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DESIGN, SYNTHESIS AND EVALUATION OF BIOLOGICAL ACTIVITY OF AMIDE DERIVATIVES OF GRAMINE

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ABSTRACT
A series of new amide derivatives of 3-(3-((dimethylamino)methyl)-1*H*-indol-1-yl)propanoic acid **2** were synthesized after converting **2** as its acid chloride **3** and reacting **3** with various bioactive amines using 1-methylimidazole as an acid scavenger by Schotten-Baumann reaction. The newly synthesized compounds were characterized by IR, NMR and mass spectral analysis. The title compounds were evaluated for their efficacy as antioxidant and antimicrobial agents *in vitro.* The synthesized amides **4c** and **4f** exhibited promising antioxidant activity and compounds **4c-g** showed high inhibitory activity against both bacteria and fungi.

KEYWORDS: Gramine; 1-methylimidazole; Schotten-Baumann reaction; antioxidant and antimicrobial activities.

1. INTRODUCTION
Free radicals are highly reactive species and have capability to damage cells, proteins, cell membrane and DNA; it leads to development of cancer and other health complications. Antioxidants are molecules, natural or synthetic, have the potential to combat against free radicals and terminate the chain reactions before the damage of essential molecules. These were gained a lot of importance as universal remedy for widespread diseases like diabetes, aging, cardiovascular, cancer and other related diseases are caused by the oxidative stress. Thus, investigating for new molecular hybrids as antioxidants gained importance.

Indole and its derivatives show varied biological activities including antioxidant[1], antimicrobial[2], antiplatelet activity[3], insecticidal and cytotoxic activities.[4] They are also able to protect various tissues against oxidative stress, principally by scavenging venomous reactive oxygen species (ROS^{-}).[5] Recent literature articulates that structure activity relationship studies revealed that indole derivatives exhibited promising antioxidant activity.[6]

Moreover, amide derivatives containing indole nucleus showed potent bioactivity and used as antioxidant agents[7], tubulin inhibitors[8], in neurological disorders[9] and central nervous system depressive activity.[10] In addition, the amide analogues of quinolinone[11], phenylcarbamoylbenzoic acid[12], exhibited potential activities.

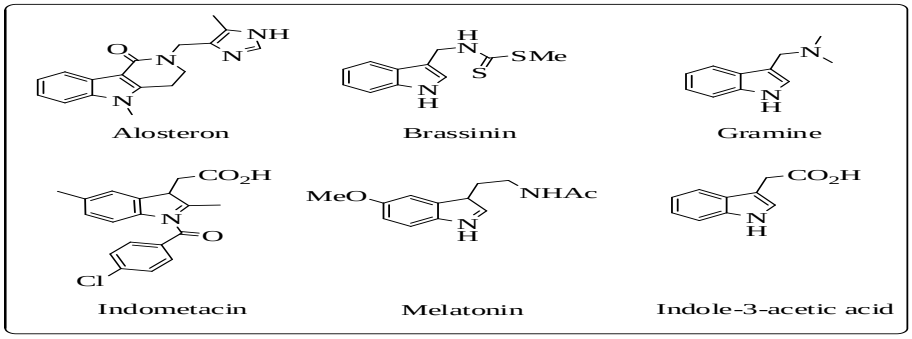

Figure 1: Some of the naturally occurring indole containing compounds.

Gramine is a natural indole containing alkaloid isolated from coal tar and raw plants and displays wide-ranging pharmaceutical activities such as blood pressure elevation, bronchial asthma[13] and anticancer.[14] It is the core moiety in many biologically important indoles containing compounds, including agents playing important roles in living organism.[15,18] As far as our knowledge; the literature survey did not reveal any work on the derivatives of gramine as antioxidants. Recently, Wei Y *et al*. reported the synthesis of its derivatives as antiviral agents.[19] The aim of this research was to design, synthesis and *in vitro* screening of antioxidant along with antibacterial and antifungal activities of amide derivatives of gramine bearing pharmacophoric moieties.

2. MATERIALS AND METHODS

Required chemicals were purchased from Sigma-Aldrich Chemicals. Melting points were determined on Guna digital melting point apparatus using a calibrated thermometer and are uncorrected. The FT-IR spectra were recorded using ALPHA, Bruker. ^{1}H and ^{13}C NMR were recorded on Bruker AMX 400 MHz using DMSO-d_6 as solvent and TMS as an internal standard. Silica gel column chromatography was performed using Merck 7734 silica gel (60-120 mesh) and Merck-made TLC plates. Liquid chromatography (LC) mass spectra were recorded on a Shimadzu LCMS 2010A. CHN analysis was performed with the Thermo Finnigan Flash EA 1112 instrument at University of Hyderabad, Hyderabad, India.

