Research Article

Biological and Pharmacological Significance of Newly Synthesized β-D-Glucuronides

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ABSTRACT

3-Methyl-5-(3`-phenyl prop-2`-enoyl)-1,2-benzisoxazoles 1a undergoes interaction with thiourea to yield 3-methyl-5-(4`-phenyl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazole **2a**, which on oxidation with $KMnO_4$ gives 5-(4`-phenyl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazole-3-carboxylic acid **3a**. Glucuronidation of these 5-(4`-phenyl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazole-3-carboxylic acid with free glucuronic acid afforded β-D-Glucuronosyl-5-(4`-phenyl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazole-3-carboxylate **4a**. The title compounds are found to have antibacterial and antifungal activities. The structures of the products have been assigned on the basis of ^{1}H NMR, ^{13}C NMR, FAB-MS, optical activity and elemental analysis. All the synthesized compounds were evaluated their antibacterial and antifungal activities by cup-plate method. The compounds showed some interesting antibacterial and antifungal activities.

Keywords: Chalcones, thiopyrimidines, Carboxylic acids, β-D-Glucuronides.

1. INTRODUCTION

In recent years thiopyrimidines have attracted much attention due to their diverse properties and wide spectrum of biological and pharmacological activities. Pyrimidines are important because they are integral part of the genetic material *viz*. DNA and RNA as nucleotides and nucleosides. Sulfa drugs containing pyrimidines moieties namely sulfadiazine, sulfamerazine and sulfadimidine are used for chemotherapy of infections. Many pyrimidines derivatives have biological properties particularly being calcium channel blockers and have been used as diuretic, therapeutic agents and possess analgesic and anti-inflammatory activities[1-5]. 1,2-Benzisoxazole derivatives have been used as potential tuberculostatic, anti-inflammatory, analgesic, sedative etc. agents[6-8]. Glucuronidation is the addition of glucuronic acid to a substrate. Glucuronidation is often involved in xenobiotic metabolism of substances such as drugs, pollutants, bilirubin, androgens, estrogens, mineralocorticoids, glucocorticoids, fatty acid derivatives, retinoids, and bile acids. These linkages involve glycosidic bonds. β-D-Glucuronides are the conjugation products of compounds possessing a carboxylic acid functional group with free D-glucuronic acid. β-D-Glucuronides are polar and chemically reactive metabolites. It form covalent adduct with protein, generating increasing interest as potential mediator of hypersensitivity reaction and it shows profound effect on drug metabolism[9-14].

2. EXPERIMENTAL

2.1 Material and methods

All the starting materials and reagents were obtained from Merk, Aldrich (USA) and Rankem Pvt Ltd (India) and were used without further purification. The course of reaction and purity were ascertained by performing Thin Layer Chromatography. Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded as KBr pallets on Shimadzu-810 IA and Perkin Elmer FTIR spectrometer and only significant absorption levels (cm^{-1}) are listed. ^{1}H NMR spectra were recorded on Bruker AC-300F (300MHz) instrument with TMS as internal standard and the chemical shift are expressed in ppm values. Mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer using *m*-nitro benzyl alcohol (NBA) matrix. The FLASH EA 1112 C, H and N analyzer, made by Thermo Finigen, Italy for elemental analysis.

2.2 Synthesis:

3-Methyl-5-(3`-phenyl prop-2`-enoyl)-1,2-benzisoxazole (1a):

A mixture of 3-methyl-5-acetyl-1,2-benzisoxazole (0.1mol, 17.5g), benzaldehyde (15mL), ethyl alcohol (25mL) and a few drops of piperidine was warmed for 1hour. It was cooled to 0^0C, the yellow solid formed was filtered, washed with distilled water and dried. It was crystallized from distilled water (9.0g, 50.9%), m.p. 120^0C. It gave dark red color with conc. H_2SO_4. IR (KBr): 1715 (C=O str aryl ketone), 1562 (C=C), 1362 (C-O), 3005 (Ar-H); ^{1}H NMR signal at δ2.2 (s, CH_3), 7.4-9.3 (8H, m, aromatic protons), 6.0-6.7 (2H, d, CH=CH); FAB-MS: M^+ 264, m/z 248, m/z 185, m/z 174, 160 and m/z 132.

Similarly, other 3-methyl-5-(3`-aryl prop-2`-enoyl)-1,2-benzisixazoles **1a-o** were synthesized.

