e-ISSN:2320-1215
p-ISSN:2322-0112

Research and Reviews: Journal of Pharmacy and Pharmaceutical Sciences

Synthesis of Some Novel 6-Nitro-8-Pyridinyl Coumarin And 6-Nitro-8-Pyranyl Coumarin Derivatives and Evaluation of Their Anti-Bacterial Activity

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Research Article

Received date: 09/12/2015

Accepted date: 19/12/2015

Published date: 29/12/2015

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Keywords: 6-Nitro-8-pyridinyl-(8-pyranyl) -coumarins, Antibacterial activity.

ABSTRACT

Two novel series of 4-methyl-6-nitro-8-(pyridin-2-yl)-2-oxo-2H-chromen-7-yl benzoates VI-VIII and 4-methyl-6-nitro-8-(pyran-6-yl)-2-oxo-2H-chromen-7-yl benzoates IX-XI were synthesized starting from the 8-acetyl-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate V. All synthesized compounds were evaluated for their antibacterial activity using paper disc diffusion technique and agar dilution technique. Among of the tested compounds, the 8-(6-Amino-4-(2-bromophenyl)-5-thio-carbamoyl-4H-pyran-2-yl)-4-methyl-6-nitro-2oxo-2H-chromen-7-yl benzoate Xh displayed the most promising antibacterial activity with MIC of 64, 512 and 128 µg/ml against *B. subtilis*, *S. aureus* and *E. coli*, respectively.

INTRODUCTION

Coumarin derivatives are a class of compounds that have great importance and interest due to their wide distribution in nature and their broad and diverse biological activities including anticoagulant[1], vasorelaxant[2], lipid lowering[3-5], antioxidant[6], antidepressant[7], anticonvulsant[8], antihistaminic[9], anticancer[10-12], antiviral[13-15], antiprotozoal[16], antimicrobial[17-20], anti-inflammatory[21,22], analgesic and antipyretic[23] activities.

Interestingly, the coumarin nucleus constitutes a basic moiety in the skeleton of several antibiotics such as Novobiocin and its derivative Clorobiocin which were discovered to possess excellent activity against gram positive pathogens[17,20]. Referring to the literature, many coumarin derivatives bearing different heterocyclic moieties at position 8[24] were reported to display promising antibacterial activity. Also, it was reported that coumarin derivatives containing nitro group in their structures possessed significant antimicrobial activity[25].

Based on this background, we achieved the synthesis and the evaluation of the antibacterial activity of two new series of coumarin derivatives bearing a nitro group at position 6 together with a pyridine or a pyran moiety at position 8 namely; 8-(pyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoates VI-VIII and 8-(pyran-6-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoates IX-XI, respectively through the reactive key intermediate; 8-acetyl-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate V. (Schemes 1 and 2).

RESULTS AND DISCUSSION

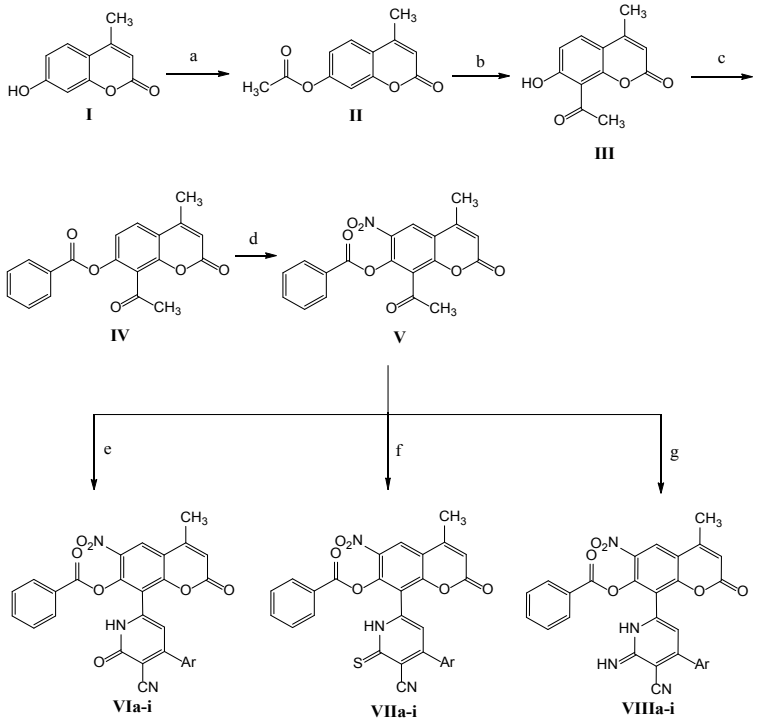
Chemistry

Scheme 1

Pechmann reaction[26] was used for the preparation of the 7-hydroxy-4-methyl-2H-chromen-2-one I. Preparation of 4-methyl-

e-ISSN:2347-7857
p-ISSN:2347-7849

2-oxo-2H-chromen-7-yl acetate II was achieved by the condensation of I with acetyl chloride according to Limaye's method[27]. Then, Fries rearrangement[28,29] was used to prepare 8-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one III. The 8-acetyl-4-methyl-2-oxo-2H-chromen-7-yl benzoate IV was prepared by direct heating of III with benzoyl chloride[30]. The 6-nitrocoumarin derivative V was prepared using a mixture of concentrated sulfuric/nitric acids **(Scheme 1).**



VI, VII, VIII	Ar
a	4-Cl-C_6H_4
b	4-NO_2-C_6H_4
c	4-OH-C_6H_4
d	4-CH_3-C_6H_4
e	3-Br-C_6H_4
f	2-Cl-C_6H_4
g	2-NO_2-C_6H_4
h	2-Br-C_6H_4
i	C_6H_5

Scheme 1.

Synthesis of the required 6-oxopyridine derivatives VIa-i was performed via the one pot reaction of the 8-acetyl coumarin V with ethyl cyanoacetate, the appropriate aromatic aldehyde and excess ammonium acetate in refluxing n-butanol **(Scheme 1).** Similarly, 6-thioxopyridine derivatives VIIa-i and 6-iminopyridine derivarives VIIIa-i were obtained by reaction with thiocyanoacetamide and malononitrile, respectively, **(Scheme 1).**

a) CH_3COCl, reflux, 1.5 h (95%)

b) $AlCl_3$, 160-170°C, 2h (75%)

c) Benzoyl chloride, 160-170°C, 1.5 h (67.5%)

d) HNO_3/H_2SO_4, rt, 1 h (72.5%), e) Ethyl cyanoacetate/Aromatic aldehyde/Ammonium acetate, n-butanol, reflux, 5 h

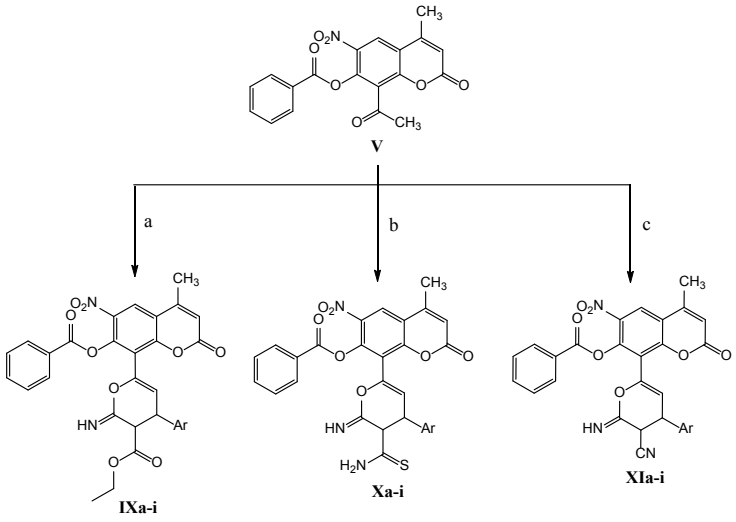
f) Thiocyanoacetamide/Aromatic aldehyde/Ammonium acetate, n-butanol, reflux, 5 h

g) Malononitrile/Aromatic aldehyde/Ammonium acetate, n-butanol, reflux, 4 h

Scheme 2

On the other hand, treatment of the 8-acetyl coumarin derivative V with the appropriate aromatic aldehyde and ethyl cyanoacetate in the presence of few drops of piperidine in refluxing n-butanol afforded the titled ethyl pyran-3-carboxylate derivatives IXa-i. In addition, 3-thiocarbamoyl pyran derivatives Xa-i and 3-cyanopyran derivatives XIa-i were obtained by a similar reaction using thiocyanoacetamide and malononitrile, respectively, **(Scheme 2).**

e-ISSN:2347-7857
p-ISSN:2347-7849



IX, X, XI	Ar
a	4-Cl-C_6H_4
b	4-NO_2-C_6H_4
c	4-OH-C_6H_4
d	4-CH_3-C_6H_4
e	3-Br-C_6H_4
f	2-Cl-C_6H_4
g	2-NO_2-C_6H_4
h	2-Br-C_6H_4
i	C_6H_5

