazole (4a):

Brownish yellow solid, Yield: 76%, m.p.: 146-148° C. IR (KBr, cm^{-1}): 3016 (Ar-H str.), 2976 (C-H str.), 1650 (C=N str.), 1593 (Ar-C=C str.), 1459 (N-O str.).^{1}H NMR (400 MHz, DMSO-d_6): δ 2.99 - 3.25 (d, 2H, isoxazole-CH_2), 3.66 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.52 - 4.56 (d, 1H, isoxazole-CH), 6.84 - 6.89 (d, 2H, Ar-H), 7.11 - 7.18 (d, 2H, Ar-H), 7.26 - 7.30 (d, 2H, Ar-H), 7.43 - 7.45 (dd, 1H, Ar-H), 7.72 - 7.80 (d, 2H, Ar-H), 7.95 (d, 1H, Ar-H).^{13}C (300 MHz, DMSO): δ 54.16, 55.19, 55.26, 101.22, 105.81, 114.05, 119.07, 124.22, 124.56, 126.81, 127.77, 127.93, 128.16, 129.86, 134.03, 134.31, 136.31, 150.92, 157.80, 158.79, 174.84. ESI-MS (m/z): 333.85 (M+1). Calc.: 333.35. HPLC purity: 97.44%.Anal. Calculated for $C_{21}H_{19}NO_3$ (333.35): C, 75.66; H, 5.74; N, 4.20; O, 14.40%.Found: C, 75.68; H, 5.76; N, 4.18; O, 14.38%.

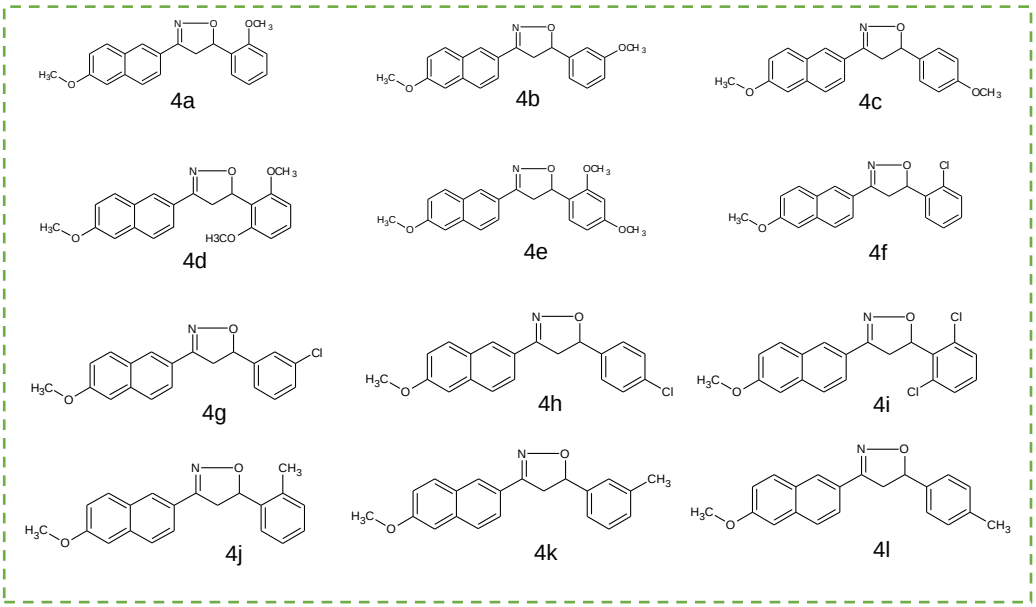


Figure 01: Structure of compounds (4a-l)

