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Synthesis, Characterization and Biological Activity of Various 3-(Substituted-Benzyl)-5-(5-Bromo-7-Methoxy-Benzofuran-2-Yl)-3h-[1, 3, 4]Oxadiazole-2-Thiones

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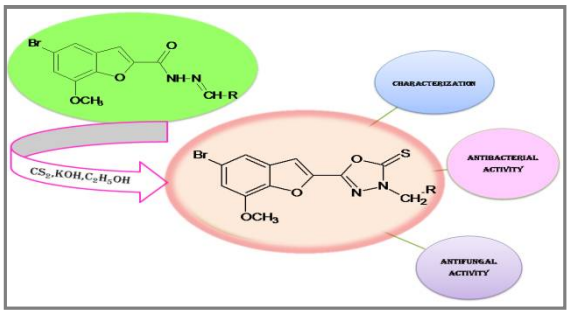
ABSTRACT

*The investigations in the development of biological activity of substituted benzofuran heterocycle and their Schiff's bases, continuation of search on biologically active benzofurans were reported the synthesis of benzofuran linked Oxadiazole-2-Thiones. The starting compound (**1**) was prepared by condensing 5-Bromo-7-methoxy-benzofuran-2-carboxylic acid hydrazide with various aldehydes. An attempt has been made to synthesis of various 3-(Substituted-Benzyl)-5-(5-Bromo-7-Methoxy-Benzofuran-2-Yl)-3h-[1,3,4]Oxadiazole-2-Thiones (**2**) by treating with carbon disulphide in presence of potassium hydroxide solution and ethanol. All the synthesized compounds were in agreement with the assigned structure which was supported by spectral and analytical data. All the titled compounds synthesized were screened for antibacterial and antifungal activity and some have exhibited appreciable activity.*

Graphical Abstract



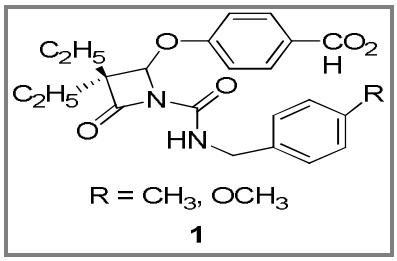
Keywords: Benzofuran, Oxadiazole, Thione, Antibacterial, Antifungal.

INTRODUCTION

The antibacterial and antifungal activity of the compound ethyl 5-bromo-7-methoxy-1-benzofuran-2-carboxylate and the compound 5-bromo-7-methoxy-1-benzofuran-2-carbohydrazide have shown

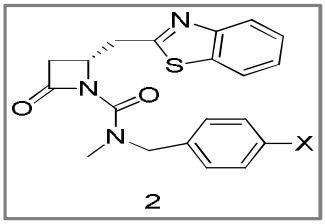
excellent activity against the various organisms, which are compared with the standard drug. And also various Schiff bases have shown considerable antibacterial and anti fungal activity [1].

The synthesis and SAR study of a series of N1-activated-4-carboxy azetidinones have showed that the conformational constrained guanidine epimers displayed potent inhibition of tryptase (IC_{50}<1.7 nM) with excellent selectivity against other serine proteases including trypsin [2]. Qian *et al.*, have carried out the highly stereo selective synthesis of the novel tryptase inhibitor of (2S,3R)-1-(4-(tert-butylcarbamoyl)piperazine-1-carbonyl)-3-(3-guanidinopropyl)-4-oxoazetidine-2-carboxylic acid [3]. Key to this synthesis was the discovery and development of a highly diastereo selective demethoxycarbonylation of diester to form the trans-azetidinone. A series of piperidine-containing N1-activated C4-carboxy azetidinones exhibited as tryptase inhibitors and the most active compound of the series with (4-benzyloxy phenyl) acetaldehyde substitution at the piperidine ring and this is highly selective for thrombin, factor Xa, and trypsin [4].The guanidine moiety at the ring C-3 position was replaced with primary or secondary amine or aminopyridine functionality has shown potent azetidinone tryptase inhibitors. In particular, the compound (2S,3R)-4-oxo-1-(4-(6-phenylhexanoyl) piperazine-1-carbonyl)-3-(2-(piperidin-3-yl)ethyl)azetidine-2-carboxylic acid was a highly potent tryptase inhibitor (IC_{50} ¼ 1.8 nM) [5]. The activity of human leukocyte elastase (HLE) by a monocyclic β-lactam has given potent and stable inhibitors of HLE. The compound **1**with a methyl or methoxy group in the para position shown excellent results [6].



