NOVEL ANTI-INFLAMMATORY AND ANALGESIC AGENTS OF 5-CHROMENO-PYRIDO[2,3-*d*]PYRIMIDINE-4-YL DERIVATIVES AND *S*-NUCLEOSIDES ANALOGOUS

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ABSTRACT

A series of new 3-formyl coumarine (**2**) was synthesized in good yields by replacement of chlorine atom by 2nd amines. In addition, 5-chromeno-pyrido[2,3-*d*]pyrimidine-4-yl derivatives (**5**,**6**) was synthesized by cyclocondensation of 6-aminouracil, the respective ketones and 3-formyl coumarine (**2**) in one pot-synthesis. Also 2-*S*-nucleosides analogous of pyrido[2,3-*d*]-pyrimidines (**12**-**15**) was synthesized by a simple method in good yields, The new compounds were evaluated for their anti-inflammatory and analgesic activity. It has been found that the derivatives **5a**, **5b**, **14a**, **14b**, **15a** and **15c** exhibited the dual pharmacological activities with superior gastrointestinal safety profile when compared to indomethacin except **5a** which resulted in ulcer lesions in many of the experimental rats.

Keywords: 5-Chromeno-pyrido[2,3-*d*]pyrimidine*; S*-Nucleosides; Anti-inflammatory and Analgesic activities.

1. INTRODUCTION

4-Hydroxycoumarin comprises the structural nucleus of many natural products, drugs and pesticides[1-4]. It is the key intermediate for various widely used anticoagulants and rodenticides[4,5] as well as drugs used as antithrombotic agents in human[6,7]. Besides, interest in coumarin derivatives is steadily increasing since they are key subjects of broad spectrum aspects of biological evaluations which include, antibacterial[8,9] antifungal[8,10], antimicrobial[11], ovicidal[12], anti-implantation[13], coronary vasodilating[14] and anti-neoplastic[15-17] activities. Moreover, many coumarin derivatives were successfully tested for controlling human immunodeficiency virus (HIV) infection and other virus-related immunodeficiency disorders[18]. Treatment of retroviral infections against HIV-protease[19,20], cell antiproliferation[21] and also antimitotics[22], were also evaluated.

Coumarone group antibiotics, such as Novobiocin, Coumermycin and Clorobiocin, are potent inhibitors of DNA gyrase. These antibiotics have been isolated from various Streptomyces species and all possess a 3-amino-4-hydroxy-coumarin moiety as their structural core. Prior labeling experiments on novobiocin established that the coumarin moiety was derived from L-tyrosine, probably via a beta-hydroxy-tyrosine (beta-OH-Tyr) intermediate. Recently the novobiocin gene cluster from Streptomyces spheroides was cloned and sequenced and allows analysis of the biosynthesis of the coumarone at the biochemical level using over expressed and purified proteins.

We recently reported the identification of pyrido[2,3-d]pyrimidines as selective anti-oxidant[23], anti-inflammatory and highly analgesic properties[24-28]. We report here a synthesis of new coumarone aldehyde, 4-piprazino-, 4-morpholino-, 5-chromeno-pyrido[2,3-d]pyrimidine and its analogous. Also, the anti-inflammatory activity was evaluated by carrageenan-induced paw edema test in rats[29] and the analgesic activity was performed by the *Armitage and Woolfe* technique[30,31].

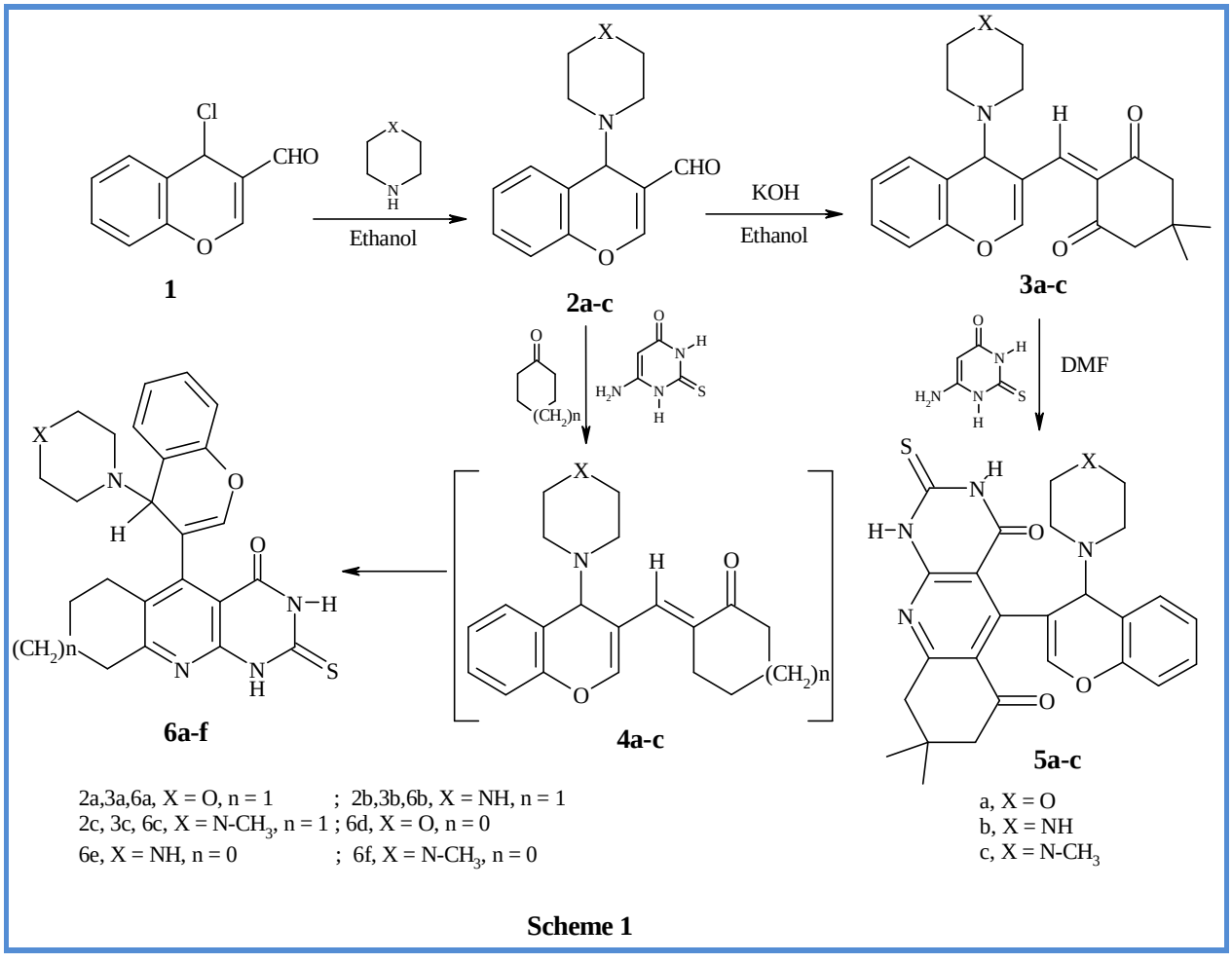
2. CHEMISTRY

The synthetic route used to synthesize various coumarin derivatives is outlined in Scheme 1. 4-chloro-3-formyl coumarine (**1**), the starting material was prepared according to the method reported in the literature[32], using *o*-hydroxy aryl alkyl ketones. The 4-substituted-3-formyl coumarine (**2a-c**) was prepared by substitution reaction of of 4-chloro-3-formyl coumarine followed by treatment with secondary amines in absolute ethanol. The reaction of (**2**) with the appropriate amines namely, morpholine, piprazine and N-methyl-piprazine gave the corresponding 4-substituted 3-formyl chromone derivatives (**2a-c**). The terminal formyl function was then condensed with the cyclicketone derivatives namly, dimedone, cyclopentanone and cyclohexanone in presence of 6-aminothiouracil in refluxing dimethylformamide to the target 5-chromeno-pyrido[2,3-*d*]-pyrimidine-4-yl derivatives (**5a-c**, **6a-f**).

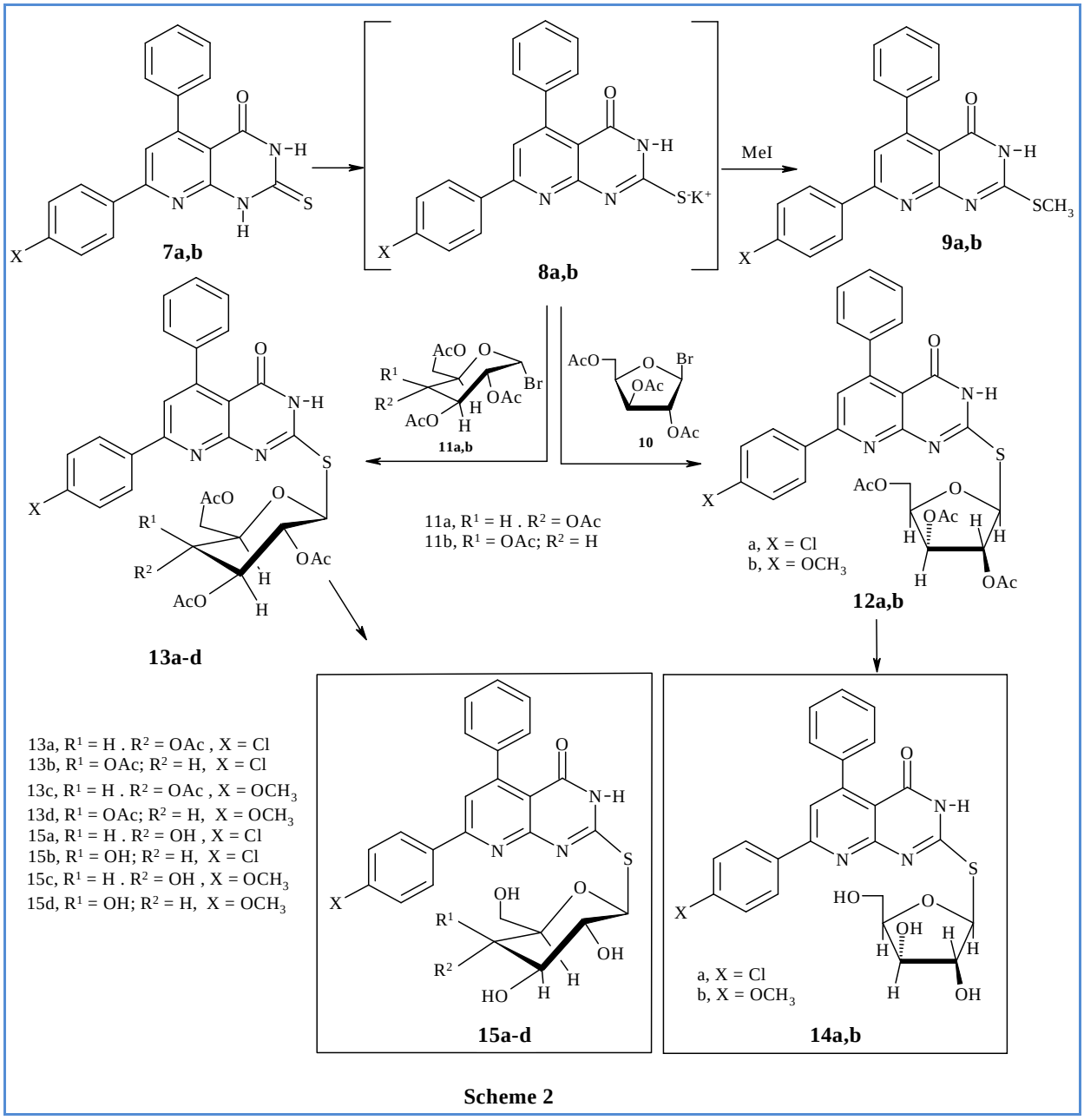
The readily available 2-thioxo-pyridopyrimidines **7a,b**, either 2-*S*-methyl (compound **9a,b**, Schemes 2) or its potassium salt (compound **8a,b**, Scheme 2) were prepared according to previously described procedures[23,24], and used as the starting material for the synthesis of 2-*S*-(β-D-glycopyranosyl)-pyrido[2,3-*d*]pyrimidine and 2-*S*-(β-D-glycofuranosyl)-pyrido[2,3-*d*]-pyrimidine derivatives. This is a modified synthetic route of 2-*S*-(β-D-glycopyranosyl/or furanosyl)-pyrido[2,3-

