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Synthesis of 3-Arylidene and 3-Arylimine Oxindole Derivatives and Evaluation of Their Src Kinase Inhibitory and Antiproliferative Activities

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Abstract

A number of novel 3-arylilidene and 3-arylimine-2-oxindole derivatives were synthesized, and their Src kinase inhibitory activities and antiproliferative activities were evaluated. Several compounds exhibited Src kinase inhibitory activity with IC_{50} values in the range of 5.3 to 211.8 μM. Compound b_{11} in 3-arylimine-2-oxindoles showed IC_{50} values of 5.3 μM against Src kinase. Compounds a_8, a_{20}, a_{38}, and b_{15} showed consistently>50% proliferation inhibition against all three cancer cell lines at a concentration of 50 μM.

Keywords: Arylilidene; Arylimine; Cytotoxicity; Indole; Src kinase

Introduction

Protein tyrosine kinases (PTKs) are a group of enzymes, which catalyze the transfer of the γ-phosphate group of ATP to tyrosine residues of proteins. PTKS play critical roles in signal transduction and cellular biochemical pathways [1]. The level of cell tyrosine phosphorylation in different proteins is normally controlled by PTKs and tyrosine phosphatases. c-Src (Src kinase) is a non-receptor PTK and an early upstream signal transduction enzyme that is activated or overexpressed in several human cancers, such as breast, lung, colon, esophagus, skin, cervix, and gastric tissues [2,3]. Thus, inhibition of c-Src kinase has become a strategy for therapeutic intervention for different types of cancer.

Several studies have provided compelling evidence that Src kinase plays a crucial role in osteoclast function [4]. Thus, Src kinase is also a potential pharmacologic target for the treatment of bone loss diseases, such as osteoporosis [5,6].

Based on the mechanism of action, current available Src kinase inhibitors can be classified into two major groups [7]: Inhibitors that compete with ATP for its binding pocket and inhibitors that work by interfering protein-protein interactions between the enzyme and its protein substrate. Competitive ATP binding site Src kinase inhibitors have shown to be more promising in terms of their potency and therapeutic applications. Several heterocyclic compounds have been used as competitive ATP binding site inhibitors, such as pyrazolo(3,4-*d*) pyrimidine (PP1), pyrrolo(2,3-*d*)pyrimidine (CGP76030), pyrido(2,3-*d*)pyrimidines (PP-166285), quinolinecarbonitrile (compound I), and indolinone derivatives (Figure 1) [8-11].

Pyrazolopyrimidine derivatives including PP1 and PP2 were found to be highly potent Src kinase inhibitors with IC_{50} values in the nanomolar range. Indole derivatives such as SU6656 and SU6657 have been also reported as selective and potent Src-inhibitors with IC_{50} values in the nanomolar range [12]. Recently, a number of 1,3-dihydroindole-2-one derivatives were reported to show Src and Yes tyrosine kinase inhibitory potency [13]. Olgen et al. have previously discovered 1-benzylindole-2-piperidinoethyl carboxylate, as a potent inhibitor of Src with IC_{50} value of 1.4 μM [14]. They have also reported a series of 3-(substituted-benzylidene)-1,3-dihydroindoline-2-thione derivatives and the corresponding indoline-2-one congeners for their ability to inhibit Src kinase [15].

More recently, Kilic et al. investigated a number of *N*-benzyl-5-phenyl indole-3-imine compounds and their corresponding amine congeners as Src kinase inhibitors. Among them, 1-(1-benzyl-5-phenyl-1H-indole-3-yl)-(4-fluorobenzyl) methanamine hydrochloride (Figure 1) was reported as promising Src kinase inhibitor with an IC_{50} value of 4.7 μM [16].

In continuation of our efforts to synthesize Src kinase inhibitors using new scaffolds and to investigate novel chemical structures as Src kinase inhibitors [17-21], a group of 3-arylilidene substituted oxindoles (a) and 3-arylimine substituted oxindoles (b) (Figure 2) were synthesized and evaluated for their inhibitory activity against Src kinase. We investigated the effect of various substituents in arylilidene and arylimine moieties at position 3 of the indole-2-one scaffold.

Experimental Protocols

General

All solvents, reagents and catalysts were purchased in analytical grade and used without further purification. The melting points (°C) were determined by open capillary method on an electro-thermal melting point apparatus and were uncorrected. The purity of compounds was confirmed by thin layer chromatography using WhatmanSil G/UV254 silica gel plates as the stationary phase and with suitable mobile phase with fluorescent indicator, and the spots were visualized under 254 and 366 nm illumination. Infrared spectra were recorded as thin films on KBr plates with υ_{max} in inverse centimeters. ^{1}H NMR spectra were recorded on a Bruker DRX-Avance (500 MHz) and

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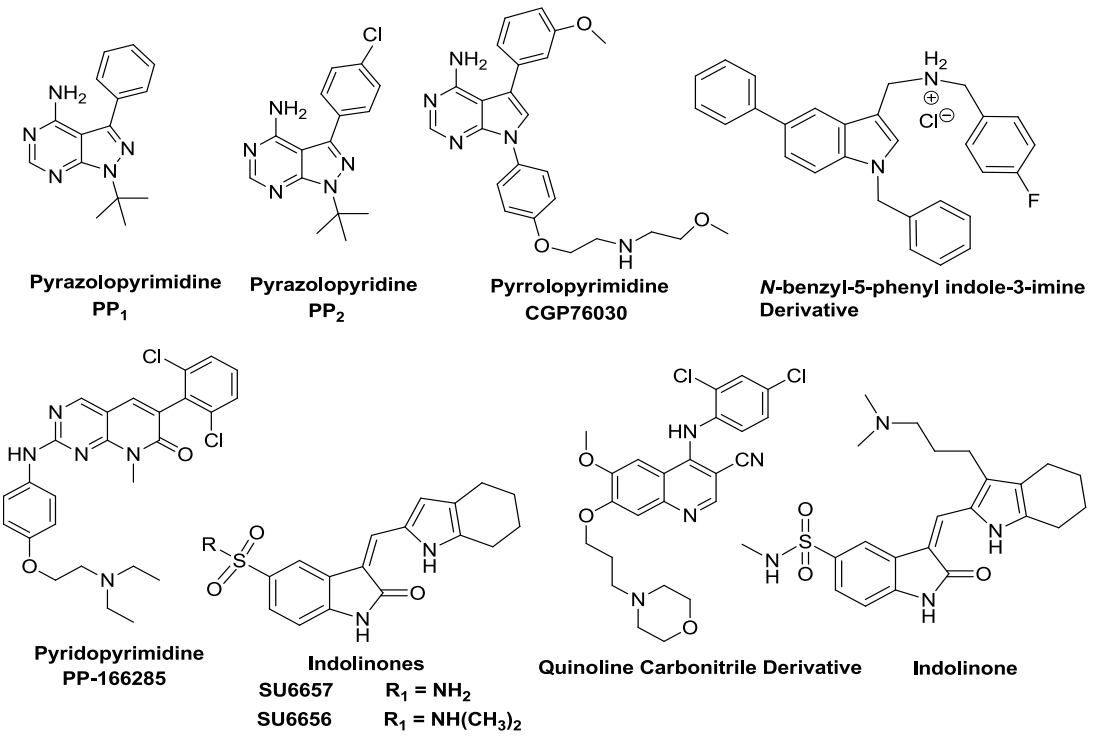


Figure 1: The chemical structure of ATP competitive Src kinase inhibitors.

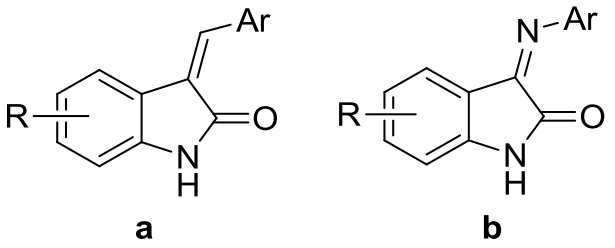


Figure 2: General structures for 3-arylilidene substituted oxindoles (a) and 3-arylimine substituted oxindoles (b).

or (250 MHz) spectrometer using DMSO-d_6 and $CDCl_3$ as solvents and chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane (TMS) as internal standard; s=singlet, d=doublet, dd=double doublet, t=triplet, q=quartet, m=multiplet, and br s=broad singlet. Mass analyses were performed with an Agilent 6400 Series mass spectrometer equipped with an electrospray ionization source (capillary voltage at 4000 V, nebulizing gas temperature at 300°C, nebulizing gas flow at 12 L/min). All the compounds were analyzed for C, H, N, and S on a Costech model 4010 and agreed with the proposed structures within ± 0.4% of the theoretical values [22-25].

General procedure for synthesis of 3-(substituted benzylidenyl)-indolin-2-one analogues (compounds a_1-a_{42}): A reaction mixture of the proper oxindole (1 equiv), aldehyde (1.2 equiv), and piperidine (0.1 equiv) in ethanol (1-2 mL/1 µmol oxindole) was stirred at 90°C for 3-5 h [16]. After the mixture cooled, the precipitate was filtered, washed with cold ethanol and hexane and recrystallized from ethanol to give the target compound.

Preparation of 4-(bromomethyl)benzonitrile (a_{30-1}): 4-Tolunitrile (0.1 mol) was added to a flask containing N-bromosuccinimide (0.11 mol) and dibenzoyl peroxide (500 mg) in dried carbon tetrachloride (200 ml). The reaction mixture was refluxed under nitrogen atmosphere overnight. Then the mixture cooled and filtered and the filtrate was concentrated and 300 ml hexane was added to this solution to form the white crystals of 4-(bromomethyl)benzonitrile [26]. The product was purified by recrystallization from chloroform. The Yield: was 50%, mp=113-115°C (lit mp=115-117).

Preparation of 4-((4-methylpiperazin-1-yl)methyl)benzonitrile (a_{30-2}): 1-(Bromo)toluenitrile (10.2 mmol) in 20 mL of chloroform was stirred at room temperature before dropwise addition of a solution of 1-methyl piperazine (28 mmol) in 5 mL chloroform. The reaction mixture was stirred at room temperature for 24 hours and the reaction was then quenched with water and further stirred for 30 min before extracting with chloroform. The organic layer was dried and concentrated [27]. In the residue, formed crystals were washed with hexane to give pure 4-((4-methylpiperazin-1-yl)methyl) benzonitrile; Yield: (35%), mp=65-67°C (lit mp=62-64°C); ESI-MS: Observed $[M+H]^+$=216. Calculated for $C_{13}H_{17}N_3$=215.2.

Preparation of 4-((4-methylpiperazin-1-yl) methyl) benzaldehyde (a_{30-3}): 4-((4-Methylpiperazin-1-yl)methyl)benzonitrile (9 mmol) was dissolved in formic acid 75% (37 mL) and raney nickel alloy (2 g) was added to this solution. The mixture was refluxed for 2 h, filtered over celite, and washed with 20 mL of cold ethanol 96°C [28]. The filtrate was concentrated to half of its volume and filtered again to remove the green colloidal impurities to give (1.8 g) crude product in the filtrate, ESI-MS: Observed $[M+H]^+$=219. Calculated for $C_{13}H_{18}N_2O$=218.29.

