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Design, synthesis and evaluation of (*E*)-α-benzylthio chalcones as novel inhibitors of BCR-ABL kinase

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ABSTRACT

Novel (*E*)-α-benzylthio chalcones are reported with preliminary in vitro activity data indicating that several of them are potent inhibitors (comparable to imatinib, the reference compound) of BCR-ABL phosphorylation in leukemic K562 cells, known to express high levels of BCR-ABL. The ability of such compounds to significantly inhibit K562 cell proliferation suggests that this scaffold could be a promising lead for the development of anticancer agents that are able to block BCR-ABL phosphorylation in leukemic cells.

1. Introduction

The Philadelphia chromosome (Ph), discovered in 1960 by Nowell and Hungerford,[1] results from a reciprocal translocation between chromosomes 9 at band q34 and chromosome 22 at band q11.[2,3] This translocation fuses the breakpoint cluster region (BCR) and the ABL genes and creates the BCR-ABL oncogene.[4] Because the BCR-ABL protein is active in greater than 90% of chronic myelogenous leukemia (CML) cases, it has been possible to synthesize small molecules that inhibit BCR-ABL kinase activity in leukemic cells without adversely affecting the normal cell population. Imatinib (STI571, Gleevec®)[5] is a small molecule inhibitor that binds to the kinase domain of BCR-ABL when the protein is in its closed, inactive conformation,[5] thereby inhibiting its activity, and is now considered as a first-line therapy for the majority of CML cases due to its high efficacy and relatively mild side effects.[6] In spite of the fact that the majority of patients receiving imatinib respond to treatment at both the hematological and cytogenetic levels, relapse occurs in a large percentage of patients.[7] Several studies have attempted to address the mechanism(s) by which CML cells acquire imatinib resistance.[8–10] Results from these studies indicate that the mechanism that accounts for a majority of imatinib-resistant leukemias, in vivo, is mutation of the BCR-ABL gene itself. Mutation within the kinase domain is the most common and to date, more than 50 different clinically relevant point mutations within this domain have been identified. It is believed that certain amino acid substitutions interfere with the ability of imatinib to interact directly with the BCR-ABL kinase domain whereas others destroy or hinder the ability of the BCR-ABL kinase domain to adopt a conformation that is required for imatinib binding.[7,11]

The challenges of mutational relapse in CML patients undergoing imatinib therapy has paved the way for the development of second generation BCR-ABL inhibitors such as PD180970,[12] CGP76030,[13] BMS-354825,[14] AMN 107 or Nilotinib[15] and more recently AP24534 (Chart 1).[16] These new BCR-ABL inhibitors are all ATP-competitive agents and therefore will potentially encounter challenges similar to imatinib via accumulation of kinase domain mutations.

Because of the frequency of mutations within the ATP-binding site, efforts are now focused on the identification of novel inhibitors that inhibit the BCR-ABL signaling pathway by mechanisms other than competing with ATP. Different approaches have recently been described to overcome this resistance in at least some CML cases. Farnesyltransferase inhibitors, such as SCH66336, and the proteasome inhibitor Bortezomib have been shown to have growth inhibitory effects on certain imatinib-resistant leukemias.[17]

Abbreviations: SAR, structure–activity relationship; FBS, fetal bovine serum.

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It has also been argued that non-ATP-competitive kinase inhibitors might constitute better drug candidates.[18] Since there is a limited number of chemotypes that act as non-ATP competitive inhibitors, we undertook the synthesis and characterization of new chemotypes that are unrelated to ATP and other purine and pyrimidine nucleosides but still possess kinase inhibitory activity.

Here we report the discovery of (*E*)-α-benzylthio chalcones as a new class of inhibitors that potently inhibit the growth of cells harboring the activated BCR-ABL protein by inhibiting the phosphorylation of the kinase.

2. Chemistry

The general method for the synthesis of novel benzylthio chalcones is outlined in Scheme 1. For the synthesis of compounds **6a**–**6t** and **6aa**–**6ag**, phenacyl bromides **2** were synthesized from acetophenones **1** and then reacted with benzylmercaptans **3** to produce phenacyl benzyl sulfides **4**.[19] Knoevenagel condensation of **4** with aromatic aldehydes **5** in glacial acetic acid in the presence of ammonium acetate yielded α-benzylthio chalcones **6**.[20] Alternatively, the condensation between **4** and **5** was also carried out in glacial acetic acid in the presence of a catalytic amount of benzylamine to obtain **6**.[21]

In Scheme 2, a method for the synthesis of new (*E*)-α-benzylsulfonyl chalcones is described. Oxidation of phenacyl benzylsulfides **4** with 30% hydrogen peroxide in the presence of glacial acetic acid gave phenacyl benzylsulfones **7**. Condensation of phenacyl benzylsulfones **7** with aldehydes **5** in glacial acetic acid and ammonium acetate provided α-benzylsulfonyl chalcones **8**. Alternatively, these sulfonyl chalcones **8** were also prepared by the oxidation of benzylthio chalcones **6** with an excess of *m*-chloroperbenzoic acid (*m*-CPBA) in chloroform. Compound **6t**, α-benzylsulfoxide chalcone was prepared by the controlled oxidation of **6c** with 1 equiv of *m*-CPBA in chloroform.

To delineate the role of benzylthio moiety in the biological activity of **6**, we synthesized a few molecules replacing the benzyl moiety with aryl group. In Scheme 3 we have outlined the synthesis of α-arylthio chalcones starting from **2**. Condensation of **2** with aryl mercaptans **9** in methanolic sodium hydroxide solution produced phenacyl arylsulfides **10**, which on Knoevenagel type condensation with aromatic aldehydes **5** in the presence of a base yielded (*E*)-α-arylthio chalcones **11**.[21]

To enhance the solubility and bioavailability of the active (*E*)-α-benzylthio chalcones, we modified the carboxylic acid group located at the *para* position of the benzoyl aromatic ring of **6** as amide substituted piperazine groups. In Scheme 4 benzylthio

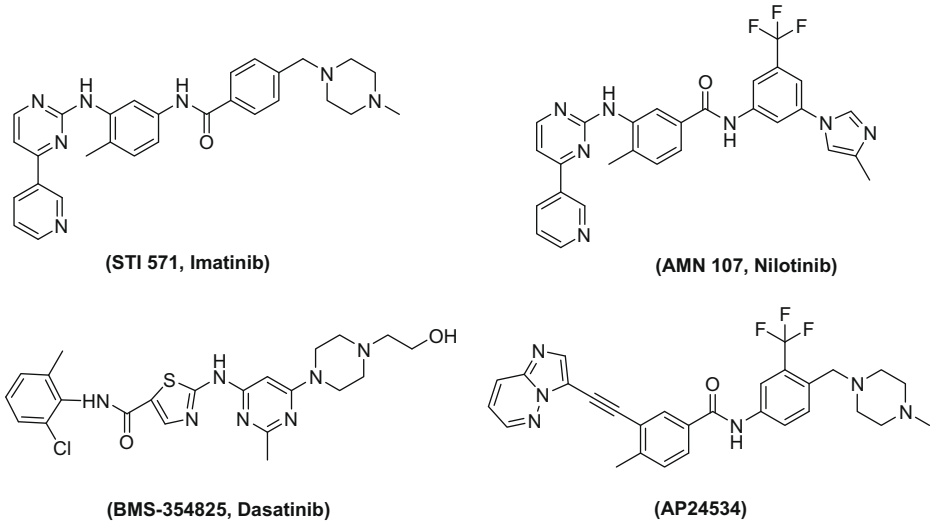
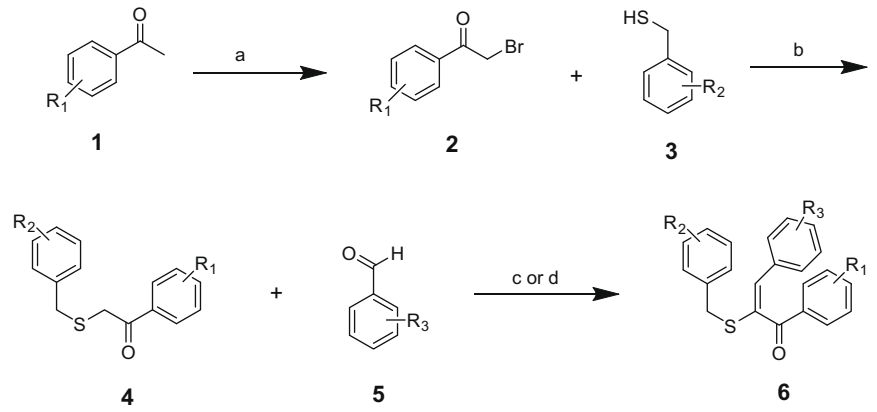
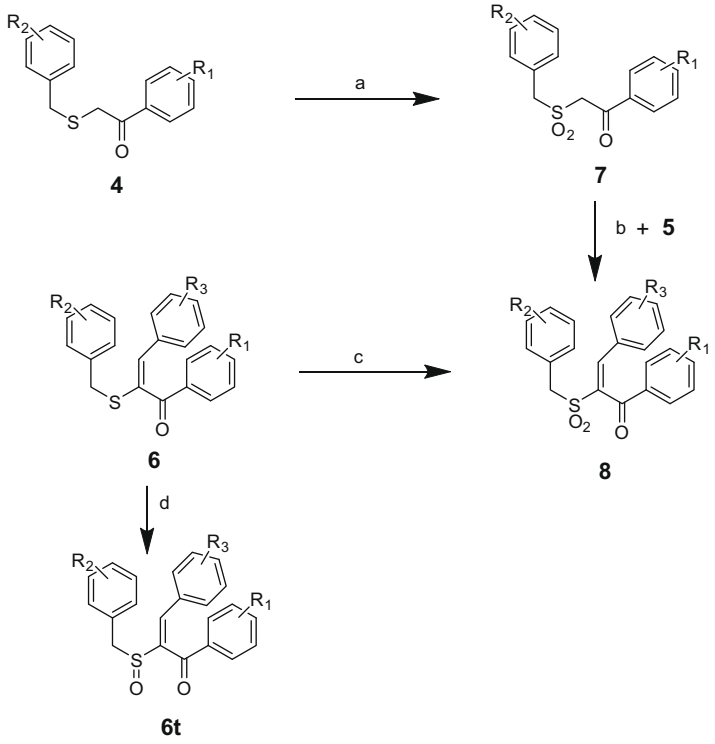