2.1. General procedure for the synthesis of 3-(3-((dimethylamino)methyl)-1H-indol-1-yl)propanoic acid (2)

Sodium hydride (0.002 mole) in THF was added to gramine, (1*H*-indol-3-yl)-N,N-dimethylmethanamine **1** (0.001 mole) in 10 mL of THF, stirred for 1h at 5-30°C. 3-Bromopropanoic acid (0.001 mole) in 10 mL of THF was added drop wise to intermediate anion, stirred for 1h at 10-45°C. It was filtered to get crude compound **2** and evaporated the solvent under vacuum, washed with hexane and ethyl acetate.

2.2. General procedure for the synthesis of 3-(3-((dimethylamino)methyl)-1H-indol-1-yl)propanoyl chloride (3)

To a stirred solution of acid **2** (0.001 mole) in dry THF (10 mL), excess of thionyl chloride (0.0015 mole) was added at 0°C in the presence of Et_3N (0.001 mole) and stirred for 1 h at 30°C to afford 3-(3-((dimethylamino) methyl)-1*H*-indolyl-1-yl)propanoyl chloride **3**. The formed Et_3N.HCl was removed by filtration, the solvent and unreacted thionyl chloride was removed in a rotaevaporator.

2.3. General procedure for the synthesis of amides 4 (a-i)

The acid chloride **3** was reacted with various bioactive amines in the presence of 1-methylimidazole as a base and THF as solvent at 10-30°C. The progress of the reaction was monitored by TLC (n-Hexane: Ethyl acetate 3:1). After completion of the reaction, water was added to the stirred mixture, which was extracted with ethyl acetate. The organic layer was washed with 5% HCl solution and 10% $NaHCO_3$ solution in order to remove unreacted amine and acid respectively. The organic phase was washed with water and brine, dried over Na_2SO_4 and concentrated in a rotaevaporator. The obtained crude product was purified by silica gel column chromatography to afford respective amides. The physical properties and spectral data of the obtained compounds are given below.

The synthetic pathway to the targeted compounds **4** (**a-i**) was sketched in **Scheme 1**. In brief, commercially available gramine (**1**), (1*H*-indol-3-yl)-N,N-dimethylmethanamine was taken as starting material. It was converted into acid (**2**) by the reaction of sodium hydride followed by 3-bromopropanoic acid in THF. Acid chloride was synthesized by the reaction of acid (**2**) with thionyl chloride, which in turn reacted with various bio-active amines in the presence of acid scavenger, 1-methylimidazole[22,24] in THF at 10-30°C.

2.3.1. 3-(3-((dimethylamino)methyl)-1H-indol-1-yl) propanoic acid (2)

White solid, yield 70%, mp: 172-173°C. IR (cm^{-1}): 3412 (COOH), 1686 (C=O). ^{1}H NMR (DMSO-d_6) δ: 2.44 (s, 6H, 2xNCH$_3$), 2.84 (t, J =7.0 Hz, 2H, C.CH$_2$), 3.20 (s, 2H, CH$_2$.N(CH$_3$)$_2$), 4.36 (t, J = 7.0 Hz, 2H, NCH$_2$), 6.35 (s, 1H), 6.88-7.28 (m, 4H, Ar-H), 11.15 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6) δ: 36.8, 41.8 (2C), 54.3, 62.4, 110.8, 114.5, 118.4, 121.6, 124.5, 126.8, 128.6, 140.5, 177.3. MS: 379 [M+H]$^+$. Anal. Calcd for $C_{21}H_{22}N_4OS$: C, 66.64; H, 5.86; N, 14.80. Found C, 66.36; H, 5.54; N, 14.65.

2.3.2. N-(benzo[d]thiazol-2-yl)-3-(3-((dimethylamino)methyl)-1H-indol-1-yl)propanamide (4a)

White solid, yield 72%, mp: 212-213°C. IR (cm^{-1}): 3215 (CONH), 1674 (C=O). ^{1}H NMR (DMSO-d_6) δ: 2.32 (s, 6H, 2xNCH$_3$), 2.72 (t, J =7.0 Hz, 2H, C.CH$_2$), 3.18 (s, 2H, CH$_2$.N(CH$_3$)$_2$), 4.32 (t, J = 7.0 Hz, 2H, NCH$_2$), 6.25 (s, 1H), 6.78-7.18 (m, 4H, Ar-H), 7.85-8.26 (m, 4H, Ar-H), 11.12 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ: 36.4, 42.5 (2C), 54.3, 61.7, 107.8, 110.8, 112.6, 117.7, 119.6, 120.5, 122.5 (2C), 124.3 (2C), 125.6 (2C), 126.4, 129.6, 135.3, 147.5, 172.5, 175.7. MS: 247 [M+H]$^+$.

