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Research Article

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Coumarin-Furoquinoline Conjugates as Potential Antitubercular Agents: Synthesis, Biological Evaluation and Molecular Docking Studies

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ABSTRACT

A series of novel coumarin-furoquinoline conjugates were designed, synthesized and screened for antitubercular activity against two mycobacterial strains (Mycobacterium tuberculosis H37 RV and Mycobacterium phlei). The preliminary bioassay results demonstrated that all tested compounds exhibited the activities with different degrees, and some compounds showed better effects than standard drugs used. The SAR results indicate that the compounds bearing hydroxyl group (8g), chlorine (8h, 8i, 9h, 9i, 10h, 10i) and bromine (8j, 9j, 10j) on coumarin and chlorine on furoquinoline are most effective. Further, molecular docking studies performed on all the title compounds, compound 8g and 10h resulted as potent. This is the first report assigning in vitro anti-mycobacterial and structure activity relationship with molecular docking studies on this new class of conjugates.

Keywords: Coumarin; Furoquinoline; Molecular docking; Quinoline; Anti-TB; SAR

INTRODUCTION

Tuberculosis (TB) is the first infectious disease declared by the World Health Organization (WHO) as a global health emergency [1]. According to statistics, which is considered as world's second highest killer by single infectious agent after HIV [2,3]. The drug treatment varies from six to nine months in the case of drug-sensitive disease, while in the case of drug-resistant disease the treatment could last up to two years [4]. Improper administration of the drugs can result in drug resistance, treatment failure or even death [5]. The World Health Organization (WHO) reported that globally 3.5% of naive infections already expressed resistance to the two most efficacious frontline agents used to treat the disease i.e., RIF (rifampicin) and INH (isoniazide), thereby classifying the infection as multidrug resistant tuberculosis (MDR-TB). A very common and deadly complication of Mtb infection is coinfection with human immunodeficiency virus (HIV) [6-10]. The worsening situation has prompted the World Health Organization (WHO) to declare tuberculosis a global public health crisis [11].

Coumarins are important class of oxygen heterocycles [12], which are predominantly found in higher plants and have diverse pharmacologic activity [13]. This privileged molecule, being a common moiety found in many biological active natural and therapeutic products and thus represents a very important pharmacophore [14]. In recent years, the actual trend in the field of chemistry of coumarins has been modification of the benzopyran-2-one system, which are of great interest for the theory of organic synthesis including heterocyclic chemistry and purposeful synthesis of new biologically active compounds centered on coumarin system have been reviewed [15-17]. Antitumor activity study is interesting and promising for these compounds [18-21]. Coumarins target a number of pathways in cancer pathogenesis, such as kinases inhibition, cell cycle arrest, angiogenesis inhibition, HSP90 inhibition, carbonic anhydrase inhibition, telomerase inhibition, etc. [22]. Recently, Dandriyal et al. reviewed the developments of C-4 substituted coumarin derivatives as anticancer agents [23]. The recent progress in the drug development of coumarin derivatives as potent antituberculosis agents have also been reviewed [24]. Quinoline scaffold plays an important role in anticancer drug development as their derivatives have shown excellent results through different mechanism of action such as growth inhibitors by cell cycle arrest, apoptosis, inhibition of angiogenesis, disruption of cell migration, and modulation of nuclear receptor responsiveness. The anticancer potential of several of these derivatives have been demonstrated on

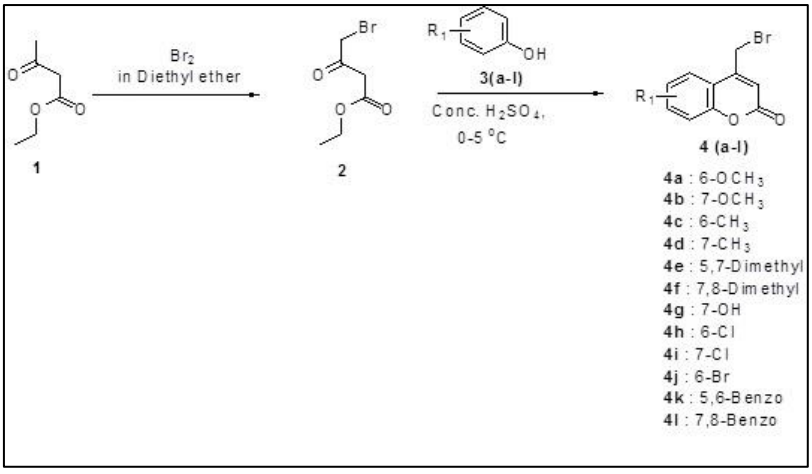
various cancer cell lines. The biological potential of quinoline derivatives have been reviewed by Bawa et al. [25,26]. In the year 2015, Afzal et al. compiled and discussed specifically the anticancer potential of quinoline derivatives on target oriented basis, which could serve as a platform for medicinal chemists to design better anticancer drugs [27]. Natural products bearing furo [2,3-*b*]quinoline skeleton and their synthetic analogues play a vital role in drug development and drug discovery. In recent years, many synthetic methods have been developed for furo [2,3-*b*]quinolines [28-31]. Molecules containing furo[2,3-*b*]quinoline scaffold show a wide range of biological activities which include vasoconstructive, antidiuretic, antiarrhythmic, spamolytic, sedative, hypothermal effects [32], antitumour, antipyretic, antiplatelet and cytotoxic activities [33,34]. Wang et al. reported a facile synthesis of furoquinoline and their effects on radical-induced oxidation of DNA [35]. The 4-anilinofuro [2,3-*b*] quinoline derivatives reported as selective and orally active compounds against non-small cell lung cancers [36].

Considering the biological significance of coumarins and quinolines, and in continuation of our interest in designing oxygen heterocycle based anticancer and anti-tuberculosis agents [37], we report herein the synthesis of new series of coumarin-furoquinoline conjugates and study their structure activity relationship with hoping that the new compounds might show significant anti-tuberculosis activity. This is the first report assigning in anti-mycobacterial and structure activity relationship with molecular docking studies on this new conjugates.

MATERIALS AND METHODS

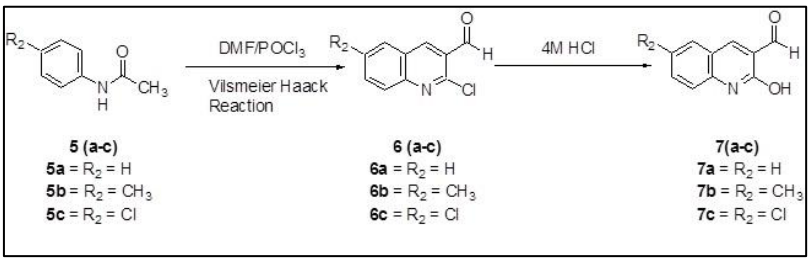
Materials

The required substituted-4-bromomethylcoumarins 4(a-l) were prepared by the Pechmann cyclisation of substituted phenols 3(a-l) with 4-bromoethylacetoacetate 2 using sulphuric acid as the condensing agent. The 4-bromoethylacetoacetate 2 in turn was obtained by the bromination of ethylacetoacetate 1 in dry ether at 0-5°C (Scheme 1).



