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Full Paper

Synthesis, Biological, and Computational Evaluation of Novel 1,3,5-Substituted Indolin-2-one Derivatives as Inhibitors of Src Tyrosine Kinase

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Several substituted indolin-2-one derivatives were synthesized and evaluated for their activities against Src kinase. Several compounds showed activity against Src, with IC_{50} values in the low micromolar range. Among them, compound **2f** showed the most significant activity with an IC_{50} value of 1.02 μM. Molecular docking studies have been performed for evaluation of the binding modes of compound **2f** into the Src active site. The docking structure of compound **2f** disclosed that the indole NH forms a hydrogen bond with the carbonyl of Met341. These results suggest that our novel compound **2f** is a promising compound for the further development of indole-based drugs targeting Src kinase.

Keywords: Cancer / Indolin-2-one / Molecular docking / Src kinase / Tyrosine kinase activity

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Introduction

The last two decades have witnessed the developments on Src kinase research and selective small molecule inhibitors [1]. Increased Src protein kinase activity in numerous human cancer cell lines has been shown as the possible reason of several types of tumors [2, 3]. Src kinase activity is usually 4- to 20-fold higher in mammary carcinomas compared to normal tissues [4–7]. The Src kinases have also been implicated in other common human cancers such as lung [8], neural (neuroblastomas, retinoblastomas) [9], ovarian [10], esophageal [11], gastric cancers [12, 13], and melanoma [14]. In addition, the Src proto-oncogene has frequently been implicated in the initiation and progression of human colorectal cancer [15, 16]. Src activity has recently been studied in pancreatic cancer. Elevated levels of Src protein and kinase activity were found in pancreatic ductal carcinomas as well as pancreatic cell lines [17]. Src has also been implicated in breast cancer [18]. Additionally, antisense Src expressed in ovarian and colon tumor cells inhibited tumor growth in nude mice and reduced vascularization [19–21] suggesting the direct relationship between Src kinase activity and cancer. On the basis of these findings, Src has been a candidate therapeutic target in the treatment of cancer.

In the last two decades, a number of heteroaromatic small-molecule inhibitor templates have been studied as Src kinase inhibitors. Several purine, pyrrolopyrimidine, pyridopyrimidine, naphthyridone, quinazoline, oxindoles, and quinoline-based inhibitors have been reported [22, 23]. The majority of these inhibitors exploit ATP-binding site competing with ATP and present one to three hydrogen bonds with the critical amino acids located in the active site of Src like adenine ring of

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ATP [24]. In general, ATP-binding site inhibitors of Src kinase contain a largely planar rigid structure, heteroaromatic ring system that occupies the adenine binding site and exhibit hydrophobic interactions with hydrophobic region of ATP binding site. Additionally, these compounds have a hydrogen bond donor/acceptor pair and form similar hydrogen bonds as ATP at the ATP-binding site of Src kinase [25].

Indole-based derivatives showed promising inhibitory properties of Src kinase. In the literature, a series of pyrrolyllactone indolinones was reported as potent Src inhibitors; among them compound **I** (Fig. 1) exhibits potent inhibitory activity against Src kinase ($IC_{50} = 1.1\,\mu M$) [26]. Recently, various substituted indolin-2-one derivatives were reported as KDR, Flt-1, PDGFR, and FGFR1 inhibitors. Compound **II** (Fig. 1) bearing amino piperidinyl at 5-position of oxindole ring also exhibited inhibitory activity for Src kinase at low concentration ($IC_{50} = 33\,nM$), when screened against a wider panel of kinases [27]. We previously found indole-based compound (**III**, Fig. 1) as potent Src inhibitor with IC_{50} of 1.34 μM [28]. Compounds **IV** and **V** (Fig. 1) of a series of 3-(substituted-benzylidene)-1,3-dihydro-indolin-2-thione derivatives and the corresponding indolin-2-one congeners were identified as moderately active Src inhibitors, with IC_{50} of 21.91 and 21.20 μM, respectively [29]. As part of our continuing efforts to develop potent Src inhibitors, we designed a series of some indole-3-imines and their corresponding amine derivatives, bearing indole as heterocyclic ring system, hydrophobic side chain, and hydrogen bond donor/acceptor group as in other ATP-competitive Src inhibitors. Among them, compound **VI** (Fig. 1) was found as a promising Src inhibitor with IC_{50} of 4.69 μM [30]. In the current study, 1,3,5-substituted-indolin-2-one derivatives were synthesized and evaluated for the effects on Src activity. In addition, the molecular docking study was carried out by AutoDock Vina to evaluate the binding properties and structure–activity relationships of the compounds.

Results and discussion

Chemistry

The target compounds (**2a–h**, **6a–h**, and **7a–h**) were prepared following the procedure reported in Scheme 1. For the synthesis of 3-(substituted-benzylidene)-5-(4-fluorophenyl) indolin-2-one derivatives (**2a–h**), 5-iodo indolin-2-one (**1**) was obtained by a Wolff–Kishner reduction of 5-iodoisatin in 50% yield [31]. Synthesis of 5-(4-fluorophenyl)indolin-2-one (**2**) was achieved by palladium-catalyzed Suzuki cross-coupling reaction of 5-iodo indolin-2-one (**1**) with *p*-fluorophenylboronic acid [32]. For the preparation of 1-(3-substituted-benzylidene-2-oxoindolin-5-yl)-3-ethylurea (**6a–h**) and 1-benzyl-3-(3-substituted-benzylidene-2-oxoindolin-5-yl)thiourea (**7a–h**) derivatives, indolin-2-one (**3**) was synthesized by Wolff–Kishner reduction of isatine with hydrazine hydrate [31]. Then, 5-nitroindolin-2-one (**4**) was prepared by stirring of indolin-2-one (**3**) with potassium nitrate in concentrated sulfuric acid at 0–5°C for 30 min [33]. 5-Aminoindolin-2-one (**5**) was generated from nitro compound (**4**) by catalytic hydrogenation in moderate yield [34]. Reaction of compound **5** with ethylisocyanate or benzylisothiocyanate afforded the corresponding ureidoindolin-2-one derivatives (compounds **6** or **7**) [35]. Finally, the target compounds (**2a–h**, **6a–h**, and **7a–h**) were prepared by condensation of **2**, **6**, and **7** with substituted benzaldehydes in ethanol at reflux [36].

The synthesis of the compounds **13a–d** was carried out as outlined in Scheme 2. At first, 5-nitroisatine (**8**) was prepared by stirring of isatine with $NaNO_3$ and sulfuric acid at 0°C for 1 h [37]. Compound **9** was synthesized by *p*-toluene sulfonic acid

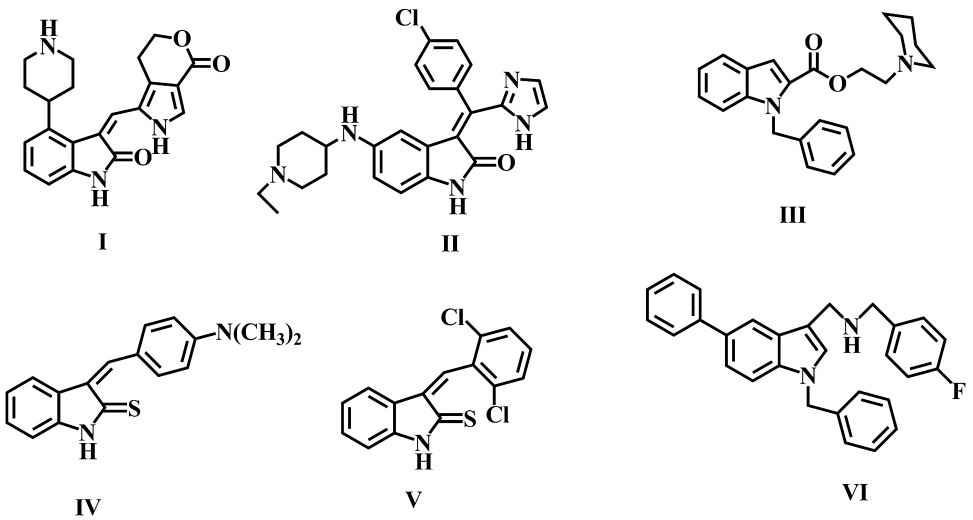
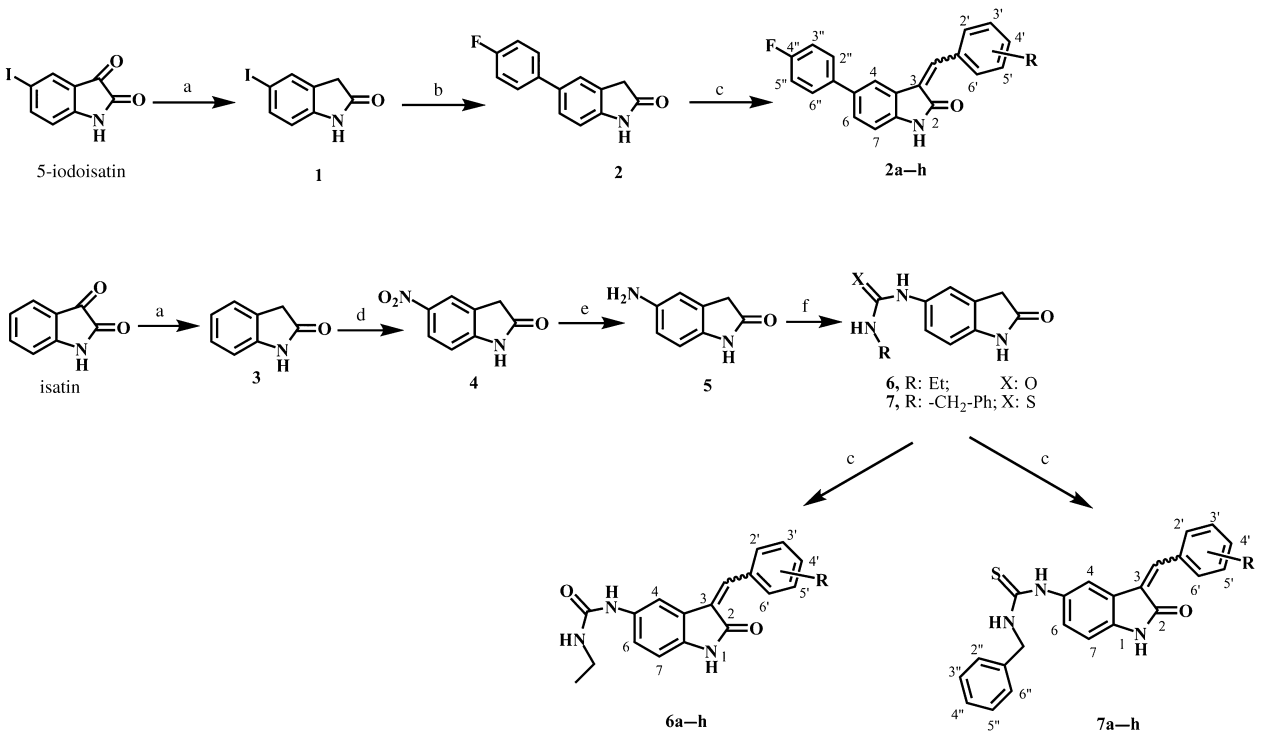
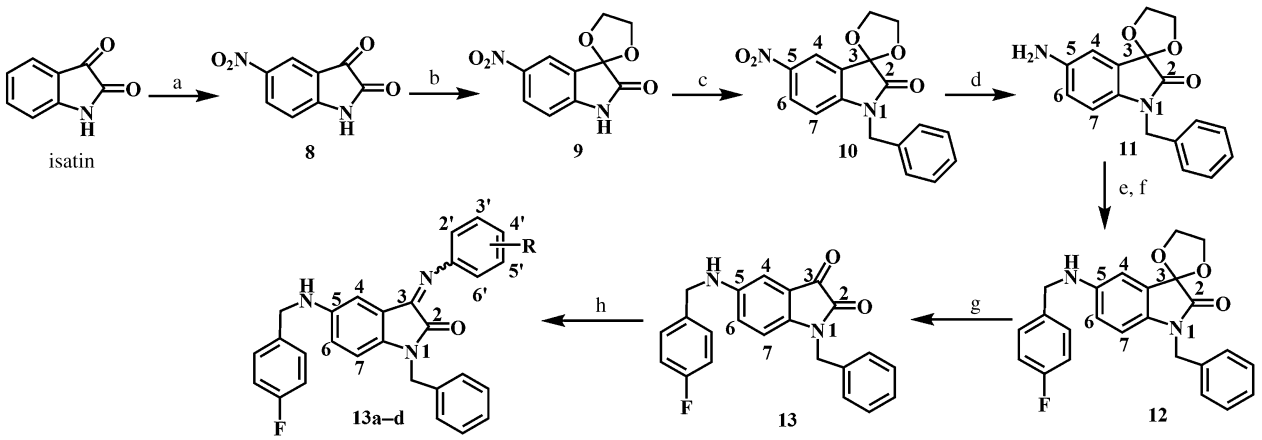


Figure 1. Indole-based Src inhibitors.



