SYNTHESIS AND CHARACTERIZATION OF NEW DERIVATIVES OF THIAZOLIDINONE 1,2-BENZISOXAZOLE WITH ANTIMICROBIAL ACTIVITY

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Abstract
1,2-Benzisoxazole's thiazolidinone derivatives were created in a number of different ways. When 4-hydroxy-2H-chromen-2-one is combined with an aromatic aldehyde, Schiff's base is produced, which, when cycled with mercaptoacetic acid, produces Benzisoxazole derivatives called thiazolidinones. The use of IR, 1HNMR, and elemental analysis to determine the structure of produced substances. TLC was used to verify the compounds' purity. By using turbidimetric techniques, the produced compounds were tested for in vitro antibacterial and antimicrobial activities. Comparing compounds 5b, 5d, 5e, and 5i to the reference standard Ciprofloxacin revealed improved antibacterial activity. Using the reference standard, compounds 5b, 5d, 5e, and 5i shown excellent antibacterial activity. ketoconazole.

Introduction
Since it is a heterocyclic substance, benzisoxazole is used in research as an intermediate2 and as a starting material1 for the production of other chemicals that are effective as medications. Thiazolidine, which is a member of a significant class of heterocyclic compounds, is the source of the thiazolidinones3 family. Thiazolidinone derivatives contain a variety of biological activities4,5,6 that have been shown in the literature, including bactericidal7, fungicidal8, anticonvulsant9,10, tuberculostatic, antithryoidal, and potentiation of pentobarbital-induced sleep duration. To explore the antibacterial action of these compounds, an attempt was undertaken to synthesis Thiazolidinone derivatives of Benzisoxazole. For assessing drug similarity, Lipinski's "rule of five" 11—which characterizes the ADME in humans—is used. The rule is crucial for drug development when a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity. The structural assignment of the product was based on IR, NMR and Mass spectral activity. The titled compounds were then screened for antibacterial activity.

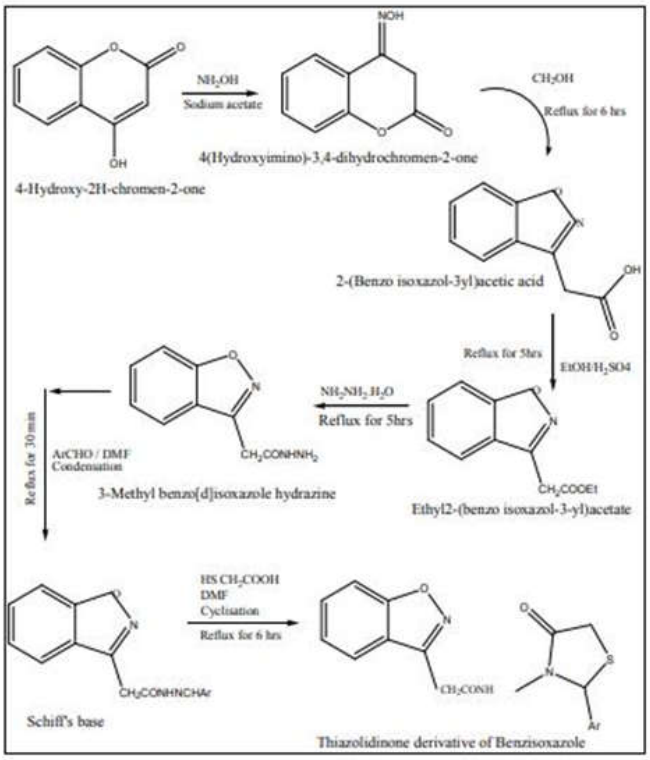
Materials and Methods
Melting points were determined by Veego's melting point apparatus and are uncorrected. The TLC of the compounds were performed on silica gel G coated glass plate with Benzene:ethanol(9:1) as solvent. Iodine chamber was used as detecting agent. Molecular formula and molecular weight were determined by using Chem-Draw software. IR spectra were recorded by KBr disc method on Perkin Elmer FT-IR instrument. 1HNMR were recorded on BrukerAvance II 500 MHz spectrophotometer. The chemical shifts were reported as parts per million downfield from tetramethylsilane using DMSO as solvent. 13CNMR spectroscopy were recorded on BrukerAvance II 500MHz spectrophotometer using DMSO as solvent. Mass spectroscopy was performed on JEOL GC mate using DMSO as solvent.

