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New acyclic nucleosides analogues as potential analgesic, anti-inflammatory, anti-oxidant and anti-microbial derived from pyrimido[4,5-*b*]quinolines

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ABSTRACT

Synthesis of 2-thioxopyrimido[4,5-*b*]quinoline **3a–c** by microwave oven was used as a base to synthesis acyclic nucleosides analogue of types, 3-(penta-*O*-acetyl-glycosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2]-pyrimido[4,5-*b*]quinolin-4-ones (**7a–c**), 2-tetra-*O*-acetyl-glycosylhydrazon-*N*3-acetyl-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-pentahydro-1*H*-pyrimido[4,5-*b*]-quinolin-4-ones (**10a–c**) and 3-(glycosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-4-ones (**8a–c**), (**12a–c**). The title compounds were investigated for analgesic, anti-inflammatory, anti-oxidant and anti-microbial activities. Compounds **8a**,**b** and **12a**,**b** exhibited highly significant activity towards Gram-negative and Gram-positive bacteria, showed more potent anti-inflammatory and analgesic activities than the acetylated glycoside derivatives **7a**,**b** and **10a**,**b** and exhibited high anti-oxidant activity when compared to the ascorbic acid.

1. Introduction

The recent literatures are enriched with progressive findings about the synthesis and pharmacological action of fused heterocycles. Heterocycles bearing a symmetrical pyrimidoquinoline moiety are reported to show a broad spectrum of pharmacological and medicinal [1,2] properties such as anti-microbial [3–7], antifungal [8], anticancer [9], antiviral [10], analgesic [11], anti-inflammatory activities [12]. Also, acyclic nucleosides of the HEPT type 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine have shown high selectivity towards HIV-1 [13].

In the new millennium free radicals become more harmful which play an important role in the pathogenesis of many diseases, accounting for continuing interest in the identification and development of novel anti-oxidants that prevent radical-induced damage. Anti-oxidants are of great interest because of their involvement in important biological industrial processes. In general, compounds with anti-oxidant activity have been found to posses anticancer, anti-cardiovascular, anti-inflammatory and many other activities [14–16].

Based on the above-mentioned evidence that, several pyrimidine derivatives have anti-microbial and pharmacological activities and also, in our previous works pyrimidine derivatives are characterized as an anti-inflammatory and anti-oxidant [11,26]. We applied microwave techniques to synthesis the start material of our work (2-thioxy-pyrimido[4,5-*b*]quinoline) derivatives. Also, we report here the discovery of pyrimidoquinoline glucoside derivatives that act as anti-microbial, anti-inflammatory, analgesic and anti-oxidant.

2. Chemistry

Microwave technology has attracted much interest for the rapid synthesis of organic compounds which are not easily accessible by conventional synthetic techniques [17]. More recently this way we extended our research program [11] to synthesize bioactive nitrogen atom containing heterocyclic compounds.

Thus, a mixture of an equimolar of α,β-unsaturated ketones **1a–c** and 6-aminothiouracil **2** in dimethylformamide was irradiated in a microwave oven at 80% power for 15 min, afforded the desired products **3a–c** in high yields, as shown in Scheme 1. Action of hydrazine hydrate on 2-thioxopyrimido[4,5-*b*]quinoline (**3b**) in ethanol afforded 2-hydrazinopyrimido-[4,5-*b*]quinolin-4-one (**4**). Structures of these compounds were supported by spectral data such as IR, NMR, Mass and Elemental analyses (Tables 7–9).

The required hydrazones **5a–c** and **9a–c** were prepared by condensation of 2-hydrazinopyrimido[4,5-*b*]quinoline **4** with the appropriate aldohexoses sugar (Scheme 2). The structures of the

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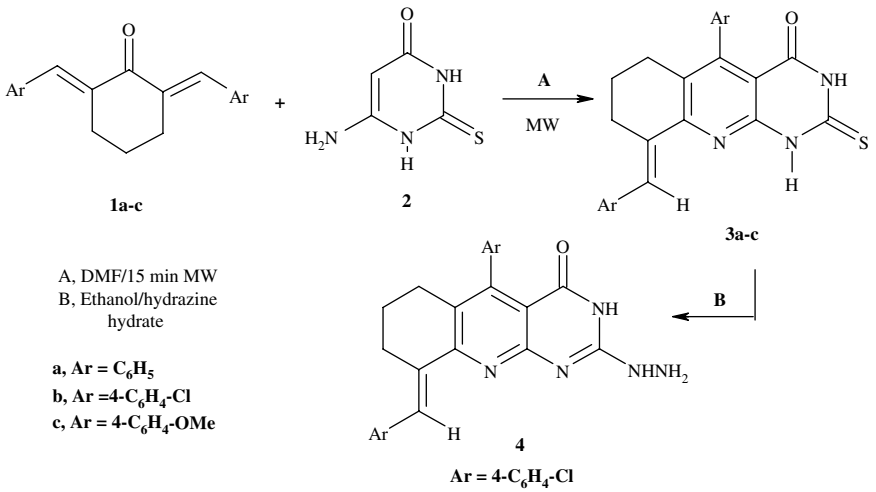


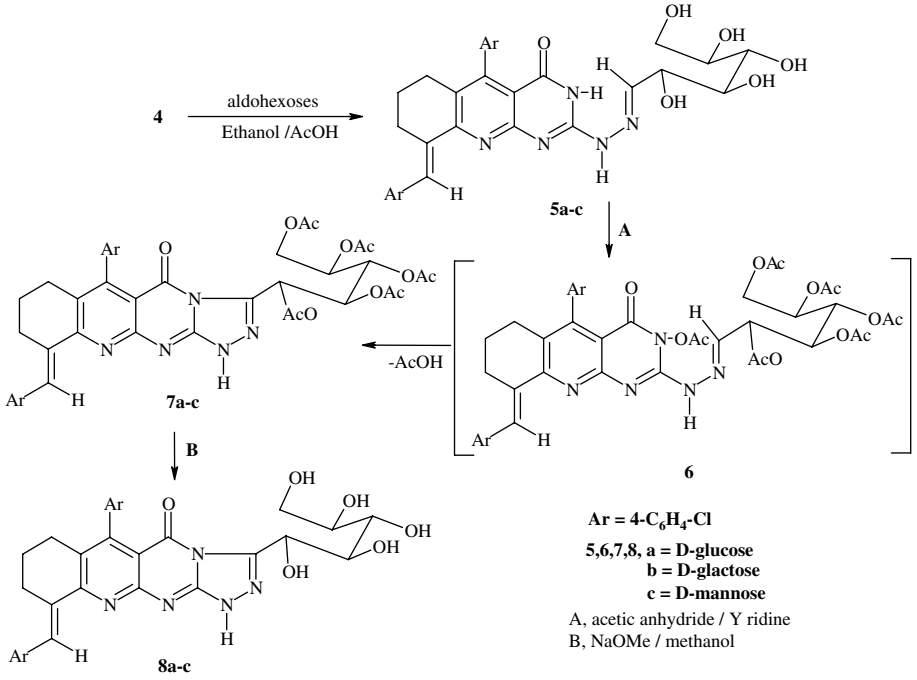
Scheme 1.

new hydrazones **5a**–**c** were confirmed by their elemental analyses and spectral (MS, IR, ^{1}H NMR (Table 8) and ^{13}C NMR) data (Table 9). Their ^{1}H NMR spectra in DMSO-d_6 exhibited, in each case, three characteristic signals: one signal due to –CH═N– proton in the region δ 7.68–7.85 as well as two others signals due to –NHN═C– and –C═NH–CO– protons in the regions 9.20–9.50 and 10.40–10.80. In addition, the spectra showed the signals of the protons of the sugar moiety at δ 3.50–5.36 and that of the phenyl protons, four doublet signals in the region 7.07–7.58. Also the spectra showed the signals supported to the three –CH_2– protons in the regions 1.65–1.69, 2.25–2.31 and 2.71–2.87. Moreover, its ^{13}C NMR spectra of these derivatives exhibited twenty-six sharp lines supported to the proposed structures (see Section 5).

Stirring of 5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-2-glycosylhydrazon-3,6,7,8,9-pentahydropyrimido[4,5-*b*]quinolin-4-one derivatives (**5a**–**c**) at room temperature in a mixture of acetic anhydride-pyridine (1:1) [18] afforded the respective 3-(penta-*O*-acetyl-glycosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-4-one derivatives (**7a**–**c**) (Scheme 2). The structures of the acetylated derivatives were deduced from their combustion analyses, spectral (MS, IR, ^{1}H NMR and ^{13}C NMR) data as depicted in Scheme 2.

Surprisingly, in the case of aldohexoses, 3-(penta-*O*-acetyl-glycosyl)-[1,2,4]triazolo[4′,3′:1,2]-pyrimido[4,5-*b*]quinolin-4-one derivatives **7** were obtained directly from the reaction of 2-glycosylhydrazones **5** with acetic anhydride without separating the 3-*N*-*O*-acetyl derivative **6** (Scheme 2), as in the case of aldopentoses in which *N*-acetyl was separated.

The ^{1}H NMR spectra of **7a**–**c** revealed the presence of five acetyl protons in the regions 1.91–2.17, and showed the signals of the sugar residue at δ 4.04–5.87, in addition to the signals characteristic of the two phenyl protons appeared at δ 7.07–7.62. Also, the spectra of these derivatives showed the absence of any absorption signal for –N═CH– protons (see Section 5). Its ^{13}C NMR spectra of these derivatives exhibited five sharp lines supported the presence of five



Scheme 2.

acetyl groups at 38.47–44.28 ppm and four methylene groups around 20.45–30.68. In addition, the ^{13}C-spectra showed the signals which supported the sugar moiety in the regions 66.81–71.04 and seventeen lines around 109.6–153.2 corresponding to 21 sp^2 carbon atoms. Also, showed the signals corresponding to the six C=O groups in the region 162–171.

