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

Venkatasubramanian H[1,2], Sarojkumar Sha[3], Hemalatha S[3], Easwaramoorthy D[*1]

[1]Department of Chemistry, B.S. Abdur Rahman Crescent Institute of Science and Technology, Vandalur, Chennai-600 048, Tamilnadu, India
[2]Department of Chemistry, MIT Campus, Anna University, Chennai-600044, Tamilnadu, India
[3]School of Life Science, B.S. Abdur Rahman Crescent Institute of Science and Technology, Vandalur, Chennai-600048, Tamilnadu, India

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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_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, ^{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.

*Corresponding Author

Name: Easwaramoorthy D
Phone: +91 9444075620
Email: easwar@crescent.education

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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_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 fasci-

nate 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-carboxylate (3)

To a stirred solution of Nicotinic acid (pyridine-3-carboxylic acid, 5g, 0.0406 mol, 1) in 50 mL dried DMF, 1-Ethyl-3-(3'-Dimethylamino) Carbodiimide hydrochloride salt (EDCI, 15.5g, 0.0812 mol) and Hydroxy Benzotriazole (HoBt, 5.48 g, 0.042 mol) were added under an inert environment at 0 °C. Ethyl-2-aminooxazole-4-carboxylate (2, 9 g, 0.0487 mol) and N, N-Diisopropyl ethyl amine (DIPEA, 21 ml, 0.125 mol) were slowly added. The mixture was agitated for 3 hours at room temperature. Ice cooled water (50 ml) was added and stirred to the reaction mixture. The filtered pale yellow solid was vacuum desiccated. 89% of the yield was obtained from this reaction. 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 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 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 25^0 °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: 232(M-1), ^{1}H -NMR (400MHz, DMSO-d6) 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_2O.

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

Pale yellow solid, m/z:309(M+1), ^{1}H-NMR (400MHz, DMSO-d6) 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, Benzylic-H), 8.4 (d, 2H, Pyridine-H, Benzylic-H), 8.3 (d,1H, Benzylic-H), 7.6 (t,2H, Benzylic-H), 7.7 (t, 2H, Pyridine-H and

Table 1: Yields of Synthesized compounds (3, 4, 5a-i)

Compound	R group	Mol.Formula (M.W)	Yield (%)	Melting point (^{0}C)
3	Ethyl group (Ester)	$C_{12}H_{11}N_3O_4$ (261)	89	248
4	Hydroxyl (Acid)	$C_{10}H_7N_3O_4$ (233)	82	240
5a	Phenyl	$C_{16}H_{12}N_4O_3$ (308)	85	298
5b	Cyclopropyl	$C_{13}H_{12}N_4O_3$ (272)	72	248
5c	Cyclobutyl	$C_{14}H_{14}N_4O_3$ (286)	75	254
5d	Cyclopentyl	$C_{15}H_{16}N_4O_3$ (300)	79	261
5e	Cyclohexyl	$C_{16}H_{18}N_4O_3$ (314)	78	278
5f	Adamantyl	$C_{20}H_{22}N_4O_3$ (366)	81	292
5g	N-Methyl piperazinyl	$C_{15}H_{17}N_5O_3$ (315)	89	277
5h	Morpholinyl	$C_{14}H_{14}N_4O_3$ (302)	90	268
5i	Thiomorpholinyl	$C_{14}H_{14}N_4O_3S$ (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

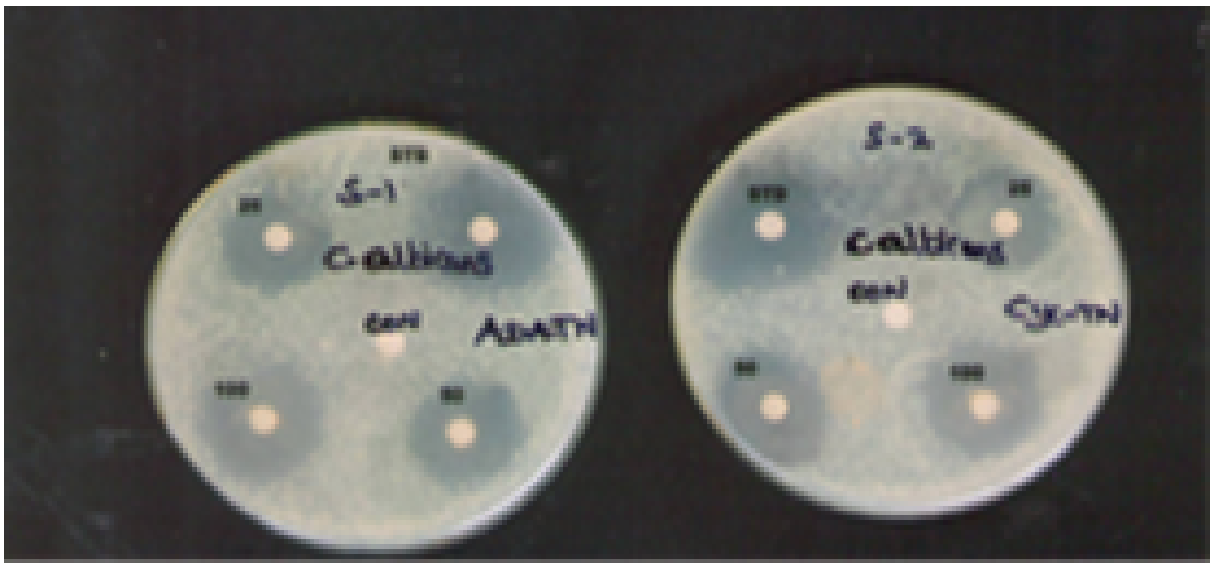
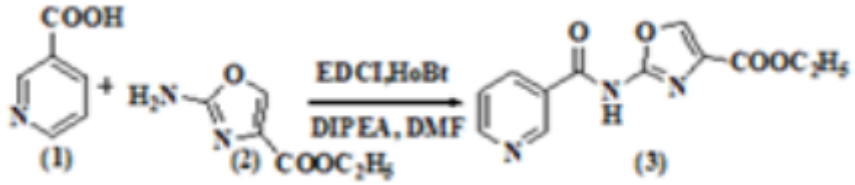
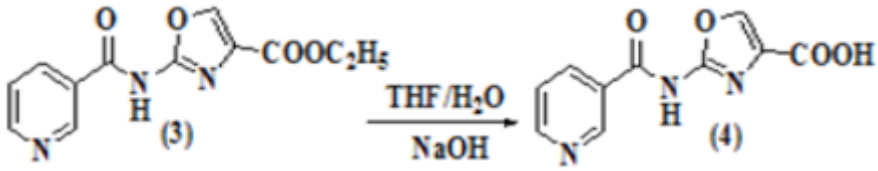


