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Synthesis and evaluation of c-Src kinase inhibitory activity of pyridin-2(1*H*)-one derivatives

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ABSTRACT

Src kinase, a prototype member of the Src family of kinases (SFKs), is over-expressed in various human tumors, and has become a target for anticancer drug design. In this perspective, a series of eighteen 2-pyridone derivatives were synthesized and evaluated for their c-Src kinase inhibitory activity. Among them, eight compounds exhibited c-Src kinase inhibitory activity with IC_{50} value of less than 25 μM. Compound 1-[2-(dimethylamino)ethyl]-5-(2-hydroxy-4-methoxybenzoyl)pyridin-2(1H)-one (**36**) exhibited the highest c-Src kinase inhibition with an IC_{50} value of 12.5 μM. Furthermore, the kinase inhibitory activity of compound **36** was studied against EGFR, MAPK and PDK, however no significant activity was observed at the highest tested concentration (300 μM). These results provide insights for further optimization of this scaffold for designing the next generation of 2-pyridone derivatives as candidate Src kinase inhibitors.

1. Introduction

Cancer is considered to be a multi-step process, occurring through an accumulation of intrinsic or extrinsic errors in responsible genes for the regulation of cell proliferation and survival. The development of human cancer can be triggered with different genes [1]. Limited abnormalities are sufficient for the development of neoplasia that is an abnormal growth of cells. The alarming increase in the cancer patients worldwide has led an unprecedented pressure on researchers to explore novel active pharmacophores with higher bioactivity, selectivity, and minimal toxicity.

PTKs are enzymes that catalyze the phosphorylation of the hydroxyl groups of tyrosine residues in various proteins by the transfer of the γ-phosphate of the ATP–Mg^{2+} complex to the said amino acid side chain [2]. PTKs are key regulators of various cell functions, such as cellular growth, proliferation, migration, differentiation, and apoptosis [3]. Due to their physiological relevance, variety and ubiquity, PTKs have become a subject of extensive study. Activation of PTKs has been shown to be critical in neoplasia progress [4]. Thus, inhibition of PTKs has become a major strategy in drug design against cancer [3].

The Src family of kinases (SFKs) are non-receptor tyrosine kinases that are involved in signal transduction in cancer cells. c-Src is a member of SFKs which has been reported to induce STATs involved in the tumorigenesis process [5]. STAT3 is a member of signal transducer and activator of transcription protein family that regulates cell growth, survival and differentiation and has been associated with various human cancers. It has been observed that the activity of c-Src kinase in human mammary carcinomas is 4 to 20-fold greater than that in normal cells [6]. Increased Src activity elevates the cell growth rate and reduces adhesion between cells, leading to the development of metastatic potential of cells [7–9]. As a result, c-Src kinase plays an important role in the genesis and progression of human cancers, including carcinomas of the breast, colon, prostate, lung, ovary, and in myeloproliferative disorders [10–12]. Thus, the design and discovery of novel and potent c-Src kinase inhibitors remains critically important.

We have previously designed and synthesized several novel derivatives of benzopyran-2-one (coumarin) [13,14] and benzopyran-4-one (chromone) [15] scaffold and evaluated their antiproliferative and c-Src kinase inhibitory activity. Finding new Src kinase inhibitors remains a challenging task. A more practical approach to such challenges encompasses modification of the structure of

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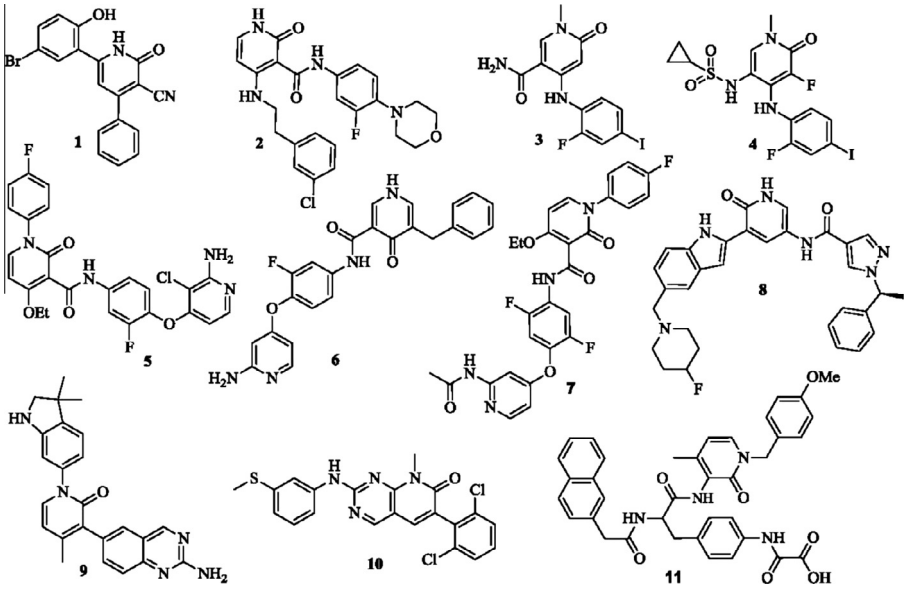


Fig. 1. Some of the 2- and 4-pyridone derivatives active against protein kinases.

existing active pharmacophores. Pyridone skeleton has been known as an ideal frame for further exploration. Recently, myriad pyridone derivatives (Fig. 1) have been tested for their potency against various protein kinases including Pim-1 kinase (**1**) [16], Focal adhesion kinase (FAK) (**2**) [17], MEK1 (**3** & **4**) [18,19], Met kinase (**5**, **6** & **7**) [20–23] and Checkpoint kinase 1 (CHK1) (**8**) [24].

Furthermore, 2-pyridone scaffold has been screened against Src kinases. Some 2-pyridone derivatives such as aryl aminoquinazolinepyridone (**9**) [25], pyrido[2,3-*d*]pyrimidine (**10**) [26,27] and pyrido-propanamide (**11**) [28] have been reported as potent Src kinase inhibitors. Thus, the wealth of information for Src kinases and pyridone skeleton obtained from literature provided a strong rationale for considering inhibition of this target using pyridones to treat cancer. In the light of the above literature reports and in continuation of our efforts to explore new scaffolds as c-Src kinase inhibitors, herein, we report the synthesis and evaluation of c-Src kinase inhibitory activity of a class of novel 2-pyridone derivatives.