Synthesis of 3-(4-((4-methylpiperazin-1-yl)methyl) benzylidene)indolin-2-one (a_{30}): A mixture of oxindole (1 equiv), 4-((4-methylpiperazin-1-yl) methyl)benzaldehyde (a_{30-3}) (1.2 equiv),

and piperidine (0.1 equiv) in ethanol (1-2 mL/1 µmol oxindole) was stirred at 90°C overnight. The solvent was evaporated, and the residue was dissolved in warm ethyl acetate and passed through a column of silica gel. The polarity of eluting solvent was increased with the addition of methanol to the ethyl acetate. The yellow liquid phase was collected and the solvent was evaporated to achieve 3-(4-((4-methylpiperazin-1-yl)methyl)benzylidene) indolin-2-one.

General Procedure for synthesis of Compounds b_1-b_{24}: A mixture of indole-2, 3-dione (0.01M) and amine (0.01M) in absolute ethanol (20 ml) was refluxed for 20 h in the presence of 2-3 drops of glacial acetic acid [24]. After cooling, the mixture was filtered and washed with hexane and recrystallized from ethanol to give compounds b_1-b_{24}.

(E)-3-Benzylideneindolin-2-one (a_1): Yield: 23%; mp: 174-175°C (dec.), ethanol; IR (KBr) υ_{max} 3203 (N-H), 1716 (C=O) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ: 9.08 (s, 1H, NH-1), 7.9 (s, 1H, H-vinyl), 7.72 (d, 2H, J=7.3 Hz, H-2', 6'), 7.68 (d, 1H, J=7.8 Hz, H-4), 7.51 (m, 3H, H-3', 4', 5'), 7.26 (dt, J=7.8 Hz, 1Hz, 1H, H-6), 6.98 (d, 1H, J=7.8 Hz, H-7), 6.91 (dt, 1H, J=7.6 Hz, 0.87, H-5); ESI-MS: Observed $[M+H]^+$=222. Calculated for $C_{15}H_{11}NO$=221; Anal. Found: C, 81.2; H, 5.02; N, 6.21; O, 6.99. Calculated: C, 81.43; H, 5.01; N, 6.33; O, 7.23%.

3-(4-Hydroxybenzylidene)indolin-2-one (a_2): Yield: 38%; mp: 295-298°C (dec.) (lit mp>300°C) [17], ethanol; IR (KBr) υ_{max} 3196 (N-H), 1668 (C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=237.9, $[M+Na]^+$=259. Calculated for $C_{15}H_{11}NO_2$=237; Anal., found: 75.91; H, 4.63; N, 5.92; O, 13.51. Calculated: C, 75.94; H, 4.67; N, 5.90; O, 13.49%.

3-(4-Methoxybenzylidene)indolin-2-one (a_3): Yield: 23%; mp: 155.5-159°C (lit mp=156-157°C) [22,23], ethanol; IR (KBr) υ_{max} 3144 (N-H), 1697 (C=O) cm^{-1}; ESI-MS: Observed $(M+H^+)$=251.9, $[M+Na]^+$=273.9. Calculated for $C_{15}H_{13}NO_2$=251; Anal., found: C, 76.45; H, 5.22; N, 5.54; O, 12.70. Calculated: C, 76.48; H, 5.21; N, 5.57; O, 12.73%.

3-(3-Methoxybenzylidene)indolin-2-one (a_4): Yield: 20%; mp: 148.5-150°C, ethanol; IR (KBr) υ_{max} 3136(N-H), 1711 (C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=252, $[M+Na]^+$=274. Calculated for $C_{15}H_{13}NO_2$=251; Anal., found: C, 76.43; H, 5.21; N, 5.55; O, 12.71. Calculated for $C_{15}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57; O, 12.73%.

4-((2-Oxoindolin-3-ylidene) methyl)benzonitrile (a_5): Yield: 40%; mp: 231-233°C, ethanol; IR (KBr) υ_{max}3177 (N-H), 1704 (C=O), 1609 cm^{-1}; ESI-MS: Observed $[M+H]^+$=246.9, $[M+Na]^+$=268.9. Calculated for $C_{16}H_{10}N_2O$=246; Anal., found: C, 78.00; H, 4.1; N, 11.35; O, 6.48. Calculated: C, C, 78.03; H, 4.09; N, 11.38; O, 6.50%.

(Z)-3-(4-Nitrobenzylidene) indolin-2-one (a_6): Yield: 88%; mp: 233.3-235.1°C, ethanol; IR (KBr) υ_{max} 3150 (N-H), 1712 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.38 (d, 2H, J=8.7 Hz, H-3', 5'), 7.85 (d, 2H, J=8.7 Hz, H-2', 6'), 7.81 (s, 1H, H-8), 7.70 (bs, 1H, NH-1), 7.48 (d, 1H, H-), 7.31 (m, 1H, H-7), 6.93 (m, 2H, H-5, 6); ESI-MS: Observed $[M+H]^+$=267. Calculated for $C_{15}H_{10}N_2O_3$=266; Anal., found: C, 67.65; H, 3.77; N, 10.50; O, 18.01. Calculated: C, 67.67; H, 3.79; N, 10.52; O, 18.03%.

(Z)-3-(3-Nitrobenzylidene) indolin-2-one (a_7): Yield: 18%; mp: 10-212°C, ethanol; IR (KBr) υ_{max} 3140 (N-H), 1695 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 10.73 (s, 1H, NH-1), 9.39 (s, 1H, H-2'), 8.64 (d, 1H, J=7.7 Hz, H-4'), 8.27 (d, 1H, J=8.2 Hz, H-6'), 7.96 (s, 1H, H-vinyl), 7.75 (m, 2H, H-, 6), 7.26 (t, 1H, J=7.6 Hz, H-5'), 7.02 (t, 1H, J=7.5 Hz, H-5), 6.85 (d, 1H, J=7.6 Hz, H-4); ESI-MS: Observed $[M+H]^+$=267. Calculated for C15H10N2O3=266; Anal., found: C, 67.68; H, 3.81; N, 10.53; O, 18.05. Calculated: C, 67.67; H, 3.79; N, 10.52; O, 18.03%.

3-(2-Nitrobenzylidene) indolin-2-one (a_8): Yield: 10%; mp: 28-231°C (lit mp: for Z isomer=239-240°C) [24], ethanol; IR (KBr) υ_{max} 3142 (N-H), 1703 (C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=267, $[M+Na]^+$=289. Calculated for $C_{15}H_{10}N_2O_3$=266; Anal., found: C, 67.69; H, 3.80; N, 10.52; O, 18.04. Calculated: C, 67.67; H, 3.79; N, 10.52; O, 18.03%.

(E)-3-(4-(Methylthio)benzylidene)indolin-2-one (a_9): Yield: 38%; mp: 84-186°C, ethanol; IR (KBr) υ_{max} 3200 (N-H), 1700 (C=O) cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ9.02 (s, 1H, NH-1), 7.82 (s, 1H, H-vinyl), 7.75 (d, 1H, J=7.7 Hz, H-4), 7.66 (d, 2H, J=8.4 Hz; H-2', 6'), 7.36 (d, 2H, J=8.3 Hz, H-3', 5'), 7.26 (t, 1H, J=7.5 Hz, H-6), 6.95 (d, 2H, J=7.7 Hz, H-7), 6.93 (t, 1H, J=7.7 Hz, H-5), 2.59 (s, 3H, CH_3); ESI-MS: Observed $[M+H]^+$=268. Calculated for $C_{16}H_{13}NOS$=267; Anal., found: C, 71.86, H, 4.92; N, 5.26; O, 5.99; S, 11.98. Calculated: C, 71.88; H, 4.90; N, 5.24; O, 5.98; S, 11.99%.

(Z)-3-(Pyridin-2-ylmethylene) indolin-2-one (a_{10}): Yield: 10%; mp: 99.5-01.5°C, ethanol; IR (KBr) υ_{max} 3194 (N-H), 1710 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 9.06 (d, 1H, J=7.8 Hz, H-3'), 7.85 (d, 1H, J=4.7 Hz, H-6'), 8.94 (bs, 1H, NH-1), 7.84 (dt, 1H, J=1.8, 7.7 Hz, H-5'), 7.76(s, 1H, H-vinyl), 7.67 (d, 1H, J=7.8 Hz; H-4), 7.37 (t, 1H, J=7.8 Hz, H-6), 7.35 (m, 2H, H-4'), 7.1 (dt, 1H, J=0.9, 7.7 Hz; H-), 6.95 (d, 1H, J=7.7 Hz, H-7); ESI-MS: Observed $[M+H]^+$=223. Calculated for $C_{14}H_{10}N_2O$=222; Anal., found: C, 75.63; H, 4.55; N, 12.61; O, 7.19. Calculated for $C_{14}H_{10}N_2O$: C, 75.66; H, 4.54; N, 12.60; O, 7.20%.

(Z)-3-(Pyridin-3-ylmethylene) indolin-2-one (a_{11}): Yield: 23%; mp: 92-194°C, ethanol; IR (KBr) υ_{max} 3134 (N-H), 1706 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ9.01 (bs, 1H, NH-1), 8.97 (s, 1H, H-2'), 8.72 (dd, 1H, J=4.8, 1.4 Hz, H-4'), 7.99 (d, 1H, J=7.9 Hz, H-4) 7.79 (s, 1H, H-vinyl), 7.55 (d, 1H, J=7.7 Hz, H-6'), 7.47 (m, 1H, H-5'), 7.28 (t, 1H, J=8.9 Hz, H-6), 6.97 (d, 1H, J=7.8 Hz, H-7), 6.92 (dt, 1H, J=7.8 Hz, H-5); ESI-MS: Observed $[M+H]^+$=223. Calculated for $C_{14}H_{10}N_2O$=222; Anal., found: C, 75.65; H, 4.54; N, 12.59; O, 7.19. Calculated: C, 75.66; H, 4.54; N, 12.60; O, 7.20%.

(E)-3-(4-Fluorobenzylidene) indolin-2-one (a_{12}): Yield: 54%; mp188-189.5°C, ethanol; IR (KBr) υ_{max} 3168 (N-H), 1696 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: .89 (s, 1H, NH-1), 7.79 (s, 1H, H- vinyl), 7.68(m, 2H, H-3', 5'), 7.62(d, 1H, J=7.9 Hz H-4), 7.25 (m, 1H, H-6), 7.19 (m, 2H, H-2', 6'), 6.91 (m, 2H, H-5, 7); ESI-MS: Observed $[M+H]^+$=240. Calculated for $C_{15}H_{10}FNO$=239; Anal., found: C, 75.31; H, 4.22; F, 7.92; N, 5.83; O, 6.70. Calculated: C, 75.30; H, 4.21; F, 7.94; N, 5.85; O, 6.69%.