Scheme 2.

a) Ethyl cyanoacetate/Aromatic aldehyde/Piperidine, n-butanol, reflux, 6 h

b) Thiocyanoacetamide/Aromatic aldehyde/Piperidine, n-butanol, reflux, 6 h

c) Malononitrile/Aromatic aldehyde/Piperidine, n-butanol, reflux, 5 h

Antibacterial screening

A preliminary *in-vitro* antibacterial evaluation was carried out on fifty five of the newly synthesized compounds, namely, 8-acetyl-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate V, 8-(pyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoates VI-VIII and 8-(pyran-6-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoates IX-XI using paper disc diffusion technique against *Bacillus subtilis ATCC 5256* and *Staphylococcus aureus ATCC 6571* (Gram-positive bacteria) and against *Escherichia coli* O_{157}:H_7 and *Pseudomonas aeruginosa ATCC 27852* (Gram-negative bacteria) using Mueller Hinton agar medium (Oxoid).

When the sensitivity test[31] was first performed for all the fifty five compounds in the concentration of 50 µg/disc, it was found that fifteen compounds V, VIf, VIIId, VIIIe, VIIIf, VIIIh, IXb, IXc, IXe, IXi, Xa, Xg, Xh, XIe and XIi possessed growth inhibitory activity against only the gram-positive bacteria *B. subtilis*, while they had no inhibitory activity against neither the gram-positive bacteria *S. aureus* nor the gram-negative bacteria *E. coli* and *P. aeruginosa*. In addition, the rest of the assessed compounds did not show growth inhibitory activity against the selected microorganisms **(Table 1).**

Table 1. *In-vitro* antibacterial activity of the newly synthesized compounds and Novobiocin using paper disc diffusion technique.

Compound No. (50 µg/disc)	Paper disc diffusion test			
	Gram-positive		Gram-negative	
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
V	++	-	-	-
VIf	+	-	-	-
VIIId	+	-	-	-
VIIIe	++	-	-	-
VIIIf	+	-	-	-
VIIIh	+	-	-	-
IXb	+	-	-	-
IXc	+	-	-	-
IXe	+	-	-	-
IXi	+	-	-	-

Xa	+	-	-	-
Xg	+	-	-	-
Xh	+	-	-	-
XIe	+	-	-	-
XIi	+	-	-	-
Novobiocin (30 µg/disc)	+++	+++	++	++
DMSO	-ve	-ve	-ve	-ve

Note:
(+): 1-8 mm
(++): 9-16 mm
(+++): 17-24 mm
(-): Compound is inactive in the used concentration
N.B: Compounds VIa-e, VIg-i, VIIa-i, VIIIa-c, VIIIg,i, IXa,d, IXf-h, Xb-f, Xi, XIa-d and XIf-h were inactive in the used concentration against all the used microorganisms.

Consequently, when the minimum inhibitory concentration[32] of the aforementioned fifteen compounds V, VIf, VIIId, VIIIe, VIIIf, VIIIh, IXb, IXc, IXe, IXi, Xa, Xg, Xh, XIe and XIi was determined using agar dilution technique against the same microorganisms, it was concluded that all of them were most active against *B. subtilis* and that they possessed variable activities against both *S. aureus* and *E. coli* while they were inactive against *P. aeruginosa* **(Table 2).**

Table 2. *In-vitro* antibacterial activity expressed as Minimum Inhibitory Concentration (MIC) µg/ml of newly synthesized compounds.

Compound No.	Minimum inhibitory concentration (MIC) in µg/ml			
	Gram-positive		Gram-negative	
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
V	64	256	1024	-
VIf	256	-	512	-
VIIId	64	-	256	-
VIIIe	64	1024	-	-
VIIIf	256	1024	-	-
VIIIh	64	-	-	-
IXb	128	-	-	-
IXc	128	-	1024	-
IXe	256	512	1024	-
IXi	256	-	256	-
Xa	128	512	512	-
Xg	128	512	512	-
Xh	64	512	128	-
XIe	128	-	512	-
XIi	256	512	512	-
DMSO	-ve	-ve	-ve	-ve

Note: (-): MIC > 1024 µg/ml

Compared to the other compounds, the 8-(4-(2-bromophenyl)-3-thiocarbamoyl-2-imino-3,4-dihydropyran-6-yl) coumarin derivative Xh could be considered as the most promising antibacterial agent with MIC of 64, 512 and 128 µg/ml against *B. subtilis*, *S. aureus* and *E. coli*, respectively.

CONCLUSION

Fifty five new coumarin derivatives were successfully synthesized and screened for their antibacterial activity. The results of the sensitivity test revealed that only fifteen compounds; V, VIf, VIIId, VIIIe, VIIIf, VIIIh, IXb, IXc, IXe, IXi, Xa, Xg, Xh, XIe and XIi possessed growth inhibitory activity against only the gram-positive bacteria *B. subtilis* while the results of the minimum inhibitory concentration of these fifteen compounds showed that all of them were most active against *B. subtilis* and that they possessed variable activities against both *S. aureus* and *E. coli* while they were inactive against *P. aeruginosa*. It was also concluded that compound Xh showed the most promising antibacterial activity against *B. subtilis*, *S. aureus* and *E. coli*.

EXPERIMENTAL

Chemistry

Melting points were measured using Electro-thermal IA 9100 apparatus and were uncorrected. Elemental microanalysis was carried out on Elmentar Vario EL apparatus at the Micro Analytical Center, Faculty of Science, Cairo University. Infrared (IR) spectra were recorded on Bruker Vector 22 FT-IR spectrometer as potassium bromide discs at the Micro Analytical Center, Faculty of Science, Cairo University. Proton nuclear magnetic resonance (^{1}HNMR) spectra were determined on Varian mercury 300 MHz

spectrometer at the Faculty of Science, Cairo University and on JEOL NMR EX-270 MHz spectrometer at the Central Services Lab, the Central Unit for Analysis and Scientific Services, National Research Center using tetramethylsilane (TMS) as an internal standard and either DMSO-d_6 or $CDCl_3$ as solvents. Mass spectra (MS) were performed on Shimadzu QP-2010 plus spectrometer using 70 eV, EI mode at the Micro Analytical Center, Faculty of Science, Cairo University. The reactions were monitored by thin layer chromatography (TLC) plates (silica gel 60 F254, aluminium sheets, Merck) using hexane/ethyl acetate (1:1 v/v) as eluent.

7-Hydroxy-4-methyl-2H-chromen-2-one (I)

In a flat bottomed flask equipped with a dropping funnel, 250 ml sulfuric acid was placed and the temperature was kept below 10°C. A solution of resorcinol (33 g, 0.3 mol) in ethyl acetoacetate (38 ml, 0.3 mol) was added dropwise. After complete addition, the reaction mixture was stirred at room temperature for 12 hours, and then poured onto ice-water. The formed precipitate was filtered off, washed with cold water, dissolved in 5% aqueous sodium hydroxide and then re-precipitated with diluted sulfuric acid. The separated product was filtered off, washed with cold water and crystallized from absolute ethyl alcohol. Yield: 90%. m.p.: 185-186°C (as reported)[26].