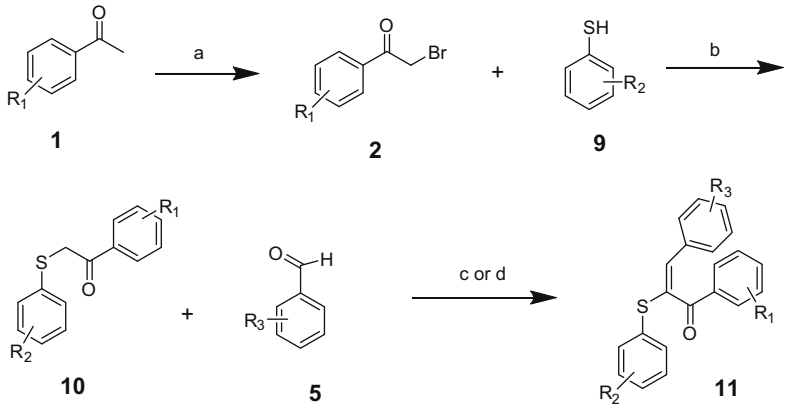
Chart 1. Structures of known BCR-ABL kinase inhibitors.



Scheme 1. General method for the synthesis of (*E*)-α-benzylthio chalcones. Reagents and conditions: (a) Br_2, AcOH, 70 °C, 3 h; (b) NaOH, MeOH, rt, 2 h; (c) NH_4OAc, AcOH, 70 °C, 3 h; (d) $PhCH_2NH_2$, AcOH, reflux, 8 h.



Scheme 2. Synthesis of (*E*)-α-benzyl sulfoxide/sulfonyl chalcones. Reagents and conditions: (a) 30% H_2O_2, AcOH, 70 °C, 2 h; (b) NH_4OAc, AcOH, reflux, 8 h; (c) *m*-CPBA (3 equiv), $CHCl_3$, rt, 2 h; (d) *m*-CPBA (1 equiv), $CHCl_3$, 0 °C to rt, 2 h.



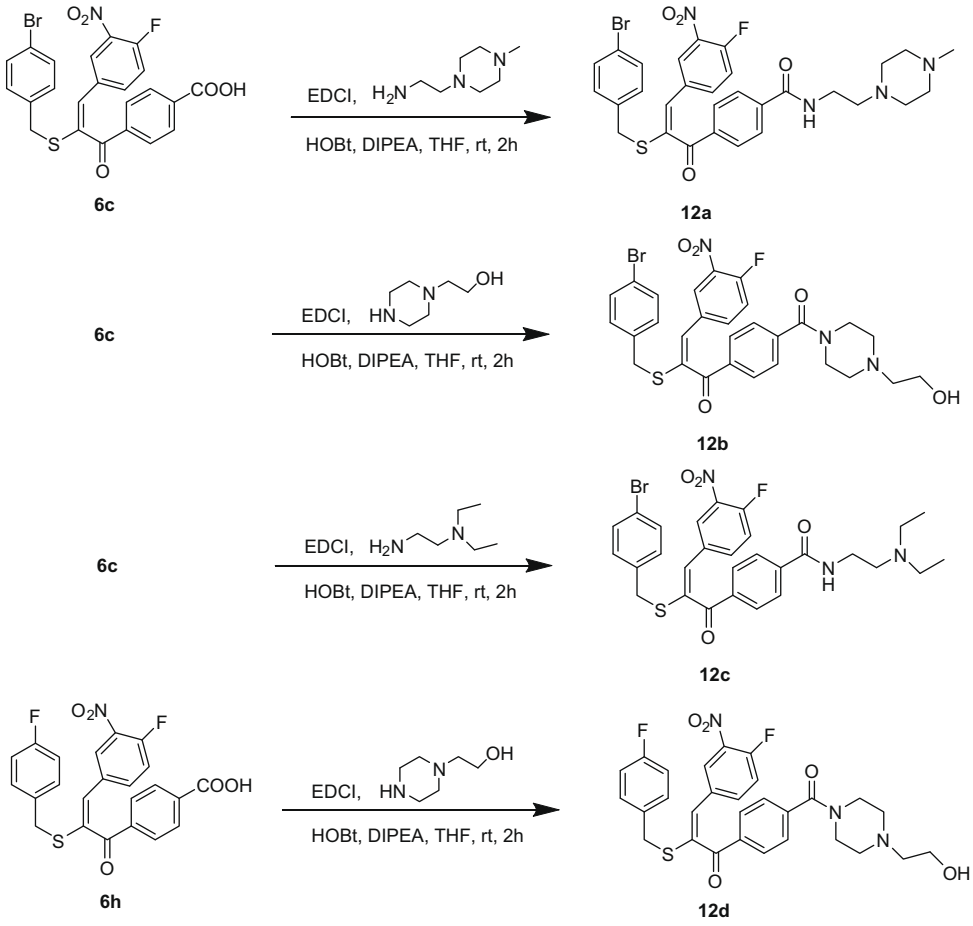
Scheme 3. Method for the synthesis of (*E*)-α-arylthio chalcones. Reagents and conditions: (a) Br_2, AcOH, 70 °C, 3 h; (b) NaOH, MeOH, rt, 2 h; (c) NH_4OAc, AcOH, reflux, 8 h; (d) $PhCH_2NH_2$, AcOH, reflux, 8 h.

chalcones, **6c** and **6h** were treated either with primary or cyclic secondary amines to produce amide substituted piperazines **12a–12d** (Scheme 4).

3. Results and discussion

3.1. Structure–activity relationships (SAR)

After the synthesis of these compounds, their in vitro cytotoxicity was assessed using K562, a BCR-ABL positive leukemic cell line. As some of these compounds are highly potent against K562 cells, their ability to inhibit the phosphorylation of BCR-ABL was assessed by western blotting of the drug treated cell lysates with phospho anti BCR-ABL antibodies. The results of this study are presented in Tables 1–3 and Figure 1. These studies show that the cytotoxicity of these compounds depends on (i) the position of the thio group, (ii) the oxidation state of the sulfur atom in the molecule and (iii) the nature and site of the substituents in the aromatic rings. During our screening of about 600 α-benzylthio and sulfonyl chalcones, we found that some of these compounds showed a very good cytotoxicity (IC_{50} = 0.3–0.9 μM) in K562 cells (Table 1). To analyze the effect of the substituents on the aromatic rings of **6**, we synthesized a number of analogs containing methoxy, halo, methyl, nitro, carboxy and hydroxy groups on these rings. Cytotoxicity analyses of these analogues on K562 cells showed that the compounds with a fluoro atom at the fourth position and a nitro group at the third position on the styryl aromatic ring exhibited the best activity in these series. To further assess the significance of the 3-nitro and 4-fluoro groups on the styryl ring in **6**, we replaced these groups in **6c** with bromo at the site of nitro and hydroxyl at the position of fluoro atom (**6ab**). Both these replacements resulted in total loss of activity. Since double



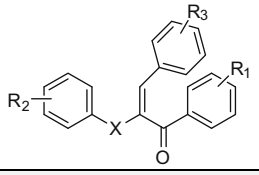
Scheme 4. Synthesis of compounds **12a–12d**.

replacement of the 3-nitro and 4-fluoro substituents on styryl ring in **6c** caused a moderate loss of activity, we then made single changes either at 3-position keeping 4-position intact or vice versa and studied the effect of these changes in cytotoxic assay in tumor cells. Modifying the fluorine atom alone on the 4-position of styryl ring in **6c** with a hydroxyl group resulted in the loss of the activity (**6ac**). The activity of **6c** was found to be unaltered when fluorine at 4-position of styryl ring was replaced with chloro (**6a**) or bromo (**6b**) atoms. This confirms that a halogen atom at that position is essential for the activity of the molecule. Any modifications replacing fluorine with atoms or groups other than halogen atoms results in total loss of activity. To understand the role of the 3-position nitro group on the cytotoxicity of the molecule, the nitro group of **6c** was replaced with a methoxy group (**6aa**). This substitution led to a drastic reduction in the cytotoxicity of the resulting molecule (**6aa**). Since both the nitro group and the fluorine atom on the ring seem to be critical for the cytotoxicity of the molecule, we tested whether their distribution on the ring other than at 3 and 4-positions has any effect on the tumor cell killing activity. To analyze the role of these two substituent locations on the ring, we synthesized **6ad**, **6ae**, **6af** and **6ag** where fluoro and nitro substituents are distributed at 2, 5 (**6ad**), 3, 4 (**6ae**), 4, 2 (**6af**) and 2, 4 (**6ag**) positions. Cytotoxicity data from K562 cells treated with these compounds showed that these alterations are drastic leading to the loss of cytotoxicity in the resulting molecules (Table 2). From this analysis it is evident that a nitro group at the third position and a fluorine atom at the fourth position on the styryl aromatic ring are critical for the anti-tumor activity of these molecules. It is also clear from Table 1 that the nature and position of the substituents on the benzoyl and benzylthio rings do not affect the cytotoxicity of the molecules as long as 3-nitro and 4-fluoro substituents are on the styryl ring. To determine the significance of sulfur oxidation state, we oxidized the sulfur atom in benzylthio group to sulfoxide and sulfone and then assayed the resulting molecules for their cytotoxicity. In both the cases, the sulfoxide (**6t**) or the sulfones (**8a** and **8b**) produced by the oxidation of **6c** were found to have significantly reduced cytotoxicity.