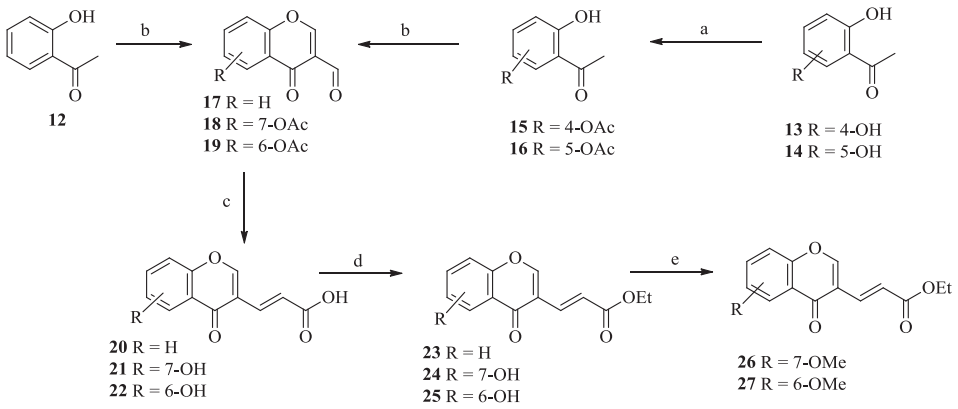
2. Results and discussion

2.1. Chemistry

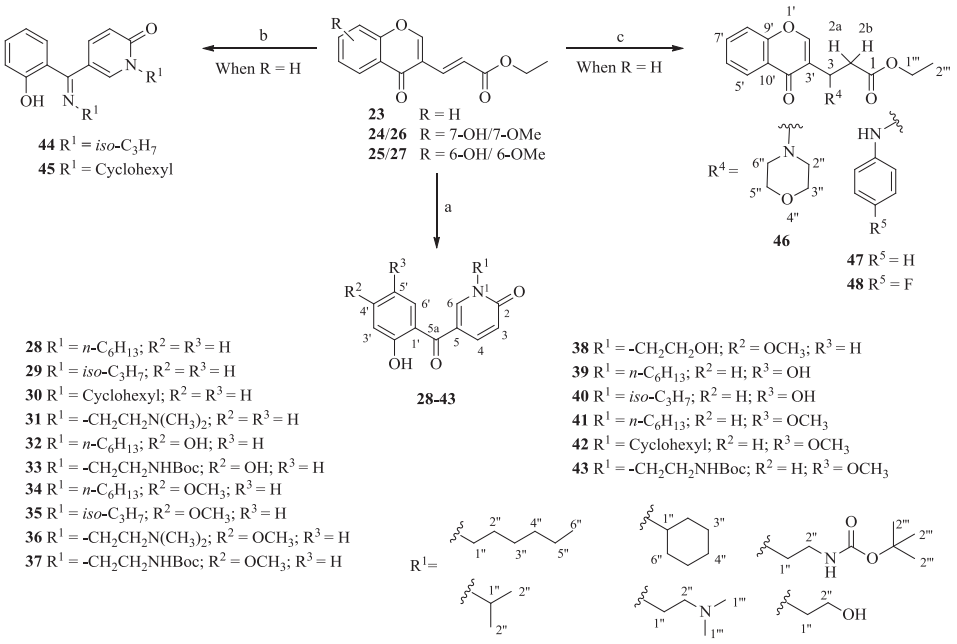
A class of novel 2-pyridone derivatives (**28**–**45**) were synthesized by reacting (*E*)-ethyl 3-(4-oxo-4*H*-chromen-3-yl)acrylates (**23**–**27**) with various alkylamines, *N*,*N*-dialkylaminoalkanes, and *t*-butyl (2-aminoethyl)carbamate in the presence of triethylamine and using ethanol as solvent (Scheme 2).

The key intermediates (**20**–**22**), in turn were synthesized from corresponding hydroxyacetophenones by following the method reported earlier from our group [29]. In the case of dihydroxyacetophenone (**13**/**14**), first mono-*O*-acetylation was carried out using acetic anhydride and pyridine while *o*-hydroxyacetophenone (**12**) was used as such. 4-Oxo-4*H*-1-chromen-3-yl-carbaldehydes (**17**–**19**) were then synthesized using Vilsmeir–Haack formylation reaction. Since *o*-hydroxyacetophenone (**12**) and its derivatives containing various substituents takes a ring form due to H-bonding and thus prohibit enolization, therefore these compounds can be doubly formylated using Vilsmeir–Haack reagent to get 3-formyl substituted chromone derivatives [30]. The formylation reaction was followed by the Knoevenagel condensation with malonic acid to yield the respective 4-oxo-4*H*-chromen-3-yl)acrylic acid (**20**–**22**) (Scheme 1).

The desired pyridone precursors i.e. compound **23**–**25** were obtained by esterification of acrylyl acid derivatives of 4-oxo-4*H*-1-benzopyran (**20**–**22**) with ethanol under acidic condition (Scheme 1). The methylation of phenolic group for compounds **24** and **25** with methyl iodide under basic conditions gave (*E*)-ethyl 3-(7/6-methoxy-4-oxo-4*H*-chromen-3-yl)acrylates (**26**/**27**). All of the compounds were well characterized from their physical and



Scheme 1. Synthesis of (*E*)-alkyl 3-(4-oxo-4*H*-chromen-3-yl)acrylate; Reagents and conditions: a) Ac_2O, pyridine, 6 h; b) $POCl_3$, DMF, 50 °C, 13 h; c) $CH_2(COOH)_2$, pyridine, 1.5 h; d) EtOH, conc. H_2SO_4 (3 or 4 drops), 12 h; e) CH_3I, K_2CO_3, anhyd. acetone, reflux, 12 h.



Scheme 2. Synthesis of pyridin-2(1*H*)-one derivatives; Reagents and conditions: a) R^1NH_2 (1.1 eq), NEt_3, C_2H_5OH, reflux, 8–10 h; b) R^1NH_2 (2.4 eq), NEt_3, C_2H_5OH, reflux, 16–17 h; c) NEt_3, C_2H_5OH, morpholine/aniline/fluoroaniline, reflux, 12–13 h.

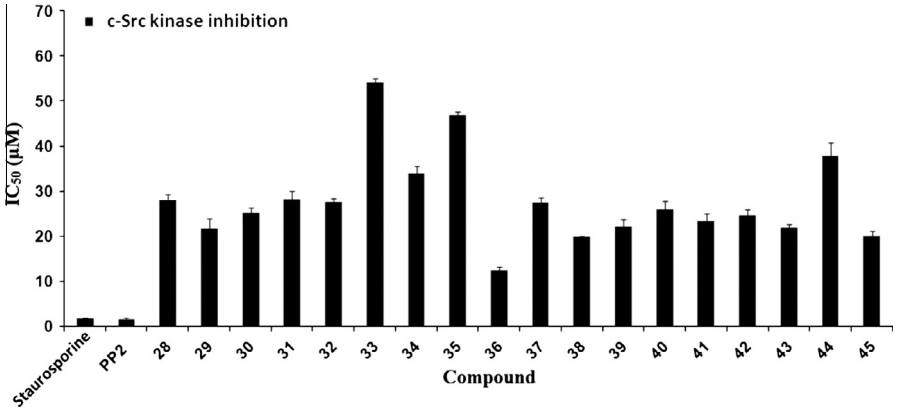


Fig. 2. c-Src Kinase inhibitory activity of 2-pyridone derivatives (**28**–**45**). IC_{50} is the concentration at which the enzyme activity is inhibited by 50% and is calculated from Graph Prism software. All the experiments were carried out in triplicate.

spectral data and by comparing the data with literature value for the known compounds.

The *t*-butyl (2-aminoethyl)carbamate used in the reaction was synthesized according to the literature procedure and characterized by comparing its physical and spectral data with the literature values [31].

When (*E*)-ethyl 3-(4-oxo-4*H*-chromen-3-yl)acrylates (**23**–**27**) were reacted with 1.1 eq. of alkyl amines, then monoalkylated products **28**–**43** were obtained (Scheme 2). However, by reacting ester **23** with alkylamines in the molar ratio of 1:2, dialkyl products **44**–**45** were obtained. Furthermore, secondary and aromatic amines e.g. morpholine, aniline, and fluoroaniline followed a different reaction pathway. For these secondary and aromatic amines we observed the nucleophilic addition of the amine across the double bond of α, β-unsaturated ester. The addition of amine occurred at the β-carbon (C-3) due to electron withdrawing influence of the adjacent carbonyl group of acrylate **23**. Thus, nucleophilic addition gave the compounds **46**–**48** (Scheme 2). The structure of these compounds were confirmed by their ^1H NMR and ^{13}C NMR spectrum, and comparing the data with the theoretical NMR obtained by Mestrenova version 5.3.

2.2. Biology

2.2.1. c-Src kinase inhibitory activity

Fig. 2 shows the c-Src inhibitory potency of all of the pyridin-2(1*H*)-one derivatives (**28**–**45**) compared to a standard protein kinase inhibitor, Staurosporine, and a Src kinase inhibitor, PP2. These compounds exhibited modest c-Src kinase inhibitory activity. Among eighteen compounds, eight were found to have IC_{50} values below 25 μM. The compound 1-[2-(dimethylamino)ethyl]-5-(2-hydroxy-4-methoxybenzoyl)pyridin-2(1*H*)-one (**36**) was found to be the most potent with IC_{50} value of 12.5 μM as shown in Table S1 (Supporting information). The compounds **38** and **45** showed also significant activity with IC_{50} values 19.9 μM and 20.1 μM, respectively.

In general, among all compounds containing pyridin-2(1*H*)-one template, compound **36** having hydroxy and methoxy groups at *meta* positions on the phenyl ring and the pyridone ring linked to 2-(dimethylamino)ethyl group was found to be the most potent. The presence of the dimethylamine was found to be important since other derivatives with similar structures including compounds **34**, **35**, and **37** showed higher IC_{50} values of 34.1, 47.0,

Table 1
Inhibitory activity of compound **36** against other kinases.