Scheme 1. Synthesis of 5-fluorophenyl-3-(substituted-benzylidene)indolin-2-one (**2a–h**), 1-(3-substituted-benzylidene-2-oxoindolin-5-yl)-3-ethylurea (**6a–h**) and 1-benzyl-3-(3-substituted-benzylidene-2-oxoindolin-5-yl)thiourea (**7a–h**) derivatives. Reagents and conditions: (a) hydrazine hydrate, 140°C, 4 h, (b) *p*-fluorophenyl boronic acid, $Pd(PPh_3)_4$, Na_2CO_3 (sat.)/DME, 85°C, 5 h, (c) substituted benzaldehydes, piperidine, ethanol, reflux, (d) KNO_3/H_2SO_4, 0–5°C, (e) Pd/C 10%, H_2/MeOH, 1 atm, 4 h, rt, (f) Et-NCO or Ph–CH_2–NCS, ethylacetate, rt.

catalyzed cyclocondensation reaction of **8** with ethylene glycol in toluene at 110°C [38]. Mixture of **9** and NaH was treated with benzyl bromide in DMF to give the desired *N*-benzylated compound **10**, which was reduced to compound **11** by catalytic hydrogenation [37, 38]. Compound **11** was treated with *p*-fluorobenzaldehyde in toluene at room temperature in the presence of molecular sieves 4 Å to afford imine derivatives of **11**. Without any work-up, the reaction of



Scheme 2. Synthesis of 1-benzyl-5-(4-fluorobenzylamino)-3-(substituted-phenylimino)indolin-2-one derivatives (**13a–d**). Reagents and conditions: (a) $NaNO_3$, H_2SO_4, 0°C, (b) *p*-toluenesulfonic acid, ethylene glycol, toluene, (c) NaH, DMF, benzyl bromide, (d) Pd/C 10%, H_2, MeOH, 1 atm, 4 h, rt, (e) *p*-fluorobenzaldehyde, molecular sieves 4 Å, toluene, 24 h, rt, (f) $NaBH_4$, MeOH, 3 h, rt, (g) conc. HCl/CH_3COOH (4:1), reflux, 1 h, (h) anilin derivatives, glacial acetic acid, ethanol, 90°C.

this product with $NaBH_4$ in methanol afforded its amine congener **12** [39]. Compound **13** was synthesized by hydrolysis of **12** with a mixture of hydrochloric and acetic acid (4:1) [40].

The synthesis of the compounds **15a–d** and **16a–d** is described in Scheme 3. *N*-Benzylation of 5-iodoisatin with benzyl bromide using NaH in DMF afforded compound **14** [41]. Synthesis of compounds **15** and **16** was achieved by palladium-catalyzed Suzuki cross-coupling reaction of 5-iodoisatin and *N*-benzyl-5-iodoisatin (**14**) with *p*-fluorophenylboronic acid [32]. Finally, the target compounds (**13a–d**, **15a–d**, and **16a–d**) were generated from **13**, **15**, or **16** by condensation reaction with various anilin derivatives in ethanol in the presence of glacial acetic acid [42].

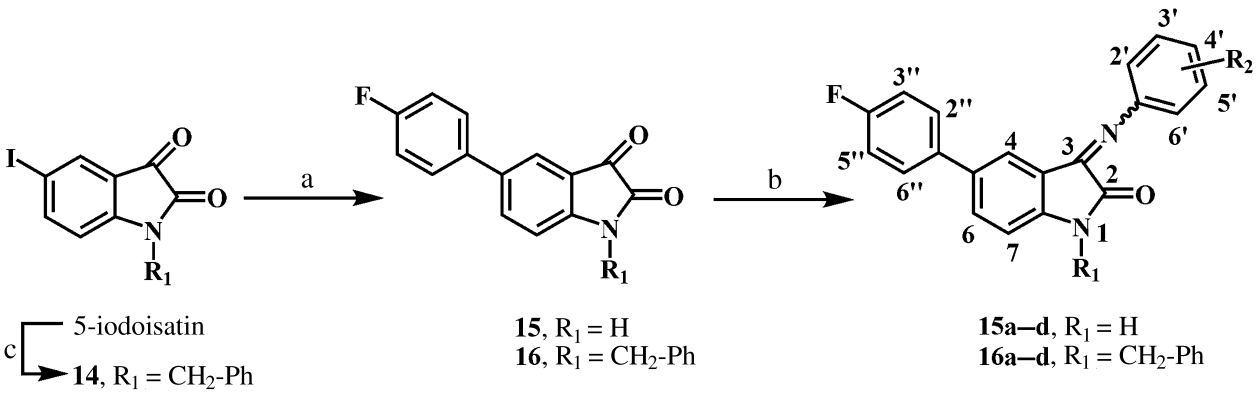
The benzylidene derivatives (**2a–h**, **6a–h**, and **7a–h)** of target compounds were obtained as the *E*- (**2a–c**, **2f**, **2h**, **6a**, **6b**, **6d**, **6g**, **7a–d**, and **7g**) or *Z*-isomer (**2e** and **7f**) or as mixture of both isomers (**2d**, **2g**, **6c**, **6e**, **6f**, **6h**, **7e**, and **7h**) while the imine derivatives (**13a–d**, **15a–d**, and **16a–d**) exist as mixture of *E*- and *Z*-isomer due to the exocyclic double bond. Due to the rapid interconversion in solution at room temperature, the mixture of isomers could not be separated by column chromatography.

The configurations of compounds were determined by a nuclear Overhauser effect (NOE) experiment and ^{1}H NMR spectra. In benzylidene derivatives, *E*-isomers have a NOESY correlation between H-4 and H-2′/H-6′, whereas the *Z*-isomers exhibit a NOESY correlation as follows: vinyl protons and H-4. Among the compounds obtained as isomer mixture, *E/Z* ratios of compounds **2g** and **6c** were determined as 2:1 using the corresponding chemical shifts and integrals in the ^{1}H NMR spectra. H-2′ and H-6′ of **2g** display a down-field shift (8.51 ppm) in the *Z*-isomer than *E*-isomer (7.79 ppm) due to the deshielding effect of the carbonyl group at the 2-position of indole ring. The signals from H-4 of *E*-isomers of **2g** and **6c** (7.87 and 7.60 ppm) were markedly shifted upfield relative to the H-4 signals of *Z*-isomers **2g** and **6c** (8.02 and 7.74 ppm) due to the shielding effect of H-2′ and H-6′ of benzylidene substituent [43, 44]. For the imine derivatives, the *E*-isomer was assigned to the major isomer because of the chemical shifts of H-4 and NOE correlation between H-4 and H-2′/H-6′. The ^{1}H NMR chemical shifts of all compounds obtained as a mixture of isomers have been reported here to prove the major *E*-isomers.

Biological evaluation

The substituted benzylidene (**2a–h**, **6a–h**, and **7a–h)** and substituted phenylimino (**13a–d**, **15a–d**, and **16a–d**) derivatives of indolin-2-one are depicted in Table 1. Compounds were evaluated for their inhibitory activity against Src kinase by universal tyrosine kinase assay that measures the changes in the enzymatic activity of Src kinase by virtue of following the alterations in the phosphorylation level of the immobilized substrate that was used for this analysis [45]. The activity of the prepared compounds was analyzed at five concentrations (0.1, 1, 10, 100, and 1000 μM) and IC_{50} values were calculated. Imatinib was tested as reference compound in this assay and PP1 (known as Src inhibitor) was used as reference compound, which was previously tested in same assay method [28]. The IC_{50} values of the compounds are shown in Table 1. Benzylidene derivative **2f** containing rigid *p*-fluorophenyl group at 5-position of indolin-2-one ring exhibited the best inhibitory activity with IC_{50} value of 1.02 μM. Compound **2f** was found 1.4- and 6-fold less potent than imatinib (IC_{50} = 0.717 μM) and PP1 (IC_{50} = 0.17 μM), respectively. Replacement of the dimethylamino group of **2f** with methoxy (**2g**) and chloro substituent (**2a**) decreased the activity to IC_{50} values of 1.98 and 10.08 μM. Structural comparison between **2a** and **2d** indicated that introduction of chloro at 3′-position of phenyl ring of benzylidene side chain in **2a** resulted in complete loss of inhibition. Compound **2b**, bearing fluoro substituent at 4′-position of benzylidene moiety did not show inhibition of Src compared with 4-dimethylaminobenzylidene derivative **2f**.

To further investigate the influence of flexible and polar group at the 5-position of indole scaffold, we kept the side chain at 3-position of indole as substituted benzylidene group



Scheme 3. Synthesis of 5-(4-fluorophenyl)-3-(substituted-phenylimino)indolin-2-one (**15a–d**) and 1-benzyl-5-(4-fluorophenyl)-3-(substituted-phenylimino)indolin-2-one (**16a–d**) derivatives. Reagents and conditions: (a) *p*-fluorophenyl boronic acid, $Pd(PPh_3)_4$, Na_2CO_3 (sat), DME, 85°C, 5 h, (b) anilin derivatives, glacial acetic acid, ethanol, 90°C, (c) NaH, DMF, benzyl bromide.

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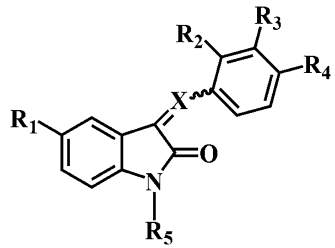
Table 1. Src tyrosine kinases inhibitory activity of 1,3,5-substituted-indolin-2-one derivatives.



Compound	R_1	R_2	R_3	R_4	R_5	X	Src enzyme IC_{50} (μM)	LogP[b)]
2a	*p*–F–Ph	H	H	Cl	H	CH	10.08	5.08
2b	*p*–F–Ph	H	H	F	H	CH	>1000	4.68
2c	*p*–F–Ph	H	F	H	H	CH	>1000	4.68
2d	*p*–F–Ph	H	Cl	Cl	H	CH	ND	5.67
2e	*p*–F–Ph	F	H	F	H	CH	417.67	4.83
2f	*p*–F–Ph	H	H	$N(CH_3)_2$	H	CH	1.02	4.80
2g	*p*–F–Ph	H	H	OCH_3	H	CH	1.98	4.39
2h	*p*–F–Ph	H	H	COOH	H	CH	>1000	4.08
6a	NH–CO–NH–Et	H	H	Cl	H	CH	ND	2.4
6b	NH–CO–NH–Et	H	H	F	H	CH	2.06	2.0
6c	NH–CO–NH–Et	H	F	H	H	CH	ND	2.0
6d	NH–CO–NH–Et	H	Cl	Cl	H	CH	ND	2.95
6e	NH–CO–NH–Et	F	H	F	H	CH	309.35	2.15
6f	NH–CO–NH–Et	H	H	$N(CH_3)_2$	H	CH	>500	2.12
6g	NH–CO–NH–Et	H	H	OCH_3	H	CH	ND	1.71
6h	NH–CO–NH–Et	H	H	COOH	H	CH	>1000	1.40
7a	NH–CS–NH–CH_2–Ph	H	H	Cl	H	CH	>500	5.08
7b	NH–CS–NH–CH_2–Ph	H	H	F	H	CH	1.38	4.68
7c	NH–CS–NH–CH_2–Ph	H	F	H	H	CH	ND	4.68
7d	NH–CS–NH–CH_2–Ph	H	Cl	Cl	H	CH	ND	5.64
7e	NH–CS–NH–CH_2–Ph	F	H	F	H	CH	1.24	4.84
7f	NH–CS–NH–CH_2–Ph	H	H	$N(CH_3)_2$	H	CH	>500	4.80
7g	NH–CS–NH–CH_2–Ph	H	H	OCH_3	H	CH	ND	4.39
7h	NH–CS–NH–CH_2–Ph	H	H	COOH	H	CH	2.01	4.08
13a	NH–CH_2–*p*–F–Ph	H	H	F	CH_2–Ph	N	>500	5.97
13b	NH–CH_2–*p*–F–Ph	H	H	Cl	CH_2–Ph	N	153.13	6.37
13c	NH–CH_2–*p*–F–Ph	H	H	SCH3	CH_2–Ph	N	>500	6.26
13d	NH–CH_2–*p*–F–Ph	H	Cl	F	CH_2–Ph	N	>500	6.53
15a	*p*–F–Ph	H	H	F	H	N	ND	4.45
15b	*p*–F–Ph	H	H	Cl	H	N	>500	4.85
15c	*p*–F–Ph	H	H	SCH3	H	N	ND	4.73
15d	*p*–F–Ph	H	Cl	F	H	N	7.48	5.01
16a	*p*–F–Ph	H	H	F	CH_2–Ph	N	>500	6.42
16b	*p*–F–Ph	H	H	Cl	CH_2–Ph	N	314.48	6.82
16c	*p*–F–Ph	H	H	SCH3	CH_2–Ph	N	>500	6.70
16d	*p*–F–Ph	H	Cl	F	CH_2–Ph	N	4.04	6.98
Imatinib							0.717	
PP1[a)]							0.17	

ND, not determined.
[a)] PP1 was tested in the previous study [28].
[b)] LogP value was calculated by ChemBioDraw Ultra 11.0.

and changed the 4-fluorophenyl moiety at 5-position of indolin-2-one ring (**2a–h**) as ethylurea (**6a–h**) and benzylthiourea (**7a–h**). Compound **6b** was found 2.8- and 12.1-fold less active than the imatinib and PP1, respectively. The insertion of one fluoro in the *ortho* position of benzylidene moiety of **6b** leading to **6e** abolished the activity. The replacement of the fluoro in **6b** with a chloro (**6a**), dimethylamino (**6f**), methoxy (**6g**), and carboxy (**6h**) resulted in either complete loss or dramatically reduction of activity. Of the benzylthiourea (**7a–h**) derivatives, 2,4-difluorophenyl substituted compound **7e** ($IC_{50} = 1.24\,\mu M$) exhibited 1.7- and 7.2-fold less activity than the imatinib and PP1, respectively. Exchanging the 2′-fluoro substituent with hydrogen was well tolerated when compounds **7e** ($IC_{50} = 1.24\,\mu M$) and **7b** ($IC_{50} = 1.38\,\mu M$) were compared. The carboxy substitution at 4′-position of the **7h** ($IC_{50} = 2.01\,\mu M$) slightly decreased Src inhibition compared with 4′-fluorobenzylidene substituted compound **7b** ($IC_{50} = 1.38\,\mu M$). Compounds, bearing chloro (**7a**), dimethylamino (**7f**), and methoxy (**7g**) substituents at the 4′-position of benzylidene moiety did not show inhibition of Src. Moving of fluoro substituent from 4′- to the 3′-position on the phenyl ring of **6b** and **7b**, leading to **6c** and **7c**, resulted in complete loss of inhibiton. From Src activity results of benzylidene derivatives, it has been suggested that hydrophobic and rigid *p*-fluorophenyl group at 5-position of indolin-2-one ring significantly increased the potency in the case of **2a**, **2f**, and **2g** relative to their ethylurea (**6a**, **6f**, and **6g**) and benzylthiourea derivatives (**7a**, **7f**, and **7g**). On the contrary, the polar and flexible ethylurea and benzylthiourea substituents at the 5-position of indole such as the cases of **6b** and **7b** significantly improved the activity than *p*-fluorophenyl substiuted compound **2b**. Although the presence of ethylurea or benzylthiourea substitution at 5-position of indole ring has no significant impact on activity when compounds **6b** and **7b** are compared, benzylthiourea moiety increased the activity of **7e** and **7h** relative to ethylurea congeners **6e** and **6h**.