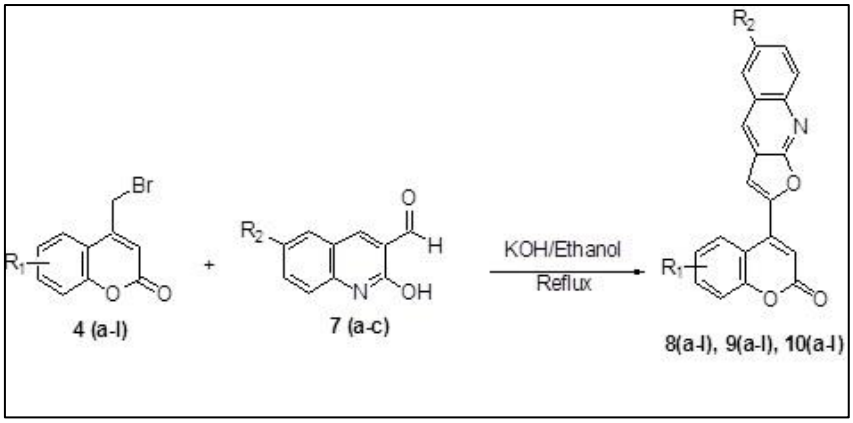
Scheme 1: Synthesis of substituted-4-bromoethylcoumarins 4(a-l)

The 6-substituted-2-chloro-3-formyl quinolines 6(a-c) were synthesized from substituted acetanilides 5(a-c) *via* Vilsmeier Haack reaction. Compounds 6(a-c) were converted to corresponding hydroxyl derivatives 7(a-c) using 4M HCl [38] as depicted in Scheme 2.



Scheme 2: Substituted-2-hydroxy-3-formyl quinolines 7(a-c)

The attempts to carry out further reaction using milder base like potassium carbonate was unsuccessful due to its weak alkalinity. Hence, potassium carbonate was replaced with KOH. One equivalent of compounds 7(a-c) were stirred at room temperature in ethanol with 2.5 equivalents of KOH, which resulted in homogeneous solution after a period of 1 hour. One equivalent of substituted-4-bromomethyl coumarins 4(a-l) were then added and stirring was continued for one hour, and then refluxed for 12 hours on a water bath. The reaction mixture was concentrated to half of its original volume and diluted with ice cold water and neutralized with few drops of dil. HCl. The separated solid was washed thoroughly with water and recrystallised from a suitable solvent to obtain title compounds, 8(a-l), 9(a-l), and 10(a-l) (Scheme 3).



Scheme 3: Synthesis of title compuounds 8(a-l), 9(a-l), 10(a-l)

Anti-Mycobacterial Screening

The *in vitro* anti-mycobacterial activity against *Mycobacterium tuberculosis* H_{37} RV and *Mycobacterium Phlei* by Microplate Alamar Blue Assay (MABA) for the determination of MIC values of all the synthesized compounds along with standard drugs streptomycin and pyrizanamide for the comparison are presented in Table 1. All the thirty six compounds were screened in the present study, MIC ranging from 1.56 to 200 µg/mL.

Table 1: Results of anti-mycobacterial activity of compounds 8(a-l), 9(a-l) and 10(a-l) MICs (µg/mL)

Compound	R_1	R_2	*Mycobacterium tuberculosis (H_{37} RV)*	*Mycobacterium Phlei*
8a	6-OCH_3	H	50	100
8b	7-OCH_3	H	50	100
8c	6-CH_3	H	200	200
8d	7-CH_3	H	200	200
8e	5,7-diCH_3	H	200	200
8f	7,8-diCH_3	H	200	200
8g	7-OH	H	6.25	3.12
8h	6-Cl	H	12.5	12.5
8i	7-Cl	H	12.5	12.5
8j	6-Br	H	6.25	12.5
8k	5,6-Benzo	H	100	200
8l	7,8-Benzo	H	100	200
9a	6-OCH_3	CH_3	25	25
9b	7-OCH_3	CH_3	25	25
9c	6-CH_3	CH_3	100	50
9d	7-CH_3	CH_3	100	50
9e	5,7-diCH_3	CH_3	100	50
9f	7,8-diCH_3	CH_3	100	50
9g	7-OH	CH_3	25	12.5
9h	6-Cl	CH_3	6.25	6.25
9i	7-Cl	CH_3	6.25	6.25
9j	6-Br	CH_3	1.56	12.5
9k	5,6-Benzo	CH_3	100	100
9l	7,8-Benzo	CH_3	100	100
10a	6-OCH_3	Cl	6.25	12.5
10b	7-OCH_3	Cl	6.25	12.5
10c	6-CH_3	Cl	100	50
10d	7-CH_3	Cl	100	50
10e	5,7-diCH_3	Cl	100	50
10f	7,8-diCH_3	Cl	100	50
10g	7-OH	Cl	12.5	6.25
10h	6-Cl	Cl	1.56	1.56
10i	7-Cl	Cl	3.12	1.56
10j	6-Br	Cl	1.56	1.56
10k	5,6-Benzo	Cl	50	100
10l	7,8-Benzo	Cl	50	100
Standard				
Streptomycin	-	-	6.25	6.25
Pyrizanamide	-	-	3.125	3.125

Mycobacterium tuberculosis (H_{37} RV)

In the series of unsubstitution on furoquinoline (R_2 = H), 8(a-l) and the presence of bromine and hydroxyl group on coumarin moiety, i.e., compounds 8g, 8j exhibited potent activity with MIC 6.25 µg/mL, which were equal to standard

drug streptomycin. The chloro derivatives (8h, 8i) exhibited moderate activity (MIC 12.5 µg/mL). The derivatives containing methoxy (8a, 8b) showed better results (MIC 50 µg/mL) than benzo derivatives (8k, 8l) with MIC 100 µg/mL. The poor results in the series were exhibited by mono-methyl (8c, 8d) and di-methyl (8e, 8f) derivatives with MIC 200 µg/mL.

In the series compounds 9(a-l) having a methyl group on furoquinoline ($R_2 = CH_3$), the potent activity was observed for bromo-compound 9j with 1.56 µg/mL which was more potent than standard drugs streptomycin (6.25 µg/mL) and pyrazinamide (3.12 µg/mL). The chloro derivatives (9h, 9i) showed equipotent to streptomycin with MIC 6.25 µg/mL. The methoxy (9a, 9b) and hydroxyl (9g) compounds exhibited moderate activity (MIC 25 µg/mL). The least activity was observed for compounds containing mono-methyl (9c, 9d), di-methyl (9e, 9f) and benzo (9k, 9l) derivatives with MIC 100 µg/mL. In the series of chloro substituted furoquinoline derivatives 10(a-l), chloro (10h) and bromo 10j derivatives exhibited potent activity (1.56 µg/mL) than chloro derivative (10i) with MIC 3.12 µg/mL which were more and equal potent compared to standard drugs used. The methoxy substituted compounds (10a, 10b) showed the MIC 6.25 µg/mL, which were more active than hydroxyl derivative 10g (MIC 12.5 µg/mL). The benzo derivatives (10k, 10l) were showed better results (MIC 50 µg/mL) than mono-methyl (10c, 10d) and di-methyl (10e, 10f) derivatives with 100 µg/mL. In the library of thirty six compounds, the bromo-compounds 9j and 10j exhibited potent activity with MIC 1.56 µg/mL than both the standard drugs, whereas the compound 8j exhibited equipotent to streptomycin (MIC 6.25 µg/mL).

Mycobacterium phlei

In the series of compounds, 8(a-l) presence of halogen on 6- or 7- positions of coumarin moiety exhibited potent activity. The change in position of chlorine from 6-to 7-position and replacement of chlorine by bromine on 6-position resulted into equal activity (8h, 8i and 8j, MIC 12.5 µg/mL). Whereas replacement of chlorine on 7-position by hydroxyl group lead to highest activity in the series (8g, MIC 3.12 µg/mL). The replacement of chlorine by methoxy group (8a and 8b) reduced to MIC 100 µg/mL. Replacement by mono-methyl group (8c and 8d), dimethyl (8e, 8f) and benzo-derivatives (8k and 8l) exhibited least activity in the series with MIC 200 µg/mL.