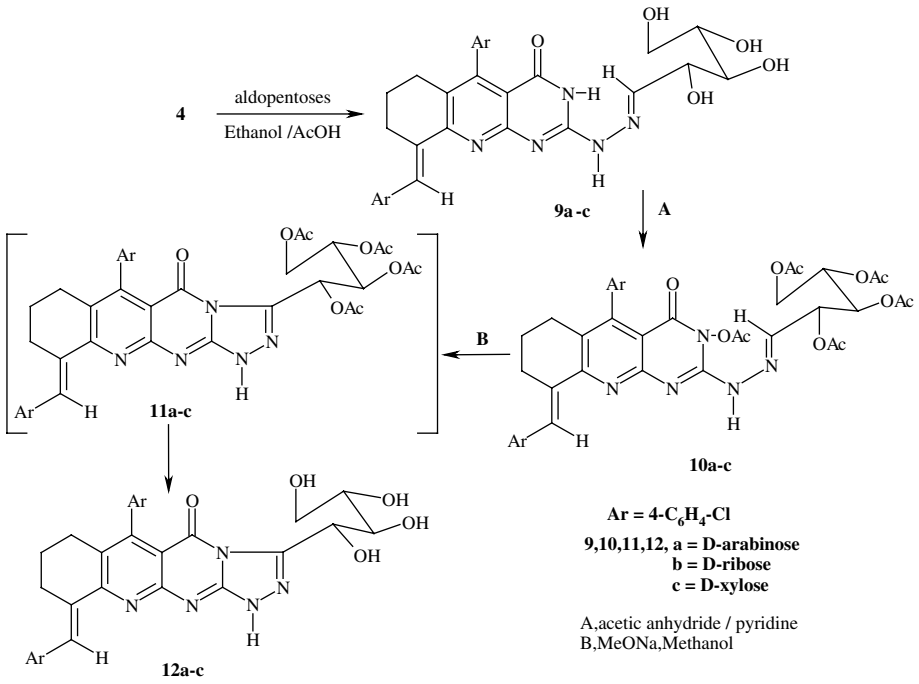
Also, we extend our work to react compound **4** with aldopentoses, namely D-arabinose, D-ribose and D-xylose. Thus, heating under reflux in boiling dioxane of a mixture of 9-(4-chlorophenylmethylene)-5-(4-chlorophenyl)-2-hydrazino-3,6,7,8,9-pentahydropyrimido[4,5-*b*]quinolin-4-one (**4**) with each of aldopentoses, in the presence of catalytic amounts of piperidine, yielded the corresponding 5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-2-glucosylhydrazon-3,6,7,8,9-pentahydropyrimido[4,5-*b*]quinolin-4-one derivatives (**9a–c**) as shown in Scheme 3. The IR spectrum of compounds **9a–c** showed the absorption bands around 3500–3450 cm^{-1} which belong to the hydroxyl groups and the band at 1682–1676 cm^{-1} corresponding to the carbonyl group. Its ^{1}H NMR in DMSO-d_6 spectrum of **9a** as an example showed 1.64–1.67 (m, 2H, CH_2), 2.26–2.31 (t, 2H, CH_2), 2.71–2.76 (t, 2H, CH_2), 3.68 (m, 4OH, D_2O exchangeable), 4.32 (m, 1H, H-3′), 4.47 (m, 1H, H-4′), 4.64 (m, 2H, H-5′, H-5″), 5.12 (dd, 1H, H-2′, $J = 7.45$ Hz), 7.60 (d, 1H, H-1′, $J = 7.50$ Hz), 7.09 (d, 2H, Ar-H, $J = 8.42$ Hz), 7.26 (d, 2H, Ar-H, $J = 8.44$ Hz), 7.45 (d, 2H, Ar-H, $J = 8.39$ Hz), 7. 60 (d, 2H, Ar-H, $J = 8.41$ Hz), 8.35 (s, 1H, vinylic proton), 10.20, 11.30 (2br s, 2NH, D_2O exchangeable) (Table 8). Also, its ^{13}C NMR exhibited signals at δ 22.29, 23.64, 27.70 and 29.25 corresponding to the four methylene groups, the signals of the sugar moiety displayed at 66.88, 69.50 and 71.54 (3CH) and seventeen lines at 110.3–155.3 supported to 21 sp^2 carbons atoms. Also the spectrum showed sharp line at 164.3 corresponding to the carbonyl group (Table 9).

De-protection of the acyclic C-nucleosides **7a–c** and **11a–c** could be achieved when they were stirred in methanolic sodium methoxide solution at room temperature to give a moderate to good yields of 3-(glycosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-4-one derivatives (**8**,**12**). Structures **8a–c** and **12a–c** were confirmed by spectral and elemental analyses (see Section 5). Their ^{1}H NMR spectra showed no absorption signals for the acetyl protons but showed the multiplet signal supported to the five hydroxyl protons in the region δ 3.65–3.70 (D_2O exchangeable), the signals due to the protons of the sugar moiety at δ 3.71–5.42. In addition, the signals supported to the three methylene and the two phenyl protons. The ^{1}H NMR spectrum of **8a**, as an example showed three multiplet absorption bands for three methylene groups around 1.63–2.80, and very broad absorption bands at 3.65 supported to the five hydroxyl groups (D_2O exchangeable) and the signals at 3.71 (m, 1H, H-4′), 4.24 (m, 2H, H-5′, H-5″), 4.48 (m, 1H, H-3′), 4.62 (m, 1H, H-2′), 5.35 (m, 1H, H-1′) which supported the CH's of the sugar moiety. Also showed the aromatic protons at δ 7.18 (d, 2H, Ar-H, $J = 8.45$ Hz), 7.29 (d, 2H, Ar-H, $J = 8.43$ Hz), 7.42 (d, 2H, Ar-H, $J = 8.39$ Hz), 7.62 (d, 2H, Ar-H, $J = 8.51$ Hz), 8.20 (s, 1H, vinylic proton) and 9.00 (br, NH, D_2O exchangeable), respectively (Table 8). Also the ^{13}C NMR spectrum showed eight lines around 22.28–70.52 corresponding to eight sp^3 carbon atoms, seventeen lines around 112.5–157.6 supported to twenty one sp^2 carbon atoms and the absorption signal corresponds to the carbonyl group at 164.5 (Table 9).

3. Biological screening

3.1. Anti-microbial activity

In vitro anti-microbial screening of the new synthesized bases and glycoside compounds was evaluated against two Gram-negative bacteria (*Escherichia coli* ATCC 23556 and *Pseudomonas aeruginosa* ATCC 10145), two Gram-positive bacteria (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923) and two fungal strains (*Candida albicans* ATCC 14053 and *Aspergillus fumigatus* ATCC 96918). Activities of the compounds and the minimum inhibitory (MIC) of the tested compounds were evaluated by agar diffusion method. Compounds **4**, **8a**,**b** and **12a**,**b** exhibited highly significant activity towards Gram-negative and Gram-positive bacteria as compared with the reference drugs (Ciprofloxacin) and also the tested compounds have moderate activity against the fungi as compared with the reference (Ketoconazole) as shown in



Scheme 3.

Table 1. Also, the MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate as shown in Table 2. We concluded that all the tested compounds with free NH group in the pyrimidine moiety showed significant biological activity against all the bacteria, especially 2-hydrazinopyrimido[4,5-*b*]quinoline **4** has high activity.

3.2. Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan-induced paw edema test in rats. Some of the tested compounds showed a reasonable inhibition of edema size in comparison with indomethacin as shown in Table 3, Compounds **8a**,**b** showed higher activity than the indomethacin. Compounds **12a**,**b** were found to be the most potent anti-inflammatory compounds, whereas compounds **3a**, **4**, **7a**,**b** and **10a**,**b** showed the least inhibitory effect. According to structure–activity relationship (SAR), it is clear that 3-glucosyltriazolopyrimido[4,5-*b*]quinoline (**8a)** and 3-glactosyltriazolopyrimido[4,5-*b*]-quinoline (**8b)** were more active than pyrimido[4,5-*b*]quinoline (**3**,**4**). Among the same ring systems (pyrimido[4,5-*b*]pyrimidines), it was noticed that, attachment of the acetylated groups to the glycosyltriazolopyrimido[4,5-*b*]quinoline moiety decreases the anti-inflammatory effect as in the case of **7a**,**b**. Also, the anti-inflammatory activity of the arabinosyl and ribosyl derivatives **12a**,**b** showed high activity as compared to the acetylated derivatives **10a**,**b**. Moreover, activity of the glycosyltriazolopyrimido[4,5-*b*]-quinoline derived from the aldohexoses more active than its derived from aldopentoses. The high activity of the glycosides may be due to the presence of the free hydroxyl groups in the molecules.

3.3. Analgesic activity

The analgesic activity of the synthesized compounds was also investigated. It was assessed by hot plate test model. Some of the pyrimido[4,5-*b*]quinoline derivatives exhibited analgesic effect as shown in Table 4. Compared with the control, the analgesic potency of compounds **8a**,**b** and **12a**,**b** was found to be the highest. While compound **4** showed moderate activity and compounds **3a**, **7a**,**b**, **10a**,**b** showed lower analgesic activity. From the structure–activity relationship (SAR) viewpoint, the effect of acetylated groups on 3-glycosyltriazolopyrimido[4,5-*b*]quinoline decreases the analgesic activity as in the case of compounds **7a**,**b** and **10a**,**b**. On the other hand, The electronic effect of the hydroxyl groups on 3-glycosides attached to triazolopyrimido[4,5-*b*]quinoline ring increases the analgesic activity as in the case of compounds **8a**,**b** and **12a**,**b**.