(E)-3-(3-Fluorobenzylidene) indolin-2-one (a_{13}): Yield: 70%; mp164-65°C, ethanol; IR (KBr) υ_{max} 3169 (N-H), 1719 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: .93 (bs, 1H, NH-1), 7.78 (s, 1H, H-2'), 7.60 (d, 1H, J=7.8 Hz, H- vinyl), 7.46 (m, 2H, H-,4'), 7.37 (d, 1H, J=10 Hz, H-'), 7.26 (m, 1H, H-6), 7.16 (m, 1H, H-5), 6.92 (t, 2H, J=7.7 Hz, H-5', 7); ESI-MS: Observed $[M+H]^+$=240. Calculated for $C_{15}H_{10}FNO$=239; Anal., found: C, 75.32; H, 4.21; F, 7.93; N, 5.84; O, 6.68. Calculated: C, 75.30; H, 4.21; F, 7.94; N, 5.85; O, 6.69%.

3-(2-Fluorobenzylidene)indolin-2-one (a_{14}): Yield: 70%; mp: 18.8-21°C, ethanol; ^1H NMR (500 MHz, $CDCl_3$) δ: .9 (bs, 1H, J=8.7, NH-1), 7.84 (s, 1H, J=8.7; H-vinyl), 7.74 (t, 1H, H-3'), 7.45 (m, 2H, H-4, 6'), 7.24 (m, H-3', 4', 5'), 6.89 (m, 2H, H-5, 7).; ESI-MS: Observed $[M+H]^+$=240. Calculated for $C_{15}H_{10}FNO$=239; Anal., found: C, 75.32; H, 4.22; F, 7.94; N, 5.86; O, 6.69. Calculated for $C_{15}H_{10}FNO$: C, 75.30; H, 4.21; F, 7.94; N, 5.85; O, 6.69%.

(E)-3-(4-Chlorobenzylidene) indolin-2-one (a_{15}): Yield: 65%; mp182-84°C, ethanol; IR (KBr) υ_{max} 3163 (N-H), 1719 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ7.77 (s, 1H, NH-1), 7.61 (m, 3H, H-2', 6', 4), 7.48 (s, 1H, NH-1), 7.47 (m, 2H, H-3', 5'), 7.25 (m, 1H, H-6),

6.89 (m, 2H, H-5, 7); ESI-MS: Observed $[M+H^+]$=256, $[M+Na^+]$=278. Calculated for $C_{15}H_{10}ClNO$=255; Anal., found: C, 70.45; H, 3.93; Cl, 13.85; N, 5.47; O, 6.24. Calculated for $C_{15}H_{10}ClNO$: C, 70.46; H, 3.94; Cl, 13.87; N, 5.48; O, 6.26%.

(E)-3-(3-Chlorobenzylidene)indolin-2-one (a_{16}): Yield: 21.5%; mp: 66.4-167.7°C, ethanol; IR (KBr) υ_{max} 3185 (N-H), 1709 (C=O) cm^{-1};^{1}H NMR (500 MHz, $CDCl_3$): δ7.75 (s, 1H, H-8), 7.66 (bs, 1H, NH-1), 7.65 (s, 1H, H-2'), 7.55 (m, 2H, H-4, 5'), 7.44(d, 2H, J=5.3, H-6', 4'), 7.26 (m, 1H, H-6), 6.91 (m, 2H, H-5, 7); ESI-MS: Observed $[M+H^+]$=256, $[M+Na^+]$=278. Calculated for $C_{15}H_{10}ClNO$=255; Anal., found: C, 70.47; H, 3.95; Cl, 13.87; N, 5.49; O, 6.27. Calculated: C, 70.46; H, 3.94; Cl, 13.87; N, 5.48; O, 6.26%.

3-(2-Chlorobenzylidene)indolin-2-one (a_{17}): Yield: 15%; mp: 82-84°C (lit mp: f Z isomere=181°C) [25], ethanol; IR (KBr) υ_{max} 3192 (N-H), 1718 (C=O) cm^{-1}; ESI-MS: Observed $[M+H^+]$=256, $[M+Na]^+$=278. Calculated for $C_{15}H_{10}ClNO$=255; Anal., found: C, 70.45; H, 3.94; Cl, 13.87; N, 5.50; O, 6.26. Calculated: C, 70.46; H, 3.94; Cl, 13.87; N, 5.48; O, 6.26%.

3-(4-Methylbenzylidene)indolin-2-one (a_{18}): Yield: 67%; mp: 89-191°C, ethanol; IR (KBr) υ_{max} 3122 (N-H), 1682(C=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ9.5 (s, 1H, NH-1), 7.78 (s, 1H, H- vinyl), 7.75 (d, 1H, J=7.7 Hz, H-4), 7.64 (d, 2H, J=7.9 Hz, H-2', 6'), 7.32 (m, 2H, H-3', 5'), 7.25 (m, 1H, H-6), 7 (d, 1H, J=7.7Hz, H-7), 6.92 (t, 1H, J=7.4 Hz, H-5), 2.48 (s, 3H, CH_3); ESI-MS: Observed $[M+H]^+$=236. Calculated for $C_{16}H_{13}NO$=235; Anal., found: C, 81.67; H, 5.55; N, 5.94; O, 6.81. Calculated: C, 81.68; H, 5.57; N, 5.95; O, 6.80%.

3-(4-Bromobenzylidene)indolin-2-one (a_{19}): Yield: 22%; mp: 95-197°C (lit mp=191-92) [29], ethanol; IR (KBr) υ_{max} 3188 (N-H), 1713(C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=300, 302. Calculated for $C_{15}H_{10}BrNO$=299; Anal., found: C, 60.00; H, 3.35; Br, 26.60; N, 4.66; O, 5.34. Calculated for $C_{15}H_{10}BrNO$: C, 60.02; H, 3.36; Br, 26.62; N, 4.67; O, 5.33%.

3-(3-Bromobenzylidene)indolin-2-one (a_{20}): Yield: 23%; mp: 63-164°C, ethanol; IR (KBr) υ_{max} 3179 (N-H), 1699 (C=O) cm^{-1}; ESI-MS: Observed $(M+H^+)$=300, 302 Calculated for $C_{15}H_{10}BrNO$=299; Anal., found: C, 60.04; H, 3.35; Br, 26.61; N, 4.68; O, 5.32. Calculated: C, 60.02; H, 3.36; Br, 26.62; N, 4.67; O, 5.33%.

3-(2-Bromobenzylidene)indolin-2-one (a_{21}): Yield: 36%; mp: 84.7-86.7°C, ethanol; IR (KBr) υ_{max} 3124 (N-H), 1709 (C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=300, 302 Calculated for $C_{15}H_{10}BrNO$=299; Anal., found: C, 60.03; H, 3.34; Br, 26.59; N, 4.69; O, 5.33. Calculated: C, 60.02; H, 3.36; Br, 26.62; N, 4.67; O, 5.33%.

(E/Z)-3-((5-(4-Fluorophenyl)pyridin-3-yl)methylene)indolin-2-one (a_{22}): Yield: 85%; mp: 04-206.9°C (dec.), ethanol; IR (KBr) υ_{max} 3160 (N-H), 1720 (C=O), 1689, 1607 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ10.70 (s, 1H, NH), 9.23 (t, 1H, J=2.0 Hz, H-2'), 9.15 (d, 1H, J=1.8 Hz, H-6'), 8.9 (m, 1H, H-4'), 7.91 (s, 1H, H-vinyl), 7.84 (m), 7.72 (t, 1H, J=7.5 Hz, H-4), 7.39 (m), 7.26 (t, 1H, J=7.5 Hz, H-5), 7.05 (t, 1H, J=7.5 Hz, H-6), 6.87 (m, 1H, H-7), mixture of Z and E isomers; ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 115.9, 116.2, 120.2, 120.7, 121.3, 122.1, 124.3, 128.9, 129, 129.1, 129.6, 129.8, 130.6, 130.7, 131.8, 132.4, 132.7, 132.8, 133.3, 133.7, 134.3, 135.6, 141.1, 143.2, 147.9, 148, 148.1, 150.9, 160.5, 164.4, 167, 168.1; ESI-MS: Observed $[M+H]^+$=317. Calculated for $C_{20}H_{13}FN_2O$=316.3. Anal., found: C, 75.96; H, 4.15; F, 6.03; N, 8.84; O, 5.05. Calculated: C, 75.94; H, 4.14; F, 6.01; N, 8.86; O, 5.06%.

(E/Z)-3-((E)-3-Phenylallylidene)indolin-2-one (a_{23}): Yield: 54%; mp: 23.7-26.7°C (dec.), ethanol; IR (KBr) υ_{max} 3167 (N-H), 1710 (C=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ7.76 (d, 1H, NH), 9.23 (t, 1H, J=2 Hz, H-2'), 9.15 (d, 1H, J=1.8 Hz, H-'), 8.9 (m, 1H, H-4'), 7.91 (s, 1H, H-vinyl), 7.84 (m), 7.72 (t, 1H, J=7.5 Hz, H-4), 7.39 (m), 7.26 (t, 1H, J=7.5, H-5), 7.05 (t, 1H, J=7.5 Hz, H-6), 6.87 (m, 1H, H-7); ESI-MS: Observed $[M+H]^+$=248. Calculated for $C_{17}H_{13}NO$=247. Anal., found: C, 82.55; H, 5.30; N, 5.65; O, 6.46. Calculated: C, 82.57; H, 5.30; N, 5.66; O, 6.47%.

(Z)-3-(3-Phenoxybenzylidene)indolin-2-one (a_{24}): Yield: 10%; mp140-41.5°C (dec.), ethanol; IR (KBr) υ_{max} 3135 (N-H), 1704 (C=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ7.77 (s, 1H, NH), 9.23 (t, 1H, J=2 Hz; H-2'), 9.15 (d, 1H, J=1.8 Hz; H-6'), 8.9 (m, 1H, H-4'), 7.91 (s, 1H, H-vinyl), 7.84 (m), 7.72 (t, 1H, J=7.5 Hz; H-4), 7.39 (m), 7.26 (t, 1H, J=7.5, H-5), 7.05 (t, 1H, J=7.5, H-6), 6.87 (m, 1H, H-7); ESI-MS: Observed $[M+H]^+$=314. Calculated for $C_{21}H_{15}NO_2$=313. Anal., found: C, 80.48; H, 4.81; N, 4.48; O, 10.20. Calculated for $C_{21}H_{15}NO_2$: C, 80.49; H, 4.82; N, 4.47; O, 10.21%.