3-Methyl-5-(4`-phenyl-2-thiopyrimidin-6`-yl)-1,2-benzisoxazole (2a):

A mixture of 3-methyl-5-(3`-phenyl prop-2`-enoyl)-1,2-benzisoxazole **1a** (0.01mol, 2.6g), thiourea (0.7g), ethyl alcohol (20mL) and KOH (0.5g) was refluxed on water bath for 5hours. It was cooled and acidified with dil. HCl (1.0mL) and was poured on ice-cold water (75mL). The yellow solid obtained was filtered, washed with cold water (150mL), dried and crystallized with aq. alcohol (yield 2.0g, 76%), m.p. 131^0C. IR (KBr): 1622 (C=N str.), 2592 (C-SH str), 3144 (N-H str), 1222 (C-O-N str vibration in isoxazole ring), 3005 (C-H str –CH_3); ^{1}H-NMR signal at δ2.2 (s, CH_3), 4.0 (s, Ar-SH), 6.2-7.7 (9H, m, aromatic protons), 6.5 (s, 1H for isoxazoles ring C_4-H); FAB-MS: M^+ 319, m/z 304, m/z 286, m/z 187, m/z 154.

Following the above procedure 3-methyl-5-(4`-aryl-2`-thiopyrimidin-6`-yl)-1,2-benzisixazoles **2a-o** were prepared and compounds gave satisfactory C, H, and N analysis (**Table 1**).

5-(4`-Phenyl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazole-3-carboxylic acid (3a):

In 100mL round flask a mixture of 3-methyl-5-(4`-phenyl-2-thiopyrimidin-6`-yl)-1,2-benzisoxazole **2a** (0.01mol, 3.19g), sodium carbonate (1.5g), $KMnO_4$ (1.5g) and water (100mL) was refluxed under water bath for 4h, until the purple color has disappeared. It was acidified with dil. H_2SO_4, the excess manganese dioxide was removed by sodium metabisulphite (0.1g), filtered, washed and crystallized with water (1.4g, 43.8%), m.p. 103^0C. IR (KBr): 3468 (OH peak), 1714 (C=O), 2563 (C-SH str), 1362 (C=N ter amine), 1222 (C-O-N str); ^{1}H-NMR signal at δ10.2 (s, COOH), 4.1 (s, Ar-SH), 6.2-7.7 (9H, m, aromatic protons); FAB-MS: M^+ 350, m/z 316, m/z 272, m/z 187 and m/z 162.

In the same way, various carboxylic acids, 5-(4`-aryl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazole-3-carboxylic acids **3a-o** were synthesized.

β-D-Glucuronosyl-5-(4`-phenyl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazole-3-carboxylate (4a):

To a solution of 5-(4`-phenyl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazole-3-carboxylic acids **3a** (0.01 mol, 3.49g) in dry pyridine (5mL), which was kept at 0^0C, D-glucuronic acid (1.94g) was added in portion with constant stirring. The reaction mixture was left at room temperature for 18h and it was poured over crushed ice. The resulting white product was filtered and washed with ice-cold water (1.90g, 54.4%). FAB-MS: M^+ 525, the base peak appearing at m/z 350 (due to loss of D-glucuronic acid moiety), m/z 316, m/z 304, m/z 272, m/z 187 and m/z 162.

Following the above procedure, others β-D-glucuronosyl-5-(4`-aryl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazole-3-carboxylate **4a-o** were prepared starting from the appropriate carboxylic acids. Compounds gave satisfactory C, H, and N analysis (**Table 2**).

