Articles

1,4-Dihydroindeno[1,2-*c*]pyrazoles with Acetylenic Side Chains as Novel and Potent Multitargeted Receptor Tyrosine Kinase Inhibitors with Low Affinity for the hERG Ion Channel

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Received October 18, 2006

The synthesis of a novel series of 1,4-dihydroindeno[1,2-*c*]pyrazoles with acetylene-type side chains is described. Optimization of those compounds as KDR kinase inhibitors identified **8**, which displayed an oral activity in an estradiol-induced murine uterine edema model (ED_{50} = 3 mg/kg) superior to Sutent (ED_{50} = 9 mg/kg) and showed potent antitumor efficacy in an MX-1 human breast carcinoma xenograft tumor growth model (tumor growth inhibition = 90% at 25 mg/kg·day po). The compound was docked into a homology model of the homo-tetrameric pore domain of the hERG potassium channel to identify strategies to improve its cardiac safety profile. Systematic interruption of key binding interactions between **8** and Phe656, Tyr652, and Ser624 yielded **90**, which only showed an IC_{50} of 11.6 μM in the hERG patch clamp assay. The selectivity profile for **8** and **90** revealed that both compounds are multitargeted receptor tyrosine kinase inhibitors with low nanomolar potencies against the members of the VEGFR and PDGFR kinase subfamilies.

Introduction

Protein kinases are a large (about 1.7% of all human genes) and diverse multigene family of enzymes, which mediate most of the signal transduction events in eukaryotic cells. They are characterized by their ability to transfer the γ-phosphate group of ATP to hydroxyl groups on their target proteins. All of the 518 protein kinases encoded in the human genome have been classified based on the phylogeny of their catalytic domains and distinct structural characteristics.[1] Approximately 90 protein kinases selectively catalyze the phosphorylation of tyrosine hydroxyl groups, and 58 of those protein tyrosine kinases were categorized as receptor tyrosine kinases (RTKs) due to the presence of a transmembrane spanning domain.[2] Further differentiation of the RTKs was achieved through comparison of their extracellular domains. For example, the members of the PDGF and VEGF receptor tyrosine kinase subfamilies contain five (class III RTKs) or seven (class V RTKs) immunoglobulin-like domains, respectively.[3,4] RTKs allosterically transmit extracellular signals through the plasma membrane and activate downstream signaling cascades which mediate growth, differentiation, and developmental signals in cells. Disregulation of those tightly controlled processes contributes significantly to the six hallmarks of cancer: self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, unlimited replicative potential, sustained angiogenesis, and tissue invasion and metastasis.[5] Members of the PDGF (PDGFR-α, PDGFR-β, FLT3, CSF1R, and cKit) and VEGF (KDR, FLT1, and FLT4) RTK subfamilies appear to play essential roles in all stages of tumor angiogenesis, are able to form autocrine loops which mediate cancer cell growth and survival, and drive hematologic malignancies.[6] Initial strategies to target these subfamilies for the development of anticancer therapies have focused on the selective inhibition of signaling by individual RTKs and have resulted in the approval of Avastin for the treatment of metastatic colorectal cancer[7] and Gleevec for cKit-driven gastrointestinal tumors.[8] However, more recent preclinical studies have demonstrated that simultaneous inhibition of multiple kinases by single-agent kinase inhibitors has the potential to greatly enhance antitumor activities, while still maintaining acceptable toxicity profiles. Proof-of-concept for this approach can be derived from Sunitinib maleate (Sutent, SU11248), a multitargeted (class III/V) RTK inhibitor, which was efficacious in clinical trials for the treatment of gastrointestinal stromal tumors and advanced renal-cell carcinoma.[9]

Recently, we disclosed 1,4-dihydroindeno[1,2-*c*]pyrazoles as a novel class of KDR kinase inhibitors.[10] Attachment of a urea-based side chain to access the hydrophobic specificity pocket in KDR kinase then led to a series of potent multitargeted (KDR, FLT1, cKit, Tie2) receptor tyrosine kinase inhibitors.[11] Disappointingly, when dosed intraperitoneally (ip) in a mouse uterine edema (UE) model, these compounds displayed only mediocre *in vivo* efficacies (Table 1). In addition, a detailed analysis of

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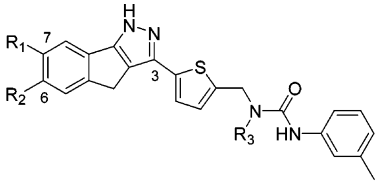
10.1021/jm061223o CCC: $37.00
Published on Web 04/11/2007

Table 1. *In Vitro* Potencies and *in Vivo* Efficacies of a Selected Set of 1,4-Dihydroindeno[1,2-*c*]pyrazoles with Urea-type Side Chains



Cmpds	R_1	R_2	R_3	IC_{50}, nM[a] KDR	IC_{50}, nM[a] KDR cell	UE, % inhib. at 25 mg/kg[b]
1	H		H	61	146[c]	89 (0.004)
2	H		Me	159	146[c]	59 (0.001)
3		H	H	48	115[d]	19 (0.050)
4		H	Me	51	64[c]	59 (0.001)

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate, variability around the mean value was <50%. [b] Intraperitoneal dosing, data reported as an average of five animals with associated p-values vs vehicle in parentheses. [c] Determined by ELISA. [d] Determined by Western blot analysis.

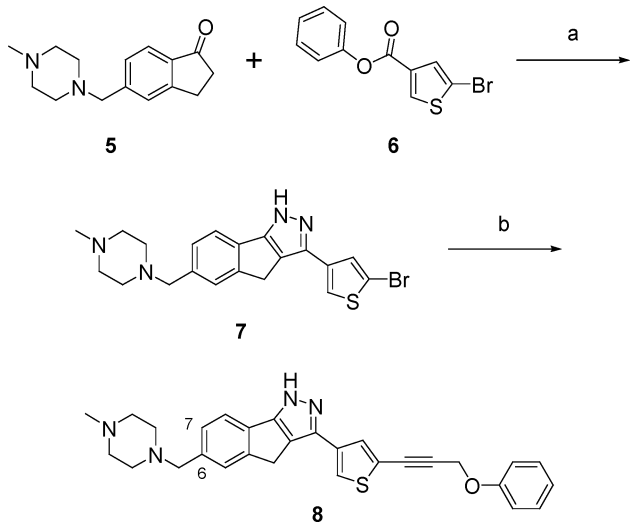
the ADMET (**A**bsorption, **D**istribution, **M**etabolism, **E**xcretion, **T**oxicity) profile of a selected set of ureas and their precursors showed that the urea-based side chain gave rise to unfavorable pharmacokinetic properties (short half-lives, low oral bioavailabilities). Attempts to substitute this side chain with urea mimetics and bioisosteres, or to replace it with substituents based on other functional groups, fortuitously led to the discovery of a new series of alkynes. In this report we now describe the structure−activity relationships (SARs) that were developed for 1,4-dihydroindeno[1,2-*c*]pyrazoles with acetylene-type side chains and our efforts to improve the cardiac safety profile of this class of compounds by minimizing their affinity for the hERG potassium channel.

Chemistry

The synthesis of 1,4-dihydroindeno[1,2-*c*]pyrazoles with acetylene-type side chains is exemplified with the synthesis of **8** (Scheme 1). Cyclization of indanone **5** with the bromothiophene ester **6** provided 1,4-dihydroindeno[1,2-*c*]pyrazole **7** as a key intermediate for the preparation of various target compounds with modified acetylenic side chains. For instance, Sonogashira coupling of **7** with phenyl propargyl ether under microwave conditions[12] afforded alkyne **8**. Alternatively, it was also possible to reverse the two synthesis steps (Scheme 2). As such, Sonogashira coupling of the bromothiophene ester **6** with phenyl propargyl ether furnished alkyne **9**, which served as a key building block for target compounds with modifications of the basic side chain. Cyclization of **9** with indanone **5**, for example, also gave access to the 1,4-dihydroindeno[1,2-*c*]-pyrazole **8**. The yields for the latter route were commonly slightly lower than for the previous approach. Analogues of **8**, with the polar side chain in the 7-position, were generated in analogy to Schemes 1 and 2.

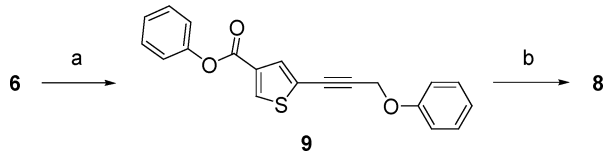
The syntheses of indanones with modified side chains are illustrated in Scheme 3. The majority of indanones were generated according to Method A, using commercially available aliphatic or aromatic heterocycles, as described previously.[10] One heterocycle, 1-methylpiperazin-2-one, was prepared according to Cusic et al.[13] Nucleophilic aromatic substitution of

Scheme 1[a]



[a] Reagents and conditions: (a) (i) NaH, benzene, reflux, (ii) $H_2NNH_2 \cdot H_2O$, HOAc, EtOH, reflux, 83%; (b) phenyl propargyl ether, $PdCl_2(PPh_3)_2$, CuI, PPh_3, Et_3N, DMF, microwave 120 °C, 62%.

Scheme 2[a]



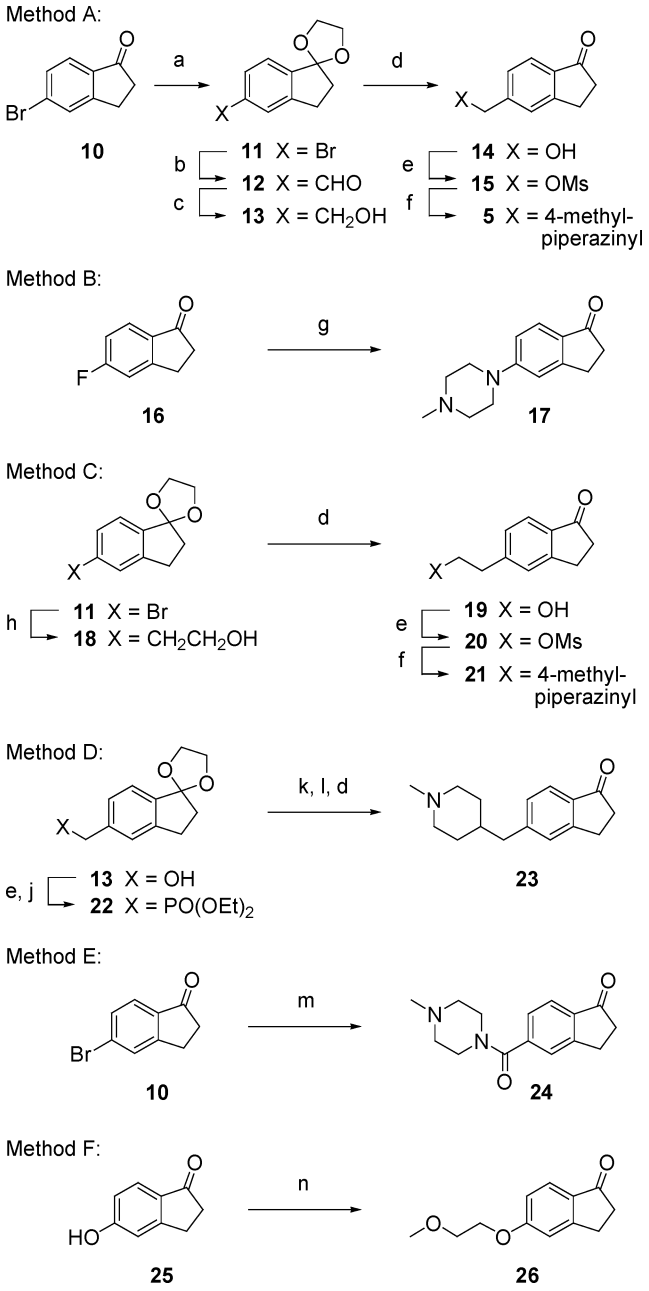
[a] Reagents and conditions: (a) phenyl propargyl ether, $PdCl_2(PPh_3)_2$, CuI, PPh_3, Et_3N, DMF, microwave 120 °C, 86%; (b) **5**, NaH, benzene, reflux, (ii) $H_2NNH_2 \cdot H_2O$, HOAc, EtOH, reflux, 54%.

5-fluoroindanone (**16**) with *N*-methylpiperazine (Method B) produced indanone **17** without a tether to the *N*-methylpiperazine group. The next higher homologue of **5** could be obtained via lithium−halogen exchange of the protected bromoindanone **11**,[10] followed by treatment with ethylene oxide[14] to furnish alcohol **18** (Method C). Hydrolysis of the dioxolane (**19**), mesylation of the OH-group (**20**), and subsequent nucleophilic substitution with *N*-methylpiperazine afforded **21** in 11% overall yield. For the preparation of an indanone with a C-linked basic side chain, the hydroxymethyl indanone **13** was mesylated and then converted to the phosphonate **22** in 78% yield (Method D). Successive Horner−Wadsworth−Emmons reaction with 1-methyl-4-piperidone followed by hydrogenation of the generated C−C-double bond and hydrolysis of the dioxolane yielded the piperidine-type indanone **23**. Palladium-mediated carboxamination of **10** gave rise to indanone **24** (Method E) with a carbonyl functionality as a tether to the *N*-methylpiperazine group. O-Alkylation of hydroxyindanone **25** with alkyl halides in the presence of potassium carbonate led to indanone ethers as, for example, **26**.

The highest yields in the coupling reaction to form the 1,4-dihydroindeno[1,2-*c*]pyrazole ring system were achieved when the aryl carboxylates were used in form of their phenol esters. Preparation of these phenol esters followed a standard protocol (DCC, DMAP, CH_2Cl_2/ether, 0 °C to r.t.). The precursor for **6**, 5-bromothiophene-3-carboxylic acid, was synthesized following Campaigne et al.'s[15] conditions for the bromination of thiophene-3-carboxylic acid.

The applied routes for the syntheses of modified acetylenes are illustrated in Scheme 4. Alcohol **28** was accessed from propargyl bromide (**27**) as described by Kitching et al. (Method 1).[16] Alkylation with **27** was the method of choice for the

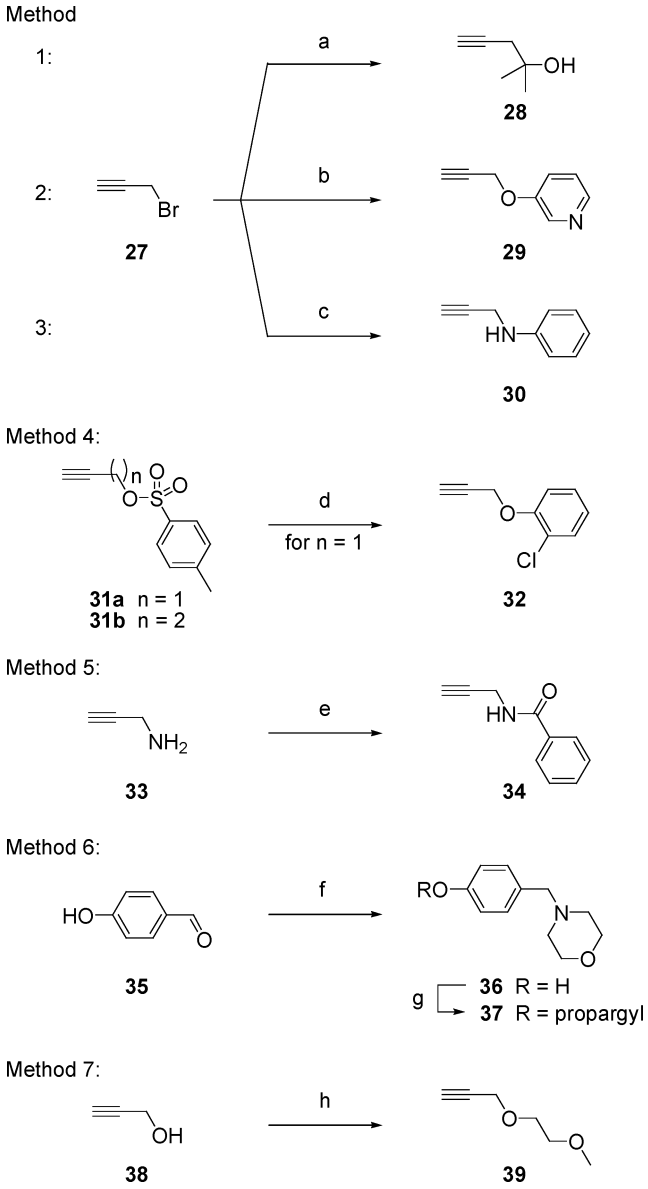
Scheme 3. Synthesis of Indanone Precursors[a]



[a] Reagents and conditions: (a) ethylene glycol, *p*-TsOH, benzene, reflux (Dean Stark trap), 82%; (b) (i) *n*-BuLi, THF, −78 °C; (ii) DMF, THF, −78 °C to r.t., 78%; (c) $NaBH_4$, MeOH/THF (10:1), 0 °C to r.t., 85%; (d) *p*-TsOH, acetone/H_2O (4:1), reflux, 69%; (e) MsCl, Et_3N, THF, 0 °C, 96%; (f) *N*-methylpiperazine, K_2CO_3, EtOH, 0 °C to r.t., 68%; (g) *N*-methylpiperazine, 100 °C, 60%; (h) (i) *n*-BuLi, THF, −78 °C; (ii) ethylene oxide, ether, −78 °C to 0 °C, 42%; (j) $HPO(OEt)_2$, NaH, THF, reflux, 78%; (k) (i) NaH, DME, 0 °C; (ii) 1-methyl-4-piperidone, reflux, 54%; (l) H_2 (60 psi), 10% Pd/C, MeOH, r.t., 89%; (m) CO (130 psi), *N*-methylpiperazine, $PdCl_2(dppf)\cdot CH_2Cl_2$, Et_3N, 110 °C, 40%; (n) 2-chloroethyl methyl ether, K_2CO_3, CH_3CN, reflux, 84%.

production of propargyl ethers of aromatic heterocycles like **29**[17] (Method 2) and secondary propargyl amines such as **30** (Method 3). In contrast, optimum yields for the synthesis of aryl propargyl ethers like **32** were obtained when the propargyl toluenesulfonate **31a** was used as alkylating agent (Method 4). Acylation of amine **33** with benzoyl chloride generated amide **34** (Method 5).[18] Reductive amination of **35** with morpholine through treatment with macroporous triethylammonium methylpolystyrene borohydride, followed by Mitsunobu reaction of the phenol **36** with

Scheme 4. Synthesis of Acetylenes[a]

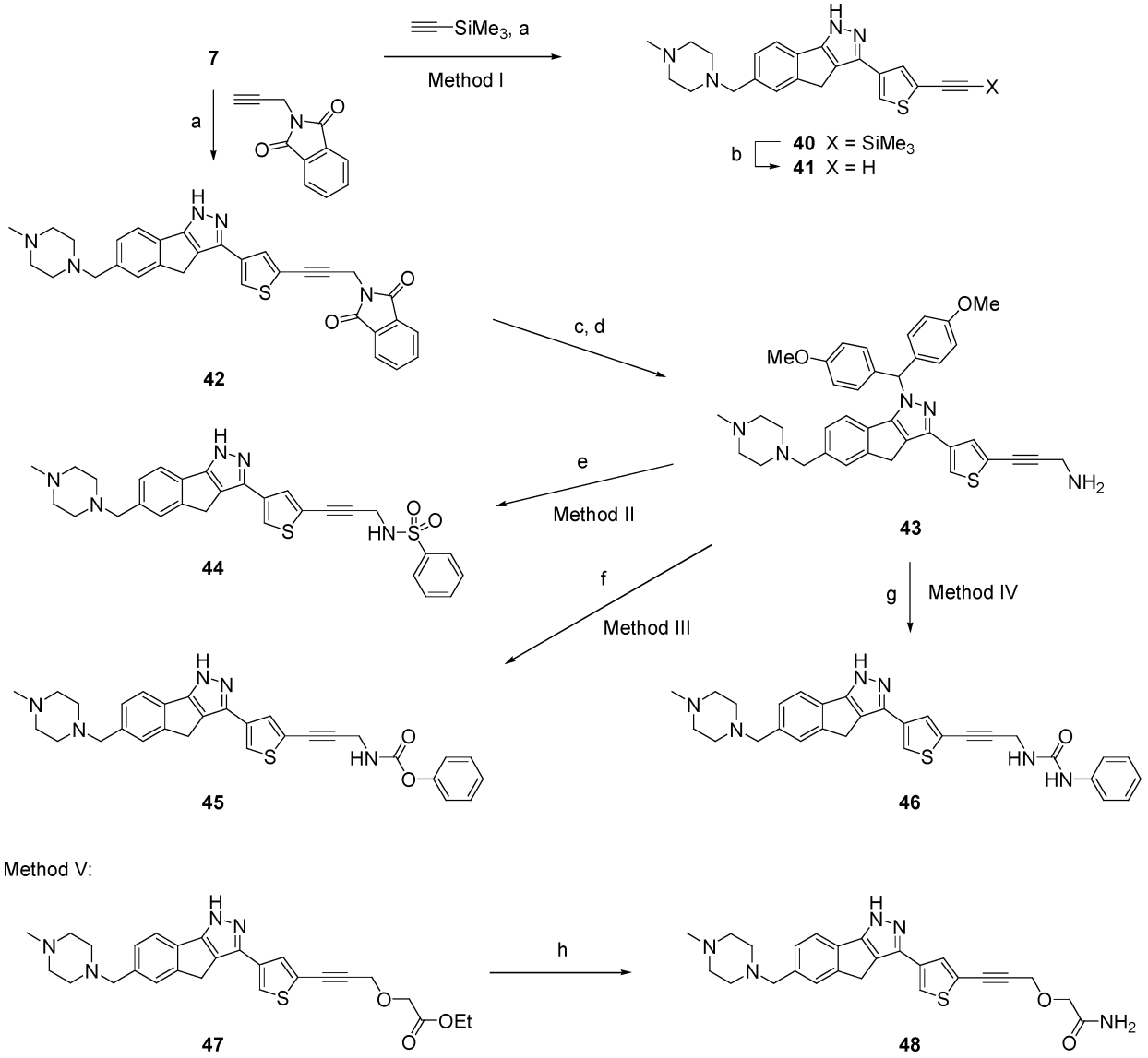


[a] Reagents and conditions: (a) (i) Al-powder, $HgCl_2$, THF, 45 °C; (ii) acetone, THF, 0 to 45 °C, 70%; (b) 3-hydroxypyridine, KOH, DMF, 5 °C to r.t., 10%; (c) aniline, EtOH, r.t., 91%; (d) 2-chlorophenol, K_2CO_3, acetone, 56 °C, 50%; (e) BzCl, Et_3N, CH_2Cl_2, 0 °C to r.t., 34%; (f) morpholine, MP-borohydride, MeOH, r.t. 98%; (g) propargyl alcohol (**38**), THF, PS−PPh_3, DEAD, r.t. 93%; (h) (i) NaH, THF, 5 °C, (ii) 2-bromoethyl methyl ether, 5 °C to r.t. 56%.

propargyl alcohol (**38**) afforded acetylene **37** (Method 6). Aliphatic ethers of propargyl alcohol, for example **39**, were prepared by alkylation of **38** with alkyl halides (Method 7).

