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"Synthesis and Biofunctional Evaluation of Novel Isoxazole, C-Nucleoside and Thioxopyrimidine Derivatives"

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ABSTRACT

Thioxopyrimidines are an important class of compounds in the fields of biology and chemistry that play significant roles in drug discovery, nucleic acid chemistry, enzyme inhibition, and various other fields of science and technology. Their structural diversity and potential bioactivities make them valuable tools for researchers. This work presents a thorough synthesis and analysis and bio-functional assessment of a variety of isoxazole C-Nucleoside, and thioxopyrimidine derivative. The structural characterization of the synthesized compounds was achieved through spectral analyses, including IR, ^{1}H NMR, and mass spectra and also deals with its antibacterial and antifungal activity.

Keywords: Glucosides, Thiopyrimidines, Benzisoxazoles, Antimicrobial activities, Pharmaceutical properties.

INTRODUCTION

Organic chemistry's thiopyrimidines constitute an intriguing subject with a wide range of practical and research applications. They make adaptable building blocks for the synthesis of diverse compounds with specific features because of their distinctive sulfur-containing structure. In contrast to regular pyrimidines, such as uracil or cytosine found in DNA and RNA, thiopyrimidines contain sulfur atoms in place of oxygen atoms within the ring.[21] Glucosides are other organic substances that can be found in both plants and animal species. They are essential for producing organic molecules more readily soluble in water and reducing the toxicity of aglyconemoieties. It has been discovered that the metabolic and antitumor properties of glucosides. The glucosylation reaction is essential for the production of complex glucosides, oligosaccharides, complex carbohydrate conjugates,

and biomolecules based on carbohydrates. They serve as the main carrier for the aglycone moiety and act as a pharmacophoric group which helps target cells in recognizing the structure. Glycosides are consisting of a sugar remnant that links up to moiety associated with aglycone, where the non-carbohydrate group is links the sugar molecule in glucosides[1-3]. Several glycosides are utilized in the production of sweetening agents, additives for food, non-ionic surfactants and antibiotics for use in pharmaceuticals, synthetic glycogen biosynthesis primers, and cosmetics[4] aglycones associated to glucose show significant effects involving cytotoxicity, antitumor, anti-inflammatory, anti-carcinogenic,[5] antiviral, antifungal,[6] antimicrobial activity. molluscicidal, and anti-hypercholesteremic.[7] steroidal glycosides have been reported to exhibit plant growth stimulant activities[8].

The alcohols and thiols are equivalent to each other with different functional groups in both, the -SH of thiols have various useful biological activities therefore studied with scientific interest. Due its reactive nature towards free radicals plays a significant role in biological activities as well as it protecting against harmful ionizing radiation and have significant role in metabolism of drugs in body.[20] The higher Molecular weight thiols are important ingredient of natural flavor's, While lower molecular weight thiols with unpleasant smell are widely used of the diagnosis of extreme thyroid storm. Thiopyrimidine scaffold abundantly present of pharmaceutical products with antiviral, analgesic, and most important compounds used in the non-destructive therapy of thyroid disease; they are the treatment of choice in pregnancy and adolescence.

Pyrimidine compounds recently came to light to have antimicrobial and antiinflammatory effects, antitumor and anticancer activities[9-12]. Pyrimidine plays a crucial role in nucleic acids and serves as a fundamental building block in numerous pharmaceutical products.[17]

Pyrimidinethione are frequently incorporated into metal-based medications intended to specifically address various biological processes or medical conditions.[18] Previously we have investigated various biological activities of these benzisoxazole derivatives aspromising class of heterocyclic compounds and show biological and pharmacological properties such as antitumor, antithrombotic, analgesic antioxidant, antimicrobial, anticancer, anticonvulsant, antipsychotic and anti-inflammatory agents[13-14]. With the broad biological and pharmacological uses of *O*-glucosides, thiopyrimidines, benzisoxazolesandextended research on heterocyclic compounds previously carried out, we produced a variety of pyrimidine and *O*-glucoside and examined their abilities to fight off microbes.[20]

EXPERIMENITAL

MATERIAL AND METHODS

All chemicals and reagents for initiation for this work were purchased from Merk, Aldrich, and Rankem. Pvt. Ltd and used directly after purchase without any additional treatment. A FT-IR spectrum from Shimadzu Ltd, the IR spectra were obtained using KBr disc. The chemical shift was reported in (δ) or ppm values in the ^{1}H NMR spectra, which were obtained using a Bruker AC-300F (300MHz) instrument using TMS as the internal standard. Mass spectrometer, the JEOL SX 102/DA-6000, was used to examine the mass spectra. TLC was carried out on silica gel plates to evaluate the purity of the synthesised compounds.

