Synthesis, characterization and biological evaluation of tryptamine based benzamide derivatives

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Abstract: Benzamides and tryptamine are biologically significant compounds, therefore, various benzamide analogous of tryptamine have been efficiently synthesized using tryptamine and different benzoyl chlorides, in order to find new biologically active compounds. The resulting products were then characterized by melting point determination, calculation of R_f values and LC-MS techniques. At last, structure activity relationship (SAR) of the synthesized compounds was evaluated against two microbial strains; *Bacillus subtilis and Aspergillus niger*. All the five prepared products have shown high yield, sharp characterization and significant resistance against the growth of tested microorganism, providing a new range of tryptamine based benzamide derivatives having significant antimicrobial activities.

Keywords: Benzamide, tryptamine, analogous, LC-MS, antimicrobial.

INTRODUCTION

The amides are simple ammonia derivatives in which one atom of hydrogen is replaced by an acyl group, as shown in fig. 1. Hudson (1988) reported different biologically active molecules containing amides as an important part. He showed that amide bond is not only common in natural compounds like peptides and proteins but it has been used in lot of synthetic transformations.

Drews (2000), Boyd (1988), Mrozik *et al*. (1996), and Katrizky *et al*. (2004) reported various biological activities of a number of amide derivatives, such as antihistamine, anticancer, antibacterial, antifungal, antibacterial, diuretic, hypoglycemic and antithyroid.

Tryptamine is a natural monoamine alkaloid present in fungi, as shown by the structure in fig. 2. Jones (1982), Greenberg (1960), Dijk (1997), and Berger *et al*. (2009) obtained chemical variants of tryptamine by synthetic modification of a functional group or a side-chain.

Kazuta and coworkers presented a significant work in medicinal and synthetic organic chemistry by the synthesis of different analogues of tyramine, histamine, serotonine, dopamine, tryptamine and melatonin. These compounds have vital function as chemical messengers in different biological processes.

Anderson (2005), Dobry (1952), Movassaghi and Hill (2006) determined the biological significance of benzamide derivatives. They utilized them in the chemical industry as significant intermediates for many types of synthetic reactions.

Similarly, by considering a variety of substances with different pharmacological activities from the generic family of benzamides, Costall (1987), and Rangappa *et al*. (2006) synthesized a new class of benzamide derivatives, bearing different bioactive moieties and evaluated their efficacy as antimicrobials *in vitro*.

Due to the vast biological significance of amides, benzamides and tryptamine, the present study was designed to synthesize the benzamide analogous of tryptamine, in order to find new biologically active compounds.

MATERIALS AND METHODS

Chemicals

Sodium hydroxide and magnesium sulphate were obtained from Merck. Dichloromethane and benzoyl chloride were obtained from BDH whereas tryptamine was obtained from Alpha acer. All other chemicals and solvents were of the highest available commercial grade and used as they were received without further purification. Nutrient Agar was obtained from Fluka Industriestrasse.

Bacterial strains

The microorganisms (*Bacillus subtilis and Aspergillus niger*) used for the study of antimicrobial activity were obtained from NIBGE, Faisalabad.

Experimental

General scheme for synthesis of substituted benzamides

Different substituted benzamides will be prepared using amine and different substituted benzoyl halides by the following reaction scheme:

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General method for the preparation of benzamides

An aqueous solution of sodium hydroxide 4M (1.2mmol) was added in tryptamine (1.2mmol) that was dissolved in organic solvent dichloromethane (3ml) and stirred for 5 minutes. Then the benzoyl chloride (1.2mmol) was added drop wise. The mixture was stirred for 5 minutes at 0°C and then for 3 hours at room temperature. After that the reaction was monitored by TLC to confirm the formation of the product then added H_2O (20mL) to reaction mixture for the separation of the product. The product was separated from reaction mixture by adding 20ml of distilled water. Using separating funnel aqueous layer was separated from the organic layer. The organic layer was dried with $MgSO_4$, filtered and evaporated.