In several cases, acetylenic side chains were further modified after coupling to the 1,4-dihydroindeno[1,2-*c*]pyrazole precursor **7** (Scheme 5). For instance, Sonogashira coupling of **7** with trimethylsilylacetylene and subsequent desilylation of **40** with tetra-*n*-butylammonium fluoride (TBAF) gave rise to compound **41** with a terminal alkyne group (Method I). The amine intermediate **43** was synthesized by coupling of **7** with *N*-propargylphthalimide (89% yield), protection of the pyrazole-NH group in **42** by employing 4,4′-dimethoxybenzhydryl chloride and deprotection of the primary amine functionality with anhydrous hydrazine. Starting from **43**, the sulfonamide **44** (Method II), the carbamate **45** (Method III) and the urea **46** (Method IV) could be obtained via application of

Scheme 5. Modifications of Acetylene Side Chains[a]



[a] Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, CuI, PPh_3, Et_3N, DMF, microwave 120 °C, 75%; (b) TBAF·H_2O, THF, r.t., 72%; (c) 4,4′-dimethoxybenzhydryl chloride, Et_3N, THF, 50 °C, 60%; (d) H_2NNH_2, EtOH/THF (1:1), r.t., 46%; (e) (i) $PhSO_2Cl$, pyridine, r.t.; (ii) 4M HCl in dioxane, r.t., 47%; (f) (i) PhOCOCl, Et_3N, CH_2Cl_2, r.t.; (ii) 37% HCl in EtOH/EtOAc, r.t., 11%; (g) (i) PhNCO, THF, r.t.; (ii) 4M HCl in dioxane, r.t., 30%; (h) 2M NH_3 in MeOH, r.t., 68%.

well-established reaction conditions. Conversion of the ester **47** to the amide **48** was achieved by stirring of the starting material for 3 days in a 2 M solution of ammonia in methanol (Method V).

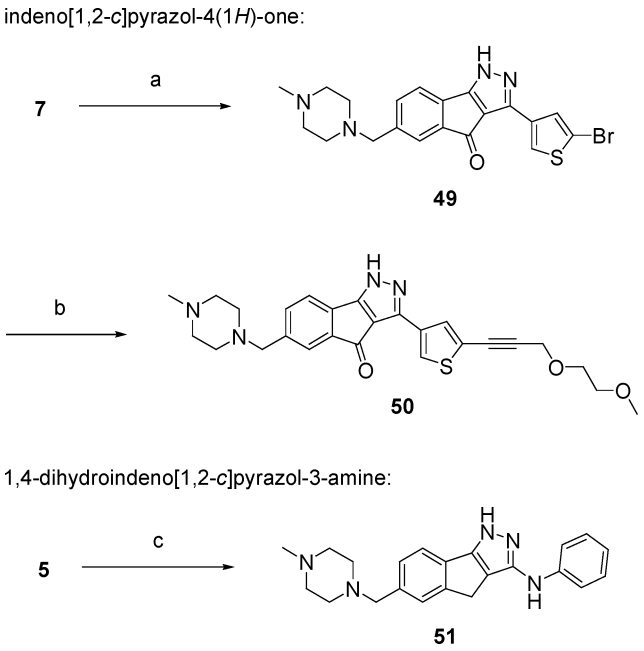
Two compounds with modifications of the general chemotype were synthesized as shown in Scheme 6. The indeno[1,2-*c*]-pyrazol-4(1*H*)-one **50** was obtained through air-oxidation of the 4-methylene group[19] in **7** and Sonogashira coupling of the resulting intermediate **49** with alkyne **39**. For the preparation of the 1,4-dihydroindeno[1,2-*c*]pyrazol-3-amine **51**, the sodium enolate of **5** was treated with phenyl isothiocyanate, and the resulting thioamide intermediate was methylated *in situ*. The crude material was then refluxed in ethanol in the presence of hydrazine to give the target compound.[20]

Results and Discussion

Structure−Activity Studies. Initially, the 1,4-dihydroindeno-[1,2-*c*]pyrazoles were optimized for inhibition of the unactivated form of human KDR kinase. The activities of the target compounds were assessed based on their ability to inhibit the phosphorylation of a biotinylated polypeptide substrate (biotin-Ahx-AEEEYFFLFA-amide) by KDR kinase in a homogeneous time-resolved fluorescence (HTRF) assay at physiological relevant concentrations (1.0 mM) of adenosine-5′-triphosphate (ATP). Potent compounds (IC_{50} < 100 nM) were then evaluated for their effectiveness to inhibit VEGF-induced KDR phosphorylation in a 3T3 murine fibroblast cell line, stably transfected with full length human KDR.

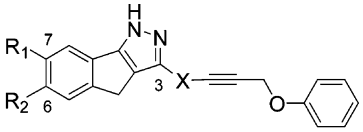
Previously established SAR demonstrated the importance of reoptimizing the substitution pattern on the thiophene spacer and the position of the basic side chain. The results are listed in Table 2. In contrast to the urea-type 1,4-dihydroindeno[1,2-*c*]pyrazoles, a 3′,5′-disubstitution pattern on the thienyl spacer (**8**) now appears to provide a better trajectory to accommodate the more rigid acetylenic side chain in the hydrophobic specificity pocket of KDR kinase. Compound **52**, with a 2′,5′-disubstituted thienyl, which was the preferred constellation for the urea-type analogues, is about 4-fold less active than **8**, and **53**, with a 2′,4′-substitution pattern on the thienyl group, is about 8-fold less potent. Attempts to replace the thienyl moiety with a phenyl group (**54** and **55**) turned out to be detrimental. A comparison between compounds **8** and **56** illustrates that the

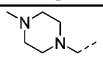
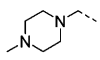
Scheme 6. Synthesis of Related Chemotypes **50** and **51**[a]



[a] Reagents and conditions: (a) air, Cs_2CO_3, DMF, 90 °C, 61%; (b) **39**, $PdCl_2(PPh_3)_2$, CuI, PPh_3, Et_3N, DMF, microwave 120 °C, 55%; (c) (i) phenyl isothiocyanate, NaH, DMF, 0 °C to r.t.; (ii) MeI, r.t.; (iii) H_2NNH_2, EtOH, reflux, 30%.

Table 2. Optimization of the Spacer in 3-Position and Identification of the Preferred Regiochemistry for the Basic Side Chain



Cmpds	R_1	R_2	X	IC_{50}, nM[a] KDR	KDR cell
8	H			6	40[b]
52	H			22	316[b]
53	H			50	99[c]
54	H			108	
55	H			962	
56		H		42	197[c]

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate, variability around the mean value was <50%. [b]Determined by ELISA. [c]Determined by Western blot analysis.

basic side chain is now preferred in the 6-position, as compared to the 7-position in the urea series. The *in vitro* (IC_{50} = 6 nM) and whole cell (IC_{50} = 40 nM) potencies of **8** were comparable to more advanced competitor compounds like Sutent (*in vitro* IC_{50} = 18 nM, whole cell IC_{50} = 22 nM), and therefore the compound was further evaluated in the uterine edema model.

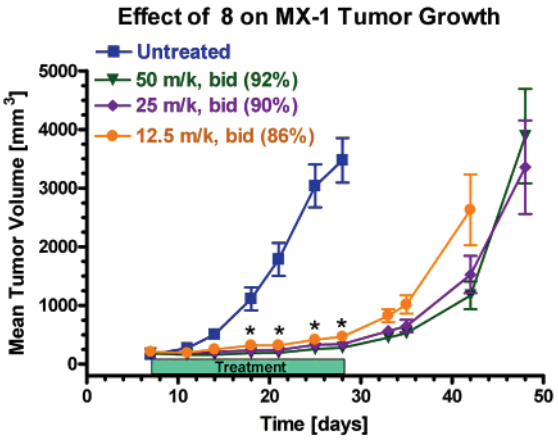
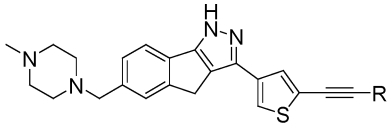


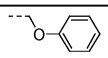
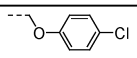
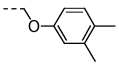
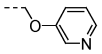
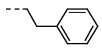
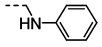
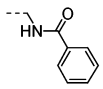
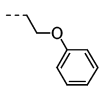
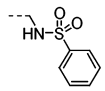
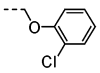
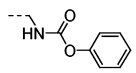
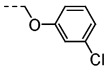
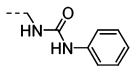
Figure 1. Effects of **8** on the growth of MX-1 human xenografts. Dosing (BID) started on day seven. Tumor volumes are expressed as means ± SEM; n = 7−10 per group. The percent inhibition on Day 28 for each dosage compared to control is shown in parentheses in the legend. Significant differences ($p < 0.01$ for all groups compared to vehicle controls (Mann−Whitney)) in mean tumor volume were observed for all treatment groups by day 18.

The model is based on the fact that a bolus injection of estradiol into a mouse uterus results in upregulation of VEGF. As a consequence, vascular permeability is increased, leading to the development of edema. The efficacy of KDR kinase inhibitors can be determined based on their capacity to reduce the acute increase in uterine weight. With an ED_{50} of 3 (0.1−5.6) mg/kg,[21] **8** turned out to be more potent than Sutent (ED_{50} = 9 mg/kg) in the same assay. This outstanding result and an acceptable pharmacokinetic profile (F = 75% after a 10 mg/kg oral (po) dose, and $T_{1/2}$ = 5.5 h after a 3 mg/kg intravenous (iv) dose into CD-1 mice)[22] qualified the compound to be evaluated for its antitumor efficacy against tumors derived from MX-1 human breast carcinoma implanted subcutaneously into nude mice. The tumor growth inhibition was determined to be 90% at 25 mg/kg·day po (Figure 1) (PTK787: ED_{75} > 100 mg/kg·day po).

Maintaining the optimized substitution pattern described for **8**, we then focused on the optimization of the acetylenic substituent of our inhibitors (Table 3). A comparison of the terminal alkyne **41** and its *n*-propyl analogue **57** showed that a minimum size of the substituent was required to maintain potent KDR activity. However, steric bulk from branched chains or aliphatic ring systems was not well tolerated. The site was also sensitive to the introduction of polar functional groups (no examples shown). One of the few functionalities that could be utilized was the alcohol **58**. The compound was extremely potent in both the enzyme and the whole cell assays but then failed to demonstrate sufficient *in vivo* efficacy in the uterine edema model (54 (0.002) % inhibition at 100 mg/kg ip).[21] Assuming that rapid metabolism of the primary hydroxy group could be responsible for this result, the geminal dimethyl analogue **59** was synthesized. However, this modification led to a dramatic deterioration of KDR activity. The methyl ether **60** was more successful with an ED_{50} of 12.6 (10−120) mg/kg[21] after oral dosing in the UE model. Homologation of **8** to **61** only resulted in diminished KDR inhibitory activity. Small hydrophobic substituents on the phenyl ether portion of **8** (**62** to **64**) were best tolerated in the meta-position, while the meta- and para-positions were preferred for disubstituted phenyl ethers (**65**). Unfortunately, evaluation of a set of compounds in the UE model demonstrated that these substitutions on the terminal phenyl ring generally resulted in reduced *in vivo* efficacies (UE

Table 3. SAR for 1,4-Dihydroindeno[1,2-*c*]pyrazoles with a Selected Set of Acetylenic Substituents



Cmpds	R	IC_{50}, nM[a] KDR	IC_{50}, nM[a] KDR cell	[^{3}H]dofetilide binding assay IC_{50}, μM[d]	Cmpds	R	IC_{50}, nM[a] KDR	IC_{50}, nM[a] KDR cell	[^{3}H]dofetilide binding assay IC_{50}, μM[d]
8		6	40[b]	1.11 (0.49)	**64**		78	419[b]	3.59 (1.20)
41	H	1000		1.41 (0.29)	**65**		33	34[c]	2.50 (0.79)
57	*n*-propyl	75	503[c]	1.80 (0.13)	**66**		54	44[c]	3.39 (0.84)
58	CH_2CH_2OH	8	40[b]	>10.00	**67**		48	542[b]	4.80 (2.12)
59		2370			**68**		36	28[b]	1.75 (0.63)
60	CH_2OCH_3	23	8[c]	5.19 (0.04)	**69**		3	5[b]	7.46 (1.78)
61		85	313[b]	0.45 (0.04)	**44**		45	109[b]	4.87 (0.47)
62		101	178[c]	0.79 (0.02)	**45**		38	51[b]	2.75 (0.77)
63		36	178[b]	2.59 (1.38)	**46**		11	15[b]	7.91 (0.80)

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate, variability around the mean value was <50%. [b]Determined by ELISA. [c]Determined by Western blot analysis. [d]Values are means of two experiments, standard deviation is given in parentheses.

(**65**): 32 (0.025) % inhibition at 10 mg/kg po).[21] Poor efficacies were also observed for heterocyclic ethers. For example the 3-pyridyl ether **66** only produced 24 (0.040) % inhibition at 10 mg/kg po[21] in the UE model. Isosteric replacement of the ether oxygen in the parent compound **8** with a methylene group led to the phenethyl analogue **67**, which exhibited low whole cell activity. Substitution with sulfur (not shown) resulted in markedly reduced potencies as well, but replacement with nitrogen appeared to be beneficial as long as the basicity of the resulting amines could be held to a minimum (**68**, **69**, and **44**−**46**). The benzamide **69** and the urea **46** were exceptionally potent but then could not reach relevant plasma concentrations after oral dosing (10 mg/kg) into CD-1 mice (F = 17%, AUC (po) = 3.5 μg·h/mL and F = 0%, respectively).[22]

Cardiac Safety Studies. Drug-induced blockade of the hERG (human ether-à-go-go)-encoded potassium channel has received increased attention over the past few years since it can lead to delayed repolarization and prolongation of the QT interval on the electrocardiogram. QT prolongation, in turn, is associated with a predisposition for the polymorphous ventricular arrhythmia *Torsades de Pointes*, which may degenerate into ventricular fibrillation and sudden death.[23] The [^{3}H]dofetilide membrane binding assay provides a useful high throughput platform to assess the potential of early lead compounds to block the hERG K^+ channel.[24] In this radioligand binding assay, the test compounds compete with [^{3}H]dofetilide for binding to homogenized membrane aliqoutes, derived from hERG-transfected HEK 293 cells. Table 3 shows that our KDR inhibitors displayed sig-

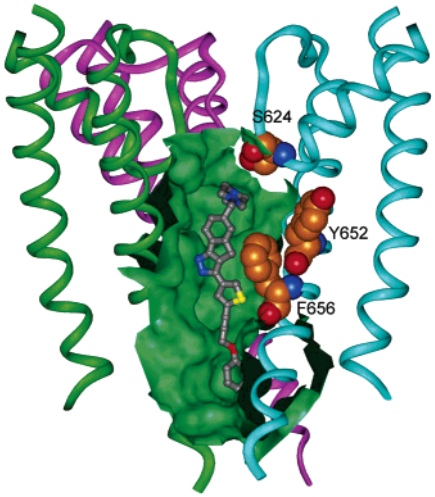


Figure 2. Model of **8** (gray) docked into the inner cavity of the hERG K^+ channel (closed state, homology model based on KcsA, PDB entry 1BL8). One of the four subunits was removed for better visibility. Key binding sensitive residues (Phe656, Tyr652, Ser624) are shown for one monomer (cyan) as space filling models (orange). Surface (green) is shown for two other monomers (light green and purple).

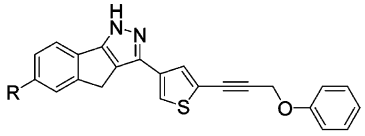
nificant hERG affinities. To address this problem, we constructed a homology model of the homo-tetrameric pore domain of hERG in its closed state (drug trapping mode), based on the crystal structure of the bacterial KcsA potassium channel (Figure 2).[25,26] The 1,4-dihydroindeno[1,2-*c*]pyrazole **8** was then docked into the inner ion conduction cavity. The best fit was obtained by orienting **8** parallel to the pore axis, such that the more hydrophobic acetylenic ether moiety was located at the intracellular mouth. This allowed the π-stacking interaction between the 1,4-dihydroindeno[1,2-*c*]pyrazole core and Phe656 in the S6 helix. The *N*-methylpiperazine-containing side chain, which is protonated under physiological pH, aligned with its internal nitrogen atom such that π-cation interactions with Tyr652 were possible, while the external nitrogen was able to interact with Ser624 in the pore helix of the selectivity filter. Based on the binding mode of **8**, three strategies were developed to interrupt the binding to the hERG K^+ channel.