d]pyrimidine which requires the salt of 2-thioxo-pyrido[2,3-*d*]pyrimidines as one of the key intermediates is developed as shown in Scheme 2.

Moreover, we describe the synthesis of 2-*S*-(*β*-D-glycopyranosyl/or furanosyl)-pyrido-[2,3-*d*]-pyrimidine is outlined in Scheme 2. The pyrido[2,3-*d*]pyrimidines **7a,b** were converted into their potassium salts **8a,b** by using of KOH in acetone and stirring at room temperature for long time with with 1-bromo-2,3,5-tri-*O*-acetyl-*α*-*D*-arabinofuranose (**10**) yielding the *S*-glycosylated nucleosides **12a,b** in good yields. Thin layer chromatography (Chloroform: Methanol, 7:3) indicated the purity of the compounds. Structures of the *S*-glycosides were confirmed by elemental analyses and spectral data (IR, ^{1}H, ^{13}CNMR). The ^{1}H NMR spectrum of compound **12a** as an example, showed the anomeric proton of the arabinose moiety as a doublet at δ 6.64 ppm with a coupling constant *J*=3.67*Hz* indicating *β*-configuration of the anomeric center. Other protons of the furanosyl ring resonated at δ 4.05-5.39 ppm, while the three acetoxy groups appeared as three singlets at δ 1.92, 1.97 and 2.00 ppm. The ^{13}C NMR spectrum revealed the absence of the thione C-2 atom around ≈ 175 ppm and a resonance of –N=C-N- carbon atom (C-2) at δ 159 ppm. The signals in the region δ 168.5-170.2 ppm are due to the three acetoxy carbonyl atoms (3 C=O). and the five signals at δ 60.82, 65.69, 67.58, 69.73, 87.59 ppm were assigned to C-5′, C-3′, C-2′, C-4' and C-1′, respectively. Moreover, the IR spectra of compounds **12** revealed the absence of the vibration band of a thione group. Similarly, the reaction of heterocycle base **8a,b** with 2,3,4,6-tetra-*O*-acetyl-*α*-*D*-glucopyranosyl bromide (**11a**) or 2,3,4,6-tetra-*O*-acetyl-*α*-*D*-galacto-pyranosyl bromide (**11b**), yielding the *S*-glycosylated nucleosides **13a-d** in good yields. Thin layer chromatography (Chloroform: Methanol, 7:3) indicated the purity of the compounds. Structures of the *S*-glycosides were confirmed by elemental analyses and spectral data (IR, ^{1}H, ^{13}CNMR). The ^{1}H NMR spectrum of compound **13a** as an example, showed the anomeric proton of the glucose moiety as a doublet at δ 5.99 ppm with a coupling constant *J*=10.60*Hz* indicating *β*-configuration of the anomeric center. Other protons of the glucopyranose ring resonated at δ 3.86-5.37 ppm, while the four acetoxy groups appeared as four singlets at δ 1.95, 1.99, 2.05 and 2.13 ppm. The ^{13}C NMR spectrum revealed the resonance of –N=C-N- carbon atom (C-2) at δ 161.0 ppm and the signals in the region δ 169.9-170.1 ppm are due to the four acetoxy carbonyl atoms (4 C=O), Also, the six signals at δ 61.40, 66.50, 67.57, 70.32, 74.63, 81.47 ppm were assigned to C-6′, C-3′, C-2′, C-4′, C-5' and C-1′, respectively (see experimental).



Scheme 1



Scheme 2

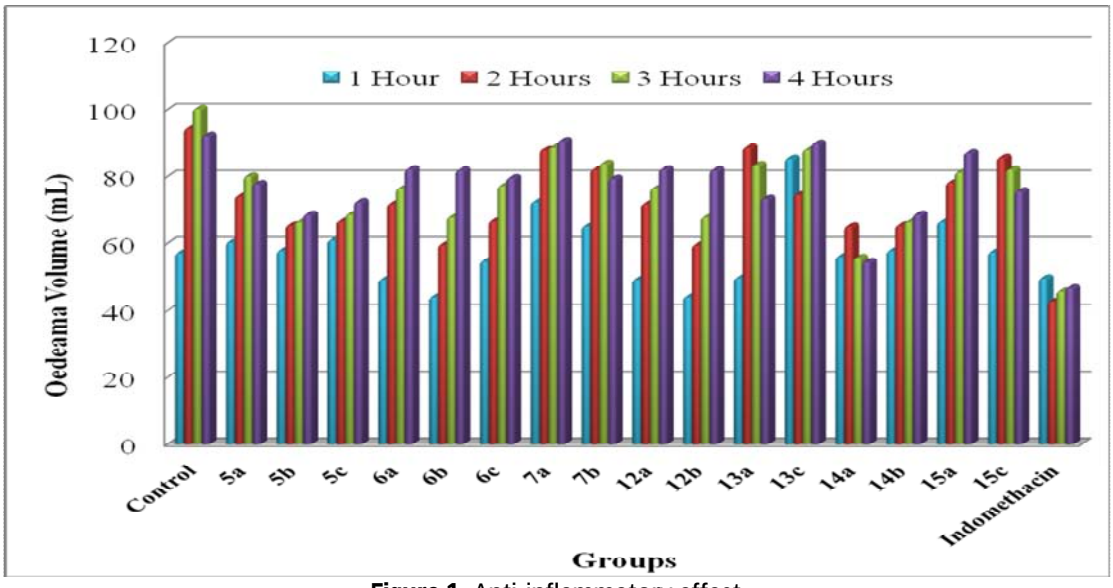


Figure 1: Anti-inflammatory effect

Table 1: Anti-inflammatory effect.

Groups	Oedeama volume (ml)			
	1h	2h	3h	4h
Control	57.3 ±6.8	94.4 ± 8.5	100.3 ± 3.3	92.3 ± 3.3
5a	60.4 ± 7.1	74.1 ± 5.4[a]	80.1 ± 6.0[a]	77.8 ± 5.5[a]
5b	57.9 ± 7.2	65.4 ± 8.8[a]	66.4 ± 6.9[a]	68.6 ± 6.3[a]
5c	61.0 ± 6.6	66.6 ± 5.9[a]	68.6 ± 7.0[a]	72.4 ± 7.4*
6a	49.4 ± 7.1	71.8 ± 6.7[a]	76.4 ± 4.8[a]	82.2 ± 5.2
6b	44.2 ± 5.1	59.6 ± 4.7[a]	67.8 ± 3.3[a]	81.9 ± 3.2
6c	54.9 ± 6.2	66.7 ± 6.9[a]	77.2 ± 6.6[a]	79.6 ± 4.9
7a	72.4 ± 4.9	88.0 ± 9.5	88.7 ± 6.5	90.7 ± 5.4
7b	65.1 ± 7.2	82.1 ± 6.9	83.9 ± 2.3	79.4 ± 7.3
12a	49.4 ± 7.1	71.8 ± 6.7[a]	76.4 ± 4.8[a]	82.2 ± 5.2
12b	44.2 ± 5.1	59.6 ± 4.7[a]	67.8 ± 3.3[a]	81.9 ± 3.2
13a	49.9 ± 2.5	88.7 ± 6.1	83.5 ± 3.7	73.4 ± 2.1[a]
13c	85.4 ± 3.1	74.6 ± 7.5[a]	88.0 ± 7.2	89.9 ± 2.1
14a	56.2 ± 9.9	65.1 ± 7.5[a]	55.9 ± 10.6[a]	54.7 ± 7.2[a]
14b	57.9 ± 7.2	65.4 ± 8.8[a]	66.4 ± 6.9[a]	68.6 ± 6.3[a]
15a	66.4 ± 7.5	78.2 ± 3.5[a]	81.3 ± 3.3	87.1 ± 2.1
15c	57.5 ± 6.3	85.7 ± 2.8	82.1 ± 1.3	75.6 ± 5.1[a]
Indomethacin	49.8 ± 5.3	42.9 ± 5.1[a]	45.9 ± 4.6[a]	46.9 ± 5.8[a]

[a] $P < 0.05$: Statistically significant from the control using one way ANOVA (Two sided Dunnett as Post Hoc test).

Deacetylation of *S*-nucleosides **12a,b** and **13a-d** proceeded smoothly *via* methanolic ammonia treatment to afford the free nucleosides **14a,b** and **15a-d** in good to excellent yields (Scheme 2). The ^{1}H-NMR data of the compounds **14** and **15** revealed the absence of the acetyl protons in the region δ 1.90-2.20 and appearance of the D_2O exchangeable OH protons in the region δ 4.60-5.56. The IR data of the compound **14a** as a typical example showed also the absence of the acetyl carbonyl function around 1700 cm^{-1} and the appearance of the characteristic OH band at 3500 (br) cm^{-1}.

