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Paper

3-(Benzo[d][1,3]dioxol-5-ylamino)-N-(4-fluorophenyl)thiophene-2-carboxamide overcomes cancer chemoresistance via inhibition of angiogenesis and P-glycoprotein efflux pump activity†‡

Ramesh Mudududdla,[a,b] Santosh K. Guru,[c] Abubakar Wani,[c] Sadhana Sharma,[⊥] Prashant Joshi,[a,b] Ram A. Vishwakarma,[a,b] Ajay Kumar,[*,c] Shashi Bhushan,[*,c] and Sandip B. Bharate[*,a,b]

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3-((Quinolin-4-yl)methylamino)-N-(4-(trifluoromethoxy)phenyl)thiophene-2-carboxamide (OSI-930, 1) is a potent inhibitor of c-kit and VEGFR2, currently under phase I clinical trials in patients with advanced solid tumors. In order to understand the structure-activity relationship, a series of 3-arylamino *N*-aryl thiophene 2-carboxamides were synthesized by modifications at both quinoline and amide domain of OSI-930 scaffold. All synthesized compounds were screened for in-vitro cytotoxicity in a panel of cancer cell lines and for VEGFR1 and VEGFR2 inhibition. Thiophene 2-carboxamides substituted with benzo[d][1,3]dioxol-5-yl and 2,3-dihydrobenzo[b][1,4]dioxin-6-yl groups 1l and 1m displayed inhibition of VEGFR1 with IC_{50} values of 2.5 and 1.9 µM, respectively. Compounds 1l and 1m also inhibited the VEGF-induced HUVEC cell migration, indicating its anti-angiogenic activity. OSI-930 along with compounds 1l and 1m showed inhibition of P-gp efflux pump (MDR1, ABCB1) with EC_{50} values in the range of 35-74 µM. The combination of these compounds with doxorubicin led to significant enhancement of the anticancer activity of doxorubicin in human colorectal carcinoma LS180 cells, which was evident by the improved IC_{50} of doxorubicin, increased activity of caspase-3 and significant reduction in colony formation ability of LS180 cells after treatment with doxorubicin. Compound 1l showed 13.8-fold improvement in the IC_{50} of doxorubicin in LS180 cells. The ability of these compounds to possess dual inhibition of VEGFR and P-gp efflux pump demonstrates the promise of this scaffold for development as multi-drug resistance-reversal agents.

Introduction

Vascular endothelial growth factor receptors (VEGFR) are cell surface receptors belonging to class-V receptor tyrosine kinase family (RTKs). VEGFRs are classified into three classes VEGFR1, 2 and VEGFR3.[1] These receptors play an important role both in cell proliferation and migration. VEGFR1 is expressed in haematopoietic endothelial, vascular endothelial cells and VEGFR2 is expressed in vascular endothelial, lymphatic endothelial cells and plays enormous role in both vasculogenesis and angiogenesis.[2] Angiogenesis is a process for the formation of new blood vessels from pre-existing vessels.[3] Tumors need blood vessels to grow and spread. The role of angiogenesis inhibitors is to prevent formation of new blood vessels, thereby stopping the spreading of tumor growth.[4] Number of angiogenesis inhibitors are in clinical development or are available in clinic. Representative examples (sorafenib, pazopanib and axitinib used for treatment of renal cell carcinoma) are shown in the Figure 1.

3-((Quinolin-4-yl)methylamino)-N-(4-(trifluoromethoxy)phenyl) thiophene-2-carboxamide (OSI-930, **1**),[5] is a potent inhibitor of closely related receptor tyrosine kinases C-kit (activated) and VEGFR2 (KDR) possessing IC_{50} values of 80 and 9 nM, respectively. It also inhibits platelet derived growth factor receptor beta (PDGF-β).[6] It is currently in phase I clinical trials for the treatment of cancer, and has shown activity in multiple tumor models that are thought to be dependent upon angiogenesis.[7]

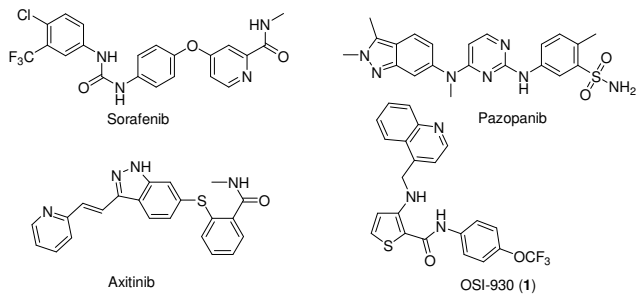

Figure 1. Examples of angiogenesis inhibitors in clinic / under clinical development

[a]*Medicinal Chemistry Division, CSIR-Indian Institute of Integrative Medicine (CSIR), Canal Road, Jammu-180001, India. Fax: +91-191-2586333; Tel: +91-191-2585006 (Extn. 345); *E-mail: sbharate@iiim.ac.in; sandipbharate@gmail.com (S.B.B.)*
[b]*Academy of Scientific & Innovative Research (AcSIR), CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu-180001, India*
[c]*Cancer Pharmacology Division, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu-180001, India. E-mail: ajaykmahajan@hotmail.com (AK), sbhushan@iiim.ac.in (S.B.)*
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Thiophene-2-carboxamides have been patented as antifibrotic agents,[8] and anticancer agents.[5, 9] The medicinal chemistry efforts on this scaffold has primarily been published in the form of a patent literature,[5, 9] where biology data has not been revealed. Korlipara and coworkers[10] have modified quinoline domain (site A) and identified amino-pyridine linked[10] and nitro-pyridine linked[11] thiophene-2-carboxamides as dual inhibitors of ABCG2 and VEGFR. Nitropyridyl and ortho-nitrophenyl analogs VKJP1 and VKJP3 (structures shown in Figure 2) were effective in reversing ABCG2-mediated MDR, as shown by the reduction in IC_{50} of mitoxantrone.[11] In the present work, we aimed to further understand the structure-activity relationship (SAR) of this scaffold by modifying both quinoline domain as well as trifluoromethoxy aniline moiety for VEGFR inhibition as shown in Figure 2. Through our efforts, we identified new thiophene-2-carboxamides possessing ability to display dual inhibition of VEGFR and ABCB1 (P-gp) efflux pump.

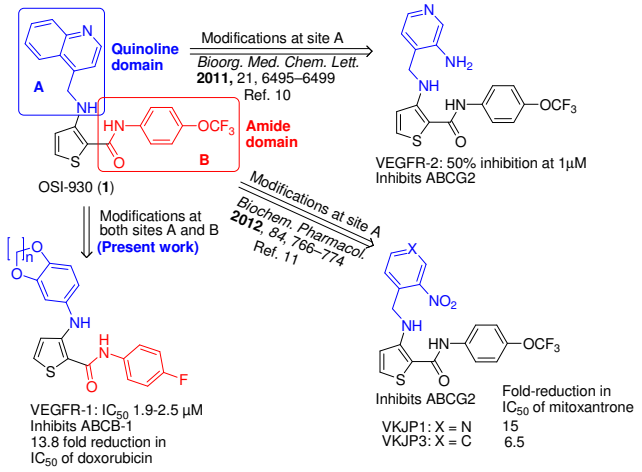
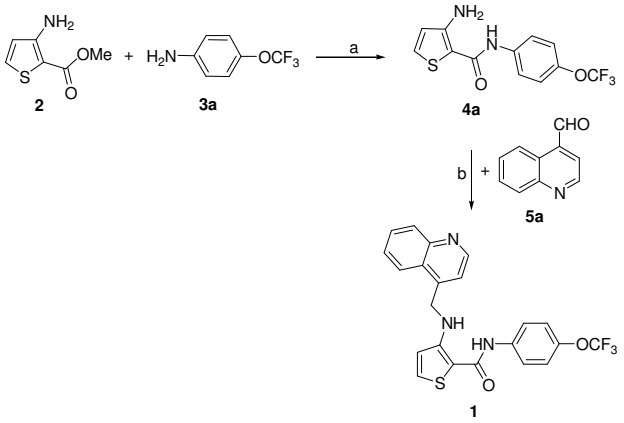


Figure 2. Medicinal chemistry of OSI-930 (**1**). The overview of literature reports and the present work.

Results and discussion

Chemistry. The parent compound OSI-930 (**1**) was synthesized using reported synthetic protocol.[5a] The coupling of 4-trifluoromethoxy aniline (**3a**) with methyl-3-aminothiophene 2-carboxylate (**2**) using $AlMe_3$ in anhydrous toluene under reflux led to formation of thiophene-2-carboxamide **4a**. The reductive amination of compound **4a** with quinoline 4-carboxaldehyde (**5a**) using TFA and triethylsilane yielded OSI-930 (**1**) in 80% yield (Scheme 1).

For synthesis of OSI-930 (**1**) analogs, initially we targeted replacement of quinoline moiety with variety of anilines **3** and heterocyclic aldehydes **5** using reductive amination strategy. The products formed by reductive amination reaction between thiophene-2-carboxamide **4a** and different substituted heterocyclic aldehydes **5**, were found to have stability issues, as we noticed degradation of these products on storage.



Scheme 1. Synthesis of OSI-930 (**1**). Reagents and conditions: a) anhyd. toluene, $AlMe_3$ (2.0 M in toluene, 1.2 equiv), 16 h, room temp., followed by addition of **3a**, (1.0 eq), reflux for 24 h, 78%; (b) TFA: DCM (1: 1), heat at reflux for 2 h under N_2 atm, Et_3SiH (2.0 eq), reflux for 16 h, 80%.

Then, we changed our strategy and targeted the direct coupling of thiophene 2-carboxamides **4a-d** with substituted arylboronic acids **6a-j**. In the latter approach, we prepared two series of compounds as shown in Table 1 and Scheme 2, respectively. 3-Amino-thiophene 2-carboxamides **4a-d** were reacted with arylboronic acids **6a-j** in presence of $Cu(OAc)_2$ and triethyl amine (Chan-Lam coupling) which produced N-arylated products **1a-s** (Table 1). In next series, 3-amino thiophene 2-carboxamide **4e** was prepared by reacting methyl-3-aminothiophene 2-carboxylate (**2**) with (4-fluorophenyl)methanamine (**3e**). The intermediate **4e** on Chan-Lam coupling with arylboronic acids **6a**, **6c**, **6k** and **6d** produced corresponding N-arylated products **1t-w** (Scheme 2).

