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Original article

Novel acylureidoindolin-2-one derivatives as dual Aurora B/FLT3 inhibitors for the treatment of acute myeloid leukemia

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ABSTRACT

A series of 6-acylureido derivatives containing a 3-(pyrrol-2-ylmethylidene)indolin-2-one scaffold were synthesized as potential dual Aurora B/FLT3 inhibitors by replacing the 6-arylureido moiety in 6-arylureidoindolin-2-one-based multi-kinase inhibitors. (*Z*)-*N*-(2-(pyrrolidin-1-yl)ethyl)-5-((6-(3-(2-fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxamide (**54**) was identified as a dual Aurora B/FLT3 inhibitor (IC_{50} = 0.4 nM and 0.5 nM, respectively). Compound **54** also exhibited potent cytotoxicity with single-digit nanomolar IC_{50} values against the FLT3 mutant-associated human acute myeloid leukemia (AML) cell lines MV4-11 (FLT3-ITD) and MOLM-13 (FLT3-ITD). Compound **54** also specifically induced extrinsic apoptosis by inhibiting the phosphorylation of the Aurora B and FLT3 pathways in MOLM-13 cells. Compound **54** had a moderate pharmacokinetic profile. The mesylate salt of **54** efficiently inhibited tumor growth and reduced the mortality of BALB/c nude mice (subcutaneous xenograft model) that had been implanted with AML MOLM-13 cells. Compound **54** is more potent than sunitinib not only against FLT3-WT AML cells but also active against sunitinib-resistant FLT3-ITD AML cells. This study demonstrates the significance of dual Aurora B/FLT3 inhibitors for the development of potential agents to treat AML.

1. Introduction

Acute myeloid leukemia (AML) is a leading cause of cancer mortality worldwide and the most common form of leukemia in children and adolescents. It is characterized by rapid proliferation of abnormal white blood cells that accumulate in the bone marrow, thereby disrupting normal hematopoiesis [1]. Faline McDonough Sarcoma like tyrosine kinase 3 (FLT3), a member of the class III receptor tyrosine kinase (RTK) family, plays a pivotal role in the proliferation, differentiation, and survival of hematopoietic cells

Abbreviations: AML, acute myeloid leukemia; FLT3, faline McDonough sarcoma like tyrosine kinase 3; RTK, receptor tyrosine kinase; ITD, internal tandem duplication; ATP, adenine triphosphate; PDGFR, platelet-derived growth factor receptor; WT, wild type; PML, human promyelocytic leukemia; PAMPA, parallel artificial membrane permeability; HH3, histone H3; STAT5, signal transducer and activator of transcription 5; hERG, human ether-à-go-go-related gene; CYP, cytochrome P450; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PBS, phosphate buffered saline; SDS, sodium dodecyl sulfate; ABL1, Abelson murine leukemia viral oncogene homolog 1; c-Met, MNNG HOS-transforming gene; CHK1, checkpoint kinase 1; EGFR, epithelial growth factor receptor; Her2, human epidermal growth factor receptor 2; FGFR1, fibroblast growth factor receptor 1; IGF-1R, insulin-like growth factor-1 receptor; JAK2, Janus-associated kinase 2; JNK2, c-jun *N*-terminal kinase 2; VEGFR-2, vascular epithelial growth factor receptor 2; Lck, lymphocyte-specific protein tyrosine kinase; MAP2K1, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; Plk1, polo-like kinase 1; ROCK1, rho-associated, coiled-coil containing protein kinase 1; Tie-2, angiopoietin receptor 2.

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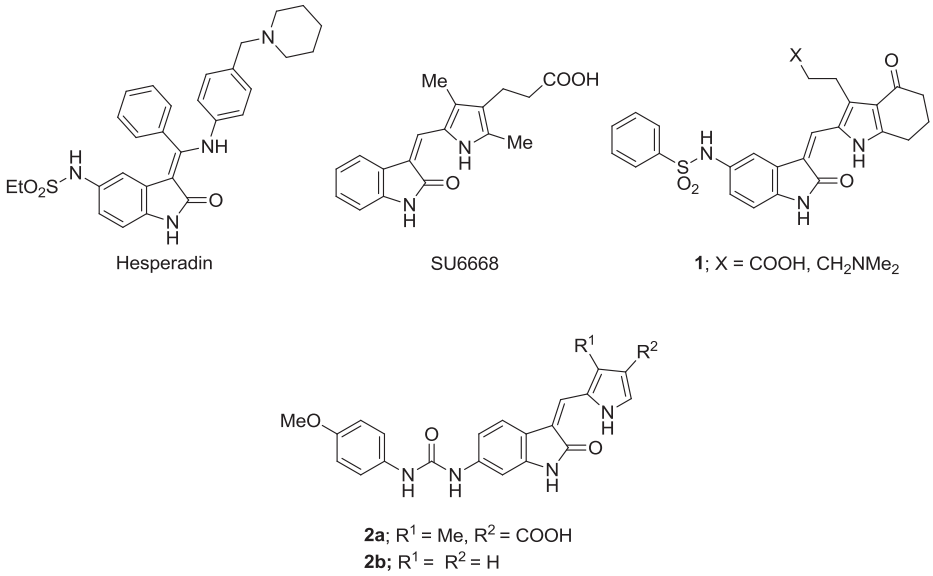


Fig. 1. Aurora kinase inhibitors containing an indolin-2-one scaffold.

[2]. Approximately 30% of AML cases result from one of two mutations in FLT3—an internal tandem duplication (ITD) of the juxtamembrane domain or a point mutation in the tyrosine kinase domain—and they lead to constitutive FLT3 activity and a poor prognosis [3–5]. Considering the importance of FLT3 in the pathophysiology of AML, much effort has been invested in the development of small-molecule inhibitors targeting its kinase domain and several FLT3 inhibitors, such as sunitinib [6], dovitinib [7], midostaurin [8], lestaurtinib [9], tandutinib [10], and quizartinib [11,12] are now under clinical investigation. However, the clinical efficacy of these FLT3 inhibitors has been limited owing to a transient response when used individually, which results in the emergence of acquired resistance [13].

Recent studies [14–17] have demonstrated that dual Aurora/FLT3 inhibitors have better single-agent efficacy than selective FLT3 inhibitors against FLT3 mutant-associated AML. Aurora kinases form a small family of three homologous serine/threonine kinases (Aurora A, Aurora B, and Aurora C) that play a crucial role in mammalian cell cycle regulation [18]. The three Aurora kinase isozymes have different subcellular locations and functions during mitosis [19]. Aurora A and Aurora B are overexpressed in a variety of solid tumors [20–24] and in hematological malignancies [25], highlighting their potential as pharmacological targets [26]. Indeed, several Aurora kinase inhibitors including the selective Aurora-A inhibitors MK-5108 [27] and alisertib [28], the selective Aurora B inhibitor barasertib (AZD1152) [29,30], and the pan-Aurora inhibitors AT9283 [31] and danusertib [32,33] are undergoing clinical trials. Recent studies [34] that assessed the antiproliferative and pro-apoptotic effects of Aurora B knockdown on AML cells demonstrated that Aurora B is functionally more significant for the survival of AML cells than is Aurora A. Moreover, in phase I–II clinical trials the Aurora B inhibitor AZD1152 showed a promising response rate of 25% in elderly AML patients (52–75 years of age) who had relapsed or shown resistance to standard treatment [35]; in comparison, the response rate was 10% for patients ≥65 years old who received standard treatment [36].

Derivatives of the indolin-2-one scaffold are a prototypical class of protein kinase inhibitors [37]. Among them, sunitinib [38] was the first multi-kinase inhibitor in clinical use for treatment of advanced renal cell carcinoma and gastrointestinal stromal tumors. Compounds containing indolin-2-one also inhibit Aurora kinases as exemplified by hesperadin [39], SU6668 [40], and 5-benzenesulfonamidoindolin-2-one derivative **1** [41] (Fig. 1). In addition, we reported 6-arylureido-3-(pyrrol-2-ylmethylidene) indolin-2-one derivatives as potent multi-RTK inhibitors [42]. Among them, compound **2a** (Fig. 1) is a potent multi-RTK inhibitor and an Aurora kinase inhibitor. Although **2a** is a potent multi-RTK inhibitor, it has a poor distribution volume and a short half-life *in vivo*, which limit efficacy.

Perusal of the literature reveals that introduction of acyl moieties adjacent to urea and guanidine moieties in certain compounds improves their bioactivity, selectivity, and pharmacokinetics [43–45]. Moreover, an acylureido moiety is present in certain anticancer compounds [46,47]. Given these observations, we designed and tested a series of indolin-2-one derivatives containing an acylureido moiety starting with **2b** (Fig. 2). To our knowledge, acylureido derivatives of indolin-2-one have not been reported. To date, only imidazo[4,5-*b*]pyridine-based dual Aurora/FLT3 inhibitors, which have a pan-Aurora kinase inhibitory profile, have been reported [14–17]. Herein, we report the design and synthesis of acylureidoindolin-2-one derivatives and evaluate their potential as dual Aurora B/FLT3 inhibitors *in vitro* and *in vivo*.

2. Results and discussion

2.1. Chemistry

Reaction of benzamides **3a**–**k** with oxalyl chloride gave the corresponding acyl isocyanates **4a**–**k** (Scheme 1) [48], which were used without further purification. Pyrrole-3-carboxylic acids **5** and **6** were treated with methyl chloroformate to give the corresponding alkoxycarbonyl ester intermediates, which were transesterified with ethanol to afford ethyl (5-formyl-2,4-dimethyl-1*H*-pyrrole)-3-carboxylate (**7**) and ethyl 3-(5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)propanoate (**8**) (Scheme 1). The amide-functionalized pyrrole aldehydes **9**–**15** were obtained by amidation of **5** or **6** with an appropriate amine in the presence of peptide-coupling reagents.

A modified Gassman oxindole synthesis (Scheme 2) was used to convert *m*-nitroaniline into 3-(methylthio)-4-nitroindolin-2-one (**16**) [49,50]. Compound **16** was reduced to give the 4-amino derivative **17**, which was desulfurized with Raney nickel to give 4-

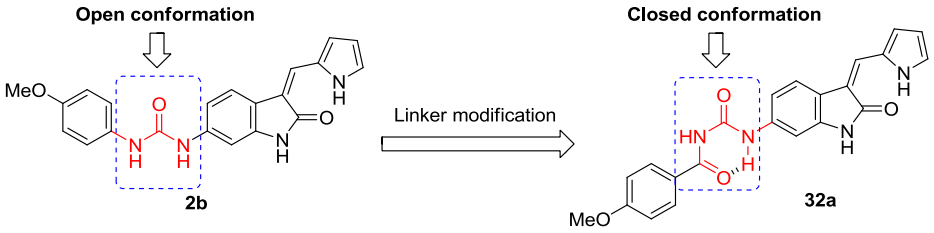
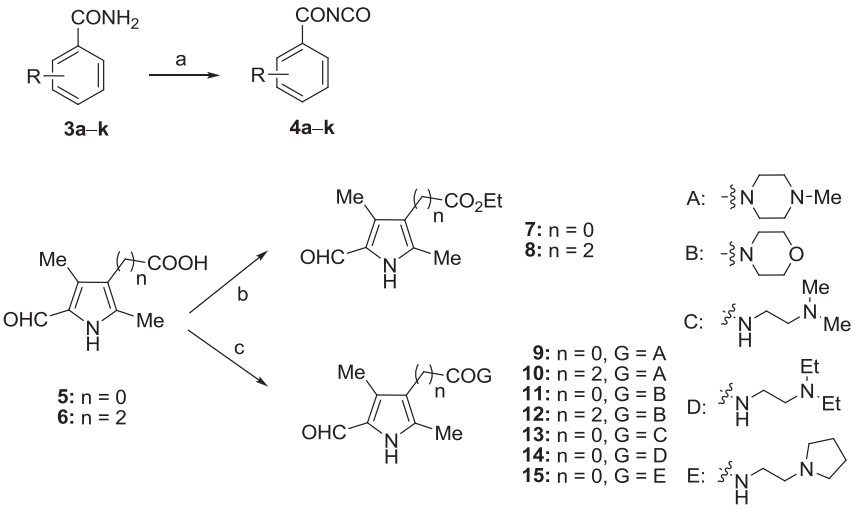


Fig. 2. Design of the benzoylureido derivatives of indoline-2-one based on **2b**.



Scheme 1. Synthesis of the benzoyl isocyanates **4a**–**k**, 3-carboxylates **7** and **8**, and 3-carboxamides **9**–**15** of 5-formyl-2,4-dimethylpyrrole. Reagents and conditions: (a) oxalyl chloride, CH_2Cl_2, 40 °C, 20–22 h; (b) i. methyl chloroformate, Et_3N, THF, rt, 8 h; ii. EtOH, reflux, 4 h; (c) amines, DMF, HOBt, Et_3N, EDCI, rt, 24 h.

aminoindolin-2-one (**18**). 5-Aminoindolin-2-one (**19**) was commercially available; 6-aminoindolin-2-one (**20**) was obtained by hydrogenation of 2,4-dinitrophenylacetic acid and subsequent cyclization under acidic conditions [51]; and 7-aminoindolin-2-one (**21**) was prepared by direct reduction of 7-nitroindolin-2-one. 6-Amino-5-fluoroindolin-2-one (**22**) was obtained by hydrogenation of methyl (5-fluoro-2,4-dinitrophenyl)acetate (**23**), which was prepared together with methyl (3-fluoro-2,6-dinitrophenyl)acetate (**24**) and methyl (5-methoxy-2,4-dinitrophenyl)acetate (**25**) by nitration and subsequent esterification of *m*-fluorophenyl acetic acid (Scheme 2) [52].

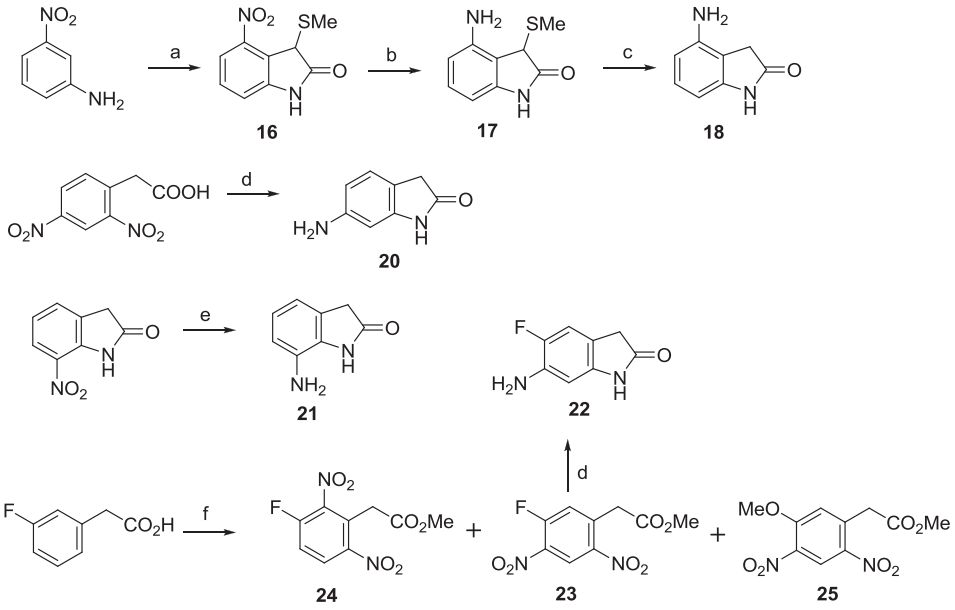
Reaction of the aminoindolin-2-ones **18**–**22** with various acyl isocyanates (**4**) afforded the corresponding acylureidoindolin-2-ones **26**–**29** (Scheme 3) [53], each of which underwent aldol condensation and subsequent dehydration with various pyrrole-2-carbaldehydes to afford the target compounds **30**–**54** (Scheme 3).

Synthesized compounds were purified by column chromatography and structures of the compounds were confirmed by FTIR, Mass spectra, ^{1}H NMR, ^{13}C NMR and elemental analysis. Several well-resolved IR bands associated with characteristic NH stretching (near 3290 cm^{-1}), carbonyl stretching (near 1690 cm^{-1}), CN stretching (near 1410 cm^{-1}), C=O out-of-plane deformation (near 840 cm^{-1}) and NH out of plane deformation (near 760 cm^{-1}) were observed for nearly all compounds. The 1H NMR spectra contained typical signals for H_4–H_7 protons of indolin-2-one in aromatic region depending on substitution pattern (d or dd). Also the two methylene protons at C3 position of indolin-2-one in intermediate compounds 26–29 were observed ~3.4 ppm. Similarly, peaks at 10–11 ppm, observable in the ^{1}H NMR spectra of the library compounds, could be attributed to the NH of acylurea and indolin-2-one. The pyrrole NH is involved in intramolecular hydrogen bond with C=O of indolin-2-one and thus in ^{1}H NMR peak for pyrrole NH was observed at ~13.0 ppm. The formation of adducts after aldol condensation were confirmed by observing sharp singlet for vinylic proton at ~7.4 ppm. In ^{13}C spectra, signals at ~150 or ~160 ppm and between ~110 and 140 ppm were observed for carbonyl and aromatic carbons respectively. Depending on the nature of the substituent on aromatic, other expected signals in both ^{1}H and ^{13}C NMR spectra were noted. For example peak for OMe was observed at 3.8 ppm and 55.5 ppm in ^{1}H NMR and ^{13}C NMR, respectively.

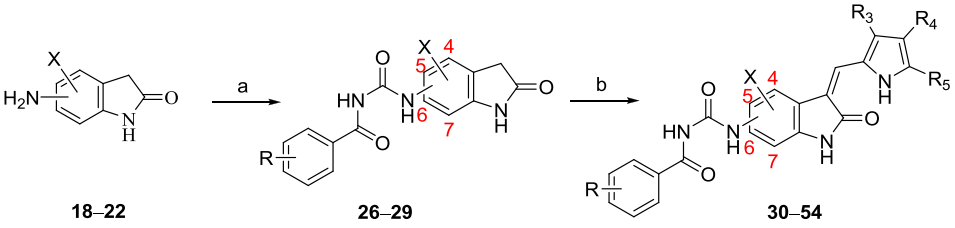
2.2. Biology

2.2.1. Structure–activity relationships

Modification of the (4-methoxyphenyl)ureido moiety in **2b** to yield the (4-methoxybenzoyl)ureido moiety in **32a** resulted in inhibitory activity against Aurora B in addition to FLT3. In an enzymatic assay using radiolabeled adenine triphosphate (ATP) and the six kinases Aurora A/B/C, platelet-derived growth factor receptor (PDGFR) α/β, and FLT3, 1 μM of **32a** inhibited 94% of the Aurora B activity and 60% of the FLT3 activity (Table S6, Supplementary data). It is generally accepted that an acylureido moiety adopts a closed pseudo-six-membered ring conformation owing to an intramolecular hydrogen bond (Fig. 2) [54–56]. In hydrogen/deuterium-exchange NMR studies [56] of **32a**, the aniline N(1)H was not exchanged on addition of D_2O in $CDCl_3$/DMSO-d_6 (9:1) while it was exchanged on addition of D_2O in DMSO-d_6 (Figure S1, Supplementary data), characterizing a relatively weak intramolecular hydrogen bond for the pseudo-six-membered ring conformation. This result may lend some supports to the fact of the kinase inhibitory profiles of acylureido derivatives are different from those of ureido-containing derivatives **2**. Thus, **32a** served as



Scheme 2. Synthesis of the Aminoindolin-2-ones **18** and **20**–**22**. Reagents and conditions: (a) i. $(MeS)CH_2CO_2Et$, SO_2Cl_2, DCM, −78 °C, 45 min; ii. Proton Sponge, 3 h; iii. Et_3N, 1 h; iv. AcOH, 52%; (b) $SnCl_2{\cdot}2H_2O$, AcOH, EtOH, 70 °C, 7 h, 96%; (c) Raney-Ni, EtOH, reflux, 4 h, 39%; (d) i. H_2, Pd/C, MeOH; ii. 1 *N* HCl, reflux, 6 h, 68% (**20**), 78% (**22**); (e) H_2, Pd/C, MeOH, 77%; (f) i. 90% HNO_3, H_2SO_4, 20–35 °C, 20 h; ii. MeOH, H^+, reflux, 7 h, 32% (**23**), 19% (**24**), 18% (**25**).