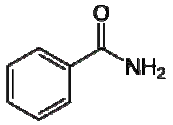


Fig. 1: Structure of benzamides

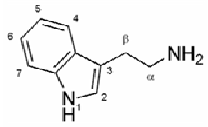


Fig. 2: Structure of tryptamine

Characterization of synthesized compounds

The synthesized products were characterized by TLC (R_f values), melting point and LC-MS techniques.

Thin layer chromatography

Commercially available TLC cards were utilized for the monitoring of reactions, which were run in 50:50 ethyl acetate/petroleum ether solvents mixture and UV lamp was used for spot locating. At the end R_f values were calculated.

Melting point

The melting points (uncorrected) of the synthesized compounds were taken on the Stuart SMP3, melting point apparatus.

LC-MS spectra

The evaluations of the LC-MS were performed on LC-MS apparatus by the following method: The sample was dissolved in dichloromethane and directly injected into MS (mass spectrometer) from where it was carried into the pump. In the ionization chamber, ESI (electron spray ionization) was performed for the ionization of the sample molecules. Then, analyzer separated the different molecular fragments on the basis of their m/z values and at the end instrument generated the mass spectrum of the compounds under study.

Biological characterization of synthesized compounds

Antimicrobial activities

For antimicrobial activities of the synthesized compounds, agar well plate method was used.

Organisms tested

- *Bacillus subtilis*
- *Aspergillus niger*

Agar well plate method for antimicrobial activity

Antimicrobial activity was determined by agar well plate technique. The test bacteria (*Bacillus subtilis)* were grown on nutrient agar while the test fungi (*Aspergillus niger)* were grown on Vogal's medium plate. The 0.01g of samples was dissolved in 200µl of methanol and 50µl of the solution was placed in the agar well. Growth inhibition was measured after incubation for 24 hours at 37ºC for bacteria and for 72 hours at room temperature for fungi. Antimicrobial activity was estimated by measuring the inhibition zone diameters.

RESULTS

In the present work, various novel tryptamine-benzamide derivatives were synthesized, characterized and their biological activities were investigated.

Experimental data

(N-(2-(1H-indol-3-yl)ethyl)benzamide) (I)

Color and Appearance; Off white and crystalline, R_f value; 0.7708, % Yield; 91.4% (290mg), M.P; 95-98ºC, IR values; C=O (1650cm^{-1}), N-H of indole ring (1550cm^{-1}), N-H of amide (3250cm^{-1}) and CH_2 (1450cm^{-1}), LC-MS values; $[M+H]^+$ = 265, $[M+Na]^+$ 287, $[M+K]^+$ =303, $[M-H]^-$ =263, $[C_{10}H_{10}N]$ =144.17, $[C_7H_6ON]$ =121.17. Structure of the respective compound I is shown in fig. 3.

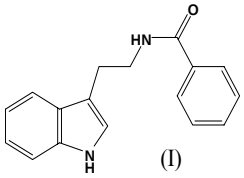


Fig. 3: Structure of (*N*-(2-(1H-indol-3-yl) ethyl) benzamide) (I)

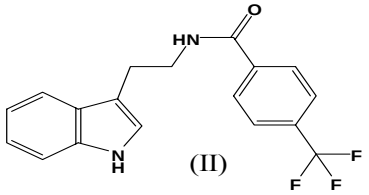


Fig. 4: Structure of *N*-(2-(1H-indol-3-yl) ethyl)-4-(trifluoromethyl) benzamide (II)

N-(2-(1H-indol-3-yl)ethyl)-4-(trifluoromethyl)benzamide (II)

Color and Appearance; white and crystalline, R_f value; 0.8958, % Yield; 88.8% (276mg), M.P; 170-175ºC, IR values; C=O (1650cm^{-1}), N-H of indole ring (1550cm^{-1}), N-H of amide (3250cm^{-1}), *p*-substituted Ar-H (850cm^{-1}) and C-F (1300cm^{-1}), LC-MS values; $[M+H]^+$ =333,

$[M+Na]^+$ =355, $[M+K]^+$ =371 and $[M-H]^-$ =331. Structure of the respective compound II is shown in fig. 4.