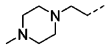
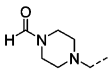
First, the basic side chain was modified (Table 4). Compound **70** demonstrated that the hERG affinity was not intrinsic to the chemotype but also that a polar side chain was required for potent KDR activity. Extending the tether to the *N*-methylpiperazine group (**72**) reduced hERG activity more than completely removing it (**71**) but at the same time led to a loss of whole cell activity. Selective isosteric replacement of either basic nitrogen atom with a CH group (**73** and **74**) revealed that both nitrogens contribute to the hERG binding; the internal nitrogen (**73**), however, appeared to play a more important role. This result is in agreement with our computer model predictions, since both nitrogens interact with the key binding-sensitive residues Tyr652 and Ser624, but interaction with Tyr652 is considered to provide the more significant contribution to the binding energy. The basicity of either nitrogen atom was then modulated through the introduction of neighboring carbonyl groups (**75** to **78**). In the case of **75** and **78**, with the carbonyl group external to the piperazine ring, the priority of the nitrogen atoms for binding to hERG was reversed compared to **73** and **74**. This might be due to conformational changes since **78** contained an sp^2-configured tether. Gratifyingly, both **76** and **77** had excellent activities against KDR but did not display any affinity for hERG. This result was confirmed for **77** in a whole cell patch clamp assay, where the inhibition of the actual hERG ionic current was measured using hERG-transfected HEK 293 cells.[27] At a concentration of 2 μM, **77** did not affect a block of the tail current in a statistically significant manner (3.7% block compared to 64% block at 0.5 μM for **8**) and only showed an IC_{50} of 53 μM.[28] Compared to **76**, **77** had a slightly better pharmacokinetic profile (F = 50% at 10 mg/kg po in CD-1 mice),[22] and the results in the UE model were comparable for both compounds (ED_{50} = 5.5 (3−7.7) mg/kg for **76** and 5.7 (3.5−10) mg/kg po for **77**).[21] Therefore, **77** was further evaluated for its antitumor activity in an HT1080 fibrosarcoma mouse flank xenograft model. However, when orally dosed twice a day with a total daily dose of 10 mg/kg, **77** inhibited tumor growth only 29% (p-value vs vehicle <0.001) compared to the vehicle control (Sutent: ED_{75} = 65 mg/kg·day po). Compounds **79** to **81** are examples where the piperazine moiety could be replaced with other aliphatic rings and chains to obtain analogues with acceptable KDR inhibitory potency but no affinity for the hERG K^+ channel. Unfortunately, none of those compounds showed sufficient efficacy in the UE model (46 (0.007) %, 8 (0.633) %, and 9 (0.510) % inhibition at 10 mg/kg po).[21] Replacements with aromatic heterocycles are exemplified with **82** to **85**. The imidazole (**82**) and 1,2,3-triazole (**84**) analogues only displayed low KDR activity, the pyrazole **83** failed to demonstrate sufficient efficacy in the UE model (14 (0.664) % inhibiton at 10 mg/kg po),[21] but the 1,2,4-triazole **85** was potent *in vitro* and *in vivo* (UE: 96 (<0.001) % inhibition at 10 mg/kg po),[21] with reduced binding to the hERG channel (patch clamp IC_{50} = 1.7 μM).[28] Nonetheless, further evaluations were put on hold because of the compound's marginal pharmacokinetic profile (Cl = 2.5 L/kg·h, $T_{1/2}$ = 0.8 h at 3 mg/kg iv in CD-1 mice).[22]

The second strategy to reduce the affinity of our KDR kinase inhibitors to bind to the hERG K^+ channel was based on increasing the polarity of the acetylenic side chain (Table 5). Attachment, for example, of a morpholine group in the para-postion of the phenyl ether (**86**) had no beneficial effect on reducing hERG affinity. Using **60**, one of the least hERG active compounds from the initial optimization of the acetylenic side chain (Table 3), as a parent compound, the methyl ether was extended with additional polar functionalities. This strategy generally led to a reduction of hERG channel affinity, but in most cases those compounds also lost their inhibitory potency against KDR (**48**). The only exception was **87**; the glycol ether moiety of this compound clearly reduced hERG binding and at the same time maintained acceptable KDR activity *in vitro*. *In vivo*, **87** exhibited 71 (0.001) % inhibition at 25 mg/kg po[21] in the uterine edema model and 76% tumor growth inhibition at 70 mg/kg·day po (p-value vs vehicle <0.001) in the MX-1 tumor xenograft model. Reoptimization of the regiochemistry of the polar side chain (**87** vs **88**) and combination with the earlier identified 1,2,4-triazole resulted in **89** and **90** (patch clamp IC_{50} > 150 μM and 11.6 μM, respectively).[28] The pharmacokinetic profile for **89** was characterized by a relatively high clearance rate, a short half-life, and low oral bioavailability (Cl = 3.4 L/h·kg and $T_{1/2}$ = 0.3 h at 3 mg/kg iv, and F = 22% at 10 mg/kg po in CD-1 mice).[22] The profile was slightly better for **90** (Cl = 2.2 L/h·kg and $T_{1/2}$ = 0.3 h at 3 mg/kg iv, and F = 64% at 10 mg/kg po in CD-1 mice)[22] and the compound achieved 57 (<0.001) % inhibition at 10 mg/kg po[21] in the uterine edema model.

Finally, we investigated whether two common variations of the 1,4-dihydroindeno[1,2-*c*]pyrazole core have the potential to disrupt the proposed π-stacking interactions with Phe656 either sterically or electronically (Figure 3). Introduction of a carbonyl

Table 4. Selected Modifications of the Polar Side Chain in **8** and their Effect on KDR Inhibitory Potency and Binding to the hERG K^+ Channel



Cmpds	R	IC_{50}, nM[a] KDR	IC_{50}, nM[a] KDR cell	[^{3}H]dofetilide binding IC_{50}, μM[d]	Cmpds	R	IC_{50}, nM[a] KDR	IC_{50}, nM[a] KDR cell	[^{3}H]dofetilide binding IC_{50}, μM[d]
70	H	4680		>10.00	**78**		44	29[b]	1.72 (0.35)
71		11	10[c]	1.74 (0.31)	**79**		24	10[c]	>10.00
72		5	58[c]	5.87 (0.80)	**80**		40	92[c]	>10.00
73		69	112[b]	0.08 (0.02)	**81**		42	10[c]	>10.00
74		11	71[c]	2.10 (0.77)	**82**		195		1.43 (0.48)
75		9	20[c]	5.36 (0.83)	**83**		20	49[c]	>10.00
76		11	18[c]	>10.00	**84**		124	148[b]	>10.00
77		7	2[c]	>10.00	**85**		62	15[c]	>10.00

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate, variability around the mean value was <50%. [b]Determined by ELISA. [c]Determined by Western blot analysis. [d]Values are means of two experiments, standard deviation is given in parentheses.

functionality into the methylene-bridge of the pharmacophore, exemplified by **50**, was tolerated but did not result in any considerable improvement (UE: 54 (0.010) % inhibition at 10 mg/kg po).[21] Insertion of an NH group between the pharmacophore and the thiophene spacer (**51** vs **91**[10]) rendered the compound inactive.

The selectivity profile of the two key compounds **8** and **90** are listed in Table 6. Both compounds can clearly be characterized as potent multitargeted (class III/V) receptor tyrosine kinase inhibitors with comparable potencies against both, the kinases of the VEGFR and PDGFR subfamilies. On the other hand, the compounds are much less active against kinases that are structurally less homologous such as Tie2, LCK, FYN, SRC, and HCK.

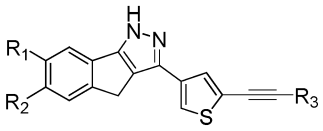
Conclusion

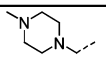
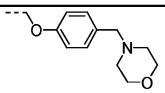
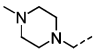
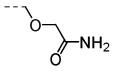
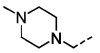
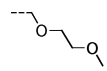
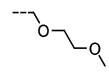
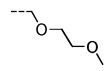
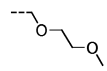
A series of 1,4-dihydroindeno[1,2-*c*]pyrazoles with acetylene-type side chains was discovered as novel KDR kinase inhibitors. Extensive SAR studies established a 3′,5′-disubstitution pattern for the thienyl spacer between the pharmacophore and the alkyne. A second substituent, containing a 4-methylpiperazine moiety, was preferred in the 6-position of the 1,4-dihydroindeno-[1,2-*c*]pyrazole core. Optimization of the acetylene-type side chain identified the phenylpropargyl ether **8**, which displayed an oral activity in the estradiol-induced murine uterine edema model (ED_{50} = 3 mg/kg) superior to Sutent (ED_{50} = 9 mg/kg) and potent antitumor efficacy against the MX-1 human breast carcinoma xenograft tumor growth model (tumor growth inhibition = 90% at 25 mg/kg·day po). However, the compound also showed a strong affinity for the hERG potassium channel. Docking of **8** into a homology model of the homo-tetrameric pore domain of hERG allowed the development of strategies to systematically interrupt key binding interactions. Replacement of the 4-methylpiperazine moiety in **8** with 1,2,4-triazole minimized interactions with Tyr652 and Ser624, and changing the phenyl propargyl ether to its methoxyethyl analogue disrupted the binding in the intracellular mouth of the hERG channel. Alternatively, attempts to reduce the π-stacking interactions between the 1,4-dihydroindeno[1,2-*c*]pyrazole core and Phe656 turned out to be less successful. Combination of the improved features ultimately led to the discovery of **90**, which, compared to **8**, displayed a strongly reduced hERG affinity (IC_{50} = 11.6 μM in the hERG patch clamp assay) but also a lower *in vivo* efficacy. The selectivity profile for the key compounds **8** and **90** revealed that both are multitargeted receptor kinase inhibitors with low nanomolar potencies against other structurally related members of the VEGFR- and PDGFR kinase subfamilies. Studies to improve the *in vivo* efficacy profile of **90** will be published in due time.

Experimental Section

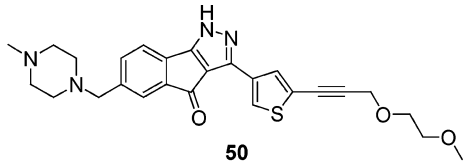
Chemistry. All reactions were carried out under inert atmosphere (N_2). Solvents and reagents were obtained commercially and were used without further purification. Normal-phase flash chromatography was carried out using Merck Kieselgel 60 (230−400 mesh) or was performed on an Analogix IntelliFlash system with prepacked columns. Reverse-phase chromatography was carried out

Table 5. Selected Modifications of the Propargyl Ether and the Polar Chain in **8** and Their Effect on KDR Inhibitory Potency and Binding to the hERG K^+ Channel



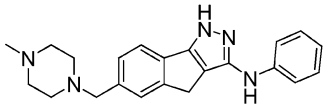
Cmpds	R_1	R_2	R_3	IC_{50}, nM[a] KDR	KDR cell	[^{3}H] dofetilide binding IC_{50}, μM[d]
86	H			84	2140[b]	2.56 (0.22)
48	H			7960		>10.00
87	H			53	25[b]	9.19 (0.33)
88		H		30	62[c]	>10.00
89	H			48	14[c]	>10.00
90		H		5	16[b]	>10.00

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate, variability around the mean value was <50%. [b]Determined by ELISA. [c]Determined by Western blot analysis. [d]Values are means of two experiments, standard deviation is given in parentheses.



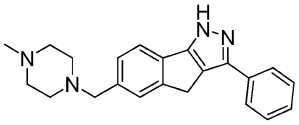
50

KDR IC_{50} = 10 nM
KDR cell IC_{50} = 13 nM
[^{3}H]dofetilide binding IC_{50} = 9.40 (0.59) μM
hERG patch clamp IC_{50} = 5.3 μM



51

KDR IC_{50} > 50 μM



91

KDR IC_{50} = 708 nM

Figure 3. Modifications of the 1,4-dihydroindeno[1,2-*c*]pyrazole core. Each KDR IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate. The IC_{50} values from the [^{3}H]dofetilide binding assay and the hERG patch clamp assay are the means of two experiments.

on a Waters HPLC system equipped with a Gilson FC204 fraction collector. Samples were purified on a Waters Symmetry C8 column (25 mm x 100 mm, 7 μm particle size) using a gradient of 10% to 100% acetonitrile and 0.1% aqueous TFA over 8 min (10 min run time) at a flow rate of 40 mL/min. ^{1}H NMR spectra were recorded on a Varian UNITY or Inova (500 MHz), Varian UNITY (400 MHz), or Varian UNITY plus or Mercury (300 MHz) spectrometer. The chemical shifts are reported as δ values (ppm) relative to tetramethylsilane as an internal standard. Mass spectra were obtained on a Finnigan MAT SSQ700 instrument. High resultion mass spectra were generated on an Agilent MSDTOF instrument. The above spectral data were obtained through the Department of Structural Chemistry at Abbott Laboratories. Elemental analysis was performed by Robertson Microlit Laboratories, Inc. Madison, NJ, or Quantitative Technologies, Inc. Whitehouse, NJ, and the results indicated by elemental symbols are within ±0.4% of theoretical values. Analytical HPLC was performed on a Waters 2690 HPLC system, peaks were acquired at λ = 254 nm (given) and 220 nm (see Supporting Information). The methods used were (A) a YMC ODS-A C18 column (4.6 × 150 mm, 5.5 μm particle size) using a gradient of 10−100% acetonitrile and 0.1% trifluoroacetic acid in water at a flow rate of 1 mL/min over 20 min and (B) a Xterra RP18 column (4.6 × 150 mm, 5 μm particle size) using a gradient of 20−100% methanol and water at a flow rate of 1 mL/min over 20 min.

3-(5-Bromothiophen-3-yl)-6-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazole (7). A mixture of **5**[10] (360 mg, 1.47 mmol), **6**[10] (416 mg, 1.47 mmol), and 60% NaH in mineral oil (147 mg, 3.68 mmol) in benzene (5.5 mL) was heated to reflux for 2.5 h. The reaction was cooled and quenched by dropwise addition of 50% aq HOAc. The solvents were evaporated, and the residue was dried in high vacuum for 1 h. The crude intermediate was taken up in ethanol (5.5 mL), hydrazine monohydrate (107 μL, 2.21 mmol), and HOAc (252 μL, 4.41 mmol) were added, and the mixture was heated to reflux for 4 h. The mixture was cooled and concentrated, and the crude product was purified by flash chromatography on silica gel eluting with 10:1 dichloromethane/methanol + 1% ammonium hydroxide to provide the title compound (524 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.32 (s, 3H), 2.4−2.65 (m, 8H), 3.58 (s, 2H), 3.70 (s, 2H), 7.29 (d, J = 7.8 Hz, 1H), 7.37 (d, J = 1.3 Hz, 1H), 7.40 (d, J = 1.6 Hz, 1H), 7.49 (s, 1H), 7.61 (d, J = 7.8 Hz, 1H). MS (ESI) *m*/*z* 429, 431 $(M + H)^+$.

6-((4-Methylpiperazin-1-yl)methyl)-3-(5-(3-phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (8). A mixture of **7** (200 mg, 0.47 mmol), phenyl propargyl ether (68 mg, 0.51 mmol), triphenylphosphine (24 mg, 0.09 mmol), dichlorobis(triphenylphosphine)palladium(II) (16 mg, 0.023 mmol), copper iodide (4 mg, 0.023 mmol), and triethylamine (0.97 mL, 6.99 mmol) in DMF (2 mL) was stirred under nitrogen in a heavy walled process vial in a microwave synthesizer at 120 °C for 25 min. The reaction mixture was concentrated, and the crude product was purified by preparative reverse phase HPLC to give the title compound (239 mg, 62%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.81 (s, 3H), 3.00−3.50 (m, 8H), 3.83 (s, 2H), 3.89 (bs, 2H), 5.11 (s, 2H), 6.99−7.02 (m, 1H), 7.05−7.06 (m, 2H), 7.33−7.37 (m, 2H), 7.40 (d, J = 7.8 Hz, 1H), 7.59 (s, 1H), 7.67 (d, J = 7.8 Hz, 1H), 7.74 (d, J = 1.3 Hz, 1H), 7.90 (d, J = 1.3 Hz, 1H). MS (ESI) *m*/*z* 481 $(M + H)^+$. Anal. ($C_{29}H_{28}N_4OS \cdot 3.5CF_3CO_2H$) C, H, N.

Phenyl 5-(3-Phenoxyprop-1-ynyl)thiophene-3-carboxylate (9). Compound **9** was prepared using the same procedure as described for the synthesis of **8** by substituting **6** for **7**. The title compound was obtained after purification by flash chromatography on silica gel using 5:1 hexane/ethyl acetate as the mobile phase (3.0 g, 86%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.10 (s, 2H), 6.98−7.01 (m, 1H), 7.04−7.05 (m, 2H), 7.25−7.27 (m, 2H), 7.29−7.36 (m, 3H), 7.45−7.48 (m, 2H), 7.76 (d, J = 1.3 Hz, 1H), 8.62 (d, J = 1.3 Hz, 1H). MS (DCI) *m*/*z* 352 $(M + NH_4)^+$.

5-(4-Methylpiperazin-1-yl)indan-1-one (17). A solution of 5-fluoro-1-indanone (3.5 g, 23.3 mmol) in 1-methylpiperazine (20 mL) was heated to 100 °C for 20 h. The reaction mixture was concentrated, and the crude product was purified by flash chromatography on silica gel eluting with ethyl acetate/methanol (97:3) to provide the title compound (3.2 g, 60%). ^{1}H NMR (300 MHz, CD_3OD) δ 2.35 (s, 3H), 2.57−2.63 (m, 6H), 3.03−3.07 (m, 2H), 3.43−3.46 (m, 4H), 6.94 (d, J = 2.4 Hz, 1H), 6.98 (dd, J = 8.6, 2.4 Hz, 1H), 7.56 (d, J = 8.6 Hz, 1H). MS (ESI) *m*/*z* 231 $(M + H)^+$.

Table 6. Kinase Inhibition Profiles of 1,4-Dihydroindeno[1,2-*c*]pyrazoles **8** and **90**

	IC_{50}, nM[a]											
	VEGFR family			PDGFR family				other families				
Cmpds	KDR	FLT1	FLT4	PDGFR[b]	FLT3	CSF1R	cKit	Tie2	LCK	FYN	SRC	HCK
8	6	2	3	58	9	8	26	4600	>50000	27300	30400	>50000
90	5	<3	4	84	<3	9	12	8100	11700	>50000	>50000	>50000

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate; variability around the mean value was <50%. [b] Values of cellular phosphorylation by ELISA assay.

2-(Spiro[1,3dioxolane-2,1′-Indane]-5′-yl)ethanol (18). To a solution of **11**[10] (470 mg, 1.84 mmol) in THF (5.5 mL) was added a 1.6 M solution of *n*-butyllithium in hexanes (1.44 mL, 2.3 mmol) dropwise at −78 °C. To this mixture was then added a solution of ethylene oxide (1.21 g, 27.5 mmol) in diethyl ether (2.1 mL) at −78 °C. The mixture was allowed to warm to 0 °C over 2 h, and stirring at 0 °C was continued for an additional 2 h. The reaction was quenched by addition of H_2O (10 mL), and the mixture was extracted four times with ethyl acetate. The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated, and the crude product was purified by flash column chromatography on silica gel using hexane/ethyl acetate (1:1) as the mobile phase to give the title compound (170 mg, 42%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.28−2.32 (m, 2H), 2.91−2.98 (m, 4H), 3.92 (t, J = 6.4 Hz, 2H), 4.05−4.21 (m, 4H), 7.25 (d, J = 7.8 Hz, 1H), 7.36 (s, 1H), 7.71 (d, J = 7.8 Hz, 1H). MS (DCI) m/z 221 $(M + H)^+$.

5-(2-Hydroxyethyl)indan-1-one (19). A mixture of **18** (170 mg, 0.77 mmol) and *p*-TsOH (147 mg, 0.77 mmol) in 4:1 acetone/H_2O (2.5 mL) was refluxed for 1 h. The reaction mixture was concentrated, diluted with H_2O, and neutralized by addition of K_2CO_3. The aqueous layer was extracted five times with ethyl acetate, and the combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated to provide the title compound (121 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.67−2.71 (m, 2H), 2.96 (t, J = 6.4 Hz, 2H), 3.10−3.14 (m, 2H), 3.92 (t, J = 6.4 Hz, 2H), 7.24 (d, J = 7.8 Hz, 1H), 7.35 (s, 1H), 7.71 (d, J = 7.8 Hz, 1H). MS (DCI) m/z 177 $(M + H)^+$.

2-(1-Oxo-indan-5-yl)ethyl Methanesulfonate (20). To a solution of **19** (110 mg, 0.62 mmol) in THF (5 mL) was added triethylamine (130 μL, 0.94 mmol), followed by methanesulfonyl chloride (58 μL, 0.75 mmol) at 0 °C. The mixture was stirred for 1 h, while being allowed to warm to ambient temperature, poured into H_2O, and extracted five times with ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and concentrated to provide the title compound (150 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.68−2.72 (m, 2H), 2.93 (s, 3H), 3.12−3.17 (m, 4H), 4.46 (t, J = 6.4 Hz, 2H), 7.25 (d, J = 7.8 Hz, 1H), 7.37 (s, 1H), 7.73 (d, J = 7.8 Hz, 1H). MS (DCI) m/z 255 $(M + H)^+$.

