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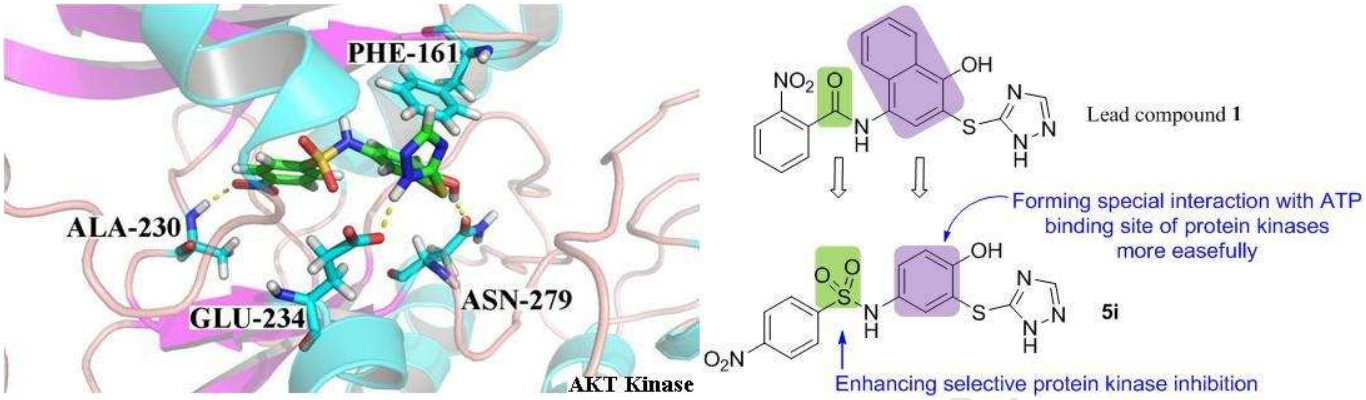
Graphical Abstract:

Key compound 5i and its calculated binding mode in the ATP-binding pocket of AKT kinase.



Discovery of 4-amino-2-(thio)phenol derivatives as novel protein kinase and angiogenesis inhibitors for the treatment of cancer: synthesis and biological evaluation. Part □

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Abstract

A novel series of 4-amino-2-(thio)phenol derivatives were well synthesized. The preliminary biological test revealed that several compounds displayed high specific protein kinase and angiogenesis inhibitory activities compared with previous work mainly because of the substitution of sulfonamide structure for amide fragment. Among which, compound **5i** was identified to inhibit protein kinase B/AKT (IC_{50} = 1.26 μM) and ABL tyrosine kinase (IC_{50} = 1.50 μM) effectively. Meanwhile, compound **5i** demonstrated competitive *in vitro* antiangiogenic activities to Pazopanib in both human umbilical vein endothelial cell (HUVEC) tube formation assay and the rat thoracic aorta rings test.

Keywords: AKT; ABL; protein kinase; HUVECs; rat thoracic aorta rings

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1. **Introduction**

Protein kinases are crucial for extensive signal transduction processes which could lead to the transformation, proliferation and metastasis of tumor cells [1]. Meanwhile, aberrant angiogenesis, the formation of new blood vessels from preexisting endothelium-lined vessels, is a prerequisite for tumor cell proliferation, invasion and metastasis [2,3]. Therefore, molecular design aimed at both different protein kinase and angiogenesis inhibitors are potential to the discovery of new antitumor drugs [4,5].

Herein five representative protein kinases, i.e. ABL, protein kinase B/AKT, ALK, EGFR and VEGFR-2, associated with tumor growth and tumor-induced angiogenesis, were selected as targets for small molecule intervention. It has been demonstrated that multitargeted cancer therapies with molecules able to inhibit different types of protein kinases could significantly increase tumor control [6]. What's more, the approval of both multitargeted protein kinase and angiogenesis inhibitors, Pazopanib [7] and Sorafenib [8], boosts the optimism of antiangiogenic agents with protein kinase inhibitory activity as an effective way to control tumor growth.

In our previous work, we have reported a series of N-(4-hydroxy-3-mercaptonaphthalen-1-yl)amide derivatives, of which the biological characterization revealed that the most potent compound **1** (see Fig. 1) displayed potent inhibitory effect and can be used as a leading antiangiogenic and anticancer agent for further structure optimization [9]. In this report, we disclose our progress on identifying a novel series of 4-amino-2-(thio)phenol derivatives based on the structure of lead **1**. The *in vitro* protein kinase and angiogenesis inhibitory activity was measured by application of kinase inhibition assay, tube formation assay using HUVECs [10,11] and the rat thoracic aorta rings model [10,12]. Among them, compound **5i** was identified to inhibit protein kinase B/AKT (IC_{50} = 1.26 μM) and ABL tyrosine kinase (IC_{50} = 1.50 μM) superior to compound **1**. Meanwhile, **5i** demonstrated competitive *in vitro* antiangiogenic activities to Pazopanib in both human umbilical vein endothelial cell (HUVEC) tube formation assay and the rat thoracic aorta rings test.

2. Design of 4-amino-2-(thio)phenol derivatives.

In order to find better protein kinase and angiogenesis inhibitors, we modified the structure of lead compound **1** as follows, Fig. 1: (i) Naphthalene ring was substituted by benzenoid framework in order to form special interaction with ATP binding site of protein kinases more easefully. (ii) Replacing amide structure with sulfonamide structure fragment on considering its broadly biological activity in medicinal chemistry [13-17]. Meanwhile, some amide structure containing molecules were also prepared for comparisons. (iii) The R_1 group corresponding to the 2-nitrobenzene group of compound **1** can be methyl or substituted aromatic fragments; the R_2 group is mainly 1*H*-1,2,4-triazole ring with few diversities to keep pace with compound **1**, as the 1*H*-1,2,4-triazole ring can play a role in the strong interaction with the active site of protein kinases.

