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Synthesis and Characterization of Antimicrobial Activity of Novel Thiazolidinone Derivatives of 1,2-Benzisoxazole

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Abstract : A series of Thiazolidinone derivatives of 1,2-Benzisoxazole were synthesized. 4-hyroxy-2H-chromen-2-one is condensed with aromatic aldehyde to yield Schiff's base which on cyclization with Mercapto acetic acid yields Thiazolidinone derivatives of Benzisoxazole . The structure of synthesized compounds has been established on the basis of their IR, ^{1}HNMR and Elemental analysis. The purity of the compounds was confirmed by TLC. The synthesized compounds were screened for *In vitro* antibacterial andantimicrobial activity by turbidimetric methods. Compounds 5b, 5d, 5e, 5i showed better antibacterial activity with the reference standard Ciprofloxacin and Compounds 5b, 5d, 5e, 5i showed good antimicrobial activity with the reference standard ketoconazole.
Key Words : Coumarin, Schiff's base, Benzisoxazole.

Introduction:

Being a heterocyclic compound, Benzisoxazole finds use in research as a starting material[1] for the synthesis of larger bioactive structures and as intermediate[2] for the synthesis of other compounds which are useful as medicines. Thiazolidinones[3] are derivatives of thiazolidine, which also belongs to an important group of heterocyclic compounds. Literature revealed that Thiazolidinone derivatives have diverse biological activities[4,5,6] such as bactericidal[7], fungicidal[8], anticonvulsant[9,10], tuberculostatic, antithryoidal, potentiation of pentobarbital induced sleeping time. Therefore an attempt was made to synthesize Thiazolidinone derivatives of Benzisoxazole and to study the antibacterial activity of synthesized compounds. Lipinski's "rule of five"[11]which describes the ADME in human is employed for evaluating the drug likeness and the rule is important for drug development where 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. ^{1}HNMR were recorded on BrukerAvance II 500 MHz spectrophotometer. The chemical shifts were reported as parts per million downfield from tetramethylsilane using DMSO as solvent. ^{13}CNMR 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 25-30°c. Sodium acetate (885g,10.80 mol) was added to the above solution lot wise in half an hour. The reaction mass was stirred at 25-30°c for half an hour, heated to reflux (65-70°c) and maintained at reflux for 5-6hrs. After completion of the reactivity (by TLC), methanol was distilled under vaccum(<50°c). After complete removal of methanol, 7.0lit of water was added to the residue and the resulting solution was cooled to 10-15°c. The pH of the reaction mass was adjusted to 2-3 with 50%HCl and stirred the reaction for one hour at 10-15°c. The solid obtained was filtered and washed with 2lit of water. The solid was dired at 55-60°c.

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% $NaHCO_3$.

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.