3-(6-methoxynaphthalen-2-yl)-5-(3-methoxyphenyl)-4,5-dihydro-1,2-oxazole (4b):
Crystalline white solid, Yield = 80%, m.p.: 141-143°C. IR (KBr, cm^{-1}): 3063 (Ar-H str.), 2982 (C-H str.), 1613 (C=N str.), 1548 (Ar-C=C str.), 1472 (N-O str.), 1430 (C-H bend).^{1}H NMR (400 MHz, DMSO-d_6):δ 3.05 - 3.26 (d, 2H, isoxazole-CH_2), 3.66 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.14 - 4.18 (d, 1H, isoxazole-CH), 6.76 - 6.78 (d, 2H, Ar-H), 7.14 - 7.19 (d, 2H, Ar-H), 7.26 - 7.29 (d, 2H, Ar-H), 7.67 - 7.73 (m, 2H, Ar-H), 7.77 - 7.86 (m, 2H, Ar-H).^{13}C (300 MHz, DMSO): δ 54.86, 55.68, 55.92, 100.86, 105.37, 114.60, 119.29, 123.61, 123.86, 125.43, 127.19, 127.42, 128.03, 129.52, 133.49, 133.73, 137.19, 152.71, 157.49, 158.72, 174.37. ESI-MS (m/z): 333.85 (M+), calc.: 333.35. HPLC purity: 99.95%.Anal. Calculated for $C_{21}H_{19}NO_3$ (333.35): C, 75.66; H, 5.74; N, 4.20; O, 14.40%. Found: C, 75.69; H, 5.72; N, 4.18; O, 14.39%.

3-(6-methoxynaphthalen-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1,2-oxazole (4c):
Off White solid, Yield = 74%, m.p.: 181-183°C, IR (KBr, cm^{-1}): 3089 (Ar-H str.), 2942 (C-H str.), 1610 (C=N str.), 1565 (Ar-C=C str.), 1465 (N-O str.), 1436 (C-H bend).^{1}H NMR (400 MHz, DMSO-d_6): δ 3.09-3.19 (d, 2H, isoxazole-CH_2), 3.61 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.21-4.29 (d, 1H, isoxazole-CH), 6.85-6.98 (d, 2H, Ar-H), 7.19-7.34 (d, 2H, Ar-H), 7.38-7.49 (d, 2H, Ar-H), 7.61-7.73 (m, 2H, Ar-H), 7.82-7.92 (d, 2H, Ar-H).^{13}C (300 MHz, DMSO):δ 54.62, 55.48, 55.81, 101.68, 105.73, 114.15, 120.91, 123.18, 123.68, 126.34, 127.41, 127.68, 129.34, 129.75, 133.16, 133.39, 137.64, 153.18, 157.92, 158.29, 174.47. ESI-MS (m/z): 333.65 (M+1), calc.: 333.35. HPLC purity: 98.62%.Anal. Calculated for $C_{21}H_{19}NO_3$ (333.35): C, 75.66; H, 5.74; N, 4.20; O, 14.40%. Found: C, 75.70; H, 5.76; N, 4.17; O, 14.37%.

***5-(2,4-dimethoxyphenyl)-3-(6-methoxynaphthalen-2-yl)-4,5-dihydro-1,2-oxazole (4d)*:**
Light brown solid, Yield = 71%, m p: 158-160°C, IR (KBr, cm^{-1}): 3093 (Ar-H str.), 2926 (C-H str.), 1618 (C=N str.), 1552 (Ar-C=C str.), 1456 (N-O str.), 1429 (C-H bend).^{1}H NMR (400 MHz, DMSO-d_6): δ 3.16-3.32 (d, 2H, isoxazole-CH_2), 3.52 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 4.36-4.42 (d, 1H, isoxazole-CH), 6.98-7.26 (m, 4H, Ar-H), 7.42-7.63 (m, 3H, Ar-H), 7.79-7.91 (d, 2H, Ar-H).^{13}C (300 MHz, DMSO): δ 53.29, 55.26, 55.61, 55.82, 105.09, 113.86, 116.47, 120.47, 123.08, 124.62, 126.17, 127.64, 127.34, 128.06, 129.28, 134.34, 134.47, 136.51, 150.92, 157.40, 158.61, 174.42. ESI-MS (m/z): 363.91 (M+1), calc.: 363.40. HPLC purity: 97.86%.Anal. Calculated for $C_{22}H_{21}NO_4$ (363.40): C, 75.66; H, 5.74; N, 4.20; O, 14.40%. Found: C, 75.68; H, 5.71; N, 4.21; O, 14.42%.