The series 9(a-l) with methyl substitution on furoquinoline moiety (R_2=CH_3), the equipotent activity compared to streptomycin was observed for chloro compounds (9h, 9i, MIC 6.25 µg/mL) than bromo compound 9j which was equipotent to hydroxyl compound 9g (MIC 12.5 µg/mL). The methoxy compounds (9a and 9b, MIC 25 µg/mL) were more active than mono-methyl-(9c, 9d) and di-methyl compounds (9e, 9f) with MIC 50 µg/mL. The least activity in the series were observed for benzo-compounds (9k and 9l, MIC 100 µg/mL). In the series of chloro substitution on furoquinoline moiety (R_2 = Cl), 10(a-l), the activity further increased for chloro-compounds 10h, 10i (MIC 1.56 µg/mL) and bromo-compound 10j (3.12 µg/mL) which were more and equipotent to standard drugs. The decrease in activity was observed for hydroxyl compound 10g (MIC 6.25 µg/mL) which was better than the methoxy compounds (10a, 10b, MIC 12.5 µg/mL). The mono-methyl (10c, 10d), and di-methyl compounds (10e and 10f) exhibited more activity (MIC 50 µg/mL) than benzo-compounds (10k and 10l, MIC 100 µg/mL).

In the library of thirty six compounds, the chloro-compounds (8h, 8i, 9h, 9i, 10h, 10i) exhibited potent activity than bromo compound except in case of 8j. The bromo-compounds (8j, 9j, and 10j) showed moderate activity.

Molecular Docking Study

The results of molecular docking with binding site interacting amino acid residues and hydrogen bond formation are provided in the following Table 2.

Table 2: **The results of molecular docking**

Compound	Orientation	Binding Energy	Docking Energy	Inhibitation Constant	Intermol Energy	Torsional Energy	Internal Energy	Rms	Hydrogen Bond
8a	5th	-9.58	-10.39	9.43×10^{-8}	-10.21	0.62	-0.19	0	1 H-bond with HIS135
8b	7th	-9.37	-9.73	1.36×10^{-7}	-9.68	0.31	0.05	0	1 H-bond with GLN336
8c	10th	-9.37	-9.8	1.36×10^{-7}	-9.68	0.31	-0.19	0	-----
8d	7th	-9.61	-9.76	9.03×10^{-8}	-9.61	0	-0.15	0	-----
8e	1th	-9.8	-10.25	6.5×10^{-8}	-10.12	0.31	0.13	0	1 H-bond with LYS418
8f	7th	-10.27	-10.44	2.98×10^{-8}	-10.27	0	-0.17	0	1 H-bond with GLN336
8g	6th	-7.87	-8.43	1.69×10^{-6}	-8.18	0.31	-0.24	0	2 H-bonds with HIS132, HIS132
8h	3rd	-7.9	-8.35	1.62×10^{-6}	-8.21	0.31	-0.14	0	1 H-bond with HIS132
8i	6th	-8.46	-8.9	6.29×10^{-7}	-8.77	0.31	-0.13	0	-----
8j	6th	-7.4	-7.92	3.75×10^{-6}	-7.71	0.31	-0.2	0	1 H-bond with LYS418
8k	4th	-9.73	-10.41	7.41×10^{-8}	-10.04	0.31	-0.37	0	-------
8l	4th	-9.85	-10.29	6.03×10^{-8}	-10.16	0.31	-0.13	0	1 H-bond with GLN336
9a	10th	-9.34	-9.77	1.43×10^{-7}	-9.65	0.31	-0.13	0	1 H-bond with ASN385
9b	4th	-8.86	-9.26	3.23×10^{-7}	-9.17	0.31	-0.09	0	---------
9c	3nd	-8.8	-9.3	3.56×10^{-7}	-9.11	0.31	-0.2	0	---------
9d	2nd	-9.56	-9.72	9.91×10^{-8}	-9.56	0	-0.17	0	1 H-bond with GLN336
9e	4th	-9.52	-10.02	1.06×10^{-7}	-9.83	0.31	-0.19	0	1 H-bond with GLN336
9f	8th	9.44	9.58	1.21×10^{-7}	-9.44	0	-0.14	0	-----
9g	1st	9.1	9.54	2.14×10^{-7}	-9.41	0.31	-0.13	0	----

9h	7th	9.09	9.56	2.16×10^{-7}	-9.41	0.31	-0.15	0	1 H-bond with HIS 132
9i	2nd	-9.19	-9.66	1.84×10^{-7}	-9.5	0.31	-0.16	0	1 H-bond with GLN336
9j	10th	-9.08	-9.57	2.21×10^{-7}	-9.39	0.31	-0.18	0	----
9k	9th	-10.52	-11.04	1.95×10^{-8}	-10.83	0.31	-0.21	0	1 H-bond with LYS134
9l	3rd	-9.78	-10.2	6.76×10^{-8}	-10.09	0.31	-0.1	0	---
10a	2nd	-8.45	-9.21	6.39×10^{-7}	-9.07	0.62	-0.13	0	1 H-bond with LEU317
10b	5th	-8.95	-9.44	2.77×10^{-7}	-9.57	0.62	0.13	0	1 H-bond with
10c	4th	-8.88	-9.35	3.08×10^{-7}	-9.19	0.31	-0.15	0	----
10d	5th	-8.85	-9.29	3.23×10^{-7}	-9.17	0.31	-0.13	0	1 H-bond with LEU317
10e	10th	-10.06	-10.54	4.2×10^{-8}	-10.37	0.31	-0.17	0	---
10f	6th	-9.55	-10	9.91×10^{-8}	-9.87	0.31	-0.14	0	----
10g	3rd	-22.06	-22.13	6.74×10^{-1}	-22.37	0.31	-0.24	0	1 H-bond with SER228
10h	7th	-9.58	-9.99	9.55×10^{-8}	-9.89	0.31	-0.1	0	2H-bonds with HIS132, SER228
10i	2nd	-8.33	-8.78	7.83×10^{-7}	-8.64	0.31	-0.14	0	LEU317
10j	6th	-8.44	-8.85	6.51×10^{-7}	-8.75	0.31	-0.1	0	--
10k	3rd	-10.83	-11.08	1.16×10^{-8}	-10.83	0	-0.26	0	--
10l	6th	-10.42	-10.52	2.29×10^{-8}	-10.42	0	-0.1	0	1 H-bond with GLN336
Streptomycin (Standard)	8th	-9.03	-14.55	2.4×10^{-7}	-12.14	3.11	-2.41	0	4 H-bonds LEU363, LEU363, SER228,SER228
Pyrizanamide (Standard)	4th	-5.21	-5.57	1.51×10^{-4}	5.52	0.31	0.05	0	----

Experimental Protocols

Chemistry

The melting points were determined by open capillary method and are uncorrected. The IR spectra (KBr disc) were recorded on a Nicolet-5700 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 500 MHz spectrometer using DMSO-d_6 as solvent and TMS as an internal standard. The chemical shifts are expressed in δ ppm. The mass spectra were recorded using Agilent-Single Quartz LC-MS. The elemental analysis was carried out using Heraus CHN rapid analyzer. The purity of the compounds was checked by TLC. All the chemicals purchased were of analytical grade, and were used without further purification unless otherwise stated, which were purchased from Sigma-Aldrich Chemicals (India) and S.D Fine Chemicals (India).

