Synthesis, characterization of new nicotinamide-oxazole analogs, and their antimicrobial activity

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ABSTRACT
Identification of a novel antimicrobial molecule is vital to research due to contaminated agro related products and harmful pathogens. Especially, candida albicans is the most common infective fungi in the world that causes hospital-acquired infections. There is a medical and biological need for the discovery of novel antimicrobial drugs with high potent in nature. This effort involves the synthesis of scaffold molecule in which vitamin B\textsubscript{3} and oxazole play vital role as pharmacophore moiety, where 2-(Nicotinamido) oxazole-4-carboxylic acid couples with pyridine–3-carboxylic acid (nicotinic acid) and 2-aminooxazole derivatives. Then, it is carried for mass spectra, \textsuperscript{1}H NMR spectroscopy, and growth control ability study against microbial targets such as fungi and bacteria. The zone of inhibition is measured in millimeters for the serially diluted solution of the compound. From the outcomes, the compound (5i) displayed 35mm of inhibition zone area, but standard fluconazole showed 29mm for 250 ppm solution. The outcome revealed that the amide bond and oxazole moiety turn as imperative pharmacophore besides showing decent inhibition activities.

INTRODUCTION
Oxazole and Thiazole moiety (Venkatasubramanian and Easwaramoorthy, 2019) is an important, pharmaceutical, chemical entity, which coexists without toxicity. It plays a significant role in antibacterial (Jayaprakash et al., 2016b), antifungal, and anticancer (Mathew et al., 2018) discovery areas, where it has been used as linker moiety. A series of oxazole moieties is present in many bio-natural products. Oxazole analogs revealed substantial activities, for instance, antimicrobial (Mohammed, 2019), antitumor, anti-HIV, analgesic, and anti-Inflammatory (Rolfe, 2014). Nicotinamides were part of a naturally occurring part of vitamin B\textsubscript{3} (Peng et al., 2017). Niacin is another name for amide linkage that holds good to enhance the biological activity. The amide linkage (Montalbetti and Falque, 2005) has a biological significance, especially towards the antimicrobial area. So, superior novel antibiotic investigation expected further consideration among the researchers to implicate in the proposal besides preparation of the novel dynamic molecules. With the variety of the reactant molecules, this effort was well-known to fascinate the diamide functionality. Microbial infections
were the main threat to mankind. Since ancient times, huge amounts of increasing events of microbial infections have been observed and the frequent use of antibacterial, antifungal, and cytotoxic drugs. Microbial species found resistance towards various existing antimicrobial agents. Superbugs were a huge threat that can inhibit all existing drugs. Slowly, we were increasing the dosage of drugs to control the infections. Afterwards, the existing drugs will not work against new infections, which were caused by superbugs, and they can rule the entire world. Hence, it is necessary to synthesize new antimicrobial agents (Sathish et al., 2018). Proton NMR, Mass spectra, and HPLC (Sathiyanarayanan et al., 2019) techniques were used to identify new series of oxazole compounds. The categorized chemical analogs’ preventing capacity was restrained by the broth dilution method against bacterial and fungal targets. The accumulated experimental conclusions were dignified in the area of inhibition with millimeter units.

**Experimental Methods**

**MATERIALS AND METHODS**

Nicotinic acid, 1-Ethyl-3-(3’-Dimethylamino) Carbodiimide, Hydroxy benzotriazole, N, N-Diisopropyl ethylamine, and DMF were obtained from Sigma Chemicals. The solvent drying process was followed by standard procedures. TLC plates were purchased from Merck. A mixture of 50% n-Hexane and Ethyl acetate was used to monitor the reaction progress. Using Jeol-JMS D-300 spectrometer, Mass spectra of the analogs were recorded. Spectra of $^1$H-NMR (400 MHZ) were analyzed using BRUCKER. Agar and dextrose agar medium nutrients were used for the antimicrobial studies.