These results show that these molecules exhibit much higher potency towards tumor cells when an un-oxidized sulfur atom is present in their structure. To understand the role of the benzyl methylene group in structure–activity relationship, we replaced the benzylthio moiety with phenylthio group where the sulfur atom is directly connected to the aromatic ring instead of having a methylene bridge between the ring and the sulfur atom (**11**). This modification seems to have an adverse effect on the molecule resulting in a fivefold reduction in cytotoxicity.

We next focused our attention on enhancing the aqueous solubility and bioavailability of the active molecules. As all the active molecules synthesized have very poor aqueous solubility (<1 mg/mL), these were dissolved in dimethyl sulfoxide (DMSO) to treat the cells or administer to the mice by intraperitoneal (IP) injection. Bioavailability and pharmacokinetic data from the mice injected with **6c** in DMSO showed very low levels of this compound in serum. To improve the solubility and bioavailability of these compounds, we converted the carboxylic acid on the benzoyl ring of **6c** and **6h** in to compounds **12a**–**12d** having secondary and tertiary amino groups. As shown in Table 3, secondary and tertiary amine salts of **6c** and **6h** were made and tested for their solubility and cytotoxicity. The

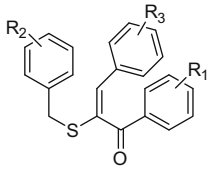
Table 1
In vitro cytotoxicity of α-aryl and benzyl thio chalcones



Compd	R_2	X	R_1	R_3	IC_{50} (μM)	
					K562	pBCR/ABL
6a[a]	4-Br	CH_2S	4-COOH	3-NO_2, 4-Cl	0.7	10
6b[a]	4-Br	CH_2S	4-COOH	3-NO_2, 4-Br	0.9	10
6c[a]	4-Br	CH_2S	4-COOH	3-NO_2, 4-F	0.3	0.5
6d[a]	4-Cl	CH_2S	4-COOH	3-NO_2, 4-F	0.5	2.5
6e[a]	4-Cl	CH_2S	4-COOH	3-NO_2, 4-Cl	0.5	10
6f[a]	4-Cl	CH_2S	4-COOH	3-NO_2, 4-Br	0.6	2.5
6g[a]	4-F	CH_2S	4-COOH	3-NO_2, 4-Cl	0.5	2.5
6h[a]	4-F	CH_2S	4-COOH	3-NO_2, 4-F	0.4	2.5
6i[a]	4-F	CH_2S	4-COOH	3-NO_2, 4-Br	0.4	15
6j[b]	4-F	CH_2S	4-F	3-NO_2, 4-F	0.6	2.5
6k[a]	2-F	CH_2S	4-COOH	3-NO_2, 4-Cl	0.8	10
6l[a]	2-Cl	CH_2S	4-COOH	3-NO_2, 4-F	0.5	10
6m[a]	2,4-Cl_2	CH_2S	4-COOH	3-NO_2, 4-Cl	0.6	2.5
6n[a]	2,4-Cl_2	CH_2S	4-COOH	3-NO_2, 4-F	0.4	2.5
6o[a]	2,4-Cl_2	CH_2S	4-COOH	3-NO_2, 4-Br	0.6	2.5
6p[a]	4-CH_3	CH_2S	4-COOH	3-NO_2, 4-F	0.3	2.5
6q[a]	4-CF_3	CH_2S	4-COOH	3-NO_2, 4-F	0.4	0.2
6r[b]	4-Br	CH_2S	4-$COOCH_3$	3-NO_2, 4-F	0.3	0.75
6s[a]	H	CH_2S	4-COOH	3-NO_2, 4-F	0.3	2.5
6t[a]	4-Br	CH_2SO	4-COOH	3-NO_2, 4-F	75	>20
8a	4-Br	CH_2SO_2	4-COOH	3-NO_2, 4-F	5	>20
8b	4-Br	CH_2SO_2	4-COOH	3-NO_2, 4-Cl	25	>20
11[c]	4-Br	S	4-COOH	3- NO_2, 4-F	1.5	20

[a] These compounds were synthesized by utilizing method A.
[b] These compounds were synthesized by utilizing method B.
[c] These compounds were synthesized by utilizing method A and method B.

Table 2
In vitro cytotoxicity of α-benzyl thio chalcones



Compd[a]	R_2	R_1	R_3	IC_{50} (μM)	
				K562	pBCR/ABL
6aa	4-Br	4-COOH	3-OCH_3, 4-F	20	>20
6ab	4-Br	4-COOH	3-Br, 4-OH	35	>20
6ac	4-Br	4-COOH	3-NO_2, 4-OH	75	>20
6ad	4-Br	4-COOH	2-F, 5-NO_2	7.5	>20
6ae	4-Br	4-COOH	3-F, 4-NO_2	5.0	>20
6af	4-Br	4-COOH	2-NO_2, 4-F	20	>20
6ag	4-Br	4-COOH	2-F, 4-NO_2	7.5	>20

[a] All the compounds were synthesized by utilizing method A.

amino hydrochloride salts (**12a**–**12d**) made from **6c** and **6h** displayed excellent aqueous solubility and retained their cytotoxicity in K562 cells. The bioavailability of these compounds is being tested.

3.2. Inhibition of BCR-ABL kinase activity

The cell based cytotoxicity assays reported above demonstrate that (*E*)-α-benzylthio chalcones exhibit nanomolar cytotoxicity against K562 cells. To assess whether these compounds directly inhibit the phosphorylation of BCR-ABL, we tested the cell lysates prepared from K562 cells treated with these compounds in western blot analysis with anti-phospho BCR-ABL antibody (Fig. 1). These results showed that while all the compounds, in general, inhibited the phosphorylation of BCR-ABL, compounds **6c**, **6q**, **6r**, **12a**, **12b** and **12d** were most effective in this assay. Imatinib[5] was used as a positive control while DMSO was used as the negative control. The results of this study showed that some of the α-benzylthio chalcones (**6c**, **6q**, **6r**, **12b**, **12c** and **12d**) inhibited the phosphorylation of BCR-ABL with an IC_{50} (0.2–0.75 μM) comparable to that of imatinib (0.75 μM).

Table 3
In vitro cytotoxicity of carboxamide derivatives of α-benzylthio chalcones

Compd	IC_{50} (μM)		Solubility (mg/mL)
	K562	pBCR/ABL	
12a	0.6	2.5	>10.0
12b	0.2	0.5	>10.0
12c	0.75	0.5	>10.0
12d	0.3	0.5	>10.0

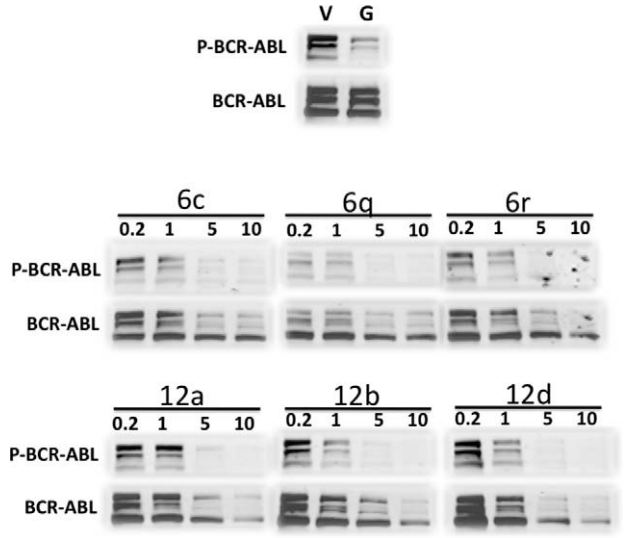


Figure 1. BCR-ABL kinase inhibition in human chronic myelogenous leukemic cells. K562 Cells expressing BCR-ABL p210, were treated with the indicated micromolar concentrations of each compound, 1 μM Gleevec(G) or Vehicle(DMSO) for 2 h. Total cellular proteins were harvested and resolved by 10%-SDS–PAGE. The gel was transferred and hybridized against antibodies specific for P-BCR-ABL or BCR-ABL. The western blot was treated with secondary antibodies conjugated with infrared dyes (LiCor) and scanned using Odyssey (LiCor) scanner. Percent inhibition was determined by quantifying each band using the software provided by LiCor, then normalizing the P-BCR-ABL signal to the parental BCR-ABL signal and determining% inhibition based on the vehicle control signal.