Screening for cytotoxicity, VEGFR inhibition and in vitro anti-angiogenesis activity: As a first step to explore the biological activity, all synthesized analogs were screened for in-vitro cytotoxicity in a panel of cancer cell lines including MIAPaCa-2, MCF-7, HCT116, LS180 and HUVEC. The preliminary cytotoxicity results indicated that most of the compounds showed growth inhibition only in HUVEC cells with weak or no effect in other cell lines (Table 2). Compounds **1d**, **1g**, **1p** and **1q** displayed growth inhibition of human umbilical vein endothelial cells (HUVEC) with IC_{50} of 4 μM, whereas the cytotoxicity in other cell lines was very weak (IC_{50} > 25 μM) (Table 2). Next, all compounds were screened for VEGFR1 and VEGFR2 inhibition activity. Few compounds **1a**, **1f**, **1l**, **1m**, **1v** showed significant inhibition (>50%) of VEGFR1 at 2 μM. Further, we determined IC_{50} of two compounds **1l** and **1m** against VEGFR1, which was found to be 2.5 and 1.9 μM, respectively. However, none of the compound showed significant inhibitory activity against VEGFR2 (Table 2) in cell-free enzyme inhibition assay.

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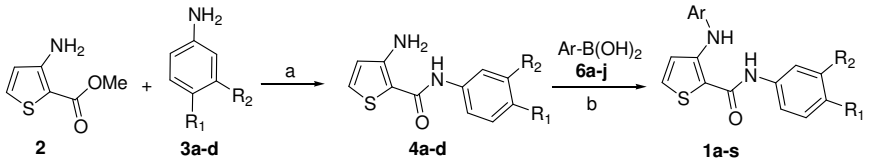
Table 1. Synthesis of thiophene 2-carboxamides **1a-s**[a]



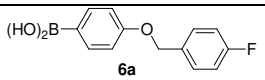
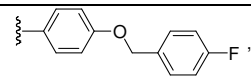
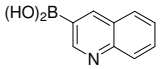
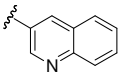
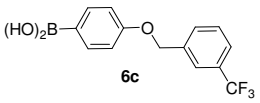
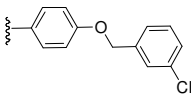
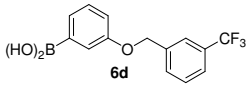
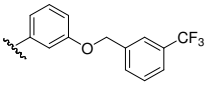
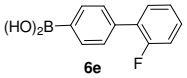
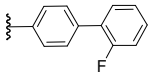
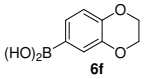
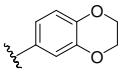
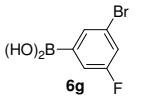
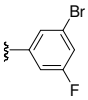
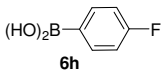
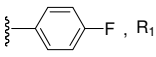
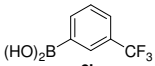
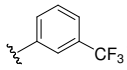
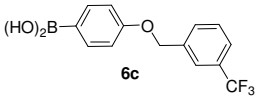
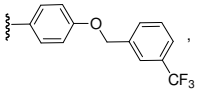
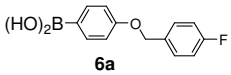
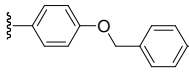
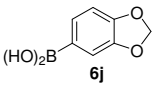
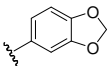
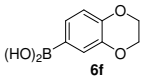
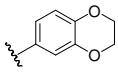
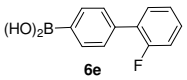
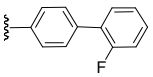
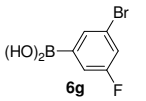
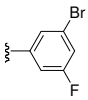
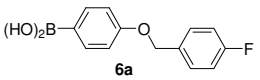
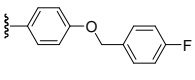
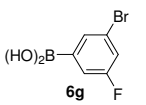
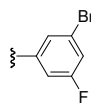
Sr No	**3a-d** and **4a-d**	Ar-B(OH)$_2$ (**6a-j**)	**Products 1a-s**[b]
1	**3a, 4a**: R_1 = OCF_3, R_2 = H	 **6a**	**1a**: Ar =  , R_1 = OCF_3, R_2 = H
2	**3a, 4a**: R_1 = OCF_3, R_2 = H	 **6b**	**1b**: Ar =  , R_1 = OCF_3, R_2 = H
3	**3a, 4a**: R_1 = OCF_3, R_2 = H	 **6c**	**1c**: Ar =  , R_1 = OCF_3, R_2 = H
4	**3a, 4a**: R_1 = OCF_3, R_2 = H	 **6d**	**1d**: Ar =  , R_1 = OCF_3, R_2 = H
5	**3a, 4a**: R_1 = OCF_3, R_2 = H	 **6e**	**1e**: Ar =  , R_1 = OCF_3, R_2 = H
6	**3a, 4a**: R_1 = OCF_3, R_2 = H	 **6f**	**1f**: Ar =  , R_1 = OCF_3, R_2 = H
7	**3a, 4a**: R_1 = OCF_3, R_2 = H	 **6g**	**1g**: Ar =  , R_1 = OCF_3, R_2 = H
8	**3a, 4a**: R_1 = OCF_3, R_2 = H	 **6h**	**1h**: Ar =  , R_1 = OCF_3, R_2 = H
9	**3a, 4a**: R_1 = OCF_3, R_2 = H	 **6i**	**1i**: Ar =  , R_1 = OCF_3, R_2 = H
10	**3b, 4b**: R_1 = F, R_2 = H	 **6c**	**1j**: Ar =  , R_1 = F, R_2 = H
11	**3b, 4b**: R_1 = F, R_2 = H	 **6a**	**1k**: Ar =  , R_1 = F, R_2 = H
12	**3b, 4b**: R_1 = F, R_2 = H	 **6j**	**1l**: Ar =  , R_1 = F, R_2 = H
13	**3b, 4b**: R_1 = F, R_2 = H	 **6f**	**1m**: Ar =  , R_1 = F, R_2 = H
14	**3b, 4b**: R_1 = F, R_2 = H	 **6e**	**1n**: Ar =  , R_1 = F, R_2 = H
15	**3b, 4b**: R_1 = F, R_2 = H	 **6g**	**1o**: Ar =  , R_1 = F, R_2 = H
16	**3c, 4c**: R_1 = CF_3, R_2 = H	 **6a**	**1p**: Ar =  , R_1 = CF_3, R_2 = H
17	**3d, 4d**: R_1 = H, R_2 = CF_3	 **6g**	**1q**: Ar =  , R_1 = H, R_2 = CF_3

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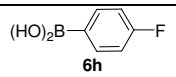
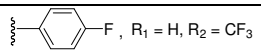
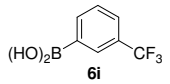
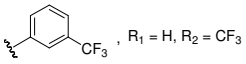
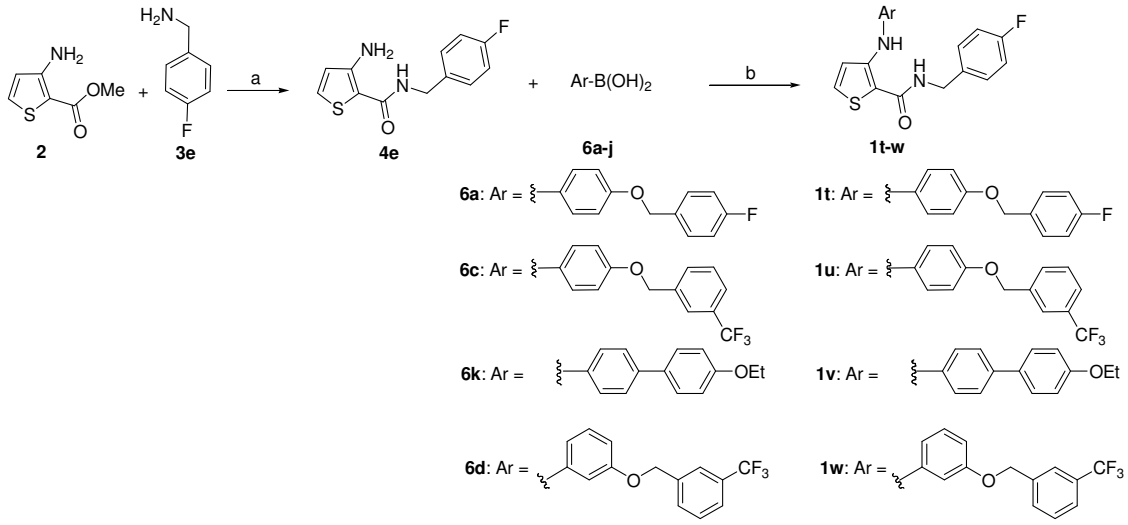
Table 1. Synthesis of thiophene 2-carboxamides **1a-s**[a]

Sr No	**3a-d** and **4a-d**	Ar-B(OH)$_2$ (**6a-j**)	**Products 1a-s**[b]
18	**3d, 4d**: R_1 = H, R_2 = CF_3	 **6h**	**1r**: Ar =  , R_1 = H, R_2 = CF_3
19	**3d, 4d**: R_1 = H, R_2 = CF_3	 **6i**	**1s**: Ar =  , R_1 = H, R_2 = CF_3

[a] Reagents and conditions: (a) **3a-d** in anhyd. toluene, $AlMe_3$ (2.0 M in toluene, 1.2 equiv), 16 h at room temp., followed by addition of **2**, (1.0 eq), reflux for 24 h, 72-78% (b) $Cu(OAc)_2$ (1.0 eq), anhydrous DCM, Et_3N (3.0 eq), O_2 atm, at room temp. for 6-8 h, 65%. [b]complete structures of all products **1a-s** are shown in ESI.



Scheme 2. Synthesis of thiophene 2-carboxamides **1t-w.** Reagents and conditions: (a) **3e** in anhyd. toluene, $AlMe_3$ (2.0 M in toluene, 1.2 equiv), 16 h at rt, followed by addition of **2**, (1.0 eq), reflux for 24 h, 72-78% (b) $Cu(OAc)_2$ (1.0 eq), anhydrous DCM, Et_3N (3.0 eq), O_2 atm, at room temp., for 6-8 h, 65%.