Kinase	IC_{50} (μM)
EGFR(h)	>300
MAPK1(h)	>300
PDK1(h)	>300

and 27.6 μM, respectively. The nature of substituent in the phenyl ring was found to be critical i.e. the methoxy group on the phenyl ring appeared to be involved in contributing to Src kinase inhibitory activity. The compound **31** that lacked a methoxy group showed higher IC_{50} value (28.2 μM) when compared with the corresponding methoxy analog **36**. Similarly, while comparing the compounds **33** and **37** which differed in terms of presence of hydroxyl and methoxy groups, respectively, at C-4 position of phenyl ring, higher c-Src kinase inhibitory activity was observed for compound **37** (IC_{50}: 27.6 μM) in comparison to compound **33** (IC_{50}: 57.8 μM). Also, by comparing the IC_{50} values of pyridones reported herein with that of coumarins [13] and chromones [15] published earlier from our group, it was observed that 2-pyridones have significantly higher c-Src kinase inhibitory activities.

2.2.2. Inhibitory activity aganist EGFR, MAPK and PDK

In order to further explore the selectivity of compound **36** (most active compound for c-Src kinase inhibition) against other kinases, three different kinases namely Epidermal Growth Factor Receptor (EGFR), Mitogen-Activated Protein Kinase (MAPK) and Phosphoinositide-Dependent Kinase (PDK) were chosen as the target. It was observed that the compound **36** did not show any kinase inhibition at the highest tested concentration of 300 μM and thus it can be established that this compound has selective activity against c-Src kinase (1).

Also, in continuation of our efforts to get further insights about the various 2-pyridone derivatives synthesized, the antiproliferative screening was carried out. However, the majority of compounds did not show significant antiproliferative potency compared to the positive control (Dox) at the concentration of 50 μM after 72 h incubation as shown in Fig. S1 (Supporting information). Compounds **35** and **41** exhibited noticeable inhibition potency with the proliferation of CCRF-CEM cells by 35% and 53%, respectively. Furthermore, compounds **33**, **38**, and **43** exhibited modest inhibitory activities in CCRF-CEM cells by 27%, 27%, and 26%, respectively, after 72 h incubation. However, these compounds did not exhibit a noticeable inhibition of the proliferation of SK-OV-3 and MCF-7 cells. These data indicate that there is a weak correlation between Src kinase inhibition and antiproliferative activity, presumably because of limited cellular uptake and contribution of other mechanisms in antiproliferative activity of these compounds.

3. Conclusion

In summary, a total of twenty one compounds including eighteen 2-pyridone derivatives and three of chromone derivatives were synthesized and fully characterized by ^{1}H NMR, ^{13}C NMR, UV, FT-IR, and high resolution mass spectroscopy (HRMS). Nineteen compounds *i.e.* **28**–**33**, and **36**–**48** are novel. Although compounds **34** and **35** were known in literature, their complete spectral data were not reported. Herein, we have reported the spectral data for all of the compounds in the experimental section. All of the synthesized 2-pyridones were evaluated for c-Src kinase inhibitory activity. Preliminary results showed that eight compounds exhibited relatively modest c-Src kinase inhibitory activities with IC_{50} values less than 25 μM. Among all of the 2-pyridone derivatives, compound **36** was found to be the most potent c-Src kinase inhibitor (IC_{50}: 12.5 μM), however it did not exhibit kinase inhibition activity against three other kinases studied namely EGFR, MAPK and PDK at the highest tested concentration of 300 μM. In the antiproliferative activity assay, a modest inhibition potency was exhibited by compounds **35** and **41** with the proliferation of CCRF-CEM cells by 35% and 53% respectively. However, none of the compounds synthesized have any significant antiproliferative activity against SK-OV-3 and MCF-7 cells, thus establishing a weak correlation between Src kinase inhibition and antiproliferative activity. Structure-activity relationship of 2-pyridone derivatives for Src kinase inhibition has not been studied extensively, hence these results can be used for further optimization of 2-pyridones for designing and investigation of the potentiality of these compounds as the lead potent and selective Src kinase inhibitors.

4. Experimental section

4.1. Materials and methods

The organic solvents were dried and distilled prior to their use. Reactions were monitored by precoated TLC plates (Merck silica gel $60F_{254}$); the spots were visualized either by UV light, or by spraying with 5% alcoholic $FeCl_3$ solution. Silica gel (100–200 mesh) was used for column chromatography. All of the chemicals and reagents were procured from Spectrochem Pvt. Ltd., India and Sigma–Aldrich Chemicals Pvt. Ltd., USA. Melting points were measured on a Buchi M-560 apparatus and are uncorrected. Infrared spectra were recorded on Perkin-Elmer FT-IR model 9 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on Jeol-400 (400 MHz, 100.5 MHz) NMR spectrometer and Avance-300 (300 MHz, 75.5 MHz) spectrometer using tetramethylsilane as internal standard. The chemical shift values are on a δ scale and the coupling constant values (J) are in Hertz. The UV data were recorded on Analytik Jena SPECORD 250 and Perkin-Elmer Lambda 35. The HRMS data were recorded on Agilent-6210 ES-TOF, JEOL JMX-SX-102A and Waters LCT Micromass-KC455.

4.2. Chemistry

*4.2.1. General procedure for the synthesis of N-substituted pyridone derivatives (**28**–**43**)*

To a solution of (4-oxo-4*H*-chromen-3-yl)acrylate (**23**–**27**) (4 mmol) and aminoalkane/diaminoalkane/*t*-butylaminoethylcarbamate (4.4 mmol) in ethanol (70 mL) was added triethylamine (2 drops), and the reaction mixture was refluxed for 8–10 h. The progress of reaction was monitored on TLC. On completion of reaction, the mixture was cooled to room temperature, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography over silica gel (100–200 mesh) in 20–40% ethyl acetate/petroleum ether to give 2-pyridone derivatives (**28**–**43**) in 74–85% yield.

*4.2.1.1. 1-Hexyl-5-(2-hydroxybenzoyl)pyridin-2(1H)-one (**28**).* The reaction of (*E*)-ethyl 3-(4-oxo-4*H*-chromen-3-yl)acrylate (**23**) (0.98 g, 4 mmol) with hexylamine (0.45 g, 4.4 mmol) gave the title compound **28** as a light yellow solid (0.97 g, 81%) by following the general procedure: mp = 73 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.89 (t, 3H, J = 6.6 Hz, H-6″), 1.34 (*brs*, 6H, H-5″, H-4″ & H-3″), 1.76–1.81 (m, 2H, H-2″), 3.99 (t, 2H, J = 7.3 Hz, H-1″), 6.61 (d, 1H, J = 9.6 Hz, H-3′), 6.94 (t, 1H, J = 7.5 Hz, H-4′), 7.07 (d, 1H, J = 8.1 Hz, H-3), 7.50–7.58 (m, 2H, H-5′ & H-6′), 7.76 (dd, 1H, J = 2.4 & 9.6 Hz, H-4), 7.93 (d, 1H, J = 2.4 Hz, H-6), 11.43 ppm (s, 1H, OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 13.92, 22.42, 26.23, 29.63, 31.26, 50.71, 117.01, 118.67, 118.83, 119.91, 131.51, 136.09, 138.84, 143.41,

161.93, 162.33, 195.17 ppm; IR (KBr): ν_{max} = 3424 (O—H str), 3063, 2956, 1669 (C=O), 1624, 1483, 1337, 1247, 1137, 838, 760, 638 cm^{-1}; UV (MeOH): λ_{max} = 293 nm; HRMS: *m/z* $[M + Na]^+$ calcd for $C_{18}H_{21}NO_3$: 322.1419, found: 322.1418.