3.4. Anti-oxidant activity screening

A number of quinoline derivatives were tested for anti-oxidant activity as reflected in the ability to inhibit lipid per-oxidation in rat brain and kidney homogenates and rate erythrocyte hemolysis. The pro-oxidant activities of the selected compounds were assessed by their effects on bleomycin-induced DNA damage the pyrimido[4,5-*b*]-quinoline derivatives, manifested potent anti-oxidative activity in the lipid per-oxidation assay. Also, the results from the erythrocyte hemolysis assay and ABTS showed that, the glycoside derivatives **8a**,**b** and **12a**,**b** exhibited high anti-oxidant activity when compared to the ascorbic acid, while the acetylated glycosides have moderate anti-oxidant activity. (Table 5). The glycoside derivatives were selected to test for bleomycin-dependent DNA damage, and showed that its ability to protect DNA from the induced damage by bleomycin (Table 6).

4. Conclusions

The prepared new ring systems seem to be interesting for biological activity studies. Furthermore, the present investigation offers rapid and effective new procedures for the synthesis of the polycondensed new heterocyclic ring systems. The new compounds were investigated for analgesic, anti-inflammatory, anti-oxidant and anti-microbial activities. Compounds **8a**,**b** and **12a**,**b** exhibited highly significant activity towards Gram-negative and Gram-positive bacteria, showed more potent anti-inflammatory and analgesic activities than the acetylated glycoside derivatives **7a**,**b** and **10a**,**b** and exhibited high anti-oxidant activity when compared to the ascorbic acid. Compounds **8a**,**b** and **12a**,**b** showed the highest inhibitory anti-oxidant activity using ABTS methods. Also, these derivatives manifested the best protective effect against DNA damage induced by Bleomycin.

5. Experimental

5.1. Chemistry

All melting points were taken on Electrothermal IA 9100 series digital melting point apparatus. Microanalytical data (in accord with the calculated values) were performed by Vario, Elementar apparatus (Shimadzu), (Table 7). The IR spectra (KBr) were recorded on a Perkin–Elmer 1650 spectrometer (USA). ^{1}H NMR spectra were determined on a JEOL EX-270 and JEOL ECA-500. Chemical shifts were expressed in ppm relative to $SiMe_4$ as internal standards and DMSO-d_6 as solvent. Mass spectra were recorded on 70 eV EI Ms-QP 1000 EX (Shimadzu, Japan), (Tables 8 and 9). The starting material

Table 1
Preliminary anti-microbial activity test for tested compounds.

Compd. no	Microorganism inhibition zone in mm diameter					
	Gram −ve bacteria		Gram +ve bacteria		Fungi	
	Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus fumigatus*
3a	17	15	15	14	11	13
4	22	20	22	18	13	11
7a	14	12	10	13	11	12
7b	14	10	12	10	11	11
8a	24	22	20	19	12	16
8b	20	19	18	20	11	7
10a	14	15	15	13	10	8
10b	14	13	14	11	7	11
12a	20	19	22	20	12	16
12b	18	18	21	22	13	13
Ciprofloxacin	25	24	25	24	–	–
Ketoconazole	–	–	–	–	21	19

Inhibition zone = 6–10 mm slight activity, 11–15 mm moderate activity, more than 15 mm high activity.

Table 2
The minimum inhibitory concentration (MIC).

Compd. no	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus fumigatus*
3a	3.1	5.3	4.2	4.1	25.0	>100
4	1.0	3.1	2.9	5.0	50.1	>100
7a	10.5	20.5	22.4	4.5	30.4	>100
7b	10.2	25.4	20.2	25.0	50.2	>100
8a	0.2	3.1	4.5	0.2	>100	30.0
8b	1.0	2.4	2.6	3.1	>100	>100
10a	5.0	5.3	8.1	4.1	>100	50.4
10b	12.0	10.0	15.0	4.2	>100	65.5
12a	1.5	2.0	2.1	25.4	45.0	50.0
12b	2.5	1.4	2.0	0.39	60.0	50.3
Ciprofloxacin	0.3	0.39	0.3	0.2	–	–
Ketoconazole	–	–	–	–	1.0	6.1

was prepared by Microwave irradiation 80% in (MW domestic type oven 1000 W with a frequency 2450 MHz).

*5.2. 9-(4-Arylmethylene)-5-aryl-2-thioxo-3,6,7,8,9-hexahydro-1*H*-pyrimido[4,5-*b*]quino-lin-4-one (**3a–c**)*

5.2.1. General procedure

A mixture of **1** (10 mmol) and 6-aminothiouracil **2** (10 mmol) in dimethylformamide (DMF) (10 mL) was irradiated in a domestic microwave for 15 min. The reaction mixture was cooled; the precipitate was filtered off, washed with ethanol, dried and crystallized from DMF.

*5.2.2. 9-Phenylmethylene-5-phenyl-2-thioxo-2,3,6,7,8,9-hexahydropyrimido[4,5-*b*]quino-lin-4-one (**3a**)*

The compound was obtained from the reaction of **1a** (10 mmol), as a yellow powder (DMF).

*5.2.3. 9-(4-Chlorophenylmethylene)-5-(4-chlorophenyl)-2-thioxo-2,3,6,7,8,9-hexahydro-pyrimido[4,5-*b*]quinolin-4-one (**3b**)*

The compound was obtained from the reaction of **1b** (10 mmol), as a yellow powder (DMF).

*5.2.4. 9-(4-Methoxyphenymethylene)-5-(4-methoxyphenyl)-2-thioxo-2,3,6,7,8,9-hexahydropyrimido[4,5-*b*]quinolin-4-one (**3c**)*

The compound was obtained from the reaction of **1c** (10 mmol), as a yellow powder (DMF).

*5.3. 5-(4-Chlorophenyl)-9-(4-chlorophenylmethylene)-2-hydrazino-3,6,7,8,9-pentahydro-1*H*-pyrimido[4,5-*b*]quinolin-4(4*H*)-one (**4**)*

A suspension of **3b** (10 mmol) in hydrazine hydrate (99–100%) (25 mL) was stirred under reflux in ethanol (30 mL, 95%) for 8 h. The reaction mixture was allowed to cool to room temperature. The solid which separated was filtered, washed with ethanol, dried as yellow powder (DMF).

*5.4. 2-(Glycosylhydrazon)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-3,6,7,8,9-pentahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**5a–c**), (**9a–c**)*

5.4.1. General procedure

A mixture of **4** (10 mmol), the appropriate mono-sacchride (10 mmol) and a catalytic amounts of piperidine was heated at reflux in dioxane (50 mL) for 12 h, the reaction mixture was allowed to cool to room temperature, the precipitate was filtered off, washed with ethanol, dried and crystallized to afford the title compounds.

*5.4.2. 2-(Glucosylhydrazon)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-3,6,7,8,9-pentahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**5a**)*

It was obtained from D-glucose (10 mmol), as a white powder (dioxane).

*5.4.3. 2-(Galactosylhydrazon)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-3,6,7,8,9-pentahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**5b**)*

It was obtained from D-galactose (10 mmol), as a white powder (dioxane).

*5.4.4. 2-(Mannosylhydrazon)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-3,6,7,8,9-pentahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**5c**)*

It was obtained from D-mannose (10 mmol), as a white powder (ethanol).

Table 3
Carrageenan-induced rat paw edema: Anti-flammatory activity.

Group	Increase in paw volume (mL) ± S.E/(% inhibition of edema)			
	1 h	2 h	3 h	4 h
3a	0.22 ± 0.016 (31.25)[b]	0.35 ± 0.024 (24.00)[b]	0.47 ± 0.013 (27.54)[b]	0.45 ± 0.017 (25.00)[b]
4	0.21 ± 0.013 (34.38)[b]	0.33 ± 0.018 (34.00)[b]	0.43 ± 0.013 (37.68)[b]	0.35 ± 0.015 (35.19)[b]
7a	0.26 ± 0.015 (18.75)[a]	0.46 ± 0.026 (14.80)[a]	0.55 ± 0.034 (13.28)[a]	0.47 ± 0.024 (19.57)[b]
7b	0.25 ± 0.006 (21.88)[a]	0.43 ± 0.018 (14.00)[a]	0.56 ± 0.014 (18.48)[a]	0.53 ± 0.014 (17.19)[a]
8a	0.12 ± 0.013 (62.50)[b]	0.28 ± 0.012 (44.00)[b]	0.40 ± 0.022 (42.03)[b]	0.36 ± 0.019 (40.62)[b]
8b	0.10 ± 0.007 (50.00)[b]	0.20 ± 0.018 (56.00)[b]	0.32 ± 0.017 (49.27)[b]	0.30 ± 0.014 (50.00)[b]
10a	0.26 ± 0.020 (18.75)[a]	0.36 ± 0.015 (28.00)[b]	0.49 ± 0.014 (28.98)[b]	0.42 ± 0.021 (22.22)[b]
10b	0.27 ± 0.006 (15.62)[a]	0.43 ± 0.021 (20.37)[b]	0.51 ± 0.027 (26.09)[b]	0.47 ± 0.022 (26.56)[b]
12a	0.18 ± 0.017 (40.62)[b]	0.19 ± 0.018 (52.00)[b]	0.39 ± 0.019 (45.31)[b]	0.16 ± 0.019 (46.67)[b]
12b	0.17 ± 0.0186 (46.88)[b]	0.41 ± 0.014 (40.58)[b]	0.39 ± 0.018 (39.06)[b]	0.32 ± 0.0156 (39.81)[b]
Control	0.32 ± 0.022	0.05 ± 0.018	0.69 ± 0.026	0.64 ± 0.017
Indomethacin	0.16 ± 0.014 (50.00)[b]	0.21 ± 0.012 (58.00)[b]	0.24 ± 0.013 (65.22)	0.27 ± 0.011 (57.80)[b]

Values represent the mean ± S.E. of six animals for each group. Data were analyzed by One-way ANOVA followed by Post-Hoc test.
[a] Represents not significance level at $P < 0.05$.
[b] Represents the significance level of $P < 0.05$.

