SYNTHESIS, CHARACTERISATION AND ANTIMICROBIAL ACTIVITY OF NEW NICOTINAMIDE-THIAZOLE DERIVATIVES

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ABSTRACT

In this era, the identification or synthesis of a new antibiotic is important research due to the pollution and harmful bacterial pathogens. Hence this work selected the already known mild active molecules such as Nicotinamide and Thiazole to enhance the activity against the disease-causing pathogens. Using peptide coupling reagent EDCI, nine compounds were prepared and characterized by ¹H NMR, Mass spectroscopy. The characterized compounds were evaluated *In vitro* antibacterial and antifungal activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumonia*, *Candida albicans* by broth dilution method and zone of inhibition measured in millimeter. All the derivatives showed good to moderate activity. Out of nine compounds *N*-(4-(thiomorpholine-4-carbonyl) thiazol-2-yl) nicotinamide showed better zone of inhibition between 27 mm and 34 mm. the outcomes of the result revealed that the diamide bond acts as an important pharmacophore and exposed the good inhibition behavior.

Keywords: Nicotinamide, Thiazole, Antimicrobial Activity, Amide Bond

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INTRODUCTION

Different infectious microbes have increased and are causing harmful diseases to human beings and are also causing death across the world.¹ So, enhanced new antibiotic research received more attention between the researchers to involve in design and synthesis of the new active molecules. Based on the society based theme, this work investigated the already reported mild active molecules such as Nicotinamide and thiazole for this research. Nicotinamide is a pyridine ring containing vitamin B₃ and prevents deficiency syndrome pellagra.^{2,3} Similarly, Thiazole ring also the active moiety and present in many bio-natural products like thiamine (vitamin B1).^{4,5} Thiazole derivatives are exhibited the therapeutic activities such as antimicrobial, antitumor, anti-HIV, analgesic and anti-inflammatory.⁶⁻¹² After the selection of the reactant molecules, this work identified the easily absorbing functional group amide from the reported researches.¹³⁻¹⁵ Using the reported researches, this research derived the new series of compounds and was characterized by ¹H-NMR, Mass spectral techniques. The characterized compounds inhibiting ability were measured by broth dilution method against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumonia*, *Candida albicans* and the outcomes were measured in zone of inhibition with the unit of millimeter.

EXPERIMENTAL

The solvents and reagents were purchased from Sigma-Aldrich (India), Biocorporals, Chennai. Solvents were dried over Type 3A molecular sieve for 72 hours, followed by distillation under reduced pressure.

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¹H-NMR spectra were recorded on a BRUCKER 400 MHZ Instrument (DMSO-d₆ used as solvent) and TMS used as an internal standard. Using Jeol-JMS D-300 spectrometer, mass spectra of the derivatives were recorded. Anti-bacterial strains of American Type Culture Collection (ATCC), Rockville, USA such as *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 14990), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13883), and *Candida albicans* (ATCC 10231) were purchased from the Department of Microbiology, Ceeal Analytical Lab, Chennai, TamilNadu.. All reactions are monitored by 60:40 ethyl acetate, hexane mixture. Nutrients agar medium and dextrose agar medium (Hi-Media Laboratories, Mumbai, India) were the media used for the study of anti-bacterial and anti-fungal respectively.

General Procedure

Synthesis of Ethyl 2-(Nicotinamido)Thiazole-4-Carboxylate (3)

5g (0.0406 mol) of Nicotinic acid (**1**) was treated with 50 mL dried DMF and 15.5g of *1-Ethyl-3-(3'-Dimethylamino) Carbodiimide hydrochloride* salt (EDCI, 0.0812 mol) then 5.48 g of Hydroxy Benzotriazole (HOBt, 0.0406 mol) were added and stirred under nitrogen atmosphere at 0°C for 30 minutes. After 15 min 9 g of ethyl-2-aminothiazole-4-carboxylate (**2**) (0.0487mol) and 20 mL of N, N-Diisopropylethylamine (DIPEA 0.122mol) were added slowly over the period of 10 min. The reaction mixture was stirred for 4 hours at room temperature and the reaction was monitored by TLC using 60% ethyl acetate with Hexane. After the reaction completion, reaction mass poured into ice water. The brown solid was filtered and dried under vacuum at 50°C. The yield obtained was 85%.

Synthesis of 2-(Nicotinamido) Thiazole-4-Carboxylic Acid (4)

The dried and characterized product was carried for the conversion of carboxylic acid. 10 g (0.034mol) of compound **3** was added in 100 mL of THF with 2.72 g of sodium hydroxide in 25 ml of water at 10°C. After the addition, the reaction mixture was stirred for 2 hours at room temperature. THF solvent was evaporated under high vacuum after the completion of the reaction. The reaction mass was cooled below 10°C and neutralized using 10 % HCl. The white precipitate formed was filtered and dried. Finally, the 78% of (7gm) compound **4** was obtained and carried for the next stage.