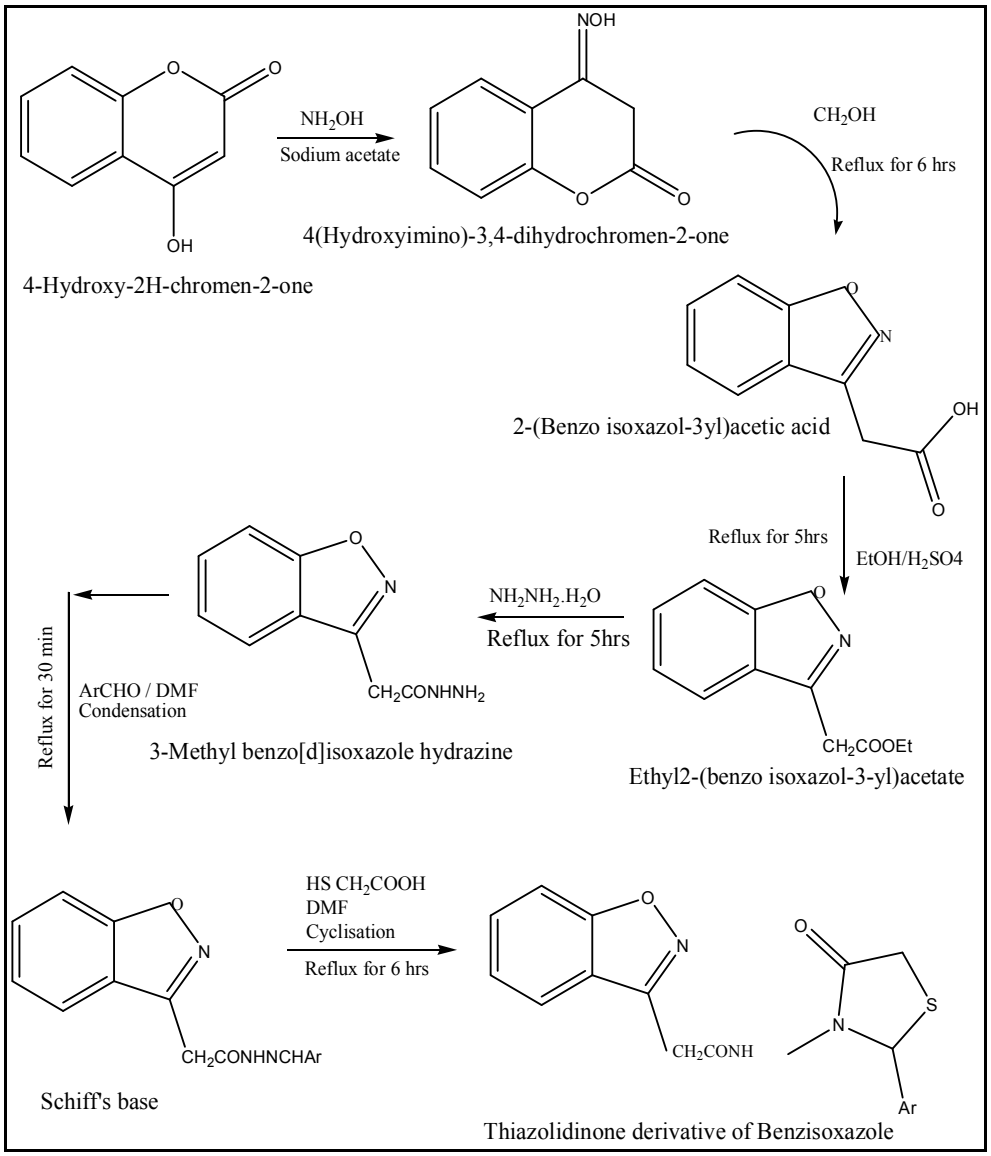

Table1: Drug likeliness properties.

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.

Table 2: Physico chemical parameters

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-CH_2CO, δ 3.2-3.3 (S,2H, Thiazolidinone ring CH_2).

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-CH_2CO,δ 3.3 (S,2H, Thiazolidinone ring CH_2).

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.

Compound 5c:

IUPAC name: 2-(Benzo{d}isoxazol-3-yl)-N- (2-(4-methoxyphenyl)-4-oxothiaxzolidin-3-yl)acetamide

IR (KBr)cm^{-1} (C=C str) 1451.51, (N-H str) 3530.51, (N-H bend)1623.25, (C=O str)1708.69, (=C-H str)3098.12, (C-N str) 1154.47, (C-S str) 683.89.

1H-NMR(δppm) :δ8.0 (S,1H,NH),δ6.2-7.5 (m,8H,Ar-H), δ 5.8(S,1H, Thiazolidinone ring)δ3.44 (S,2H,3-CH_2CO,δ 3.3 (S,2H, Thiazolidinone ring CH_2).

Elemental analysis: Calculated for C 65.45 and found 65.69, Calculated for H 5.09 and found 5.88,Calculated for N 6.90 and found 7.31.

Compound 5d:

IUPAC name: 2-(Benzo{d}isoxazol-3-yl)-N- (2-(3-hydroxyphenyl)-4-oxothiaxzolidin-3-yl)acetamide

IR (KBr)cm^{-1} : (N-H str) 3510.85, (N-H bend)1607.88, (C=O str)1708.73, (=C-H str)3068.01, (C-N str) 1154.38, (C-S str) 683.82, (O-H str)3530.64 .

1H-NMR(δppm) :δ 7.8 (S,1H,NH),δ6.4-7.6 (m,8H,Ar-H), δ 5.8(S,1H, Thiazolidinone ring)δ3.6 (S,2H,3-CH_2CO,δ 3.3 (S,2H, Thiazolidinone ring CH_2).

Compound 5e:

IUPAC name: 2-(Benzo{d}isoxazol-3-yl)-N- (2-(2-chlorophenyl)-4-oxothiaxzolidin-3-yl)acetamide

IR (KBr)cm^{-1} (C- Clstr) 751.97, (N-H str) 3510.77, (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),δ6.9-7.5 (m,8H,Ar-H), δ5.9(S,1H, Thiazolidinone ring)δ3.5 (S,2H,3-CH_2CO,δ 3.2(S,2H, Thiazolidinone ring CH_2).

Compound 5f:

IUPAC name: 2-(Benzo{d}isoxazol-3-yl)-N- (2-(3-nitrophenyl)-4-oxothiaxzolidin-3-yl)acetamide

IR (KBr)cm^{-1} (C-N str) 1328.06, (N-H str) 3510.60, (N-H bend)1562.27, (C=O str)1622.27, (=C-H str)3083.97, (C-N str) 1106.93, (C-S str) 710.52.