3.3. Discussion

Our current study shows that (*E*)-α-benzylthio chalcones exert a cytotoxic effect by inhibiting BCR-ABL phosphorylation in leukemic cell lines that over express this protein, thereby causing growth arrest and cell death. We describe the development of novel small molecules that inhibit autophosphorylation of BCR-ABL at a concentration of 0.2–0.75 μM. While assessing the kinase inhibitory profile of **6c** against other kinases implicated in cancer, we found **6c** to be a specific inhibitor of BCR-ABL. **6c** exhibited 10–15-fold higher selectivity for BCR-ABL over other tyrosine kinases and more than 40-fold higher selectivity for BCR-ABL over serine/threonine kinases (Table 4). The optimization of **6c** to improve its bioavailability and pharmacokinetics is ongoing. The in vivo efficacy of the optimized version of **6c** needs to be tested in xenograft mouse models of leukemia and ex vivo on patient samples.

Table 4
Kinase inhibition profile of **6c**

Kinase	IC_{50} (μM) **6c**
BCR-ABL	0.75
Lyn B	17.0
Src	13.2
ErbB2	>10.0
EGFR	>20.0
Plk-1	>50.0
Cdk-1	>50.0

Studies with kinase inhibitors have identified three general mechanisms for pharmacological inhibition of kinase activity: (1) direct binding in the ATP-binding site, (2) binding in the substrate-binding site, and (3) engagement of an allosteric site which results in the altered conformation of the kinase causing a block in proper substrate phosphorylation. The first kinase inhibitor to reach the market was Imatinib (Gleevec®),[5] an inhibitor of BCR-ABL tyrosine kinase that has been a remarkable success for the treatment of Philadelphia chromosome positive (Ph+) chronic myelogenous leukemia's (CMLs). This was followed by the development of a series of kinase inhibitors, which include gefitinib (Iressa),[22] erlotinib (OSI-1774; Tarceva),[23] lapatinib (GW-572016),[24] canertinib (CI 1033),[25] semaxinib (SU5416),[26] vatalanib (PTK787/ZK222584),[27] sorafenib (BAY 43-9006),[28] sutent (SU11248),[29] and leflunomide (SU101).[30] All of these compounds are ATP-competitive inhibitors and an understanding of the pharmacological properties and anticancer activities of these compounds have resulted in a rapid advance to our understanding of the advantages and limitations associated with this class of therapeutic agents. One of the important facts that has emerged in the past few years has been the realization that tumor cells often develop resistance to ATP-competitive kinase inhibitors as a result of accumulating mutations in the ATP-binding site of the kinase, which has been observed in patients undergoing treatment with imatinib[31] as well as gefitinib[32] and erlotinib.[33]

Compound **6c** and its analogs do not resemble typical ATP mimetics in structure. Hence **6c** and its analogs are expected to target regions outside the ATP-binding site of their target kinases and offer the potential to be unaffected by mutations in the kinase domain that make tumor cells resistant to ATP-competitive inhibitors.

4. Conclusion

In conclusion, we describe here, for the first time, the discovery and synthesis of a novel class of compounds, (*E*)-α-benzylthio chalcones, which possess potent kinase inhibitory activity and exhibit cytotoxicity against human tumor cells that express the oncogenic kinase BCR-ABL. While these compounds are comparable to imatinib in their in vitro efficacy they do not resemble typical ATP-mimetics. Hence, they offer the potential to be unaffected by mutations in the kinase domain that make tumor cells resistant to ATP-competitive inhibitors. These compounds possess a simple molecular structure and are easy to synthesize which makes them very attractive for further exploration as kinase inhibitors with application in cancer therapy.

5. Experimental

5.1. Chemistry

5.1.1. General information

All reagents and solvents were obtained from commercial suppliers and used without further purification unless otherwise stated. Solvents were dried using standard procedures and reactions requiring anhydrous conditions were performed under N_2 atmosphere. Reactions were monitored by Thin Layer Chromatography (TLC) on precoated Silica Gel F254 plates (Sigma–Aldrich) with a UV indicator. Column chromatography was performed with Merck 70–230 mesh Silica Gel 60 Å. Yields were of purified product and were not optimized. Melting points were determined using an Electro thermal Mel-Temp® 3.0 micro melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Bruker AM 300 and 400 MHz spectrometer. The chemical shifts are reported in parts per million (δ) downfield using tetramethyl silane (Me_4Si) as internal standard and. Spin multiplicities are given as s (singlet), d (doublet), br s (broad singlet), m (multiplet), and q (quartet). Coupling constants (*J* values) were measured in hertz (Hz). The purity of the final compounds was determined by HPLC and is 95% or higher unless specified otherwise. Phenacylbromides were prepared according to the procedure reported in the literature.[34]

5.1.2. General procedure for the preparation of 2-(benzyl/aryl thio)-1-arylethanone (4 and 10)

To a solution of sodium hydroxide (20 mmol) in methanol (50 mL) benzylthiol **3** (20 mmol) or arylthiol **9** (20 mmol) was added and the contents were stirred at room temperature for 10 min. To this reaction mixture phenacylbromide **2** (20 mmol) was added and stirred for further 1–2 h. After completion of the reaction, the contents were cooled, poured on to ice-water and the solid obtained was collected by filtration. The crude product was recrystallized from 2-propanol to get pure **4** or **10**. The following 2-(benzyl/aryl thio)-1-arylethanones **4** and **10** were prepared using the above procedure.

5.1.2.1. 4-(2-(4-Bromobenzylthio)acetyl)benzoic acid (4a). Yield: 95%; white solid, mp 195–197 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.72 (s, 2H, CH_2), 3.95 (s, 2H, CH_2), 7.27–8.05 (m, 8H, Ar–H), 13.40 (br s, 1H, COOH). HRMS found *m*/*z* 364.78. Calcd for $C_{16}H_{13}BrO_3S$ $(M+H)^+$ *m*/*z* 364.98.

5.1.2.2. 4-(2-(4-Chlorobenzylthio)acetyl)benzoic acid (4b). Yield: 92%; white solid, mp 194–196 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.78 (s, 2H, CH_2), 4.05 (s, 2H, CH_2), 7.28–8.20 (m, 8H, Ar–H), 13.35 (br s, 1H, COOH). HRMS found *m*/*z* 321.08. Calcd for $C_{16}H_{13}ClO_3S$ $(M+H)^+$ *m*/*z* 321.04.

5.1.2.3. 4-(2-(4-Fluorobenzylthio)acetyl)benzoic acid (4c). Yield: 88%; white solid, mp 200–202 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.67 (s, 2H, CH_2), 3.92 (s, 2H, CH_2), 7.21–8.05 (m, 8H, Ar–H), 13.38 (br s, 1H, COOH). HRMS found *m*/*z* 305.10. Calcd for $C_{16}H_{13}FO_3S$ $(M+H)^+$ *m*/*z* 305.06.

5.1.2.4. 2-(4-Fluorobenzylthio)-1-(4-fluorophenyl)ethanone (4d). Yield: 84%; white solid, mp 68–70 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.60 (s, 2H, CH_2), 3.63 (s, 2H, CH_2), 7.18–7.85 (m, 8H, Ar–H). HRMS found *m*/*z* 279.06. Calcd for $C_{15}H_{12}F_2OS$ $(M+H)^+$ *m*/*z* 279.07.

5.1.2.5. 4-(2-(2-Fluorobenzylthio)acetyl)benzoic acid (4e). Yield: 72%; white solid, mp 189–190 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.82 (s, 2H, CH_2), 4.02 (s, 2H, CH_2), 7.15–8.14 (m, 8H, Ar–H), 13.35 (br s, 1H, COOH). HRMS found *m*/*z* 304.58. Calcd for $C_{16}H_{13}FO_3S$ $(M+H)^+$ *m*/*z* 304.58.

5.1.2.6. 4-(2-(2-Chlorobenzylthio)acetyl)benzoic acid (4f). Yield: 84%; white solid, mp 190–192 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.71 (s, 2H, CH_2), 3.93 (s, 2H, CH_2), 7.11–8.10 (m, 8H, Ar–H), 13.25 (br s, 1H,

COOH). HRMS found *m/z* 321.02. Calcd for $C_{16}H_{13}ClO_3S$ $(M+H)^+$ *m/z* 321.04.

5.1.2.7. 4-(2-(2,4-Dichlorobenzylthio)acetyl)benzoic acid (4g). Yield: 89%; white solid, mp 215–217 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 3.88 (s, 2H, CH_2), 4.12 (s, 2H, CH_2), 7.28–8.21 (m, 7H, Ar–H), 13.55 (br s, 1H, COOH). HRMS found *m/z* 354.97. Calcd for $C_{16}H_{12}Cl_2O_3S$ $(M+H)^+$ *m/z* 354.99.

5.1.2.8. 4-(2-(4-Methylbenzylthio)acetyl)benzoic acid (4h). Yield: 93%; white solid, mp 196–198 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 2.27 (s, 3H, CH_3), 3.68 (s, 2H, CH_2), 3.91 (s, 2H, CH_2), 7.10–8.04 (m, 8H, Ar–H), 13.25 (br s, 1H, COOH). HRMS found *m/z* 301.07. Calcd for $C_{17}H_{16}O_3S$ $(M+H)^+$ *m/z* 301.09.