(**Fig. 1** should be listed here)

3. Chemistry

In scheme 1, commercially available 4-aminophenol hydrochloride **2** dissolved in pyridine reacted with various substituted sulfonyl chlorides to generate compounds **3a-j**, which were then oxidated using $NaIO_4/SiO_2$ [18] to obtain quinone type structures **4a-j**. At last, compounds **4a-j** took Michael addition reaction [19] to gain the target compounds **5a-j** and **5i1-5i4** with different arylthiol groups.

As shown in scheme 2, we utilized THF/H_2O (50/1) as the reagent first to gain N-(4-hydroxyphenyl)-4-nitrobenzamide **3k**. The following steps for the preparation of compounds **5k** and **5k1-5k4** are the same as that in scheme 1. The spectral characterization of all target compounds is the chemical shift of three active hydrogens (1 × phenolic O<u>H</u> and 2 × aromatic N<u>H</u>) with (DMSO-d_6) δ 8.74-14.33.

(**Scheme 1** should be listed here)

(**Scheme 2** should be listed here)

4. Results and discussion

4.1. In vitro protein kinase inhibition

In vitro kinase inhibitory activities of the 4-amino-2-(thio)phenol derivatives against AKT, ABL, ALK and EGFR kinases were evaluatede by measuring the phosphorylation levels of the kinase-specific ligand peptides at 10 μM; The pan-kinase inhibitor Staurosporine was used as a positive control. As shown in Table 1, most of the compounds showed weak AKT, ABL, ALK and EGFR inhibition, while the exceptions were compounds **5e, 5i** and **5k4** which possessed remarkable potency against AKT and ABL kinases (the corresponding inhibition rates (%) were 56.39, 100, 64.63 to AKT and 62.60, 97.42, 68.17 to ABL). The IC_{50} values of compound **5i** against AKT and ABL were further determined, which were 1.26 and 1.50 μM, respectively. Comparing the data, we obtained some basic structure–activity relationships (SAR) as the following: (1) The size and shape of R_1 group may influence the protein kinase inhibitory activity: **a**. The phenyl group of R_1 contributed more to the activity rather than bigger substitutes (such as 1-naphthyl and 2-naphthyl groups) or smaller substitutes (such as methyl group). For example, compounds **5a**, **5d** and **5h** showed less inhibition activities against AKT and ABL than compounds **5c**, **5e**, **5i** and **5k**. **b**. Compounds containing electron-withdrawing groups (for instance, -NO_2/-Cl group of **5i**/**5e**) attached to the phenyl ring of R_1 displayed much more inhibitory performances against AKT and ABL than compounds with electron-donating groups (for instance, -OMe/-Me group of **5b**/**5c**). (2) The sulfuryl structure may be critical to the activity. When the sulfuryl structure was replaced by carbonyl, compound **5i**, the best potency compound, almost lost its kinase inhibition activity (compared with **5l**). (3) Comparing compounds **5i** and **5i1-5i4** (or **5k** and **5k1-5k4**), if the R_2 group was changed, compounds even containing electron-withdrawing group

on R_1 almost lost AKT, ABL and EGFR kinase inhibitory efficacy, revealing that R_2 group is more important than R_1 group for the contribution to kinase inhibition.
(**Table 1** should be listed here)

4.2. In vitro antiangiogenesis assay

4.2.1. HUVEC tuber formation and rat thoracic aorta rings (TARs) assay

To further investigate the anti-cancer activity of compounds **5e, 5i** and **5k4**, HUVEC tube formation had been used first as a model to test *in vitro* angiogenesis, as vascular endothelial tube network formed *in vitro* has many similarities with capillary vessels formed *in vivo* [20-24]. While the HUVECs were plated, elongated and robust tube-like structures were well established after incubation for 6 h in the negative control group; but treatment of HUVECs with compounds **5e, 5i** and **5k4** at the concentration of 0.1, 1 and 10 μM inhibited the formation of tubular structures in different ranks. Encouragingly, compound **5i** exhibited dose-dependent inhibition of tube formation which was comparable to Pazopanib, the reference standard (see Fig. 2). But both compounds **5e** and **5k4** (data not shown) showed no obvious tuber formation inhibitory activity even at the concentration of 10 μM.

(**Fig. 2** should be listed here)

Considering that rat thoracic aorta rings (TARs) model is more close to *in vivo* condition compared with HUVEC tuber formation model in view of multi-steps involved in angiogenesis: sprouting, proliferation, migration and differentiation, TARs assay was then employed to evaluate the antiangiogenic activity of compounds **5e, 5i** and **5k4**. As predicted, compounds **5e** and **5k4** (data not shown) having weak HUVECs tuber formation inhibitory efficacy still could not reduce microvessel outgrowth at 10 μM in the TARs assay, while Compound **5i** appeared to reduce microvessel outgrowth in a concentration-dependent manner. As we can see in Fig. 3, compound **5i** demonstrated almost complete inhibition of angiogenesis at 1 μM, and comparable angiogenesis inhibitory activity to pazopanib at 0.1 μM.