Table 2. Cytotoxicity, kinase inhibition and P-gp inhibition data of thiophene-2-carboxamides **1a-w**

Entry	Cytotoxicity (IC_{50}, μM)					VEGFR inhibition (%)[abc]		P-gp inhibition
	MIAPaCa2	MCF-7	HCT-116	HUVEC	LS180	VEGFR1[a]	VEGFR2[b]	(% of Rh123 accumulation in LS180 cells[def])
Control	0	0	0	0	0	0	0	100
Elacridar, 10 μM	nd	nd	nd	nd	nd	nd	nd	234
OSI-930 (1)	18	22	9	1.9	>100	99.2	72.3	128
1a	60	>100	>100	>100	>100	50.4	0	nd
1b	>100	>100	>100	>100	>100	4.8	3.6	nd
1c	65	>100	90	25	>100	25.6	5.4	84
1d	45	>100	30	4	>100	37.6	5.3	116
1e	38	>100	50	20	>100	36.2	2.8	116
1f	62	>100	58	10	>100	60.3	7.5	121
1g	30	60	25	4	>100	11	3.6	103
1h	65	54	60	15	>100	38.3	5.9	109
1i	60	54	50	7	>100	-1.8	4.2	98
1j	58	>100	>100	40	>100	51.8	4.2	93
1k	25	>100	>100	>100	>100	44	2.2	109
1l	80	>100	>100	25	>100	60.5	4.7	152
1m	>100	60	30	25	>100	66.1	6.0	152
1n	58	>100	>100	20	>100	51.4	6.0	98
1o	70	55	65	20	>100	7.4	3.3	136
1p	20	80	25	4	>100	18.3	1.09	96
1q	22	70	50	4	>100	-3.1	6.7	107
1r	50	65	25	6	>100	48.2	3.8	118
1s	22	>100	25	60	>100	6.3	1.2	99
1t	18	>100	>100	>100	>100	24.8	2.2	96
1u	11	>100	>100	>100	>100	15.9	4.2	101
1v	12	>100	>100	80	>100	77	10.3	92
1w	65	98	58	18	>100	-12.0	4.9	97

nd: not determined; [a] tested at 2 μM; [b]tested at 1 μM; [c]cell free assay for inhibition of VEGFR1 and 2; [d]*in vitro* assay for inhibition of P-gp activity in LS180 cells. [e]Increase in intracellular level of rhodamine-123 of treated samples in comparison to control indicate inhibition of P-gp activity; [f] compound **1a-w** were tested at 50 μM in P-gp inhibition assay.

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Compounds which showed good VEGFR1 inhibition in enzyme assay were selected for further studies such as western-blot and cell migration assay. Although compound **1a** showed 50% inhibition of VEGFR2 at 2 μM, it was not selected for further studies as it was inactive in HUVEC cells (IC_{50} > 100 μM). The effect of compounds **1c**, **1f**, **1l**, **1m** and **1r** on VEGFR1 and VEGFR2 expression was checked by western-blot experiment in HUVEC cell line at their respective IC_{50} concentrations in this cell line. As shown in Figure 3, the compounds **1f**, **1l, 1m** and **1r** displayed significant inhibition of VEGFR2 in HUVEC cells. Similarly, compound **1r** also showed significant inhibition of VEGFR1.

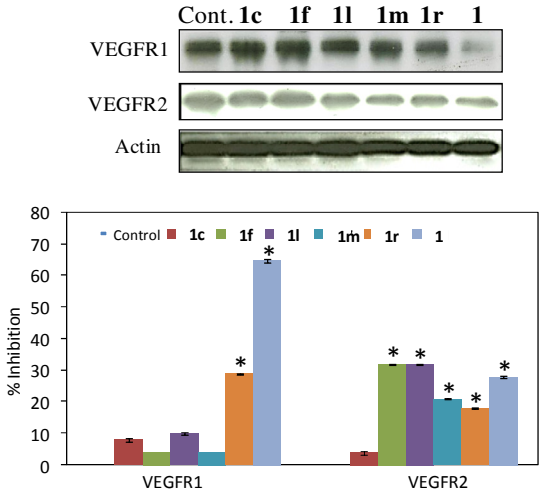

Figure 3. Western-blot experiment to check effect of compounds on VEGFR expression in HUVEC cell line (Time: 24 h, Concentration: IC_{50} value). Data were mean ± S.D. of three independent experiments. *p* values *<0.001 were considered significant.

To appraise the *in vitro* anti-angiogenic property of compounds **1c**, **1f**, **1l**, **1m** and **1r** along with OSI-930 (**1**), we examined chemotactic motility and microvessel sprouting of HUVEC cells using the wound-healing migration assay. It was observed that compounds **1f**, **1l**, **1m, 1r** and **1** significantly inhibited VEGF-induced HUVEC migration and decreased number of migrated cells percentage from 100% to 20% at their IC_{50} values (Figure 4a-b).

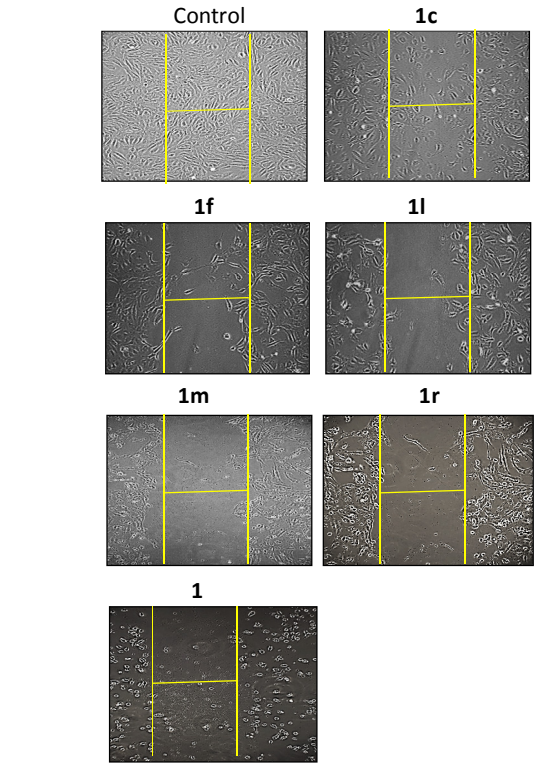

Figure 4. Effect of compounds on angiogenesis-dependent cell migration in HUVEC cells. Data were mean ± S.D. of three independent experiments. *p* values *<0.001 were considered significant.

Screening for P-glycoprotein (P-gp) inhibition and for ability of compounds to overcome chemoresistance in cancer: OSI-930 (**1**) and its analogs have been reported to inhibit ABCG2 (BCRP) mediated drug resistance.[10-11] The third generation efflux pump inhibitors are known to inhibit both BCRP and P-gp efflux pumps,[12] therefore with the known ability of this scaffold to inhibit BCRP,[11-12] it was worthwhile to investigate its P-gp inhibition activity. Thus, we decided to investigate the effect of OSI-930 and synthesized analogs for P-gp mediated drug resistance. All synthesized compounds were tested for P-gp inhibition activity at 50 μM in LS180 cells using Rh123 as a P-gp substrate. Interestingly, OSI-930 and several analogs showed significant P-gp inhibitory activity, which was reflected by

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increased intracellular accumulation of rhodamine-123 in LS180 cells. OSI-930 was able to increase the intracellular level of Rh-123 by 27%, whereas, compounds **1l** and **1m** were better as indicated by 51.6% increase in Rh-123 accumulation in LS180 cells (Table 2). The EC_{50} of OSI-930 (**1**) and compounds **1l** and **1m** for P-gp inhibition was found to be 35, 40 and 74 μM, respectively.

In general it was observed that all synthesized analogs (with the removal of $-CH_2$ from quinoline domain of OSI-930, resulted in significant reduction in VEGFR inhibition activity (e.g. **1** *vs* **1b**, a close structural analog). Based on the obtained screening results, a precise structure-activity relationship could not be established; however it was interesting to note that analogs where quinoline domain of OSI-930 was replaced with benzo[d][1,3]dioxol-5-yl (analog **1l**) and 2,3-dihydrobenzo[b][1,4]dioxin-6-yl (analog **1m**) groups displayed significant inhibition of VEGFR1 as well as P-gp efflux pump; and these analogs were better than other prepared analogs.

The human P-gp is a 170 kD, transmembrane ATPase efflux pump, present in cancer cells and is responsible for the efflux of anticancer agents including the anthracyclins,[13] taxol derivatives,[13b, 14] colchicinoids[15] and tyrosine kinase inhibitor imatinib.[16] Our data indicated that on account of high activity of P-gp in LS180 cells in comparison to other cancer cells, the P-gp substrate anticancer drugs like doxorubicin generally shows higher IC_{50} value in LS180 cells (Table 3).

Table 3. MTT assay was done in different cancer cell lines after treatment with different concentrations of doxorubicin to calculate its IC_{50} value

Cell line	IC_{50} (nM)
LS180	840
K562	190
T47D	100
HL-60	370
HCT116	190
A431	48
THP-1	49

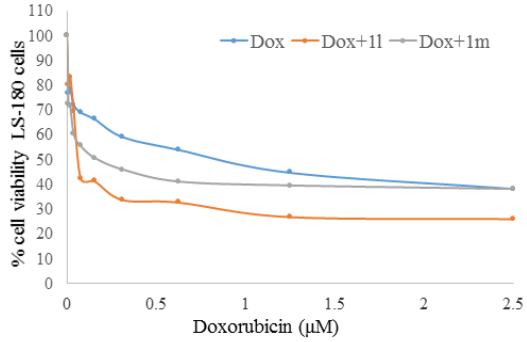
Based on these observations, we selected LS180 cells to demonstrate the effect of P-gp inhibition on the cytotoxic activity of doxorubicin. Our initial experiments showed that pre-treatment of cells with 100 μM of compounds **1l** or **1m** significantly increased the intracellular accumulation of doxorubicin by 18.7 and 28.1% respectively (Table 4).

Table 4. Assay for intracellular accumulation of doxorubicin[a]

Entry	Control	Doxo	**1l**	**1m**
P-gp inhibitor concentration, μM	0	0	100	100
Doxorubicin concentration, μM	0	10	10	10
Intracellular doxorubicin level (ng/ml)	0	177.8	211.0	227.8
% Intracellular doxorubicin level	0	100	118.7	128.1

[a]LS180 cells were co-treated with doxorubicin and **1l** or **1m** for 90 minutes. Cells were washed with PBS and lysed before quantitation by LCMS.

Due to increased accumulation of doxorubicin, it was hypothesized that both compounds **1l** and **1m** may potentiate the cytotoxicity of doxorubicin in LS180 cells. Therefore, IC_{50} value of doxorubicin was calculated in presence or absence of 50 μM of compounds **1l** and **1m**. The results clearly indicated a significant improvement in the IC_{50} value of doxorubicin, as it is changed from 840 nM to 61 and 160 nM, respectively (Figure 5). Compound **1l** at 50 μM led to 13.8 fold increase in sensitivity of LS180 cells towards doxorubicin. It is noteworthy to mention that compounds **1l** and **1m** did not display any cytotoxicity in LS180 cells even at high concentration of 100 μM (Table 2). Therefore, it is clear that the potentiation of cytotoxicity of doxorubicin must be caused by the P-gp inhibitory effect of compounds **1l** and **1m**.



Treatments	IC_{50} (nM)	DMF
Doxorubicin alone	840	-
Doxorubicin + **1l**	61	13.8
Doxorubicin + **1m**	160	5.3

Figure 5. Combined treatment of doxorubicin and compounds **1l** and **1m** displayed higher efficacy of doxorubicin in LS180 cells. MTT assay was done in LS180 cells after 48 h treatment with doxorubicin in presence or absence of 50 μM of compounds **1l** or **1m**. The viability of control cells was considered as 100% and the concentration at which the cell viability was reduced to 50% was taken as IC_{50} of doxorubicin. Data were mean ± S.D. of three independent experiments. DMF: Dose-modifying factor was the ratio of IC_{50} value of doxorubicin in LS180 cells without an inhibitor to IC_{50} value of doxorubicin in LS180 cells with an inhibitor.