4-Methyl-2-oxo-2H-chromen-7-yl acetate (II)

A mixture of I (14.08 g, 0.08 mol) and acetyl chloride (36 ml, 0.5 mol) was refluxed for one and half hours. The reaction mixture was then poured onto ice-water and the separated product was filtered off, washed with cold water and crystallized from absolute ethyl alcohol. Yield: 95%. m.p.: 150- 151°C (as reported) [27,28].

8-Acetyl-7-hydroxy-4-methyl-2H-chromen-2-one (III)

A mixture of the 7-acetoxycoumarin derivative II (32.7 g, 0.15 mol) and anhydrous aluminium chloride (66.67 g, 0.5 mol) was heated in an oil bath at 160-170°C for 2 hours. After cooling down, the reaction mixture was placed in an ice bath and decomposed with diluted hydrochloric acid. The formed solid was then filtered off, washed with cold water, dissolved in 5% aqueous sodium hydroxide and then re-precipitated with diluted hydrochloric acid. The obtained precipitate was filtered off, washed with cold water and crystallized from absolute ethyl alcohol. Yield: 75%. m.p.: 162-163°C (as reported)[29].

8-Acetyl-4-methyl-2-oxo-2H-chromen-7-yl benzoate (IV)

A mixture of the 8-acetyl-7-hydroxycoumarin III (17.44 g, 0.08 mol) and benzoyl chloride (59 ml, 0.5 mol) was refluxed for one and half hours at 160-170°C. The reaction mixture was cooled then poured onto ice-water. The water layer was then removed by decantation and the obtained gray sticky mass was suspended in the least amount of ethyl alcohol, filtered off, washed with diethyl ether and crystallized from glacial acetic acid. Yield: 67.5%. m.p.: 188-189°C (as reported)[30].

8-Acetyl-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (V)

To a solution of the 8-acetyl-7-benzoyloxycoumarin derivative IV (32.2 g, 0.1 mol) in sulfuric acid (30 ml) placed in an ice-salt bath to keep the temperature below 5°C, a mixture of nitric acid (8 ml) and sulfuric acid (10 ml), kept at the same temperature, was added drop wise with stirring while keeping the temperature below 5°C. After complete addition, the reaction mixture was stirred at room temperature for 1 hour then poured onto ice-water. The obtained precipitate was then filtered off, washed with cold water and crystallized from absolute ethyl alcohol. Yield: 72.5%. m.p.: 98-100°C. Analysis for $C_{19}H_{13}NO_7$, M.wt.: 367.31. Calcd.: %C, 62.13; H, 3.57; N, 3.81. Found: %C, 61.92; H, 3.61; N, 3.73. IR (KBr, ū, cm^{-1}): 3065 (CH, aromatic), 2928 (CH, aliphatic), 1735 (3 C=O), 1615 and 1482 (C=C), 1546 and 1374 (NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.36 (s, 3H, CH_3), 2.53 (s, 3H, CO$\underline{CH_3}$), 6.16 (s, 1H, C-3 proton), 7.46-8.57 (m, 6H, aromatic protons). MS; m/z (R.I. %): [M^+] 367 (0.34), 80 (100).

8-(4-Aryl-5-cyano-6-oxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoates (VIa-i)

A mixture of the 8-acetyl-7-benzoyloxy-6-nitrocoumarin V (0.36 g, 0.001 mol), the appropriate aromatic aldehyde (0.001 mol), ethyl cyanoacetate (0.1 ml, 0.001 mol) and ammonium acetate (0.62 g, 0.008 mol) in n-butanol (5 ml) was refluxed for 5 hours. After cooling down, the obtained precipitate was filtered off, washed s with diethyl ether and crystallized from absolute ethyl alcohol.

8-(4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIa)

Yield: 60%. m.p.: 176-178°C. Analysis for $C_{29}H_{16}ClN_3O_7$, M.wt.: 553.91. Calcd.: %C, 62.88; H, 2.91; N, 7.59. Found: %C, 62.97; H, 2.75; N, 7.71. IR (KBr, ū, cm^{-1}): 3413 (NH), 2211 (C≡N), 1725 (3 C=O), 1530 and 1377 (NO_2). MS; m/z (R.I. %): [M^+] 553 (9.02), [M^++2] 555 (2.16), 80 (100).

8-(5-Cyano-4-(4-nitrophenyl)-6-oxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIb)

Yield: 60%. m.p.: 174-175°C. Analysis for $C_{29}H_{16}N_4O_9$, M.wt.: 564.46. Calcd.: %C, 61.71; H, 2.86; N, 9.93. Found: %C, 61.52; H, 2.99; N, 9.80. IR (KBr, ū, cm^{-1}): 3375 (NH), 2208 (C≡N), 1723 (3 C=O), 1521 and 1389 (2 NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.32 (s, 3H, CH_3), 5.90 (s, 1H, C-3 proton), 6.41 (s, 1H, oxopyridine proton), 7.42-8.38 (m, 10H, aromatic protons, 1H, NH, D_2O exchangeable).

8-(5-Cyano-4-(4-hydroxyphenyl)-6-oxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIc)

Yield: 70%. m.p.: 166-168°C. Analysis for $C_{29}H_{17}N_3O_8$, M.wt.: 535.46. Calcd.: %C, 65.05; H, 3.20; N, 7.85. Found: %C, 65.24; H, 3.42; N, 7.68. IR (KBr, ū, cm^{-1}): 3376 (NH), 3193 (OH), 2206 (C≡N), 1720 (3 C=O), 1530 and 1383 (NO_2).

8-(5-Cyano-6-oxo-4-p-tolyl-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VId)

Yield: 82%. m.p.: 188-190°C. Analysis for $C_{30}H_{19}N_3O_7$, M.wt.: 533.49. Calcd.: %C, 67.54; H, 3.59; N, 7.88. Found: %C, 67.58; H, 3.63; N, 7.71. IR (KBr, ū, cm^{-1}): 3425 (NH), 2213 (C≡N), 1732 (3 C=O), 1522 and 1371 (NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.32 (s, 3H, CH_3), 2.35 (s, 1H, p-CH_3), 5.96 (s, 1H, C-3 proton), 6.50 (s, 1H, oxopyridine proton), 7.05-8.75 (m, 10H, aromatic protons, 1H, NH, D_2O exchangeable). MS; m/z (R.I. %): [M^+] 533 (62.31), 86 (100).

8-(4-(3-Bromophenyl)-5-cyano-6-oxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIe)

Yield: 62%. m.p.: 159-160°C. Analysis for $C_{29}H_{16}BrN_3O_7$, M.wt.: 597.36. Calcd.: %C, 58.21; H, 2.70; N, 7.02. Found: %C, 58.02; H, 2.55; N, 7.22. IR (KBr, ū, cm^{-1}): 3426 (NH), 2208 (C≡N), 1728 (3 C=O), 1526 and 1381 (NO_2). MS; m/z (R.I. %): [M^+] 597 (22.14), [M^++2] 599 (10.84), 406 (100).

8-(4-(2-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIf)

Yield: 52%. m.p.: 150-151°C. Analysis for $C_{29}H_{16}ClN_3O_7$, M.wt.: 553.91. Calcd.: %C, 62.88; H, 2.91; N, 7.59. Found: %C, 62.67; H, 3.05; N, 7.56. IR (KBr, ū, cm^{-1}): 3430 (NH), 2210 (C≡N), 1731 (3 C=O), 1527 and 1390 (NO_2).

