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Synthesis and Antimicrobial evaluation of Azole Based (p-nitro Benzoic Acid) derivatives

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Abstract

This study includes the Synthesis of Azoles Based p-nitro Benzoic Acid Derivative. The compounds were prepared by reaction of p-nitro 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, H^1NMR and screened for their antibacterial (S. aureus, B. subtilis, E. coli, and P. aerugiosa) and antifungal activity (A. Niger and C. albicans).

Key Words: Azole derivative, p-nitro Benzoic Acid derivative, antimicrobial activity.

Introduction

Medicine is defined as a system of scientific knowledge and practical activities aimed at preserving and improving the health of society including prevention and treatment of human disease. This would serve as the basis for ensuring good health for a community Tradition Antimicrobial resistance is becoming a serious threat to global public health. As per WHO around 45-50 thousand people face the drug resistance in tuberculosis disease.[1] As per all Indian medicine-a conglomerate of Ayurveda, Sidha and Unani has a long history The 4-thiazolidinones that do not contain aryl or higher alkyl substituents are somewhat soluble in water or thiazolidinone that do not contain aryl or higher alkyl substituents are somewhat less soluble in water[2] Thiadiazole is a heterocyclic compound featuring both a nitrogen and sulphur atom as a part of the aromatic five membered rings.

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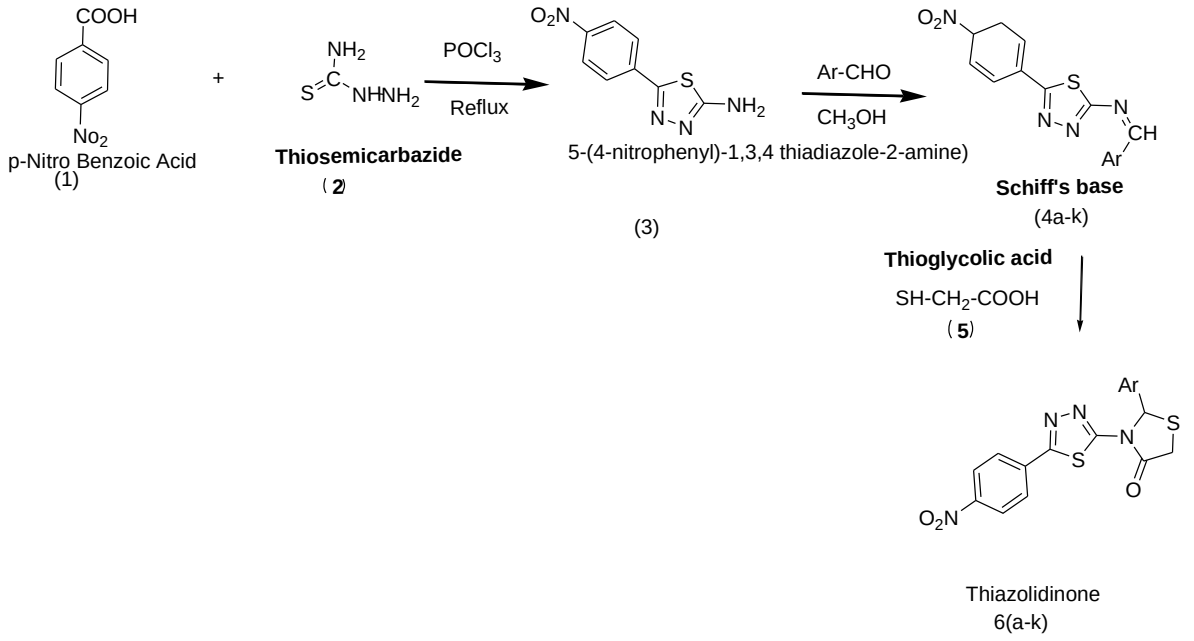
It acts as Hydrogen bonding domain and two electron donor moiety along with ability of Bio-isosteric replacement of thiazole moiety. Bioisosteres are atoms or group of molecules that fit the broadest definition for isosteres. [3-5]. Thiazole is resistant to oxidizing agents; even hot nitric acid has 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 catalytical 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 B_1 [7]. The literature survey showed that the Thiadiazole moiety exhibit antimicrobial, anti cancer, anti inflammatory, antioxidant Anti Fungal, anti depressant, anti-Anthelmintic activity. [8-13]. Five membered heterocyclic compounds show various types of biological activities. Among these, Thiadiazole are associated with diverse pharmacological actions probably by virtue of –N=C-S- grouping. Thiadiazole moiety acts as a "hydrogen binding domain" and "two-electron donor system". Thiadiazole acts as a bioisosteric replacement of thiazole moiety. So, it acts as third and fourth generation cephalosporin and hence can be used in antibiotic preparations [19].The numbering of monocyclic azole systems begins with the heteroatom that is in the highest group in the periodic table and with the element of lowest atomic

weight in that group. Hence, the numbering of 1, 3, 4-thiadiazole is done in the following manner. This designated that one sulphur group is present in the ring [20].