(**Fig. 3** should be listed here)

4.2.2. Phosphorylation inhibition of VEGFR-2

The inhibition against the phosphorylation of VEGFR-2 was tested in cell level utilizing western blotting analysis. Compounds **5i, 5k4** and **5e** showed inhibition against the phosphorylation of VEGFR-2 to different degrees. As shown in Fig. 4, Pazopanib can inhibit the phosphorylation of VEGFR-2 at the concentration of 1 μg/mL; compound **5i** can partly inhibit the phosphorylation of VEGFR at 1 μg/mL. At the concentration of 5 μg/mL, Pazopanib and compound **5i** can completely inhibit the phosphorylation of VEGFR-2, while compound **5k4** can partly inhibit it; compound **5e** has no phosphorylation inhibition of VEGFR-2 (data not shown). The moderate VEGFR-2 inhibition activity indicated that compound **5i** could perform its antiangiogenic activity through blocking ABL-(PI3-K)/AKT signaling pathway rather

than VEGFR-2. The data demonstrated that compound **5i** would be a promising lead compound in discovery of novel antiangiogenesis agents but with moderate VEGFR-2 inhibition activity for the treatment of cancer.

(**Fig. 4** should be listed here)

4.3. Molecular docking

To further investigate the protein kinase and angiogenesis inhibitory mechanism of compound **5i**, a molecular docking study of **5i** in the ATP-binding pocket of the AKT (PDB ID: 3CQU), ABL (PDB ID: 2HYY), ALK (PDB ID: 3LCS) and VEGFR-2 (PDB ID: 2QU5) kinase domains was performed using FlexX module of SYBYL 8.0 (see Fig. 5). Compound **5i** interacted with the ATP binding pocket of the catalytic site of the four kinases mainly through hydrophobic interaction and hydrogen bonds. As shown in Fig. 5A, **5i** was nicely bound to the AKT binding domain, of which the p-nitro group formd a hydrogen bond with the crucial residue Ala230. It was consistent with the result that compound **5i** showed more potency than any other p-nitro free compound as an AKT kinase inhibitor. The –NH– on triazole ring and –OH of compound **5i** formed the other two hydrogen bonds with Glu234 and Asn279, respectively. More over, a type of face to face aromatic–aromatic (π–π stacking) interaction between the two benzene rings of compound **5i** and Phe161 was observed, which may enhance the AKT binding affinity and stability of compound **5i**. Fig. 5B showed that compound **5i** penetrated deeply into the ABL ATP binding domain with $–SO_2–$, –NH– and =N– groups forming significant hydrogen bonds with Asp381, Glu286 and Thr315 respectively. Molecular docking of compound **5i** with ALK and VEGFR-2 kinases displayed in Fig. 5C and 5D illustrated that compound **5i** could form few hydrogen bonds with amino acid side chains of the two kinases. The computational data is consistent with the *in vitro* experimental protein kinase inhibition results and supports the further investigation of this class of compounds as potential AKT and ABL kinase inhibitors.

(**Fig. 5** should be listed here)

5. Conclusions

In general, we designed and synthesized a series of 4-amino-2-(thio)phenol derivatives for the first time. In light of the biological activity assay results, it is concluded that some of these compounds are potent against AKT and ABL protein kinases, microvessel tube formation using HUVECs, endothelial sprouting of TARs. The SAR indicated that electron-withdrawing substituent at the benzene ring of R_1, the sulfuryl structure and 1*H*-1,2,4-triazole ring (R_2) contributed much to the kinase inhibitory activity. The results of kinase assay and molecular docking study exhibited that 4-amino-2-(thio)phenol derivatives are novel AKT and ABL kinases inhibitors. Among all the compounds, **5i** comprehensively showed strong inhibitory effect, which deserved further *in vitro* and *in vivo* studies on its mechanism of anti-cancer activity.

6. Experimental Part

6.1 Chemistry

Unless otherwise noted, all solvents and reagents were commercially available and used without further purification. All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60 GF-254) and visualized with UV light, or iodine vapor. IR spectra were recorded with a Nexus 470 FTIR spectrometer. Proton NMR spectrums were determined on a Brucker DRX spectrometer (600/400 MHz), δ in parts per million and J in Hertz, using TMS as an internal standard. Measurements were made in DMSO-d_6 solutions. ESI-MS was determined on an API 4000 spectrometer. Melting points were determined on an electrothermal melting point apparatus and were uncorrected.

*6.1.1. N-(4-hydroxyphenyl)naphthalene-2-sulfonamide (**3a**)*

Under the condition of ice bath, 4-aminophenol hydrochloride **2** (1.46 g, 10 mmol) was dissolved in pyridine (50 mL). After 2-Naphthalenesulfonyl chloride (2.72 g, 12 mmol) was added drop by drop, the mixture was stirred at room temperature for 5 h (detected by TLC). Solvents were evaporated with the residues being taken up in EtOAc (50 mL). Then the organic layer was washed with saturated 1 N HCl (20 mL × 2), distilled water (20 mL × 2), brine (20 mL × 2), dried over anhydrous $MgSO_4$ and evaporated under vacuum. The residues were purified by EtOH/H_2O to give desired intermediate **3a**. Yield: 78 %. ^{1}H NMR (DMSO-d_6) δ 6.56 (d, J = 15.60 Hz, 2H, aromatic H), 6.85 (d, J = 15.60 Hz, 2H, aromatic H), 7.63 (t, J = 7.50 Hz, 1H, aromatic H), 7.68 (t, J = 7.80 Hz, H, aromatic H), 7.71 (d, J = 9.00 Hz, 1H, aromatic H), 8.00 (d, J = 3.48 Hz, 1H, aromatic H), 8.08 (t, J = 15.60 Hz, 2H, aromatic H), 8.28 (s, 1H, aromatic H), 9.27 (s, 1H, phenolic OH), 9.84 (s, 1H, aromatic NH). ESI-MS *m/z* 300.3 $[M+H]^+$.