8-(5-Cyano-4-(2-nitrophenyl)-6-oxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIg)

Yield: 56%. m.p.: 173-175°C. Analysis for $C_{29}H_{16}N_4O_9$, M.wt.: 564.46. Calcd.: %C, 61.71; H, 2.86; N, 9.93. Found: %C, 61.56; H, 2.91; N, 9.80. IR (KBr, ū, cm^{-1}): 3414 (NH), 2210 (C≡N), 1722 (3 C=O), 1523 and 1382 (2 NO_2).

8-(4-(2-Bromophenyl)-5-cyano-6-oxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIh)

Yield: 56%. m.p.: 170-172°C. Analysis for $C_{29}H_{16}BrN_3O_7$, M.wt.: 597.36. Calcd.: %C, 58.21; H, 2.70; N, 7.02. Found: %C, 58.04; H, 2.56; N, 7.16. IR (KBr, ū, cm^{-1}): 3419 (NH), 2210 (C≡N), 1729 (3 C=O), 1527 and 1385 (NO_2).

8-(5-Cyano-6-oxo-4-phenyl-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIi)

Yield: 67%. m.p.: 190-192°C. Analysis for $C_{29}H_{17}N_3O_7$, M.wt.: 519.46. Calcd.: %C, 67.05; H, 3.30; N, 8.09. Found: %C, 66.95; H, 3.41; N, 8.28. IR (KBr, ū, cm^{-1}): 3424 (NH), 2211 (C≡N), 1715 (3 C=O), 1511 and 1373 (NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.34 (s, 3H, CH_3), 5.90 (s, 1H, C-3 proton), 6.56 (s, 1H, oxopyridine proton), 7.45-8.19 (m, 11H, aromatic protons, 1H, NH, D_2O exchangeable).

8-(4-Aryl-5-cyano-6-thioxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoates (VIIa-i)

A mixture of 8-acetyl-7-benzoyloxy-6-nitrocoumarin V (0.36 g, 0.001 mol), the appropriate aromatic aldehyde (0.001 mol), thiocyanoacetamide (0.1 g, 0.001 mol) and ammonium acetate (0.62 g, 0.008 mol) in n-butanol (5 ml) was refluxed for 5 hours. The reaction mixture was cooled; the formed precipitate was filtered off, washed with diethyl ether and crystallized from absolute ethyl alcohol.

8-(4-(4-Chlorophenyl)-5-cyano-6-thioxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIa)

Yield: 91%. m.p.: 198-199°C. Analysis for $C_{29}H_{16}ClN_3O_6S$, M.wt.: 569.97. Calcd.: %C, 61.11; H, 2.83; N, 7.37. Found: %C, 60.01; H, 2.85; N, 7.50. IR (KBr, ū, cm^{-1}): 3425 (NH), 2210 (C≡N), 1726 (2 C=O), 1500 and 1316 (NO_2), 1269 (C=S). MS; m/z (R.I. %): [M^+] 569 (46.61), [M^++2] 571 (11.18), 179 (100).

8-(5-Cyano-4-(4-nitrophenyl)-6-thioxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIb)

Yield: 74 %. m.p.: 216-217°C. Analysis for $C_{29}H_{16}N_4O_8S$, M.wt.: 580.52. Calcd.: %C, 60.00; H, 2.78; N, 9.65. Found: %C, 61.13; H, 2.64; N, 9.62. IR (KBr, ū, cm^{-1}): 3355 (NH), 2207 (C≡N), 1718 (2 C=O), 1519 and 1387 (2 NO_2), 1267 (C=S). MS; m/z (R.I. %): [M^+] 580 (61.32), [M^++1] 581 (5.66), 265 (100).

8-(5-Cyano-4-(4-hydroxyphenyl)-6-thioxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIc)

Yield: 61%. m.p.: 194-195°C. Analysis for $C_{29}H_{17}N_3O_7S$, M.wt.: 551.53. Calcd.: %C, 63.15; H, 3.11; N, 7.62. Found: %C, 63.20; H, 3.18; N, 7.55. IR (KBr, ū, cm^{-1}): 3343 (NH), 3202 (OH), 2205 (C≡N), 1717 (2 C=O), 1517 and 1379 (NO_2), 1270 (C=S). MS; m/z (R.I. %): [M^+] 551 (20.60), [M^++1] 552 (19.29), 162 (100).

8-(5-Cyano-6-thioxo-4-p-tolyl-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIId)

Yield: 51%. m.p.: 184-186°C. Analysis for $C_{30}H_{19}N_3O_6S$, M.wt.: 549.55. Calcd.: %C, 65.57; H, 3.48; N, 7.65. Found: %C, 65.52; H, 3.42; N, 7.55. IR (KBr, ū, cm^{-1}): 3348 (NH), 2205 (C≡N), 1722 (2 C=O), 1530 and 1379 (NO_2), 1268 (C=S).

8-(4-(3-Bromophenyl)-5-cyano-6-thioxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIe)

Yield: 51%. m.p.: 160-162°C. Analysis for $C_{29}H_{16}BrN_3O_6S$, M.wt.: 613.42. Calcd.: %C, 56.69; H, 2.62; N, 6.84. Found: %C, 56.71; H, 2.46; N, 6.77. IR (KBr, ū, cm^{-1}): 3334 (NH), 2207 (C≡N), 1719 (2 C=O), 1541 and 1378 (NO_2), 1268 (C=S). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.46 (s, 3H, CH_3), 6.20 (s, 1H, C-3 proton), 6.46 (s, 1H, thioxopyridine proton), 7.00-8.45 (m, 10H, aromatic protons, 1H, NH, D_2O exchangeable).

8-(4-(2-Chlorophenyl)-5-cyano-6-thioxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIf)

Yield: 81%. m.p.: 202-204°C. Analysis for $C_{29}H_{16}ClN_3O_6S$, M.wt.: 569.97. Calcd.: %C, 61.11; H, 2.83; N, 7.37. Found: %C, 60.87; H, 2.73; N, 7.32. IR (KBr, ū, cm^{-1}): 3328 (NH), 2210 (C≡N), 1718 (2 C=O), 1533 and 1380 (NO_2), 1271 (C=S).

8-(5-Cyano-4-(2-nitrophenyl)-6-thioxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIg)

Yield: 83%. m.p.: 140-142°C. Analysis for $C_{29}H_{16}N_4O_8S$, M.wt.: 580.52. Calcd.: %C, 60.00; H, 2.78; N, 9.65. Found: %C, 60.12; H, 2.68; N, 9.67. IR (KBr, ū, cm^{-1}): 3326 (NH), 2206 (C≡N), 1722 (2 C=O), 1527 and 1376 (2 NO_2), 1267 (C=S). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.32 (s, 3H, CH_3), 6.19 (s, 1H, C-3 proton), 6.46 (s, 1H, thioxopyridine proton), 7.05-8.65 (m, 10H, aromatic protons, 1H, NH, D_2O exchangeable).

8-(4-(2-Bromophenyl)-5-cyano-6-thioxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIh)

Yield: 66%. m.p.: 144-146°C. Analysis for $C_{29}H_{16}BrN_3O_6S$, M.wt.: 613.42. Calcd.: %C, 56.69; H, 2.62; N, 6.84. Found: %C, 56.82; H, 2.66; N, 6.80. IR (KBr, ū, cm^{-1}): 3345 (NH), 2209 (C≡N), 1724 (2 C=O), 1532 and 1378 (NO_2), 1273 (C=S). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.40 (s, 3H, CH_3), 6.21 (s, 1H, C-3 proton), 6.50 (s, 1H, thioxopyridine proton), 7.07-8.40 (m, 10H, aromatic protons, 1H, NH, D_2O exchangeable).