Among the imine derivatives, **16d** exhibited the moderate inhibitory activity against Src with IC_{50} values of 4.04 μM. Replacement of indole hydrogen of **15d** ($IC_{50} = 7.48\,\mu M$) with benzyl group, **16d**, brought approximately twofold increase of Src inhibitory activity. Introduction of chloro substituent in 3′ positions of **15a** and **16a**, leading to **15d** and **16d**, significantly increased potency, while the activity was not effected by similar modification of **13a** to corresponding **13d**. Changing the rigid *p*-fluorophenyl group of **16d** to flexible benzylamino as in **13d** resulted in dramatic reduction of Src inhibitory activity. On the contrary, **13b** exhibited weak inhibition against Src compared with **16b**. Compounds **13a–d**, **15a–c**, and **16a–c** did not inhibit Src kinase.

In general, the hydrophobic compounds with logP value ranging from 4 to 7 (Table 1) were found more active than hydrophilic compounds (logP = 1.4–2.95). This indicates that lipophilicity of compounds would help improve inhibitory activity and binding affinity with hydrophobic interactions. When imine and benzylidene derivatives are structurally compared, it was observed that introduction of nitrogen instead of CH of benzylidene derivatives resulted in less Src inhibitory activity.

Molecular docking

In this study, the active compounds (**2f**, **2g**, **6b**, **7b**, **7e**, **7h**, **15d**, and **16d**) were docked into the active site of Src tyrosine kinase to understand their binding modes by the docking program AutoDock Vina 1.0.2 software package. At the first step, the validation of the scoring function implemented in AutoDock Vina was done by docking the native ligand (PP2) into its binding site. The docked results were compared to the crystal structure of the bound PP2–Src complex (PDB code: 3GEQ). The obtained success rate is excellent as the docked PP2 appeared to be superimposed almost exactly on the native ligand. The RMSD of docked ligand (PP2) was 0.104 Å. Binding mode of PP2 in Src kinase has been extensively studied. Two highly conserved hydrogen bonds are created by N5 of PP2 and amino group of C4 of PP2 with NH group of Met 341 and carbonyl of Glu 339, respectively [46]. The docked PP2 exhibited one hydrogen bond between N5 of PP2 and NH of Met 341, whereas the native ligand exhibited two hydrogen bonds with Met 341 and Glu 339.

Molecular insights based on molecular docking indicated that favorable binding interactions of the active compounds with the binding site of Src were visually analyzed. The best active compound **2f** located in somewhat similar position to the PP2 with overlapping *p*-dimethylaminobenzylidene at 3-position of indole ring and *p*-chlorophenyl group of PP2 and showed a hydrogen bond with Met341 of critical amino acids for the binding to the Src active site. Dimethylaminobenzylidene moiety of **2f** inserted into the hydrophobic pocket surrounded by Val281, Lys295, Ile336, Ala403, and Phe405. Moreover, *p*-fluorophenyl group at 5-position of indole ring was directed toward the solvent accessible region as *tert*-butyl group of PP2 (Fig. 2). In addition to hydrophobic interactions, hydrogen bond interaction with critical amino acid Met341 may account for the inhibitory activity of **2f** at low micromolar level comparable with PP1. Compound **2g** occupied the ATP-binding site with no hydrogen bond being formed with backbone of the hinge region residues Glu339 and Met341. Compound **6b** formed a hydrogen bond between carbonyl of ethylurea moiety and NH of Lys295. Compounds **7b** and **7h** located in the active site of Src kinase by forming hydrogen bonding interactions between their side chain NH group at 5-position of indole ring and backbone carbonyl groups of Leu273. Compound **7e** showed a hydrogen bond contact between its indole carbonyl and hydroxy of Thr338. Among the active compounds, imine derivatives **15d** and **16d** were not involved in direct hydrogen bonding interactions with Src kinase. 4-Fluoro-3-chlorophenylimino side chain of these compounds overlapped *p*-chlorophenyl group of PP2 and fitted into the hydrophobic pocket like **2f**. When conformations of **15d** and **16d** were compared with **2f**, the slight difference was observed in position of *p*-fluorophenyl group of **15d** and **16d,** lying in the

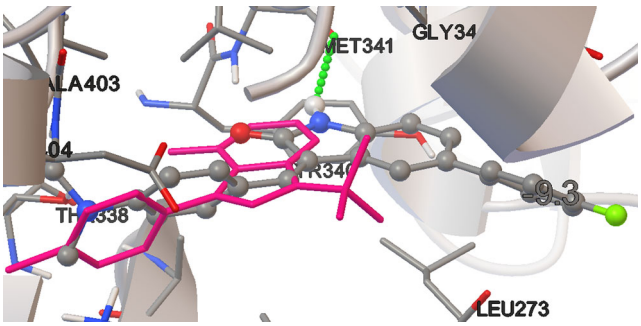


Figure 2. Comparison of PP2 (pink) and compound **2f** (colored by element) binding properties in the active site of Src. H-bond is represented as yellow dashed lines.

vertical direction to the *p*-fluorophenyl of **2f**. The decrease in activity of **15d** and **16d** relative to **2f** can be the result of different conformations from **2f**. In general, the binding modes predicted by AutoDock modeling between the Src and active compounds were correlated with inhibitory activity.

Conclusion

In this study, we designed and synthesized novel 1,3,5-substituted indolin-2-one derivatives as Src kinase inhibitors. Some of the compounds (**2f**, **2g**, **6b**, **7b**, **7e**, **7h**, **15d**, and **16d**) exhibited single digit micromolar inhibition of Src. The best activity was obtained by compound **2f** with IC_{50} value of 1.02 μM. The compound **2f** showed comparable activity with PP1 and imatinib in Src kinase assay. In addition, the most active compound **2f** was docked into the Src active site and favorable protein–ligand interactions were visually analyzed. Results of a virtual docking study of compound **2f** demonstrated that it located into the active site of Src similar to PP2 and formed a hydrogen bond interaction with Met341 backbone amino acid of Src like PP2. The results reported here provide a foundation for discovery of more active novel indolin-2-one compounds as promising compounds for Src kinase target in the future.

Experimental

Chemistry

$Pd(PPh_3)_4$, *p*-fluorophenyl boronic acid, DME, hydrazine hydrate, $NaHCO_3$, ethylacetate, Na_2SO_4, molecular sieves 4 Å, anhydrous DMF, NaH, $NaNO_3$, methanol, *p*-toluenesulfonic acid, and ethylene glycol were purchased from Merck. 4-Fluorobenzaldehyde, 2,4-difluorobenzaldehyde, 4-chlorobenzaldehyde, *N*,*N*-dimethylaminobenzaldehyde, 3,4-dichlorobenzaldehyde, 4-carboxybenzaldehyde, potassium nitrate, 5-iodoisatin, ethylisocyanate, benzylisothiocyanate, 5-iodoisatin, dichlorometane, *p*-fluoroanilin, *p*-chloroanilin, toluene, Pd/C 10%, and $NaBH_4$ were purchased from Aldrich. 4-Fluoro-3-chloroanilin and 4-methylthioanilin were purchased from Acros and Lancaster, respectively. HCl, piperidine, glacial acetic acid, and ethanol were purchased from Riedel de Haen. 4-Methoxybenzaldehyde, 3-fluorobenzaldehyde from Fluka were used for reactions. Solvents and reagents were used without further purification. Analytical TLC was carried out on Merck 0.2 mm pre-coated silica gel (60 F-254) aluminum sheets (Merck), visualization by irradiation with an UV lamp. Takara Universal Tyrosine Assay Kit (from Takara-Bio Inc., Shiga, Japan) was used to test our compounds for their Src kinase inhibition. The contents of a kit: PTK substrate immobilized microplate (8 wells × 12), kinase reacting solution (11.0 mL), 40 mM ATP-2Na (0.55 mL), extraction buffer (11.0 mL), PTK control (0.50 mL), anti-phoshotyrosine (PY20-HRP, for 5.5 mL/H_2O), blocking solution (11.0 mL), horseradish peroxidase (HRP) coloring solution (tetramethylbenzidine, TMBZ,12.0 mL).

Melting points were measured with a capillary melting point apparatus (BUCHI Melting Point B-540). The nuclear magnetic resonance (^1H NMR and ^{13}C NMR) spectra were recorded on Varian Mercury 400 NMR spectrometer 400 MHz (Varian Inc., Palo Alto, CA, USA). The chemicals shift values were expressed in parts per million (ppm) relative to tetramethylsilane as an internal standard. Mass spectra were recorded on a Waters ZQ Micromass LC-MS spectrometer (Waters Corporation, Milford, MA, USA). Elemental analysis was taken on a Leco-932 CHNS-O analyzer (Leco, St. Joseph, MI, USA).

Synthesis of compounds 1–16

*5-Iodoindolin-2-one (**1**)*

A stirred solution of 5-iodoisatin (0.01 mol; 3.06 g) in hydrazine hydrate (15 mL) was heated to 140°C for 4 h. The reaction was cooled to room temperature, poured into ice-cold water, and acidified to pH 2 with 6 N hydrochloric acid. After standing at room temperature for 2 days the precipitate was collected by vacuum filtration, washed with water, and dried *in vacuo* to give pure compound **1**. Yield 50% (1.45 g), mp 175–178°C (lit. [47], 170–172°C).

*5-(4-Fluorophenyl)indolin-2-one (**2**)*

5-Iodoindolin-2-one (**1**, 9.25 mmol; 2.39 g) was dissolved in 150 mL DME. $Pd(PPh_3)_4$ (0.46 mmol; 0.53 g), *p*-fluorophenylboronic acid (0.01 mol; 1.55 g), and Na_2CO_3 solution (0.01 mol; 1.55 g in 30 mL H_2O) were added, respectively. The mixture was boiled at 80°C for 10 h. DME was removed under reduced pressure, then the water layer was extracted by ethylacetate (3 × 100 mL). The organic layer was washed with H_2O (2 × 10 mL), brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated at reduced pressure. The crude product was purified by column chromatography (hexane/ethylacetate = 6:3) to give pure compound **2**. Yield: 26% (0.55 g), mp 211–212°C [48].

*Indolin-2-one (**3**)*
Indolin-2-one was synthesized from isatin according to the same procedure of compound **1**. Yield: 41% (2.58 g), mp 127°C (lit.[31], 127–129°C).

*5-Nitroindolin-2-one (**4**)*
Isatin (0.03 mol; 5 g) was dissolved in 28 mL of cold concentrated sulfuric acid at 0°C. After complete dissolution, potassium nitrate (0.03 mol; 3.87 g) was added as a portion. The temperature of the mixture should not exceed 5°C. After further stirring for 30 min, the mixture was added to 200 mL of crushed ice. The precipitate was collected by filtration, washed with water, and dried. The raw product was purified by recrystallization from acetic acid (50%) to give pure compound **4**. Yield: 35% (2.37 g), mp 240–243°C (lit. [33], 249–254°C).

*5-Aminoindolin-2-one (**5**)*
Ten percent Pd/C (0.092 g) was added to a solution of 5-nitroindolin-2-one (**4**, 2.07 mmol; 0.36 g) in 5 mL of methanol. The mixture was hydrogenated (45 psi H_2) for 3 h at room temperature. Then the solution was filtered with celite and filtrate was evaporated. The crude product was purified by column chromatography (CH_2Cl_2/MeOH = 9.5:0.5) to give compound **5**. Yield: 64% (0.19 g), mp 202–204°C (lit.[49], 213–214°C).