Scheme 3. Synthesis of the 4/5/6/7-(Benzoylureido)-3-(pyrrol-2-ylmethylidene)indolin-2-ones **30**–**54**. Reagents and conditions: (a) benzoyl isocyanates **4**, MeCN, 70–80 °C, 3 h, 42–89%; (b) substituted pyrrole-2-carbaldehydes, pyrrolidine, EtOH, microwave irradiation, 100 °C, 15 min, 34–91% (**34**–**54**).

the lead compound for optimization of dual Aurora B/FLT3 inhibitors. All synthesized compounds were also assessed for cellular activities using the human AML cell lines MV4-11 (FLT3-ITD), MOLM-13 (FLT3-ITD), and THP-1 (FLT3-wild type (WT)) and the human promyelocytic leukemia cell (PML) line HL-60 (FLT3-WT).

Initial evaluation utilized the 4/5/6/7-(4-methoxybenzoyl)ureido derivatives **30**, **31**, **32a**, and **33** (Table 1) and the 3-aryl/heteroarylmethylidene derivatives **S1**–**S11** (Table S7, Supplementary data). Compound **32a** with a 6-(4-methoxybenzoyl)ureido moiety and a 3-pyrrolylmethylidene substituent on indolin-2-one exhibited the best inhibitory activity with IC_{50} values of 14.9 nM and 333.9 nM against Aurora B and FLT3, respectively. Encouragingly, **32a** demonstrated cellular activities with IC_{50} values of 29.3 nM, 18.1 nM, 68.4 nM, and 29.5 nM against the leukemia cell lines MV4-11, MOLM-13, THP-1, and HL-60, respectively.

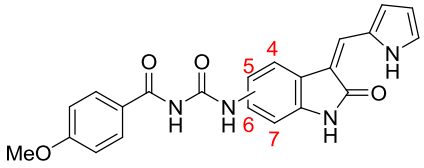
To verify the role of the 4-methoxy group on the benzoyl moiety, **32a**–**j** with optionally substituted benzoyl groups were investigated. Compounds bearing the 4-H (**32b**), 4-Cl (**32c**), 3,4-diCl (**32d**), or 2-F (**32e**) showed very potent Aurora B inhibitory activity with IC_{50} values between 5.3 and 7.7 nM, whereas they did not inhibit FLT3 (Table 2). Compound **32f** (4-CF_3, Aurora B, IC_{50} = 40.9 nM; FLT3, IC_{50} > 1 μM) was less active than **32a**, whereas **32g** (3-CF_3, 4-Cl; Aurora B, IC_{50} = 15.0 nM; FLT3, IC_{50} = 384.8 nM) had similar inhibitory activities against both kinases.

Again, **32h** (4-CH_3) and **32i** (3,5-diOCH_3) with electron-donating groups were potent Aurora B inhibitors (IC_{50} values of 18.4 nM and 29.8 nM, respectively) but weak FLT3 inhibitors (IC_{50} values >1 μM). Although **32j** (4-$N(CH_3)_2$) had improved FLT3 inhibitory potency (IC_{50} = 133.5 nM), suggesting that −σ effect was favorable, its Aurora B inhibitory activity (IC_{50} = 158.7 nM) decreased. In general, compounds with little FLT3 inhibitory activity, e.g., **32b**, **32e**, **32f**, **32h**, and **32i**, exhibited poor cytotoxicity against leukemia cell lines (Table 2). Further modifications, therefore, focused on the pyrrole ring of **32a**.

Compound **34** with the pyrrole ring bearing the 3′,5′-diCH_3 and 4′-CO_2H moieties had improved inhibitory activities against Aurora B (IC_{50} = 1.9 nM) and FLT3 (IC_{50} = 179.8 nM) relative to those of **32a** (Table 2), and it displayed improved cytotoxicity against MV4-11 (IC_{50} = 12.0 nM), MOLM-13 (IC_{50} = 9.0 nM), THP-1 (IC_{50} = 78.4 nM), and HL60 (IC_{50} = 15.8 nM) cells. Removal of the 4′-CO_2H moiety (**35**) resulted in loss of Aurora B inhibitory activity (IC_{50} = 55.5 nM) and FLT3 inhibitory activity (IC_{50} > 1 μM). Concurrent with its loss of enzyme inhibitory activity, **35** had somewhat poorer cytotoxicity (MV4-11, IC_{50} = 62.8 nM; MOLM-13, IC_{50} = 42.8 nM; THP-1, IC_{50} = 115.8 nM; HL60, IC_{50} = 45.8 nM). Similarly, **36** (4′-Ph) and **37** (5′-CO_2H) lost kinase inhibitory activity (IC_{50} > 1 μM) and cytotoxicity (IC_{50} > 500 nM), indicating that the 4′-CO_2H moiety is important for inhibition of Aurora B and FLT3.

The effect of elongation of the 4′-CO_2H side chain of **34** was assessed. Insertion of a methylene (**38**, IC_{50} = 1.8 nM) or an ethylene (**39**, IC_{50} = 1.9 nM) between the CO_2H and pyrrole ring provided comparable Aurora B inhibition. Notably, **38** and **39** showed three-

Table 1
Kinase inhibitory activities and cytotoxicities of the 4/5/6/7-(4-methoxybenzoyl)ureido-3-(pyrrol-2-ylmethylidene)indolin-2-ones **30**, **31**, **32a**, and **33**.



30,31, 32a & 33

Cpd	Position	Kinase inhibition IC_{50}[a] (nM)		Cytotoxicity IC_{50}[b] (nM)			
				AML			PML
		Aur B	FLT3	MV4-11	MOLM-13	THP-1	HL-60
30	4	(11.0%)[c]	58.2	357.2 ± 6.5	302.7 ± 4.2	355.8 ± 1.8	334.5 ± 1.0
31	5	(39.0%)[c]	201.1	256.3 ± 6.4	222.0 ± 2.7	373.2 ± 6.0	300.7 ± 3.4
32a	6	14.9	333.9	29.3 ± 3.2	18.1 ± 2.1	68.4 ± 4.9	29.5 ± 2.4
33	7	(7.0%)[c]	(7.0%)[c]	>500 (78%)	>500 (72%)	>500 (53%)	488
Staurosporine		1.8	<1.0				
AZD1152		0.3[d]		8.6 ± 0.3	9.7 ± 0.5	25.1 ± 0.1	12.7 ± 0.4
Sunitinib				24.3 ± 0.4	17.7 ± 0.3	45.7 ± 0.3	36.8 ± 0.3

[a] Mean value from two independent dose–response curves with a variation of <30%.
[b] Mean value ± S.D. ($n > 2$).
[c] Mean value of the percentage enzyme inhibition at 1.0 μM ($n = 2$).
[d] Value from Ref. [30].

and eight-fold improved FLT3 inhibitory activity (IC_{50} = 31.7 nM and 2.7 nM, respectively). Furthermore, **38** and **39** exhibited potent cytotoxicity against leukemia cells in agreement with their improved enzyme inhibitory activities (Table 2).

Fluoro substituents are known to substantially improve lipophilicity, bioavailability, and solubility [57–59]. To enhance the physicochemical properties of **34** and **39**, fluorinated acetanilide and benzamide moieties as described by Meanwell [58] were individually incorporated. Introduction of a fluorine atom at C5 of indoline-2-one in **34** and **39** yielded **40** and **41**, respectively. Compounds **40** and **41** retained Aurora B inhibitory activity (IC_{50}, 1.5 nM and 3.0 nM, respectively) but showed decreased FLT3 inhibitory activity ($IC_{50} \geq 1$ μM) (Table 3). In agreement with their decreased FLT3 inhibitory activities, **40** and **41** exhibited decreased potency against the tested leukemia cells. The effect of the benzanilide moiety was also investigated by introducing a fluorine atom at the 2-position of the benzoyl group of **34** and **39** to form **42** and **43**, respectively. Interestingly, **42** and **43** retained inhibitory activity against both Aurora B (IC_{50} = 2.3 nM and 1.3 nM, respectively) and FLT3 (IC_{50} = 111.1 nM and 10.0 nM, respectively). Both **42** and **43** showed potent cytotoxicity against the tested leukemia cells with IC_{50} values in the low nanomolar range (Table 3). Notably, the 2-fluoro-substituted benzoyl derivatives of **40** and **41**, namely **44** and **45**, regained FLT3 inhibitory activity (IC_{50} = 405.5 nM and 16.3 nM, respectively) while retaining Aurora B inhibitory activity (IC_{50} = 3.0 nM and 1.5 nM, respectively). Compounds **44** and **45** still had slightly decreased FLT3 inhibitory activity compared with their nonfluorinated parent compounds **34** and **39**, respectively, indicating that the 2-fluoro substituent on the 4-methoxybenzoyl moiety was needed for optimal activity against both kinases.

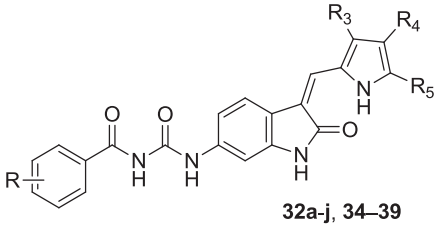
The effects of fluoro substitution on the permeability coefficients of **32a**, **34**, **40**, and **42** were evaluated by a standard parallel artificial membrane permeation assay (PAMPA) targeting passive gastrointestinal absorption at pH 7.4. Interestingly, the introduction of a fluorine atom at the C5 of the indolin-2-one to form **40** ($P_e = 2.46 \times 10^{-6}$ cm s^{-1}) or at the 2-position of the benzoyl moiety in **42** ($P_e = 2.49 \times 10^{-6}$ cm s^{-1}) resulted in greater permeability than that of **34** ($P_e = 0.23 \times 10^{-6}$ cm s^{-1}, Table 4). In sum, the 2-fluoro substituent on the 4-methoxybenzoyl moiety benefits solubility (Table S8, Supplementary data) and permeability while retaining kinase inhibitory activity and cytotoxicity.

Efficacy of compounds bearing carboxylic groups have been reported to be affected by serum proteins [60]. To avoid this drawback, the effectiveness of the carboxylic acid derivatives of **42** and **43** was assessed. Unexpectedly, ethyl esters **46** and **47** had decreased inhibitory activity against Aurora B and FLT3 (Table 3). Amide derivatives containing the cyclic *N*-methylpiperidino group (**48** and **49**) or morpholino group (**50** and **51**) exhibited decreased inhibitory activity against Aurora B and FLT3 (IC_{50} = 20–60 nM) relative to their respective parent analogs. Notably, **52** and **53**, amide derivatives with long chain amines, regained Aurora B and FLT3 inhibitory activities (Table 3), and **53** containing the *N*-(2-(*N*,*N*-diethylamino)ethyl) carboxamide was a better inhibitor than was **53** containing the *N*-(2-(*N*,*N*-dimethylamino)ethyl) carboxamide. Surprisingly, the *N*-(2-(pyrrolidin-1-yl)ethyl) carboxamide derivative **54**, the cyclic analog of **53**, potently inhibited Aurora B and FLT3 (IC_{50} = 0.4 nM and 0.5 nM, respectively) and was very cytotoxic (IC_{50} = 2.4 nM, 0.7 nM, 48.9 nM, and 2.8 nM against MV4-11, MOLM-13, THP-1, and HL60 cells, respectively). Compound **54** also had good solubility (Table S8, Supplementary data) and permeability (Table 4) in this series of compounds. Kinase profiling of **54** against 25 additional kinases (Table 5) showed that it was also a potent inhibitor of c-Kit (IC_{50} = 14.0 nM) and Lck (IC_{50} = 2.3 nM) and a fair inhibitor of ABL, Lyn, ROCK1, and PDGFRα/β (50–75% inhibition at 1 μM).

2.2.2. Cellular inhibition mechanism

To determine the cytotoxic mechanism of **54**, **54**-treated MOLM-13 cells were characterized for the phosphorylation states of Aurora B and FLT3, and their respective direct downstream substrates histone H3 (HH3) and the signal transducer and activator of transcription 5 (STAT5) using a high-throughput dot-blotting system. Compound **54** showed concentration-dependent reduction in the levels of the phosphorylated forms of HH3 and STAT5 in MOLM-13 cells (Fig. 3B and D), with the reduction being the consequence of the dual inhibition of Aurora B and FLT3 as evidenced by the

Table 2
Kinase inhibitory activities and cytotoxicities of the 6-acylureido-3-(pyrrol-2-yl)methylideneindolin-2-ones **32a–j** and **34–39**.



32a-j, 34–39

Cpd	R	R_3	R_4	R_5	Kinase inhibition IC_{50}[a] (nM)		Cytotoxicity, IC_{50}[b] (nM)			
							AML			PML
					Aur B	FLT3	MV4-11	MOLM-13	THP-1	HL-60
32a	4-OMe	H	H	H	14.9	333.9	29.3 ± 3.2	18.1 ± 2.1	68.4 ± 4.9	29.5 ± 2.4
32b	H	H	H	H	5.3	>1000.0	239.9 ± 5.9	238.3 ± 8.5	293.2 ± 5.3	249.9 ± 3.9
32c	4-Cl	H	H	H	6.5	675.8	19.3 ± 5.3	19.1 ± 2.3	81.2 ± 7.1	21.6 ± 3.8
32d	3,4-Cl	H	H	H	7.7	>1000.0	31.6 ± 1.1	29.0 ± 1.2	90.9 ± 1.7	35.0 ± 2.2
32e	2-F	H	H	H	6.5	>1000.0	233.5 ± 8.4	232.4 ± 4.4	331.3 ± 2.1	268.0 ± 7.5
32f	4-CF_3	H	H	H	40.9	>1000.0	190.7 ± 3.8	195.7 ± 2.4	326.3 ± 1.9	292.6 ± 2.8
32g	4-Cl,3-CF_3	H	H	H	15.0	384.8	14.1 ± 0.9	11.1 ± 0.6	68.8 ± 4.0	23.2 ± 2.3
32h	4-Me	H	H	H	18.4	>1000.0	284.9 ± 26.8	212.0 ± 4.2	355.7 ± 5.4	301.2 ± 10.1
32i	3,5-OMe	H	H	H	29.8	>1000.0	149.9 ± 2.8	113.7 ± 8.4	299.9 ± 2.3	250.9 ± 7.4
32j	4-NMe_2	H	H	H	158.7	133.5	261.4 ± 6.9	260.3 ± 7.6	262.2 ± 12.9	269.3 ± 31.0
34	4-OMe	Me	CO_2H	Me	1.9	179.8	12.0 ± 0.2	9.0 ± 1.2	78.4 ± 3.8	15.8 ± 1.1
35	4-OMe	Me	H	Me	55.5	>1000.0	62.8 ± 9.4	42.8 ± 0.7	115.8 ± 3.5	45.8 ± 7.6
36	4-OMe	Me	Ph	Me	>1000.0	>1000.0	>500 (51%)	>500 (51%)	>500 (73%)	>500 (65%)
37	4-OMe	H	H	CO_2H	>1000.0	>1000.0	>500 (62%)	>500 (55%)	>500 (65%)	>500 (65%)
38	4-OMe	Me	CH_2CO_2H	Me	1.8	31.7	4.2 ± 0.3	3.1 ± 0.2	69.0 ± 1.3	8.1 ± 0.3
39	4-OMe	Me	$(CH_2)_2CO_2H$	Me	1.2	2.7	5.3 ± 0.9	5.2 ± 0.7	52.8 ± 0.8	6.4 ± 1.4
Staurosporine					1.8	<1.0				
AZD1152					0.3[c]		8.6 ± 0.3	9.7 ± 0.6	25.1 ± 0.1	12.7 ± 0.4
Sunitinib							24.3 ± 0.4	17.7 ± 0.3	45.7 ± 0.3	36.8 ± 0.3

[a] Average value from two independent dose–response curves with variations of ≤30%.
[b] Mean value ± S.D. ($n > 2$).
[c] Value from Ref. [30].

concomitant reduction in phosphorylated (activated) Aurora B and FLT3 in MOLM-13 cells (Fig. 3A and C). Additionally, cell cycle arrest at the G2/M phase was observed in MOLM-13 cells treated with **54**, but not for MOLM-13 cells treated with the multi-RTK inhibitor sunitinib (Fig. 4B and C).

Recent studies have suggested that Aurora and/or FLT3 kinase-type inhibitors exert their antitumor effects by inducing apoptosis [61–63]. Thus, apoptotic induction by **54** was evaluated using fluorometric assays for caspase 3/8/9 activation in MOLM-13 and THP-1 cells. Compound **54** dramatically triggered the activation of caspases 3 and 8 in a concentration-dependent manner in both MOLM-13 and THP-1 cells (Fig. 5). The activation was greater in MOLM-13 cells than in THP-1. Moreover, caspase 8 activation was more pronounced for MOLM-13 and THP-1 cells, suggesting that a caspase 8-dependent apoptosis mechanism is involved. Similar results for caspase 8 activation were also observed for the human non-small-cell lung cancer cells A549 treated with **54** (Figure S3, Supplementary data). These results are in agreement with the observations that caspase 8 activity is increased by the Aurora B inhibitor reversine in oral squamous cell carcinoma cells or by the FLT3 inhibitor PKC412 in AML cells [64,65]. Because caspase 8 is a downstream effector of the extrinsic apoptosis pathway, which is triggered by the binding of Fas ligand (FasL) to Fas, the level of FasL was investigated by dot blotting. The FasL level in **54**-treated MOLM-13 cells increased (Fig. 3E) compared with the level in untreated cells, suggesting that **54** exerted its cytotoxicity by activating the extrinsic apoptosis pathway via inhibition of Aurora B and FLT3. Compound **54** was more effective against FLT3 mutant-containing MOLM-13 cell viability than for wild-type FLT3-containing THP-1 cells by inhibiting the Aurora B/FLT3-activated signals and activating the extrinsic apoptosis pathway, suggesting the novel Aurora B/FLT3 dual inhibitors are more effective against FLT3 mutant-containing AML cells, which are prone to develop resistance against singly selective kinase inhibitors [66].