(E)-N-(4-((2-Oxoindolin-3-ylidene)methyl)phenyl)acetamide (a_{25}): Yield: 17%; mp: 77-90°C (dec.); ethanol; IR (KBr) υ_{max} 3285 (N-H), 3071 (NH of acetamide), 1710 (C=O), 1658(C=O of acetamide), 1592 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.56 (s, 1H, NH-1), 10.22 (s, 1H, NH of acetamide), 7.74 (d, 2H, J=8.5 Hz, H-2', 6'), 7.69 (d, 2H, J=8.5 Hz, H-3', 5'), 7.65 (d, 1H, J=7.6 Hz, H-4), 7.56 (s, 1H, H-vinyl), 7.22 (t, 1H, J=7.6 Hz, H-6), 6.88 (m, 2H, H-5, 7), 2.10 (s, 3H, $NHCOCH_3$-'); ESI-MS: Observed $[M+H]^+$=278. Calculated for $C_{17}H_{14}N_2O_2$=278. Anal., found: C, 73.35; H, 5.06; N, 10.05; O, 11.49. Calculated: C, 73.37; H, 5.07; N, 10.07; O, 11.50%.

N-(2-Fluoro-4-((2-oxoindolin-3-ylidene)methyl)phenyl) acetamide (a_{26}): Yield: 15%; mp: 49-252°C (dec.), ethanol; IR (KBr) υ_{max} 3185 (NH), 3175 (NH of acetamide), 1710 (C=O), 1660(C=O of acetamide), 1613 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ10.69 (s, 1H, NH-1), 10 (s, 1H, NH of acetamide), 8.14 (dt, 1H, J=8.5, 1.8 Hz; H-6), 7.72(m), 7.23 (dd, 2H, J=15, 7.5 Hz, H-5', 6'), 6.9 (m), 2.14(s, 3H, $NHCOCH_3$-4'), mixture of Z and E isomers; ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 109.3, 110.1, 116.2, 116.5, 117.5, 117.8, 118.6, 119.6, 120.6, 121.1, 121.2, 122.3, 123, 124, 125.6, 126.3, 127.4, 127.5, 127.7, 128.9, 129.5, 129.7, 130.2, 130.4, 130.5, 130.7, 134.2, 140.6, 142.9, 150.5, 154.4, 167.2, 168.5, 169, 169.1; ESI-MS: Observed $[M+H]^+$=297. Calculated for $C_{17}H_{13}FN_2O_2$=296 Anal., found: C, 68.93; H, 4.41; F, 6.43; N, 9.47; O, 10.82. Calculated: C, 68.91; H, 4.42; F, 6.41; N, 9.45; O, 10.80%.

N-(2-Chloro-4-((2-oxoindolin-3-ylidene)methyl)phenyl) acetamide (a_{27}): Yield: 56%; mp: 20-228°C (dec.), ethanol; IR (KBr) υ_{max} 3184 (N-H), 3082 (NH of acetamide), 1702 (C=O), 1662(C=O of acetamide), 1611 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ10.65 (s, 1H, NH-1), 9.66 (s, 1H, NH of acetamide), 7 (m), 2.15 (s, 3H, $NHCOCH_3$ -4'), mixture of Z and E isomers; ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 23.5, 23.6, 109.4, 110.2, 119.8, 120.6, 121.1, 121.2, 122.2, 124, 124.4, 124.6, 125, 125.4, 126.8, 127.8, 128.2, 129.1, 130.2, 130.3, 131.2, 131.5, 131.7, 132.1, 133.8, 134.6, 136, 136.5, 140.7, 142.9, 167.1, 168.5, 169; ESI-MS: Observed $[M+H]^+$=313. Calculated for $C_{17}H_{13}ClN_2O_2$=312; Anal., found: C, 65.31; H, 4.2; Cl, 11.36; N, 8.95; O, 10.21. Calculated for $C_{17}H_{13}ClN_2O_2$: C, 65.29; H, 4.19; Cl, 11.34; N, 8.96; O, 10.23%.

(Z)-3-(Thiophen-2-ylmethylene)indolin-2-one (a_{28}): Yield: 54%; mp: 08-209°C (dec.), ethanol; IR (KBr) υ_{max} 3171 (N-H), 1677 (C=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ8.14 (s, 1H, NH), 7.89 (d, 1H, J=3.6 Hz; H-3'), 7.78 (s, 1H, H-vinyl), 7.7 (d, 1H, J=5.1 Hz, H-5'), 7.56 (d, 1H, J=7.6 Hz H-4), 7.27 (t, 1H, J=7.6 Hz, H-4'), 7.22 (t, 1H, J=4.4 Hz; H-6), 7.09 (t, 1H, J=7.7 Hz, H-5), 6.94 (d, 1H, J=7.7Hz, H-7); ESI-MS: Observed $[M+H]^+$=228. Calculated for $C_{13}H_9NOS$=227. Anal., found: C, 68.71; H, 3.98; N, 6.17; O, 7.03; S, 14.10. Calculated for $C_{13}H_9NOS$: C, 68.70; H, 3.99; N, 6.16; O, 7.04; S, 14.11%.

(E/Z)-3-(Furan-2-ylmethylene)indolin-2-one (a_{29}): Yield: 22%; mp: 83.9-85.09°C (dec.), ethanol; IR (KBr) υ_{max} 3131 (N-H), 1697 (C=O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.49 (d, 1H, J=7.76, H-4), 8.08 (m, 1H, NH-1), 7.8 (d, 1H, J=1.8 Hz; H-5'), 7.48 (s, 1H, H-vinyl), 7.28 (m, 2H, H-6, 3'), 7.1 (t, 1H, J=6.8 Hz, H-5), 6.95 (d, 1H, J=3.4 Hz, H-5), 6.91 (d, 1H, J=7.8 Hz, H-7), 6.65 (dd, 1H, J=2.6, 1.8 Hz, H-4'); ESI-MS: Observed $[M+H]^+$=212. Calculated for $C_{13}H_9NO_2$=211. Anal., found: C, 73.91; H, 4.28; N, 6.64; O, 15.16. Calculated: C, 73.92; H, 4.29; N, 6.63; O, 15.15%.

3-(4-((4-methylpiperazin-1-yl)methyl)benzylidene)indolin-2-one (a_{30}): Yield: 38%; mp: 64-269°C (dec.), ethanol; ^1H NMR (500 MHz, DMSO-d_6) δ10.58 (s, 1H, NH-1), 7.66 (d, 2H, J=8 Hz, H-2', 6'), 7.59 (s , 1H, H- vinyl), 7.56 (d, J=8 Hz , 1H, H-4), 7.43 (d, 2H, J=8, H-3', 5'), 7.21 (t, 1H, J=7.5 Hz, H-6), 6.85 (m, 2H, H-5, 7), 3.52 (s, 2H, CH_2), 2.37(m, 8H, Piperazine CH_2), 2.16 (s, 3H, CH_3); ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 45.2, 52.1, 54.3, 61.5, 110.1, 120.8, 121.1, 122.3, 127.1, 128.5, 129, 129.2, 130, 131.8, 132.9, 135.7, 140.2, 142.8, 164.6, 168.6; ESI-MS: Observed $[M+H]^+$=334. Calculated for $C_{21}H_{23}N_3O$=333.43; Anal., found: C, 75.70; H, 6.93; N, 12.58; O, 4.78. Calculated: C, 75.65; H, 6.95; N, 12.60; O, 4.80%.

(E/Z)-5-Chloro-3-(4'-methybenzylidene)indolin-2-one (a_{31}): Yield: 74%; mp: 12-16°C (dec.) (lit mp=220-223) [24], ethanol; ESI-MS: Observed $[M+H]^+$=270, $[M+Na]^+$=292. Calculated for $C_{16}H_{12}ClNO$=269; Anal., found: C, 71.26; H, 4.49; Cl, 13.15; N, 5.2; O, 5.94. Calculated: 71.25; H, 4.48; Cl, 13.14; N, 5.19; O, 5.93%.

(E/Z)-3-(4-hydroxybenzylidene)-5-chloroindolin-2-one (a_{33}): Yield: 74%; mp: 77-278°C (dec.), ethanol; IR (KBr) υ_{max} 3282 (N-H), 1674(C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=272, $[M+Na]^+$=294. Calculated for $C_{15}H_{10}ClN_2O$=271; Anal., found: C, 66.30; H, 3.72; Cl, 13.02; N, 5.14; O, 11.76. Calculated: C, 66.31; H, 3.71; Cl, 13.05; N, 5.16; O, 11.78%.

(E/Z)-5-chloroindolin-2-one (a_{33}): Yield: 74%; mp: 77-278°C (dec.), ethanol; IR (KBr) υ_{max} 3282 (N-H), 1674(C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=272, $[M+Na]^+$=294. Calculated for $C_{15}H_{10}ClN_2O$=271; Anal., found: C, 66.30; H, 3.72; Cl, 13.02; N, 5.14; O, 11.76. Calculated: C, 66.31; H, 3.71; Cl, 13.05; N, 5.16; O, 11.78%.

3-(4-Bromobenzylidene)-5-chloroindolin-2-one (a_{34}): Yield: 30%; mp: 42-47.7°C (dec.), ethanol; ESI-MS: Observed $[M+H]^+$=335, $[M+Na]^+$=357. Calculated for $C_{16}H_9ClN_2O$=334; Anal., found: C, 53.81; H, 2.70; Br, 23.86; Cl, 10.62; N, 4.18; O, 4.77. Calculated: C, 53.84; H, 2.71; Br, 23.88; Cl, 10.60; N, 4.19; O, 4.78%.

3-(4-Bromobenzylidene)-5-methylindolin-2-one (a_{35}): Yield: 51%; mp: 18-20.1°C (dec.), ethanol; ESI-MS: Observed $[M+H]^+$=313, $[M+Na]^+$=335. Calculated for $C_{16}H_{12}BrNO$=312; Anal., found: C, 61.15; H, 3.83; Br, 25.41; N, 4.45; O, 5.07. Calculated: C, 61.17; H, 3.85; Br, 25.43; N, 4.46; O, 5.09%.

4-((5-Bromo-2-oxoindolin-3-ylidene)methyl)benzonitrile (a_{36}): Yield: 58%; mp: 67-272.5°C (dec.), ethanol; IR (KBr) υ_{max} 3195 (NH), 2246 (nitrile), 1712 (C=O), 1613 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ10.86 (s, 1H, NH), 8.4 (d, 2H , J=8.25 Hz, H-3', 5'), 7.9 (m), 7.71 (s, 1H, H- vinyl), 7.4 (m), 6.83 (dd, 2H, J=15 Hz, 8.25; H-2', 6'), mixture of Z and E isomers; ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 116.2, 116.7, 116.9, 117.5, 117.9, 123.2, 123.4, 127.1, 127.9, 129.5, 131.2, 133.1, 133.3, 134.6, 136.6, 136.8, 137.3, 137.8, 140, 140.7, 142.6, 143.6, 145, 147.1, 171.1, 172.4; ESI-MS: Observed $[M+H]^+$=325, 327, $[M+Na]^+$=347, 349. Calculated for $C_{16}H_9BrN_2O$=325.16; Anal., found: C, 59.21; H, 2.78; Br, 24.59; N, 8.60; O, 4.91. Calculated for $C_{16}H_9BrN_2O$: C, 59.10; H, 2.79; Br, 24.57; N, 8.62; O, 4.92%.