There are only few cells among cancer cell population with ability to form colonies which defines the clonogenic potential of given type of cancer. Therefore, the ability of a chemotherapeutic agent to target these clonogenic cells is an essential feature of successful chemotherapy. Inhibition of P-gp can thus contribute to eradicate even the chemo-resistant cells which can reproduce to lead cancer recurrence. With this view, we treated the LS180 cells with doxorubicin (100 nM) in presence or absence of compound **1l** and **1m** (50 μM each) for 48 h and analysed the formation of colonies. After 15-days of treatment, the number of colonies formed by cells treated in combination with **1l** or **1m** was significantly reduced as compared to the cells treated with doxorubicin alone (Figure 6A).

Doxorubicin is a topoisomerase-IIα inhibitor,[17] however, it is also known to form adduct with the DNA, resulting in induction of apoptosis and leading to the activation of caspases and apoptotic fragmentation of DNA. In this context, further studies revealed that the potentiation of cytotoxicity of doxorubicin is caused by

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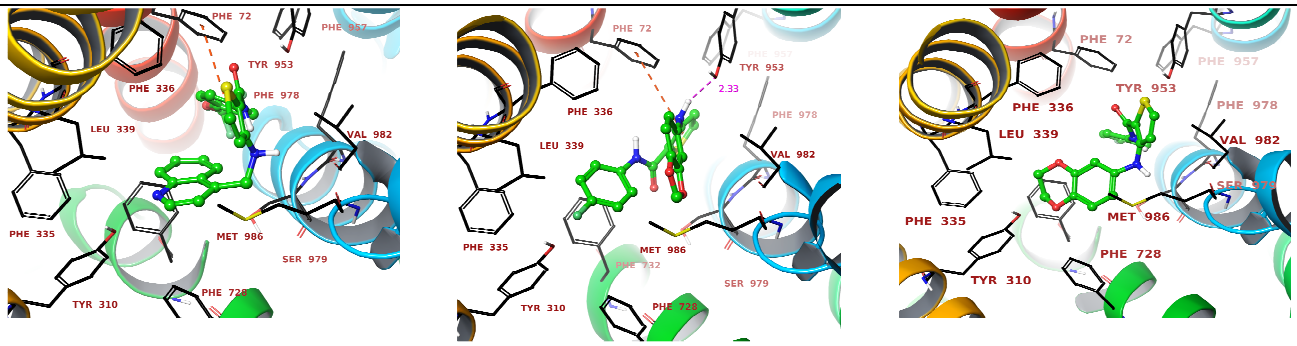

Figure 7. Proposed hypothetical binding sites and interaction pattern of compounds **1**, **1l** and **1m** with P-gp.

increased activation of caspase-3, which was evident by the abrogated expression of procaspase-3 after 48 h treatment of LS180 cells with doxorubicin in combination with compounds **1l** and **1m** (Figure 6B). Treatment of cells with compound **1l** and **1m** also led to the cleavage of DNA repairing enzyme Poly ADP-ribose polymerase 1 (PARP1) and inhibitor of caspase activated Dnase (ICAD), which are down-stream targets of caspase-3 (Figure 6B).

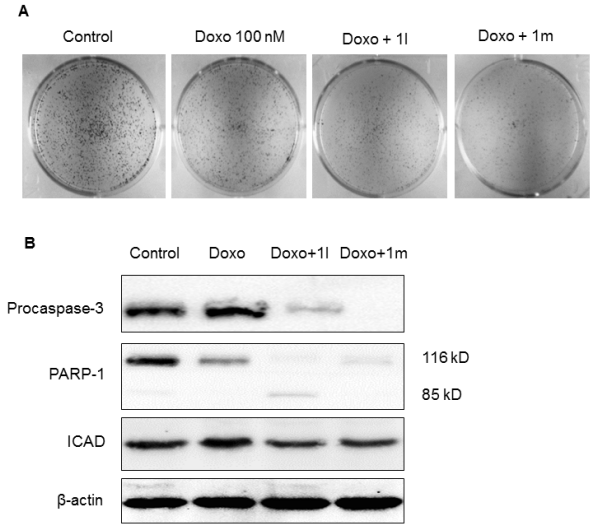

Figure 6 (A) Colony formation assay. Combined treatment of doxorubicin (100 nM) with 50 μM of compounds **1l** and **1m** significantly reduced the number of colonies in LS180 cells, when compared to treatment with doxorubicin alone. (B) Western-blot analysis. Compounds **1l** and **1m** at 50 μM potentiated the apoptotic effect of doxorubicin (5 μM) by enhancing the cleavage of procaspase-3, PARP-1 and ICAD in LS180 cells.

Molecular modelling with P-gp: The process of substrate or ligand transport across biological membranes by efflux pumps is a complex dynamic process and it requires energy in form of ATP.[18 19] Recently, it was observed that the P-gp pump is capable of binding more than one ligands simultaneously at drug-binding pocket, although the exact binding site for substrate and ligands to P-gp may vary because of the multiple drug transport active sites.[20] Therefore, based on the molecular docking studies[21] at verapamil binding site[22] of human P-gp homology model,[23] plausible P-gp binding site for OSI-930 (**1**) and its analog **1l** and **1m** has been proposed. It was observed that OSI-930 (**1**) interacts with P-gp in similar fashion as that of verapamil by purely hydrophobic vander waal and π-π interactions. OSI-930 (**1**) and its analog **1l** interact with the Phe72, Tyr310, Leu332, Phe335, Phe336, Leu339, Phe-728, Phe732, Met948, Tyr953, Phe957, Leu975, Val982 and Phe983 and Met986 residues by hydrophobic interactions as shown in Figure 7.

Interestingly, the secondary amino group of compound **1l** was found to interact with the Tyr953 phenolic hydroxyl group via polar H-bonding (2.33 Å). The interactions of compound **1l** with verapamil binding site of P-gp is thought to restricts the flexibility of P-gp transmembrane domains and ultimately block conformational changes in P-gp structure required for the substrate Rh123 or doxorubicin translocation across the membrane. Although compound **1m** does not show any polar H-bonding, however it showed purely hydrophobic interactions like OSI-930, which appears to be enough to block the efflux function of the pump.

Conclusion

In summary, we have synthesized a new series of OSI-930 analogs and evaluated for in vitro cytotoxicity, VEGFR1/2 inhibition and P-gp inhibition activity. Two analogs **1l** and **1m** substituted with benzo[d][1,3]dioxol-5-yl and 2,3-dihydrobenzo[b][1,4]dioxin-6-yl groups displayed significant inhibition of VEGFR1 along with inhibition of P-gp efflux pump. Further, we have shown that these compounds led to increased intracellular doxorubicin accumulation inside tumor cells and hence resulting in potentiation of its cytotoxic effect. These compounds also enhanced ability of doxorubicin to activate executioner caspase-3 and its downstream ICAD. The dual antiangiogenic and P-gp inhibition activity against cancer makes these compounds suitable candidates for further studies for development of effective anticancer therapeutics.

Experimental section

General. All chemicals were obtained from Sigma-Aldrich Company and used as received. ^{1}H, ^{13}C and DEPT NMR spectra were recorded on Brucker-Avance DPX FT-NMR 500 and 400 MHz instruments. Chemical data for protons are reported in parts per million (ppm) downfield from tetramethylsilane and are referenced to the residual proton in the NMR solvent ($CDCl_3$, 7.26 ppm). Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 125 MHz or 100 MHz: chemical data for

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carbons are reported in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonance of the solvent ($CDCl_3$, 77 ppm). ESI-MS and HR-ESIMS spectra were recorded on Agilent 1100 LC-Q-TOF and HRMS-6540-UHD machines. IR spectra were recorded on Perkin-Elmer IR spectrophotometer. Melting points were recorded on digital melting point apparatus. HPLC analysis was done using Shimadzu LC 10-AT HPLC system connected with a PDA detector. HPLC methods include: Method A: isocratic flow (water: acetonitrile - 10: 90), 0.4 ml/min, Merck 5 u, 4 × 250 mm C18 column, run time: 45 min. Method B: isocratic flow (water: methanol - 30:70), 1 ml/min, 3.5 µ, 4.6× 250 mm Inertsil C8 column, run time: 30 min.

General procedure for the preparation of 3-amino thiophene-2-carboxamides 4a-e. To a stirred solution of substituted aniline/benzyl amine **3a-e** (7.8 g, 44.5 mmol) in toluene (50 ml), under nitrogen was added trimethyl aluminium (2.0 M in toluene, 26.7 ml, 53.4 mmol). The mixture was stirred at room temperature for 16 h. Methyl 3-amino-2-thiophene carboxylate (**2**, 44.5 mmol) was added and the resulting solution was stirred at reflux at 130 °C under nitrogen for 24 h. After cooling to room temperature saturated sodium bicarbonate solution (100 ml) was added dropwise with caution and the mixture was stirred at room temperature for 30 min. The product was extracted into DCM (3 x 100 ml), and the organic layer was dried over Na_2SO_4, concentrated under vacuum and purified with silica gel using 20% EtOAc: n-Hexane to yield compounds **4a-e** in 85-92% yield.

*3-Amino-N-(4-(trifluoromethoxy)phenyl)thiophene-2-carboxamide (**4a**).* Light brown semisolid; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.54 (d, J = 8.8 Hz, 2H), 7.23-7.16 (m, 4H), 6.58 (d, J = 5.2 Hz, 1H), 5.71 (bs, 2H); IR ($CHCl_3$): ν_{max} 3788, 3459, 3349, 2923, 2852, 1709, 1633, 1593, 1537, 1509, 1447, 1406, 1319, 1262, 1242, 1221, 1200, 1161, 1084, 1017 cm^{-1}; ESI-MS: *m/z* 303.0 $[M+H]^+$.

*N-(4-Fluorophenyl) 3-aminothiophene-2-carboxamide (**4b**).* Light Brown solid; m.p.78-80 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.48 (dd, J = 4.8, 8.8 Hz, 2H), 7.20 (d, J = 5.2 Hz, 1H), 7.13 (bs, 1H), 7.05 (t, J = 8.8 Hz, 2H), 6.59 (d, J = 5.2 Hz, 1H), 5.69 (bs, 2H); IR ($CHCl_3$): ν_{max} 3851, 3743, 3460, 3415, 3340, 3105, 2923, 2852, 1882, 1632, 1592, 1537, 1507, 1446, 1403, 1316, 1260, 1212, 1156, 1122, 1083, 1014 cm^{-1}; ESI-MS: *m/z* 237.0 $[M+H]^+$.