3. BIOLOGICAL EVALUATION

3.1. Antiinflammatory effect: The anti-inflammatory activity of sixteen of the newly synthesized compounds: **5a-c**, **6a-c**, **7a,b**, **12a,b**, **13a,c**, **14a,b**, **15a,c** were evaluated by applying carrageenan-induced paw oedema bioassay in rats[29] using indomethacin as a reference standard. Results were expressed as mean ± S.E. Difference between vehicle control and treatment groups were tested using one way ANOVA followed by the least significant difference (L.S.D.). Methods of statistical analysis were done according to *Armitage et al*. [30]. According to Table 1, administration of many of tested compounds 60min prior to carrageenan injection at dose of 9 mg/kgb wt caused significant inhibition of paw oedema response. Compounds **5a**,**5b**, **5c**, **14a** and **14b** caused significant decrease in paw oedeama after 2, 3, 4 h after drug administration, while **6a**, **6b** and **6c** gave their response after 2 h of administration and continued to the third hour. Compounds **13c** and **15a** showed the effect only after 2 h but compounds **13a**, **15c** significantly decreased the paw oedeama after 4 h post administration. On the other hand compounds **7a** and **7b** were inactive towards carrageenan-induced oedema in comparison to the standard reference indomethacin which markedly and significantly inhibited the pawoedema after 2, 3, 4 h of carrageenan injection. Thus, compounds **5a**, **5b**, **5c**, **6a**, **6b**, **6c**, **13c**, **14a**, **15a** and **15c** have good anti-inflammatory activity and compound **14a** was the most potent derivative. Results are illustrated by Fig. 1.

3.2. Analgesic activity: The analgesic activity of the above mentioned sixteen derivatives was also evaluated by applying Hot plate test[31] using Tramadol as a standard reference. Results were expressed as mean ± S.E. Difference between vehicle control and treatment groups were tested using one way ANOVA followed by the least significant difference (L.S.D.). Methods of statistical analysis were done according to Armitage et al. [30]. According to Table 2, compounds **14a**, **14b**, **15a** and **15c** showed significant analgesic activity higher than that obtained by Tramadol 1 h and 2 h post administration. While compounds **6a**, **6b** and **6c** exhibited equipotent analgesic effects or slightly less than that of Tramadol after 1 and 2 h of their administration. Compounds **5a**, **5b** and **5c** exhibited significant analgesic activity higher than or slightly equipotent to Tramadol only after 2 h of administration. Compounds **12a**, **12b**, **13a** and **13c** exhibited the analgesic effect after 1 h of administration only. Compounds **7a** and **7b** have no analgesic activity in comparison to the base line of the same group 1 and 2 h post administration. Thus, it can be concluded that, compounds **5a**, **5c**, **6a**, **6b**, **12a**, **12b**, **14a**, **14b**, **15a** and **15c** have significant analgesic activity and compound **14a** is the most potent one. Results are illustrated by Fig. 2.

Table 2: Analgesic effect

Groups	Reaction time (sec.)		
	Basal	1h	2h
Control	12.2 ± 0.63	12.1 ± 0.83	12.2 ± 1.18
5a	12.9 ± 0.95	15.0 ± 1.02	20.5 ± 1.19[a]
5b	10.3 ± 1.03	14.6 ± 0.93	18.4 ± 1.47[a]
5c	13.3 ± 1.33	16.9 ± 1.18	18.1 ± 1.36[a]
6a	9.6 ± 1.11	17.1 ± 1.52[a]	17.0 ± 1.25[a]
6b	9.5 ± 0.68	17.5 ± 1.33[a]	17.2 ± 1.11[a]
6c	9.1 ± 1.00	17.0 ± 1.11[a]	17.3 ± 1.13[a]
7a	9.2 ± 0.83	10.9 ± 0.56	15.3 ± 1.17
7b	11.8 ± 0.82	13.5 ± 0.75	11.5 ± 0.85
12a	9.5 ± 0.49	17.0 ± 1.89[a]	9.32 ± 0.89
12b	9.4 ± 0.51	17.0 ± 0.43[a]	13.6 ± 1.14
13a	9.6 ± 1.11	17.1 ± 1.52[a]	9.32 ± 0.89
13c	9.1 ± 1.00	17.0 ± 1.11[a]	17.3 ± 1.13
14a	15.1 ± 1.34	24.9 ± 1.38[a]	19.4 ± 0.61[a]
14b	11.0 ± 0.91	19.1 ± 1.46[a]	19.2 ± 1.00[a]
15a	13.0 ± 0.85	24.1 ± 1.65[a]	17.8 ± 2.64[a]
15c	12.6 ± 1.13	21.0 ± 2.47[a]	18.4 ± 0.69[a]
Tramadol	13.1 ± 0.78	17.6 ± 0.32[a]	18.2 ± 0.28[a]

Values represent the mean ± S.E. of six animals for each groups.
[a] P < 0.05: Statistically significant from Control. (Dunnett's test).

Figure 2: Analgesic effect.

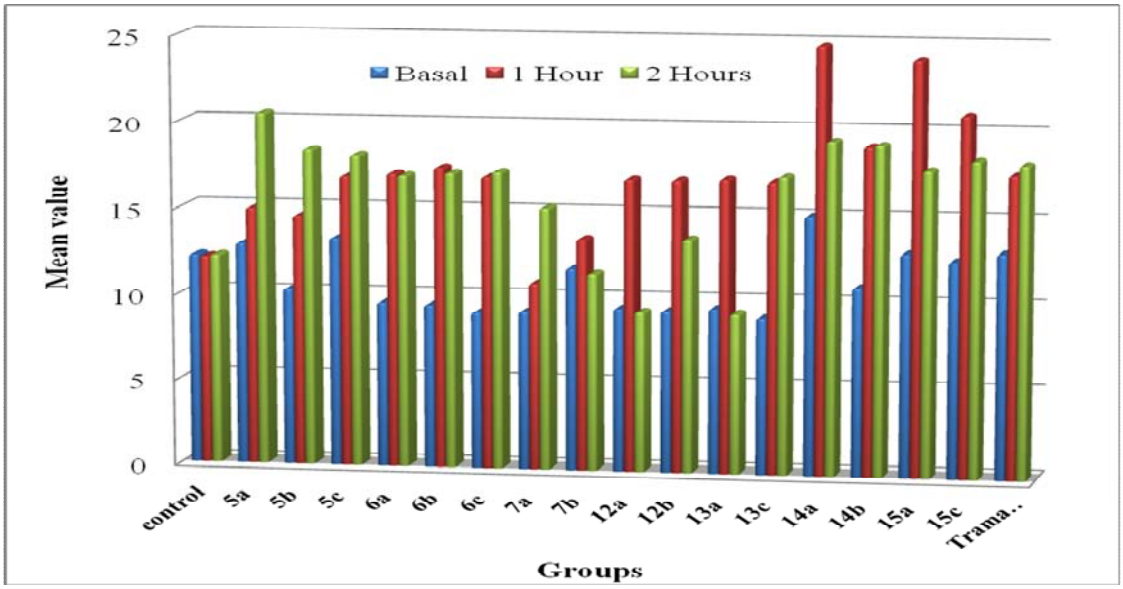


Table 3: Ulcerogenic effect.

Group	Ulcer index		No. Of rats with ulcer/5
	No. of ulcer	Severity of ulcer	
Control (ethanol)	8.2 ± 0.86	19.4 ± 2.20	5
5a	7.0 ± 1.64	10.4 ± 2.86	5
5b	1.6 ± 1.36[a]	2.0 ± 1.76[a]	2
14a	0.0 ± 0.00[a]	0.0 ± 0.00[a]	0
14b	0.4 ± 0.40[a]	0.6 ± 0.60[a]	1
15a	2.6 ± 1.66[a]	3.2 ± 1.96[a]	2
15c	0.4 ± 0.40[a]	0.4 ± 0.40a	1
Indomethacin	5.8 ± 1.77	9.8 ± 3.23[a]	5

Values represent the mean ± S.E. of five animals for each group.
[a] P < 0.05: Statistically significant from ethanol treated rats. (Kruskal Wallis, followed by Mann Whitney test).

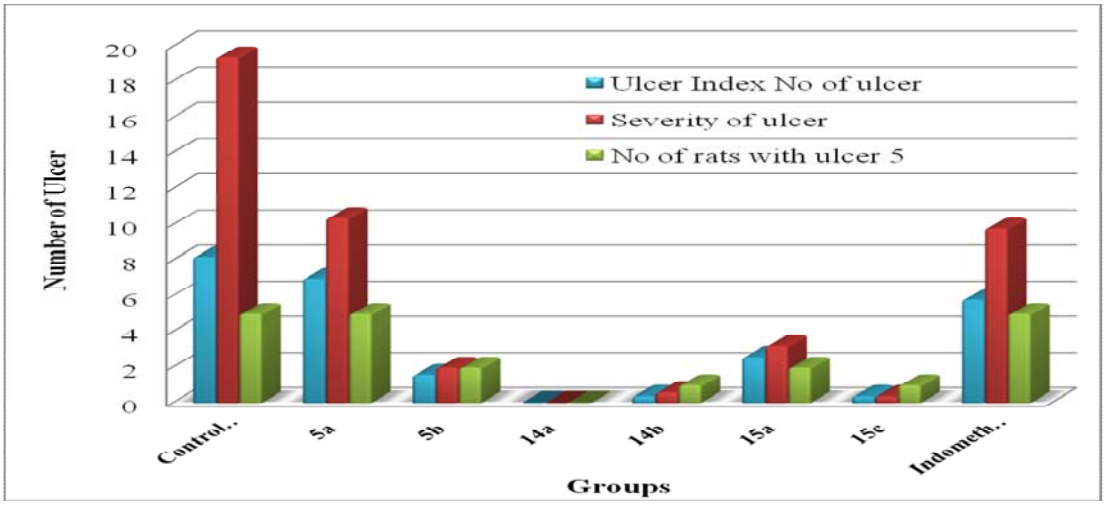


Figure 3: Ulcerogenic effect.

3.3. Ulcerogenic effect

The ulcerogenic effect of the most active anti-inflammatory and analgesic derivatives: **5a**, **5b**, **14a**, **14b**, **15a**, **15c** was evaluated[33]. According to Table 3, it has been found that compounds **5b**, **14b**, **15a**, **15c** have very little ulcerogenic effect with better safety margin in comparison to indomethacin. Interestingly, compound **14a** exhibited no ulcerogenic effect in all of the experimental animals. On the other hand compound **5a** resulted in ulcer lesions in many of the experimental rats. Therefore, the potential medicinal value of these compounds as anti-inflammatory and analgesic agents, that they have better safety margin than indomethacin on gastric mucosa. Results are illustrated by Fig. 3.

