Egyptian Journal of Chemistry

Design, Synthesis, Molecular Docking of Some New Polyhydrobenzothieno-thiazolopyrimidinedione Glycoside Derivatives with Double Anti-microbial-Anti-inflammatory Action

Nesrin M. Morsy [a*], **Khadiga M. Abu-Zied** [b] , **Ahmed S. Aly** [b], **Abdelbaset M. Elgamal** [c]

a*Organometallic and Organometalloid Chemistry Department, National Research Centre, Dokki, Cairo, 12622, Egypt.
b Photochemistry Department, National Research Centre, Dokki, Cairo, 12622, Egypt.
C Chemistry of Microbial and Natural Products Department, National Research Centre, Dokki, Cairo, 12622, Egypt.

Abstract

A novel series of thienopyrimidinone glycoside derivatives **7a–e** or **8a–e** were synthesized from the reaction of 2- mercapto-theino-pyrimidinone derivtives **3** and **4** or theino-thiazolopyrimidinone **5** and **6** with different aldo-sugars either hexoses or pentoses, The structure of the new resulted compounds were elucidated using IR, [1]-HNMR, ^{13}C-NMRspectra and elemental analysis. The compounds **2a**, **2b**,**3**,**4**,**7a-e** and **8a–e** were screened against seven of pathogenic bacteria and one of fungus using Cprofloxacin and Bavistin as standard respectively. Also, measurement of Minimum Inhibitory Concentration (MIC) was made and showing that compounds **2a**, **7a** and **7d** had the height results and near to the standard. Anti-inflammatory study was made for the fourteen compounds against Lipoxygenase "LOX",cyclooxygenase "COX 1" cyclooxygenase "COX 2" which revealed that compounds **2a**, **7a** and **7d** had the best values among the series and good results when compared with the standard. Ipubrofen and indomethacin. Moreover molecular docking studies were done on the three compounds **2a**, **7a** and **7d**. This study confirmed the dual action of our compounds as antimicrobial and anti-inflammatory agents

Keywords: Synthesis , Design ,Thiazolopyrimidinedione, Antibacterial, Antifungal, Anti-inflammatory, Molecular Docking

1-Introduction

The development of new antimicrobial drugs becomes very important due to the presence of drug resistance and appearance of new generations of pathogenic microorganisms [1,2]. The recent researches showed the bioactive effect of many heterocyclic compounds [3,4]. Some derivatives which containing substituted theinopyrimidinone moieties appears to have potent bioactivity as antibacterial, antifungal and antiviral agents activities [5,6]. The presence of pyrimidine ring in our compounds gives it heigh pharmacological activity such as antibacterial [7-13], antileshmanial [14],

anti-inflammatory [15,16], analgesic [17], antihypertensive [18,19], antiviral [20-22]. Many marketed drugs containing pyrimidine nucleus such as some antibacterial drugs like Brodiprim (**I**) [23] and Trimethoprim (**II**) which is selective antibacterial DHFR [24]. Otherwise, pyrimidine moiety was recorded as antifungal drugs such as Flucytosine (**III**) which is used for treatment of Candida and Cryptococcus [25,26] and Hexetidine (**IV**)which is used for the treatment of ophthous ulceration [27] (Figure1).

The presence of sugar moiety in the prepared products exceeding the activity while carbohydrates are bioactive compounds acting as antibacterial, antifungal, antiviral and antiprotozoal [28]. Many marketed drugs containing the sugar moiety (sweet

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antibiotics) such as Streptomycin (**V**) , Dihydrostreptomycin (**VI**) and Gentamycin (**VII**) which are antibacterial drugs [29] (Figure 1).

Theinopyrimidines are well known with its high pharmaceutical applications [30-33]. It was important to discover and develop new non selective anti-inflammatory drugs (NSAIDs) due to the undesirable side effects of marketed anti-inflammatory drugs [34,35].Thus many literatures represented the roles of theinopyrimidinone derivatives as anti-inflammatory agents [36-39]. The compound N4-(3-(dimethylamino)propyl)-N2-methyl-6-(4-morpholinophenyl-)thieno[3,2-*d*] pyrimi-dine-2,4-diamine which is theinopyrimidinone derivative (**V**) was found to have antimalarial potential [40,41],while the other compound, 6-(4-chlorophenyl)-3-(2-(dimethyl-amino)-1-(3-(pyrrolidin-1-yl)propyl)-1*H*-benzo[*d*]-imidazol-5-yl)thieno[3,2-d]pyrimidin-4(3H)-one (**VI**) was marketed by Glaxosmithklino as antiplasmodial drug [42]. The compound N-(3-((2-((4-(4-methylpiperazin-1-yl)phenyl)amino)thieno[3,2-d]pyrimidin-4-yl)oxy)phenyl)acrylamide which isknown as Olmutinib (**VII**) is a marketed drug and has the role of inhibition of epidermal growth factor receptor (EGFR) of nonsmall cell lung cancer (NSCLC) [43–46] (Figure 2).

From the above, we observed the importance of the synthesis and studying the pharmacological properties of new theinopyrimidinone derivatives . A promising results were found in the resulting compounds when investigating the antimicrobial and anti-inflammatory activity .That we discovered the dual action of these derivatives which is very important in the treatment of patients who suffering from Kidney or liver dysfunction and it had economically advantages . In this article and in continuation of our researches [47-68] we designed and synthesized new theinopyrimidinone derivatives attached to some sugars to give **7a-e** and **8a-e** ,confirmed the chemical structure using ^{1}H-NMR, ^{13}C-NMR, IR spectra and elemental analysis and we investigated its double biological action as antimicrobial and anti-inflammatory agents followed by molecular docking study.
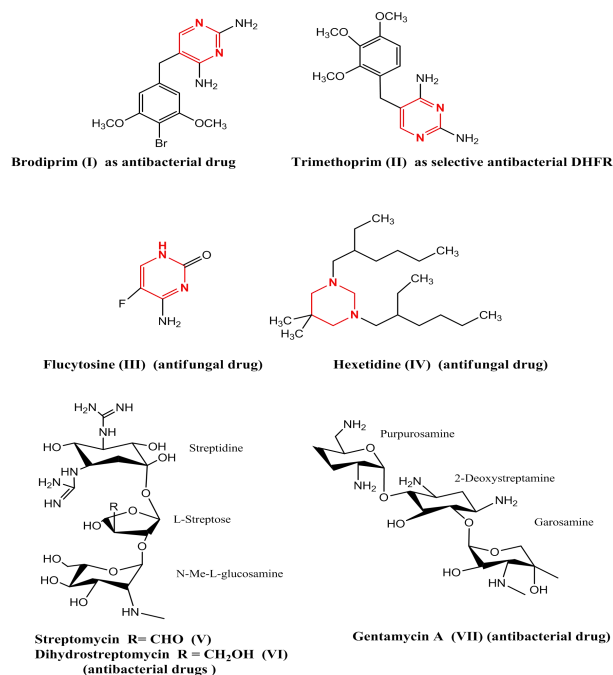

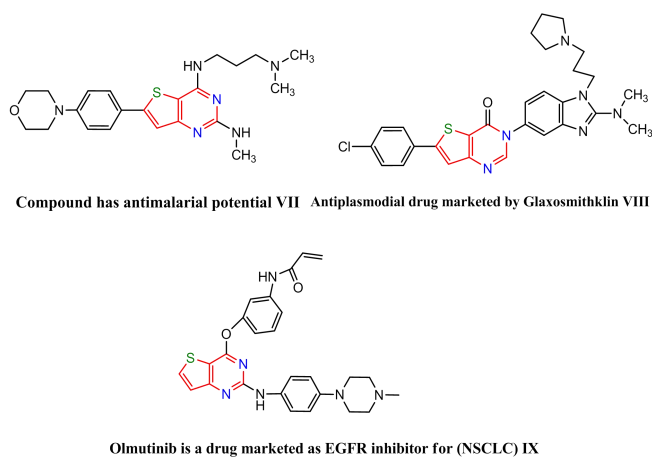**Fig. 1**: Drugs containing pyrimidine moiety and others containing sugar moiety

Fig. 2 : Some pyrimidine and theinopyrimidine derivatives as drugs

2. Results and Discussion:

2.1. Chemistry:

Our team work play on exploring new compounds with expected high biological activity thus we found that theinopyrimidinone derivatives with some pentoses and hexoses give great results when applied as antifungal, antibacterial and anti-inflammatory agents. Upon adding ethylcyano= acetate and sulphur to cyclopentanone or cyclo-hexanone in presence of diethyl amine; the product was either ethyl-2-amino-5,6-dihydro-4*H*cyclopent-[*b*]-thiophene-3-carboxylate **2a** or ethyl-2-amino-4,5,6,7-tetrahydro-benzo[*b*]-thiophene-3-carboxylate **2b**. When refluxing compounds **2a** or **2b** with potassium thiocyanate the compounds 2-mercapto-6,7dihydro-3*H*-cyclopenta[4,5]thieno[2,3-*d*] pyrimidine-4(5*H*)-one **(3)** or 2-mercapto-5,6,7,8-tetrahydrobenzo-[4,5]theino[2,3-*d*]pyrimidine-4(3*H*)-one**(4)**were isolated, respectively.(Scheme 1).