2.3.3. N-(4-chlorobenzo[d]thiazol-2-yl)-3-(3-((dimethylamino)methyl)-1H-indol-1-yl)propanamide (4b)

White solid, yield 76%, mp: 232-233°C. IR (cm^{-1}): 3211 (CONH), 1671 (C=O). ^{1}H NMR (DMSO-d_6) δ: 2.15 (s, 6H, 2xNCH$_3$), 2.54 (t, J =7.0 Hz, 2H, C.CH$_2$), 3.64 (s, 2H, CH$_2$.N(CH$_3$)$_2$), 4.42 (t, J = 7.0 Hz, 2H, NCH$_2$), 6.38 (s, 1H), 6.94-7.34 (m, 4H, Ar-H), 7.8-8.12 (m, 3H, Ar-H), 10.18 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ: 35.3,

44.3 (2C), 53.4, 58.5, 111.3, 113.4, 117.7, 119.3, 121.3, 122.7, 123.8, 124.5, 125.7, 126.8, 128.4, 130.5, 136.8, 148.3, 169.5, 173.4. MS: 414 $[M+2]^+$. Anal. Calcd for $C_{21}H_{21}ClN_4OS$: C, 61.08; H, 5.13; N, 13.57. Found C, 60.86; H, 4.96; N, 13.24.

2.3.4. 3-(3-((dimethylamino)methyl)-1H-indol-1-yl)-N-(4,6-difluorobenzo[d]thiazol-2-yl) propanamide (4c)

White solid, yield 78%, mp: 241-242 °C. IR (cm^{-1}): 3223 (CONH), 1662 (C=O). ^{1}H NMR (DMSO-d_6) *δ:* 2.24 (s, 6H, 2xNCH$_3$), 2.48 (t, *J* =7.0 Hz, 2H, C.CH$_2$), 3.54 (s, 2H, CH$_2$.N(CH$_3$)$_2$), 4.38 (t, *J* = 7.0 Hz, 2H, NCH$_2$), 6.32 (s, 1H), 6.90-7.24 (m, 4H, Ar-H), 6.82 (s, 1H, Ar-H), 7.46 (s, 1H, Ar-H), 10.26 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) *δ*: 34.5, 46.2 (2C), 52.4, 58.3, 98.7, 104.5, 110.3, 114.2, 118.5, 121.4, 124.5, 126.8, 128.3, 130.4, 132.2, 138.5, 155.7, 159.4, 168.5, 173.2. MS: 415 $[M+H]^+$.

2.3.5. 3-(3-((dimethylamino)methyl)-1H-indol-1-yl)-N-(6-nitrobenzo[d]thiazol-2-yl)propan amide (4d)

Yellow solid yield 80%, mp: 202-203°C. IR (cm^{-1}): 3218 (CONH), 1665 (C=O), 1548 and 1354 (NO$_2$). ^{1}H NMR (DMSO-d_6) *δ:* 2.18 (s, 6H, 2xNCH$_3$), 2.65 (t, *J* =7.0 Hz, 2H, C.CH$_2$), 3.68 (s, 2H, CH$_2$.N(CH$_3$)$_2$), 4.35 (t, *J* = 7.0 Hz, 2H, NCH$_2$), 6.42 (s, 1H), 6.92-7.24 (m, 4H, Ar-H), 8.52 (d, *J* = 8.4 Hz, 2H, Ar-H), 9.14 (s, 1H, Ar-H), 10.25 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) *δ*: 36.4, 42.5 (2C), 54.3, 61.6, 106.4, 108.8, 114.4, 117.2, 119.4, 122.3, 124.5, 127.8, 129.4, 132.7, 13 8.2, 142.5, 153.2, 172.3, 174.5. MS: 424 $[M+H]^+$. Anal. Calcd for $C_{21}H_{21}N_5O_3S$: C, 59.56; H, 5.00; N, 16.54. Found C, 59.15; H, 4.86; N, 16.14.

