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Synthesis and structure–activity relationship of 6-arylureido-3-pyrrol-2-ylmethylideneindolin-2-one derivatives as potent receptor tyrosine kinase inhibitors

Rahul R. Khanwelkar [a], Grace Shiahuy Chen [b], Hsiao-Chun Wang [a], Chao-Wu Yu [a], Chiung-Hua Huang [a], On Lee [c], Chih-Hung Chen [c], Chrong-Shiong Hwang [c], Ching-Huai Ko [c], Nien-Tzu Chou [c], Mai-Wei Lin [c], Ling-mei Wang [c], Yen-Chun Chen [c,d], Tzong-Hsiung Hseu [d], Chia-Ni Chang [c], Hui-Chun Hsu [c], Hui-Chi Lin [c], Ying-Chu Shih [c], Shuen-Hsiang Chou [c], Hsiang-Wen Tseng [c], Chih-Peng Liu [c], Chia-Mu Tu [c], Tsan-Lin Hu [c], Yuan-Jang Tsai [c], Ji-Wang Chern [a,e,*]

[a] School of Pharmacy, College of Medicine, National Taiwan University, Taipei 10051, Taiwan
[b] Department of Applied Chemistry, Providence University, Shalu 43301, Taiwan
[c] Biomedical Engineering Research Laboratories, Industrial Technology Research Institute, Hsinchu 30011, Taiwan
[d] Institute of Biotechnology, National Tsing Hua University, Hsinchu 30013, Taiwan
[e] Department of Life Science, National Taiwan University, Taipei 10027, Taiwan

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ABSTRACT

A series of new ureidoindolin-2-one derivatives were synthesized and evaluated as inhibitors of receptor tyrosine kinases. Investigation of structure–activity relationships at positions 5, 6, and 7 of the oxindole skeleton led to the identification of 6-ureido-substituted 3-pyrrolemethylidene-2-oxindole derivatives that potently inhibited both the vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) families of receptor tyrosine kinases. Several derivatives showed potency against the PDGFR inhibiting both its enzymatic and cellular functions in the single-digit nanomolar range. Among them, compound **35** was a potent inhibitor against tyrosine kinases, including VEGFR and PDGFR families, as well as Aurora kinases. Inhibitor **36** (non-substituted on the pyrrole or phenyl ring) had a moderate pharmacokinetic profile and completely inhibited tumor growth initiated with the myeloid leukemia cell line, MV4-11, in a subcutaneous xenograft model in BALB/c nude mice.

1. Introduction

Angiogenesis refers to the formation of new blood vessels from the endothelium of preexisting vasculature.[1] Pathological angiogenesis has been implicated in a wide array of vascular hyperproliferative disorders, most notably cancer,[1] rheumatoid arthritis,[2] diabetic retinopathy,[3] endometriosis,[4] age-related macular degeneration,[5] and ocular neovascularization.[6] Solid tumors, in particular, are dependant on angiogenesis for growth beyond a certain critical size via the induction of new capillaries sprouting from existing blood vessels, thereby securing a supply of nutrients and oxygen and enabling waste removal.[1,7] In addition, angiogenesis also promotes metastasis.[8]

Receptor tyrosine kinases (RTKs) represent a large family of membrane-bound enzymes that play key roles in tumor growth, survival, and metastasis. RTK activity is tightly regulated in normal cells, but aberrant RTK activation, in particular that of vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR), has been linked to the development and progression of variety of human cancers.[9] The VEGFR family comprises FLT1 (VEGFR1), KDR (VEGFR2), and FLT4 (VEGFR3), which play pivotal roles in angiogenesis and contribute to tumor progression through their ability to mediate tumor angiogenesis and lymphangiogenesis, and to enhance vascular permeability.[10] The PDGFR family comprises PDGFRα, PDGFRβ, colony stimulating factor 1 receptor (CSF1R), c-KIT, and FLT3, which promote tumor cell growth and metastasis through modification of the tumor microenvironment.[11] Additionally, mutants of FLT3 and c-KIT are directly associated with proliferation of acute myeloid leukemia blast cells[12] and gastrointestinal stromal tumor cells,[13] respectively.

Inhibition of RTK pathways has become an important approach for the discovery of new anticancer drugs.[14] The approval of bevacizumab,[15] an antibody against VEGF, by the United States Food and Drug Administration (FDA) for the treatment of first-line metastatic colorectal cancer in combination with chemotherapy,[16] has

* Corresponding author. Tel.: +886 223939462; fax: +886 223934221.
E-mail address: jwchern@ntu.edu.tw (J.-W. Chern).

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promoted greater interest in this field. Several selective KDR inhibitors, including SU5416,[17] PTK787,[18] CP-547632,[19] SU6668,[20] and ZD6474,[21] are in late-stage clinical trials. Although VEGFR and PDGFR are compelling cancer targets individually, tumors are capable of secreting multiple angiogenic factors, and they depend on these factors at different stages of progression.[22] As a result of the complex and redundant cellular signaling network associated with RTKs, broad-acting and multitargeted RTK inhibitors may be more advantageous than selective agents, owing to their ability to block multiple signaling pathways associated with tumor survival.[23] This is evidenced by the FDA's recent approval of multitargeted kinase inhibitors such as imatinib [inhibits Abelson tyrosine kinase; (Abl), c-KIT protein (CD117), and PDGFR; approved for chronic myelogenous leukemia,[24] gastrointestinal stromal tumors, and a number of other malignancies], sunitinib [inhibits KDR, PDGFR2, PDGFRβ, c-KIT and FLT3; approved for the treatment of renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumors],[25] sorafenib [inhibits Raf kinase, KDR, PDGFRβ, and c-KIT; approved for the treatment of advanced renal cell carcinoma],[26] and lapatinib [inhibits EGFR, and ErbB-2; approved for advanced metastatic breast cancer in conjugation with chemotherapy].[27] These agents demonstrate clinical benefits with manageable side effects.

The activity of inhibitors having a urea moiety,[28] especially the diphenyl urea motif,[29] against kinases such as VEGFR, PDGFR, Tie-2, Raf, and p38 has been described. These candidates, which include sorafenib,[26] PD-173074,[28b,c] CP-547632,[19] KRN633,[28c] and ABT-869[29a], either have been approved by the FDA or are in late-stage clinical trials (Fig. 1). Similarly, there are many reports on oxindole derivatives as potential RTK inhibitors;[30] these include sunitinib,[25,30a] SU5416,[17,30b] and SU6668[20,30c] (Fig. 1). However, structure–activity relationship (SAR) studies on ureido-substituted oxindoles against VEGFR and PDGFR have been less explored.[31] As part of our efforts toward the discovery and biological evaluation of new anticancer agents,[32] we report here the synthesis and SAR studies of a series of 6-ureido/thioureido-substituted indolin-2-ones as potent multitargeted RTK inhibitors. This study has led to the identification of potential kinase inhibitors for members of both the VEGFR and PDGFR families.

2. Chemistry

The 5-, 6-, and 7-arylureido-substituted 3-arylmethylidene-2-oxindole derivatives **13**–**54** were prepared according to a general method (Scheme 1). 5-Amino-2-oxindole (**6**) is commercially available, whereas a direct reduction of 7-nitro-2-oxindole led to 7-amino-2-oxindole (**7**) in high yield. Hydrogenation followed by cyclization of 2,4-dinitrophenyl acetic acid (**8**) afforded 6-amino-2-oxindole (**9**) in moderate yield. Reaction of compounds **6**, **7**, and **9** with aryl isocyanates afforded the corresponding arylureidoindolin-2-one derivatives **10**–**12** in excellent yield. Condensation of derivatives **10**–**12** with aryl/heteroaryl aldehydes afforded the corresponding 3-arylmethylidene-2-oxindole derivatives **13**–**54** in moderate yield.

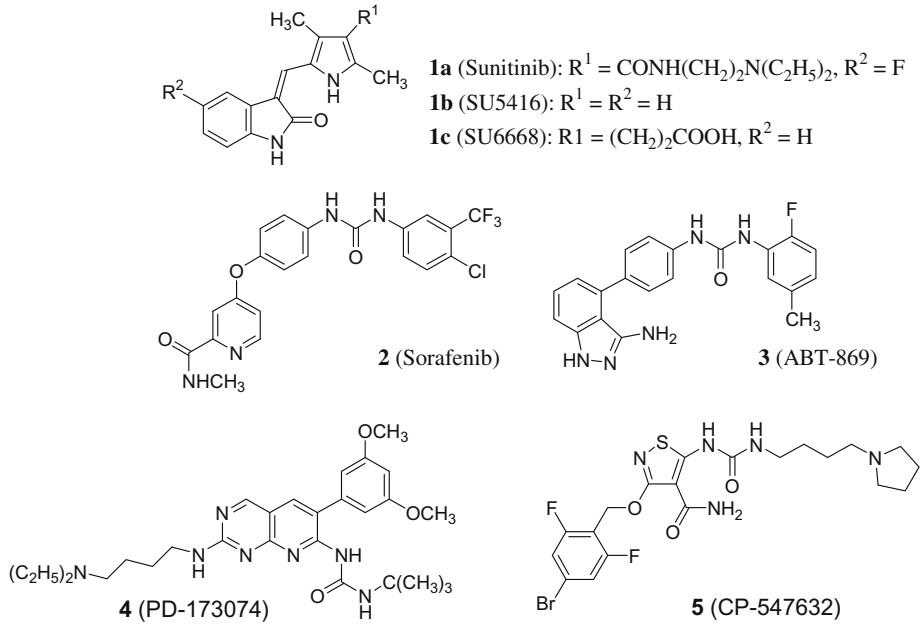
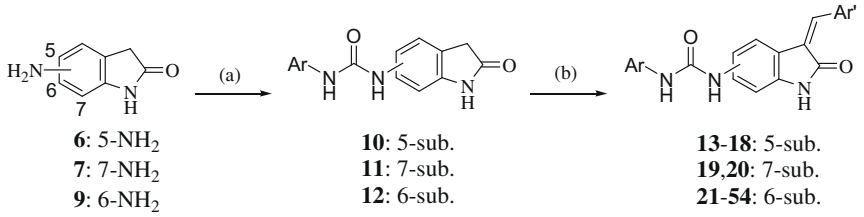


Figure 1. Structures of RTK inhibitors.



Scheme 1. Reagents and conditions: (a) Ar-NCO, DCM/methanol, 10 °C–rt, 3–4 h; (b) Ar′CHO, pyrrolidine, ethanol, reflux, 1.5–5 h.

3. Results and discussion

3.1. In vitro kinase activity

All prepared compounds were screened for inhibitory activity against a panel of RTKs that included c-KIT, FLT3, KDR and VEGFR3. We first estimated the percent inhibition of each kinase in the presence of 0.1 μM of each compound (Table 1). Derivatives **13** and **14**, each containing a 5-arylureido substituent in conjunction with a 3-phenylmethylidene group on the indolin-2-one ring, exhibited poor inhibition of all kinases tested. Replacement of the phenylmethylidene group with pyrrol-2-ylmethylidene at the 3-position, **15**, improved the inhibition to 30–60% (e.g., a 50% inhibitory concentration (IC_{50}) value of 82 nM against VEGFR3 (Table 1). Addition of methyl and phenyl group substituents on the pyrrole ring of **15**, leading to **16**, reduced the inhibitory activity. The furan-2-ylmethylidine at the 3-position, **17**, did not inhibit kinases. Interestingly, an allylic substituent at the 4-positon of phenylureido moiety (compound **18**) was tolerated (compare with **15**). The two analogs **19** and **20** with an arylureido moiety at the 7-position of the 2-oxindole did not inhibit the kinases.