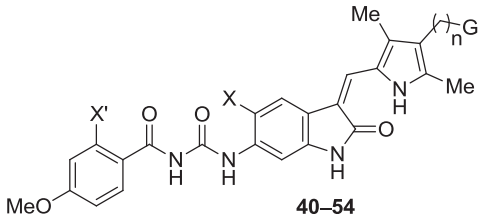
2.2.3. Safety and pharmacokinetics

In vitro safety assessments revealed that **54** had weak affinity for membrane-bound human Ether-à-go-go-Related Gene (hERG) (IC_{50} = 9.6 μM) and does not significantly inhibit the major cytochrome P450 (CYP) isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) at a concentration of 10 μM (Table 6). Moreover, 10 μM of **54** was found to be non-toxic to normal Vero cells, as were the other acylureido derivatives. An *in vivo* pharmacokinetic study of **54** was also performed (Table 7). Intravenous administration of **54** at 2 mg/kg in mice showed a large distribution volume (22.2 L/kg), a minimal clearance rate (0.56 L/h/kg), and a long half-life (27.6 h). However, **54** showed a poor oral pharmacokinetic profile including low bioavailability (2.6% at 10 mg/kg).

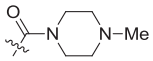
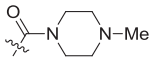
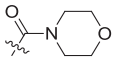
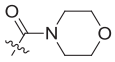
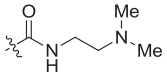
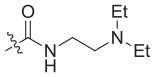
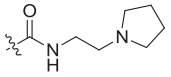
2.2.4. In vivo antitumor activity

Compound **54** was evaluated for its *in vivo* antitumor activity using a human AML (MOLM-13) subcutaneous xenograft model. The mesylate salt of **54**, administered intraperitoneally, is correlated with reduced tumor growth in a dose-dependent manner (Fig. 6A). A more pronounced reduction in tumor growth was

Table 3
Kinase inhibitory activities and cytotoxicities of the carboxylic acid derivatives of the fluorinated 5-(6-(4-methoxybenzoyl)ureido-2-oxoindolin-3-ylidene)methyl-2,4-dimethyl-1*H*-pyrroles **40**–**54**.



40–54

Cpd.	X	X′	*n*	G	Kinase inhibition IC_{50}[a] (nM)		Cytotoxicity, IC_{50}[b] (nM)			
							AML			PML
					Aur B	FLT3	MV4-11	MOLM-13	THP-1	HL-60
40	F	H	0	$-CO_2H$	1.5	908.9	34.6 ± 5.7	25.3 ± 2.2	81.2 ± 2.1	32.2 ± 5.6
41	F	H	2	$-CO_2H$	3.0	>1000.0	26.1 ± 3.3	30.1 ± 1.5	64.7 ± 1.1	28.8 ± 2.3
42	H	F	0	$-CO_2H$	2.3	111.1	5.3 ± 0.9	5.2 ± 0.7	79.5 ± 2.8	6.4 ± 1.4
43	H	F	2	$-CO_2H$	1.3	10.0	6.1 ± 0.8	4.0 ± 0.3	61.3 ± 1.4	16.7 ± 1.8
44	F	F	0	$-CO_2H$	3.0	405.5	35.9 ± 6.2	17.8 ± 0.8	76.3 ± 1.7	28.6 ± 2.6
45	F	F	2	$-CO_2H$	1.5	16.3	6.5 ± 0.6	2.3 ± 0.4	55.9 ± 2.4	8.9 ± 0.2
46	H	F	0	$-CO_2Et$	>1000.0	>1000.0	11.3 ± 1.1	9.8 ± 0.3	74.0 ± 2.4	13.0 ± 1.5
47	H	F	2	$-CO_2Et$	217.9	>1000.0	15.1 ± 1.1	10.9 ± 1.4	53.9 ± 1.2	29.9 ± 1.2
48	H	F	0		30.6	57.1	10.6 ± 0.4	6.9 ± 1.4	95.1 ± 3.2	15.7 ± 0.8
49	H	F	2		18.4	41.4	11.0 ± 0.6	9.2 ± 0.3	65.0 ± 2.1	15.7 ± 1.3
50	H	F	0		16.8	79.5	16.8 ± 0.4	10.8 ± 0.5	102.2 ± 1.2	24.0 ± 0.8
51	H	F	2		16.4	106.0	17.9 ± 0.8	16.1 ± 0.8	70.3 ± 2.0	31.2 ± 0.9
52	H	F	0		10.5	16.2	8.1 ± 0.3	5.3 ± 0.6	63.7 ± 0.8	14.1 ± 1.0
53	H	F	0		7.6	4.4	17.7 ± 1.2	12.9 ± 0.3	41.2 ± 1.9	20.3 ± 2.6
54	H	F	0		0.4	0.5	2.4 ± 0.6	0.7 ± 0.0	48.9 ± 3.1	2.8 ± 0.5
Mesylate salt of **54**							2.0 ± 0.8	0.5 ± 0.1	32.2 ± 1.2	2.8 ± 0.3
Staurosporine					1.8	<1.0				
AZD1152					0.3[c]		8.6 ± 0.3	9.7 ± 0.6	25.1 ± 0.1	12.7 ± 0.4
Sunitinib							24.3 ± 0.4	17.7 ± 0.3	45.7 ± 0.3	36.8 ± 0.3

[a] Average value from two independent dose–response curves with variations of <30%.
[b] Mean value ± S.D. ($n > 2$).
[c] Value from Ref. [30].

observed at a dose of 10 mg/kg (i.p., q.d.). Notably, tumor volume decreased significantly (>90%) when a dose of 20 mg/kg was administered (i.p., q.d.). Administration of the salt correlated with reduced tumor volume and mortality of MOLM-13 tumor-bearing mice (Fig. 6B) without affecting body weight (Fig. 6C). A significant reduction in tumor mass (up to 95%) was observed after treatment with the mesylate salt of **54** (Fig. 6D and Figure S4, Supplementary data) at a dose of 10 and 20 mg/kg, when compared to that of vehicle treated animal group. A calculated ED_{50} of 54 for the reduction of MOLM-13 tumor mass *in vivo* was 2.6 mg/kg, whereas the LD50 for 54 was >20 mg/kg. The therapeutic index (as expressed in the ratio of LD50/ED50) of 54 is much larger than 10.

3. Conclusions

A series of acylureido derivatives of indolin-2-one was developed to serve as a pool of potential dual Aurora B/FLT3 inhibitors. Some of these compounds inhibit the viability of AML cells and are more cytotoxic to AML cells with FLT3-ITD than those with FLT3-WT. Structure–activity relationship studies demonstrated the significance of the 6-acylureido and 3-pyrrolemethylidene moieties attached to the indolin-2-one scaffold with respect to potent kinase inhibitory activity and cytotoxicity. The presence/absence of the indolin-2-one C5-fluoro substituent modulates the selectivity profile for Aurora B and FLT3. Our ligand-based molecular engineering led to the identification of **54** as a potent inhibitor of dual

Table 4
Permeability of **32a**, **34**, **40**, **42**, and **54** as determined by the PAMPA-GIT assay.

Cpd.	P_e[a]	BCS code[b]
32a	0.67 ± 0.09	Low
34	0.23 ± 0.06	Low
40	2.46 ± 0.10	High
42	2.49 ± 1.51	High
54	1.78 ± 0.35	High

[a] Effective permeability coefficient P_e ($\times 10^{-6}$ cm s^{-1}) assessed by a PAMPA assay targeting gastrointestinal absorption at pH 7.4 ($n = 3$).
[b] Biopharmaceutics Classification System (BCS) Code: High ($P_e > 1.5 \times 10^{-6}$ cm s^{-1}), Low ($P_e < 1.5 \times 10^{-6}$ cm s^{-1}).

Aurora B/FLT3 kinases. In addition to inhibition of Aurora B and FLT3, **54** inhibits c-Kit and LCK. Compound **54** exhibits good pharmacological and safety profiles *in vitro* and *in vivo* and an acceptable *in vivo* pharmacokinetic profile when administered intravenously. Further development of **54** for the treatment of hematological malignancies, such as AML, is under active investigation and will be reported in due course.

4. Experimental

4.1. Chemistry

4.1.1. General methods

All solvents were ACS grade, obtained from Merck, ECHO, or Mallinckrodt, and used without further purification. An SP-1 standalone solvent purification system (LC Technology Solutions) was used to dry solvents, which then contained <10 ppm water as determined by Karl-Fischer moisture analysis. Chemicals were purchased from Acros, Aldrich, Alfa Aesar, Carbosynth, Matrix, Ryss, or Zealing. Thin layer chromatography was performed using precoated silica gel plates (60 F254, Merck). Flash column chromatography used Merck silica gel 60 (40–63 μm). IR spectra were collected by Thermoscientific Nicolet iS5 FT-IR spectrometer equipped with iD3 attenuated total reflection (ATR) accessory (ZnSe). ^{1}H and ^{13}C NMR spectra were obtained using a Bruker Avance 400-MHz or 200-MHz NMR spectrometer. Chemical shifts were referenced to the central peak of DMSO-d_6 (2.49 ppm and 39.4 ppm for ^{1}H and ^{13}C NMR, respectively). Elemental analyses used a Heraeus VariaEL-III elemental analyzer. Melting points were determined using a Mel-Temp melting-point apparatus (Laboratory Devices Inc.) and are reported uncorrected. MS was performed using a Bruker Esquire 2000 spectrometer.

Table 5
Kinase inhibitory activity of **54** against various kinases.

Kinase	Inhibition[a] (IC_{50})	Kinase	Inhibition[a] (IC_{50})
ABL1	65%	IGF1R	10%
AKT1	40%	JAK2	NI[b]
Aurora A	21%	JNK2	32%
Aurora B	95% (0.4 nM)	KDR/VEGFR2	20%
Aurora C	20%	Lck	97% (2.3 nM)
BRAF	NI[b]	Lyn	62%
c-Kit	89% (14.0 nM)	MEK1/MAP2K1	38%
c-Met	40%	mTOR/FRAP1	NI[b]
c-Src	20%	PDGFRα	57%
CHK1	18%	PDGFRβ	74%
EGFR	NI[b]	Plk1	13%
ERBB2/Her2	2%	ROCK1	55%
FGFR1	23%	TEK/Tie-2	32%
FLT3	95% (0.5 nM)		

ABL1, Abelson murine leukemia viral oncogene homolog 1; c-Met, MNNG HOS-transforming gene; CHK1, checkpoint kinase 1; EGFR, epithelial growth factor receptor; Her2, human epidermal growth factor receptor 2; FGFR1, fibroblast growth factor receptor 1; IGF-1R, insulin-like growth factor-1 receptor; JAK2, Janus-associated kinase 2; JNK2, c-Jun *N*-terminal kinase 2; VEGFR-2, vascular epithelial growth factor receptor 2; Lck, lymphocyte-specific protein tyrosine kinase; MAP2K1, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; Plk1, polo-like kinase 1; ROCK1, rho-associated, coiled-coil containing protein kinase 1; Tie-2, angiopoietin receptor 2.
[a] Mean value ($n = 2$) for percentage inhibition of enzyme activity after treatment with **54** at 1.0 μM.
[b] NI, no inhibition.

4.1.2. Ethyl (5-formyl-2,4-dimethyl-1H-pyrrole)-3-carboxylate (*7*) and ethyl 3-(5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)propanoate (*8*)

Methyl chloroformate (0.101 g, 1.076 mmol) was added into a stirred solution of 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (**5**) or 3-(5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid (**6**) (0.897 mmol) and triethylamine (0.181 g, 1.794 mmol) in THF (10 mL). Reaction mixture was stirred at room temperature for 4 h, and then ethanol (0.2 mL, 3.588 mmol) was added. The resulting mixture was heated at reflux for 2 h. The reaction mixture was cooled to room temperature and evaporated *in vacuo*. After evaporation, the residue was extracted with chloroform and washed first with 0.1 *N* HCl, and then with saturated $NaHCO_3$ and brine. Organic extract was dried over anhydrous $MgSO_4$, filtered through Celite and evaporated to give the corresponding crude ethyl esters **7** [67] and **8** [68], which were used for the aldol condensations without purification.

4.1.3. Preparation of the 5-formylpyrrole-3-carboxamides *9*–*15*

N-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (1.92 mmol), hydroxybenzotriazole hydrate (1.92 mmol), triethylamine (1.85 mL, 13.26 mmol) and the appropriate amine (1.60 mmol) were added into a stirred solution of 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (**5**) or (5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid (**6**) (1.28 mmol) in DMF (5 mL). The mixtures were stirred at room temperature for 12 h and then diluted with water (5 mL). The resultant mixture was adjusted to pH 10 with 10 *N* NaOH and extracted with 10% (v/v) methanol in CH_2Cl_2. The organic extracts were dried over $MgSO_4$, filtered through Celite, and evaporated to give the 5-formylpyrrole-carboxamides **9**–**15**, which were used for the aldol condensations without purification.

4.1.4. 3-(Methylthio)-4-nitroindolin-2-one (*16*)

Ethyl (methylthio)acetate (2.91 g, 21.72 mmol) was added into CH_2Cl_2 (150 mL) at −78 °C under argon after which sulfuryl chloride (1.75 mL, 21.72 mmol) was added. After stirring for 35 min, a solution of *m*-nitroaniline (3 g, 21.72 mmol) and Proton Sponge (4.65 g, 21.72 mmol) in CH_2Cl_2 (100 mL) was then added over the course of 1 h. The mixture was stirred for 2 h, and then triethylamine (3.01 mL, 21.72 mmol) in CH_2Cl_2 (10 mL) was added dropwise at −78 °C over the course of 1 h with stirring. The mixture was allowed to warm to room temperature and then washed three times with water (100 mL each). The combined aqueous layers were back-extracted with CH_2Cl_2 (100 mL). The organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated to yield ethyl 2-(2-amino-6-nitrophenyl)-2-(methylthio)acetate as a brown solid, which was dissolved in glacial acetic acid (200 mL) and stirred for 5 h. Acetic acid was removed by evaporation to yield a brown tacky solid that was extracted with EtOAc. The extract was sequentially washed with saturated $KHCO_{3(aq)}$ and brine, dried over $MgSO_4$, filtered, and evaporated to give **16**, which was recrystallized from methanol (2.5 g, 52%). mp: 228–230 °C (Lit. [49] 228–230 °C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.88 (s, 3H), 4.83 (s, 1H), 7.22 (d, $J = 8.0$ Hz, 1H), 7.50 (dd, $J = 8.0$, 8.0 Hz, 1H), 7.694 (d, $J = 8.4$ Hz, 1H), 10.99 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.8, 45.6, 115.0, 116.6, 122.3, 130.3, 144.4, 144.7, 174.7. MS (ESI): m/z 222.8 $[M-H]^-$.

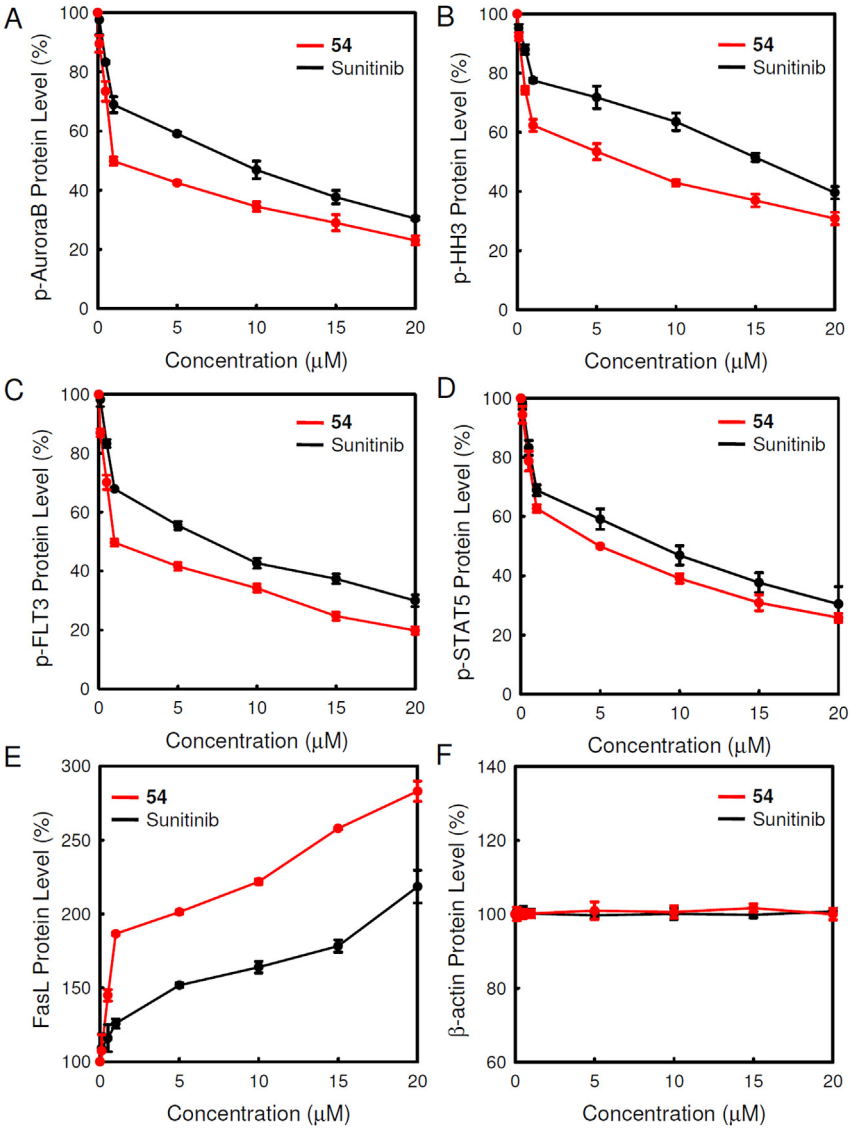


Fig. 3. Levels of the *p*-Aurora B, *p*-HH3, *p*-FLT3, *p*-STAT5, FasL and actin in MOLM-13 cells. Data are expressed as the mean ± SEM of three independent determinations.

*4.1.5. 4-Amino-3-(methylthio)indolin-2-one (**17**)*

Compound **16** (1 g, 4.45 mmol) and stannous chloride dihydrate (5.03 g, 26.5 mmol) in EtOH/AcOH 5:1 (36 mL) was heated at 70 °C under argon for 7 h. After cooling, solvent was evaporated to give a sticky solid, to which water was added. The mixture was extracted with chloroform, and saturated $NaHCO_{3(aq)}$ was added dropwise to the water layer, which was then again extracted with chloroform. The organic layers were combined, sequentially washed with saturated $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$, filtered, and evaporated to give the crude product, which was washed with toluene (5 mL) and used unpurified in the next step (1.2 g, 96%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.89 (s, 3H), 4.30 (s, 1H), 5.12 (s, 2H), 6.05 (d, J = 7.6 Hz, 1H), 6.25 (d, J = 8.4, 1H), 6.89 (dd, J = 7.6, 8.0 Hz, 1H), 10.23 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.3, 44.4, 98.0, 106.3, 109.0, 129.4, 143.0, 144.8, 176.3. MS (ESI): m/z 217.0 $[M+Na]^+$.

*4.1.6. 4-Aminoindolin-2-one (**18**)*

Literature reported procedure was followed to afford **18** (39%), mp:181–183 °C (Lit. [69] 182–183 °C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.15 (s, 2H), 5.01 (s, 2H), 6.05 (d, J = 7.6 Hz, 1H), 6.21 (d, J = 8.0, 1H), 6.83 (dd, J = 7.6, 8.0 Hz, 1H), 10.09 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 33.8, 97.9, 107.6, 108.3, 127.9, 143.8, 144.0, 176.4. MS (ESI): m/z 146.8 $[M-H]^-$.