3-(4-Hydroxybenzylidene)-5-bromoindolin-2-one (a_{37}): Yield: 75%; mp: 91-92°C (dec.), ethanol; IR (KBr) υ_{max} 3293(NH), 1679 (C=O) cm^{-1}; ESI-MS: $[M+H]^+$=316, 318 $[M+Na]^+$=338, 340. Calculated for $C_{15}H_{10}BrNO_2$=316; Anal., found: C, 56.95; H, 3.18; Br, 25.23; N, 4.42; O, 10.11. Calculated: C, 56.99; H, 3.19; Br, 25.27; N, 4.43; O, 10.12%.

3-(4-Bromobenzylidene)-5-bromoindolin-2-one (a_{38}): Yield: 35%; mp: 35-37°C (dec.), ethanol; ESI-MS: $[M+H]^+$=379.5, $[M+Na]^+$=401.6. Calculated for $C_{15}H_9Br_2NO$=379; Anal., found: C, 47.51; H, 2.38; Br, 42.15; N, 3.72; O, 4.21. Calculated: C, 47.53; H, 2.39; Br, 42.16; N, 3.70; O, 4.22%.

6-Chloro-3-(4-methylbenzylidene)-indolin-2-one (a_{39}): Yield: 37%; mp: 214-18°C,, ethanol; ESI-MS: $[M+H]^+$=271, $[M+Na]^+$=293. Calculated for $C_{16}H_{12}ClNO$=270; Anal., found: C, 71.26; H, 4.47; Cl, 13.13; N, 5.18; O, 5.92. Calculated: C, 71.25; H, 4.48; Cl, 13.14; N, 5.19; O, 5.93%.

4-((6-Chloro-2-oxoindolin-3-ylidene)methyl)benzonitrile (a_{40}): Yield: 54%; mp: 06-310°C (dec.), ethanol; ESI-MS: $[M+H]^+$=282, $[M+Na]^+$=304. Calculated for $C_{16}H_9ClN_2O$=281; Anal., found: C, 68.43; H, 3.22; Cl, 12.60; N, 9.96; O, 5.71. Calculated: C, 68.46; H, 3.23; Cl, 12.63; N, 9.98; O, 5.70%.

3-(4-Hydroxybenzylidene)-6-chloroindolin-2-one (a_{41}): Yield: 10%; mp: 65-72°C (dec.), ethanol; IR (KBr) υ_{max} 3164 (NH), 1692 (C=O) cm^{-1}; ESI-MS: $[M+H]^+$=271.9, $[M+Na]^+$=293.8. Calculated for $C_{15}H_{10}ClNO_2$=271; Anal., found: C, 66.30; H, 3.70; Cl, 13.03; N, 5.15; O, 11.77. Calculated: C, 66.31; H, 3.71; Cl, 13.05; N, 5.16; O, 11.78%.

3-(4-Bromobenzylidene)-6-chloroindolin-2-one (a_{42}): Yield: 84%; mp: 80-95°C (dec.), ethanol; ESI-MS: $[M+H]^+$=335, 337, $[M+Na]^+$=357, 359. Calculated for $C_{15}H_9BrClNO$=336; Anal., found: C, 53.82; H, 2.70; Br, 23.87; Cl, 10.61, N, 4.18; O, 4.77. Calculated: C, 53.84; H, 2.71; Br, 23.88; Cl, 10.60; N, 4.19; O, 4.78%.

3-(Phenylimino)indolin-2-one (b_1): Yield: 10%; mp: 22-24°C (dec.), ethanol; IR (KBr) υ_{max} 3156 (NH), 1733 (C=O) cm^{-1}; ESI-MS: $[M+H]^+$=223, $[M+Na]^+$=244.9. Calculated for $C_{14}H_{10}N_2O$=222; Anal., found: C, 75.63; H, 4.54; N, 12.62; O, 7.21. Calculated: C, 75.66; H, 4.54; N, 12.60; O, 7.20%.

3-(4-Fluorophenylimino)indolin-2-one (b_2): Yield: 10%; mp: 20-22°C (dec.); ethanol; IR (KBr) υ_{max} 3159 (NH), 1725 (C=O) cm^{-1}; ESI-MS: $[M+H]^+$=240.9, $[M+Na]^+$=262.9. Calculated for $C_{14}H_9FN_2O$=222; Anal., found: C, 69.97; H, 3.77; F, 7.90; N, 11.67; O, 6.67. Calculated: C, 69.99; H, 3.78; F, 7.91; N, 11.66; O, 6.66%.

3-(Pyridin-4-ylimino)indolin-2-one (b_3): Yield: 10%; mp: 70-275°C (dec.), ethanol; ESI-MS: Observed $[M+H]^+$=223.9, $[M+Na]^+$=245.9. Calculated for $C_{13}H_9N_3O$=223; Anal., found: C, 69.93; H, 4.05; N, 18.80; O, 7.16. Calculated: C, 69.95; H, 4.06; N, 18.82; O, 7.17%.

3-(Pyridin-4-ylimino) indolin-2-one (b_4): Yield: 18%; mp: 29.5-31°C (dec.), ethanol; IR (KBr) υ_{max}3052 (NH), 1710 (C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=223.9, $[M+Na]^+$=245.9. Calculated for $C_{13}H_9N_3O$=223; Anal., found: C, 69.92; H, 4.05; N, 18.80; O, 7.16. Calculated: C, 69.95; H, 4.04; N, 18.83; O, 7.15%.

3-(4-Nitrophenylimino) indolin-2-one (b_5): Yield: 10%; mp278-79.5°C (dec.), ethanol; IR (KBr) υ_{max} 3283 (NH) 1739 (C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=267.9, $[M+Na]^+$=289.9. Calculated for $C_{14}H_9N_3O_3$=223; Anal., found: C, 62.93; H, 3.38; N, 15.71; O, 17.95. Calculated: C, 62.92; H, 3.39; N, 15.72; O, 17.96%.

3-(3-Nitrophenylimino)indolin-2-one (b_6): Yield: 10%; mp: 28-228°C (dec.), ethanol; IR (KBr) υ_{max}3212 (NH) 1717 (C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=267.9, $[M+Na]^+$=289.9. Calculated for $C_{14}H_9N_3O_3$=223; Anal., found: C, 62.91; H, 3.37; N, 15.73; O, 17.97. Calculated: C, 62.92; H, 3.39; N, 15.72; O, 17.96%.

5-Nitro-3-(phenylimino)indolin-2-one (b_7): Yield: 79%; mp: 40-42°C (dec.), ethanol; IR (KBr) υ_{max} 3253 (NH) 1737 (C=O) cm^{-1}; ESI-MS: Observed $[M+H^+]$=267.9, $[M+Na]^+$=289.9. Calculated for $C_{14}H_9N_3O_{3=}$223; Anal., found: C, 62.91; H, 3.37; N, 15.73; O, 17.97. Calculated: C, C, 62.92; H, 3.39; N, 15.72; O, 17.96%.

3-(4-Chlorophenylimino)-5-nitroindolin-2-one (b_8): Yield: 26%; mp: 12-28°C (dec.), ethanol; IR (KBr) υ_{max} 3095 (NH) 1733 (C=O)cm^{-1}; ESI-MS: Observed $[M+H]^+$=301.8, $[M+Na]^+$=323.8. Calculated for $C_{14}H_8ClN_3O_3$=300; Anal., found: C, 55.73; H, 2.66; Cl, 11.77; N, 13.91; O, 15.90. Calculated: C, 55.74; H, 2.67; Cl, 11.75; N, 13.93; O, 15.91%.

5-Nitro-3-(p-tolylimino)indolin-2-one (b_9): Yield: 26%; mp: 87-90°C (dec.), ethanol; ESI-MS: Observed $[M+H]^+$=281.9, $[M+Na]^+$=303.9. Calculated for $C_{15}H_{11}N_3O_3$=281; Anal., found: C, 64.03; H, 3.93; N, 14.93; O, 17.08. Calculated: C, 64.05; H, 3.94; N, 14.94; O, 17.07%.

3-(4-Hydroxyphenylimino)-5-nitroindolin-2-one (b_{10}): Yield: 70%; mp: 52-59°C (dec.), ethanol; IR (KBr) υ_{max} 3380 (OH), 3095 (NH), 1734 (C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=283.9, $[M+Na]^+$=305.8. Calculated for $C_{14}H_9N_3O_4$=283; Anal., found: C, 59.37; H, 3.20; N, 14.84; O, 22.59. Calculated: C, 59.37; H, 3.20; N, 14.84; O, 22.59%.

N-(4-(5-Nitro-2-oxoindolin-3-ylideneamino)phenyl)acetamide (b_{11}): Yield: 74%; mp: 98-05°C (dec.), ethanol; IR (KBr) υ_{max} 3195 (NH), 2246 (nitrile), 1712 (C=O), 1613 cm^{-1}; ESI-MS: Observed $[M+H]^+$=325, $[M+Na]^+$=347. Calculated for $C_{16}H_{12}N_4O_4$=324; Anal., found: C, 59.25; H, 3.71; N, 17.27; O, 19.71. Calculated: C, 59.26; H, 3.73; N, 17.28; O, 19.73%.

4-(5-Nitro-2-oxoindolin-3-ylideneamino)benzamide (b_{12}): Yield: 20%; mp: 20-30°C (dec.), ethanol; ESI-MS: Observed $[M+H]^+$=311, $[M+Na]^+$=333. Calculated for $C_{15}H_{10}N_4O_4$=310; Anal., found: C, 58.05; H, 3.24; N, 18.07; O, 20.62. Calculated: 58.07; H, 3.25; N, 18.06; O, 20.63%.

5,7-Dichloro-3-(4-chlorophenylimino)indolin-2-one (b_{13}): Yield: 30%; mp: 71.2-73°C (dec.), ethanol; IR (KBr) υ_{max} 3168 (NH), 1716 (C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=324.7, 326.7, 328.7, $[M+Na]^+$=346.7, 348.7, 350.7. Calculated for $C_{14}H_7Cl_3N_2O$=325.5; Anal., found: C, 51.62; H, 2.16; Cl, 32.65; N, 8.62; O, 4.90. Calculated: C, 51.65; H, 2.17; Cl, 32.67; N, 8.60; O, 4.91%.

5,7-Dichloro-3-(p-tolylimino)indolin-2-one (b_{14}): Yield: 44%; mp: 230-37°C (dec.), ethanol; IR (KBr) υ_{max} 3162 (NH), 1718 (C=O) cm^{-1}; ESI-MS: Observed $(M+H^+)$=304.8, 306.8 $[M+Na]^+$=326.8, 328.8. Calculated for $C_{15}H_{10}Cl_2N_2O$=305; Anal., found: C, 59.00; H, 3.31; Cl, 23.22; N, 9.17; O, 5.22. Calculated: C, 59.04; H, 3.30; Cl, 23.24; N, 9.18; O, 5.24%.