Synthesis of substituted-4-bromomethylcoumarins 4(a-l):

The required substituted-4-bromomethyl-coumarins [39,40] have been synthesized by the Pechmann cyclization of substituted phenols with 4-bromoethylacetoacetate [41].

Synthesis of 2-chloro-3-formyl quinolines 6(a-c):

DMF (0.125 M) was cooled to 0°C in an R. B. flask equipped with a drying tube $POCl_3$ (0.35 M) was added drop wise with stirring. After a few minutes formation of the Vilsmeier-Haack adduct was observed. To this mixture, substituted acetanilides 5(a-c) (0.05 M) was added slowly and stirred well. The mixture was then heated to reflux for 16.5 h on steam bath. The contents of flask were poured into one litre of ice water and stirred for half an hour. The brownish yellow solid 6(a-c) separated was washed well with water dried and recrystallised from ethyl acetate [38].

Synthesis of 2-hydroxy-3-formyl quinolines 7(a-c):

One mol of 2-chloro-3-formyl quinoline 6(a-c) was treated with 4 M hydrochloric acid. The mixture was refluxed for 3-4 h on water bath. This mixture was poured in crushed ice. The separated solid 7(a-c) was filtered, washed with cold water and recrystallised from acetic acid [38].

General procedure for the synthesis of 4-furo(2,3-b]quinolin-2-yl--chromen-2-ones 8(a-l), 9(a-l), 10(a-l)

The 0.001 M of 2-hydroxy-3-formyl quinoline 7(a-c) and 0.0025 M KOH were taken in 25 mL absolute alcohol and stirred at room temperature for an hour and then 0.001 M of substituted-4-bromomethyl coumarin 4(a-l) was added to the reaction mixture and stirred for one hour. The reaction mixture was then refluxed at 80-90°C for 12 hours, (completion of reaction was monitored by TLC), then the reaction mixture was concentrated to half and diluted with ice cold water and neutralized with few drops of dil. HCl. The separated precipitate was then filtered, washed thoroughly with water and dried. Recrystallisation from suitable solvent.

4-Furo (2,3-b)quinolin-2-yl-6-methoxy-chromen-2-one (8a):

Recrystallised by EtOH+EtOAc; m.p. 255-56°C; yield 72%; IR (KBr, ν in cm^{-1}): 1715 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.41 (*s*, 3H, C6-OCH_3), 6.71 (*s*, 1H, coumarin C3-H), 7.26-8.24 (*m*, 9H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 56.50, 115.87, 116.89, 118.59, 123.11, 124.95, 126.05, 131.36, 133.29, 133.53, 133.60, 133.64, 134.13,

134.22, 134.29, 141.60, 142.86, 151.13, 151.55, 151.64, 161.89. LCMS *m/z:* 343 (M+); Anal.calcd. for $C_{21}H_{13}NO_4$; C, 73.46; H, 3.82; N, 4.08. Found: C, 73.44; H, 3.81; N, 4.07.

4-Furo (2,3-b)quinolin-2-yl-7-methoxy-chromen-2-one (8b):
Recrystallised by EtOH+EtOAc; m.p. 216-17°C; yield 75%; IR (KBr, ν in cm^{-1}): 1712 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.57 (*s*, 3H, C7-OCH_3), 6.72 (*s*, 1H, coumarin C3-H), 7.29-8.32 (*m*, 9H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 56.89, 116.63, 116.94, 118.68, 123.98, 125.16, 126.46, 131.64, 133.62, 133.84, 133.91, 133.96, 134.45, 134.67, 134.42, 141.74, 142.97, 151.34, 151.71, 151.87, 162.02. LCMS *m/z:* 343 (M+); Anal.calcd. for $C_{21}H_{13}NO_4$; C, 73.46; H, 3.82; N, 4.08. Found: C, 73.42; H, 3.81; N, 4.08.

4-Furo (2,3-b)quinolin-2-yl-6-methyl-chromen-2-one (8c):
Recrystallised by EtOH+EtOAc; m.p. 260-261°C; yield 70%; IR (KBr, ν in cm^{-1}): 1710 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.39 (*s*, 3H, C6-CH_3), 6.72 (*s*, 1H, coumarin C3-H), 7.22-8.99 (*m*, 9H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 21.20, 114.63, 115.93, 117.11, 123.02, 124.08, 125.84, 130.93, 131.98, 132.14, 133.12, 133.16, 134.01, 134.08, 134.12, 141.23, 142.54, 151.01, 151.12, 151.18, 161.42. LCMS *m/z:* 328 (M+1); Anal.calcd. for $C_{21}H_{13}NO_3$; C, 77.05; H, 4.00; N, 4.88. Found: C, 77.01; H, 4.00; N, 4.87.

4-Furo(2,3-b)quinolin-2-yl-7-methyl-chromen-2-one (8d):
Recrystallised by EtOH+EtOAc; m.p. 208-210°C; yield 67%; IR (KBr, ν in cm^{-1}): 1714 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.71 (*s*, 3H, C7-CH_3), 6.74 (*s*, 1H, coumarin C3-H), 7.24-9.06 (*m*, 9H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 22.52, 115.04, 115.98, 117.56, 123.61, 124.54, 125.92, 130.97, 132.04, 132.19, 133.23, 133.22, 134.08, 134.14, 134.61, 141.62, 142.72, 151.51, 151.68, 151.71, 161.58. LCMS *m/z:* 328 (M+1); Anal.calcd. for $C_{21}H_{13}NO_3$; C, 77.05; H, 4.00; N, 4.88. Found: C, 77.00; H, 4.00; N, 4.87.

4-Furo(2,3-b)quinolin-2-yl-5,7-dimethyl-chromen-2-one (8e):
Recrystallised by EtOH+EtOAc; m.p. 216-217°C; yield 73%; IR (KBr, ν in cm^{-1}): 1709 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.37 (*s*, 3H, CH_3), 2.48 (*s*, 3H, CH_3), 6.72 (*s*, 1H, coumarin C3-H), 6.80 (*s*, 1H, C6-H), 7.15 (*s*, 1H, C8-H), 7.21-8.88 (*m*, 6H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 19.25, 22.06, 116.19, 116.28, 117.62, 123.92, 124.59, 126.52, 131.16, 132.62, 132.59, 133.42, 133.57, 134.68, 134.76, 134.82, 141.66, 142.74, 152.08, 152.86, 152.92, 162.66. LCMS *m/z:* 342 (M+1); Anal.calcd. for $C_{22}H_{15}NO_3$; C, 77.41; H, 4.43; N, 4.10. Found: C, 77.35; H, 4.42; N, 4.09.

4-Furo(2,3-b)quinolin-2-yl-7,8-dimethyl-chromen-2-one (8f):
Recrystallised by EtOH+EtOAc; m.p. 204-205°C; yield 76%; IR (KBr, ν in cm^{-1}): 1718 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.34 (*s*, 3H, CH_3), 2.48 (*s*, 3H, CH_3), 6.75 (*s*, 1H, coumarin C3-H), 7.24-8.92 (*m*, 6H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 20.41, 22.15, 116.22, 116.31, 117.68, 123.99, 124.68, 126.71, 131.65, 132.82, 132.94, 133.58, 133.69, 134.85, 134.96, 135.04, 141.92, 142.89, 152.54, 152.63, 152.98, 162.74. LCMS *m/z:* 342 (M+1); Anal.calcd. for $C_{22}H_{15}NO_3$; C, 77.41; H, 4.43; N, 4.10. Found: C, 77.36; H, 4.42; N, 4.09.