8-(5-Cyano-4-phenyl-6-thioxo-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIi)

Yield: 79%. m.p.: 183-185°C. Analysis for $C_{29}H_{17}N_3O_6S$, M.wt.: 535.53. Calcd.: %C, 65.04; H, 3.20; N, 7.85. Found: %C, 64.06; H, 3.29; N, 7.83. IR (KBr, ū, cm^{-1}): 3319 (NH), 2207 (C≡N), 1717 (2 C=O), 1550 and 1387 (NO_2), 1270 (C=S). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.34 (s, 3H, CH_3), 6.27 (s, 1H, C-3 proton), 6.42 (s, 1H, thioxopyridine proton), 7.18-8.27 (m, 11H, aromatic protons, 1H, NH, D_2O exchangeable).

8-(4-Aryl-5-cyano-6-imino-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoates (VIIIa-i)

A mixture of 8-acetyl-7-benzoyloxy-6-nitrocoumarin V (0.36 g, 0.001 mol), the appropriate aromatic aldehyde (0.001 mol), malononitrile (0.066 g, 0.001 mol) and ammonium acetate (0.62 g, 0.008 mol) in n-butanol (5 ml) was refluxed for 4 hours then cooled. The formed precipitate was filtered off, washed with diethyl ether and crystallized from absolute ethyl alcohol.

8-(4-(4-Chlorophenyl)-5-cyano-6-imino-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIIa)

Yield: 90%. m.p.: 223-224°C. Analysis for $C_{29}H_{17}ClN_4O_6$, M.wt.: 552.92. Calcd.: %C, 62.99; H, 3.10; N, 10.13. Found: %C, 62.86; H, 3.13; N, 10.21. IR (KBr, ū, cm^{-1}): 3441 and 3341 (2 NH), 2199 (C≡N), 1707 (2 C=O), 1622 (C=N), 1571 and 1381 (NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.44 (s, 3H, CH_3), 6.25 (s, 1H, C-3 proton), 6.89 (s, 1H, iminopyridine proton), 7.23-8.87 (m, 10H, aromatic protons, 2H, 2NH, D_2O exchangeable). MS; m/z (R.I. %): [M^+] 552 (1.39), [M^++2] 554 (0.33), 64 (100).

8-(5-Cyano-6-imino-4-(4-nitrophenyl)-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIIb)

Yield: 91%. m.p.: 228-229°C. Analysis for $C_{29}H_{17}N_5O_8$, M.wt.: 563.47. Calcd.: %C, 61.81; H, 3.04; N, 12.43. Found: %C, 61.87; H, 3.11; N, 12.32. IR (KBr, ū, cm^{-1}): 3418 and 3337 (2 NH), 2203 (C≡N), 1713 (2 C=O), 1642 (C=N), 1571 and 1387 (2 NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.41 (s, 3H, CH_3), 6.32 (s, 1H, C-3 proton), 6.94 (s, 1H, iminopyridine proton), 7.02-8.33 (m, 10H, aromatic protons, 2H, 2NH, D_2O exchangeable).

8-(5-Cyano-4-(4-hydroxyphenyl)-6-imino-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIIc)

Yield: 88%. m.p.: 242-244°C. Analysis for $C_{29}H_{18}N_4O_7$, M.wt.: 534.48. Calcd.: %C, 65.17; H, 3.39; N, 10.48. Found: %C, 65.02; H, 3.45; N, 10.55. IR (KBr, ū, cm^{-1}): 3435 and 3339 (2 NH), 3212 (OH), 2194 (C≡N), 1706 (2 C=O), 1619 (C=N), 1552 and 1386 (NO_2). MS; m/z (R.I. %): [M^+] 534 (77.78), 175 (100).

8-(5-Cyano-6-imino-4-p-tolyl-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIId)

Yield: 95%. m.p.: 170-172°C. Analysis for $C_{30}H_{20}N_4O_6$, M.wt.: 532.50. Calcd.: %C, 67.67; H, 3.79; N, 10.52. Found: %C, 67.74; H, 3.61; N, 10.57. IR (KBr, ū, cm^{-1}): 3436 and 3342 (2 NH), 2197 (C≡N), 1706 (2 C=O), 1620 (C=N), 1553 and 1383 (NO_2). MS; m/z (R.I. %): [M^+] 532 (20.00), 110 (100).

8-(4-(3-Bromophenyl)-5-cyano-6-imino-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIIe)

Yield: 89%. m.p.: 210-211°C. Analysis for $C_{29}H_{17}BrN_4O_6$, M.wt.: 596.37. Calcd.: %C, 58.31; H, 2.87; N, 9.38. Found: %C, 58.43; H, 2.82; N, 9.50. IR (KBr, ū, cm^{-1}): 3437 and 3342 (2 NH), 2198 (C≡N), 1712 (2 C=O), 1627 (C=N), 1568 and 1379 (NO_2).

^{1}HNMR (DMSO-d_6, δ, ppm): 2.33 (s, 3H, CH_3), 6.27 (s, 1H, C-3 proton), 6.94 (s, 1H, iminopyridine proton), 7.14-8.85 (m, 10H, aromatic protons, 2H, 2NH, D_2O exchangeable).

8-(4-(2-Chlorophenyl)-5-cyano-6-imino-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIIf)

Yield: 91%. m.p.: 180-182°C. Analysis for $C_{29}H_{17}ClN_4O_6$, M.wt.: 552.92. Calcd.: %C, 62.99; H, 3.10; N, 10.13. Found: %C, 63.11; H, 3.04; N, 10.23. IR (KBr, ū, cm^{-1}): 3448 and 3345 (2 NH), 2198 (C≡N), 1714 (2 C=O), 1620 (C=N), 1570 and 1381 (NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.40 (s, 3H, CH_3), 6.19 (s, 1H, C-3 proton), 6.93 (s, 1H, iminopyridine proton), 7.06-8.16 (m, 10H, aromatic protons, 2H, 2NH, D_2O exchangeable).

8-(4-(2-Nitrophenyl)-5-cyano-6-imino-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIIg)

Yield: 89%. m.p.: 248-249°C. Analysis for $C_{29}H_{17}N_5O_8$, M.wt.: 563.47. Calcd.: %C, 61.81; H, 3.04; N, 12.43. Found: %C, 61.69; H, 3.15; N, 12.34. IR (KBr, ū, cm^{-1}): 3438 and 3376 (2 NH), 2199 (C≡N), 1708 (2 C=O), 1624 (C=N), 1562 and 1382 (2 NO_2).

8-(4-(2-Bromophenyl)-5-cyano-6-imino-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIIh)

Yield: 99%. m.p.: 200-202°C. Analysis for $C_{29}H_{17}BrN_4O_6$, M.wt.: 596.37. Calcd.: %C, 58.31; H, 2.87; N, 9.38. Found: %C, 58.22; H, 2.85; N, 9.47. IR (KBr, ū, cm^{-1}): 3447 and 3341 (2 NH), 2200 (C≡N), 1705 (2 C=O), 1620 (C=N), 1561 and 1383 (NO_2).

8-(5-Cyano-6-imino-4-phenyl-1,6-dihydropyridin-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (VIIIi)

Yield: 98%. m.p.: 188-189°C. Analysis for $C_{29}H_{18}N_4O_6$, M.wt.: 518.48. Calcd.: %C, 67.18; H, 3.50; N, 10.81. Found: %C, 67.01; H, 3.55; N, 10.90. IR (KBr, ū, cm^{-1}): 3447 and 3342 (2 NH), 2201 (C≡N), 1710 (2 C=O), 1617 (C=N), 1560 and 1384 (NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.43 (s, 3H, CH_3), 6.48 (s, 1H, C-3 proton), 6.89 (s, 1H, iminopyridine proton), 7.06-8.07 (m, 11H, aromatic protons, 2H, 2NH, D_2O exchangeable).

Ethyl 4-aryl-6-(7-(benzoyloxy)-4-methyl-6-nitro-2-oxo-2H-chromen-8-yl)-2-imino-3,4-dihydro-2H-pyran-3-carboxylates (IXa-i)°C

A mixture of 8-acetyl-7-benzoyloxy-6-nitrocoumarin V (0.36 g, 0.001 mol), the appropriate aromatic aldehyde (0.001 mol), ethyl cyanoacetate (0.1 ml, 0.001 mol) and 5 drops piperidine in n-butanol (5 ml) was refluxed for 6 hours. After cooling down, the formed precipitate was filtered off, washed with diethyl ether and crystallized from absolute ethyl alcohol.