Materials and Methods

The chemical used for experimental were of synthetic grade and procured from Nice Laboratory, Loba Chemie Private 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. H^1NMR spectra were recorded on were recorded in CDCl3/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%).

Scheme



Ar =(a)= 4-Cl-C_6H_5-, (b) 2-F-C_6H_5-, (c) 2-NO_2-C_6H_5-, (d) 3-OH-C_6H_5- , (e) 4-OH-C_6H_5- ,(f) 2-OCH_3-C_6H_5-
(g) 4-OCH_3-C_6H_5- (h) 4-OH-C_6H_5- (i) 2,4-Cl-C_6H_5- (j) 4-NO_2-C_6H_5- (k) 2-Br-C_6H_5-

Synthetic procedure

Synthesis of 5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine

A mixture of equimolar quantity of p-Nitro Benzoic Acid (50mmol) and thiosemicarbazide (50 mmol) were refluxed in 20 ml POCl3 solution for 2 h at 75^0C. 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-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine (compound 3) so obtained was filtered and recrystallized using ethanol. (Bhatia et al)

Synthesis of Schiff bases

In this step compound 3 i.e. 5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine 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). [21-23] and Kumar et al 2018]

Synthesis of Thiazolidinone

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 thaizolidinone (6a-k) was recrystallized from ethanol. [Sharma et al 2010].

Characterization of synthesized compounds:

Compound 1

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

IR (KBr) (cm^{-1}) 3013.18 (C-H str, aromatic), 1672.7 (C=O str.), 1639.1 (C=N str.), 1297 (C-N), 831.42(C-Cl). ^{1}H NMR (DMSO) (δ-ppm): 7.1-8.15 (8H, Ar H); 5.6 (1H, N-CH); 3.5 (1H, CH-S)

Compound 2

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

IR (KBr) (cm^{-1}) 3019.15 (C-H str, aromatic), 1674.5 (C=O str.), 1643.9 (C=N str.), 1292.1 (C-N), 830.47(C-F). ^{1}H NMR (DMSO) (δ-ppm): 7.0-8.18 (8H, Ar H); 5.58 (1H, N-CH); 3.68 (1H, CH-S)

Compound 3

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

IR (KBr) (cm^{-1}) 3015.6 (C-H str, aromatic), 1667.6 (C=O str.), 1640.1(C=N str.), 1285.7 (C-N),

^{1}H NMR (DMSO) (δ-ppm): 7.27-8.24 (8H, Ar H); 5.71 (1H, N-CH); 3.73 (1H, CH-S)

Compound 4

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

IR (KBr) (cm^{-1}): 3381.10 (O-H str.), 3004.3 (C-H str, aromatic), 1667.1 (C=O str.), 1641.5(C=N str.), 1293 (C-N),

^{1}H NMR (DMSO) (δ-ppm): 6.90-7.90 (8H, Ar -H); 5.9 (1H, N-CH); 4.94 (Ar-OH), 3.78 (1H, CH-S)

Compound 5

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

IR (KBr) (cm^{-1}): 3385.6 (O-H str.), 3007.1(C-H str, aromatic), 1673.4 (C=O str.), 1642.9(C=N str.), 1291 (C-N),

^{1}H NMR (DMSO) (δ-ppm): 6.90-8.10 (8H, Ar -H); 5.84 (1H, N-CH); 4.90 (Ar-OH), 3.68 (1H, CH-S)

Compound 6

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

IR (KBr) (cm^{-1}) 3010.4 (C-H str, aromatic), 1669.5 (C=O str.), 1642.4(C=N str.), 1288.3 (C-N), 1232.5 (OCH3)

^{1}H NMR (DMSO) (δ-ppm): 6.90-7.95 (8H, Ar -H); 5.85 (1H, N-CH); 3.97 (-OCH_3), 3.56 (1H, CH-S)

Compound 7

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

IR (KBr) (cm^{-1}) 3010.5(C-H str, aromatic), 1666.7 (C=O str.), 1639.1(C=N str.), 1285.5 (C-N), 1233.2 (OCH3)

^{1}H NMR (DMSO) (δ-ppm): 6.85-8.10 (8H, m, Ar -H); 5.82 (1H, N-CH); 3.82 (1H, s, -OCH_3), 3.50 (1H, s, CH-S)

Compound 8

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

IR (KBr) (cm^{-1}) 3006.9(C-H str, aromatic), 1665.0 (C=O str.), 1641.7(C=N str.), 1286.2 (C-N),

^{1}H NMR (DMSO) (δ-ppm): 7.05-8.18 (9H, m, Ar -H); 5.85 (1H, s, N-CH), 3.51 (1H, s, CH-S)

Compound 9

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

IR (KBr) (cm^{-1}) 3004.0(C-H str, aromatic), 1658.2 (C=O str.), 1643.5(C=N str.), 1284.8 (C-N),

^{1}H NMR (DMSO) (δ-ppm): 6.95-7.98(7H, m, Ar -H); 5.88 (1H, s, N-CH), 3.43 (1H, s, CH-S)