4-Furo(2,3-b)quinolin-2-yl-7-hydroxy-chromen-2-one (8g):
Recrystallised by EtOH+EtOAc; m.p. 221-222°C; yield 76%; IR (KBr, ν in cm^{-1}): 1713 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.74 (*s*, 1H, coumarin C3-H), 7.26-9.12 (*m*, 9H, Ar-H), 10.11 (*s*, 1H, OH, D_2O exchangeable). ^{13}C NMR (125 MHz, $CDCl_3$): 115.18, 116.14, 117.54, 124.12, 124.65, 126.16, 130.05, 131.98, 132.21, 133.24, 133.62, 134.18, 134.29, 134.38, 141.42, 142.33, 151.17, 151.24, 151.33, 161.45. LCMS *m/z:* 330 (M+1); Anal.calcd. for $C_{20}H_{11}NO_4$; C, 72.95; H, 3.37; N, 4.25. Found: C, 72.87; H, 3.37; N, 4.26.

6-Chloro-4-furo(2,3-b)quinolin-2-yl-chromen-2-one (8h):
Recrystallised by EtOH+EtOAc; m.p. 234-235°C; yield 68%; IR (KBr, ν in cm^{-1}): 1716 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.70 (*s*, 1H, coumarin C3-H), 7.22-8.89 (*m*, 9H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 113.02, 114.08, 115.24, 122.63, 122.71, 124.41, 128.12, 130.99, 131.26, 132.35, 132.72, 133.38, 133.44, 133.52, 139.14, 140.38, 150.10, 151.26, 151.42, 160.23. LCMS *m/z:* 348 (M+1); Anal.calcd. for $C_{20}H_{10}ClNO_3$; C, 69.08; H, 2.90; N, 4.03. Found: C, 69.01; H, 2.90; N, 4.03.

7-Chloro-4-furo(2,3-b]quinolin-2-yl-chromen-2-one (8i):
Recrystallised by EtOH+Dioxane; m.p. 210-211°C; yield 66%; IR (KBr, ν in cm^{-1}): 1710 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.74 (*s*, 1H, coumarin C3-H), 7.20-8.84 (*m*, 9H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 112.14, 113.23, 115.18, 122.22, 122.63, 124.52, 128.05, 130.28, 131.04, 132.62, 132.82, 133.97, 134.16, 134.48, 139.10, 141.02, 151.01, 151.58, 151.87, 160.08. LCMS *m/z:* 348 (M+1); Anal.calcd. for $C_{20}H_{10}ClNO_3$; C, 69.08; H, 2.90; N, 4.03. Found: C, 69.03; H, 2.90; N, 4.03.

6-Bromo-4-furo(2,3-b)quinolin-2-yl-chromen-2-one (8j):
Recrystallised by EtOH+Dioxane; m.p. 242-243°C; yield 63%; IR (KBr, ν in cm^{-1}): 1708 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 6.73 (*s*, 1H, coumarin C3-H), 7.25-8.86 (*m*, 9H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 112.14, 113.35, 114.35, 121.36, 121.84, 124.05, 128.17, 130.15, 131.54, 132.32, 132.65, 133.20, 133.32, 133.48, 139.08, 141.02, 150.02, 151.18, 151.32, 160.18. LCMS *m/z:* 391, 393 (M+, M+2); Anal.calcd. for $C_{20}H_{10}BrNO_3$; C, 61.25; H, 2.57; N, 3.57 Found: C, 61.18; H, 2.57; N, 3.57.

1-Furo(2,3-b)quinolin-2-yl-benzo(f)chromen-3-one (8k):
Recrystallised by EtOH+Dioxane; m.p. 260-261°C; yield 78%; IR (KBr, ν in cm^{-1}): 1714 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 6.72 (*s*, 1H, coumarin C3-H), 7.14-9.10 (*m*, 12H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 111.10, 111.23, 111.73, 111.95, 112.10, 113.28, 114.39, 121.41, 121.63, 123.84, 127.38, 130.06, 130.32, 132.16, 132.48, 133.10, 133.64, 133.71, 139.14, 140.94, 149.53, 150.98, 151.16, 160.61. LCMS *m/z:* 364 (M+1); Anal.calcd. for $C_{24}H_{13}NO_3$; C, 79.33; H, 3.61; N, 3.85 Found: C, 79.28; H, 3.61; N, 3.85.

4-Furo(2,3-b)quinolin-2-yl-benzo(h)chromen-2-one (8l):
Recrystallised by EtOH+Ethyl acetate; m.p. 245-246°C; yield 76%; IR (KBr, ν in cm^{-1}): 1710 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 6.74 (*s*, 1H, coumarin C3-H), 7.10-9.18 (*m*, 12H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 111.42, 111.58, 111.78, 111.99, 112.18, 113.32, 114.45, 121.36, 121.81, 123.68, 127.10, 129.84, 130.12, 132.01, 132.25, 133.61, 133.82, 133.93, 139.28, 140.82, 149.18, 150.31, 151.04, 160.40. LCMS *m/z:* 364 (M+1); Anal.calcd. for $C_{24}H_{13}NO_3$; C, 79.33; H, 3.61; N, 3.85 Found: C, 79.28; H, 3.61; N, 3.85.

6-Methoxy-4-(6-methyl-furo(2,3-b]quinolin-2-yl)-chromen-2-one (9a):
Recrystallised by EtOH; m.p. 234-235°C; yield 76%; IR (KBr, ν in cm^{-1}): 1708 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 2.43 (*s*, 3H, C6-CH_3), 3.43 (*s*, 3H, C6-OCH_3), 6.73 (*s*, 1H, coumarin C3-H), 7.22-8.28 (*m*, 8H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 21.20, 56.62, 114.10, 115.82, 117.43, 122.94, 124.58, 125.96, 131.28, 132.85, 133.46, 133.82, 133.98, 134.25, 134.38, 134.46, 141.24, 142.93, 152.20, 152.68, 152.74, 162.94. LCMS *m/z:* 358 (M+1); Anal.calcd. for $C_{22}H_{15}NO_4$; C, 73.94; H, 4.23; N, 3.92. Found: C, 73.92; H, 4.23; N, 3.92.

7-Methoxy-4-(6-methyl-furo(2,3-b]quinolin-2-yl)-chromen-2-one (9b):
Recrystallised by EtOH; m.p. 248-249°C; yield 71%; IR (KBr, ν in cm^{-1}): 1714 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 2.41 (*s*, 3H, C6-CH_3), 3.60 (*s*, 3H, C7-OCH_3), 6.73 (*s*, 1H, coumarin C3-H), 7.25-8.28 (*m*, 8H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 21.01, 56.88, 115.42, 115.65, 117.58, 122.95, 124.18, 124.25, 130.82, 133.17, 133.84, 133.91, 133.98, 134.08, 134.26, 134.39, 141.52, 142.84, 151.08, 151.68, 151.91, 162.18. LCMS *m/z:* 358 (M+1); Anal.calcd. for $C_{22}H_{15}NO_4$; C, 73.94; H, 4.23; N, 3.92. Found: C, 73.92; H, 4.23; N, 3.92.