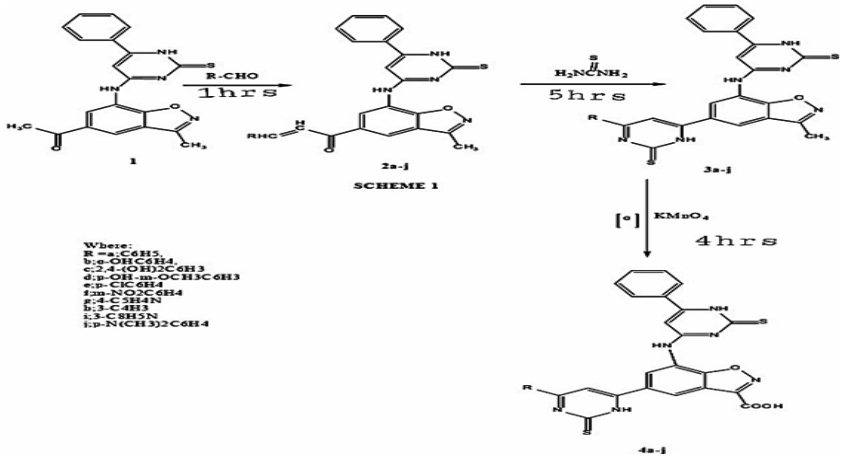

Fig. 1. Scheme

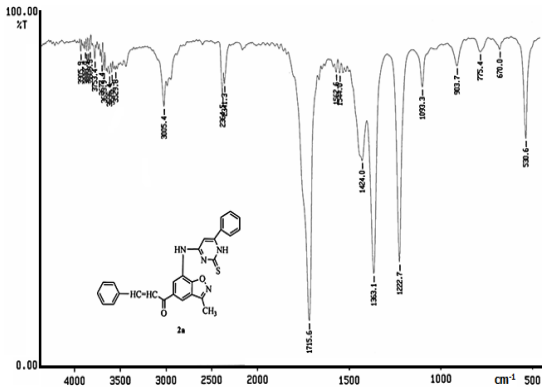

Fig. 2. FTIR-2a

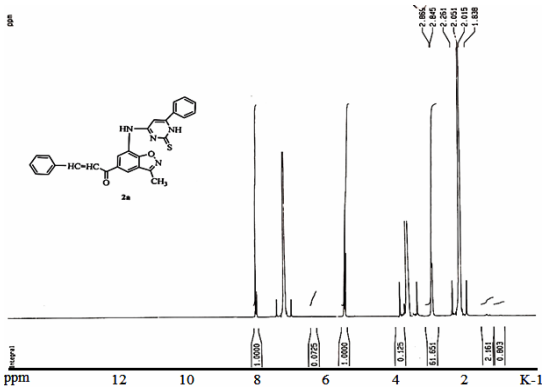

Fig. 3. NMR-2a

(DPBIMBO-MBIP)* (2a)