5.1.2.9. 4-(2-(4-(Trifluoromethyl)benzylthio)acetyl)benzoic acid (4i). Yield: 78%; white solid, mp 162–164 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 3.68 (s, 2H, CH_2), 3.95 (s, 2H, CH_2), 7.11–8.10 (m, 8H, Ar–H), 13.35 (br s, 1H, COOH). HRMS found *m/z* 355.04. Calcd for $C_{17}H_{13}F_3O_3S$ $(M+H)^+$ *m/z* 355.06.

5.1.2.10. Methyl 4-(2-(4-bromobenzylthio)acetyl)benzoate (4j). Yield: 90%; white solid, mp 75–77 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 3.72 (s, 2H, CH_2), 3.92 (s, 3H, OCH_3), 4.05 (s, 2H, CH_2), 7.26–8.15 (m, 8H, Ar–H). HRMS found *m/z* 379.00. Calcd for $C_{17}H_{15}BrO_3S$ $(M+H)^+$ *m/z* 379.00.

5.1.2.11. 4-(2-(Benzylthio)acetyl)benzoic acid (4k). Yield: 93%; white solid, mp 182–184 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 3.88 (s, 2H, CH_2), 4.02 (s, 2H, CH_2), 6.98–8.04 (m, 9H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m/z* 287.07. Calcd for $C_{16}H_{14}O_3S$ $(M+H)^+$ *m/z* 287.07.

5.1.3. 4-(2-(4-Bromobenzylsulfonyl)acetyl)benzoic acid (7)

To a mixture of **4a** (50 mmol) in glacial acetic acid (100 mL), 30% hydrogen peroxide (60 mL) was added and the contents were refluxed for 1 h. After completion of the reaction, the cooled reaction contents were poured on to ice-water and the separated solid was filtered and dried. The crude product was recrystallized from methanol to obtain pure sample of **7**.

Yield: 80%; white solid, mp 270–272 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 3.82 (s, 2H, CH_2), 4.05 (s, 2H, CH_2), 7.36–8.15 (m, 8H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m/z* 396.98. Calcd for $C_{16}H_{13}BrO_5S$ $(M+H)^+$ *m/z* 396.98.

5.1.4. 4-(2-(4-Bromophenylthio)acetyl)benzoic acid (10a)

Yield: 94%; white solid, mp 188–190 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 3.85 (s, 2H, CH_2), 7.21–8.15 (m, 8H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m/z* 350.98. Calcd for $C_{15}H_{11}BrO_3S$ $(M+H)^+$ *m/z* 350.97.

5.1.5. General procedure for the preparation of (*E*)-2-(benzylthio)-1,3-diphenylprop-2-en-1-one (6) and (*E*)-1,3-diphenyl-2-(phenylthio)prop-2-en-1-one (11)

Method A: A mixture of 2-(benzyl/aryl thio)-1-arylethanone (**4** or **10**) (10 mmol), araldehyde (**5**) (12.5 mmol) and ammonium acetate (25 mmol) in glacial acetic acid (10 mL) was refluxed for 5–8 h. After completion of the reaction, reaction mixture was cooled and separated product was filtered, washed with 2-propanol and petroleum ether and dried. If solid was not formed, the reaction mixture was poured on to crushed ice, extracted with ethyl acetate, washed with water, brine and dried over sodium sulfate. After concentration, crude product obtained was recrystallized in 2-propanol to yield analytically pure sample of **6** or **11**.

Method B: A mixture of 2-(benzyl/aryl thio)-1-arylethanone (**4** or **10**) (10 mmol), araldehyde (**5**) (10 mmol), glacial acetic acid (5 mL), and a catalytic amount (100 μL) of benzyl amine was refluxed for 5–8 h. After completion of the reaction (TLC monitoring, $CHCl_3$/MeOH on silica gel plate), the contents were cooled to room temperature, the precipitated product was filtered, washed with 2-propanol and petroleum ether and dried. If solid was not formed, the reaction mixture was poured on to ice-water and extracted with ethyl acetate. The organic layer was dried over sodium sulfate, filtered and concentrated under vacuum to obtain the crude **6** or **11**. The crude product was recrystallized form 2-propanol to yield an analytically pure sample of **6** or **11**.

5.1.5.1. (*E*)-4-(2-(4-Bromobenzylthio)-3-(4-chloro-3-nitrophenyl)acryloyl)benzoic acid (6a). Yield: 78%; light yellow solid, mp 148–150 °C. ^1H NMR ($CDCl_3$, 400 MHz): δ 3.92 (s, 2H, CH_2), 6.84–6.94 (m, 3H, Ar–H), 7.17–7.23 (m, 5H, Ar–H), 7.22 (s, 1H, CH), 7.55–7.57 (dd, 2H, Ar–H), 7.58–7.62 (m, 1H, Ar–H), 7.85–7.87 (d, 2H, *J* = 8.0 Hz, Ar–H), 8.04–8.15 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m/z* 533.98. Calcd for $C_{23}H_{15}BrClNO_5S$ $(M+H)^+$ *m/z* 533.96.

5.1.5.2. (*E*)-4-(2-(4-Bromobenzylthio)-3-(4-bromo-3-nitrophenyl)acryloyl)benzoic acid (6b). Yield: 80%; light yellow solid, mp 168–170 °C. ^1H NMR ($CDCl_3$, 400 MHz): δ 3.90 (s, 2H, CH_2), 6.85–6.92 (m, 3H, Ar–H), 7.22–7.28 (m, 5H, Ar–H), 7.25 (s, 1H, CH), 7.62–7.65 (dd, 2H, Ar–H), 7.60–7.62 (m, 1H, Ar–H), 7.82–7.84 (d, 2H, *J* = 8.0 Hz, Ar–H), 8.02–8.09 (dd, 1H, Ar–H), 13.40 (br s, 1H, COOH). HRMS found *m/z* 577.92. Calcd for $C_{23}H_{15}Br_2NO_5S$ $(M+H)^+$ *m/z* 577.91.

5.1.5.3. (*E*)-4-(2-(4-Bromobenzylthio)-3-(4-fluoro-3-nitrophenyl)acryloyl)benzoic acid (6c). Yield: 72%; light yellow solid, mp 170–172 °C. ^1H NMR ($CDCl_3$, 400 MHz): δ 3.72 (s, 2H, CH_2), 6.68–6.75 (m, 3H, Ar–H), 6.98–7.06 (m, 5H, Ar–H), 7.01 (s, 1H, CH), 7.30–7.37 (dd, 2H, Ar–H), 7.54–7.58 (m, 1H, Ar–H), 7.87–7.89 (d, 2H, *J* = 8.3 Hz, Ar–H), 8.13–8.16 (dd, 1H, Ar–H), 13.33 (br s, 1H, COOH). HRMS found *m/z* 518.10. Calcd for $C_{23}H_{15}BrFNO_5S$ $(M+H)^+$ *m/z* 517.99.

5.1.5.4. (*E*)-4-(2-(4-Chlorobenzylthio)-3-(4-fluoro-3-nitrophenyl)-acryloyl)benzoic acid (6d). Yield: 82%; light yellow solid, mp 165–168 °C. ^1H NMR ($CDCl_3$, 400 MHz): δ 3.75 (s, 2H, CH_2), 6.71–6.76 (m, 3H, Ar–H), 6.95–7.06 (m, 5H, Ar–H), 7.05 (s, 1H, CH), 7.28–7.35 (dd, 2H, Ar–H), 7.48–7.55 (m, 1H, Ar–H), 7.78–7.85 (d, 2H, *J* = 8.2 Hz, Ar–H), 8.10–8.14 (dd, 1H, Ar–H), 13.36 (br s, 1H, COOH). HRMS found *m/z* 472.05. Calcd for $C_{23}H_{15}ClFNO_5S$ $(M+H)^+$ *m/z* 472.04.

5.1.5.5. (*E*)-4-(2-(4-Chlorobenzylthio)-3-(4-chloro-3-nitrophenyl)acryloyl)benzoic acid (6e). Yield: 77%; light yellow solid, mp 170–162 °C. ^1H NMR ($CDCl_3$, 400 MHz):δ 3.82 (s, 2H, CH_2), 6.69–6.71 (m, 3H, Ar–H), 6.82–7.08 (m, 5H, Ar–H), 7.01 (s, 1H, CH), 7.18–7.24 (dd, 2H, Ar–H), 7.35–7.55 (m, 1H, Ar–H), 7.79–7.91 (d, 2H, *J* = 8.2 Hz, Ar–H), 8.04–8.10 (dd, 1H, Ar–H), 13.40 (br s, 1H, COOH). HRMS found 488.05. Calcd for $C_{23}H_{15}Cl_2NO_5S$ $(M+H)^+$ *m/z* 488.01.

5.1.5.6. (*E*)-4-(2-(4-Chlorobenzylthio)-3-(4-bromo-3-nitrophenyl)acryloyl)benzoic acid (6f). Yield: 85%; light yellow solid, mp 189–191 °C. ^1H NMR ($CDCl_3$, 400 MHz): δ 3.80 (s, 2H, CH_2), 6.68–6.75 (m, 3H, Ar–H), 6.82–7.08 (m, 5H, Ar–H), 7.11 (s, 1H, CH), 7.22–7.28 (dd, 2H, Ar–H), 7.41–7.56 (m, 1H, Ar–H), 7.81–7.91 (d, 2H, *J* = 8.0 Hz, Ar–H), 8.15–8.22 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m/z* 533.95. Calcd for $C_{23}H_{15}BrClNO_5S$ $(M+H)^+$ *m/z* 533.96.

