Research Article

Synthesis, characterization and antimicrobial evaluation of novel azole based (Benzoic Acid) derivatives

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Abstract

Objective: The aim of present study was the Synthesis and characterization of Azoles Based Benzoic Acid Derivative. **Materials and Methods:** The compounds were prepared by reaction of Benzoic Acid with thiosemicarbazide in the presence of Phosphorus oxychloride to yield 1, 3, 4-thiadiazole nucleus. Further this thiadiazole was treated with different aromatic aldehydes in presence of methanol to yield corresponding Schiff's bases. All the Schiff's bases thus obtained were reacted with Thioglycolic acid to yield the title compounds. Synthesized derivatives were characterized by IR, ¹H NMR and screened for their antibacterial (*S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*) and antifungal activity (*A. Niger* and *C. albicans*). **Results and conclusion:** The experimental data of present research work reveals that the synthesized azole derivative (1, 2, 9, and 11) demonstrates significant Antibacterial and Antifungal Activity as shown in table no. 1 and out of all synthesized compounds the halogen substituted thiazolidinones derivatives are found most active.

Keywords: Azole derivative, Benzoic acid derivative, antimicrobial activity

Introduction

Medicine is a system of scientific knowledge and practical activities aimed at preserving and improving the health of people, prevention and treatment of human disease. History of medicine dates back practically to the existence of human civilization (Patwardhan et al., 2004). This would serve as the basis for ensuring good health for a community Traditional Indian medicine-a conglomerate of Ayurveda, Sidha and Unani has a long history and is one of the greatest living traditions, as it finds a highly respectable place in the officially recognized health care system of the country (Ogundele et al., 2007).

Thiadiazole is a heterocyclic compound featuring both a nitrogen and sulphur atom as parts of the aromatic five membered rings. It acts as Hydrogen bonding domain and two

electron donor moiety along with ability of Bio-isosteric replacement of thiazole moiety (Siddiqui et al., 2009; Al-Amiery et al., 2009). Thiazole is resistant to oxidizing agents; even hot nitric acid has little effect. It is either unaffected by reducing agents or at most some ring opening occurs it is therefore not possible to make dihydrothiazoles or thiazolidines by catalytic dehydrogenation but they must need to be prepared by means of condensation reactions. Thiazole is of great biological importance as it is used as an intermediate to manufacture synthetic drugs, fungicides and dyes. A thiazole ring is found in the essential vitamin B₁ (Singh et al., 2011). The literature survey showed that the Thiadiazole moiety exhibit antimicrobial, anti cancer, anti inflammatory, antioxidant Anti Fungal, anti depressant, anti-Anthelmintic activity (Patel et al., 2010; Shrivastava et al., 2010; Chatrabhuji et al., 2010; Bhatt et al., 1994; Shih et al., 2004; Havrylyuk et al., 2009).

Materials and Methods

The chemical used for experimental were of synthetic grade and purchased from Nice Laboratory, Loba Chemie Private

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Limited, CDH New Delhi and Research Lab Fine Chem industries Mumbai. The melting points of the synthesized compounds were determined in open glass capillaries and are uncorrected. IR spectra were recorded on FTIR Spectrometer. ^1H NMR spectra were recorded on were recorded in $\text{CDCl}_3/\text{DMSO}$ solution on a Bruker Avance II 400 MHz NMR spectrometer taking Tetramethylsilane (TMS) as internal standard. Progress of reaction was monitored by TLC using solvent Hexane: Ethyl acetate (40%) & Methanol: Chloroform (1%).

Synthetic route of thiazolidinones

Synthesis of 5-Phenyl-[1, 3, 4]-thiadiazole-2-ylamine

A mixture of equimolar quantity of benzoic acid (50mmol) and thiosemicarbazide (50 mmol) were refluxed in 20 ml POCl_3 solution for 2 h at 75°C . After cooling down to room temperature, cold water was added. The mixture was again refluxed for 4 h. The content was cooled down at room temperature and made alkaline at pH 8.0 by addition of 50 % NaOH solution. The solid (5-Phenyl-[1, 3, 4]-thiadiazole-2-ylamine) (compound 3) so obtained was filtered and recrystallized using ethanol (Bhatia et al., 2014).