5-(2-(4-Methylpiperazin-1-yl)ethyl)indan-1-one (21). To a suspension of **20** (150 mg, 0.59 mmol) and potassium carbonate (163 mg, 1.18 mmol) in 1:1 ethanol/THF (8 mL) was added 1-methylpiperazine (131 μL, 1.18 mmol). The mixture was refluxed overnight and concentrated, and the crude product was purified by flash column chromatography on silica gel eluting with 10:1 dichloromethane/methanol + 1% ammonium hydroxide to provide the title compound (20 mg, 13%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.33 (s, 3H), 2.45−2.67 (m, 8H), 2.63−2.67 (m, 2H), 2.67−2.69 (m, 2H), 2.87−2.90 (m, 2H), 3.09−3.12 (m, 2H), 7.21 (d, J = 7.8 Hz, 1H), 7.30 (s, 1H), 7.67 (d, J = 7.8 Hz, 1H). MS (DCI) m/z 259 $(M + H)^+$.

Diethyl (Spiro[1,3dioxolane-2,1′-indane]-5′-yl)methylphosphonate (22). A solution of methanesulfonyl chloride (0.38 mL, 4.9 mmol) in dichloromethane (5 mL) was added dropwise to a solution of **13**[10] (1.0 g, 4.85 mmol) and triethylamine (0.73 mL, 5.25 mmol) in dichloromethane (10 mL) at 0 °C. The mixture was stirred for 30 min, while being allowed to warm to ambient temperature and was then washed once with a saturated aqueous solution of sodium bicarbonate. The organic layer was dried (Na_2SO_4) and evaporated. The residue was taken up in THF (5 mL) and was slowly added to a mixture of diethyl phosphite (0.82 mL, 6.4 mmol) and a 60% suspension of NaH in mineral oil (252 mg, 6.3 mmol) in THF (5 mL) at room temperature. The reaction mixture was refluxed for 1 h, cooled, and quenched with H_2O. The layers were separated, and the aqueous phase was extracted three times with ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and evaporated, and the crude product was purified by flash column chromatography on silica gel using ethyl acetate as the mobile phase to provide the title compound (954 mg, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, J = 7.1 Hz, 6H), 2.27−2.32 (m, 2H), 2.91−2.95 (m, 2H), 3.14 (d, J = 31.7 Hz, 2H), 3.95−4.05 (m, 4H), 4.06−4.21 (m, 4H), 7.16−7.19 (m, 2H), 7.29 (d, J = 8.1 Hz, 1H). MS (DCI) m/z 327 $(M + H)^+$.

4-((Spiro[1,3dioxolane-2,1′-indane]-5′-yl)methylene)-1-methylpiperidine (92). To a 60% suspension of NaH in mineral oil (90 mg, 2.24 mmol) in 1,2-dimethoxyethane (3.5 mL) was added dropwise a solution of **22** (665 mg, 2.04 mmol) in 1,2-dimethoxyethane (2.0 mL) at 0 °C. The mixture was stirred for 15 min before a solution of 1-methyl-4-piperidone (231 mg, 2.04 mmol) in 1,2-dimethoxyethane (2.0 mL) was added. The mixture was heated to reflux overnight, cooled, quenched with H_2O, and concentrated. The residue was partitioned between ethyl acetate and H_2O. The organic layer was separated, dried ($MgSO_4$), and concentrated. The crude product was purified by flash column chromatography on silica gel using 10:1 dichloromethane/methanol + 1% ammonium hydroxide as the mobile phase to provide the title compound (313 mg, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.28−2.33 (m, 2H), 2.38 (s, 3H), 2.43−2.67 (m, 8H), 2.91−2.96 (m, 2H), 4.05−4.22 (m, 4H), 6.33 (s, 1H), 7.05−7.08 (m, 2H), 7.31 (d, J = 7.5 Hz, 1H). MS (DCI) m/z 286 $(M + H)^+$.

4-((Spiro[1,3dioxolane-2,1′-indane]-5′-yl)methyl)-1-methylpiperidine (93). To a solution of **92** (350 mg, 1.35 mmol) in methanol (70 mL) was added 10% palladium on charcoal (350 mg), and the mixture was hydrogenated at 60 psi for 2 h. The mixture was filtered through Celite, and the filtrate was concentrated to provide the title compound (344 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49−1.57 (m, 2H), 1.68−1.72 (m, 2H), 2.02−2.16 (m, 1H), 2.27−2.31 (m, 4H), 2.41 (s, 3H), 2.55−2.57 (m, 2H), 2.89−2.94 (m, 2H), 3.01−3.05 (m, 2H), 4.05−4.12 (m, 2H), 4.13−4.21 (m, 2H), 7.00−7.03 (m, 2H), 7.27 (d, J = 7.5 Hz, 1H). MS (DCI) m/z 288 $(M + H)^+$.

5-((1-Methylpiperidin-4-yl)methyl)indan-1-one (23). A solution of **93** (340 mg, 1.18 mmol) and *p*-toluenesulfonic acid monohydrate (225 mg, 1.18 mmol) in a mixture of 5:1 acetone/H_2O (6 mL) was refluxed for 1 h. The mixture was cooled, concentrated, and partitioned between ethyl acetate and a saturated aqueous solution of potassium carbonate. The layers were separated, and the aqueous phase was extracted five times with ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and evaporated, and the crude product was purified by flash column chromatography on silica gel using 10:1 dichloromethane/methanol + 1% ammonium hydroxide as the mobile phase to provide the title compound (166 mg, 58%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43−1.68 (m, 5H), 1.95−2.03 (m, 2H), 2.33 (s, 3H), 2.63 (d, J = 6.8 Hz, 2H), 2.66−2.70 (m, 2H), 2.91−2.95 (m, 2H), 3.09−3.13 (m, 2H), 7.16 (d, J = 7.8 Hz, 1H), 7.24 (s, 1H), 7.68 (d, J = 7.8 Hz, 1H). MS (DCI) m/z 244 $(M + H)^+$.

5-(4-Methylpiperazine-1-carbonyl)indan-1-one (24). To **10** (497 mg, 2.35 mmol) were added triethylamine (10 mL), 1-methylpiperazine (0.5 mL, 4.5 mmol), and [1,1′bis(diphenylphosphino)-ferrocene]dichloropalladium(II) complex with dichloromethane

(1:1) (41.3 mg, 0.05 mmol), and the mixture was carbonylated at 130 psi and about 110 °C for 20 h. The mixture was filtered through Celite and concentrated, and the crude product was purified by flash column chromatography on silica gel using dichloromethane/methanol (20:1) as the mobile phase to provide the title compound (250 mg, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (s, 3H), 2.40−2.58 (m, 4H), 2.66−2.69 (m, 2H), 3.12−3.15 (m, 2H), 3.25−3.35 (m, 4H), 7.40 (d, J = 7.7 Hz, 1H), 7.58 (s, 1H), 7.69 (d, J = 7.7 Hz, 1H). MS (DCI) m/z 259 $(M + H)^+$.

5-(2-Methoxyethoxy)indan-1-one (26). To a solution of **25** (870 mg, 5.9 mmol) in DMF (20 mL) were added 1-bromo-2-methoxyethane (1.8 g, 12.9 mmol) and potassium carbonate (2.0 g, 14.5 mmol), and the mixture was heated to reflux for 2 h. The solvent was evaporated, and the crude product was purified by flash column chromatography on silica gel using hexane/ethyl acetate (9:1) as the mobile phase to provide the title compound (1.0 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.65−2.69 (m, 2H), 3.06−3.10 (m, 2H), 3.46 (s, 3H), 3.77−3.80 (m, 2H), 4.18−4.21 (m, 2H), 6.92−6.95 (m, 2H), 7.68 (d, J = 9.5 Hz, 1 H). MS (ESI) m/z 207 $(M + H)^+$.

***N*-(Prop-2-ynyl)aniline (30).** A solution of **27** (0.11 mL, 1 mmol) and aniline (0.46 mL, 5 mmol) in ethanol (2 mL) was stirred at room temperature for 5 d. The solvent was evaporated, and the crude product was purified by flash chromatography on silica gel using hexane/ethyl acetate (20:1) as eluent to provide the title compound (120 mg, 91%). ^{1}H NMR (500 MHz, CD_3OD) δ 2.47 (t, J = 2.5 Hz, 1H), 3.87 (d, J = 2.5 Hz, 2H), 6.66−6.71 (m, 3H), 7.11−7.14 (m, 2H). MS (ESI) m/z 132 $(M + H)^+$.

1-Chloro-2-(prop-2-ynyloxy)benzene (32). A mixture of **31a** (1.0 g, 4.76 mmol), 2-chlorophenol (0.6 g, 4.76 mmol), and potassium carbonate (1.0 g, 7.14 mmol) in acetone (25 mL) was stirred at 56 °C for 2 d. The mixture was cooled, filtered, and the solvent was carefully distilled off at atmospheric pressure. The crude product was purified by flash chromatography on silica gel using diethyl ether/*n*-pentane (1:9) as eluent to provide the title compound (400 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.53 (t, J = 2.5 Hz, 1H), 4.76 (d, J = 2.5 Hz, 2H), 6.94 (dt, J = 1.5, 7.8, 7.8 Hz, 1H), 7.07 (dd, J = 8.3, 1.5 Hz, 1H), 7.21 (ddd, J = 8.3, 7.8, 1.5 Hz, 1H), 7.36 (dd, J = 7.8, 1.5 Hz, 1H). MS (DCI) m/z 166, 168 $(M + H)^+$.

4-(Morpholinomethyl)phenol (36). To a solution of **35** (500 mg, 4.09 mmol) and morpholine (356 mg, 4.09 mmol) in methanol (10 mL) was added macroporous triethylammonium methylpolystyrene borohydride (2.0 g, 6.0 mmol), and the mixture was stirred at ambient temperature for 2 d. The mixture was filtered and evaporated in vacuum to provide the title compound (780 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.44−2.48 (m, 4H), 3.44 (s, 2H), 3.70−3.73 (m, 4H), 6.70−6.73 (m, 2H), 7.14−7.17 (m, 2H). MS (ESI) m/z 194 $(M + H)^+$.

4-(4-(Prop-2-ynyloxy)benzyl)morpholine (37). To a solution of **36** (780 mg, 4.04 mmol) in THF (20 mL) were added propargyl alcohol (229 mg, 4.09 mmol), diphenylphosphino-polystyrene (5.1 g, 8.18 mmol), and diethyl azodicarboxylate (1.06 g, 6.13 mmol). After being stirred overnight at room temperature, the mixture was filtered and the filtrate was concentrated. The crude product was purified by flash chromatography on silica gel using diethyl ether/*n*-pentane (1:3) as eluent to provide the title compound (869 mg, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42−2.43 (m, 4H), 2.52 (t, J = 2.4 Hz, 1H), 3.45 (s, 2H), 3.69−3.72 (m, 4H), 4.68 (d, J = 2.4 Hz, 2H), 6.92−6.94 (m, 2H), 7.24−7.27 (m, 2H). MS (DCI-NH_3) m/z 232 $(M + H)^+$.

3-(2-Methoxyethoxy)prop-1-yne (39). A solution of **38** (5.0 g, 89.2 mmol) in THF (20 mL) was added dropwise to a 60% suspension of NaH in mineral oil (3.55 g, 89.2 mmol) in THF (100 mL) at 5 °C, and the mixture was stirred for 30 min. Then, 2-bromoethyl methyl ether (5.6 mL, 59.5 mmol) was added, and the mixture was stirred overnight at room temperature. The reaction was quenched by addition of H_2O, and the layers were separated. The aqueous layer was extracted with diethyl ether, and the combined organic extracts were dried ($MgSO_4$) and filtered. The organic solvents were carefully distilled off at atmospheric pressure, and the crude product was purified by flash chromatography on silica gel using diethyl ether/*n*-pentane (1:3) as eluent to provide the title compound (3.8 g, 56%). ^{1}H NMR (300 MHz, CD_3OD) δ 2.74 (t, J = 2.4 Hz, 1H), 3.37 (s, 3H), 3.47−3.56 (m, 2H), 3.64−3.74 (m, 2H), 4.15 (d, J = 2.4 Hz, 2H). MS (DCI) m/z 115 $(M + H)^+$.

6-((4-Methylpiperazin-1-yl)methyl)-3-(5-((trimethylsilyl)ethynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole (40). Compound **40** was prepared using the same procedure as described for the synthesis of **8** by substituting trimethylsilylacetylene for phenyl propargyl ether. The title compound was obtained after purification by flash chromatography on silica gel using 10:1 dichloromethane/methanol + 1% ammonium hydroxide as the mobile phase (150 mg, 75%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.00 (s, 9H), 2.54 (s, 3H), 2.75−3.2 (m, 8H), 3.58 (s, 2H), 3.68 (bs, 2H), 7.13 (d, J = 7.8 Hz, 1H), 7.32 (s, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 1.3 Hz, 1H), 7.62 (d, J = 1.3 Hz, 1H). MS (ESI) m/z 447 $(M + H)^+$.

3-(5-Ethynylthiophen-3-yl)-6-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazole (41). A solution of **40** (130 mg, 0.29 mmol) and tetrabutylammonium fluoride hydrate (250 mg, 0.96 mmol) in THF (3.0 mL) was stirred at room temperature for 30 min. The solvent was evaporated, and the crude product was purified by flash column chromatography on silica gel using ethyl acetate/methanol (95:5) as the mobile phase to provide the title compound (78 mg, 72%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.14 (s, 3H), 2.22−2.45 (m, 8H), 3.49 (s, 2H), 3.78 (s, 2H), 4.63 (s, 1H), 7.27 (d, J = 7.8 Hz, 1H), 7.46 (s, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.73 (d, J = 1.3 Hz, 1H), 7.83 (d, J = 1.3 Hz, 1H). MS (ESI) m/z 375 $(M + H)^+$. HRMS (FAB) m/z 375.16506; calcd, $C_{22}H_{23}N_4S$: 375.16379 $(M + H)^+$. HPLC 99% purity (A), 99% purity (B).

2-(3-(4-(6-((4-Methylpiperazin-1-yl)methyl)-1,4-dihydroindeno-[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)prop-2-ynyl)isoindoline-1,3-dione (42). Compound **42** was prepared using the same procedure as described for the synthesis of **8** by substituting *N*-propargylphthalimide for phenyl propargyl ether. The title compound was obtained after purification by flash chromatography on silica gel using 10:1 dichloromethane/methanol + 1% ammonium hydroxide as the mobile phase (2.2 g, 89%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.15 (s, 3H), 2.25−2.45 (m, 8H), 3.50 (s, 2H), 3.78 (s, 2H), 4.70 (s, 2H), 7.27 (d, J = 7.8 Hz, 1H), 7.47 (s, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 1.7 Hz, 1H), 7.81 (d, J = 1.7 Hz, 1H), 7.87−7.90 (m, 2H), 7.93−7.96 (m, 2H). MS (ESI) m/z 534 $(M + H)^+$.

2-(3-(4-(1-(Bis(4-methoxyphenyl)methyl)-6-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)prop-2-ynyl)isoindoline-1,3-dione (94). To 4,4′-dimethoxybenzhydrol (1.8 g, 7.3 mmol) was carefully added thionyl chloride (6.3 mL, 86.9 mmol), and the mixture was heated to reflux for 1 h. The solution was cooled, evaporated to dryness, and the residue was taken up in THF (15 mL). This solution was then added to a solution of **42** (2.2 g, 4.1 mmol) and triethylamine (1.7 mL, 12.4 mmol) in THF (25 mL). The reaction mixture was heated to 50 °C for 2 h, cooled, diluted with H_2O, and extracted six times with ethyl acetate. The combined organic extracts were dried (Na_2SO_4) and evaporated, and the crude product was purified by flash chromatography on silica gel using dichloromethane/methanol (10:1) as eluent to provide the title compound (1.9 g, 60%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.14 (s, 3H), 2.20−2.45 (m, 8H), 3.47 (s, 2H), 3.72 (s, 6H), 3.77 (s, 2H), 4.69 (s, 2H), 6.90−6.93 (m, 4H), 7.10 (s, 1H), 7.17 (d, J = 7.9 Hz, 1H), 7.25−7.27 (m, 4H), 7.37 (d, J = 7.9 Hz, 1H), 7.47 (s, 1H), 7.50 (d, J = 1.5 Hz, 1H), 7.72 (d, J = 1.5 Hz, 1H), 7.87−7.89 (m, 2H), 7.93−7.95 (m, 2H). MS (ESI) m/z 760 $(M + H)^+$.

3-(4-(1-(Bis(4-methoxyphenyl)methyl)-6-((4-methylpiperazin-1-yl)methyl)-1 ,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)prop-2-yn-1-amine (43). A solution of **94** (2.9 g, 3.8 mmol) and anhydrous hydrazine (0.53 mL, 16.9 mmol) in 1:1 ethanol/THF (60 mL) was stirred at ambient temperature overnight. THF (30 mL) was added, and the mixture was filtered. The filtrate was

concentrated, and the crude product was purified by flash chromatography on silica gel using 10:1 dichloromethan/methanol + 1% ammonium hydroxide as eluent to provide the title compound (1.1 g, 46%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.14 (s, 3H), 2.20−2.45 (m, 8H), 3.47 (s, 2H), 3.54 (s, 2H), 3.72 (s, 6H), 3.78 (s, 2H), 6.91−6.93 (m, 4H), 7.09 (s, 1H), 7.17 (d, J = 7.8 Hz, 1H), 7.25−7.27 (m, 4H), 7.37 (d, J = 7.8 Hz, 1H), 7.47 (s, 1H), 7.50 (d, J = 1.3 Hz, 1H), 7.68 (d, J = 1.3 Hz, 1H). MS (ESI) m/z 630 $(M + H)^+$.

***N*-(3-(4-(6-((4-Methylpiperazin-1-yl)methyl)-1,4-dihydroindeno-[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)prop-2-ynyl)benzenesulfonamide TFA Salt (44).** To benzenesulfonyl chloride (11 μL, 0.09 mmol) was added a solution of **43** (50 mg, 0.08 mmol) in pyridine (1 mL), and the mixture was agitated at room temperature for 2.5 h. The mixture was evaporated in vacuum. To the residue was added a 4 M solution of hydrogen chloride in 1,4-dioxane (1 mL), and the mixture was agitated at room temperature for 6 h. The mixture was concentrated, and the crude product was purified by preparative reverse phase HPLC to give the title compound (18 mg, 47%). ^{1}H NMR (500 MHz, CD_3OD) δ 2.28 (s, 3H), 2.40−2.65 (m, 8H), 3.61 (s, 2H), 3.78 (s, 2H), 4.09 (s, 2H), 7.34 (d, J = 7.8 Hz, 1H), 7.38 (s, 1H), 7.55−7.66 (m, 6H), 7.92−7.94 (m, 2H). MS (ESI) m/z 544 $(M + H)^+$. HRMS (FAB) m/z 544.18469; calcd, $C_{29}H_{30}N_5O_2S_2$: 544.18354 $(M + H)^+$. HPLC 97% purity (A), 96% purity (B).

Phenyl 3-(4-(6-((4-Methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)prop-2-ynylcarbamate TFA Salt (45). To phenyl chloroformate (11 μL, 0.09 mmol) was added a solution of **43** (50 mg, 0.08 mmol) and triethylamine (22 μL) in dichloromethane (0.7 mL), and the mixture was shaken at room temperature overnight. The mixture was concentrated, and the residue was taken up in ethyl acetate (1 mL). A 37% solution of hydrochloric acid in ethanol (1 mL) was added and the mixture was shaken at room temperature overnight. The mixture was concentrated, and the crude product was purified by preparative reverse phase HPLC to give the title compound (4 mg, 11%). ^{1}H NMR (500 MHz, CD_3OD) δ 2.28 (s, 3H), 2.42−2.63 (m, 8H), 3.61 (s, 2H), 3.80 (s, 2H), 4.25 (s, 2H), 7.13−7.16 (m, 2H), 7.22−7.23 (m, 1H), 7.34 (d, J = 8.1 Hz, 1H), 7.36−7.39 (m, 2H), 7.55 (s, 1H), 7.63 (s, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.69 (s, 1H). MS (ESI) m/z 524 $(M + H)^+$. Anal. ($C_{30}H_{29}N_5O_2S\cdot3.5CF_3CO_2H$) C, H, N.