In order to obtain compounds **7a-e** or **8a-e** ; there are two methods, one of them is to react **3** or **4** with chloroacetic acid, acetic anhydride, anhydrous sodium acetate and aldo-sugars in one pot reaction or the second method which included two steps , the first step is to prepare compounds **5** and **6** as presented in Scheme 2 and then reacted with aldo-sugars in absolute ethanol and few drops of pyridine/piperidine mixture as presented in scheme 2 .

The resulted products **7a-e** or **8a-e** (scheme 2) were crystallized from the proper solvent and then investigated by different spectroscopic methods like

IR spectrum, ^{1}H-NMR, ^{13}C-NMR spectra, for example compound **7b** has elucidated by elemental analysis and IR (KBr, cm^{-1}) showing bands at 3434 for (OHs) group, 2857 for (CHs) group;1666; 1660 corresponded to two of the carbonyl groups. The ^{1}H-NMR spectrum (DMSO-d_6, 500 MHz , δ ppm : showed the signals ; 2.3(m, 2H, CH$_2$); 2.49 (t, 2H, CH$_2$); 2.5 (t, 2H, CH$_2$); 3.05 (m, 1H, H-3$^\backslash$) ; 3.11 (m, 2H, H-6$^\backslash$, H- 6$^{\backslash\backslash}$); 3.4 (t, 1H, H-4$^\backslash$); 3.16 (m, 5H, 5 (OH), D2O exchangeable); 3.52 (m, 1H, H-2$^\backslash$); 5.96 (d, 1H,H-1$^\backslash$, *J* = 7.5 Hz). ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm) 24.9, 25.8 and 31.9 (CH$_2$), 63.3, 64.4, 70.9, 72.7, 74.6 (sugar carbon atoms), 117.6 (C=C, pyrimidine), 125.4 (C= C-S, pent.), 126.0 (C=C-S, thiazole),139.4 (S-C-N, pent.) , 148.2 (sugar carbon atom), 155.5 (C= C, pyrimidine), 158.3 (N-C=N), 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole).

2. 2. Biological screening:

2. 2. 1. Antimicrobial Screening and Minimum Inhibition Concentration:

The method was used to determine the antibacterial activity is the measuring of the inhibition growth zone. The inhibition zones of compounds **2a**,**2b**, **3**, **4**,**7a-e** and **8a- e** were investigated against five types of bacteria , *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Salmonella typhi*, and *Escherichia coli*, all tested compounds gave good results but the values of compounds **2a**, **7a** and **7d** was the closest when compared with the standard drug Ciprofloxacin (CPR) . Also the same series of compounds were studied as antifungal against seven types of fungi, Shigella sp and Pseudomonas aeruginosa 1204. all tested compounds gave good results but the values of compounds **2a**, **7a** and **7d** was the closest when compared with the standard drug Ciprofloxacin (CPR) . Also the same series of compounds were studied as antifungal against one type of fungi, *Trichophyton schoenleinii*, the tested compounds had valuable results especially compounds **2a**, **7a** and **7d** showed the optimum results compared to the standard drug Bavistin as shown in (table 1). The minimum inhibition concentration also was studied for all compounds and compound **2a** , **7a** and **7d** have the lowest values. (Table 2) .
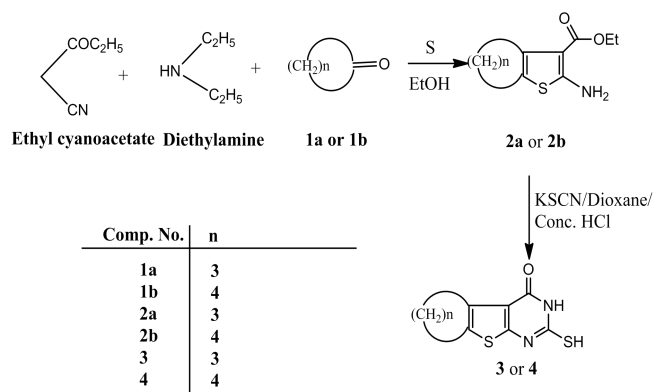
Scheme 1 Synthesis of mercapto-theinopyrimidine derivatives.
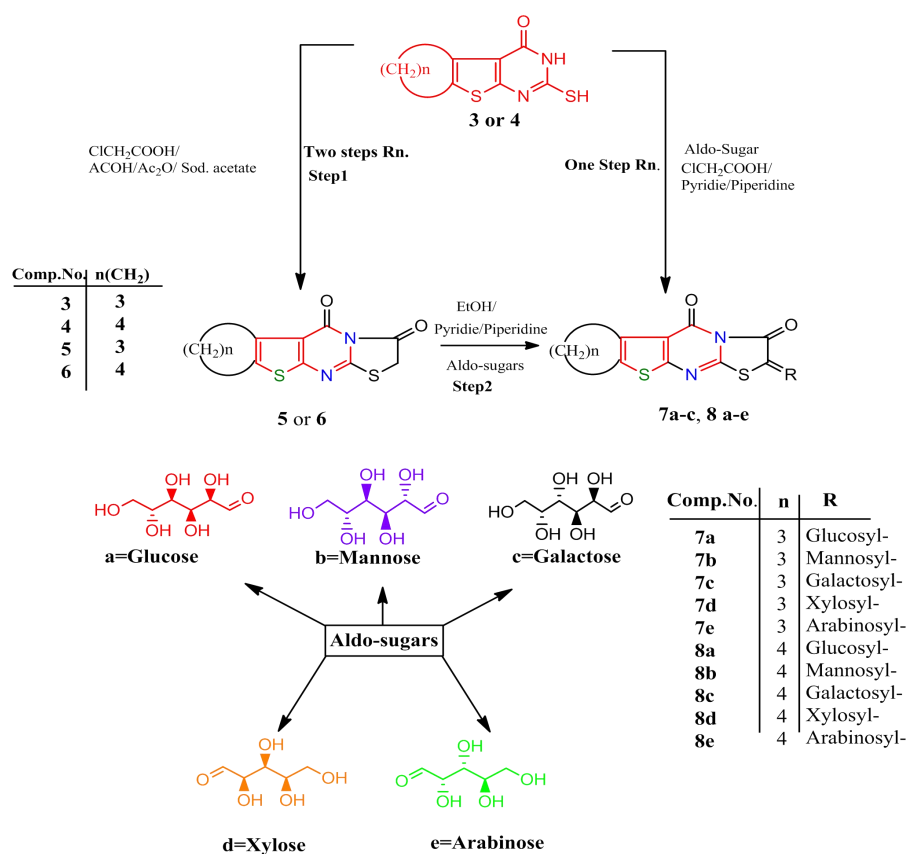
Scheme 2 Synthesis of thienopyrimidinone glycoside derivatives **7a-e, 8a-e**

Table (1) : Screening of Compounds 2a, 2b , 3, 4, 7a-e and 8a- e (µg/ml) Against Bacterial and Fungal Strains:

