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Research Article

BIOLOGICAL STUDIES OF 4-AMINO BENZAMIDE DERIVED 1,2,3-TRIAZOLE LINKED CHALCONE AND ITS PYRAZOLINE DERIVATIVES

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ABSTRACT

Objectives: The present work planned to investigate the anti-bacterial, anti-fungal, and anti-oxidant activity against 4-amino benzamide derived 1,2,3-triazole linked chalcone and pyrazoline derivatives.

Methods: Anti-microbial activity for pyrazoline derivatives was accomplished by serial dilution method. The test organisms of bacterial strains were (*Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas fluorescence*) and the fungal cultures (*Aspergillus niger*, *Aspergillus terreus,* and *Trichoderma harzanium*). The method used in the anti-oxidant activity is 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide scavenging method.

Results: All the compounds showed good to moderate anti-bacterial and anti-fungal activities. The results of IC_{50} values showed lower potent inhibition activity with DPPH and higher potent inhibition activity in nitric oxide scavenging method.

Conclusion: All the compounds had exhibited capricious growth inhibitory effect on anti-bacterial, anti-fungal, and anti-oxidant activities.

Keywords: 1,2,3-triazole, Chalcone, Pyrazoline, Anti-microbial activity and anti-oxidant activity.

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INTRODUCTION

Triazole is an important unit in the molecular structure of numerous naturally occurring products as well as synthetic molecules [1]. Due to the structural features of this triazole, they found application in optically active materials as well as in the medicinal field such as anticancer agents [2]. The wide application of 1,2,3-triazole plays an important role in the field of biochemistry and production of materials in pharmacological studies [3-6]. The stable reduction and oxidation properties, metabolic reactions, dipole interactions, and hydrogen bonding supported and led to excellent pharmacological activities [7,8]. Chalcones are the primary component of innate product and also significant synthetic maneuvering ancestor [9]. This is due to the presence of carbonyl functions with double bond in chalcones [10]. The anti-microbial activities of 1,2,3-triazole linked compounds have been reported by more researches [11-15]. We have already reported a series of novel 4-amino benzamide derived 1,2,3-triazole linked pyrazoline derivatives have been synthesized by 1,3-dipolar cycloaddition reaction through their chalcone precursor and characterized with analytical methods such as FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectral analysis [1].

MATERIALS AND METHODS

Anti-microbial assay

The newly synthesized compounds were screened for anti-bacterial properties by the serial dilution method [16] against Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative bacteria (*Klebsiella pneumoniae* and *Pseudomonas fluorescence*) and also evaluated for fungicidal activity against (*Aspergillus niger, Aspergillus terreus,* and *Trichoderma harzanium*) in DMSO solvent and acting as a negative control. All the inoculated test tubes of synthesized compounds and the standard drugs Streptomycin and Ketoconazole incubated for 24 h at 37° C. Then, the test compounds were screened with the expectant anti-bacterial and anti-fungal activity was selected for minimum inhibitory concentration (MIC) studies which are calculated using the equation 1.

$$\text{Minimum Inhibitory Concentration} = \frac{\text{Volume of solvent(ml)} \times \text{Concentration}(\mu g / ml)}{\text{Potency of powder}(\mu g / mg)} \quad (1)$$

Anti-oxidant assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The electron donation ability or hydrogen atom of some compounds were dignified from bleaching of purple colored methanol solution of DPPH [17]. The spectrophotometric assay practices the stable radical DPPH as a reagent. 1 mL of various concentrations of the test compounds (5, 10, 25, 50, and 100 µg/mL) in methanol was added to 2 mL of 0.004% (w/v) Methanoic solution of DPPH. The reaction mixture was incubated at 37°C. The scavenging activity on DPPH was determined by measuring the absorbance at 517 nm at 30 min. All tests were done in triplicate and the mean values were calculated using the equation 2.

$$\% \text{ of scavenging} = [(A_{control} - A_{sample})/(A_{sample} \times 100)]. \quad (2)$$

Nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction [18]. Sodium nitroprusside (5 mM) in standard phosphate buffer saline solution (0.025 M, pH 7.4) was incubated with different concentrations (5, 10, 25, 50, and 100 µg/mL) of the test compounds dissolved in phosphate buffer saline (0.025 M, pH 7.4) and the tubes were incubated at 25°C for 5 h. Control experiments were showed in the identical manner using the equivalent amount of buffer solution. After 5 h, 0.5 mL of the sample was diluted with

0.5 mL of Griess' reagent (1% sulfanilamide, 2 % o-phosphoric acid, and 0.1% napthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and its subsequent coupling with napthyl ethylene diamine was read at 546 nm. The experiments were repeated in triplicate and the mean values were calculated using the equation 3.

$$\text{Nitric oxide scavenged}(\%) = \frac{A_{control} - A_{test}}{A_{control}} \times 100 \quad (3)$$

The chemical entity which was used in this research work is shown in Figs. 1 and 2.