Synthesis of Schiff bases

In this step compound 3 i.e. 5-Phenyl-[1, 3, 4]-thiadiazole-2-ylamine was refluxed with different substituted aromatic aldehydes (0.025M) [a-k] using methanol (50 mL) as solvent in presence of small amount of glacial acetic acid for 2 hrs. The mixture was cooled and poured in ice water. The content was put

undisturbed for 24h. The solid thus obtained was separated by filtration and recrystallized from Methanol to give the Corresponding Schiff bases (a-k) (Kumar et al., 2011; Kumar et al., 2018).

Synthesis of thiazolidinones

A mixture of Synthesized Schiff base (0.02M) (a-k) and Thioglycolic acid (0.02M) in 50ml DMF, containing a pinch of anhydrous ZnCl_2 were refluxed for about 6 hrs. The reaction mixture was cooled and poured on to crushed ice. The solid thus obtained was filtered, washed with water and the product thiazolidinones (6a-k) was recrystallized from ethanol (Sharma et al., 2010).

Characterization of synthesized compounds

2-(4-chlorophenyl)-3-(5-phenyl-[1, 3, 4]-thiadiazol-2-yl)-thiazolidin-4-one (Compound 1)

IR (KBr) (cm^{-1}) 3011.15 (C-H str, aromatic), 1670 (C=O str.), 1638 (C=N str.), 1296 (C-N), 835.40 (C-Cl). ^1H NMR (DMSO) (δ -ppm): 7.1-7.79 (9H, Ar H); 5.4 (1H, N-CH); 3.8 (1H, CH-S).

2-(2-Fluorophenyl)-3-(5-phenyl-[1, 3, 4]-thiadiazol-2-yl)-thiazolidin-4-one (Compound 2)

IR (KBr) (cm^{-1}) 3017.11 (C-H str, aromatic), 1674 (C=O str.), 1643 (C=N str.), 1291 (C-N), 831.45 (C-F). ^1H NMR (DMSO) (δ -ppm): 7.04-7.68 (9H, Ar H); 5.56 (1H, N-CH); 3.73 (1H, CH-S).

Ar = (a) 4-Cl-C₆H₅-, (b) 2-F-C₆H₅-, (c) 2-NO₂-C₆H₅-, (d) 3-OH-C₆H₅-, (e) 4-OH-C₆H₅-, (f) 2-OCH₃-C₆H₅-, (g) 4-OCH₃-C₆H₅-, (h) 4-OH-C₆H₅-, (i) 2,4-Cl-C₆H₅-, (j) 4-NO₂-C₆H₅-, (k) 2-Br-C₆H₅-

Figure 1. Synthetic route of thiazolidinones

2-(2-Nitrophenyl)-3-(5-phenyl-[1, 3, 4]-thiadiazol-2-yl)-thiazolidin-4-one (Compound 3)

IR (KBr) (cm^{-1}) 3014.3 (C-H str, aromatic), 1668 (C=O str.), 1639(C=N str.), 1287 (C-N),

^1H NMR (DMSO) (δ -ppm): 7.21-7.94 (8H, Ar H); 5.84 (1H, N-CH); 3.76 (1H, CH-S).

2-(3-Hydroxyphenyl)-3-(5-phenyl-[1, 3, 4]-thiadiazol-2-yl)-thiazolidin-4-one (Compound 4)

IR (KBr) (cm^{-1}) 3002.3 (C-H str, aromatic), 1670.8 (C=O str.), 1644.2(C=N str.), 1294 (C-N),

^1H NMR (DMSO) (δ -ppm): 7.05-7.68 (8H, Ar -H); 5.89 (1H, N-CH); 4.87 (Ar-OH), 3.81 (1H, CH-S).

2-(4-Hydroxyphenyl)-3-(5-phenyl-[1, 3, 4]-thiadiazol-2-yl)-thiazolidin-4-one (Compound 5)

IR (KBr) (cm^{-1}) 3004.7 (C-H str, aromatic), 1673.3 (C=O str.), 1644.9(C=N str.), 1292 (C-N),

^1H NMR (DMSO) (δ -ppm): 6.95-7.57 (9H, Ar -H); 5.94 (1H, N-CH); 4.95 (Ar-OH), 3.67 (1H, CH-S).