Cpd. No.	Conc. (µg/ml)	Strains							
		Staphylo- coccus aureus ATCC 23235	*Streptococcus pyogenes* ATCC 19315	*Staphylo- coccus epidermidis* ATCC 12228	*Salmonella typhi* ATCC 35664	*Escherichia coli* ATCC 25922	*Shigella sp*	*Pseudomonas aeruginosa* 12040	*Trichophyton schoenleinii* 22766
2a	10	4.70±0.90	4.35±1.00	4.40±1.50	4.50±0.90	4.30±1.00	4.10±0.75	4.25±1.00	4.10±1.00
	5	3.5±1.50	3.20±0.75	4.0±1.50	3.70±0.50	3.50±1.00	3.50±0.85	3.50±0.90	3.50±0.75
	2.5	3.0±0.90	2.90±1.00	3.0±1.20	3.0±1.00	3.00±0.90	3.00±1.00	2.90±1.00	2.90±0.90
2b	10	3.20±1.00	3.10±0.90	3.30±110	3.00±0.75	3.20±1.00	3.50±1.00	3.30±0.90	3.00±0.75
	5	2.50±0.90	2.75±0.75	2.50±0.55	2.60±0.85	2.50±0.75	2.40±0.45	2.20±0.80	2.80±0.90
	2.5	2.00±0.50	1.90±0.40	1.50±0.45	2.00±0.55	1.90±0.75	2.00±0.45	1.50±0.55	1.75±0.60
3	10	3.5±1.00	3.00±0.75	3.10±0.90	2.90±1.50	2.30±1.00	3.00±0.90	3.40±1.20	3.00±1.00
	5	2.40±1.00	2.50±1.00	2.40±0.80	2.00±0.75	2.00±1.20	2.20±0.75	2.80±1.20	2.10±0.90
	2.5	2.00±0.90	2.10±0.80	2.10±0.50	1.50±.0.90	1.90±1.00	1.70±0.85	2.00±1.00	1.90±1.00
4	10	3.00±0.90	2.90±0.75	2.75±0.80	2.50±0.60	2.80±0.70	2.90±0.90	2.70±0.65	2.75±0.60
	5	2.10±0.75	2.00±0.45	1.90±0.55	1.50±0.40	2.00±0.55	2.00±0.55	2.00±0.50	2.10±0.55
	2.5	1.50±0.55	1.20±0.50	1.20±0.45	1.10±0.30	1.20±0.45	1.50±0.45	1.60±0.55	1.50±0.45
7a	10	4.60±1.50	4.10±1.00	4.10±0.90	4.10±1.00	4.00±1.00	4.00±0.90	4.05±1.00	3.90±1.00
	5	3.50±1.00	3.50±0.75	3.40±0.75	3.00±0.90	3.00±0.75	3.30±1.00	2.90±0.90	2.75±1.50
	2.5	2.70±0.90	2.80±0.75	2.50±0.80	2.50±0.75	2.40±0.90	2.50±1.10	2.00±1.20	2.00±1.00
7b	10	3.50±0.90	3.20±0.75	3.60±0.95	3.50±0.1.00	3.70±1.00	3.50±0.90	3.30±0.85	3.20±1.00
	5	3.00±0.75	2.95±0.80	3.00±0.75	3.10±0.90	2.90±0.75	2.50±0.75	2.40±0.90	2.30±0.90
	2.5	2.20±0.80	2.00±0.55	1.90±0.70	1.75±0.80	2.00±0.85	1.90±0.80	2.00±0.75	1.90±0.75
7c	10	2.75±0.90	2.80±0.75	3.00±1.00	2.90±0.75	3.00±1.00	2.95±0.75	2.80±0.70	3.00±1.00
	5	2.00±0.55	2.10±0.45	2.00±0.45	190±0.55	2.10±0.60	1.80±0.40	2.00±0.55	1.90±0.45
	2.5	1.50±0.30	1.60±0.45	1.50±0.35	1.30±0.25	1.50±0.35	1.50±0.30	145±0.40	155±0.25
7d	10	4.50±1.00	4.20±0.90	4.30±1.00	4.20±0.75	4.20±1.00	4.00±0.90	4.20±1.00	4.00±1.00
	5	4.00±1.50	3.00±1.50	3.20±0.90	3.00±1.50	3.30±1.50	3.30±0.80	3.00±1.20	3.20±0.75
	2.5	3.50±0.90	2.90±0.75	2.50±1.50	2.70±1.10	2.50±0.90	2.20±1.00	2.00±1.50	2.10±1.50
7e	10	3.90±1.00	3.75±0.90	3.80±1.50	3.95±0.75	3.50±1.00	3.60±0.90	3.50±1.00	3.80±1.50
	5	3.00±1.00	2.90±1.00	2.75±1.50	2.70±0.90	2.90±1.00	2.50±0.80	2.80±1.20	3.00±1.00
	2.5	2.50±1.00	2.00±0.90	1.90±1.00	2.00±1.20	2.10±110	1.90±1.00	2.10±1.50	1.75±0.80
8a	10	2.50±1.00	2.25±0.55	2.00±0.40	2.10±0.55	2.20±0.45	2.25±0.45	2.00±0.45	2.10±0.40
	5	1.70±0.55	1.60±0.25	1.50±0.25	1.50±0.25	1.45±0.35	1.50±0.30	1.50±035	1.45±0.35
	2.5	1.20±0.25	1.10±0.20	100±0.20	1.10±0.30	1.00±0.25	1.10±0.20	1.00±0.20	1.00±0.25
8b	10	2.95±0.90	3.00±1.00	2.95±1.00	2.90±0.95	2.95±0.80	3.00±0.90	3.10±0.75	3.00±0.85
	5	2.00±0.55	2.20±0.45	2.10±0.35	2.00±0.45	2.20±0.40	2.00±0.25	2.20±0.35	2.15±0.25
	2.5	1.50±0.35	1.55±0.25	1.50±0.30	1.30±0.270	1.50±0.25	1.30±0.20	1.50±0.25	1.50±0.25
8c	10	3.00±1.00	2.90±0.75	2.75±0.95	3.00±1.00	3.10±0.75	3.20±1.00	3.00±1.00	3.10±0.95
	5	2.20±0.90	2.00±075	2.10±0.85	2.30±0.90	2.50±0.45	2.60±0.55	2.50±0.45	2.30±0.55
	2.5	1.75±0.45	1.70±0.55	1.80±0.35	1.75±0.45	1.80±0.55	2.00±0.60	1.90±035	2.00±0.35

Table (1) continue:

| Cpd. No. | Conc. (µg/ml) | Strains | | | | | | | |
		Staphylo-coccus aureus ATCC 23235	*Strepto-coccus pyogenes ATCC 19315*	*Staphylo-coccus epidermidis ATCC 12228*	*Salmonella typhi ATCC 35664*	*Escherichia coli ATCC 25922*	*Shigella sp*	*Pseudomo-nas aeruginosa 12040*	*Trichophyton schoenleinii 22766*
8d	10	3.50±1.00	3.20±0.90	3.40±0.75	3.30±0.80	3.20±1.00	3.00±0.90	3.20±1.00	3.00±1.00
	5	2.20±0.90	2.00±0.75	2.10±0.80	2.00±0.75	1.90±0.90	2.10±0.70	2.50±0.60	2.20±0.75
	2.5	1.90±0.75	1.70±0.80	1.50±0.75	1.60±0.55	1.30±0.60	1.50±0.45	1.30±0.50	1.60±0.75
8e	10	3.00±1.50	3.10±0.90	2.90±1.00	2.75±1.50	2.50±0.90	2.75±0.80	2.90±1.20	3.00±1.00
	5	2.20±1.00	2.00±0.90	1.75±0.80	1.90±0.70	2.00±0.75	1.90±0.85	1.80±0.70	2.00±0.90
	2.5	1.90±0.80	1.50±0.75	1.50±0.60	1.60±0.75	1.40±0.40	1.50±0.50	1.30±0.45	1.50±0.50
DMSO	0.1	NI	NI	NI	NI	NI	NI	NI	NI
CPR	10	4.60±1.50	4.30±1.55	4.35±1.20	4.30±1.90	4.20±1.50	4.00±1.00	4.20±1.00	NT
BAVISTIN	10	NT	NT	NT	NT	NT	NT	NT	4.16±0.90

Table (2): Minimum Inhibition Concentration (µg/mL) for Compounds 2a,2b,3,4 ,7a-e and **8a- e**

Cpd. No. / Strains	2a (µg/mL)	2b (µg/mL)	3 (µg/mL)	4 (µg/mL)	7a (µg/mL)	7b (µg/mL)	7c (µg/mL)	7d (µg/mL)	7e (µg/mL)	8a (µg/mL)	8b (µg/mL)	8c (µg/mL)	8d (µg/mL)	8e (µg/mL)	CRP (µg/mL)
Staphylo Coccus aureus ATCC 23235	9.50	10.50	10.20	10.50	9.00	11.00	10.20	9.20	10.50	10.20	10.10	10.20	11.00	11.00	12.13
Strepto coccus pyogenes ATCC 19315	9.30	10.80	11.50	10.90	9.10	10.85	11.00	9.40	10.30	11.80	11.10	11.95	10.80	10.90	11.93
Staphylococcus Epidermidis ATCC 12228	9.60	10.95	10.30	11.00	9.20	10.90	11.50	9.00	10.25	11.95	11.10	12.00	11.00	11.10	12.40
Salmonella typhi ATCC 35664	9.50	11.50	10.50	11.50	9.00	11.00	11.60	9.20	10.00	11.95	11.20	11.50	11.10	10.80	12.62
Escherichia coli ATCC 25922	9.70	11.00	11.00	11.20	9.10	10.95	11.50	9.30	10.10	11.90	11.10	11.55	11.00	10.90	11.89
Shigella sp	9.40	11.20	10.30	11.45	9.00	11.10	11.60	9.00	10.20	11.90	10.95	11.50	11.20	10.95	11.30
Pseudomonas aeruginosa 12040	9.80	10.95	10.10	11.45	9.20	11.00	11.70	9.10	10.10	12.00	11.00	11.50	11.20	11.00	12.20
Trichophyton schoenleinii 22766	9.40	11.50	10.30	11.45	9.00	11.00	11.50	9.20	10.00	11.95	10.95	11.50	11.10	11.00	11.90

2. 2. 2. Antiinflammatory Screening:

Investigation of compounds **2a**, **2b**,**3**,**4**,**7a-e** and **8a-e** was completed by using Lipoxygenase (LOX), Cyclooxgenase (COX-1) and Cyclooxgenase (COX-2) and all compounds gave respectable results (Table 3)

Table (3) : Anti-inflammatory Screening for Compounds 2a,2b,3,4,7a-e,8 a-e Using LOX[a], COX-1[b], COX-2[c] Using Ibuprofen as Standard and Membrane Stabilization Method Using Indomethacin as Standards.