Compounds **3b**-**3j** were synthesized following the procedure described above.

*6.1.1.1. N-(4-hydroxyphenyl)-4-methoxybenzenesulfonamide (**3b**)*: Yield: 80 %. ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 3H, OCH_3), 6.59 (d, J = 8.76 Hz, 2H, aromatic H), 6.82 (d, J = 8.76 Hz, 2H, aromatic H), 7.02 (d, J = 8.88 Hz, 2H, aromatic H), 7.57 (d, J = 8.84 Hz, 2H, aromatic H), 9.27 (s, 1H, phenolic OH), 9.56 (s, 1H, aromatic NH). ESI-MS *m/z* 280.3 $[M+H]^+$.

*6.1.1.2. N-(4-hydroxyphenyl)-4-methylbenzenesulfonamide (**3c**)*: Yield: 87 %. ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H, CH_3), 6.59 (d, J = 8.76 Hz, 2H, aromatic H), 6.83 (d, J = 8.76 Hz, 2H, aromatic H), 7.31 (d, J = 8.12 Hz, 2H, aromatic H), 7.53 (d, J = 8.20 Hz, 2H, aromatic H), 9.28 (s, 1H, phenolic OH), 9.64 (s, 1H, aromatic NH). ESI-MS *m/z* 264.3 $[M+H]^+$.

*6.1.1.3. N-(4-hydroxyphenyl)naphthalene-1-sulfonamide (**3d**)*: Yield: 68 %. ^{1}H NMR (DMSO-d_6) δ 6.52 (d, J = 8.84 Hz, 2H, aromatic H), 6.74 (d, J = 8.80 Hz, 2H, aromatic H), 7.55 (t, J = 7.80 Hz, 1H, aromatic H), 7.64-7.74 (m, 2H, aromatic H), 8.72 (d, J = 8.52 Hz, 2H, aromatic H), 9.23 (s, 1H, phenolic OH), 10.09 (s, 1H, aromatic NH). ESI-MS *m/z* 300.3 $[M+H]^+$.

*6.1.1.4. 4-chloro-N-(4-hydroxyphenyl)benzenesulfonamide (**3e**)*: Yield: 75 %. ^{1}H NMR (DMSO-d_6) δ 6.61 (d, J = 8.80 Hz, 2H, aromatic H), 6.82 (d, J = 8.76 Hz, 2H,

aromatic H), 7.59-7.64 (m, 4H, aromatic H), 9.35 (s, 1H, phenolic OH), 9.80 (s, 1H, aromatic NH). ESI-MS *m/z* 284.5 $[M+H]^+$.

*6.1.1.5. 2,6-dichloro-N-(4-hydroxyphenyl)benzenesulfonamide (**3f**)*: Yield: 79 %. ^{1}H NMR (DMSO-d_6) δ 6.61 (d, J = 6.84 Hz, 2H, aromatic H), 6.90 (d, J = 6.88 Hz, 2H, aromatic H), 7.51 (t, J = 7.96 Hz, 1H, aromatic H), 7.59 (d, J = 7.36 Hz, 2H, aromatic H), 9.34 (s, 1H, phenolic OH), 10.21 (s, 1H, aromatic NH). ESI-MS *m/z* 318.4 $[M+H]^+$.

*6.1.1.6. N-(4-hydroxyphenyl)-2,4,6-trimethylbenzenesulfonamide (**3g**)*: Yield: 65 %. ^{1}H NMR (DMSO-d_6) δ 2.21 (s, 3H, CH_3), 2.50 (s, 6H, 2 × CH_3), 6.59 (d, J = 9.00 Hz, 2H, aromatic H), 6.76 (d, J = 8.40 Hz, 2H, aromatic H), 6.95 (s, 1H, aromatic H), 9.30 (s, 1H, phenolic OH), 9.49 (s, 1H, aromatic NH). ESI-MS *m/z* 292.3 $[M+H]^+$.

*6.1.1.7. N-(4-hydroxyphenyl)methanesulfonamide (**3h**)*: Yield: 68 %. ^{1}H NMR (DMSO-d_6) δ 2.84 (s, 3H, CH_3), 6.72 (d, J = 13.14 Hz, 2H, aromatic H), 7.03 (d, J = 13.08 Hz, 2H, aromatic H), 9.17 (s, 1H, phenolic OH), 9.36 (s, 1H, aromatic NH). ESI-MS *m/z* 188.4 $[M+H]^+$.

*6.1.1.8. N-(4-hydroxyphenyl)-4-nitrobenzenesulfonamide (**3i**)*: Yield: 88 %. ^{1}H NMR (DMSO-d_6) δ 6.63 (d, J = 9.16 Hz, 2H, aromatic H), 6.84 (d, J = 8.72 Hz, 2H, aromatic H), 7.88 (d, J = 8.84 Hz, 2H, aromatic H), 8.36 (d, J = 13.20 Hz, 2H, aromatic H), 9.40 (s, 1H, phenolic OH), 10.06 (s, 1H, aromatic NH). ESI-MS *m/z* 295.3 $[M+H]^+$.

*6.1.1.9. N-(4-hydroxyphenyl)-2,4,6-triisopropylbenzenesulfonamide (**3j**)*: Yield: 75 %. ^{1}H NMR (DMSO-d_6) δ 1.06 (d, J = 10.08 Hz, 12H, 4 × -CH_3), 1.17 (d, J = 10.38 Hz, 6H, 2 × CH_3), 6.62 (d, J = 13.08 Hz, 2H, aromatic H), 6.82 (d, J = 13.08 Hz, 2H, aromatic H), 7.15 (s, 2H, aromatic H), 9.32 (s, 1H, phenolic OH), 9.51 (s, 1H, aromatic NH). ESI-MS *m/z* 376.4 $[M+H]^+$.

