2014

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Available at: http://dx.doi.org/10.1016/j.bioorg.2014.02.001

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PII: S0045-2068(14)00009-1
DOI: http://dx.doi.org/10.1016/j.bioorg.2014.02.001
Reference: YBIOO 1698

To appear in: Bioorganic Chemistry

Received Date: 23 September 2013


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Synthesis and Evaluation of c-Src Kinase Inhibitory Activity of Pyridin-2(1H)-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. Further the kinase inhibitory potential of compound 36 was studied for 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.

Keywords: chromone; pyridin-2(1H)-one; synthesis; c-Src kinase inhibition

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 existing active pharmacophores. Pyridone skeleton has been known as an ideal frame for further exploration. Recently, myriad pyridone derivatives (Figure 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].

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

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-4H-chromen-3-yl)acrylates (23-27) with various alkylamines, N,N-dialkylaminoalkanes, and t-buty1 (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-4H-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-4H-chromen-3-yl)acrylic acid (20-22) (Scheme 1).

**Scheme 1.** Synthesis of (E)-alkyl 3-(4-oxo-4H-chromen-3-yl)acrylate; Reagents and conditions: a) Ac₂O, pyridine, 6 h; b) POCl₃, DMF, 50 °C, 13 h; c) CH₂(COOH)₂, pyridine, 1.5 h; d) EtOH, conc. H₂SO₄ (3 or 4 drops), 12 h; e) CH₃I, K₂CO₃, anhyd. acetone, reflux, 12 h.
The desired pyridone precursors i.e. compound 23-25 were obtained by esterification of acrylyl acid derivatives of 4-oxo-4H-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-4H-chromen-3-yl)acrylates (26/27). All of the compounds were well characterized from their physical and spectral data and by comparing the data with literature value for the known compounds.

The t-buty 1(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].

**Scheme 2.** Synthesis of pyridin-2(1H)-one derivatives; Reagents and conditions: a) R\(^{1}\)NH\(_{2}\) (1.1 eq), NEt\(_{3}\), C\(_{2}\)H\(_{5}\)OH, reflux, 8-10 h; b) R\(^{1}\)NH\(_{2}\) (2.4 eq), NEt\(_{3}\), C\(_{2}\)H\(_{5}\)OH, reflux, 16-17 h; c) NEt\(_{3}\), C\(_{2}\)H\(_{5}\)OH, morpholine/aniline/ fluoroaniline, reflux, 12-13 h.

When (E)-ethyl 3-(4-oxo-4H-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 ¹³C 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

Figure 2 shows the c-Src inhibitory potency of all of the pyridin-2(1H)-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 IC₅₀ values below 25 µM. The compound 1-[2-(dimethylamino)ethyl]-5-(2-hydroxy-4-methoxybenzoyl)pyridin-2(1H)-one (36) was found to be the most potent with IC₅₀ value of 12.5 µM as shown in Table S1 (Supporting Information). The compounds 38 and 45 too showed significant activity with IC₅₀ values 19.9 µM and 20.1 µM respectively.

Figure 2. c-Src Kinase inhibitory activity of 2-pyridone derivatives (28-45). IC₅₀ 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.

In general, among all compounds containing pyridin-2(1H)-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 IC₅₀ values of 34.1, 47.0, 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 IC₅₀ 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 (IC₅₀: 27.6 μM) in comparison to compound 33 (IC₅₀: 57.8 μM). Also, by comparing the IC₅₀ 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 against 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 (Table 1).