6-Methyl-4-(6-methyl-furo(2,3-b]quinolin-2-yl)-chromen-2-one (9c):
Recrystallised by EtOH+EtOAc; m.p. 212-23°C; yield 69%; IR (KBr, ν in cm^{-1}): 1706 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 2.38 (*s*, 3H, C6-CH_3), 2.41 (*s*, 3H, C6-CH_3), 6.71 (*s*, 1H, coumarin C3-H), 7.21-8.57 (*m*, 8H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 21.12, 21.39, 112.54, 114.28, 116.66, 122.45, 124.21, 124.68, 130.19, 131.58, 132.25, 133.38, 133.46, 134.15, 134.29, 134.41, 141.63, 142.71, 151.15, 151.22, 151.37, 162.02. LCMS *m/z:* 342 (M+1); Anal.calcd. for $C_{22}H_{15}NO_3$; C, 77.41; H, 4.43; N, 4.10. Found: C, 77.40; H, 4.43; N, 4.10.

7-Methyl-4-(6-methyl-furo(2,3-b)quinolin-2-yl)-chromen-2-one (9d):
Recrystallised by EtOH+Dioxane; m.p. 223-224°C; yield 65%; IR (KBr, ν in cm^{-1}): 1711 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 2.41 (*s*, 3H, C6-CH_3), 2.65 (*s*, 3H, C7-CH_3), 6.73 (*s*, 1H, coumarin C3-H), 7.21-8.82 (*m*, 8H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 21.30, 22.61, 112.25, 114.97, 116.10, 122.21, 124.02, 125.41, 129.82, 131.90, 132.02, 133.12, 133.37, 134.29, 134.50, 134.68, 142.05, 142.64, 151.18, 151.49, 151.82, 161.78. LCMS *m/z:* 342 (M+1); Anal.calcd. for $C_{22}H_{15}NO_3$; C, 77.41; H, 4.43; N, 4.10. Found: C, 77.40; H, 4.43; N, 4.10.

5,7-Dimethyl-4-(6-methyl-furo(2,3-b)quinolin-2-yl)-chromen-2-one (9e):
Recrystallised by EtOH; m.p. 200-201°C; yield 71%; IR (KBr, ν in cm^{-1}): 1715 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 2.37 (*s*, 3H, CH_3), 2.41 (*s*, 3H, CH_3), 2.48 (*s*, 3H, CH_3), 6.71 (*s*, 1H, coumarin C3-H), 6.82 (*s*, 1H, C6-H), 7.19 (*s*, 1H, C8-H), 7.24-8.80 (*m*, 5H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 19.25, 22.05, 22.14, 115.84, 116.20, 117.55, 123.81, 124.42, 126.26, 131.08, 132.67, 132.74, 133.48, 133.58, 134.81, 134.92, 135.10, 141.78, 142.86, 152.23, 152.90, 152.98, 162.72. LCMS *m/z:* 356 (M+1); Anal.calcd. for $C_{23}H_{17}NO_3$; C, 77.73; H, 4.82; N, 3.94. Found: C, 77.72; H, 4.82; N, 3.94.

7,8-Dimethyl-4-(6-methyl-furo(2,3-b)quinolin-2-yl)-chromen-2-one (9f):
Recrystallised by EtOH+Dioxane; m.p. 215-216°C; yield 72%; IR (KBr, ν in cm^{-1}): 1710 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 2.34 (*s*, 3H, CH_3), 2.42 (*s*, 3H, CH_3), 2.48 (*s*, 3H, CH_3), 6.72 (*s*, 1H, coumarin C3-H), 7.22-8.86 (*m*, 5H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 20.48, 21.91, 22.20, 116.10, 116.28, 118.40, 123.52, 124.48, 126.63, 131.12, 132.89,

133.45, 133.62, 133.96, 134.25, 134.97, 135.16, 141.95, 142.92, 152.68, 152.72, 153.09, 162.88. LCMS *m/z:* 356 (M+1); Anal.calcd. for $C_{23}H_{17}NO_3$; C, 77.73; H, 4.82; N, 3.94. Found: C, 77.72; H, 4.82; N, 3.94.

7-Hydroxy-4-(6-methyl-furo(2,3-b)quinolin-2-yl)-chromen-2-one (9g):
Recrystallised by EtOH+EtOAc; m.p. 232-233°C; yield 72%; IR (KBr, ν in cm^{-1}): 1710 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.42 (*s*, 3H, CH_3), 6.73 (*s*, 1H, coumarin C3-H), 7.23-8.86 (*m*, 8H, Ar-H), 10.20 (*s*, 1H, OH, D_2O exchangeable). ^{13}C NMR (125 MHz, $CDCl_3$): 21.41, 115.52, 116.23, 117.42, 124.10, 124.14, 126.18, 130.14, 131.99, 132.25, 133.28, 133.78, 134.24, 134.68, 134.76, 141.18, 142.55, 151.24, 151.48, 151.65, 161.82. LCMS *m/z:* 344 (M+1); Anal.calcd. for $C_{21}H_{13}NO_4$; C, 73.46; H, 3.82; N, 4.08. Found: C, 73.45; H, 3.82; N, 4.08.

6-Chloro-4-(6-methyl-furo(2,3-b)quinolin-2-yl)-chromen-2-one (9h):
Recrystallised by EtOH; m.p. 210-211°C; yield 64%; IR (KBr, ν in cm^{-1}): 1709 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.41 (*s*, 3H, CH_3), 6.71 (*s*, 1H, coumarin C3-H), 7.24-8.85 (*m*, 8H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 21.27, 113.14, 114.42, 115.16, 122.72, 122.87, 124.63, 128.25, 131.18, 131.42, 132.53, 132.78, 133.60, 133.78, 133.92, 139.45, 140.85, 150.28, 151.54, 151.80, 161.14. LCMS *m/z:* 362 (M+1); Anal.calcd. for $C_{21}H_{12}ClNO_3$; C, 69.72; H, 3.34; N, 3.87. Found: C, 69.70; H, 3.34; N, 3.87.

7-Chloro-4-(6-methyl-furo(2,3-b)quinolin-2-yl)-chromen-2-one (9i):
Recrystallised by EtOH; m.p. 205-206°C; yield 69%; IR (KBr, ν in cm^{-1}): 1712 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.41 (*s*, 3H, CH_3), 6.72 (*s*, 1H, coumarin C3-H), 7.22-8.82 (*m*, 8H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 21.20, 112.20, 113.42, 115.22, 122.48, 122.75, 124.64, 128.42, 130.50, 131.21, 132.80, 132.90, 134.12, 134.35, 134.80, 139.52, 141.58 151.56, 151.84, 151.98, 160.52. LCMS *m/z:* 362 (M+1); Anal.calcd. for $C_{21}H_{12}ClNO_3$; C, 69.72; H, 3.34; N, 3.87. Found: C, 69.70; H, 3.34; N, 3.87.

6-Bromo-4-(6-methyl-furo(2,3-b)quinolin-2-yl)-chromen-2-one (9j):
Recrystallised by EtOH+Dioxane; m.p. 216-217°C; yield 60%; IR (KBr, ν in cm^{-1}): 1710 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.41 (*s*, 3H, CH_3), 6.71 (*s*, 1H, coumarin C3-H), 7.22-8.78 (*m*, 8H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 21.02, 112.82, 113.54, 114.63, 121.42, 121.98, 124.54, 128.82, 130.65, 131.96, 132.54, 132.87, 133.45, 133.62, 133.68, 139.24, 141.18, 150.64, 151.65, 151.86, 160.42. LCMS *m/z:* 406, 408 (M+, M+2); Anal.calcd. for $C_{21}H_{12}BrNO_3$; C, 62.09; H, 2.98; N, 3.45 Found: C, 62.08; H, 2.98; N, 3.45.