Samples Param-eters	Standard (µmol)	2a (µmol)	2b (µmol)	3 (µmol)	4 (µmol)	7a (µmol)	7b (µmol)	7c (µmol)	7d (µmol)	7e (µmol)	8a (µmol)	8b (µmol)	8c (µmol)	8d (µmol)	8e (µmol)
Lipoxyg-enase (LOX)	Ibuprofen 1.5±0.90	1.5±0.85	1.8±1.00	1.8±0.75	2.4±1.00	1.7±1.00	1.6±0.90	1.9±0.95	1.6±1.50	1.8±0.75	17±1.50	5±1.00	1.9±1.20	1.7±1.40	1.8±1.00
Cycloox-egenase (COX1)	10.2±1.20 Ibuprofen	10.7±0.90	12.1±0.75	12±1.50	11.9±1.00	10.8±0.75	11.3±1.00	11.5±0.80	10.8±1.00	11±1.20	19±1.10	11±1.00	22±1.50	11.7±0.95	12±1.00
Cycloox-egenase (COX2)	12.7±0.85 Ibuprofen	12.4±1.50	13.1±1.00	25±1.50	12.9±0.90	12.7±0.80	13.7±1.00	13.8±1.00	12.7±0.90	13.7±1.10	13.5±1.50	21±1.00	13.2±0.75	13.9±1.00	14.2±0.85
Membr-ane Stabili-zation	14.3±1.50 Indometha-cin	15.2±1.50	18.9±1.00	19.9±1.00	18.7±0.85	16.2±1.00	19.8±1.20	18.2±0.90	15.9±0.90	19.8±0.85	17.5±1.20	16.9±0.75	16.2±1.50	19.6±0.75	18.4±1.20

[a]Lipoxygenase enzyme
[b]Human recombinant COX-2 enzyme.
[c]Human COX-1 enzyme from human

3. Molecular Docking of Antimicrobial:
3. 1. Active Site Prediction for Antibacterial :

The active informatics-site was predicted by using the finder option of using MOE 14.0901 Software .The site finder option was used to manage calculation of   possible active sites in (bacterial gyrase enzyme and fungal CYP 51) . Calculations were managed to determine the potential sites for ligand binding, docking   and restriction sets for rendering partial molecular surfaces. In bacterial gyrase enzyme critical active site contain DC13, DG9, Mn2000, Ser1084, Glu477 and Arg458. Whereas fungal CYP 51 critical active site contain Phe385, Met512. Ser383, His382 and Pro239 [69].

The binding mode of the candidate compound 2a exhibited a binding energy of -6.57 kcal/mol. Which a 5,6-dihydro-4H-cyclopenta[*b*]-thiophene nucleus formed one Pi-Pi interaction with His1081. While NH2 group formed two hydrogen bonds through Asp512, Asp510 with distance of 2.28, 2.89 °A respectively and carbonyl group formed metal-ion interaction with Mn2492 (Figure 2).

The binding module of the selected compound **7c** exhibited a binding energy of -12.07 kcal/mol. Which a 5*H*-thiazolo [3,2-*a*] pyrimidine-3,5(2*H*)-dione ring interacted with DNA nucleotides DC13, DC12 and DG9 by Pi-Pi interactions. While 5,6-dihydro-4*H*-cyclopenta[*b*]thiophene   ring   formed   Pi-Alkyl interactions with Arg458. The hydroxyl groups in hept-6-ene-1,2,3,4,5-pentaol tail formed one hydrogen

bonding through Ser1084 with distance of 2.68 °A and two metal-ion interactions with Mn2000 (Figure 3) .The binding module of the selected compound **7d** exhibited an energy binding of -12.69 kcal/mol. Which a 7,8-dihydro-5*H*,6*H*-cyclopenta[4,5]thieno-[2,3-*d*]thiazolo-[3,2-*a*]pyrimidine-3,5(2*H*)-dione nucleus formed Pi-Pi

interactions with DA13, DG9 and DT8, while a carbonyl groups formed two hydrogen bond through Gly459 with a distance of 2.61, 2.20 °A. Thehydroxyl group in hept-6-ene-1,2,3,4,5-tetraol moiety formed another ionic interaction with Mn2492. (Figure 4). Molecular mapping surfaces showing **7c** and **7d** occupying the active pocket of bacterial gyrase enzyme were illustrated. (Figure 5) Flexible alignment between Ciprofloxacin and candidate **7d** inside the active pocket of bacterial gyrase enzyme show complete matching (Figure 6).
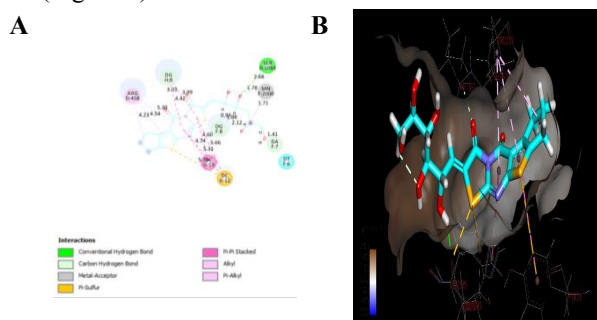

Fig. 3 : The two and three dimensional structures for **7c** (A) and (B) respectively docked in bacterial gyrase enzyme, hydrogen bonds (green) and the pi interactions are represented in Pu.
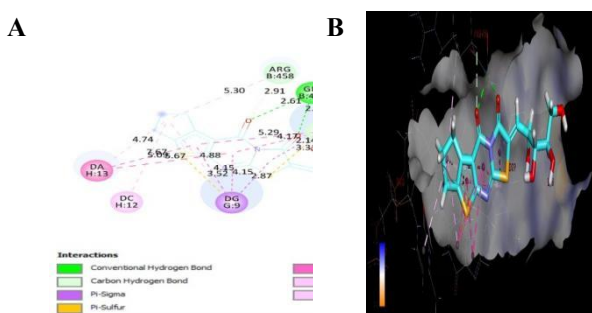

Fig. 4 : The two and three dimensional structures for **7d** (A) and interactions are represented in Purple lines.
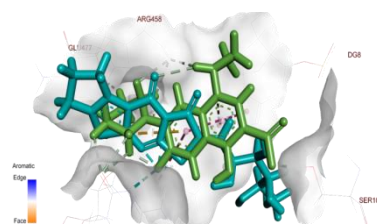

Fig. 5: Flexible alignment between Ciprofloxacin and candidate **7d** inside the active pocket of bacterial gyrase enzyme show complete matching.
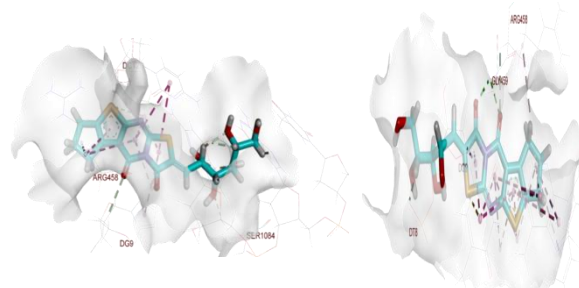

Fig. 6 : Mapping surface showing **7c** and **7d** occupying the active pocket of bacterial gyrase enzyme.

3. 2. Molecular Docking of Antifungal:

The binding module of the selected compound **7c** exhibited binding energy of -8.36 kcal/mol. The 7,8-dihydro-5*H*,6*H*-cyclopenta[4,5]thieno[2,3-d]thiazolo-[3,2-*a*]pyrimidine-3,5(2*H*)-dione nucleus formed Pi-Pi interactions and Pi-alkyl interaction with His382, Pro239 and Tyr73. While hydroxyl group in hept-6-ene-1, 2, 3, 4, 5-pentaol moiety formed one hydrogen bond with Ser383 with distance of 1.86 °A. sulfur groups formed extra bonds with Phe385, Tyr73 and Ser383 (Figure 7).

The binding module of the selected compound **7d** exhibited binding energy of -9.34 kcal/mol. The 7,8-dihydro-5*H*,6*H*-cyclopenta[4,5]thieno[2,3-d]thiazolo-[3,2-*a*]pyrimidine-3,5(2*H*)-dione nucleus formed Pi-Pi interactions and Pi-alkyl interactions with Leu381, Met512 and Tyr127.while hydroxyl groups in hept-6-ene-1,2,3,4,5-tetraol moiety formed two hydrogen bonds with Ser511 and His382 with distance of 2.25,

2.12 °A respectively. While a carbonyl group interacted with Ser383 by hydrogen bonding with distance of 2.42 °A. (Figure 8).
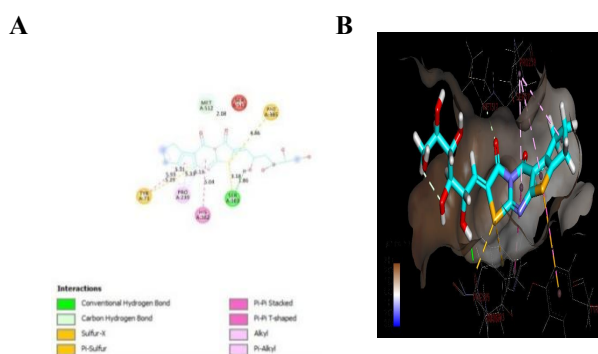
A   B

Fig. 7: The two and threedimensional structure of **7c** (A) and (B) respectively docked in fungal CYP 51 targeted pocket, hydrogen bonds (green) and the pi interactions are represented in Purple lines.