2.3.6. 3-(3-((dimethylamino)methyl)-1H-indol-1-yl)-1-(4-(4-nitrophenyl)piperazin-1-yl) propan-1-one (4e)

Yellow solid, yield 82%, mp: 186-187°C. IR (cm^{-1}): 1654 (C=O), 1542 and 1348 (NO$_2$). ^{1}H NMR (DMSO-d_6) *δ:* 2.15 (s, 6H, N(CH$_3$)$_2$), 2.75 (t, *J* =6.8 Hz, 2H, C.CH$_2$), 3.18 (t, *J* = 7.2 Hz, 4H, (NCH$_2$)$_2$), 3.56 (t, *J* = 7.2 Hz, 4H, (NCH$_2$)$_2$), 3.78 (s, 2H, CH$_2$.N(CH$_3$)$_2$), 4.48 (t, *J* = 7.0 Hz, 2H, NCH$_2$), 6.25 (s, 1H), 6.50 (d, *J* = 8.6 Hz, 2H, Ar-H), 6.88-7.18 (m, 4H, Ar-H), 8.14 (d, *J* = 8.6 Hz, 2H, Ar-H). ^{13}C NMR (DMSO-d_6) *δ*: 36.2, 43.4, 45.4, 51.7, 56.3, 61.5, 108.4, 110.3, 113.8, 117.7, 119.5, 121.3, 124.7, 127.8, 129.4, 136.4, 139.3, 154.5, 171.3. MS: 436 $(M+H)^+$. Anal. Calcd for $C_{24}H_{29}N_5O_3$: C, 66.19; H, 6.71; N, 16.08. Found C, 65.86; H, 6.42; N, 15.88.

2.3.7. 3-(3-((dimethylamino)methyl)-1H-indol-1-yl)-1-(4-(4-fluorophenyl)piperazin-1-yl) propan-1-one (4f)

White solid, yield 76%, mp: 192-193°C. IR (cm^{-1}): 1663 (C=O). ^{1}H NMR (DMSO-d_6) *δ:* 2.16 (s, 6H, 2x NCH$_3$), 2.66 (t, *J* =6.8 Hz, 2H, C.CH$_2$), 3.24 (t, *J* = 7.2 Hz, 4H, 2x NCH$_2$), 3.52 (t, *J* = 7.2 Hz, 4H, 2xNCH$_2$), 3.68 (s, 2H, CH$_2$.N(CH$_3$)$_2$), 4.54 (t, *J* = 7.0 Hz, 2H, NCH$_2$), 6.34 (s, 1H), 6.44 (d, *J* = 8.6 Hz, 2H, Ar-H), 6.75 (d, *J* = 8.6 Hz, 2H, Ar-H), 6.95-7.26 (m, 4H, Ar-H). ^{13}C NMR (DMSO-d_6) *δ*: 31.3, 43.4 (2C), 45.7, (2C), 47.8 (2C), 52.3, 56.8, 109.8, 112.7, 114.7 (2C), 116.3 (2C), 118.5, 120.7, 124.3, 126.7, 128.6, 137.2, 144.5, 153.2, 172.3. MS: 409 $[M+H]^+$. Anal. Calcd for $C_{24}H_{29}FN_4O$: C, 70.56; H, 7.16; N, 13.71. Found C, 70.28; H, 7.04; N, 13.48.

2.3.8. 3-(3-((dimethylamino)methyl)-1H-indol-1-yl)-1-(4-(pyrimidin-2-yl)piperazin-1-yl)propan-1-one (4g)

White solid, yield 72%, mp: 197-198 °C. IR (cm^{-1}): 1655 (C=O). ^{1}H NMR (DMSO-d_6) *δ:* 2.12 (s, 6H, 2x NCH$_3$), 2.72 (t, *J* =7.0 Hz, 2H, C.CH$_2$), 3.26 (t, *J* = 7.0 Hz, 4H, 2x NCH$_2$), 3.58 (t, *J* = 7.0 Hz, 4H, 2x NCH$_2$), 3.7 (s, 2H, CH$_2$.N(CH$_3$)$_2$), 4.64 (t, *J* = 7.0 Hz, 2H, NCH$_2$), 6.38 (s, 1H), 6.46 (dd, *J* = 3.2, 8.4 Hz, 1H, Ar-H), 8.26 (d, *J* = 8.6 Hz, 2H, Ar-H). ^{13}C NMR (DMSO-d_6) *δ:* 30.7, 42.4 (2C), 45.3 (2C), 48.7 (2C), 51.6, 57.4, 108.5, 110.7, 113.2, 118.3, 121.4, 123.2, 125.8, 128.7, 136.4, 158.5 (2C), 163.7, 172.3. MS: 393 $[M+H]^+$.