*1-Ethyl-3-(2-oxoindolin-5-yl)urea (**6**)*
Ethylisocyanate (3.24 mmol; 0.25 mL) was added to a solution of 5-aminoindolin-2-one (**5**, 2.71 mmol; 0.4 g) in 5 mL of ethylacetate. The mixture was stirred at the room temperature for 24 h. The precipitate was filtered, washed with diethylether, and dried to give compound **6**. Yield: 85% (0.5 g), mp 183°C. ^{1}H NMR (DMSO-d_6) δ: 0.99 (t, 3H, CH_3), 3.02–3.05 (m, 2H, –$\underline{CH_2}$–CH_3), 3.37 (s, 2H, H-3), 5.93 (t, 1H, NH–CO–$\underline{NH}C_2H_5$), 6.62 (d, 1H, J_o = 8.4 Hz, H-7), 7.05 (dd, 1H, J_o = 8.4 Hz, J_m = 2.0 Hz, H-6), 7.27 (s, 1H, H-4), 8.14 (s, 1H, $\underline{NH}$–CO–NHC_2H_5), 10.13 (s, 1H, indole-NH). MS (ESI): *m/z* 220.6 (M+H).

*1-Benzyl-3-(2-oxoindolin-5-yl)thiourea (**7**)*
The compound was prepared from **5** (2.22 mmol; 0.33 g) and benzylisothiocyanate (2.67 mmol; 0.35 mL) according to the same procedure of compound **6**. Yield: 67% (0.44 g), mp 248–250°C. ^{1}H NMR (DMSO-d_6) δ: 4.72 (d, 2H, J = 4.4 Hz, CH_2), 6.80 (d, 1H, J_o = 8.8 Hz, H-7), 7.10 (dd, 1H, J_o = 8.8 Hz, J_m = 1.6 Hz, H-6), 7.20–7.40 (m, 5H, aromatic benzyl protons), 7.60 (s, 1H, H-4), 8.01 (s, 1H, $\underline{NH}$–CH_2–Ph), 9.45 (s, 1H, –NH–C=S), 10.63 (s, 1H, indole-NH). MS (ESI): *m/z* 298.6 (M+H).

*5-Nitroisatine (**8**)*
A solution of $NaNO_3$ (0.034 mol; 2.91 g) in H_2SO_4 (50 mL) at 0°C was added dropwise over 1 h to a solution of isatin (0.034 mol; 5.04 g) in H_2SO_4 (58 mL). The reaction mixture then was poured onto ice and the resulting precipitate was filtered and washed with water to yield 5-nitroisatin (**8**). Yield: 72% (4.77 g), mp 254–256°C (lit. [40], 252–254°C).

*5′-Nitrospiro[[1,3]dioxolane-2,3′-indolin]-2′-one (**9**)*
Ethylene glycol (0.093 mol; 5.20 mL) and a catalytic amount of *p*-toluenesulfonic acid (0.63 mmol; 0.12 g) was added to a suspension of 5-nitroisatin (**8**, 0.024 mol; 4.77 g) in toluene (300 mL). The reaction mixture then was refluxed for 8 h using a Dean–Stark apparatus. The reaction mixture was cooled, and the solution was concentrated *in vacuo* and kept overnight at room temperature. The crude product was recrystallized from toluene to give ketal of 5-nitroisatin (**9**). Yield: 81% (4.64 g), mp 216°C (lit. [38], 216–217°C).

*1′-Benzyl-5′-nitrospiro[[1,3]dioxolane-2,3′-indolin]-2′-one (**10**)*
5′-Nitrospiro[[1,3]dioxolane-2,3′-indolin]-2′-one (**9**, 0.019 mol; 4.64 g) and NaH (0.059 mol; 1.41 g) were stirred in anhydrous dimethylformamide (10 mL) at 0°C, then stirred for 15 min at room temperature. To the mixture, benzyl bromide (0.019 mol; 2.35 mL) was added at 0°C. After being stirred for 5 h at room temperature, the mixture was poured onto ice and the product filtered. The crude product was washed with cold water and dried to give **10**. Yield: 72% (3.53 g), mp 164–166°C. ^{1}H NMR (DMSO-d_6, 400 MHz): 4.42 (s, 4H, –$(CH_2)_2$–), 4.95 (s, 2H, –$\underline{CH_2}$–Ph), 7.25 (d, J = 8.8 Hz, 1H, H-7), 7.29–7.31 (m, 3H, aromatic protons), 7.35–7.38 (m, 2H, aromatic protons), 8.25 (d, J = 2.4 Hz, 1H, H-4), 8.32 (dd, J_o = 8.0 Hz, J_m = 2.4 Hz, 1H, H-6). MS (ESI): *m/z* 327.45 (M+H).

*5′-Amino-1′-benzylspiro[[1,3]dioxolane-2,3′-indolin]-2′-one (**11**)*
Compound **10** (3.11 mmol; 1.01 g) was dissolved in methanol (20 mL) and palladium on activated carbon (0.14 g; % 10) added to the solution at room temperature. The reaction mixture was stirred under a hydrogen atmosphere for 4 h. The catalyst was then removed by filtration over celite. The filtrate was concentrated *in vacuo* to give crude product, which was recrystallized from ethanol to give **11**. Yield: 59% (0.55 g), mp 136–137°C. ^{1}H NMR (DMSO-d_6, 400 MHz): 4.24 (t, J = 6.8 Hz, 2H, –CH_2–), 4.39 (t, J = 6.8 Hz, 2H, –CH_2–), 4.73 (s, 2H, –$\underline{CH_2}$–Ph), 5.00 (s, 2H, –NH_2), 6.50 (dd, J_o = 8.0 Hz, J_m = 2.4 Hz, 1H, H-6), 6.61 (d, J = 8 Hz, 1H, H-7), 6.65 (d, J_m = 2.4 Hz, 1H, H-4), 7.24–7.27 (m, 3H, Ar-H), 7.31–7.35 (m, 2H, Ar–H). MS (ESI): *m/z* 297.6 (M+H).

*1′-Benzyl-5′-(4-fluorobenzylamino)spiro[[1,3]dioxolane-2,3′-indolin]-2′-one (**12**)*
To a suspension of compound **11** (2.27 mmol; 0.67 g) and molecular sieves 4 Å (1 g) in dry toluene (15 mL) was added *p*-fluorobenzaldehyde (2.27 mmol; 0.24 mL), and then the mixture was stirred at room temperature for 48 h. The mixture was mixed with 10 mL of methanol and sodium borohydride (4.55 mmol; 0.17 g) was added in portions at room temperature. After completion of the reaction, the mixture was filtered. Then, the filtrate was concentrated and 5% $NaHCO_3$ was added. The organic material was extracted with dichloromethane, the combined organic layer was

washed with water and brine, then dried over anhydrous Na_2SO_4. The solvent was evaporated and the residual solid was crystallized from hot ethanol to afford compound **12**. Yield: 64% (0.59 g), mp 150°C. ^{1}H NMR (DMSO-d_6, 400 MHz): 4.16 (d, $J = 5.6$ Hz, 2H, –NH–$\underline{CH_2}$–), 4.20 (t, $J = 6.8$ Hz, 2H, –CH_2–), 4.35 (t, $J = 6.8$ Hz, 2H, –CH_2–), 4.68 (s, 2H, –$\underline{CH_2}$–Ph), 6.10 (t, $J = 6$ Hz, 1H, –NH–), 6.42 (dd, $J_o = 8.0$ Hz, $J_m = 2.4$ Hz, 1H, H-6) 6.61 (d, $J = 8.8$ Hz, 1H, H-7), 6.65 (d, $J = 2.4$ Hz, 1H, H-4), 7.08 (t, $J = 8.8$ Hz, 2H, aromatic protons), 7.21–7.33 (m, 7H, aromatic protons). MS (ESI): *m/z* 405.9 (M+H).

*1-Benzyl-5-(4-fluorobenzylamino)indoline-2,3-dione (**13**)*
Compound **12** (1.46 mmol; 0.59 g) was heated at reflux in a mixture of concentrated hydrochloric acid (12 mL) and acetic acid (3 mL) for 1 h. The reaction mixture was cooled to room temperature. Removal of hydrochloric acid and acetic acid under reduced pressure gave a crude product which was recrystallized from ethanol to afford compound **13**. Yield: 81% (0.43 g), mp 234°C. ^{1}H NMR (DMSO-d_6, 400 MHz): 4.25 (s, 2H, –$\underline{CH_2}$–NH–), 4.80 (s, 2H, –$\underline{CH_2}$–Ph), 6.07 (t, 1H, $J = 6.0$ Hz, –NH–), 6.72 (d, $J = 8$ Hz, 1H, H-7), 6.84 (t, $J = 8.4$ Hz, 2H, aromatic protons), 7.14 (t, $J = 8.8$ Hz, 2H, aromatic protons), 7.24–7.39 (m, 7H, aromatic protons). MS (ESI): *m/z* 361.7 (M+H).

*1-Benzyl-5-iodoindoline-2,3-dione (**14**)*
5-Iodoisatin (2.51 mmol; 0.68 g) was dissolved in anhydrous dimethylformamide (3 mL) and NaH (7.54 mmol; 0.18 g) was added at 0°C, then stirred for 15 min at room temperature. Benzyl bromide (2.51 mmol; 0.3 mL) was added to this mixture at 0°C. After being stirred for 2 h at room temperature, the mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, then dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude product was crystallized from ethanol to afford compound **14**. Yield: 41% (0.38 g), mp 150–153°C (lit. [41], 149–152°C).

*5-(4-Fluorophenyl)indoline-2,3-dione (**15**)*
$Pd(PPh_3)_4$ (0.42 mmol; 0.49 g), *p*-fluorophenyl boronic acid (0.01 mol; 1.43 g), and sodium hydrogen carbonate (0.017 mol; 143 g) in H_2O (30 mL) were added into a solution of 5-iodoisatin (8.5 mmol; 2.33 g) in DME (145 mL) under nitrogen. The reaction mixture was refluxed for 5 h. The organic solvent was removed under reduced pressure. The residue was extracted with ethylacetate and the combined organic layer was washed with water and brine, then dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography (hexane/ethylacetate = 6:3) to afford compound **15**. Yield 25% (0.5 g), mp 239–240°C [50].

*1-Benzyl-5-(4-fluorophenyl)indoline-2,3-dione (**16**)*
$Pd(PPh_3)_4$ (9.66 mmol; 0.11 g), *p*-fluorophenyl boronic acid (2.32 mmol; 0.32 g), and sodium hydrogen carbonate (3.86 mmol; 0.32 g) in H_2O (6.5 mL) were added into a solution of 1-benzyl-5-iodoindoline-2,3-dione (**14**, 1.93 mmol; 0,7 g) in DME (50 mL) under nitrogen. The reaction mixture was refluxed for 7 h. The organic solvent was removed under reduced pressure. The residue was extracted with ethylacetate and the combined organic layer was washed with water and brine, then dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography (hexane/ethylacetate = 6:3) to afford compound **16**. Yield: 41% (0.26 g), mp 162–163°C. ^{1}H NMR (DMSO-d_6, 400 MHz): 4.95 (s, 2H, –CH_2), 7.04 (d, 1H, $J_o = 8.4$ Hz, H-7), 7.24–7.31 (m, 3H, aromatic protons), 7.35 (t, 2H, $J = 7.6$ Hz, H-3′, H-5′), 7.46 (d, 2H, $J_o = 7.2$ Hz, aromatic protons), 7.68–7.73 (m, 2H, aromatic protons), 7.83 (d, 1H, $J_m = 2.0$ Hz, H-4), 7.87 (dd, 1H, $J_o = 8.0$ Hz; $J_m = 2.0$ Hz, H-6). MS (ESI): *m/z* 332.8 (M+H).

*General procedure for the synthesis of target compounds **2a–h**, **6a–h**, and **7a–h***
A catalytic amount of piperidine (0.01 mmol) was added into a solution of compounds **2**, **6**, and **7** (1 mmol) and substituted benzaldehyde (1.1 mmol) in ethanol (5 mL). The mixture was heated at reflux for 3–5 h. After cooling at room temperature, the precipitate was filtered, washed with ethanol, and dried at room temperature.

*(E)-3-(4-Chlorobenzylidene)-5-(4-fluorophenyl)indolin-2-one (**2a**)*
Yield: 80%, mp 190–192°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.97 (d, 1H, $J_o = 8.4$ Hz, H-7), 7.25 (t, 2H, $J = 8.8$ Hz, H-3″, H-5″), 7.48–7.52 (m, 3H, H-6, H-2″, H-6″), 7.62 (d, 2H, $J_o = 8.4$ Hz, H-2′, H-6′), 7.65 (s, 1H, H-vinyl), 7.69 (d, 1H, $J_m = 2.0$ Hz, H-4), 7.83 (d, 2H, $J_o = 8.4$ Hz, H-3′, H-5′), 10.74 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 168.54, 162.71, 160.29, 142.51, 136.73, 134.88, 134.31, 133.60, 133.23, 132.54, 131.27, 129.03, 128.80, 128.11, 128.03, 127.97, 121.30, 120.70, 115,80, 115.69, 110.55. MS (ESI): *m/z* 350.8 (M+H). Elemental analysis calculated (%) for $C_{21}H_{13}ClFNO \cdot 0.04\ H_2O$: C 71.96, H 3.76, N 3.99. Found: C 71.64, H 3.54, N 4.22.