1H-NMR(δppm) :δ8.0 (S,1H,NH),δ6.9-7.6 (m,8H,Ar-H), δ 5.8(S,1H, Thiazolidinone ring)δ3.5 (S,2H,3-CH_2CO,δ 3.3 (S,2H, Thiazolidinone ring CH_2).

Compound 5g:

IUPAC name: 2-(Benzo{d}isoxazol-3-yl)-N- (2-(2-nitrophenyl)-4-oxothiaxzolidin-3-yl)acetamide

IR (KBr)cm^{-1} (C- N str) 1379.37, (N-H str) 3440.65, (N-H bend)1563.66, (C=O str)1710.73, (=C-H str)3073.97, (C-N str) 1144.95, (C-S str) 683.92, (C=Cstr) 1451.86.

1H-NMR(δppm) :δ8.0 (S,1H,NH),δ6.9-7.8 (m,8H,Ar-H), δ 5.8(S,1H, Thiazolidinone ring)δ3.4 (S,2H,3-CH_2CO,δ 3.5 (S,2H, Thiazolidinone ring CH_2).

Compound 5h:

IUPAC name: 2-(Benzo{d}isoxazol-3-yl)-N- (2-(3,4- dimethoxy)-4-oxothiaxzolidin-3-yl)acetamide

IR (KBr)cm^{-1} (C=Cstr) 1495.48, (N-H str) 3510.66, (N-H bend)1560.40, (C=O str)1623.27, (=C-H str)3067.81, (C-N str) 1154.35, (C-O str) 2816.40.

1H-NMR(δppm) :δ8.2(S,1H,NH),δ6.5-7.4 (m,8H,Ar-H), δ 5.8(S,1H, Thiazolidinone ring)δ3.5 (S,2H,3-CH_2CO,δ 3.3(S,2H, Thiazolidinone ring CH_2).

Compound 5i:

IUPAC name: 2-(Benzo{d}isoxazol-3-yl)-N- (2-(4-chlorophenyl)-4-oxothiaxzolidin-3-yl)acetamide

IR (KBr)cm^{-1} (C- Clstr) 751.77, (N-H str) 3510.88, (N-H bend)1607.89, (C=O str)1708.16, (=C-H str)3067.80, (C-N str) 1154.29, (C-S str) 683.69, (C=Cstr) 1495.29.

1H-NMR(δppm) :δ8.0 (S,1H,NH),δ6.9-7.4 (m,8H,Ar-H), δ 5.9(S,1H, Thiazolidinone ring)δ3.4 (S,2H,3-CH_2CO,δ 3.2 (S,2H, Thiazolidinone ring CH_2).

Compound 5j:

IUPAC name: 2-(Benzo{d}isoxazol-3-yl)-N- (2-(2-hydroxyphenyl)-4-oxothiaxzolidin-3-yl)acetamide

IR (KBr)cm^{-1} (C=N str) 1622.70, (N-H str) 3466.19, (N-H bend)1607.71, (C=O str)1708.41, (=C-H str)3068.07, (C-N str) 1154.41, (C-S str) 683.65, (C=Cstr) 1495.42.

1H-NMR(δppm) :δ 7.9 (S,1H,NH),δ6.5-7.4 (m,8H,Ar-H), δ 5.8(S,1H, Thiazolidinone ring)δ3.4 (S,2H,3-CH_2CO,δ 3.3 (S,2H, Thiazolidinone ring CH_2).

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. Turbidimetric[12-16] 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 activity[17] using *Aspergillus niger*. Control experiment was carried out under similar condition using ketoconazole as standard. Turbidimetric[18-22]method 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 NO_2 groups. 5a and 5h showed moderately active.

Results and Discussion:

In the present work 10 different thiazolidinone derivatives (5a-5j) were synthesized. Thin layer chromatography was performed on pre-coated silica gel G, glass plates using benzene:ethanol (9:1) solvent systems to ascertain the purity of these compounds. Spots were visualized by iodine chamber. The structure of the synthesized compounds were confirmed by IR and 1HNMR. Infrared spectroscopy showed the characteristic absorption bands of NH stretching, C=C stretching, No2 functional group, OCH3 group, C=O stretching. The 1H NMR spectra of the synthesized compounds showed chemical shifts, which are characteristics of the anticipated structure of compounds. Based on these studies, we have taken up the compounds for synthesis and evaluated forantibacterial activity. Antibacterial screening of newly synthesized compounds was carried out against *E. coli, S. aureus B.subtilis.* The synthesized compounds have found to be betterantimicrobial activity than parent compound. All the synthesised compounds have shown mild to good activity against the pathogenic bacteria and fungi.

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