*3-Amino-N-(4-(trifluoromethyl)phenyl)thiophene-2-carboxamide (**4c**).* Light brown solid, m.p. 74-76 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.47 (d, J = 8.4 Hz, 2H), 7.20 (m, 3H), 6.55 (d, J = 8.4 Hz, 1H); IR ($CHCl_3$): ν_{max} 3855, 3392, 3043, 2926, 2854, 1907, 1622, 1595, 1520, 1449, 1409, 1320, 1260, 1234, 1180, 1161, 1112, 1065, 1013 cm^{-1}; ESI-MS: *m/z* 287.0 $[M+H]^+$.

*3-Amino-N-(4-chloro-3-(trifluoromethyl)phenyl)thiophene-2-carboxamide (**4d**).* Light brown solid; m.p. 90-91 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.88 (s, 1H), 7.71 (d, J = 8.8 Hz, 1H), 7.42 (d, J=8.8 Hz, 1H), 7.23 (d, J = 5.6 Hz, 1H), 6.60 (d, J = 5.2 Hz, 1H), 5.73 (bs, 2H); IR ($CHCl_3$): ν_{max} 3854, 3745, 3470, 3415, 3353, 3113, 2926, 2854, 1633, 1594, 1537, 1483, 1446, 1412, 1262, 1234, 1176, 1086, 1033 cm^{-1}; ESI-MS: *m/z* 321.0 $[M+H]^+$.

*N-(4-Fluorobenzyl) 3-aminothiophene-2-carboxamide (**4e**).* Light cream colored solid; m.p. 148-150 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.30 (m, 2H), 7.14 (d, J = 5.6 Hz, 1H), 7.05 (m, 2H),6.57 (d, J = 5.6 Hz, 1H), 5.72 (bs, 1H), 5.63 (s, 2H), 4.54 (d, J = 6.0 Hz, 2H); IR ($CHCl_3$): ν_{max} 3855, 3438, 3342, 2923, 2850, 1884, 1537, 1593, 1508, 1447, 1418, 1312, 1268, 1219, 1155, 1097, 1017 cm^{-1}; ESI-MS: *m/z* 251.0 $[M+H]^+$.

General procedure for the preparation of 3-(arylamino)-N-arylthiophene 2-carboxamides 1a-w. The mixture of N-aryl thiophene 2-carboxamide **4a-e** (100 mg, 1 equiv.) and aryl boronic acid **6a-j** (1.05 equiv.) in anhydrous DCM (10 ml) under oxygen atmosphere was stirred at room temperature. Then to this mixture was added $Cu(OAc)_2$ (1.1 equiv.), TEA (3.0 equiv.) and stirred at room temperature for 6-8 hrs. Reaction was monitored by TLC and the product was extracted with DCM (2 x 25 ml). The organic layer was dried over Na_2SO_4, concentrated under vacuum and purified with silica gel using 20% EtOAc: hexane to yield **1a-w** in 65-73% yield.

*3-((4-((4-Fluorobenzyl)oxy)phenyl)amino)-N-(4-(trifluoromethoxy)phenyl)thiophene-2-carboxamide (**1a**).* Light yellow solid; m.p. 115-116 °C; HPLC purity: 100% (t_R = 10.82 min – Method A); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.17 (s, 1H), 7.58 (d, J = 9.2 Hz, 2H), 7.42 (dd, J = 5.6, 8.4 Hz, 2H), 7.28 (d, J = 5.6 Hz, 1H), 7.22 (s, 1H), 7.20 (d, J = 4.8 Hz, 2H), 7.13-7.06 (m, 4H), 7.00 (d, J = 5.6 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 5.01 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.5 (d, $^1J_{CF}$ = 244.5 Hz), 163.2 155.1, 152.5, 145.3, 136.5, 135.1, 132.7, 129.4 (d, $^2J_{CF}$ = 7.8 Hz), 128.1, 123.0, 121.8, 121.6, 119.3, 115.7, 115.6, 115.4, 103.1, 69.7; IR($CHCl_3$): ν_{max} 3306, 2920, 2850, 1593, 1563, 1504, 1407, 1376, 1299, 1209, 1166, 1067 cm^{-1}; ESI-MS: *m/z* 503.0 $[M+H]^+$; HRMS: *m/z* 503.0907 calcd for $C_{25}H_{18}F_4N_2O_3S$ + H^+ (503.1047).

*3-(Quinolin-3-ylamino)-N-(4-(trifluoromethoxy)phenyl) thiophene-2-carboxamide (**1b**).* Brown colored solid; m.p. 215-217 °C; HPLC purity: 100% (t_R = 9.19 min– Method A); ^{1}H NMR (CD_3OD, 400 MHz): δ 8.74 (bs, 1H), 7.98 (m, 2H), 7.82 (d, J = 7.6 Hz, 1H), 7.79 (s, 1H), 7.73 (d, J = 9.2 Hz, 2H), 7.67 (d, J = 5.6 Hz, 1H), 7.61-7.50 (m, 2H), 7.39 (d, J = 5.6 Hz, 1H), 7.24 (d, J = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$ + CD_3OD, 100 MHz): δ 163.4, 149.5, 145.5, 144.0, 136.5, 135.7, 129.8, 129.1, 127.8, 127.6, 126.9, 122.4, 122.3, 121.7, 121.6, 120.9, 119.5, 119.2, 108.2; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -58.09 (s, 3F); IR ($CHCl_3$): ν_{max} 3440, 2954, 2924, 2853, 2358, 1733, 1629, 1579, 1540, 1509, 1456, 1410, 1377, 1266, 1246, 1218, 1155, 1082, 1019 cm^{-1}; ESI-MS: *m/z* 430.07 $[M+H]^+$, HRMS: *m/z* 430.0834 calcd for $C_{21}H_{14}F_3N_3O_2S$ + H^+ (430.0832).

*N-(4-(Trifluoromethoxy)phenyl)-3-((4-((3-(trifluoromethyl)benzyl)oxy)phenyl)amino)thiophene-2-carboxamide (**1c**).* Light grey solid; m.p. 87-88 °C; HPLC purity: 100% (t_R = 12.91 min– Method A); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.40 (s, 1H), 7.58 (d, J = 9.0 Hz, 2H), 7.41-7.34 (m, 4H), 7.25-7.18 (m, 7H), 7.15 (d, J = 8.5 Hz, 1H), 5.04 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.1, 158.8, 150.5, 145.4, 141.7, 136.3, 132.0 (d, $^1J_{CF}$ = 32.0 Hz), 130.4, 130.0, 129.1, 128.3, 125.1, 121.8, 121.5, 119.8, 119.7, 118.2, 117.7, 117.6, 111.7, 105.5, 70.0; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -58.10 (s, 3F), -62.7 (s,

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3F); IR ($CHCl_3$): ν_{max} 3307, 2923, 2852, 1589, 1562, 1509, 1449, 1411, 1381, 1328, 1262, 1241, 1201, 1163, 1125, 1096, 1066, 1017 cm^{-1}; ESI-MS: *m/z* 553.09 $[M+H]^+$; HRMS: *m/z* 553.1036 calcd for $C_{26}H_{18}F_6N_2O_3S+H^+$ (553.1015).

*N-(4-(Trifluoromethoxy)phenyl)-3-((3-((3-(trifluoromethyl)benzyl) oxy)phenyl)amino) thiophene-2-carboxamide (**1d**).* Light brown semisolid; HPLC purity: 97.2% (t_R = 13.2 min– Method A); ^1H NMR: ($CDCl_3$, 400 MHz): δ 9.38 (s, 1H), 7.57 (d, *J* = 8.8 Hz, 2H), 7.41-7.28 (m, 4H), 7.25- 7.19 (m, 4H), 7.13-7.08 (m, 4H), 5.08 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.1, 158.8, 156.0, 150.6, 145.4, 142.1, 138.1, 137.8, 136.2, 132.1 (d, $^1J_{CF}$ = 32.2 Hz), 130.1 (m), 129.7, 128.2, 121.8, 121.7, 119.7 (m), 118.7, 118.3, 117.7, 115.1, 114.2, 111.7, 105.6, 70.0; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -58.10 (s, 3F), -62.68 (s, 3H); IR ($CHCl_3$): ν_{max} 3337, 2920, 2851, 1592, 1566, 1509, 1492, 1449, 1411, 1383, 1328, 1262, 1242, 1221, 1202, 1164, 1125, 1096, 1066, 1018 cm^{-1}; ESI-MS: *m/z* 553.0 $[M+H]^+$, 575.0 $[M+Na]^+$; HRMS: *m/z* 553.1022 calcd for $C_{26}H_{18}F_6N_2O_3S+H^+$ (553.1015).

*3-((3-Fluoro-[1,1'-biphenyl]-4-yl)amino)-N-(4-(trifluoromethoxy)phenyl)thiophene-2-carboxamide (**1e**).* Light yellow solid; m.p. 116-118 °C; HPLC purity: 99.7% (t_R = 9.73 min – Method B); ^1H NMR ($CDCl_3$, 400 MHz): δ 9.46 (s, 1H), 7.57-7.47 (m, 4H), 7.44-7.40 (m, 2H), 7.38-7.31 (m, 3H), 7.25 (1H, *J* = 4 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 2H), 6.95 (d, *J* = 8.0 Hz, 2H), 6.65 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.0, 161.2 (d, $^1J_{CF}$ = 246.3 Hz), 149.7, 145.5, 142.4, 136.1, 135.5, 131.3, 128.7, 128.5, 128.4, 127.3, 123.0, 121.9, 121.8, 119.8, 115.3, 111.6, 106.5 (d, $^2J_{CF}$ = 26.2 Hz), 103.7 (d, $^2J_{CF}$ = 25.8 Hz); ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -58.09 (s, 3F), 116.06 (m, 1F); IR ($CHCl_3$): ν_{max} 3400, 2918, 2850, 1624, 1586, 1508, 1486, 1411, 1308, 1259, 1219, 1201, 1162, 1018 cm^{-1}; ESI-MS: *m/z* 472.9 $[M+H]^+$; HRMS: *m/z* 473.0944 calcd for $C_{24}H_{16}F_4N_2O_2S+H^+$ (473.0941).