Compound 10

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

IR (KBr) (cm^{-1}) 2996.0(C-H str, aromatic), 1663.1 (C=O str.), 1645.9(C=N str.), 1278.8 (C-N),

^{1}H NMR (DMSO) (δ-ppm): 7.25-8.05 (8H, m, Ar -H); 5.90 (1H, s, N-CH), 3.45 (1H, s, CH-S)

Compound 11

2-(2-Bromo-phenyl)-3-(5-94-nitro-phenyl-[1, 3, 4]-thiadiazol-2-yl)-thiazolidin-4-one

IR (KBr) (cm^{-1}) 2998.7(C-H str, aromatic), 1664.2 (C=O str.), 1644.9(C=N str.), 1279.3(C-N),

^{1}H NMR (DMSO) (δ-ppm): 7.05-8.10 (8H,m, Ar -H); 5.85 (1H,s, N-CH), 3.49 (1H, s, CH-S)

Biological Activity

Keeping in view the activity profile of Thiazolidinone and 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.

Antibacterial activity

The antibacterial activities of compounds were determined *in vitro* against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis)* and Gram-negative *(Escherichia coli*, *P. aeuroginosa*) 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. [18] The observed MIC is presented in Table 1.

Table-1: Minimum Inhibitory Concentration (MIC)

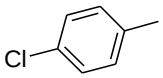
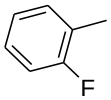
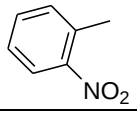
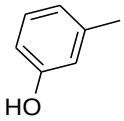
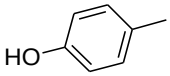
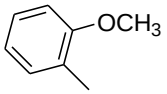
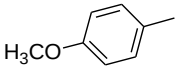
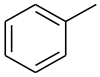
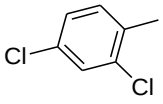
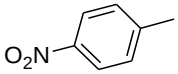
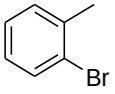
Comp-ounds	**Minimum Inhibitory Concentration (MIC)**					
	Bacterial strains				**Fungal strains**	
	Gram Positive		**Gram Negative**			
	S. aureus	***B. subtilis***	***P. aeruginosa***	***E.coli***	***C. albicans***	***A. niger***
1	1.610	1.610	1.610	1.610	1.610	1.610
2	**0.842**	**0.842**	**0.842**	**0.842**	**0.842**	**0.842**
3	1.570	1.570	**0.785**	**0.785**	1.570	1.570
4	1.689	1.689	1.689	1.689	1.689	1.689
5	1.689	1.689	1.689	1.689	1.689	1.689
6	1.74	1.74	1.74	1.74	**0.870**	**0.870**
7	1.74	1.74	1.74	1.74	**0.870**	**0.870**
8	1.77	1.77	1.77	1.77	1.77	1.77
9	**0.740**	**0.740**	**0.740**	**0.740**	1.48	1.48
10	1.570	1.570	**0.785**	**0.785**	1.570	1.570
11	**0.723**	**0.723**	**0.723**	**0.723**	**0.723**	**0.723**
Std.	0.471	0.471	0.471	0.471	0.510	0.510

Standard: ciprofloxacin (antibacterial) and fluconazole (antifungal).

Result and discussion

The Title compounds were synthesized as per the procedure mentioned under heading Synthetic procedure. The synthesized 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 2, 3, 6, 7,9,10, 11 showed the significant antimicrobial activity against different strains of microbes.

Table 2: Characterization (Physiochemical) data of compounds

Compound no	Ar-	Molecular formula	Molecular weight	Melting point	% yield
1		$C_{17}H_{11}ClN_4O_3S_2$	418.88	206-208	71
2		$C_{17}H_{11}FN_4O_3S_2$	402.42	200-203	74
3		$C_{17}H_{11}N_5O_5S_2$	429.43	180-182	76
4		$C_{17}H_{12}N_4O_4S_2$	400.43	185-187	76
5		$C_{17}H_{12}N_4O_4S_2$	400.43	184-187	75
6		$C_{18}H_{14}N_4O_4S_2$	414.46	174-176	74
7		$C_{18}H_{14}N_4O_4S_2$	414.46	172-174	76
8		$C_{17}H_{12}N_4O_3S_2$	384.43	180-183	72
9		$C_{17}H_{19}Cl_2N_4\ O_3S_2$	453.32	190-193	67
10		$C_{17}H_{11}N_5O_5S_2$	429.43	190-192	70
11		$C_{17}H_{11}BrN_4\ O_3S_2$	463.33	194-197	64

Conclusion

In the present work two azole containing moiety i.e. Thiadiazole and Thiazolidinone were clubbed to obtain their synergistic effect and evaluated for their antimicrobial effect. The experimental data of the present research work reveals that synthesized azole derivatives of demonstrate significant Antibacterial and Antifungal Activity. Compound 2, 3, 6, 7,9,10, 11 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.

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