*4.2.1.2. 5-(2-Hydroxybenzoyl)-1-isopropylpyridin-2(1H)-one (**29**).* The reaction of (*E*)-ethyl 3-(4-oxo-4*H*-chromen-3-yl)acrylate (**23**) (0.98 g, 4 mmol) with isopropylamine (0.26 g, 4.4 mmol) gave the title compound **29** as a light yellow solid (0.81 g, 79%) by following the general procedure: mp = 109 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38 (d, 6H, *J* = 6.8 Hz, H-2″), 5.25–5.28 (m, 1H, H-1″), 6.58 (d, 1H, *J* = 9.2 Hz, H-3'), 6.91 (t, 1H, *J* = 7.6 Hz, H-4′), 7.05 (d, 1H, *J* = 8.4 Hz, H-3), 7.47–7.53 (m, 2H, H-5′ & H-6′), 7.70 (d, 1H, *J* = 9.6 Hz, H-4), 7.97 (s, 1H, H-6), 11.44 ppm (s, 1H, OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 22.05, 47.50, 117.46, 118.85, 118.96, 118.97, 119.74, 131.58, 136.20, 138.30, 139.45, 161.78, 162.54, 195.28 ppm; IR (KBr): ν_{max} = 3336 (O—H str), 3053, 2981, 1658 (C=O), 1621, 1589, 1440, 1338, 1245, 1135, 763, 640 cm^{-1}; UV (MeOH): λ_{max} = 297 nm; HRMS: *m/z* $[M + Na]^+$ calcd for $C_{15}H_{15}NO_3$: 280.0950, found: 280.0949.

*4.2.1.3. 1-Cyclohexyl-5-(2-hydroxybenzoyl)pyridin-2(1H)-one (**30**).* The reaction of (*E*)-ethyl 3-(4-oxo-4*H*-chromen-3-yl)acrylate (**23**) (0.98 g, 4 mmol) with cyclohexylamine (0.44 g, 4.4 mmol) gave the title compound **30** as a light yellow solid (0.90 g, 76%) by following the general procedure: mp = 101 °C; ^{1}H NMR (300 MHz; $CDCl_3$): δ = 1.18–1.78 (m, 6H, H-3″, H-4″ & H-5″), 1.90–2.00 (m, 4H, H-2″ & H-6″), 4.89 (m, 1H, H-1″), 6.60 (d, 1H, *J* = 9.2 Hz, H-3′), 6.94 (t, 1H, *J* = 7.6 Hz, H-4′), 7.08 (d, 1H, *J* = 8.2 Hz, H-3), 7.50–7.54 (m, 2H, H-5′ & H-6′), 7.72 (dd, 1H, *J* = 2.8 & 9.6 Hz, H-4), 7.99 (d, 1H, *J* = 2.3 Hz, H-6), 11.48 ppm (s, 1H, OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 25.38, 25.88, 32.83, 54.98, 117.34, 118.96, 119.06, 119.09, 119.75, 131.71, 136.29, 138.34, 140.15, 161.93, 162.69, 195.61 ppm; IR (KBr): ν_{max} = 3430 (O—H str), 3047, 2921, 1629 (C=O), 1570, 1533, 1451, 1096, 974, 850, 750 cm^{-1}; UV (MeOH): λ_{max} = 265 and 305 nm; HRMS: *m/z* $[M + H]^+$ calcd for $C_{18}H_{19}NO_3$: 298.1443, found: 298.1460.

*4.2.1.4. 1-[2-(Dimethylamino)ethyl]-5-(2-hydroxybenzoyl)pyridin-2(1H)-one (**31**).* The reaction of (*E*)-ethyl 3-(4-oxo-4*H*-chromen-3-yl)acrylate (**23**) (0.98 g, 4 mmol) with N^1,N^1-dimethylethane-1, 2-diamine (0.39 g, 4.4 mmol) gave the title compound **31** as a light yellow solid (0.94 g, 82%) by following the general procedure: mp = 147 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.30 (s, 6H, H-1‴), 2.66 (t, 2H, *J* = 5.4 Hz, H-2″), 4.05 (t, 2H, *J* = 5.4 Hz, H-1″), 6.61 (d, 1H, *J* = 9.3 Hz, H-3′), 6.91 (t, 1H, *J* = 7.5 Hz, H-4′), 7.07 (d, 1H, *J* = 8.4 Hz, H-3), 7.51 (t, 1H, *J* = 7.5 Hz, H-5′), 7.70 (d, 1H, *J* = 7.5 Hz, H-6′), 7.82 (dd, 1H, *J* = 2.1 & 9.6 Hz, H-4), 7.99 (d, 1H, *J* = 1.8 Hz, H-6), 11.53 ppm (s, 1H, OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 45.50, 47.27, 57.79, 116.11, 118.64, 118.69, 119.03, 119.82, 131.81, 135.98, 138.98, 145.08, 161.98, 162.53, 195.24 ppm; IR (KBr): ν_{max} = 3430 (O—H str), 3048, 1666 (C=O), 1624, 1590, 1336, 1246, 1174, 1138, 1048, 855, 764, 699 cm^{-1}; UV (MeOH): λ_{max} = 268 & 310 nm; HRMS: *m/z* $[M + H]^+$ calcd for $C_{16}H_{18}N_2O_3$: 287.1396, found: 287.1398.

*4.2.1.5. 5-(2,4-Dihydroxybenzoyl)-1-hexylpyridin-2(1H)-one (**32**).* The reaction of (*E*)-ethyl 3-(7-hydroxy-4-oxo-4*H*-chromen-3-yl)acrylate (**24**) (1.04 g, 4 mmol) with hexylamine (0.45 g, 4.4 mmol) gave the title compound **32** as a light yellow solid (1.07 g, 85%) by following the general procedure: mp = 166–168 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.81 (t, 3H, *J* = 6.8 Hz, H-6″), 1.23 (brm, 6H, H-3″–H-5″), 1.58–1.61 (m, 2H, H-2″), 3.91 (t, 2H, *J* = 7.2 Hz, H-1″), 6.32–6.36 (m, 2H, H-5′ & H-3′), 6.41 (d, 1H, *J* = 9.2 Hz, H-3), 7.38 (d, 1H, *J* = 8.4 Hz, H-6′), 7.67 (dd, 1H, *J* = 2.4 & 9.6 Hz, H-4), 8.18 (d, 1H, *J* = 2.4 Hz, H-6), 10.45 (brs, 1H, OH); 11.33 ppm (brs, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ = 14.40, 22.52, 26.12, 29.12, 31.34, 49.70, 103.30, 108.40, 114.22, 117.27, 119.07, 134.25, 139.58, 144.97, 161.76, 162.59, 164.24, 192.93 ppm; IR (KBr): ν_{max} = 3200 (O—H str), 2929, 2854, 1663 (C=O), 1629, 1602, 1432, 1340, 1236, 1117, 846, 721, 597, 554, 467 cm^{-1}; UV ($CHCl_3$): λ_{max} = 284, 298 and 333 nm; HRMS: *m/z* $[M + Na]^+$ calcd for $C_{18}H_{21}NO_4$: 338.1368, found: 338.1378.

*4.2.1.6. t-Butyl [2-{5-(2,4-dihydroxybenzoyl)-2-oxopyridin-1(2H)-yl}ethyl]carbamate (**33**).* The reaction of (*E*)-ethyl 3-(7-hydroxy-4-oxo-4*H*-chromen-3-yl)acrylate (**24**) (1.04 g, 4 mmol) with *t*-butyl (2-aminoethyl)carbamate (0.70 g, 4.4 mmol) gave the title compound **33** as a light yellow solid (1.23 g, 82%) by following the general procedure: mp = 180–182 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.24 (s, 9H, H-2‴), 3.24–3.25 (m, 2H, H-2″), 3.96 (t, 2H, *J* = 4.8 Hz, H-1″), 6.34–6.40 (m, 2H, H-3′ & H-5′), 6.45 (d, 1H, *J* = 9.5 Hz, H-3), 6.95 (t, 1H, *J* = 5.92 Hz, CONH, D_2O exchanged), 7.49 (d, 1H, *J* = 8.8 Hz, H-6′), 7.72 (dd, 1H, *J* = 2.2 & 9.5 Hz, H-4), 7.99 (d, 1H, *J* = 2.2 Hz, H-6) 10.52 (brs, 1H, OH, D_2O exchanged), 11.54 ppm (brs, 1H, OH, D_2O exchanged); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ = 28.04, 38.25, 49.62, 77.93, 102.86, 107.95, 113.16, 116.09, 118.79, 133.95, 139.10, 145.20, 155.71, 161.39, 162.66, 163.94, 192.49 ppm; IR (KBr): ν_{max} = 3368 (O—H str), 2976, 2698, 1686 (NHCOO—), 1647 (C=O), 1586, 1521, 1335, 1271, 1173, 851, 619, 584, cm^{-1}; UV ($CHCl_3$): λ_{max} = 288 and 334 nm; HRMS: *m/z* $[M + H]^+$ and $[M + K]^+$ calcd for $C_{19}H_{22}N_2O_6$: 375.1556 and 413.1115, found: 375.1482 and 413.1033 respectively.