*6.1.1.10. N-(4-hydroxyphenyl)-4-nitrobenzamide (**3k**)*: Under the condition of ice bath, 4-aminophenol hydrochloride **2** (1.46 g, 10 mmol) was added to a solution of $NaHCO_3$ (2.52 g, 30mmol) in THF/H_2O (50 mL/1mL). After 4-nitrobenzoyl chloride (2.23 g, 12 mmol) was added drop by drop, the mixture was stirred at room temperature for 5 h (detected by TLC) [25]. Solvents were evaporated with the residues being taken up in EtOAc (50 mL). Then the organic layer was washed with saturated 1 N HCl (20 mL × 2), distilled water (20 mL × 2), brine (20 mL × 2), dried over anhydrous $MgSO_4$ and evaporated under vacuum. The residues were purified by EtOH/H_2O to give 1.91 g of desired compound **3k** as yellow solid. Yield: 74 %. ^{1}H NMR (DMSO-d_6) δ 6.66 (d, J = 6.76 Hz, 2H, aromatic H), 6.92 (d, J = 6.76 Hz, 2H, aromatic H), 7.84 (d, J = 7.64 Hz, 2H, aromatic H), 8.07 (d, J = 7.64 Hz, 2H, aromatic H), 9.43 (s, 1H, phenolic OH), 10.15 (s, 1H, aromatic NH). ESI-MS *m/z* 259.4 $[M+H]^+$.

*6.1.2. N-(4-oxocyclohexa-2,5-dien-1-ylidene)naphthalene-2-sulfonamide (**4a**)*: To a solution of compounds **3a** (2.99 g, 10.0 mmol) in 200 mL of CH_2Cl_2, was added 22 g (15 mmol) of $NaIO_4/SiO_2$(0.68mmol of NaIO4/1g of SiO_2) [18]. After stirring at room temperature for 2 h, the solid particles were filtered from the solution and washed with 20 mL of CH_2Cl_2, and the filtrates were evaporated in vacuum to obtain reddish-brown crude compound **4a** which was then purified by recrystallization using

EtOAc. Yield: 89 %. ^{1}H NMR (DMSO-d_6) δ 6.83-6.92 (m, 2H, aromatic H), 7.73 (d, J = 5.60 Hz, 1H, aromatic H), 7.78 (d, J = 5.60 Hz, 1H, aromatic H), 7.89 (d, J = 5.60 Hz, 1H, aromatic H), 7.99 (d, J = 6.00 Hz, 1H, aromatic H), 8.03 (d, J = 5.60 Hz, 1H, aromatic H), 8.10-8.15 (m, 3H, aromatic H), 8.44 (s, 1H, aromatic H). ESI-MS *m/z* 298.3 $[M+H]^+$.

Compounds **4b**-**4k** were synthesized following the procedure described above.

*6.1.2.1. 4-methoxy-N-(4-oxocyclohexa-2,5-dien-1-ylidene)benzenesulfonamide (**4b**)*: Yield: 91 %. ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 3H, OC$\underline{H}_3$), 6.66-6.70 (m, 2H, aromatic H), 6.98 (dd, J = 10.52 Hz, 2.24 Hz, 1H, aromatic H), 7.04 (d, J = 8.96 Hz, 2H, aromatic H), 7.95 (d, J = 7.08 Hz, 2H, aromatic H), 8.23 (dd, J = 10.20 Hz, 2.24 Hz, 1H, aromatic H). ESI-MS *m/z* 278.4 $[M+H]^+$.

*6.1.2.2. 4-methyl-N-(4-oxocyclohexa-2,5-dien-1-ylidene)benzenesulfonamide (**4c**)*: Yield: 85 %. ^{1}H NMR (DMSO-d_6) δ 2.46 (s, 3H, C$\underline{H}_3$), 6.69 (d, J = 11.40 Hz, 2H, aromatic H), 6.98 (dd, J = 10.56 Hz, 3.00 Hz, 1H, aromatic H), 7.38 (d, J = 8.16 Hz, 2H, aromatic H), 7.90 (d, J = 8.28 Hz, 2H, aromatic H), 8.21 (dd, J = 10.08 Hz, 3.08 Hz, 2H, aromatic H). ESI-MS *m/z* 262.3 $[M+H]^+$.

*6.1.2.3. N-(4-oxocyclohexa-2,5-dien-1-ylidene)naphthalene-1-sulfonamide (**4d**)*: Yield: 78 %. ^{1}H NMR (DMSO-d_6) δ 6.66-6.73 (m, 2H, aromatic H), 6.95 (dd, J = 10.04 Hz, 2.68 Hz, 1H, aromatic H), 7.58-7.70 (m, 3H, aromatic H), 7.97 (d, J = 7.60 Hz, 1H, aromatic H), 8.15 (d, J = 8.24 Hz, 1H, aromatic H), 8.28 (dd, J = 10.24 Hz, 2.68 Hz, 1H, aromatic H), 8.39 (dd, J = 7.36 Hz, 1.12 Hz, 1H, aromatic H), 8.56 (d, J = 8.64 Hz, 1H, aromatic H). ESI-MS *m/z* 298.3 $[M+H]^+$.