1

Finke *et al.* have carried out the stereospecific synthesis of the series 4-(((2S,3R)-3-ethyl-3-methyl-4-oxo-1-((1-phenylpropyl)carbamoyl)azetidin-2-yl)oxy)benzoic acidand evaluated their *in vitro* inhibitory potency for human leukocyte elastase (HLE), and their *in vivo* oral efficiency in an HLE-mediated hamster lung haemorrhage assay [7]. Cvetovich *et.al* reported the convergent synthesis of azetidinones derivatives and evaluated human leukocyte elastase inhibitor activity [8]. Many monocyclic β-lactams have shown potent and selective inhibitors of the human cytomegalovirus protease (HCMV) with an IC_{50} of 0.07 mM. The synthesis of novel inhibitors of HCMV protease incorporating a carbon side chain at C-4 and a urea function at N-1[9] and Potent activity was exhibited by benzyl substitution of the urea moiety at the para position. Borthwick *et al* have developed mechanism based inhibitors of HCMV protease based on the monocylic β-lactam template that are stable to hydrolysis, have low mM activity against the viral enzyme and have selectivity over acetylcholine esterase, mammalian serine proteases elastase and chymotrypsin [10]. The compound **2** was shown to inhibit the HCMV protease activity 80-fold inside cells by using a cell transfection assay, indicating that antiviral activity in the plaque reduction assay could be attributed to protease inhibition [11].



2

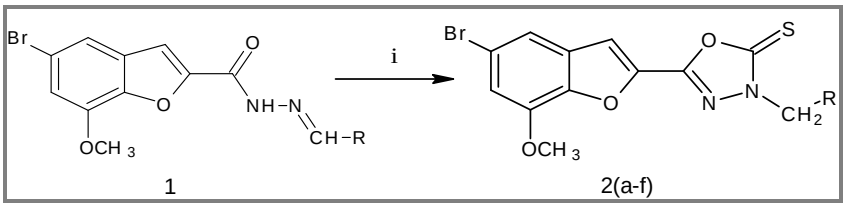
In the presence of an acylenzyme intermediate suggesting that β-lactams were hydrolyzed by the enzyme, cause inhibition by competing with substrate [12]. The compound 3-(3-chloro-2-oxo-4-

substitutedaryl-azetidin-1-yl)-2-(5-pyridin-4-yl-[1,3,4]-oxadiazol-2-yl-sulfanylmethyl)-substituted-3H quinazolin-4-ones derivatives have shown excellent anti-inflammatory activity [**13**]. The compound 3-chloro-2-oxo-4-[(substituted phenyl) azetidine-1-yl] thiourea and derivatives are evaluated for their antiparkinsonian activity, result were shown that the phenyl and 2-chlorophenyl substitution at 4-position of azetidinone decrease rigidity by 80% while L-dopa decrease rigidity upto only 20% at the same dose. Substituted phthalocyanine has shown excellent results against antibacterial and anti fungal activities of various organisms [**14**].

MATERIALS AND METHODS

All the chemicals used were of analytical grade. Melting point was determined in open capillary tubes and was not corrected. IR spectra were recorded on Perkin Elmer Spectrum Two spectrophotometer instrument recorded from 4000-450cm^{-1} by KBr pallet technique. H^1 NMR were recorded on Brucker 400MHz Spectrometer in DMSO and $CDCl_3$. Chemical shift were recorded in parts per million.

3-(substituted-benzyl)-5-(5-bromo-7-methoxy-benzofuran-2-yl)-3H-[1,3,4]oxadiazole-2-thiones (2a-f): 5-Bromo-7-methoxy-benzofuran-2-carboxylic acid hydrazide (substituted benzylidine) hydrazide **1**(0.01 mol) were taken in ethanol (20 mL) (Scheme 1). To this solution potassium hydroxide (0.05 g, 0.008 mol) and carbon disulphide (1mL, 0.013 mol) were added. The reaction mixture was refluxed on a steam bath for 10hr. the solution was allowed to cool overnight and then dissolved in 150 mL ice cold water. The resulting solution acidified with dil.HCl and allowed to stand for 12 h. The solid thus obtained was filtered, air dried and recrystallized from the suitable solvent (Table 1).