2.3.9.N-(2,6-dichloropyrimidin-4-yl)-3-(3-((dimethylamino)methyl)-1H-indol-1-yl)propan amide (4h)

White solid yield 64%, mp: 220-221°C. IR (cm^{-1}): 3217 (CONH), 1664 (C=O). ^{1}H NMR (DMSO-d_6) *δ:* 2.24 (s, 6H, 2xNCH$_3$), 2.75 (t, *J* =7.0 Hz, 2H, C.CH$_2$), 3.78 (s, 2H, CH$_2$.N(CH$_3$)$_2$), 4.46 (t, *J* = 7.0 Hz, 2H, NCH$_2$), 6.25 (s, 1H), 7.14-7.64 (m, 4H, Ar-H), 8.12 (s, 1H, Ar-H), 10.24 (s, 1H, CONH). ^{13}C NMR (DMSO-d_6) *δ:* 32.5, 43.8 (2C), 52.4, 57.8, 106.7, 110.5, 113.6, 116.8, 120.4, 122.7, 125.8, 128.4, 136.7, 152.5, 158.5, 163.2, 172.7. MS: 395 $[M+4]^+$.

2.3.10. 3-(3-((dimethylamino)methyl)-1H-indol-1-yl)-N-(1H-indazol-6-yl)propanamide (4i)

White solid, yield 68%, mp: 227-228 °C. IR (cm^{-1}): 3221 (CONH), 3211 (NH), 1674 (C=O). ^{1}H NMR (DMSO-d_6) *δ:* 2.15 (s, 6H, 2xNCH$_3$), 2.54 (t, *J* =7.0 Hz, 2H, C.CH$_2$), 3.64 (s, 2H, CH$_2$.N(CH$_3$)$_2$), 4.42 (t, *J* = 7.0 Hz, 2H, NCH$_2$), 6.38 (s, 1H), 6.94-7.34 (m, 4H, Ar-H), 7.28-7.56 (m, 4H, Ar-H),7.64 (s, 1H, Ar-H), 7.86 (d, *J* = 8.0 Hz,1H, Ar-H), 7.94 (d, *J* = 8.0 Hz, 1H, Ar-H), 8.34 (s,1H, =CH), 10.34 (s, 1H, CONH), 12.64 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) *δ*: 36.2, 46.3 (2C), 52.4, 56.7, 97.3, 108.5, 112.3, 115.4, 117.5, 118.7, 119.4, 121.3, 123.8, 126.8, 128.4, 132.5, 136.8, 138.4, 142.6, 172.2. MS: 362 $[M+H]^+$.

2.4. *Antioxidant activity*

The radical scavenging activity of the newly synthesized compounds was measured by DPPH method[25] and Super oxide radical scavenging[26,27] at two different concentrations 50 and 100 µg/mL. Scavenging capacity was measured spectrophotometric- ally by monitoring the decrease in absorbance at 517 nm.

DPPH radical scavenging activity

To 5 mL of 0.004% w/v methanol solution of DPPH, 2 mL of different concentrations of the test samples (50 and 100 µg/mL) in methanol were added. The test tubes were incubated at room temperature for 30 minutes. The solution was rapidly mixed and scavenging capacity was measured spectrometrically by monitoring the decrease

in absorbance at 517 nm. Ascorbic acid was used as a standard to compare the activity of the test samples. The tests were carried out in triplicate and mean of the values were summarized in **Table 1**. The % inhibition of free radical production from DPPH was evaluated by using the following formula.

DPPH radical scavenging activity (%) = [(1-a)/b] x 100

a= Absorbance of sample at 517 nm, b= Absorbance of control 517 nm.

Super oxide radical Scavenging activity

Superoxide radicals were determined using spectrophotometric measurement of the effects of different concentrations of test compounds on the reduction of nitrobluetetrazolium (NBT). Super oxide radicals were g enerated in a non-enzymatic phenazinemethosulfate/ nicotinamide adenine dinucleotide (PMS/NADH) system. The non-enzymatic generation of superoxide radicals were measured in reaction mixtures containing various concentrations of test compounds (50 and 100 µg/mL), PMS (15 µM), NADH (73 µM) and NBT (50 µM) in phosphate buffer (20 mM, p^H 7.4). After incubation for 5 minutes at ambient temperature, the color was read at 560 nm against blank samples.

