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Synthesis, Spectral Characterization, Antibacterial, Antifungal and Anticancer Evaluation of *N*-[4-(1,3-Benzothiazol-2-ylcarbamoyl)phenyl]pyrazine-2-carboxamide

Gopal Senthilkumar[1,✉], Chinnusamy Umarani[1] and Dhurairaj Satheesh[2]

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ABSTRACT

A new organic compound, *N*-[4-(1,3-benzothiazol-2-ylcarbamoyl)-phenyl]pyrazine-2-carboxamide was synthesized through the reaction between 4-amino-*N*-(benzo[*d*]thiazol-2-yl)benzamide and pyrazine-2-carboxylic acid. The synthesized compound has been characterized by spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR, FT-IR and mass spectroscopy. The synthesized compound was screened to antibacterial (*Staphylococcus aureus, Klebsiella pneumonia* and *Escherichia coli*), antifungal (*Candida albicans* and *Aspergillus niger*) activities. The anticancer activity of the title compound was also evaluated against MDA-MB-231 breast cancer cells.

KEYWORDS

Benzothiazole, Quinoline, Carboxamide, Antibacterial activity, Antifungal activity, Anticancer activity.

INTRODUCTION

The allylic alcohols, cyclic sulfides, thioketones and physiologically active compounds such as β-hydroxysulfides are used as an attractive precursors in the medicinally important compounds [1-5]. Upon the oxidation of β-hydroxysulfones, the product formed are useful as the reagents in the synthesis of lactones [6], 2,5-disubstituted tetrahydrofuranes [7] and vinyl sulfones [8]. In the synthesis of organic compounds containing phenylsulfonyl fragment are also widely used in organic synthesis [9]. Various 5- and 6-substituted 1,3-dioxepanes are relatively scarce chemotype in medicinal chemistry due to synthetic difficulties and stability issues under physiological conditions. By showing more interest in the development of antibacterial agents with slow and gradual growth towards their bacterial resistance by a variety of agents and a multi-drug resistance bacterial species [10]. By means antimicrobial activity against Gram-positive (*S. aureus* and *S. pyogenes*), Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and strains of fungi (*C. albicans*, *A. niger* and *A. clavatus*) a study was evaluated on synthesis of a series of 1-(3-(1*H*-benzoimidazol-2-yl)-5-aryl-4,5-dihydro-1*H*-pyrazol-1-yl)-2-napthalene-1-loxy)ethanones (**5a-l**) [11]. For a medicinal chemists, they have to design by synthesizing and characterization of novel antimicrobial agents

Author affiliations:

[1]Department of Chemistry, Government Arts College (Autonomous), Salem-7, India
[2]PG and Research Department of Chemistry, L.N. Government College (Autonomous), Ponneri-601204, India

✉To whom correspondence to be addressed:

E-mail: 72000senthil@gmail.com

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which has lesser toxicity and should show more potent effect in time consumption. Compound such as furamizole, nasapadil, tazobactam, antimicrobial activity [12] and cefatrizine. Benzimidazoles have various types of pharmacological effects, which includes antiprolifretive [13] and anthelmatics [14]. By the condensation of *o*-phenylenediamine with acids, nitriles, *o*-ester and imidates gives the derivatives of synthesized benzimidazole compounds. A numerous illustrations of benzimidazole and its derivatives have shown their importance in the development of antimicrobial agent [15]. In this present investigation, a new organic compound *N*-[4-(1,3-benzothiazol-2-yl-carbamoyl)-phenyl]pyrazine-2-carboxamide has been synthesized and characterized by spectral studies and also evaluated the antimicrobial and anticancer activities.

EXPERIMENTAL

All the starting materials, reagents and solvent used in the work were commercially procured from Sigma-Aldrich, S.D. Fine Chemicals, Merck and Spectrochem. The solvents and reagents were of AR grade and used without further purification.