5,7-Dichloro-3-(4-hydroxyphenylimino)indolin-2-one (b_{15}): Yield: 26%; mp: 260-62°C (dec.), ethanol; IR (KBr) υ_{max} 3354 (OH), 3203 (NH), 1713 (C=O) cm^{-1}; ESI-MS: Observed $[M+H]^+$=306.8, 308.8, $[M+Na]^+$=328.8, 330.8. Calculated for $C_{14}H_8Cl_2N_2O_2$=307; Anal., found: C, 54.74; H, 2.61; Cl, 23.07; N, 9.11; O, 10.40. Calculated: C, 54.75; H, 2.63; Cl, 23.09; N, 9.12; O, 10.42%.

4-(5,7-Dichloro-2-oxoindolin-3-ylideneamino)benzamide (b_{16}): Yield: 58%; mp: 22-325°C (dec.), ethanol; IR (KBr) υ_{max} 3460 (NH_2), 3339 (N-H), 1733(C=O Oxindole), 1662(C=O benzamide), 1610(C=N) cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ11.65 (s, 1H, NH-1), 11.51 (s, 2H, $CONH_2$-4'), 7.3 (m), 6.18 (d, 1H, J=1.8 Hz, H-4), mixture of Z and E isomers; ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 112.4, 115.8, 116.6, 116.9, 117.9, 118.4, 120.6, 120.8, 121.3, 122.8, 123.3, 123.9, 125.6, 126.8, 127, 127.9, 129, 129.2, 130.3, 131, 133, 135, 142.2, 143.6, 146.5, 151.2, 151.6, 152, 153.4, 158.1, 159.4, 163, 167.2, 167.5, 168; ESI-MS: Observed $[M+H]^+$=335. Calculated for $C_{15}H_9Cl_2N_3O_2$=334; Anal., found: C, 53.91; H, 2.70; Cl, 21.24; N, 12.58; O, 9.59. Calculated: C, 53.91; H, 2.71; Cl, 21.22; N, 12.57; O, 9.58%.

3-(4-Chlorophenylimino)-5-(trifluoromethoxy)indolin-2-one (b_{17}): Yield: 43%; mp: 30-240°C (dec.), ethanol; IR (KBr) υ_{max} 3282 (N-H), 1746 (C=O), 1628 (C=N) cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ11.21 (s, 1H, NH-1), 7.46 (m), 7.06 (m), 6.20 (d, 1H, J=1.25 Hz; H-4), mixture of Z and E isomers; ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 112, 112.7, 116, 117.8, 119.3, 121.1, 127.3, 127.4, 128.2, 129.4, 129.5, 142.1, 143.3, 144.7, 146, 147.3, 148.7, 152.9, 154.8, 158.4, 163.3.6; ESI-MS: Observed $[M+H]^+$=342. Calculated for $C_{15}H_8ClF_3N_2O_2$=340.7; Anal., found C, 52.92; H, 2.36; Cl, 10.43; F, 16.75; N, 8.21; O, 9.37. Calculated: C, 52.88; H, 2.37; Cl, 10.41; F, 16.73; N, 8.22; O, 9.39%.

3-(p-Tolylimino)-5-(trifluoromethoxy)indolin-2-one (b_{18}): Yield: 59%; mp: 66.5-268.5°C (with dec.); ethanol; IR (KBr) υ_{max} 3254 (N-H), 1741 (C=O), 1619(C=N) cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ11.3 (s, 1H, NH-1), 7.15(m, 6H, H-6, 7, 2', 3', 5', 6'), 6.25 (d, 1H, J=1.3 Hz, H-4), 2.36 (s, 3H, CH_3-4'), mixture of Z and E isomers; ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 20.41, 20.55, 111.8, 112.6, 116.1, 117.3, 117.8, 119.9, 127.1, 128.7, 129.9, 134.7, 142.1, 145.7, 147.4, 154.1, 163.4; ESI-MS: Observed $[M+H]^+$=321. Calculated for $C_{16}H_{11}F_3N_2O_2$=320.2; Anal., found: C, 60.2; H, 3.45; F, 17.81; N, 8.74; O, 9.97. Calculated: C, 60.00; H, 3.46; F, 17.80; N, 8.75; O, 9.99%.

3-(4-Hydroxyphenylimino)-5-(trifluoromethoxy)indolin-2-one (b_{19}): Yield: 80%; mp: 30-238°C (dec.), ethanol; IR (KBr) υ_{max} 3314 (O-H), 3209 (N-H), 1732 (C=O), 1627 (C=N) cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ11.13 (1H, s, NH-1), 9.66 (s, 1H, OH-4'), 7.37 (m), 6.89(m), 6.60 (d, 1H , J=1.25 Hz; H-4), mixture of Z and E isomers; ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 111.5, 112.4, 114.7, 115.9, 116.3, 117.5, 119.9, 123.8, 124.8, 125.8, 126.8, 138.7, 141, 142.1, 143.4, 145.6, 149.1, 153.2, 155.9, 157.1, 158.9, 163.7; ESI-MS: Observed $[M+H]^+$=323. Calculated for $C_{15}H_9F_3N_2O_3$=322.2; Anal., found: C, 55.93; H, 2.84; F, 17.67; N, 8.69; O, 14.92. Calculated for $C_{15}H_9F_3N_2O_3$: C, 55.91; H, 2.82; F, 17.69; N, 8.69; O, 14.90%.

4-(2-Oxo-5-(trifluoromethoxy)indolin-3-ylideneamino) benzamide (b_{20}): Yield: 49%; mp: 96-304°C (dec.), ethanol; IR (KBr) υ_{max} 3429 (NH_2), 3121 (N-H), 1727 (C=O Oxindole), 1697 (C=O benzamide), 1608 (C=N) cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ11.22 (brs, 3H, NH-1, $CONH_2$-'), 7.48 (m, 6H, H-6, 7, 2', 3', 5', 6'), 6.10 (s, 1H, H-4), mixture of Z and E isomers; ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 112, 112.7, 116, 116.8, 118, 118.3, 121.8, 122.1, 127.4, 127.9, 129.1, 130.1, 130.8, 142.1, 143.3, 144.8, 145.9, 151.5, 152.5, 154.4, 158.4, 163.2, 167.1, 167.6; ESI-MS: Observed $[M+H]^+$=350. Calculated for $C_{16}H_{10}F_3N_3O_3$=349.26; Anal., found: C, 55.05; H, 2.88; F, 16.30; N, 12.05; O, 13.72. Calculated: C, 55.02; H, 2.89; F, 16.32; N, 12.03; O, 13.74%.

3-(4-Chlorophenylimino)-5-fluoroindolin-2-one (b_{21}): Yield: 22%; mp: 44.6-46°C (dec.); ethanol; IR (KBr) υ_{max} 3198 (N-H), 1714 (C=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ7.95 (bs, 1H, NH-1), 7.47 (d, 2H, J=8.56; H-3', 5'), 7.36 (m), 6.89 (m), 6.60 (d, 1H , J=1.3 Hz; H-4); ESI-MS: Observed $[M+H]^+$=275. Calculated for $C_{14}H_8ClFN_2O$=274; Anal., found: C, 61.22; H, 2.94; Cl, 12.91; F, 6.92; N, 10.20; O, 5.82. Calculated: C, 61.22; H, 2.94; Cl, 12.91; F, 6.92; N, 10.20; O, 5.82%.

5-Fluoro-3-(p-tolylimino)indolin-2-one (b_{22}): Yield: 38%; mp:

64-269°C (dec.), ethanol; IR (KBr) υ_{max} 3256 (N-H), 1741 (C=O), 1652 (C=N), 1613 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ11.03 (s, 1H, NH-1), 7.15 (m, 6H, H-, 7, 2', 3', 5', 6'), 6.14 (dd, 1H, J=8.5, 2.5 Hz; H-4), 2.37(s, 3H, CH_3-4'), mixture of Z and E isomers; ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 20.5, 111.5, 111.9, 112.5, 112.6, 115.9, 116, 117.4, 119.8, 120.5, 120.9, 128.7, 130, 134.6, 143.2, 147.2, 154.3, 154.8, 158.5, 163.5; ESI-MS: Observed $[M+H]^+$=255 Calculated for $C_{15}H_{11}FN_2O$=254.2; Anal., found: C, 70.89; H, 4.31; F, 7.45; N, 11.03; O, 6.27. Calculated: C, 70.86; H, 4.36; F, 7.47; N, 11.02; O, 6.29%.

5-Fluoro-3-(4-hydroxyphenylimino)indolin-2-one (b_{23}): Yield: 74%; mp: 07-320°C (dec.), ethanol; IR (KBr) υ_{max} 3298, 1711 (C=O), 1619, 1599 (C=N) cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ10.97 (s, 1H, NH-1), 9.66 (s, 1H, OH-4'), 7.26 (m), 6.84 (m), 6.44 (dd, 1H, J=8.5, 2.5 Hz; H-4), mixture of Z and E isomers; ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 108.6, 109, 111.1, 111.3, 111.5, 112.3, 112.4, 114.7, 115.9, 116, 116.2, 119, 119.4, 119.9, 120.1, 120.5, 124.6, 138.8, 140.7, 141, 142.9, 143, 153.4, 154.8, 155.8, 156.9, 158.6, 158.9, 163.7; ESI-MS: Observed $[M+H]^+$=257. Calculated for $C_{14}H_9FN_2O_2$=256.23; Anal., found: C, 65.68; H, 3.53; F, 7.42; N, 10.91; O, 12.47. Calculated: C, 65.62; H, 3.54; F, 7.41; N, 10.93; O, 12.49%.

4-(5-Fluoro-2-oxoindolin-3-ylideneamino)benzamide (b_{24}): Yield: 19%; mp: 77-310°C (dec.), ethanol; IR (KBr) υ_{max} 3394 (NH_2), 3232 (N-H), 1741 (C=O Oxindole), 1673 (C=O benzamide), 1630 (C=N) cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ11.09 (s, 1H, NH-1), 7.94 (m), 7.33 (m), 7.06 (m), 6.92 (m), 5.97 (dd, 1H, J=8.25, 2.5; H-4), mixture of Z and E isomers; ^{13}C-NMR (62.9 MHz, DMSO-d_6) δ 109.7, 110.1, 111.7, 112.1, 112.7, 112.8, 115.8, 115.9, 116.9, 118.2, 120.9, 121.3, 127.9, 129.2, 130.8, 143.4, 152.4, 163.3, 167.3, 167.6; ESI-MS: Observed $[M+H]^+$=284. Calculated for $C_{15}H_{10}FN_3O_2$=283.2; Anal., found: C, 63.70; H, 3.55; F, 6.72; N, 14.85; O, 11.33. Calculated: C, 63.60; H, 3.56; F, 6.71; N, 14.83; O, 11.30%.

Sodium(Z/E)-3-(4-chlorophenylimino)-2-oxoindoline-5-sulfonate (b_{25}): Yield: 30%; mp: >300°C (dec.), ethanol; ^{1}H NMR (250 MHz, DMSO-d_6) δ11.13 (1H, s, NH-1), 7.88 (dd, 1H, J=6.5;H-), 7.61 (m, 1H, H-), 7.51 (d, 2H, J=8.8 Hz, H-', 6'), 7.06 (m, 2H, H-3', 5'), 6.86 (m, 2H, H-), mixture of Z and E isomers.