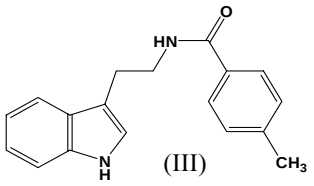


Fig. 5: Structure of *N*-(2-(1H-indol-3-yl) ethyl)-4-methylbenzamide (III)

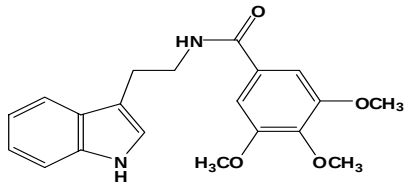


Fig. 6: Structure of *N*-(2-(1H-indol-3-yl) ethyl)-3, 4, 5-trimethoxybenzamide (IV)

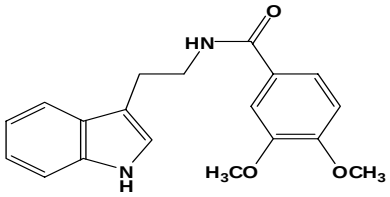


Fig. 7: Structure of *N*-(2-(1H-indol-3-yl) ethyl)-3, 4-dimethoxybenzamide (V)

Table 1: Results of inhibition zones of synthesized compounds against bacterial strain (*Bacillus subtilis*)

Compounds	Zones of inhibition against (*Bacillus subtilis)*
I	10.55±0.05
II	11.1±0.07
III	10.17±0.14
IV	10.62±0.12
V	12.07±0.08

Zones of inhibition were measured in millimeters
Samples were taken in mg/mL

N-(2-(1H-indol-3-yl)ethyl)-4-methylbenzamide (III)

Color and Appearance; white and crystalline, R_f value; 0.7446, % Yield; 80.9% (270mg), M.P; 131-134ºC, IR values; C=O (1600cm^{-1}), N-H of indole ring (1520cm^{-1}), N-H of amide (3300cm^{-1}), *p*-substituted Ar-H (800cm^{-1}) and CH_3 (2950cm^{-1}), LC-MS values; $[M+H]^+$=279, $[M+Na]^+$ =301, $[M+K]^+$ =317, $[M-H]^-$ =277, $[C_{10}H_{10}N]$ = 144.17, $[C_7H_7]$ =89.17. Structure of the respective compound III is shown in fig. 5.

N-(2-(1H-indol-3-yl)ethyl)-3,4,5-trimethoxybenzamide (IV)

Color and Appearance; yellowish white and crystalline, R_f value; 0.4468, % Yield; 73.9% (314mg), M.P; 204-207ºC, IR values; C=O (1600cm^{-1}), N-H of indole ring (1520cm^{-1}), N-H of amide (3350cm^{-1}), substituted Ar-H (970cm^{-1}), Ar-H (1250cm^{-1}), C-O (970 cm^{-1}) and -CH_3 (1470cm^{-1}), LC-MS values; $[M+H]^+$ =355, $[M+Na]^+$ =377, $[M+K]^+$ = 393, $[M-H]^-$ =353, $[C_{10}H_{11}O]$ =197.17, $[C_{10}H_{10}N]$ = 144.17 and $[C_{10}H_{12}O_4N]$ =211.17. Structure of the respective compound IV is shown in fig. 6.

Table 2: Results of minimum inhibitory concentration (MIC) of synthesized compounds against bacterial strain (*Bacillus subtilis*)

Compounds	Minimum Inhibitory Concentration (MIC) against (*Bacillus subtilis)*
I	0.0023±0.0007
II	0.0046±0.0015
III	0.0011±0.0003
IV	0.0023±0.0007
V	0.0011±0.0003

Amounts of samples were taken in mg/mL
MIC was taken on Eliza Reader Gen 5 at the wavelength of 500nm.