Compound (1a) have been synthesized and reported previously[16-17]. (MPTABE)*1 (0.1mol, 3.7 g), benzaldehyde (15 mL), A mixture of C_2H_5OH (25 mL) and some drops of $C_5H_{11}N$ was heated an hour. The solution was then cooled to 0°C, resulting in the formation of yellow solid. The solid was isolated by filtration, washed with distilled water, and dried. Finally, purified through recrystallization from dil. water(3.1 g, 83.7%), m. p. 118ºC. It gave dark red colour with conc. H_2SO_4. FT-IR (KBr): 1715 (C=O str. aryl ketone), 2364 (C-SH str.), 1544 and 1562 (chalcone, C=C), 1363 (C-O), 3329-3673 (Ar-H), 1562 (C=N), 3005 (N-H str.) 1222 (C-O-N isoxazole ring); ^{1}H-NMR signal at δ 3.2 (s, CH_3), 3.7 (s, Ar-SH), 2.8 (s, –NH), 7.4 (s, isoxazole ring), 5.4 (s, –CH, pyrimidine ring), 7.9-8.3 (aromatic protons), 6.9-7.3 (2H, d, CH=CH); ^{13}CNMR (22.6 MHz, $CDCl_3$): δ C 15.9 (CH_3), 118.4-132.0 ((-C=C, benzisoxazole), 155 (N=C, benzisoxazole), 145.2 (C=C, ethylene), 128 (C-H, benzene), 189.7 (C=O), 164 (C-NH), 180.4 (C=S, thioamide), 115.5 (-C=O), 135.2 (C-C), 118.4 (=C-C), 121.1 (NH–C), 164.0 (NH–C), 126.4 (C-N, thioamide); FAB-MS: M^+ 464, m/z 362, 334, 300, 258, 203 and 190. In the same way, other (DPBIMBO-MBIP)*(2a-j) were prepared.

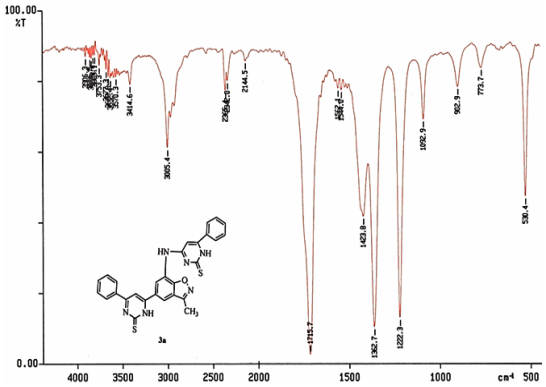

Fig. 4. FTIR-3a

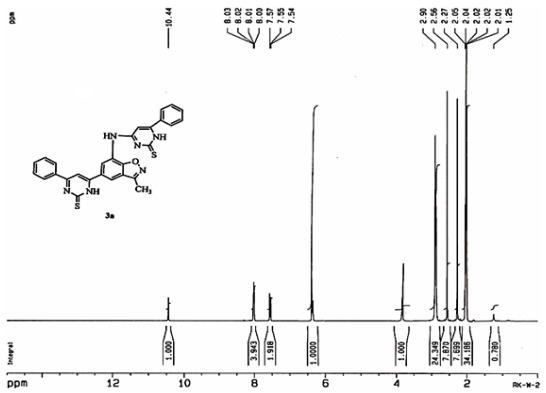

Fig. 5. NMR-3a

(DPBIMBO-PPT)* (3a)

Mixture of(DPBIMBO-MBIP)*2a (0.01 mole, 4.64 g), thiourea (0.76 g), ethanol and KOH (0.5 g) was refluxed for 5 h After cooling, the reaction mixture was acidified with HCl and placed into cold water. Obtained solid was filtered, washed,desiccated and solidified from alcohol (yield 3.2 g, 68%), m. p. 113ºC. IR (KBr): 2365 (C-SH str), 2950-3005 (two N-H str), 1222 (C-O-N str. isoxazole ring), 1715 (C=N), 3414 (N-H), 3570-3677 (Ar-H) cm^{-1}; ^{1}H NMR ($CDCl_3$): signal at 2.0 (s, –CH_3), 7.54-7.57 (m, aromatic), 3.8 (s, Ar-SH), 2.9 (s, –NH), 6.4 (s, isoxazole ring), 5.4 (s, –CH, pyrimidine ring); ^{13}CNMR (22.6 MHz, $CDCl_3$): δδC 15.9 (CH_3), 114.9-148.5 (-C=C, benzisoxazole), 155 (N=C, benzisoxazole), 176.3 (C-N, ethylene), 128.0-131.1 (C-H, benzene), 180.4 (2C=S, thioamide), 112.0 (C-NH), 176.3 (C=C), 85.7 (-C-C), 164.6 (-C=N), 148.5 (=C-N), 85.7 (C=C), 126.4 (-C=C), 164.3 (C-NH); FAB-MS: M^+ 520, m/z 506,490, 444, 334, 319, 258 and 148. Similarly,(DPBIMBO-PPT)* (3a-j) were obtained.