Table 4
Central analgesic activity (Hot plate test).

Group	Reaction time (s)			
	0	30	60	90
3a	2.350 ± 0.117	2.700 ± 0.770	2.650 ± 0.117	2.583 ± 0.149
4	2.500 ± 0.129	3.700 ± 0.141[b]	4.067 ± 0.166[b]	6.617 ± 0.188[b]
7a	2.350 ± 0.117	2.483 ± 0.083	2.667 ± 0.098	2.633 ± 0.066
7b	2.600 ± 0.112	2.650 ± 0.117	2.667 ± 0.111	2.483 ± 0.149
8a	2.550 ± 0.170	4.650 ± 0.299[b]	7.400 ± 0.498[b]	9.567 ± 0.240[b]
8b	2.983 ± 0.147	3.767 ± 0.190[b]	5.450 ± 0.275[b]	6.767 ± 0.391[b]
10a	2.383 ± 0.175	3.667 ± 0.135[b]	3.483 ± 0.181[a]	4.583 ± 0.210[b]
10b	2.483 ± 0.124	3.500 ± 0.139[a]	3.483 ± 0.181	4.033 ± 0.128[b]
12a	2.667 ± 0.158	3.600 ± 0.308[b]	5.933 ± 0.375[b]	8.433 ± 0.334[b]
12b	2.800 ± 0.123	3.550 ± 0.140[b]	5.350 ± 117[b]	6.667 ± 0.143[b]
Control	2.683 ± 0.132	2.600 ± 0.150	2.650 ± 0.154	2.667 ± 0.189
Acetyl salicylic acid	2.433 ± 0.120	5.100 ± 0.216[b]	7.683 ± 0.291[b]	8.650 ± 0.325[b]

Statistical analysis: the statistical analysis was performed by One-way ANOVA followed by Dunnett's test.
[a] Statistically significant for $P < 0.05$.
[b] Statistically significant for $P < 0.01$.

*5.4.5. 2-(Arabinosylhydrazon)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-3,6,7,8,9-pentahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**9a**)*

It was obtained from D-arabinose (10 mmol), as a white powder (ethanol).

*5.4.6. 2-(Ribosylhydrazon)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-3,6,7,8,9-pentahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**9b**)*

It was obtained from D-ribose (10 mmol), as a white powder (ethanol).

*5.4.7. 2-(Xylosylhydrazon)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-3,6,7,8,9-pentahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**9c**)*

It was obtained from D-xylose (10 mmol), as a white powder (ethanol).

*5.5. 3-(Penta-*O*-acetyl-glycosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9-10-tetrahydro[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-4-ones (**7a–c**), 2-tetra-*O*-acetyl-glycosylhydrazon-*N*3-acetyl-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-pentahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**10a–c**)*

5.5.1. General procedure

A solution from each of **5a–c**, **9a–c** (10 mmol) in a mixture of acetic anhydride-pyridine (40 mL, 1:1) was stirred at room temperature for 24 h, poured onto water (100 mL). The mixture was then extracted with chloroform several times (150 mL), after the removal of chloroform under reduced pressure; the precipitate was filtered off, dried, and crystallized from the proper solvent to obtain **7a–c**, **10a–c**.

Table 5
Anti-oxidant assays by erythrocyte hemolysis and ABTS method.

Methods	Erythrocyte hemolysis		ABTS	
	$A/B \times 100$		$A_{control} - A_{test}/A_{control} \times 100$	
Compounds	Abs samples (A)	Hemolysis (%)	Abs samples (A)	Inhibition (%)
Complete hemolysis with Dist-H_2O (B)	0.660	–	–	–
Control of ABTS	–	–	0.54	0
Ascorbic acid	0.026	3.93	0.06	88.9
3a	0.236	35.75	0.30	44.4
4	0.084	5.45	0.22	59.3
7a	0.054	8.18	0.32	40.7
7b	0.055	8.33	0.31	42.6
8a	0.038	4.23	0.10	81.5
8b	0.052	4.01	0.18	83.7
10a	0.203	30.75	0.35	35.2
10b	0.040	6.06	0.32	40.7
12a	0.048	3.51	0.18	66.7
12b	0.041	3.42	0.20	68.8

Table 6
Assay for bleomycin-dependent DNA damage (DNA).

Methods	Bleomycin-dependent DNA damage
Compounds	Absorbance of samples
Ascorbic acid	0.026
8a	0.029
8b	0.019
12a	0.050
12b	0.045

*5.5.2. 3-(1′,2′,3′,4′,5′-Penta-*O*-acetylglucosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-4-one (**7a**)*

It was obtained from **5a** as white powder (ethanol).

*5.5.3. 3-(1′,2′,3′,4′,5′-Penta-*O*-acetylgalactosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-4-one (**7b**)*

It was obtained from **5b** as white powder (ethanol).

Table 7
Physical constants of newly synthesized compounds.

No.	Yield (%)	m.p. (°C)	Mol. form. (Mol. wt)	Microanalysis		
				C	H	N
3a	88	220–223	$C_{24}H_{19}N_3OS$ (397.5)	72.52	4.82	10.57
				72.54	4.81	10.55
3b	90	295–297	$C_{24}H_{17}Cl_2N_3OS$ (466.4)	61.80	3.67	9.01
				61.78	3.65	9.06
3c	87	280–282	$C_{26}H_{23}N_3O_3S$ (457.5)	68.25	5.07	9.18
				68.19	5.08	9.14
4	83	278–280	$C_{24}H_{19}Cl_2N_5O$ (464.3)	62.08	4.12	15.08
				62.11	4.09	15.03
5a	65	254–257	$C_{30}H_{29}Cl_2N_5O_6$ (626.5)	57.51	4.66	11.17
				57.49	4.62	11.14
5b	69	241–243	$C_{30}H_{29}Cl_2N_5O_6$ (626.5)	57.51	4.66	11.17
				57.47	4.63	11.19
5c	68	156–158	$C_{30}H_{29}C_{l2}N_5O_6$ (626.5)	57.51	4.66	11.17
				57.53	4.64	11.13
9a	69	192–195	$C_{29}H_{27}Cl_2N_5O_5$ (596.5)	58.39	4.56	11.74
				58.41	4.54	11.68
9b	63	209–211	$C_{29}H_{27}Cl_2N_5O_5$ (596.5)	58.39	4.56	11.74
				58.37	4.53	11.76
9c	56	219–221	$C_{29}H_{27}Cl_2N_5O_5$ (596.5)	58.39	4.56	11.74
				58.41	4.54	11.68
7a	68	176–178	$C_{40}H_{37}Cl_2N_5O_{11}$ (834.6)	57.56	4.47	8.39
				57.53	4.39	8.41
7b	61	198–201	$C_{40}H_{37}Cl_2N_5O_{11}$ (834.6)	57.56	4.47	8.39
				57.51	4.40	8.36
7c	63	171–173	$C_{40}H_{37}Cl_2N_5O_{11}$ (834.6)	57.56	4.47	8.39
				57.58	4.42	8.36
10a	70	156–159	$C_{39}H_{37}Cl_2N_5O_{11}$ (822.6)	56.93	4.53	8.51
				56.89	4.56	8.53
10b	59	148–150	$C_{39}H_{37}Cl_2N_5O_{11}$ (822.6)	56.93	4.53	8.51
				56.88	4.49	8.56
10c	60	163–165	$C_{39}H_{37}Cl_2N_5O_{11}$ (822.6)	56.93	4.53	8.51
				56.87	4.52	8.55
8a	67	237–240	$C_{30}H_{27}Cl_2N_5O_6$ (624.5)	57.69	4.36	11.21
				57.71	4.34	11.18
8b	69	253–255	$C_{30}H_{27}Cl_2N_5O_6$ (624.5)	57.69	4.36	11.21
				57.67	4.31	11.23
8c	65	271–273	$C_{30}H_{27}Cl_2N_5O_6$ (624.5)	57.69	4.36	11.21
				57.70	4.41	11.23
12a	53	243–245	$C_{29}H_{25}Cl_2N_5O_5$ (594.4)	58.59	4.24	11.78
				58.41	4.23	11.73
12b	49	211–213	$C_{29}H_{25}Cl_2N_5O_5$ (594.4)	58.59	4.24	11.78
				58.53	4.27	11.76
12c	59	231–233	$C_{29}H_{25}Cl_2N_5O_5$ (594.4)	58.59	4.24	11.78
				58.61	4.26	11.75

Table 8
Mass, IR, ^{1}H NMR spectral data of newly synthesized compounds.