Table 3: Results of inhibition zones of synthesized compounds against fungal strain (*Aspergillus niger*)

Compounds	Zones of inhibition against (*Aspergillus niger)*
I	10.02±0.14
II	10.27±0.12
III	11.27±0.08
IV	12.2±0.1
V	10.55±0.08

Zones of inhibition were measured in millimeters
Samples were taken in mg/mL

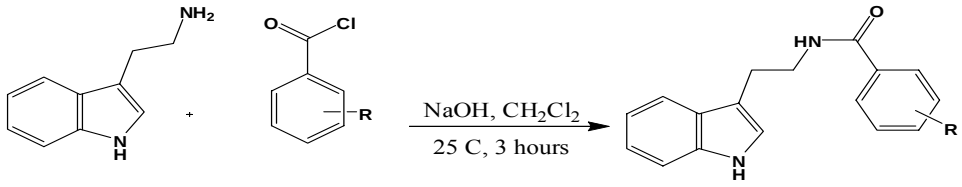
Table 4: Results of minimum inhibitory concentration (MIC) of synthesized compounds against fungal strain (*Aspergillus niger*)

Compounds	Minimum Inhibitory Concentration (MIC) against (*Aspergillus niger)*
I	0.0046±0.0015
II	0.0046±0.0015
III	0.0023±0.0007
IV	0.0023±0.0007
V	0.0046±0.0015

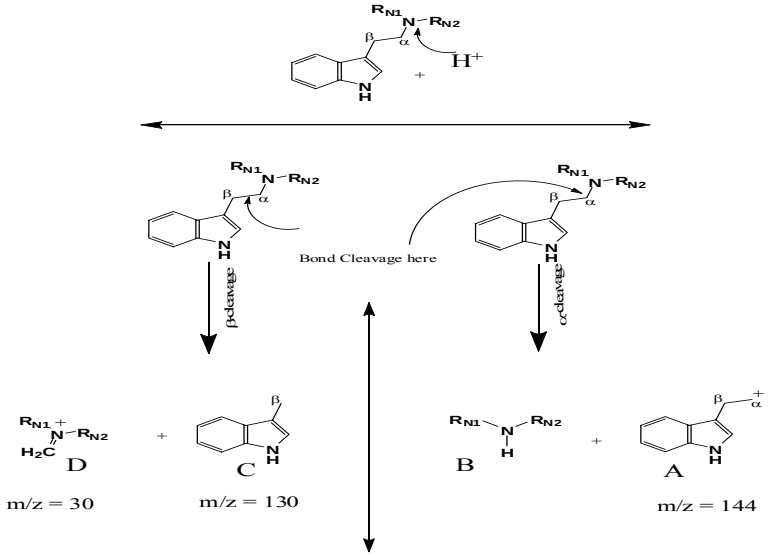
Amounts of samples were taken in mg/mL
MIC was taken on Eliza Reader Gen 5 at the wavelength of 500nm

N-(2-(1H-indol-3-yl) ethyl)-3, 4-dimethoxybenzamide (V)

Color and Appearance; yellowish brown and crystalline, R_f value; 0.6818, % Yield; 92.8% (361mg), M.P; 168-171ºC, IR values; C=O (1600cm^{-1}), N-H of indole ring (1500cm^{-1}), N-H of amide (3350cm^{-1}), tri-substituted Ar-



Scheme 1: Synthesis of substituted benzamides



Scheme 2: Mass fragmentation pattern of tryptamine

H (1000cm^{-1}), Ar-O (1250 cm^{-1}), C-O (970cm^{-1}) and -CH_3 (1470cm^{-1}), LC-MS values; $[M+H]^+$ =325, $[M+Na]^+$ =347, $[M+K]^+$ =363, $[M-H]^-$ =323, $[C_{10}H_{10}N]$ = 144.08 and $[C_9H_{10}O_4N]$ =181.17. Structure of the respective compound V is shown in fig. 7.