5-(2,6-dimethoxyphenyl)-3-(6-methoxynaphthalen-2-yl)-4,5-dihydro-1,2-oxazole (4e):
Brown semi-solid, Yield=79%, m. p.: 196-198°C. IR (KBr, cm^{-1}): 3086 (Ar-H str.), 2932 (C-H str.), 1611 (C=N str.), 1565 (Ar-C=C str.), 1446 (N-O str.), 1428 (C-H bend).^{1}H NMR (400 MHz, DMSO-d_6): δ 3.21-3.36 (d, 2H, isoxazole-CH_2), 3.46 (s, 6H, OCH_3), 3.68 (s, 3H, OCH_3), 4.59-4.66 (d, 1H, isoxazole-CH), 6.89-7.31 (m, 4H, Ar-H), 7.48-7.69 (dd, 2H, Ar-H), 7.81-7.98 (m, 3H, Ar-H).^{13}C (300 MHz, DMSO): δ 53.57, 55.35, 55.60, 55.94, 105.17, 113.58, 116.74, 120.87, 123.16, 123.47, 126.13, 127.09, 127.41, 128.22, 129.47, 133.41, 134.72, 136.42, 151.27, 157.14, 158.70, 175.21. ESI-MS (m/z): 363.13 (M+1), calc.: 363.40. HPLC purity: 97.16%.Anal. Calculated for $C_{22}H_{21}NO_4$ (363.40): C, 75.66; H, 5.74; N, 4.20; O, 14.40%. Found: C, 75.71; H, 5.75; N, 4.18; O, 14.39%.

5-(2-chlorophenyl)-3-(6-methoxynaphthalen-2-yl)-4,5-dihydro-1,2-oxazole (4f):
Yellow solid, Yield = 73%, m. p.: 186-188°C. IR (KBr, cm^{-1}): 3038 (Ar-H str.), 2952 (C-H str.), 1658 (C=N str.), 1589 (Ar-C=C str.), 1453 (N-O str.), 1429 (C-H bend.).^{1}H NMR (400 MHz, DMSO-d_6):δ 3.13-3.26 (d, 2H, isoxazole-CH_2), 3.49 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.41-4.49 (d, 1H, isoxazole-CH), 6.92-7.09 (d, 2H, Ar-H), 7.29-7.56 (m, 4H, Ar-H), 7.74-7.91 (m, 4H, Ar-H).^{13}C (300 MHz, DMSO): δ 53.62, 55.42, 105.49,110.69, 113.40, 117.27, 120.72, 123.62, 123.73, 126.39, 127.67, 128.34, 129.30, 133.19, 134.28, 136.74,

152.45, 157.38, 158.09, 176.27. ESI-MS (m/z): 338.28 (M+1), Calc.: 337.79. HPLC purity: 98.71%.Anal. Calculated for$C_{20}H_{16}ClNO_2$ (337.79): C, 71.11; H, 4.77; N, 4.15; Cl, 10.49; O, 9.47%. Found: C, 71.16; H, 4.75; N, 4.14; Cl, 10.51; O, 9.45%.

5-(3-chlorophenyl)-3-(6-methoxynaphthalen-2-yl)-4,5-dihydro-1,2-oxazol(4g):

Pale yellow solid, Yield = 78%, m. p.: 186-188° C. IR (KBr, cm^{-1}): 3085 (Ar-H str.), 2936 (C-H str.), 1628 (C=N str.), 1548 (Ar-C=C str.), 1443 (N-O str.), 1437 (C-H bend.).^{1}H NMR (400 MHz, DMSO-d_6): δ 3.07 - 3.14 (d, 2H, Isoxazole-CH_2), 3.67 (s, 3H, OCH_3), 4.75 - 4.79 (d, 1H, Isoxazole-CH), 6.86 - 7.06 (d, 2H, Ar-H), 7.16 - 7.39 (m, 4H, Ar-H), 7.48 - 7.81 (m, 4H, Ar-H).^{13}C (300 MHz, DMSO): δ 53.71, 55.34, 101.67, 105.48,110.74, 116.64, 120.46, 122.87, 123.62, 125.08, 127.81, 128.34, 129.29, 132.38, 133.45, 137.61, 151.42, 156.91, 158.38, 174.26. ESI-MS (m/z): 338.42 (M+1), Calc.: 337.79. HPLC purity: 98.42%.Anal. Calculated for $C_{20}H_{16}ClNO_2$ (337.79): C, 71.11; H, 4.77; N, 4.15; Cl, 10.49; O, 9.47%. Found: C, 71.13; H, 4.78; N, 4.13; Cl, 10.47; O, 9.46%.