Super oxide radical Scavenging activity (%) = [(1-a)/b] x 100

a=Absorbance of control, b= Absorbance of test samples

Reactive oxygen species (ROS), such as superoxide anion radical (O^{2-}), hydroxy radicals ($OH^{\cdot}$) and peroxy radical ($ROO^{\cdot}$) are produced as a part of normal metabolic processes. The compounds **4c, 4f** and **4e** showed significant antioxidant activity by scavenging the free radicals and super oxide radicals may be due to the presence of high electronegative halogens (F, Cl) and NO_2 group. The antioxidant activity evaluation with the 1,1-diphenyl-2-picryl-hydrazyl (DPPH), radical scavenging assay was carried out and the results are presented in **Table 1.**

2.5. Antimicrobial activity

Bacterial cultures were prepared on Nutrient Agar Medium (NAM) and for fungal test Potato Dextrose Agar (PDA) medium was used. 10 mL of distilled water was used to scrape conidia from 10 days culture and the spores were collected after filtration. The spores were resuspended in sterile distilled water and were used as inoculum. For bacterial culture plates a 100 µL of the cell suspension (10^6 cells/ mL) was used to prepare bacterial lawn. Anti-microbial tests were done by disc diffusion technique.[28,29] Discs were prepared with Whatman No.1 filter paper (6 mm diameter) and was impregnated with 100 µg/ disc of each compound and placed on the inoculated microbial plates. And all the plates were subjected to incubation at 37°C for 24 h for bacterial culture and 72 h for fungal cultures respectively. Chloramphenicol was used as positive control and was placed in the center of all the plates for bacterial cultures and nystatin was used as a positive control for fungal cultures. Anti-microbial activity was evaluated by measuring the Zone of inhibition against the tested organisms and the results are summarized in **Table 2** (antibacterial) and **Table 3** (antifungal). Minimum inhibitory concentration (MIC) values (µg/mL) evaluated using broth micro dilution assay method. The lowest concentration (highest) of test compound that produced no visible signs of microbial growth (no turbidity) when compared with the control tubes were regarded as MICs (**Tables 2** and **3**).

Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organisms and the results are summarized in **Table 2** (antibacterial) and **Table 3** (antifungal). The title compounds were dissolved in DMSO at 100 µg/mL and each test was carried out three times and mean values of inhibition zone diameter are taken. All the synthesized compounds were screened for their *in vitro* antibacterial activity against the following bacterial strains: Gram negative bacteria; *Escherichia coli (E.Coli)* and *Klebsiella pneumoniae (K.pneumoniae)*, Gram positive bacteria; *Bacillus subtilis (B.subtilis)* and *Staphylococcus aureus (S.aureus)*. Moderate to good antibacterial activity was exhibited by the synthesized compounds. Among the synthesized amides **4c-g** showed high antibacterial activity against the growth of *E. coli* and *S. aureus* than that of others when compared to standard Chloramphenicol.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The reaction of different amines with the acid chloride *3-(3-((dimethylamino)methyl)-1H-indol-1-yl) propanoyl chloride* **3** gave moderate yields in the presence of triethylamine as an acid scavenger but with 1-methylimidazole gave high yields[20] (78-86%). All the newly synthesized compounds were characterized by IR, NMR and mass spectral analysis. Amide carbonyl group stretching band is observed in the region 1654-1686cm^{-1}. A band in the region 3211-3221 cm^{-1} corresponds to the amide NH stretching for the compounds **4a-d** and no absorption band was observed in this region for the compounds **4e-h**, because lack of NH bond in that compounds.