Sodium-3-(4-hydroxyphenylimino)-2-oxoindoline-5-sulfonate (b_{26}): Yield: 70%; mp>300°C (dec.), ethanol; ^{1}H NMR (250 MHz, DMSO-d_6) δ11. 01(1H, s, NH-1), 7.61 (dd, 1H, J=6.5; H-), 7.35 (d, 2H, J=1.3 Hz, H-), 6.95 (d, J=6.3 Hz, 2H, H-2', 6'), 6.84 (m, H-', 5', 7), mixture of Z and E isomers.

Sodium(Z/E)-3-(4-acetamidophenylimino)-2-oxoindoline-5-sulfonate (b_{27}): Yield: 22%; mp>300°C (dec.), ethanol; ^{1}H NMR (250 MHz, DMSO-d_6) δ11.05 (1H, s, NH-acetamide), 10.13 (s, 1H, NH-), 7.76 (d, 2H, J=1, H-', 6'), 7.63 (d, 1H, J=0.8 Hz, H-), 7.15 (s, 1H, H-4), 6.99 (d, 2H, J=0.8 Hz, H-', 5'), 6.87 (d, 1H, J=0.8 Hz, H-), mixture of Z and E isomers.

Cell culture

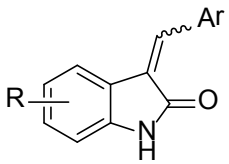
Human ovarian adenocarcinoma (SK-OV3, ATCC no.HTB-77), breast adenocarcinoma (MCF-7, ATCC no.HTB-22), and colon adenocarcinoma (HT-29, ATCC no. HTB-38) cell lines were obtained from American Type Culture Collection. Cells were grown on 75 cm^2 cell culture flasks with EMEM (Eagle's minimum essential medium), supplemented with 10% fetal bovine serum, and 1% penicillin/streptomycin solution (10,000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO_2, 95% air at 37°C.

Cell proliferation assay

Antiproliferative assay was performed using Cell Titer 96 aqueous one solution cell proliferation assay kit (Promega, USA). In a brief, after reaching 75-80% confluency of cells under microscope, cells (5000 cells/well) were seeded in 96-well microplates in media (100 µL). After 24 h, compounds (50 µM) were added to wells in triplicate. Doxorubicin (10 µM) and DMSO were tested in the assay as positive and negative controls. After 72 h incubation, CellTiter 96 aqueous solution (20 µL) was added into wells. The plate was kept at 37°C for 1-2 h. The formazan product absorbance at 490 nm was measured by 96-well plate reader. The blank control was recorded by measuring the absorbance at 490 nm with wells containing medium mixed with CellTiter 96 aqueous solution but no cells. Results were expressed as the percentage of the control (without compound set at 100%). The results of the inhibition of MCF-7, SK-OV- and HT-9 cells by compounds (a_1-a_{42}) and (b_1-b_{27}) Series (50 µM) after 72 h incubation is demonstrated in Table 1. All the experiments were performed in triplicate.

Src kinase activity assay

The effect of synthesized compounds on the activity of *c*-Src kinase was assessed by Transcreener® ADP² FI Assay, from Bell Brook Labs, Madison, WI, (catalogue no. 3013-1K) according to manufacturer's protocol. 384-well Low volume Black non-binding surface round bottom microplate was purchased from Corning (#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 His_6-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 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



No.	R	Ar	No.	R	Ar
a_1	H	Ph	a_{22}	H	5-(4-F-Ph)-3-pyridyl
a_2	H	4-OH-Ph	a_{23}	H	-CH=CH-Ph
a_3	H	4-OCH_3-Ph	a_{24}	H	3-OPh-Ph
a_4	H	3-OCH_3-Ph	a_{25}	H	4-CH_3-CO-NH-Ph
a_5	H	4-CN-Ph	a_{26}	H	3-F-4-CH_3-CO-NHPh
a_6	H	4-NO_2-Ph	a_{27}	H	3-Cl-4-CH_3-CO-NHPh
a_7	H	3-NO_2-Ph	a_{28}	H	2-Thienyl
a_8	H	2-NO_2-Ph	a_{29}	H	2-Furyl
a_9	H	4-SCH_3-Ph	a_{30}	H	4-Me-Piperazinyl-NBzl
a_{10}	H	2-Pyridyl	a_{31}	5-Cl	4-CH_3-Ph
a_{11}	H	3-Pyridine	a_{32}	5-Cl	4-CN-Ph
a_{12}	H	4-F-Ph	a_{33}	5-Cl	4-OH-Ph
a_{13}	H	3-F-Ph	a_{34}	5-Cl	4-Br-Ph
a_{14}	H	2-F-Ph	a_{35}	5-Br	4-CH_3-Ph
a_{15}	H	4-Cl-Ph	a_{36}	5-Br	4-CN-Ph
a_{16}	H	3-Cl-Ph	a_{37}	5-Br	4-OH-Ph
a_{17}	H	2-Cl-Ph	a_{38}	5-Br	4-Br-Ph
a_{18}	H	4-CH_3-Ph	a_{39}	6-Cl	4-CH_3-Ph
a_{19}	H	4-Br-Ph	a_{40}	6-Cl	4-CN-Ph
a_{20}	H	3-Br-Ph	a_{41}	6-Cl	4-OH-Ph
a_{21}	H	2-Br-Ph	a_{42}	6-Cl	4-Br-Ph

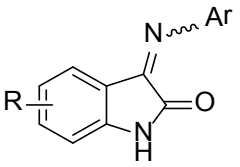
Table 1: Chemical structures 3-arylidene-2-oxindole derivatives (a_{1-42}).

2-mercaptoethanol (43 mM). 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 microplate shaker. Src optimal peptide (AEEEIYGEFEAKKKK) was used as the substrate for the kinase reaction. 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 ADP2 Antibody-IRDye® QC-1 (10 µg/mL) and ADP Alexa594 Tracer (8 nM) to Stop and Detect Buffer B (1X). Fluorescence Intensity measurements were performed using fluorescence intensity optical module using the excitation of 580 nm and emission of 630 nm with band widths of 10 nm by Optima, BMG Labtechmicroplate reader. IC_{50} 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 the experiments were carried out in triplicate.

Results and Discussion

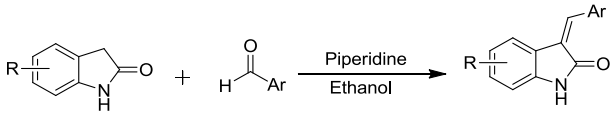
The synthetic pathways to prepare 3-arylidene-2-oxindole derivatives (a_{1-42}) (Table 1) and 3-arylimino-2-oxindoles (b_{1-27}) (Table 2) are depicted in Schemes 1 and 3. 3-Arylidene-2-oxindoles a_1 to a_{42} were synthesized by the reaction of proper 2-oxindoles with different aryl aldehydes in the presence of piperidine in absolute ethanol (Scheme 1, Table 1).

In the case of compound a_{30}, the aldehyde was synthesized in three steps starting with bromination of 4-tolunitrile, with N-bromosuccinimide (NBS), reaction with 4-methylpiperazine, followed by conversion of nitrile group to aldehyde in the presence of Raney Nickel and formic acid, respectively (Scheme 2).

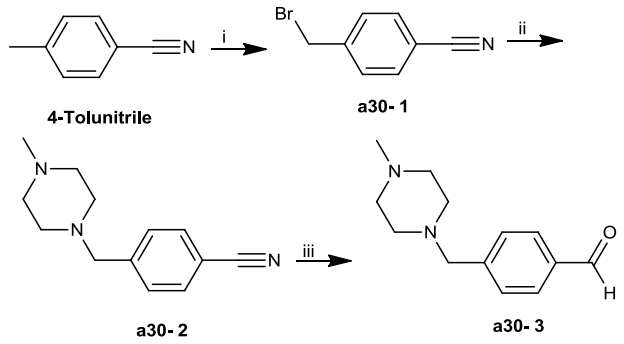


No.	R	Ar	No.	R	Ar
b_1	H	Ph	b_{15}	5,7-DiCl	4-OH-Ph
b_2	H	4-F-Ph	b_{16}	5,7-DiCl	4-NH_2CO-Ph
b_3	H	4-Pyridyl	b_{17}	F_3CO	4-Cl-Ph
b_4	H	3-pyridyl	b_{18}	F_3CO	4-CH_3-Ph
b_5	H	4-NO_2-Ph	b_{19}	F_3CO	4-OH-Ph
b_6	H	3-NO_2-Ph	b_{20}	F_3CO	4-NH_2CO-Ph
b_7	5-NO_2	Ph	b_{21}	F	4-Cl-Ph
b_8	5-NO_2	4-Cl-Ph	b_{22}	F	4-CH_3-Ph
b_9	5-NO_2	4-CH_3-Ph	b_{23}	F	4-OH-Ph
b_{10}	5-NO_2	4-OH-Ph	b_{24}	F	4-NH_2CO-Ph
b_{11}	5-NO_2	4-MeCONHPh	b_{25}	SO_3Na	4-Cl-Ph
b_{12}	5-NO_2	4-NH_2CO-Ph	b_{26}	SO_3Na	4-OH-Ph
b_{13}	5,7-DiCl	4-Cl-Ph	b_{27}	SO_3Na	4-MeCONHPh
b_{14}	5,7-DiCl	4-CH_3-Ph			

Table 2: Chemical structures of 3-arylimino-2-oxindoles (b_{1-27}).



Scheme 1: Synthesis of 3-arylidene-2-oxindole derivatives (a_{1-42}).



Scheme 2: Synthesis of 4-((4-methylpiperazin-1-yl) methyl)benzaldehyde (a30-3): (i) dibenzoyl peroxide, NBS, CCl4, reflux, 24 h; (ii) 4-methylpiperazine, CHCl3, 24 h; (iii) Raney Nickel alloy, formic acid 75%; reflux, 2 h.

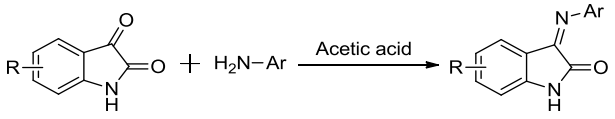
The synthesis of 3-arylimino-2-oxindoles was achieved by the reaction of an appropriate isatin with different aryl amines in the presence of catalytic amount of acetic acid in absolute ethanol (Scheme 3, Table 2).

Both arylilidene and arylimine derivatives were obtained as colored crystalline or powdered products, and they were purified by crystallization. Attempts to separate the cis/trans isomers were unsuccessful due to interconvention of cis and trans isomers during the dissolution of the separated compounds in ethanol and other polar solvents as the extracting solvents. The structure of all the synthesized compounds was confirmed by using IR, ^{1}H NMR, ^{13}C NMR, ESI-Mass spectra, and CHNS elemental analysis.