Compd. no.	Mass (m/z) (%)	IR (ν, cm^{-1})	^{1}H NMR (δ, ppm) (DMSO-d_6)
3a	[M$^+$], 397 (100)	3380 (br s, NH) 3018 (CH aryl) 2907 (CH alkyl) 1688 (CO)	δ 1.63–1.67 (m, 2H, CH_2), 2.28–2.32 (t, 2H, CH_2) 2.77–2.92 (t, 2H, CH_2), 7.06–7.12 (m, 3H, Ar-H), 7.18–7.26 (m, 4H, Ar-H), 7.33–7.42 (m, 3H, Ar-H), 8.21 (s, 1H, vinylic proton) and 11.30, 12.10 (two broad bands, 2NH)
3b		3380 (br, NH's) 3025 (CH aryl) 2921 (CH alkyl) 1688 (CO)	δ 1.64–1.67 (m, 2H, CH_2), 2.29–2.32 (t, 2H, CH_2), 2.75–2.97 (t, 2H, CH_2), 7.10–7.12 (d, 2H, Ar-H, J = 8.3 Hz), 7.15 (d, 2H, Ar-H, J = 8.4 Hz), 7.18 (2d, 4H, Ar-H, J = 8.4, 8.5 Hz), 8.19 (s, 1H, vinylic proton), 10.30, 11.50 (2br s, 2NH)
3c	[M$^+$], 456 (100)	3348 (br s, NH) 3029 (CH aryl) 2907 (CH alkyl) 1693 (CO)	δ 1.65–1.68 (m, 2H, CH_2), 2.27–2.30 (t, 2H, CH_2) 2.74–2.95 (t, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 7.12 (d, 2H, Ar-H, J = 8.3 Hz), 7.15 (d, 2H, Ar-H, J = 8.4 Hz), 7.38 (d, 2H, Ar-H, J = 8.4 Hz), 7.55 (d, 2H, Ar-H, J = 8.4 Hz), 8.23 (s, 1H, vinylic proton) and 11.20, 12.32 (two broad bands, 2NH)
4		3198 (br s, NH) 3036 (CH aryl) 2934 (CH alkyl) 1676 (CO) 1620 (C=N)	δ 1.64–1.69 (m, 2H, CH_2), 2.29–2.32 (t, 2H, CH_2), 2.76–2.78 (t, 2H, CH_2), 7.09 (d, 2H, Ar-H, J = 8.4 Hz), 7.40 (d, 2H, Ar-H, J = 8.5 Hz), 7.43–7.47 (2d, 4H, Ar-H, J = 8.3, 8.4 Hz), 8.02 (s, 1H, vinylic proton), 8.75 (br s, NH_2, D_2O exchangeable), 9.50, 11.00 (2 br s, 2NH, D_2O exchangeable)
5a	[M$^+$ + 2], 628 (64), [M$^+$], 626 (100)	3500 (br s, OH) 3385 (br, NH's) 3056 (CH aryl) 2924 (CH alkyl) 1686 (CO) 1635 (C=N)	δ 1.65–1.67 (m, 2H, CH_2), 2.27–2.31 (t, 2H, CH_2), 2.73–2.87 (t, 2H, CH_2), 3.56 (m, 5OH, D_2O exchangeable), 3.74 (m, 1H, H-5′), 4.28 (m, 2H, H-6′, H-6″), 4.44 (m, 1H, H-4′), 4.56 (m, 1H, H-3′), 5.38 (m, 1H, H-2′), 7.07 (d, 2H, Ar-H, J = 8.4 Hz), 7.27 (d, 2H, Ar-H, J = 8.4 Hz), 7.45 (d, 2H, Ar-H, J = 8.3 Hz), 7.57 (d, 2H, Ar-H, J = 8.5 Hz), 7.85 (m, 1H, H-1′), 8.15 (s, 1H, vinylic proton), 9.50, 10.40 (2 br s, 2NH, D_2O exchangeable)
5b	[M$^+$ + 2], 628 (56), [M$^+$], 626 (43), [M$^+$ − $C_6H_{12}N_2O_5$] 433 (100)	3520 (br s, OH), 3390 (br, NH's) 3029 (CH aryl) 2908 (CH alkyl) 1681 (CO), 1620 (C=N)	δ 1.66–1.69 (m, 2H, CH_2), 2.26–2.30 (t, 2H, CH_2), 2.71–2.82 (t, 2H, CH_2), 3.65 (m, 5OH, D_2O exchangeable), 3.80 (m, 1H, H-5′), 4.24 (m, 2H, H-6′, H-6″), 4.40 (m, 1H, H-4′), 4.53 (m, 1H, H-3′), 5.34 (d, 1H, H-2′, J = 7.4 Hz), 7.11 (d, 2H, Ar-H, J = 8.3 Hz), 7.26 (d, 2H, Ar-H, J = 8.3 Hz), 7.40 (d, 2H, Ar-H, J = 8.4 Hz), 7.53 (d, 2H, Ar-H, J = 8.4 Hz), 7.68 (m, 1H, H-1′), 8.20 (s, 1H, vinylic proton), 9.20, 10.80 (2 br s, 2NH, D_2O exchangeable)
5c	[M$^+$ + 2], 628 (64), [M$^+$], 626 (100)	3480 (br s, OH) 3390 (br, NH's) 3023 (CH aryl) 2921 (CH alkyl) 1687 (CO) 1620 (C=N)	δ 1.66–1.69 (m, 2H, CH_2), 2.25–2.30 (t, 2H, CH_2), 2.71–2.79 (t, 2H, CH_2), 3.50 (m, 5OH, D_2O exchangeable), 3.77 (m, 1H, H-5′), 4.24 (m, 2H, H-6′, H-6″), 4.41 (m, 1H, H-4′), 4.56 (m, 1H, H-3′), 5.36 (m, 1H, H-2′), 7.09 (d, 2H, Ar-H, J = 8.3 Hz), 7.28 (d, 2H, Ar-H, J = 8.5 Hz), 7.44 (d, 2H, Ar-H, J = 8.3 Hz), 7.58 (d, 2H, Ar-H, J = 8.5 Hz), 7.85 (m, 1H, H-1′) 8.30 (s, 1H, vinylic proton), 9.30, 10.45 (2 br s, 2NH, D_2O exchangeable)
9a	[M$^+$ + 2], 598 (43), [M$^+$], 596 (65), [M$^+$ − $C_5H_{11}N_2O_4$], 433 (100)	3450 (br s, OH) 3365 (br, NH's) 3019 (CH aryl) 2908 (CH alkyl) 1678 (CO) 1610 (C=N)	δ 1.64–1.67 (m, 2H, CH_2), 2.26–2.31 (t, 2H, CH_2), 2.71–2.76 (t, 2H, CH_2), 3.68 (m, 4OH, D_2O exchangeable), 4.32 (m, 1H, H-3′), 4.47 (m, 1H, H-4′), 4.64 (m, 2H, H-5′, H-5″), 5.12 (dd, 1H, H-2′, J = 7.45 Hz), 7.60 (d, 1H, H-1′, J = 7.50 Hz), 7.09 (d, 2H, Ar-H, J = 8.42 Hz), 7.26 (d, 2H, Ar-H, J = 8.44 Hz), 7.45 (d, 2H, Ar-H, J = 8.39 Hz), 7. 60 (d, 2H, Ar-H, J = 8.41 Hz), 8.35 (s, 1H, vinylic proton), 10.20, 11.30 (2 br s, 2NH, D_2O exchangeable)
9b	[M$^+$ + 2], 598 (51), [M$^+$], 596 (100), [M$^+$ − $C_5H_{11}N_2O_4$], 433 (75).	3490 (br s, OH) 3370 (br, NH's) 3029 (CH aryl) 2921 (CH alkyl) 1682 (CO) 1615 (C=N)	δ 1.67–1.70 (m, 2H, CH_2), 2.29–2.32 (t, 2H, CH_2), 2.74–2.82 (t, 2H, CH_2), 3.74 (m, 4OH), 4.36 (m, 1H, H-3′), 4.45 (m, 1H, H-4′), 4.69 (m, 2H, H-5′, H-5″), 5.20 (dd, 1H, H-2′, J = 7.47 Hz), 7.62 (d, 1H, H-1′, J = 7.39 Hz), 7.12 (d, 2H, Ar-H, J = 8.43 Hz), 7.28 (d, 2H, Ar-H, J = 8.44 Hz), 7.48 (d, 2H, Ar-H, J = 8.41 Hz), 7.64 (d, 2H, Ar-H, J = 8.43 Hz), 8.40 (s, 1H, vinylic proton), 9.80, 11.20 (2 br s, 2NH)
9c	[M$^+$ + 2], 598 (48), [M$^+$], 596 (72), [M$^+$ − $C_5H_{11}N_2O_4$], 433 (83)	3500 (br s, OH) 3340 (br, NH's) 3028 (CH aryl) 2916 (CH alkyl) 1676 (CO) 1600 (C=N)	δ 1.62–1.67 (m, 2H, CH_2), 2.24–2.31 (t, 2H, CH_2), 2.67–2.73 (t, 2H, CH_2), 3.78 (m, 4OH), 4.29 (m, 1H, H-3′), 4.40 (m, 1H, H-4′), 4.72 (m, 2H, H-5′, H-5″), 5.33 (dd, 1H, H-2′, J = 7.45 Hz), 7.60 (d, 1H, H-1′, J = 7.36 Hz), 7.13 (d, 2H, Ar-H, J = 8.41 Hz), 7.25 (d, 2H, Ar-H, J = 8.42 Hz), 7.50 (d, 2H, Ar-H, J = 8.40 Hz), 7.68 (d, 2H, Ar-H, J = 8.41 Hz), 8.35 (s, 1H, vinylic proton), 9.15, 11.00 (2 br s, 2NH)
7a	[M$^+$], 834 (53)	3390 (br, NH) 3024 (CH aryl) 2908 (CH alkyl) 1760–1735 (5 CO) 1678 (CO) 1630 (C=N)	δ 1.62–1.67 (m, 2H, CH_2), 1.92, 1.98, 2.02, 2.05, 2.12 (5s, 5CH_3), 2.27–2.31 (t, 2H, CH_2), 2.76–2.83 (t, 2H, CH_2), 4.04 (m, 2H, H-5′, H-5″), 5.06 (t, 1H, H-4′, J = 8.41 Hz), 5.28-5.35 (m, 2H, H-2′, H-3′), 5.87 (d, 1H, H-1′, J = 8.24 Hz), 7.07 (d, 2H, Ar-H, J = 8.45 Hz), 7.27 (d, 2H, Ar-H, J = 8.46 Hz), 7.44 (d, 2H, Ar-H, J = 8.43 Hz), 7.58 (d, 2H, Ar-H, J = 8.44 Hz), 8.45 (s, 1H, vinylic proton), 9.50 (br, NH, D_2O exchangeable)
7b	[M$^+$], 834 (67)	3410 (br, NH) 3032 (CH aryl) 2923 (CH alkyl) 1755–1730 (5 CO) 1682 (CO) 1625 (C=N)	δ 1.63–1.67 (m, 2H, CH_2), 1.91, 1.96, 2.01, 2.06, 2.11 (5s, 5CH_3), 2.29–2.34 (t, 2H, CH_2), 2.77–2.84 (t, 2H, CH_2), 4.06–4.12 (m, 2H, H-5′, H-5″), 5.07 (t, 1H, H-4′, J = 8.39 Hz), 5.30–5.39 (m, 2H, H-2′, H-3′), 5.85 (d, 1H, H-1′, J = 8.31 Hz), 7.11 (d, 2H, Ar-H, J = 8.44 Hz), 7.29 (d, 2H, Ar-H, J = 8.43 Hz), 7.48 (d, 2H, Ar-H, J = 8.46 Hz), 7.60 (d, 2H, Ar-H, J = 8.44 Hz Hz), 8.24 (s, 1H, vinylic proton), 10.15 (br, NH, D_2O exchangeable)
7c	[M$^+$], 834 (46)	3370 (br, NH) 3029 (CH aryl) 2921 (CH alkyl) 1750–1725 (5 CO) 1679 (CO) 1610 (C=N)	δ 1.63–1.68 (m, 2H, CH_2), 1.93, 1.97, 2.04, 2.06, 2.13 (5s, 5CH_3), 2.28–2.34 (t, 2H, CH_2), 2.78–2.85 (t, 2H, CH_2), 4.04–4.16 (m, 2H, H-5′, H-5″), 5.03 (t, 1H, H-4′, J = 8.40 Hz), 5.23–5.31 (m, 2H, H-2′, H-3′), 5.76 (d, 1H, H-1′, J = 8.30 Hz), 7.14 (d, 2H, Ar-H, J = 8.43 Hz), 7.30 (d, 2H, Ar-H, J = 8.41 Hz), 7.48 (d, 2H, Ar-H, J = 8.45 Hz), 7.62 (d, 2H, Ar-H, J = 8.44 Hz), 8.35 (s, 1H, vinylic proton), 9.00 (br, NH, D_2O exchangeable)
10a	[M$^+$], 822 (82)	3370 (br, NH) 3043 (CH aryl) 2931 (CH alkyl) 1755–1720 (5 CO) 1682 (CO) 1610 (C=N)	δ 1.66–1.72 (m, 2H, CH_2), 1.91, 1.97, 2.01, 2.05, 2.17 (5CH_3), 2.29–2.33 (t, 2H, CH_2), 2.76–2.84 (t, 2H, CH_2), 4.23–4.34 (m, 2H, H-4′, H-4″), 5.25 (t, 1H, H-3′, J = 8.56 Hz), 5.40 (t, 1H, H-2″, J = 8.76 Hz), 5.60 (d, 1H, H-1′, J = 8.49 Hz), 7.09 (d, 2H, Ar-H, J = 8.39 Hz), 7.28 (d, 2H, Ar-H, J = 8.40 Hz), 7.45 (d, 2H, Ar-H, J = 8.42 Hz), 7.60 (d, 2H, Ar-H, J = 8.43 Hz), 7.78 (s, 1H, azomethine), 8.20 (s, 1H, vinylic proton), 10.40 (br, NH)
10b	[M$^+$], 822 (56)	3410 (br, NH) 3061 (CH aryl) 2935 (CH alkyl) 1752–1725 (5 CO) 1675 (CO) 1600 (C=N)	δ 1.64–1.70 (m, 2H, CH_2), 1.93, 1.98, 2.02, 2.07, 2.15 (5CH_3 of acetyl), 2.31–2.37 (t, 2H, CH_2), 2.74–2.80 (t, 2H, CH_2), 4.27–4.38 (m, 2H, H-4′, H-4″), 5.21 (t, 1H, H-3′, J = 8.66 Hz), 5.43 (t, 1H, H-2″, J = 8.79 Hz), 5.58 (d, 1H, H-1′, J = 8.69 Hz), 7.17 (d, 2H, Ar-H, J = 8.40 Hz), 7.33 (d, 2H, Ar-H, J = 8.38 Hz), 7.49 (d, 2H, Ar-H, J = 8.43 Hz), 7.67 (d, 2H, Ar-H, J = 8.42 Hz), 7.90 (s, 1H, azomethine), 8.35 (s, 1H, vinylic proton), 10.20 (br, NH)