*4.2.1.7. 1-Hexyl-5-(2-hydroxy-4-methoxybenzoyl)pyridin-2(1H)-one (**34**)* [32]. The reaction of (*E*)-ethyl 3-(7-methoxy-4-oxo-4*H*-chromen-3-yl)acrylate (**26**) (1.1 g, 4 mmol) with hexylamine (0.45 g, 4.4 mmol) gave the title compound **34** as a light yellow solid (1.11 g, 84%) by following the general procedure: mp = 72–74 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.85 (t, 3H, *J* = 7.3 Hz, H-6″), 1.28–1.33 (m, 6H, H-3″–H-5″), 1.72–1.76 (m, 2H, H-2″), 3.83 (s, 3H, OCH_3), 3.95 (t, 3H, *J* = 7.7 Hz, H-1″), 6.43 (dd, 1H, *J* = 2.2 & 8.8 Hz, H-5′), 6.47 (d, 1H, *J* = 2.2 Hz, H-3′), 6.55 (d, 1H, *J* = 9.5 Hz, H-6′), 7.45 (d, 1H, *J* = 8.8 Hz, H-3), 7.66 (dd, 1H, *J* = 2.9 & 9.5 Hz, H-4), 7.83 (d, 1H, *J* = 2.2 Hz, H-6), 12.19 ppm (brs, 1H, OH); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 13.89, 22.40, 26.21, 29.17, 31.23, 50.58, 55.59, 101.32, 107.48, 112.49, 117.28, 119.79, 133.24, 138.83, 142.50, 161.92, 165.67, 166.03, 193.87 ppm; IR (KBr): ν_{max} = 3427 (O—H str), 2929, 2258, 1675 (C=O), 1628, 1583, 1347, 1285, 1159, 1026, 828, 784, 622 cm^{-1}; UV ($CHCl_3$): λ_{max} = 290 and 337 nm; HRMS: *m/z* $[M + H]^+$ calcd for $C_{19}H_{23}NO_4$: 330.1705, found: 330.1628.

*4.2.1.8. 5-(2-Hydroxy-4-methoxybenzoyl)-1-isopropylpyridin-2(1H)-one (**35**)* [33]. The reaction of (*E*)-ethyl 3-(7-methoxy-4-oxo-4*H*-chromen-3-yl)acrylate (**26**) (1.1 g, 4 mmol) with isopropyl amine (0.26 g, 4.4 mmol) gave the title compound **35** as a light yellow solid (0.95 g, 83%) by following the general procedure: mp = 141–143 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.37 (d, 6H, *J* = 6.6 Hz, H-2″), 3.83 (s, 3H, OCH_3), 5.21–5.28 (m, 1H, H-1″), 6.42–6.45 (m, 1H, H-5′), 6.48 (d, 1H, *J* = 2.2 Hz, H-3′), 6.55 (d, 1H, *J* = 9.5 Hz, H-6′), 7.43 (d, 1H, *J* = 8.8 Hz, H-3), 7.64 (dd, 1H, *J* = 2.9 & 9.5 Hz, H-4), 7.89 (d, 1H, *J* = 2.9 Hz, H-6), 11.57 ppm (brs, 1H, OH); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 21.88, 47.17, 55.59, 101.36, 107.44, 112.48, 117.60, 119.48, 133.18, 138.15, 138.36, 161.63, 165.69, 166.01, 194.03 ppm; IR (KBr): ν_{max} = 3462 (O—H str), 2982, 2851, 1664 (C=O), 1617, 1588, 1438, 1348, 1262, 1211, 1112, 937, 836, 606 cm^{-1}; UV ($CHCl_3$): λ_{max} = 283, 294 and 336 nm; HRMS: *m/z* $[M + H]^+$ calcd for $C_{16}H_{17}NO_4$: 288.1236, found: 288.1159.

*4.2.1.9. 1-[2-(Dimethylamino)ethyl]-5-(2-hydroxy-4-methoxybenzoyl) pyridin-2(1H)-one (**36**).* The reaction of (*E*)-ethyl 3-(7-methoxy-

4-oxo-4*H*-chromen-3-yl)acrylate (**26**) (1.1 g, 4 mmol) with N^1,N^1-dimethylethane-1,2-diamine (0.39 g, 4.4 mmol) gave the title compound **36** as a light yellow solid (1.03 g, 81%) by following the general procedure: mp = 150–152 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.27 (s, 6H, H-1‴), 2.63 (t, 2H, *J* = 5.5 Hz, H-2″), 3.84 (s, 3H, OCH_3), 4.02 (t, 2H, *J* = 5.5 Hz, H-1″), 6.41 (dd, 1H, *J* = 2.9 & 8.8 Hz, H-5′), 6.48 (d, 1H, *J* = 2.9 Hz, H-3′), 6.58 (d, 1H, *J* = 9.5 Hz, H-6′), 7.61 (d, 1H, *J* = 8.8 Hz, H-3), 7.73 (dd, 1H, *J* = 3.0 & 9.5 Hz, H-4), 7.88 (d, 1H, *J* = 2.2 Hz, H-6), 12.30 ppm (brs, 1H, OH); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 45.43, 47.09, 55.58, 57.71, 101.12, 107.38, 112.57, 116.25, 119.66, 133.47, 138.98, 144.07, 161.91, 165.76, 165.92, 193.87 ppm; IR (KBr): ν_{max} = 3427 (O—H str), 2944, 2778, 1669 (C=O), 1616, 1440, 1344, 1261, 1116, 1027, 921, 818, 608, 588 cm^{-1}; UV ($CHCl_3$): λ_{max} = 286 and 331 nm; HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{17}H_{20}N_2O_4$: 317.1501, found: 317.1425.

*4.2.1.10. t-Butyl [2-{5-(2-hydroxy-4-methoxybenzoyl)-2-oxopyridin-1(2H)-yl}ethyl]carbamate (**37**).* The reaction of (*E*)-ethyl 3-(7-methoxy-4-oxo-4*H*-chromen-3-yl)acrylate (**26**) (1.1 g, 4 mmol) with *t*-butyl (2-aminoethyl)carbamate (0.70 g, 4.4 mmol) gave the title compound **37** as a light yellow solid (1.29 g, 83%) by following the general procedure: mp = 164–166 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.31 (s, 9H, H-2‴), 3.44–3.46 (m, 2H, H-2″), 3.81 (s, 3H, OCH_3), 4.09 (brs, 2H, H-1″), 5.17 (brs, 1H, CONH), 6.41 (brm, 1H, H-5′), 6.43–6.45 (m, 1H, H-3′), 6.53 (d, 1H, *J* = 8.8 Hz, H-6′), 7.48 (d, 1H, *J* = 8.8 Hz, H-3), 7.68 (d, 1H, *J* = 9.6 Hz, H-4), 7.80 (d, 1H, *J* = 2.2 Hz, H-6), 12.14 ppm (brs, 1H, OH); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 28.14, 39.38, 49.98, 55.57, 79.73, 101.36, 107.51, 112.45, 117.36, 119.73, 133.35, 139.31, 143.23,156.03,162.19, 165.59, 166.04, 193.62 ppm; IR (KBr): ν_{max} = 3363 (O—H str), 2979, 2962, 1663 (NHCOO—), 1627 (C=O), 1508, 1436, 1342, 1291, 1160, 857, 776, 621, cm^{-1}; UV ($CHCl_3$): λ_{max} = 285 and 332 nm; HRMS: *m*/*z* $[M+H]^+$ and $[M+Na]^+$ calcd for $C_{20}H_{24}N_2O_6$: 389.1713 and 411.1532, found: 389.1654 and 411.1486 respectively.