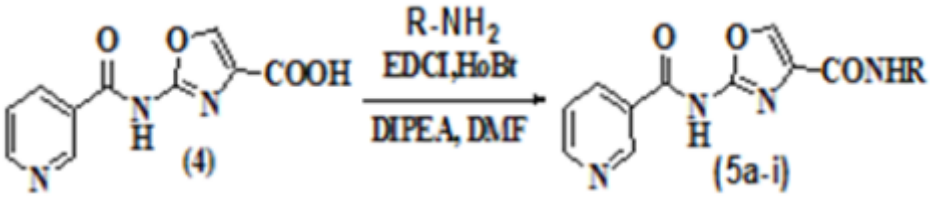
Figure 1: The antifungal discs of the 5i exhibited



Scheme 1: Preparation of Ethyl 2 - (nicotinamido) oxazole – 4 – carboxylate



Scheme 2: Preparation of 2- (nicotinamido) oxazole – 4 -carboxylic acid



Scheme 3: Preparation of N-Substituted 2-(nicotinamido) oxazole – 4 – carboxamide

oxazole-H).

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- CH_2), 1.0 (h,2H, Cyclopropyl-CH_2).

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- CH_2), 1.7 (m,2H, Cyclobutyl-CH_2), 1.5 (m,2H, Cyclobutyl- CH_2).

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, Cyclopenyl-CH), 1.8 (m,4H, Cyclopenyl-2CH_2), 1.65 (m,2H, Cyclopenyl-CH_2), 1.62 (q,2H, Cyclopenyl-CH_2).

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), 3.5 (d,1H, Cylohexyl-CH), 1.6 (m,2H Cylohexyl-CH_2), 1.4 (m,9H, Cylohexyl-CH_2).

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-CH_2), 1.8 (m,10H, Adamantyl-CH_2), 0.5 (h,2H, Adamantyl-CH_2).

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-CH_2), 3.2 (m,4H, N-Methylpiperazyl-CH_2), 2.7 (dd,2H, N-Methylpiperazyl-CH_2), 2.2 (s, 3H,Nitrogen-CH_3).

N-(4-(morpholine-4-carbonyl) oxazol-2-yl) nicotinamide (5h)

Pale brown solid, m/z:303(M+1), 1H-NMR (400MHz, DMSO-d6) δ (ppm) 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,8H. Morpholinyl-CH_2).

N-(4-(thiomorpholine-4-carbonyl) oxazol-2-yl)nicotinamide (5i)

Pale brown solid, m/z:319(M-1), 1H-NMR (400MHz, DMSO-d6) δ (ppm) 12.4 (s, 1H, -NH amide), 9.1 (s, 1H, Pyridine-H), 8.75 (s,1H, Pyridine-H), 8.30 (t,2H, Pyridine-H), 7.6 (q,1H, oxazole-H), 3.3 (q, 4H, Thiomorpholinyl-CH_2), 3.1 (q, 4H, Thiomorpholinyl-CH_2).

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 R_f value modification and physical properties, all prepared analogs were analyzed for Mass and ^{1}H-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 ^{1}H-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 ^{1}H-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.

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 decent outcomes.

CONCLUSIONS

In this study of new analogs of Nicotinamide-Oxazole, derivatives were successfully prepared, characterized, and subjected to antimicrobial bioassay. Totally, eight analogs were biologically effective, while analogs such as 5a, 5e, 5h, and 5i) were found to be highly active. On the other hand, Nicotinamide-oxazole ester (3) and carboxylic acid (4) were moderately active, and that need to be considered. Further investigation established that diamide holding heterocyclic analogs were found to be better biological potent. The present outcomes

encouraged us towards new antifungal agents.

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Conflict of interest None

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