4- CONCLUSION

This study includes the synthesis of two series of novel derivatives of pyrido[2,3-*d*]pyrimidines attached to various aromatic and/or heterocyclic ring systems such as: morhpoline, piprazines and their *S*-glycosides. Different sixteen derivatives were evaluated as anti-inflammatory and analgesic agents in experimental animals. It has been found that the derivatives **5a**, **5b**, **14a**, **14b,** **15a**, and **15b** exhibited the dual pharmacological activities with superior gastrointestinal safety profile when compared to indomethacin except **5a** which resulted in ulcer lesions in many of the experimental rats.

Surprisingly, compound **14a** exhibited no ulcerogenic effect in all of the experimental animals. Thus, it can be concluded that pyridopyrimidine moiety, 4-chlorophenyl and arabinofuranosyl ring systems are important for both anti-inflammatory and analgesic activity of potent safety margin profiles towards G.I.T. Compounds **7a** and **7b** has no activity but by placing an acetylated arabinofuranosyl and glucopyranosyl group at C-2 in the pyrimidine ring on the pyridopyrimidine resulted in good activity of compound 12a,b and 13a,c in comparison to the standard drug (Indomethacine, Tramadol) while the deacetylated of S-glycoside compounds in pyridopyrimidine resulted in **14b, 15a,c** showed the higher activity than the acetylated glycosides. Especially compound **14a** have the most potent activity as anti-inflammatory and analgesic activities. Also has no ulcerogenic effect. Compounds **5a-c** has good activity as Anti-inflammatory and analgesic activity due to the presence of coumarine moiety which attached to the piprazine and morpholine moiety which increases the anti-inflammatory and analgesic activities. Also compounds **6a-c** have good activity due to the presence of substituted Quinoline attached to the coumarine which attached to the piprazine and morpholine moiety so increases the analgesic and anti-inflammatory activities

5-MATERIALS AND METHODS

5.1. Chemistry

All reactions were performed with commercially available reagents and they were used without further purification. Solvents were dried by standard methods and stored over molecular sieves.

All reactions were monitored by thin-layer chromatography (TLC) carried on fluorescent precoated plates and detection of the components was made by short UV light. Melting points were determined in open capillaries using MEL-TEMP II and Buchi B-540 Melting Point apparatus and are uncorrected. NMR The ^{1}H NMR spectra were measured with a Jeol ECA 500 MHz (Japan) in DMSO-d_6 or $CDCl_3$ and chemical shifts were recorded in δ ppm relative to TMS. Mass spectra (EI) were run at 70 eV with a Finnigan SSQ 7000 spectrometer (Thermo-Instrument System Incorporation, USA). Elemental analyses were performed by the Microanalytical Unit, Faculty of Science Cairo University.

General procedure for the preparation of 4-substituted coumarin aldehyde (2a-c). The respective amine (10 mmol), together with the respective 4-chlorocoumarin aldehyde (**1**) (10 mmol) was refluxed absolute ethanol (30 ml) for 8 to 10 h. The precipitate obtained was filtered,

washed with ethyl alcohol and dried. For the purification purpose, the precipitate was subjected either to re-crystallization from benzene.

4-Morpholino-3-coumarin aldehyde (2a). It was obtained from **1** and morpholine, IR (cm^{-1}, ν); 2978 (CH alkyl), 1720 (CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.25 (t, 4H, morpholinyl 2 NCH_2, *J* = 5.2 Hz), 3.87 (t, 4H, morpholinyl 2 OCH_2, *J* = 5.3), 4.89 (s, H, C4-*H*), 7.35 (m, 2H, phenyl), 7.52 (d, 1H, phenyl), 7.73 (d, 1H, phenyl), 8.04 (s, H, pyran-*H*); Its MS (m/z), 244 (M^+, 100%).

Table 4: Characterization data of compounds **2-15**

Comp. No.	M.p. [°C]	Yield [%]/ solvent	Mol. Fromula[a] (Mol.Wt.)
2a	154-156	72 (Benzene)	$C_{14}H_{14}NO_3$ (244.2)
2b	163-165	76 (Benzene)	$C_{14}H_{15}N_2O_2$ (243.2)
2c	134-137	79 (Benzene)	$C_{15}H_{17}N_2O_2$ (257.3)
3a	129-131	68 (Ethanol)	$C_{22}H_{24}NO_4$ (366.4)
3b	142-144	70 (Ethanol)	$C_{22}H_{25}N_2O_3$ (365.4)
3c	119-121	74 (Ethanol)	$C_{23}H_{27}N_2O_3$ (379.4)
5a	278-280	65 (DMF+Ethanol)	$C_{26}H_{25}N_4O_4S$ (489.5)
5b	254-256	68 (DMF+Ethanol)	$C_{26}H_{26}N_5O_3S$ (488.5)
5c	261-263	71 (DMF+Ethanol)	$C_{27}H_{28}N_5O_3S$ (502.6)
6a	296-298	81 (DMF)	$C_{24}H_{22}N_4O_3S$ (446.5)
6b	288-290	83 (DMF)	$C_{24}H_{23}N_5O_2S$ (445.5)
6c	301-303	80 (DMF)	$C_{25}H_{25}N_5O_2S$ (459.5)
6d	273-275	69 (DMF)	$C_{23}H_{21}N_4O_3S$ (433.5)
6e	292-294	72 (DMF)	$C_{23}H_{22}N_5O_2S$ (432.5)
6f	312-314	77 (DMF)	$C_{24}H_{24}N_5O_2S$ (446.5)
9a	226-228	87 (Dioxane)	$C_{20}H_{15}ClN_3OS$ (380.8)
9b	202-204	82 (Dioxane)	$C_{21}H_{18}N_3O_2S$ (376.4)
12a	178-180	70 (Diethylether)	$C_{30}H_{25}ClN_3O_8S$ (623.0)
12b	163-165	67 (Diethylether)	$C_{31}H_{28}N_3O_9S$ (618.6)
13a	179-181	72 (Diethylether)	$C_{33}H_{30}ClN_3O_{10}S$ (696.1)
13b	192-193	74 (Diethylether)	$C_{33}H_{30}ClN_3O_{10}S$ (696.1)
13c	179-181	75 (Diethylether)	$C_{24}H_{33}N_3O_{11}S$ (691.7)
13d	188-190	72 (Diethylether)	$C_{24}H_{33}N_3O_{11}S$ (691.7)
14a	229-231	52 (Diethylether)	$C_{24}H_{20}ClN_3O_5S$ (497.9)
14b	251-253	49 (Diethylether)	$C_{25}H_{23}N_3O_6S$ (493.5)
15a	282-284	56 (Diethylether)	$C_{25}H_{22}ClN3O_6S$ (527.9)
15b	261-263	59 (Diethylether)	$C_{25}H_{22}ClN_3O_6S$ (527.9)
15c	249-251	54 (Diethylether)	$C_{26}H_{25}N_3O_7S$ (523.5)
15d	271-273	58 (Diethylether)	$C_{26}H_{25}N_3O_7S$ (523.5)

[a] Analysis for C, H, N and the results were within ± 0.4% of the theoretical values.

4-Piprazino-3-coumarin aldehyde (2b). It was obtained from **1** and piprazine, IR (cm^{-1}, ν); 3380 (NH), 2985 (CH alkyl), 1722 (CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.58 (br, s, 4H, piprazinyl 2 CH_2), 3.22 (br, s, 4H, piprazinyl 2 NCH_2), 4.95 (s, H, C4-*H*), 7.37 (m, 2H, phenyl), 7.58 (d, 1H, phenyl), 7.80 (d, 1H, phenyl), 8.10 (s, H, pyran-*H*); Its MS (m/z), 243 (M^+, 100%).

4-N-Methyl-piprazino-3-coumarin aldehyde (2c). It was obtained from **1** and N-methyl-piprazine, IR (cm^{-1}, ν); 2988 (CH alkyl), 1718 (CO), 1230; ^{1}H NMR (DMSO-d_6, δ, ppm): 2.24 (s, 3H, piperazinyl NCH_3), 2.53 (br, s, 4H, piperiazinyl 2 NCH_2), 3.34 (br, s, 4H, piperazinyl 2 NCH_2), 4.98 (s, H, C4-*H*), 7.34 (m, 2H, phenyl), 7.50 (d, 1H, phenyl), 7.71 (d, 1H, phenyl), 8,06 (s, H, pyran-*H*); Its MS (m/z), 257 (M^+, 100%).

General procedure for the preparation of α,β-unsaturated ketones (3a-c). The respective ketone (1 mmol), together with the respective coumarin aldehyde (**2**) (1 mmol), were dissolved in ethanol (30 ml) in presence of triethylamine (1 mL), put under reflux for 3 h. The precipitate obtained was filtered, washed with ethyl alcohol and dried. For the purification purpose, the precipitate was subjected either to re-crystallization from a mixture of ethanol.

α,β-Unsaturated ketones (3a). It was obtained from **2a**, IR (cm^{-1}, ν); 2980 (CH alkyl), 1710, 1725 (2CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 0.96 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 2.08 (d, 2H, CH_2), 2.17 (d, 2H, CH_2), 3.27 (t, 4H, morpholinyl 2 NCH_2, *J* = 5.1 Hz), 3.85 (t, 4H, morpholinyl 2 OCH_2, *J* = 5.1), 5.00 (s, 1H, C4-*H*), 7.35 (m, 2H, phenyl), 7.52 (d, 1H, phenyl), 7.73 (d, 1H, phenyl), 8.06 (s, H), 8.14 (s, H, pyran-*H*); Its MS (m/z), 366 (M^+, 100%).

α,β-Unsaturated ketones (3b). It was obtained from **2b**, IR (cm^{-1}, ν); 3430 (br, NH), 2982 (CH alkyl), 1712, 1720 (2CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 0.94 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 2.05 (d, 2H, CH_2), 2.14 (d, 2H, CH_2), 2.53 (br,

s, 4H, piprazinyl 2 CH_2), 3.20 (br, s, 4H, piprazinyl 2 NCH_2), 4.98 (s, 1H, C4-*H*), 7.41 (m, 2H, phenyl), 7.59 (d, 1H, phenyl), 7.82 (d, 1H, phenyl), 8.12 (s, H), 8.16 (s, H, pyran-*H*); Its MS (m/z), 365 (M^+, 100%).

α,6-Unsaturated ketones (3c). It was obtained from **2c**, IR (cm^{-1}, ν); 2986 (CH alkyl), 1708, 1712 (2CO); ^1H NMR (DMSO-d_6, δ, ppm): 0.98 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 2.13 (d, 2H, CH_2), 2.18 (d, 2H, CH_2), 2.28 (s, 3H, piperazinyl N*CH*$_3$), 2.57 (br, s, 4H, piperiazinyl 2 N*CH*$_2$), 3.36 (br, s, 4H, piperazinyl 2 N*CH*$_2$), 5.01 (s, 1H, C4-*H*), 7.33 (m, 2H, phenyl), 7.50 (d, 1H, phenyl), 7.70 (d, 1H, phenyl), 8.09 (s, H), 8.13 (s, H, pyran-*H*); Its MS (m/z), 379 (M^+, 100%).