*4.2.1.11. 5-(2-Hydroxy-4-methoxybenzoyl)-1-(2-hydroxyethyl)pyridin-2(1H)-one (**38**).* The reaction of (*E*)-ethyl 3-(7-methoxy-4-oxo-4*H*-chromen-3-yl)acrylate (**26**) (1.1 g, 4 mmol) with ethanolamine (0.27 g, 4.4 mmol) gave the title compound **38** as a light yellow solid (0.88 g, 76%) by following the general procedure: mp = 176–178 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.60 (brm, 2H, H-2″), 3.77 (s, 3H, OCH_3), 3.99 (t, 2H, *J* = 5.5 Hz, H-1″), 4.93 (*brs*, 1H, OH, D_2O exchanged), 6.43–6.47 (m, 2H, H-5′ & H-3′), 6.49 (brs, 1H, H-6′), 7.49 (d, 1H, *J* = 8.0 Hz, H-3), 7.71 (dd, 1H, *J* = 2.2 & 9.5 Hz, H-4), 8.11 (d, 1H, *J* = 2.2 Hz, H-6), 11.32 ppm (brs, 1H, OH, D_2O exchanged); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ = 51.79, 55.57, 58.48,101.42, 106.45, 115.17, 115.97, 118.43, 133.10, 139.16, 146.02,161.36, 161.45, 164.37, 192.33 ppm; IR (KBr): ν_{max} = 3336 (O—H str), 2917, 1660 (C=O), 1623 (C=O), 1577, 1351, 1290, 1163, 1023, 832, 790, 625, cm^{-1}; UV ($CHCl_3$): λ_{max} = 287 and 333 nm; HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{15}H_{15}NO_5$: 290.1028, found: 290.0988.

*4.2.1.12. 5-(2,5-Dihydroxybenzoyl)-1-hexylpyridin-2(1H)-one (**39**).* The reaction of (*E*)-ethyl 3-(6-hydroxy-4-oxo-4*H*-chromen-3-yl)acrylate (**25**) (1.04 g, 4 mmol) with hexylamine (0.45 g, 4.4 mmol) gave the title compound **39** as a light yellow solid (1.02 g, 81%) by following the general procedure: mp = 87–89 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.85 (t, 3H, *J* = 5.8 Hz, H-6″), 1.26 (brm, 6H, H-5″, H-4″ & H-3″), 1.58–1.62 (m, 2H, H-2″), 3.94 (t, 2H, *J* = 7.3 Hz, H-1″), 6.44 (d, 1H, *J* = 9.5 Hz, H-3), 6.67 (d, 1H, *J* = 2.9 Hz, H-6′), 6.79 (d, 1H, *J* = 8.0, Hz, H-3′), 6.83 (dd, 1H, *J* = 2.9 & 8.8 Hz, H-4′), 7.73 (dd, 1H, *J* = 2.4 & 9.6 Hz, H-4), 8.19 (d, 1H, *J* = 2.9 Hz, H-6), 9.07 (brs, 1H, OH); 9.46 ppm (brs, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ = 13.90, 21.98, 25.54, 28.69, 30.81, 49.18, 115.26, 116.87, 117.59, 118.39, 119.94, 125.03, 138.68, 145.64, 148.33, 149.85, 161.40, 191.66 ppm; IR (KBr): ν_{max} = 3285 (O—H str), 2921, 2854, 1664 (C=O), 1602, 1446, 1208, 1137, 994, 831, 790, 644, 551 cm^{-1}; UV ($CHCl_3$): λ_{max} = 281 and 328 nm; HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{18}H_{21}NO_4$: 316.1549, found: 316.1588.

*4.2.1.13. 5-(2,5-Dihydroxybenzoyl)-1-isopropylpyridin-2(1H)-one (**40**).* The reaction of (*E*)-ethyl 3-(6-hydroxy-4-oxo-4*H*-chromen-3-yl)acrylate (**25**) (1.04 g, 4 mmol) with isopropyl amine (0.26 g, 4.4 mmol) gave the title compound **40** as a light yellow solid (0.87 g, 80%) by following the general procedure: mp = 158–160 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.28 (d, 6H, *J* = 6.6 Hz, H-2″), 4.96–5.03 (m, 1H, H-1″), 6.45 (d, 1H, *J* = 9.5 Hz, H-3), 6.70 (d, 1H, *J* = 2.2 Hz, H-6′), 6.80 (d, 1H, *J* = 8.8 Hz, H-3′), 6.84 (dd, 1H, *J* = 2.9 & 8.8 Hz, H-4′), 7.71 (dd, 1H, *J* = 2.9 & 9.5 Hz, H-4), 8.10 (d, 1H, *J* = 2.9 Hz, H-6), 9.08 (brs, 1H, OH), 9.54 ppm (brs, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ = 21.06, 21.20, 47.49, 115.44, 116.98, 117.62, 118.40, 120.23, 124.85, 138.09, 141.36, 148.37, 149.95,161.05, 191.54 ppm; IR (KBr): ν_{max} = 3417 (O—H str), 3057, 2986, 1654, (C=O), 1612 (C=O), 1571, 1438, 1350, 1236, 1140, 795, 643, 574 cm^{-1}; UV ($CHCl_3$): λ_{max} = 286 and 337 nm; HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{15}H_{15}NO_4$: 274.1079, found: 274.1009.

*4.2.1.14. 1-Hexyl-5-(2-hydroxy-5-methoxybenzoyl)pyridin-2(1H)-one (**41**).* The reaction of (*E*)-ethyl 3-(6-methoxy-4-oxo-4*H*-chromen-3-yl)acrylate (**27**) (1.1 g, 4 mmol) with hexylamine (0.45 g, 4.4 mmol) gave the title compound **41** as a light yellow solid (1.12 g, 85%) by following the general procedure: mp = 60–62 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86 (t, 3H, *J* = 6.8 Hz, H-6″), 1.28–1.36 (m, 6H, H-3″-H-5″), 1.72–1.79 (m, 2H, H-2″), 3.74 (s, 3H, OCH_3), 3.96 (t, 2H, *J* = 7.3 Hz, H-1″), 6.57 (d, 1H, *J* = 9.2 Hz, H-3), 6.97–7.00 (m, 2H, H-3′ & H-6′), 7.12 (dd, 1H, *J* = 2.8 & 8.7 Hz, H-4′), 7.73 (dd, 1H, *J* = 2.9 & 9.5 Hz, H-4), 7.93 (d, 1H, *J* = 2.9 Hz, H-6), 10.88 ppm (brs, 1H, OH); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 14.05, 22.51, 26.37, 29.44, 31.43, 50.85, 56.04, 114.09, 117.17, 118.67, 119.63,120.06, 123.47, 138.86, 143.48, 151.75, 156.53,162.05, 194.89 ppm; IR (KBr): ν_{max} = 3235 (O—H str), 2930, 2858, 1654 (C=O), 1599, 1422, 1316, 1286, 1213, 1131, 1039, 837, 803, 646, 423 cm^{-1}; UV ($CHCl_3$): λ_{max} = 289 and 330 nm; HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{19}H_{23}NO_4$: 330.1705, found: 330.1629.

*4.2.1.15. 1-Cyclohexyl-5-(2-hydroxy-5-methoxybenzoyl)pyridin-2(1H)-one (**42**).* The reaction of (*E*)-ethyl 3-(6-methoxy-4-oxo-4*H*-chromen-3-yl)acrylate (**27**) (1.1 g, 4 mmol) with cyclohexylamine (0.44 g, 4.4 mmol) gave the title compound **42** as a light yellow solid (1.09 g, 83%) by following the general procedure: mp = 105–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.18–1.74 (m, 6H, H-3″–H-5″), 1.86–1.96 (m, 4H, H-2″ & H-6″), 3.72 (s, 3H, OCH_3), 4.82–4.88 (m, 1H, H-1″), 6.56 (d, 1H, *J* = 9.5 Hz, H-3), 6.94 (d, 1H, *J* = 2.9 Hz, H-6′), 6.95–6.98 (m, 1H, H-3′), 7.09 (dd, 1H, *J* = 2.2 & 8.8 Hz, H-4′), 7.70 (dd, 1H, *J* = 2.2 & 9.5 Hz, H-4), 7.98 (d, 1H, *J* = 2.2 Hz, H-6), 10.93 ppm (brs, 1H, OH); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 25.08, 25.55, 32.54, 54.65, 55.77, 114.09, 117.02, 118.43, 119.48, 123.65, 137.96, 139.79, 151.55, 156.42,161.58, 194.82 ppm; IR (KBr): ν_{max} = 3437 (O—H str), 3061, 2941, 1667 (C=O), 1626, 1587, 1442, 1276, 1167, 1123, 1026, 846, 789, 626 cm^{-1}; UV ($CHCl_3$): λ_{max} = 283 and 337 nm; HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{19}H_{21}NO_4$: 328.1549, found: 328.1488.