*(E)-3-(4-Fluorobenzylidene)-5-(4-fluorophenyl)indolin-2-one (**2b**)*
Yield: 72%, mp 249–252°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.97 (d, 1H, $J_o = 8.4$ Hz, H-7), 7.24 (t, 2H, $J = 8.8$ Hz, H-3″, H-5″), 7.40 (t, 2H, $J = 8.8$ Hz, H-3′, H-5′), 7.48–7.52 (m, 3H, H-6, H-2″, H-6″), 7.67 (s, 1H, H-vinyl), 7.71 (d, 1H, $J_m = 1.6$ Hz, H-4), 7.84–7.88 (m, 2H, H-2′, H-6′), 10.73 (s, 1H, NH). MS (ESI): *m/z* 334.6 (M+H). Elemental analysis calculated (%) for $C_{21}H_{13}F_2NO$: C 75.67, H 3.93, N 4.20. Found: C 75.46, H 4.17, N 4.36.

*(E)-3-(3-Fluorobenzylidene)-5-(4-fluorophenyl)indolin-2-one (**2c**)*
Yield: 75%, mp 230–234°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.95 (d, 1H, $J_o = 8.4$ Hz, H-7), 7.22 (t, 2H, $J = 8.8$ Hz, H-3″, H-5″), 7.32 (t, 1H, H-4′ or H-5′), 7.42–7.46 (m, 2H, H-2″, H-6″), 7.49 (dd, 1H, $J_o = 8.0$ Hz, $J_m = 1.6$ Hz, H-6), 7.55–7.62 (m, 3H, H-2′, H-6′, H-4′, or H-5′), 7.64 (s, 1H, H-vinyl), 7.65 (d, 1H, $J_m = 1.6$ Hz, H-4),

10.74 (s, 1H, NH). MS (ESI): *m/z* 334.69 (M+H). Elemental analysis calculated (%) for $C_{21}H_{13}F_2NO$: C 75.67, H 3.93, N 4.20. Found: C 75.53, H 3.86, N 4.29.

*(E)-3-(3,4-Dichlorobenzylidene)-5-(4-fluorophenyl)-indolin-2-one (**2d**)*
Yield: 86%, mp 220–222°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: major *E*-isomer: 6.97 (d, 1H, $J_o = 8.0$ Hz, H-7), 7.25 (t, 2H, $J = 8.8$ Hz, H-3″, H-5″), 7.50–7.54 (m, 3H, H-2″, H-6″, H-6), 7.61 (s, 1H, H-vinyl), 7.69 (d, 1H, $J_m = 1.2$ Hz, H-4), 7.79 (d, 2H, $J_o = 8.0$ Hz, H-5′, H-6′), 8.10 (d, 1H, $J_m = 1.2$ Hz, H-2′), 10.76 (s, 1H, indole-NH). MS (ESI): *m/z* 385.79 (M+H). Elemental analysis calculated (%) for $C_{21}H_{12}Cl_2FNO$: C 65.64, H 3.15, N 3.65. Found: C 65.80, H 3.02, N 3.80.

*(Z)-3-(2,4-Difluorobenzylidene)-5-(4-fluorophenyl)-indolin-2-one (**2e**)*
Yield: 24%, mp 245–247°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.90 (d, 1H, $J_o = 8.0$ Hz, H-7), 7.19 (t, 1H, H-3′ or H-5′), 7.28 (t, 2H, $J = 8.4$ Hz, H-3″, H-5″), 7.36 (t, 1H, H-3′ or H-5′), 7.53 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2.0$ Hz, H-6), 7.72–7.76 (m, 2H, H-2″, H-6″), 7.92 (s, 1H, H-vinyl), 8.08 (d, 1H, $J_m = 1.6$ Hz, H-4), 8.56–8.62 (m, 1H, H-6′), 10.72 (s, 1H, NH). MS (ESI): *m/z* 352.69 (M+H). Elemental analysis calculated (%) for $C_{21}H_{12}F_3NO \cdot 0.9\ H_2O$: C 68.62, H 3.78, N 3.81. Found: C 68.31, H 3.47, N 3.61.

*(E)-3-(4-(Dimethylamino)benzylidene)-5-(4-fluorophenyl)-indolin-2-one (**2f**)*
Yield: 94%, mp 269–271°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.03 (s, 6H, $N(CH_3)_2$), 6.84 (d, 2H, $J_o = 9.2$ Hz, H-3′, H-5′), 6.94 (d, 1H, $J_o = 8.0$ Hz, H-7), 7.25 (t, 2H, H-3″, H-5″), 7.44 (dd, 1H, $J_o = 8.0$ Hz, $J_m = 1.6$ Hz, H-6), 7.55–7.58 (m, 2H, H-2″, H-6″), 7.57 (s, 1H, H-vinyl), 7.72 (d, 2H, $J_o = 8.8$ Hz, H-2′, H-6′), 8.00 (d, 1H, $J_m = 1.6$ Hz, H-4), 10.55 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 169.46, 162.62, 160.20, 151.47, 141.48, 137.90, 137.21, 137.18, 132.19, 132.08, 132.02, 128.15, 128.07, 127.33, 122.51, 121.95, 120.95, 119.89, 115.77, 115.56, 111.41, 110.04, 39.65. MS (ESI): *m/z* 359.7 (M+H). Elemental analysis calculated (%) for $C_{23}H_{19}FN_2O$: C 77.08, H 5.34, N 7.82. Found: C 77.40, H 5.43, N 7.59.

*(E:Z = 2:1)-3-(4-Methoxybenzylidene)-5-(4-fluorophenyl)-indolin-2-one (**2g**)*
Yield: 94%, mp 269–271°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: *E*-isomer: 3.80 (s, 3H, OCH_3), 6.96 (d, 1H, $J_o = 8.4$ Hz, H-7), 7.12 (d, 2H, $J_o = 8.8$ Hz, H-3′, H-5′), 7.23–7.31 (m, 2H, H-3″, H-5″), 7.48–7.54 (m, 3H, H-2″, H-6″, H-6), 7.64 (s, 1H, H-vinyl), 7.79 (d, 2H, $J_o = 8.8$ Hz, H-2′, H-6′), 7.87 (d, 1H, $J_m = 1.6$ Hz, H-4), 10.66 (s, 1H, NH). *Z*-isomer: 3.80 (s, 3H, OCH_3), 6.89 (d, 1H, $J_o = 8.4$ Hz, H-7), 7.06 (d, 2H, $J_o = 8.8$ Hz, H-3′, H-5′), 7.23–7.31 (m, 2H, H-3″, H-5″), 7.48–7.54 (m, 1H, H-6), 7.71–7.74 (m, 2H, H-2″, H-6″), 7.94 (s, 1H, H-vinyl), 8.02 (d, 1H, $J_m = 1.6$ Hz, H-4), 8.51 (d, 2H, $J_o = 8.8$ Hz, H-2′, H-6′), 10.66 (s, 1H, NH). MS (ESI): *m/z* 346.8 (M+H). Elemental analysis calculated (%) for $C_{22}H_{16}FNO_2$: C 76.51, H 4.67, N 4.06. Found: C 76.67, H 4.51, N 4.05.

*(E)-3-(4-Carboxybenzylidene)-5-(4-fluorophenyl)indolin-2-one (**2h**)*
Yield: 81%, mp 275–279°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.98 (d, 1H, $J_o = 8.0$ Hz, H-7), 7.22–7.26 (t, 2H, H-3″, H-5″), 7.47–7.52 (m, 3H, H-2″, H-6″, H-6), 7.70 (s, 1H, H-vinyl), 7.75 (d, 1H, $J_m = 1.6$ Hz, H-4), 7.81 (d, 2H, $J_o = 7.6$ Hz, H-2′, H-6′), 8.04 (d, 2H, $J_o = 8.4$ Hz, H-3′, H-5′), 10.78 (s, 1H, NH), 13.74 (s, 1H, COOH). MS (ESI): *m/z* 360.8 (M+H). Elemental analysis calculated (%) for $C_{22}H_{14}FNO_3 \cdot 0.5\ H_2O$: C 71.73, H 4.10, N 3.80. Found: C 71.60, H 4.12, N 3.82.

*(E)-1-(3-(4-Chlorobenzylidene)-2-oxoindolin-5-yl)-3-ethylurea (**6a**)*
Yield: 76%, mp 285–287°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.01 (t, 3H, CH_3), 3.04–3.07 (m, 2H, CH_2), 5.93 (t, 1H, NH–CO–$\underline{NH}C_2H_5$), 6.74 (d, 1H, $J_o = 8.0$ Hz, H-7), 7.30 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 1.6$ Hz, H-6), 7.53 (s, 1H, H-vinyl), 7.56 (d, 2H, $J_o = 8.4$ Hz, H-2′, H-6′), 7.60 (d, 1H, $J_m = 2.0$ Hz, H-4), 7.74 (d, 2H, $J_o = 8.4$ Hz, H-3′, H-5′), 8.18 (s, 1H, $\underline{NH}$–CO–NHC_2H_5), 10.42 (s, 1H, indole-NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 168.52, 155.28, 137.36, 136.43, 134.47, 134.00, 133.91, 133.26, 131.15, 128.79, 128.65, 128.55, 120.61, 120.48, 113.40, 109.95, 33.95, 15.40. MS (ESI): *m/z* 342.5 (M+H), 344.9 (M+H+2). Elemental analysis calculated (%) for $C_{18}H_{16}ClN_3O_2$: C 63.25, H 4.72, N 12.29. Found: C 63.51, H 4.78, N 12.27.

*(E)-1-(3-(4-Fluorobenzylidene)-2-oxoindolin-5-yl)-3-ethylurea (**6b**)*
Yield: 85%, mp 275–277°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.98 (t, 3H, CH_3), 2.98–3.05 (m, 2H, CH_2), 5.89 (t, 1H, NH–CO–$\underline{NH}C_2H_5$), 6.70 (d, 1H, $J_o = 8.8$ Hz, H-7), 7.23 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 1.6$ Hz, H-6), 7.31 (t, 2H, $J = 8.8$ Hz, H-3′, H-5′), 7.52 (s, 1H, H-vinyl), 7.60 (d, 1H, $J_m = 2.0$ Hz, H-4), 7.72–7.76 (m, 2H, H-2′, H-6′), 8.13 (s, 1H, $\underline{NH}$–CO–NHC_2H_5), 10.38 (s, 1H, indole-NH). MS (ESI): *m/z* 326.8 (M+H). Elemental analysis calculated (%) for $C_{18}H_{16}FN_3O_2$: C 66.45, H 4.96, N 12.92. Found: C 66.35, H 5.08, N 12.93.

*(E:Z = 2:1)-1-(3-(3-Fluorobenzylidene)-2-oxoindolin-5-yl)-3-ethylurea (**6c**)*
Yield: 73%, mp 320–325°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: *E*-isomer: 0.97 (t, 3H, CH_3), 2.99–3.02 (m, 2H, CH_2), 5.89 (t, 1H, NH–CO–$\underline{NH}C_2H_5$), 6.71 (d, 1H, $J_o = 8.8$ Hz, H-7), 7.22 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2.0$ Hz, H-6), 7.25–7.31 (m, 1H, aromatic proton), 7.51 (s, 1H, H-vinyl), 7.45–7.53 (m, 3H, aromatic protons), 7.60 (d, 1H, $J_m = 2.0$ Hz, H-4), 8.13 (s, 1H, $\underline{NH}$–CO–NHC_2H_5), 10.40 (s, 1H, indole-NH); *Z*-isomer: 1.02 (t, 3H, CH_3), 3.02–3.10 (m, 2H, CH_2), 6.02 (t, 1H, NH–CO–$\underline{NH}C_2H_5$), 6.67 (d, 1H, $J_o = 8.8$ Hz, H-7), 7.07 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2.0$ Hz, H-6), 7.62 (s, 1H, H-vinyl), 7.45–7.53 (m, 2H, H-4′, H-5′), 7.74 (d, 1H, $J_m = 2.0$ Hz, H-4), 7.97 (d, 1H, $J_o = 8.0$ Hz, aromatic proton), 8.19 (s, 1H, $\underline{NH}$–CO–NHC_2H_5), 8.50 (d, 1H, $J_o = 8.4$ Hz, $J_m = 2.0$ Hz, aromatic proton), 10.47 (s, 1H, indole-NH). MS (ESI): *m/z* 326.9 (M+H). Elemental analysis calculated (%) for $C_{18}H_{16}FN_3O_2$: C 66.45, H 4.96, N 12.92. Found: C 66.48, H 5.13, N 12.85.

*(E)-1-(3-(3,4-Dichlorobenzylidene)-2-oxoindolin-5-yl)-3-ethylurea (**6d**)*
Yield: 73%, mp 335–337°C (decomp). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.98 (t, 3H, CH_3), 3.00–3.03 (m, 2H, CH_2), 5.90 (t, 1H, NH–CO–NHC$_2$H$_5$), 6.71 (d, 1H, J_o = 8.4 Hz, H-7), 7.25 (dd, 1H, J_o = 8.4 Hz, J_m = 2.0 Hz, H-6), 7.47 (s, 1H, H-vinyl), 7.52 (d, 1H, J_m = 2.0 Hz, H-4), 7.67 (dd, 1H, J_o = 8.4 Hz, J_m = 1.6 Hz, H-6′), 7.72 (d, 1H, J_o = 8.0 Hz, H-5′), 7.90 (d, 1H, J_m = 1.6 Hz, H-2′), 8.14 (s, 1H, NH–CO–NHC$_2$H$_5$), 10.41 (s, 1H, indole-NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 168.31, 155.24, 137.48, 135.20, 134.59, 132.41, 131.77, 131.65, 131.13, 130.83, 129.55, 129.16, 120.83, 120.39, 113.39, 110.07, 33.94, 15.42. MS (ESI): *m/z* 376.7 (M^+). Elemental analysis calculated (%) for $C_{18}H_{15}Cl_2N_3O_2 \cdot 0.07\ H_2O$: C 57.27, H 4.04, N 11.13. Found: C 56.90, H 4.04, N 11.12.