*4.1.7. 6-Aminoindolin-2-one (**20**)*

Literature reported procedure was followed to afford **20** (68%), mp: 193–195 °C (Lit. [51] 194–195 °C), R_f = 0.36 (EtOAc/hexane 7:3). ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.23 (s, 2H), 5.00 (s, 2H), 6.09 (dd, J = 2.2, 6.2 Hz, 1H), 6.10 (d, J = 2.0 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 10.09 (s, 1H). MS (ESI) m/z 146.8 $[M-H]^-$.

*4.1.8. 7-Aminoindolin-2-one (**21**)*

Literature reported procedure was followed to afford **21** (77%). mp: 228–229 °C (Lit. [42] 228–229 °C), R_f = 0.6 ($CHCl_3$/MeOH 9:1). ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.38 (s, 2H), 4.79 (s, 2H), 6.45–6.50 (m, 2H), 6.67 (t, J = 8.0 Hz, 1H), 9.90 (s, 1H). MS (ESI): m/z 146.8 $[M-H]^-$.

*4.1.9. Methyl (5-fluoro-2,4-dinitrophenyl)acetate (**23**)*

A solution of 90% (v/v) HNO_3 (18 mL) and conc. H_2SO_4 (22.5 mL) was added dropwise into a stirred solution of *m*-fluorophenyl acetic acid (15.00 g, 97.31 mmol) in conc. H_2SO_4 (30 mL) while

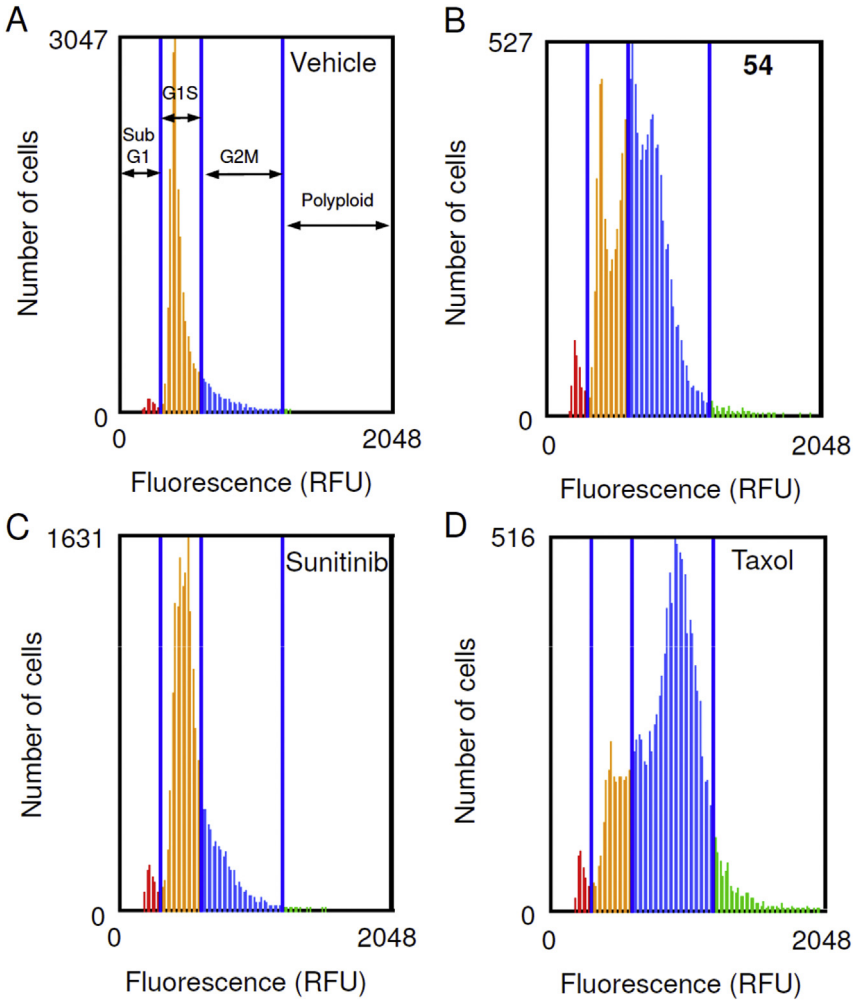


Fig. 4. Effects on the cell cycle detected by the Tali image-based cytometer against MOLM-13 cells. Taxol was used as a positive control to illustrate the G2/M arrest.

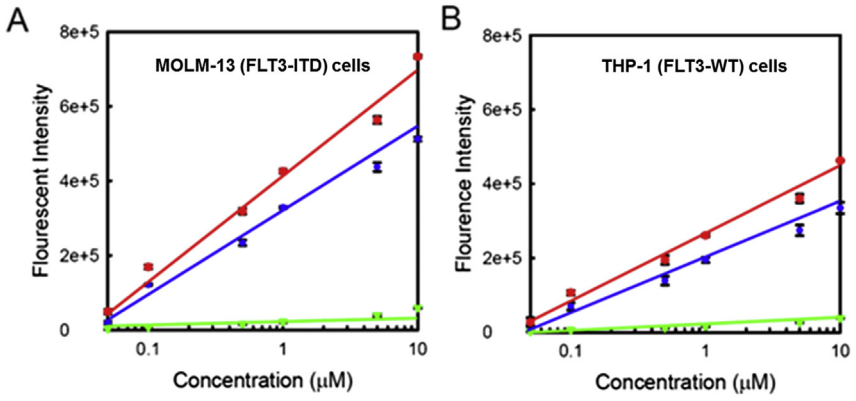


Fig. 5. Caspase activation in MOLM-13 (A) and THP-1 (B) cells. Data are expressed as the mean ± SEM of three independent determinations.

Table 6
Toxicity/safety of **54**.

Cpd.	IC_{50} (μM)					IC_{50} (μM) hERG-binding assay
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4	
54	>10	>10	>10	>10	>10	9.670
Ketoconazole	0.06	2.83	3.33	9.81		
Furafylline					1.52	
E-4031						0.001

maintaining the temperature between 20 and 35 °C. The mixture was stirred at 35 °C for 20 h. The resultant yellow slurry was poured onto ice and filtered to give an off-white solid (21 g). H_2SO_4 (1 mL) was then added into a solution of this solid in methanol (250 mL), and the mixture was heated at reflux for 5 h. Then, the mixture was adjusted to pH 5 with 2.5 *N* NaOH in an ice bath. After removal of methanol *in vacuo*, the resulting mixture was extracted three times with 100-mL volumes of EtOAc. The organic extract was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to give a

Table 7
Pharmacokinetic profile of **54**.

Parameters	54	
	iv[a]	po[b]
Dose	2 mg/kg	10 mg/kg
V_{ss} (L/kg)	22.2 ± 4.9	
CL (L/h/kg)	0.5 ± 0.02	
$T_{1/2}$ (h)	27.6 ± 7.3	8.6 ± 0.5
T_{max} (h)	0.1 ± 0	1.7 ± 1.2
C_{max} (ng/mL)	653.5 ± 101.1	20.9 ± 6.8
AUC_{0-24} (h × ng/mL)	1965.0 ± 266.0	252.1 ± 44.1
F (%)		2.6

V_{ss}, volume of distribution; CL, clearance; $T_{1/2}$, half-life; T_{max}, time to peak concentration; C_{max}, peak concentration; AUC, area under the curve; F, bioavailability.
[a] $n = 2$.
[b] $n = 3$.

light-brown oil containing methyl (5-fluoro-2,4-dinitrophenyl)acetate (**23**), methyl (3-fluoro-2,6-dinitrophenyl)acetate (**24**), and methyl (5-methoxy-2,4-dinitrophenyl)acetate (**25**). The mixture was separated by flash column chromatography (EtOAc/hexane, 15–50%). **23**: Dark brown oil (8.00 g, 32%). $R_f = 0.39$ (EtOAc/hexane 3:7). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.64 (s, 3H), 4.22 (s, 2H), 7.92 (d, J = 11.6 Hz, 1H), 8.83 (d, J = 6.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.1, 52.0, 123.3 (J = 23.5 Hz), 123.7, 135.4 (J = 8.6 Hz), 138.6 (J = 10.9 Hz), 143.6 (J = 4.0 Hz), 155.9 (J = 268.1 Hz), 169.0. Anal. ($C_9H_7FN_2O_6 \cdot 1/9CH_3COOC_2H_5$) C, H, N. MS (ESI) m/z 256.8 $[M-H]^-$. **24**: Yellow oil (3.85 g, 19%). $R_f = 0.52$ (EtOAc/hexane 3:7). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.65 (s, 3H), 4.06 (s, 2H), 7.91 (dd, J = 8.8, 9.2 Hz, 1H), 8.49 (dd, J = 7.6, 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 33.4, 52.3, 118.1 (J = 21.2 Hz), 125.5, 130.2 (J = 10.3 Hz), 140.0 (J = 15.1 Hz), 145.4 (J = 3.3 Hz), 155.2 (J = 262.3 Hz), 176.4. MS (ESI) m/z 256.8 $[M-H]^-$. **25**: Yellow solid (4.80 g, 18%). mp: 91–94 °C (Lit. [52] 90–93 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.64 (s, 3H), 4.05 (s, 3H), 4.21 (s, 2H), 7.63 (s, 1H), 8.71 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 39.1, 51.9, 57.8, 119.1, 123.0, 137.0, 137.4, 139.8, 159.2, 169.6. MS (ESI) m/z 268.9 $[M-H]^-$.

*4.1.10. 6-Amino-5-fluoroindolin-2-one (**22**)*

Palladium on charcoal (500 mg, 10% w/v) was added into a solution of **23** (6.00 g, 23.25 mmol) in ethanol (200 mL). The mixture was stirred under a hydrogen atmosphere at rt for 12 h. After filtration through Celite, the filtrate was evaporated to give the diamino derivative as an oil. Hydrochloric acid (1 *N*, 50 mL) was added into the oil, and the mixture was refluxed by heating for 1 h. After cooling, the mixture was neutralized with 2.5 *N* NaOH (20 mL) and then extracted three times with EtOAc (100 mL). The organic extract was washed with brine, dried over anhydrous $MgSO_4$, and evaporated *in vacuo* to give a greenish brown solid (3.08 g, 78%). mp: 185–187 °C (Lit. [52] 185–187 °C), $R_f = 0.45$ ($CHCl_3$/MeOH 9:1). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.27 (s, 2H), 5.03 (s, 2H), 6.29 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 10.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.4, 97.5 (J = 3.7 Hz), 111.6 (J = 21.4 Hz), 111.7 (J = 7.9 Hz), 135.5 (J = 14.3 Hz), 139.8 (J = 1.7 Hz), 146.3 (J = 227.0 Hz), 176.9. MS (ESI) m/z 164.8 $[M-H]^-$.

4.1.11. Preparation of the benzoylureidoindolin-2-one intermediates

Oxalyl chloride (6.61 mmol) was added dropwise into a stirred solution of the appropriate benzamide (2.6 mmol) in CH_2Cl_2 (10 mL). The mixtures were heated at reflux for 20–22 h. Then, solvents were evaporated *in vacuo* to give the respective crude benzoyl isocyanates **4a**–**k**, which were used immediately in the next step without purification. Aminoindolin-2-one (2.8 mmol) was added into a stirred solution of the appropriate benzoyl isocyanate (2.8 mmol) in acetonitrile (10 mL). After addition, each mixture was heated at 70–80 °C for 2–3 h. The solid products were each filtered, washed with acetonitrile, and air dried.

*4.1.11.1. N-(2-Oxoindolin-4-ylcarbamoyl)-4-methoxybenzamide (**26**).* Reaction of **18** (0.450 g, 2.8 mmol) with 4-methoxybenzoyl isocyanate (**4a**, 0.500 g, 2.8 mmol) afforded **26** (0.696 g, 76%). mp: 276–279 °C. IR (ATR): 3440, 3310, 1710, 1675, 1475, 838, 759 cm^{-1}. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.45 (s, 2H), 3.84 (s, 3H), 6.61 (d, J = 7.6 Hz, 1H), 7.05 (d, J = 9.2 Hz, 2H), 7.17 (t, J = 8.0 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 8.05 (d, J = 8.8 Hz, 2H), 10.44 (s, 1H), 10.84 (s, 1H), 10.93 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 34.2, 55.7, 105.3, 113.6, 114.0, 115.6, 124.1, 128.4, 130.7, 133.8, 144.2, 151.2, 163.3, 168.3, 175.7. Anal. ($C_{17}H_{15}N_3O_4 \cdot 1/2H_2O$) C, H, N. MS (ESI): m/z 324.0 $[M-H]^-$.

*4.1.11.2. N-(2-Oxoindolin-5-ylcarbamoyl)-4-methoxybenzamide (**27**).* Reaction of **19** (0.450 g, 2.8 mmol) with **4a** (0.500 g, 2.8 mmol) afforded **27** (0.722 g, 79%). mp: 287–290 °C. IR (ATR): 3437, 3312, 1705, 1670, 1465, 838, 760 cm^{-1}. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.48 (s, 2H), 3.83 (s, 3H), 6.77 (d, J = 8.0 Hz, 1H), 7.04 (d, J = 8.0 Hz, 2H), 7.28 (d, J = 8.4 Hz, 1H), 7.50 (brs, 1H), 8.03 (d, J = 8.0 Hz, 2H), 10.31 (s, 1H), 10.78 (s, 1H), 10.79 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 36.0, 55.5, 109.0, 113.8, 117.2, 119.3, 124.0, 126.3, 130.4, 131.5, 139.8, 151.3, 163.0, 167.8, 176.2. Anal. ($C_{17}H_{15}N_3O_4 \cdot 1/2H_2O$) C, H, N. MS (ESI): m/z 324.0 $[M-H]^-$.

*4.1.11.3. N-(2-Oxoindolin-6-ylcarbamoyl)-4-methoxybenzamide (**28a**).* Reaction of **20** (0.450 g, 2.8 mmol) with **4a** (0.500 g, 2.8 mmol) afforded **28a** (0.786 g, 86%). mp: 283–285 °C. IR (ATR): 3433, 3312, 1700, 1662, 1456, 829, 748 cm^{-1}. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.42 (s, 2H), 3.83 (s, 3H), 6.91 (dd, J = 1.8, 8.6 Hz, 1H), 7.06 (d, J = 9.0 Hz, 2H), 7.14 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 1.8 Hz, 1H), 8.04 (d, J = 8.8 Hz, 2H), 10.40 (s, 1H), 10.87 (s, 1H), 10.95 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 35.3, 55.6, 101.2, 112.1, 113.9, 120.7, 124.0, 124.6, 130.5, 137.0, 144.2, 151.2, 163.0, 167.9, 176.7. Anal. ($C_{17}H_{15}N_3O_4 \cdot 1/2H_2O$) C, H, N. MS (ESI) m/z 324.0 $[M-H]^-$.

*4.1.11.4. N-(2-Oxoindolin-6-ylcarbamoyl)benzamide (**28b**).* Reaction of **20** (0.450 g, 2.8 mmol) with benzoyl isocyanate (**4b**, 0.411 g, 2.8 mmol) afforded **28b** (0.648 g, 78%). mp: 277–279 °C. IR (ATR): 3442, 3320, 1710, 1675, 1470, 842, 745 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.42 (s, 2H), 6.93 (dd, J = 1.2, 7.6 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.35 (brs, 1H), 7.53 (dd, J = 7.6, 8.0 Hz, 2H), 7.64 (dd, J = 7.2, 7.6 Hz, 1H), 8.01 (d, J = 8.0 Hz, 2H), 10.38 (s, 1H), 10.83 (s, 1H), 11.00 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.3, 101.2, 112.1, 120.7, 124.5, 128.2, 128.4, 132.2, 132.9, 137.0, 144.2, 150.9, 168.6, 176.6. Anal. ($C_{16}H_{13}N_3O_3 \cdot 1/6H_2O$) C, H, N. MS (ESI) m/z 293.9 $[M-H]^-$.

*4.1.11.5. N-(2-Oxoindolin-6-ylcarbamoyl)-4-chlorobenzamide (**28c**).* Reaction of **20** (0.450 g, 2.8 mmol) with 4-chlorobenzoyl isocyanate (**4c**, 0.508 g, 2.8 mmol) afforded **28c** (0.736 g, 80%). mp: 275–278 °C. IR (ATR): 3445, 3318, 1709, 1675, 1463, 840, 755 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.42 (s, 2H), 6.92 (dd, J = 1.6, 8.0 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.36 (d, J = 1.6 Hz, 1H), 7.60 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.4 Hz, 2H), 10.39 (s, 1H), 10.74 (s, 1H), 11.08 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.3, 101.2, 112.1, 120.8, 124.5, 128.5, 130.1, 131.0, 136.9, 137.8, 144.2, 150.7, 167.6, 176.6. Anal. ($C_{16}H_{12}ClN_3O_3 \cdot 1/2H_2O$) C, H, N. MS (ESI) m/z 327.9 $[M-H]^-$.

*4.1.11.6. N-(2-Oxoindolin-6-ylcarbamoyl)-3,4-dichlorobenzamide (**28d**).* Reaction of **20** (0.450 g, 2.8 mmol) with 3,4-dichlorobenzoyl isocyanate (**4d**, 0.604 g, 2.8 mmol) afforded **28d** (0.882 g, 87%). mp: 278–280 °C. IR (ATR): 3452, 3317, 1695, 1665, 1450, 838, 749 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.41 (s, 2H), 3.83 (s, 3H), 6.90 (dd, J = 2.0, 8.0 Hz, 1H), 7.13 (d, J = 8.0 Hz, 1H), 7.32 (brs, 1H), 7.79 (d,

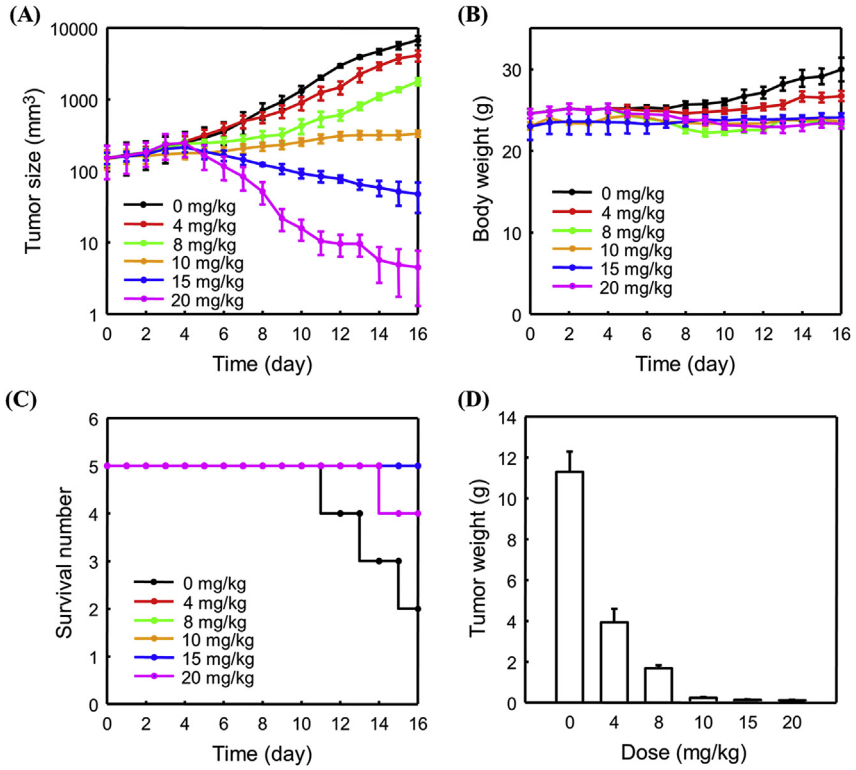


Fig. 6. Treatment with the mesylate salt of **54** inhibits the growth of FLT3-ITD AML xenografts *in vivo*. Mice were assessed for tumor growth (A), body weight (B), and survival (C). After sacrificing the mice, tumors were excised and tumor mass (D) determined. Mean tumor volume ± SEM is shown.