Ethyl 2-amino-6-(7-(benzoyloxy)-4-methyl-6-nitro-2-oxo-2H-chromen-8-yl)-4-(4-chlorophenyl)-4H-pyran-3-carboxylate (IXa)

Yield: 57%. m.p.: 90-92°C. Analysis for $C_{31}H_{23}ClN_2O_9$, M.wt.: 602.98. Calcd.: %C, 61.75; H, 3.84; N, 4.65. Found: %C, 61.82; H, 4.02; N, 4.54. IR (KBr, ū, cm^{-1}): 3438 (NH), 1713 (3 C=O), 1611 (C=N), 1566 and 1391 (NO_2).

Ethyl 2-amino-6-(7-(benzoyloxy)-4-methyl-6-nitro-2-oxo-2H-chromen-8-yl)-4-(4-nitrophenyl)-4H-pyran-3-carboxylate (IXb)

Yield: 62%. m.p.: 80-82°C. Analysis for $C_{31}H_{23}N_3O_{11}$, M.wt.: 613.53. Calcd.: %C, 60.69; H, 3.78; N, 6.85. Found: %C, 60.62; H, 3.86; N, 6.72. IR (KBr, ū, cm^{-1}): 3439 (NH), 1726 (3 C=O), 1617 (C=N), 1521 and 1382 (2 NO_2).

Ethyl 2-amino-6-(7-(benzoyloxy)-4-methyl-6-nitro-2-oxo-2H-chromen-8-yl)-4-(4-hydroxyphenyl)-4H-pyran-3-carboxylate (IXc)

Yield: 62 %. m.p.: 110-111°C. Analysis for $C_{31}H_{24}N_2O_{10}$, M.wt.: 584.53. Calcd.: %C, 63.70; H, 4.14; N, 4.79. Found: %C, 63.79; H, 4.20; N, 4.83. IR (KBr, ū, cm^{-1}): 3418 (NH), 3260 (OH), 1727 (3 C=O), 1603 (C=N), 1519 and 1379 (NO_2). MS; m/z (R.I. %): [M^+] 584 (36.93), [M^++1] 585 (52.27), 129 (100).

Ethyl 2-amino-6-(7-(benzoyloxy)-4-methyl-6-nitro-2-oxo-2H-chromen-8-yl)-4-p-tolyl-4H-pyran-3-carboxylate (IXd)

Yield: 73%. m.p.: 84-86°C. Analysis for $C_{32}H_{26}N_2O_9$, M.wt.: 582.56. Calcd.: %C, 65.98; H, 4.50; N, 4.81. Found: %C, 66.18; H, 4.58; N, 4.77. IR (KBr, ū, cm^{-1}): 3437 (NH), 1726 (3 C=O), 1602 (C=N), 1528 and 1378 (NO_2). MS; m/z (R.I. %): [M^+] 582 (8.16), 64 (100).

Ethyl 2-amino-6-(7-(benzoyloxy)-4-methyl-6-nitro-2-oxo-2H-chromen-8-yl)-4-(3-bromophenyl)-4H-pyran-3-carboxylate (IXe)

Yield: 64%. m.p.: 98-100°C. Analysis for $C_{31}H_{23}BrN_2O_9$, M.wt.: 646.43. Calcd.: %C, 57.51; H, 3.58; N, 4.33. Found: %C, 57.57; H, 3.42; N, 4.43. IR (KBr, ū, cm^{-1}): 3435 (NH), 1724 (3 C=O), 1598 (C=N), 1532 and 1380 (NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 1.58 (t, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.98 (d, 1H, iminopyran C-3 proton), 3.99 (t, 1H, iminopyran C-4 proton), 4.12 (q, 2H, OCH_2), 6.20 (s, 1H, C-3 proton), 6.97 (d, 1H, iminopyran C-5 proton), 7.18-8.57 (m, 10H, aromatic protons, 1H, NH, D_2O exchangeable).

Ethyl 2-amino-6-(7-(benzoyloxy)-4-methyl-6-nitro-2-oxo-2H-chromen-8-yl)-4-(2-chlorophenyl)-4H-pyran-3-carboxylate (IXf)

Yield: 65%. m.p.: 100-102°C. Analysis for $C_{31}H_{23}ClN_2O_9$, M.wt.: 602.98. Calcd.: %C, 61.75; H, 3.84; N, 4.65. Found: %C, 61.65; H, 3.91; N, 4.48. IR (KBr, ū, cm^{-1}): 3430 (NH), 1724 (3 C=O), 1603 (C=N), 1540 and 1382 (NO_2).

Ethyl 2-amino-6-(7-(benzoyloxy)-4-methyl-6-nitro-2-oxo-2H-chromen-8-yl)-4-(2-nitrophenyl)-4H-pyran-3-carboxylate (IXg)

Yield: 57%. m.p.: 86-88°C. Analysis for $C_{31}H_{23}N_3O_{11}$, M.wt.: 613.53. Calcd.: %C, 60.69; H, 3.78; N, 6.85. Found: %C, 60.73; H, 3.85; N, 6.74. IR (KBr, ū, cm^{-1}): 3433 (NH), 1730 (3 C=O), 1607 (C=N), 1543 and 1378 (2 NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm):

1.58 (t, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.95 (d, 1H, iminopyran C-3 proton), 3.89 (t, 1H, iminopyran C-4 proton), 4.00 (q, 2H, OCH_2), 6.19 (s, 1H, C-3 proton), 6.96 (d, 1H, iminopyran C-5 proton), 7.19-8.57 (m, 10H, aromatic protons, 1H, NH, D_2O exchangeable). MS; m/z (R.I. %): [M^+] 613 (24.94), 69 (100).

Ethyl 2-amino-6-(7-(benzoyloxy)-4-methyl-6-nitro-2-oxo-2H-chromen-8-yl)-4-(2-bromophenyl)-4H-pyran-3-carboxylate (IXh)

Yield: 55%. m.p.: 112-114°C. Analysis for $C_{31}H_{23}BrN_2O_9$, M.wt.: 646.43. Calcd.: %C, 57.51; H, 3.58; N, 4.33. Found: %C, 57.70; H, 3.52; N, 4.37. IR (KBr, ū, cm^{-1}): 3398 (NH), 1727 (3 C=O), 1599 (C=N), 1550 and 1380 (NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 1.58 (t, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.04 (d, 1H, iminopyran C-3 proton), 4.04 (t, 1H, iminopyran C-4 proton), 4.40 (q, 2H, OCH_2), 6.16 (s, 1H, C-3 proton), 6.98 (d, 1H, iminopyran C-5 proton), 7.18-8.58 (m, 10H, aromatic protons, 1H, NH, D_2O exchangeable).

Ethyl 2-amino-6-(7-(benzoyloxy)-4-methyl-6-nitro-2-oxo-2H-chromen-8-yl)-4-phenyl-4H-pyran-3-carboxylate (IXi)

Yield: 52%. m.p.: 88-90°C. Analysis for $C_{31}H_{24}N_2O_9$, M.wt.: 568.53. Calcd.: %C, 65.49; H, 4.25; N, 4.93. Found: %C, 65.61; H, 4.31; N, 4.88. IR (KBr, ū, cm^{-1}): 3436 (NH), 1732 (3 C=O), 1606 (C=N), 1527 and 1379 (NO_2).