Our attention was drawn by the arylureido substitution at the 6-position of the oxindole. We first investigated derivatives with an arylmethylidene group at the 3-position, **21**–**27**. In general, this series of compounds showed better kinase inhibition than the 5-arylureido-substituted analogs **13** and **14**. For most of them, however, inhibition was <50% (at 0.1 μM). One exception was the vanillin derivative **24**, which inhibited c-KIT by 87% and FLT3 by 71%. Derivative **27**, with a 2,3,4-trimethoxyphenyl methylidene substituent, showed selective inhibition of 70% against c-KIT. We thus investigated derivatives with a heteroarylmethylidene group at the 3-position of the oxindole, **28**–**35**. A 3-[5-(3-nitrophenyl)-furan-2-yl]-methylidene (**28**–**30**) or 3-pyridyl (**31** and **32**) moiety did not enhance inhibition compared with the arylmethylidene derivatives. Interestingly, 0.1 μM of **33**, with a thiophen-3-ylmethylidene moiety, inhibited c-KIT and FLT3 by ~80%, which is comparable with sunitinib (**1a**). Derivatives **34** and **35** each contained a pyrrolylmethylidene group. Compound **34**, with a 3,5-dimethyl-4-phenyl-substituted pyrrole moiety, did not show good inhibition of the kinases, as was the case for **16**. Surprisingly, 0.1 μM of compound **35**, with a 4-hydroxycarbonyl-3-methylpyrrole moiety, showed near-complete inhibition of FLT3 and KDR. Also, **35** showed 52% of inhibition against VEGFR3, the best inhibitory activity against VEGFR3 among all the compounds. The IC_{50} values of **35** for FLT3, KDR, and VEGFR3 were 0.5 nM, 0.5 nM, and 0.8 nM, respectively; these values are much better than those for sunitinib (**1a**) (Table 2).

The results presented above demonstrated the importance of the arylureido and pyrrol-2-ylmethylidene groups at positions 6 and 3, respectively, on the indolin-2-one skeleton. Therefore, we focused on the optimization of the pyrrole ring together with the 6-arylureido moiety. To better understand the SARs, we synthesized compounds **36**–**53** and tested them for kinase inhibition (Table 2). The parent compound **36**, with no substitution on the pyrrole or phenyl ring, exhibited high potency with IC_{50} values of 5.1 nM, 2.3 nM, <1 nM, and 22.4 nM for c-KIT, FLT3, KDR, and VEGFR3, respectively. To explore the role of the 6-arylureido moiety, methoxy, 3,4-dimethoxy, methyl, chloro, phenoxy, phenyl, and even naphth-2-yl substituents were introduced on the phenyl ring (**37**–**43**). Derivatives **37**–**43** exhibited selective potency with IC_{50} values of ~1 nM for FLT3. Substitution on the phenylureido moiety was well tolerated. Even a bulky group such as naphthalene (compound **43**) did not affect inhibition. These results suggested that the kinases contain a large hydrophobic binding region that interacts with the arylureido moiety of the compounds. In general, compounds **37**–**43**, containing substituents on the phenyl ring of the arylureido moiety, showed moderate inhibition of KDR and VEGFR3, but their potency was clearly decreased compared with the parent compound **36**. Overall, substitution on the phenyl ring increased the inhibition of and selectivity for FLT3.

Investigation of substitutions on the pyrrole ring generated distinct SARs. Structural comparison between **35** and **37** indicated that the presence of a methyl group at C-3′ and a carboxylate group at C-4′ in **35** substantially enhanced kinase inhibition compared with **37** (Table 2). Further introduction of a methyl group at C-5′ on the pyrrole ring (**44**–**47**) did not increase inhibition (Table 2), but these compounds indeed retained the ability to inhibit all the kinases. The only exception was **47** (containing a chloro group), which had substantially reduced potency for FLT3. Introduction of a methylene (**48** and **49**) or ethylene (**50**) group between the carboxylate group and pyrrole ring at the C-4′ position was tolerated. Surprisingly, **48** showed a tremendous boost (IC_{50} = 0.9 nM) in potency against KDR but a drastically decreased potency against FLT3 and VEGFR3 compared to **44**. Compound **48** thus exhibited good selectivity for KDR, as the IC_{50} values for VEGFR3 and FLT3 were 38-fold and 60-fold higher, respectively. Further lengthening of the chain on the pyrrole ring at the C-4′ position (**50**) led to a minor change in the inhibition profile compared with **49**. Shifting of the carboxylate group from C-4′ to C-5′ on the pyrrole ring (**51**–**53**) resulted in complete loss of inhibition. The potent multikinase inhibitors (**35**–**37**, **39**–**42**, **44**, **45**, and **50**) also were investigated for their inhibitory potential against c-KIT, and they exhibited high potency with IC_{50} values in the nanomolar range (Table 2).

In view of compound **35** possessing potent inhibitory activity against both the VEGFR (KDR and VEGFR3) and PDGFR (c-KIT and FLT3) families, it was subjected to screening for the percent inhibition of each kinase at a concentration of 10 μM against a panel of 19 kinases including Polo-like kinases. Compound **35** was found to maintain some specificity, as it did not inhibit other 14 protein kinases tested such as AKT, RAF, EGFR, PLK, and MEK (Table 3). In accord to the inhibitory activity on PDGFR (c-KIT and FLT3), compound **35** showed 89% and 80% inhibition on PDGFRα and PDGFRβ, respectively. In addition, compound **35** exhibited high potency on aurora kinases. At a concentration of 10 μM, compound **35** almost totally inhibited the activity of Aurora A and Aurora B (Table 3). IC_{50} values of **35** for Aurora A and Aurora B were 2.1 nM and 1.2 nM, respectively. As a result, compound **35** is a potent inhibitor which inhibits Aurora kinases as well as tyrosine kinases that drive tumor vascularization, including VEGFR (KDR and VEGFR3) and PDGFR (c-KIT and FLT3) families.

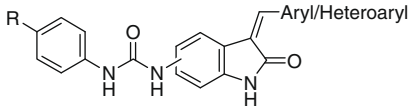
3.2. Docking model of 36 to KDR

To better understand the SAR observed, inhibitor **36** was docked into the active site of KDR in its inactive conformation by molecular modeling (Fig. 2). The oxindole core mimics the adenine of ATP in its interaction with the hinge region of KDR, and the phenylureido moiety extends into the hydrophobic pocket of the kinase domain. Two hydrogen bonds are formed by the NH group of the oxindole to Glu917 and the C═O of the oxindole core to Cys919. Importantly, the nitrogen atom of the pyrrole is located very close (2.89 Å) to the NH of Cys919. The phenyl group of the phenylurea unit of **36** accesses the hydrophobic pocket adjacent to ATP-binding site. The urea unit is nicely bound to the region with the carbonyl group situated within H-bonding distance to Asp1046, and one urea NH forms an H-bond with Glu885.

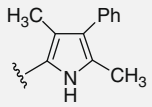
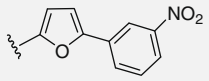
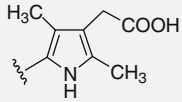
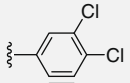
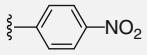
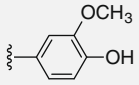
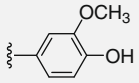
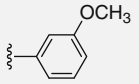
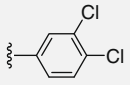
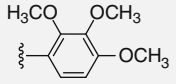
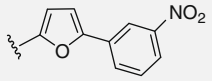
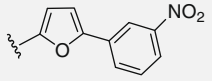
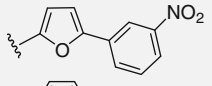
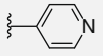
3.3. In vitro cellular activity

Initially, the potent kinase inhibitors **36, 37**, **44**, and **45** were tested for their cytotoxicity toward four human cell lines: MV4-

Table 1
Inhibition of kinase activity by arylureido-substituted 3-aryl/heteroarylmethylideneindolin-2-one derivatives **13**–**35**[a]

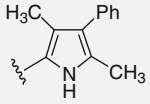
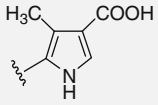


13 - 35

Compd	[b]	R	Aryl/heteroaryl	[c]	% Inhibition at 0.1 μM			
					c-KIT	FLT3	KDR	VEGFR3
1a	—	—	—	—	90.1	78.8	74.6	81.3
13	5	Cl		*E*	7.8	0	0	12
14	5	OCH_3		*E*	2.4	0	12.1	0
15	5	H		*Z*	30.1	59.8	41.9	48[d]
16	5	H		*Z*	18.9	5	15.1	35
17	5	OCH_3		*E*	—	16.7	1.7	nd
18	5	$CH_2CH{=}CH_2$		*Z*	2013	42.5	48	nd
19	7	OCH_3		*Z*	0.9	1.1	0	nd
20	7	OCH_3		*Z*	8	0	6.5	nd
21	6	H		*E*	2.1	26.8	11.5	23
22	6	H		*Z*	17.5	15.9	11.3	0
23	6	H		*Z*	32.5	28.5	0	nd
24	6	OCH_3		*Z*	86.7	71.4	5.0	nd
25	6	OCH_3		*E*	nd	2.5	nd	nd
26	6	OCH_3		*E*	5.8	50.3	0	42
27	6	OCH_3		*E*	70.2	35.5	1.6	15
28	6	H		*E*	0	19.2	0	0
29	6	Cl		*E*	50	22.9	6.5	20
30	6	OCH_3		*E*	32.7	49.3	0	0
31	6	OCH_3		*Z*	42.4	36.2	1.0	33

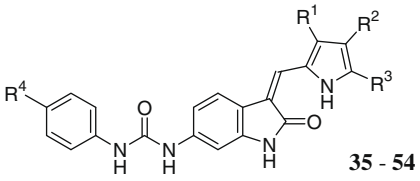
(*continued on next page*)

Table 1 (*continued*)

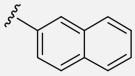
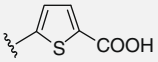
Compd	[b]	R	Aryl/heteroaryl	[c]	% Inhibition at 0.1 μM			
					c-KIT	FLT3	KDR	VEGFR3
32	6	OCH_3		*Z*	17.8	18.1	3.6	4
33	6	OCH_3		*Z*	82.4	76.8	nd	35.7
34	6	OCH_3		*Z*	23.0	17.5	7.4	0
35	6	OCH_3		*Z*	nd	96.5	99.3	52

[a] The values represent the percentage of inhibition of kinases at 0.1 μM concentration of the compounds. nd: not determined.
[b] Position of ureido group at the indolin-2-one.
[c] Configuration of the methylidene group.
[d] IC_{50} value of 82 nM for compound **15** against VEGFR3 was determined for seven concentrations and in duplicate.

Table 2
Kinase inhibitory activity of 6-arylureido-3-pyrrolylmethylideneindolin-2-ones **35–54**[a]



35 - 54

Compd	R^1	R^2	R^3	R^4	IC_{50} (nM)			
					c-KIT	FLT3	KDR	VEGFR3
1a	—	—	—	—	13.1	6.5	22.6	8.9
MLN 518	—	—	—	—	nd	86.5	2070	nd
2	—	—	—	—	nd	nd	179.7	3.0
35	CH_3	CO_2H	H	OCH_3	3.1	0.5	0.5	0.8
36	H	H	H	H	5.1	2.3	<1	22.4
37	H	H	H	OCH_3	1.5	1.4	30.6	12.8
38	H	H	H	3,4-$(OCH_3)_2$	nd	1.2	27.0	92.0
39	H	H	H	CH_3	14.4	0.9	271.9	146.8
40	H	H	H	Cl	9	1.9	13.7	8.7
41	H	H	H	OPh	16.2	0.8	31.5	68
42	H	H	H	Ph	7.2	0.7	20.4	88
43	H	H	H		nd	1.5	22.1	13
44	CH_3	CO_2H	CH_3	H	6.0	0.2	7.6	5.8
45	CH_3	CO_2H	CH_3	OCH_3	3.3	14.9	3.4	32.9
46	CH_3	CO_2H	CH_3	CH_3	nd	7.3	10.2	39
47	CH_3	CO_2H	CH_3	Cl	nd	97.8	7.9	42
48	CH_3	CH_2CO_2H	CH_3	H	nd	51.2	0.9	34
49	CH_3	CH_2CO_2H	CH_3	OCH_3	nd	4.0	4.7	11
50	CH_3	$(CH_2)_2CO_2H$	CH_3	OCH_3	4.5	2.7	10	13
51	CH_3	H	CO_2H	H	nd	>1 μM	>1 μM	>1 μM
52	CH_3	H	CO_2H	Cl	nd	>1 μM	>1 μM	>1 μM
53	H	H	CO_2H	OCH_3	nd	>1 μM	>1 μM	>1 μM
54				OCH_3	nd	17.3	nd	>1 μM

[a] Value was determined for seven concentrations and in triplicate. nd: not determined.