A   B

Fig. 8: The two and three dimensional structure of **7c** (A) and (B) respectively docked in fungal CYP 51 targeted pocket, hydrogen bonds (green) and the pi interactions are represented in Purple lines.
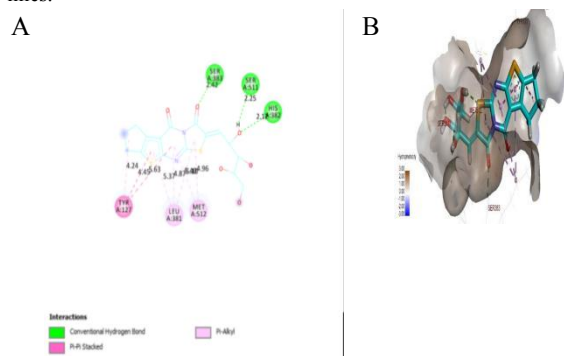
Table (4) : ADME and Physicochemical Properties for 7d.

Properties of 7d	
c log P	0.71
c log S	-2.27
H bond acceptor	7
H bond donor	4
Bioavailability score	0.55
Skin permeation (log Kp)	-8.84 cm/s
Human gastrointestinal absorption	low
Mutagenic	None
Tumorigenic	None
Irritant	None
Drug likeness	1
TPSA	188.84 A°
BBB permeation	NO

3. 3. Molecular Docking of Anti-inflammatory

Using the molecular targets by comparing our targeted compound with crystal ligands and determination the features that can binding with critical amino acid in a target site, target site selection has been done by RCSB protein data bank. Our compound tested against many target sites then good result determine suitable protein for managing docking studies.

3.3.1. Molecular Modeling:
Active Site Prediction:

The active site was predicted by using the site finder option of using MOE 14.0901 Software .The site finder option was used to calculate possible active sites in (Human **5-Lipoxygenase**) [70]. Calculations were made to determine potential sites for ligand binding and docking, and restriction sets for rendering partial molecular surfaces. *In* Human **5-Lipoxygenase** critical active site contain *His372, Trp599, His600, Ile406, Ala410, Gln363 and Arg596* amino acid residues.

The binding module of the candidate compound **7c** exhibited an binding energy of -5.47 kcal/mol. Which a 7,8-dihydro-5*H*,6*H*-cyclopenta[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5(2*H*)-dione nucleus interacted with Leu368, Ala410, His372 and Ile406 by six Pi-Pi interactions and Pi-alkyl interactions . While the hydroxyl groups in hept-6-ene-1,2,3,4,5-pentaol tail formed five hydrogen bonding through Arg596, His600, His432 and Gln363 with distance of 2.55, 2.61, 2.66, 1.96 and 2.72 °A respectively (Figure 9).

The binding module of the selected compound **7d** showed binding energy of -5.99 kcal/mol. Which a 7,8-dihydro-5*H*,6*H*-cyclopenta[4,5]thieno[2,3-*d*]thiazolo-[3,2-*a*]pyrimidine-3,5(2*H*)-dione nucleus formed Pi-Pi interactions and sulfur-Pi interactions with Leu607, Ile406, Ala410 and Gln363 respectively, while the hydroxyl group in hept-6-ene-

1,2,3,4,5-tetraol moiety formed four hydrogen bonding with Arg596, Pro569, His360 and His432 with a distance of 2.06, 2.52, 2.18 and 1.85 °A respectively (Figure 10).

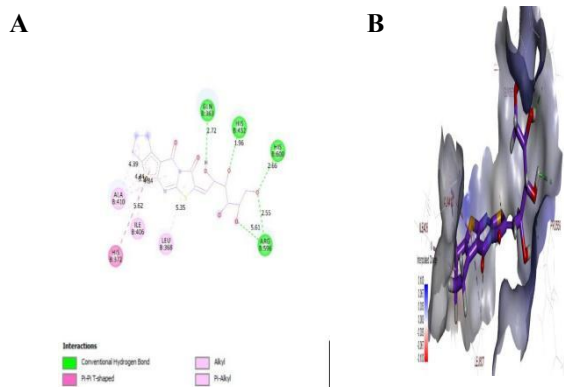**A**   **B**

Fig. 9 : The two and three dimensional structure of compounds **7c** (A) and (B) respectively docked in Human 5-Lipoxygenase, hydrogen bonds (green) and the pi interactions are represented in Purple lines

A   **B**

Fig. 10: The two and three dimensional structure of compounds **7d** (A) and (B) respectively docked in Human 5-Lipoxygenase, hydrogen bonds (green) and the pi interactions are represented in Purple lines
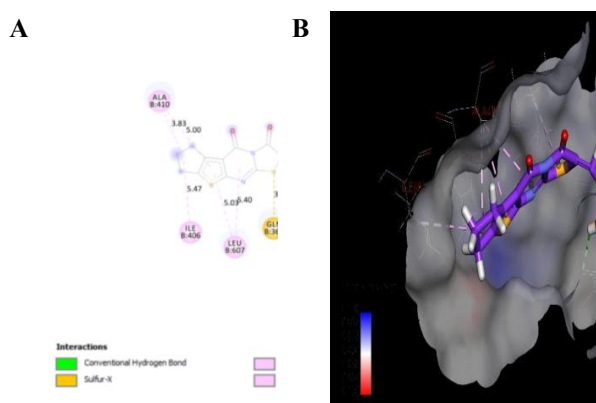
4. Conclusion:

In this study, we prepared new theinopyrimidinone glycoside derivatives **7a-e or 8a-e** and fourteen compounds were experimented with three dilutions of each compound as antibacterial and antifungal agents, where the **2a**,**7c** and **7d** compounds at a concentration of 10 micrograms per ml were the best results which were close to the results of the antibiotic ciprofloxacin as an antibiotic for the seven strains of tested bacteria. Also, the dilution of 10

microgrms per milliliter for the **2a** , **7c** and **7d** compounds as anti-fungal was closed to the results of the anti-fungal Bavistin**.** The compounds **2a**,**7c** and **7d** are considered the optimum compounds as anti-inflammatory agents also because the native energy is very low for them and the anti-inflammatory results for these compounds are closed to the standard anti-inflammatory drugs ibuprofen and indomethacin. The results of lipoxygenase "LOX" , cyclooxygenase "COX-1" and cyclooxygenase "COX-2"method indicated to achieve better results with compounds **2a** , **7c** and **7d** compared to the standard**.**

5. Experimental :
5. 1. Chemistry

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected .^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Varian spectrometer using DMSO-d_6 as a solvent and TMS as an internal standard. Chemical shifts are reported in ppm. Coupling constants (J) are expressed in Hz. Mass spectra were recorded on a Varian MAT 112 spectrometer at 70 elV. Elemental analyses were performed at the Micro Analytical Centre, Cairo University, Egypt. Progress of the reactions was monitored by thin-layer chromatography (TLC) using aluminum sheets coated with silica gel F254 (Merck), viewing under a short-wavelength UV lamp effected detection. All evaporations were carried out under reduced pressure at 40°C.

The starting materials were synthesized according to to the references [71-74].

Ethyl-2-amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate (2a) m.p. 111-112ºC.

Ethyl-2amino4,5,6,7tetrahydrobenzo[b]thiophene-3-carboxylate(2b),m.p.92ºC.

2-**Mercapto-6,7-dihydro-3*H*-cyclopenta [4,5]thieno[2,3-*d*]pyrimidin-4(5*H*)-one (3)**.m.p.340-342ºC.

3-**Mercapto-5,6,7,8tetrahydr-obenzo-[4,5]theino[2,3*d*]pyrimidine4(3*H*)-one(4)** m.p.>300ºC

4-.**7,8Dihydrocyclopenta[4,5]thieno[2,3-*d*]thiazolo[3,2-a]pyrimidine-3,5(2H,6H)-dione (5)** m.p.>300ºC.

5-**6,7,8,9-Tetrahydro-2H-benzo[4,5] thieno[2,3-*d*]thiazolo[3,2-a]pyrimidine-3,5-dione (6),** m.p.>300ºC.

Synthesis of Thienopyrimidinone Glycoside Derivatives 7, 8 (a–e)

Method 1:

As a one pot reaction a solution of compound **3** or **4** (10 mmole) in 30 mL pyridine was added to the proper aldo-sugars (10 mmole) and they allowed to heated under reflux for 8 hrs. after addition of chloroacetic acid (10 mmole, 00.93 gm) and a few drops of piperidine . The reaction was monitored by using TLC technique and the reaction was stopped after completed. The reaction mixture was poured onto ice-water, the precipitate was collected and recrystallized from the proper solvent to produce compounds7a-e and 8a-e, respectively.