J = 8.0 Hz, 1H), 7.93 (dd, J = 2.0, 8.4 Hz, 1H), 8.23 (d, J = 8.0 Hz, 1H), 10.37 (s, 1H), 10.63 (s, 1H), 11.13 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.3, 101.2, 112.2, 120.9, 124.5, 128.4, 130.2, 130.8, 131.4, 132.7, 135.7, 136.8, 144.2, 150.5, 166.4, 176.6. Anal. ($C_{16}H_{11}Cl_2N_3O_3 \cdot 1/2H_2O$) C, H, N. MS (ESI) m/z 361.8 $[M-H]^-$.

*4.1.11.7. N-(2-Oxoindolin-6-ylcarbamoyl)-2-fluorobenzamide (**28e**).* Reaction of **20** (0.450 g, 2.8 mmol) with 2-fluorobenzoyl isocyanate (**4e**, 0.462 g, 2.8 mmol) afforded **28e** (0.576 g, 66%). mp: 258–260 °C. IR (ATR): 3460, 3328, 1715, 1685, 1455, 840, 762 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.42 (s, 2H), 6.93 (d, J = 8.0 Hz, $1H_5$), 7.14 (d, J = 8.0 Hz, 1H), 7.30–7.36 (m, 3H), 7.59–7.64 (m, 1H), 7.67–7.70 (m, 1H), 10.40 (s, 1H), 10.49 (s, 1H), 11.02 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.1, 101.0, 112.0, 116.0 (J = 21.4 Hz), 120.7, 122.3 (J = 13.6 Hz), 124.3 (J = 3.4 Hz), 124.3, 129.8 (J = 2.0 Hz), 133.6 (J = 8.7 Hz), 136.7, 144.0, 150.1, 158.9 (J = 249.7 Hz), 166.1, 176.4. Anal. ($C_{16}H_{12}FN_3O_3 \cdot 1/4H_2O$) C, H, N. MS (ESI) m/z 311.9 $[M-H]^-$.

*4.1.11.8. N-(2-Oxoindolin-6-ylcarbamoyl)-4-(trifluoromethyl)benzamide (**28f**).* Reaction of **20** (0.450 g, 2.8 mmol) with 4-(trifluoromethyl)benzoyl isocyanate (**4f**, 0.602 g, 2.8 mmol) afforded **28f** (0.901 g, 89%). mp: 288–290 °C. IR (ATR): 3452, 3331, 1726, 1700, 1464, 850, 758 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.42 (s, 2H), 6.93 (dd, J = 2.0, 8.0 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.34 (d, J = 1.6 Hz, 1H), 7.89 (d, J = 8.4 Hz, 2H), 8.17 (d, J = 8.0 Hz, 2H), 10.39 (s, 1H), 10.68 (s, 1H), 11.24 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.3, 101.2, 112.2, 120.8, 123.6 (J = 271.0 Hz), 124.5, 125.4 (J = 3.7, 7.5 Hz), 129.1, 132.3 (J = 31.9 Hz), 136.2, 136.9, 144.2, 150.6, 167.5, 176.6. Anal. ($C_{17}H_{12}F_3N_3O_3 \cdot 1/4H_2O$) C, H, N. MS (ESI) m/z 361.9 $[M-H]^-$.

*4.1.11.9. N-(2-Oxoindolin-6-ylcarbamoyl)-4-chloro-3-trifluoromethyl)benzamide (**28g**).* Reaction of **20** (0.450 g, 2.8 mmol) with 4-chloro-3-(trifluoromethyl)benzoyl isocyanate (**4g**, 0.698 g, 2.8 mmol) afforded **28g** (0.736 g, 66%). mp: 262–264 °C. IR (ATR): 3453, 3292, 1715, 1690, 1448, 842, 767 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.42 (s, 2H), 6.92 (d, J = 7.6 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.33 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 8.44 (s, 1H), 10.38 (s, 1H), 10.64 (s, 1H), 11.32 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.9, 101.8, 112.8, 121.5, 123.0 (J = 271.7 Hz), 125.2, 127.3 (J = 31.2 Hz), 128.4 (J = 5.4, 10.7 Hz), 132.4, 132.6, 134.5, 135.8 (J = 1.7 Hz), 137.4, 144.8, 151.2, 167.0, 177.2. Anal. ($C_{17}H_{11}ClF_3N_3O_3$) C, H, N. MS (ESI) m/z 395.9 $[M-H]^-$.

*4.1.11.10. N-(2-Oxoindolin-6-ylcarbamoyl)-4-methylbenzamide (**28h**).* Reaction of **20** (0.450 g, 2.8 mmol) with 4-methylbenzoyl isocyanate (**4h**, 0.451 g, 2.8 mmol) afforded **28h** (0.553 g, 64%). mp: 294–296 °C. IR (ATR): 3432, 3319, 1705, 1670, 1458, 838, 761 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H), 3.42 (s, 2H), 6.92 (d, J = 7.6 Hz, 1H), 7.13 (d, J = 8.0 Hz, 1H), 7.32 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 2.4 Hz, 1H), 7.92 (d, J = 8.0 Hz, 2H), 10.38 (s, 1H), 10.87 (s, 1H), 10.91 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.0, 35.3, 101.2, 112.1, 120.7, 124.5, 128.3, 129.0, 129.3, 137.0, 143.4, 144.2, 151.084, 168.5, 176.6. Anal. ($C_{17}H_{15}N_3O_3 \cdot 1/3H_2O$) C, H, N. MS (ESI) m/z 308.0 $[M-H]^-$.

*4.1.11.11. N-(2-Oxoindolin-6-ylcarbamoyl)-3,5-dimethoxybenzamide (**28i**).* Reaction of **20** (0.450 g, 2.8 mmol) with 3,5-dimethoxybenzoyl isocyanate (**4i**, 0.580 g, 2.8 mmol) afforded **28i** (0.530 g, 53%). mp: 248–251 °C. IR (ATR): 3461, 3318, 1700, 1675, 1463, 841, 765 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.41 (s, 2H), 3.80 (s, 6H), 6.73 (brs, 1H), 6.92 (d, J = 7.6 Hz, 1H), 7.13 (d, J = 8.0 Hz, 1H), 7.19 (brs, 2H), 7.34 (brs, 1H), 10.38 (s, 1H), 10.83 (s, 1H), 11.08 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.3, 55.5, 101.2, 105.2, 105.9, 112.1, 120.8, 124.6, 134.0, 137.0, 144.2, 150.8, 160.3, 168.0, 176.6. Anal. ($C_{18}H_{17}N_3O_5 \cdot 1/4H_2O$) C, H, N. MS (ESI) m/z 354.0 $[M-H]^-$.

*4.1.11.12. N-(2-Oxoindolin-6-ylcarbamoyl)-4-(dimethylamino)benzamide (**28j**).* Reaction of **20** (0.450 g, 2.8 mmol) with 4-(dimethylamino)benzoyl isocyanate (**4j**, 0.532 g, 2.8 mmol) afforded **28j** (0.533 g, 56%). mp > 300 °C. IR (ATR): 3456, 3321, 1710, 1685, 1465, 842, 758 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.02 (s, 6H), 3.42 (s, 2H), 6.74 (d, J = 9.2 Hz, 2H), 7.40 (s, 1H), 7.14 (s, 2H), 7.84 (d, J = 9.2 Hz, 2H), 10.41 (s, 1H), 10.66 (s, 1H), 11.28 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 36.0, 40.1, 102.0, 111.4, 113.1, 117.9, 121.9, 125.0, 131.0, 138.0, 144.6, 154.1, 160.6, 165.5, 177.2. Anal. ($C_{18}H_{18}N_4O_3 \cdot 4/5H_2O$) C, H, N. MS (ESI) m/z 338.1 $[M]^+$

*4.1.11.13. N-(2-Oxoindolin-6-ylcarbamoyl)-2-fluoro-4-methoxybenzamide (**28k**).* Reaction of **20** (0.450 g, 2.8 mmol) with 2-fluoro-4-methoxybenzoyl isocyanate (**4k**, 0.546 g, 2.8 mmol) afforded **28k** (0.406 g, 42%). mp: 257–260 °C. IR (ATR): 3448, 3328, 1721, 1701, 1469, 845, 762 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.42 (s, 2H), 3.84 (s, 3H), 6.87–6.92 (m, 2H), 6.96 (dd, J = 2.4, 12.8 Hz, 1H), 7.13 (d, J = 8.0 Hz), 7.31 (d, J = 1.6 Hz, 1H), 7.68 (dd, J = 8.4, 8.8 Hz, 1H), 10.38 (s, 1H), 10.58 (s, 1H), 10.71 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.2, 55.9, 101.1, 101.8 (J = 25.6 Hz), 110.5 (J = 2.6 Hz), 112.0, 113.9 (J = 12.7 Hz), 120.6, 124.4, 131.4 (J = 3.7 Hz), 136.8, 144.0, 150.3, 160.8 (J = 250.7 Hz), 163.5 (J = 11.7 Hz), 165.6 (J = 2.0 Hz), 176.5. Anal. ($C_{17}H_{14}FN_3O_4 \cdot 1/3H_2O$) C, H, N. MS (ESI) m/z 341.9 $[M-H]^-$.

*4.1.11.14. N-(5-Fluoro-2-oxoindolin-6-ylcarbamoyl)-4-methoxybenzamide (**28l**).* Reaction of **22** (0.465 g, 2.8 mmol) with **4a** (0.500 g, 2.8 mmol) afforded **28l** (0.758 g, 79%). mp: 185–187 °C. IR (ATR): 3452, 3330, 1700, 1670, 1455, 828, 750 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.45 (s, 2H), 3.84 (s, 3H), 7.05 (d, J = 8.8 Hz, 2H), 7.21 (d, J = 10.4 Hz, 1H), 7.80 (d, J = 6.4 Hz, 1H), 8.05 (d, J = 8.4 Hz, 2H), 10.36 (s, 1H), 11.04 (s, 1H), 11.32 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.8, 55.5, 101.8, 111.8 (J = 22.6 Hz), 113.8, 120.4 (J = 8.7 Hz), 123.8, 124.8 (J = 11.6 Hz), 130.5, 139.9 (J = 2.0 Hz), 147.6 (J = 233.4 Hz), 151.1, 163.1, 168.2, 176.3. Anal. ($C_{17}H_{14}FN_3O_4 \cdot H_2O$) C, H, N. MS (ESI) m/z 341.9 $[M-H]^-$.

*4.1.11.15. N-(5-Fluoro-2-oxoindolin-6-ylcarbamoyl)-2-fluoro-4-methoxybenzamide (**28m**).* Reaction of **22** (0.465 g, 2.8 mmol) with **4k** (0.546 g, 2.8 mmol) afforded **28m** (0.602 g, 60%). mp: 270–273 °C. IR (ATR): 3440, 3317, 1710, 1685, 1450, 835, 758 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.45 (s, 2H), 3.83 (s, 3H), 6.88 (d, J = 2.4, 8.8 Hz, 1H), 6.96 (dd, J = 2.4, 12.8 Hz, 1H), 7.20 (d, J = 10.4 Hz, 1H), 7.70 (dd, J = 8.4, 8.8 Hz, 1H), 7.75 (d, J = 6.8 Hz), 10.36 (s, 1H), 10.94 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 36.0, 56.3, 102.1, 102.3, 110.8 (J = 2.7 Hz), 112.0 (J = 22.2 Hz), 113.9 (J = 12.3 Hz), 120.9 (J = 8.8 Hz), 124.8 (J = 11.7 Hz), 131.9 (J = 3.6 Hz), 140.1 (J = 2.1 Hz), 147.8 (J = 233.6 Hz), 150.6, 161.2 (J = 251.1 Hz), 164.0 (J = 11.8 Hz), 166.3 (J = 2.0 Hz), 176.5. Anal. ($C_{17}H_{13}F_2N_3O_4 \cdot 1/5H_2O$) C, H, N. MS (ESI) m/z 359.9 $[M-H]^-$.

*4.1.11.16. N-(2-oxoindolin-7-ylcarbamoyl)-4-methoxybenzamide (**29**).* Reaction of **21** (0.450 g, 2.8 mmol) with **4a** (0.500 g, 2.8 mmol) afforded **29** (0.673 g, 73%). mp: 284–286 °C. IR (ATR): 3437, 3312, 1705, 1670, 1465, 838, 757 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 3.53 (s, 2H), 3.84 (s, 3H), 6.93 (t, J = 7.6 Hz, 1H), 7.05–7.10 (m, 2H), 7.17 (d, J = 8.0 Hz, 1H), 8.05 (d, J = 8.0 Hz, 2H), 10.24 (s, 1H), 10.35 (s, 1H), 10.81 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 36.0, 55.5, 113.8, 119.0, 121.1, 121.8, 124.3, 124.6, 126.7, 130.3, 138.6, 152.0, 162.9, 167.4, 176.0. Anal. ($C_{17}H_{15}N_3O_4 \cdot 1/2H_2O$) C, H, N. ESI-MS: m/z 348.1 $[M+Na]^+$.

4.1.12. General procedure for the synthesis of benzoylureido-3-(pyrrol-2-ylmethylidene)indolin-2-one derivatives

A catalytic amount of pyrrolidine (0.001 mmol) was added into a stirred ethanol solution (5 mL) containing benzoylureidoindolin-2-one (1 mmol) and the appropriate pyrrole-2-carbaldehyde (1.2 mmol). The reaction was irradiated with microwaves (CEM, Discover) at 100 °C for 15 min. The crude product precipitated with cooling.

*4.1.12.1. N-(3-(1H-Pyrrol-2-ylmethylidene)-2-oxoindolin-4-ylcarbamoyl)-4-methoxybenzamide (**30**).* Condensation of **26** (0.081 g, 0.25 mmol) with 1*H*-pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **30** (0.065 g, 66%). mp: 290–295 °C (dec.). IR (ATR): 3438, 3305, 1705, 1670, 1465, 828, 759 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 3.86 (s, 3H), 6.34–6.36 (m, 1H), 6.72–6.73 (m, 1H), 6.75 (d, J = 7.6 Hz, 1H), 7.10 (d, J = 9.2 Hz, 2H), 7.16 (t, J = 8.0 Hz, 1H), 7.35 (brs, 1H), 7.44 (d, J = 8.0 Hz, 1H), 8.04 (s, 1H), 8.13 (d, J = 8.8 Hz, 2H) 10.89 (s, 1H), 11.03 (s, 2H), 13.38 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 55.3, 106.3, 111.2, 113.7, 115.6, 116.0, 117.5, 121.0, 123.8, 125.6, 126.6, 127.7, 129.2, 130.3, 130.4, 139.7, 151.5, 162.9, 168.0, 168.6. Anal. ($C_{22}H_{18}N_4O_4 \cdot 1/2H_2O$) C, H, N. ESI-MS: m/z 400.9 $[M-H]^-$.

*4.1.12.2. N-(3-(1H-pyrrol-2-ylmethylidene)-2-oxoindolin-5-ylcarbamoyl)-4-methoxybenzamide (**31**).* Condensation of **27** (0.081 g, 0.25 mmol) with 1*H*-pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **31** (0.074 g, 74%). mp: 285–290 °C (dec.). IR (ATR): 3440, 3315, 1710, 1680, 1450, 845, 765 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.84 (s, 3H), 6.36 (brs, 1H), 6.87 (brs, 1H), 6.86 (d, J = 8.4 Hz, 1H), 7.059 (d, J = 8.8 Hz, 2H), 7.36 (brs, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.77 (s, 1H), 7.79 (brs, 1H), 8.06 (d, J = 8.8 Hz, 2H), 10.83 (s, 1H), 10.86 (s, 1H), 10.88 (s, 1H), 13.43 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.7, 109.7, 111.4, 111.6, 114.0, 116.8, 119.4, 120.8, 124.3, 125.8, 126.0, 126.9, 129.7, 130.6, 131.8, 135.5, 151.6, 163.2, 168.1, 169.4. Anal. ($C_{22}H_{18}N_4O_4 \cdot 1/3H_2O$) C, H, N. ESI-MS: m/z 401.0 $[M-H]^-$.

*4.1.12.3. N-(3-(1H-pyrrol-2-ylmethylidene)-2-oxoindolin-6-ylcarbamoyl)-4-methoxybenzamide (**32a**).* Condensation of **28a** (0.081 g, 0.25 mmol) with 1*H*-pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **32a** (0.080 g, 80%). mp: 278–283 °C (dec.). IR (ATR): 3450, 3320, 1710, 1685, 1455, 840, 747 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.83 (s, 3H), 6.32–6.34 (m, 1H), 6.78–6.79 (m, 1H), 6.99 (dd, J = 1.6, 8.2 Hz, 1H), 7.05 (d, J = 8.8 Hz, 2H), 7.32 (brs, 1H), 7.45 (d, J = 2 Hz, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.63 (s, 1H), 8.04 (d, J = 8.8 Hz, 2H), 10.87 (s, 1H), 10.90 (s, 1H), 11.03 (s, 1H), 13.23 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.5, 101.3, 111.2, 112.5, 113.8, 116.7, 119.0, 119.7, 120.6, 124.0, 125.1, 125.2, 129.6, 130.4, 136.7, 139.5, 151.1, 163.0, 167.9, 169.5. Anal. ($C_{22}H_{18}N_4O_4$) C, H, N. MS (ESI) m/z 401.0 $[M-H]^-$.

*4.1.12.4. N-(3-(1H-pyrrol-2-ylmethylidene)-2-oxoindolin-6-ylcarbamoyl)benzamide (**32b**).* Condensation of **28b** (0.073 g, 0.25 mmol) with 1*H*-pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **32b** (0.072 g, 78%). mp: 290–295 °C (dec.). IR (ATR): 3450, 3320, 1708, 1665, 1463, 852, 749 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 6.33 (brs, 1H), 6.79 (brs, 1H), 7.02 (dd, J = 1.6, 8.4 Hz, 1H), 7.32 (brs, 1H), 7.45 (brs, 1H), 7.53 (t, J = 7.6 Hz, 2H), 7.58 (d, J = 8.4 Hz, 1H), 7.63–7.66 (m, 2H), 8.02 (d, J = 7.6 Hz, 2H), 10.90 (s, 1H), 10.92 (s, 1H), 11.04 (s, 1H), 13.23 (s, 1H), ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.3, 111.2, 112.6, 116.7, 119.0, 120.7, 125.2, 125.2, 128.2, 128.5, 129.5, 132.2, 133.0, 136.6, 139.5, 150.8, 168.6, 169.5. Anal. ($C_{21}H_{16}N_4O_3 \cdot 1/5H_2O$) C, H, N. MS (ESI) m/z 371.1 $[M-H]^-$.