RESULTS

The synthesized 4-amino benzamide derived chalcone and its pyrazoline derivatives were evaluated for *in vitro* anti-bacterial, anti-fungal, and anti-oxidant activities. The efficacy of antibiotic molecule was explained based on inhibition of microbial growth.

DISCUSSION

The anti-bacterial activity of 4-amino benzamide derived 1,2,3-triazole linked chalcone and its pyrazoline derivatives were examined against *E. faecalis*, *S. aureus*, *Klebsiella pneumonia,* and *P. fluorescence* by standard serial dilution method. Streptomycin was used as standard a drug for anti-bacterial analysis. MIC in µg/mL of all the compounds is depicted in Table 1. The anti-bacterial activity of compound 3.1 and 4.1 exhibited MIC 6.0 µg/ml against *E. faecalis*. Compound 3.2, 3.3, 4.2, and 4.3 also showed low and moderate anti-bacterial activity with 6.5 MIC against *E. faecalis*. Six compounds (3.1–3.3, 4.1–4.3) show the lowest anti-bacterial activity against *S. aureus* and *Klebsiella pneumonia* with (MIC) 6.0 µg/ml. The antibacterial activity of compound 3.3, 4.2 and 4.3 exhibited MIC 6.0 µg/ml against *P. fluorescence*. Compound 3.1, 3.2, and 4.1 also showed lowest MIC 6.5 µg/ml against *P. fluorescence*.

The anti-fungal activity of 4-aminobenzamide derived 1,2,3-triazole linked chalcone and its pyrazoline derivatives was examined against *A. niger*, *A. terreus,* and *T. harzanium* by serial dilution method and it is shown in Table 2. Ketoconazole was used as a standard drug for anti-fungal analysis. The effect of anti-fungal activity of compounds (3.3, 4.2, and 4.3) showed moderate anti-fungal activity against with MIC 5.0 µg/mL and the remaining compounds exit low anti-fungal properties. The compound 3.2 and 4.1 showed good to moderate anti-bacterial and anti-fungal activities compared with other compounds.

Anti-oxidants play a significant role in preventing aging and also oxidative stress-related diseases. The synthesized compounds have shown promising anti-oxidant activity and have potential to develop as lead compounds. Thus, it is necessary to estimate the synthesized compounds for their anti-oxidant activity. Anti-oxidant activities of the synthesized compounds 3.1–3.3 and 4.1–4.3 were measured using DPPH radical scavenging assay and nitric oxide scavenging assay. The lowest IC_{50} value indicates the highest anti-oxidant activity. The IC_{50} (concentration required to scavenge 50% of the radicals) were calculated to evaluate the potential anti-oxidant activities.

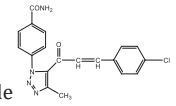
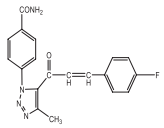
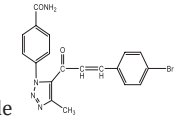
Compound Code	4-Amino benzamide derived chalcone series
3.1	4-(5-(3-(4-chlorophenyl) acryloyl)-4-methyl-1*H*-1,2,3-triazol-1-yl) benzamide 
3.2	4-(5-(3-(4-fluorophenyl) acryloyl)-4-methyl-1*H*-1,2,3-triazol-1-yl) benzamide 
3.3	4-(5-(3-(4-bromophenyl) acryloyl)-4-methyl-1*H*-1,2,3-triazol-1-yl) benzamide 

Fig. 1: Chalcone series

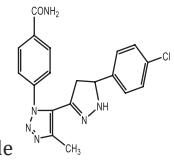
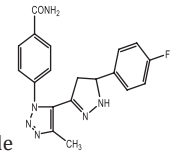
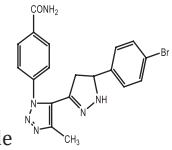
Compound Code	4-Amino benzamide derived chalcone and its pyrazoline derivative series
4.1	4-(5-(5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-4-methyl-1*H*-1,2,3-triazol-1-yl) benzamide 
4.2	4-(5-(5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-4-methyl-1*H*-1,2,3-triazol-1-yl) benzamide 
4.3	4-(5-(5-(4-bromophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-4-methyl-1*H*-1,2,3-triazol-1-yl) benzamide 