**Synthetic Scheme**

**Preparation of Ethyl 2-(Nicotinamido)oxazole-4-carboxylic acid (3)**

To a stirred solution of Nicotinic acid (pyridine-3-carboxylic acid, 5g, 0.0406 mol), in 50 mL dried DMF, 1-Ethyl-3-(3’-Dimethylamino) Carbodiimide hydrochloride salt (EDCI, 15.5g, 0.0812 mol) and Hydroxy Benzotriazole (HOBt, 1g, 0.0076 mol) were added under an inert environment at 0°C. After stirring for 3 hours at room temperature, TLC was used to observe the reaction development. The solvents were distilled out in condensed pressure. The aqueous reaction mass was cooled to about 10°C. The filtered yellow precipitate was dried. YIELD: 7 gm (82%). The synthetic route is represented in Scheme 1.

**Preparation of 2-(Nicotinamido)oxazole-4-carboxylic acid (4)**

To a stirred solution of compound 3 (10 g, 0.034 mol) in THF (100ml), aqueous sodium hydroxide (2.72g) was added at 0°C. After stirring for 3 hours at room temperature, TLC was used to observe the reaction development. The reaction was examined by TLC. The aqueous reaction mass was cooled to about 10°C. The filtered yellow precipitate was washed. YIELD: 2 gm (85%). The synthetic route is represented in Scheme 2.

**Preparation of N-Substituted 2-(nicotinamido)oxazole-4-carboxamide (5)**

The compound 4 (1 g, 0.005 mol) was stirred in dry DMF (20 ml), to which EDCI (2.9g, 0.015 mol) and HOBt (1g, 0.0076 mol) were added in a nitrogen atmosphere at 0°C for 0.5 hrs. Then, cyclohexylamine (0.7g, 0.006 mol) was added and followed by DIPEA (3.2 ml, 0.025 mol) and stirring was continued for about 4 hours at 25°C. The progress of the reaction was examined by TLC. Ice cooled water (30 ml) was added and stirred to the reaction mixture. The filtered pale white precipitate was dried in reduced pressure. YIELD: 1.3 gm (78%). Similarly, all other analogs were prepared with the same procedure by substituted amines. The synthetic route is represented in Scheme 3.

**Spectral Data**

Ethyl 2-(Nicotinamido)oxazole-4-carboxylate (3)

Pale yellow solid, m/z: 262 (M+1). $^1$H-NMR (400MHz, CDCl$_3$) δ (ppm) 12.0 (s, br, 1H, NH amide), 9.2 (d, 1H, Pyridine-H), 8.9 (d, 1H, Pyridine-H), 8.4 (d, 1H, Pyridine-H), 7.7 (t,2H, Pyridine-H and oxazole-H), 4.6 (q, 2H, -CH$_2$), 1.5 (t, 3H, -CH$_3$).

2-(nicotinamido) oxazole-4-carboxylic acid (4)

Pale white solid, m/z: 323(M-1). $^1$H-NMR (400MHz, DMSO-d$_6$) δ (ppm) 12.9(b,1H, -COOH), 9.2 (d, 1H, Pyridine-H), 8.8 (q, 1H, Pyridine-H), 8.4 (q, 1H, Pyridine-H), 7.7 (q,2H, Pyridine-H and oxazole-H). Deuterium exchange experiment was done using D$_2$O.

2-(nicotinamido)-N-phenyloxazole-4-carboxamide (5a)