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

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 Figure 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 showed 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-4H-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-4H-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; ¹H NMR (300 MHz, CDCl₃): 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); ¹³C NMR (75.5 MHz, CDCl₃): δ = 13.92, 22.42, 26.23, 29.63, 31.26, 50.71, 117.01, 118.67, 118.83, 119.91, 131.51, 136.01, 138.84, 143.41, 161.93, 162.33, 195.17 ppm; IR (KBr): νmax = 3424 (O-H str), 3063, 2956, 1669 (C=O), 1624, 1483, 1337, 1247, 1137, 838, 760, 638 cm⁻¹; UV (MeOH): λmax = 293 nm; HRMS: m/z [M + Na]⁺ calcd for C₁₈H₂₁NO₃: 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-4H-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; ¹H NMR (300 MHz, CDCl₃): 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); ¹³C NMR (75.5 MHz, CDCl₃): 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): νmax = 3336 (O-H str), 3053, 2981, 1658 (C=O), 1621, 1589, 1440, 1338, 1245, 1135, 763, 640 cm⁻¹; UV (MeOH): λmax = 297 nm; HRMS: m/z [M + Na]⁺ calcd for C₁₅H₁₅NO₃: 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-4H-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; 1H NMR (300 MHz; CDCl₃): 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); 13C NMR (75.5 MHz, CDCl₃): 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): νmax = 3430 (O-H str), 3047, 2921, 1629 (C=O), 1570, 1533, 1451, 1096, 974, 850, 750 cm⁻¹; UV (MeOH): λmax = 265 and 305 nm; HRMS: m/z [M + H]+ calcd for C₁₈H₁₉NO₃: 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-4H-chromen-3-yl)acrylate (23) (0.98 g, 4 mmol) with N¹,N¹-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; 1H NMR (300 MHz, CDCl₃): 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); 13C NMR (75.5 MHz, CDCl₃): 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): νmax = 3430 (O-H str), 3048, 1666 (C=O), 1624, 1590, 1336, 1246, 1174, 1138, 1048, 855, 764, 699 cm⁻¹; UV (MeOH): λmax = 268 & 310 nm; HRMS: m/z [M + H]+ calcd for C₁₆H₁₈N₂O₃: 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-4H-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; 1H NMR (400 MHz, DMSO-d₆): 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); 13C NMR (100.5 MHz, DMSO-d₆): 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): νmax = 3200 (O-H str), 2929, 2854, 1663 (C=O), 1629, 1602, 1432, 1340, 1236, 1117, 846, 721, 597, 554, 467 cm⁻¹; UV (CHCl₃): λmax = 284, 298 and 333 nm; HRMS: m/z [M + Na]+ calcd for C₁₈H₂₂N₄O₄: 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-4H-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; 1H NMR (400 MHz, DMSO-d6): 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, D2O 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 (dd, 1H, J = 2.2 Hz, H-6) 10.52 (brs, 1H, OH, D2O exchanged), 11.54 ppm (brs, 1H, OH, D2O exchanged); 13C NMR (100.5 MHz, DMSO-d6): 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): v_max = 3368 (O-H str), 2976, 2968, 1686 (NHCOO-), 1647 (C=O), 1586, 1585, 1521, 1335, 1271, 1173, 851, 584, cm⁻¹; UV (CHCl₃): λ_max = 288 and 334 nm; HRMS: m/z [M + H]⁺ and [M + K]⁺ calcd for C₁₉H₂₂N₂O₆: 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-4H-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; 1H NMR (400 MHz, CDCl₃): 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, OCH₃), 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); 13C NMR (100.5 MHz, CDCl₃): 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): v_max = 3427 (O-H str), 2929, 2258, 1675 (C=O), 1628, 1583, 1347, 1285, 1159, 1026, 828, 784, 622 cm⁻¹; UV (CHCl₃): λ_max = 290 and 337 nm; HRMS: m/z [M + H]⁺ calcd for C₁₉H₂₃NO₄: 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-4H-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; 1H NMR (400 MHz, CDCl₃): δ = 1.37 (d, 6H, J = 6.6 Hz, H-2″), 3.83 (s, 3H, OCH₃), 5.21-5.28 (m, 1H, H-1″), 6.42-6.46 (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); 13C NMR (100.5 MHz, CDCl₃): 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): v_max = 3462 (O-H str), 2982, 2851, 1664 (C=O), 1617, 1588, 1438, 1348, 1262, 1211, 1112, 937, 836, 606 cm⁻¹; UV (CHCl₃): λ_max = 283, 294 and 336 nm; HRMS: m/z [M + H]⁺ calcd for C₁₆H₁₇NO₄: 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-4H-chromen-3-yl)acrylate (26) (1.1 g, 4 mmol) with N,N′-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; 1H 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); 13C NMR (100.5 MHz, CDCl$_3$): δ = 45.43, 47.09, 55.58, 57.71, 101.12, 107.38, 112.57, 116.25, 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$_2$O$_4$: 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-4H-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; 1H 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); 13C 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$_2$O$_6$: 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-4H-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; 1H 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$_2$O 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$_2$O exchanged); 13C 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), 2979, 1660 (C=O), 1508, 1436, 1342, 1291, 1160, 857, 776, 621, cm$^{-1}$; UV (CHCl$_3$): λ$_{max}$ = 287 and 333 nm; HRMS: m/z [M + H]$^+$ and [M + Na]$^+$ calcd for C$_{15}$H$_{15}$NO$_5$: 290.1028, found: 290.0988.