*(E)-1-(3-(2,4-Difluorobenzylidene)-2-oxoindolin-5-yl)-3-ethylurea (**6e**)*
Yield: 76%, mp > 360°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: major *E*-isomer 0.98 (t, 3H, CH_3), 3.01–3.06 (m, 2H, CH_2), 5.90 (t, 1H, NH–CO–NHC$_2$H$_5$), 6.72 (d, 1H, J_o = 8.0 Hz, H-7), 7.22–7.28 (m, 2H, aromatic protons), 7.35 (s, 1H, H-vinyl), 7.41–7.47 (m, 2H, H-4, aromatic proton), 7.79–7.82 (m, 1H, aromatic proton), 8.15 (s, 1H, NH–CO–NHC$_2$H$_5$), 10.45 (s, 1H, indole-NH). MS (ESI): *m/z* 344.8 (M+H). Elemental analysis calculated (%) for $C_{18}H_{15}F_2N_3O_2 \cdot 0.5\ H_2O$: C 61.35, H 4.57, N 11.92. Found: C 61.75, H 4.96, N 11.93.

*(E)-1-(3-(4-Dimethylaminobenzylidene)-2-oxoindolin-5-yl)-3-ethylurea (**6f**)*
Yield: 74%, mp 255–258°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: major *E*-isomer 1.03 (t, 3H, CH_3), 3.03–3.09 (m, 8H, CH_2, N$(CH_3)_2$), 5.94 (t, 1H, NH–CO–NHC$_2$H$_5$), 6.72 (d, 1H, J_o = 8.8 Hz, H-7), 6.80 (d, 2H, J_o = 8.4 Hz, H-3′, H-5′), 7.22 (dd, 1H, J_o = 8.0 Hz, J_m = 2.0, H-6), 7.47 (s, 1H, H-vinyl), 7.66 (d, 2H, J_o = 9.2 Hz, H-2′, H-6′), 7.85 (s, 1H, H-4), 8.17 (s, 1H, NH–CO–NHC$_2$H$_5$), 10.25 (s, 1H, indole-NH). MS (ESI): *m/z* 351.8 (M+H). Elemental analysis calculated (%) for $C_{20}H_{22}N_4O_2$: C 68.55, H, 6.33, N 15.99. Found: C 68.45, H 6.28, N 15.49.

*(E)-1-(3-(4-Methoxybenzylidene)-2-oxoindolin-5-yl)-3-ethylurea (**6g**)*
Yield: 86%, mp 285–290°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.99 (t, 3H, CH_3), 3.02–3.05 (m, 2H, CH_2), 3.82 (s, 3H, OCH_3), 5.90 (t, 1H, NH–CO–NHC$_2$H$_5$), 6.70 (d, 1H, J_o = 8.8 Hz, H-7), 7.04 (d, 2H, J_o = 8.8 Hz, H-3′, H-5′), 7.22 (dd, 1H, J_o = 8.4 Hz, J_m = 2.0 Hz, H-6), 7.50 (s, 1H, H-vinyl), 7.69 (d, 2H, J_o = 8.8 Hz, H-2′, H-6′), 7.72 (d, 1H, J_m = 2.0 Hz, H-4), 8.14 (s, 1H, NH–CO–NHC$_2$H$_5$), 10.31 (s, 1H, indole–NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 168.98, 168.87 160.41, 155.34, 137.02, 135.64, 134.31, 131.52, 126.87, 126.59, 125.98, 121.16, 120.04, 114.22, 113.36, 109.70, 55.35, 33.95, 15.47. MS (ESI): *m/z* 338.8 (M+H). Elemental analysis calculated (%) for $C_{19}H_{19}N_3O_3$: C 67.64, H 5.68, N 12.46. Found: C 67.70, H 5.56, N 12.46.

*(E)-1-(3-(4-Carboxybenzylidene)-2-oxoindolin-5-yl)-3-ethylurea (**6h**)*
Yield: 81%, mp 290–295°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: major *E*-isomer 1.01 (t, 3H, CH_3), 3.04–3.07 (m, 2H, CH_2), 6.30 (t, 1H, NH–CO–NHC$_2$H$_5$), 6.74 (d, 1H, J_o = 8.0 Hz, H-7), 7.45 (dd, 1H, J_o = 8.4 Hz, J_m = 2.0 Hz, H-6), 7.58 (s, 1H, H-vinyl), 7.64 (d, 1H, J_m = 2.0 Hz, H-4), 7.75 (d, 2H, J_o = 8.4 Hz, H-2′, H-6′), 8.05 (d, 2H, J_o = 8.4 Hz, H-3′, H-5′), 8.44 (s, 1H, NH–CO–NHC$_2$H$_5$), 10.42 (s, 1H, indole–NH), 13.70 (s, 1H, COOH). MS (ESI): *m/z* 352.7 (M+H). Elemental analysis calculated (%) for $C_{19}H_{17}N_3O_4 \cdot 0.5\ H_2O$: C 63.32, H 5.03, N 11.66. Found: C 63.62, H 5.27, N 11.38.

*(E)-1-Benzyl-3-(3-(4-chlorobenzylidene)-2-oxoindolin-5-yl)thiourea (**7a**)*
Yield: 75%, mp 227–228°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.70 (d, 2H, J = 4.4 Hz, CH_2), 6.84 (d, 1H, J_o = 8.8 Hz, H-7), 7.13 (dd, 1H, J_o = 8.8 Hz, J_m = 1.6 Hz, H-6), 7.25–7.34 (m, 5H, benzyl protons), 7.50 (d, 2H, J_o = 8.4 Hz, H-2′, H-6′), 7.59 (s, 1H, H-vinyl), 7.64 (s, 1H, H-4), 7.77 (d, 2H, J_o = 8.4 Hz, H-3′, H-5′), 8.03 (s, 1H, –NH–CH$_2$–Ph), 9.44 (s, 1H, –NH–C=S), 10.64 (s, 1H, indole–NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 181.07, 168.60, 140.15, 139.10, 136.11, 135.30, 134.58, 134.21, 134.24, 133.63, 133.09, 132.42, 131.29, 128.80, 128.17, 127.95, 127.33, 126.80, 126.52, 120.56, 119.65, 110.02, 47.18. MS (ESI): *m/z* 420.2 (M+H); 422.1 (M+H+2). Elemental analysis calculated (%) for $C_{23}H_{18}ClN_3OS$: C 65.78, H 4.32, N 10.01, S 7.64. Found: C 65.43, H 4.44, N 9.91, S7.44.

*(E)-1-Benzyl-3-(3-(4-fluorobenzylidene)-2-oxoindolin-5-yl)thiourea (**7b**)*
Yield: 76%, mp 220–222°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.70 (d, 2H, J = 4.4 Hz, CH_2), 6.84 (d, 1H, J_o = 8.4 Hz, H-7), 7.12 (dd, 1H, J_o = 8.4 Hz, J = 2.0 Hz, H-6), 7.23–7.33 (m, 7H, benzyl protons, H-3′, H-5′), 7.61 (s, 1H, H-vinyl), 7.66 (s, 1H, H-4), 7.79–7.83 (m, 2H, H-2′, H-6′), 8.03 (s, 1H, –NH–CH$_2$–Ph), 9.44 (s, 1H, –NH–C=S), 10.63 (s, 1H, indole=NH). MS (ESI): *m/z* 404.1 (M+H). Elemental analysis calculated (%) for $C_{23}H_{18}FN_3OS$: C 68.47, H 4.50, N 10.41, S 7.95. Found: C 68.49, H 4.67, N 10.41, S 7.74.

*(E)-1-Benzyl-3-(3-(3-fluorobenzylidene)-2-oxoindolin-5-yl)thiourea (**7c**)*
Yield: 70%, mp 191–193°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.69 (d, 2H, J = 4.4 Hz, CH_2), 6.85 (d, 1H, J_o = 8.4 Hz, H-7), 7.12 (dd, 1H, J_o = 8.4 Hz, J_m = 2.0 Hz, H-6), 7.22–7.33 (m, 6H, aromatic protons), 7.47–7.58 (m, 3H, aromatic protons), 7.61 (s, 1H, H-vinyl), 7.65 (s, 1H, H-4), 8.01 (s, 1H, –NH–CH$_2$–Ph), 9.44 (s, 1H, –NH–C=S), 10.65 (s, 1H, indole=NH). MS (ESI): *m/z* 404.2 (M+H). Elemental analysis calculated (%) for $C_{23}H_{18}FN_3OS \cdot 0.2H_2O$: C 67.86, H 4.55, N 10.32, S 7.87. Found: C 67.94, H 4.59, N 10.28, S 7.73.

*(E)-1-Benzyl-3-(3-(3,4-dichlorobenzylidene)-2-oxoindolin-5-yl)thiourea (**7d**)*
Yield: 78%, mp 225–227°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.70 (d, 2H, J = 5.2 Hz, CH_2), 6.85 (d, 1H, J_o = 8.0 Hz, H-7), 7.14

(dd, 1H, $J_o = 8.4$ Hz, $J_m = 2.0$ Hz, H-6), 7.24–7.33 (m, 5H, benzyl protons), 7.56 (s, 1H, H-vinyl), 7.61 (s, 1H, H-4), 7.66 (d, 1H, $J_o = 8.4$ Hz, H-5′), 7.74 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2.0$ Hz, H-6′), 7.97 (d, 1H, $J_m = 2.0$ Hz, H-2′), 8.02 (s, 1H, –NH–CH_2–Ph), 9.44 (s, 1H, –NH–C=S), 10.67 (s, 1H, indole–NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 181.07, 168.40, 167.16, 140.30, 138.85, 139.10, 135.03, 133.12, 132.95, 131.97, 131.68, 131.30, 130.81, 129.20, 128.94, 128.16, 127.29, 126.78, 120.32, 119.68, 110.14, 109.68, 47.16. MS (ESI): *m/z* 454.3 (M+H); 456.2 (M+H+2). Elemental analysis calculated (%) for $C_{23}H_{17}Cl_2N_3OS$: C 60.80, H 3.77, N 9.25, S 7.06. Found: C 60.69, H 3.93, N 9.24, S 6.91.

*(E)-1-Benzyl-3-(3-(2,4-difluorobenzylidene)-2-oxoindolin-5-yl)thiourea (**7e**)*
Yield: 29%, mp 190–193°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: major *E*-isomer 4.68 (d, 2H, $J = 5.6$ Hz, CH_2), 6.85 (d, 1H, $J_o = 8.0$ Hz, H-7), 7.08–7.33 (m, 6H, aromatic protons), 7.42–7.47 (m, 2H, aromatic protons), 7.49 (s, 1H, H-vinyl), 7.67 (s, 1H, H-4), 7.84–7.90 (m, 1H, aromatic protons), 7.99 (s, 1H, –NH–CH_2–Ph), 9.42 (s, 1H, –NH–C=S), 10.67 (s, 1H, indole–NH). MS (ESI): *m/z* 422.36 (M+H). Elemental analysis calculated (%) for $C_{23}H_{17}F_2N_3OS \cdot 0.01\ H_2O$: C 65.51, H 4.06, N 9.96, S 7.60. Found: C 65.66, H 4.05, N 10.35, S 7.89.

*(Z)-1-Benzyl-3-(3-(4-dimethylaminobenzylidene)-2-oxoindolin-5-yl)thiourea (**7f**)*
Yield: 78%, mp 226–228°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.04 (s, 6H, $N(CH_3)_2$), 4.73 (d, 2H, $J = 5.6$ Hz, CH_2), 6.75 (d, 2H, $J_o = 8.0$ Hz, H-3′, H-5′), 6.78 (d, 1H, $J_o = 8.8$ Hz, H-7), 6.97 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 2.0$ Hz, H-6), 7.22–7.25 (m, 1H, H-4″), 7.33 (d, 4H, $J = 4.4$ Hz, H-2″, H-3″, H-5″, H-6″), 7.54 (d, 1H, $J_m = 1.6$ Hz, H-4), 7.61 (s, 1H, H-vinyl), 7.91 (s, 1H, –NH–CH_2–Ph), 8.46 (d, 2H, $J_o = 9.2$ Hz, H-2′, H-6′), 9.42 (s, 1H, –NH–C=S), 10.47 (s, 1H, indole-NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 181.41, 167.79, 151.83, 139.88, 139.45, 138.62, 137.36, 134.85, 131.99, 131,87, 131.55, 128.13, 127.27, 126.66, 125.12, 124.89, 121.98, 119.95, 116.94, 116.72, 116.53, 111.05, 108.96, 108.85, 47.36. MS (ESI): *m/z* 429.3 (M+H). Elemental analysis calculated (%) for $C_{25}H_{24}N_4OS$: C 70.07, H 5.64, N 13.07, S 7.48. Found: C 69.73, H 5.92, N 12.92, S 7.32.