Pale yellow solid, m/z:309(M+1), $^1$H-NMR (400MHz, DMSO-d$_6$) δ (ppm) 12.6 (b, 1H, -NH amide), 12.1 (b, 1H, -NH amide), 9.2 (d, 1H, Pyridine-H), 8.9 (t, 2H, Pyridine-H, Benzyl-H), 8.4 (d, 2H, Pyridine-H, Benzyl-H), 8.3 (d,1H, Benzyl-H), 7.6 (t,2H, Benzyl-H), 7.7 (t, 2H, Pyridine-H and oxazole-H).
Table 1: Yields of Synthesized compounds (3, 4, 5a-i)

| Compound | R group          | Mol.Formula (M.W) | Yield (%) | Melting point (°C) |
|----------|-----------------|-------------------|-----------|--------------------|
| 3        | Ethyl group (Ester) | C_{12}H_{11}N_{3}O_{4} (261) | 89        | 248                |
| 4        | Hydroxyl (Acid)  | C_{10}H_{7}N_{3}O_{4} (233) | 82        | 240                |
| 5a       | Phenyl           | C_{18}H_{12}N_{4}O_{3} (308) | 85        | 298                |
| 5b       | Cyclopropyl      | C_{13}H_{12}N_{4}O_{3} (272) | 72        | 248                |
| 5c       | Cyclobutyl       | C_{14}H_{14}N_{4}O_{4} (286) | 75        | 254                |
| 5d       | Cyclopentyl      | C_{17}H_{16}N_{4}O_{3} (300) | 79        | 261                |
| 5e       | Cyclohexyl       | C_{16}H_{18}N_{4}O_{3} (314) | 78        | 278                |
| 5f       | Adamantyl        | C_{20}H_{22}N_{4}O_{3} (366) | 81        | 292                |
| 5g       | N-Methyl piperazinyl | C_{15}H_{17}N_{5}O_{3} (315) | 89        | 277                |
| 5h       | Morpholinyl      | C_{14}H_{14}N_{4}O_{3} (302) | 90        | 268                |
| 5i       | Thiomorpholinyl  | C_{14}H_{14}N_{4}O_{3}S (319) | 91        | 272                |

Table 2: Study of Antimicrobial activity of potential compounds

| Organism      | STD    | 3     | 4     | 5a    | 5e    | 5h    | 5i    |
|---------------|--------|-------|-------|-------|-------|-------|-------|
| Staphylococcus Aureus | 32 mm | 20 mm | 28 mm | 17 mm | 19 mm | 22 mm | 20 mm |
| Staphylococcus Epidermidis | 29 mm | 19 mm | 24 mm | 15 mm | 17 mm | 20 mm | 22 mm |
| Escherichia Coli | 31 mm | 15 mm | 23 mm | 14 mm | 17 mm | 22 mm | 23 mm |
| Klebsiella Pneumoniae | 28 mm | 12 mm | 20 mm | 13 mm | 15 mm | 18 mm | 19 mm |
| Candida Albicans | 29 mm | 21 mm | 25 mm | 17 mm | 19 mm | 31 mm | 35 mm |

Figure 1: The antifungal discs of the 5i exhibited
N-cyclopropyl-2-(nicotinamido) oxazole-4-carboxamide (5b)
Pale white solid, m/z:273(M+1), 1H-NMR (400MHz, DMSO-d6) δ (ppm) 12.6 (b, 1H, -NH amide), 12.3 (b,1H, -NH amide), 9.1 (s, 1H, Pyridine-H), 8.75 (d,1H, Pyridine-H), 8.30 (d,1H, Pyridine-H), 7.5 (t,1H, oxazole-H), 3.0 (m,1H, Cyclopropyl-CH), 1.4 (m,2H, Cyclopropyl-CH₂), 1.0 (h,2H, Cyclopropyl-CH₂).

N-cyclobutyl-2-(nicotinamido) oxazole-4-carboxamide (5c)
Pale white solid, m/z:287(M+1), 1H-NMR (400MHz, DMSO-d6) δ (ppm) 12.6 (b, 1H, -NH amide), 12.3 (b,1H, -NH amide), 9.1 (s, 1H, Pyridine-H), 8.75 (d,1H, Pyridine-H), 8.30 (d,1H, Pyridine-H), 7.5 (t,1H, oxazole-H), 4.2 (m,1H Cyclobutyl-CH), 2.0 (m,2H, Cyclobutyl-CH₂), 1.7 (m,2H, Cyclobutyl-CH₂), 1.5 (m,2H, Cyclobutyl-CH₂).