(*continued on next page*)

Table 8 (*continued*)

Compd. no.	Mass (*m*/*z*) (%)	IR (ν, cm^{-1})	^1H NMR (δ, ppm) (DMSO-d_6)
10c	[M^+], 822 (46)	3365 (br, NH) 3029 (CH aryl) 2918 (CH alkyl) 1750–1715 (5 CO) 1679 (CO) 1600 (C=N)	δ 1.64–1.71 (m, 2H, CH_2), 1.90, 1.96, 2.00, 2.06, 2.16 (5CH_3 of acetyl), 2.26–2.31 (t, 2H, CH_2), 2.75–2.82 (t, 2H, CH_2), 4.27–4.35 (m, 2H, H-4′, H-4″), 5.26 (t, 1H, H-3′, J = 8.54 Hz), 5.38 (t, 1H, H-2″, J = 8.78 Hz), 5.57 (d, 1H, H-1′, J = 8.47 Hz), 7.12 (d, 2H, Ar-H, J = 8.37 Hz), 7.31 (d, 2H, Ar-H, J = 8.41 Hz), 7.46 (d, 2H, Ar-H, J = 8.40 Hz), 7. 62 (d, 2H, Ar-H, J = 8.41 Hz), 7.75 (s, 1H, azomethine), 8.23 (s, 1H, vinylic proton), 9.40 (br, NH, D_2O exchangeable)
8a	[$M^+ + 2$], 626 (38) [M^+], 624 (100)	3480 (br s, OH) 3355 (br, NH) 3037 (CH aryl) 2919 (CH alkyl) 1691 (CO) 1620 (C=N)	δ 1.63–1.67 (m, 2H, CH_2), 2.26–2.30 (t, 2H, CH_2), 2.71–2.80 (t, 2H, CH_2), 3.65 (m, 5OH, D_2O exchangeable), 3.71 (m, 1H, H–4′), 4.24 (m, 2H, H-5′, H-5″), 4.48 (m, 1H, H-3′), 4.62 (m, 1H, H-2′), 5.35 (m, 1H, H-1′), 7.18 (d, 2H, Ar-H, J = 8.45 Hz), 7.29 (d, 2H, Ar-H, J = 8.43 Hz), 7.42 (d, 2H, Ar-H, J = 8.39 Hz), 7.62 (d, 2H, Ar-H, J = 8.51 Hz), 8.20 (s, 1H, vinylic proton), 9.00 (br, NH, D_2O exchangeable)
8b	[$M^+ + 2$], 626 (52) [M^+], 624 (100)	3510 (br s, OH) 3340 (br, NH) 3023 (CH aryl) 2918 (CH alkyl) 1688 (CO) 1625 (C=N)	δ 1.69–1.73 (m, 2H, CH_2), 2.28–2.36 (t, 2H, CH_2), 2.74–2.83 (t, 2H, CH_2), 3.70 (m, 5OH, D_2O exchangeable), 3.78 (m, 1H, H-4′), 4.20 (m, 2H, H-5′, H-5″), 4.36 (m, 1H, H-3′), 4.55 (m, 1H, H-2′), 5.30 (d, 1H, H-1′, J = 7.43 Hz), 7.16 (d, 2H, Ar-H, J = 8.39 Hz), 7.29 (d, 2H, Ar-H, J = 8.36 Hz), 7.44 (d, 2H, Ar-H, J = 8.42 Hz), 7.58 (d, 2H, Ar-H, J = 8.42 Hz), 8.26 (s, 1H, vinylic proton), 9.20 (br, NH, D_2O exchangeable)
8c	[$M^+ + 2$], 626 (46) [M^+], 624 (71)	3490 (br s, OH) 3340 (br, NH) 3027 (CH aryl) 2909 (CH alkyl) 1693 (CO) 1600 (C=N)	δ 1.66–1.70 (m, 2H, CH_2), 2.26–2.31 (t, 2H, CH_2), 2.72–2.80 (t, 2H, CH_2), 3.73 (m, 5OH, D_2O exchangeable), 3.82 (m, 1H, H-4′), 4.30 (m, 2H, H-5′, H-5″), 4.52 (m, 1H, H-3′), 4.63 (m, 1H, H-2′), 5.42 (d, 1H, H-1′, J = 7.56 Hz), 7.20 (d, 2H, Ar-H, J = 8.38 Hz), 7.26 (d, 2H, Ar-H, J = 8.41 Hz), 7.48 (d, 2H, Ar-H, J = 8.39 Hz), 7.64 (d, 2H, Ar-H, J = 8.50 Hz), 8.22 (s, 1H, vinylic proton), 9.50 (br, NH, D_2O exchangeable);
12a	[$M^+ + 2$], 596 (43) [M^+], 594 (65) [$M^+ - C_5H_{11}N_2O_4$], 431 (100)	3495 (br s, OH) 3340 (br, NH) 3039 (CH aryl) 2928 (CH alkyl) 1687 (CO) 1600 (C=N)	δ 1.68–1.73 (m, 2H, CH_2), 2.28–2.35 (t, 2H, CH_2), 2.70–2.75 (t, 2H, CH_2), 3.83 (m, 4OH, D_2O exchangeable), 4.30 (m, 1H, H-3′), 4.54 (m, 2H, H-4′, H-4″), 5.15 (t, 1H, H-2′, J = 7.59 Hz), 5.61 (d, 1H, H-1′, J = 7.87 Hz), 7.11 (d, 2H, Ar-H, J = 8.40 Hz), 7.28 (d, 2H, Ar-H, J = 8.42 Hz), 7.47 (d, 2H, Ar-H, J = 8.40 Hz), 7.69 (d, 2H, Ar-H, J = 8.43 Hz), 8.12 (s, 1H, vinylic proton), 10.30 (br, NH, D_2O exchangeable)
12b	[$M^+ + 2$], 596 (39) [M^+], 594 (100) [$M^+ - C_4H_9O_4$], 473 (81).	3500 (br s, OH) 3350 (br, NH) 3053 (CH aryl) 2929 (CH alkyl) 1688 (CO) 1620 (C=N)	δ 1.65–1.71 (m, 2H, CH_2), 2.25–2.32 (t, 2H, CH_2), 2.73–2.81 (t, 2H, CH_2), 3.66 (m, 4OH, D_2O exchangeable), 4.33 (m, 1H, H-3′), 4.62 (m, 2H, H-4′, H-4″), 5.25 (t, 1H, H-2′, J = 7.46 Hz), 5.68 (d, 1H, H-1′, J = 7.86 Hz), 7.16 (d, 2H, Ar-H, J = 8.40 Hz), 7.26 (d, 2H, Ar-H, J = 8.43 Hz), 7.50 (d, 2H, Ar-H, J = 8.43 Hz), 7.71 (d, 2H, Ar-H, J = 8.40 Hz), 8.18 (s, 1H, vinylic proton), 10.40 (br, NH, D_2O exchangeable)
12c	[$M^+ + 2$], 596 (38) [M^+], 594 (47) [$M^+ - C_4H_9O_4$], 473 (100).	3490 (br s, OH) 3385 (br, NH) 3035 (CH aryl) 2927 (CH alkyl) 1686 (CO) 1610 (C=N)	δ 1.66–1.69 (m, 2H, CH_2), 2.25–2.32 (t, 2H, CH_2), 2.71–2.78 (t, 2H, CH2), 3.70 (m, 4OH, D_2O exchangeable), 4.32 (m, 1H, H-3′), 4.60 (m, 2H, H-4′, H-4″), 5.04 (t, 1H, H-2′, J = 7.52 Hz), 5.68 (d, 1H, H-1′, J = 8.46 Hz), 7.18 (d, 2H, Ar-H, J = 8.40 Hz), 7.25 (d, 2H, Ar-H, J = 8.41 Hz), 7.50 (d, 2H, Ar-H, J = 8.42 Hz), 7. 68 (d, 2H, Ar-H, J = 8.44 Hz), 8.16 (s, 1H, vinylic proton), 10.30, 11.00 (br s, NH, D_2O exchangeable)