11 (myeloid leukemia cells), HepG2 (liver cancer cells), COLO 205 (colon carcinoma cells), and H 460 (lung cancer cells). As shown in Table 4, all four compounds were highly cytotoxic for MV4-11, with IC_{50} values in nanomolar range—comparable with sunitinib (**1a**). However, **36, 37**, **44**, and **45** only showed moderate cytotoxicity against lines HepG2, COLO 205, and H460. These kinase inhibitors exhibited high selective cytotoxicity against acute leukemia cells. Thus, all the other potent multikinase inhibitors were subjected to the cytotoxicity assay with various acute leukemia cell lines including MV4-11, MOLM-13, RS4-11, THP-1, U-937, and Kasumi-1. Interestingly, 6-arylureido-3-pyrrol-2-ylmethylidene-2-oxindole derivatives **38**–**43** showed high potency with IC_{50} values in the range of 1–9 nM for the acute monocytic leukemia cell lines MV4-11 and MOLM-13 (Table 5). One key structural

Table 3
Kinase inhibitory activity of **35** against a panel of 19 Kinases

Kinase	% Inhibition at 10 μM[a]	Kinase	% Inhibition at 10 μM[a]	IC_{50}[b] (nM)
AKT1	0	Aurora A	97.2	2.09
AKT2	0	Aurora B	98.5	1.19
AKT3	0	Aurora C	84.2	
ARAF	0	PDGFRα	88.5	0.98
BRAF	1.5	PDGFRβ	79.6	
RAF1	0	PLK1	0	
EGFR	1.9	PLK2	0	
IGF-1R	0	PLK3	3.5	
TIE2/TEK	25.5	MEK1	0.9	
		MEK2	0	

[a] Percent inhibitions were determined in relative to DMSO controls. Compounds were tested in single dose duplicate at a concentration of 10 μM.
[b] Compound **35** and control compound staurosporine were tested in 10-dose IC_{50} mode with threefold serial dilution starting at 10 μM. Reactions were carried out at 20 μM ATP.

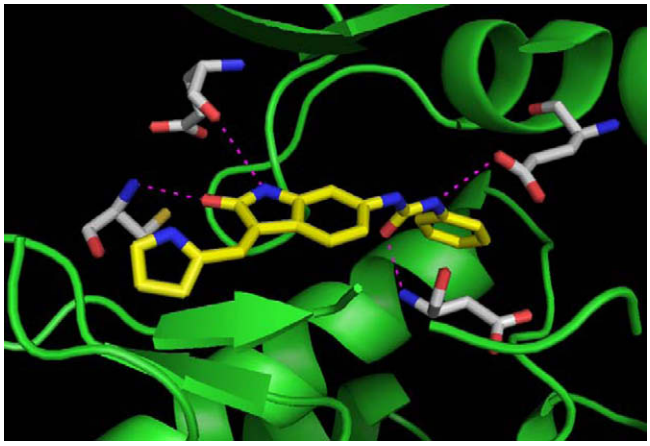


Figure 2. Model of compound 36 bound to the inactive conformation of KDR kinase. Docking simulation was performed using the program GOLD and the crystal structure of KDR kinase (PDB code 2QU5). Hydrogen bonds in magenta-colored dashed lines are shown between the oxindole core NH and carbonyl of Glu919, between the oxindole core carbonyl and the Cys919 NH, between the urea carbonyl and Asp1046 NH, and between the urea NH and Glu885 carboxylate.

Table 4
Cytotoxicity of derivatives **36**, **37**, **44**, and **45** against various cancer cells[a]

Compd	IC_{50} (μM)			
	MV4-11	HepG2	COLO 205	H 460
1a	0.009	3.81	>4	>4
36	0.028	4.01	1.68	3.28
37	< 0.05	3.46	1.4	3.01
44	0.043	0.52	6.15	2.83
45	0.015	3.74	3.23	3.60

[a] Values were determined for seven concentrations and in duplicate.

feature is that **38**–**43** lack substituents on the pyrrole moiety. Substituents on the arylureido group were well tolerated; compounds **38** and **41**–**43**, with a bulky substituent on the arylureido side, all retained high potency with IC_{50} values in the range of 1–3 nM for MV4-11 and MOLM-13. Interestingly, **36**, with no substituent on the arylureido group, showed 10-fold weaker cytotoxicity in this series of compounds. The substituent on arylureido moiety might have a hydrophobic effect. Introduction of 3′,5′-dimethyl and 4′-carboxylate substituents on the pyrrole ring (**44**) decreased cytotoxicity by 1.5-fold against MV4-11 compared with **36**. Again, methoxy (**45**), methyl (**46**), and chloro (**47**) substituents at the 4-position of the arylureido moiety increased the cytotoxicity against MV4-11 in this series of compounds (Table 5). It appeared that the

Table 5
Cytotoxicity of compounds against various leukemia cancer cells[a]

Compd	IC_{50} (μM)					
	MV4-11	MOLM-13	RS4-11	THP-1	U-937	Kasumi-1
1a	0.009	nd	1–5	0.5–1	>5	0.016
MLN 518	0.119	0.081	>5	1–5	>5	0.022
2	0.015	nd	>5	0.310	>5	0.015
35	0.017	0.230	0.213	>5	1–5	nd
36	0.028	nd	>5	>5	>5	0.12
38	0.002	0.002	>5	0.204	nd	nd
39	0.003	0.002	0.1	0.227	0.1	0.022
40	0.009	nd	1–5	1–5	1.310	nd
41	0.001	0.002	1	0.1–0.5	nd	nd
42	0.002	0.003	1	0.1–0.5	nd	nd
43	0.002	0.003	1	0.346	nd	nd
44	0.043	nd	1.253	>5	>5	nd
45	0.015	nd	>5	>5	>5	nd
46	0.008	0.035	0.112	0.1	nd	nd
47	0.009	0.038	0.123	0.1	nd	nd
48	1	1–5	0.929	1–5	nd	nd
49	0.030	nd	>5	>5	>5	0.019
50	0.008	0.097	1	0.010	nd	nd
54	>5	>5	>5	>5	>5	>5

[a] Value was determined for seven concentrations and in duplicate. nd: not determined.

C-5′ methyl group on the pyrrole ring did not affect cytotoxicity (**35** vs **45**). Increasing the length of carboxy group at the 4′-position led to a twofold decrease in activity (**48** vs **44**, **49** vs **45**). Interestingly, further elongation by one carbon restored cytotoxicity (**50**) against MV4-11. The same trend was found for the MOLM-13 line. In general, compounds **35**–**50** exhibited moderate potency against RS4-11 and THP-1 (except **50**, IC_{50} = 10 nM). Compound **39** had moderate cytotoxicity against U-937 and Kasumi-1 (IC_{50} of 0.1 and 0.02 μM, respectively), and **49**, which had an IC_{50} of 0.02 μM against Kasumi-1. Replacement of the pyrrole moiety with thiophene (**54**) caused a complete loss of cytotoxicity.

Compound **36** was assessed for its cell permeability and inhibition of cellular phosphorylation by western blot analysis. Exposure of Kasumi-1 cells to **36** (0.01–1 μmol/L) for 45 min blocked autophosphorylation of c-KIT in a dose-dependent manner (data not shown). Additionally, autophosphorylation of FLT3 in RS4-11 cells and KDR in human umbilical vein endothelial (HUVEC) cells was also inhibited by **36** (0.01–1 μmol/L, 45 min). Furthermore, **36** showed substantial ability to enter cells and to compete with ATP for binding to kinases (data not shown); each of these attributes could potentially contribute to the efficacy of this compound for the treatment of tumor cells expressing c-KIT, FLT3, and KDR. As shown in Table 6, cellular kinase phosphorylation was significantly inhibited at low concentrations of **36** (IC_{50} values <10 nM). Taken together, the in vitro kinase and cytotoxicity results demon-

Table 6
Cellular kinase phosphorylation inhibition of **36**[a]

Compd	IC_{50} (nM)			
	KDR	c-KIT	FLT3	PDGFRβ
36	<1	<10	<10	230

[a] Each IC_{50} determination was performed by Western blot analysis.

strate that **36** is a potent and multi-RTK inhibitor that functions to inhibit kinase phosphorylation both in vitro and in cells.

3.4. Pharmacokinetic studies and in vivo efficacy of 36

Based on the outstanding cytotoxicity of the 6-arylureido-pyrrolyl-methylidene-2-oxindole derivatives against acute myeloid leukemia cell lines, the pharmacokinetic profiles and antitumor activity of compound **36** was evaluated. To ascertain if the in vitro effects of **36** correlated with tumor growth inhibition, the in vivo efficacy of **36** was examined against MV4-11 tumor xenografts in BALB/c nude mice. Mice were implanted subcutaneously with MV4-11 tumor cells, and **36** dosing (20 mg/kg per day for seven days) was initiated at post-implantation day 14 when tumor size was 271 ± 22 mm.[3] As shown in Figure 3, MV4-11 tumor growth was halted, and the overall decrease in tumor volume at the end of the experiment was 46 ± 2.3%. The average body weight loss was about 10%, and no animal died during the treatment period. A pharmacokinetic analysis of **36** was undertaken in rats with a single intravenous or oral dose (Table 7). Compound **36**, when administered intravenously, showed low-volume distribution and slow clearance, with a half-life of ~1.1 h. The poor oral pharmacokinetic profile with low bioavailability (2.2%) might be due to the low solubility of **36**.

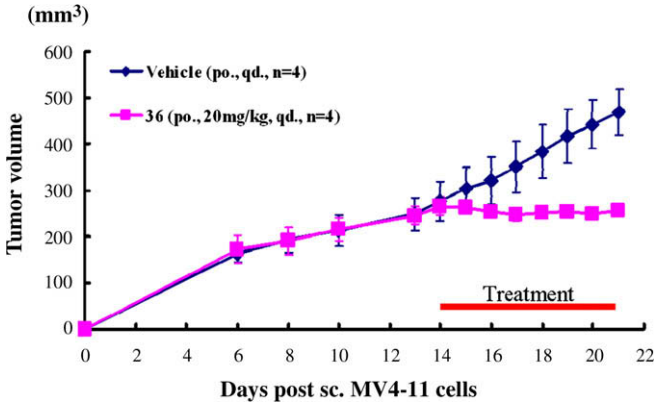


Figure 3. Antitumor growth effects of inhibitor 36 in MV4-11 subcutaneous (sc) xenograft tumor models in BALB/c nude mice. Treatment with 36 at dose of 20 mg/kg by oral gavage (po) once-daily (qd) was initiated on day 14 and continued to day 20 after sc implantation of MV4-11 human leukemia cells. No mice died during the treatment. Tumor volumes are expressed as means ± SEM ($n = 4$).

Table 7
Pharmacokinetic profiles of multikinase inhibitor **36** in Sprague-Dawley rats[a]

Parameters	iv (0.5 mg/kg)	po (10 mg/kg)
CL (L/h/kg)	0.4	13.7
$T_{1/2}$ (h)	1.1	4.1
V_d (L/kg)	0.5	79.5
$AUC_{0-24\ h}$ (h ng/mL)	1327.7	590.8
C_{max} (ng/mL)	NA	211.1
T_{max} (h)	NA	0.8
F (%)	NA	2.2

[a] NA: Not available.