H-1 hydrogen of indole ring was proved to be exchangeable in the presence of few drops of deuterium oxide in ^{1}H NMR spectra. Scrutinizing the ^{1}H NMR for the compounds studied in the present study revealed that for all 3-arylilidene-2-oxindoles and 3-arylimine-2-oxindoles, H-4 hydrogen of indole ring appears at around 6 ppm in E isomers and around 6.8-7 ppm in Z isomers. This phenomenon can be explained by the anisotropic effect of aryl ring on H-4 hydrogen of indole ring in E isomers (Figure 3).

All 69 compounds were evaluated for their inhibitory activity against Src tyrosine kinase and antiproliferative activities. IC_{50} values of the compounds against Src kinase were determined using a fluorescence intensity assay. The results are shown in Tables 3 and 4. The most potent compounds against Src kinase were among 3-arylimine-2-oxindole derivatives. Among all compounds, b_{11}, b_{16}, and b_{26} showed IC_{50} values of 5.3, 10.4 and 17 µM, respectively, against Src kinase (Figure 4). All the compounds were among 3-arylimine-2-oxindoles. Only one 3-arylilidene-2-oxindole (compound a_1) showed modest Src kinase inhibitory activity (IC_{50}=12.9 µM).

Both 3-arylilidene-2-oxindoles and 3-arylimine-2-oxindoles were also tested for their cytotoxic effects against three tumor cell lines: human ovarian adenocarcinoma (SK-OV3), breast adenocarcinoma (MCF-7), and colon adenocarcinoma (HT-29) cell lines at 50 µM concentration, and the results were obtained in a percentage of inhibition of proliferation (Tables 3 and 4). As it is shown in Table 3, a number of the 3-arylilidene-2-oxindole derivatives showed the inhibitory potency higher than 50% in cells Among the three cancer cell lines used in this study, HT-29 was found to be the most sensitive cell line. Nineteen compounds showed greater than 50% proliferation inhibition in this cell line. Thirteen out of these nineteen compounds are among arylidene derivatives. Thus, it appears that arylidenes are more potent cytotoxic agents against colon cancer cell lines.



Scheme 3: Synthesis of 3-arylimino-2-oxindoles (b_{1-27}).

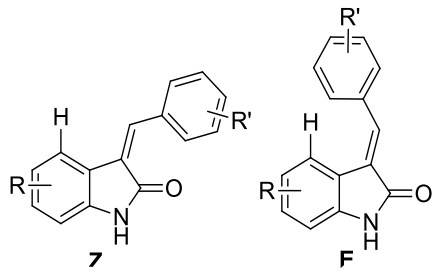


Figure 3: E and Z stereoisomers of oxindoles.

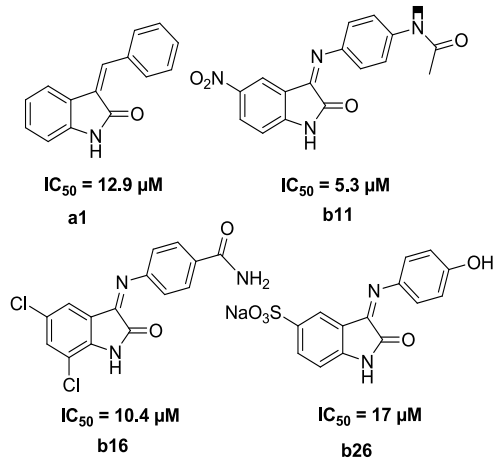


Figure 4: Compounds b11, b16, and b26 from 3-arylimine-2-oxindoles and a1 from 3-arylilidene-2-oxindoles were the most potent derivatives against Src kinase.

Compounds a_8, a_{20}, a_{38}, and b_{15} showed consistently>50% proliferation inhibition against all three cancer cell lines. Among 3-arylidene-2-oxindole derivatives, compounds a_{22}, a_{38}, and a_{15} were the most potent compounds against HT-29, SK-OV-3, and MCF-7 cells, respectively.

While 5,7-dichloro- derivative b15 in 3-arylimine substituted oxindoles showed high antiproliferative activity against HT-29 and SK-OV-3 cell lines.

Src is a protein tyrosine kinase that is involved in the regulation of multiple signal transduction pathways that are critical to cell survival and proliferation. Here, the Src kinase inhibition assay revealed that four compounds a1, b11, b16, and b26 showed the highest inhibitory activity by IC_{50} values of 12.9, 5.3, 10.4, and 17 µM, respectively. A comparison among the chemical structures of b11, b16, and b26 showed that all these compounds carry an electron withdrawing group, such as nitro, dichloro, and SO_3Na, as a substituents R group. Moreover, the presence of an electron donating groups like hydroxyl, methyl, or amine functional groups on the aryl ring was found to be facilitating the interaction with the binding site. However, as it was described above, there are additional factors such as molecular flexibility, the orientation of chemical functional groups, and proximity to binding sites that contribute to kinase inhibitory potency. Thus, further modeling investigations are required to determine the appropriate functional groups for generating more optimal Src kinase inhibition potency.

Furthermore, the antiproliferative activity of compounds in three different cancer cell lines including HT-29, SK-OV-3, and MCF-7 showed that the activity was cell-dependent. Among all compounds, a8, a38, a20, b15, a22, a36, and a15 showed the highest antiproliferative potency by 70%, 67%, 70%, 76%, 76%, 76%, and 77%, respectively, in various types of cells. However, a8 and a38 were more potent in SK-OV-3 cells compared to other types of cells. A similar pattern was observed for compounds a20 and a22 in HT-29 cells and a15 and b15

Compound	c-Src kinase Inhibition IC_{50} (µM)[a]	Proliferation inhibition (%)		
		HT-29	SK-OV-3	MCF-7
a_1	12.9	70	42	NA[c]
a_2	21	52	<30	NA
a_3	ND[b]	<30	<30	<30
a_4	ND	<30	NA	<30
a_5	>300	52	40	35
a_6	ND	44	35	<30
a_7	30.5	45	<30	35
a_8	ND	62	70	69
a_9	ND	4	<30	NA
a_{10}	ND	66	52	<30
a_{11}	ND	40	35	NA
a_{12}	ND	62	35	NA
a_{13}	27.5	37	<30	<30
a_{14}	ND	33	<30	<30
a_{15}	25.3	<30	<30	77
a_{16}	36.1	52	47	45
a_{17}	>300	45	<30	NA
a_{18}	ND	52	<30	NA
a_{19}	ND	NA	<30	<30
a_{20}	35.2	67	62	50
a_{21}	156.4	NA	<30	<30
a_{22}	21.2	76	68	35
a_{23}	65.3	45	35	NA
a_{24}	63.1	60	<30	76
a_{25}	ND	48	<30	NA
a_{26}	ND	ND	ND	ND[b]
a_{27}	97.2	67	48	<30
a_{28}	ND	42	37	<30
a_{29}	95.1	<30	<30	<30
a_{30}	46.5	60	60	<30
a_{31}	ND	<30	<30	<30
a_{32}	ND	40	32	<30
a_{33}	ND	NA	<30	<30
a_{34}	39.2	50	35	40
a_{36}	46.2	55	38	<30
a_{37}	178.9	33	<30	<30
a_{38}	30.3	68	76	55
a_{39}	>300	<30	<30	<30
a_{40}	ND	<30	NA	<30
a_{41}	69.1	<30	NA	<30
a_{42}	33.7	40	30>	<30

[a]The concentration that inhibited enzyme activity by 50%; [b]Not determined; [c]No activity. All data are average of triplicate experiments.

Table 3: The biological activity of compounds a_{1-42}.

Compound	c-Src kinase Inhibition IC_{50} (μM)[a]	Proliferation inhibition (%)		
		HT-29	SK-OV-3	MCF-7
b1	ND[b]	<30	<30	NA
b_2	41.4	<30	<30	NA
b_3	ND	50.0	NA	60
b_4	ND	<30	<30	NA
b_5	40.5	41.0	<30	<30
b_6	ND	NA[c]	<30	NA
b_7	ND	<30	NA	NA
b_8	ND	40	<30	NA
b_9	ND	NA	<30	<30
b_{10}	36.5	NA	<30	NA
b_{11}	5.3	ND	ND	ND
b_{12}	148.4	<30	NA	<30
b_{13}	29.9	32	<30	47.0
b_{14}	ND	<30	NA	<30
b_{15}	50.8	82.0	68	<70
b_{16}	10.4	<30	NA	<30
b_{17}	ND	NA	NA	<30
b_{18}	23.9	NA	<30	NA
b_{19}	ND	<30	<30	58.0
b_{20}	211.8	<30	NA	<30
b_{21}	ND	<30	NA	<30
b_{22}	ND	<30	NA	<30
b_{23}	61.1	<30	NA	<30
b_{24}	27.7	<30	NA	<30
b_{25}	ND	<30	<30	NA
b_{26}	17.0	77.0	NA	48.0
b_{27}	27.7	<30	<30	NA

[a]The concentration that inhibited enzyme activity by 50%; [b]Not determined; [c]No activity. All data are average of triplicate experiments.

Table 4: The biological activity of compounds $\mathbf{b}_{1\text{-}27}$.

in MCF-7 cells. Comparing the chemical structures of compounds revealed that the majority of them carry electron withdrawing groups including Br, Cl, and NO_2 either as the R substituent or on the aryl ring. Several factors contribute to the antiproliferative activity of a compound, such as cellular uptake and mechanism of action. Further investigations are needed to determine the mechanism of action like intercalating ability with DNA, radical generating property, apoptosis pathway, and/or cell necrosis.

A direct correlation between Src kinase inhibitory potency and cytotoxicity of all compounds individually was not discovered. However, comparing the results obtained in Src kinase inhibitory and cytotoxic studies revealed the following different trends: In general, arylilidenes were more cytotoxic agents than arylimines, possibly due to the presence of α,β-unsturated amide and a different cytotoxicity mechanism. On the other hand, arylimines exhibited higher Src kinase inhibitory activity than arylilidenes. We postulate that the arylimines are modestly active against Src kinase and less active in antiproliferative assays possibly because of limited cellular permeability. Compound a_1 was the only arylilidene derivative with high potency against Src kinase along with modest activities against HT-29 and SK-OV-3 cell lines. Further studies are required to optimize the Src kinase inhibitory and antiproliferative activities of these compounds to find an optimized one that works both as Src kinase inhibitor and antiproliferative agent for potential cancer therapy.

Conclusions

In conclusion, a number of novel 3-arylilidene- and 3-arylimine-2-oxindole [30] derivatives were synthesized and evaluated for Src kinase inhibitory and antiproliferative activities. In general, arylilidenes exhibited higher antiproliferative activity than arylimines. Compound b_{11} in 3-arylimine-2-oxindoles showed IC_{50} values of 5.3 μM against Src kinase. These data suggest that 3-arylilidene and 3-arylimine-2-oxindole chemical scaffolds can be used as new scaffolds for further structure optimization for generating compounds with higher antiproliferative or Src kinase inhibitory activities, respectively.

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