1-(3-(4-(6-((4-Methylpiperazin-1-yl)methyl)-1,4-dihydroindeno-[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)prop-2-ynyl)-3-phenylurea TFA Salt (46). To phenyl isocyanate (10 μL, 0.09 mmol) was added a solution of **43** (50 mg, 0.08 mmol) in THF (1 mL), and the mixture was agitated at room temperature overnight. The mixture was concentrated; the residue was suspended in a 4 M solution of hydrogen chloride in 1,4-dioxane (1 mL) and was agitated at room temperature overnight. The mixture was concentrated, and the crude product was purified by preparative reverse phase HPLC to give the title compound (32 mg, 30%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.80 (s, 3H), 3.00−3.50 (m, 8H), 3.83 (s, 2H), 3.96 (bs, 2H), 4.20 (d, J = 5.5 Hz, 2H), 6.67 (t, J = 5.5 Hz, 1H), 6.91−6.94 (m, 1H), 7.23−7.26 (m, 2H), 7.38 (d, J = 7.6 Hz, 1H), 7.41−7.43 (m, 2H), 7.58 (s, 1H), 7.66−7.67 (m, 2H), 7.84 (s, 1H), 8.70 (s, 1H). MS (ESI m/z 523 $(M + H)^+$. Anal. ($C_{30}H_{30}N_6OS\cdot4.5CF_3CO_2H$) C, H, N.

Ethyl 2-(Prop-2-ynyloxy)acetate (95). To a solution of ethyl glycolate (3.12 g, 30.0 mmol) in THF (80 mL) was added a 60% suspension of NaH in mineral oil (1.28 g, 32.0 mmol), and the mixture was stirred for 1 h at ambient temperature. Propargyl bromide (4.08 g, 35.0 mmol) was added dropwise, and stirring was continued for 3 d. The mixture was concentrated; the residue was taken up in diethyl ether (150 mL) and was washed twice with H_2O. The organic layer was dried ($MgSO_4$) and evaporated, and the crude product was purified by flash chromatography on silica gel using pentane/diethyl ether (5:1) as eluent to provide the title compound (2.7 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (t, J = 7.1 Hz, 3H), 2.48 (t, J = 2.4 Hz, 1H), 4.20 (s, 2H), 4.24 (q, J = 7.1 Hz, 2H), 4.32 (d, J = 2.4 Hz, 2H). MS (ESI) m/z 143 (M + H)$^+$.

Ethyl 2-(3-(4-(6-((4-Methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)prop-2-ynyloxy)acetate (47). Compound **47** was prepared using the same procedure as described for the synthesis of **8** by substituting **95** for phenyl propargyl ether. The title compound was obtained after purification by flash chromatography on silica gel using 10:1 dichloromethane/methanol + 1% ammonium hydroxide as the mobile phase (270 mg, 59%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.22 (t, J = 7.2 Hz, 3H), 2.81 (s, 3H), 3.00−3.50 (m, 8H), 3.84 (s, 2H), 4.02 (bs, 2H), 4.14 (s, 2H), 4.16 (q, J = 7.2 Hz, 2H), 4.53 (s, 2H), 7.41 (d, J = 7.8 Hz, 1H), 7.60 (s, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.75 (s, 1H), 7.90 (s, 1H). MS (ESI) m/z 491 $(M + H)^+$.

2-(3-(4-(6-((4-Methylpiperazin-1-yl)methyl)-1,4-dihydroindeno-[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)prop-2-ynyloxy)acetamide TFA Salt (48). To **47** (120 mg, 0.24 mmol) was added a 2 M solution of ammonia in methanol (2 mL), and the mixture was stirred at room temperature for 3 d. The mixture was concentrated, and the crude product was purified by preparative reverse phase HPLC to give the title compound (130 mg, 68%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.81 (s, 3H), 3.00−3.50 (m, 8H), 3.84 (s, 2H), 3.95 (s, 2H), 4.00 (bs, 2H), 4.53 (s, 2H), 7.26 (m, 2H), 7.40 (d, J = 7.8 Hz, 1H), 7.60 (s, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.75 (s, 1H), 7.90 (s, 1H). MS (ESI) m/z 462 $(M + H)^+$. Anal. ($C_{25}H_{27}N_5O_2S\cdot3.2CF_3CO_2H$) C, H, N.

3-(5-Bromothiophen-3-yl)-6-((4-methylpiperazin-1-yl)methyl)-indeno[1,2-*c*]pyrazol-4(1*H*)-one (49). To a solution of **7** (800 mg, 1.87 mmol) in DMF (180 mL) was added cesium carbonate (2 g, 6.1 mmol), and the mixture was heated to 90 °C. Air was bubbled through the vigorously stirred mixture, and heating was continued overnight. The reaction mixture was cooled and filtered, and the filtrate was evaporated. The crude product was purified by flash chromatography on silica gel using 9:1 ethyl acetate/methanol + 1% ammonium hydroxide as eluent to provide the title compound (503 mg, 61%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.15 (s, 3H), 2.22−2.47 (m, 8H), 3.50 (s, 2H), 7.43−7.50 (m, 2H), 7.51 (s, 1H), 7.87 (d, J = 1.7 Hz, 1H), 8.35 (d, J = 1.7 Hz, 1H). MS (ESI) m/z 443, 445 $(M + H)^+$.

3-(5-(3-(2-Methoxyethoxy)prop-1-ynyl)thiophen-3-yl)-6-((4-methylpiperazin-1-yl)methyl)indeno[1,2-*c*]pyrazol-4(1*H*)-one TFA Salt (50). Compound **50** was prepared using the same procedure as described for the synthesis of **8** by substituting **49** for **7** and **39** for phenyl propargyl ether (59 mg, 55%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.79 (s, 3H), 2.95−3.15 (m, 8H), 3.27 (s, 3H), 3.50−3.51 (m, 2H), 3.63−3.64 (m, 2H), 3.74 (s, 2H), 4.47 (s, 2H), 7.51−7.56 (m, 2H), 7.61 (s, 1H), 7.97 (s, 1H), 8.37 (s, 1H), 13.79 (bs, 1H). MS (ESI) m/z 477 $(M + H)^+$. HRMS (FAB) m/z 477.19585; calcd, $C_{26}H_{29}N_4O_3S$: 477.19549 $(M + H)^+$. HPLC 96% purity (A), 95% purity (B).

6-((4-Methylpiperazin-1-yl)methyl)-*N*-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazol-3-amine TFA Salt (51). To a solution of **5**[10] (200 mg, 0.82 mmol) and phenyl isothiocyanate (98 μL, 0.82 mmol) in DMF (0.6 mL) was added a 60% suspension of NaH in mineral oil (36 mg, 0.9 mmol) at 0 °C. The mixture was then stirred for 1.5 h at room temperature, methyl iodide (56 μL, 0.9 mmol) was added, and stirring was continued for 1 h. The mixture was concentrated; the residue was taken up in diethyl ether (10 mL) and was washed with H_2O and brine. The organic layer was dried ($MgSO_4$) and concentrated. The crude intermediate was dissolved in ethanol (1.4 mL), hydrazine monohydrate (60 μL, 1.23 mmol) was added, and the mixture was refluxed overnight. The solvent was evaporated, and the crude product was purified by preparative reverse phase HPLC to provide the title compound (200 mg, 30%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.80 (s, 3H), 2.90−3.40 (m, 8H), 3.49 (s, 2H), 3.94 (bs, 2H), 6.74−6.78 (m, 1H), 7.16−7.23 (m, 4H), 7.37 (d, J = 7.7 Hz, 1H), 7.55−7.58 (m, 2H), 8.45 (bs, 1H). MS (ESI) m/z 360 $(M + H)^+$. Anal. ($C_{22}H_{25}N_5\cdot3.4CF_3CO_2H$) C, H, N.

3-(5-Bromothiophen-2-yl)-6-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazole (97). Compound **97** was prepared using the same procedure as described for the synthesis of **7** by substituting phenyl 5-bromothiophene-2-carboxylate[10] for **6** (2.4

g, 55%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.15 (s, 3H), 2.23−2.48 (m, 8H), 3.50 (s, 2H), 3.73 (s, 2H), 7.18−7.35 (m, 3H), 7.49 (s, 1H), 7.52−7.62 (m, 1H). MS (ESI) *m*/*z* 429, 431 (M + H)$^+$.

6-((4-Methylpiperazin-1-yl)methyl)-3-(5-(3-phenoxyprop-1-ynyl)thiophen-2 -yl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (52). Compound **52** was prepared using the same procedure as described for the synthesis of **8** by substituting **97** for **7** (141 mg, 74%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.79 (s, 3H), 3.00−3.50 (m, 8H), 3.78 (s, 2H), 3.84 (bs, 2H), 5.10 (s, 2H), 6.94−7.06 (m, 3H), 7.11 (s, 1H), 7.28 (s, 1H), 7.32−7.42 (m, 3H), 7.58 (s, 1H), 7.63 (d, J = 7.8 Hz, 1H). MS (ESI) *m*/*z* 481 (M + H)$^+$. Anal. ($C_{29}H_{28}N_4OS\cdot 2.9CF_3CO_2H$) C, H, N.

3-(4-Bromothiophen-2-yl)-6-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazole (98). Compound **98** was prepared using the same procedure as described for the synthesis of **7** by substituting phenyl 4-bromothiophene-2-carboxylate for **6** (850 mg, 87%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.15 (s, 3H), 2.25−2.45 (m, 8H), 3.51 (s, 2H), 3.77 (s, 2H), 7.30 (d, J = 7.8 Hz, 1H), 7.40 (s, 1H), 7.49 (s, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.68 (s, 1H). MS (ESI) *m*/*z* 429, 431 (M + H)$^+$.

6-((4-Methylpiperazin-1-yl)methyl)-3-(4-(3-phenoxyprop-1-ynyl)thiophen-2 -yl)-1,4-dihydroindeno[1,2-*c*]pyrazole (53). Compound **53** was prepared using the same procedure as described for the synthesis of **8** by substituting **98** for **7**. The title compound was obtained after purification by flash chromatography on silica gel using 10:1 dichloromethane/methanol + 1% ammonium hydroxide as the mobile phase (35 mg, 31%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.23 (s, 3H), 2.35−2.55 (m, 8H), 3.76 (s, 2H), 4.01 (s, 2H), 5.05 (s, 2H), 6.98−7.01 (m, 1H), 7.04−7.06 (m, 2H), 7.29 (d, J = 7.7 Hz, 1H), 7.32−7.36 (m, 3H), 7.49 (s, 1H), 7.52−7.61 (m, 1H), 7.76 (bs, 1H), 13.22 (s, 1H). MS (ESI) *m*/*z* 481 (M + H)$^+$. Anal. ($C_{29}H_{28}N_4OS\cdot 0.4CH_2Cl_2$) C, H, N.

Phenyl 3-(3-Phenoxyprop-1-ynyl)benzoate (99). Compound **99** was prepared using the same procedure as described for the synthesis of **9** by substituting phenyl 3-bromobenzoate for **6** (160 mg, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.94 (s, 2H), 6.98−7.05 (m, 3H), 7.19−7.22 (m, 2H), 7.26−7.35 (m, 3H), 7.41−7.49 (m, 3H), 7.69 (dt, J = 7.8, 1.7, 1.7 Hz, 1H), 8.15 (dt, J = 7.8, 1.7, 1.7 Hz, 1H), 8.28 (t, J = 1.7 Hz, 1H). MS (ESI) *m*/*z* 329 (M + H)$^+$.

6-((4-Methylpiperazin-1-yl)methyl)-3-(3-(3-phenoxyprop-1-ynyl)phenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (54). Compound **54** was prepared using the same procedure as described for the synthesis of **7** by substituting **99** for **6**. The crude product was purified by preparative reverse phase HPLC to provide the title compound (125 mg, 42%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.79 (s, 3H), 3.00−3.50 (m, 8H), 3.86 (bs, 2H), 3.89 (s, 2H), 5.09 (s, 2H), 6.99−7.02 (m, 1H), 7.07−7.09 (m, 2H), 7.34−7.39 (m, 3H), 7.43 (d, J = 7.8 Hz, 1H), 7.53 (t, J = 7.8 Hz, 1H), 7.58 (s, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.89 (s, 1H). MS (ESI) *m*/*z* 475 (M + H)$^+$. HRMS (FAB) *m*/*z* 475.25057; calcd, $C_{31}H_{31}N_4O$: 475.24924 (M + H)$^+$. HPLC 97% purity (A), 96% purity (B).

Phenyl 4-(3-Phenoxyprop-1-ynyl)benzoate (100). Compound **100** was prepared using the same procedure as described for the synthesis of **9** by substituting phenyl 4-bromobenzoate for **6** (2.2 g, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.95 (s, 2H), 7.00−7.05 (m, 3H), 7.20−7.21 (m, 2H), 7.25−7.29 (m, 1H), 7.32−7.35 (m, 2H), 7.41−7.44 (m, 2H), 7.55−7.57 (m, 2H), 8.13−8.15 (m, 2H). MS (ESI) *m*/*z* 329 (M + H)$^+$.

6-((4-Methylpiperazin-1-yl)methyl)-3-(4-(3-phenoxyprop-1-ynyl)phenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (55). Compound **55** was prepared using the same procedure as described for the synthesis of **7** by substituting **100** for **6**. The crude product was purified by preparative reverse phase HPLC to provide the title compound (110 mg, 70%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.78 (s, 3H), 3.00−3.50 (m, 8H), 3.88 (s, 4H), 5.06 (s, 2H), 6.98−7.01 (m, 1H), 7.05−7.06 (m, 2H), 7.32−7.35 (m, 2H), 7.37 (d, J = 7.5 Hz, 1H), 7.56−7.57 (m, 3H), 7.66 (d, J = 7.5 Hz, 1H), 7.81−7.83 (m, 2H). MS (ESI) *m*/*z* 475 (M + H)$^+$. HRMS (FAB) *m*/*z* 475.24940; calcd, $C_{31}H_{31}N_4O$: 475.24924 (M + H)$^+$. HPLC 99% purity (A), 99% purity (B).

3-(5-Bromothiophen-3-yl)-7-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazole (101). Compound **101** was prepared using the same procedure as described for the synthesis of **7** by substituting 6-((4-methylpiperazin-1-yl)methyl)indan-1-one[10] for **5** (3.25 g, 86%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.15 (s, 3H), 2.25−2.45 (m, 8H), 3.52 (s, 2H), 3.78 (s, 2H), 7.20 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.58 (s, 1H), 7.64 (s, 1H), 7.84 (s, 1H). MS (ESI) *m*/*z* 429, 431 (M + H)$^+$.

7-((4-Methylpiperazin-1-yl)methyl)-3-(5-(3-phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (56). Compound **56** was prepared using the same procedure as described for the synthesis of **8** by substituting **101** for **7** (57 mg, 34%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.81 (s, 3H), 3.00−3.50 (m, 8H), 3.84 (s, 2H), 4.07 (bs, 2H), 5.11 (s, 2H), 6.99−7.02 (m, 1H), 7.05−7.06 (m, 2H), 7.33−7.37 (m, 3H), 7.59 (d, J = 7.7 Hz, 1H), 7.74 (m, 2H), 7.89 (d, J = 1.3 Hz, 1H). MS (ESI) *m*/*z* 481 (M + H)$^+$. Anal. ($C_{29}H_{28}N_4OS\cdot 3.7CF_3CO_2H$) C, H, N.

6-((4-Methylpiperazin-1-yl)methyl)-3-(5-(pent-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (57). Compound **57** was prepared using the same procedure as described for the synthesis of **8** by substituting 1-pentyne for phenyl propargyl ether (49 mg, 32%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.99−1.02 (m, 3H), 1.55−1.62 (m, 2H), 2.15 (s, 3H), 2.25−2.45 (m, 8H), 2.46−2.48 (m, 2H), 3.50 (s, 2H), 3.79 (m, 2H), 7.27 (d, J = 7.8 Hz, 1H), 7.47 (s, 1H), 7.56−7.63 (m, 2H), 7.75 (s, 1H). MS (ESI) *m*/*z* 417 (M + H)$^+$. HRMS (FAB) *m*/*z* 417.21150; calcd, $C_{25}H_{29}N_4S$: 417.21074 (M + H)$^+$. HPLC 95% purity (A), 95% purity (B).

4-(4-(6-((4-Methylpiperazin-1-yl)methyl)-1,4-dihydroindeno-[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)but-3-yn-1-ol TFA Salt (58). Compound **58** was prepared using the same procedure as described for the synthesis of **8** by substituting 3-butyn-1-ol for phenyl propargyl ether (25 mg, 28%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.62 (t, J = 6.5 Hz, 2H), 2.79 (s, 3H), 3.00−3.50 (m, 8H), 3.60 (t, J = 6.5 Hz, 2H), 3.82 (s, 2H), 3.91 (bs, 2H), 7.37 (d, J = 7.8 Hz, 1H), 7.56 (s, 1H), 7.61 (d, J = 1.2 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.78 (d, J = 1.2 Hz, 1H). MS (ESI) *m*/*z* 419 (M + H)$^+$. Anal. ($C_{24}H_{26}N_4OS\cdot 2.7CF_3CO_2H$) C, H, N.

2-Methyl-5-(4-(6-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)pent-4-yn-2-ol TFA Salt (59). Compound **59** was prepared using the same procedure as described for the synthesis of **8** by substituting 2-methylpent-4-yn-2-ol[16] for phenyl propargyl ether (190 mg, 60%). ^{1}H NMR (500 MHz, CD_3OD) δ 1.36 (s, 6H), 2.63 (s, 2H), 2.90 (s, 3H), 3.02−3.14 (m, 4H), 3.34−3.42 (s, 4H), 3.82 (s, 2H), 4.00 (s, 2H), 7.43 (d, J = 7.8 Hz, 1H), 7.54 (s, 1H), 7.63 (s, 2H), 7.73 (d, J = 7.8 Hz, 1H). MS (ESI) *m*/*z* 447 (M + H)$^+$. HRMS (FAB) *m*/*z* 447.22172; calcd, $C_{26}H_{31}N_4OS$: 447.22131 (M + H)$^+$. HPLC 96% purity (A), 96% purity (B).

3-(5-(3-Methoxyprop-1-ynyl)thiophen-3-yl)-6-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (60). Compound **60** was prepared using the same procedure as described for the synthesis of **8** by substituting methyl propargyl ether for phenyl propargyl ether (88 mg, 54%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.81 (s, 3H), 3.20−3.45 (m, 8H), 3.35 (s, 3H), 3.84 (s, 2H), 3.99 (bs, 2H), 4.39 (s, 2H), 7.40 (d, J = 7.8 Hz, 1H), 7.60 (s, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.74 (d, J = 1.3 Hz, 1H), 7.89 (d, J = 1.3 Hz, 1H). MS (ESI) *m*/*z* 419 (M + H)$^+$. HRMS (FAB) *m*/*z* 419.19022; calcd, $C_{24}H_{27}N_4OS$: 419.19001 (M + H)$^+$. HPLC 96% purity (A), 97% purity (B).

(But-3-ynyloxy)benzene (102). Compound **102** was prepared using the same procedure as described for the synthesis of **32** by substituting 3-butynyl *p*-toluenesulfonate for propargyl *p*-toluenesulfonate and phenol for 2-chlorophenol (170 mg, 52%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.04 (t, J = 2.7 Hz, 1H), 2.68 (dt, J = 7.1, 2.7 Hz, 2H), 4.08 (t, J = 7.1 Hz, 2H), 6.90−6.99 (m, 3H), 7.26−7.31 (m, 2H). MS (ESI) *m*/*z* 147 (M + H)$^+$.

6-((4-Methylpiperazin-1-yl)methyl)-3-(5-(4-phenoxybut-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (61). Compound **61** was prepared using the same procedure as described for the synthesis of **8** by substituting **102** for phenyl propargyl ether (106 mg, 55%). ^{1}H NMR (500 MHz, DMSO-d_6) δ

2.78 (s, 3H), 2.98 (t, J = 6.6 Hz, 2H), 3.00−3.50 (m, 8H), 3.82 (s, 4H), 4.18 (t, J = 6.6 Hz, 2H), 6.95−6.98 (m, 1H), 6.99−7.00 (m, 2H), 7.30−7.33 (m, 2H), 7.36 (d, J = 7.5 Hz, 1H), 7.55 (s, 1H), 7.64−7.66 (m, 2H), 7.80 (s, 1H). MS (ESI) m/z 495 $(M + H)^+$. Anal. ($C_{30}H_{30}N_4OS \cdot 2.6CF_3CO_2H$) C, H, N.