5.1.5.7. (*E*)-4-(2-(4-Fluorobenzylthio)-3-(4-chloro-3-nitrophenyl)acryloyl)benzoic acid (6g). Yield: 68%; light yellow solid, mp 164–166 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.69 (s, 2H, CH_2), 6.65–6.75 (m, 3H, Ar–H), 6.80–7.09 (m, 5H, Ar–H), 7.08 (s, 1H, CH), 7.15–7.30 (dd, 2H, Ar–H), 7.35–7.48 (m, 1H, Ar–H), 7.78–7.89 (d, 2H, J = 8.1 Hz, Ar–H), 8.05–8.18 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found m/z 472.05. Calcd for $C_{23}H_{15}ClFNO_5S$ $(M+H)^+$ m/z 472.04.

5.1.5.8. (*E*)-4-(2-(4-Fluorobenzylthio)-3-(4-fluoro-3-nitrophenyl)acryloyl)benzoic acid (6h). Yield: 82%; light yellow solid, mp 160–162 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.97 (s, 2H, CH_2), 6.93–6.97 (m, 3H, Ar–H), 7.11–7.15 (m, 5H, Ar–H), 7.21 (s, 1H, CH), 7.67–7.69 (dd, 2H, Ar–H), 7.94–7.99 (m, 1H, Ar–H), 8.03–8.07 (d, 2H, J = 8.1 Hz, Ar–H), 8.48–8.50 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found m/z 456.05. Calcd for $C_{23}H_{15}F_2NO_5S$ $(M+H)^+$ m/z 456.07.

5.1.5.9. (*E*)-4-(2-(4-Fluorobenzylthio)-3-(4-bromo-3-nitrophenyl)acryloyl)benzoic acid (6i). Yield: 75%; light yellow solid, mp 185–187 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.93 (s, 2H, CH_2), 6.88–6.99 (m, 3H, Ar–H), 7.18–7.25 (m, 5H, Ar–H), 7.26 (s, 1H, CH), 7.55–7.68 (dd, 2H, Ar–H), 7.87–7.89 (m, 1H, Ar–H), 7.98–8.02 (d, 2H, J = 8.1 Hz, Ar–H), 8.35–8.40 (dd, 1H, Ar–H), 13.40 (br s, 1H, COOH). HRMS found m/z 517.98. Calcd for $C_{23}H_{15}FNO_5S$ $(M+H)^+$ m/z 517.99.

5.1.5.10. (*E*)-3-(4-Fluoro-3-nitrophenyl)-2-(4-fluorobenzylthio)-1-(4-fluorophenyl)prop-2-en-1-one (6j). Yield: 79%; light yellow solid, mp 88–90 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.02 (s, 2H, CH_2), 6.98–7.02 (m, 2H, Ar–H), 7.15–7.19 (m, 2H, Ar–H), 7.25 (s, 1H, CH), 7.62–7.67 (dd, 2H, Ar–H), 7.71–7.73 (d, 1H, J = 8.0 Hz, Ar–H), 7.98–8.03 (d, 2H, J = 8.1 Hz, Ar–H), 8.07–8.11 (dd, 1H, Ar–H), 8.52–8.54 (dd, 1H, Ar–H). HRMS found m/z 430.06. Calcd for $C_{22}H_{14}F_3NO_3S$ $(M+H)^+$ m/z 430.07.

5.1.5.11. (*E*)-4-(3-(4-Chloro-3-nitrophenyl)-2-(2-fluorobenzylthio)acryloyl)benzoic acid (6k). Yield: 66%; white solid, yield: 78%; mp 138–140 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.04 (s, 2H, CH_2), 6.95–7.02 (m, 2H, Ar–H), 7.09–7.18 (m, 2H, Ar–H), 7.20 (s, 1H, CH), 7.34–7.32 (dd, 2H, Ar–H), 7.73–7.80 (m, 2H, Ar–H), 7.95–8.06 (d, 2H, J = 8.1 Hz, Ar–H), 8.16–8.22 (dd, 1H, Ar–H), 8.44–8.59 (dd, 1H, Ar–H), 13.57 (br s, 1H, COOH). HRMS found m/z 472.01. Calcd for $C_{23}H_{15}Cl$ FNO_5S $(M+H)^+$ 472.03.

5.1.5.12. (*E*)-4-(2-(2-Chlorobenzylthio)-3-(4-fluoro-3-nitrophenyl)acryloyl)benzoic acid (6l). Yield: 76%; white solid, mp 198–200 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.15 (s, 2H, CH_2), 7.03–7.14 (m, 2H, Ar–H), 7.22–7.28 (m, 2H, Ar–H), 7.20 (s, 1H, CH), 7.58–7.62 (dd, 2H, Ar–H), 7.78–7.83 (d, 1H, J = 8.0 Hz, Ar–H), 7.98–8.03 (d, 2H, J = 8.0 Hz, Ar–H), 8.05–8.10 (dd, 1H, Ar–H), 8.43–8.54 (dd, 1H, Ar–H), 13.47 (br s, 1H, COOH). HRMS found 472.04. Calcd for $C_{23}H_{15}ClFNO_5S$ $(M+H)^+$ m/z 472.04.

5.1.5.13. (*E*)-4-(3-(4-Chloro-3-nitrophenyl)-2-(2,4-dichlorobenzylthio)acryloyl)benzoic acid (6m). Yield: 85%; white solid, mp 126–130 °C. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 4.10 (s, 2H, CH_2), 6.93–7.12 (m, 2H, Ar–H), 7.24–7.29 (m, 2H, Ar–H), 7.26 (s, 1H, CH), 7.55–7.60 (dd, 2H, Ar–H), 7.82–7.89 (d, 1H, J = 8.1 Hz, Ar–H), 8.03–8.08 (d, 2H, J = 8.0 Hz, Ar–H), 8.11–8.18 (dd, 1H, Ar–H), 8.51–8.59 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found m/z 523.97. Calcd for $C_{23}H_{14}Cl_3NO_5S$ $(M+H)^+$ m/z 523.97.

5.1.5.14. (*E*)-4-(3-(4-Fluoro-3-nitrophenyl)-2-(2,4-dichlorobenzylthio)acryloyl)benzoic acid (6n). Yield: 80%; white solid, mp 124–128 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.92 (s, 2H, CH_2), 6.92–6.98 (m, 2H, Ar–H), 7.12–7.19 (m, 2H, Ar–H), 7.20 (s, 1H, CH), 7.25–7.33 (dd, 2H, Ar–H), 7.52–7.59 (d, 1H, J = 8.1 Hz, Ar–H), 7.68–7.73 (d, 2H, J = 8.0 Hz, Ar–H), 7.81–7.88 (dd, 1H, Ar–H), 8.10–8.16 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found m/z 506.00. Calcd for $C_{23}H_{14}Cl_2FNO_5S$ $(M+H)^+$ m/z 506.00.

5.1.5.15. (*E*)-4-(3-(4-Bromo-3-nitrophenyl)-2-(2,4-dichlorobenzylthio)acryloyl)benzoic acid (6o). Yield: 74%; white solid, mp 160–162 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.95 (s, 2H, CH_2), 6.90–6.98 (m, 2H, Ar–H), 7.15–7.21 (m, 2H, Ar–H), 7.25 (s, 1H, CH), 7.28–7.32 (dd, 2H, Ar–H), 7.49–7.53 (d, 1H, J = 8.1 Hz, Ar–H), 7.62–7.70 (d, 2H, J = 8.0 Hz, Ar–H), 7.78–7.84 (dd, 1H, Ar–H), 8.15–8.20 (dd, 1H, Ar–H), 13.48 (br s, 1H, COOH). HRMS found m/z 567.92. Calcd for $C_{23}H_{14}BrCl_2NO_5S$ $(M+H)^+$ m/z 567.92.

5.1.5.16. (*E*)-4-(3-(4-Fluoro-3-nitrophenyl)-2-(4-methylbenzylthio)acryloyl)benzoic acid (6p). Yield: 85%; white solid, mp 148–150 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.25 (s, 3H, CH_3), 3.90 (s, 2H, CH_2), 6.83–6.92 (m, 2H, Ar–H), 6.98–7.12 (m, 2H, Ar–H), 7.20 (s, 1H, CH), 7.25–7.30 (dd, 2H, Ar–H), 7.38–7.43 (d, 1H, J = 8.1 Hz, Ar–H), 7.67–7.70 (d, 2H, J = 8.0 Hz, Ar–H), 7.85–7.89 (dd, 1H, Ar–H), 8.22–8.33 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found m/z 452.11. Calcd for $C_{24}H_{18}FNO_5S$ $(M+H)^+$ m/z 452.10

5.1.5.17. (*E*)-4-(3-(4-Fluoro-3-nitrophenyl)-2-(4-(trifluoromethyl)benzylthio)acryloyl)benzoic acid (6q). Yield: 68%; white solid, mp 183–186 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.87 (s, 2H, CH_2), 6.73 (s, 1H, CH), 7.03–7.11 (m, 2H, Ar–H), 7.17–7.19 (d, 2H, Ar–H), 7.30–7.37 (d, 1H, J = 8.1 Hz, Ar–H), 7.59–7.65 (dd, 2H, J = 8.0 Hz, Ar–H), 7.88–7.98 (dd, 1H, Ar–H), 8.19–8.21 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found m/z 506.07. Calcd for $C_{24}H_{15}F_4NO_5S$ $(M+H)^+$ m/z 506.07.