Experimental Condition: i. CS_2, KOH, C2H5OH, **R: a**=C_6H_5,**b**= $C_6H_5NO_2$ (p),**c**=C_6H_5Cl (O),**d**= C_6H_5Cl (m),**e**= C_6H_5OH (O), **f**= C_6H_5OH (P)

Scheme 1. Synthesis of 3-(Substituted-Benzyl)-5-(5-Bromo-7-Methoxy-Benzofuran-2-Yl)-3h-[1, 3, 4] Oxadiazole-2-Thiones.

Table 1. Analytical data of the compounds 2(a-f)

Compounds	Substituent	M P°C	Yield %	Solvent	Molecular Formula
2a	C_6H_5	190	85	Ethanol	$C_{18}H_{13}BrN_2O_3S$
2b	$C_6H_5NO_2$(p)	205	77	Ethanol	$C_{18}H_{12}BrN_3O_5S$
2c	C_6H_5Cl (O)	170	75	Ethanol	$C_{18}H_{12}BrClN_2O_3S$
2d	C_6H_5Cl (m)	189	73	Ethanol	$C_{18}H_{12}BrClN_2O_3S$
2e	C_6H_5OH(O)	186	70	Aq.Ethanol	$C_{18}H_{13}BrN_2O_4S$
2f	C_6H_5OH (P)	225	81	Ethanol	$C_{18}H_{13}BrN_2O_4S$

RESULTS AND DISCUSSION

2a: IR (KBr) *v* cm^{-}1: 1581 (C=N), 1300 (C-N),1062 (C=S),1H-NMR(400 MHz, DMSO-d6): δ 3.83 (s, 3H), 6.74 (s, 2H), 7.01 (q, J = -2.40 Hz, 2H), 8.19 (q, J = 0.00 Hz, 2H).

2b: IR (KBr) *v* cm^{-}1:1583(C=N), 1306 (C-N),1062 (C=S), 1H-NMR(400 MHz, DMSO-d6): δ 3.79 (s, 3H), 6.76 (s, 2H), 7.01 (q, J = -1.60 Hz, 2H), 8.18 (q, J = 8.00 Hz, 2H).

2c: IR (KBr) *v* cm-1: 1583 (C=N), 1305 (C-N),1060 (C=S), 1H-NMR(400 MHz, DMSO-d6): δ 3.73 (s,3H), 6.66 (s, 2H), 7.02 (q, J = -0.80 Hz, 2H), 8.20 (q, J = 0.40 Hz, 2H).

2d: IR (KBr) *v* cm-1: 1550 (C=N), 1309 (C-N),1066 (C=S), 1H-NMR(400 MHz, DMSO-d6): δ 3.73 (s,3H), 6.66 (s, 2H), 7.02 (q, J = -0.80 Hz, 2H), 8.20 (q, J = 0.40 Hz, 2H).

2e: IR (KBr) *v* cm-1: 1588 (C=N), 1305 (C-N),1061 (C=S), 1H-NMR(400 MHz, DMSO-d6): δ 3.73 (s,3H), 6.66 (s, 2H), 7.02 (q, J = -0.80 Hz, 2H), 8.20 (q, J = 0.40 Hz, 2H).

2f: IR (KBr) *v* cm-1: 1582 (C=N), 1304 (C-N),1060(C=S),1H-NMR(400 MHz, DMSO-d6): δ 3.73 (s,3H), 6.66 (s,2H), 7.02 (q,J = -0.80Hz,2H),8.20 (q, J = 0.40 Hz, 2H).

Biological assay

Antibacterial Activity, Cup-Plate Method: This method depends on the diffusion of an antibiotic from a cavity through the solidified agar layer in a petridish to an extent such that growth of the added microorganism is prevented entirely in a circular area or zone around the cavity containing a solution of standard. The standard antibiotic used was Azithromycin.