3-(5-(3-(2-Chlorophenoxy)prop-1-ynyl)thiophen-3-yl)-6-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (62). Compound **62** was prepared using the same procedure as described for the synthesis of **8** by substituting **32** for phenyl propargyl ether (106 mg, 55%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.76 (s, 3H), 3.00−3.50 (m, 8H), 3.73 (s, 2H), 3.81 (s, 2H), 5.24 (s, 2H), 7.03 (dt, J = 1.5, 7.8, 7.8 Hz, 1H), 7.31 (dd, J = 7.8, 1.5 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 7.38 (ddd, J = 8.1, 7.8, 1.5 Hz, 1H), 7.47 (dd, J = 8.1, 1.5 Hz, 1H), 7.53 (s, 1H), 7.63 (d, J = 7.8 Hz, 1H), 7.75 (d, J = 1.2 Hz, 1H), 7.90 (d, J = 1.2 Hz, 1H). MS (ESI) m/z 515, 517 $(M + H)^+$. HRMS (FAB) m/z 515.16690; calcd, $C_{29}H_{28}ClN_4OS$: 515.16669 $(M + H)^+$. HPLC 95% purity (A), 95% purity (B).

1-Chloro-3-(prop-2-ynyloxy)benzene (103). Compound **103** was prepared using the same procedure as described for the synthesis of **32** by substituting 3-chlorophenol for 2-chlorophenol (280 mg, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.55 (t, J = 2.4 Hz, 1H), 4.73 (d, J = 2.4 Hz, 2H), 7.15 (dd, J = 8.1, 2.4 Hz, 1H), 7.21−7.27 (m, 2H), 7.42 (t, J = 8.1 Hz, 1H). MS (DCI) m/z 166, 168 $(M + H)^+$.

3-(5-(3-(3-Chlorophenoxy)prop-1-ynyl)thiophen-3-yl)-6-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (63). Compound **63** was prepared using the same procedure as described for the synthesis of **8** by substituting **103** for phenyl propargyl ether (78 mg, 45%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.81 (s, 3H), 3.00−3.50 (m, 8H), 3.83 (s, 2H), 3.98 (bs, 2H), 5.17 (s, 2H), 7.04 (dd, J = 8.4, 0.6 Hz, 1H), 7.07 (dd, J = 7.8, 0.6 Hz, 1H), 7.16 (t, J = 2.2 Hz, 1H), 7.37 (t, J = 8.1 Hz, 1H), 7.40 (d, J = 7.5 Hz, 1H), 7.59 (s, 1H), 7.67 (d, J = 7.5 Hz, 1H), 7.75 (d, J = 1.3 Hz, 1H), 7.91 (d, J = 1.3 Hz, 1H). MS (ESI) m/z 515, 517 $(M + H)^+$. HRMS (FAB) m/z 515.16773; calcd, $C_{29}H_{28}ClN_4OS$: 515.16669 $(M + H)^+$. HPLC 97% purity (A), 97% purity (B).

1-Chloro-4-(prop-2-ynyloxy)benzene (104). Compound **104** was prepared using the same procedure as described for the synthesis of **32** by substituting 4-chlorophenol for 2-chlorophenol (297 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.52 (t, J = 2.4 Hz, 1H), 4.66 (d, J = 2.4 Hz, 2H), 6.88−6.93 (m, 2H), 7.22−7.28 (m, 2H). MS (DCI) m/z 166, 168 $(M + H)^+$.

3-(5-(3-(4-Chlorophenoxy)prop-1-ynyl)thiophen-3-yl)-6-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (64). Compound **64** was prepared using the same procedure as described for the synthesis of **8** by substituting **104** for phenyl propargyl ether (54 mg, 31%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.80 (s, 3H), 3.00−3.50 (m, 8H), 3.83 (s, 2H), 3.97 (bs, 2H), 5.13 (s, 2H), 7.07−7.11 (m, 2H), 7.39−7.41 (m, 3H), 7.59 (s, 1H), 7.67 (d, J = 7.8 Hz, 1H), 7.74 (s, 1H), 7.91 (s, 1H). MS (ESI) m/z 515, 517 $(M + H)^+$. HRMS (FAB) m/z 515.16774; calcd, $C_{29}H_{28}ClN_4OS$: 515.16669 $(M + H)^+$. HPLC 97% purity (A), 98% purity (B).

1,2-Dimethyl-4-(prop-2-ynyloxy)benzene (105). Compound **105** was prepared using the same procedure as described for the synthesis of **32** by substituting 3,4-dimethylphenol for 2-chlorophenol (400 mg, 49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.19 (s, 3H), 2.24 (s, 3H), 2.49 (t, J = 2.4 Hz, 1H), 4.65 (d, J = 2.4 Hz, 2H), 6.72 (dd, J = 8.1, 2.7 Hz, 1H), 6.77 (d, J = 2.7 Hz, 1H), 7.05 (d, J = 8.1 Hz, 1H). MS (DCI) m/z 161 $(M + H)^+$.

3-(5-(3-(3,4-Dimethylphenoxy)prop-1-ynyl)thiophen-3-yl)-6-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (65). Compound **65** was prepared using the same procedure as described for the synthesis of **8** by substituting **105** for phenyl propargyl ether (42 mg, 49%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H), 2.21 (s, 3H), 2.82 (s, 3H), 3.00−3.50 (m, 8H), 3.83 (s, 2H), 4.06 (bs, 2H), 5.04 (s, 2H), 6.77 (dd, J = 8.1, 2.4 Hz, 1H), 6.85 (d, J = 2.4 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.62 (s, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.74 (d, J = 1.3 Hz, 1H), 7.90 (d, J = 1.3 Hz, 1H). MS (ESI) m/z 509 $(M + H)^+$. HRMS (FAB) m/z 509.23907; calcd, $C_{31}H_{33}N_4OS$: 509.23696 $(M + H)^+$. HPLC 96% purity (A), 95% purity (B).

6-((4-Methylpiperazin-1-yl)methyl)-3-(5-(3-(pyridin-3-yloxy)-prop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (66). Compound **66** was prepared using the same procedure as described for the synthesis of **8** by substituting 3-(prop-2-ynyloxy)pyridine for phenyl propargyl ether (85 mg, 52%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.78 (s, 3H), 3.00−3.50 (m, 8H), 3.81 (s, 2H), 3.90 (bs, 2H), 5.24 (s, 2H), 7.37 (d, J = 7.2 Hz, 1H), 7.50 (dd, J = 8.4, 4.7 Hz, 1H), 7.56 (s, 1H), 7.63−7.66 (m, 2H), 7.74 (d, J = 1.3 Hz, 1H), 7.90 (d, J = 1.3 Hz, 1H), 8.30 (d, J = 4.7 Hz, 1H), 8.46 (d, J = 2.8 Hz, 1H). MS (ESI) m/z 482 $(M + H)^+$. Anal. ($C_{28}H_{27}N_5OS \cdot 4.5CF_3CO_2H$) C, H, N.

6-((4-Methylpiperazin-1-yl)methyl)-3-(5-(4-phenylbut-1-ynyl)-thiophen-3-yl) -1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (67). Compound **67** was prepared using the same procedure as described for the synthesis of **8** by substituting 4-phenyl-1-butyne for phenyl propargyl ether (38 mg, 40%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.77−2.80 (m, 5H), 2.88 (t, J = 7.5 Hz, 2H), 3.00−3.50 (m, 8H), 3.81 (s, 2H), 3.89 (bs, 2H), 7.22−7.25 (m, 1H), 7.31−7.33 (m, 4H), 7.38 (d, J = 7.7 Hz, 1H), 7.57 (m, 2H), 7.67 (d, J = 7.7 Hz, 1H), 7.77 (s, 1H). MS (ESI) m/z 479 $(M + H)^+$. Anal. ($C_{30}H_{30}N_4S \cdot 2.4CF_3CO_2H$) C, H, N.

***N*-(3-(4-(6-((4-Methylpiperazin-1-yl)methyl)-1,4-dihydroindeno-[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)prop-2-ynyl)aniline TFA Salt (68).** Compound **68** was prepared using the same procedure as described for the synthesis of **8** by substituting **30** for phenyl propargyl ether (38 mg, 40%). ^{1}H NMR (500 MHz, CD_3OD) δ 2.33 (s, 3H), 2.45−2.65 (m, 8H), 3.61 (s, 2H), 3.77 (s, 2H), 4.17 (s, 2H), 6.70−6.73 (m, 1H), 6.77−6.78 (m, 2H), 7.16−7.19 (m, 2H), 7.33 (d, J = 7.5 Hz, 1H), 7.53 (s, 1H), 7.54 (s, 1H), 7.63 (m, 2H). MS (ESI) m/z 480 $(M + H)^+$. HRMS (FAB) m/z 480.22241; calcd, $C_{29}H_{30}N_5S$: 480.22164 $(M + H)^+$. HPLC 95% purity (A), 96% purity (B).

***N*-(3-(4-(6-((4-Methylpiperazin-1-yl)methyl)-1,4-dihydroindeno-[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)prop-2-ynyl)benzamide TFA Salt (69).** To benzoyl chloride (10 μL, 0.09 mmol) was added a solution of **43** (50 mg, 0.08 mmol) in pyridine (1 mL), and the mixture was agitated for 2.5 h. The mixture was evaporated, a 4 M solution of hydrogen chloride in 1,4-dioxane (1 mL) was added, and the mixture was agitated at room temperature for 6 h. The mixture was concentrated, and the crude product was purified by preparative reverse phase HPLC to provide the title compound (14 mg, 39%). ^{1}H NMR (500 MHz, CD_3OD) δ 2.28 (s, 3H), 2.40−2.65 (m, 8H), 3.60 (s, 2H), 3.79 (s, 2H), 4.44 (s, 2H), 7.33 (d, J = 7.8 Hz, 1H), 7.47−7.50 (m, 2H), 7.54−7.57 (m, 2H), 7.62 (s, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.68 (s, 1H), 7.86−7.88 (m, 2H). MS (ESI) m/z 508 $(M + H)^+$. HRMS (FAB) m/z 508.21644; calcd, $C_{30}H_{30}N_5OS$: 508.21656 $(M + H)^+$. HPLC 97% purity (A), 97% purity (B).

3-(5-(3-Phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno-[1,2-*c*]pyrazole (70). Compound **70** was prepared using the same procedure as described for the synthesis of **7** by substituting 1-indanone for **5** and **9** for **6** (46 mg, 36%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.82 (s, 2H), 5.11 (s, 2H), 6.99−7.02 (m, 1H), 7.05−7.06 (m, 2H), 7.28−7.30 (m, 1H), 7.33−7.38 (m, 3H), 7.56 (d, J = 7.8 Hz, 1H), 7.66 (m, 1H), 7.74 (bs, 1H), 7.89 (bs, 1H). MS (ESI) m/z 369 $(M + H)^+$. Anal. ($C_{23}H_{16}N_2OS \cdot 0.1H_2O$) C, H, N.

6-(4-Methylpiperazin-1-yl)-3-(5-(3-phenoxyprop-1-ynyl)-thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (71). Compound **71** was prepared using the same procedure as described for the synthesis of **7** by substituting **17** for **5** and **9** for **6**. The crude product was purified by preparative reverse phase HPLC to provide the title compound (49 mg, 30%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.87 (s, 3H), 2.98−3.02 (m, 2H), 3.15−3.21 (m, 2H), 3.52−3.55 (m, 2H), 3.74 (s, 2H), 3.87−3.90 (m, 2H), 5.09 (s, 2H), 6.97−7.01 (m, 2H), 7.03−7.05 (m, 2H), 7.22 (s, 1H), 7.32−7.35 (m, 2H), 7.51 (d, J = 7.8 Hz, 1H), 7.70 (d, J = 1.3 Hz, 1H), 7.84

(d, $J = 1.3$ Hz, 1H). MS (ESI) *m/z* 467 $(M + H)^+$. Anal. ($C_{28}H_{26}N_4OS \cdot 2.7CF_3CO_2H$) C, H, N.

6-(2-(4-Methylpiperazin-1-yl)ethyl)-3-(5-(3-phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole (72). Compound **72** was prepared using the same procedure as described for the synthesis of **7** by substituting **21** for **5** and **9** for **6** (32 mg, 28%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.14 (s, 3H), 2.25−2.48 (m, 8H), 2.50−2.54 (m, 2H), 2.75−2.78 (m, 2H), 3.75 (s, 2H), 5.09 (s, 2H), 6.98−7.01 (m, 1H), 7.04−7.05 (m, 2H), 7.20 (d, $J =$ 7.8 Hz, 1H), 7.32−7.35 (m, 2H), 7.39 (s, 1H), 7.53 (d, $J = 7.8$ Hz, 1H), 7.71 (s, 1H), 7.86 (s, 1H), 13.05 (s, 1H). MS (ESI) *m/z* 495 $(M + H)^+$. HRMS (FAB) *m/z* 495.22215; calcd, $C_{30}H_{31}N_4OS$: 495.22131 $(M + H)^+$. HPLC 98% purity (A), 98% purity (B).

5-((4-Methylpiperidin-1-yl)methyl)indan-1-one (106). To a suspension of **15**[10] (200 mg, 0.83 mmol) and potassium carbonate (276 mg, 2.0 mmol) in ethanol (6 mL) was added 4-methylpiperidine (0.24 mL, 2.0 mmol) dropwise at 0 °C. The mixture was stirred at room temperature for 3 h, concentrated, diluted with H_2O, and extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated. The crude product was purified by flash column chromatography on silica gel using dichloromethane/methanol (5:1) as the mobile phase to provide the title compound (195 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92 (d, $J = 6.6$ Hz, 3H), 1.22−1.30 (m, 2H), 1.34−1.39 (m, 1H), 1.59 (t, $J = 13.7$ Hz, 2H), 1.98 (t, $J = 10.6$ Hz, 2H), 2.67−2.70 (m, 2H), 2.82−2.84 (m, 2H), 3.12 (t, $J = 5.6$ Hz, 2H), 3.54 (s, 2H), 7.33 (d, $J = 7.8$ Hz, 1H), 7.45 (s, 1H), 7.69 (d, $J = 7.8$ Hz, 1H). MS (ESI) *m/z* 244 $(M + H)^+$.

6-((4-Methylpiperidin-1-yl)methyl)-3-(5-(3-phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (73). Compound **73** was prepared using the same procedure as described for the synthesis of **7** by substituting **106** for **5** and **9** for **6**. The crude product was purified by preparative reverse phase HPLC to provide the title compound (48 mg, 54%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.91 (d, $J = 6.6$ Hz, 3H), 1.28−1.36 (m, 2H), 1.55−1.66 (m, 2H), 1.80−1.82 (m, 3H), 2.91−2.98 (m, 2H), 3.87 (s, 2H), 4.33 (s, 2H), 5.11 (s, 2H), 6.99−7.02 (m, 1H), 7.05−7.06 (m, 2H), 7.33−7.37 (m, 2H), 7.48 (d, $J = 7.8$ Hz, 1H), 7.68 (s, 1H), 7.73−7.75 (m, 2H), 7.91 (s, 1H). MS (ESI) *m/z* 480 $(M + H)^+$. HRMS (FAB) *m/z* 480.21062; calcd, $C_{30}H_{30}N_3OS$: 480.21041 $(M + H)^+$. HPLC 97% purity (A), 98% purity (B).

6-((1-Methylpiperidin-4-yl)methyl)-3-(5-(3-phenoxyprop-1-ynyl)thiophen-3 -yl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (74). Compound **74** was prepared using the same procedure as described for the synthesis of **7** by substituting **23** for **5** and **9** for **6**. The crude product was purified by preparative reverse phase HPLC to provide the title compound (47 mg, 22%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.31−1.44 (m, 2H), 1.78−1.82 (m, 3H), 2.60 (d, $J = 6.1$ Hz, 2H), 7.73 (s, 3H), 2.81−2.93 (m, 2H), 3.37−3.41 (m, 2H), 3.78 (s, 2H), 5.11 (s, 2H), 6.98−7.03 (m, 1H), 7.04−7.07 (m, 2H), 7.17 (d, $J = 7.4$ Hz, 1H), 7.31−7.38 (m, 3H), 7.57 (d, $J = 7.4$ Hz, 1H), 7.73 (d, $J = 1.4$ Hz, 1H), 7.88 (d, $J = 1.4$ Hz, 1H). MS (ESI) *m/z* 480 $(M + H)^+$. HRMS (FAB) *m/z* 480.21245; calcd, $C_{30}H_{30}N_3OS$: 480.21041 $(M + H)^+$. HPLC 97% purity (A), 96% purity (B).

5-((4-Formylpiperazin-1-yl)methyl)indan-1-one (107). Compound **107** was prepared using the same procedure as described for the synthesis of **106** by substituting 1-formylpiperazine for 4-methylpiperidine (200 mg, 77%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.63−2.65 (m, 2H), 2.75−2.76 (m, 4H), 3.09−3.11 (m, 2H), 3.42−3.44 (m, 4H), 3.73 (s, 2H), 7.37 (d, $J = 7.8$ Hz, 1H), 7.52 (s, 1H), 7.62 (d, $J = 7.8$ Hz, 1H), 8.03 (s, 1H). MS (ESI) *m/z* 259 $(M + H)^+$.

4-((3-(5-(3-Phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazol-6-yl)methyl)piperazine-1-carbaldehyde (75). Compound **75** was prepared using the same procedure as described for the synthesis of **7** by substituting **107** for **5** and **9** for **6** (50 mg, 24%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.33−2.42 (m, 4H), 3.35−3.41 (m, 4H), 3.56 (s, 2H), 3.81 (s, 2H), 5.11 (s, 2H), 6.98−7.03 (m, 1H), 7.04−7.07 (m, 2H), 7.30 (d, $J = 7.5$ Hz, 1H), 7.32−7.38 (m, 2H), 7.49 (s, 1H), 7.61 (d, $J = 7.5$ Hz, 1H), 7.74 (s, 1H), 7.89 (s, 1H), 7.99 (s, 1H), 13.10 (bs, 1H). MS (ESI) *m/z* 495 $(M + H)^+$. HRMS (FAB) *m/z* 495.18527; calcd, $C_{29}H_{27}N_4O_2S$: 495.18492 $(M + H)^+$. HPLC 99% purity (A), 99% purity (B).

1-Methyl-4-((1-oxo-indan-5-yl)methyl)piperazin-2-one (108). Compound **108** was prepared using the same procedure as described for the synthesis of **106** by substituting 1-methylpiperazin-2-one[13] for 4-methylpiperidine (380 mg, 30%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.69−2.73 (m, 4H), 2.88 (s, 3H), 3.11−3.17 (m, 4H), 3.31−3.39 (m, 2H), 3.64 (s, 2H), 7.35 (d, $J = 7.8$ Hz, 1H), 7.46 (s, 1H), 7.72 (d, $J = 7.8$ Hz, 1H). MS (ESI) *m/z* 259 $(M + H)^+$.

1-Methyl-4-((3-(5-(3-phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazol-6-yl)methyl)piperazin-2-one TFA Salt (76). Compound **76** was prepared using the same procedure as described for the synthesis of **7** by substituting **108** for **5** and **9** for **6**. The crude product was purified by preparative reverse phase HPLC to provide the title compound (50 mg, 27%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.87 (s, 3H), 3.44−3.51 (m, 4H), 3.76−3.80 (m, 2H), 3.87 (s, 2H), 4.32 (bs, 2H), 5.11 (s, 2H), 6.98−7.03 (m, 1H), 7.04−7.07 (m, 2H), 7.32−7.38 (m, 2H), 7.47 (d, $J = 7.5$ Hz, 1H), 7.67 (s, 1H), 7.72−7.75 (m, 2H), 7.91 (d, $J = 1.3$ Hz, 1H). MS (ESI) *m/z* 495 $(M + H)^+$. Anal. ($C_{29}H_{26}N_4O_2S \cdot 1.7CF_3CO_2H$) C, H, N.

4-Methyl-1-((1-oxo-indan-5-yl)methyl)piperazin-2-one (109). Compound **109** was prepared using the same procedure as described for the synthesis of **106** by substituting 4-methylpiperazin-2-one for 4-methylpiperidine (380 mg, 55%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.38 (s, 3H), 2.66−2.72 (m, 4H), 3.11−3.14 (m, 2H), 3.25 (s, 2H), 3.29−3.33 (m, 2H), 4.68 (s, 2H), 7.25 (d, $J = 7.8$ Hz, 1H), 7.38 (s, 1H), 7.72 (d, $J = 7.8$ Hz, 1H). MS (DCI) *m/z* 259 $(M + H)^+$.