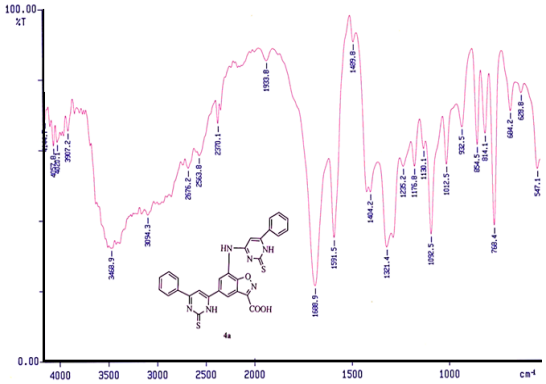

Fig. 6. FTIR- 4a

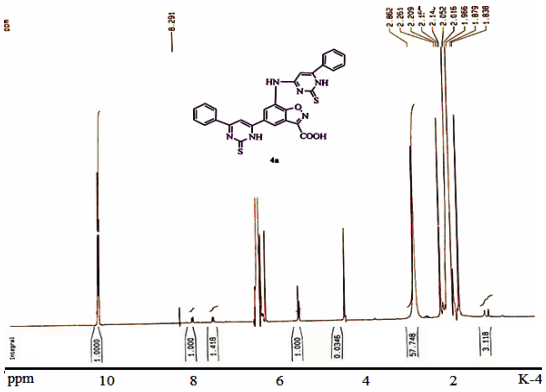

Fig. 7. NMR-4a

(DTBIO-CA)* (4a)

Oxidation of(DPBIMBO-PPT)*3a(0.01mol, 5.20 g), $NaCO_3$ (1.5 g), $KMnO_4$ (1.5 g) refluxed for 4 h with water and acidified with dil. H_2SO_4. After the reaction, any excess manganese dioxide was eliminated by adding sodium metabisulfite. The resulting mixture then filtered, and solid wasrinse with water. Finally, desired compound was obtained through crystallization from water (4.5 g, 86.5%), m. p. 110^{0}C.The product seemed to dissolve in dil. $NaHCO_3$ with bubbling. IR (KBr): 3094 (OH peak), 1688 (C=O ketones), 2563 (C-SH str.), 1321 (C=N 30–NH_2), 1235 (C-O-N isoxazole str.), 2563 and 2676 (two N-H str), 1321 (C-O-N str. isoxazole), 1591 (C=N), 3468 (N-H), 3907 (Ar-H), 2950 (–CH_3 str.); ^{1}H-NMR signal at ð10.4 (s, COOH), 4.6 (s, Ar-SH), 6.4-8.2 (m, aromatic), 2.0 (s, –NH), 6.3 (s, isoxazole ring), 5.6 (s, –CH, pyrimidine ring); ^{13}CNMR (22.6 MHz, $CDCl_3$): δ C 167.5 (=C=O, carboxyl), 114.9-148.5 (-C=C, benzisoxazole), 176.3 (C=C, ethylene), 164.0 (C=N, imine), 128.0-131.1 (C-H, benzene), 180.4 (2C=S, thioamide), 112.0 (C-NH), 176.3 (C=C), 85.7 (-C-C), 164.0 (-C-NH), 148.5 (=C-N), 133.2 (-C=N), 104.2 (C=C), 176.3 (C=C); FAB-MS of the compound showed a peak at m/z 550 $[C_{28}H_{18}N_6O_3S_2]^+$, m/z 506, 474, 398, 364, 349, 203 and 119.(DTBIO-CA)* (4a-j) were synthesized (Scheme I, Table 1).