5.1.5.18. (*E*)-Methyl4-(2-(4-bromobenzylthio)-3-(4-fluoro-3-nitrophenyl)acryloyl)benzoate (6r). Yield: 76%; white solid, mp 143–145 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.83 (s, 3H, OCH_3), 3.92 (s, 2H, CH_2), 6.83 (s, 1H, CH), 7.06–7.12 (m, 2H, Ar–H), 7.20–7.28 (d, 2H, Ar–H), 7.30–7.37 (d, 1H, J = 8.1 Hz, Ar–H), 7.55–7.65 (dd, 2H, J = 8.0 Hz, Ar–H), 7.78–7.88 (dd, 1H, Ar–H), 8.12–8.20 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found m/z 532.01. Calcd for $C_{24}H_{17}BrFNO_5S$ $(M+H)^+$ m/z 532.01.

5.1.5.19. (*E*)-4-(2-(Benzylthio)-3-(4-fluoro-3-nitrophenyl)acryloyl)benzoic acid (6s). Yield: 85%; white solid, mp 148–150 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.87 (s, 2H, CH_2), 6.76 (s, 1H, CH), 6.96–7.00 (m, 4H, Ar–H), 7.08–7.14 (m, 2H, Ar–H), 7.40–7.43 (d, 2H, J = 12 Hz, Ar–H), 7.67–7.71 (m, 1H, Ar–H), 7.93–7.95 (d, 2H, J = 8 Hz, Ar–H), 8.20–8.22 (dd, 1H, J = 8.0 Hz, Ar–H), 13.50 (br s, 1H, COOH). HRMS found m/z 438.08. Calcd for $C_{23}H_{16}FNO_5S$ $(M+H)^+$ m/z 438.08.

5.1.5.20. (*E*)-4-(2-(4-Bromobenzylsulfinyl)-3-(4-fluoro-3-nitrophenyl)acryloyl)benzoic acid (6t). To a mixture of **6c** (0.51 g, 10 mmol) in dry chloroform (10 mL), *m*-chloroperoxybenzoic acid (0.22 g, 10 mmol) in 5 mL dry chloroform was added drop wise at 0 °C. After the addition, the reaction was continued at this temperature for 1 h and at room temperature for an additional 1 h. The separated solid was filtered and recrystallized from chloroform to obtain a pure sample of **6t**. Yield: 78%; white solid, mp 213–215 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.47–4.63 (q, 2H, CH_2), 7.22–7.24 (d, 2H, Ar–H), 7.36–7.39 (d, 2H, Ar–H), 7.47–7.56 (m, 2H, Ar–H), 7.63–7.67 (m, 1H, CH), 7.81–7.98 (m, 1H, Ar–H), 8.02–8.05 (dd, 1H, Ar–H), 8.11–8.24 (m, 3H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found m/z 531.90. Calcd for $C_{23}H_{15}BrFNO_6S$ $(M+H)^+$ m/z 531.99.

5.1.6. (*E*)-4-(2-(4-Bromobenzylsulfonyl)-3-(4-fluoro-3-nitrophenyl)acryloyl)benzoic acid (8a)

This compound was prepared from **7** and 3-nitro-4-fluorobenzaldehyde in 62% yield as described method A. Alternatively, this compound was also prepared by the oxidation of **6c** with excess (3 equiv) of *m*-chloroperoxybenzoic acid as described in **6t**. Yield: 62%; white solid, mp 178–180 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.82 (s, 2H, CH_2), 7.39–7.49 (m, 2H, Ar–H), 7.51–7.67 (m, 4H, Ar–H), 7.81 (s, 1H, CH), 7.91–7.99 (m, 3H, Ar–H), 8.10–8.14 (m, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m*/*z* 549.98. Calcd for $C_{23}H_{15}BrFNO_7S$ $(M+H)^+$ *m*/*z* 549.98.

5.1.6.1. (*E*)-4-(2-(4-Bromobenzylsulfonyl)-3-(4-chloro-3-nitrophenyl)acryloyl)benzoic acid (8b). Yield: 48%; white solid, mp 148–150 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.53 (s, 2H, CH_2), 7.20–7.24 (m, 3H, Ar–H), 7.30–7.36 (m, 5H, Ar–H), 7.38 (s, 1H, CH), 7.43–7.55 (dd, 2H, Ar–H), 7.51–7.59 (m, 1H, Ar–H), 7.95–7.98 (d, 2H, *J* = 8.6 Hz, Ar–H), 8.09–8.21 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m*/*z* 565.92. Calcd for $C_{23}H_{15}BrClNO_7S$ $(M+H)^+$ *m*/*z* 565.95.

5.1.7. (*E*)-4-(2-(4-Bromophenylthio)-3-(4-fluoro-3-nitrophenyl)-acryloyl)benzoic acid (11)

Yield: 89%; white solid, mp 145–147 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.12–7.25 (m, 2H, Ar–H), 7.28–7.31 (d, 2H, Ar–H), 7.65 (s, 1H, CH), 7.88–7.90 (d, 1H, *J* = 8.1 Hz, Ar–H), 8.00–8.03 (dd, 2H, *J* = 8.0 Hz, Ar–H), 8.24–8.27 (dd, 1H, Ar–H), 8.65–8.67 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m*/*z* 502.97. Calcd for $C_{22}H_{13}BrFNO_5S$ $(M+H)^+$ *m*/*z* 502.97.

5.1.7.1. (*E*)-4-(2-(4-Bromobenzylthio)-3-(4-fluoro-3-methoxyphenyl)acryloyl)benzoic acid (6aa). Yield: 65%; white solid, mp 72–74 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.85 (s, 3H, OCH_3), 4.04 (s, 2H, CH_2), 6.84–6.94 (m, 2H, Ar–H), 7.18–7.27 (m, 5H, Ar–H), 7.29 (s, 1H, CH), 7.55–7.57 (dd, 2H, Ar–H), 7.58–7.62 (m, 1H, Ar–H), 7.85–7.87 (d, 2H, *J* = 8.0 Hz, Ar–H), 8.04–8.15 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m*/*z* 502.34. Calcd for $C_{24}H_{18}BrFO_4S$ $(M+H)^+$ *m*/*z* 502.36.

5.1.7.2. (*E*)-4-(2-(4-Bromobenzylthio)-3-(4-hydroxy-3-bromo) acryloyl)benzoic acid (6ab). Yield: 60%; yellow solid: mp 158–160 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.45 (br s, 1H, OH), 3.98 (s, 2H, CH_2), 7.20–7.26 (m, 4H, Ar–H), 7.30–7.37 (m, 2H, Ar–H), 7.62–7.69 (m, 4H, Ar–H), 7.95–8.04 (dd, 1H, *J* = 12.0, 8.0 Hz, Ar–H), 8.52–8.61 (m, 1H, Ar–H), 13.48 (br s, 1H, COOH). HRMS found *m*/*z* 549.30. Calcd for $C_{23}H_{16}Br_2O_4S$ $(M+H)^+$ *m*/*z* 549.25.

5.1.7.3. (*E*)-4-(2-(4-Bromobenzylthio)-3-(4-hydroxy-3-nitrophenyl)acryloyl)benzoic acid (6ac). Yield: 78%; yellow solid: mp 200–202 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.35 (br s, 1H, OH), 4.05 (s, 2H, CH_2), 7.00–7.04 (m, 2H, Ar–H), 7.11–7.21 (m, 4H, Ar–H), 7.63–7.65 (d, 2H, *J* = 8.0, Ar–H), 7.90–7.93 (dd, 1H, *J* = 12.0, 8.0 Hz, Ar–H), 8.00–8.02 (d, 2H, *J* = 8.0, Ar–H), 8.38–8.39 (d, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m*/*z* 515.32. Calcd for $C_{23}H_{16}BrNO_6S$ $(M+H)^+$ *m*/*z* 515.36.

5.1.7.4. (*E*)-4-(2-(4-Bromobenzylthio)-3-(2-fluoro-5-nitrophenyl)acryloyl)benzoic acid (6ad). Yield: 68%; yellow solid: mp 223–225 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.00 (s, 2H, CH_2), 6.95–7.02 (m, 2H, Ar–H), 7.12–7.19 (m, 2H, Ar–H), 7.29–7.35 (m, 2H, Ar–H), 7.38–7.43 (d, 1H, *J* = 8.1 Hz, Ar–H), 7.61–7.70 (d, 2H, *J* = 8.0 Hz, Ar–H), 7.92–7.98 (m, 2H, Ar–H), 8.28–8.33 (dd, 1H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m*/*z* 517.32. calcd for $C_{23}H_{15}BrFNO_5S$ $(M+H)^+$ *m*/*z* 517.35.

5.1.7.5. (*E*)-4-(2-(4-Bromobenzylthio)-3-(3-fluoro-4-nitrophenyl)-acryloyl)benzoic acid (6ae). Yield: 90%; yellow solid, mp 198–200 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.01 (s, 2H, CH_2), 6.52 (s, 1H, CH), 6.92–7.09 (m, 2H, Ar–H), 7.16–7.27 (m, 4H, Ar–H), 7.38–7.46 (m, 1H, Ar–H), 7.62–7.67 (m, 2H, Ar–H), 8.11–8.36 (m, 2H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m*/*z* 517.98. Calcd for $C_{23}H_{15}BrFNO_5S$ $(M+H)^+$ *m*/*z* 517.99.