4-Methyl-1-((3-(5-(3-phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazol-6-yl)methyl)piperazin-2-one TFA Salt (77). Compound **77** was prepared using the same procedure as described for the synthesis of **7** by substituting **109** for **5** and **9** for **6**. The crude product was purified by preparative reverse phase HPLC to provide the title compound (17 mg, 23%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.86 (s, 3H), 3.48−3.53 (m, 4H), 3.81 (s, 2H), 3.92−4.08 (m, 2H), 4.62−4.73 (m, 2H), 5.11 (s, 2H), 6.98−7.03 (m, 1H), 7.04−7.07 (m, 2H), 7.29 (d, $J = 7.8$ Hz, 1H), 7.32−7.38 (m, 2H), 7.49 (s, 1H), 7.64 (d, $J = 7.8$ Hz, 1H), 7.73 (d, $J = 1.4$ Hz, 1H), 7.88 (d, $J = 1.4$ Hz, 1H). MS (ESI) *m/z* 495 $(M + H)^+$. Anal. ($C_{29}H_{26}N_4O_2S \cdot 1.8CF_3CO_2H$) C, H, N.

(4-Methylpiperazin-1-yl)(3-(5-(3-phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazol-6-yl)methanone TFA Salt (78). Compound **78** was prepared using the same procedure as described for the synthesis of **7** by substituting **24** for **5** and **9** for **6**. The crude product was purified by preparative reverse phase HPLC to provide the title compound (130 mg, 53%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.20 (s, 3H), 2.28−2.38 (m, 4H), 3.30−3.65 (m, 4H), 3.86 (s, 2H), 5.11 (s, 2H), 6.98−7.02 (m, 1H), 7.05−7.07 (m, 2H), 7.31−7.39 (m, 3H), 7.57 (s, 1H), 7.69 (d, $J = 7.7$ Hz, 1H), 7.74 (s, 1H), 7.90 (s, 1H). MS (ESI) *m/z* 495 $(M + H)^+$. Anal. ($C_{29}H_{26}N_4O_2S \cdot 0.5CF_3CO_2H$) C, H, N.

5-(Thiazolidin-3-ylmethyl)indan-1-one (110). Compound **110** was prepared using the same procedure as described for the synthesis of **106** by substituting thiazolidine for 4-methylpiperidine (660 mg, 69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.69−2.73 (m, 2H), 2.97−3.01 (m, 2H), 3.12−3.16 (m, 4H), 3.67 (s, 2H), 4.06 (s, 2H), 7.40 (d, $J = 7.8$ Hz, 1H), 7.53 (s, 1H), 7.73 (d, $J = 7.8$ Hz, 1H). MS (DCI) *m/z* 234 $(M + H)^+$.

3-((3-(5-(3-Phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazol-6-yl)methyl)thiazolidine TFA Salt (79). Compound **79** was prepared using the same procedure as described for the synthesis of **7** by substituting **110** for **5** and **9** for **6**. The crude product was purified by preparative reverse phase HPLC to provide the title compound (112 mg, 28%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.00−3.08 (m, 2H), 3.23−3.34 (m, 2H), 3.84 (s, 2H), 4.02 (bs, 2H), 4.18 (s, 2H), 5.11 (s, 2H), 6.98−7.03 (m, 1H), 7.04−7.07 (m, 2H), 7.32−7.38 (m, 2H), 7.41 (d, $J = 7.8$ Hz, 1H), 7.62 (s, 1H), 7.67 (d, $J = 7.8$ Hz, 1H), 7.74 (d, $J = 1.4$ Hz, 1H), 7.89

(d, $J = 1.4$ Hz, 1H). MS (ESI) *m/z* 470 $(M + H)^+$. Anal. ($C_{27}H_{23}N_3OS_2 \cdot CF_3CO_2H$) C, H, N.

6-(2-Methoxyethoxy)-3-(5-(3-phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole (80). Compound **80** was prepared using the same procedure as described for the synthesis of **7** by substituting **26** for **5** and **9** for **6** (37 mg, 33%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.31 (s, 3H), 3.66−3.68 (m, 2H), 3.75 (s, 2H), 4.12−4.14 (m, 2H), 5.09 (s, 2H), 6.92 (d, $J = 8.1$ Hz, 1H), 6.98−7.01 (m, 1H), 7.03−7.05 (m, 2H), 7.15 (s, 1H), 7.32−7.35 (m, 2H), 7.51 (d, $J = 8.1$ Hz, 1H), 7.69 (s, 1H), 7.83 (s, 1H). MS (ESI) *m/z* 443 $(M + H)^+$. HRMS (FAB) *m/z* 443.14265; calcd, $C_{26}H_{23}N_2O_3S$: 443.14239 $(M + H)^+$. HPLC 97% purity (A), 98% purity (B).

***N,N*-Dimethyl-2-(1-oxo-indan-5-yloxy)acetamide (111).** Compound **111** was prepared using the same procedure as described for the synthesis of **26** by substituting 2-chloro-*N,N*-dimethylacetamide for 1-bromo-2-methoxy-ethane (1.4 g, 60%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.57−2.59 (m, 2H), 2.85 (s, 3H), 3.00 (s, 3H), 3.02−3.05 (m, 2H), 4.95 (s, 2H), 6.94 (dd, $J = 8.6$, 2.1 Hz, 1H), 7.04 (d, $J = 2.1$ Hz, 1H), 7.54 (d, $J = 8.6$ Hz, 1H). MS (ESI) *m/z* 234 $(M + H)^+$.

***N,N*-Dimethyl-2-(3-(5-(3-phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazol-6-yloxy)acetamide TFA Salt (81).** Compound **81** was prepared using the same procedure as described for the synthesis of **7** by substituting **111** for **5** and **9** for **6**. The crude product was purified by preparative reverse phase HPLC to provide the title compound (15 mg, 21%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.86 (s, 3H), 3.02 (s, 3H), 3.77 (s, 2H), 4.84 (s, 2H), 5.11 (s, 2H), 6.92 (dd, $J = 8.2$, 2.1 Hz, 1H), 6.99−7.02 (m, 1H), 7.05−7.06 (m, 2H), 7.14 (d, $J = 2.1$ Hz, 1H), 7.33−7.36 (m, 2H), 7.52 (d, $J = 8.2$ Hz, 1H), 7.71 (d, $J = 1.3$ Hz, 1H), 7.85 (d, $J = 1.3$ Hz, 1H). MS (ESI) *m/z* 470 $(M + H)^+$. HRMS (FAB) *m/z* 470.15328; calcd, $C_{27}H_{24}N_3O_3S$: 470.15329 $(M + H)^+$. HPLC 98% purity (A), 97% purity (B).

5-((1*H*-Imidazol-1-yl)methyl)indan-1-one (112). Compound **112** was prepared using the same procedure as described for the synthesis of **106** by substituting imidazole for 4-methylpiperidine (650 mg, 75%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.61−2.63 (m, 2H), 3.06−3.08 (m, 2H), 5.32 (s, 2H), 6.94 (s, 1H), 7.21 (s, 1H), 7.27 (d, $J = 7.8$ Hz, 1H), 7.38 (s, 1H), 7.62 (d, $J = 7.8$ Hz, 1H), 7.79 (s, 1H). MS (ESI) *m/z* 213 $(M + H)^+$.

6-((1*H*-Imidazol-1-yl)methyl)-3-(5-(3-phenoxyprop-1-ynyl)thiophen-3-yl)-1, 4-dihydroindeno[1,2-*c*]pyrazole (82). Compound **82** was prepared using the same procedure as described for the synthesis of **7** by substituting **112** for **5** and **9** for **6** (50 mg, 37%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.83 (s, 2H), 5.11 (s, 2H), 5.48 (s, 2H), 6.99−7.02 (m, 1H), 7.04−7.06 (m, 2H), 7.33−7.35 (m, 2H), 7.42 (d, $J = 8.4$ Hz, 1H), 7.63 (s, 1H), 7.68−7.70 (m, 2H), 7.73 (s, 1H), 7.81 (s, 1H), 7.89 (s, 1H), 9.23 (s, 1H). MS (ESI) *m/z* 449 $(M + H)^+$. HRMS (FAB) *m/z* 449.14303; calcd, $C_{27}H_{21}N_4OS$: 449.14306 $(M + H)^+$. HPLC 99% purity (A), 99% purity (B).

5-((1*H*-Pyrazol-1-yl)methyl)indan-1-one (113). Compound **110** was prepared using the same procedure as described for the synthesis of **106** by substituting pyrazole for 4-methylpiperidine (582 mg, 45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.66−2.70 (m, 2H), 3.08−3.12 (m, 2H), 5.41 (s, 2H), 6.36 (t, $J = 2.0$ Hz, 1H), 7.19 (d, $J = 7.8$ Hz, 1H), 7.22 (s, 1H), 7.45 (d, $J = 2.4$ Hz, 1H), 7.58 (d, $J = 1.7$ Hz, 1H), 7.71 (d, $J = 7.8$ Hz, 1H). MS (DCI) *m/z* 213 $(M + H)^+$.

6-((1*H*-Pyrazol-1-yl)methyl)-3-(5-(3-phenoxyprop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (83). Compound **83** was prepared using the same procedure as described for the synthesis of **7** by substituting **113** for **5** and **9** for **6**. The crude product was purified by preparative reverse phase HPLC to provide the title compound (42 mg, 39%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.79 (s, 2H), 5.11 (s, 2H), 5.39 (s, 2H), 6.28 (t, $J = 2.0$ Hz, 1H), 6.97−7.02 (m, 1H), 7.04−7.06 (m, 2H), 7.22 (d, $J = 7.8$ Hz, 1H), 7.32−7.37 (m, 2H), 7.40 (s, 1H), 7.47 (dd, $J = 2.0$, 0.7 Hz, 1H), 7.60 (d, $J = 7.8$ Hz, 1H), 7.72 (d, $J = 1.4$ Hz, 1H), 7.85 (dd, $J = 2.4$, 0.7 Hz, 1H), 7.87 (d, $J = 1.4$ Hz, 1H). MS (ESI) *m/z* 449 $(M + H)^+$. HRMS (FAB) *m/z* 449.14307; calcd, $C_{27}H_{21}N_4OS$: 449.14306 $(M + H)^+$. HPLC 98% purity (A), 99% purity (B).

5-((1*H*-1,2,3-Triazol-1-yl)methyl)indan-1-one (114). Compound **114** was prepared using the same procedure as described for the synthesis of **106** by substituting 1,2,3-triazole for 4-methylpiperidine (120 mg, 45%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61−2.64 (m, 2H), 3.06−3.09 (m, 2H), 5.75 (s, 2H), 7.30 (d, $J = 7.8$ Hz, 1H), 7.43 (s, 1H), 7.63 (d, $J = 7.8$ Hz, 1H), 7.78 (d, $J = 0.9$ Hz, 1H), 8.23 (d, $J = 0.9$ Hz, 1H). MS (DCI) *m/z* 214 $(M + H)^+$.

6-((1*H*-1,2,3-Triazol-1-yl)methyl)-3-(5-(3-phenoxyprop-1-ynyl)-thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole (84). Compound **84** was prepared using the same procedure as described for the synthesis of **7** by substituting **114** for **5** and **9** for **6** (92 mg, 36%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.81 (s, 2H), 5.10 (s, 2H), 5.68 (s, 2H), 6.99−7.02 (m, 1H), 7.04−7.06 (m, 2H), 7.31 (d, $J = 7.8$ Hz, 1H), 7.33−7.36 (m, 2H), 7.50 (s, 1H), 7.64 (d, $J = 7.8$ Hz, 1H), 7.72 (s, 1H), 7.75 (d, $J = 0.9$ Hz, 1H), 7.87 (s, 1H), 8.21 (d, $J = 0.9$ Hz, 1H), 13.16 (bs, 1H). MS (ESI) *m/z* 450 $(M + H)^+$. Anal. ($C_{26}H_{19}N_5OS$) C, H, N.

5-((1*H*-1,2,4-Triazol-1-yl)methyl)inden-1-one (115). Compound **115** was prepared using the same procedure as described for the synthesis of **106** by substituting 1,2,4-triazole for 4-methylpiperidine (8.6 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.69−2,73 (m, 2H), 3.11−3.15 (m, 2H), 5.44 (s, 2H), 7.26 (d, $J = 7.8$ Hz, 1H), 7.32 (s, 1H), 7.76 (d, $J = 7.8$ Hz, 1H), 8.02 (s, 1H), 8.21 (s, 1H). MS (ESI) *m/z* 214 $(M + H)^+$.

6-((1*H*-1,2,4-Triazol-1-yl)methyl)-3-(5-(3-phenoxyprop-1-ynyl)-thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole (85). Compound **85** was prepared using the same procedure as described for the synthesis of **7** by substituting **115** for **5** and **9** for **6** (20 mg, 13%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.80 (s, 2H), 5.11 (s, 2H), 5.48 (s, 2H), 6.99−7.02 (m, 1H), 7.04−7.06 (m, 2H), 7.29 (d, $J = 7.8$ Hz, 1H), 7.33−7.36 (m, 2H), 7.48 (s, 1H), 7.62 (d, $J = 7.8$ Hz, 1H), 7.72 (s, 1H), 7.87 (s, 1H), 8.00 (s, 1H), 8.70 (s, 1H). MS (ESI) *m/z* 450 $(M + H)^+$. Anal. ($C_{26}H_{19}N_5OS$) C, H, N.

4-(4-(3-(4-(6-((4-Methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)thiophen-2-yl)prop-2-ynyloxy)benzyl)-morpholine TFA Salt (86). Compound **86** was prepared using the same procedure as described for the synthesis of **8** by substituting **37** for phenyl propargyl ether (80 mg, 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.84 (s, 3H), 3.00−3.50 (m, 12H), 3.60−3.70 (m, 2H), 3.84 (s, 2H), 3.90−4.00 (m, 2H), 4.15 (bs, 2H), 4.31 (s, 2H), 5.16 (s, 2H), 7.14−7.16 (m, 2H), 7.44 (d, $J = 7.9$ Hz, 1H), 7.48−7.50 (m, 2H), 7.64 (s, 1H), 7.70 (d, $J = 7.9$ Hz, 1H), 7.76 (d, $J = 1.2$ Hz, 1H), 7.92 (d, $J = 1.2$ Hz, 1H). MS (ESI) *m/z* 580 $(M + H)^+$. HRMS (FAB) *m/z* 580.27470; calcd, $C_{34}H_{38}N_5O_2S$: 580.27407 $(M + H)^+$. HPLC 97% purity (A), 97% purity (B).

3-(5-(3-(2-Methoxyethoxy)prop-1-ynyl)thiophen-3-yl)-6-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (87). Compound **86** was prepared using the same procedure as described for the synthesis of **8** by substituting **39** for phenyl propargyl ether (60 mg, 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.83 (s, 3H), 3.26 (s, 3H), 3.00−3.50 (m, 8H), 3.48−3.50 (m, 2H), 3.62−3.64 (m, 2H), 3.83 (s, 2H), 4.19 (s, 2H), 4.44 (s, 2H), 7.44 (d, $J = 7.9$ Hz, 1H), 7.64 (s, 1H), 7.69 (d, $J = 7.9$ Hz, 1H), 7.73 (d, $J = 1.2$ Hz, 1H), 7.88 (d, $J = 1.2$ Hz, 1H). MS (ESI) *m/z* 463 $(M + H)^+$. HRMS (FAB) *m/z* 463.21700; calcd, $C_{26}H_{31}N_4O_2S$: 463.21622 $(M + H)^+$. HPLC 98% purity (A), 97% purity (B).

3-(5-(3-(2-Methoxyethoxy)prop-1-ynyl)thiophen-3-yl)-7-((4-methylpiperazin-1-yl)methyl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (88). Compound **88** was prepared using the same procedure as described for the synthesis of **8** by substituting **101** for **7** and **39** for phenyl propargyl ether. The crude product was purified by preparative reverse phase HPLC to provide the title compound (24 mg, 23%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.84 (s, 3H), 3.00−3.50 (m, 8H), 3.27 (s, 3H), 3.50−3.52 (m, 2H), 3.63−3.66 (m, 2H), 3.86 (s, 2H), 4.21 (s, 2H), 4.46 (s, 2H), 7.37 (d, $J = 7.9$ Hz, 1H), 7.62 (d, $J = 7.9$ Hz, 1H), 7.74 (s, 1H), 7.78 (s, 1H),

7.89 (s, 1H). MS (ESI) *m*/*z* 463 $(M + H)^+$. HRMS (FAB) *m*/*z* 463.21634; calcd, $C_{26}H_{31}N_4O_2S$: 463.21622 $(M + H)^+$. HPLC 95% purity (A), 96% purity (B).

6-((1*H*-1,2,4-Triazol-1-yl)methyl)-3-(5-bromothiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole (116). Compound **116** was prepared using the same procedure as described for the synthesis of **7** by substituting **115** for **5** (1.3 g, 70%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.80 (s, 2H), 5.48 (s, 2H), 7.29 (d, $J = 7.8$ Hz, 1H), 7.48 (s, 1H), 7.62−7.64 (m, 2H), 7.83 (s, 1H), 7.99 (s, 1H), 8.69 (s, 1H), 13.24 (bs, 1H). MS (ESI) *m*/*z* 398, 400 $(M + H)^+$.

6-((1*H*-1,2,4-Triazol-1-yl)methyl)-3-(5-(3-(2-methoxyethoxy)-prop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole (89). Compound **89** was prepared using the same procedure as described for the synthesis of **8** by substituting **116** for **7** and **39** for phenyl propargyl ether (90 mg, 49%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.26 (s, 3H), 3.48−3.50 (m, 2H), 3.62−3.64 (m, 2H), 3.80 (s, 2H), 4.44 (s, 2H), 5.47 (s, 2H), 7.28 (d, $J = 7.8$ Hz, 1H), 7.47 (s, 1H), 7.63 (d, $J = 7.8$ Hz, 1H), 7.70 (s, 1H), 7.85 (s, 1H), 7.98 (s, 1H), 8.67 (s, 1H), 13.16 (bs, 1H). MS (ESI) *m*/*z* 432 $(M + H)^+$. Anal. ($C_{23}H_{21}N_5O_2S$) C, H, N.

6-((1*H*-1,2,4-Triazol-1-yl)methyl)indan-1-one (117). Compound **117** was prepared using the same procedure as described for the synthesis of **106** by substituting (3-oxo-indan-5-yl)methyl methanesulfonate for **15** and 1,2,4-triazole for 4-methylpiperidine (3.7 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.70−2.74 (m, 2H), 3.13−3.17 (m, 2H), 5.40 (s, 2H), 7.50−7.51 (m, 2H), 7.67 (s, 1H), 7.98 (s, 1H), 8.10 (s, 1H). MS (DCI) *m*/*z* 214 $(M + H)^+$.

7-((1*H*-1,2,4-Triazol-1-yl)methyl)-3-(5-bromothiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole (118). Compound **118** was prepared using the same procedure as described for the synthesis of **7** by substituting **117** for 5 (3.0 g, 75%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.79 (s, 2H), 5.50 (s, 2H), 7.24 (d, $J = 7.8$ Hz, 1H), 7.53−7.55 (m, 2H), 7.63 (s, 1H), 7.83 (s, 1H), 8.00 (s, 1H), 8.71 (s, 1H), 13.21 (bs, 1H). MS (ESI) *m*/*z* 398, 400 $(M + H)^+$.

7-((1*H*-1,2,4-Triazol-1-yl)methyl)-3-(5-(3-(2-methoxyethoxy)-prop-1-ynyl)thiophen-3-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole TFA Salt (90). Compound **90** was prepared using the same procedure as described for the synthesis of **8** by substituting **118** for **7** and **39** for phenyl propargyl ether. The crude product was purified by preparative reverse phase HPLC to provide the title compound (46 mg, 22%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.27 (s, 3H), 3.49−3.51 (m, 2H), 3.63−3.65 (m, 2H), 3.81 (s, 2H), 4.45 (s, 2H), 5.50 (s, 2H), 7.24 (d, $J = 7.8$ Hz, 1H), 7.53−7.57 (m, 2H), 7.72 (s, 1H), 7.87 (s, 1H), 7.99 (s, 1H), 8.70 (s, 1H), 13.18 (s, 1H). MS (ESI) *m*/*z* 432 $(M + H)^+$. HRMS (FAB) *m*/*z* 432.14888; calcd, $C_{23}H_{22}N_5O_2S$: 432.14887 $(M + H)^+$. HPLC 98% purity (A), 97% purity (B).