Preparation of N-Substituted-2-(Nicotinamido) Thiazole-4-Carboxamide (5a-i)

As discussed in compound **3** preparation, the reaction carried for the preparation of 5a to 5i derivatives. 1: 1.2 equivalents of the compound **4** and substituted amines were used for the synthesis. The reaction mass poured into ice water and the brown color solid was filtered and dried under vacuum at 60°C for 3 hrs.

¹H- NMR Characterization of Compounds

Ethyl 2-(nicotinamido) thiazole-4-carboxylate (3)

¹H-NMR (400MHz, CDCl₃) δ (ppm) 12.3 (b, 1H), 9.15 (s, 1H), 8.75 (d, 1H), 8.30 (d, 1H), 8.10 (s,1H), 7.50 (t,1H), 4.4 (q, 2H), 1.30 (t,3H) ; m/z: 278(M+1).

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

¹H-NMR (400MHz, DMSO-d₆) δ (ppm) 12.5 (b, 1H), 11.1 (s, 1H), 9.15 (s, 1H), 8.75 (d, 1H), 8.30 (d, 1H), 8.20 (s, 1H), 7.50 (t, 1H); m/z: 248(M-1)

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

¹H-NMR (400MHz, DMSO-d₆) δ (ppm) 12.3 (b, 1H), 9.1 (s, 1H), 8.74 (t, 2H), 8.30 (d, 1H), 8.10 (s,1H), 7.7 (d,2H), 7.5 (t,1H), 7.30 (t,2H) , 7.05 (t,1H); m/z:325(M+1)

N-cyclopropyl-2-(nicotinamido) thiazole-4-carboxamide (5b)

¹H-NMR (400MHz, DMSO-d₆) δ (ppm) 12.3 (b,1H), 9.1 (s, 1H), 8.75 (d,1H), 8.30 (d,1H), 8.0 (s,1H), 7.55 (d,1H), 7.5 (t,1H), 3.0 (m,1H), 0.8 (m,2H), 0.5 (h,2H) ; m/z:289(M+1)

N-cyclobutyl-2-(nicotinamido) thiazole-4-carboxamide (5c)

¹H-NMR (400MHz, DMSO-d₆) δ (ppm) 12.3 (b,1H), 9.1 (s, 1H), 8.75 (d,1H), 8.30 (d,1H), 8.0 (s,1H), 7.5 (t,1H), 6.8 (d,1H), 4.2 (m,1H), 2.0 (m,2H), 1.7 (m,2H), 1.5 (m,2H) ; m/z:303(M+1)

N-cyclopentyl-2-(nicotinamido) thiazole-4-carboxamide (5d)

¹H-NMR (400MHz, DMSO-d₆) δ (ppm) 12.3 (s,1H), 9.1 (s, 1H), 8.7 (d,1H), 8.05 (s,1H), 7.5 (t,1H), 7.15 (d,1H), 4.15 (m,1H), 1.8 (m,4H), 1.68 (d,1H), 1.65 (m,2H), 1.62 (q,2H); m/z:317(M+1)

N-cyclohexyl-2-(nicotinamido) thiazole-4-carboxamide (5e)

¹H-NMR (400MHz, DMSO-d₆) δ (ppm) 12.3 (s,1H), 9.1 (s, 1H), 8.75 (d,1H), 8.30 (d,1H), 8.0 (s,1H), 7.5 (t,1H), 6.5 (d,1H), 3.5 (d,1H), 1.6 (m,2H), 1.4 (m,9H); m/z:331(M+1)

N-((1r, 3r, 5r, 7r)-adamantan-2-yl)-2-(nicotinamido) thiazole-4-carboxamide (5f)

¹H-NMR (400MHz, DMSO-d₆) δ (ppm) 12.3 (s,1H), 9.1 (s, 1H), 8.75 (d,1H), 8.30 (d,1H), 8.05 (s,1H), 7.5 (t,1H), 4.05 (q,1H), 2.1 (m,2H), 1.8 (m,13H), 0.5 (h,2H) ; m/z:383(M+1)

N-(4-(4-methylpiperazine-1-carbonyl) thiazol-2-yl) nicotinamide (5g)

¹H-NMR (400MHz, DMSO-d₆) δ (ppm) 12.2 (s,1H), 9.1 (s, 1H), 8.75 (d,1H), 8.28 (d,1H), 8.0 (s,1H), 7.5 (t,1H), 3.7 (m,2H), 3.2 (m,4H), 2.7 (dd,2H), 2.2 (s, 3H); m/z:332(M+1)

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

¹H-NMR (400MHz, DMSO-d₆) δ (ppm) 12.25 (s, 1H), 9.1 (s, 1H), 8.75 (d, 1H), 8.2 (d, 1H), 8.0 (s,1H), 7.5 (t,1H), 3.7 (m,8H) ; m/z:319(M+1)