5.1.7.6. (*E*)-4-(2-(4-Bromobenzylthio)-3-(4-fluoro-2-nitrophenyl)acryloyl)benzoic acid (6af). Yield: 86%; yellow solid: mp 194–196 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.89 (s, 2H, CH_2), 6.59 (s, 1H, CH), 6.91–7.06 (m, 2H, Ar–H), 7.16–7.27 (m, 4H, Ar–H), 7.40–7.49 (m, 1H, Ar–H), 7.68–7.74 (m, 2H, Ar–H), 8.16–8.36 (m, 2H, Ar–H), 13.49 (br s, 1H, COOH). HRMS found *m*/*z* 517.97. Calcd for $C_{23}H_{15}BrFNO_5S$ $(M+H)^+$ *m*/*z* 517.99.

5.1.7.7. (*E*)-4-(2-(4-Bromobenzylthio)-3-(2-fluoro-4-nitrophenyl)acryloyl)benzoic acid (6ag). Yield: 87%; yellow solid, mp 196–199 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.89 (s, 2H, CH_2), 6.52 (s, 1H, CH), 6.92–7.09 (m, 2H, Ar–H), 7.16–7.27 (m, 4H, Ar–H), 7.38–7.46 (m, 1H, Ar–H), 7.62–7.67 (m, 2H, Ar–H), 8.11–8.36 (m, 2H, Ar–H), 13.50 (br s, 1H, COOH). HRMS found *m*/*z* 517.98. Calcd for $C_{23}H_{15}BrFNO_5S$ $(M+H)^+$ *m*/*z* 517.99.

5.1.8. (*E*)-4-(2-(4-Bromobenzylthio)-3-(4-fluoro-3-nitrophenyl) acryloyl)-*N*-(2-(4-methylpiperazin-1-yl)ethyl)benzamide (12a)

A mixture of **6c** (0.155 g, 3 mmol), 1-hydroxybenzotriazole hydrate (0.040 g, 3 mmol), *N*,*N*-diisopropylethylamine (157 μL, 9 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.064 g, 3.3 mmol) in dry tetrahydrofuran (10 mL) were stirred at room temperature under nitrogen atmosphere for 30 min. To this reaction mixture, 2-(4-methylpiperazin-1-yl)ethanamine (0.039 g, 3 mmol) in THF (2 mL) was added drop wise and continued stirring for additional 2 h. The progress of the reaction was monitored by TLC (9:1 chloroform/methanol on silica gel plate). After completion of the reaction, THF was removed under reduced pressure, diluted with water and extracted with ethyl acetate. The ethyl acetate layer was washed with saturated sodium bicarbonate solution, water and brine and dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure afforded a crude product which was purified by silica gel column chromatography, eluting with chloroform/methanol (9:0.5), to yield compound **12a**. Yield: 75%; light orange solid, mp 60–62 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.19 (s, 3H, CH_3), 2.25–2.54 (m, 10H), 3.45–346, (d, 2H, NH–CH_2), 3.98 (s, 2H, CH_2), 6.80–6.95 (m, 2H, Ar–H), 7.12–7.25 (m, 3H, Ar–H), 7.39–7.49 (dd, 2H, Ar–H), 7.63–7.78 (m, 2H, Ar–H), 8.28–7.29 (d, 2H, *J* = 8.0 Hz, Ar–H), 8.58 (br s, 1H, NH). HRMS found *m*/*z* 643.12. Calcd for $C_{30}H_{30}BrFN_4O_4S$ $(M+H)^+$ *m*/*z* 643.12.

5.1.8.1. (*E*)-2-(4-Bromobenzylthio)-3-(4-fluoro-3-nitrophenyl)-1-(4-(4-(2-hydroxy-ethyl)piperazine-1-carbonyl)phenyl)prop-2-en-1-one (12b). Yield: 70%; white solid, mp 117–119 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.44–2.54 (m, 6H), 2.76 (br s, 1H, OH), 3.34–3.37 (d, 2H), 3.57–3.61 (m, 2H), 3.74–3.79 (m, 2H,), 3.89 (s, 2H, CH_2), 6.84–6.85 (s, 1H, Ar–H), 6.92–6.94 (d, 2H, Ar–H), 7.14–7.16 (d, 2H, Ar–H), 7.20–7.26 (m, 2H, Ar–H), 7.34–7.36 (d, 2H, Ar–H), 7.50–7.52 (d, 2H, Ar–H), 7.74–7.77 (m, 1H, Ar–H). HRMS found *m*/*z* 630.08. Calcd for $C_{29}H_{27}BrFN_3O_5S$ $(M+H)^+$ *m*/*z* 630.09.

5.1.8.2. (*E*)-4-(2-(4-Bromobenzylthio)-3-(4-fluoro-3-nitrophenyl)-acryloyl)-N-(2-(diethylamino)ethyl)benzamide (12c). Yield: 72%; white solid, mp 60–61 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.44–2.54 (m, 6H), 2.76 (br s, 1H, OH), 3.34–3.37 (d, 2H), 3.57–3.61 (m, 2H), 3.74–3.79 (m, 2H,), 3.89 (s, 2H, CH_2), 6.84–6.85 (s, 1H, Ar–H), 6.92–6.94 (d, 2H, Ar–H), 7.14–7.16 (d, 2H, Ar–H),

7.20–7.26 (m, 2H, Ar–H), 7.34–7.36 (d, 2H, Ar–H), 7.50–7.52 (d, 2H, Ar–H), 7.74–7.77 (m, 1H, Ar–H). HRMS found *m/z* 616.10. Calcd for $C_{29}H_{29}BrFN_3O_4S$ $(M+H)^+$ *m/z* 616.11.

5.1.8.3. (*E*)-3-(4-Fluoro-3-nitrophenyl)-2-(4-fluorobenzylthio)-1-(4-(4-(2-hydroxyethyl)piperazine-1-carbonyl)phenyl)prop-2-en-1-one (12d). Yield: 75%; light yellow solid, mp 68–70 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.21 (br s, 2H), 2.28–2.33 (m, 6H), 3.13 (br s, 1H, OH), 3.35–3.38 (t, 2H), 3.54 (br s, 2H,), 3.71 (s, 2H, CH_2), 6.51–6.55 (m, 2H, Ar–H), 6.65 (s, 1H, Ar–H), 6.80–6.86 (m, 2H, Ar–H), 6.98–7.03 (dd, 2H, Ar–H), 7.10–7.15 (m, 2H, Ar–H), 7.33–7.35 (d, 2H, Ar–H), 7.50–7.53 (m, 1H, Ar–H), 8.12–8.14 (dd, 1H, Ar–H). HRMS found *m/z* 568.15. Calcd for $C_{29}H_{27}F_2N_3O_5S$ $(M+H)^+$ *m/z* 568.16.

5.1.9. Biological assay methods, cells and culture conditions

5.1.9.1. Cell culture and growth inhibition assays. K562 cells were purchased from ATCC and maintained at 37 °C under 5% CO_2 in RPMI medium supplemented with 10% fetal bovine serum (Cell Generation, Co.) and penicillin–streptomycin. For growth inhibition studies, K562 cells were plated at 1×10^5 cells/mL and incubated with varying concentrations of each compound. DMSO was used as a negative control. After 96 h of treatment the cell viability was determined by Trypan blue exclusion and expressed as percent of DMSO control to determine GI_{50} values.

5.1.9.2. Western blotting. Exponentially growing K562 cells were treated for 2 h with increasing concentration of compound (dissolved in DMSO at 10 mM stock concentrations and diluted in DMSO to 1000× working stock solutions. Whole cell protein lysate was collected and 50 μg of the clarified lysate was resolved by 10%-SDS–PAGE and western blotted. The blots were sequentially probed against antibodies specific for phosphorylated BCR-ABL (Santa Cruz Biotechnologies sc-885) and BCR-ABL (Santa Cruz Biotechnologies N-20), respectively, using the protocol provided by Li-Cor, Inc, Co. The western was treated with secondary antibodies conjugated with infrared dyes (LiCor) and scanned using Odyssey (LiCor) scanner. Percent inhibition was determined by quantifying each band using the software provided by LiCor, then normalizing the P-BCR-ABL signal to the parental BCR-ABL signal and determining% inhibition based on the vehicle control signal.

5.1.9.3. Kinase assays and IC_{50} determination. For Src (Invitrogen P3044), LynB (Invitrogen P2907), Plk-1 (Invitrogen PV3501) and Cdk-1/cyclinB (Upstate 14–450) kinase assays 10 ng of recombinant kinase was used with 1 μg GST-Sam68 (aa 331–443; Santa Cruz Biotechnologies sc-4249), dephosphorylated α-Casein (Sigma C8032) or Histone H1 (Roche Diagnositics 223549) as protein substrate, respectively. The kinase reactions were initiated by the addition of 1 μg recombinant substrate, 20 μM ATP and 20 μCi γ-32P-ATP. The reactions were incubated at 30 °C for 20 min, terminated by the addition of 2× Laemmli sample buffer, boiled for 2 min, resolved by 12% acrylamide SDS–PAGE and subjected to autoradiography. The autoradiograms were scanned and the band corresponding to phosphorylation of substrate was quantitated using MacBas software. The densitometric values obtained were plotted as a function of log drug concentration using Prism 4 Graphpad software and IC_{50} values determined by plotting sigmoidal non-linear regression curves with variable slope. ErbB2 and EGFR assays were performed using cell based immunoprecipitation assays like those performed by us earlier.[35]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.01.051.

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