Computational Analysis. The hERG pore homology model in its closed state was constructed (Insight II software, HOMOLOGY module, Accelrys, San Diego, CA) using the homologous pore region of the KcsA potassium channel (PDB entry 1BL8) following methodologies described earlier.[25,26] The structure of **8** was built in Insight II and was energy minimized. Its piperazine moiety, which is protonated at physiological pH, was maintained in a protonated form. Compound **8** was docked manually into the hERG pore homology model, following previously established orientations for compounds with comparable shape and charge.[25,26] Energy minimization using the CFF force field (25 iterations with the static pore model, followed by 50 iterations of full ligand−protein minimization) provided the final model shown if Figure 2. No solvation was included.

Homogeneous Time-Resolved Fluorescence (HTRF) Assays of Receptor Tyrosine Kinases.[29] Assays were performed in a total of 40 μL in 96-well Costar black half-volume plates using HTRF technology. A peptide substrate (biotin-Ahx-AEEEYFFLFA-amide) at 4 μM, 1 mM ATP, enzyme, and inhibitors were incubated for 1 h at ambient temperature in 50 mM HEPES/NaOH (pH 7.5), 10 mM $MgCl_2$, 2 mM $MnCl_2$, 2.5 mM DTT, 0.1 mM orthovanadate, and 0.01% bovine serum albumin (BSA). Inhibitors were added to the wells at a final concentration of 3.2 nM to 50 μM with 5% DMSO added as a cosolvent. The reactions were stopped with 10 μL/well of 0.5 M ethylenediaminetetraacetic acid (EDTA), and then 75 μL of buffer containing streptavidin−allophycocyanin (Prozyme) (1.1 μg/mL) and PT66 antibody europium cryptate (Cis-Bio) (0.1 μg/mL) was added to each well. The plates were read from 1 to 4 h after addition of the detection reagents, and the time-resolved fluorescence (665:615 ratio) was measured using a Packard Discovery instrument. The amount of each tyrosine kinase added to the wells was calibrated to give a control (no inhibitor) to background (prequenched with EDTA) ratio of 10−15, and it was shown to be in the low nanomolar concentration range for each kinase. The inhibition of each well was calculated using the control and background readings for that plate. Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.

Cellular KDR Phosphorylation Assay. KDR Phosphorylation Determined by ELISA.[29] NIH3T3 cells stably transfected with full length human KDR (VEGFR2) were maintained in a Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 500 μg/mL geneticin. KDR cells were plated at 20000 cells per well into duplicate 96-well tissue culture plates and cultured overnight in an incubator at 37 °C with 5% CO_2 and 80% humidity. The growth medium was replaced with a serum-free growth medium for 2 h prior to compound addition. Compounds in DMSO were diluted in the serum-free growth medium (final DMSO concentration 1%) and added to cells for 20 min prior to stimulation for 10 min with VEGF (50 ng/mL). Cells were lysed by addition of RIPA buffer (50 mM Tris-HCl (pH 7.4), 1% IGEPAL, 150 mM NaCl, 1 mM EDTA, and 0.25% sodium deoxycholate) containing protease inhibitors (Sigma cocktail), NaF (1 mM), and Na_3VO_4 (1 mM) and placed in a microtiter plate shaker for 10 min. The lysates from duplicate wells were combined, and 170 μL of the combined lysate was added to the KDR ELISA plate. The KDR ELISA plate was prepared by adding an anti-VEGFR2 antibody (1 μg/well, R&D Systems) to an unblocked plate and incubated overnight at 4 °C. The plate was then blocked for at least 1 h with 200 μL/well of 5% dry milk in PBS. The plate was washed two times with PBS containing 0.1% Tween 20 (PBST) before addition of the cell lysates. Cell lysates were incubated in the KDR ELISA plate with constant shaking on a microtiter plate shaker for 2 h at room temperature. The cell lysate was then removed, and the plate was washed five times with PBST. Detection of phospho-KDR was performed using a 1:2000 dilution of biotinylated 4G10 anti-phosphotyrosine (UBI, Lake Placid, NY). The wells were incubated with constant shaking for 1.5 h at room temperature and washed five times with PBST, and for detection, a 1:2000 dilution of streptavidin−HRP (UBI, Lake Placid, NY) was added and incubated with constant shaking for 1 h at room temperature. The wells were then washed five times with PBST, and K-Blue HRP ELISA substrate (Neogen) was added to each well. The development time was monitored at 650 nm in a SpectraMax Plus plate reader until 0.4−0.5 absorbance units were obtained (approximately 10 min) in the VEGF only wells. Phosphoric acid (1 M) was added to stop the reaction, and the plate was read at 450 nm. The percent inhibition was calculated using the VEGF only wells as 100% controls and wells containing 5 μM pan-kinase inhibitor as 0% controls (no VEGF wells were used to monitor the endogenous phosphorylation state of the cells). IC_{50} values were calculated by nonlinear regression analysis of the concentration response curve. Each IC_{50} determination was performed with five concentrations, and each assay point was determined in duplicate.

KDR Phosphorylation Determined by Western Blot Analysis.[29] NIH3T3 cells stably transfected with full length human KDR (VEGFR2) were cultured in T75 or T150 flasks; compounds were added in the serum-free growth medium for 20 min prior to stimulation for 3 min with VEGF (50 ng/mL). The cells were washed one time with PBS and lysed with 1 mL/flask of RIPA buffer containing protease inhibitors, NaF, and Na_3VO_4 for 5 min on ice. Cells were scraped, and the lysate was incubated for an additional 20 min in ice with occasional mixing in a 2 mL microcentrifuge tube. The lysate was centrifuged at 20000*g* for 20 min at 4 °C, and the supernatant was used to immunoprecipitate

KDR. The lysate, 500 μg, was precleared with Protein-G agarose beads (Calbiochem) for 30 min at 4 °C while rotating. The lysate was centrifuged (20000*g* for 1 min), and the supernatant was immunoprecipitated with 4 μg of an anti-VEGFR2 antibody (R&D Systems) overnight at 4 °C while rotating. After incubation, 50 μL of Protein-G agarose was added and incubated for 1 h at 4 °C while rotating. Beads were washed five times with PBS containing Na_3VO_4 (1 mM) and protease inhibitors (Sigma). The washed beads were resuspended in 50 μL of sample buffer, electrophoresed on a 8−16% Tris-glycine gel (Invitrogen), transferred to a nitrocellulose membrane, blocked with 5% milk in PBST, and probed with an anti-KDR antibody (sc-315, Santa Cruz). KDR was visualized with enhanced chemoluminescence detection, and IC_{50} values were determined by comparison of the treated samples to the vehicle (DMSO) treated samples.

Estradiol-Induced Murine Uterine Edema Assay.[29] Twelve week old balb/c female mice (Taconic, Germantown, NY) were pretreated with 10 units of pregnant mare's serum gonadotropin (PMSG) (Calbiochem) intraperitoneally (ip) administered 72 and 24 h prior to estradiol. Mice were randomized the day of the experiment. Test compounds were formulated in a variety of vehicles and administered po 30 min prior to stimulation with an intraperitoneal injection of water-soluble 17β-estradiol (20−25 μg/mouse). Animals were sacrificed and uteri removed 2.5 h following estradiol stimulation by cutting just proximal to the cervix and at the fallopian tubes. After the removal of fat and connective tissue, uteri were weighed, squeezed between filter paper to remove fluid, and weighed again. The difference between wet and blotted weights represented the fluid content of the uterus. Compound treated groups were compared to vehicle treated groups of animals after subtracting the background water content of unstimulated uteri. The experimental group size was five or six.

HT1080 Tumor Growth Inhibition Model.[29] The 1080 human fibrosarcoma cells were obtained from the American Type Tissue Culture Collection and maintained in DMEM, supplemented with 10% fetal bovine serum and antibiotics. For tumor xenograft studies, cells were suspended in PBS, mixed with an equal volume of matrigel (phenol red free) to a final concentration of 2 million cells/mL, and inoculated (0.25 mL) into the flank of SCID-beige mice. One week after inoculation, tumor-bearing animals were divided into groups (n = 10), and administration of vehicle (2% EtOH, 5% Tween 80, 20% PEG 400, and 73% saline) or inhibitor at the indicated dose was initiated. Tumor growth was assessed every 2−3 days by measuring tumor size and calculating tumor volume using the formula [length × width^2]/2.

MX-1 Tumor Growth Inhibition Model. MX-1 human mammary carcinoma was obtained from the tumor repository of the National Cancer Institute (Bethesda, MD). Solid tumors were maintained *in vivo* by serial passage in specific pathogen free female athymic Ncr-nude mice (Taconic). Donor tumors were harvested when they reached 1−2 grams and 50 mg pieces of non-necrotic tissue were implanted sc into anesthetized recipient mice through a small skin incision in the flank which was subsequently closed with a wound clip. Animals were randomized into groups of 5−10 when tumors reached a size of approximately 100 mg (5−7 days) and treatment with vehicle or inhibitor was initiated. Tumor growth was monitored 2−3 times per week by caliper measurements and converted to volume using the formula: V = width^2 × length/2. All animal procedures were reviewed and approved by the Animal Care and Use Committee.

[^{3}H]Dofetilide/HEK-293 Membrane Competition Binding Assay.[24] The affinity of test drugs for the hERG cardiac K^+ channel was determined by their ability to displace tritiated dofetilide (a class III antiarrhythmic drug and potent hERG blocker) in membrane homogenates from HEK-293 cells heterogenously expressing the hERG channel. Drug dilutions were prepared from 10 mM DMSO stocks and the following were added to a 96-well polystyrene plate (Perkin-Elmer Optiplate): 20 μL of assay binding buffer (for total bounds), or 1 μM astemizole (for nonspecific bounds), or test drug, 50 μL of [^{3}H]dofetilide (20 nM, 85 Ci/mmol specific activity), and 130 μL of membrane homogenate (final protein concentration of 30 μg per well). The plates were incubated at ambient temperature for 45 min, aspirated onto GF/B filter plates (Perkin-Elmer), and washed with 2 mL of cold wash buffer. After allowing the plates to dry, 50 μL of scintillant (Perkin-Elmer MicroScint 20) was added to each well, and the radioactivity was counted in a Perkin-Elmer Topcount NXT scintillation counter. IC_{50} determinations were calculated from competition curves using six drug concentrations, half-log apart, starting at a high concentration of 100 μM (final assay DMSO concentration = 1%) using a four-parameter logistic equation.

hERG Patch Clamp Assay. hERG channels stably expressed in human embryonic kidney cells (HEK-293 cells)[30] were maintained in culture. For electrophysiology recordings, cells were briefly trypsinized to release the cells from the plates, pelleted by centrifugation (1000*g*), resuspended in culture media, and studied within 8 h. HERG current was recorded using standard patch clamp techniques as described previously.[31] Currents were recorded using either an Axopatch 200A or Axopatch Multiclamp 700A amplifier along with pClamp data acquisition software (version 8, Molecular Devices). Drug effects were assessed using a voltage clamp protocol that sequentially stepped to −25, 0, 25 or 50 mV for 3 s, followed by a step to −50 mV for 4 s from a holding potential of −80 mV; clamp pulses were applied once every 15 s. IC_{50} values for block of hERG current were calculated from tail currents measured at −50 mV following conditioning pulses to 0 mV. Patch pipettes were constructed with borosilicate glass capillary tubes (resistance 1.8−3.8 M-ohm)). The pipet solution contained (in mM): K^+ aspartate 125, KCl 20, EGTA 10, $MgCl_2$ 1, HEPES 5, MgATP 5 (pH = 7.3). The bath solution contained the following (in mM): NaCl 140, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 5, HEPES 20, pH = 7.4. Experiments were performed at 36.5−37 °C.

Supporting Information Available: Combustion analysis and high resolution MS and HPLC data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. The Protein Kinase Complement of the Human Genome. *Science* **2002**, *298*, 1912−1934.

(2) Robinson, D. R.; Wu, Y.-M.; Lin, S.-F. The Protein Tyrosine Kinase Family of the Human Genome. *Oncogene* **2000**, *19*, 5548−5557.

(3) Ullrich, A.; Schlessinger, J. Signal Transduction by Receptors with Tyrosine Kinase Activity. *Cell* **1990**, *61*, 203−212.

(4) Rousset, D.; Agnes, F.; Lachaume, P.; Andre, C.; Galibert, F. Molecular Evolution of the Genes Encoding Receptor Tyrosine Kinase with Immunoglobulinlike Domains. *J. Mol. Evol.* **1995**, *41*, 421−429.

(5) Hanahan, D.; Weinberg, R. The Hallmarks of Cancer. *Cell* **2000**, *100*, 57−70.

(6) Potapova, O.; Laird, A. D.; Nannini, M. A.; Barone, A.; Li, G.; Moss, K. G.; Cherrington, J. M.; Mendel, D. B. Contribution of Individual Targets to the Antitumor Efficacy of the Multitargeted Receptor Tyrosine Kinase Inhibitor SU11248. *Mol. Cancer Ther.* **2006**, *5*, 1280−1289.

(7) Rueegg, C.; Hasmim, M.; Lejeune, F. J.; Alghisi, G. C. Antiangiogenic Peptides and Proteins: From Experimental Tools to Clinical Drugs. *Biochim. Biophys. Acta* **2006**, *1765*, 155−177.

(8) Dagher, R.; Cohen, M.; Williams, G.; Rothmann, M.; Gobburu, J.; Robbie, G.; Rahman, A.; Chen, G.; Staten, A.; Griebel, D.; Pazdur, R. Approval Summary: Imatinib Mesylate in the Treatment of Metastatic and/or unresectable Malignant Gastrointestinal Stromal Tumors. *Clin. Cancer Res.* **2002**, *8*, 3034−3038.

(9) Atkins, M.; Jones, C.; A.; Kirkpatrick, P. Sunitinib Maleate. *Nature Rev., Drug Discovery* **2006**, *5*, 279−280.

(10) Dinges, J.; Akritopoulou-Zanze, I.; Arnold, L. D.; Barlozzari, T.; Bousquet, P. F.; Cunha, G. A.; Ericsson, A. M.; Iwasaki, N.; Michaelides, M. R.; Ogawa, N.; Phelan, K. M.; Rafferty, P.; Sowin, T. J.; Stewart, K. D.; Tokuyama, R.; Xia, Z.; Zhang, H. Q. Hit-to-Lead Optimization of 1,4-Dihydroindeno[1,2-*c*]pyrazoles as a Novel Class of KDR Kinase Inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4371−4375.

(11) Dinges, J.; Ashworth, K. L.; Akritopoulou-Zanze, I.; Arnold, L. D.; Baumeister, S. A.; Bousquet, P. F.; Cunha, G. A.; Davidsen, S. K.; Djuric, S. W.; Gracias, V. J.; Michaelides, M. R.; Rafferty, P.; Sowin, T. J.; Stewart, K. D.; Xia, Z.; Zhang, H. Q. 1,4-Dihydroindeno[1,2-*c*]pyrazoles as Novel Multitargeted Receptor Tyrosine Kinase Inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4266−4271.

(12) Erdélyi, M.; Gogoll, A. Rapid Homogeneous-Phase Sonogashira Coupling Reactions Using Controlled Microwave Heating. *J. Org. Chem.* **2001**, *66*, 4165.
(13) Cusic, J. W.; Lowrie, H. S.; Sause, H. W. U.S. Patent 2,787,617, 1957.
(14) Hosoya, T.; Takashiro, E.; Matsumoto, T.; Suzuki, K. Total Synthesis of the Gilvocarcins. *J. Am. Chem. Soc.* **1994**, *116*, 1004−1015.
(15) Campaigne, E.; Bourgeois, R. C. 3-Substituted Thiophenes. VI. Substitution Reactions of 3-Thenoic Acid. *J. Am. Chem. Soc.* **1954**, *76*, 2445−2447.
(16) Tu, Y. Q.; Hübener, A., Zhang, H., Moore, C. J.; Fletcher, M. T.; Hayes, P.; Dettner, K.; Francke, W.; McErlean, C. S. P.; Kitching, W. Synthesis and Stereochemistry of Insect Derived Spiroacetals with Branched Carbon Skeletons. *Synthesis* **2000**, *13*, 1956−1978.
(17) Rawat, D. S.; Zaleski, J. M. A Convenient Method for the Synthesis of 1,8-*Bis*(pyridin-3-oxy)oct-4-ene-2,6-diyne. *Synth. Commun.* **2002**, *32*, 1489−1494.
(18) Wipf, P.; Aoyama, Y.; Benedum. T. E. A Practical Method for Oxazole Synthesis by Cycloisomerization of Propargyl Amides. *Org. Lett.* **2004**, *6*, 3593−3595.
(19) Tao, Z.-F.; Sowin, T. J.; Lin, N.-H. A facile Synthesis of Antitumoral Indeno[1,2-*c*]pyrazol-4-one by mild Oxidation with molecular Oxygen. *Tetrahedron Lett.* **2005**, *46*, 7615−7618.
(20) Reich, S. H.; Wallace, M. B. PCT Int. Appl. WO 079198, 2001.
(21) Data reported as an average of five animals; p-values vs vehicle are given in parentheses for percent inhibition data, and 95% confidence levels are given in parenthesis for ED_{50} values.
(22) Pharmacokinetic data are reported as an average of three animals. Variability around the mean value was <40 %. Vehicle: iv, 2.5 % DMSO, 2.5% Tween-80, 25% PEG400, 70% PBS; po, 5% Tween-80, 2.5% ethanol, 25% PEG400, 67.5% PBS.
(23) Recanatini, M.; Poluzzi, E.; Masetti, M.; Cavalli, A.; De Ponti, F. QT Prolongation Through hERG K^+ Channel Blockade: Current Knowledge and Strategies for the Early Prediction During Drug Development. *Med. Res. Rev.* **2005**, *25*, 133−166.
(24) Diaz, G. J.; Daniell, K.; Leitza, S. T.; Martin, R. L.; Su, Z.; McDermott, J. S.; Cox, B. F.; Gintant, G. A. The [^{3}H]Dofetilide Binding is a predictive Screening Tool for hERG Blockade and Proarrythmia: Comparison of intact Cell and Membrane Preparations and Effect of altering $[K^+]_o$. *J. Pharmacol. Toxicol. Meth.* **2004**, *50*, 187−199.
(25) Mitcheson, J. S.; Chen, J.; Lin, M.; Culberson, C.; Sanguinetti, M. C. A Structural Basis for Drug-Induced long QT Syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *24*, 12329−12333.
(26) Witchel, H. J.; Dempsey, C. E.; Sessions, R. B.; Perry, M.; Milnes, J. T.; Hancox, J. C.; Mitcheson, J. S. The Low-Potency, Voltage-Dependent hERG Blocker Propafenone − Molecular Determinants and Drug Trapping. *Mol. Pharmacol.* **2004**, *66*, 1201−1212.
(27) Lacerda, A. E.; Kramer, J.; Shen, K.-Z.; Thomas, D.; Brown, A. M. Comparison of block among cloned cardiac potassium channels by non-antiarrhytmic drugs. *Eur. Heart J. Suppl.* **2001**, *3*, K23−K30.
(28) The hERG patch clamp IC_{50} values are means of two experiments; variability around the mean value was <50%.
(29) Dai, Y.; Guo, Y.; Frey, R. R.; Ji, Z.; Curtin, M. L.; Ahmed, A. A.; Albert, D. H.; Arnold, L.; Arries, S. S.; Barlozzari, T.; Bauch, J. L.; Bouska, J. J.; Bousquet, P. F.; Cunha, G. A.; Glaser, K. B.; Guo, J.; Li, J.; Marcotte, P. A.; Marsh, K. C.; Moskey, M. D.; Pease, L. J.; Stewart, K. D.; Stoll, V. S.; Tapang, P.; Wishart, N.; Davidsen, S. K.; Michaelides, M. R. Thienopyrimidine Ureas as Novel and Potent Multitargeted Receptor Tyrosine Kinase Inhibitors. *J. Med. Chem.* **2005**, *48*, 6066−6083.
(30) Zhou, Z.; Gong, Q.; Ye, B.; Fan, Z.; Makielski, J. C.; Robertson, G. A.; January, C. T. Properties of hERG Channels Stably Expressed in HEK 293 Cells Studied at Physiological Temperature. *Biophys. J.* **1998**, *74*, 230−241.
(31) Martin, R. L.; McDermott, J. S.; Salmen, H. J.; Pallmatier, J.; Cox, B. F.; Gintant, G. A. The Utility of hERG and Repolarization Assays in Evaluating Delayed Cardiac Repolarization: Influence of Multi-Channel Block. *J. Cardiovasc. Pharmacol.* **2004**, *43*, 369−379.

JM061223O