2-(2-Methoxyphenyl)-3-(5-phenyl-[1, 3, 4]-thiadiazol-2-yl)-thiazolidin-4-one (Compound 6)

IR (KBr) (cm^{-1}) 3008.1 (C-H str, aromatic), 1669.5 (C=O str.), 1642.4(C=N str.), 1288.3 (C-N), 1230.2 (-OCH₃). ^1H NMR (DMSO) (δ -ppm): 6.91-7.64 (9H, Ar -H); 5.83 (1H, N-CH); 4.01 (-OCH₃), 3.57 (1H, CH-S).

2-(4-Methoxyphenyl)-3-(5-phenyl-[1, 3, 4]-thiadiazol-2-yl)-thiazolidin-4-one (Compound 7)

IR (KBr) (cm^{-1}) 3008.8(C-H str, aromatic), 1669.3 (C=O str.),

1642.4(C=N str.), 1287.5 (C-N), 1232.4 (-OCH₃). ^1H NMR (DMSO) (δ -ppm): 6.84-7.73 (9H, Ar -H); 5.78 (1H, N-CH); 3.9 (-OCH₃), 3.54 (1H, CH-S).

2-phenyl-3-(5-phenyl-[1, 3, 4]-thiadiazol-2-yl)-thiazolidin-4-one (Compound 8)

IR (KBr) (cm^{-1}) 3009.7(C-H str, aromatic), 1667.6 (C=O str.), 1644.5(C=N str.), 1286.9 (C-N). ^1H NMR (DMSO) (δ -ppm): 7.05-7.83 (10H, Ar -H); 5.85 (1H, N-CH), 3.51 (1H, CH-S).

2-(2, 4-Dichlorophenyl)-3-(5-phenyl-[1,3,4]-thiadiazol-2-yl)-thiazolidin-4-one(Compound 9)

IR (KBr) (cm^{-1}) 3007.1(C-H str, aromatic), 1665.2 (C=O str.), 1642.6(C=N str.), 1282.2 (C-N), ^1H NMR (DMSO) (δ -ppm): 6.95-7.64 (8H, Ar -H); 5.89 (1H, N-CH), 3.43 (1H, CH-S).

2-(2-Nitrophenyl)-3-(5-phenyl-[1, 3, 4]-thiadiazol-2-yl)-thiazolidin-4-one (Compound 10)

IR (KBr) (cm^{-1}) 2998.5(C-H str, aromatic), 1668.4 (C=O str.), 1645.5(C=N str.), 1284.3 (C-N), ^1H NMR (DMSO) (δ -ppm): 7.10-7.94 (8H, Ar -H); 5.88 (1H, N-CH), 3.47 (1H, CH-S).

2-(2-Bromo-phenyl)-3-(5-phenyl-[1, 3, 4]-thiadiazol-2-yl)-thiazolidin-4-one (Compound 11)

IR (KBr) (cm^{-1}) 3005.8(C-H str, aromatic), 1666.1 (C=O str.), 1640.5(C=N str.), 1282.0 (C-N), ^1H NMR (DMSO) (δ -ppm): 7.05-7.61 (8H, Ar -H); 5.85 (1H, N-CH), 3.49 (1H, CH-S).

Antibacterial activity

Keeping in view the activity profile of Thiazolidinone and

Table 1. MIC of synthesized antimicrobial compounds

Compounds	Minimum Inhibitory Concentration (MIC)					
	Bacterial strains				Fungal strains	
	Gram Positive		Gram Negative			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>A. niger</i>
1	0.835	0.835	0.835	0.835	1.670	1.670
2	0.890	0.890	0.890	0.890	0.890	0.890
3	1.627	1.627	1.627	1.627	1.627	1.627
4	1.760	1.760	1.760	1.760	1.760	1.760
5	1.760	1.760	1.760	1.760	1.760	1.760
6	1.689	1.689	1.689	1.689	1.689	1.689
7	1.689	1.689	1.689	1.689	1.689	1.689
8	1.838	1.838	1.838	1.838	1.838	1.838
9	0.765	0.765	0.765	0.765	0.765	0.765
10	1.623	1.623	0.811	0.811	1.623	1.623
11	0.747	0.747	0.747	0.747	0.747	0.747
Std.	0.471	0.471	0.471	0.471	0.510	0.510

Standard: Ciprofloxacin (antibacterial) and Fluconazole (antifungal)

Thiadiazole itself, it was thought sensible to carry out biological screening of all the newly synthesized compounds. Therefore all the synthesized compounds were subjected to Antimicrobial evaluation.