General procedure for the preparation of (5a-c). A mixture of α,β-unsaturated ketone **3** (10 mmol), and 6-aminothiouracil (10 mmol) was refluxed in 50 mL dimethylformamide (DMF) for 8-10 h (under TLC control). The reaction mixture was cooled; the precipitate was filtered off, washed with ethanol, dried, and crystallized from DMF /Ethanol (1:10).

5-(4-Morpholinocoumarin-3-yl)-2-thioxo-8-dimethyl-6,7,8,9-tetrahydropyrimido[4,5-b]-quinoline-4,6-dione (5a). It was obtained from **3a**, IR (cm^{-1}, ν); 3450 (br, NH's), 2987 (CH alkyl), 1708, 1688 (2CO), 1230 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 0.98 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 2.12 (d, 2H, CH_2), 2.19 (d, 2H, CH_2), 3.23 (t, 4H, morpholinyl 2 NCH_2, *J* = 5.0 Hz), 3.82 (t, 4H, morpholinyl 2 OCH_2, *J* = 5.0), 5.02 (s, 1H, C4-*H*), 7.32 (m, 2H, Ar-*H*), 7.49 (d, 1H, Ar-*H*), 7.69 (d, 1H, Ar-*H*), 8.09 (s, H, pyran-*H*), 9.80,10.30 (2br, 2NH, D_2O exchangeable); Its MS (m/z), 489 (M^+, 100%).

5-(4-Piprazinocoumarin-3-yl)-2-thioxo-8-dimethyl-6,7,8,9-tetrahydropyrimido[4,5-b]-quinoline-4,6-dione (5b). It was obtained from **3b**, IR (cm^{-1}, ν); 3415 (br, NH's), 2984 (CH alkyl), 1710, 1689 (2CO), 1228 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 1.00 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 2.09 (d, 2H, CH_2), 2.16 (d, 2H, CH_2), 2.55 (br, s, 4H, piprazinyl 2 CH_2), 3.26 (br, s, 4H, piprazinyl 2 NCH_2), 4.98 (s, 1H, C4-*H*), 7.35 (m, 2H, Ar-*H*), 7.53 (d, 1H, Ar-*H*), 7.74 (d, 1H, Ar-*H*), 8.12 (s, H, pyran-*H*), 9.40,10.35 (2br, 2NH, D_2O exchangeable); Its MS (m/z), 488 (M^+, 100%).

5-(4-N-Methylpiprazinocoumarin-3-yl)-2-thioxo-8-dimethyl-6,7,8,9-tetrahydro-pyrimido[4,5-b]quinoline-4,6-dione (5c). It was obtained from **3c**, IR (cm^{-1}, ν); 3447 (br, NH,s), 2986 (CH alkyl), 1706, 1684 (2CO), 1234 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 0.99 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 2.18 (d, 2H, CH_2), 2.21 (d, 2H, CH_2), 2.25 (s, 3H, piperazinyl N*CH*$_3$), 2.55 (br, s, 4H, piperiazinyl 2 N*CH*$_2$), 3.37 (br, s, 4H, piperazinyl 2 N*CH*$_2$), 5.00 (s, 1H, C4-*H*), 7.32 (m, 2H, Ar-*H*), 7.50 (d, 1H, Ar-*H*), 7.70 (d, 1H, Ar-*H*), 8.05 (s, H, pyran-*H*), 10.20,10.80 (2br, 2NH, D_2O exchangeable); Its MS (m/z), 502 (M^+, 100%).

General procedure for the preparation of (6a-f). A mixture of ketone (10 mmol), coumarin aldehyde **2** and 6-aminothiouracil (10 mmol) was refluxed in 50 mL dimethylformamide (DMF) for 12-15 h (under TLC control). The reaction mixture was cooled; the precipitate was filtered off, washed with ethanol, dried, and crystallized from DMF.

5-(4-Morpholinocoumarin-3-yl)-2-thioxo-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline-4,6-dione(6a). It was obtained from **2a** and cyclohexanone, IR (cm^{-1}, ν); 3425 (br, NH), 2987 (CH alkyl), 1689 (CO), 1231 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 1.69 (m, 4H, $2CH_2$), 2.94 (m, 2H, CH_2), 3.36 (m, 2H, CH_2), 3.20 (t, 4H, morpholinyl 2 NCH_2, *J* = 5.2 Hz), 3.84 (t, 4H, morpholinyl 2 OCH_2, *J* = 5.2), 5.00 (s, 1H, C4-*H*), 7.35 (m, 2H, Ar-*H*), 7.51 (d, 1H, Ar-*H*), 7.72 (d, 1H, Ar-*H*), 8.06 (s, H, pyran-*H*), 9.30,10.00 (2br, 2NH, D_2O exchangeable); Its MS (m/z), 446 (M^+, 78%).

5-(4-Piprazinocoumarin-3-yl)-2-thioxo-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline-4,6-dione (6b). It was obtained from **2b** and cyclohexanone, IR (cm^{-1}, ν); 3445 (br, NH's), 2985 (CH alkyl), 1678 (CO), 1227 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 1.67 (m, 4H, $2CH_2$), 2.92 (m, 2H, CH_2), 3.30 (m, 2H, CH_2), 2.56 (br, s, 4H, piprazinyl 2 CH_2), 3.28 (br, s, 4H, piprazinyl 2 NCH_2), 4.97 (s, 1H, C4-*H*), 7.37 (m, 2H, Ar-*H*), 7.51 (d, 1H, Ar-*H*), 7.72 (d, 1H, Ar-*H*), @ (s, H, pyran-*H*), 9.60,10.40 (2br, 2NH, D_2O exchangeable); Its MS (m/z), 445 (M^+, 83%).

5-(4-N-Methyylpiprazinocoumarin-3-yl)-2-thioxo-6,7,8,9-tetrahydropyrimido[4,5-b]-quinoline-4,6-dione (6c). It was obtained from **2c** and cyclohexanone, IR (cm^{-1}, ν); 3438 (br, NH's), 2982 (CH alkyl), 1688 (CO), 1235 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 1.69 (m, 4H, $2CH_2$), 2.96 (m, 2H, CH_2), 3.34 (m, 2H, CH_2), 2.28 (s, 3H, piperazinyl N*CH*$_3$), 2.59 (br, s, 4H, piperiazinyl 2 N*CH*$_2$), 3.40 (br, s, 4H, piperazinyl 2 N*CH*$_2$), 5.03 (s, 1H, C4-*H*), 7.36 (m, 2H, Ar-*H*), 7.49 (d, 1H, Ar-*H*), 7.73 (d, 1H, Ar-*H*), 8.12 (s, H, pyran-*H*), 10.35,10.90 (2br, 2NH, D_2O exchangeable); Its MS (m/z), 459 (M^+, 100%).

5-(4-Morpholinocoumarin-3-yl)-2-thioxo-6,7,8-trihydrocyclopentenopyrido[2,3-d]-pyrimidin-4-one (6d). It was obtained from **2a** and cyclopentanone, IR (cm^{-1}, ν); 3430 (br, NH's), 2983 (CH alkyl), 1683 (3CO), 1231 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 2.54 (t, 2H, CH_2), 2.73 (m, 2H, CH_2), 2.88 (t,2H, CH_2), 3.28 (t, 4H, morpholinyl 2 NCH_2, *J* = 5.0 Hz), 3.81 (t, 4H, morpholinyl 2 OCH_2, *J* = 5.0), 5.04 (s, 1H, C4-*H*), 7.38 (m, 2H, Ar-*H*), 7.54 (d, 1H, Ar-*H*), 7.77 (d, 1H, Ar-*H*), 8.00 (s, H, pyran-*H*), 9.70,11.00 (2br, 2NH, D_2O exchangeable); Its MS (m/z), 433 (M^+, 76%).

5-(4-Piprazinocoumarin-3-yl)-2-thioxo-6,7,8-trihydrocyclopentenopyrido[2,3-d]-pyrimidin-4-one(6e). It was obtained from **2b** and cyclopentanone, IR (cm^{-1}, ν); 3445 (br, NH's), 2985 (CH alkyl), 1679 (CO), 1228 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 2.56 (t, 2H, CH_2), 2.76 (m, 2H, CH_2), 2.91 (t,2H, CH_2), 2.53 (br, s, 4H, piprazinyl 2 CH_2), 3.31 (br, s, 4H, piprazinyl 2 NCH_2), 4.98 (s, 1H, C4-*H*), 7.39 (m, 2H, Ar-*H*), 7.54 (d, 1H, Ar-*H*), 7.76 (d, 1H, Ar-*H*), 8.04 (s, H, pyran-*H*), 9.50,10.30 (2br, 2NH, D_2O exchangeable); Its MS (m/z), 432 (M^+, 69%).

5-(4-N-Methyylpiprazinocoumarin-3-yl)--2-thioxo-6,7,8-trihydrocyclopentenopyrido-[2,3-d]-pyrimidin-4-one (6f). It was obtained from **2c** and cyclopentanone, IR (cm^{-1}, ν);

3435 (br, NH's), 2987 (CH alkyl), 1683 (CO), 1232 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.57 (t, 2H, CH_2), 2.75 (m, 2H, CH_2), 2.90 (t,2H, CH_2), 2.27 (s, 3H, piperazinyl NCH_3), 2.53 (br, s, 4H, piperiazinyl 2 NCH_2), 3.34 (br, s, 4H, piperazinyl 2 NCH_2), 4.93 (s, 1H, C4-*H*), 7.30 (m, 2H, Ar-*H*), 7.45 (d, 1H, Ar-*H*), 7.67 (d, 1H, Ar-*H*), 8.08 (s, H, pyran-*H*), 9.20,10.35 (2br, 2NH, D_2O exchangeable); Its MS (m/z), 446 (M^+, 100%).

General procedure for the preparation of 7-aryl-5-phenyl-2-methylthiopyrido[2,3-*d*]-pyrimidine-4-one (9a,b). To a warmed ethanolic KOH solution (prepared by dissolving 0.01 mol of KOH in 50 mL ethanol) **7** (10 mmol) was added, the heating was continued for 30 min, the mixture was allowed to cool to room temperature methyliodide (0.012 mol) was added. The mixture was stirred under reflux for 5 h, then cooled to room temperature and poured into cold water (100 mL). The solid product precipitated was filtered off, washed with water (100 mL), the product was dried and crystallized from dioxane (30 mL).