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

¹H-NMR (400MHz, DMSO-d₆) δ (ppm) 12.40 (s, 1H), 9.14 (s, 1H), 8.75 (d, 1H), 8.31-8.27 (d, 2H), 7.60-7.58 (s, 1H), 3.34-3.32 (t, 4H), 3.14-3.12 (t, 4H); ¹³C-NMR (128MHz, DMSO-d₆) δ(ppm) 165.2, 161.8, 160.7, 152.2, 149.6, 149.1, 136.5, 133.6, 131.5, 124.5, 117.7, 47, 28.2; m/z:335(M+1)

RESULTS AND DISCUSSION**Chemistry**

This research work successfully synthesized the series of nine diamide and one amide compound from the thiamine and thiazole amine ester for the antibacterial study as per Scheme-1. For the synthesis, this work used EDCI reagent which is easy to work-up, good yield with good purity. The reaction yield, molecular weight, Melting Point and TLC Rf Value are presented in Table-1.

Scheme-1: Preparation of Nicotinamide Thiazole Compounds 5a-5i

From the table result, this work identified that polarity of the derivatives is lower than the starting material and their melting points are somehow higher due to the diamide group and molecular weight of the compounds. Based on the Rf value difference and physical properties, all synthesized derivatives were carried for ¹H-NMR and Mass spectral characterization. The ¹H-NMR results exposed that the pyridine nitrogen neighboring singlet proton identified between 12.4 ppm and 12.2 ppm. Amide protons were identified between 8.7 ppm and 8.3 ppm in all compounds.¹⁶ Similarly triplet and doublet protons of compound 3 vanished in compound 4. This absent confirmed the ester hydrolyzed product (4). Compound

Table-1: Compounds Yield, Melting Point and TLC R_f Value

Comp.ID	R group	Molecular formula	Molecular weight	Yield in %	Melting point (°C)	R _f Value
3	---	C ₁₂ H ₁₁ N ₃ O ₃ S	277	85	252-255	0.60
4	---	C ₁₀ H ₇ N ₃ O ₃ S	249	78	245-248	0.40
5a	C ₆ H ₅	C ₁₆ H ₁₂ N ₄ O ₂ S	324	88	275-278	0.75
5b	Cyclopropyl	C ₁₃ H ₁₂ N ₄ O ₂ S	288	84	255-258	0.80
5c	Cyclobutyl	C ₁₄ H ₁₄ N ₄ O ₂ S	302	82	263-268	0.85
5d	Cyclopentyl	C ₁₅ H ₁₆ N ₄ O ₂ S	316	80	273-278	0.90
5e	Cyclohexyl	C ₁₆ H ₁₈ N ₄ O ₂ S	330	89	295-300	0.95
5f	Adamantyl	C ₂₀ H ₂₂ N ₄ O ₂ S	382	81	350-355	0.95
5g	N-methyl piperazinyl	C ₁₅ H ₁₇ N ₅ O ₂ S	331	86	268-273	0.65
5h	Morphinyl	C ₁₄ H ₁₄ N ₄ O ₃ S	318	89	272-275	0.70
5i	Thiomorphinyl	C ₁₄ H ₁₄ N ₄ O ₂ S ₂	334	88	288-293	0.75

Fig.-1: ^1H -NMR of 5i

Fig.-2: ^{13}C -NMR of 5i

The derived compounds were carried for the inhibition study using the paper disc diffusion method to evaluate the *in-vitro* anti-microbial activity in terms of inhibition zone in millimeter. The anti-microbial activity of the synthesized compounds was screened against the following bacteria and fungi using the reported methods.¹⁷ Ciprofloxacin and Ketoconazole were used as standard drugs for the inhibition assay. Ciprofloxacin (5 mcg/disc) and Ketoconazole (50mcg/disc) were used as standard for anti-bacterial and anti-fungal activities and compared with the 200 ppm. The results have shown in Table-2.

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when compared to the fungus standard. Most of the compounds showed good results when compared to starting materials. The antimicrobial plates of the **5i** have shown in Fig.-3.

Table- 2: Antimicrobial Zone of Inhibition Results for the Derivatives

Compounds	<i>S.aureus</i>	<i>S.epidermidis</i>	<i>E. coli</i>	<i>K.pneumonia</i>	<i>C.albicans</i>
3	24	26	24	15	18
4	25	27	26	16	21
5a	25	28	18	20	24
5b	24	27	20	18	23
5c	25	26	24	16	24
5d	26	25	25	18	25
5e	27	28	28	20	27
5f	28	29	28	18	28
5g	28	29	26	24	27
5h	32	30	30	25	29
5i	34	32	32	27	31
Ciprofloxacin	35	30	32	28	---
Ketaconazole	---	---	---	---	30

Fig.-3: Antimicrobial Test Plates of 5i

CONCLUSION

In this research, synthesized and characterized the Nicotinamide-thiazole derivatives were successfully investigated for their antibacterial and antifungal ability. All the compounds were bio-active and **5i** was found to possess significant activity. Finally, this research concluded that the diamide containing heterocyclic molecules have shown good biological activity results. In addition to the biological work, this work will be extended for their anti-cancer activity on cell lines in future.

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