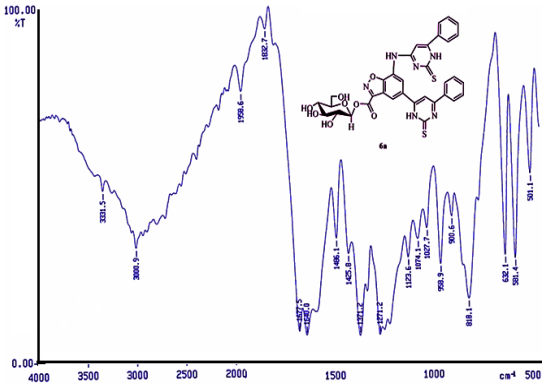

Fig. 8. FTIR-6a

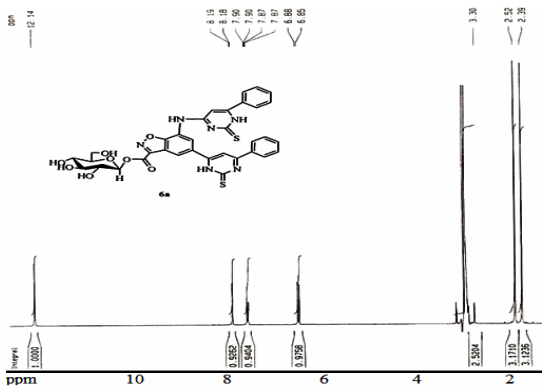

Fig. 9. NMR-6a

(GUP-DHPPA-DHDPTPB-ISb1OX-CA)*(6a)

Glucosylation

To a solution of (DTBIO-CA)*4a (0.01 mol, 5.50 g) and (TAGBr, (3 g) inCH_2Cl_2 was added$C_{16}H_{36}BrN$ (0.32 g) with stirring at 5°C. NaOH (10%, 10 mL)was gradually added to it over a duration of 30 min, agitating the reaction mixture for an additional 24 hours. The non-aqueous layer of (TAOA-Glu-DHPTPB-ISOX-CA)*(5a) had been separated rinsed with water, 5% dil $NaHCO_3$, and finally rinsed with water further before being dried.

Deacetylation

In (TAOA-Glu-DHPTPB-ISOX-CA)*(5a) added (26 mL) absolute methanol, (1.5 mL) of 0.5% CH_3NaO and kept at RT for 50 minute. The reaction blend was neutralized, dried and purified.A gelatinous mass of (GUP-DHPPA-DHDPTPB-ISOX-

CA)*(6a) was obtained. crystallized it from ethanol shortly after purification on a silica gel column. (3.2 g, 58.1%). Monosaccharides show in the range of 958-1271 cm^{-1}. Noabsorption band at 1175-1140 cm-1 showsthe polysaccharide's glycoside linkage, which shows the breakage of linkage. Sharp bands at 1271 and 1371 cm^{-1} were obtainedbecause ofester linkage of formed glycoside. A band at 3331 cm^{-1} is due to –OH groups. Sharp absorption peak at 2890-3000 cm^{-1} correspond to the alkyl CAH stretched oscillations. Bands at 1640 and 1677 cm^{-1} are due to C-O stretching and CAHdistortion. ^{1}H-NMR spectra of 6a product showing a series of signals between δ 3.73-3.15 shows sugar moiety and the down field signal at 12.14 (1H, s) and doublets at δ 6.88 and 6.85 which assigned H-8 and H-6, 7.87-8.19 (m, phenyl unit). ^{13}CNMR (22.6 MHz, $CDCl_3$): δ C 74.3-95.9 (C-H, tetrahydropyran), 59.0 (CH_2, carbohydrate), 147.1-148.5 (-C=C, benzisoxazole), 167.9 (carboxyl), 176.3 (C=C, ethylene), 164.0 (C=N, imine), 128.0-131.1 (C-H, benzene), 180.4 (2C=S, thioamide), 112.0 (C-NH), 176.3 (C=C), 85.7 (-C-C), 164.0 (-C-NH), 133.2 (=C-N), 104.2 (C=C), 126.4 (=NH). The resulting compound was discovered to be optically active and to have a specific rotation of +38.2° in water. Applying the same methodology, (GUP-DHPPA-DHDPTPB-ISOX-CA)*(6a-j) were ready and provided significant C, H, and N analyses.(Scheme II, Table 2).