Mass fragmentation pattern of tryptamine

General schematic outline for the α-cleavage and β-cleavage of tryptamine is shown in the scheme 2.

The above scheme shows that, if tryptamine acquires a proton that will form a $[M+H]^+$ ion. The α-cleavage would progress at the C_α -N bond and as a result a cation, indole containing group (A) and a small neutral amine (B) would be formed. However, the proton itself could also be donated to the C_β atom (the β carbon), leading to C_α-C_β bond breakage i.e. β-cleavage. As a result, an iminium ion (D) and a neutral indole-containing fragment (C) would be formed. Only three conspicuous peaks would be produced; at m/z =189, 144 and 58, corresponding to $[M+H]^+$ ion and fragments from α- and β-cleavage respectively. The ratio of the peaks at m/z =144 and 58 could be changed, depending on the conditions used such as voltage (positive and negative), temperature for ESI (electrospray ionization).

Antimicrobial activity of synthesized compounds

Antibacterial activity

The antibacterial results of the synthesized compounds indicated that all these compounds have inhibited bacterial activity to some extent. Compound (V) had shown maximum inhibition against the *Bacillus subtilis* strain. Other synthesized compounds had also prevented the growth of bacteria to the lesser extent. The results are presented in table 1 and 2.

Antifungal activity

The fungal strains used for determining antifungal activity were of *Aspergillus niger*. All the synthesized compounds from I to V showed prevention of growth of tested micro organisms. Compounds (III) and (IV) had shown better inhibition than other synthesized compounds against *Aspergillus niger*. The growth inhibitions of other synthesized compounds against this fungal strain are nearly similar to one another. The results are presented in tables 3 and 4.

DISCUSSION

The search for new antimicrobial agents is a result of the increasing number of multiresistant pathological microorganisms. (Khedkar *et al*., 2007). In the current

work, substituted benzamides were synthesized and their antimicrobial activity was evaluated. Among all the five synthesised compounds, compound (V) had showed maximum inhibition against microbes which may be attributed to the presence of two methoxy groups on the benzene ring of benzoyl chloride moiety in the compound structure. This is in accordance with the work done by Nuta *et al*., (2013) who presented the synthesis of new benzanilides, derived from 2,6-dimethylaniline. They reported that *in vitro* antimicrobial activity depends on the substituents in the benzoyl ring and the presence of fluoro- and trifluoromethyl substituents is correlated with an optimal antimicrobial activity. Whereas, compounds (III) and (V) have shown better inhibition than other synthesized compounds leading to a conclusion that different groups on the benzene ring of benzoyl chloride moiety in all synthesized compounds had imparted characteristics significance to the antimicrobial activities of the synthesized compounds. Similarly, Rai and Singh (2011) have reported the Synthesis and antibacterial activity of benzamides and sulfonamide derived from2-amino- 5-bromo/nitropyridine against bacterial strains isolated from clinical patients and concluded that molecules with a combination of benzamide and sulfonamide linkages and halogen substituents are supposed to be highly potent antibacterial agents. Oren *et al*. (2004) ave also reported the synthesis and microbiological activity of some substituted N-(2−hydroxy-4-nitrophenyl) benzamides and phenylacetamides as possible metabolites of antimicrobial active benzoxazoles and both amides and their cyclic analogues showed comparable activities.

CONCLUSION

The development of new and different antimicrobial drugs is an important objective and much of research program efforts are directed towards the design of new agents. In this work, different substituted benzamides have been synthesized and characterized through melting point, R_f values and LC-MS. At last their biological evaluation is done through antimicrobial (antifungal and antibacterial) activities. The products obtained have shown high yield, good characterization and a trend that these compounds can resist the growth of tested microorganism up to a certain limit. The biological activities of the synthesized compounds have suggested the substituted benzamides are useful addition in the future design of antimicrobial drugs.

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