*3-((2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)amino)-N-(4-(trifluoromethoxy)phenyl)thiophene-2-carboxamide (**1f**).* Light brown solid; m.p. 98-99 °C; HPLC purity: 99.0% (t_R = 5.07 min– Method B); ^1H NMR ($CDCl_3$, 400 MHz): δ 9.11 (s, 1H), 7.57 (d, *J* = 8 Hz, 2H), 7.26-7.18 (m, 3H), 7.04 (d, *J* = 8 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 6.71 (s, 1H), 6.66 (d, *J* = 4.0 Hz, 1H), 4.25 (t, *J* = 8.0 Hz, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.2, 152.1, 145.2, 143.8, 139.9, 136.4, 135.4, 128.1, 121.7, 121.6, 121.4, 119.5, 117.6, 114.8, 110.4, 103.5, 64.4, 64.2; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -58.10 (s, 3F); IR ($CHCl_3$): ν_{max} 3325, 2919, 2846, 1594, 1563, 1506, 1411, 1300, 1262, 1241, 1201, 1164, 1067, 1017 cm^{-1}; ESI-MS: *m/z* 436.9 $[M+H]^+$; HRMS: *m/z* 437.0785 calcd for $C_{20}H_{16}F_3N_2O_4S + H^+$ (437.0777).

*3-((3-Bromo-5-fluorophenyl)amino)-N-(4-(trifluoromethoxy)phenyl) thiophene-2-carboxamide (**1g**).* Light brown colored solid; m.p. 93-94 °C; HPLC purity: 99.6% (t_R = 16.44 min– Method B); ^1H NMR ($CDCl_3$, 400 MHz): δ 9.38 (S, 1H), 7.48 (m, 2H), 7.31 (m, 1H), 7.13 (m, 3H), 6.97 (s, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 6.70 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.6 (d, $^1J_{CF}$ = 247.7 Hz), 162.8, 148.8, 145.6, 144.3, 136.0, 128.5, 123.2 (d, $^1J_{CF}$ = 12.1 Hz), 121.9, 121.8, 119.8, 119.2, 117.6, 112.7 (d, $^2J_{CF}$ = 24.8 Hz), 107.8, 104.8 (d, $^2J_{CF}$ =24.6 Hz); ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -58.09 (s, 3F), -109.90 (m, 1F); IR ($CHCl_3$): ν_{max} 3306, 2919, 2850, 1604, 1587, 1563, 1524, 1508, 1459, 1378, 1311, 1262, 1244, 1214, 1201, 1158, 1091, 1033, 1018 cm^{-1}; ESI-MS: *m/z* 474.8 $[M+H]^+$; HRMS: *m/z* 474.9748 calcd for $C_{18}H_{12}BrF_4N_2O_2S+H^+$ (474.9734).

*3-((4-Fluorophenyl)amino)-N-(4-(trifluoromethoxy)phenyl)thiophene-2-carboxamide (**1h**).* Light yellow solid, mp 102-104 °C; HPLC purity: 100% (t_R = 10.36 min– Method B); ^1H NMR (400 MHz, $CDCl_3$): δ 9.17 (s, 1H), 7.57 (d, *J* = 12.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 2H), 7.04 (m, 2H), 6.95 (m, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.1, 159.0 (d, $^1J_{CF}$ = 241.0 Hz), 150.7, 144.3, 136.7, 135.3, 127.2, 121.5, 120.8, 120.5, 118.4, 118.2, 115.1 (d, $^2J_{CF}$ = 22.5 Hz), 103.1; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -58.10 (s, 3F), -119.54 (m, 1F); IR ($CHCl_3$): ν_{max} 3307, 2920, 2850, 1629, 1601, 1566, 1507, 1437, 1411, 1376, 1243, 1217, 1201, 1160, 1094, 1017 cm^{-1}; ESI-MS: *m/z* 397.0 $[M+H]^+$; HRMS: *m/z* 397.0628 calcd for $C_{18}H_{13}F_4N_2O_2S+H^+$ (397.0628).

*N-(4-(Trifluoromethoxy)phenyl)-3-((3-(trifluoromethyl)phenyl)amino)thiophene-2-carboxamide (**1i**).* Light brown colored solid; m.p. 105-106 °C; HPLC purity: 100% (t_R = 12.35 min– Method B); ^1H NMR: ($CDCl_3$, 400 MHz): δ 9.42 (s, 1H), 7.48 (d, *J* = 9.2 Hz, 2H), 7.33-7.25(m, 3H), 7.20 (t, *J* = 8.4 Hz, 2H), 7.12 (t, *J* = 10 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.0, 148.7, 144.5, 141.2, 135.1, 130.9 (d, $^1J_{CF}$ = 32.1 Hz), 128.9, 127.4, 124.0, 121.8, 121.4, 120.8, 120.5,118.4, 118.0, 114.8, 105.5; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -58.10 (s, 3F), -62.84 (s, 3F); IR ($CHCl_3$): ν_{max} 3306, 2919, 2850, 1597, 1565, 1509, 1454, 1412, 1333, 1264, 1243, 1219, 1202, 1163, 1069, 1018 cm^{-1}; ESI-MS: *m/z* 447.0 $[M+H]^+$; HRMS: *m/z* 447.0609 calcd for $C_{19}H_{12}F_6N_2O_2S+H^+$ (447.0596).

*N-(4-Fluorophenyl)-3-((4-((3-(trifluoromethyl)benzyl)oxy)phenyl)amino)thiophene-2-carboxamide (**1j**).* Light yellow solid; m.p. 149-150 °C; HPLC purity: 97.2% (t_R = 19.88 min– Method B); ^1H NMR ($CDCl_3$, 400 MHz): δ 9.06 (s, 1H), 7.84 (d, *J* = 4.0 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.33 (m, 3H), 7.22 (m, 2H), 7.18-6.98 (m, 4H), 6.91-6.85 (m, 3H), 4.94 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.5 (d, $^1J_{CF}$ =244.8 Hz), 163.1, 155.3, 153.0, 136.8, 134.9, 132.5, 131.9, 129.4 (d, $^2J_{CF}$ = 8.1 Hz), 128.6, 124.1, 123.3, 119.3, 119.2 (d, $^1J_{CF}$ = 5.2Hz), 119.1, 115.7, 115.7, 115.6, 115.4, 102.6, 69.7; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -62.75 (s, 1F), -114.13 (m, 1F); IR ($CHCl_3$): ν_{max} 3434, 2919, 2850, 1636, 1509, 1412, 1321, 1230, 1018 cm^{-1}; ESI-MS; *m/z* 487.0 $[M+H]^+$.

*3-((4-((4-Fluorobenzyl)oxy)phenyl)amino)-N-(4-fluorophenyl)thiophene-2-carboxamide (**1k**).* Light brown solid; m.p. 114-115 °C; HPLC purity: 96.7% (t_R = 10.82 min– Method B); ^1H NMR ($CDCl_3$, 400 MHz): δ 9.07 (s, 1H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.14 (d, *J* = 4.0 Hz, 2H), 7.0-6.88 (m, 6H), 6.82 (d, *J* =12.0 Hz, 2H), 6.64 (m, 1H), 4.88 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.5 (d, $^1J_{CF}$ = 244.7 Hz), 163.4, 160.5 (d, $^1J_{CF}$ = 242.2 Hz), 155.0, 152.2, 135.2, 133.6, 132.8, 129.4 (d, $^2J_{CF}$ = 8.2 Hz), 128.1, 122.8, 119.3, 116.1, 116.0, 115.8, 115.6 (d, $^2J_{CF}$ = 3.5 Hz), 115.4, 69.7; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -114.17 (m, 1F), -118.0 (m, 1F); IR ($CHCl_3$):

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ν_{max} 3411, 2923, 2851, 1569, 1507, 1407, 1222, 1017 cm^{-1}; ESI-MS: *m/z* 437.0 $[M+H]^+$, 459.0 $[M+Na]^+$; HRMS: *m/z* 437.1119 calcd for $C_{24}H_{18}F_2N_2O_2S+H^+$ (437.1130).

*3-(Benzo[d][1,3]dioxol-5-ylamino)-N-(4-fluorophenyl)thiophene-2-carboxamide (**Il**).* Light yellow solid; m.p. 124-125 °C; HPLC purity: 98.9% (t_R = 7.62 min– Method B); ^1H NMR ($CDCl_3$, 400 MHz): δ 9.16 (s, 1H), 7.50-7.47 (m, 2H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.15 (s, 1H), 7.07-7.00 (m, 3H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.17 (s, 1H), 6.63 (d, *J* = 8.0 Hz, 1H), 5.88 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.3, 160.7 (d, $^1J_{CF}$ = 242.2 Hz), 152.1, 148.2, 143.9, 136.1, 133.6, 127.9, 122.6, 119.5, 115.8 (d, $^2J_{CF}$ = 22.4 Hz), 114.5, 108.5, 103.7, 101.3; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -117.97 (m, 1F); IR ($CHCl_3$): ν_{max} 3400, 2918, 2846, 1568, 1507, 1488, 1407, 1218, 1019 cm^{-1}; ESI-MS: *m/z* 357.0 $[M+H]^+$, 379.0 $[M+Na]^+$; HRMS: *m/z* 357.0699 calcd for $C_{24}H_{17}F_2N_2O_2S+H^+$ (357.0704).

*3-((2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)amino)-N-(4-fluorophenyl)thiophene-2-carboxamide (**Im**).* Light brown yellow semisolid; HPLC purity: 98.1% (t_R = 4.91 min – Method B); ^1H NMR ($CDCl_3$, 400 MHz): δ 9.06 (s, 1H), 7.42 (dd, *J* = 4.8, 9.2 Hz, 2H), 7.19 (d, *J* = 8.0 Hz, 1H), 7.0- 6.94 (m, 3H), 6.74 (d, *J* = 8.0 Hz, 1H), 6.64 (d, *J* = 4.0 Hz, 1H), 6.58 (d, *J* = 8.8 Hz, 1H), 4.17 (t, *J* = 4.0 Hz, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.3, 160.4 (d, $^1J_{CF}$ = 242.0 Hz), 151.9, 143.8, 139.8, 135.5, 133.6, 127.9, 122.6, 119.5, 117.6, 115.8 (d, $^2J_{CF}$ = 22.3 Hz), 114.8, 110.3, 103.5, 64.5, 64.3; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -118.05 (m, 1F); IR ($CHCl_3$): ν_{max} 3435, 2921, 2850, 1621, 1505, 1408, 1300, 1210, 1019 cm^{-1}; ESI-MS: *m/z* 371.0 $[M+H]^+$; HRMS: *m/z* 371.0863 calcd for $C_{19}H_{15}FN_2O_3S+H^+$ (371.0860).