Characterization: Infrared measurements were recorded using KBr discs (4000-400 cm^{-1}) on Perkin-Elmer. The ^{1}H & ^{13}C NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ solvents on Bruker 300 MHz NMR using tetramethylsilane as an internal standard. The mass spectra were recorded on a Waters-Synapt G2 by electro spray ionization (ESI) technique with a flow rate of 0.5 mL/min on C-18 column and total run time of 45 min. The sample used for recording the mass spectrum was prepared by dissolving 0.4 mg of compound in 10 mL of methanol:acetonitrile (7:3).

Synthesis of *N*-[4-(1,3-benzothiazol-2-ylcarbamoyl)-phenyl]pyrazine-2-carboxamide: The compound was obtained by the reaction between 4-amino-*N*-(benzo[*d*]thiazol-2-yl)benzamide and pyrazine-2-carboxylic acid as described previously (**Scheme-I**). TLC technique was used to follow the chemical reaction.

Biological evaluation

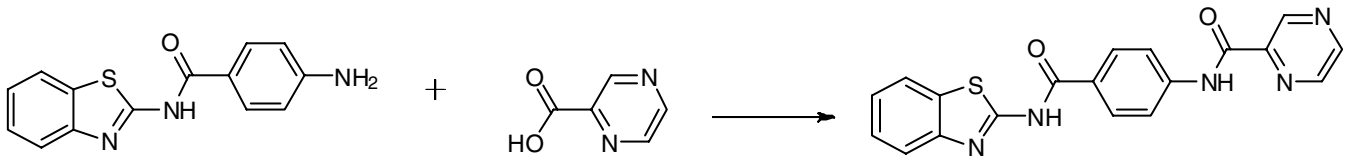
Antibacterial and antifungal screening: Three bacterial strains *Staphylococcus aureus, Klebsiella pneumonia* and *Escherichia coli* while two fungal strains *Candida albicans* and *Aspergillus niger* were selected in this study. All the bacterial cultures and fungal strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

Screening and preparation of inoculums: The young bacterial broth cultures were prepared before the screening procedure. Stock cultures were maintained at 4 ºC on slopes of nutrient agar. Active cultures of the experiment were prepared by transferring a loopful of cells from the stock cultures to test-tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 h at 37 ºC and 25 ºC, respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0×10^6 colony forming units (CFU/mL) for bacteria.

Antimicrobial susceptibility testing: The disc diffusion method [16] was used to screen the antimicrobial activity. *in vitro* Antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 mL of molten media into sterile petriplates. The plates were allowed to solidify for 5 min and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 min. The concentration of extracts is 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 min and the plates were kept for incubation at 37 ºC for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Determination of antifungal activity: The agar well diffusion method [17] was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (methanol, ethyl acetate and hexane). Standard antibiotic (fucanazole, concentration 1 mg/mL) was used as positive control and fungal plates were incubated at 37 ºC for 72 h. The diameters of zone of inhibition observed in mm were measured.

Anticancer screening: MDA-MB-231 breast cancer cells were plated on a 96-well plate at a seeding density of 1×10^5 cells per well in a growth medium consisting of DMEM with 10% FBS and incubated at 37 ºC for 24 h for the cells to attach and spread. Then the spent medium was removed and the cells were washed with PBS. Various concentrations of the samples (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg/mL) in serum-free medium were added to the cells and incubated for 24 h at 37 ºC. MTT solution (50 µL) and serum-free media (50 µL) were added to the wells and incubated at 37 ºC for 3 h to allow MTT to form Formosan crystals by reacting with metabolically active cells. The supernatants were then aspirated and were replaced with 300 µL DMSO in each well and the plates were incubated for 15 min at room temperature with continuous shaking. The optical density was measured using an ELISA plate reader at 570 nm.