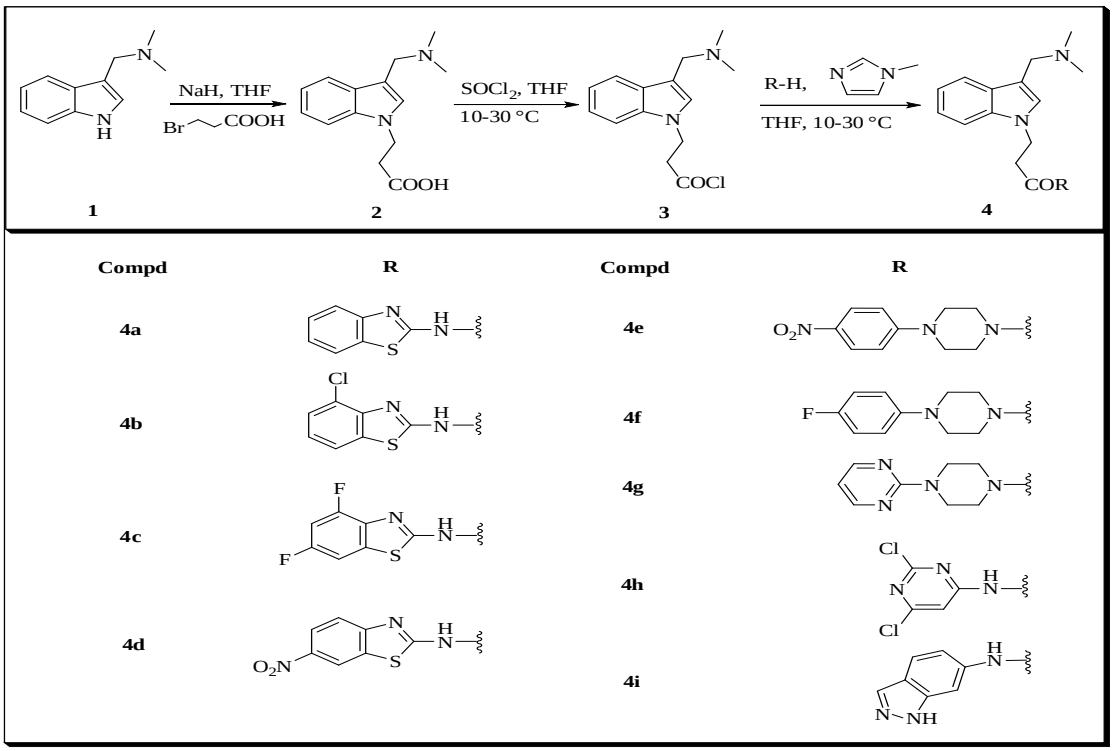

Figure 1: Synthesis of amide derivatives of Gramine.

In ^{1}H NMR, a signal at δ 2.42-4.48 indicates the methylene protons and signals due to amide NH protons were observed in the region δ 10.96-11.25 for the compounds **4a-d.** Signals in the range of δ 6.28-8.92 correspond to aromatic protons. In ^{13}C NMR spectra, signals at δ 34.2-38.5 confirmed the presence of methylene carbons and peaks in the region at δ 169.6-173.7 indicate the carbonyl group of amide. EIMS were recorded for a few representative compounds; they gave $(M+H)^{+}$ ions at their respective molecular masses. CHN analysis was obtained for a few title compounds and the data confirmed their elemental composition.

4. Biological assay

The radical scavenging activity of the title compounds was carried out by DPPH method and Superoxide radical scavenging at two different concentrations 50 and 100 µg/mL. The results of the antioxidant screening (radical scavenging activity) are summarized in **Table 1**. Most of the title compounds exhibited moderate to good antioxidant activity when compared to natural antioxidant ascorbic acid as a standard.

The compounds **4a-d** acted as potent scavengers when compared to the **4e-g**, it may be due to the presence of fused benzothiazole heterocyclic moiety. On the other hand, piperazine linked hybrids showed somewhat less antioxidant activity. Among the benzothiazole series, compounds **4c** and **4d** displayed excellent radical scavenging activity in super oxide method due to the presence of two F atoms and NO_2 group and the scavenging power follows the order **4c**>**4d**>**4b**>**4a** with percentage inhibition of 78.45>72.2>66.4>62.3 (100 µg/mL) respectively. The compound **4i** showed more antioxidant activity than that of **4h**.

Table 1. Antioxidant activity of synthesized compounds 4 (a-i).

Product	DPPH Scavenging		Superoxide Scavenging	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
4a	50.82±1.14	52.45±1.16	56.64±1.22	64.28±1.04
4b	52.54±1.16	58.34±1.18	58.25±1.15	66.42±1.20
4c	56.34±1.12	64.42±1.28	66.24±1.14	78.45±1.16
4d	53.18±1.10	60.14±1.12	62.54±1.26	72.22±1.12
4e	46.42±1.08	56.54±1.04	54.64±1.12	65.32±1.04
4f	48.14±1.22	58.34±1.14	58.42±1.08	68.24±1.18
4g	42.64±1.14	54.25±1.22	52.56±1.13	60.64±1.08
4h	38.42±1.25	46.74±1.05	48.84±1.20	58.84±1.10
4i	42.34±1.12	50.46±1.04	52.65±1.14	62.82±1.18
Std	54.25±1.16	62.46±1.12	64.42±1.10	76.34±1.0

Std: Ascorbic acid.