*3-((3-Fluoro-[1,1'-biphenyl]-4-yl)amino)-N-(4-fluorophenyl)thiophene-2-carboxamide (**In**).* Light yellow solid; m.p. 126-127 °C; HPLC purity: 98.8% (t_R = 9.64 min– Method B); ^1H NMR ($CDCl_3$, 400 MHz): δ 9.44 (s, 1H), 7.47-7.40 (m, 4H), 7.37-7.31 (m, 4H), 7.29-7.18 (m, 2H), 7.01 (m, 2H), 6.88 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.1, 161.2 (d, $^1J_{CF}$ =246.2 Hz), 160.6 (d, $^1J_{CF}$ = 242.6 Hz), 149.5, 142.6 (d, $^2J_{CF}$ = 10.5 Hz), 135.5, 133.4, 131.3, 128.8, 128.5, 128.2, 127.3, 122.9 (d, $^2J_{CF}$ = 7.8 Hz), 122.7, 119.8, 115.9 (d, $^2J_{CF}$ = 22.5 Hz), 115.3, 106.6, 106.4; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -116.13 (m, 1F), -117.49 (m, 1F); IR ($CHCl_3$): ν_{max} 3330, 2923, 2853, 1744, 1713, 1623, 1586, 1555, 1508, 1486, 1465, 1408, 1305, 1220, 1156, 1019 cm^{-1}; ESI-MS: *m/z* 405.0 $[M-H]^+$; HRMS: *m/z* 407.1023 calcd for $C_{23}H_{15}F_2N_2OS+H^+$ (407.1024).

*3-((3-Bromo-5-fluorophenyl)amino)-N-(4-fluorophenyl)thiophene-2-carboxamide (**Io**).* White amorphous solid; m.p. 140-141 °C; HPLC purity: 99.2% (t_R = 11.92 min– Method B); ^1H NMR ($CDCl_3$, 400 MHz): δ 9.41 (s, 1H), 7.41 (m, 2H), 7.30 (d, *J* = 4.0 Hz, 1H), 7.14 (m, 1H), 6.99 (m, 3H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.69 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 164.3 (d, $^1J_{CF}$ = 247.6 Hz), 162.9, 160.7 (d, $^1J_{CF}$ = 243.0 Hz), 148.6, 144.4, 133.2, 128.2, 123.1, 122.8, 119.8, 117.5, 115.9 (d, $^2J_{CF}$ = 22.5 Hz), 112.6 (d, $^2J_{CF}$ = 24.7 Hz), 107.8, 104.7 (d, $^2J_{CF}$ = 24.6 Hz); ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -109.98 (m, 1F), -117.26 (m, 1F); IR ($CHCl_3$): ν_{max} 3306, 2918, 1605, 1585, 1562, 1528, 1507, 1460, 1408, 1307, 1215, 1156, 1019 cm^{-1}; ESI-MS: *m/z* 410.7 $[M+H]^+$; HRMS: *m/z* 408.9814 calcd for $C_{18}H_{10}BrF_4N_2O_2S + H^+$ (408.9816).

*3-((4-((4-Fluorobenzyl)oxy)phenyl)amino)-N-(4-(trifluoromethyl)phenyl)thiophene-2-carboxamide (**Ip**).* Light yellow semisolid; HPLC purity: 93.1% (t_R = 5.10 min– Method B); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.40 (d, *J* = 8.4 Hz, 3H), 7.33 (dd, *J* = 5.2, 8.4 Hz, 2H), 7.24 (m, 2H), 7.01-7.6.97 (m, 4H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.66 (d, *J* = 8.8 Hz, 2H), 5.70 (bs, 1H), 4.92 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.8 (d, $^1J_{CF}$ = 244.9 Hz), 155.3, 154.8, 147.3, 134.1, 132.7, 130.3, 129.3, 126.1, 125.8, 124.5 (d, $^1J_{CF}$ =128 Hz, CF_3), 123.8, 123.4, 123.1, 120.9, 115.8, 115.6 (d, $^2J_{CF}$ = 88 Hz), 114.1, 69.8; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -61.7 (s, 3F), -114.1 (m, 1F); IR ($CHCl_3$): ν_{max} 3400, 2918, 2850, 1593, 1405, 1088, 1019 cm^{-1}; ESI-MS: *m/z* 487.1 $[M+H]^+$.

*3-((3-Bromo-5-fluorophenyl)amino)-N-(4-chloro-3-(trifluoromethyl)phenyl)thiophene-2-carboxamide (**Iq**).* Light yellow solid; HPLC purity: 99.0% (t_R = 23.67 min– Method B); m.p. 112-114 °C; ^1H NMR ($CDCl_3$, 400 MHz): δ 9.41 (s, 1H), 7.88 (s, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.43 (m, 3H), 7.19 (d, *J* = 4.0 Hz, 1H), 7.03 (s, 1H), 6.87 (d, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 164.3 (d, $^1J_{CF}$ = 248 Hz), 162.8, 149.3, 144.1, 136.4, 132.0, 128.9, 127.1, 124.3, 123.6, 123.2 (d, $^1J_{CF}$ = 12.1 Hz), 121.1, 119.8, 119.4, 117.8, 113.0 (d, $^2J_{CF}$ = 24.6 Hz), 107.1, 104.9 (d, $^2J_{CF}$ = 24.5 Hz); ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -62.79 (s, 3F), -109.79 (m, 1F); IR ($CHCl_3$): ν_{max} 3305, 2918, 2850, 1585, 1562, 1523, 1482, 1413, 1321, 1261, 1240, 1211, 1143, 1033 cm^{-1}; ESI-MS: *m/z* 494.8 $[M+H]^+$; HRMS-MS: *m/z* 494.9386 calcd for $C_{18}H_{10}BrF_4N_2O_2S + H^+$ (494.9386).

*N-(4-chloro-3-(trifluoromethyl)phenyl)-3-((4-fluorophenyl)amino)thiophene-2-carboxamide (**Ir**).* Light yellow solid; m.p. 96-97 °C; ^1H NMR ($CDCl_3$, 400 MHz): δ 9.17 (s, 1H), 7.84 (s, 1H), 7.68 (m, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.1-7.05 (m, 2H), 6.98- 6.94 (m, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.2 , 159.29 (d, $^1J_{CF}$ = 241.3 Hz), 151.1, 150.1, 144.4, 136.6, 135.7, 131.0, 127.7, 125.8, 123.2, 121.8, 120.8, 118.3, 115.2 (d, $^2J_{CF}$ = 22.5 Hz), 103.2; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -62.79 (s, 3F), 109.80 (m, 1F); IR ($CHCl_3$): ν_{max} 3307, 2922, 1566, 1412, 1321, 1217, 1016 cm^{-1}; ESI-MS: *m/z* 414.9 $[M+H]^+$; HRMS: *m/z* 415.0301 calcd for $C_{18}H_{11}ClF_4N_2OS + H^+$ (414.0290).

*N-(4-Chloro-3-(trifluoromethyl)phenyl)-3-((3-(trifluoromethyl)phenyl)amino)thiophene-2-carboxamide (**Is**).* Light brown solid; m.p. 111-112 °C; HPLC purity: 99.6% (t_R = 18.06 min– Method B); ^1H NMR ($CDCl_3$, 400 MHz): δ 9.38 (s, 1H), 7.82 (d, *J* = 2.4 Hz, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 7.37-7.28 (m, 4H), 7.22 (m, 2H), 7.09 (d, *J* = 5.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.9, 150.2, 142.0, 136.5, 132.0, 131.7, 130.0, 129.0, 128.9, 128.7, 127.0, 124.9, 124.3, 123.6, 122.7, 121.4, 119.4, 116.1, 106.0; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -62.78 (s, 3F), -62.83 (s, 3F); IR ($CHCl_3$): ν_{max} 3307, 2920, 2850, 1568, 1524, 1482, 1413, 1321, 1236, 1127, 1019; ESI-MS: *m/z* 465.0 $[M+H]^+$; HRMS: *m/z* 465.0274 calcd for $C_{19}H_{11}ClF_6N_2O S + H^+$ (465.0258).

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N-(4-Fluorobenzyl)-3-((4-((4-fluorobenzyl)oxy)phenyl)amino)thiophene-2-carboxamide (***1t***). Light grey solid; m.p. 114-116 °C; HPLC purity: 100% (t_R = 8.34 min– Method B); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.19 (s, 1H), 7.42 (dd, *J* = 5.6, 8.4 Hz, 2H), 7.34 (dd, *J* = 5.2, 8.4 Hz, 2H), 7.19 (d, *J* = 5.6 Hz, 1H), 7.10-7.00 (m, 6H), 6.97 (d, *J* = 5.6 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 2H), 5.79 (s, 1H), 5.0 (s, 2H), 4.56 (d, *J* = 5.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.02, 163.7 (d, $^1J_{CF}$ = 244.7 Hz), 161.3 (d, $^1J_{CF}$ = 244.2 Hz), 154.8, 151.4, 135.5, 134.3, 132.8 , 129.4, 129.3, 127.2, 122.7, 119.1, 115.7 (d, $^2J_{CF}$ = 7.7 Hz), 115.6, 115.4 (d, *J* = 7.9 Hz) , 103.4, 69.8, 42.7; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -114.25 (m, 1F), -115.09 (m, 1F); IR ($CHCl_3$): ν_{max} 3423, 2922, 2852, 1743, 1608, 1589, 1563, 1507, 1465, 1437, 1410, 1382, 1225, 1156, 1096, 1015 cm^{-1}; ESI-MS: *m/z* 451.0 $[M+H]^+$; HRMS: *m/z* 451.1290 calcd for $C_{25}H_{20}F_2N_2O_2S + H^+$ (451.1286).

N-(4-Fluorobenzyl)-3-((4-((3-(trifluoromethyl)benzyl)oxy)phenyl)amino)thiophene-2-carboxamide (***1u***). White solid; m.p. 78-79 °C; HPLC purity: 100% (t_R = 9.59 min– Method B); ^{1}H NMR: ($CDCl_3$, 400 MHz): δ 9.45 (s, 1H), 7.40-7.29 (m, 5H), 7.25-7.20 (m, 3H), 7.18-712 (m, 4H), 7.04 (t, *J* = 8.8 Hz, 2H), 5.89 (s, 1H), 5.02 (s, 2H), 4.56 (d, *J* = 6.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.4, 162.7 (d, $^1J_{CF}$ = 244.2 Hz), 158.4, 149.0, 141.6, 133.7, 131.5 (d, $^1J_{CF}$ = 32.5 Hz), 129.5, 129.4, 128.8, 128.6, 126.9, 124.6, 122.4, 119.1, 117.8, 117.2, 115.2 (d, $^2J_{CF}$ = 21.3 Hz), 111.3, 105.4, 69.6, 42.3; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -62.68 (s, 3F), -114.96 (m, 1F); IR ($CHCl_3$): ν_{max} 3430, 2921, 2850, 1614, 1587, 1562, 1510, 1448, 1409, 1328, 1263, 1226, 1165, 1124, 1065 cm^{-1}; ESI-MS: *m/z* 501.12 $[M+H]^+$; HRMS: *m/z* 501.1249 calcd for $C_{26}H_{20}F_4N_2O_2S + H^+$ (501.1288).