1-(6-Methyl-furo(2,3-b)quinolin-2-yl)-benzo(f)chromen-3-one (9k):
Recrystallised by EtOH; m.p. 196-197°C; yield 71%; IR (KBr, ν in cm^{-1}): 1708 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.42 (*s*, 3H, CH_3), 6.71 (*s*, 1H, coumarin C3-H), 7.10-8.42 (*m*, 11H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 21.4, 110.21, 110.42, 110.58, 110.87, 111.64, 112.57, 113.14, 120.92, 121.01, 121.83, 126.26, 130.14, 130.27, 132.10, 132.43, 133.06, 133.52, 133.67, 138.12, 140.63, 148.14, 150.15, 151.20, 160.42. LCMS *m/z:* 378 (M+1); Anal.calcd. for $C_{25}H_{15}NO_3$; C, 79.56; H, 4.01; N, 3.71 Found: C, 79.54; H, 4.01; N, 3.71.

4-(6-Methyl-furo(2,3-b)quinolin-2-yl)-benzo(h)chromen-2-one (9l):
Recrystallised by EtOH+Ethyl acetate; m.p. 210-211°C; yield 74%; IR (KBr, ν in cm^{-1}): 1716 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.41 (*s*, 3H, CH_3), 6.72 (*s*, 1H, coumarin C3-H), 7.08-8.80 (*m*, 11H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 22.14, 111.16, 111.28, 111.83, 112.06, 112.34, 113.39, 114.14, 121.58, 121.88, 122.61, 127.02, 129.71, 130.10, 132.18, 132.29, 133.51, 133.85, 134.03, 139.68, 140.80, 149.11, 150.86, 151.94, 161.12. LCMS *m/z:* 378 (M+1); Anal.calcd. for $C_{25}H_{15}NO_3$; C, 79.56; H, 4.01; N, 3.71 Found: C, 79.55; H, 4.01; N, 3.71.

4-(6-Chloro-furo(2,3-b)quinolin-2-yl)-6-methoxy-chromen-2-one (10a):
Recrystallised by EtOH+Ethyl acetate ; m.p. 210-211°C; yield 70%; IR (KBr, ν in cm^{-1}): 1710 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.40 (*s*, 3H, C6-OCH_3), 6.71 (*s*, 1H, coumarin C3-H), 7.21-8.20 (*m*, 8H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 56.01, 113.25, 114.16, 116.28, 121.18, 123.85, 124.84, 130.34, 132.15, 133.58, 133.67, 133.91, 134.18, 134.39, 134.95, 140.88, 142.17, 152.04, 152.52, 152.68, 162.14. LCMS *m/z:* 377.5 (M+); Anal.calcd. for $C_{21}H_{12}ClNO_4$; C, 66.77; H, 3.20; N, 3.71. Found: C, 66.69; H, 3.20; N, 3.71.

4-(6-Chloro-furo(2,3-b)quinolin-2-yl)-7-methoxy-chromen-2-one (10b):
Recrystallised by EtOH; m.p. 223-224°C; yield 72%; IR (KBr, ν in cm^{-1}): 1718 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.64 (*s*, 3H, C7-OCH_3), 6.72 (*s*, 1H, coumarin C3-H), 7.22-8.40 (*m*, 8H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 56.16, 114.16, 115.21, 117.16, 122.40, 124.10, 124.87, 130.41, 132.58, 133.62, 133.81, 133.90, 134.02, 134.80, 134.92, 141.04, 142.98, 151.06, 151.80, 151.86, 162.06. LCMS LCMS *m/z:* 377.5 (M+); Anal.calcd. for $C_{21}H_{12}ClNO_4$; C, 66.77; H, 3.20; N, 3.71. Found: C, 66.70; H, 3.20; N, 3.71.

4-(6-Chloro-furo(2,3-b)quinolin-2-yl)-6-methyl-chromen-2-one (10c):

Recrystallised by EtOH+EtOAc; m.p. 201-202°C; yield 68%; IR (KBr, ν in cm^{-1}): 1710 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.40 (*s*, 3H, C6-CH_3), 6.73 (*s*, 1H, coumarin C3-H), 7.24-8.60 (*m*, 8H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 21.40, 114.16, 115.20, 116.01, 121.32, 123.69, 123.90, 130.02, 131.81, 132.16, 134.08, 134.34, 134.60, 134.72, 134.86, 141.52, 142.59, 150.85, 151.16, 152.25, 162.18. LCMS *m/z:* 361 (M+); Anal.calcd. for $C_{21}H_{12}ClNO_3$; C, 69.72; H, 3.34; N, 3.87. Found: C, 69.71; H, 3.34; N, 3.87.

4-(6-Chloro-furo(2,3-b)quinolin-2-yl)-7-methyl-chromen-2-one (10d):
Recrystallised by EtOH; m.p. 196-197°C; yield 76%; IR (KBr, ν in cm^{-1}): 1716 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.62 (*s*, 3H, C7-CH_3), 6.72 (*s*, 1H, coumarin C3-H), 7.24-8.86 (*m*, 8H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 22.74, 113.81, 115.16, 116.42, 122.65, 124.38, 125.94, 129.14, 132.13, 132.44, 133.58, 133.84, 134.76, 134.90, 134.98, 143.14, 143.28, 151.90, 151.99, 152.08, 162.06. LCMS *m/z:* 361 (M+); Anal.calcd. for $C_{21}H_{12}ClNO_3$; C, 69.72; H, 3.34; N, 3.87. Found: C, 69.71; H, 3.34; N, 3.87.

4-(6-Chloro-furo(2,3-b)quinolin-2-yl)-5,7-dimethyl-chromen-2-one (10e):
Recrystallised by EtOH; m.p. 211-212°C; yield 73%; IR (KBr, ν in cm^{-1}): 1708 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.42 (*s*, 3H, CH_3), 2.49 (*s*, 3H, CH_3), 6.72 (*s*, 1H, coumarin C3-H), 6.84 (*s*, 1H, C6-H), 7.21 (*s*, 1H, C8-H), 7.26-8.86 (*m*, 5H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 22.14, 22.29, 116.18, 116.63, 117.84, 123.94, 124.62, 126.14, 131.28, 132.84, 132.98, 133.58, 134.08, 134.96, 135.08, 135.28, 142.06, 142.98, 152.84, 152.97, 153.54, 162.98. LCMS *m/z:* 375 (M+); Anal.calcd. for $C_{22}H_{14}ClNO_3$; C, 70.31; H, 3.75; N, 3.73. Found: C, 70.30; H, 3.75; N, 3.73.

4-(6-Chloro-furo(2,3-b)quinolin-2-yl)-7,8-dimethyl-chromen-2-one (10f):
Recrystallised by EtOH+Dioxane; m.p. 208-209°C; yield 76%; IR (KBr, ν in cm^{-1}): 1713 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.43 (*s*, 3H, CH_3), 2.50 (*s*, 3H, CH_3), 6.73 (*s*, 1H, coumarin C3-H), 7.26-8.84 (*m*, 5H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): 22.08, 22.41, 116.81, 116.92, 118.16, 123.10, 124.12, 125.68, 131.98, 132.60, 133.87, 133.92, 134.18, 134.62, 135.08, 135.23, 142.02, 142.96, 152.23, 152.54, 152.85, 162.91. LCMS *m/z:* 375 (M+); Anal.calcd. for $C_{22}H_{14}ClNO_3$; C, 70.31; H, 3.75; N, 3.73. Found: C, 70.30; H, 3.75; N, 3.73.