***5-(4-chlorophenyl)-3-(6-methoxynaphthalen-2-yl)-4,5-dihydro-1,2-oxazole(4h)*:**

Pale yellow solid, Yield = 74%. m. p.: 178-180° C. IR (KBr, cm^{-1}): 3062 (Ar-H str.), 2926 (C-H str.), 1625 (C=N str.), 1556 (Ar-C=C str.), 1438 (N-O str.), 1428 (C-H bend.).^{1}H NMR (400 MHz, DMSO-d_6): δ 3.11 - 3.17 (d, 2H, Isoxazole-CH_2), 3.52 (s, 3H, OCH_3), 4.88 - 4.92 (d, 1H, Isoxazole-CH), 6.93 - 7.15 (d, 2H, Ar-H), 7.24 - 7.36 (d, 2H, Ar-H), 7.46 - 7.53 (d, 2H, Ar-H), 7.59 - 7.71 (m, 2H, Ar-H), 7.80 - 7.91 (m, 2H, Ar-H).^{13}C (300 MHz, DMSO): δ 53.22, 55.60, 102.42, 105.19, 111.64, 116.24, 120.69, 122.37, 123.29, 125.82, 127.26, 128.49, 129.67, 132.80, 133.70, 137.27, 152.14, 155.68, 158.53, 174.09. ESI–MS (m/z): 338.53(M+1), Calc.: 337.79. HPLC purity: 98.86%.Anal. Calculated for $C_{20}H_{16}ClNO_2$ (337.79): C, 71.11; H, 4.77; N, 4.15; C, 10.49; O, 9.47%. Found: C, 71.15; H, 4.76; N, 4.14; Cl, 10.50; O, 9.44%.

5-(2,6-dichlorophenyl)-3-(6-methoxynaphthalen-2-yl)-4,5-dihydro-1,2-oxazole (4i):

Yellow semi-solid, Yield = 70%. M. p.: 206-208°C. IR (KBr, cm^{-1}): 3089 (Ar-H str.), 2943 (C-H str.), 1619 (C=N str.), 1549 (Ar-C=C str.), 1461 (N-O str.), 1436 (C-H bend.).^{1}H NMR (400 MHz, DMSO-d_6): δ 3.19-3.27 (d, 2H, Isoxazole-CH_2), 3.63 (s, 3H, OCH_3), 4.82-4.89 (d, 1H, Isoxazole-CH), 6.98-7.12 (d, 2H, Ar-H), 7.19-7.30 (d, 2H, Ar-H), 7.39-7.51 (d, 2H, Ar-H), 7.67-7.86 (m, 3H, Ar-H).^{13}C (300 MHz, DMSO): δ 54.19, 55.86, 101.24, 106.27, 111.55, 116.59, 119.38, 120.81, 123.02, 123.51, 127.48, 127.63, 128.91, 129.70, 132.71, 133.05, 137.63, 152.44, 155.49, 157.81, 176.49. ESI-MS (m/z): 372.89 (M+1), Calc.: 372.24. HPLC purity: 97.79%.Anal. Calculated for $C_{20}H_{15}Cl_2NO_2$ (372.24): C, 64.19; H, 4.58; N, 3.74; Cl, 18.94; O, 8.55%. Found: C, 64.21; H, 4.56; N, 3.72; Cl, 18.97; O, 8.52%.

***3-(6-methoxynaphthalen-2-yl)-5-(2-methylphenyl)-4,5-dihydro-1,2-*oxazole*(4j)*:**

White solid, Yield = 77%, m. p.: 169-171°C. IR (KBr, cm^{-1}): 3059 (Ar-H str.), 2939 (C-H str.), 1626 (C=N str.), 1552 (Ar-C=C str.), 1468 (N-O str.), 1426 (C-H bend.).^{1}H NMR (400 MHz, DMSO-d_6): δ 2.18 (s, 3H, CH_3), 3.08-3.13 (d, 2H, isoxazole-CH_2), 3.69 (s, 3H, CH_3), 4.59-4.63 (d, 1H, isoxazole-CH), 6.94-7.09 (d, 2H, Ar-H), 7.29-7.42 (d, 2H, Ar-H), 7.58-7.67 (d, 2H, Ar-H), 7.71-7.94 (m, 4H, Ar-H).^{13}C (300 MHz, DMSO): δ 28.67, 56.61, 102.08, 105.56, 112.19, 117.92, 119.47, 121.66, 123.08, 123.26, 125.35, 127.31, 127.59, 129.16, 132.28, 133.53, 136.38, 152.26, 155.64, 158.28, 174.19. ESI-MS (m/z): 317.63 (M+1), Calc.: 317.38. HPLC purity: 98.87%.Anal. Calculated for $C_{21}H_{19}NO_2$ (317.38): C, 79.47; H, 6.03; N, 4.41; O, 10.08%. Found: C, 79.52; H, 6.05; N, 4.39; O, 10.06%.