*(E)-1-Benzyl-3-(3-(4-methoxybenzylidene)-2-oxoindolin-5-yl)thiourea (**7g**)*
Yield: 80%, mp 207–209°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.80 (s, 3H, OCH_3), 4.66 (d, 2H, $J = 5.6$ Hz, CH_2), 6.80 (d, 1H, $J_o = 8.0$ Hz, H-7), 7.00 (d, 2H, $J_o = 8.8$ Hz, H-3′, H-5′), 7.06 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 1.6$ Hz, H-6), 7.17–7.30 (m, 5H, benzyl protons), 7.55 (s, 1H, H-vinyl), 7.72 (d, 2H, $J_o = 8.8$ Hz, H-2′, H-6′), 7.73 (s, 1H, H-4), 7.98 (s, 1H, –NH–CH_2–Ph), 9.39 (s, 1H, –NH–C=S), 10.52 (s, 1H, indole–NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ: 181.13, 169.06, 160.60, 139.88, 139.25, 139.16, 136.34, 134.53, 132.20, 131.72, 128.15, 127.30, 126.99, 126.74, 126.45, 126.09, 125.31, 121.17, 119.53, 114.26, 113.98, 109.83, 55.38, 47.21. MS (ESI): *m/z* 416.3 (M+H). Elemental analysis calculated (%) for $C_{24}H_{21}N_3O_2S$: C 69.37, H 5.09, N 10.11, S 7.72. Found: C 69.27, H 5.03, N 10.06, S 7.58.

*(E)-1-Benzyl-3-(3-(4-carboxybenzylidene)-2-oxoindolin-5-yl)thiourea (**7h**)*
Yield: 72%, mp 170–173°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: major *E* isomer 4.74 (d, 2H, $J = 5.2$ Hz, CH_2), 6.81 (d, 1H, $J_o = 8.4$ Hz, H-7), 7.20 (dd, 1H, $J_o = 8.4$ Hz, $J_m = 1.6$ Hz, H-6), 7.28–7.33 (m, 5H, benzyl protons), 7.60 (s, 1H, H-vinyl), 7.63 (s, 1H, H-4), 7.72 (d, 2H, $J_o = 8.0$ Hz, H-2′, H-6′), 8.06 (d, 2H, $J_o = 8.0$ Hz, H-3′, H-5′), 7.98 (s, 1H, –NH–CH_2–Ph), 9.39 (s, 1H, –NH–C=S), 10.54 (s, 1H, indole–NH), 13.50 (s, 1H, COOH). MS (ESI): *m/z* 430.4 (M+H). Elemental analysis calculated (%) for $C_{24}H_{19}N_3O_3S.0.5\ H_2O$: C 65.73, H 4.59, N 9.58, S 7.31. Found: C 65.55, H 4.55, N 10.33, S 7.70.

*General synthesis of 1-benzyl-5-(4-fluorobenzylamino)-3-(substituted-phenylimino)indolin-2-one (**13a–d**), 5-(4-fluorophenyl)-3-(substituted-phenylimino)indolin-2-one (**15a–d**) and 1-benzyl-5-(4-fluorophenyl)-3-(substituted-phenylimino)indolin-2-one (**16a–d**) derivatives*
A mixture of compounds **13**, **15**, or **16** (0.01 mol) and anilin derivatives (0.01 mol) in ethanol (5 mL) in the presence of glacial acetic acid (0.2 mL) were refluxed for 12–24 h. On cooling, the crystalline product was collected by filtration, dried to afford **13a–d**, **15a–d**, and **16a–d**. The analytical data reported here are for the major isomer. The data for the minor isomer are not reported.

*(E)-1-Benzyl-5-(4-fluorobenzylamino)-3-(4-fluorophenylimino)indolin-2-one (**13a**)*
Yield: 68%, mp 202–204°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.90 (d, 2H, $J = 6$ Hz, –NH–CH_2), 4.86 (s, 2H, –CH_2Ph), 5.80 (d, 1H, $J = 2.4$ Hz, H-4), 6.22 (t, 1H, $J = 6.0$ Hz, –NH), 6.59 (dd, 1H, $J_o = 8.0$ Hz, $J_m = 2.4$ Hz, H-6), 6.74 (d, 1H, $J_o = 8.4$ Hz, H-7), 6.96–6.99 (m, 2H, aromatic protons), 7.06–7.38 (11H, m, aromatic protons). MS (ESI): *m/z* 454.9 (M+H). Elemental analysis calculated (%) for $C_{28}H_{21}F_2N_3O$: C 74.16, H 4.67, N 9.27. Found: C 73.97, H 4.55, N 9.15.

*(E)-1-Benzyl-3-(4-chlorophenylimino)-5-(4-fluorobenzylamino)indolin-2-one (**13b**)*
Yield: 77%, mp 213°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.89 (d, 2H, $J = 6$ Hz, –NH–CH_2), 4.86 (s, 2H, –CH_2Ph), 5.80 (d, 1H, $J_m = 2.4$ Hz, H-4), 6.27 (t, 1H, $J = 6.0$ Hz, –NH), 6.59 (dd, 1H, $J_o = 8.0$ Hz, $J_m = 2.4$ Hz, H-6), 6.74 (d, 1H, $J_o = 8.4$ Hz, H-7), 6.97 (d, 2H, $J_o = 8.0$ Hz, H-2′ and H-6′), 7.07 (d, 2H, $J_o = 8.0$ Hz, aromatic protons), 7.13 (t, 1H, $J = 8.8$ Hz, aromatic proton), 7.25–7.36 (m, 6H, aromatic protons), 7.43 (d, 2H, $J = 8.4$ Hz, H-3′ and H-5′). MS (ESI): *m/z* 470 (M+H). Elemental analysis calculated (%) for $C_{28}H_{21}ClFN_3O$: C 71.56, H 4.50, N 8.94. Found: C 71.36, H 4.40, N 8.92.

*(E)-1-Benzyl-5-(4-fluorobenzylamino)-3-(4-(methylthio)phenylimino)indolin-2-one (**13c**)*
Yield: 49%, mp 221–222°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.53 (s, 3H, $-CH_3$), 3.87 (d, 2H, $J = 6$ Hz, –NH–CH_2), 4.86 (s, 2H, –CH_2Ph), 5.96 (d, 1H, $J_m = 2.4$ Hz, H-4), 6.23 (t, 1H, $J = 6.4$ Hz, –NH), 6.58 (dd, 1H, $J_o = 8.0$ Hz, $J_m = 2.4$ Hz, H-6), 6.73 (d, 1H,

J_o = 8.0 Hz, H-7), 6.94 (d, 2H, J_o = 8.0 Hz, H-2′ and H-6′), 7.06 (d, 2H, J_o = 8 Hz, H-3′ and H-5′), 7.13 (t, 1H, J = 8.8 Hz, aromatic proton), 7.20–7.37 (m, 8H, aromatic protons). MS (ESI): *m/z* 482.9 (M+H). Elemental analysis calculated (%) for $C_{29}H_{24}FN_3OS$: C 72.33, H 5.02, N 8.73, S 6.66. Found: C 72.68, H 4.99, N 8.78, S 6.78.

*(E)-1-Benzyl-3-(3-chloro-4-fluorophenylimino)-5-(4-fluorobenzylamino)indolin-2-one (**13d**)*
Yield: 79%, mp 196–197°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.94 (d, 2H, J = 6 Hz, –NH–CH_2), 4.86 (s, 2H, -CH_2Ph), 5.80 (d, 1H, J = 2.4 Hz, H-4), 6.26 (t, 1H, J = 6.0 Hz, -NH), 6.60 (dd, 1H, J_o= 8.0, J_m= 2.4 Hz, H-6), 6.68 (s, 1H, H-2′), 6.75 (d, 1H, J = 8.8 Hz, H-7), 6.94–7.11 (m, 5H, aromatic protons), 7.13 (t, 1H, J = 8.8 Hz, aromatic proton), 7.24-7.42 (m, 5H, aromatic protons). MS (ESI): *m/z* 488 (M+H). Elemental analysis calculated (%) for $C_{28}H_{20}ClF_2N_3O$: C 68.92, H 4.13, N 8.61. Found: C 69.19, H 3.91, N 8.68.

*(E)-5-(4-Fluorophenyl)-3-(4-fluorophenylimino)indolin-2-one (**15a**)*
Yield: 14%, mp 274–275°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.65 (d, 1H, J_m = 1.2 Hz, H-4), 7.05 (d, 1H, J_o = 8.0 Hz, H-7), 7.15–7.35 (m, 6H, aromatic protons), 7.40 (t, 2H, J = 8.8 Hz, aromatic protons), 7.69 (dd, 1H, J_o = 8.0, J_m = 2.0 Hz, H-6), 11.20 (s, 1H, NH). MS (ESI): *m/z* 335.47 (M+H). Elemental analysis calculated (%) for $C_{20}H_{12}F_2N_2O \cdot 2CH_3COOH$: C 63.43, H 4,43, N 6,16. Found: C 63.50, H 4.17, N 6.56.

*(E)-3-(4-Chlorophenylimino)-5-(4-fluorophenyl)indolin-2-one (**15b**)*
Yield: 16%, mp 280–282°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.57 (d, 1H, J_m = 2.0 Hz, H-4), 7.05 (d, 1H, J = 8.0 Hz, H-7), 7.16 (d, 2H, J_o = 8.0 Hz, H-2′-6′), 7.24–7.35 (m, 4H, H-2″, H-3″, H-5″, H-6″), 7.62 (d, 2H, J_o = 8.0 Hz, H-3′, H-5′), 7.70 (dd, 1H, J_o = 8.0 Hz, J_m = 1.6 Hz, H-6), 11.30 (s, 1H, NH). MS (ESI): *m/z* 351.47 (M+H). Elemental analysis calculated (%) for $C_{20}H_{12}ClFN_2O \cdot 1.8CH_3COOH$: C 61.77, H 4.21, N 6.10. Found: C 61.92, H 4.14, N 6.50.

*(E)-5-(4-Fluorophenyl)-3-(4-(methylthio)phenylimino)-indolin-2-one (**15c**)*
Yield: 12%, mp 279–283°C (decomp). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.60 (s, 3H, SCH_3), 6.60 (d, 1H, J = 2.0 Hz, H-4), 6.98 (d, 1H, J_o = 8.0 Hz, H-7), 7.04 (d, 2H, J_o = 8.0 Hz, H-2′ and H-6′), 7.17–7.27 (m, 4H, H-2″, H-3″, H-5″, H-6″), 7.43 (dd, 2H, J_o = 8.0 Hz, H-3′ and H-5′), 7.63 (dd, 1H, J_o = 8.0 Hz, J_m = 1.6 Hz, H-6), 11.09 (s, 1H, NH). MS (ESI): *m/z* 363.6 (M+H). Elemental analysis calculated (%) for $C_{21}H_{15}FN_2OS \cdot 1.6CH_3COOH$: C 63.39, H 4.70, N 6.10, S 6.99. Found: C 63.25, H 4.51, N 6.40, S 7.31.

*(E)-3-(3-Chloro-4-fluorophenylimino)-5-(4-fluorophenyl)indolin-2-one (**15d**)*
Yield: 21%, mp 236°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.63 (d, 1H, J = 2.0 Hz, H-4), 7.00 (d, 1H, J = 8.0 Hz, H-7), 7.05–7.08 (m, 1H, H-2′), 7.23–7.30 (m, 4H, H-2″, H-3″, H-5″, H-6″), 7.42 (dd, 1H, J_o = 8.0 Hz; J_m = 2.4 Hz, H-6′), 7.57 (t, 1H, J = 8.0 Hz, H-5′), 7.64 (dd, 1H, J_o = 8.0; J_m = 2.0 Hz, H-6), 11.10 (s, 1H, NH). MS (ESI): *m/z* 369.7 (M+H). Elemental analysis calculated (%) for $C_{20}H_{11}ClF_2N_2O \cdot 0.2H_2O$: C 64.51, H 3.08, N 7.52. Found: C 64.18, H 3.02, N 7.38.

*(E)-1-Benzyl-5-(4-fluorophenyl)-3-(4-fluorophenylimino)-indolin-2-one (**16a**)*
Yield: 64%, mp 194–195°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.00 (s, 2H, –CH_2), 6.62 (d, 1H, J = 2.0 Hz, H-4), 7.07 (d, 1H, J = 8.0 Hz, H-7), 7.11–7.42 (m, 13H, aromatic protons), 7.61 (dd, 1H, J_o = 8.0 Hz, J_m = 2.0 Hz, H-6). MS (ESI): *m/z* 425.9 (M+H). Elemental analysis calculated (%) for $C_{27}H_{18}F_2N_2O \cdot 0.3H_2O$: C 75.44, H 4.36, N 6.51. Found: C 75.21, H 4.76, N 6.16.

*(E)-1-Benzyl-3-(4-chlorophenylimino)-5-(4-fluorophenyl)-indolin-2-one (**16b**)*
Yield: 62%, mp 196–197°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.03 (s, 2H, –CH_2), 6.57 (d, 1H, J = 1.6 Hz, H-4), 7.11 (d, 1H, J = 8.0 Hz, H-7), 7.14–7.45 (m, 11H, aromatic protons), 7.56 (dd, 2H, J_o = 8.8 Hz, H-3′ and H-5′), 7.65 (dd, 1H, J_o = 8.2 Hz, J_m = 2.0 Hz, H-6). MS (ESI): *m/z* 441.8 (M+H). Elemental analysis calculated (%) for $C_{27}H_{18}ClFN_2O \cdot 0.6H_2O$: C 71.79, H 4.28, N 6.20. Found: C 71.63, H 4.10, N 6.12.