*6.1.2.4. 4-chloro-N-(4-oxocyclohexa-2,5-dien-1-ylidene)benzenesulfonamide (**4e**)*: Yield: 81 %. ^{1}H NMR (DMSO-d_6) δ 6.69-6.73 (m, 2H, aromatic H), 6.98 (dd, J = 10.52 Hz, 2.28 Hz, 1H, aromatic H), 7.56 (d, J = 8.64 Hz, 2H, aromatic H), 7.96 (d, J = 8.64 Hz, 2H, aromatic H), 8.16 (dd, J = 10.20 Hz, 2.28 Hz, 1H, aromatic H). ESI-MS *m/z* 282.4 $[M+H]^+$.

*6.1.2.5. 2,6-dichloro-N-(4-oxocyclohexa-2,5-dien-1-ylidene)benzenesulfonamide (**4f**)*: Yield: 79 %. ^{1}H NMR (DMSO-d_6) δ 6.73 (t, J = 11.78 Hz, 2H, aromatic H), 7.06 (d, J = 9.72 Hz, 1H, aromatic H), 7.41-7.45 (m, 1H, aromatic H), 7.50-7.52 (m, 2H, aromatic H), 8.11 (d, J = 10.28 Hz, 1H, aromatic H). ESI-MS *m/z* 316.4 $[M+H]^+$.

*6.1.2.6. 2,4,6-trimethyl-N-(4-oxocyclohexa-2,5-dien-1-ylidene)benzenesulfonamide (**4g**)*: Yield:87 %. ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3H, C$\underline{H}_3$), 2.62 (s, 6H, 2 × -C$\underline{H}_3$), 6.64-6.69 (m, 2H, aromatic H), 6.98 (d, J = 9.60 Hz, 3H, aromatic H), 8.17 (d, J = 10.80 Hz, 1H, aromatic H). ESI-MS *m/z* 290.3 $[M+H]^+$.

*6.1.2.7. N-(4-oxocyclohexa-2,5-dien-1-ylidene)methanesulfonamide (**4h**)*: Yield: 83 %. ^{1}H NMR (DMSO-d_6) δ 3.29 (s, 3H, C$\underline{H}_3$), 6.65 (dd, J = 10.36 Hz, 2.20 Hz, 1H, aromatic H), 6.74 (dd, J = 10.04 Hz, 2.96 Hz, 1H, aromatic H), 7.02 (dd, J = 10.04 Hz, 2.64 Hz, 1H, aromatic H), 7.96 (dd, J = 10.40 Hz, 2.64 Hz, 1H, aromatic H). ESI-MS *m/z* 186.4 $[M+H]^+$.

*6.1.2.8. 4-nitro-N-(4-oxocyclohexa-2,5-dien-1-ylidene)benzenesulfonamide (**4i**)*: Yield: 68 %. 6.75 (d, J = 10.36 Hz, 2H, aromatic H), 6.99 (dd, J = 10.60 Hz, 2.96 Hz, 1H, aromatic H), 8.12 (dd, J = 10.00 Hz, 2.96 Hz, 1H, aromatic H), 8.22 (d, J = 8.92 Hz, 2H, aromatic H), 8.44 (d, J = 8.88 Hz, 2H, aromatic H). ESI-MS *m/z* 293.4 $[M+H]^+$.

*6.1.2.9. 2,4,6-triisopropyl-N-(4-oxocyclohexa-2,5-dien-1-ylidene)benzenesulfonamide (**4j**)*: Yield: 77 %. ^{1}H NMR (DMSO-d_6) δ 1.27 (s, 12H, 4 × C$\underline{H}_3$), 1.29 (s, 6H, 2 × C$\underline{H}_3$), 6.67 (dd, J = 10.36 Hz, 2.20 Hz, 1H, aromatic H), 6.71 (dd, J = 10.04 Hz, 2.16 Hz, 1H, aromatic H), 7.00 (dd, J = 9.92 Hz, 2.60 Hz, 1H, aromatic H), 8.15 (dd, J = 10.36 Hz, 2.60 Hz, 1H, aromatic H). ESI-MS *m/z* 374.5 [M+H] $^{+}$.

*6.1.2.10. 4-nitro-N-(4-oxocyclohexa-2,5-dien-1-ylidene)benzamide (**4k**)*: Yield: 71 %. ^{1}H NMR (DMSO-d_6) δ 6.71-6.79 (m, 2H, aromatic H), 7.03 (dd, J = 9.80 Hz, 2.44 Hz, 1H, aromatic H), 7.84-7.86 (m, 2H, aromatic H), 7.97-7.99 (m, 1H, aromatic H), 8.12 (dd, J = 10.36 Hz, 2.44 Hz, 1H, aromatic H), 8.32-8.34 (m, 1H, aromatic H). ESI-MS *m/z* 257.3 [M+H] $^{+}$.

*6.1.3. N-(3-((1H-1,2,4-triazol-5-yl)thio)-4-hydroxyphenyl)naphthalene-2-sulfonamide (**5a**)*: To a solution of 1*H*-1,2,4-triazole-5-thiol (2.53 g, 25 mmol) in 10 mL of DMF, was added 2.97 g (10 mmol) of compounds **4a**, slowly [19]. After stirring at room temperature for 4 h, the solution was poured into 100 mL of distilled water with slight yellow solid precipitation. After standing for 20 minutes the slight yellow solid was filtered with suction, pressed to remove excess water, and allowed to dry in air. The dry residues were recrystallized with MeOH to give title compound **5a** as white or yellow powder. Yield: 90 %, mp: 206.8-207.8 □. ^{1}H NMR (DMSO-d_6) δ 6.83-6.86 (m, 1H, aromatic H), 6.91 (dd, J = 10.80 Hz, 2.40 Hz, 1H, aromatic H), 7.16 (dd, J = 10.20 Hz, 2.40 Hz, 1H, aromatic H), 7.73 (t, J = 7.20 Hz, 1H, aromatic H), 7.79 (d, J = 7.20 Hz, 1H, aromatic H), 7.98 (dd, J = 9.00 Hz, 2.40 Hz, 1H, aromatic H), 8.09-8.12 (m, 2H, aromatic H), 8.24 (d, J = 8.40 Hz, 1H, aromatic H), 8.27 (d, J = 7.80 Hz, 1H, aromatic H), 8.74 (s, 1H, aromatic N$\underline{H}$), 9.28 (s, 1H, phenolic O$\underline{H}$), 9.84 (s, 1H, aromatic N$\underline{H}$). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 115.9, 122.4, 122.6, 124.0, 128.0, 128.2, 128.3, 129.3, 129.6, 129.7, 129.8, 131.9, 134.6, 136.8, 152.4. ESI-MS *m/z* 399.2 [M+H] $^{+}$.