8-(4-Aryl-3-thiocarbamoyl-2-imino-3,4-dihydro-2H-pyran-6-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoates (Xa-i)

A mixture of 8-acetyl-7-benzoyloxy-6-nitrocoumarin V (0.36 g, 0.001 mol), the appropriate aromatic aldehyde (0.001 mol), thiocyanoacetamide (0.1 g, 0.001 mol) and 5 drops piperidine in n-butanol (5 ml) was refluxed for 6 hours. The reaction mixture was left to cool; the formed precipitate was filtered off, washed with diethyl ether and crystallized from absolute ethyl alcohol.

8-(6-Amino-5-thiocarbamoyl-4-(4-chlorophenyl)-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (Xa)

Yield: 79%. m.p.: 120-122°C. Analysis for $C_{29}H_{20}ClN_3O_7S$, M.wt.: 589.00. Calcd.: %C, 59.04; H, 3.42; N, 7.12. Found: %C, 59.12; H, 3.47; N, 7.01. IR (KBr, ū, cm^{-1}): 3441 and 3400 (NH_2), 3280 (NH), 1724 (2 C=O), 1592 (C=N), 1525 and 1375 (NO_2), 1257 (C=S). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.32 (s, 3H, CH_3), 2.96 (d, 1H, iminopyran C-3 proton), 3.57 (t, 1H, iminopyran C-4 proton), 6.20 (s, 1H, C-3 proton), 6.98 (d, 1H, iminopyran C-5 proton), 7.23-8.57 (m, 10H, aromatic protons, 1H, exchangeable NH), 9.98 (s, 2H, NH_2, D_2O exchangeable).

8-(6-Amino-5-thiocarbamoyl-4-(4-nitrophenyl)-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (Xb)

Yield: 83%. m.p.: 116-118°C. Analysis for $C_{29}H_{20}N_4O_9S$, M.wt.: 600.56. Calcd.: %C, 58.00; H, 3.36; N, 9.33. Found: %C, 58.14; H, 3.42; N, 9.53. IR (KBr, ū, cm^{-1}): 3432 and 3372 (NH_2), 3292 (NH), 1724 (2 C=O), 1595 (C=N), 1519 and 1377 (2 NO_2), 1271 (C=S).

8-(6-Amino-5-thiocarbamoyl-4-(4-hydroxyphenyl)-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (Xc)

Yield: 78%. m.p.: 154-155°C. Analysis for $C_{29}H_{21}N_3O_8S$, M.wt.: 571.56. Calcd.: %C, 60.94; H, 3.70; N, 7.35. Found: %C, 61.06; H, 3.71; N, 7.44. IR (KBr, ū, cm^{-1}): 3446 and 3401 (NH_2), 3302 (NH), 3197 (OH), 1717 (2 C=O), 1593 (C=N), 1514 and 1374 (NO_2), 1263 (C=S). MS; m/z (R.I. %): [M^+] 571 (23.27), [M^++1] 572 (12.16), 69 (100).

48-(6-Amino-5-thiocarbamoyl-4-p-tolyl-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (Xd)

Yield: 84%. m.p.: 86-88°C. Analysis for $C_{30}H_{23}N_3O_7S$, M.wt.: 569.58. Calcd.: %C, 63.26; H, 4.07; N, 7.38. Found: %C, 63.33; H, 4.17; N, 7.30. IR (KBr, ū, cm^{-1}): 3429 and 3384 (NH_2), 3298 (NH), 1726 (2 C=O), 1597 (C=N), 1520 and 1376 (NO_2).1262 (C=S). MS; m/z (R.I. %): [M^+] 569 (54.41), 199 (100).

8-(6-Amino-4-(3-bromophenyl)-5-thiocarbamoyl-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (Xe)

Yield: 79%. m.p.: 116-118°C. Analysis for $C_{29}H_{20}BrN_3O_7S$, M.wt.: 633.45. Calcd.: %C, 54.90; H, 3.18; N, 6.62. Found: %C, 56.84; H, 3.23; N, 6.47. IR (KBr, ū, cm^{-1}): 3431 and 3380 (NH_2), 3292 (NH), 1726 (2 C=O), 1594 (C=N), 1527 and 1375 (NO_2), 1255 (C=S).

8-(6-Amino-5-thiocarbamoyl-4-(2-chlorophenyl)-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (Xf)

Yield: 87%. m.p.: 106-108°C. Analysis for $C_{29}H_{20}ClN_3O_7S$, M.wt.: 589.00. Calcd.: %C, 59.04; H, 3.42; N, 7.12. Found: %C, 59.24; H, 3.55; N, 7.15. IR (KBr, ū, cm^{-1}): 3429 and 3372 (NH_2), 3287 (NH), 1727 (2 C=O), 1596 (C=N), 1530 and 1377 (NO_2), 1266 (C=S).

8-(6-Amino-5-thiocarbamoyl-4-(2-nitrophenyl)-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (Xg)

Yield: 72%. m.p.: 130-132°C. Analysis for $C_{29}H_{20}N_4O_9S$, M.wt.: 600.56. Calcd.: %C, 58.00; H, 3.36; N, 9.33. Found: %C, 58.05; H, 3.41; N, 9.25. IR (KBr, ū, cm^{-1}): 3430 and 3379 (NH_2), 3293 (NH), 1723 (2 C=O), 1601 (C=N), 1530 and 1378 (2 NO_2), 1263 (C=S).

8-(6-Amino-4-(2-bromophenyl)-5-thiocarbamoyl-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (Xh)

Yield: 90%. m.p.: 88-90°C. Analysis for $C_{29}H_{20}BrN_3O_7S$, M.wt.: 633.45. Calcd.: %C, 54.90; H, 3.18; N, 6.62. Found: %C, 55.02; H, 3.12; N, 6.54. IR (KBr, ū, cm^{-1}): 3429 and 3369 (NH_2), 3287 (NH), 1727 (2 C=O), 1596 (C=N), 1531 and 1375 (NO_2),

1262 (C=S). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.32 (s, 3H, CH_3), 2.95 (d, 1H, iminopyran C-3 proton), 3.85 (t, 1H, iminopyran C-4 proton), 6.32 (s, 1H, C-3 proton), 6.93 (d, 1H, iminopyran C-5 proton), 7.15-8.58 (m, 10H, aromatic protons, 1H, exchangeable NH), 10.19 (s, 2H, NH_2, D_2O exchangeable).

8-(6-Amino-5-thiocarbamoyl-4-phenyl-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (XIi)

Yield: 83%. m.p.: 92-94°C. Analysis for $C_{29}H_{21}N_3O_7S$, M.wt.: 555.56. Calcd.: %C, 62.70; H, 3.81; N, 7.56. Found: %C, 62.75; H, 3.91; N, 7.45. IR (KBr, ū, cm^{-1}): 3432 and 3381 (NH_2), 3297 (NH), 1727 (2 C=O), 1597 (C=N), 1528 and 1376 (NO_2), 1266 (C=S). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.32 (s, 3H, CH_3), 2.95 (d, 1H, iminopyran C-3 proton), 3.80 (t, 1H, iminopyran C-4 proton), 6.26 (s, 1H, C-3 proton), 6.96 (d, 1H, iminopyran C-5 proton), 7.19-8.40 (m, 11H, aromatic protons, 1H, exchangeable NH), 9.99 (s, 2H, NH_2, D_2O exchangeable). MS; m/z (R.I. %): [M^+] 555 (0.80), 91 (100).

8-(4-Aryl-3-cyano-2-imino-3,4-dihydro-2H-pyran-6-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoates (XIa-i)

A mixture of 8-acetyl-7-benzoyloxy-6-nitrocoumarin V (0.36 g, 0.001 mol), the appropriate aromatic aldehyde (0.001 mol), malononitrile (0.066 g, 0.001 mol) and 5 drops piperidine in n-butanol (5 ml) was refluxed for 5 hours. After cooling, the formed precipitate was filtered off, washed with diethyl ether and crystallized from absolute ethyl alcohol

8-(6-Amino-4-(4-chlorophenyl)-5-cyano-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (XIa)

Yield: 92%. m.p.: 182-183°C. Analysis for $C_{29}H_{18}ClN_3O_7$, M.wt.: 555.92. Calcd.: %C, 62.65; H, 3.26; N, 7.56. Found: %C, 62.60; H, 3.33; N, 7.49. IR (KBr, ū, cm^{-1}): 3334 (NH), 2195 (C≡N), 1709 (2 C=O), 1616 (C=N), 1560 and 1379 (NO_2).