4. Conclusion

We developed a new series of 6-arylureido-3-pyrrol-2-yl-indolin-2-one derivatives as multitargeted RTK inhibitors that potently inhibit members of both the VEGFR and PDGFR families, each of which plays a major role in angiogenesis. SAR studies of these compounds revealed that the arylureido moiety at the 6-position plays a key role in kinase inhibition. Incorporation of a pyrrolylmethylidene moiety at the 3-position generated a series of compounds with potent kinase inhibitory activity and cytotoxicity with IC_{50} values in the nanomolar range. Compound **35** is a potent inhibitor against tyrosine kinases as well as Aurora kinases. Compound **36** demonstrated significant in vivo efficacy for inhibition of tumor growth. Results of a virtual docking study of compound **36** to the enzyme KDR suggested that this compound binds in the ATP-binding pocket, with the oxindole core forming a bidentate H-bonding interaction with the hinge region of the enzyme together with the arylureido group occupying the hydrophobic pocket.

5. Experimental section

5.1. Gemera; experimental procedures

Melting points were measured using open capillaries on an electrothermal apparatus and were uncorrected. NMR spectra were recorded on Bruker AMX series spectrometers at 298 K. Mass spectra were recorded on a Finnigan MAT TSQ-7000 mass spectrometer. Elemental analyses for C, H, N and S were carried out on a Heraeus VariaEL-III elemental analyzer. The thin layer chromatographic analyses were performed using precoated silica gel plates (60 F254, Merck), and the spots were examined under UV light. Column chromatography was carried out on Merck Silica Gel 60 (70–230 mesh). The purity of compounds were tested to be ⩾95% by LC/MS by HPLC.

5.2. 7-Amino-2-oxindole (7)

A solution of 7-nitrooxindole (3 mmol) and 10% Pd–C (30 wt %) in ethanol (50 mL) was degassed three times at room temperature. The mixture was stirred under H_2 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 washed with diethyl ether to afford pure compound **7**. Yield 77%; mp 228–229 °C (lit.[33] 247–249 °C). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.40 (s, 2H), 4.80 (s, 2H), 6.44–6.50 (m, 2H), 6.69 (t, J = 8.0 Hz, 1H), 9.89 (s, 1H).

5.3. 2,4-Diaminophenylacetic acid

A solution of 2,4-dinitrophenylacetic acid (4 mmol) and 10% Pd–C (10 wt %) in methanol (50 mL) was hydrogenated as in the method above to give the brown solid 2,4-diamino phenyl acetic acid. It was stored in inert atmosphere and used immediately for the synthesis of 6-amino-2-oxindole without further purification.

5.4. 6-Amino-2-oxindole (9)

To a solution of 2,4-diamino phenyl acetic acid (4 mmol) in methanol (30 mL), 5 mL aqueous 2 N HCl was added. The mixture was heated at reflux for 4 h. After the removal of methanol, the obtained mixture was adjusted to pH 10 with 10 N aqueous NaOH. Product was extracted in ethyl acetate, and the organic layer was washed with water. After drying with anhydrous Na_2SO_4, ethyl acetate was removed in vacuo to obtain the desired product. Yield 65%; mp 193 °C (lit.[34] 194–195 °C). ^{1}H NMR (200 MHz, DMSO-d_6):

δ 3.23 (s, 2H), 5.01 (s, 2H), 6.08–6.11 (m, 2H), 6.79 (d, J = 8.0 Hz, 1H), 10.09 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 36.0, 96.9, 107.5, 112.9, 125.4, 145.2, 149.4, 178.2 ppm.

5.5. General procedure for the synthesis of 5-, 6-, and 7-arylureidoindolin-2-one derivatives 10–12

To a solution of **6**, **7**, or **9** (1 mmol) in 10 mL dichloromethane/methanol (90:10), the appropriate aromatic/allyl isocyanate (1.1 mmol) was added slowly at 10 °C. The mixture was stirred at the room temperature for 4 h. Solid product **10**–**12** was separated out from the mixture. It was filtered, washed with dichloromethane, and air dried.

5.5.1. 1-(2-Oxo-2,3-dihydro-1*H*-indol-5-yl)-3-phenylurea (10a)

Yield 94%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.40 (s, 2H), 6.71 (d, J = 8.0 Hz, 1H), 6.93 (t, J = 6.0 Hz, 1H), 7.16 (dd, J = 2.0, 8.0 Hz, 1H), 7.25 (t, J = 8.0, 8.2 Hz, 2H), 7.36–7.44 (m, 3H), 8.47 (s, 1H), 8.58 (s, 1H), 10.24 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 37.0, 56.0, 109.8, 114.8, 116.9, 118.6, 120.8, 127.1, 133.8, 134.8, 139.1, 153.8, 155.2, 177.1 ppm. Anal. ($C_{15}H_{13}N_3O_2$) C, H, N.

5.5.2. 1-(4-Chlorophenyl)-3-(2-oxo-2,3-dihydro-1*H*-indol-5-yl)-urea (10b)

Yield 99%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.44 (s, 2H), 6.70 (d, J = 8.0 Hz, 1H), 7.16 (dd, J = 2.0, 8.0 Hz, 1H), 7.26 (s, 1H), 7.32 (d, J = 10.0 Hz, 2H), 7.45 (d, J = 8.0 Hz, 2H), 8.56 (s, 1H), 8.78 (s, 1H), 10.23 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 37.0, 109.9, 117.1, 118.9, 120.4, 126.0, 127.1, 129.5, 134.3, 139.4, 139.8, 153.5, 177.1 ppm. Anal. ($C_{15}H_{12}ClN_3O_2$) C, H, N.

5.5.3. 1-(4-Methoxyphenyl)-3-(2-oxo-2,3-dihydro-1*H*-indol-5-yl)-urea (10c)

Yield 95%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.44 (s, 2H), 3.70 (s, 3H), 6.70 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 8.0 Hz, 2H), 7.14 (dd, J = 2.0, 8.0 Hz, 1H), 7.32 (d, J = 9.1 Hz, 3H), 8.36 (s, 2H), 10.22 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 37.0, 56.0, 109.8, 114.8, 116.9, 118.6, 120.8, 127.1, 133.8, 134.8, 153.8, 155.2, 177.1 ppm. Anal. ($C_{16}H_{15}N_3O_3$) C, H, N.

5.5.4. 1-Allyl-3-(2-oxo-2,3-dihydro-1*H*-indol-5-yl)urea (10d)

Yield 93%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.41 (s, 2H), 3.69 (d, J = 6.0 Hz, 2H), 5.01–5.18 (m, 2H), 5.75–5.94 (m, 1H), 6.14 (t, J = 4.0 Hz, 1H), 6.65 (d, J = 8.2 Hz, 1H), 7.09 (d, J = 8.0 Hz, 1H), 7.31 (s, 1H), 8.29 (s, 1H), 10.19 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 37.0, 42.4, 109.8, 115.4, 116.6, 118.2, 127.0, 135.4, 137.3, 138.7, 156.2, 177.1 ppm. Anal. ($C_{12}H_{13}N_3O_2$) C, H, N.

5.5.5. 1-(4-Methoxyphenyl)-3-(2-oxo-2,3-dihydro-1*H*-indol-7-yl)urea (11)

Yield 83%; mp 298–299 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.51 (s, 2H), 3.70 (s, 3H), 6.83–6.97 (m, 4H), 7.23 (d, J = 7.6 Hz, 1H), 7.39 (d, J = 8.6 Hz, 2H), 8.10 (s, 1H), 8.58 (s, 1H), 10.05 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 36.9, 56.0, 114.8, 120.5, 121.1, 122.2, 122.6, 122.7, 127.5, 133.8, 136.5, 154.0, 155.3, 176.9 ppm. Anal. ($C_{16}H_{15}N_3O_3\cdot0.5H_2O$) C, H, N.

5.5.6. 1-(2-Oxo-2,3-dihydro-1*H*-indol-6-yl)-3-phenylurea (12a)

Yield 85%; mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.34 (s, 2H), 6.76 (dd, J = 2.0, 7.8 Hz, 1H), 6.93 (t, J = 7.2, 7.6 Hz, 1H), 7.04 (d, J = 8.0 Hz, 1H), 7.22 (d, J = 2.0 Hz, 1H), 7.24 (d, J = 7.6 Hz, 2H), 7.40 (d, J = 7.6 Hz, 2H), 8.54 (s, 1H), 8.60 (s, 1H), 10.28 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 35.9, 100.5, 110.6, 111.2, 115.1, 118.7, 118.8, 129.3, 139.7, 140.2, 153.0, 158.2, 177.4 ppm. Anal. ($C_{15}H_{13}N_3O_2\cdot0.6H_2O$) C, H, N.

5.5.7. 1-(4-Methoxyphenyl)-3-(2-oxo-2,3-dihydro-1*H*-indol-6-yl)urea (12b)

Yield 87%; mp 290–292 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.32 (s, 2H), 3.70 (s, 3H), 6.77 (dd, J = 1.8, 7.7 Hz, 1H), 6.85 (d, J = 9.2 Hz, 2H), 7.05 (d, J = 8.0 Hz, 1H), 7.23 (br, 1H), 7.33 (d, J = 9.0 Hz, 2H), 8.39 (s, 1H), 8.57 (s, 1H), 10.30 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 35.9, 55.7, 100.5, 111.1, 114.5, 119.0, 120.6, 125.0, 133.2, 140.0, 144.6, 153.2, 155.0, 177.4 ppm. Anal. ($C_{16}H_{15}N_3O_3\cdot0.3H_2O$) C, H, N.

5.5.8. 1-(3,4-Dimethoxyphenyl)-3-(2-oxo-2,3-dihydro-1*H*-indol-6-yl)urea (12c)

Yield 83%; mp 270–271 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.37 (s, 2H), 3.69 (s, 3H), 3.72 (s, 3H), 6.76 (dd, J = 2.4, 8.0 Hz, 1H), 6.81–6.86 (m, 2H), 7.05 (d, J = 8.0 Hz, 1H), 7.21 (d, J = 2.4 Hz, 1H), 7.27 (d, J = 2.4 Hz, 1H), 8.41 (s, 1H), 8.55 (s, 1H), 10.28 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 35.9, 55.9, 56.4, 100.5, 104.4, 110.7, 111.1, 113.1, 119.0, 124.9, 133.8, 139.9, 144.5, 144.6, 149.3, 153.1, 177.4 ppm. Anal. ($C_{17}H_{17}N_3O_1\cdot0.1H_2O$) C, H, N.

5.5.9. 1-(2-Oxo-2,3-dihydro-1*H*-indol-6-yl)-3-p-tolylurea (12d)

Yield 89%; mp 271–272 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.23 (s, 3H), 3.54 (s, 2H), 6.76 (dd, J = 2.0, 8.0 Hz, 1H), 7.04–7.08 (m, 3H), 7.24 (d, J = 1.6 Hz, 1H), 7.31 (d, J = 8.0 Hz, 2H), 8.47 (s, 1H), 8.60 (s, 1H), 10.32 (s, 1H). Anal. ($C_6H_{15}N_3O_2\cdot0.2H_2O$) C, H, N.

5.5.10. 1-(4-Chlorophenyl)-3-(2-oxo-2,3-dihydro-1*H*-indol-6-yl)urea (12e)

Yield 92%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.38 (s, 2H), 6.79 (dd, J = 1.9, 8.0 Hz, 1H), 7.07 (d, J = 8.0 Hz, 1H), 7.21 (d, J = 1.8 Hz, 1H), 7.31 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 8.70 (s, 1H), 8.74 (s, 1H), 10.33 (s, 1H). Anal. ($C_{15}H_{12}ClN_3O_2\cdot0.3H_2O$) C, H, N.

5.5.11. 1-(2-Oxo-2,3-dihydro-1*H*-indol-6-yl)-3-(4-phenoxyphenyl)urea (12f)

Yield 88%; mp 294–295 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.38 (s, 2H), 6.78 (dd, J = 1.9, 8.0 Hz, 1H), 6.92–6.99 (m, 4H), 7.04–7.11 (m, 2H), 7.25 (d, J = 1.8 Hz, 1H), 7.31–7.39 (m, 2H), 7.45 (d, J = 8.9 Hz, 2H), 8.61 (s, 1H), 8.64 (s, 1H), 10.33 (s, 1H). Anal. ($C_{21}H_{17}N_3O_3$) C, H, N.