Method 2 :

A mixture of acetic anhydride, acetic acid, chloroacetic acid and anhydrous sodium acetate was added to (10 mmole) of compounds **3** and **4** then refluxed for 4 hrs., poured into cooled water and the collected precipitate was crystallized from ethanol to give brown precipitate with 70% yield of compounds **5** and **6**. Aldo-sugars (10 mmole) were added to a solution of **5** or **6** and refluxed for 3 hrs., poured into water , filteredoff and crystallized from the proper solvent to give the same product **7a-e, 8 a-e** as two steps of the reaction.

2-Glucosyl-7,8-dihydrocyclopenta [4,5] thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5(2*H*,6*H*)-dione (7a) .

Brown crystals (ethanol), m. p. 207-209 ºC, yield: 65%, IR (KBr, cm^{-1}): 3422 (OH); 2856 (CH); 1662, 1641 (2CO) ;^{1}H-NMR (DMSO-d_6, 500 MHZ , δ ppm) 2.37 (m, 2H, H-6$^{\backslash}$, H- 6$^{\backslash\backslash}$); 2.34 (m, 1H, H5$^{\backslash}$); 2.83 (t, 2H, CH$_2$), 2.5 (t, 1H, H-4$^{\backslash}$); 2.83 (t, 1H, H-3$^{\backslash}$);   2.87 (t,2H, CH$_2$); 2.87 (m, 1H, H-2$^{\backslash}$); 3.58 (m, 5H, 5 (OH), D$_2$O exchangeable); 6.67 (d, 1H,H-1$^{\backslash}$, *J* = 7.5 Hz) .^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 24.9, 25.6, 31.9 (CH$_2$), 63.3 , 64.4, 70.9, 72.7, 74.6 (carbon atoms of sugar), 117.6 (C=C=O, pyrimidine), 125.4 (C= C-S, pent), 126.0 (C=C-S, thiazole),139.4 (C=C=O, pyrimidine) , 148.2 (carbon atom of sugar), 155.5 (C= C-S, pent.), 158.3 (N-C=N, pyrimidine), 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole). Anal. Calcd. (%); C$_{17}$H$_{18}$N$_2$O$_7$S$_2$ (426.46) : C, 47.88; H, 4.25; N, 6.57; S, 15.04. Found (%) ; C,47.90; H,4.21; N,6.59; S, 15.1.

2-Mannosyl-7,8-dihydrocyclopenta[4,5] thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5(2*H*,6*H*)-dione (7b) .

Brown crystals (ethanol), m. p. 200-202ºC , yield: 52 %, IR (KBr, cm^{-1}): 3434 (OH), 2857 (CH);1666; 1660 (2 CO).^{1}H-NMR (DMSO-d_6) , 500 MHz , δ ppm : 2.3(m, 2H, CH$_2$); 2.49 (t, 2H, CH$_2$); 2.5 (t, 2H, CH$_2$); 3.05 (m, 1H, H-3$^{\backslash}$) ; 3.11 (m, 2H, H-6$^{\backslash}$, H-6$^{\backslash\backslash}$); 3.4 (t, 1H, H-4$^{\backslash}$); 3.16 (m, 5H, 5 (OH), D$_2$O exchangeable); 3.52 (m, 1H, H-2$^{\backslash}$); 5.96 (d, 1H,H-1$^{\backslash}$, *J* = 7.5 Hz). ^{13}C- NMR (DMSO-d_6, 100 MHz, δ ppm) 24.9, 25.8 and 31.9 (CH$_2$), 63.3, 64.4, 70.9, 72.7, 74.6 (carbon atoms of sugar), 117.6 (C=C, pyrimidine), 125.4 (C= C-S, pent.), 126.0 (C=C-S, thiazole),139.4 (S-C-N, pent.) , 148.2 (carbon atom of sugar), 155.5 (C= C, pyrimidine), 158.3 (N-C=N), 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole). Anal. Calcd. (%) for C$_{17}$H$_{18}$N$_2$O$_7$S$_2$ (426.46) : C, 47.88; H, 4.25;

N, 6.57; O, 26.26; S, 15.04. Found (%) : C, 47.49 ; H,4.30 ; N,6.48; S,14.49.

2-Galctosyl-7,8-dihydrocyclopenta[4,5]thieno[2,3-d]thiazolo[3,2-a]pyrimidine-3,5(2H,6H)-dione (7c) .

Brown crystals (ethanol),m. p. 334-336 °C , yield: 49 %, IR (KBr, cm⁻¹): 3758 (OH), 2860 (CH); 1668; 1666 (2 CO). ¹H-NMR (DMSO-d_6, 500 MHz , δ ppm) 2.34 (m, 1H, H5'); 2.48 (t, 2H, CH₂); 2.49 (t, 2H, CH₂); 2.83 (m, 2H, H-6', H- 6''); 2.85 (m, 3H-3', H-4'); 3.56 (m, 5H, 5 (OH), D₂O exchangeable); 4.2 (dd, 1H, H-2', J= 7.5 Hz); ; 6.6 (d, 1H,H-1', J = 7.5 Hz). Anal. Calcd. (%): for $C_{17}H_{18}N_2O_7S_2$ (426.46):Cal; C, 47.88; H, 4.25; N, 6.57; O, 26.26; S, 15.04. Found (%) ; C,47.90 ; H,4.0 ; N,6.59; S,15.01.

2-Xylosyl-7,8-dihydrocyclopenta[4,5]thieno[2,3-d]thiazolo[3,2-a]pyrimidine-3,5(2H,6H)-dione (7d) .

Pale brown (crystals),(ethanol) . M. P. 240-242°C, yield: 66 %, IR (KBr, cm⁻¹): 3152 (OH); 2865 (CH); 1651; 1539 (2 CO) ;¹H-NMR (DMSO-d_6, 500 MHz , δ ppm) ; 2.33 (m, 2H, CH₂); 2.81 (t, 2H, CH₂); 2.85 (t, 2H, CH₂); 3.38 (m, 1H, H-4'); 3.41 (t, 2H, H-5', H-5''); 3.56 (d, 1H, H-3', J = 5Hz); 3.58 (m, 4H, 4 (OH), D₂O exchangeable); 5.8 (dd, 1H, H-2', J = 7.5 Hz); 6.68 (d, 1H,H-1', J = 7.5 Hz).¹³C-NMR (DMSO-d_6, 100 MHz, δ ppm) : 24.9, 25.8 and 31.9 (CH₂), 63.0, 72.4, 64.5, 76.8 (Sugar carbon atom), 117.6 (C=C, pyrimidine), 125.4 (C= C, pent.), 126.0 (C=C-S, thiazole),139.4 (C=C, pyrimidine), 148.2 (Sugar carbon atom), 155.5 (C= C, pent.), 158.3 (N-C=N), 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole). Anal. Calcd. (%) for $C_{16}H_{16}N_2O_6S_2$ (396.44): Cal; C, 48.47; H, 4.07; N, 7.07; O, 24.21; S, 16.18. Found (%): C, 48.50; H, 4.06; N, 7.05, S, 16.30 .

2-Arabinosyl-7,8dihydrocyclopenta[4,5]thieno[2,3-d]- thiazolo[3,2-a]pyrimidine-3,5(2H,6H)-dione (7e) .

Brown crystals (ethanol). M. P. 216-217 °C, yield: 69%, IR (KBr, cm⁻¹): 3423 (OH); 2527 (CH); 1649, 1541 (2CO) ;¹H-NMR (DMSO, 500 MHz , δ ppm) : 2.33 (m, 2 H, CH₂); 2.35 (t, 2H, CH₂); 2.49 (t, 2H, CH₂); 3.38 (m, 1H, H-4'); 4.46 (t, 2H, H-5', H- 5''); 4.98 (m, 1H, H-3') ; 3.4 (m, 4H, 4 (OH), D₂O exchangeable); 4.89 (dd, 1H, H-2', J= 7.5 HZ); 6.62 (d, 1H,H-1', J= 7.5 Hz). Anal. Calcd. (%) for $C_{16}H_{16}N_2O_6S_2$ (396.44) : C, 48.47; H, 4.07; N, 7.07; O, 24.21; S, 16.18 Found (%): C, 48.39 ; H, 4.00; N, 7.09; S,16.21.

2-Glucosyl-6,7,8,9-tetrahydro-2H-benzo[4,5] thieno[2,3-d]thiazolo[3,2-a]pyrimidine-3,5-dione (8a) .

Pale yellow crystals (ethanol), m. p. 220-222 °C , yield: 49 %, IR (KBr, cm⁻¹): 3935 (OH), 2854 (CH); 1671, 1540 (2 CO). ¹H-NMR (DMSO-d_6, 500 MHz , δ ppm) :1.22 (m, 2H, CH₂); 1.55 (m, 2H, CH₂); 2.33 (m, 1H, H5'); 2.34 (t, 2H, CH₂); 2.35 (m, 2H, H-6', H- 6''); 2.37 (t, 1H, H-4'); 2.52 (t, 1H, H-3'); 2.83 (t, 2H, CH₂); 2.89 (m, 1H, H-2'); 3.38 (m, 5H, 5 (OH), D₂O exchangeable); 6.68 (d, 1H,H-1', J = 7.5 HZ). Anal. Calcd. (%) For $C_{18}H_{20}N_2O_7S_2$ (440.49) : C, 49.08; H, 4.58; N, 6.36; O, 25.43; S, 14.56. Found (%): C,49.00; H,4.55; N, 6.40; S, 25.49.