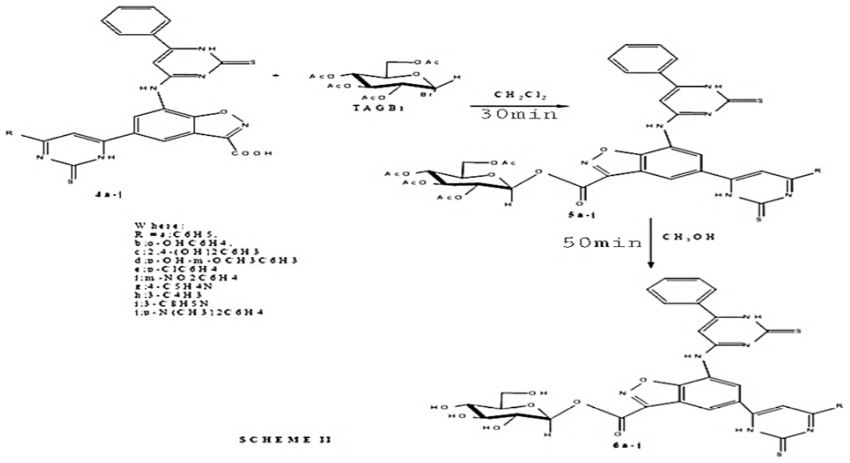

Fig. 10. Scheme II

RESULTS AND DISCUSSION

Thiopyrimidines and their *O*-glucosides were successfully synthesized with high yields, as outlined in Schemes I and II. The structural confirmation of the products was accomplished through spectral and elemental analyses and Melting point and boiling and optical properties which are given in Table 1, 2 including FT-IR was taken at 4000 cm^{-1} to 500 cm^{-1} confirms functional groups and bonds presentshow in the range of 958-1271 cm^{-1}. Noabsorption band at 1175-1140 cm^{-1} shows the polysaccharide's glycoside linkage, which shows the breakage of linkage. Sharp bands at 1271 and 1371 cm^{-1} were obtainedbecause ofester linkage of formed glycoside. A band at 3331 cm^{-1} is due to –OH groups. Sharp absorption peak at 2890-3000 cm^{-1} correspond to the alkyl CAH stretched oscillations. Bands at 1640 and 1677 cm^{-1} are due to C-O stretching and CAHdistortion. ^{1}H-NMR spectra of 6a product shows a series of signals between δ 3.73-3.15 Confirms sugar moiety and the down field signal at δ12.14(1H, s) and doublets at δδ 6.88 and 6.85 which assigned H-8 and H-6, δ 7.87-8.19 (m, phenyl unit)and ^{13}CNMR (22.6 MHz, $CDCl_3$): δ C 74.3-95.9 (C-H, tetrahydropyran), 59.0 (CH_2, carbohydrate), 147.1-148.5 (-C=C, benzisoxazole), 167.9 (carboxyl), 176.3 (C=C, ethylene), 164.0 (C=N, imine), 128.0-131.1 (C-H, benzene), 180.4 (2C=S, thioamide), 112.0 (C-NH), 176.3 (C=C), 85.7 (-C-C), 164.0 (-C-NH), 133.2 (=C-N), 104.2 (C=C), 126.4 (=NH). FAB-MS results which are given in Table 1, 2 confirmers molecular mass and structure of compounds. The Compound evaluated for Biological activity, as antibacterial and antifungal on species such as *Candida albicans* and *Aspergillus niger* (fungal) and *S. aureus* and *E. coli* (Bacterial) using different concentrations of (6a-j) and screening results showed excellent (22-28mm),

moderate (15-21mm) and poor (11-14mm) growth against the both organisms. The different zone of Inhibition by the action of compound against bacterial and fungal strains show in Table 3.