5.5.12. 1-Biphenyl-4yl-3-(2-oxo-2,3-dihydro-1*H*-indol-6-yl)urea (12g)

Yield 87%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.38 (s, 2H), 6.81 (dd, J = 1.7, 8.0 Hz, 1H), 7.07 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 1.3 Hz, 1H), 7.31 (d, J = 7.0 Hz, 1H), 7.43 (t, J = 7.3 Hz, 2H), 7.50–7.64 (m, 6H), 8.70 (s, 1H), 8.71 (s, 1H), 10.35 (s, 1H). Anal. ($C_{21}H_{17}N_3O_2$) C, H, N.

5.5.13. 1-Naphthalen-2-yl-3-(2-oxo-2,3-dihydro-1*H*-indol-6-yl)-urea (12h)

Yield 85%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.39 (s, 2H), 6.81 (dd, J = 1.9, 8.0 Hz, 1H), 7.08 (d, J = 7.9 Hz, 1H), 7.29–7.48 (m, 4H), 7.75–7.84 (m, 3H), 8.09 (s, 1H), 8.75 (s, 1H), 8.82 (s, 1H), 10.36 (s, 1H). Anal. ($C_{19}H_{15}N_3O_2\cdot0.3H_2O$) C, H, N.

5.6. General procedure for the synthesis of arylureido-substituted 3-arylmethylidene-2-oxindole derivatives 13–54

A catalytic amount of pyrrolidine (0.01 mmol) was added into a solution of 5-, 6-, or 7-arylureidoindolin-2-one derivatives **10**–**12** (1 mmol) and aryl/heteroaryl aldehyde (1.1 mmol) in ethanol (5 mL). The mixture was heated at reflux for 1.5–5 h. After cooling to room temperature, crude product precipitated from the

solution and was collected by filtration, washed with ethanol, and air dried.

5.6.1. (*E*)-1-[3-Benzylidene-2-oxo-2,3-dihydro-1*H*-indol-5-yl]-3-(4-chlorophenyl)urea (13)

Yield 55%; mp 273–275 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.80 (d, J = 8.4 Hz, 1H), 7.29 (d, J = 8.8 Hz, 2H), 7.33 (d, J = 8.2 Hz, 1H), 7.42 (d, J = 9.2 Hz, 2H), 7.48–7.53 (m, 3H), 7.61 (s, 1H), 7.70–7.73 (m, 3H), 8.46 (s, 1H), 8.65 (s, 1H), 10.47 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 110.9, 115.1, 120.5, 121.8, 122.1, 126.0, 128.6, 129.5, 129.7, 130.3, 130.7, 134.1, 135.2, 136.7, 139.0, 139.7, 153.5, 169.6. Anal. ($C_{22}H_{16}ClN_3O_2$) C, H, N.

5.6.2. (*E*)-1-[3-Benzylidene-2-oxo-2,3-dihydro-1*H*-indol-5-yl]-3-(4-methoxyphenyl)-urea (14)

Yield 70%; mp 286–287 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.69 (s, 3H), 6.76–6.85 (m, 3H), 7.28–7.35 (m, 3H), 7.49–7.52 (m, 3H), 7.60 (s, 1H), 7.71–7.75 (m, 3H), 8.43 (s, 1H), 8.47 (s, 1H), 10.45 (s, 1H). Anal. ($C_{23}H_{19}N_3O_3\cdot 0.3H_2O$) C, H, N.

5.6.3. (*Z*)-1-[2-Oxo-3-(1*H*-pyrrol-2-ylmethylene)-2,3-dihydro-1*H*-indol-5-yl]-3-phenyl-urea (15)

Yield 70%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.32–6.36 (m, 1H), 6.79 (d, J = 8.3 Hz, 1H), 6.90–6.93 (m, 1H), 6.95 (d, J = 7.3 Hz, 1H), 7.08 (dd, J = 1.9, 8.3 Hz, 1H), 7.26 (t, J = 8.0 Hz, 2H), 7.34–7.37 (m, 1H), 7.46 (d, J = 7.6 Hz, 2H), 7.65 (s, 1H), 7.81 (d, J = 1.9 Hz, 1H), 8.66 (s, 1H), 8.82 (s, 1H), 10.78 (s, 1H), 13.36 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 110.5, 111.0, 112.3, 118.0, 119.0, 119.1, 121.4, 122.5, 126.3, 126.5, 127.1, 129.7, 130.4, 134.6, 135.2, 140.8, 153.8, 170.2. MS (ESI) m/z 344 [M]$^+$. Anal. ($C_{20}H_{16}N_4O_2\cdot 0.1H_2O$) C, H, N.

5.6.4. (*Z*)-1-[3-(3,5-Dimethyl-4-phenyl-1*H*-pyrrol-2-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indol-5-yl]-3-phenylurea (16)

Yield 60%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.29 (s, 3H), 2.34 (s, 3H), 6.85 (d, J = 8.7 Hz, 1H), 6.93 (t, J = 7.4 Hz, 2H), 7.12 (d, J = 7.5 Hz, 1H), 7.26–7.33 (m, 5H), 7.48–7.53 (m, 4H), 7.79 (s, 1H,), 8.51 (s, 1H), 8.71 (s, 1H), 10.74 (s, 1H), 13.71 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 11.2, 13.6, 110.3, 111.0, 114.9, 118.7, 119.0, 122.5, 124.1, 125.9, 127.0, 129.0, 129.2, 129.6, 130.4, 134.2, 134.3, 134.6, 135.5, 140.9, 154.0, 170.4 ppm. Anal. ($C_{28}H_{24}N_4O_2$) C, H, N.

5.6.5. (*E*)-1-(4-Methoxyphenyl)-3-{3-[5-(3-nitrophenyl)-furan-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indol-5-yl}urea (17)

Yield 95%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.69 (s, 3H), 6.73–6.84 (m, 2H), 7.11–7.17 (m, 2H), 7.35 (s, 1H), 7.46 (d, J = 1.8 Hz, 1H,), 7.53–7.62 (m, 2H), 8.02 (d, J = 8.0 Hz, 1H), 8.37 (d, J = 8.0 Hz, 1H), 8.48 (d, J = 8.0 Hz, 1H), 8.69 (s, 2H), 10.49 (s, 1H). MS (ESI) m/z 496 [M]$^+$. Anal. ($C_{27}H_{20}N_4O_6\cdot 0.5H_2O$) C, H, N.

5.6.6. (*Z*)-1-[2-Oxo-3-(1*H*-pyrrol-2-ylmethylene)-2,3-dihydro-1*H*-indol-5-yl]-3-allyl-urea (18)

Yield 50%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.72 (d, J = 6.0 Hz, 2H), 5.03–5.20 (m, 2H), 5.77–5.93 (m, 1H), 6.26–6.28 (m, 1H), 6.33–6.36 (m, 1H), 6.74 (d, J = 8.2 Hz, 1H), 6.87–6.89 (m, 1H), 7.02 (d, J = 8.2 Hz, 1H), 7.33 (s, 1H), 7.59 (s, 1H), 7.74 (s, 1H), 8.36 (s, 1H), 10.73 (s, 1H), 13.36 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 42.4, 110.04, 110.4, 112.2, 115.5, 118.2, 118.6, 121.3, 126.1, 126.4, 130.4, 134.6, 135.5, 137.4, 156.3, 170.1. Anal. ($C_{17}H_{16}N_4O_2\cdot 0.2H_2O$) C, H, N.

5.6.7. (*Z*)-(5-{7-[3-(4-Methoxyphenyl)-ureido]-2-oxo-1,2-dihydroindol-3-ylidene-methyl}-2,4-dimethyl-1*H*-pyrrol-3-yl) acetic acid (19)

Yield 90%; mp 277–278 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.23 (s, 3H), 2.32 (s, 3H), 3.30 (s, 2H), 3.70 (s, 3H), 6.87–6.93 (m, 4H), 7.40–7.47 (m, 4H), 8.62 (s, 1H, NH), 9.06 (s, 1H), 10.54 (s, 1H), 13.46 (s, 1H). Anal. ($C_{25}H_{24}N_4O_5\cdot 1.1H_2O$) C, H, N.

5.6.8. (*Z*)-1-(4-Methoxyphenyl)-3-(2-oxo-3-thiophen-3-ylmethylene-2,3-dihydro-1*H*-indol-7-yl)urea (20)

Yield 57%; mp 286–287 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.70 (s, 3H), 6.88–6.91 (m, 3H), 7.29 (d, J = 8.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 2H), 7.48 (s, 1H), 7.53–7.55 (m, 1H), 7.56 (s, 1H), 7.75–7.77 (m, 1H), 8.04 (s, 1H), 8.12 (s, 1H), 8.56 (s, 1H), 10.30 (s, 1H). Anal. ($C_{21}H_{17}N_3O_3S\cdot 0.3H_2O$) C, H, N, S.

5.6.9. (*E*)-1-[3-(3,4-Dichlorobenzylidene)-2-oxo-2,3-dihydro-1*H*-indol-6-yl]-3-phenyl-urea (21)

Yield 80%; mp 268–270 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.69 (dd, J = 2.0, 8.0 Hz, 1H), 6.97 (t, J = 7.2 Hz, 1H), 7.25 (d, J = 8.3 Hz, 2H), 7.27 (d, J = 3.4 Hz, 1H), 7.36–7.46 (m, 4H, Ar-H), 7.68 (dd, J = 1.8, 8.0 Hz, 1H), 7.76 (d, J = 8.3 Hz, 1H,), 7.92 (d, J = 1.6 Hz, 1H), 8.69 (s, 1H), 8.94 (s, 1H, NH), 10.61 (s, 1H, NH). MS (ESI) m/z 423 [M]$^+$. Anal. ($C_{22}H_{15}N_3O_2Cl_2\cdot 0.3H_2O$) C, H, N.

5.6.10. (*Z*)-1-[3-(4-Nitrobenzylidene)-2-oxo-2,3-dihydro-1*H*-indol-6-yl]-3-phenylurea (22)

Yield 65%; mp 282–284 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.65 (dd, J = 2.0, 8.4 Hz, 1H), 6.94 (t, J = 7.2 Hz, 1H), 7.25 (dd, J = 7.9, 7.9 Hz, 2H), 7.34–7.43 (m, 5H), 7.91 (d, J = 8.4 Hz, 2H), 8.31 (d, J = 8.8 Hz, 2H), 8.68 (s, 1H), 8.97 (s, 1H), 10.64 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 100.8, 111.4, 114.5, 119.2, 123.0, 124.6, 124.8, 129.7, 130.3, 130.7, 131.3, 140.2, 142.8, 143.4, 145.6, 147.0, 153.0, 169.8 ppm. MS (ESI) m/z 400 [M]$^+$. Anal. ($C_{22}H_{16}N_4O_4\cdot 0.2H_2O$) C, H, N.

5.6.11. (*Z*)-1-[3-(4-Hydroxy-3-methoxybenzylidene)-2-oxo-2,3-dihydro-1*H*-indol-6-yl]-3-phenylurea (23)

Yield 77%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.83 (s, 3H), 6.82 (d, J = 8.1 Hz, 1H), 6.85 (d, J = 8.1, 2.0 Hz, 1H), 6.96 (t, J = 7.3 Hz, 1H), 7.23–7.31 (m, 3H), 7.42–7.54 (m, 4H), 7.66 (d, J = 8.4 Hz, 1H), 8.61 (d, J = 2.0 Hz, 1H), 8.65 (s, 1H), 8.79 (s, 1H), 9.73 (br, 1H), 10.49 (s, 1H). MS (ESI) m/z 401 [M]$^+$. Anal. ($C_{23}H_{19}N_3O_4\cdot 0.2H_2O$) C, H, N.