*5.5.4. 3-(1′,2′,3′,4′,5′-Penta-*O*-acetylmannosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-4-one (**7c**)*

It was obtained from **5c** as white powder (ethanol).

*5.5.5. 2-(1′,2′,3′,4′-Tetra-*O*-acetyl-arabinosylhydrazon)-*N*3-acetyl-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**10a**)*

It was obtained from **9a** as a yellow powder (ethanol).

*5.5.6. 2-(1′,2′,3′,4′-Tetra-*O*-acetyl-ribosylhydrazon)-*N*3-acetyl-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**10b**)*

It was obtained from **9b** as a white powder (ethanol).

*5.5.7. 2-(1′,2′,3′,4′-Tetra-*O*-acetyl-xylosylhydrazon)-*N*3-acetyl-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**10c**)*

It was obtained from **9c** as a white powder (ethanol).

*5.6. 3-(Glycosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro-[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-4-ones (**8a–c**), (**12a–c**)*

5.6.1. General procedure

A solution from each of **7a–c** or **10a–c** (10 mmol) in sodium methoxide solution (10 mmol) of sodium metal in methanol (100 mL), was stirred at room temperature for 24 h, and then neutralized with hydrochloric acid solution (pH control). The precipitate formed was filtered off, washed with cold water, dried and crystallized from ethanol (60–100 mL) to obtain **8a–c**, **12a–c** respectively.

*5.6.2. 3-(Glucosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro-[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-4-one (**8a**)*

It was obtained from **7a** as a white powder.

*5.6.3. 3-(Galactosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2] pyrimido[4,5-*b*]quinolin-4-one (**8b**)*

It was obtained from **7b**, as a white powder.

*5.6.4. 3-(Mannosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro-[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-4-one (**8c**)*

It was obtained from **7c** as white powder.

*5.6.5. 3-(Arabinosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2] pyrimido[4,5-*b*]quinolin-4-one (**12a**)*

It was obtained from **10a**, as a white powder.

*5.6.6. 3-(Ribosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro-[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-4-one (**12b**)*

It was obtained from **10b** as a white powder.

*5.6.7. 3-(Xylosyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro-[1,2,4]-triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-4-one (**12c**)*

It was obtained from **10c** as a white powder.

Table 9
^{13}C NMR Spectral data of some newly synthesized compounds.

Compd. no.	^{13}C NMR (δ, ppm) (DMSO-d_6)
3b	δ 22.27, 26.73, 27.18 ($3CH_2$), 108.3, 127.9, 128.2, 128.5, 128.6, 129.4, 130.1, 131.1, 132.3, 132.4, 135.5, 135.7, 136.2, 149.7, 158.4 (15C, sp^2 with four symmetric carbon atoms), 162.2 (CO), 176.4 (CS).
4	δ 22.27, 22.73, 27.19 ($3CH_2$), 109.1, 127.7, 127.8, 128.3, 129.3, 129.4, 131.1, 131.6, 136.1, 136.3, 137.9, 150.0, 155.1 (16 sp^2 carbons with four symmetric carbon atoms), 160.2 (CO).
5a	δ 22.23, 22.76, 27.08 and 28.95 ($4CH_2$) 67.7, 68.4, 69.2 and 69.5 (4CH), 109.5, 126.3, 127.6, 128.4, 129.1, 129.5, 130.2, 131.8, 136.2, 136.7, 137.9, 150.0, 155.1, 157.3 (17 sp^2 with four symmetric and glucose C-1′ carbon atoms) and 166.2 (CO)
5b	δ 22.25, 22.66, 27.02 and 28.86 ($4CH_2$) 67.3, 68.0, 68.7 and 69.4 (4CH), 108.2, 125.9, 126.8, 128.2, 128.7, 129.3, 130.5, 131.7, 135.8, 136.6, 137.5, 148.6, 154.2, 156.3 (17 sp^2 with four symmetric and galactose C-1′ carbon atoms) and 163.5 (CO)
5c	δ 22.26, 22.82, 27.12 and 29.40 ($4CH_2$) 66.81, 68.57, 70.11 and 70.52 (4CH), 108.8, 125.9, 127.8, 128.3, 129.3, 129.7, 130.8, 132.2, 136.4, 136.9, 137.5, 151.1, 154.8, 157.5 (17 sp^2 with four symmetric and mannose C-1′ carbon atoms) and 163.2 (CO)
9a	δ 22.29, 23.64, 27.70 and 29.25 ($4CH_2$), 66.88, 69.50 and 71.54 (3CH), 110.3, 124.6, 127.3, 128.5, 129.7, 130.7, 131.4, 132.6, 136.3, 137.6, 138.5, 149.3, 153.5, 155.3 (17 sp^2 with four symmetric and arabinose C-1′ carbon atoms) and 164.3 (CO)
9b	δ 22.27, 23.62, 27.69 and 29.18 ($4CH_2$), 67.85, 69.48 and 70.84 (3CH), 112.6, 126.2, 127.8, 128.9, 129.5, 130.2, 131.7, 133.1, 137.3, 138.6, 139.5, 148.7, 152.6, 153.8 (17 sp^2 with four symmetric and ribose C-1′ carbon atoms) and 161.6 (CO)
9c	δ 23.21, 23.69, 27.70 and 30.06 ($4CH_2$), 66.90, 67.65 and 69.53 (3CH), 109.6, 116.2, 121.7, 126.8, 128.3, 129.1, 130.7, 132.3, 137.6, 138.9, 139.8, 148.2, 151.9, 152.4 (17 sp^2 with four symmetric and xylose C-1′ carbon atoms) and 163.2 (CO)
7a	δ 20.45, 23.69, 26.87 and 30.66 ($4CH_2$), 38.58, 39.20, 39.81, 40.43, 44.25 ($5CH_3$ of ester) 67.01, 67.72, 69.81 and 70.85 (4CH), 113.6, 119.2, 121.5, 126.7, 127.9, 129.0, 130.8, 132.5, 137.1, 138.9, 139.8, 148.6, 151.3, 152.5 (17 sp^2 with four symmetric and C-1′ carbon atoms) and 162.7 (CO), 168.9, 169.4, 169.6, 169.9 and 170.0 (5 carbonyl ester)
7b	δ 21.47, 23.72, 26.88 and 30.56 ($4CH_2$), 38.47, 39.26, 39.87, 40.34, 44.19 ($5CH_3$ of ester) 66.81, 67.75, 69.84 and 71.04 (4CH), 115.6, 118.6, 122.3, 126.4, 127.6, 129.2, 130.7, 132.8, 137.5, 138.6, 140.2, 148.3, 151.6, 152.4 (17 sp^2 with four symmetric and C-1′ carbon atoms) and 161.6 (CO), 167.8, 168.9, 169.3, 169.8 and 171.1 (5 carbonyl ester)
7c	δ 21.15, 23.59, 26.67 and 30.68 ($4CH_2$), 38.63, 39.29, 39.87, 40.51, 44.28 ($5CH_3$ of ester) 67.05, 67.78, 69.83 and 70.69 (4CH), 109.6, 115.2, 121.7, 126.5, 127.6, 129.3, 130.6, 132.3, 137.5, 138.6, 139.6, 148.7, 151.6, 153.2 (17 sp^2 with four symmetric and C-1′ carbon atoms) and 163.2 (CO), 168.4, 169.0, 169.3, 169.8 and 170.3 (5 carbonyl ester)
10a	δ 22.22, 23.56, 26.60 and 31.64 ($4CH_2$), 38.70, 39.21, 39.85, 40.56, 45.35 ($5CH_3$ of ester) 67.15, 69.68 and 70.91 (3CH), 110.3, 116.2, 122.7, 126.4, 127.1, 129.4, 130.8, 132.9, 137.3, 138.6, 139.3, 148.5, 151.2, 153.7 (17 sp^2 with four symmetric and C-1′ carbon atoms), 162.6 (CO), 168.3, 169.1, 169.6, 169.9 and 174.7 (5 carbonyl ester)
10b	δ 22.18, 23.46, 26.55 and 31.76 ($4CH_2$), 39.20, 39.89, 40.02, 40.76, 46.15 ($5CH_3$ of ester), 68.10, 69.75 and 71.23 (3CH), 108.9, 116.5, 122.9, 126.1, 127.4, 129.7, 130.3, 132.2, 137.8, 138.4, 139.1, 148.3, 151.3, 151.5 (17 sp^2 with four symmetric and C-1′ carbon atoms), 160.9 (CO), 168.6, 169.3, 169.5, 170.3 and 172.9 (5 carbonyl ester)
10c	δ 22.25, 23.59, 26.63 and 31.69 ($4CH_2$), 38.73, 39.28, 39.75, 40.68, 45.39 ($5CH_3$ of ester) 67.25, 69.72 and 70.76 (3CH), 111.5, 116.5, 123.5, 126.9, 127.3, 129.8, 130.6, 132.3, 137.7, 138.3, 139.6, 148.8, 151.4, 153.3 (17 sp^2 with four symmetric and C-1′ carbon atoms) and 164.2 (CO), 168.5, 169.6, 169.9, 170.1 and 173.5 (5 carbonyl ester)
8a	δ 22.28, 22.73, 27.21 and 30.85 ($4CH_2$) 67.70, 68.45, 69.24 and 70.52 (4CH), 112.5, 126.4, 127.5, 128.9, 129.3, 129.6, 130.6, 131.4, 136.7, 136.8, 137.5, 151.2, 155.4, 157.6 (17 sp^2 with four symmetric carbon atoms) and 164.5 (CO)
8b	δ 22.30, 22.65, 27.05 and 29.86 ($4CH_2$) 67.30, 68.04, 68.77 and 69.46 (4CH), 113.2, 124.7, 126.5, 128.3, 128.8, 129.8, 130.5, 132.5, 135.4, 136.6, 137.9, 148.3, 153.2, 156.2 (17 sp^2 with four symmetric carbon atoms) and 163.7 (CO)
8c	δ 22.27, 22.90, 27.36 and 29.50 ($4CH_2$), 66.84, 68.77, 70.13 and 70.56 (4CH), 118.3, 125.5, 127.6, 128.7, 128.9, 129.5, 131.3, 132.5, 136.6, 136.8, 137.2, 151.4, 154.6, 157.3 (17 sp^2 with four symmetric carbon atoms) and 163.8 (CO)
12a	δ 21.38, 23.51, 27.67 and 29.95 ($4CH_2$), 67.83, 69.45 and 71.59 (3CH), 113.4, 125.7, 127.9, 128.7, 129.4, 130.3, 131.2, 132.9, 136.3, 137.3, 138.8, 149.5, 154.2, 154.9 (17 sp^2 with four symmetric carbon atoms) and 162.6 (CO)
12b	δ 22.39, 23.67, 27.81 and 30.28 ($4CH_2$), 66.91, 69.32 and 70.41 (3CH), 114.5, 126.4, 127.6, 128.3, 129.6, 130.6, 131.9, 133.3, 138.0, 138.5, 139.7, 148.6, 154.6, 151.5 (17 sp^2 with four symmetric carbon atoms) and 162.3 (CO)
12c	δ 22.19, 23.56, 27.39 and 32.02 ($4CH_2$), 67.31, 67.86 and 69.78 (3CH), 117.3, 120.2, 124.5, 126.5, 128.6, 129.3, 130.9, 132.7, 137.3, 138.4, 139.6, 148.9, 151.6, 153.7 (17 sp^2 with four symmetric carbon atoms) and 162.9 (CO)