Antimicrobial activities

All Media and agar required for the bacterial and fungal growth are of highest grade and from Indian supplier. Synthesized compounds(6a-j)shows huge antibacterial and antifungal action on different species of bacteria and fungi therefore examined for antibacterial activities by using the cup plate procedure counter to *S. aureus* and *E. coli* at concentration of 150 µg/mL in DMF. The screening gave excellent outcomes. (13-16mm),(9-12mm) and inactive (below 8mm) growth counter to both the microbial strain. The antibacterial action of (6a-j) and derivatives is due inhibition of bacterial RNA synthesis and replication of DNA which inhibits the proteins syntheses.[21] The compound also dispute the bacterial cell wall by interacting with lipoproteins and leads to leakage of cellular fluids which is responsible for the death of bacteria.[19] Antifungal activity was determined by the same cup-plate method counter to *Candida albicans* and *Aspergillus niger* at a concentration of 100 µm/mL in DMF. The screening results showed excellent (22-28mm), moderate (15-21mm) and poor (11-14mm) growth against the both organisms. Norfloxacin 100 µg/mL was standard against *E. coli* and *S. aureus*, and Griseofulvin 100 µm/mL was standard against *A. niger* and *C. albicans* Table 3. The mechanism of action on fungal speciesis same as that for bacteria, one of the important essential ergosterol[22] which need for fungal cell wall synthesis is inhibited by (6a-j) leds to antifungal activity of the Compound.

Table: Abbreviation*

Sr. No	Abbreviation	Name
1	GUP-DHPPA-DHDPTPB-ISOX-CA	β-D-glucuropyranosyl-7-(1,2-dihydro-6-phenyl-2-thioxopyrimidin-4-ylamino)-5-(2,3-dihydro-6-phenyl-2-thioxopyrimidin-4-yl)benzoisoxazole-3-carboxylate
2	TAOA-Glu-DHPTPB-ISOX-CA	7-(2,3,4,6-tetra-O-acetyl-3-acetyl-δD-glucopyranosyl)-1,2-dihydro-6-phenyl-2-thioxopyrimidin-4-ylamino-5-(2,3-dihydro-6-phenyl-2-thioxopyrimidin-4-yl) benzoisoxazole-3-carboxylate
3	MPTABE	1-{3-Methyl-7-[(6-phenyl-2-thioxo-1,2-dihydropyrimidin-4-yl)amino]-1,2-benzisoxazol-5-yl}ethanone
4	DPBIMBO-PPT	6-(7-(1,2-dihydro-6-phenyl-2-thioxopyrimidin-4-ylamino)-3-methylbenzoisoxazol-5-yl) -4-phenylpyrimidine-2(1H)-thione
5	DPBIMBO-MBIP	(2E)-1-(7-(1,2-Dihydro-6-phenyl-2-thioxopyrimidin-4-ylamino)-3-methylbenzoisoxazol-5-yl) -3-phenylprop-2-en-1-one
6	TAGBr	2,3,4,6-tetra-O-acetyl-δD-glucopyranosyl bromide
7	DTBIO-CA	7-(1,2-dihydro-6-aryl-2-thioxopyrimidin-4-ylamino)-5-(2,3-dihydro-6-phenyl-2-thioxopyrimidin-4-yl)benzoisoxazole-3-carboxylic acids

Table 1: Physical data of (DTBIO-CA)* (4a-j)

Compound	R	Mol. F	Mol. Wt.	m. p. (°C)	Calculated (Found)%		
					C	H	N
4a	C_6H_5	$C_{28}H_{18}N_6O_3S_2$	550.6	114	61.08 -61.01	3.3 -3.28	15.26 -15.12
4b	*o*-OHC_6H_4	$C_{28}H_{18}N_6O_4S_2$	566.6	113	59.35 -59.35	3.2 -3.19	14.83 -14.8
4c	2,4-$(OH)_2C_6H_3$	$C_{28}H_{18}N_6O_5S_2$	582.6	111	57.72 -57.71	3.11 -3.09	14.42 -14.41
4d	*p*-OH-m-$OCH_3C_6H_3$	$C_{29}H_{20}N_6O_5S_2$	596.6	119	58.38 -58.36	3.38 -3.36	14.09 -14
4e	*p*-ClC_6H_4	$C_{28}H_{17}N_6O_3S_2Cl$	585.1	118	57.48 -57.48	2.93 -2.91	14.36 -14.35
4f	*m*-$NO_2C_6H_4$	$C_{28}H_{17}N_7O_5S_2$	595.6	113	56.46 -56.44	2.88 -2.85	16.46 -16.45
4g	4-C_5H_4N	$C_{27}H_{17}N_7O_3S_2$	551.5	110	58.79 -58.78	3.11 -3.1	17.78 -1.72
4h	3-C_4H_3O	$C_{26}H_{16}N_6O_4S_2$	540.5	117	57.77 -57.74	2.98 -2.97	15.55 -15.51
4i	3-C_8H_5N	$C_{30}H_{19}N_7O_3S_2$	589.6	114	61.11 -61.07	3.25 -3.23	16.63 -16.61
4j	*p*-$N(CH_3)_2C_6H_4$	$C_{30}H_{23}N_7O_3S_2$	593.6	123	60.69 -60.71	3.9 -3.89	16.52 -16.52