4.2.1.12. 5-(2,5-Dihydroxybenzoyl)-1-hexylpyridin-2(1\(H\))-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-\(d_6\)): \(\delta = 0.85\) (t, 3H, \(J = 5.8\) Hz, H-6\(\"\)), 1.26 (brm, 6H, H-5\(\"\) & 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); \(^1\)C NMR (100.5 MHz, DMSO-\(d_6\)): \(\delta = 13.90, 21.98, 25.54, 28.69, 30.81, 49.18, 115.26, 116.87, 117.59, 118.39, 119.94, 125.03, 138.68, 145.64, 148.33, 149.85, 161.40, 191.66\) ppm; IR (KBr): \(\nu_{\max} = 3285\) (O-H str), 2921, 2854, 1664 (C=O), 1602, 1446, 1208, 1137, 994, 831, 790, 644, 551 cm\(^{-1}\); UV (CHCl\(_3\)): \(\lambda_{\max} = 281\) 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(1\(H\))-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-\(d_6\)): \(\delta = 1.28\) (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, \(J = 2.9\) & 9.5 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); \(^1\)C NMR (100.5 MHz, DMSO-\(d_6\)): \(\delta = 21.06, 21.20, 47.49, 115.44, 116.98, 117.62, 118.40, 120.23, 124.85, 138.09, 141.36, 148.37, 149.95, 161.05, 191.54\) ppm; IR (KBr): \(\nu_{\max} = 3417\) (O-H str), 3057, 2986, 1654 (C=O), 1612, 1438, 1350, 1236, 1140, 795, 643, 574 cm\(^{-1}\); UV (CHCl\(_3\)): \(\lambda_{\max} = 286\) 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(1\(H\))-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\(_3\)): \(\delta = 0.86\) (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); \(^1\)C NMR (100.5 MHz, CDCl\(_3\)): \(\delta = 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): \(\nu_{\max} = 3235\) (O-H str), 3057, 2986, 1654 (C=O), 1612 (C=O), 1571, 1438, 1350, 1236, 1140, 795, 643, 574 cm\(^{-1}\); UV (CHCl\(_3\)): \(\lambda_{\max} = 286\) and 337 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-4H-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\(_3\)): \(\delta = 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, \(J = 9.5 \text{ Hz}, H-3\)), 6.94 (d, 1H, \(J = 2.9 \text{ Hz}, H-6\)), 6.95-6.98 (m, 1H, H-3'), 7.09 (dd, 1H, \(J = 2.2 \& 8.8 \text{ Hz}, H-4'\)), 7.70 (dd, 1H, \(J = 2.2 \& 9.5 \text{ Hz}, H-4\)), 7.98 (d, 1H, \(J = 2.2 \text{ Hz}, H-6\)), 10.93 ppm (brs, 1H, OH); \(^{13}\)C NMR (100.5 MHz, CDCl\(_3\)):\(\delta = 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 \text{ ppm}; IR (KBr): \(\nu_{\text{max}} = 3437 (O-H \text{ str}), 3061, 2941, 1667 (C=O), 1626, 1587, 1442, 1276, 1167, 1123, 1026, 846, 789, 626 \text{ cm}^{-1}\); UV (CHCl\(_3\)): \(\lambda_{\text{max}} = 283 \text{ and } 337 \text{ nm}\); 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-4H-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\(_3\)):\(\delta = 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, \(J = 9.5 \text{ Hz}, H-3\)), 6.97 (d, 1H, \(8.8 \text{ Hz}, H-3'\)), 7.03 (brs, 1H, H-6'), 7.10 (dd, 1H, \(J = 2.2 \& 8.8 \text{ Hz}, H-4'\)), 7.79 (d, 1H, \(J = 2.2 \text{ Hz}, H-6\)), 10.85 ppm (brs, 1H, OH); \(^{13}\)C NMR (100.5 MHz, CDCl\(_3\)):\(\delta = 28.17, 39.39, 49.92, 56.05, 79.74, 114.09, 118.43, 119.48, 123.65, 137.96, 139.79, 151.55, 156.42, 161.58, 194.82 \text{ ppm}; IR (KBr): \(\nu_{\text{max}} = 3365 (O-H \text{ str}), 3061, 2926, 1702 (NHCOO-), 1667 (C=O), 1626, 1587, 1442, 1276, 1167, 1123, 1026, 846, 789, 626 \text{ cm}^{-1}\); UV (CHCl\(_3\)): \(\lambda_{\text{max}} = 283 \text{ and } 337 \text{ nm}\); HRMS: \(m/z [M]^+\) calcd for C\(_{20}\)H\(_{24}\)N\(_2\)O\(_6\): 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-(6-methoxy-4-oxo-4H-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)(isopropyliminomethyl)-1-isopropyl pyridin-2(1H)-one (44): The reaction of \((E)\)-ethyl 3-(6-methoxy-4-oxo-4H-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\(_3\)): \(\delta = 1.25\) (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\(_3\)): \(\delta = 21.97, 24.26, 46.60, 52.04, 112.26, 117.65, 118.29, 119.54, 121.03, 130.60, 131.97, 132.66, 133.94, 161.20, 163.28, 167.12\) ppm ; IR (KBr): \(\nu_{\text{max}} = 3433\) (O-H str), 3053, 2966, 1661 (C=O), 1595, 1522, 1438, 1304, 1257, 926, 754 cm\(^{-1}\) ; UV (MeOH): \(\lambda_{\text{max}} = 260\) and 322 nm ; HRMS: \([M + H]^+\) calcd for C\(_{18}\)H\(_{22}\)N\(_2\)O\(_2\): 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-4H-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\(_3\)): \(\delta = 1.14-1.99\) (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\(_3\)): \(\delta = 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): \(\nu_{\text{max}} = 3441\) (O-H str), 2932, 2856, 1668, 1624, 1582, 1337, 1133, 836, 758 cm\(^{-1}\) ; UV (MeOH): \(\lambda_{\text{max}} = 265\) & 305 nm ; HRMS: \([M + H]^+\) calcd for C\(_{24}\)H\(_{30}\)N\(_2\)O\(_2\): 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 fluoroanilin) (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-4H-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\(_3\)): \(\delta = 1.20\) (t, 3H, \(J = 7.0\) Hz, 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) , 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$_3$): $\delta = 14.20, 36.33, 50.22, 57.58, 60.51, 67.23, 118.04, 120.47, 124.05, 125.26, 126.09, 133.70, 154.56, 156.02, 171.46, 177.35$ ppm; IR (KBr): $\nu_{\text{max}} = 3092, 2892$ (C-H), 2756, 1733 (C=O ester), 1638 (C=O), 1570, 1464, 1356, 1030, 913, 853, 767 cm$^{-1}$; UV (MeOH): $\lambda_{\text{max}} = 297$ 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-4H-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$_3$): $\delta = 1.23$ (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, NH); $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta = 14.18, 41.34, 60.78, 75.71, 103.65, 116.40, 118.01, 121.78, 122.82, 123.93, 126.47, 134.65, 139.97, 141.57, 157.56, 170.21, 182.02 ppm; IR (Nujol): $\nu_{\text{max}} = 3235, 2970$ (C-H), 1747 (C=O ester), 1647 (C=O), 1597, 1542, 1453, 1230, 1143, 956, 742 and 635 cm$^{-1}$; UV (MeOH): $\lambda_{\text{max}} = 298$ nm; HRMS: $m/z$ [M$^+$]$^+$ 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-4H-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$_3$): $\delta = 1.24$ (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, NH); $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta = 14.08, 41.34, 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, 182.02 ppm; IR (Nujol): $\nu_{\text{max}} = 3078, 2982$ (C-H), 1731 (C=O ester), 1651 (C=O), 1515, 1469, 1371, 1283, 1029, 945 and 759 cm$^{-1}$; UV (MeOH): $\lambda_{\text{max}} = 297$ 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 (AEEEIYGEFEAkkkkk) 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$^5$ 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 KTFCGTPEYLAPEVRREPRILSEEQQEMFRDFDYIADWC (PDK tide). The incubation was followed by the addition of 10 mM magnesium acetate and [$\gamma$-$^{33}$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.