*4.2.1.16. t-Butyl [2-{5-(2-hydroxy-5-methoxybenzoyl)-2-oxopyridin-1(2H)-yl}ethyl]carbamate (**43**).* The reaction of (*E*)-ethyl 3-(6-methoxy-4-oxo-4*H*-chromen-3-yl)acrylate (**27**) (1.1 g, 4 mmol) with *t*-butyl (2-aminoethyl)carbamate (0.70 g, 4.4 mmol) gave the title

compound **43** as a light yellow solid (1.26 g, 81%) by following the general procedure: mp = 145–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.32 (s, 9H, H-2‴), 3.44–3.49 (m, 2H, H-2″), 3.74 (s, 3H, OCH_3), 4.13 (brs, 2H, H-1″), 4.95 (brs, 1H, CONH), 6.58 (d, 1H, *J* = 9.5, H-3), 6.97 (d, 1H, 8.8 Hz, H-3′), 7.03 (brs, 1H, H-6′), 7.10 (dd, 1H, *J* = 2.9 & 8.8 Hz, H-4′), 7.77 (dd, 1H, *J* = 2.2 & 9.5 Hz, H-4), 7.91 (d, 1H, *J* = 2.2 Hz, H-6), 10.85 ppm (brs, 1H, OH); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 28.17, 39.39, 49.92, 56.05, 79.74, 114.78, 117.19,119.06, 119.63, 119.71, 123.13, 139.22, 144.18, 151.67, 155.98, 156.15,162.21, 194.51 ppm; IR (KBr): ν_{max} = 3365 (O—H str), 3061, 2926, 1702 (NHCOO—), 1667 (C=O), 1634, 1578, 1479, 1327, 1246, 1048, 946, 839 cm^{-1}; UV ($CHCl_3$): λ_{max} = 282 and 336 nm; HRMS: *m*/*z* $[M]^+$ calcd for $C_{20}H_{24}N_2O_6$: 388.1634, found: 388.1649.

*4.2.2. General procedure for the synthesis of N,N-dialkylimino pyridin-2(1H)-one (**44**–**45**)*

To a solution of (*E*)-ethyl 3-(4-oxo-4*H*-chromen-3-yl)acrylate (**23**) (4 mmol) in ethanol (45 mL), primary amines (10 mmol) and few drops of triethylamine were added and stirred under reflux for about 16 h. The mixture was then cooled to room temperature, and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography over silica gel using petroleum ether-ethyl acetate (15–20%) as eluent to give the desired analogues of 2-pyridone (**44**–**45**) in 75–80% yield.

*4.2.2.1. (E)-5-[(2-Hydroxyphenyl)(isopropylimino)methyl]-1-isopropylpyridin-2(1H)-one (**44**).* The reaction of (*E*)-ethyl 3-(4-oxo-4*H*-chromen-3-yl)acrylate (**23**) (0.98 g, 4 mmol) with isopropyl amine (0.59 g, 10 mmol) gave the title compound **44** as a light yellow solid (0.94 g, 79%) by following the general procedure: mp = 183 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.25 (d, 6H, *J* = 6.3 Hz, H-2″), 1.37 (d, 6H, *J* = 6.9 Hz, H-2‴), 3.64–3.73 (m, 1H, H-1″), 5.29–5.38 (m, 1H, H-1‴), 6.66–6.76 (m, 2H, H-3′ & H-4′), 6.96–6.99 (m, 2H, H-3 & H-5′), 7.15 (d, 1H, *J* = 9.3 Hz, H-6′), 7.26–7.31 (m, 2H, H-4 & H-6), 15.48 ppm (s, 1H, OH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 21.97, 24.26, 46.60, 52.04, 112.26, 117.65, 118.29, 119.54, 121.03,130.60, 131.97, 132.66, 137.94, 161.20, 163.28, 167.12 ppm; IR (KBr): ν_{max} = 3433 (O—H str), 3053, 2966, 1661 (C=O), 1595, 1522, 1438, 1304, 1257, 926, 754 cm^{-1}; UV (MeOH): λ_{max} = 260 and 322 nm; HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{18}H_{22}N_2O_2$: 299.1760, found: 299.1775.

*4.2.2.2. (E)-1-Cyclohexyl-5-[(cyclohexylimino)(2-hydroxyphenyl) methyl]pyridin-2(1H)-one (**45**).* The reaction of (*E*)-ethyl 3-(4-oxo-4*H*-chromen-3-yl)acrylate (**23**) (0.98 g, 4 mmol) with cyclohexyl amine (0.99 g, 10 mmol) gave the title compound **45** as a yellow low melting solid (1.18 g, 78%) by following the general procedure: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.14–1.99 (m, 20H, H-2″–H-6″ & H-2‴–H-6‴), 3.31–3.38 (m, 1H, H-1″), 4.89–4.97 (m, 1H, H-1‴), 6.66–6.73 (m, 2H, H-3′ & H-4′), 6.95–6.97 (m, 2H, H-3 & H-5′), 7.13 (dd, 1H, *J* = 2.3 & 9.2 Hz, H-6′), 7.23–7.24 (m, 1H, H-4), 7.25–7.30 ppm (m, 1H, H-6); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 23.98, 25.24, 25.32, 25.65, 32.60, 54.08, 59.44, 111.83, 117.44, 118.38, 119.40, 120.82, 130.57, 132.64, 132.75, 137.93, 161.27, 163.68, 167.24 ppm; IR (Nujol): ν_{max} = 3441 (O—H str), 2932, 2856, 1668, 1624, 1582, 1337, 1133, 836, 758 cm^{-1}; UV (MeOH): λ_{max} = 265 & 305 nm; HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{24}H_{30}N_2O_2$: 379.2386, found: 379.2407.

*4.2.3. General procedure for the synthesis of ethyl 3-morpholin-3-(4-oxo-4H-chromen-3-yl)propanoate (46), ethyl 3-(4-oxo-4H-chromen-3-yl)-3(phenylamino)propanoate (47) and ethyl 3-(fluorophenylamino)-3-(4-oxo-4H-chromen-3-yl)propanoate (**48**)*

To a solution of chromone ester (**23**) (4 mmol) in ethanol (45 mL), secondary or aromatic amines (morpholine, aniline and fluoroaniline) (16 mmol) and few drops of triethylamine were added and stirred under reflux for 12–13 h. After the mixture was cooled to room temperature, and the solvent removed, the crude product was purified by column chromatography over silica gel using petroleum ether–ethyl acetate (15–20%) as eluent to give analogues of 2-pyridone (**46**–**48**) in 68–79% yield.