***3-(6-methoxynaphthalen-2-yl)-5-(3-methylphenyl)-4,5-dihydro-1,2-*oxazole*(4k)*:**

White solid, Yield = 73%, m. p.: 123-125°C. IR (KBr, cm^{-1}), 3092 (Ar-H str.), 2927 (C-H str.), 1621 (C=N str.), 1563 (Ar-C=C str.), 1454 (N-O str.), 1431 (C-H bend.).^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, CH_3), 3.13-3.19 (d, 2H, isoxazole-CH_2), 3.57 (s, 3H, OCH_3), 4.92-4.99 (d, 1H, isoxazole-CH), 6.89-7.06 (m, 4H, Ar-H), 7.21-7.29 (d, 2H, Ar-H), 7.47-

7.56 (d, 2H, Ar-H), 7.88-7.96 (d, 2H, Ar-H).^{13}C (300 MHz, DMSO): δ 28.06, 56.31, 101.49, 105.38, 112.38, 117.53, 119.18, 120.57, 123.27, 123.60, 125.49, 127.16, 127.70, 129.93, 131.57, 132.36, 135.62, 154.61, 155.15, 158.43, 175.08. ESI-MS (m/z): 317.93 (M+1), Calc.: 317.38. HPLC purity: 97.83%.Anal. Calculated for $C_{21}H_{19}NO_2$ (317.38): C, 79.47; H, 6.03; N, 4.41; O, 10.08%. Found: C, 79.50; H, 6.02; N, 4.38; O, 10.09%.

***3-(6-methoxynaphthalen-2-yl)-5-(4-methylphenyl)-4, 5-dihydro-1, 2*-oxazole *(4l)*:**

White to brown solid, Yield = 77%, m. p.: 167-169°C. IR (KBr, cm^{-1}), 3062 (Ar-H str.), 2936 (C-H str.), 1629 (C=N str.), 1552 (Ar-C=C str.), 1438 (N-O str.), 1430 (C-H bend.).^{1}H NMR (400 MHz, DMSO-d_6): δ 2.19 (s, 3H, CH_3), 3.09-3.16 (d, 2H, Isoxazole-CH_2), 3.68 (s, 3H, OCH_3), 4.86-4.91 (d, 1H, Isoxazole-CH), 6.92-7.11 (d, 2H, Ar-H), 7.23-7.42 (m, 4H, Ar-H), 7.62-7.74 (d, 2H, Ar-H), 7.81-7.92 (d, 2H, Ar-H).^{13}C (300 MHz, DMSO): δ 28.19, 55.14, 102.06, 105.53, 112.18, 117.33, 119.34, 121.08, 123.41, 123.69, 125.25, 127.37, 127.84, 129.16, 129.64, 132.18, 136.62, 153.92, 155.31, 157.27, 175.26. ESI-MS (m/z): 317.46 (M+1), Calc.: 317.38. HPLC purity: 98.41%.Anal. Calculated for $C_{21}H_{19}NO_2$ (317.38): C, 79.47; H, 6.03; N, 4.41; O, 10.08%. Found: C, 79.51; H, 6.01; N, 4.40; O, 10.06%.

Biological section

The National Committee for Clinical Laboratory Standards (NCCLS) method was followed for the antimicrobial activity of the synthesised isoxazole series. The strains were procured from the Institute of Microbial Technology, Chandigarh, and employed for the activity. Mueller Hinton broth was used as a nutrient medium to grow and dilute the drug suspension for the test. To get the desired concentration of compounds dimethyl sulfoxide (DMSO) was used as the diluent upon the standard bacterial strains.

Antibacterial activity was screened against two Gram-negative E. coli (Escherichia coli) strains, MTCC-443 (MTCC-Micro Type Culture Collection) and P. Aeruginosa (Pseudomonas aeruginosa) MTCC 1688, and two Gram-positive S. Aureus (Staphylococcus aureus) and S. Pyogenes (Streptococcus pyogenes) MTCC 442 bacteria. Chloramphenicol and ciprofloxacin are used as the standard antibacterial drugs. Three fungal species, C. albicans (Candida albicans) MTCC 227, A. niger (Aspergillus niger) MTCC 282, and A. clavatus (Aspergillus clavatus) MTCC 1323, were used to determine the antifungal activity of the synthesised isoxazole series. Nystatin and griseofulvin were used as the standard antifungal drugs.