The antibacterial activity of the compounds **4a-d** against tested bacterial strains is as follows, **4a<4b<4d<4c**. Compounds **4b-d** displayed more antibacterial activity than that of **4a** due to the presence of substitutents in the former ones and lacks in the latter compound. Substitutions like halogens (Cl, F) and nitro groups at 4, 6 positions on the benzene ring in the compounds **4b-d** and *p*-position on the phenyl ring in the case of compounds **4e** and **4f** enhances the zone of inhibition**.** The compound **4c** showed more antibacterial activity with MIC **(**2.35 µg/mL**)** than **4b (**6.15 µg/mL**)** against *Staphylococcus aureus*, due to fluorine substitution[21] which enhances the activity. Similar trend is observed in the case of **4e-h** analogs, **4h<4g<4e<4f.** The zone of inhibition of the compound **4h<4g,** due to the presence of pyrimidine ring in **4g**. MIC values (16.42 and 17.24 µg/mL) were attributed to analog **4i** against *Staphylococcus aureus* and *Escherichia coli*, respectively.

On the other hand, study of the antifungal activity of the synthesized compounds revealed that all the analogs exhibited substantial growth inhibitory activity against *Trichoderma viride (T.viride), Aspergillus niger (A.niger), Aspergillus flavus (A.flavus)* and *Penicillium chrysogenum* (*P.chrysogenum)* by disc diffusion method at 100 µg/mL concentrations.

Table 2. Antibacterial activity of synthesized compounds 4 (a-i).

Product	Gram negative				Gram positive			
	E.coli		*K.pneumoniae*		*B.subtilis*		*S.aureus*	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
4a	22	9.35	16	11.45	22	12.85	22	10.42
4b	23	5.75	18	6.85	32	4.85	24	6.15
4c	28	3.15	24	4.24	32	2.65	28	2.35
4d	27	6.25	20	8.25	28	5.15	26	4.85
4e	26	12.15	22	12.45	26	8.35	28	12.45
4f	25	14.45	23	10.35	24	6.25	26	8.14
4g	21	16.15	15	15.45	25	16.35	20	14.45
4h	18	18.45	14	18.15	18	18.35	16	20.28
4i	20	17.24	16	16.24	21	15.35	18	16.42
Std	30	7.25	26	14.25	34	10.45	32	12.25

IZ: Inhibition zone in (mm), Std: Chloramphenicol.

Table 3. Antifungal activity of synthesized compounds 4 (a-i).

Product	*A. niger*		*T. viride*		*A. flavus*		*P. chrysogenum*	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
4a	14	6.45	22	10.45	16	10.85	14	8.45
4b	18	3.25	25	4.75	22	4.24	16	6.25
4c	25	2.15	30	3.14	20	3.65	18	5.35
4d	20	2.45	25	4.64	18	5.25	15	4.25
4e	16	4.65	24	6.15	15	6.75	16	10.45
4f	22	3.35	28	8.25	22	5.55	14	12.24
4g	16	8.45	22	12.25	15	12.35	13	16.34
4h	12	10.55	20	16.36	12	14.45	10	18.45
4i	15	9.45	23	14.42	14	13.65	13	15.25
Std	28	5.25	32	12.45	26	8.44	20	6.25

Std: Nystatin, IZ: Inhibition zone in (mm).

When compared the antibacterial activity of the compounds, it is clear that the effect of substitution plays major role in inhibition of the growth of the bacterial and fungal strains. Nystatin was used as a standard antifungal agent. The synthesized analogs showed high activity towards *Trichoderma viride* and *Aspergillus flavus*. In particular, compounds **4c** and **4f** (3.14 and 8.25 µg/mL) displayed the highest zone of inhibition when compared to standard antifungal agent against *Trichoderma viride*.

CONCLUSION

In summary, it was designed, synthesized the amide derivatives of gramine through simple procedure in high yield using 1-methylimidazole as a base instead of triethyl amine as an acid scavenger. Among all the synthesized analogs, compound **4c** showed augmented radical scavenging activity, it may be due to the presence of benzothiazole moiety with fluorine substitution. Further, *in vitro* antimicrobial activities of the compounds containing benzothiazole ring residues showed better zone of inhibition when compared to others, particularly antibacterial and antifungal activity of **4c** high good. Based on these findings, derivatives of gramine paved the way for the synthesis of potential antioxidants and antimicrobials and lead scaffolds for the pharmaceutical industry.

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