Scheme-I: Synthesis of *N*-[4-(1,3-benzothiazol-2-ylcarbamoyl)phenyl]pyrazine-2-carboxamide

RESULTS AND DISCUSSION

The synthesized organic compound *N*-[4-(1,3-benzothiazol-2-ylcarbamoyl)phenyl]pyrazine-2-carboxamide was characterized by FT-IR, ^{1}H & ^{13}C NMR and mass spectroscopies.

The key bands of the synthesized compound are given in Table-1. A vibrational frequency at 3328 cm^{-1} is attributed to the stretching vibration of the amide -NH group. The aromatic CH stretching vibration at 3051 cm^{-1} and the C=O stretching vibration at 1679 cm^{-1} are also observed. The frequency at 1537 cm^{-1} is due to the amide NH bending vibration, while 1450 cm^{-1} is assigned to the C≡N stretching vibration. The vibrational frequency observed at 1017 cm^{-1} and 697 cm^{-1} are attributed to the CH bending vibrations in-plane and out of plane, respectively. The CS stretching vibration is also observed at 747 cm^{-1} (Fig. 1).

TABLE-1
FT-IR SPECTRUM PEAK OF *N*-[4-(1,3-BENZOTHIAZOL-2-YLCARBAMOYL)PHENYL]PYRAZINE-2-CARBOXAMIDE

Wavenumber (cm^{-1})	Assignment(s)
3328	NH stretch, amide
3051	CH stretch, aromatic
1679	C=O stretch, amide
1537	NH bend, amide
1450	C–N stretch, amide
1017	CH bend, in-plane
747	C–S stretch
697	CH bend, out-of-plane

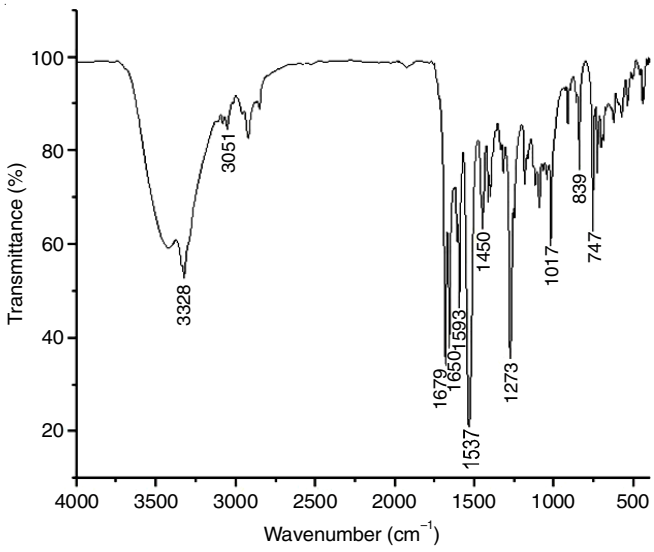


Fig. 1. FT-IR spectrum of compound *N*-[4-(1,3-benzothiazol-2-ylcarbamoyl)phenyl]pyrazine-2-carboxamide

^{1}H & ^{13}C NMR spectral characterization: The ^{1}H & ^{13}C NMR spectra of the synthesized compound were recorded in DMSO-d_6 and shown in Figs. 2 and 3, respectively. ^{1}H NMR (300 MHz, DMSO-d_6, δ in ppm): 7.34 (t, 1H, *J* = 7.2 Hz), 7.47 (td, 1H, *J* = 7.6, 1.2 Hz), 7.79 (d, 1H, *J* = 8.1 Hz), 8.02 (d, 1H, *J* = 7.8 Hz), 8.19 (d, 2H, *J* = 8.7 Hz), 8.12 (d, 2H, *J* = 8.7 Hz), 8.85-8.86 (m, 1H), 8.97 (d, 1H, *J* = 2.4 Hz), 9.34 (d, 1H, *J* = 1.2 Hz), 11.11 (s, 1H), 12.84 (s, 1H). ^{13}C NMR (300 MHz, DMSO-d_6, δ in ppm): 120.3, 120.7, 122.1, 124.0, 126.6, 127.5, 129.7, 131.9, 142.7, 143.7, 144.7, 145.2, 148.4, 162.7.