N-cyclopentyl-2-(nicotinamido) oxazole-4-carboxamide (5d)
Pale white solid, m/z:301(M+1), 1H-NMR (400MHz, DMSO-d6) δ (ppm) 12.6 (b, 1H, -NH amide), 12.3 (b,1H, -NH amide), 9.1 (s, 1H, Pyridine-H), 8.75 (d,1H, Pyridine-H), 8.30 (d,1H, Pyridine-H), 7.5 (t,1H, oxazole-H), 4.15 (m,1H, Cyclophenyl-CH), 1.8 (m,4H, Cyclophenyl-2CH₂), 1.65 (m,2H, Cyclophenyl-CH₂), 1.62 (q,2H, Cyclophenyl-CH₂).

N-cyclohexyl-2-(nicotinamido) oxazole-4-carboxamide (5e)
Pale white solid, m/z:315(M+1), 1H-NMR (400MHz, DMSO-d6) δ (ppm) 12.6 (b, 1H, -NH amide), 12.3 (b,1H, -NH amide), 9.1 (s, 1H, Pyridine-H), 8.75 (d,1H, Pyridine-H), 8.30 (d,1H, Pyridine-H), 7.5 (t,1H, oxazole-H), 4.05 (q,1H, Adamantyl-CH), 2.1 (m,2H, Adamantyl-CH₂), 1.8 (m,10H, Adamantyl-CH₂), 0.5 (h,2H, Adamantyl-CH₂).

N-((1r, 3r, 5r, 7r)-adamantan-2-yl)-2-(nicotinamido) oxazole-4-carboxamide (5f)
Brown solid, m/z:367(M+1), 1H-NMR (400MHz, DMSO-d6) δ (ppm) 12.6 (b, 1H, -NH amide), 12.3 (b,1H, -NH amide), 9.1 (s, 1H, Pyridine-H), 8.75 (d,1H, Pyridine-H), 8.30 (d,1H, Pyridine-H), 7.5 (t,1H, oxazole-H), 4.05 (q,1H, Adamantyl-CH), 2.1 (m,2H, Adamantyl-CH₂), 1.8 (m,10H, Adamantyl-CH₂), 0.5 (h,2H, Adamantyl-CH₂).

N-(4-(4-methylpiperazine-1-carbonyl) oxazol-2-yl)nicotinamide (5g)
Pale brown solid, m/z:316(M+1), 1H-NMR (400MHz, DMSO-d6) δ (ppm) 12.6 (b, 1H, -NH amide), 12.3 (b,1H, -NH amide), 9.1 (s, 1H, Pyridine-H), 8.75 (d,1H, Pyridine-H), 8.30 (d,1H, Pyridine-H), 7.5 (t,1H, oxazole-H), 3.7 (m,2H, N-Methylpiperazyl-
In the current investigation, we have aimed to synthesize novel nicotinamide analogs. The second step is the hydrolysis of ethyl 2-amino oxazole-4-carboxylate. The second step is the hydrolysis of ethyl 2-(nicotinamido)oxazole-4-carboxylate (3), to get 2-(nicotinamido) oxazole-4-carboxylic acid (4), which was prime scaffold. The scaffold (4) coupled with various amines to get other derivatives (5a-i) were synthesized. The molecular formula, molecular weight, yield, and melting point of the synthesized compounds are presented in Table 1.