3. Microbial activities

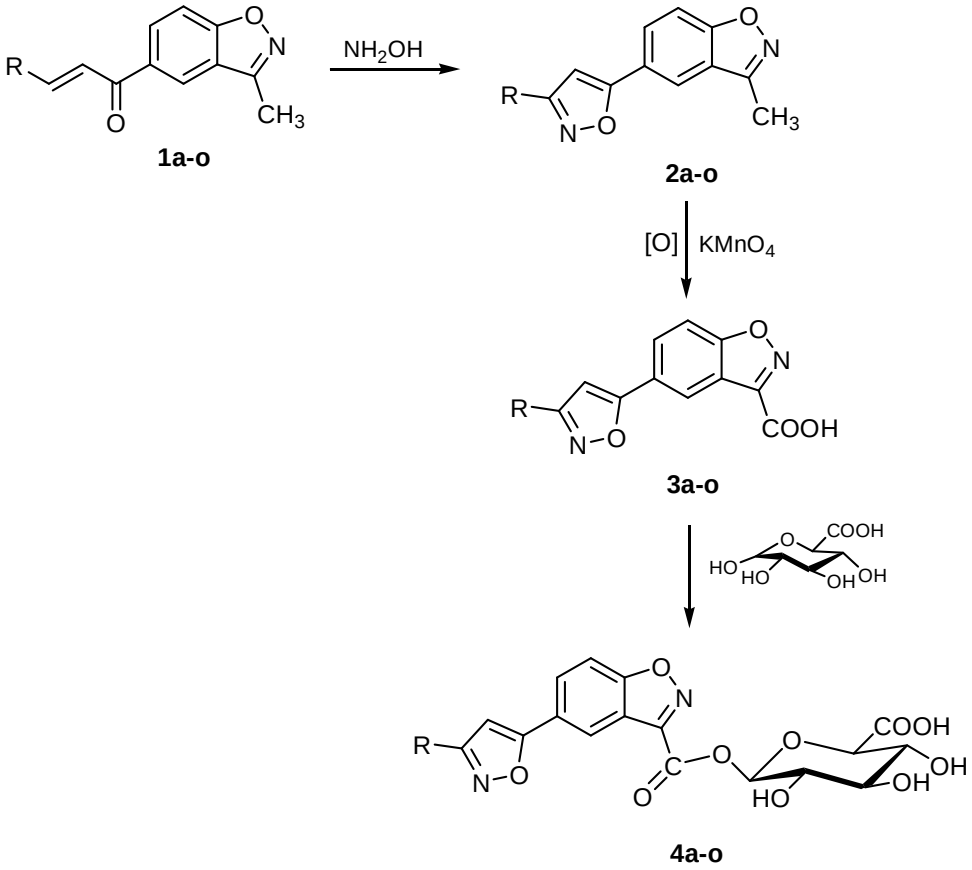
3.1 Antimicrobial Activity

The synthesized compounds were tested for their antibacterial activities by the using the cup-plate method against *Bacillus subtilis* (gram-positive) and *Escherichia coli* (gram-negative) at concentration of 100μg/mL in DMF. Pure Norfloxacin was used as standard antibiotic for the comparison of the results. The sterilized Mullier-Hinton agar medium 50mL was inoculated with test organism and poured into petridishes. Then four holes of 6mm were completely filled with different test solution. The plates were then incubated for 24h at 37^0C and zones of inhibitions were measured. Similar procedure was adopted for pure Norfloxacin and the corresponding zone diameters were compared. Screening results indicate that compounds **4a-o** showed to excellent bacteriocidal activities against both organisms (**Table 3**).

3.2 Antifungal Activity

The antifungal activity of synthesized compounds was evaluated by the using above

same procedure (cup-plate) against *Aspergillus niger* and *Candida albicans* at a concentration 100μm/mL in DMF. The plates were incubated for 8 days at 37^0C. The zones of inhibitions were measured. A commercial fungicide griseofulvin was also tested under similar condition with a view of comparing the results. The compounds showed significant fungi toxicity against both the fungi (**Table 3**).



Scheme

R = **a**; C_6H_5 **f**; *o*-ClC_6H_4 **k**; 3-C_5H_4N

b; *o*-OHC_6H_4 **g**; *p*-ClC_6H_4 **l**; 4-C_5H_4N

c; *p*-OHC_6H_4 **h**; *o*-$NO_2C_6H_4$ **m**; 3-C_4H_3O

d; 2,4-$(OH)_2C_6H_3$ **i**; *m*-$NO_2C_6H_4$ **n**; 3-C_8H_5N

e; *p*-OH-*m*-$OCH_3C_6H_3$ **j**; 2-C_5H_4N **o**; *p*-*N*$(CH_3)_2C_6H_4$

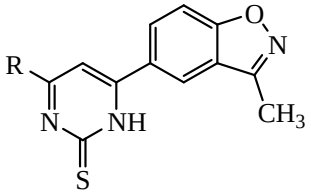
4. RESULT AND DISCUSSION

In view of pronounced biological and pharmacological applications of some β-D-Glucuronosyl-5-(4`-aryl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazole-3-carboxylates **4a-o** have been prepared with a view of study their biological significance by the glucuronidation of 5-(4`-aryl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazole-3-carboxylic acids **3a-o** with free D-glucuronic acid using dry pyridine. The above acids were synthesized by the oxidation of 3-methyl-5-(4`-aryl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazoles **2a-o** using alkaline $KMnO_4$ and the products **2a-o** were prepared by cyclization of 3-methyl-5-(3`-aryl prop-2`-enoyl)-1,2-benzisoxazoles **1a-o** and thiourea and compounds **1a-o** were obtained by the condensation of 3-methyl-5-acetyl-1,2-benzisoxazole with different aldehydes using piperidine by Claisen-Schmidt condensation.