*4.1.12.5. N-(3-(1H-pyrrol-2-ylmethylidene)-2-oxoindolin-6-ylcarbamoyl)-4-chlorobenzamide (**32c**).* Condensation of **28c** (0.082 g, 0.25 mmol) with 1*H*-pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **32c** (0.082 g, 81%). mp: 280–285 °C (dec.). IR (ATR): 3440, 3322, 1712, 1685, 1447, 840, 758 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 6.33 (d, J = 2.4 Hz, 1H), 6.79 (s, 1H), 7.00 (d, J = 8.4 Hz, 1H), 7.320 (s, 1H), 7.44 (s, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 8.8 Hz, 2H), 7.65 (s, 1H), 8.02 (d, J = 8.4 Hz, 2H), 10.82 (s, 1H), 10.90 (s, 1H), 11.10 (s, 1H), 13.22 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.4, 111.3, 112.6, 116.7, 119.0, 119.8, 120.8, 125.3, 128.6, 129.6, 130.2, 131.1, 136.5, 137.9, 139.5, 150.7, 167.7, 169.5. Anal. ($C_{21}H_{15}ClN_4O_3$) C, H, N. MS (ESI) m/z 405.1 $[M-H]^-$.

*4.1.12.6. N-(3-(1H-pyrrol-2-ylmethylidene)-2-oxoindolin-6-ylcarbamoyl)-3,4-dichlorobenzamide (**32d**).* Condensation of **28d** (0.091 g, 0.25 mmol) with 1*H*-pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **32c** (0.081 g, 73%). mp: 295–300 °C (dec.). IR (ATR): 3437, 3312, 1709, 1664, 1462, 819, 769 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 6.33 (brs, 1H), 6.79 (brs, 1H), 7.00 (d, J = 8.4 Hz, 1H), 7.32 (brs, 1H), 7.43 (s, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.64 (brs, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.95 (d, J = 8.4 Hz, 1H), 8.25 (s, 1H), 10.72 (s, 1H), 10.89 (s, 1H), 13.23 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 102.0, 111.9, 113.2, 117.3, 119.6, 120.4, 121.4, 125.9, 129.1, 130.2, 130.8, 131.4, 132.0, 133.3, 136.4, 137.1, 140.1, 151.1, 167.1, 170.1. Anal. ($C_{21}H_{14}Cl_2N_4O_3$) C, H, N. MS (ESI) m/z 438.9 $[M-H]^-$.

*4.1.12.7. N-(3-(1H-pyrrol-2-ylmethylidene)-2-oxoindolin-6-ylcarbamoyl)-2-fluorobenzamide (**32e**).* Condensation of **28e** (0.078 g, 0.25 mmol) with 1*H*-pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **32e** (0.075 g, 77%). mp: 276–281 °C (dec.). IR (ATR): 3456, 3327, 1712, 1669, 1465, 848, 769 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 6.33 (brs, 1H), 6.79 (brs, 1H), 7.01 (d, J = 8.0 Hz, 1H), 7.33–7.37 (m, 3H), 7.41 (s, 1H), 7.58 (d, J = 8.0 Hz, 1H), 7.61–7.71 (m, 3H), 10.55 (s, 1H), 10.90 (s, 1H), 11.05 (s, 1H), 13.22 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.4, 111.2, 112.6, 116.2 (J = 21.3 Hz), 116.6, 119.0, 119.8, 120.8, 122.5 (J = 13.5 Hz), 124.5 (J = 3.5 Hz), 125.2, 129.5, 130.0 (J = 2.0 Hz), 133.8 (J = 8.2 Hz), 136.4, 139.5, 150.2, 159.0 (J = 249.7 Hz), 166.3, 169.4. Anal. ($C_{21}H_{15}FN_4O_3 \cdot 1/5H_2O$) C, H, N. MS (ESI) m/z 389.0 $[M-H]^-$.

*4.1.12.8. N-(3-(1H-pyrrol-2-ylmethylidene)-2-oxoindolin-6-ylcarbamoyl)-4-(trifluoromethyl)-benzamide (**32f**).*
Condensation of **28f** (0.090 g, 0.25 mmol) with pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **32f** (0.072 g, 66%). mp > 300 °C. IR (ATR): 3445, 3320, 1712, 1671, 1460, 855, 763 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 6.33 (brs, 1H), 6.79 (brs, 1H), 7.02 (dd, J = 1.2, 8.4 Hz, 1H), 7.32 (brs, 1H), 7.45 (s, 1H), 7.58 (d, J = 8.0 Hz, 1H), 7.65 (s, 1H), 7.90 (d, J = 8.4 Hz, 2H), 8.18 (d, J = 8.4 Hz, 2H), 10.76 (s, 1H), 10.90 (s, 1H), 11.26 (s, 1H), 13.22 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.4, 111.2, 112.6, 116.6, 119.0, 119.8, 120.8, 123.6 (J = 271.1 Hz), 125.2, 125.4 (J = 3.7, 7.3 Hz), 129.1, 129.5, 132.3 (J = 31.9 Hz), 136.2, 136.4, 139.5, 150.5, 167.6, 169.4. Anal. ($C_{22}H_{15}F_3N_4O_3$) C, H, N. MS (ESI) m/z 439.1 $[M-H]^-$.

*4.1.12.9. N-(3-(1H-pyrrol-2-ylmethylidene)-2-oxoindolin-6-ylcarbamoyl)-4-chloro-3-(trifluoromethyl)benzamide (**32g**).*
Condensation of **28g** (0.099 g, 0.25 mmol) with pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **32g** (0.059 g, 50%). mp: 280–285 °C (dec.). IR (ATR): 3447, 3322, 1710, 1668, 1458, 852, 758 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 6.32–6.34 (m, 1H), 6.79 (brs, 1H), 7.00 (dd, J = 1.6, 8.0 Hz, 1H), 7.32 (brs, 1H), 7.43 (d, J = 1.6 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 8.255 (d, J = 8.4 Hz, 1H), 8.45 (s, 1H), 10.72 (s, 1H), 10.89 (s, 1H), 11.35 (s, 1H), 13.21 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.4, 111.3, 112.6, 116.6, 119.0, 119.8, 120.8, 122.4 (J = 271.1 Hz), 125.3, 126.7 (J = 31.2), 127.7 (J = 5.3, 10.0 Hz), 129.5, 131.7, 132.0, 133.9, 135.2 (J = 1.6 Hz), 136.4, 139.5, 150.5, 166.5, 169.5. Anal. ($C_{22}H_{14}ClF_3N_4O_3$) C, H, N. MS (ESI) m/z 472.9 $[M-H]^-$.

*4.1.12.10. N-(3-(1H-pyrrol-2-ylmethylidene)-2-oxoindolin-6-ylcarbamoyl)-4-methylbenzamide (**32h**).* Condensation of **28h** (0.077 g, 0.25 mmol) with 1*H*-pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **32h** (0.087 g, 91%). mp: 290–295 °C (dec.). IR (ATR): 3443, 3317, 1706, 1662, 1455, 848, 753 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H), 6.32–6.34 (m, 1H), 6.78–6.79 (m, 1H), 7.00 (dd, J = 2.0, 8.6 Hz, 1H), 7.32 (brs, 1H), 7.33 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 1.6 Hz, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.64 (s, 1H), 7.94 (d, J = 8.4 Hz, 2H), 10.89 (s, 1H), 10.94 (s, 1H), 10.96 (s, 1H), 13.22 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.8, 101.1, 111.0, 112.3, 116.5, 118.8, 119.5, 120.4, 124.9, 125.0, 128.1, 128.8, 129.1, 129.3, 136.4, 139.3, 143.2, 150.7, 168.2, 169.3. Anal. ($C_{22}H_{18}N_4O_3 \cdot 1/3H_2O$) C, H, N. ESI-MS: m/z 409.1 $[M+Na]^+$

*4.1.12.11. N-(3-(1H-pyrrol-2-ylmethylidene)-2-oxoindolin-6-ylcarbamoyl)-3,5-dimethoxybenzamide (**32i**).* Condensation of **28i** (0.088 g, 0.25 mmol) with 1*H*-pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **32i** (0.078 g, 72%). mp: 276–281 °C (dec.). IR (ATR): 3448, 3312, 1706, 1667, 1448, 852, 758 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.81 (s, 6H), 6.33 (brs, 1H), 7.65 (s, 1H), 6.75 (s, 1H), 6.79 (brs, 1H), 7.00 (dd, J = 1.2, 8.4 Hz, 1H), 7.20 (d, J = 2 Hz, 2H), 7.33 (brs, 1H), 7.44 (brs, 1H), 7.58 (d, J = 8.4 Hz, 1H), 10.90 (s, 1H), 10.91 (s, 1H), 13.22 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.4, 101.2, 105.1, 105.8, 111.1, 112.4, 116.5, 118.9, 119.6, 120.6, 125.1, 125.1, 129.4, 133.9, 136.4, 139.4, 150.6, 160.2, 167.9, 169.3. Anal. ($C_{23}H_{20}N_4O_5 \cdot 1/3H_2O$) C, H, N. MS (ESI) m/z 431.1 $[M-H]^-$.

*4.1.12.12. N-(3-(1H-pyrrol-2-ylmethylidene)-2-oxoindolin-6-ylcarbamoyl)-4-(dimethylamino)benzamide (**32j**).* Condensation of **28j** (0.084 g, 0.25 mmol) with 1*H*-pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **32j** (0.083 g, 80%). mp: 285–290 °C (dec.). IR (ATR): 3453, 3318, 1700, 1665, 1465, 830, 758 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.02 (s, 6H), 6.34 (d, J = 2.8 Hz, 1H), 6.75 (d, J = 8.8 Hz, 2H), 6.80 (brs, 1H), 7.22 (d, J = 8.0 Hz, 1H), 7.33 (brs, 1H), 7.50 (s, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 8.8 Hz, 2H), 10.74 (s, 1H), 10.93 (s, 1H), 11.29 (s, 1H), 13.23 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 40.1, 102.2, 111.4, 111.9, 113.4, 115.4, 117.3, 117.9, 119.4, 120.5, 121.7, 125.9, 130.2, 131.0, 137.6, 139.9, 154.1, 160.5, 165.5, 170.1. Anal. ($C_{23}H_{21}N_5O_3 \cdot 5/4H_2O$) C, H, N. ESI-MS: m/z 437.1 $[M-H+Na]^+$.

*4.1.12.13. N-(3-(1H-Pyrrol-2-ylmethylidene)-2-oxoindolin-7-ylcarbamoyl)-4-methoxybenzamide (**33**).* Condensation of **29** (0.081 g, 0.25 mmol) with 1*H*-pyrrole-2-carbaldehyde (0.026 g, 0.28 mmol) afforded **33** (0.079 g, 80%). mp: 278–283 °C (dec.). IR (ATR): 3440, 3310, 1700, 1668, 1462, 832, 757 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.83 (s, 3H), 6.32–6.34 (m, 1H), 6.78–6.79 (m, 1H), 7.0 (dd, J = 1.6 Hz, 8.2 Hz, 1H), 7.05 (d, J = 8.8 Hz, 2H) 7.32 (brs, 1H), 7.45 (d, J = 2 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.63 (s, 1H), 8.04 (d, J = 8.8 Hz, 2H), 10.87 (s, 1H), 10.89 (s, 1H), 11.03 (s, 1H), 13.23 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.5, 101.3, 111.2, 112.5, 113.8, 116.7, 119.0, 119.7, 120.6, 124.0, 125.1, 125.2, 129.6, 130.4, 136.7, 139.5, 151.1, 163.0, 167.9, 169.5. Anal. ($C_{22}H_{18}N_4O_4$) C, H, N. ESI-MS: m/z 401.0 $[M-H]^-$.

*4.1.12.14. 5-((6-(3-(4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (**34**).* Condensation of **28a** (0.081 g, 0.25 mmol) with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (0.046 g, 0.28 mmol) afforded **34** (0.061 g, 52%). mp > 300 °C. IR (ATR): 3455, 3330, 1701, 1660, 1458, 838, 759 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.47 (s, 3H), 2.52 (s, 3H), 3.84 (s, 3H), 6.98 (dd, J = 1.6, 8.4 Hz, 1H), 7.05 (d,

J = 8.8 Hz, 2H), 7.46 (d, J = 1.6 Hz, 1H), 7.57 (s, 1H), 7.74 (d, J = 8.4 Hz, 1H), 8.04 (d, J = 8.8 Hz, 2H), 10.86 (s, 1H), 10.94 (s, 1H), 11.02 (s, 1H), 13.70 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.3, 14.4, 55.5, 101.3, 112.5, 113.8, 116.1, 119.4, 120.8, 122.1, 124.0, 126.0, 130.4, 136.6, 139.1, 139.8, 151.0, 163.0, 166.0, 167.9, 169.8. Anal. ($C_{25}H_{22}N_4O_6 \cdot 2/3H_2O$) C, H, N. MS (ESI) m/z 473.0 $[M-H]^-$.

*4.1.12.15. N-(3-((3,5-dimethyl-1H-pyrrol-2-yl)methylidene)-2-oxoindolin-6-ylcarbamoyl)-4-methoxybenzamide (**35**).* Condensation of **28a** (0.081 g, 0.25 mmol) with 3,5-dimethyl-1*H*-pyrrole-2-carbaldehyde (0.034 g, 0.28 mmol) afforded **35** (0.071 g, 67%). mp: 293–298 °C (dec.). IR (ATR): 3447, 3315, 1715, 1698, 1438, 839, 756 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 3H), 2.30 (s, 3H), 3.84 (s, 3H), 5.97 (s, 1H), 6.96 (dd, J = 1.6, 8.4 Hz, 1H), 7.06 (d, J = 9.2 Hz, 2H), 7.44 (brs, 1H), 7.47 (s, 1H), 7.66 (d, J = 8.0 Hz, 1H), 8.05 (d, J = 8.8 Hz, 2H), 10.79 (s, 1H), 10.85 (s, 1H), 11.00 (s, 1H), 13.22 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.2, 13.4, 55.5, 101.2, 112.3, 112.6, 113.8, 118.5, 121.4, 122.3, 124.0, 126.5, 130.4, 130.9, 135.1, 135.7, 138.6, 151.0, 163.0, 167.8, 169.6. Anal. ($C_{24}H_{22}N_4O_4 \cdot 1/3H_2O$) C, H, N. ESI-MS: m/z 453.1 $[M+Na]^+$

*4.1.12.16. N-(3-((3,5-Dimethyl-4-phenyl-1H-pyrrol-2-yl)methylidene)-2-oxoindolin-6-ylcarbamoyl)-4-methoxybenzamide (**36**).* Condensation of **28a** (0.081 g, 0.25 mmol) with 3,5-dimethyl-4-phenyl-1*H*-pyrrole-2-carbaldehyde (0.055 g, 0.28 mmol) afforded **36** (0.073 g, 58%). mp > 300 °C. IR (ATR): 3456, 3339, 1705, 1669, 1450, 840, 760 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 3H), 2.33 (s, 3H), 3.84 (s, 3H), 6.98 (dd, J = 1.6, 8.2 Hz, 1H), 7.06 (d, J = 8.8 Hz, 2H), 7.28–7.35 (m, 3H), 7.43 (t, J = 7.2 Hz, 2H), 7.47 (d, J = 2.0 Hz, 1H), 7.59 (s, 1H), 7.71 (d, J = 8.0 Hz, 1H), 8.05 (d, J = 8.8 Hz, 2H), 10.85 (s, 2H), 11.02 (s, 1H), 13.54 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.2, 12.6, 55.5, 101.2, 112.3, 113.6, 113.8, 118.8, 121.3, 122.4, 124.0, 124.8, 126.1, 127.6, 128.2, 129.4, 130.4, 132.6, 134.6, 135.9, 138.7, 151.0, 163.0, 167.9, 169.7. Anal. ($C_{30}H_{26}N_4O_4 \cdot 1/3H_2O$) C, H, N. MS (ESI) m/z 505.0 $[M-H]^-$.

*4.1.12.17. 5-((6-(3-(4-Methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-1H-pyrrole-2-carboxylic acid (**37**).* Condensation of **28a** (0.081 g, 0.25 mmol) with 5-formyl-1*H*-pyrrole-2-carboxylic acid (0.038 g, 0.28 mmol) afforded **37** (0.065 g, 59%). mp: 286–291 °C (dec.). IR (ATR): 3457, 3336, 1707, 1675, 1474, 838, 758 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.84 (s, 3H), 6.78 (s, 1H), 6.87 (s, 1H), 7.03–7.07 (m, 3H), 7.48 (s, 1H), 7.63 (d, J = 8.0 Hz, 1H), 7.69 (s, 1H), 8.04 (d, J = 8.8 Hz, 2H), 10.90 (s, 1H), 11.03 (s, 1H), 11.06 (s, 2H), 13.68 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.5, 101.5, 112.8, 113.8, 115.7, 119.1, 119.7, 120.0, 121.5, 123.8, 124.0, 126.8, 130.4, 132.3, 137.9, 140.5, 151.1, 161.2, 163.0, 167.9, 169.2. Anal. ($C_{23}H_{18}N_4O_6 \cdot H_2O$) C, H, N. MS (ESI) m/z 445.0 $[M-H]^-$.

*4.1.12.18. 2-(5-((6-(3-(4-Methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)acetic acid (**38**).* Condensation of **28a** (0.081 g, 0.25 mmol) with 2-(5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)acetic acid (0.050 g, 0.28 mmol) afforded **38** (0.057 g, 48%). mp: 240–245 °C (dec.). IR (ATR): 3447, 3335, 1705, 1680, 1472, 841, 762 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.19 (s, 3H), 2.45 (s, 3H), 3.27 (s, 2H), 3.81 (s, 3H), 6.97 (dd, J = 2.0, 8.4 Hz, 1H), 7.04 (d, J = 9.2 Hz, 2H), 7.37 (d, J = 1.6 Hz, 1H), 7.45 (s, 1H), 7.62 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 8.8 Hz, 2H), 10.77 (s, 1H), 10.93 (s, 1H), 13.23 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.8, 12.2, 31.0, 55.8, 101.6, 112.5, 112.8, 114.1, 118.0, 118.7, 121.9, 122.7, 124.3, 125.9, 130.3, 130.7, 134.6, 135.7, 138.6, 151.3, 163.3, 168.3, 169.9, 173.4. Anal. ($C_{26}H_{24}N_4O_6 \cdot H_2O$) C, H, N. MS (ESI) m/z 487.0 $[M-H]^-$.

*4.1.12.19. 3-(5-((6-(3-(4-Methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)propanoic acid (**39**).* Condensation of **28a** (0.081 g, 0.25 mmol) with 3-(5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid (0.054 g, 0.28 mmol) afforded **39** (0.065 g, 52%). mp: 240–245 °C (dec.). IR (ATR): 3450, 3339, 1699, 1670, 1468, 842, 761 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 3H), 2.24 (s, 3H), 2.34 (t, J = 7.6 Hz, 2H), 2.63 (t, J = 7.6 Hz, 2H), 3.84 (s, 3H), 6.95 (dd, J = 1.6, 8.4 Hz, 1H), 7.06 (d, J = 8.8 Hz, 2H), 7.43 (d, J = 1.6 Hz, 1H), 7.47 (s, 1H), 7.66 (d, J = 8.4 Hz, 1H), 8.04 (d, J = 8.8 Hz, 2H), 10.76 (s, 1H), 10.99 (s, 1H), 12.17 (brs, 1H), 13.26 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.3, 11.8, 19.4, 34.5, 55.5, 101.2, 112.2, 112.3, 113.8, 118.4, 121.6, 121.7, 122.3, 124.0, 125.7, 129.0, 130.4, 133.3, 135.5, 138.4, 151.0, 163.0, 167.9, 169.6, 173.9. Anal. ($C_{27}H_{26}N_4O_6 \cdot 1/2H_2O$) C, H, N. MS (ESI) m/z 501.0 $[M-H]^-$.