3-((4'-Ethoxy-[1,1'-biphenyl]-4-yl)amino)-N-(4-fluorobenzyl)thiophene-2-carboxamide (***1v***). White amorphous solid; m.p. 152-154 °C; HPLC purity: 100% (t_R = 5.14 min– Method B); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.44 (s, 1H), 7.50 (d, *J* = 8.8 Hz, 4H), 7.33 (dd, *J* = 5.2, 8.4 Hz, 2H), 7.25 (d, *J* = 5.2 Hz, 1H), 7.20 (t, *J* = 8.4 Hz, 3H), 7.05 (t, *J* =8.4 Hz, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 5.87 (t, *J* = 5.2 Hz, 1H), 4.57 (d, *J* = 5.6 Hz, 2H), 4.09 (q, *J* = 7.2 Hz, 2H), 1.45 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.9, 163.4 (d, $^1J_{CF}$ = 251.3 Hz), 158.2, 149.9, 140.7, 135.1, 134.2, 133.1, 129.4, 127.6, 127.4, 127.2, 120.1, 119.6, 115.7 (d, $^2J_{CF}$ = 21.3 Hz), 114.8, 105.2, 63.5, 42.8, 14.9; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -115.03 (m, 1F); IR ($CHCl_3$): ν_{max} 3435, 2914, 2846, 1613, 1499, 1088, 1019 cm^{-1}; ESI-MS: *m/z* 447.0 $[M+H]^+$, 469.0 $[M+Na]^+$; HRMS: *m/z* 447.1533 calcd for $C_{26}H_{23}FN_2O_2S + H^+$ (447.1537).

N-(4-Fluorobenzyl)-3-((3-((3-(trifluoromethyl)benzyl)oxy)phenyl)amino)thiophene-2-carboxamide (***1w***). Brown colored semisolid; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.44 (s, 1H), 7.41 (t, *J* = 8.4 Hz, 1H), 7.33-7.29 (m, 3H), 7.25 (m, 4H), 7.17 (m, 3H), 7.05 (m, 3H), 5.87 (t, *J* = 5.2 Hz, 1H), 5.07 (s, 2H), 4.56 (d, *J* = 5.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.7, 163.0 (d, $^1J_{CF}$ = 245.5 Hz), 158.6, 149.3, 142.3, 137.5, 134.1, 131.8 (q, $^1J_{CF}$ = 32 Hz), 129.9, 129.6, 129.2, 127.3, 124.9, 122.8, 121.1, 119.4, 118.1, 117.6, 115.5 (d, $^2J_{CF}$ = 21.3 Hz), 111.7, 105.7, 69.8, 42.6; ^{19}F NMR ($CDCl_3$, 376.50 MHz): δ -62.69 (s, 3F), -114.98 (m, 1F); IR ($CHCl_3$): ν_{max} 3307, 2921, 2854, 1725, 1606, 1592, 1566, 1509, 1493, 1449, 1418, 1386, 1328, 1271, 1226, 1166, 1125, 1096, 1065, 1016 cm^{-1}; ESI-MS: *m/z* 501.1 $[M+H]^+$; HRMS: *m/z* 501.1249 calcd for $C_{26}H_{20}F_4N_2O_2S + H^+$ (501.1254).

Cell culture, growth conditions, and treatment. MIAPaCa-2 pancreatic cancer, MCF-7 human breast cancer cells, HCT-116 human colon carcinoma, HUVEC Human Umbilical Vein Endothelial cells and LS180 colonic adenocarcinoma cells were obtained from the National Cancer Institute (NCI), Bethesda, USA. The cells were grown in RPMI-1640 or MEM medium supplemented with 10% heat inactivated fetal bovine serum (FBS), penicillin (100 units/mL), streptomycin (100 µg/mL), L-glutamine (0.3 mg/mL), pyruvic acid (0.11 mg/mL), and 0.37% $NaHCO_3$. Cells were grown in a CO_2 incubator (Thermocon Electron Corporation, MA, USA) at 37 ^oC in an atmosphere of 95% air and 5% CO_2 with 98% humidity. Camptothecin was used as a positive control in this study.

Cell proliferation assay: MTT assay was performed to determine the cell viability. Cells were seeded in 96 well plates and exposed to different concentrations of synthesized compounds for 48 h. MTT dye (10 µl of 2.5 mg/ml in PBS) was added to each well 4 hours priors to experiment termination. The plates were then centrifuged at 1500 RPM for 15 min and the supernatant was discarded, while the MTT formazan crystals were dissolved in 150 µL of DMSO. The OD measured at 570 nm with reference wavelength of 620 nm.[24] For MTT assay of combined treatment of doxorubicin and P-gp inhibitors **1l** and **1m**, different concentrations of doxorubicin (ranging from 2.5 µM to 0.0097 µM along with 50 µM of P-gp inhibitors were used (details are provided in ESI).

VEGFR Screening. This screening was done at International Center for Kinase Profiling, University of Dundee, UK on commercial basis. VEGFR (5-20mU diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 1 mg/ml BSA) is assayed against a substrate peptide (KKKSPGEYVNIEFG) in a final volume of 25.5 µl containing 50mM Tris pH 7.5, 300µM substrate peptide, 10 mM magnesium acetate and 0.02 mM [33P-g-ATP] (50-1000 cpm/pmole) and incubated for 30 min at room temperature. Assays are stopped by addition of 5 µl of 0.5 M (3%) orthophosphoric acid and then harvested onto P81 Unifilter plates with a wash buffer of 50 mM orthophosphoric acid.

In-vitro **screening of OSI-930 analogs for P-gp inhibitory activity.** Colorectal LS180 cells were seeded at a density of 2×10^4 per well of 96 well plate and allowed to grow for next 24 h. Cells were incubated with the test compounds diluted to a final concentration of 50 µM and elacridar (positive control) to a final concentration of 10 µM in HANKS buffer containing 10 µM of Rh123 as a P-gp substrate for 90 minutes. The final concentration of DMSO was kept at 0.1%. Test compounds were removed and cells were washed four times with cold PBS followed by cell lysis for 1 h using 200 µl of lysis buffer (0.1% Triton X 100 and 0.2 N NaOH). A total of 100 µl lysate was used for reading the fluorescence of Rh123 at 485/529 nm. All samples were normalized by dividing fluorescence of each sample with total protein present in the lysate. IC_{50} value for each of selected compound was calculated by using Graphpad Prism software.

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Data is expressed as mean ± SD or representative of one of three similar experiments unless otherwise indicated.

Colony formation assay in LS180 cells. LS180 cells were treated with doxorubicin (100 nM) for 24 h in presence or absence of compounds (50 μM each). Cells were trypsinized, viable cells were counted and 500 cells were plated into each well of a 6-well plate to determine the effect of treatments on colonogenic survival. Cells were incubated for 15 days at 37 °C in 5% CO_2 and 95% humidity. The colonies were fixed in 4% formaldehyde for 15-20 min and stained with 1% crystal violet before being photographed.

Cell migration studies in HUVEC cells. The cell migration assay was performed as described previously.[26] Briefly, HUVEC cells were treated with mitomycin-C to inactivate cell proliferation, wounded by microtip, washed with PBS, supplemented with fresh medium and treated with the IC_{50} value of compounds for 24 h. Images of the cells were taken after 24 h of incubation and the percentage of wound closure was expressed with respect to untreated cells consider 100%.

Assay for intracellular of accumulation doxorubicin: LS180 cells were seeded at a density of 0.2×10^6 per well of a 6 well plate and left overnight in the CO_2 incubator. Cells were treated with 10 μM of doxorubicin in presence or absence of 100 μM of **II** and **1m** for a period of 90 minutes. At the end of treatment cell were washed four times with cold PBS to remove any traces of extracellular doxorubicin. Cells were lysed with 200 μM of lysis buffer containing 0.1% of triton X-100 and 0.2N of NaOH and intracellular quantity of doxorubicin was calculated by mass spectroscopy.

Western-blot studies for procaspase-3, PARP-1 and ICAD in LS180 cells and for VEGFR1 and VEGFR2 in HUVEC cells.

Preparation of cell lysates for western-blot analysis. The western-blot analysis for VEGFR1 and VEGFR2 was done in HUVEC cells; and for protein procaspase -3, PARP-1 and ICAD in LS180 cells. Cells were treated with different concentration of compounds for 24 h. Cells were collected at 400 × g at 4 ºC, washed in PBS twice and cell pellets were incubated with cold RIPA buffer (Sigma Aldrich, India)) containing 50 mM NaF, 0.5 mM $NaVO_4$, 2 mM PMSF and 1% protease inhibitor cocktail for 40 min. Cells were centrifuged at 12000 x g for10 min at 4 ºC and the supernatant was collected as whole cell lysates for western-blot analysis of various proteins

Western-blot analysis. Protein content was measured by using Bio-Rad protein assay reagent and protein lysates (70 μg) were subjected to discontinuous SDS-PAGE analysis. Proteins were electro-transferred to PVDF membrane for 90 min at 4 °C at 100V. Non-specific binding was blocked by incubation with 5% non-fat milk or 3% BSA in tris-buffered saline containing 0.1% Tween-20 (TBST), for 1 h at room temperature. The blots were probed with respective primary antibodies for 3 h and washed three times with TBST. Blots were incubated with horse-radish peroxidase conjugated secondary antibodies for 1 h and washed three times with TBST. Blots were incubated with ECL plus reagent and signals were detected by using BioRad ChemiDoc XRS system.[25]

Statistical analysis. Data is expressed as mean ± SD of three independent experiments unless otherwise indicated. The comparisons were made between control and treated groups or the entire intra-group using Bonferroni test through Instat-2 software. p -values *<0.5 were considered significant.

Molecular modelling studies of 1 (OSI-930), 1l and 1m with P-gp. Molecular modeling studies were performed using human P-gp homology model developed using *C. elegans* crystal structure (PDB: 4AZF)[27] by Prof. Jue Chen. Homology model was prepared by protein preparation wizard module of Schrodinger (Schrodinger, Inc., New York, NY, 2009) under default conditions. The prepared protein was further utilized to construct grid file by selecting verapamil interacting residues to murine P-gp.[28] All ligand structures were sketched, minimized and docked using GLIDE XP, and minimized using macromodel.

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Abbreviations

ABCG2, ATP-binding cassette sub-family G member 2; A431, human epithelial carcinoma cell line; BCRP, breast cancer resistant protein; HCT116, human colon carcinoma cells; HL-60, human promyelocytic leukemia cells; HUVEC, human umbilical vein endothelial cells; K562, human erythromyeloblastoid leukemia cell line; LS180, human colon adenocarcinoma cell line; MIAPaCa-2, human pancreatic tumor cell line; MCF-7, is the acronym of Michigan Cancer Foundation and is a human breast adenocarcinoma cell line; MDR, multidrug resistance; P-gp, p-glycoprotein; RTKs, receptor tyrosine kinases; SAR, structure-activity relationship; THP-1, human monocytic leukemia cell line; T47D, human ductal breast epithelial tumor cell line; VEGFR, Vascular endothelial growth factor receptor.

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