5. CONCLUSION

In conclusion, I have synthesized 5-(4`-aryl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazole-3-carboxylic acids and their glucuronides. These novel compounds were evaluated for in vitro antibacterial activity against *Escherichia coli* and *Bacillus subtilis* strains as well as for antifungal activity against *Candida albicans* and *Aspergillus niger* strains using cup-plate technique. Some β-D-glucuronides gives excellent results against bacterial and fungal strain. All synthesized compounds are confirmed by FT-IR, ^{1}H-NMR, FAB-MS, optical activity and elemental analysis.

Table 1: Characterization data of 3-methyl-5-(4`-aryl-2`-thiopyrimidin-6`-yl)-1,2-benzisoxazoles

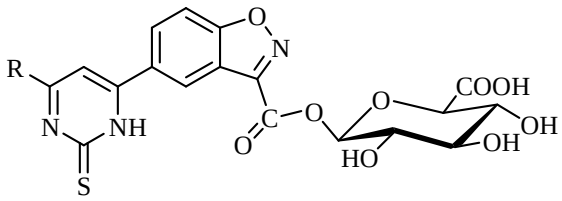


2a-o

Compd	R	Molecular Formula	Mol. wt.	M.P. ^{0}C	Yield %	R_f value	Found (calcd) % C	H	N
1.	C_6H_5-	$C_{18}H_{13}N_3OS$	319	131	76.0	0.29	67.68 (67.69	4.09 4.10	13.15 13.16)
2.	*o*-OHC_6H_4-	$C_{18}H_{13}N_3O_2S$	335	187	71.5	0.36	64.45 (64.46	3.90 3.91	12.92 12.94)
3.	*p*-OHC_6H_4-	$C_{18}H_{13}N_3O_2S$	335	105	78.5	0.37	64.45 (64.46	3.89 3.91	12.93 12.94)
4.	2,4-$(OH)_2C_6H_3$-	$C_{18}H_{13}N_3O_3S$	351	219	67.8	0.24	61.50 (61.53	3.70 3.73	11.95 11.96)
5.	*p*-OH--*m*-$OCH_3C_6H_3$-	$C_{19}H_{15}N_3O_3S$	365	181	83.5	0.34	62.43 (62.45	4.14 4.14	11.49 11.50)
6.	*o*-ClC_6H_4-	$C_{18}H_{12}ClN_3OS$	353	211	74.0	0.30	61.08 (61.10	3.41 3.42	11.87 11.88)
7.	*p*-ClC_6H_4-	$C_{18}H_{12}ClN_3O_3S$	353	191	84.0	0.22	61.10 (61.10	3.40 3.42	11.86 11.88)
8.	*o*-$NO_2C_6H_4$-	$C_{18}H_{12}N_4O_3S$	364	141	86.5	0.33	59.32 (59.33	3.30 3.32	15.37 15.38)
9.	*m*-$NO_2C_6H_4$-	$C_{18}H_{12}N_4O_3S$	364	161	80.0	0.23	59.35 (59.33	3.33 3.32	15.36 15.38)

10.	2-C_5H_4N-	$C_{17}H_{12}N_4OS$	320	155	8.0	0.24	63.70	3.76	17.37
							(63.73	3.78	17.49)
11.	3-C_5H_4N-	$C_{17}H_{12}N_4OS$	320	112	75.8	0.36	63.72	3.78	17.50
							(63.73	3.78	17.49)
12.	4-C_5H_4N-	$C_{17}H_{12}N_4OS$	320	116	90.0	0.28	63.71	3.75	17.46
							(63.73	3.78	17.49)
13.	3-C_4H_3O-	$C_{16}H_{11}N_3O_2S$	309	172	71.5	0.40	62.10	3.55	13.57
							(62.12	3.58	13.58)
14.	3-C_8H_5N-	$C_{20}H_{14}N_4OS$	358	176	93.5	0.29	67.00	3.94	15.62
							(67.02	3.94	15.63)
15.	*p*-$N(CH_3)_2$-C_6H_4-	$C_{20}H_{18}N_4OS$	362	159	83.5	0.27	66.27	5.05	15.47
							(66.28	5.01	15.46)

Table 2: Characterization data of β-D-glucuronosyl-5-(4`-aryl-2`-thiopyrimidin-6-yl)--1,2-benzisoxazole-3-carboxylates.