5.6.12. (*Z*)-1-[3-(4-Hydroxy-3-methoxybenzylidene)-2-oxo-2,3-dihydro-1*H*-indol-6-yl]-3-(4-methoxyphenyl)urea (24)

Yield 60%; mp 276–279 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.70 (s, 3H), 3.83 (s, 3H), 6.80–6.91 (m, 4H), 7.18–7.37 (m, 4H), 7.48–7.52 (m, 1H), 7.65 (d, J = 8.1 Hz, 1H), 8.47 (s, 1H), 8.61 (d, J = 2.0 Hz, 1H), 8.72 (s, 1H), 9.85 (br, 1H), 10.48 (s, 1H). MS (ESI) m/z 431 [M]$^+$. Anal. ($C_{24}H_{21}N_3O_5\cdot 0.65H_2O$) C, H, N.

5.6.13. (*E*)-1-[3-(3-Methoxybenzylidene)-2-oxo-2,3-dihydro-1*H*-indol-6-yl]-3-(4-methoxyphenyl)urea (25)

Yield 50%; mp 273–275 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.70 (s, 3H), 3.79 (s, 3H), 6.68 (dd, J = 1.9, 8.6 Hz, 1H), 6.86 (d, J = 8.9 Hz, 2H), 7.01 (dd, J = 2.4, 7.6 Hz, 1H), 7.23–7.49 (m, 8H), 8.50 (s, 1H), 8.82 (s, 1H), 10.55 (s, 1H). MS (ESI) m/z 415 [M]$^+$. Anal. ($C_{24}H_{21}N_3O_4\cdot 0.2H_2O$) C, H, N.

5.6.14. (*E*)-1-[3-(3,4-Dichlorobenzylidene)-2-oxo-2,3-dihydro-1*H*-indol-6-yl]-3-(4-methoxyphenyl)urea (26)

Yield 85%; mp 261–263 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.70 (s, 3H), 6.68 (dd, J = 2.0, 8.0 Hz, 1H), 6.86 (d, J = 8.8 Hz, 2H), 7.33–7.34 (m, 3H), 7.38 (d, J = 2.0 Hz, 1H), 7.67–7.77 (m, 3H), 7.91 (s, 1H), 8.49 (s, 1H), 8.85 (s, 1H), 10.58 (s, 1H). MS (ESI) m/z 453 [M]$^+$. Anal. ($C_{23}H_{17}N_3O_3Cl_2\cdot 0.2H_2O$) C, H, N.

5.6.15. (*E*)-1-(4-Methoxyphenyl)-3-[2-oxo-3-(2,3,4-trimethoxybenzylidene)-2,3-di-hydro-1*H*-indol-6-yl]urea (27)

Yield 78%; mp 262–264 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.68 (s, 3H), 3.76 (s, 3H), 3.79 (s, 3H), 3.85 (s, 3H), 6.65 (dd, J = 1.8, 8.6 Hz, 1H), 6.83 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 1H), 7.30–7.33 (m, 3H), 7.38–7.45 (m, 3H), 8.43 (s, 1H), 8.75 (s, 1H), 10.47 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 55.7, 56.6, 61.0, 61.9, 100.3, 108.3, 110.7, 114.6, 115.0, 120.6, 121.5, 123.4, 124.9, 126.8, 128.6, 132.9, 142.1, 142.3, 144.2, 152.8, 152.9, 155.1, 155.5, 169.9 ppm. MS (ESI) m/z 475 [M]$^+$. Anal. ($C_{26}H_{25}N_3O_6\cdot0.2H_2O$) C, H, N.

5.6.16. (*E*)-1-{3-[5-(3-Nitrophenyl)-furan-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indol-6-yl}phenylurea (28)

Yield 81%; mp 278–280 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.94–6.98 (m, 2H), 7.19 (s, 1H), 7.25–7.36 (m, 3H), 7.45–7.49 (m, 3H), 7.58 (d, J = 3.8 Hz, 1H), 7.83 (dd, J = 8.0, 8.0 Hz, 1H), 8.24 (dd, J = 2.0, 8.0 Hz, 1H), 8. 33 (d, J = 8.4 Hz, 1H), 8.39 (d, J = 8.5 Hz, 1H), 8.73 (s, 1H), 8.88 (s, 1H), 9.22 (s, 1H), 10.60 (s, 1H). MS (ESI) m/z 466 [M]$^+$. Anal. ($C_{26}H_{18}N_4O_5\cdot0.3H_2O$) C, H, N.

5.6.17. (*E*)-1-(4-Chlorophenyl)-3-{3-[5-(3-nitrophenyl)-furan-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indol-6-yl}urea (29)

Yield 90%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.96 (dd, J = 1.8, 8.0 Hz, 1H), 7.19 (s, 1H), 7.33 (d, J = 8.9 Hz, 2H), 7.35 (d, J = 3.5 Hz, 1H), 7.45 (d, J = 1.7 Hz, 1H), 7.50 (d, J = 8.9 Hz, 2H), 7.56 (d, J = 3.7 Hz, 1H), 7.82 (dd, J = 8.0, 8.0 Hz, 1H), 8.23 (dd, J = 1.5, 9.6 Hz, 1H), 8.32 (d, J = 7.8 Hz, 1H), 8.38 (d, J = 7.8 Hz, 1H), 8.70 (s, 1H), 8.84 (s, 1H), 9.08 (s, 1H), 10.60 (s, 1H). MS (ESI) m/z 500 [M]$^+$. Anal. ($C_{26}H_{17}N_4O_5Cl\cdot0.3H_2O$) C, H, N.

5.6.18. (*E*)-1-(4-Methoxyphenyl)-3-{3-[5-(3-nitrophenyl)-furan-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indol-6-yl}urea (30)

Yield 83%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.71 (s, 3H), 6.88 (d, J = 9.1 Hz, 2H), 6.95 (s, 1H), 7.19 (s, 1H), 7.49 (d, J = 2.0 Hz, 1H), 7.58 (d, J = 3.6 Hz, 1H), 7.83 (dd, J = 7.7, 8.1 Hz, 1H), 8.23–8.41 (m, 3H), 8.52 (s, 1H), 8.73 (s, 1H), 8.99 (s, 1H), 10.59 (s, 1H). MS (ESI) m/z 496 [M]$^+$. Anal. ($C_{27}H_{20}N_4O_6\cdot0.7H_2O$) C, H, N.

5.6.19. (*Z*)-1-(4-Methoxyphenyl)-3-(2-oxo-3-pyridin-4-ylmethylene-2,3-dihydro-1*H*-indol-6-yl)urea (31)

Yield 71%; mp 262–264 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.70 (s, 3H), 6.66 (d, J = 8.4 Hz, 1H), 6.86 (d, J = 8.8 Hz, 2H), 7.31–7.36 (m, 4H), 7.39 (d, J = 2.0 Hz, 1H), 7.61 (d, J = 6.2 Hz, 2H), 8.50 (s, 1H), 8.68 (d, J = 6.0 Hz, 2H), 8.89 (s, 1H), 10.64 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 56.0, 109.9, 111.5, 114.4, 114.9, 121.4, 124.1, 124.5, 129.7, 130.9, 132.9, 143.3, 143.6, 145.2, 150.8, 153.3, 155.6, 169.8 ppm. MS (ESI) m/z 386 [M]$^+$. Anal. ($C_{22}H_{18}N_4O_3\cdot0.4H_2O$) C, H, N.

5.6.20. (*Z*)-1-(4-Methoxyphenyl)-3-(2-oxo-3-pyridin-2-ylmethylene-2,3-dihydro-1*H*-indol-6-yl)urea (32)

Yield 73%; mp 292–294 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ .71 (s, 3H), 6.78 (dd, J = 2.0, 8.0 Hz, 1H), 6.87 (d, J = 9.0 Hz, 2H), 7.33–7.45 (m, 5H), 7.79 (d, J = 7.7 Hz, 1H), 7.91 (td, J = 1.8, 8 Hz, 1H), 8.53 (s, 1H), 8.84–8.88 (m, 2H), 8.92 (s, 1H), 10.56 (s, 1H). MS (ESI) m/z 386 [M]$^+$. Anal. ($C_{22}H_{18}N_4O_3\cdot0.4H_2O$) C, H, N.

5.6.21. (*Z*)-1-(4-Methoxyphenyl)-3-(2-oxo-3-thiophen-3-ylmethylene-2,3-dihydro-1*H*-indol-6-yl)urea (33)

Yield 58%; mp 266–267 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.70 (s, 3H), 6.75 (dd. J = 2.0, 8.3 Hz, 1H), 6.86 (d, J = 8.9 Hz, 2H), 7.27–7.33 (m, 4H), 7.49 (d, J = 5.0 Hz, 1H), 7.70–7.74 (m, 1H), 8.06 (d, J = 1.5 Hz, 1H), 8.49 (s, 1H), 8.85 (s, 1H), 10.52 (s, 1H). MS (ESI) m/z 391 [M]$^+$. Anal. ($C_{21}H_{17}N_3O_3S$) C, H, N, S.

5.6.22. (*Z*)-1-[3-(3,5-Dimethyl-4-phenyl-1*H*-pyrrol-2-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indol-6-yl]-3-(4-methoxyphenyl)-urea (34)

Yield 85%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.27 (s, 3H), 2.32 (s, 3H), 3.71 (s, 3H), 6.85–6.87 (m, 3H), 7.29–7.32 (m, 4H), 7.35 (d, J = 8.8 Hz, 2H), 7.43 (dd, J = 8.0, 8.0 Hz, 2H), 7.50 (s, 1H), 7.61 (d, J = 8.4 Hz, 1H), 8.41 (s, 1H), 8.64 (s, 1H), 10.77 (s, 1H), 13.49 (s, 1H). MS (ESI) m/z 478 [M]$^+$. Anal. ($C_{29}H_{26}N_4O_3\cdot0.3H_2O$) C, H, N.

5.6.23. (*Z*)-5-{6-[3-(4-Methoxyphenyl)-ureido]-2-oxo-1,2-dihydroindol-3-ylidene-methyl}-4-methyl-1*H*-pyrrole-3-carboxylic acid (35)

Yield 79%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.56 (s, 3H), 3.70 (s, 3H), 6.83–6.88 (m, 3H), 7.33–7.37 (m, 3H), 7.52 (s, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 2.0 Hz, 1H), 8.58 (s, 1H), 8.85 (s, 1H), 10.94 (s, 1H), 13.60 (s, 1H). MS (ESI) m/z 432 [M]$^+$. Anal. ($C_{23}H_{20}N_4O_5\cdot0.4H_2O$) C, H, N.

5.6.24. (*Z*)-1-[2-Oxo-3-(1*H*-pyrrol-2-ylmethylene)-2,3-dihydro-1*H*-indol-6-yl]-3-phenyl-urea (36)

Yield 86%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.31–6.32 (m, 1H), 6.74–6.76 (m, 1H), 6.86 (dd, J = 1.8, 8.0 Hz, 1H), 6.96 (dd, J = 7.2, 7.2 Hz, 1H), 7.25 (d, J = 7.8 Hz, 2H), 7.29–7.31 (m, 1H), 7.35 (d, J = 1.6 Hz, 1H), 7.45 (d, J = 7.6 Hz, 2H), 7.50 (d, J = 9.8 Hz, 1H), 7.56 (s, 1H), 8.65 (s, 1H), 8.77 (s, 1H), 10.86 (s, 1H), 13.21 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 100.5, 110.1, 111.6, 111.7, 115.1, 117.8, 118.7, 119.4, 119.8, 122.4, 124.8, 125.4, 129.3, 130.2, 139.6, 140.1, 140.2, 152.9, 170.2 ppm. MS (ESI) m/z 344 [M]$^+$. Anal. ($C_{20}H_{16}N_4O_2\cdot0.1H_2O$) C, H, N.