2-Mannosyl-6,7,8,9-tetrahydro-2H-benzo[4,5] thieno[2,3-d]thiazolo[3,2-a]pyrimidine-3,5-dione (8b).

Brown crystals (ethanol), m. p. 243-244 °C, yield: 65 %, IR (KBr, Cm⁻¹) 3419 (OH), 2853 (CH); 1671, 1530 (2 CO) ;¹H-NMR (DMSO-d_6, 500 MHZ , δ ppm) :1.79 (m, 2H, CH₂); 1.8 (m, 2H, CH₂); 2.33 (m, 1H, H5'); 2.72 (t, 2H, CH₂); 2.83 (t, 2H, CH₂); 3.11 (m, 2H, H-6', H- 6''); 3.40 (t, 1H, H-4'); 3.52 (m, 5H,

5 (OH), D$_2$O exchangeable); 3.68 (m, 1H, H-2$^\backslash$); 3.75 (t, 1H, H-3$^\backslash$); 6.20 (d, 1H, H-1$^\backslash$, J = 7.5 Hz). ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm) : 23.0, 25.4 and 24.5 (CH$_2$), 63.3, 64.4, 70.9, 72.7 and 74.6 (carbon atoms of sugar), 117.6 (C=C, pyrimidine), 125.4 (C=C, hex.), 126.0 (C=C-S, thiazole),139.4 (C=C, hex.) , 148.2 (carbon atom of sugar), 155.5 (C= C, pyrimidine), 158.3 (N-C=N), 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole). Anal. Calcd. (%) For C$_{18}$H$_{20}$N$_2$O$_7$S$_2$ (440.49) : C, 49.08; H, 4.58; N, 6.36; O, 25.43; S, 14.56. Found (%): C,49.10; H, 4.55; N,6.29.

2-Galactosyl-6,7,8,9-tetrahydro-2*H*-benzo[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-dione (8c) .

Gray crystals (ethanol), m. p. 209-211°C, yield: 55%, IR (KBr, cm^{-1}) 3419 (OH), 2853 (CH); 1671, 1530 (2 CO) ; ^{1}H-NMR (DMSO-d_6, 500 MHz , δ ppm) :1.74 (m, 2H, CH$_2$); 1.94 (m, 2H, CH$_2$); 2.33 (m, 1H, H5$^\backslash$); 2.49 (t, 2H, CH$_2$); 2.5 (t, 2H, CH$_2$); 2.83 (m, 2H, H-6$^\backslash$, H- 6$^{\backslash\backslash}$); 2.85 (m, 3H,H-3$^\backslash$, H-4$^\backslash$, H-5$^\backslash$); 3.56 (m, 5H, 5 (OH), D$_2$O exchangeable); 4.2 (dd, 1H, H-2$^\backslash$, J = 7.5 Hz); 6.6 (d, 1H,H-1$^\backslash$, J = 7.5 Hz). Anal. Calcd. (%) for C$_{18}$H$_{20}$N$_2$O$_7$S$_2$ (440.49) : C, 49.08; H, 4.58; N, 6.36; O, 25.43; S, 14.56. Found: (%) C,48.99; H, 4.44; N, 6.46; 25.41; S, 14.46.

2-Xylosyl-6,7,8,9-tetrahydro-2*H*-benzo[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-dione (8d) .

Gray crystals (ethanol), m. p. 215-217 °C , yield: 70 %, IR (KBr, Cm^{-1}) 3457 (OH), 2457 (CH); 1637, 1531,1542 (2 CO) ; ^{1}H-NMR (DMSO-d_6, 500 MHz , δ ppm) :1.78 (m, 2H, CH$_2$); 1.94 (m, 2H, CH$_2$); 1.8 (t, 2H, CH$_2$); 3.95 (t, 2H, CH$_2$); 3.38 (m, 1H, H-4$^\backslash$); 3.39 (t, 2H, H-5$^\backslash$, H- 5$^{\backslash\backslash}$); 3.49 (d, 1H, H-3$^\backslash$, J = 5Hz); 3.56 (m, 4H, 4 (OH), D$_2$O exchangeable); 5.98 (dd, 1H, H-2$^\backslash$, J = 7.5 Hz); 6.7 (d, 1H,H-1$^\backslash$, J = 7.5 Hz). ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm) : 23.0, 23.4 and 24.5 (CH$_2$), 63.0, 72.4, 64.5, 76.8 (sugar carbon atom), 117.6 (C=C, pyrimidine), 125.4 (C= C, hex.), 126.0 (C=C-S, thiazole),139.4 (C=C, hex.) , 148.2 (sugar carbon atom), 155.5 (C= C, pyrimidine.), 158.3 (N=C-N, pyrimidine), 168.6 (C=O, pyrimidine), 171.3 (N-C=O, thiazole). Anal. Calcd. (%) for C$_{17}$H$_{18}$N$_2$O$_6$S$_2$ (410.46) : C, 49.74; H, 4.42; N, 6.82; O, 23.39; S, 15.62 Found (%) : C, 49.60 ; H, 4.39; N, 6,79, S, 1564.

2-Arabinosyl-6,7,8,9-tetrahydro-2*H*-benzo[4,5]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidine-3,5-dione (8e).

Gray crystals (ethanol), m. p. 233-235 °C, yield: 43% , IR (KBr, Cm^{-1}): 3423 (OH) ; 2854 (CH); 1669, 1529 (2 CO) ;^{1}H-NMR (DMSO-d_6, 500 MHz, δ ppm) 1.77 (m, 2H, CH$_2$); 1.79 (m, 2H, CH$_2$); 2.5 (t, 2H, CH$_2$); 2,67 (t, 2H, CH$_2$), 3.38 (m, 1H, H-4$^\backslash$); 4.5 (t, 2H, H-5$^\backslash$, H- 5$^{\backslash\backslash}$); 4.00 (m, 1H, H-3$^\backslash$) ; 3.41 (m, 4H, 4 (OH), D$_2$O exchangeable); 4.9 (dd, 1H, H-2$^\backslash$, J= 7.5 Hz); 6.60 (d, 1H,H-1$^\backslash$, J = 7.5 Hz). Anal. Calcd. (%) for C$_{17}$H$_{18}$N$_2$O$_6$S$_2$ (410.46) : C, 49.74; H, 4.42; N, 6.82; O, 23.39; S, 15.62, Found (%) : C, 49.81; H, 4.39; N, 6.79; S, 15.55.

5. 2. Biological Screening:

5.2.1. Antimicrobial Screening:

Materials:

The bacterial and fungal microorganisms used in this study were *Salmonella typhi, Salmonella paratyphi, Salmonella typhimurium, Shigella species, Pseudomonas aeruginosa, Staphylococcus aureus,* and Escherichia coli and one fungi (*Trichophyton schoenleinii* ,which were donated ATCC collection culture-USA.

Methods:

1. Preparation of Inoculums:

The bacteria used for the study were prepared by inoculating isolates into nutrient broth and incubated at 37° C for 24hr. Fungal strains used for the study

were prepared by inoculating isolates into SDA and incubated at 30 ℃.

2. Antimicrobial Sensitivity Testing. Agar well diffusion method was used to determine zone of inhibition. Mueller-Hinton agar for bacteria, SDA for *Trichophyton choenleinii* was used. About 20–25 mL of molten medium cooled to 45^0C and was added to sterilized plates (150 mm in size). After these 16–

24-hour-old cultures of bacterial species, and 7-day-old cultures of *Trichophyton schoenleinii* were spread using a sterile cotton swab and each microbe evenly spread over the entire surface of agar plate to obtain a uniform plate surface growth. Petri plates were allowed to dry. About 3- 4 wells in each plate of 15 mm diameter and 5mm depth were punched in agar surface with the help of a sterilized borer for placing the extracted oil samples. About 50 µL of the undiluted essential oil of two samples was dispensed into respective wells and 10 mg Ciprofloxacin was used as a positive control for bacteria and Bavistin was used as positive control for fungus. Dimethyl sulfoxide (DMSO) was used as negative control. The plates were then left at room temperature for 30 minutes and then incubated at 30^0C for 7 days for Trichophyton choenleinii. and plates with bacteria were incubated for 24 hours at 37℃. After incubation, the zones of inhibition were measured using a ruler and the results reported in millimeters (mm). All the tests were run in triplicate and the average result was taken [75,76].