4-(6-Chloro-furo(2,3-b)quinolin-2-yl)-7-hydroxy-chromen-2-one (10g):
Recrystallised by EtOH+Dioxane; m.p. 211-212°C; yield 70%; IR (KBr, ν in cm^{-1}): 1712 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.70 (*s*, 1H, coumarin C3-H), 7.25-8.84 (*m*, 8H, Ar-H), 10.12 (*s*, 1H, OH, D_2O exchangeable). ^{13}C NMR (125 MHz, $CDCl_3$): 113.42, 115.10, 116.24, 123.44, 123.98, 126.01, 131.22, 131.63, 132.28, 133.64, 133.92, 134.72, 134.84, 134.92, 141.62, 142.78, 151.38, 151.64, 151.82, 161.18. LCMS *m/z:* 363 (M+); Anal.calcd. for $C_{20}H_{10}ClNO_4$; C, 66.04; H, 2.77; N, 3.85. Found: C, 66.01; H, 2.77; N, 3.85.

6-Chloro-4-(6-chloro-furo(2,3-b)quinolin-2-yl)-chromen-2-one (10h):
Recrystallised by EtOH+Dioxane; m.p. 219-220°C; yield 68%; IR (KBr, ν in cm^{-1}): 1711 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.72 (*s*, 1H, coumarin C3-H), 7.22-8.84 (*m*, 8H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 114.20, 114.60, 115.41, 122.82, 122.98, 124.74, 128.40, 131.56, 131.68, 132.70, 132.85, 133.40, 133.52, 133.86, 139.14, 141.12, 150.01, 151.25, 151.43, 161.32. LCMS *m/z:* 382 (M+); Anal.calcd. for $C_{20}H_9Cl_2NO_3$; C, 62.85; H, 2.37; N, 3.66. Found: C, 62.82; H, 2.37; N, 3.66.

7-Chloro-4-(6-chloro-furo(2,3-b)quinolin-2-yl)-chromen-2-one (10i):
Recrystallised by EtOH; m.p. 214-215°C; yield 66%; IR (KBr, ν in cm^{-1}): 1714 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.70 (*s*, 1H, coumarin C3-H), 7.21-8.80 (*m*, 8H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 111.45, 112.94, 114.12, 122.32, 122.61, 124.22, 127.32, 130.62, 131.04, 132.70, 132.84, 133.92, 134.10, 134.62, 139.80, 141.10 151.40, 151.52, 151.64, 161.18. LCMS *m/z:* 382 (M+); Anal.calcd. for $C_{20}H_9Cl_2NO_3$; C, 62.85; H, 2.37; N, 3.66. Found: C, 62.82; H, 2.37; N, 3.66.

6-Bromo-4-(6-chloro-furo(2,3-b)quinolin-2-yl)-chromen-2-one (10j):
Recrystallised by EtOH; m.p. 186-187°C; yield 64%; IR (KBr, ν in cm^{-1}): 1711 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.70 (*s*, 1H, coumarin C3-H), 7.24-8.74 (*m*, 8H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 113.02, 113.86, 121.02, 121.54, 124.08, 128.14, 130.30, 131.82, 132.63, 133.14, 133.86, 133.95, 134.04, 139.10, 140.82, 150.48, 151.08, 151.24, 160.82. LCMS *m/z:* 425, 427 (M+, M+2); Anal.calcd. for $C_{20}H_9BrClNO_3$; C, 56.30; H, 2.13; N, 3.28 Found: C, 56.28; H, 2.13; N, 3.28.

1-(6-Chloro-furo(2,3-b)quinolin-2-yl)-benzo(f)chromen-3-one (10k):
Recrystallised by EtOH; m.p. 204-205°C; yield 74%; IR (KBr, ν in cm^{-1}): 1705 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.71 (*s*, 1H, coumarin C3-H), 7.14-8.54 (*m*, 11H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 110.32, 111.34, 111.84, 111.92, 112.08, 112.62, 112.98, 121.01, 121.68, 121.86, 125.32, 130.22, 130.45, 132.32, 132.84, 133.15, 133.84, 133.96, 138.10, 140.54, 148.22, 150.38, 151.84, 161.02. LCMS *m/z:* 398 (M+1); Anal.calcd. for $C_{24}H_{12}ClNO_3$; C, 72.46; H, 3.04; N, 3.52 Found: C, 72.43; H, 3.04; N, 3.52.

4-(6-Chloro-furo(2,3-b)quinolin-2-yl)-benzo(h)chromen-2-one (10l):
Recrystallised by EtOH+Ethyl acetate; m.p. 164-165°C; yield 72%; IR (KBr, ν in cm^{-1}): 1708 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.71 (*s*, 1H, coumarin C3-H), 7.10-8.74 (*m*, 11H, Ar-H). ^{13}C NMR (125 MHz, $CDCl_3$): 111.32, 111.82, 111.94, 112.12, 112.44, 113.30, 114.10, 121.41, 121.63, 122.74, 127.80, 129.47, 130.28, 132.64, 132.76, 133.25, 133.42, 134.12, 139.74, 140.88, 149.24, 150.63, 151.98, 161.30. LCMS *m/z:* 398 (M+1); Anal.calcd. for $C_{24}H_{12}ClNO_3$; C, 72.46; H, 3.04; N, 3.52 Found: C, 72.43; H, 3.04; N, 3.52.

Anti-Mycobacterial Activity
The anti-mycobacterial activity of compounds was assessed against *M. tuberculosis* H_{37}RV and *M. phlei* using Microplate Alamar Blue Assay (MABA) [42]. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µL of sterile deionized water was added to all outer perimeter wells of sterile 96-well plate to minimize evaporation of medium in the test wells during incubation. The 96-well plate received 100 µL of the Middlebrook 7H9 broth, and serial dilution of compounds was made directly on plate. The final drug concentrations tested were 100-0.2 µg/mL. Plates were covered and sealed with parafilm and incubated at 37°C for 5 days. After this time, 25 µL of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 were added to the plate and incubated for 24 h. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as lowest drug concentration that prevented the colour change from blue to pink.

RESULTS AND DISCUSSION

In compound 8a (R_1 = 6-OCH_3, R_2 = H), the presence of strong band at 1715 cm^{-1} in the IR spectrum indicated the presence of lactone carbonyl group. Absence of aldehydic stretching band of quinoline at 1686 cm^{-1} confirmed the cyclization. In ^{1}H-NMR spectrum, a singlet at 3.41 ppm corresponding to three protons attributed to the presence of C6 methoxy group. Peak at 6.71 ppm is due to C3-H of coumarin. The remaining protons were resonated as multiplet in between 7.26 to 8.24 ppm. Further, absence of signals for N-H and aldehydic and methylene protons confirmed the annulations. The ^{13}C NMR indicated presence of methoxy carbon with peak at δ 56.50, and absence of methylene carbon and while the carbonyl carbon resonated at δ 161.89. Peaks for remaining carbons were resonated in the aromatic region from δ 115.87 to 151.64, underlined the presence of required aromatic carbon skeleton. Formation of the compound 8a was further supported by appearance of molecular ion peak at *m/z* 343.

CONCLUSION

We have disclosed in the present work the SAR of coumarin-furoquinoline conjugates by synthesis and their preliminary anti-tuberculosis evaluations of 36 structural variants by modifying the groups/substituents on coumarin and furoquinoline. It is interesting to note that, the compounds having the hydroxyl group on 7-position of coumarin (8g), chlorine on coumarin at 6-position (8h, 9h, 10h) and 7-position (8i, 9i, 10i) and bromine at 6-position (8j, 9j, 10j) and chlorine on furoquinoline exhibited potent activity compared to methyl, methoxy, hydroxyl and benzo-substitutions in anti-tuberculosis activities. The potent activity of the compounds 8g and 10h supported by molecular docking studies.

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