5.6.25. (*Z*)-1-(4-Methoxyphenyl)-3-[2-oxo-3-(1*H*-pyrrol-2-ylmethylene)-2,3-dihydro-1*H*-indol-6-yl]urea (37)

Yield 75%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.70 (s, 3H), 6.30–6.32 (m, 1H), 6.74–6.88 (m, 4H), 7.29–7.37 (m, 4H), 7.48 (d, J = 9.2 Hz, 1H), 7.55 (s, 1H), 8.46 (s, 1H), 8.70 (s, 1H), 10.85 (s, 1H), 13.20 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 56.0, 100.7, 110.1, 111.8, 112.0, 114.9, 118.2, 119.5, 119.9, 120.1, 120.9, 125.0, 125.7, 130.5, 133.5, 140.2, 140.6, 153.4, 155.4, 170.6 ppm. MS (ESI) m/z 374 [M]$^+$. Anal. ($C_{21}H_{18}N_4O_3\cdot0.2H_2O$) C, H, N.

5.6.26. (*Z*)-1-(3,4-Dimethoxyphenyl)-3-[2-oxo-3-(1*H*-pyrrol-2-ylmethylene)-2,3-dihydro-1*H*-indol-6-yl]urea (38)

Yield 97%; mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.70 (s, 3H), 3.73 (s, 3H), 6.30–6.32 (m, 1H), 6.74–6.75 (m, 1H), 6.83–6.85 (m, 3H), 7.22 (d, J = 2.0 Hz, 1H), 7.29 (s, 1H), 7.35 (d, J = 2.0 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.55 (s, 1H), 8.46 (s, 1H), 8.66 (s, 1H), 10.80 (s, 1H), 13.19 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 55.9, 56.4, 100.5, 104.5, 110.7, 111.6, 111.7, 113.1, 117.8, 119.3, 119.6, 119.7, 124.7, 125.3, 130.2, 133.8, 139.8, 140.2, 144.6, 149.3, 153.0, 170.2 ppm. MS (ESI) m/z 404 [M]$^+$. Anal. ($C_{22}H_{20}N_4O_4\cdot0.2H_2O$) C, H, N.

5.6.27. (*Z*)-1-[2-Oxo-3-(1*H*-pyrrol-2-ylmethylene)-2,3-dihydro-1*H*-indol-6-yl]-3-(*p*-tolyl)-urea (39)

Yield 87%; mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.21 (s, 3H), 6.27–6.30 (m, 1H), 6.72–6.73 (m, 1H), 6.83 (dd, J = 2.2, 8.0 Hz, 1H), 7.05 (d, J = 8.0 Hz, 2H), 7.26 (s, 1H), 7.30 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 7.52 (s, 1H), 8.49 (s, 1H), 8.68 (s, 1H), 10.79 (s, 1H), 13.17 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 20.9, 100.5, 111.6, 111.7, 117.8, 118.9, 119.3, 119.6, 119.7, 124.7, 125.3, 129.7, 130.2, 131.2, 137.6, 139.7, 140.2, 152.9, 170.2 ppm. MS (ESI) m/z 358 [M]$^+$. Anal. ($C_{21}H_{18}N_4O_2\cdot0.25H_2O$) C, H, N.

5.6.28. (*Z*)-1-(4-Chlorophenyl)-3-[2-oxo-3-(1*H*-pyrrol-2-ylmethylene)-2,3-dihydro-1*H*-indol-6-yl]urea (40)

Yield 85%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.29–6.34 (m, 1H), 6.74–6.76 (m, 1H), 6.87 (dd, J = 2.0, 8.0 Hz, 1H), 7.29–7.34 (m, 4H), 7.48 (d, J = 8.9 Hz, 2H), 7.5 (d, J = 8.2 Hz, 1H), 7.56 (s, 1H), 8.80 (s, 1H), 8.81 (s, 1H), 10.86 (s, 1H), 13.20 (s, 1H). MS (ESI) m/z 378 $[M]^+$. Anal. ($C_{20}H_{15}N_4O_2Cl{\cdot}0.1H_2O$) C, H, N.

5.6.29. (*Z*)-1-[2-Oxo-3-(1*H*-pyrrol-2-ylmethylene)-2,3-dihydro-1*H*-indol-6-yl]-3-(4-phenoxyphenyl)urea (41)

Yield 92%; mp 293–295 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.30–6.34, (m, 1H), 6.74–6.76 (m, 1H), 6.86 (d, J = 8.3 Hz, 1H), 6.92–6.99 (m, 4H), 7.07 (t, J = 7.4 Hz, 1H), 7.29–7.51 (m, 7H), 7.55 (s, 1H), 8.66 (s, 1H), 8.75 (s, 1H), 10.84 (s, 1H), 13.20 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 100.5, 111.6, 111.7, 117.8, 118.2, 119.4, 119.6, 119.8, 120.3, 120.5, 123.3, 124.8, 125.3, 130.2, 130.4, 136.2, 139.6, 140.2, 151.2, 153.0, 158.2, 170.2 ppm. MS (ESI) m/z 436 $[M]^+$. Anal. ($C_{26}H_{20}N_4O_3{\cdot}0.1H_2O$) C, H, N.

5.6.30. (*Z*)-1-Biphenyl-4-yl-3-[2-oxo-3-(1*H*-pyrrol-2-ylmethylene)-2,3-dihydro-1*H*-indol-6-yl]urea (42)

Yield 97%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.30–6.34 (m, 1H), 6.75–6.77 (m, 1H), 6.88 (dd, J = 1.8, 8.3 Hz, 1H), 7.27–7.36 (m, 3H), 7.41 (d, J = 7.4 Hz, 2H), 7.47–7.65 (m, 9H), 8.77 (s, 1H), 8.82 (s, 1H), 10.87 (s, 1H), 13.21 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 100.6, 111.6, 111.7, 117.8, 119.1, 119.5, 119.6, 119.8, 124.8, 125.4, 126.6, 127.3, 127.5, 129.4, 130.2, 134.1, 139.6, 139.7, 140.2, 140.4, 152.9, 170.2 ppm. MS (ESI) m/z 420 $[M]^+$. Anal. ($C_{26}H_{20}N_4O_2{\cdot}0.2H_2O$) C, H, N.

5.6.31. (*Z*)-1-Naphthalen-2-yl-3-[2-oxo-3-(1*H*-pyrrol-2-ylmethylene)-2,3-dihydro-1*H*-indol-6-yl]urea (43)

Yield 88%; mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.31–6.33 (m, 1H), 6.76–6.77 (m, 1H), 6.91 (dd, J = 1.7, 8.0 Hz, 1H), 7.29 (m, 1H), 7.35 (dd, J = 8.0, 8.0 Hz, 1H), 7.40 (dd, J = 2, 8 Hz, 1H), 7.44 (dd, J = 8.0, 8.0 Hz, 1H), 7.48 (dd, J = 2.0, 8.0 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.57 (s, 1H), 7.79 (d, J = 8.0 Hz, 1H), 7.83 (d, J = 8.0 Hz, 2H), 8.10 (d, J = 2.4 Hz, 1H), 8.85 (s, 1H), 8.86 (s, 1H), 10.86 (s, 1H), 13.20 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 100.6, 111.6, 111.7, 114.0, 117.8, 119.5, 119.6, 119.8, 120.2, 124.5, 124.9, 125.4, 126.9, 127.5, 128.0, 129.0, 129.6, 130.2, 134.3, 137.8, 139.5, 140.2, 153.0, 170.2 ppm. MS (ESI) m/z 394 $[M]^+$. Anal. ($C_{24}H_{18}N_4O_2$) C, H, N.

5.6.32. (*Z*)-2,4-Dimethyl-5-[2-oxo-6-(3-phenylureido)-1,2-dihydroindol-3-ylidene-methyl]-1*H*-pyrrole-3-carboxylic acid (44)

Yield 84%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.52 (s, 6H), 6.92–6.96 (m, 2H), 7.23–7.28 (m, 2H), 7.36 (s, 1H), 7.47–7.50 (m, 3H), 7.65 (d, J = 8.3 Hz, 1H), 9.07 (s, 1H), 9.24 (s, 1H), 10.87 (s, 1H), 13.64 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 12.3, 15.3, 100.8, 111.9, 117.2, 119.0, 119.9, 120.2, 122.1, 122.6, 126.8, 129.6, 131.8, 140.0, 140.1, 140.2, 140.8, 153.4, 156.0, 163.7, 170.9 ppm. MS (ESI) m/z 416 $[M]^+$. Anal. ($C_{23}H_{20}N_4O_4{\cdot}0.7H_2O$) C, H, N.

5.6.33. (*Z*)-5-{6-[3-(4-Methoxyphenyl)-ureido]-2-oxo-1,2-dihydroindol-3-ylidene-methyl}-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (45)

Yield 85%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.67 (s, 6H), 3.71 (s, 3H), 6.84–6.88 (m, 3H), 7.34–7.37 (m, 3H), 7.50 (s, 1H), 7.75 (d, J = 8.0 Hz, 1H), 8.59 (s, 1H), 8.84 (s, 1H), 10.90 (s, 1H), 11.50–12.40 (br, 1H), 13.67 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 12.3, 15.3, 56.0, 100.7, 111.8, 114.8, 117.5, 119.3, 119.7, 120.3, 120.9, 122.0, 126.9, 131.8, 133.6, 140.1, 140.2, 140.3, 153.5, 155.3, 170.9, 177.8 ppm. MS (ESI) m/z 446 $[M]^+$. Anal. ($C_{24}H_{22}N_4O_5$) C, H, N.

5.6.34. (*Z*)-2,4-Dimethyl-5-[2-oxo-6-(3-*p*-tolylureido)-1,2-dihydroindol-3-ylidene-methyl]-1*H*-pyrrole-3-carboxylic acid (46)

Yield 69%; mp >300 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.22 (s, 3H), 2.41 (s, 3H), 2.49 (s, 3H), 6.85 (d, J = 8.0 Hz, 1H), 7.04 (d, J = 8.0 Hz, 2H), 7.26–7.28 (m, 3H), 7.45 (s, 1H), 7.60 (d, J = 7.0 Hz, 1H), 8.56 (s, 1H), 8.76 (s, 1H), 10.89 (s, 1H), 13.59 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 12.3, 15.3, 21.2, 110.0, 112.2, 114.8, 117.4, 119.3, 119.9, 122.2, 126.9, 130.1, 131.8, 132.0, 137.8, 139.9, 140.1, 140.6, 153.4, 161.6, 167.2, 170.3 ppm. MS (ESI) m/z 430 $[M]^+$. Anal. ($C_{24}H_{22}N_4O_4{\cdot}1.2H_2O$) C, H, N.

5.6.35. (*Z*)-5-{6-[3-(4-Chlorophenyl)-ureido]-2-oxo-1,2-dihydroindol-3-ylidene-methyl}-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (47)

Yield 60%; mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.23 (s, 3H), 2.50 (s, 3H), 6.91 (dd, J = 2.0, 8.0 Hz, 1H), 7.27 (d, J = 8.0 Hz, 2H), 7.33 (s, 1H), 7.46 (s, 1H), 7.50 (d, J = 8.0 Hz, 2H), 7.61 (d, J = 8.0 Hz, 1H), 9.47 (s, 1H), 9.51 (s, 1H), 10.83 (s, 1H), 13.59 (s, 1H). MS (ESI) m/z 450 $[M]^+$. Anal. ($C_{23}H_{19}N_4O_4Cl{\cdot}0.7H_2O$) C, H, N.

5.6.36. (*Z*)-{2,4-Dimethyl-5-[2-oxo-6-(3-phenylureido)-1,2-dihydroindol-3-ylidene-methyl]-1*H*-pyrrol-3-yl} acetic acid (48)

Yield 85%; mp 226–228 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.23 (s, 3H), 2.28 (s, 3H), 3.22 (s, 2H), 6.82–6.86 (m, 1H), 6.90–6.94 (m, 1H), 7.22–7.27 (m, 2H), 7.33 (d, J = 2.0 Hz, 1H), 7.37 (s, 1H), 7.44–7.48 (m, 2H), 7.52 (d, J = 8.0 Hz, 1H), 9.35 (s, 1H), 9.37 (s, 1H), 10.64 (s, 1H), 13.19 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 10.7, 13.1, 25.0, 110.0, 113.3, 119.0, 119.1, 120.5, 122.4, 125.5, 126.4, 129.5, 130.1, 134.3, 139.2, 139.3, 140.7, 141.2, 144.9, 153.7, 170.7, 176.0 ppm. MS (ESI) m/z 430 $[M]^+$. Anal. ($C_{24}H_{22}N_4O_4{\cdot}0.5H_2O$) C, H, N.