8-(6-Amino-5-cyano-4-(4-nitrophenyl)-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (XIb)

Yield: 84%. m.p.: 160-161°C. Analysis for $C_{29}H_{18}N_4O_9$, M.wt.: 566.47. Calcd.: %C, 61.49; H, 3.20; N, 9.89. Found: %C, 61.52; H, 3.27; N, 9.91. IR (KBr, ū, cm^{-1}): 3338 (NH), 2196 (C≡N), 1725 (2 C=O), 1611 (C=N), 1563 and 1384 (2 NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.39 (s, 3H, CH_3), 2.97 (d, 1H, iminopyran C-3 proton), 3.56 (t, 1H, iminopyran C-4 proton), 6.17 (s, 1H, C-3 proton), 6.99 (d, 1H, iminopyran C-5 proton), 7.04-8.59 (m, 10H, aromatic protons, 1H, NH, D_2O exchangeable).

8-(6-Amino-5-cyano-4-(4-hydroxyphenyl)-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (XIc)

Yield: 85%. m.p.: 210-212°C. Analysis for $C_{29}H_{19}N_3O_8$, M.wt.: 537.48. Calcd.: %C, 64.80; H, 3.56; N, 7.82. Found: %C, 64.87; H, 3.68; N, 7.64. IR (KBr, ū, cm^{-1}): 3335 (NH), 3192 (OH), 2195 (C≡N), 1707 (2 C=O), 1614 (C=N), 1557 and 1375 (NO_2). MS; m/z (R.I. %): [M^+] 537 (23.18), [M^++1] 538 (25.75), 56 (100).

8-(6-Amino-5-cyano-4-p-tolyl-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (XId)

Yield: 92%. m.p.: 245-247°C. Analysis for $C_{30}H_{21}N_3O_7$, M.wt.: 535.50. Calcd.: %C, 67.29; H, 3.95; N, 7.85. Found: %C, 67.34; H, 3.88; N, 7.75. IR (KBr, ū, cm^{-1}): 3336 (NH), 2196 (C≡N), 1720 (2 C=O), 1614 (C=N), 1563 and 1377 (NO_2). MS; m/z (R.I. %): [M^+] 535 (7.23), 80 (100).

8-(6-Amino-4-(3-bromophenyl)-5-cyano-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (XIe)

Yield: 96%. m.p.: 202-204°C. Analysis for $C_{29}H_{18}BrN_3O_7$, M.wt.: 599.37. Calcd.: %C, 58.02; H, 3.02; N, 7.00. Found: %C, 58.13; H, 3.16; N, 6.92. IR (KBr, ū, cm^{-1}): 3339 (NH), 2199 (C≡N), 1727 (2 C=O), 1608 (C=N), 1572 and 1377 (NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.41 (s, 3H, CH_3), 2.99 (d, 1H, iminopyran C-3 proton), 3.39 (t, 1H, iminopyran C-4 proton), 6.38 (s, 1H, C-3 proton), 6.82 (d, 1H, iminopyran C-5 proton), 7.15-8.59 (m, 10H, aromatic protons, 1H, NH, D_2O exchangeable).

8-(6-Amino-4-(2-chlorophenyl)-5-cyano-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (XIf)

Yield: 88%. m.p.: 213-215°C. Analysis for $C_{29}H_{18}ClN_3O_7$, M.wt.: 555.92. Calcd.: %C, 62.65; H, 3.26; N, 7.56. Found: %C, 62.80; H, 3.32; N, 7.61. IR (KBr, ū, cm^{-1}): 3334 (NH), 2196 (C≡N), 1724 (2 C=O), 1617 (C=N), 1562 and 1379 (NO_2). ^{1}HNMR (DMSO-d_6, δ, ppm): 2.45 (s, 3H, CH_3), 3.01 (d, 1H, iminopyran C-3 proton), 3.35 (t, 1H, iminopyran C-4 proton), 6.21 (s, 1H, C-3 proton), 6.97 (d, 1H, iminopyran C-5 proton), 7.25-8.64 (m, 10H, aromatic protons, 1H, NH, D_2O exchangeable).

8-(6-Amino-5-cyano-4-(2-nitrophenyl)-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (XIg)

Yield: 88%. m.p.: 250-252°C. Analysis for $C_{29}H_{18}N_4O_9$, M.wt.: 566.47. Calcd.: %C, 61.49; H, 3.20; N, 9.89. Found: %C, 61.44; H, 3.11; N, 9.74. IR (KBr, ū, cm^{-1}): 3333 (NH), 2199 (C≡N), 1713 (2 C=O), 1618 (C=N), 1563 and 1381 (2 NO_2). MS; m/z (R.I. %): [M^+] 566 (27.93), 80 (100).

8-(6-Amino-4-(2-bromophenyl)-5-cyano-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (XIh)

Yield: 98%. m.p.: 246-248°C. Analysis for $C_{29}H_{18}BrN_3O_7$, M.wt.: 599.37. Calcd.: %C, 58.02; H, 3.02; N, 7.00. Found: %C, 57.85; H, 2.95; N, 7.16. IR (KBr, ū, cm^{-1}): 3334 (NH), 2201 (C≡N), 1724 (2 C=O), 1622 (C=N), 1563 and 1385 (NO_2).

8-(6-Amino-5-cyano-4-phenyl-4H-pyran-2-yl)-4-methyl-6-nitro-2-oxo-2H-chromen-7-yl benzoate (XIi)

Yield: 94%. m.p.: 242-244°C. Analysis for $C_{29}H_{19}N_3O_7$, M.wt.: 521.48. Calcd.: %C, 66.79; H, 3.67; N, 8.06. Found: %C, 66.71; H, 3.54; N, 8.01. IR (KBr, ū, cm^{-1}): 3341 (NH), 2198 (C≡N), 1723 (2 C=O), 1614 (C=N), 1568 and 1384 (NO_2).

Antibacterial screening

The antibacterial screening was performed at and all the used microorganisms were obtained from the culture collection of the Department of Microbiology and Immunology, Faculty of Pharmacy, Helwan University.

Sensitivity test using paper disc diffusion technique

The medium was sterilized at 120°C for 30 minutes using the autoclave then each 100 ml of it was inoculated with 1 ml of each microorganism suspension (10^5 cfu/ml) at 40-50°C and poured into Petri dishes to give a depth of 3-4 mm. Non sterile powders of the fifty five tested compounds were dissolved in DMSO (dimethyl sulphoxide) giving a concentration of 10,000 µg/ml. Sterile filter paper discs each of 6 mm diameter were impregnated with 5 µl (50 µg) of the above solutions and then placed over the surface of the nutrient agar containing standardized inoculums of the test microorganisms. Novobiocin was used as a reference standard in the form of standard discs each of 30 µg/disc concentration. The plates were incubated at 37°C for 24 hours. The observed zones of inhibition after incubation were measured and compared to those of Novobiocin **(Table 1).**

Minimum inhibitory concentration (MIC) using agar dilution method

MIC of each of the synthesized compounds was determined by agar dilution method. A stock solution of each compound was prepared in the concentration of 1024 µg/ml DMSO and was two folds serially diluted up to 1 µg/ml. The test compounds were incorporated in specified quantity of molten sterile nutrient agar for anti-bacterial activity. A specified quantity of the medium containing the compound was poured into a Petri dish to give a depth of 3-4 mm and allowed to solidify. The microorganisms were applied using the multipoint inoculator to the plates with serially diluted compounds in DMSO to be tested and incubated at 37°C for 24 hours. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate. The observed MIC is presented in **(Table 2).**

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