4a-o

Compd	R	Molecular Formula	Mol. wt.	$[\alpha]^{25}_D$ (^{0})	Yield %	Rf value	Found (calcd) % C	H	N
1.	C_6H_5-	$C_{24}H_{19}N_3O_9S$	525	+45.2	54.44	0.27	54.84	3.63	8.08
							(54.85	3.64	8.00)
2.	*o*-OHC_6H_4-	$C_{24}H_{19}N_3O_{10}S$	541	+41.3	47.94	0.15	53.20	3.53	7.75
							(53.23	3.54	7.76)
3.	*p*-OHC_6H_4-	$C_{24}H_{19}N_3O_{10}S$	541	+48.9	43.83	0.20	53.22	3.52	7.73
							(53.23	3.54	7.76)
4.	2,4-$(OH)_2$ C_6H_3-	$C_{24}H_{19}N_3O_{11}S$	557	+49.1	49.86	0.19	51.67	3.51	7.53
							(51.70	3.44	7.54)
5.	*p*-OH-*m*-$OCH_3C_6H_3$-	$C_{25}H_{21}N_3O_{10}S$	571	+39.2	53.16	0.21	52.55	3.71	7.34
							(52.54	3.70	7.35)
6.	*o*-ClC_6H_4-	$C_{24}H_{18}ClN_3O_9S$	559	+48.6	46.99	0.22	51.47	3.20	7.49
							(51.48	3.24	7.51)
7.	*p*-ClC_6H-	$C_{24}H_{18}ClN_3O_9S$	559	+48.8	45.69	0.16	51.48	3.23	7.50
							(51.48	3.24	7.51)
8.	*o*-$NO_2C_6H_4$-	$C_{24}H_{18}N_4O_{11}S$	570	+39.9	46.95	0.26	50.51	3.23	7.50
							(50.52	3.24	7.51)

9.	*m*-$NO_2C_6H_4$-	$C_{24}H_{18}N_4O_{11}S$	570	+38.7	45.68	0.22	50.51 (50.52	3.20 3.24	7.48 7.51)
10.	2-C_5H_4N-	$C_{24}H_{18}N_4O_9S$	526	+47.1	47.14	0.13	52.40 (52.47	3.21 3.45	10.61 10.64)
11.	3-C_5H_4N-	$C_{24}H_{18}N_4O_9S$	526	+37.8	48.57	0.24	52.44 (52.47	3.42 3.45	10.61 10.64)
12.	4-C_5H_4N-	$C_{24}H_{18}N_4O_9S$	526	+48.0	51.42	0.19	52.45 (52.47	3.41 3.45	10.63 10.64)
13.	3-C_4H_3O-	$C_{22}H_{17}N_3O_{10}S$	515	+37.9	47.19	0.24	51.25 (51.26	3.32 3.32	8.18 8.15)
14.	3-C_8H_5N-	$C_{26}H_{20}N_4O_9S$	564	+43.7	48.96	0.17	55.30 (55.31	3.58 3.57	9.92 9.93)
15.	*p*- $N(CH_3)_2$ C_6H_4-	$C_{26}H_{24}N_4O_9S$	568	+47.8	56.12	0.14	54.91 (54.92	4.25 4.26	9.85 9.85)

Table 3: Data for in vitro antibacterial and antifungal activities of compounds 4a-o

	Diameter of Inhibition Zone (in mm) Against			
	Bacterial Strains		Fungal Strain	
Products	***E. Coli***	***B. subtilis***	***A. niger***	***C. albicans***
4a	15	14	16	14
4b	13	14	12	11
4c	15	15	13	16
4d	--	10	08	22
4e	16	11	17	21
4f	14	--	22	23
4g	10	16	11	13
4h	17	14	--	18
4i	13	09	15	28
4j	12	14	23	--
4k	15	15	13	16
4l	10	13	23	23
4m	17	16	15	18
4n	--	12	21	18
4o	11	15	19	22

-- = No inhibition of growth. Diameter of zone of inhibition from 13-16 (in mm) shows excellent activity and that of 9-12 (in mm) exhibit moderate activity for bacterial strains. Diameter of zone of inhibition from 22-28 (in mm) shows excellent activity, that of 15-21 (in mm) exhibits moderate activity and that of 11-14 (in mm) shows poor activity for fungal strains. Norfloxacin 100µg/mL used as standard against *E. coli* and *B. subtilis* diameter of zone of inhibition is 20. Griseofulvin 100µm/mL used as standard against *A. niger* and *C. albicans* diameter of zone of inhibition is 32.

6. ACKNOWLEDGMENT

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