Table 2: Physical data of (GUP-DHPPA-DHDPTPB-ISOX-CA)*(6a-j)

Compound	R	Mol. F	Mol. Wt.	0	Rf ValueCalculated (Found) %			
						C	H	N
6a	C_6H_5	$C_{34}H_{28}N_6O_8S_2$	712.7	38.2	0.3	57.29	3.96	11.79
						-57.28	-3.96	-11.76
6b	*o*-OHC_6H_4	$C_{34}H_{28}N_6O_9S_2$	728.7	42.1	0.32	56.04	3.87	11.53
						-56.01	-3.85	-11.51
6c	2,4-$(OH)_2C_6H_3$	$C_{34}H_{28}N_6O_{10}S_2$	744.7	41.1	0.28	54.83	3.79	11.28
						-54.82	-3.78	-11.29
6d	*p*-OH-m-$OCH_3C_6H_3$	$C_{35}H_{30}N_6O_{10}S$	758.7	38.4	0.31	55.4	3.99	11.08
						-55.38	-3.98	-11.04
6e	*p*-ClC_6H_4	$C_{34}H_{27}N_6O_8S_2Cl$	747.1	42.6	0.32	54.65	3.64	11.25
						-54.64	-3.63	-11.23
6f	*m*-$NO_2C_6H_4$	$C_{34}H_{27}N_7O_{10}S_2$	757.7	37.8	0.29	53.89	3.59	12.94
						-52.93	-3.58	-12.92
6g	4-C_5H_4N	$C_{33}H_{27}N_7O_8S_2$	713.7	40.5	0.28	55.53	3.81	13.74
						-55.51	-3.8	-13.73
6h	3-C_4H_3O	$C_{32}H_{26}N_6O_9S_2$	702.7	42.1	0.3	54.69	3.73	11.96
						-54.68	-3.69	-11.96
6i	3-C_8H_5N	$C_{36}H_{29}N_7O_8S_2$	751.7	43.3	0.31	57.51	3.89	13.04
						-57.5	-3.84	-13.01
6j	*p*-$N(CH_3)_2C_6H_4$	$C_{36}H_{33}N_7O_8S_2$	755.8	42.4	0.32	57.21	4.4	12.97
						-57.2	-4.37	-12.96

Table 3: Antimicrobial activities of material synthesis(6a-j)

	Area of Inhibition (in mm) Against			
	Bacteriavariants		Fungiivariants	
Compounds	*E. coli*	*S. aureus*	*A. niger*	*C. albicans*
6a	16	19	20	30
6b	17	12	28	22
6c	18	13	18	24
6d	12	--	30	19
6e	--	16	22	--
6f	16	18	--	26
6g	18	16	23	28
6h	16	14	26	24
6i	--	18	18	26
6j	14	14	24	--

CONCLUSION

The successful synthesis of isoxazole, C-Nucleoside, and thioxopyrimidine compound, characterized and confirms by IR, NMR and other instrumentation techniques. Our research has demonstrated the potential of this compound as development of novel antimicrobial agent. This exploration is driven by their distinct pharmaceutical applications, characterized by specified mechanisms of action and significant biological activities, against bacterial and fungal strains. As a result, pharmaceutical companies have ample opportunities to further explore novel and potent therapeutic agents with promising potential.

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Conflict of interests

As per the authors, there are no known financial conflicts of interest or close personal connections that may have appeared to impact the research presented in this wrok.

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