7-(4-Chlorophenyl)-5-phenyl-2-methylthiopyrido[2,3-d]pyrimidine-4-one (9a). It was obtained from **7a**, IR (cm^{-1}, ν); 2985 (CH alkyl), 1678 (CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.24 (s, 3H SCH_3), 7.30 (m, 2H, Ar-*H*), 7.45 (d, 2H, Ar-*H*, *J* = 8.12 *Hz*), 7.59 (m, 3H, Ar-*H*), 8.11 (d, 2H, Ar-*H*, *J* = 8.10 *Hz*), 8.24 (s, H, pyr-*H*), 9.80, 10.60 (2br, 2NH, D_2O exchangeable); Its MS (m/z), 380 (M^+, 100%).

7-(4-Anisyl)-5-phenyl-2-methylthiopyrido[2,3-d]pyrimidine-4-one (9b). It was obtained from **7b**, IR (cm^{-1}, ν); 2983 (CH alkyl), 185 (CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.19 (s, 3H, SCH_3), 3.86 (s, 3H, OCH_3), 7.30 (m, 2H, Ar-*H*), 7.45 (d, 2H, Ar-*H*, *J* = 8.12 *Hz*), 7.59 (m, 3H, Ar-*H*), 8.11 (d, 2H, Ar-*H*, *J* = 8.10 *Hz*), 8.21 (s, H, pyr-*H*), 9.90, 10.85 (2br, 2NH D_2O exchangeable), Its MS (m/z), 376 (M^+, 100%).

General procedure for the preparation of (12a,b) and (13a-d). To a solution of **7a,b** (0.01 mol) in aqueous potassium hydroxide (0.56 g, 0.01 mol) in distilled water (5 ml) was added a solution of 2,3,5-tri-*O*-acetyl-β-D-arabinopyranosyl bromide **10** (0.011 mol) or 2,3,4,6-tetra-*O*-acetyl-α-D-gluco- or galactopyranosyl bromide **11a,b** in acetone (30 ml). The reaction mixture was stirred at room temperature for 24 h (under TLC control). The solvent was evaporated under reduced pressure at 40 ^oC, and the crude product was filtered off and washed with distilled water to remove KBr formed. The product was dried, and crystallized from diethyl ether as pale yellow powder in a good yield.

7-(4-Chlorophenyl)-5-phenyl-2-(2′,3′,5′-tri-O-acetyl-β-D-arabinopyranosylthio)pyrido-[2,3-d]-pyrimidine-4-one (12a). It was obtained from **7a** and 2,3,5-tri-*O*-acetyl-*α*-*D*-arabinofuranosyl)-bromide (**10**); IR (cm^{-1}, ν); 3355 (br, NH), 2986 (CH alkyl), 1685 (CO), 1745 (3CO), 1231 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.92, 1.97, 2.00 (3s, 9H, 3CH_3CO), 4.05 (m, 1H, *H*-4'), 4.17 (m, 2H, *H*-5', *H*-5''), 5.28 (m, 1H, *H*-3'), 5.39 (m, 1H, *H*-2'), 6.64 (d, 1H, *J* = 3.67 *Hz*, *H*-1'), 7.30 (m, 2H, Ar-*H*), 7.45 (d, 2H, Ar-*H*, *J* = 8.12 *Hz*), 7.59 (m, 3H, Ar-*H*), 8.11 (d, 2H, Ar-*H*, *J* = 8.10 *Hz*), 8.22 (s, H, pyr-*H*), 10.25 (br, 1H, NH); ^{13}C. NMR: 20.39, 21.37, 21.42 (3CH_3), 60.82 (C-5'), 65.69 (C-3'), 67.58 (C-2'), 69.73 (C-4'), 87.24 (C-1'),114.6, 115.2, 127.2, 128.5, 129.6, 130.9, 132.9, 135.6, 137.9, 143.2, 146.8, 148.7, 152.9 (carbons of the 2Ar + pyridopyrimidine ring), 159.6 (C-S), 165.4 (C=O imide) 168.5, 169.6, 170.2, (3C=O); Its MS (m/z), 623 (M^+, 63%).

7-(4-Anisyl)-5-phenyl-2-(2′,3′,5′-tri-O-acetyl-β-D-arabinopyranosylthio)pyrido[2,3-d]-pyrimidine-4-one (12b). It was obtained from **7b** and 2,3,5-tri-*O*-acetyl-*α*-*D*-arabinofuranosyl)-bromide (**10**); IR (cm^{-1}, ν); 3425 (br, NH), 2980 (CH alkyl), 1679 (CO), 1738 (3CO), 1226 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.90, 1.97, 2.00 (3s, 9H, 3CH_3CO), 3.89 (s, 3H, OCH_3), 4.07 (m, 1H, *H*-4'), 4.13 (m, 2H, *H*-5', *H*-5''), 5.26 (m, 1H, *H*-3'), 5.34 (m, 1H, *H*-2'), 6.72 (d, 1H, *J* = 3.67 *Hz*, *H*-1'), 7.25 (m, 2H, Ar-*H*), 7.36 (d, 2H, Ar-*H*, *J* = 8.16 *Hz*), 7.65 (m, 3H, Ar-*H*), 8.02 (d, 2H, Ar-*H*, *J* = 8.18 *Hz*), 8.27 (s, H, pyr-*H*), 11.00 (br, *NH*); Its MS (m/z), 618 (M^+, 28%).

7-(4-Chlorophenyl)-5-phenyl-2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosylthio)-pyrido[2,3-d]pyrimidine-4-one (13a). It was obtained from **7a** and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **11a**, IR (cm^{-1}, ν); 3420 (br, NH), 2983 (CH alkyl), 1682 (CO), 1725 (4CO), 1230 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.95, 1.99, 2.05, 2.13 (4s, 12H, 4CH_3CO), 3.86 (m, 1H, *H*-5'), 4.13 (m, 2H, *H*-6', *H*-6''), 4.29 (m, 1H, *H*-4'), 4.92 (t, 1H, *H*-2'), 5.37 (t, 1H, *J* = 9.56 *Hz*, *H*-3'), 5.99 (d, 1H, *J* = 10.60 *Hz*, *H*-1'), 7.34 (m, 2H, Ar-*H*), 7.49 (d, 2H, Ar-*H*, *J* = 8.11 *Hz*), 7.70 (m, 3H, Ar-*H*), 8.13 (d, 2H, Ar-*H*, *J* = 8.10 *Hz*), 8.32 (s, H, pyr-*H*), 9.80 (br, *NH*); ^{13}C. NMR: 20.29, 20.34, 20.37, 20.42 (4CH_3), 61.40 (C-6'), 66.50 (C-3'), 67.57 (C-2'), 70.32 (C-4'), 74.63 (C-5'), 81.47 (C-1'), 114.1, 114.3, 127.6, 128.8, 129.6, 130.8, 130.9, 135.4, 137.3, 142.4, 146.3, 148.2, 152.2 (carbons of the 2Ar + pyridopyrimidine ring), 161.0 (C-S), 163.2 (C=O imide),169.6, 169.8, 170.0, 170.1 (4C=O); Its MS (m/z), 696 (M^+, 57%).

7-(4-Chlorophenyl)-5-phenyl-2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosylthio)-pyrido[2,3-d]pyrimidine-4-one (13b). It was obtained from **7a** and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **11b**, IR (cm^{-1}, ν); 3410 (br, NH), 2986 (CH alkyl), 1685 (CO), 1736 (4CO), 1228 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.95, 2.00, 2.11, 2.15 (4s, 12H, 4CH_3CO), 3.97 (m, 1H, *H*-5'), 4.16 (m, 2H, *H*-6', *H*-6''), 4.41 (m, 1H, *H*-4'), 4.90 (t, 1H, *H*-2'), 5.23 (t, 1H, *J* = 9.64 *Hz*, *H*-3'), 6.07 (d, 1H, *J* = 10.63 *Hz*, *H*-1'),7.31 (m, 2H, Ar-*H*), 7.50 (d, 2H, Ar-*H*, *J* = 8.12 *Hz*), 7.62 (m, 3H, Ar-*H*), 8.21 (d, 2H, Ar-*H*, *J* = 8.11 *Hz*), 8.28 (s, H, pyr-*H*), 10.35 (br, *NH*); Its MS (m/z), 696 (M^+, 48%).

7-(4-Anisyl)-5-phenyl-2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosylthio)-pyrido[2,3-d]-pyrimidine-4-one (13c). It was obtained from **7b** and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **11a**, IR (cm^{-1}, ν); 3390 (br, NH), 2979 (CH alkyl), 1682 (CO), 1740 (4CO), 1230 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.91, 1.98, 2.05, 2.14 (4s, 12H,

*4CH$_3$*CO), 3.82 (m, 1H, *H*-5'), 3.90 (s, 3H, O*CH$_3$*), 4.14 (m, 2H, *H*-6', *H*-6''), 4.28 (m, 1H, *H*-4'), 4.89 (t, 1H, *H*-2'), 5.27 (t, 1H, *J* = 9.63 *Hz*, *H*-3'), 5.97 (d, 1H, *J* = 10.57 *Hz*, *H*-1'), 7.29 (m, 2H, Ar-*H*), 7.41 (d, 2H, Ar-*H*, *J* = 8.10 *Hz*), 7.53 (m, 3H, Ar-*H*), 8.21 (d, 2H, Ar-*H*, *J* = 8.10 *Hz*), 8.22 (s, H, pyr-*H*), 10.00 (br, *NH*); ^{13}C. NMR: 20.26, 20.31, 20.36, 20.40 (4CH$_3$), 54.8 (O*CH$_3$*), 62.26 (C-6'), 65.97 (C-3'), 67.61 (C-2'), 71.16 (C-4'), 73.52 (C-5'), 80.89 (C-1'), 113.7, 114.8, 127.8, 128.6, 129.7, 130.5, 131.2, 134.9, 137.6, 142.7, 146.4, 148.5, 152.0 (carbons of the 2Ar + pyridopyrimidine ring), 160.8 (C-S), 164.2 (C=O imide) 169.4, 169.7, 170.2, 170.4 (4C=O); Its MS (m/z), 696 (M$^+$, 42%).

7-(4-Anisyl)-5-phenyl-2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosylthio)-pyrido-[2,3-d]pyrimidine-4-one (13d). It was obtained from **7b** and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **11b**, IR (cm^{-1}, ν); 3432 (br, NH), 2976 (CH alkyl), 1678 (CO), 1728 (4CO), 1234 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.99, 2.04, 2.13, 2.19 (4s, 12H, *4CH$_3$*CO), 3.86 (s, 3H, OCH$_3$), 3.98 (m, 1H, *H*-5'), 4.21 (m, 2H, *H*-6', *H*-6''), 4.38 (m, 1H, *H*-4'), 4.92 (t, 1H, *H*-2'), 5.20 (t, 1H, *J* = 9.59 *Hz*, *H*-3'), 6.11 (d, 1H, *J* = 10.71 *Hz*, *H*-1'), 7.37 (m, 2H, Ar-*H*), 7.49 (d, 2H, Ar-*H*, *J* = 8.12 *Hz*), 7.63 (m, 3H, Ar-*H*), 8.24 (d, 2H, Ar-*H*, *J* = 8.10 *Hz*), 8.30 (s, H, pyr-*H*), 10.50 (br, *NH*); Its MS (m/z), 696 (M$^+$, 39%).