Method of Synthesis
Step One: Synthesis of 1,2-Benziosazole-3-acetic acid Hydroxylamine hydrochloride (75g,1.08mol) was added to a stirred solution of 4-Hydroxy coumarin(500g, 3.086mol) in methanol(5.0lit)at 25-30ºc. Sodium acetate (885g,10.80 mol) was added to the above solution lot wise in half an hour. The reaction mass was stirred at 25-

30ºc for half an hour, heated to reflux (65-70ºc) and maintained at reflux for 5-6hrs. After completion of the reactivity (by TLC), methanol was distilled under vaccum((<50ºc). After complete removal of methanol, 7.0lit of water was added to the residueand the resulting solution was cooled to 10-15ºc. The pH of the reaction mass was adjusted to 2-3 with 50%HCland stirred the reaction for one hour at 10-15ºc. The solid obtained was filtered and washed with 2lit of water.The solid was dired at 55-60ºc.

Step Two: Synthesis of Ethyl 2-(benzo[d]isoxazol-3-yl)acetate:

The acid was converted to ester by means of simple esterification procedure, by using ethanol(0.2mole), sulphuric acid (0.1mole) and reflux for 5-6 hrs. Excess alcohol was distilled off and excess acid was neutralized with 10% NaHCO3. Step Three: Synthesis of –(Benzo[d]isoxazol- 3-yl)acetohydrazide: A solution of ester (3.08gm, 0.01mole) and hydrazine hydrate (0.75gm, 0.015mole) in ethanol (25ml) was refluxed for 5hrs. The reaction mixture was cooled and poured on to ice-cold water with stirring. The separated solid was filtered, washed with water and recrystallized from ethanol. Step Four: Synthesis of –(Benzo[d]isoxazol-3-yl)-N'-benzylideneacetohydrazide: A mixture of acid hydrazide (2.94gm,0.01mole) and benzaldehyde (1.06gm, 0.01mole) in DMF (20ml) was heated on a water bath for half an hour, cooled and poured onto crushed ice. The precipitate thus obtained was filtered, washed with water and recrystallized from ethanol. Step Five: Synthesis of –(Benzo[d]isoxazol-3-yl)-N-(4-oxo-2-phenylthiazolidin-3-yl)acetamide. A mixture of Schiff's base (0.01mole) and mercapto acetic acid (0.01mole) in DMF (30ml) containing a pinch of anhydrous zinc chloride was refluxed for 6hrs. The reaction mixture was cooled and poured onto crushed ice. The solid thus obtained was filtered, washed with water and recrystallized from DMF to yield cyclized product.



S. No.	Aromatic Aldehyde	Log P	Molecular weight	No.of H atoms		No.of violation
				Acceptor	*Donor*	
1	Benzaldehyde	2.535	353.403	6	1	0
2	4-Hydroxybenzaldehyde	2.056	369.402	7	2	0
3	4-Methoxybenzaldehyde	2.591	383.429	7	1	0
4	3-Hydroxybenzaldehyde	2.032	369.402	7	2	0
5	2-Chlorobenzaldehyde	3.165	387.848	6	1	0
6	3-Nitrobenzaldehyde	2.470	398.400	9	1	0
7	2-Nitrobenzaldehyde	2.446	398.400	9	1	0
8	3,4-Dimethoxybenzaldehyde	2.181	413.455	8	1	0
9	4-Chlorobenzaldehyde	3.213	387.848	6	1	0
10	2-Hydroxybenzaldehyde	2.475	369.402	7	2	0

From the above values which are obtained by customized software all compounds showed good bioavailability because according toLipniski's rule of five number of violation is less than1. From this all these compounds were selected for synthesis. The reaction was monitored by TLC. The physico chemical parameters of the synthesized compounds are listed in Table2.

Compound	M.P (°c)	Appearance	Yield %	Rf Value
5a	97	Creamish	72	0.75
5b	92	Light brown	69	0.6
5c	95	Creamish	80	0.6
5d	98	Reddish brown	75	0.7
5e	120	Pale yellow	60	0.7
5f	105	Black	65	0.6
5g	102	Creamish yellow	60	0.5
5h	95	Creamish yellow	74	0.9
5i	101	Yellow	82	0.8
5j	110	Creamish	59	0.6

Spectral Data Analysis Compound 5a:

IUPAC name: 2-(Benzo{d}isoxazol-3-yl)-N- (4-oxo-2-phenylthiazolidn-3-yl)acetamide IR (KBr)cm^{-}1 (C=C str) 1458.76, (N-H str) 3417.62, (N-H bend)1540.66, (C=O str)1654.25, (=C-H str)3079.90, (C-N str) 1150.91, (C-S str) 699.46. 1H-NMR(δppm) :δ8.0(S,1H,NH),δ6.2-7.9 (m,9H,Ar-H), δ 5.9 (S,1H, Thiazolidinone ring), δ3.4 (S,2H,3- CH2CO, δ 3.2-3.3 (S,2H, Thiazolidinone ring CH2). Elemental analysis: Calculated for C 68.52 and found 68.74, Calculated for H 4.98 and found 5.28,Calculated for N 7.10 and found 7.70. Compound 5b: IUPAC name: 2-(Benzo{d}isoxazol-3-yl)-N- (2-(4-hydroxyphenyl)-4-oxothiaxzolidin-3-yl)acetamide IR (KBr)cm^{-}1 (C=C str) 1450.71, (N-H str) 3459.00, (N-H bend)1623.12, (C=O str)1708.19, (=C-H str)3067.97, (C-N str) 1154.38, (C-S str) 751.93. 1H-NMR(δppm) :δ8.1 (S,1H,NH),δ6.2-7.9 (m,8H,Ar-H), δ 6.0(S,1H, Thiazolidinone ring)δ3.5 (S,2H,3- CH2CO,δ 3.3 (S,2H, Thiazolidinone ring CH2). Elemental analysis: Calculated for C 65.32 and found 65.69, Calculated for H 5.09 and found 5.50,Calculated for N 7.70 and found 7.60.

Invitro Antibacterial Activity

The synthesized compounds were screened for their antibacterial activity using Escherichia coli, Bacillus subtilis, Staphylococcus aureus. Control experiment was carried out under similar condition using Ciprofloxacin as standard. Turbidimetric12-16 method was used to check antibacterial activity of the synthesized compounds at different concentration using ciprofloxacin as the positive control and DMSO as the negative control. The inhibition zone measure in mm showed that compounds like 5b,5d, 5e,5i,showed better inhibition as compared

to the standard ciprofloxacin against the three bacterial strains Escherichia coli, Bacillus subtilis, Staphylococcus aureus.

Table 3: % Inhibition of compounds 5a-5j against various bacteria

Compound code	Bacteria and Fungi along with the zone of Inhibition(mm)			
	E.coli	B.subtilis	S.aureus	A.niger
5a	50	49	52	43
5b	70	75	81	80
5c	58	52	68	55
5d	72	73	77	74
5e	76	86	86	85
5f	55	64	46	62
5g	50	68	48	64
5h	64	60	59	40
5i	74	88	83	82
5j	72	70	72	76
Standard	85	90	89	90

Invitro Antimicrobial Activity

The synthesized compounds were screened for their antimicrobial activity17 using Aspergillus niger. Control experiment was carried out under similar condition using ketoconazole as standard. Turbidimetric18- 22method was used to check antimicrobial activity of the synthesized compounds at different concentration using ketoconazole as the positive control and DMSO as the negative control. Antifungal screening revealed that the test compounds showed good to moderate activity against Aspergillusniger. % inhibition for antifungal activity revealed that some of the test compounds like 5b,5d, 5e,5i,showed good inhibition as compared to the standard ketoconazole due to substitution with OH, Cl, and NO2 groups. 5a and 5h showed moderately active.

Results and Discussion

Ten distinct thiazolidinone derivatives (5a-5j) were produced in the current investigation. To determine the purity of these compounds, thin layer chromatography was carried out using benzene:ethanol (9:1) solvent solutions on pre-coated silica gel G, glass plates. Iodine chambers were used to see the spots. By using IR and 1HNMR, the structures of the produced compounds were verified. The distinctive absorption bands of the NH stretching, C=C stretching, No2 functional group, OCH3 group, and C=O stretching were visible using infrared spectroscopy. The synthetic compounds' 1H NMR spectra displayed chemical shifts, which are indicators of a compound's expected structure. These investigations served as the foundation for our decision to synthesize the compounds and test them for antibacterial activity. Newly synthesized compounds were tested for antibacterial activity against E. coli, S. aureus, and B. subtilis. The synthesized compounds have found to be better antimicrobial activity than parent compound. All the synthesised compounds have shown mild to good activity against the pathogenic bacteria and fungi.

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