5.2.2. Determination of Minimum Inhibition Concentration (MIC):

Minimum inhibition concentration was determined using the agar dilution method. The MIC was evaluated on plant extracts that showed antimicrobial activity in the agar well diffusion assay on any

organism. Overnight incubated suspension of each organism in nutrient broth was prepared and 50 L was added to all the test tubes and preparations were incubated at 37℃ for 24 hours. After incubation, using a sterile cotton swab, suspension of each tube was inoculated on nutrient agar to see if bacterial growth was inhibited or not. Growth of bacteria on solid media indicated that a particular concentration of extract was unable to inhibit the bacteria. The MIC

was defined as the lowest concentration of an antimicrobial that inhibited the visible growth of a microorganism after overnight incubation [77]. All the measurements were replicated three times for each assay and the results are presented as mean ±SD. Data was analyzed using windows SPSS version 26.0 and descriptive statistic was used.

5. 2. 3. Method of Docking Process for Antimicrobial:

Preparation of receptor for virtual screening, after choosing protein of target site some processes should have done to give insights of molecular binding modes of the Tested compound inside the pockets of (bacterial gyrase enzyme complexed with DNA strands and fungal CYP 51) [78] by using MOE 14.0901 Software. The binding sites were generated from the co-crystallized ligand, within crystal protein (PDB codes: 2XCT – 5JLC) (https://www.rcsb.org). At first water molecules have been removed from the complex. Then, crystallographic disorders and unfilled valence atoms were corrected using protein report and utility and clean protein options. Protein energy was minimized by applying MMFF94 force fields. The rigid of binding Site was structure of protein was obtained by applying fixed atom constraint. The protein essential Amino acids defined and prepared for docking process. 2D structures of

tested compounds were drawn using Chem-Bio Draw Ultra16.0 and saved in MDL-SD file format , From MOE 14.0901 Software, the saved file was opened, 3D structures were protonated and energy Was minimized by applying .05 RMSD kcal/mol MMFF94 force field. Then, the minimized Structures were prepared for docking using prepares ligand protocol. Molecular Docking process was carried out using CDOCKER protocol. The receptor was held rigid while the ligands were allowed to be flexible during the refinement each molecule was allowed to produce ten different interaction poses with the protein. Then docking scores (-CDOCKER interaction energy) of the best-fitted poses with the active site at (bacterial gyrase enzyme and fungal CYP 51) was recorded and 3D view was generated by Discovery Studio 2016 Client software. We use all these processes to predict the proposed binding mode, affinity, preferred orientation of each docking pose and binding Free energy (ΔG) of the tested compounds with (bacterial gyrase enzyme and fungal CYP 51).

5.2.4. Method of Docking of Anti-inflammatory:

Preparation of receptor for virtual screening, after choosing protein of target site some processes should have done to give insights of molecular binding modes of the Tested compound inside the pockets of (Human 5-Lipoxygenase) by using MOE 14.0901 Software. The binding sites were generated from the co-crystallized ligand, within crystal protein (PDB codes: 6N2W) (https://www.rcsb.org). At first water molecules have been removed from the complex. Then, crystallographic disorders and unfilled valence atoms were corrected using protein report and utility and clean protein options. Protein energy was minimized by applying MMFF94 force fields. The rigid of binding Site was structure of protein was

obtained by applying fixed atom constraint. The protein essential Amino acids defined and prepared for docking process. 2D structures of tested compounds were drawn using Chem-Bio Draw Ultra16.0 and saved in MDL-SD file format , From MOE 14.0901 Software, the saved file was opened, 3D structures were protonated and energy was minimized by applying .05 RMSD kcal/mol. MMFF94 force field. Then, the minimized Structures were prepared for docking using prepares ligand protocol. Molecular Docking process was carried out using CDOCKER protocol. The receptor was held rigid while the ligands were allowed to be flexible during the refinement each molecule was allowed to produce ten different interaction poses with the protein. Then docking scores

(-CDOCKER interaction energy) of the best-fitted poses with the active site at (Human 5-Lipoxygenase) was recorded and 3D view was generated by Discovery Studio 2016 Client software. We use all these processes to predict the proposed binding mode, affinity, preferred orientation of each docking pose and binding Free energy (ΔG) of the tested compounds with (Human 5-Lipoxygenase).

5.2.5.Anti-inflammatory: Membrane Stabilization:
Preparation of erythrocyte suspension: Whole blood was obtained with heparinized syringes from rats through cardiac puncture. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4). The blood was centrifuged for 10 minutes at 3000 g. Hypotonic solution-induced erythrocyte haemolysis: Membrane stabilizing activity of the samples was assessed using hypotonic solution-induced erythrocyte hemolysis [79]. The test sample consisted of stock erythrocyte (RBCs) suspension (0.50 ml)

mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4). containing the extract (1000-7.81 μg/ml) or indomethacin. The control sample consisted of 0.5 ml of RBC mixed with hypotonic-buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000 g. In 96 well plates, the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of hemolysis or membrane stabilization was calculated according to modified method [79]. Inhibition of heamolysis (membrane stabilization%) = {OD_1-OD_2/OD1} x 100 Where: OD_1 = Optical density of hypotonic-buffered saline solution alone OD_2 = Optical density of test sample in hypotonic solution. The IC50 value was defined as the concentration of the sample to inhibit 50% RBCs haemolysis under the assay conditions [80].

5.2.6. In Vitro Lipoxygenase (LOX) Inhibition Assay:

The samples and the reference compound (Ibuprofen) were tested in order to investigate the anti-inflammatory response by inhibiting the LOX enzyme from Glycine max (type I-B). This assay was performed according to (Granica et al., 2013) with slight modifications. Briefly, in 96 well plates 100 μl of soybean LOX solution (1000 U/ml in borate buffer solution, pH 9) and 200 μl of borate buffer were mixed together with varying concentrations of the samples to a final concentration range of 0.98-125 μg /ml at 25° C for 15 min. Samples were pre-incubated with 100 μl of linoleic acid (substrate) to start the reaction. The inhibitory activity was determined by monitoring the absorbance's increase at 234 nm using a microplate reader (BIOTEK; USA). The inhibitory percentages were calculated according to the formula:

Inhibitory activity (%) = (1 − As /Ac) ×100, where, As is the absorbance in the presence of test substance and Ac is the absorbance of control. IC50 values – the inhibitory concentration of the samples required to decrease by 50% the enzyme's activity – were determined from the plotted graphs of enzyme inhibition (%) against the concentrations of the samples [81].

5.2.7. In Vitro Cyclooxygenase (COX1 and COX 2) Inhibition Assay:

The samples at the concentration range of 0.98-125 μg /mL were tested in order to investigate the anti-inflammatory response by inhibiting the COX-1 or COX-2 enzyme. The COX (EC 1.14.99.1) activity was monitored, as the result of the N,N,N,N-tetramethyl-p-phenylenediamine (TMPD) oxidation reaction with arachidonic acid. This assays were performed according to Petrovic and Murray (2010) and Amessis-Ouchemoukh et al. (2014) with slight modifications. The inhibitory activity was determined by monitoring the absorbance's increase at 611 nm using a microplate reader (BIOTEK; USA).   The inhibitory percentages were calculated according to the formula: Inhibitory activity (%) = (1 − As /Ac) ×100, where, As is the absorbance in the presence of test substance and Ac is the absorbance of control. The efficacy of extracts and reference compound (ibuprofen) to inhibit COX-1 or cox -2 isoenzymes were determined as the concentration causing 50% enzyme inhibition (IC50) [82]

6. **Structure Activity Relationship**:

From the above study we observed that compounds containing five membered ring were owed the heist

activity among the examined series **2a,2b,3,4 ,7a-e** and **8a-e** . Compound **2a** has the best results due to the presence of five membered ring, free amino group(electron donor group) and simple structure when comparing with compound 3 while ring closure decreased the activity. Sugar moiety increased the activity, especially for compounds peering five membered rings **7a-e** compared with compounds **8a-e**. Compounds **7a** and 7d gave the best biological activity due to the orientation of the hydroxyl groups (Figure 11).
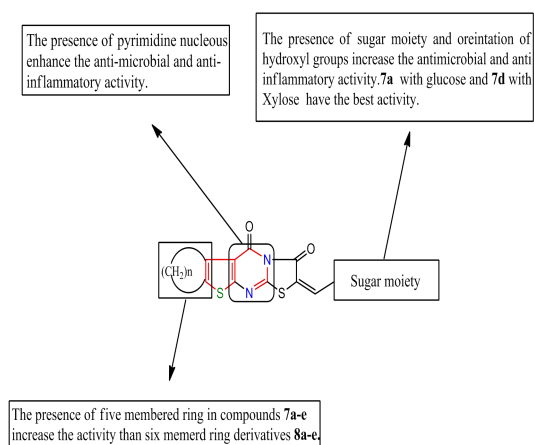

The presence of pyrimidine nucleous enhance the anti-microbial and anti-inflammatory activity.

The presence of sugar moiety and oreintation of hydroxyl groups increase the antimicrobial and anti inflammatory activity.**7a** with glucose and **7d** with Xylose have the best activity.

Sugar moiety

The presence of five membered ring in compounds **7a-e** increase the activity than six memerd ring derivatives **8a-e**.

Fig. 11: Structure activity relationship of compounds **7a-e** & **8a-e**

Acknowledgment:

Conflict of interest

No conflict of interest.

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