A previously liquefied medium was inoculated appropriate to the assay with the requisite quantity of the suspension of the microorganisms (*Staphylococcus aureus*, *Staphylococcus albus* and *Klebsiella pneumoniae*) between 40-50°C and the incubated medium was poured into Petri dishes to give a depth of 3 to 4 mm. ensuring that the layers of medium were uniform in thickness by placing the dishes on a leveled surface [**15**].

The petridishes thus prepared were stored in a manner so as to ensure that no significant growth or death of the test organism occurs before the dishes were used and the surface of the agar layer was dry at the time of use. With the help of a sterile cork borer, three cups of each 6 mm diameter were punched and scooped out of the set agar in each petridish (three cups were numbered for the particular compounds, solvent and a standard) using sterile pipettes, the standard (10 µg well^{-1}) and the sample of known concentration (10 mg mL^{-1}) were fed into the bored cups. The dishes were left standing for 2 h. at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time among the application of different solutions. These were then incubated for 24 h at 37°C. The zone of inhibition developed, if any, was then accurately measured and recorded. Each zone of inhibition recorded was average of three measurements. Zone of inhibition for dimethylsuphoxide was done separately.

The antimicrobial activities of different extracts and fractions were compared with standard antibacterial agent Azithromycin. The zone of inhibition was calculated by measuring the minimum dimensions of the zone of no bacteria. The results are incorporated in Table 2.

Table 2. Antibacterial activity of the compounds 2(a-f)

Synthetic Compounds	Diameter of Zone of inhibition (mm)		
	Staphylococcus aureus	*Staphylococcus albus*	*Klebsiella pneumoniae*
DMSO	--	--	--
2a	12	11	--
2b	12	10	10
2c	12	8	12
2d	--	--	14
2e	12	8	--
2f	14	12	12
Azithromycin (10 µg wel^{-1})	**20**	**25**	**30**

Antifungal Activity, Mic Method: The anti-fungal activity of the synthesized compounds was performed against standard fungal strains *Candida albicans, Aspergillus niger*, in DMSO by broth micro dilution method. The MIC determination of the tested compounds was investigated in comparison with fluconazole by broth micro dilution method. Double dilutions of the test compounds and reference drugs were prepared in Sabouraud's dextrose broth. 10 mg of each test compounds were dissolved in 1mL of dimethylsulfoxide (DMSO) separately to prepare stock solution. Further progressive dilutions with Sabouraud's dextrose broth were performed to obtain the required concentrations of 100, 50, 25, 10 µg mL^{-1}. The petridishes were inoculated with 1-5x104 colonies forming units (cfu mL^{-1}) and incubated at 25°C for 48-72 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the tested compound that yield no visible growth on the plates. To ensure that the solvent had no effect on the fungal growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments [**15, 16**]. The results were shown in the table 3.

Table 3. Antifungal activity of the compounds 2(a-f)

Synthetic Compounds	Fungal Strain	
	Candida albiacans	Aspergillus niger
DMSO	--	--
2a	6.00	6.00
2b	10.0	10.0
2c	10.5	10.5
2d	6.25	6.25
2e	4.00	4.00
2f	6.50	6.50
fluconazole	12.5	12.5

APPLICATION

The antibacterial activity of the synthesized compounds were screened by cup plate method using a gram-positive and a gram-negative organisms *Staphylococcus aureus, Staphylococcus albus* and *Klebsiella pneumoniae* and which are compared with the standard drug Azithromycin. The antifungal activity of the compounds **2(a-f)** were screened against two fungi *Candida albiacans, Aspergillus nigre* by MIC method. All the compounds were compared with the standard drug fluconazole.