General Procedure for the preparation of (14a,b) and (15a-d). Dry gaseous ammonia was passed through a solution of protected glycosides **12a,b** or **13a-d** (0.5 gm) in dry methanol (20 ml) at room temperature for 10 min. The mixture was stirred overnight (followed by TLC). The resulting mixture was then evaporated under reduce pressure to afford a solid residue that was crystallized from diethyl ether as pale yellow in a 40-56% yield.

7-(4-Chlorophenyl)-5-phenyl-2-(β-D-arabinofyranosylthio)-pyrido[2,3-d]pyrimidin-4-one (14a). It was obtained from **12a**, IR (cm^{-1}, ν); 3430 (brs, OH), 2981 (CH alkyl), 1676 (CO), 1230 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.73 (m, 2H, *H*-5', *H*-5''), 4.04 (m, 1H, *H*-4'), 4.74 (t, 1H, *H*-2'), 5.16 (t, *J* = 5.40 *Hz*, *J* = 4.92 *Hz*, *OH*-C(5'), 5.21 (d, *J* = 4.48 *Hz*, *OH*-C(3'), 5.43 (d, *J* = 5.89 *Hz*, *OH*-C(2'), 5.67 (t, 1H, *J* = 9.80 *Hz*, *H*-3'), 6.98 (d, 1H, *J* = 5.67 *Hz*, *H*-1'), 7.26 (m, 2H, Ar-*H*), 7.39 (d, 2H, Ar-*H*, *J* = 8.11 *Hz*), 7.54 (m, 3H, Ar-*H*), 8.18 (d, 2H, Ar-*H*, *J* = 8.10 *Hz*), 8.26 (s, H, pyr-*H*), 9.80 (br, *NH*); ^{13}C. NMR: 60.82 (C-5'), 65.38 (C-3'), 66.88 (C-2'), 68.89 (C-4'), 86.65 (C-1'),114.3, 114.9, 126.8, 128.2, 129.8, 131.0, 133.2, 135.8, 138.7, 143.7, 147.3, 149.3, 152.2 (carbons of the 2Ar + pyridopyrimidine ring), 160.2 (C-S), 166.0 (C=O imide); Its MS (m/z), 497 (M$^+$, 70%), 498 (M$^+$ +1, 27%).

7-(4-Anisyl)-5-phenyl-2-(β-D-arabinofyranosylthio)-pyrido[2,3-d]pyrimidin-4-one (14b). It was obtained from **12b**, IR (cm^{-1}, ν); 3460 (brs, OH), 2980 (CH alkyl), 1682 (CO), 1227 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.71 (m, 2H, *H*-5', *H*-5''), 3.89 (s, 3H, O*CH$_3$*), 4.13 (m, 1H, *H*-4'), 4.82 (t, 1H, *H*-2'), 5.16 (t, *J* = 5.40 *Hz*, *J* = 4.95 *Hz*, *OH*-C(5'), 5.18 (d, *J* = 4.45 *Hz*, *OH*-C(3'), 5.39 (d, *J* = 5.96 *Hz*, *OH*-C(2'), 5.67 (t, 1H, *J* = 9.78 *Hz*, *H*-3'), 6.97 (d, 1H, *J* = 5.64 *Hz*, *H*-1'), 7.27 (m, 2H, Ar-*H*), 7.41 (d, 2H, Ar-*H*, *J* = 8.10 *Hz*), 7.54 (m, 3H, Ar-*H*), 8.14 (d, 2H, Ar-*H*, *J* = 8.10 *Hz*), 8.30 (s, H, pyr-*H*), 10.00 (br, *NH*); Its MS (m/z), 493 (M$^+$, 69%).

7-(4-Chlorophenyl)-5-phenyl-2-(β-D-glucopyranosylthio)-pyrido[2,3-d]pyrimidin-4-one (15a). It was obtained from **13a**, IR (cm^{-1}, ν); 3465 (brs, OH), 2978 (CH alkyl), 1680 (CO), 1235 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.89 (m, 1H, *H*-5'), 4.07 (m, 2H, *H*-6', *H*-6''), 4.26 (m, 1H, *H*-4'), 4.53 (br, H, D$_2$O exchangeable *OH*), 4.88 (t, 1H, *H*-2'), 5.07 (brs, 1H, D$_2$O exchangeable *OH*), 5.16 (t, 1H, *J* = 9.59 *Hz*, *H*-3'), 5.16 (d, 1H, *J* = 4.78 *Hz*, D$_2$O exchangeable *OH*), 5.59 (br, H, D$_2$O exchangeable *OH*), 6.13 (d, 1H, *J* = 10.60 *Hz*, *H*-1'), 7.36 (m, 2H, Ar-*H*), 7.49 (d, 2H, Ar-*H*, *J* = 8.11 *Hz*), 7.62 (m, 3H, Ar-*H*), 8.17 (d, 2H, Ar-*H*, *J* = 8.12 *Hz*), 8.24 (s, H, pyr-*H*), 10.60 (br, *NH* D$_2$O exchangeable); ^{13}C NMR: 60.57 (C-6'), 65.53 (C-3'), 66.86 (C-2'), 68.52 (C-4'), 73.61 (C-5'), 82.17 (C-1'), 115.3, 115.9, 127.4, 128.5, 129.9, 130.4, 132.4, 135.5, 137.6, 142.7, 147.2, 148.8, 153.4 (carbons of the 2Ar + pyridopyrimidine ring), 162.4 (C-S), 164.0 (C=O imide); Its MS (m/z), 527 (M$^+$, 65%), 528 (M$^+$+1, 21%).

7-(4-Chlorophenyl)-5-phenyl-2-(β-D-galactopyranosyl-thio)-pyrido[2,3-d]pyrimidin-4-one (15b). It obtained from **13b**, IR (cm^{-1}, ν); 3480 (brs, OH), 2985 (CH alkyl), 1676 (CO), 1229 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.88 (m, 1H, *H*-5'), 4.04 (m, 2H, *H*-6', *H*-6''), 4.30 (m, 1H, *H*-4'), 4.89 (t, 1H, *H*-2'), 4.57 (br, H, D$_2$O exchangeable *OH*), 4.97 (brs, 1H, D$_2$O exchangeable *OH*), 5.13 (d, 1H, *J* = 4.82 *Hz*, D$_2$O exchangeable *OH*), 5.19 (t, 1H, *J* = 9.58 *Hz*, *H*-3'), 5.49 (br, H, D$_2$O exchangeable *OH*), 6.14 (d, 1H, *J* = 10.61 *Hz*, *H*-1'), 7.29 (m, 2H, Ar-*H*), 7.48 (d, 2H, Ar-*H*, *J* = 8.11 *Hz*), 7.63 (m, 3H, Ar-*H*), 8.14 (d, 2H, Ar-*H*, *J* = 8.10 *Hz*), 8.34 (s, H, pyr-*H*), 10.85 (br, *NH* D$_2$O exchangeble); Its MS (m/z), 527 (M$^+$, 72%), 528 (M$^+$+1, 23%).

7-(4-Anisyl)-5-phenyl-2-(β-D-glucopyranosylthio)-pyrido[2,3-d]pyrimidin-4-one (15c). It obtained from **13c**, IR (cm^{-1}, ν); 3430 (brs, OH), 2988 (CH alkyl), 1682 (CO), 1225 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.84 (s, 3H, O*CH$_3$*), 3.92 (m, 1H, *H*-5'), 4.09 (m, 2H, *H*-6', *H*-6''), 4.25 (m, 1H, *H*-4'), 4.51 (br, H, D$_2$O exchangeable *OH*), 4.93 (t, 1H, *H*-2'), 5.13 (brs, 1H, D$_2$O exchangeable *OH*), 5.21 (t, 1H, *J* = 9.56 *Hz*, *H*-3'), 5.28 (d, 1H, *J* = 4.80 *Hz*, D$_2$O exchangeable *OH*), 5.63 (br, H, D$_2$O exchangeable *OH*), 6.17 (d, 1H, *J* = 10.56 *Hz*, *H*-1'), 7.34 (m, 2H, Ar-*H*), 7.45 (d, 2H, Ar-*H*, *J* = 8.12 *Hz*), 7.60 (m, 3H, Ar-*H*), 8.21 (d, 2H, Ar-*H*, *J* = 8.12 *Hz*), 8.31 (s, H, pyr-*H*), 10.30 (br, *NH* D$_2$O exchangeable); Its MS (m/z), 523 (M$^+$, 54%).

7-(4-Anisyl)-5-phenyl-2-(β-D-galactopyranosylthio)-pyrido[2,3-d]pyrimidin-4-one (15d). It obtained from **13d**, IR (cm^{-1}, ν); 3485 (brs, OH), 2980 (CH alkyl), 1679 (3CO), 1232 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.85 (s, 3H, O*CH$_3$*), 3.90 (m, 1H, *H*-5'), 4.03 (m, 2H, *H*-6', *H*-6''), 4.30 (m, 1H, *H*-4'), 4.58 (br, H, D$_2$O exchangeable *OH*), 4.93 (t, 1H, *H*-2'), 5.12 (brs, 1H, D$_2$O exchangeable *OH*), 5.20 (d, 1H, *J* = 4.83 Hz, D$_2$O exchangeable *OH*), 5.31 (t, 1H, *J* = 9.60 *Hz*, *H*-3'), 5.60 (br, H, D$_2$O-exchangeable *OH*), 6.09 (d, 1H, *J* = 10.53 *Hz*, *H*-1'), 7.26 (m, 2H, Ar-*H*), 7.41 (d, 2H, Ar-*H*, *J* = 8.13 *Hz*), 7.63 (m, 3H, Ar-*H*), 8.17 (d, 2H, Ar-*H*, *J* = 8.11

Hz), 8.34 (s, H, pyr-*H*), 10.70 (br, NH, D_2O exchangeable); Its MS (m/z), 523 (M^+, 49%).

5.2. Biological Screening

Materials and methods: Animals-Adult rats of both sexes weighing 150–200 g and adult mice weighing 20–25 g were used in the experiments. Animals were housed under standardized conditions for light and temperature and received standard rat chow and tap water and libitum. Animals were randomly assigned to different experimental groups, each kept in a separate cage. All animal procedures were performed after approval from the Ethics committee of the National Research Center and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No.85-23, revised 1985). Carrageenan lambda Sigma-Aldrich chemical company (USA), indomethacin Khahira Pharmaceutical and Chemical Company (Cairo, Egypt) and tramadol October Pharma (Cairo, Egypt).