Fig. 2: Pyrazoline series

Table 1: Minimum inhibitory concentrations (µg/ml) against the bacterial strains

Microorganisms	Chemical Compounds						Streptomycin
	3.1	3.2	3.3	4.1	4.2	4.3	
Enterococcus faecalis	6.0	6.5	6.5	6.0	6.5	6.5	3.5
Staphylococcus aureus	6.0	6.0	6.0	6.0	6.0	6.0	3.5
Klebsiella pneumonia	6.0	6.0	6.0	6.0	6.0	6.0	3.5
Pseudomonas fluorescence	6.5	6.5	6.0	6.5	6.0	6.0	3.5

Table 2: Minimum inhibitory concentration (µg/ml) against the fungal strains

Microorganisms	Chemical Compounds						Ketoconazole
	3.1	3.2	3.3	4.1	4.2	4.3	
Aspergillus niger	5.5	5.5	5.0	5.5	5.0	5.0	2.5
Aspergillus terreus	5.5	5.0	5.5	5.0	5.5	5.5	2.5
Trichoderma harzanium	5.0	5.0	5.0	5.0	5.0	5.0	2.0

Table 3: Anti-oxidant activity

Chemical Compounds	DPPH radical scavenging activity	Nitric Oxide scavenging activity
3.1	20.69±21.54	1.88±2.13
3.2	44.72±45.79	2.00±2.32
3.3	28.84±29.23	1.61±1.85
4.1	27.64±27.88	2.58±2.79
4.2	24.16±24.74	2.13±2.31
4.3	31.39±32.63	1.69±1.90
Ascorbic acid	75.32±76.57	5.20±5.47

The anti-oxidant properties of 4-amino benzamide derived 1,2,3-triazole linked chalcone and its pyrazoline derivatives are expressed in terms of IC_{50} using DPPH method which is shown in Table 3. Six compounds were tested for free radical scavenging activity using DPPH. Compound 3.1 showed that IC_{50} at 20.54±20.91 µM compared to that of the standard ascorbic acid with IC_{50} of 75.32±76.57 µM. The anti-oxidant studies of compounds (3.1, 3.2, 3.3, 4.1, 4.2, and 4.3) using DPHH showed IC_{50} at 20.54±20.91 µM, 44.71±45.87 µM, 28.84±29.23 µM, 27.64±27.88 µM, 24.16±24.74 µM, and 31.39±32.63 µM, respectively, when compared to that of the ascorbic acid standard with 75.32±76.57 µM. Fifty percentages of inhibition in *in vitro* chemical reaction of compounds are adept to exhibit anti-oxidant properties.

The anti-oxidant studies of 4-amino benzamide derived 1,2,3-triazole linked chalcone and pyrazoline derivatives using nitric oxide scavenging method, the values of IC_{50} are also summarized in Table 3. The ascorbic acid was used as a standard drug and having IC_{50} at 5.20±5.47 µM. The anti-oxidant studies of compounds (3.1, 3.2, 3.3, 4.1, 4.2, and 4.3) using nitric oxide assay showed IC_{50} at 1.88±2.13 µM, 2.00±2.32 µM, 1.61±1.85 µM, 2.58±2.79 µM, 2.13±2.31 µM, and 1.69±1.90 µM, respectively, when compared to that of the ascorbic acid standard at 5.20±5.47 µM. The 4-aminobenzamide derived 1,2,3-triazole linked chalcone and its pyrazoline derivatives ranged from 1.6 to 2.7 indicates strong nitric acid scavenging activity of all compounds. Reducing power assay indicated in DPPH with lower IC_{50} values, meanwhile, highest values observed in nitric oxide scavenging method. The attachment point of bromo groups in main ring (3.3 and 4.3) showed significant variations in anti-oxidant properties. All the substituted 4-aminobenzamide derived 1,2,3-triazole linked pyrazoline derivatives were enhance the antioxidant properties compared to 4-aminobenzamide derived 1,2,3-triazole linked chalcone.

CONCLUSION

All the compounds had exhibited capricious growth inhibitory effect on anti-bacterial, anti-fungal and anti-oxidant activities. 4-aminobenzamide derived 1,2,3-triazole linked chalcone and pyrazoline derivatives shows moderate antimicrobial activities against fungal and bacterial strains. The anti-oxidant activities of these compounds could lead us further research; to study the reaction mechanism and structure activity relationship of the compounds.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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