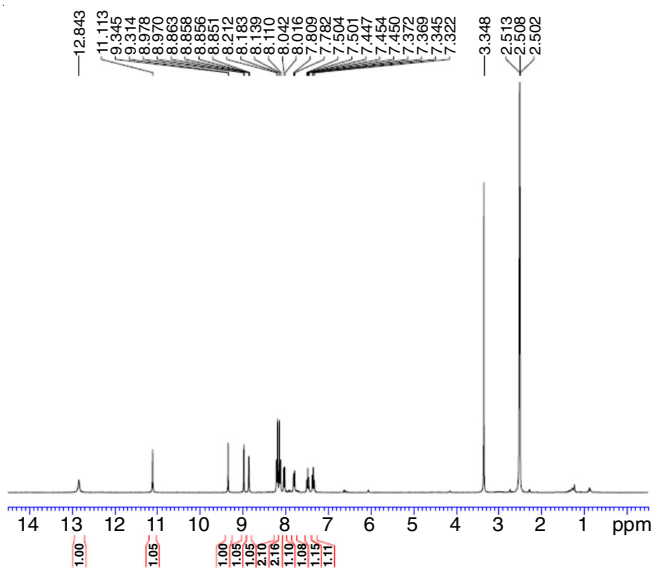


Fig. 2. ^{1}H NMR spectrum of compound *N*-[4-(1,3-benzothiazol-2-ylcarbamoyl)phenyl]pyrazine-2-carboxamide

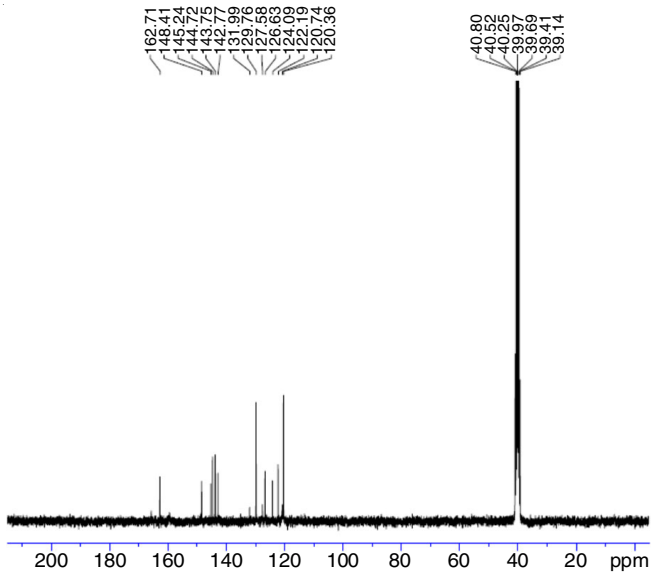


Fig. 3. ^{13}C NMR spectrum of compound *N*-[4-(1,3-benzothiazol-2-ylcarbamoyl)phenyl] pyrazine-2-carboxamide

In the ^{1}H NMR spectrum of the synthesized compound, the two different amide protons are shown at δ 12.84 and 11.11 ppm, which are assigned to benzo[*d*]thiazol and pyrazine moieties, respectively (Fig. 2). A singlet peak is shown at 9.34 ppm, the doublet peak at 8.97 ppm and multiplet peak at 8.85-8.86 ppm are due to aromatic protons of the pyrazine moiety. The aromatic protons of the phenylene ring and benzo[*d*]-thiazol moiety are shown in the range between δ 8.12-7.34 ppm.

In the ^{13}C NMR spectrum of the synthesized compound, the chemical shift of two different amide carbonyl carbon (C=O) are shown at δ 162.7 ppm, which is attributed to the benzo[*d*]-thiazol and pyrazine moieties (Fig. 3). The chemical shift value is obtained at 148.4 and 145.2 ppm are assigned to aromatic C=N and C-N group of the pyrazine moiety, respectively (Fig. 3). The peaks in the range between δ 144.7 ppm to δ 120.3 ppm are assigned to aromatic carbons of the benzo[*d*]thiazol, phenylene and pyrazine moieties, respectively.