Acknowledgements

The financial support from Defence Research Development Organization (DRDO), Delhi and Council of Scientific and Industrial Research (CSIR) is gratefully acknowledged. We also acknowledge the financial
support from the American Cancer Society Grant # RSG-07-290-01-CDD and Grant Number 8 P20 GM103430-12 for sponsoring the core facility. The author KC is thankful to CSIR for the Senior Research Fellow award.

References


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

Figure 2. c-Src Kinase inhibitory activity of 2-pyridone derivatives (28-45)
Scheme 1. Synthesis of (E)-alkyl 3-(4-oxo-4H-chromen-3-yl)acrylate

Scheme 2. Synthesis of pyridin-2(1H)-one derivatives
Kinase | IC$_{50}$ (µM)  
--- | ---  
EGFR(h) | >300  
MAPK1(h) | >300  
PDK1(h) | >300  

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

**Graphical Abstract**

![Graphical Abstract](image_url)

MeO

OH

36 $R = -\text{CH}_2\text{CH}_2\text{N(CH}_3)_2$

38 $R = -\text{CH}_2\text{CH}_2\text{OH}$

Among eighteen pyridin-2(1H)-one derivatives synthesized, compounds 36 and 38 were found to be the most potent c-Src kinase inhibitors with IC$_{50}$ values of 12.5 µM and 19.9 µM, respectively.

**Highlights:**

- Eighteen novel 2-pyridones were synthesized and characterized from spectral data.
- These compounds were screened for c-Src kinase inhibition.
- Eight compounds exhibited IC$_{50} \leq 25$ µM for Src kinase inhibition.
- Antiproliferative activity of compounds were screened against 3 cancer cell lines.
- Results can be used to design next generation of 2-pyridones as Src kinase inhibitors.