5.7. Pharmacological screening

5.7.1. Anti-microbial screening

The bacterial isolates representing Gram-negative and Gram-positive bacteria were recovered on Nutrient and Macconky agar. The two fungal isolates *C. albicans* and *A. fumigatus* were isolated on Sabouraud's dextrose agar (oxoid). They are isolated from clinical samples and identified to the species level according to different API systems (biomerilux). The selected compounds were tested *in vitro* using the agar disc diffusion method taking Ciprofloxacin and Ketoconazole as reference drugs for bacteria and fungi, respectively The anti-microbial potentialities of the tested compounds were estimated by placing presterilized filter paper disks (5 mm in diameter) impregnated with 100 μg/disk using dimethylsulfoxide (DMSO) as solvent which showed no inhibition zones. The inhibition zones of the tested compounds were measured after 24–28 h incubation at 37 °C for bacteria and at 28 °C after 5 days for fungi. The minimal inhibitory concentration (MIC) determination method of the biologically active compounds (Table 2) was applied using different concentrations per disk against Gram-negative, Gram-positive and Fungi. Reference Ketoconazole and Ciprofloxacin acid disks were supplied from Pasteur laboratory in Egypt by concentration 100 units and 30 μg respectively.

5.7.2. Animals and reagents

Both sex of Swiss mice weighing 25–30 g used in analgesic activity and adult female of Sprague-Dawley rats weighing between 150 and 180 g were used in anti-inflammatory activity, taking into account international principle and local regulations concerning the care and use of laboratory animals [19]. The animals had free access to standard commercial diet and water adlibitum and were kept in rooms maintained at 22 ± 1 °C with 12 h light dark cycle. DNA (Type 1. Calf Thymus), Bleomycin sulfate, Butylated hydroxyanisole (BHA) and L-ascorbic acid were obtained from Sigma. 2,2′-azo-bis-(2-amidinopropane) dihydrochloride (AAPH), 2,2′-azino-bis-3-ethyl benzthiazoline-6-sulfonic acid (ABTS) were purchased from Wak. All other chemicals were of the highest quality available.

5.7.3. Analgesic an anti-inflammatory activities

The experiments were carried out as described by Turner [20], Winter et al. [21] and the standard was administered indomethacin in a dose of 10 mg/kg, orally as aqueous suspension [22].

Methodology of the pharmacological assays is described in detail in pervious article [26].

5.8. Anti-oxidant screening

5.8.1. Assay for erythrocyte hemolysis

Blood was obtained from rats by cardiac puncture and collected in heparinized tubes. Erythrocytes were separated from plasma and the buffy coat was washed three times with 10 vol of 0.15 M NaCl. During the last washing, the erythrocytes were centrifuged at 2500 rpm for 10 min to obtain a constantly packed cell preparation. Erythrocyte hemolysis was mediated by peroxyl radicals in this assay system [23]. A 10% suspension of erythrocytes in pH 7.4 phosphate buffered saline (PBS) was added to the same volume of 200 mM 2.2′-azo-bis-(2-amidinopropane) dihydrochloride (AAPH) solution (in PBS) containing samples to be tested at different concentrations. The reaction mixture was shaken gently while being incubated at 37 °C for ∼1 h. The reaction mixture was then removed, diluted with 8 vol of PBS and centrifuged at 2500 rpm for 10 min. The absorbance A of the supernatant was read at 540 nm. Similarly, the reaction mixture was treated with 8 vol of distilled water to achieve complete hemolysis, and the absorbance B of the supernatant obtained after centrifugation was measured at 540 nm. The percentage hemolysis was calculated by the equation $(1 - A/B) \times$ I00%. The data were expressed as mean standard deviation, L-ascorbic acid was used as a positive control (Table 5).

5.8.2. Anti-oxidant activity screening assay: ABTS method

For each of the investigated compounds 2 mL of ABTS solution (60 μM) was added to 3 M MnO_2 solution (25 mg/mL) all prepared in phosphate buffer (PH.7, 0.1 M). The mixture was shaken, centrifuged, filtered, and the absorbance ($A_{control}$) of the resulting green-blue solution (ABTS radical solution) was adjusted at ca. 0.5 at λ 734 nm. Then, 50 μL of 2 mM solution of the test compound in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance (A_{test}) was measured and the reduction in color intensity was expressed as % inhibition. The % inhibition for each compound is calculated from the equation, the % inhibition $= A_{control} - A_{test}/A_{control} \times 100$ [24]. Ascorbic acid (Vitamin C) was used as standard anti-oxidant (Positive control). Blank sample was run without ABTS and using MeOH/Phosphate buffer (1:1) instead of sample. Negative control sample was run with MeOH/phosphate buffer (1:1) instead of tested compound (Table 5).

5.8.3. Bleomycin-dependent DNA damage

The assay was done according to Aeschlach et al. [25] with minor modifications. The reaction mixture (0.5 mL) contained DNA (0.5 mg/mL), bleomycin sulfate (0.05 mg/mL), $MgCl_2$ (5 mM), $FeCl_3$ (50 μM) and samples to be tested at different concentrations. L-Ascorbic acid was used as a positive control. The mixture was incubated at 37 °C for 1 h. The reaction was terminated by addition of 0.05 mL EDTA (0.1 M). The color was developed by adding 0.5 mL thiobarbituric acid (TBA) (1%, w/v) and 0.5 mL HCI (25%, v/v) followed by heating at 80 °C for 10 min. After centrifugation, the extent of DNA damage was measured by the increase in absorbance at 532 nm (Table 6).

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