LC-MS studies: In the mass spectrum, *N*-[4-(1,3-benzothiazol-2-ylcarbamoyl)phenyl]pyrazine-2-carboxamide showed a molecular ion peak at *m/z* = 373.9 (M-H), corresponding to the molecular formula $C_{19}H_{13}N_5O_2S$ (Fig. 4).

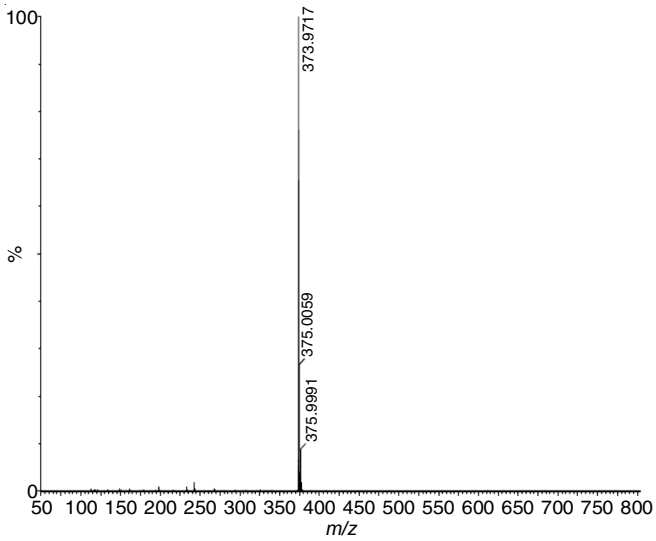


Fig. 4. Mass spectrum of compound *N*-[4-(1,3-benzothiazol-2-ylcarbamoyl)phenyl]pyrazine-2-carboxamide

Biological evaluation

Antibacterial evaluation: The synthesized compound has been screened for its antibacterial activity against three bacterial strains (*Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli*) in four different concentrations (30, 40, 50 and 60 µL) and zone of inhibition is summarized in Table-2. Chloramphenicol (30 mcg) is used as a standard drug for this present investigation. The synthesized compound inhibits 12 mm in the concentration of 30 µL, 14 mm in 40 µL, 17 mm in 50 µL and 19 mm in 60 µL against *Staphylococcus aureus* (Fig. 5). The standard drug is controlled at 21 mm in 30 mcg against of it. The zone of inhibition at 12, 13, 16 and 18 mm are shown in the concentration of 30, 40, 50 and 60 µL against *Klebsiella pneumonia*, respectively (Fig. 5). The standard drug is controlled at 22 mm against of it. Against *Escherichia coli*, the compound shown 13, 15, 17 and 18 mm antibacterial activity at the concentration of 30, 40, 50 and 60 µL, respectively. The standard drug is controlled at 21 mm against of it (Fig. 5).

Antifungal evaluation: The synthesized title compound has been screened for its antifungal activity against two fungal strains (*Candida albicans* and *Aspergillus niger*) in four different concentrations (30, 40, 50 and 60 µL) and zone of inhibition is summarized in Table-2. Clotrimazole (10 mcg) is used as a standard drug in this present investigation. The synthesized compound inhibits 8 mm in the concentration of 30 µL, 10 mm in 40 µL, 12 mm in 50 µL and 14 mm in 60 µL against *Aspergillus niger* (Fig. 6). The standard drug is controlled at 18 mm in 10 mcg against of it. The zone of inhibition at 9, 12, 14 and 15 mm are shown in the concentration of 30, 40, 50 and 60 µL against *Candida albicans*, respectively. The standard drug is controlled at 18 mm against of it (Fig. 6).