The antibacterial activities of compounds were determined *in vitro* against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *P. aeruginosa*) bacteria by tube dilution method. Double strength nutrient broth-(I.P.) media and sabourand's glucose broth media (I.P.) were used for bacteria and fungi respectively. The samples were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 d (*A. Niger*) and at 37 °C for 48 h (*C. albicans*) and the results were recorded in terms of minimum inhibitory concentration (MIC). Ciprofloxacin and fluconazole were taken as standard drugs for antibacterial and antifungal activity, respectively.

Determination of MIC

MIC of compounds was determined by two fold serial dilution technique. Dilution of test compound and standard drugs were prepared in test medium to give a concentration of 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml from stock solution (100µg/ml). All the samples were inoculated with 0.1ml suspension of bacteria in saline and incubated at required temperature. MIC was determined by lowest concentration of sample that prevents the development of turbidity (Kumar et al., 2018). The observed MIC is presented in table 1.

Result and discussion

The target compounds were synthesized starting from Benzoic

acid, In the first step Benzoic acid (50mmol) was reacted with Thiosemicarbazide (50mmol) in presence of POCl₃ (Bhatia et al., 2014). In the next step Thiadiazole was reacted with different (a-k) aromatic aldehydes in methanol (50 mL) in presence of small amount of glacial acetic acid for 2 hrs to give the Corresponding Schiff bases (a-k) (Kumar et al., 2018). Further all the Synthesized Schiff base (a-k) and appropriate quantity of Thioglycolic acid in dimethyl formamide (DMF) containing a pinch of anhydrous ZnCl₂ were refluxed for about 6 hrs. The reaction mixture was cooled and poured on to crushed ice. The solid thus obtained was filtered, washed with water and the product (6a-k) was recrystallized from ethanol (Sharma et al., 2010). By adopting this method total 11 compounds have been synthesized. The synthesized title compounds were recrystallized and purity of the compounds was ascertained by using appropriate solvent systems (ethyl acetate: benzene: water). The compounds were characterized by their IR and NMR spectroscopic data. The result of IR and HNMR data are in good agreement with their theoretical values. The Physiochemical data of Synthesized Thiazolidinone derivatives is presented in table 2. Compounds 1, 2, 9, 10, 11 showed the significant antimicrobial activity against different strains of microbes.

Conclusion

In the present work two azole containing moiety i.e. Thiadiazole and Thiazolidinone were clubbed to obtain

Table 2. Characterization data of compounds

Compound No	Ar-	Molecular formula	Molecular weight	Melting point	% yield
1		C ₁₇ H ₁₂ ClN ₃ O ₂ S ₂	373.88	206-208	71
2		C ₁₇ H ₁₂ ClN ₃ O ₂ S ₂	351.43	211-213	68
3		C ₁₇ H ₁₂ N ₄ O ₃ S ₂	384.43	186-189	67
4		C ₁₇ H ₁₃ N ₃ O ₂ S ₂	355.43	181-184	80
5		C ₁₇ H ₁₃ N ₃ O ₂ S ₂	355.43	187-190	73
6		C ₁₈ H ₁₅ N ₃ O ₂ S ₂	369.46	173-177	79
7		C ₁₈ H ₁₅ N ₃ O ₂ S ₂	369.46	168-175	83
8		C ₁₇ H ₁₃ N ₃ O ₂ S ₂	339.43	178-184	70
9		C ₁₇ H ₁₁ Cl ₂ N ₃ O ₂ S ₂	408.32	193-195	64
10		C ₁₇ H ₁₂ N ₄ O ₃ S ₂	384.43	185-188	69
11		C ₁₇ H ₁₂ BrN ₃ O ₂ S ₂	418.33	196-198	61

their synergistic effect and evaluated for their antimicrobial effect. The experimental data of the present research work reveals that synthesizedazole derivatives of demonstrate significant Antibacterial and Antifungal Activity. Compound 1, 2 9 & 11 were showed significant antimicrobial activity (table 1). Further, it has been observed that Thiadiazole which are substituted by halogen (-Cl, -Br, -F) with electron withdrawing property exhibits more Antimicrobial activity than the other.

Conflicts of interest: Not declared.

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