This effort identified that analogs’ polarization and melting points are higher than that of the parent molecules because of the molecular weight and diamide group of the compounds. Generally, the amide bond will have a greater affinity towards polarity and temperature withstanding capacity. Therefore, the expected melting point and polarity of the novel derivatives are higher than the calculated values of the new derivatives. Established on the Rf value modification and physical properties, all prepared analogs were analyzed for Mass and 1H-NMR spectra. The proton (singlet, which was besides neighbouring pyridine nitrogen) was recognized in the range of 12.6 to 12.1 ppm based on the exposed 1H-NMR outcomes. Between 8.7 ppm and 8.3 ppm, amide protons were recognized in all analogs. Likewise, protons of analog 3 (triplet and doublet) disappeared as well as confirmed in analog 4. However, the number of protons improved near the aliphatic regions established because of another amide peak. Later, the analogs confirmed the molecular ion peak by mass spectroscopy. The modification detected among the analogs 3 and 4 is 30 a.m.u., which authorize the ester hydrolyzed carboxylic acid. Similarly, the analogs were successfully characterized using Mass and 1H-NMR spectra. The categorized analogs were approved for biological studies. Antimicrobial Activity

Paper disc diffusion technique was used to estimate the in vitro antimicrobial activity of New nicotinamide-Oxazole analogs (Kakkar and Narasimhan, 2019). They were tested against in vitro antibacterial (Ubaid and Hemalatha, 2017) and antifungal activity (Saroj et al., 2019). Among all, four compounds were taken to a detailed antimicrobial activity (Jayaprakash et al., 2016a) with various concentrations to get a clear picture of efficiency.

The categorized analogs were approved for biological studies. All the prepared analogs were screened against gram-positive organisms (Saroj et al., 2018) such as Staphylococcus aureus and Staphylococcus epidermidis and gram-negative organisms such as Escherichia coli and Klebsiella pneumonia, and fungi like Candida albican were bio-assayed. Ciprofloxacin (5 mcg/disc) was used as a standard antibacterial drug for biological activity. Ketoconazole (50mcg/disc) was used as a standard antifungal drug for the inhibition assay. But Analogs like 5a, 5e, 5h, and 5i were shown highly active towards microbial studies. The results are displayed in Table 2. This effort exposed that the diamide (thiomorphinyl attached) showed worthy inhibition capacity. The antifungal disc of the 5i is exhibited in Figure 1. Moreover, analog 5i exhibited excellent results counter to the fungus. Especially, derivatives (5a, 5e, 5h, and 5i) were potentially active and all the analogs exposed recent outcomes.

RESULTS AND DISCUSSION

Chemistry

In the current investigation, we have aimed to synthesize novel nicotinamide analogs (Venkatasubramanian et al., 2019). The first step is that pyridine-3-carboxylic acid (Nicotinic acid) was coupled with ethyl-2-amino oxazole-4-carboxylate. The second step is the hydrolysis of ethyl 2-(nicotinamido)oxazole-4-carboxylate (3), to get 2-(nicotinamido) oxazole-4-carboxylic acid (4), which was prime scaffold. The scaffold (4) coupled with various amines to get other derivatives (5a-i) were synthesized. The molecular formula, molecular weight, yield, and melting point of the synthesized compounds are presented in Table 1.

This effort identified that analogs’ polarization and melting points are higher than that of the parent molecules because of the molecular weight and diamide group of the compounds. Generally, the amide bond will have a greater affinity towards polarity and temperature withstanding capacity. Therefore, the expected melting point and polarity of the novel derivatives are higher than the calculated values of the new derivatives. Established on the Rf value modification and physical properties, all prepared analogs were analyzed for Mass and 1H-NMR spectra. The proton (singlet, which was besides neighbouring pyridine nitrogen) was recognized in the range of 12.6 to 12.1 ppm based on the exposed 1H-NMR outcomes. Between 8.7 ppm and 8.3 ppm, amide protons were recognized in all analogs. Likewise, protons of analog 3 (triplet and doublet) disappeared as well as confirmed in analog 4. However, the number of protons improved near the aliphatic regions established because of another amide peak. Later, the analogs confirmed the molecular ion peak by mass spectroscopy. The modification detected among the analogs 3 and 4 is 30 a.m.u., which authorize the ester hydrolyzed carboxylic acid. Similarly, the analogs were successfully characterized using Mass and 1H-NMR spectra. The categorized analogs were approved for biological studies.
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