*4.1.12.20. 5-((5-Fluoro-6-(3-(4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (**40**).* Condensation of **28l** (0.085 g, 0.25 mmol) with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (0.046 g, 0.28 mmol) afforded **40** (0.089 g, 74%). mp: 290–295 °C (dec.). IR (ATR): 3457, 3333, 1712, 1685, 1439, 839, 745 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.45 (s, 3H), 2.48 (s, 3H), 3.80 (s, 3H), 7.01 (d, J = 8.8 Hz, 2H), 7.60 (s, 1H), 7.83–7.86 (m, 2H), 8.02 (d, J = 8.8 Hz, 2H), 10.87 (s, 1H), 11.00 (s, 1H), 11.36 (d, J = 2.4 Hz, 1H), 13.69 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.0, 15.0, 56.1, 102.5, 106.6 (J = 23.5 Hz), 114.3, 114.6, 116.3 (J = 3.0 Hz), 121.0 (J = 9.0 Hz), 123.9, 124.3, 124.8 (J = 12.3 Hz), 126.6, 131.1, 133.1, 135.4 (J = 1.6 Hz), 140.9, 148.8 (J = 232.8 Hz), 151.5, 163.7, 166.5, 168.6. 170.2. Anal. ($C_{25}H_{21}FN_4O_6 \cdot 4/3H_2O$) C, H, N. MS (ESI) m/z 491.0 $[M-H]^-$.

*4.1.12.21. 3-(5-((5-Fluoro-6-(3-(4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)-methyl)-2,4-dimethyl-1H-pyrrol-3-yl)propanoic acid (**41**).* Condensation of **28m** (0.085 g, 0.25 mmol) with 3-(5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid (0.054 g, 0.28 mmol) afforded **41** (0.060 g, 46%). mp: 265–270 °C. IR (ATR): 3455, 3340, 1709, 1678, 1441, 839, 757 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.25 (s, 3H), 2.28 (s, 3H), 2.34 (t, J = 7.2 Hz, 2H), 2.63 (t, J = 6.4 Hz, 2H), 3.84 (s, 3H), 7.05 (d, J = 8.4 Hz, 2H), 7.54 (s, 1H), 7.79–7.85 (m, 2H), 8.06 (d, J = 8.4 Hz, 2H), 10.72 (s, 1H), 11.02 (s, 1H), 11.35 (s, 1H), 13.32 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.3, 11.8, 19.4, 34.4, 55.5, 101.8, 105.1 (J = 22.7 Hz), 111.8 (J = 3.1 Hz), 113.8, 121.2 (J = 9.2 Hz), 122.0, 123.0 (J = 12.4 Hz), 123.5, 123.8, 125.8, 130.1, 130.5, 134.1 (J = 30.5 Hz), 148.2 (J = 232.2 Hz), 150.9, 163.1, 168.1, 169.4, 173.9. Anal. ($C_{27}H_{25}FN_4O_6 \cdot 7/3H_2O$) C, H, N. MS (ESI) m/z 519.0 $[M-H]^-$.

*4.1.12.22. 5-((6-(3-(2-Fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (**42**).* Condensation of **28k** (0.085 g, 0.25 mmol) with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (0.046 g, 0.28 mmol) afforded **42** (0.090 g, 74%). mp: 295–300 °C (dec.). IR (ATR): 3458, 3340, 1717, 1696, 1470, 846, 755 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.47 (s, 3H), 2.52 (s, 3H), 3.84 (s, 3H), 6.89 (dd, J = 2.0, 8.8 Hz, 1H), 6.96 (dd, J = 2.0, 13.2 Hz, 1H), 6.99 (dd, J = 2.0, 8.6 Hz, 1H), 7.42 (d, J = 1.6 Hz, 1H), 7.57 (s, 1H), 7.69 (dd, J = 8.4, 8.8 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 10.65 (s, 1H), 10.72 (s, 1H), 10.94 (s, 1H), 12.07 (brs, 1H), 13.69 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.9, 14.0, 55.6, 100.9, 101.5 (J = 25.6 Hz), 110.2 (J = 2.6 Hz), 112.1, 113.4, 113.5 (J = 12.5 Hz), 115.7, 119.0, 120.5, 121.7, 125.6, 131.1 (J = 3.5 Hz), 131.2, 136.0, 138.7, 139.4, 150.0, 160.5 (J = 250.9 Hz), 163.3 (J = 11.9 Hz), 165.3 (J = 2.1 Hz), 165.5, 169.4. Anal. ($C_{25}H_{21}FN_4O_6 \cdot 5/4H_2O$) C, H, N. MS (ESI) m/z 491.0 $[M-H]^-$.

*4.1.12.23. 3-(5-((6-(3-(2-Fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)propanoic acid (**43**).* Condensation of **28k** (0.085 g, 0.25 mmol) with 3-(5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid (0.054 g, 0.28 mmol) afforded **43** (0.063 g, 49%). mp: 255–260 °C (dec.). IR (ATR): 3456, 3341, 1716, 1687, 1472, 844, 758 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.24 (s, 3H), 2.28 (s, 3H), 2.34 (t, J = 7.6 Hz, 2H), 2.63 (t, J = 7.6 Hz, 2H), 3.84 (s, 3H), 6.90 (dd, J = 2.0, 8.6 Hz, 1H), 6.95–6.98 (m, 2H), 7.40 (d, J = 1.6 Hz, 1H), 7.48 (s, 1H), 7.64–7.71 (m, 2H), 10.63 (s, 1H), 10.76 (s, 1H), 13.26 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.3, 11.8, 19.4, 34.6, 56.0, 101.2, 102.0 (J = 25.6 Hz), 110.6 (J = 2.6 Hz), 112.2, 114.0 (J = 12.5 Hz), 118.4, 121.7, 122.3, 125.7, 129.1, 131.5 (J = 3.6 Hz), 133.4, 135.3, 138.4, 150.4, 160.9 (J = 250.7 Hz), 163.7 (J = 11.6 Hz), 165.7 (J = 1.8 Hz), 169.6, 173.9. Anal. ($C_{27}H_{25}FN_4O_6 \cdot 1/2H_2O$) C, H, N. MS (ESI) m/z 519.0 $[M-H]^-$.

*4.1.12.24. 5-((5-Fluoro-6-(3-(2-fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (**44**).* Condensation of **28m** (0.090 g, 0.25 mmol) with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (0.046 g, 0.28 mmol) afforded **44** (0.096 g, 76%). mp: 297 to >300 °C. IR (ATR): 3460, 3348, 1709, 1670, 1465, 864, 770 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.50 (s, 3H), 2.52 (s, 3H), 3.85 (s, 3H), 6.90 (dd, J = 2.4, 8.8 Hz, 1H), 6.97 (dd, J = 2.4, 12.8 Hz, 1H), 7.66 (s, 1H), 7.66 (dd, J = 8.8, 8.8 Hz, 1H), 7.84 (d, J = 6.4 Hz, 1H), 7.91 (d, J = 11.2 Hz, 1H), 10.93 (s, 1H), 11.03 (s, 1H), 11.04 (s, 1H), 13.73 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.0, 15.0, 56.7, 102.5, 102.6, 102.7, 111.2 (J = 2.3 Hz), 114.3 (J = 12.3 Hz), 114.7, 116.3 (J = 3.1 Hz), 121.2 (J = 9.2 Hz), 124.2, 124.6 (J = 12.5 Hz), 126.7, 132.3 (J = 3.7 Hz), 133.3, 135.4 (J = 1.4 Hz), 141.1, 148.8 (J = 232.8 Hz), 150.9, 161.6 (J = 251.1 Hz), 164.4 (J = 11.5 Hz), 166.5, 166.7 (J = 2.0 Hz), 170.3. Anal. ($C_{25}H_{20}F_2N_4O_6 \cdot 1/2H_2O$) C, H, N. MS (ESI) m/z 509.1 $[M-H]^-$.

*4.1.12.25. 3-(5-((5-Fluoro-6-(3-(2-fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl) propanoic acid (**45**).* Condensation of **28m** (0.090 g, 0.25 mmol) with 3-(5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid (0.054 g, 0.28 mmol) afforded **45** (0.072 g, 54%). mp: 275–280 °C (dec.). IR (ATR): 3451, 3343, 1714, 1682, 1469, 849, 760 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.25 (s, 3H), 2.28 (s, 3H), 2.34 (t, J = 7.6 Hz, 2H), 2.63 (t, J = 7.6 Hz, 2H), 3.84 (s, 3H), 6.90 (d, J = 2.0 Hz, 8.8 Hz, 1H), 6.97 (dd, J = 2.4, 13.2 Hz, 1H), 7.56 (s, 1H), 7.70 (dd, J = 8.8, 8.4 Hz, 1H), 7.80 (d, J = 6.8 Hz, 1H), 7.81 (d, J = 11.2 Hz, 1H), 10.74 (s, 1H), 10.98 (s, 1H), 10.99 (s, 1H), 13.32 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 8.9, 11.4, 19.0, 34.0, 55.6, 101.4, 101.7, 104.8 (J = 23.1 Hz), 110.2 (J = 2.4 Hz), 111.3 (J = 2.9 Hz), 113.4 (J = 12.3 Hz), 121.0 (J = 9.2 Hz), 121.7, 122.4 (J = 12.4 Hz), 123.2, 125.4, 129.8, 131.2 (J = 3.6 Hz), 133. 6, 134.0, 147.8 (J = 232.0 Hz), 149.9, 160.5 (J = 251.0 Hz), 163.4 (J = 11.7 Hz), 165.7 (J = 1.9 Hz), 169.0, 173.5. Anal. ($C_{27}H_{24}F_2N_4O_6 \cdot 2/3H_2O$) C, H, N. MS (ESI) m/z 537.0 $[M-H]^-$.

*4.1.12.26. Ethyl 3-(5-((6-(3-(2-fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrole-3-carboxylate (**46**).* Condensation of **28k** (0.085 g, 0.25 mmol) with **7** (0.054 g, 0.28 mmol) afforded **46** (0.081 g, 63%). mp: 278–283 °C (dec.). IR (ATR): 3455, 3330, 1716, 1663, 1470, 835, 745 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.29 (t, J = 7.0 Hz, 3H), 2.48 (s, 3H), 2.52 (s, 3H), 3.84 (s, 3H), 4.20 (q, J = 7.2 Hz, 2H), 6.90 (dd, J = 2.2, 8.4 Hz, 1H), 6.95–7.00 (m, 2H), 7.43 (d, J = 1.6 Hz, 1H), 7.58 (s, 1H), 7.67 (dd, J = 8.4, 8.8 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 10.66 (s, 1H), 10.73 (s, 1H), 10.97 (s, 1H), 13.75 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.3, 14.2, 14.4, 56.0, 59.0, 101.3, 102.0 (J = 25.6 Hz), 110.6 (J = 2.4 Hz), 112.6, 113.1, 114.0 (J = 12.4 Hz), 116.5, 119.5, 120.8, 122.0, 126.0, 131.1, 131.5 (J = 3.4 Hz), 136.5, 139.2, 139.7, 150.4, 160.9 (J = 251.0 Hz), 163.7 (J = 11.7 Hz), 164.3, 165.7 (J = 1.8 Hz), 169.8. Anal. ($C_{27}H_{25}FN_4O_6$) C, H, N. MS (ESI) m/z 501.1 $[M-H]^-$.

*4.1.12.27. Ethyl 3-(5-((6-(3-(2-fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)propanoate (**47**).* Condensation of **28k** (0.085 g, 0.25 mmol) with **8** (0.062 g, 0.28 mmol) afforded **47** (0.061 g, 45%). mp: 250–255 °C (dec.). IR (ATR): 3449, 3342, 1712, 1670, 1466, 838, 751 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.15 (t, J = 7.0 Hz, 3H), 2.23 (s, 3H), 2.27 (s, 3H), 2.42 (t, J = 7.6 Hz, 2H), 2.66 (t, J = 7.4 Hz, 2H), 3.84 (s, 3H), 4.03 (q, J = 7.2 Hz, 2H), 6.90 (dd, J = 2.0, 8.4 Hz, 1H), 6.95–6.98 (m, 2H), 7.39 (brs, 1H), 7.48 (s, 1H), 7.65–7.71 (m, 2H), 10.62 (s, 1H), 10.73 (s, 1H), 10.76 (s, 1H), 13.26 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 8.9, 11.3, 13.6, 18.9, 33.9, 55.6, 59.3, 100.7, 101.5 (J = 25.6 Hz), 110.2 (J = 2.7 Hz), 111.8, 111.9, 113.6 (J = 12.7 Hz), 118.0, 120.9, 121.2, 121.9, 125.3, 128.6, 131.1 (J = 3.7 Hz), 132.9, 134.9, 138.0, 149.9, 160.5 (J = 250.6 Hz), 163.2 (J = 11.6 Hz), 165.3 (J = 2.0 Hz), 169.1, 171.8. Anal. ($C_{29}H_{29}FN_4O_6$) C, H, N. MS (ESI) m/z 547.0 $[M-H]^-$.

*4.1.12.28. (5-((6-(3-(2-Fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl) 4-methylpiperazin-1-yl ketone (**48**).* Condensation of **28k** (0.085 g, 0.25 mmol) with (5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl) 4-methylpiperazin-1-yl ketone **9** (0.069 g, 0.28 mmol) afforded **48** (0.049 g, 35%). mp: 250–255 °C (dec.). IR (ATR): 3449, 3329, 1706, 1670, 1462, 841, 752 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.17 (s, 3H), 2.22 (brs, 4H), 2.26 (s, 6H), 3.44 (brs, 4H), 3.83 (s, 3H), 6.89 (dd, J = 2.4, 8.8 Hz, 1H), 6.94–6.99 (m, 2H), 7.41 (d, J = 2.0 Hz, 1H), 7.52 (s, 1H), 7.68 (dd, J = 8.4, 8.8 Hz, 1H), 7.70 (d, J = 8.0 Hz, 1H), 10.64 (s, 1H), 10.72 (s, 1H), 10.88 (s, 1H), 13.43 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.0, 12.3, 45.6, 56.0, 101.3, 102.0 (J = 25.7 Hz), 110.6 (J = 2.6 Hz), 112.5, 114.0 (J = 12.6 Hz), 114.9, 119.1, 119.7, 121.1, 122.2, 125.9, 127.4, 131.5 (J = 3.7 Hz), 132.9, 136.1, 138.9, 150.4, 160.9 (J = 250.7 Hz), 163.7 (J = 11.8 Hz), 165.3, 165.7 (J = 1.9 Hz), 169.7. Anal. ($C_{30}H_{31}FN_6O_5 \cdot 3/2H_2O$) C, H, N. MS (ESI) m/z 573.1 $[M-H]^-$.

*4.1.12.29. 2-(5-((6-(3-(2-Fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)ethyl 4-methylpiperazin-1-yl ketone (**49**).* Condensation of **28k** (0.085 g, 0.25 mmol) with 2-(5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)ethyl 4-methylpiperazin-1-yl ketone **10** (0.077 g, 0.28 mmol) afforded **49** (0.054 g, 37%). mp: 229–234 °C (dec.). IR (ATR): 3451, 3340, 1700, 1679, 1457, 842, 751 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.11 (s, 3H), 2.12–2.14 (m, 2H), 2.19–2.20 (m, 2H), 2.23 (s, 3H), 2.27 (s, 3H), 2.41 (t, J = 7.6 Hz, 2H), 2.62 (t, J = 7.6 Hz, 2H), 3.42 (brs, 4H), 3.84 (s, 3H), 6.90 (dd, J = 2.4, 8.4 Hz, 1H), 6.95–6.99 (m, 2H), 7.41 (brs, 1H), 7.48 (s, 1H), 7.65–7.71 (m, 2H), 10.63 (s, 1H), 10.76 (s, 2H), 13.27 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.3, 11.8, 19.9, 32.7, 40.8, 44.8, 45.5, 54.2, 54.5, 56.0, 101.2 (J = 2.2 Hz), 102.0 (J = 25.8 Hz), 110.6 (J = 2.5 Hz), 112.1, 112.3, 114.0 (J = 12.6 Hz), 118.4, 120.8, 121.9 (J = 31.8 Hz), 124.5, 125.7, 129.2, 131.5 (J = 3.7 Hz), 133.4, 135.3, 136.9, 138.3, 144.1, 150.4, 160.9 (J = 250.6 Hz), 163.7 (J = 11.7 Hz), 165.7, 169.6, 170.1, 176.6. Anal. ($C_{32}H_{35}FN_6O_5 \cdot 5/4H_2O$) C, H, N. MS (ESI) m/z 601.1 $[M-H]^-$.

*4.1.12.30. (5-((6-(3-(2-Fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl) morpholin-4-yl ketone (**50**).* Condensation of **28k** (0.085 g, 0.25 mmol) with (5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl) morpholin-4-yl ketone **11** (0.066 g, 0.28 mmol) afforded **50** (0.053 g, 39%). mp: 273–278 °C (dec.). IR (ATR): 3455, 3330, 1716, 1660, 1460, 835, 745 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.25 (s, 3H), 2.28 (s, 3H), 3.46 (brs, 4H), 3.57 (brs, 4H), 3.84 (s, 3H), 6.90 (dd, J = 2.4, 8.4 Hz, 1H), 6.95–7.00 (m, 2H), 7.42 (d, J = 1.6 Hz, 1H), 7.53 (s, 1H), 7.67–7.73 (m, 2H), 10.65 (s, 2H), 10.90 (s, 1H), 13.45 (s, 1H). ^{13}C NMR (100 MHz,

DMSO-d_6): δ 10.1, 12.5, 56.1, 66.5, 101.4, 102.1 (J = 25.4 Hz), 110.7 (J = 2.4 Hz), 112.6, 113.3, 114.1 (J = 12.6 Hz), 115.1, 119.2, 119.4, 121.2, 122.3, 126.0, 127.6, 131.7 (J = 3.8 Hz), 133.2, 136.3, 139.0, 150.5, 161.0 (J = 250.7 Hz), 163.8 (J = 11.5 Hz), 165.6, 165.8 (J = 2.0 Hz), 169.8. Anal. ($C_{29}H_{28}FN_5O_6 \cdot 1/2H_2O$) C, H, N. MS (ESI) m/z 560.0 $[M-H]^-$.

*4.1.12.31. 2-(5-((6-(3-(2-Fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrol-3-yl)ethyl morpholin-4-yl ketone (**51**).* **28k** (0.085 g, 0.25 mmol) on condensation with 2-(5-formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)ethyl morpholin-4-yl ketone **12** (0.074 g, 0.28 mmol) afforded **51** (0.049 g, 34%). mp: 248–253 °C (dec.). IR (ATR): 3456, 3329, 1711, 1667, 1456, 838, 751 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.24 (s, 3H), 2.28 (s, 3H), 2.42 (t, J = 7.6 Hz, 2H), 2.63 (t, J = 7.6 Hz, 2H), 3.34–3.49 (m, 8H), 3.84 (s, 3H), 6.90 (d, J = 8.4 Hz, 1H), 6.95–6.98 (m, 2H), 7.40 (brs, 1H), 7.48 (s, 1H), 7.65–7.71 (m, 2H), 10.63 (s, 1H), 10.72 (s, 1H), 10.76 (s, 1H), 13.27 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 8.9, 11.4, 19.3, 32.2, 40.9, 45.0, 55.6, 65.5, 65.5, 100.7, 101.5 (J = 25.5 Hz), 110.2 (J = 2.3 Hz), 111.7, 111.8, 113.6 (J = 12.7 Hz), 118.0, 121.3, 121.6, 121.9, 125.3, 128.8, 131.1 (J = 3.5 Hz), 133.0, 134.9, 137.9, 149.9, 160.5 (J = 250.7 Hz), 163.2 (J = 11.7 Hz), 165.3, 169.1, 170.0. Anal. ($C_{31}H_{32}FN_5O_6 \cdot 2/3H_2O$) C, H, N. MS (ESI) m/z 588.1 $[M-H]^-$.