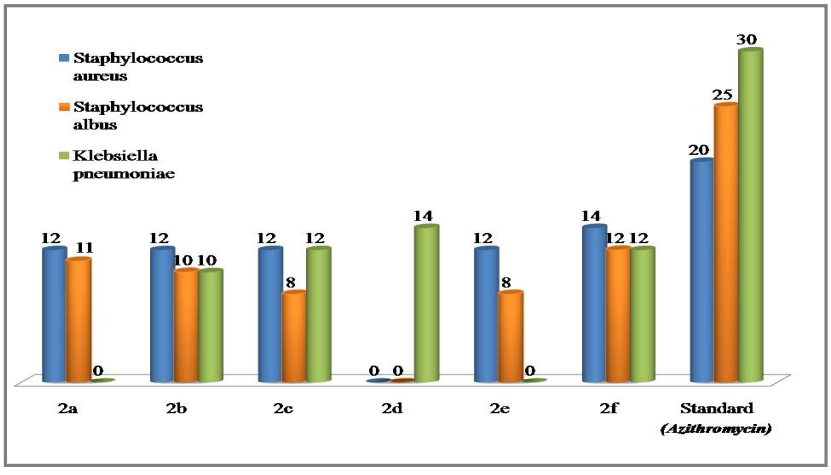


Figure 1. Antibacterial activity of the compounds 2(a-f) using a gram-positive and a gram-negative organisms *Staphylococcus aureus, Staphylococcus albus* and *Klebsiella pneumonia*, comparison with the standard drug (Azithromycin).

Results revealed that, the compound **2a** shows significant activity against *Staphylococcus aureus, Staphylococcus albus*, but it has not shown any activity against the organism *Klebsiella pneumoniae.*

Meanwhile compound 2**b** which contains NO_2 group at para position and the compound **2c** which contains Cl group at ortho position have shown good antibacterial activity. Further the compound 2**d** is inactive against two organisms *Staphylococcus aureus, Staphylococcus albus*, and it has shown promising activity against the organism *Klebsiella pneumoniae*. The compound ***2*e** which contains –OH group at ortho position has shown potential results against *Staphylococcus aureus, Staphylococcus albus*, which are compared with the standard drug Azithromycin. The compound **2f** which contains -OH group at the para position exhibits excellent activity against all the three organisms and were compared with the standard drug Azithromycin were shown in figure 1.

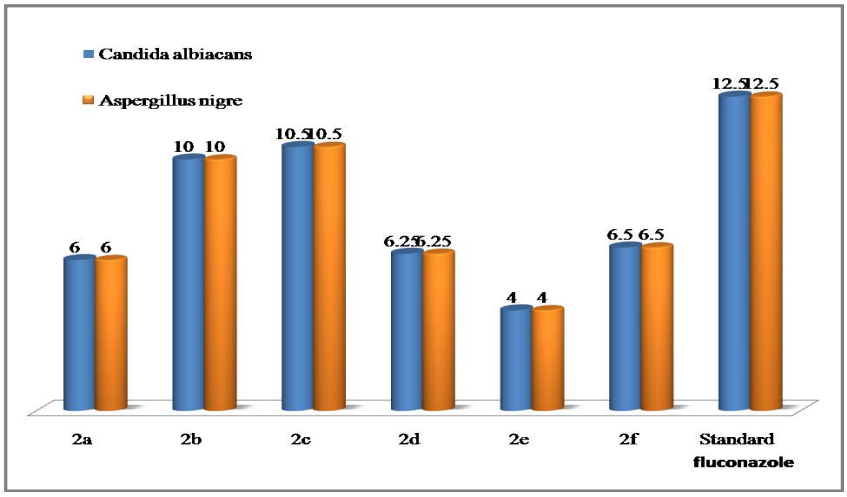


Figure 2. Antifungal activity of the compounds 2(a-f) using the organisms *Candida albiacans, Aspergillus niger* with the standard drug (*Fluconazole*).

Among the compounds **2(a-f)**, **2b** and **2c** have shown excellent activity against both the *Candida albiacans, Aspergillus niger*. It may be due to the presence of -NO_2, -Cl groups at para and ortho position respectively. The compounds **2a** and 2**d** exhibits significant activity, in which **2d** contains -Cl group at meta position. The -OH group of the compound **2e** and **2f** have shown favorable activity against the two fungi compared with the standard drug fluconazole which were shown in figure 2.

CONCLUSION

All the newly synthesized compounds **v**arious 3-(Substituted-Benzyl)-5-(5-Bromo-7-Methoxy-Benzofuran-2-Yl)-3h-[1,3,4] Oxadiazole-2-Thiones have shown excellent antibacterial and antifungal activity, which are Compared with the standard drug. All the synthesized compounds were identified by IR, H^1NMR.

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