Anticancer evaluation: The title compound was screened for its anticancer activity against MDA-MB-231 breast cancer cells in the ten different concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg/mL) and its IC_{50} value is shown in Fig. 7. It is also observed that the anticancer activity of the synthesized compounds increases with increasing concentration (Table-3).

TABLE-2
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF THE TITLE COMPOUND

Strains	Species	Zone of inhibition (mm)				
		Control	Concentration (µL)			
			30	40	50	60
Bacterial strains	*S. aureus*	21	12	14	17	19
	K. Pneumonia	22	12	13	16	18
	E. coli	21	13	15	17	18
Fungal strains	*A. niger*	18	8	10	12	14
	C. albicans	18	9	12	14	15

Control: Bacteria = Chloramphenicol (30 mcg); Fungal = Clotrimazole (10 mcg)

Fig. 5. Zone of inhibition of the title compound against bacterial strains

TABLE-3
ANTICANCER ACTIVITY OF THE TITLE COMPOUND

Abs	Concentration (µg/mL)										
	0	1	2	3	4	5	6	7	8	9	10
B1	0.802	0.737	0.606	0.589	0.458	0.508	0.421	0.393	0.379	0.375	0.405
B2	1.307	0.693	0.547	0.483	0.448	0.474	0.431	0.409	0.354	0.385	0.330
	0.406	–	–	–	–	–	–	–	–	–	–
	0.428	–	–	–	–	–	–	–	–	–	–
B	0.736	0.715	0.577	0.536	0.453	0.491	0.426	0.401	0.367	0.380	0.368

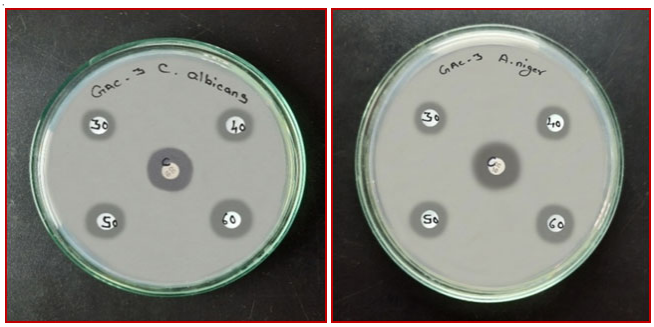


Fig. 6. Zone of inhibition of the title compound against fungal strains

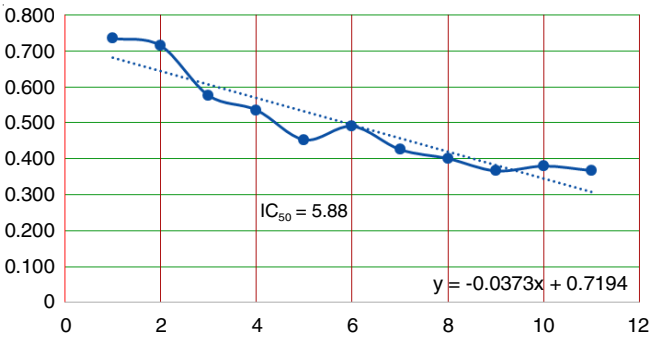


Fig. 7. Anticancer activity of the title compound

Conclusion

In conclusion, a new organic compound, *N*-[4-(1,3-benzothiazol-2-ylcarbamoyl)phenyl]pyrazine-2-carboxamide was successfully synthesized through the reaction between 4-amino-*N*-(benzo[*d*]thiazol-2-yl)benzamide and pyrazine-2-carboxylic acid. The synthesized compound has been characterized by spectroscopic techniques (^{1}H NMR, ^{13}C NMR, FT-IR and mass spectroscopy). The title compound showed an excellent antibacterial activity against all the studied three microorganisms (*Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli*) and also shown good antifungal activity against *Aspergillus niger* and an excellent activity against *Candida albicans*. Moreover, the synthesized compound have also shown an acceptable anticancer activity.

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