*4.2.3.1. Ethyl 3-morpholin-3-(4-oxo-4H-chromen-3-yl)propanoate (**46**).* The reaction of (*E*)-ethyl 3-(4-oxo-4*H*-chromen-3-yl)acrylate (**23**) (0.98 g, 4 mmol) with morpholine (1.39 g, 16 mmol) gave the title compound **46** as a light yellow solid (1.05 g, 79%) by following the general procedure: mp = 110 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.20 (t, 3H, *J* = 7.0, H-2‴), 2.49–2.62 (m, 4H, H-2″ & H-6″), 2.81–2.89 (dd, 1H, *J* = 15.0 & *J* = 7.5 Hz, H-2a/H-2b), 2.95–3.02 (dd, 1H, *J* = 15.0 & *J* = 7.8 Hz, H-2a/H-2b), 3.67 (*brs*, 4H, H-3″ & H-5″), 4.11 (q, 2H, *J* = 6.9 Hz, H-1‴), 4.30 (t, 1H, *J* = 7.3 Hz, H-3), 7.39–7.47(m, 2H, H-6′ & H-7′), 7.68 (d, 1H, *J* = 7.8 Hz, H-8′), 7.89 (s, 1H, H-2′), 8.22 ppm (d, 1H, *J* = 7.8 Hz, H-5′); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.20, 36.33, 50.22, 57.58, 60.51, 67.23, 118.04, 120.47, 124.05, 125.26, 126.09, 133.70, 154.56, 156.02, 171.46, 177.35 ppm; IR (KBr): ν_{max} = 3092, 2892 (C—H), 2756, 1733 (C=O ester), 1638 (C=O), 1570, 1464, 1356, 1030, 913, 853, 767 cm^{-1}; UV (MeOH): λ_{max} = 297 and 307 nm; HRMS: *m*/*z* $[M]^+$ calcd for $C_{18}H_{21}NO_5$: 331.1420, found: 331.1218.

*4.2.3.2. Ethyl 3-(4-oxo-4H-chromen-3-yl)-3-(phenylamino)propanoate (**47**).* The reaction of (*E*)-ethyl 3-(4-oxo-4*H*-chromen-3-yl)acrylate (**23**) (0.98 g, 4 mmol) with aniline (1.49 g, 16 mmol) gave the title compound **47** as a light yellow low melting solid (0.94 g, 70%) by following the general procedure: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.23 (t, 3H, *J* = 7.0 Hz, H-2‴), 2.71–2.79 (dd, 1H, *J* = 15.3 & *J* = 7.2 Hz, H-2a/H-2b), 2.93–3.00 (dd, 1H, *J* = 15.0 & *J* = 6.9 Hz, H-2a/H-2b), 4.15 (q, 2H, *J* = 7.0 Hz, H-1‴), 5.47 (t, 1H, *J* = 7.0 Hz, H-3), 6.92 (d, 1H, *J* = 8.1 Hz, H-4″), 7.03–7.10 (m, 4H, H-2″, H-3″, H-5″ & H-6″), 7.26–7.46 (m, 4H, H-2′, H-6′, H-7′, H-8′), 7.93 (d, 1H, *J* = 7.8 Hz, H-5′), 11.83 ppm (d, 1H, *J* = 12.0 Hz, N*H*); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.18, 41.34, 60.78, 75.71, 103.65, 116.40, 118.01, 121.78, 122.82, 123.93, 126.47, 129.82, 134.65, 139.97, 141.57, 157.56, 170.21, 182.02 ppm; IR (Nujol): ν_{max} = 3235, 2970 (C—H), 1747 (C=O ester), 1647 (C=O), 1597, 1542, 1453, 1230, 1143, 956, 742 and 635 cm^{-1}; UV (MeOH): λ_{max} = 298 nm; HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{20}H_{19}NO_4$: 338.1392, found: 338.1267.

*4.2.3.3. Ethyl 3-(4-fluorophenylamino)-3-(4-oxo-4H-chromen-3-yl) propanoate (**48**).* The reaction of (*E*)-ethyl 3-(4-oxo-4*H*-chromen-3-yl)acrylate (**23**) (0.98 g, 4 mmol) with fluoroaniline (1.78 g, 16 mmol) gave the title compound **48** as a light yellow low melting solid (0.97 g, 68%) by following the general procedure: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.24 (*brs*, 3H, H-2‴), 2.71–2.79 (dd, 1H, *J* = 14.7 & *J* = 6.9 Hz, H-2a/H-2b), 2.92–2.99 (dd, 1H, *J* = 15.3 & *J* = 7.2 Hz, H-2a/H-2b), 4.14 (*brs*, 2H, H-1‴), 5.46 (t, 1H, *J* = 6.3 Hz, H-3), 6.92 (*brs*, 1H, H-2′), 7.04–7.06 (m, 5H, H-2″, H-3″, H-5″, H-6″ & H-6′), 7.32–7.42(m, 2H, H-7′ & H-8′), 7.92 (d, 1H, *J* = 7.2 Hz, H-5′), 11.83 ppm (s, 1H, *J* = 11.4 Hz, N*H*); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.08, 41.26, 60.78, 75.59, 103.65, 116.43, 116.74, 117.89, 118.01, 121.80, 122.74, 126.46, 134.70, 136.36, 142.04, 157.57, 157.85, 161.08, 170.22, 182.05 ppm; IR (Nujol): ν_{max} = 3078, 2982 (C—H), 1731 (C=O ester), 1651 (C=O), 1515, 1469, 1371, 1283, 1029, 945 and 759 cm^{-1}; UV (MeOH): λ_{max} = 297 nm; HRMS: *m*/*z* $[M]^+$ calcd for $C_{20}H_{18}FNO_4$: 355.1220, found: 355.1121.

4.3. Biology

4.3.1. c-Src kinase inhibitory activity assay

The effect of synthesized compounds on the activity of c-Src kinase was assessed by Transcreener ADP^2 FI assay (Bell Brook Labs, Madison, Wisconsin; catalogue no. 3013-1K) according to manufacturer's protocol. A 384-well low-volume black non binding surface round-bottom microplate was purchased from Corning (No. 3676). In summary, the kinase reaction was started in 384-well low-volume black microplate with the incubation of the 2.5 μL of the reaction cocktail (0.7 nM of His6-Src kinase domain in kinase buffer) with 2.5 μL of prediluted compounds (dissolved in 10% DMSO, 4X target concentration) for 10 min at room temperature using a microplate shaker. The reaction cocktail was made using the kinase buffer HEPES (200 mM, pH 7.5), $MgCl_2$ (16 mM), EGTA (8 mM), DMSO (4%), Brij-35 (0.04%), and 2-mercaptoethanol (43 mM). The kinase reaction was started by adding 5 μL of ATP/substrate (40 μM/600 μM) cocktail and incubated for 30 min at room temperature on a microplate shaker. Src optimal peptide (AEEEIYGEFEAKKKK) was used as the substrate for the kinase reaction. The kinase reaction was stopped by adding 10 μL of the 1X ADP detection mixture to the enzyme reaction mixture and mixed using a plate shaker. The mixture was incubated at room temperature for 1 h, and the fluorescence intensity was measured. The 1X ADP detection mixture was prepared by adding ADP^2 Antibody-IRDyeR QC-1 (10 μg/mL) and ADP Alexa594 Tracer (8 nM) to Stop & Detect Buffer B (1X). Fluorescence intensity measurements were performed using a fluorescence intensity optical module using an excitation of 580 nm and an emission of 630 nm with band widths of 10 nm by an Optima-BMG Labtechmicroplate reader. IC_{50} values of the compounds were calculated using ORIGIN 6.0 (origin lab) software. IC_{50} is the concentration of the compound that inhibited enzyme activity by 50%. All of the experiments were carried out in triplicate.

4.3.2. EGFR, MAPK and PDK inhibitory activity assay

The inhibitory activity of compound **36** was determined against EGFR(h), MAPK1(h) and PDK1(h) according to the KinaseProfiler protocol from Millipore described in www.millipore.com/drugdiscovery/KinaseProfiler. In brief, EGFR (h) was incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 10 mM $MnCl_2$, 0.1 mg/mL poly(Glu, Tyr) 4:1. MAPK1 (h) was incubated with 25 mM Tris pH 7.5, 0.02 mM EGTA, 250 μM substrate peptide (MAPK1-peptide, Merck Millipore, Dundee, UK), whereas PDK1 (h) was incubated with 50 mM Tris pH 7.5, 100 μM KTFCGTPEYLAPEVRREPRILSEEEQEMFRDFDYIADWC (PDKtide). The incubation was followed by the addition of 10 mM magnesium acetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, 10 μM) to each kinase. The kinase reactions were initiated with the addition of Mg:ATP mixture. ATP concentration was 10 μM. Kinase reactions were stopped after 40 min of incubation with the addition of 3% phosphoric acid solution. 10 μL of each kinase reaction was spotted onto a Filtermat A (for EGFR) or P30 filtermat (for MAPK1 and PDK1 Kinases) and washed three times for 5 min. in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2014.02.001.

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