*4.1.12.32. (N-(2-(Dimethylamino)ethyl)-5-((6-(3-(2-fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrole-3-carboxamide (**52**).* Condensation of **28k** (0.085 g, 0.25 mmol) with *N*-(2-(dimethylamino)ethyl)-5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxamide (**13**, 0.066 g, 0.28 mmol) afforded **52** (0.059 g, 43%). mp: 298–300 °C (dec.). IR (ATR): 3457, 3331, 1709, 1670, 1453, 841, 754 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.18 (s, 6H), 2.36–2.40 (m, 5H), 2.41 (s, 3H), 3.28–3.29 (m, 2H), 3.84 (s, 3H), 6.90 (dd, J = 2.4, 8.6 Hz, 1H), 6.95–7.00 (m, 2H), 7.42 (d, J = 1.6 Hz, 1H), 7.44 (t, J = 5.6 Hz, 1H), 7.54 (s, 1H), 7.67–7.73 (m, 2H), 10.65 (s, 1H), 10.73 (s, 1H), 10.90 (s, 1H), 13.49 (s, 1H). Anal. ($C_{29}H_{31}FN_6O_5$) C, H, N. MS (ESI) m/z 589.1 $[M-H]^-$.

*4.1.12.33. N-(2-(Diethylamino)ethyl)-5-((6-(3-(2-fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrole-3-carboxamide (**53**).* Condensation of **28k** (0.085 g, 0.25 mmol) with *N*-(2-(diethylamino)ethyl)-5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxamide (**14**, 0.074 g, 0.28 mmol) afforded **53** (0.052 g, 36%). mp: 275–280 °C (dec.). IR (ATR): 3452, 3332, 1708, 1672, 1457, 841, 753 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 0.97 (t, J = 7.0 Hz, 6H), 2.39 (s, 3H), 2.43 (s, 3H), 2.52–2.56 (m, 4H), 3.25–3.28 (m, 2H), 3.84 (s, 3H), 6.90 (dd, J = 2.4, 8.8 Hz, 1H), 6.95–7.00 (m, 2H), 7.38 (t, J = 5.4 Hz, 1H), 7.42 (d, J = 2.0 Hz, 1H), 7.55 (s, 1H), 7.69 (dd, J = 8.4, 8.8 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 10.65 (s, 1H), 10.74 (s, 1H), 10.90 (s, 1H), 13.50 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.1, 11.4, 12.8, 36.5, 46.1, 51.2, 55.7, 101.0, 101.6 (J = 25.7 Hz), 110.3 (J = 2.6 Hz), 112.2, 113.6 (J = 12.6 Hz), 114.6, 118.8, 119.8, 120.7, 122.0, 125.3, 128.1, 131.2 (J = 3.7 Hz), 135.0, 135.7, 138.5, 150.0, 160.5 (J = 250.7 Hz), 163.3 (J = 11.5 Hz), 164.2, 165.4 (J = 1.9 Hz), 169.4. Anal. ($C_{31}H_{35}FN_6O_5 \cdot 2/3H_2O$) C, H, N. MS (ESI) m/z 589.1 $[M-H]^-$.

*4.1.12.34. N-(2-(Pyrrolidin-1-yl)ethyl)-5-((6-(3-(2-fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrole-3-carboxamide (**54**).* Condensation of **28k** (0.085 g, 0.25 mmol) with *N*-(2-(pyrrolidin-1-yl)ethyl)-5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxamide (**15**, 0.073 g, 0.28 mmol) afforded **54** (0.072 g, 48%). mp: 291–296 °C (dec.). IR (ATR): 3455, 3330, 1703, 1673, 1439, 850, 760 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.68 (brs, 4H), 2.38 (s, 3H), 2.41 (s, 3H), 2.57 (t, J = 6.8 Hz, 2H), 3.84 (s, 3H), 6.90 (dd, J = 2.0, 8.8 Hz, 1H), 6.95–7.00 (m, 2H), 7.42 (d, J = 2.0 Hz, 1H), 7.480 (t, J = 5.6 Hz, 1H), 7.54 (s, 1H), 7.67–7.73 (m, 2H), 10.65 (s, 1H), 10.73 (s, 1H), 10.90 (s, 1H), 13.49 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.6, 13.3, 23.3, 38.1, 53.6, 54.9, 56.3, 101.5, 102.2 (J = 25.8 Hz), 110.8 (J = 2.7 Hz), 112.7, 114.2 (J = 12.5 Hz), 115.2, 119.3, 120.5, 121.3, 122.6, 125.9, 128.7, 131.8 (J = 3.6 Hz), 135.4, 136.3, 139.1, 150.6, 161.1 (J = 250.6 Hz), 163.9 (J = 11.8 Hz), 164.8, 166.0 (J = 2.0 Hz), 170.0. Anal. ($C_{31}H_{33}FN_6O_5 \cdot 1/2H_2O$) C, H, N. MS (ESI) m/z 587.1 $[M-H]^-$.

*4.1.12.35. Mesylate salt of N-(2-(Pyrrolidin-1-yl)ethyl)-5-((6-(3-(2-fluoro-4-methoxybenzoyl)ureido)-2-oxoindolin-3-ylidene)methyl)-2,4-dimethyl-1H-pyrrole-3-carboxamide (**54** mesylate salt).* Compound **54** (0.4 g, 0.67 mmol) was dissolved in DMSO (2 mL) and treated with $MeSO_3H$ (44 μL, 0.67 mmol). The resulting reaction mixture was stirred at room temperature for 12 h, and then the solvent was evaporated under reduced pressure (−2 mmHg) to give sticky residue. To this sticky residue was added acetone, and the resultant precipitates were collected by filtration and dried to give **54** as monomesylate salt (0.25 g, 59%). IR (ATR): 3453, 3328, 1705, 1680, 1441, 847, 757 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.87 (brs, 2H), 2.02 (brs, 2H), 2.30 (s, 3H), 2.43 (s, 3H), 2.46 (s, 3H), 3.04–3.06 (m, 2H), 3.53–3.57 (m, 2H), 3.65 (brs, 2H), 3.84 (s, 3H), 6.90 (dd, J = 2.0, 8.8 Hz, 1H), 6.96–7.01 (m, 2H), 7.44 (d, J = 2.0 Hz, 1H), 7.58 (s, 1H), 7.66–7.76 (m, 3H), 9.43 (s, 1H), 10.67 (s, 1H), 10.78 (s, 1H), 10.95 (s, 1H), 13.58 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.5, 13.4, 22.3, 35.2, 39.5, 53.4, 53.5, 55.9, 101.2, 101.9 (J = 25.5 Hz), 110.5 (J = 2.8 Hz), 112.4, 113.9 (J = 12.5 Hz), 115.4, 119.0, 119.2, 120.9, 122.2, 125.7, 128.5, 131.5 (J = 3.7 Hz), 135.7, 136.2, 138.9, 150.3, 160.8 (J = 250.8 Hz), 163.6 (J = 11.5 Hz), 165.4, 165.6 (J = 2.0 Hz), 169.7. Anal. ($C_{32}H_{37}FN_6O_8S$) C, H, N, S. MS (ESI) m/z 683.2 $[M-H]^-$.

4.2. Biology

4.2.1. In vitro kinase, hERG, and metabolic enzyme activity assays

4.2.1.1. In vitro kinase assay. All three assays were performed at Reaction Biology Corp., Malvern, PA under contract. Kinase inhibition assays were performed using a radioisotope-based P81 filter-binding assay. Compounds (10 mM stock DMSO solutions) were tested in the single-dose mode at 1 μM or in the 10-dose IC_{50} mode with three-fold serial dilutions starting at 10 μM with the final DMSO concentration of less than 1% (v/v). Each enzyme and its specific substrate were diluted in 20 mM HEPES, pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA, 0.02% (v/v) Brij35, 0.02 mg/mL BSA, 0.1 mM Na_3VO_4, 2 mM DTT, 1% (v/v) DMSO), and then a test compound was delivered into the mixture. Each reaction was initiated by adding radiolabeled [^{33}P]ATP (final concentration, 10 μM), held at room temperature, and stopped after 2 h by spotting the mixtures onto P81 ion-exchange paper (Whatman #3698–915). Unreacted [^{33}P] ATP was washed away with phosphoric acid (0.75% [v/v]) before detection. Staurosporine served as the control. IC_{50} values are reported as the mean of two independent reactions with coefficients of variation <30%. Inhibition of kinase activity in the presence of a test compound at 1 μM is reported as the percentage inhibition, with that of the vehicle blank defined as 0%. The peptides H-LRRASLG and EAIYAAPFAKKK served as the substrate for Aurora B and FLT3, respectively.

4.2.1.2. In vitro hERG binding assay. The hERG-binding assay involved a binding competition between the fluorescently labeled tracer Predictor hERG Tracer Red (1 nM) and a test compound for membrane-bound hERG (Predictor hERG Membrane). Each compound was tested in the 10-dose IC_{50} mode with three-fold serial dilutions starting at 100 μM. Each solution containing a compound was added into a membrane mixture by Acoustic Technologies,

followed by tracer addition, and gently mixed in dark. Fluorescence of each membrane preparation was measured after incubation at room temperature for 3 h and reported as the millipolarization units (mP, excitation, 531 nm; emission, 595 nm). The control compound, E-4031, was tested under the same conditions but its initial concentration was 30 μM. The assay mixture contained 25 mM HEPES, pH 7.5, 15 mM KCl, 1 mM $MgCl_2$, 0.05% (**v**/**v**) PF-127 (Acronyms), and 1% (v/v) DMSO.

4.2.1.3. In vitro metabolic enzyme activity assays. For the cytochrome P450 inhibition assays, each compound was examined in the 10-dose IC_{50} mode with three-fold serial dilutions starting at 10 μM. Ketoconazole and furafylline (for CYP1A2) served as controls and were tested under the same conditions. 3-Cyano-7-ethoxycoumarin served as the substrate for CYP1A2, CYP2C19, and CYP2D6. The Vivid OOMR substrate was used for the CYP2C9 and CYP3A4 assays.

4.2.2. Cell culture and survival assay

MV4-11 and MOLM-13 cells (**American Tissue Culture Collection:**CRL-9591 and CRL3002, respectively) were kindly provided by Dr. Hsiang-Wen Tseng, and HL-60 (BCRC 60027) and THP-1 (BCRC 60430) cells were obtained from Bioresource Collection and Research Center (Taiwan). All cells were maintained at 37 °C in a 5% CO_2, humidified incubator. The culture medium for MV4-11, MOLM-13 and THP-1 cells was RPMI1640 and that for HL-60 cells was IMDM. Medium was supplemented with 10% v/v fetal bovine serum, 1% w/v penicillin and 1% w/v streptomycin. Cells were seeded at a density of 5×10^3 cells per well with the appropriate medium in wells of 96-well plates and serum starved overnight. Cell samples were then treated with one of the compounds at various concentrations ranging from 0.01 to 10 μM (final DMSO concentration, 5% (v/v)) for 48 h in medium containing 5% (v/v) fetal bovine serum. Cell viability in all leukemia cells was determined by mitochondria activity assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (A_{600}) [70]. Cells that were treated with only vehicle served as the controls (100% activity). IC_{50} values for MTT assay were determined according to the relative viability of the cells after incubation with the compounds at the different concentrations by regression analysis.

4.2.3. Dot-blot analyses of phospho-signaling Aurora B and FLT3 paths and FasL

GH Polypro membranes (0.2 μM hydrophilic polypropylene, AcroPrep, Pall) each in a well of a 96-well microplate were wet with 70% (v/v) ethanol (100 μL) for 15 s and then with water (100 μL) under vacuum using an Aurum Vacuum manifold (Bio-Rad). Cell lysates (20 μg protein equivalent in 50 μL phosphate buffered saline (PBS)), prepared as described below, were added into each well and allowed to stand at rt for 10 min. The microplate was shaken on a plate shaker (Thermo Scientific) at a low speed at rt for 10 min. Plates were then washed with PBS, and the membranes were blocked with 0.5% BSA in PBS for 5 min while shaken. After removal of the solvent, primary antibodies were added into each well and incubated with the cell lysate while shaking at 4 °C for 4 h. Anti-phospho-FLT3 (Cell Signaling Technology, 50 μL, 1:200), anti-phospho-STAT5 (Cell Signaling Technology, 50 μL, 1:200), anti-phospho-Aurora B (Genetex, 50 μL, 1:200), anti-phospho-histone H3 (Genetex, 50 μL, 1:200), anti-Fas (Santa Cruz Biotechnology, 50 μL, 1:200) and anti-actin (Santa Cruz, 50 μL, 1:200) were used. Then, samples were washed twice with PBS (100 μL). Goat anti-rabbit IgG-conjugated horseradish peroxidase (Genetex, 50 μL, 1:500) was added into each well, and the plates were incubated for 10 min with shaking. The samples were again washed twice with PBS (100 μL), and then 100 μL of diaminobenzidine solution (ImmPACT, Vector Laboratories) was added into each well. After incubated for a few minutes with shaking, a brown color appeared as the result of oxidation and polymerization of diaminobenzidine. Plates were washed with PBS and water, and then the blots in 96-well plate were scanned from top by scanner, and the blotting images were analyzed using Un-Scan-It gel software for quantification of the reactions. Three independent experiments, each in triplicate, were performed.

To prepare the cell lysates, cells that had been treated with 10 μM of **54** or AZD1152 at 37 °C for 4 h were lysed in 25 mM Tris–HCl, pH 7.6, 150 mM NaCl, 1% (v/v) NP-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), supplemented with a final concentration of 1X protease and phosphatase inhibitors (Roche Applied Science). Samples were kept on ice for 5 min with intermittent swirling and then centrifuged at 16,000 × *g* at 4 °C for 15 min to remove cell debris. The soluble portion of each cell lysate was stored at −20 °C as 50 μL aliquots.

4.2.4. Cell cycle G2M arrest analysis

Cells were cultured in 6-well plates, at a density of 8×10^4 cells per well, and exposed to 10 μM of **54** and sunitinib or to taxol as positive control (for G2M arrest). The cells were then washed with ice-cold PBS and collected and were stained for 20 min on ice in cold PBS containing 1.5 μM of PI (Sigma). For each sample 25 μL of cell suspension was loaded into one Tali™ Cellular Analysis Slide's chamber (Invitrogen) and analyzed in the Tali™ Image-Based Cytometer (Invitrogen). For each sample, 20 fields were analyzed. Counting parameters were set at 0.6 for sensitivity and 8 for circularity to reduce the background of cell debris. These settings led to an average of 5000 cells analyzed per sample. The experiment was repeated and the data are represented as the mean ± SEM of three independent experiments.

4.2.5. Caspase activation assays

The caspase 3/CPP32, caspase 8/FLICE and caspase 9/fluorometric assay kits (Enzo) were used to study apoptosis triggered by **54**. The MOLM-13 cells, at a density of 8×10^4 cells per well, were seeded into the wells of 6-well microplate. Cells treated with **54** in a concentration dependent manner for 24 h, and cells without treatment were taken as negative control. Cells were then harvested and lysed, and protein levels were detected. 50 ng/well protein was used for carrying out the caspase activation according to the manufacturer's protocol. The fluorescent intensity was measured by a Paradigm Microplate Reader (Molecular devices). Each fluorescent intensity value is presented as mean ± SEM of three independent experiments. Each experiment was done in triplicate.

4.2.6. PAMPA assay [71,72]

Effective permeability (P_e) was determined using a standard PAMPA assay conducted on a PAMPA Explorer instrument (pION Inc., Woburn, MA) as follows: 5 μL of stock solution (5 mM) in DMSO was diluted with 1 mL of system solution buffer, pH 7.4 to make 25 μM of diluted test solution. Then, 150 μL of the test solution was transferred to a UV-quartz plate, and the UV spectrum (200–500 nm) was read as the reference plate. The membrane (pore size 0.45, thickness 125 μm) of a STIRWELL PAMPA sandwich was coated with 4 μL of GIT lipid. The acceptor chamber was then filled with 200 μL of acceptor solution buffer, and the donor chamber was filled with 200 μL of tested solution. The STIRWELL PAMPA sandwich was assembled, and incubated at room temperature for 16 h without stirring. Then, it was carefully disassembled and both acceptor and donor chamber solutions (150 μL each) were transferred into UV-quartz plates. The UV spectrum (200–500 nm)

of the donor and the acceptor were read. The permeability coefficient was calculated using PAMPA Explorer command software (Version 3.7) based on the AUC of the reference plate, the donor plate, and the acceptor plate. All compounds were tested in triplicate, and the data are presented as an average value. Verapamil was used as standard for this assay. The 10 μL stock solution of verapamil in DMSO (10 mM) was diluted with system solution buffer to make 25 μM verapamil solution. All values are mean ± SEM of three independent experiments. Each experiment was done in duplicate.

4.2.7. *In vivo* pharmacokinetic studies

The *in vivo* pharmacokinetic profile of **54** was investigated using BALB/c mice, which were randomly assigned into three dosing groups. A single dose of **54** was given intravenously (2 mg/kg) or orally (10 and 30 mg/kg) in 10% (v/v) *N*-methyl-2-pyrrolidone (Sigma–Aldrich), 20% (v/v) Cremophor EL (Sigma–Aldrich), 70% (w/v) saline. Plasma was sampled for a total of six time points (0.5, 1, 3, 5, 8, and 24 h) and analyzed by liquid chromatography-coupled tandem mass spectroscopy. Pharmacokinetic parameters were characterized using WinNonlin 5.2 (Pharsight) and non-compartmental analysis.

4.2.8. *In vivo* tumor xenograft efficacy study

Animal studies were carried out in accordance with the guidelines set out by Ethical Committee for Animal Research, National Taiwan University and are in compliance with Animal Research of Agriculture Council, Republic of China. Male nude mice (BALB/cAnN.Cg-Foxnlnu/CrlNarl, 6–8 weeks old) were purchased from the National Applied Research laboratories (Taiwan). Each male BALB/C nude mice were implanted subcutaneously with 6×10^7 MOLM-13 (FLT3-ITD) leukemia cells in 0.2 mL of growth medium RPMI1640 (Sigma) containing antibiotics 100 IU penicillin/mL and supplemented with 10% fetal bovine serum (FBS) + 50% Matrigel through a 1 cm long 25G needle. After tumor volumes reached 150 mm^3, the mice were randomly assigned to 6 groups, and treatments were initiated. The mesylate salt of **54** (suspended in 0.2 mL saline) was administered i.p. at five doses (0, 4, 8, 10, 15 and 20 mg/kg) and orally at 50 mg/kg, once daily ($n \geq 5$ per group). The sizes of the tumors were measured by two perpendicular diameters (Length and Width) and tumor volume (mm^3) was calculated using the formula [73,74]: tumor volume $= 0.5 \times [\text{length} \times (\text{width})^2]$. After 16 days of treatment, the animals were sacrificed by cervical dislocation. Tumor sample were harvested from animals and post-fixed in 4% paraformaldehyde and weighted.

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Appendix A. Supplementary data

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