*(E)-1-Benzyl-5-(4-fluorophenyl)-3-(4-(methylthio)-phenylimino)indolin-2-one (**16c**)*
Yield: 63%. mp 161°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.52 (s, 3H, –CH_3), 5.03 (s, 2H, –CH_2), 6.67 (d, 1H, J_m = 2.0 Hz, H-4), 7.10 (d, 1H, J_o = 8.0 Hz, H-7), 7.16–7.46 (m, 13H, aromatic protons), 7.64 (dd, 1H, J_m = 8.0 Hz, J_o = 2.0 Hz, H-6). MS (ESI): *m/z* 453.9 (M+H). Elemental analysis calculated (%) for $C_{28}H_{21}FN_2OS \cdot 0.8H_2O$: C 72.01, H 4.87, N 5.99, S 6.86. Found: C 71.69, H 4.54, N 5.90, S 7.00.

*(E)-1-Benzyl-3-(3-chloro-4-fluorophenylimino)-5-(4-fluorophenyl)indolin-2-one (**16d**)*
Yield: 50%, mp 176–178°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.03 (s, 2H, –CH_2), 6.70 (d, 1H, J_m = 1.6 Hz, H-4), 7.12 (d, 1H, J_o = 8.0 Hz, H-7), 7.17–7.49 (m, 11H, aromatic protons), 7.58 (t, 1H, J = 8.8 Hz, H-3′), 7.67 (dd, 1H, J_o = 8.0 Hz, J_m = 2.0 Hz, H-6). MS (ESI): *m/z* 459.8 (M+H). Elemental analysis calculated (%) for $C_{27}H_{17}ClF_2N_2O \cdot 1H_2O$: C 67.99, H 4.01, N 5.87. Found: C 67.89, H 3.95, N 5.72.

Src kinase assay

The activity of protein tyrosine kinase was determined by enzyme-linked immunosorbent assay (ELISA) according to the instructions of Universal Tyrosine Kinase Assay Kit (Takara, MK410, Japan). This assay kit monitors the transfer of γ-phosphate residue from ATP to peptide substrates immobilized on plate. Briefly, the test reagents were applied on appropriate wells and phosphorylation of tyrosine was started by ATP-2Na. The plate was incubated

at 37°C for 30 min and the wells were blocked with blocking solution. The phosphorylation of tyrosine was started by ATP-2Na and the phosphorylation level of substrate was probed with HRP-conjugated anti-phosphotyrosine (PY20) antibody. The inhibitory activities of compounds against Src tyrosine kinase were monitored by the diminished activity of kinase at 450 nm. The kinase assay was performed at 37°C and concentrations of the Src used to construct the calibration curve were as follows: 0.88, 0.44, 0.22, 0.11, 0.06, 0.03, and 0.015 U/μL for Src. The test compounds were applied at a concentration range between 1×10^{-3} M and 1×10^{-7} M (0.1, 1, 10, 100, and 1000 μM). The Src tyrosine kinase activity is measured as the difference between the total activity of blank (DMSO) and the activity of enzyme in the presence of test compounds. The measuring range of the kit is from 32 fmol/well to 2 pmol/well (2.16×10^{-5} to 135×10^{-5} U/μL). To determine IC_{50} value, the increasing concentrations of PTK control were applied to the wells and a dose–response curve (linear regression) was constructed. IC_{50} value was then estimated using this formula: $y = ax + b$, $IC_{50} = (0.5 - b)/a$. IC_{50} value was determined as the concentration of a compound required to achieve 50% inhibition of Src tyrosine kinase activity.

Molecular docking study

The advanced docking program AutoDock Vina was used to evaluate the binding properties of the synthesized compounds into the Src kinase. 2D structures of compounds were established by using ChemBioDrawUltra 11.0, then they were energitically minimized with HyperChem 8.0.7 using Semi Emperical Hamiltonian AM1 and saved in mol2 format with ChemBio3D Ultra 11.0. The rigid root and rotatable bonds of ligands were defined by AutoDock Tools (ADT, version 1.5.6). The resulting files were saved as pdbqt files. The crystal structure of Src kinase in complex with PP2 was extracted from the protein data bank (PDB entry 3GEQ). ADT tools were employed to set up the enzyme: all bounds of water and ligand were removed from the protein. Polar hydrogens were added to the protein and generated pdbqt files were saved. The docking area was defined by a grid box, centered on the PP2. Grid points of $30 \times 30 \times 30$ with a spacing of 1 Å were created. Exhaustiveness was set to 30. The docking results from each calculation were clustered on the basis of root-mean-square deviation (RMSD), which was measured as distance between the centroids of the docked ligand and the native ligand (PP2), and were ranked according to the binding free energy. The mode of interaction of the native ligand within Src was used as a standard docked model as well as for RMSD calculation.

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References

[1] N. Rucci, M. Susa, A. Teti, *Anticancer Agents Med. Chem.* **2008**, *8*, 342–349.
[2] O. Alper, E. T. Bowden, *Curr. Pharm. Des.* **2005**, *11*, 1119–1130.
[3] R. B. Irby, T. J. Yeatman, *Oncogene* **2000**, *19*, 5636–5642.
[4] S. K. Muthuswamy, P. M. Siegel, D. L. Dankort, M. A. Webster, W. J. Muller, *Mol. Cell Biol.* **1994**, *14*, 735–743.
[5] S. K. Muthuswamy, W. J. Muller, *Adv. Cancer Res.* **1994**, *64*, 111–123.
[6] B. S. Verbeek, T. M. Vroom, S. S. Adriaansen-Slot, S. S. Driaansen-Slot, A. E. Ottenhoff-Kalff, J. G. Geertzema, A. Hennipman, G. Rijksen, *J. Pathol.* **1996**, *180*, 383–388.
[7] J. Araujo, C. Logothetis, *Cancer Treat. Rev.* **2010**, *36*, 492–500.
[8] N. N. Mazurenko, E. A. Kogan, I. B. Zborovskaya, F. L. Kisseljov, *Eur. J. Cancer* **1992**, *28*, 372–377.
[9] C. Bjelfman, F. Hedborg, I. Johansson, M. Nordenskjoeld, S. Paahlman, *Cancer Res.* **1990**, *50*, 6908–6914.
[10] J. R. Wiener, K. Nakano, R. P. Kruzelock, C. D. Bucana, R. C. Bast, G. E. Gallick, *Clin.Cancer Res.* **1999**, *5*, 2164–2170.
[11] S. Kumble, M. B. Omary, C. A. Cartwright, G. Triadafilopoulos, *Gastroenterology* **1997**, *112*, 348–356.
[12] N. Tegtmeyer, S. Backert, *Eur. J. Cell Bio.* **2011**, *90*, 880–890.
[13] M. Suzuki, H. Mimuro, T. Suzuki, M. Park, T. Yamamoto, C. Sasakawa, *J. Exp. Med.* **2005**, *202*, 1235–1247.
[14] G. Niu, T. Bowman, M. Huang, S. Shivers, D. Reintgen, A. Daud, A. Chang, A. Kraker, R. Jove, H. Yu, *Oncogene* **2002**, *21*, 7001–1010.
[15] C. Lieu, S. Kopetz, *Clin. Colorectal Cancer* **2010**, *9*, 89–94.
[16] J. Chen, A. Elfiky, M. Han, C. Chen, M. W. Saif, *Clin. Colorectal Cancer* **2014**, *13*, 5–13.
[17] M. P. Lutz, I. B. S. Esser, B. B. Flossmann-Kast, R. Vogelmann, H. Lührs, H. Friess, M. W. Büchler, G. Adler, *Biochem. Biophys. Res.* **1998**, *243*, 503–508.
[18] C. Egan, A. Pang, D. Durda, H. C. Cheng, J. H. Wang, D. J. Fujita, *Oncogene* **1999**, *18*, 1227–1237.
[19] J. R. Wiener, K. Nakano, R. P. Kruzelock, C. D. Bucano, R. C. Bast, G. E. Gallick, *Clin. Cancer Res.* **1999**, *5*, 2164–2170.
[20] C. A. Staley, N. U. Parikh, G. E. Gallick, *Cell Growth Diff.* **1997**, *8*, 269–274.
[21] L. M. Ellis, C. A. Staley, W. Liu, R. Y. Fleming, N. U. Parikh, C. D. Bucana, G. E. Gallick, *J. Biol. Chem.* **1998**, *273*, 1052–1057.
[22] A. Fallah-Tafti, A. Foroumadi, R. Tiwari, A. Nasrolahi Shirazi, D. G. Hangauer, Y. Bu, T. Akbarzadeh, K. Parang, A. Shafiee, *Eur. J. Med. Chem.* **2011**, *46*, 4853–4858.
[23] K. Lee, J. Kim, K. W. Jeong, K. W. Lee, Y. Lee, J. Y. Song, M. S. Kim, G. S. Lee, Y. Kim, *Bioorg. Med. Chem.* **2009**, *17*, 3152–3161.
[24] J. Zhang, P. L. Yang, N. S. Gray, *Nat. Rev.* **2009**, *9*, 28–39.
[25] K. Parang, G. Sun, *Expert Opin. Ther. Pat.* **2005**, *15*, 1183–1207.

[26] X. Li, P. Huang, J. J. Cui, J. Zhang, C. Tang, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1939–1942.
[27] M. H. Kim, A. L. Tsuhako, E. W. Co, D. T. Aftab, F. Bentzien, J. Chen, W. Cheng, S. Engst, L. Goon, R. R. Klein, D. T. Le, M. Mac, J. J. Parks, F. Qian, M. Rodriquez, T. J. Stout, J. H. Till, K. A. Won, X. Wu, F. M. Yakes, P. Yu, W. Zhang, Y. Zhao, P. Lamb, J. M. Nuss, W. Xu, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4979–4985.
[28] S. Olgen, E. Akaho, D. Nebioğlu, *J. Enzyme Inhib. Med. Chem.* **2003**, *18*, 485–490.
[29] S. Olgen, E. Akaho, D. Nebioğlu, *Farmaco* **2005**, *60*, 497–506.
[30] Z. Kilic, Y. G. Işgör, S. Ölgen, *Arch. Pharm. Chem. Life Sci.* **2009**, *342*, 333–343.
[31] F. J. Carlo, *J. Am. Chem. Soc.* **1944**, *66*, 1420.
[32] N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457–2483.
[33] T. Kniess, M. Kuchar, F. Wuest, *Synth. Commun.* **2008**, *38*, 3017–3022.
[34] D. Arnaiz, J. Bryant, Y. Chou, *US0090541*, **2005**.
[35] H. W. Pauls, B. T. Forrest, R. Laufer, *WO079767*, **2009**.
[36] L. Sun, N. Tran, F. Tang, H. App, P. Hirth, G. McMahon, C. Tang, *J. Med. Chem.* **1998**, *41*, 2588–2603.
[37] P. Magiatis, P. Polychronopoulos, O. Skaltsounis, O. Lozach, L. Meijer, L. D. B. Miller, J. P. O'callaghan, *Neurotoxicol. Teratol.* **2010**, *32*, 212–219.
[38] M. Rajopadhye, F. D. Popp, *J. Med. Chem.* **1988**, *31*, 1001–1005.
[39] Y. Kitaura, F. Ito, R. W. Stevens, N. Asai, US4835166 A, **1989**.
[40] K. L. Vine, J. M. Locke, M. Ranson, S. G. Pyne, J. B. Bremner, *Bioorg. Med. Chem.* **2007**, *15*, 931–938.
[41] L. Zhou, Y. Liu, W. Zhang, P. Wei, C. Huang, J. Pei, Y. Yuan, L. Lai, *J. Med. Chem.* **2006**, *49*, 3440–3443.
[42] S. K. Sridhar, A. Ramesh, *Biol. Pharm. Bull.* **2001**, *24*, 1149–1152.
[43] L. Mologni, R. Rostagno, S. Brussolo, P. P. Knowless, S. Kjaer, J. Murray-Rust, E. Rosso, A. Zambon, L. Scapozza, N. Q. McDonald, V. Lucchini, C. Gambacorti-Passerini, *Bioorg. Med. Chem.* **2010**, *18*, 1482–1496.
[44] C. Hung, M. Hsu, L. Huang, C. S. Hwang, O. Lee, C. Y. Wu, C. H. Chen, S. C. Kuo, *Bioorg. Med. Chem.* **2008**, *16*, 4222–4232.
[45] V. C. Taylor, C. D. Buckley, M. Douglas, A. J. Cody, D. L. Simmons, S. D. Freeman, *Biol. Chem.* **1999**, *274*, 11505–11512.
[46] F. Manetti, G. A. Locatelli, G. Maga, S. Schenone, M. Modugno, S. Forli, F. Corelli, M. Botta, *J. Med. Chem.* **2006**, *49*, 3278–3286.
[47] F. Bouchikhi, F. Anizon, P. Moreau, *Eur. J. Med. Chem.* **2008**, *43*, 755–762.
[48] M. Abelman, N. Chu, R. Jiang, J. Zablocki, WO056985, **2011**.
[49] M. S. Kisteneva, *Zh. Obshchei Khim.* **1956**, *26*, 2019–2025.
[50] C. H. Wang, A. R. White, S. N. Schwartz, S. Alluri, T. M. Cattabiani, L. K. Zhang, T. M. A. V. Chan, A. V. Buevich, A. K. Ganguly, *Tetrahedron* **2012**, *68*, 9750–9762.