Compounds **5b**-**5k** were synthesized following the procedure described above.

6.1.3.1.

*N-(3-((1H-1,2,4-triazol-5-yl)thio)-4-hydroxyphenyl)-4-methoxybenzenesulfonamide (**5b**)*: Yield: 92 %, mp: 220.8-222.3 □. ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H, OC$\underline{H}_3$), 6.70-6.72 (m, 2H, aromatic H), 6.98-7.03 (m, 2H, aromatic H), 7.50-7.55 (m, 2H, aromatic H), 8.67 (s, 1H, aromatic H), 9.63 (s, 1H, aromatic N$\underline{H}$), 9.88 (s, 1H, phenolic O$\underline{H}$), 14.30 (s, 1H, aromatic N$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.5, 114.1, 115.4, 119.9, 121.8, 123.4, 128.7, 129.5, 131.0, 145.4, 151.8, 162.2. ESI-MS *m/z* 379.3 [M+H] $^{+}$.

6.1.3.2.

*N-(3-((1H-1,2,4-triazol-5-yl)thio)-4-hydroxyphenyl)-4-methylbenzenesulfonamide (**5c**)*: Yield: 82 %, mp: 227.1-229.7 □. ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 3H, C$\underline{H}_3$), 6.67-6.76 (m, 3H, aromatic H), 7.29 (d, J = 8.10 Hz, 2H, aromatic H), 7.48 (d, J = 8.10 Hz, 2H, aromatic H), 7.95 (s, 1H, aromatic H), 8.55 (s, 1H, aromatic H), 9.71 (s, 1H, phenolic O$\underline{H}$), 13.43 (s, 1H, aromatic N$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.4, 115.9, 122.3, 123.8, 127.1, 129.8, 129.9, 136.9, 140.9, 143.4, 152.3, 165.9. ESI-MS *m/z* 363.4 [M+H] $^{+}$.

6.1.3.3.

*N-(3-((1H-1,2,4-triazol-5-yl)thio)-4-hydroxyphenyl)naphthalene-1-sulfonamide (**5d**)*: Yield: 90 %, mp: 251.2-252.4 □. ^{1}H NMR (DMSO-d_6) δ 6.58-6.65 (m, 3H, aromatic H), 7.53-7.71 (m, 3H, aromatic H), 7.94 (d, J = 7.80 Hz, 1H, aromatic H), 8.06 (d, J = 9.00 Hz, 1H, aromatic H), 8.18 (d, J = 8.10 Hz, 1H, aromatic H), 8.53 (s, 1H, aromatic H), 8.63 (d, J = 7.80 Hz, 1H, aromatic H), 10.18 (s, 1H, phenolic OH), 14.24 (s, 1H, aromatic NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.3, 120.1, 121.0, 122.3, 124.3, 126.8, 127.4, 127.9, 128.9, 129.0, 129.6, 133.6, 134.1, 134.3, 146.7, 151.5. ESI-MS *m/z* 399.2 [M+H] $^{+}$.

6.1.3.4.

*N-(3-((1H-1,2,4-triazol-5-yl)thio)-4-hydroxyphenyl)-4-chlorobenzenesulfonamide (**5e**)*: Yield: 92 %, mp: 202.1-203.9 □. ^{1}H NMR (DMSO-d_6) δ 6.71-6.76 (m, 2H, aromatic H), 7.59 (s, 4H, aromatic H), 8.25 (s, 1H, aromatic H), 8.56 (s, 1H, aromatic H), 9.89 (s, 1H, aromatic NH),13.48 (s, 1H, phenolic OH), 14.17 (s, 1H, aromatic NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.5, 119.9, 122.4, 124.0, 128.5, 128.8, 129.1, 137.5, 138.1, 140.3, 165.5. ESI-MS *m/z* 383.3 [M+H] $^{+}$.

6.1.3.5.

*N-(3-((1H-1,2,4-triazol-5-yl)thio)-4-hydroxyphenyl)-2,6-dichlorobenzenesulfonamide (**5f**)*: Yield: 95 %, mp: 220.1-222.6 □. ^{1}H NMR (DMSO-d_6) δ 6.67-6.74 (m, 2H, aromatic H), 6.84 (dd, J = 8.70 Hz, 2.40 Hz, 1H, aromatic H), 7.48-7.58 (m, 3H, aromatic H), 8.62 (s, 1H, aromatic H), 10.03 (s, 1H, aromatic NH), 10.20 (s, 1H, phenolic OH), 14.26 (s, 1H, aromatic NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.9, 122.3, 123.6, 128.5, 132.1, 134.1, 134.4, 134.7, 152.6. ESI-MS *m/z* 417.3 [M] $^{+}$.

6.1.3.6.