Antiinflammatory testing: The carrageenan rat paw oedema model of inflammation was used to evaluate the anti-inflammatory properties of the tested compounds. Rats were randomly assigned to treatment groups and sterile carrageenan lambda (100 ul of a 1% solution in saline) was injected sub-planter into right hind paw of the rat. Carrageenan caused visible redness and pronounced swelling that was well developed by 4 h and persisted formore than 48 h. Right hind pawwas measured with a planimeter[34,35] before, and at 1,2,3 and 4 h after carrageenan injection. Due to water insolubility of the tested compounds, they were dissolved in DMSO then injected i.p (9 mg/kg b wt)[36]. The control animals were injected (i.p) with appropriate volume of DMSO. The standard drug was indomethacin (10 mg/kg b wt). Different compounds or indomethacin were given 1hr before carrageenan injection.

Analgesia testing: The hot-plate test was performed on mice by using an electronically controlled hot-plate (ugo Basile, Italy) heated to 52 ^oC (± 0.1 ^oC), for possible centrally mediated analgesic effect of the drugs. Nineteen groups of rats each were given vehicle and/or the different compounds and the last group received tramadol (20 mg/Kg b wt) 60 min prior to testing. Latency to lick a hind paw or jumping[37] was recorded sequentially before and at 1, 2 h post treatment.

Ulcerogenic effects: Groups of 5 male Wistar rats with a weight between 150 and 175 g are used. They are starved 48 h prior to drug administration. The test compounds are administered orally in 10 mL/kg as aqueous suspension. Doses are chosen which are highly active in the activity (9 mg/kg) and used. The animals are sacrificed after 7 h. Stomachs are removed and placed on saline soaked filter paper until inspection. A longitudinal incision along the greater curvature is made with fine scissor. The stomach is inverted over the index finger and the presence or the absence of gastric irritation is determined. The presence of a single or multiple lesions (erosion, ulcer or perforation) is considered to be positive[33]. The number of ulcers and the occurrence of hyperemia is noted (determine ulcer index).

6-REFERENCES

1. Feuer G, The metabolism and biological actions of coumarins. Prog. Med. Chem. 58,1973, 85-91 .
2. Obaseki A O, Porter W R, 4-Hydroxycoumarin / 2-hydroxychromone tauto-merism: infrared spectra of 2-13C and 3-D labeled 4-hydroxycoumarin and its anion J. Heterocyclic Chem., 19, 1982, 385-90.
3. Yakout E M A, Ibrahim N M, Ghoneim Kh M, Mahran M R H, Reactions of 4-Hydroxycoumarin and 4-Hydroxyfurocoumarins with α,β-Unsaturated Nitriles. Mass Spectrometry of the New γ-Pyrano-α-pyran Derivatives J. Chem. Res. (S), 652, 1999, 2818-47.
4. Manolov I, Danchev N D, Synthesis, toxicological, and pharmacological assessment of some oximes and aldehyde condensation products of 4-hydroxycoumarin. Arch. Pharm. (Weinheim). 332, 1999, 243-48
5. Das Gupta A K, Chatterjee R M, Das, K R, A facile one-step synthesis of 4-hydroxycoumarin, Indian J. Chem. 20B, (1981) 511.
6. Martin J. T, Baker E. A, Bydre R J W, The fungitoxicities of plant furocoumarins; Ann. Appl. Biol., 57, 1966, 501.
7. Bingham J B, Meyer O O, Pohle F J, Studies on the hemorrhagic agent 3,3' methylenebis (4-hydroxycoumarin). I: its effect on the prothrombin and coagulation time of the blood of dogs and humans. Am J Med Sci, 202 1941, 563-78.
8. Patonay T, Litkei G, Bognar R, Erdei J, Miszti C, Antibacterial and Antifungal Activity of 4-Hydroxycoumarin Derivatives, Analogues of Novobiocin, Pharmazie, 39,1984, 86-91.
9. Yang B, Sutcliffe J, Dutton C J, 4-hydroxy coumarin derivatives., U. S. US 5985912, 1999.
10. Ali U. Shaikh A, Claudiu T S, Antibacterial, antifungal and cytotoxic properties of novel N-substituted sulfonamides from 4-hydroxycoumarin, Journal of Enzyme Inhibition and Medicinal Chemistry, 21, 2006, 741 - 748
11. Cingolani G M, Gualtrieri F, Pigini M, Notes. Researches in the Field of Antiviral Compounds. Mannich Bases of 3-Hydroxycoumarin, J. Med. Chem., 12, 1969, 531-532.
12. David L. Dreyer, Kenneth C. Jones, Leonard Jurd, Bruce C. Campbell , Feeding deterrency of some 4-hydroxycoumarins and related compounds: Relationship to host-plant resistance of alfalfa towards pea aphid (*Acyrthosiphon pisum*)., J.

Chem., Ecol., 13, 925-30 (1987); C. A., 107, (1987) 20953.

13. Abortion effect of 3-(acetonylbenzyl)-4-hydroxycoumarin (warfarin) in mice, International J of Contraception 3, 1971, 279-283
14. Bariana D S, Coumarin derivatives as coronary vasodilators. J. Med. Chem., 13, 1970, 544-46.
15. Shah A, Naliapara Y, Sureja D, Motohashi N, Kawas M, Miskolci C, Szabo D, Molnar J, Biological activity of 6,12-dihydro-1-benzopyrano[3,4-b]-[1,4]benzothiazin-6-ones. Anticancer Res., 18 (4c), 1998, 61-3.
16. Yamato M, Jpn. Kokai. Takkyo Koho JP61, 215341,1986.; C. A., 106, (1987) 66807.
17. Tzeng C C, Lee K H, ChenY L, Huang B R, U. S. US 5 962460,1999.; C. A., 131, (1999) 243173.
18. Bourinbaiar A S, U. S. US 5 811390,1998.; C. A., 129, (1998) 255529.
19. Kawano M,Chiba T, Akita Kogyo Koto Senmon Gakko Kenkyu Kiyo, 35, 64 (2000); C. A., 133, (2000) 232374.
20. Hariprasad V, Talele T T and Kulkarni V M, Crystal-Structure-Based Design and Synthesis of Novel Series of Non-peptidic Potential HIV-1 Protease Inhibitors. Pharm.Pharmcol. Commun. 4, 1998, 365-372.
21. Huang H C, Lai M W, Wang H R, Chung Y L, Hsieh L M,Chen C C, Antiproliferative effect of esculetin on vascular smooth muscle cells: possible roles of signal transduction pathways.Eur. J. Pharmacol., 237, 1995, 39-44.
22. Podbielkowska M, Kupidlowska E, Waleza M, Dobrzynska K, Louis S A, Keightley A, Zobel A M, Coumarins as Antimitotics., Int. J. Pharmacogen., 32, 1994, 262-273.
23. El-Gazzar A B A, Youssef M M, Youssef A M S, Abu-Hashem A A, Badria F A, Design and synthesis of azolopyrimidoquinolines, pyrimidoquinazolines as anti-oxidant, anti-inflammatory and analgesic activities., Euro. J. of Med. Chem., 44, 2008, 609-624.
24. El-Gazzar A B A, El-Enany M A, Youssif M N, Synthesis, analgesic, anti-inflammatory, and antimicrobial activity of some novel pyrimido[4,5-*b*]quinolin-4-ones, Bioorg and Med. Chem., 16 (2008) 3261-3273.
25. El-Gazzar A B A, Hafez H N, Abbas H S, *S*- and *C*-nucleosidoquinazoline as new nucleoside analogs with potential analgesic and anti-inflammatory activity Euro. J. Med. Chem., 44, 2009, 4249-4258.
26. El-Gazzar A B A, Hafez H N, Nawwar G A, New acyclic nucleosides analogues as potential analgesic, anti-inflammatory, anti-oxidant and anti-microbial derived from pyrimido[4,5-*b*]-quinolines., Euro. J. Med. Chem., 44, 2009, 1427-1436.
27. El-Gazzar A B A, Hafez H N, Synthesis and antitumor activity of substituted triazolo[4,3-*a*]pyrimidin-6-sulfonamide with an incorporated thiazolidinone moiety., Bioog. Med. Chem. Lett., 19, 2009, 4143-4147.
28. Hafez H N, El-Gazzar A B A, Synthesis of 4-substituted pyrido[2,3-*d*]-pyrimidin-4(1*H*)-one as analgesic and anti-inflammatory agents., Bioorg. Med. Chem. Lett., 19, 2009, 3392-3397.
29. Winter G A, Rislfy E A, Nuss G W, Proc. Soc. Exp. Biol. Med.111,1962, 544-547.
30. Armitage P, Statistical Methods in Medical Research, 1 Ed. Blackwell Scientific Publ., Oxford, London, 1971, 147.
31. Woolfe G, MacDonald A D, The evaluation of the analgesic action of pethidine hydrochloride (Demerol)., J. Pharmacol. Exp. Ther. 80,1944, 300-307.
32. Rajanna K C, Florence Solomon, Mir Moazzam Ali, Saiprakash P K., Kinetics and mechanism of Vilsmeier-Haack synthesis of 3-formyl chromones derived from *o*-hydroxy aryl alkyl ketones: A structure reactivity study, Tetrahedron, 52, 1996, 3669-3682
33. Amr A E, Abdulla M M, Synthesis and Anti-inflammatory Activities of New Cyanopyrane Derivatives Fused with Steroidal Nuclei, Arch. Pharm. Chem. Life Sci. 339, 2006, 88–95.
34. Obukowics M G, Walseh D J, Salsgiver W J, Martin-Berger C L, Chinn K S, Duffin K L, Ras A, Needleman P, Novel, Selective Delta 6 or Delta 5 Fatty Acid Desaturase Inhibitors as Antiinflammatory Agents in Mice, J. Pharmacol. Exp. Ther. 287, 1998, 157-166.
35. Meng L, Mohan R, Kwok B H B, Elofssof M, Sin N, Crews C M, Proteasome Inhibitor and Antiinflammatory Activity., Proc. Natl. Acad. Sci. USA 96, 1999, 10403–10408.
36. Fathalla O M, Kassem E M M, Ibrahem N M, Kamel M M, Synthesis of some new quinazolin-4-one derivatives and evaluation of their antimicrobial and antiinflammatory effects., Acta Pol. Pharm. Drug Res. 65 (1), 2008, 11-20.
37. Eaton M, Common animal models for spasticity and pain., J. Rehabil Res. Dev. 40, 2003, 41–54.

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