5.6.37. (*Z*)-(5-{6-[3-(4-Methoxyphenyl)-ureido]-2-oxo-1,2-dihydroindol-3-ylidene-methyl}-2,4-dimethyl-1*H*-pyrrol-3-yl) acetic acid (49)

Yield 50%; mp 222–224 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.22 (s, 3H), 2.27 (s, 3H), 3.30 (s, 2H), 3.69 (s, 3H), 6.82 (s, 1H), 6.88 (d, J = 8.0 Hz, 2H), 7.32–7.39 (m, 4H), 7.55 (d, J = 8.0 Hz, 1H), 9.08 (s, 1H), 9.28 (s, 1H), 10.73 (s, 1H), 13.25 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 10.5, 12.9, 31.9, 56.0, 100.5, 111.6, 114.0, 114.8, 118.4, 119.3, 120.3, 120.6, 122.3, 126.5, 129.8, 134.0, 134.1, 139.5, 139.6, 153.7, 155.1, 170.7, 174.7 ppm. MS (ESI) m/z 460 $[M]^+$. Anal. ($C_{25}H_{24}N_4O_5{\cdot}1.8H_2O$) C, H, N.

5.6.38. (*Z*)-3-(5-{6-[3-(4-Methoxyphenyl)-ureido]-2-oxo-1,2-dihydroindol-3-ylidene-methyl}-2,4-dimethyl-1*H*-pyrrol-3-yl) propionic acid (50)

Yield 42%; mp 270–272 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.22 (s, 3H), 2.27 (s, 3H), 2.32 (t, J = 8.0 Hz, 2H), 2.63 (t, J = 8.0 Hz, 2H), 3.71 (s, 3H), 6.83–6.86 (m, 3H), 7.28 (d, J = 2.0 Hz, 1H), 7.35 (d, J = 8.0 Hz, 2H), 7.39 (s, 1H), 7.5 (d, J = 8.0 Hz, 1H), 8.50 (s, 1H), 8.70 (s, 1H), 10.67 (s, 1H), 13.19 (s, 1H). MS (ESI) m/z 474 $[M]^+$. Anal. ($C_{26}H_{16}N_4O_5{\cdot}0.4H_2O$) C, H, N.

5.6.39. (*Z*)-4-Methyl-5-[2-oxo-6-(3-phenylureido)-1,2-dihydro-indol-3-ylidenemethyl]-1*H*-pyrrole-2-carboxylic acid (51)

Yield 55%; mp 218–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.30 (s, 3H), 6.38–6.43 (m, 1H), 6.93–7.09 (m, 2H), 7.26 (s, 2H), 7.41 (s, 2H), 7.50–7.61 (m, 3H), 9.82 (s, 1H, NH), 9.93 (s, 1H), 10.75 (s, 1H), 13.18 (s, 1H). MS (ESI) m/z 402 $[M]^+$. Anal. ($C_{22}H_{18}N_4O_4{\cdot}0.9H_2O$) C, H, N.

5.6.40. (*Z*)-5-{6[3-(4-Chlorophenyl)-ureido]-2-oxo-1,2-dihydro-indol-3-ylidene-methyl}-4-methyl-1*H*-pyrrole-2-carboxylic acid (52)

Yield 78%; mp 202–204 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.30 (s, 3H), 6.44 (s, 1H), 7.07 (d, J = 8.0 Hz, 1H), 7.30 (d, J = 8.0 Hz, 2H), 7.33 (s, 1H), 7.42 (s, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.62 (s, 1H), 10.02 (s, 1H), 10.08 (s, 1H), 10.74 (s, 1H), 13.17 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): 12.4, 100.9, 110.0, 112.1, 114.7, 117.8, 119.8, 120.0, 120.4, 120.5, 122.1, 125.7, 128.0, 129.4, 135.2, 140.4, 140.5, 153.8, 166.2, 170.5 ppm. MS (ESI) *m/z* 436 $[M]^+$. Anal. ($C_{22}H_{17}N_4O_4Cl\cdot0.9H_2O$) C, H, N.

5.6.41. (*Z*)-5-{6-[3-(4-Methoxyphenyl)-ureido]-2-oxo-1,2-dihydroindol-3-ylidene-methyl}-1*H*-pyrrole-2-carboxylic acid (53)

Yield 62%; mp 244–246 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.71 (s, 3H), 6.60–6.61 (m, 1H), 6.67–6.68 (m, 1H), 6.86 (d, J = 10.0 Hz, 2H), 6.99 (dd, J = 2.0, 8.0 Hz, 1H), 7.39–7.51 (m, 5H), 9.51 (s, 1H), 9.72 (s, 1H), 10.81 (s, 1H), 13.19 (s, 1H). MS (ESI) *m/z* 418 $[M]^+$. Anal. ($C_{22}H_{18}N_4O_5\cdot0.9H_2O$) C, H, N.

5.6.42. (*Z*)-5-{6-[3-(4-Methoxyphenyl)-urido]-2-oxo-1,2-dihydro-indol-3-ylidene-methyl}thiophen-2-carboxylic acid (54)

Yield 98%; mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.71 (s, 3H), 6.84 (d, J = 2.0 Hz, 1H), 6.87 (d, J = 8.0 Hz, 2H), 7.36 (d, J = 12.0 Hz, 2H), 7.47 (s, 1H), 7.53 (s, 1H), 7.72 (d, J = 4.0 Hz, 1H), 7.78 (d, J = 4.0 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 8.56 (s, 1H), 8.97 (s, 1H), 10.63 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 55.7, 100.0, 110.9, 113.6, 114.3, 115.1, 120.3, 124.0, 124.3, 124.9, 129.3, 134.6, 137.0, 140.2, 144.1, 144.4, 153.9, 154.5, 166.5, 170.7 ppm. MS (ESI) *m/z* 435 $[M]^+$. Anal. ($C_{22}H_{17}N_3O_5S$) C, H, N, S.

5.7. In vitro kinase assays

The IC_{50} values for the inhibition of kinases VEGFR3, FLT3, KDR, and c-kit by compounds were determined in a time-resolved fluorescence format (Cell Signaling Technology, USA), measuring the inhibition of phosphate transfer to a substrate by the respective enzyme. VEGFR3, FLT3, KDR, and c-KIT were assayed in 60 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid), pH 7.5, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 3 μM Na_3VO_4, 1.25 mM dithiothreitol, 1.5 μM biotinylated peptide substrate (MYDKEYYS, EGPWLEEEEEAYGWMDF, ANASPEC, biotinylated gastrin (1–17)) and 20 μM ATP. Reactions were incubated at room temperature for 30 min, and the phosphorylated peptide captured on streptavidin-coated microtiter plates (PerkinElmer Life Sciences, USA) containing reaction stop buffer (50 mM EDTA, pH 8). Phosphorylated peptide was measured with the ULTRA Evolution Multi-Detection Microplate Reader from Tecan (Tecan Group Ltd, Switzerland) using phosphotyrosine mouse monoclonal antibody (P-Try-100) and europium-labeled anti-mouse IgG. The IC_{50} value of compound was calculated using nonlinear regression with SigmaPlot 10.0.

5.8. Cell lines

Human MV4-11 (FLT3-ITD), MOLM-13 (FLT3-ITD), RS4-11 (FLT3-WT), THP-1 (FLT3-WT) leukemic cells and HepG2, COLO205, and H460 solid tumor cells were obtained from American Tissue Culture Collection. The cells were grown in RPMI 1640 containing 10% fetal bovine serum, 1 mmol/L sodium pyruvate, and 10 mmol/L HEPES (pH 7.4). All the cells were grown and maintained in a humidified atmosphere at 37 °C and 5% CO_2. Kasumi-1 (BCRC-60505) and human umbilical vein endothelial cells (H-UV001) were obtained from the Bioresource Collection and Research Center (Taiwan) and cultured in RPMI 1640 and Medium 199 containing 10% fetal bovine serum. Unless otherwise indicated, cell culture reagents were obtained from GIBCO™/Invitrogen Life Technologies (USA). All the cells were grown and maintained in a humidified atmosphere at 37 °C and 5% CO_2.

5.9. Cell proliferation assays

Each cell line was plated in 96-well microtiter plates (10,000 cells per well), and serial dilutions of indicated compounds were added. At the end of the incubation period (72 h at 37 °C), cell viability was determined by a tetrazolium dye, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Promega, USA). The formazan crystals were dissolved in DMSO, and the absorbance at 600 nm was recorded using an ELISA plate reader. IC_{50} values were calculated using nonlinear regression and defined as the concentration needed for a 50% reduction in absorbance of treated versus untreated control cells.

5.10. Western blot analysis

Cells were plated at 2–5 × 10^5 cells per well in six-well plates (Orange Scientific, Belgium). Cells were then cultured for 24 h, and then incubated in serum-free medium overnight before preparation of cell lysates. Cells were treated with compounds in serum-free medium for 45 min, followed by addition of ligands, stem cell factor (50 ng/ml), VEGF (VEGF-162, 50 ng/mL), FLT-3 ligand (40 ng/mL), which were obtained from R&D Systems (USA) for 5–15 min. Lysates were prepared by the addition of cell lysis buffer (Cell Signaling Technology, USA); the lysates were clarified by centrifugation (14,000*g*, 10 min, 4 °C). Lysate protein content was quantified using the Pierce protein assay kit (USA) according to the manufacturer's instructions with bovine serum albumin as the reference standard. Proteins were separated by electrophoresis on SDS-polyacrylamide gels, electroblotted onto NC membranes (Hybond-C Extra, Amersham Biosciences, USA), and then probed using primary anti-phosphotyrosine antibody (PY99, Santa Cruz Biotechnology, USA). Western blots were developed using enhanced chemiluminescence with horseradish peroxidase–labeled secondary antibodies and the SuperSignal® HRP substrate (Pierce, USA). The membranes were stripped (Western Blot Stripping Buffer, Pierce, USA), washed, and reprobed with anti-c-KIT, anti-KDR (Santa Cruz Biotechnology), and anti-FLT3 (R&D Systems) and developed as described above. The IC_{50} value of each compound was calculated using nonlinear regression with GraphPad Prism v5.0.

5.11. In vivo xenograft tumor model

The procedure for establishing tumor xenografts and the dosing of **36** were carried out in accordance with Industrial Technology Research institutional animal care and use committee. Female nude mice (BALB/cAnN.Cg-Foxn1nu/CrlNarl, 6–8 weeks old) were purchased from the National Laboratory Animal Center (Taiwan). Female BALB/c nude mice were implanted subcutaneously in the right flank with 1 × 10^7 MV4-11 cells (human leukemia FLT3-ITD) per mouse. Treatments were initiated when tumor sizes were 200–300 mm^3. Mice were randomly assigned into cohorts (four mice per group for efficacy studies). Compound **36** at 20 mg/kg, once-daily (qd), and vehicle (10% NMP + 20% Cremophor EL + 70% saline) were given via oral gavage (po.) for seven days. Tumor volumes were assessed every day, and body weights were assessed twice weekly. Caliper measurements of tumors were converted into mean tumor volume using the formula: 0.5 × [length × $(width)^2$].

5.12. In vivo pharmacokinetics

The pharmacokinetic profile was investigated in overnight fasted male Sprague-Dawley rats following administration of a single intravenous or oral dose of **36**. Compound **36** was administered as a solution in 20% *N*-methyl-2-pyrrolidone, 10% cremophor EL, in 0.9% normal saline. At 0.083, 0.25, 0.5, 1, 2, 4, 6, and 8 h post-dose, blood was collected and processed to plasma by centrifugation and stored at −80 °C until analysis. Samples were analyzed by liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) technique. Pharmacokinetics were analyzed by WinNonlin 5.2 (Pharsight, CA) via noncompartmental analysis.

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