In vitro antibacterial activity: For E. coli, compounds **(4b and 4c)** had activity MIC-62.50 ug/ml and MIC-50 ug/ml, respectively. These show good activity when compared with standard chloramphenicol MIC-50 ug/ml but moderate with standard ciprofloxacin (MIC-25 ug/ml). For P. aeruginosa, compounds **(4g and 4j)**, each having an activity MIC of 62.50 ug/ml, perform moderate activity compared with ciprofloxacin and chloramphenicol as standard drugs. For S. aureus, compounds **(4c and 4h)** each show activity MIC-62.50 ug/ml, and for S. progenies, compounds **(4b)** (MIC-62.50 ug/ml) &**(4g)** (MIC-50 ug/ml) show promising antibacterial activity using chloramphenicol and ciprofloxacin as standard drugs. Results are shown in **Table 01**.

In vitro antifungal activity: The synthesised compounds **(4a-l)** show moderate antifungal activities using fungal species C. albicans, A. niger, and A. clavatus in comparison with nystatin & griseofulvin as standard drugs. However, compound **(4e)** (MIC-250 ug/ml) could be a good antifungal compound. Results are shown in **Table 01**.

Biological evolution conclusion: The newly synthesised sequence **(4a–l)** was evaluated for antibacterial and antifungal evolution studies. Compound (**4b, 4c, and 4g)** shows good antibacterial activity. However, series **(4a–l)** had moderate antifungal activity, except for compound **(4e)**, which had good antifungal activity.

Table 01: Antibacterial and Antifungal activity (MIC µgm/ml)

Antibacterial Activity						Antifungal Activity		
Entry	*E. Coli*	*P. Aeruginosa*	*S. Aureus*	*S. Pyogenes*		*C. Albicans*	*A. Niger*	*A. Clavatus*
4a	125	100	200	100		500	500	500
4b	62.5	100	100	62.5		1000	500	500
4c	50	100	62.5	100		1000	500	500
4d	100	250	100	250		1000	1000	1000
4e	250	100	250	250		250	1000	1000
4f	200	125	125	200		500	1000	1000
4g	100	62.5	100	50		1000	500	500
4h	100	125	62.5	100		1000	500	500
4i	100	200	100	100		1000	1000	1000
4j	125	62.5	100	125		500	1000	1000
4k	100	100	125	125		1000	500	500
4l	100	125	100	250		500	1000	1000
Chloramphenicol	50	50	50	50		NA	NA	NA
Ciprofloxacin	25	25	50	50		NA	NA	NA
Nystatin	NA	NA	NA	NA		100	100	100
Griseofulvin	NA	NA	NA	NA		500	100	100

RESULTS AND DISCUSSION

Chemistry: Ketone compound **(1)** and aldehyde compound **(2)** were dissolved in ethanol, followed by the addition of sodium hydroxide. The reaction was maintained overnight at room temperature to give chalcone scaffold E-1 (6-methoxynaphthalene-2-yl)-3-(substituted phenyl) prop-2-en-1-one **(3a-l)**. Compounds **(3a-l)** and hydroxylamine hydrochloride cyclized at hot conditions to corresponding isoxazole compounds **(4a-l)**. The formation of compounds **(4a-l)** was confirmed by IR, ^{1}H NMR, ^{13}C NMR, and mass spectra. Synthesised compounds were screened for antibacterial and antifungal activity.

CONCLUSION

A series of dihydro isoxazole derivatives had been synthesised by cyclisation of chalcone scaffolds with hydroxylamine hydrochloride. The compounds were synthesised in a simple way; no harsh conditions were employed, no catalyst was used, and no hazardous solvents were utilised. Synthesised molecules were confirmed with the help of spectral techniques. Series **(4a-l)** was screened for antimicrobial activities. Antibacterial activity results of compounds **(4b, 4c, and 4g)** indicate that these molecules could be good antibacterial agents. Compound **(4e)**, which had good antifungal activity, was the rest of the compounds that showed moderate antifungal activity. These compounds may be helpful for further designing, developing, and biologically screening more potent active agents.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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