*N-(3-((1H-1,2,4-triazol-5-yl)thio)-4-hydroxyphenyl)-2,4,6-trimethylbenzenesulfonamide (**5g**)*: Yield: 85 %, mp: 229.4-231.0 □. ^{1}H NMR (DMSO-d_6) δ 2.22 (s, 3H, CH_3), 2.39 (s, 6H, 2 × CH_3), 6.64 (s, 1H, aromatic H), 6.69 (d, J = 7.80 Hz, 2H, aromatic H), 6.93 (m, 2H, aromatic H), 7.96 (s, 1H, aromatic H), 9.58 (s, 1H, aromatic NH), 13.29 (s, 1H, phenolic OH), 13.47 (s, 1H, aromatic NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 29.5, 35.8, 116.7, 119.4, 123.4, 124.9, 125.7, 128.3, 135.5, 142.9, 146.2, 150.8, 151.4, 152.9, 154.3, 155.1. ESI-MS *m/z* 391.4 [M+H] $^{+}$.

*6.1.3.7. N-(3-((1H-1,2,4-triazol-5-yl)thio)-4-hydroxyphenyl)methanesulfonamide (**5h**)*: Yield: 94 %, mp: 204.8-205.4 □. ^{1}H NMR (DMSO-d_6) δ 2.83 (s, 3H, CH_3), 6.81-6.98 (m, 2H, aromatic H), 8.66 (s, 1H, aromatic H), 9.22 (s, 1H, aromatic H), 9.98 (s, 1H, aromatic NH), 10.18 (s, 1H, phenolic OH), 14.28 (s, 1H, aromatic NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.7, 119.9, 122.5, 124.5, 129.8, 145.2, 146.2, 152.4, 155.9. ESI-MS *m/z* 287.2 [M+H] $^{+}$.

6.1.3.8.

*N-(3-((1H-1,2,4-triazol-5-yl)thio)-4-hydroxyphenyl)-4-nitrobenzenesulfonamide (**5i**)*: Yield: 93 %, mp: 201.2-203.7 □. ^{1}H NMR (DMSO-d_6) δ 7.44 (s, 1H, aromatic H), 7.57-7.60 (m, 2H, aromatic H), 7.89-7.95 (m, 1H, aromatic H), 8.27-8.31 (m, 1H, aromatic H), 8.55 (s, 2H, aromatic H), 9.02-9.07 (m, 1H, aromatic H), 9.23-9.26 (m, 2H, aromatic H), 10.91 (s, 1H, phenolic OH), 14.25 (s, 1H, aromatic NH). ^{13}C NMR

(150 MHz, DMSO-d_6) δ: 121.5, 123.2, 123.9, 125.7, 126.0, 126.4, 127.5, 128.6, 130.7, 137.4, 147.0, 148.6, 152.3. ESI-MS *m/z* 394.2 $[M+H]^+$.

6.1.3.9.

*N-(3-((1H-1,2,4-triazol-5-yl)thio)-4-hydroxyphenyl)-2,4,6-triisopropylbenzenesulfonamide (**5j**)*: Yield: 86 %, mp: 224.2-225.7 □. ^{1}H NMR (DMSO-d_6) δ 1.04 (s, 12H, 4 × C$\underline{H}_3$), 1.17 (d, J = 6.90 Hz, 6H, 2 × C$\underline{H}_3$), 2.80-2.94 (m, 1H, aromatic H), 3.85-3.99 (m, 2H, aromatic H), 6.70-6.77 (m, 3H, aromatic H), 7.12 (s, 2H, aromatic H), 8.55 (s,1H, aromatic H), 9.56 (s, 1H, aromatic N$\underline{H}$), 9.96 (s, 1H, phenolic O$\underline{H}$), 14.24 (s,1H, aromatic N$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 23.8, 25.0, 29.3, 33.6, 115.6, 120.9, 123.7, 123.9, 125.7, 128.8, 133.3, 140.9, 145.7, 150.4, 152.4, 152.7, 153.2, 155.8. ESI-MS *m/z* 475.4 $[M+H]^+$.

*6.1.3.10. N-(3-((1H-1,2,4-triazol-5-yl)thio)-4-hydroxyphenyl)-4-nitrobenzamide (**5k**)*: Yield: 91 %, mp: 186.8-187.2 □. ^{1}H NMR (DMSO-d_6) δ 6.71 (d, J = 2.40 Hz, 1H, aromatic H), 6.75 (d, J = 8.40 Hz, 1H, aromatic H), 6.82 (d, J = 7.20 Hz, 1H, aromatic H), 7.71-7.78 (m, 2H, aromatic H), 7.82-7.88 (m, 2H, aromatic H), 7.94 (d, J = 9.00 Hz, 1H, aromatic H), 8.67 (s, 1H, aromatic H), 10.09 (s, 1H, aromatic H), 10.16 (s, 1H, phenolic O$\underline{H}$), 14.33 (s, 1H, aromatic N$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.9, 123.4, 124.9, 128.3, 129.3, 130.4, 131.7, 132.7, 133.1, 132.7, 133.1, 133.9, 134.8, 136.2, 147.6, 148.2, 152.9. ESI-MS *m/z* 358.2 $[M+H]^+$.

6.1.4.

*N-(4-hydroxy-3-((5-methyl-1,3,4-thiadiazol-2-yl)thio)phenyl)-4-nitrobenzenesulfonamide (**5i1**)*: To a solution of 5-methyl-1,3,4-thiadiazole-2-thiol (3.31 g, 25 mmol) in 10 mL of DMF, was added 2.92 g (10 mmol) of compound **4i**, slowly [19]. After stirring at room temperature for 4 h, the solution was poured into 100 mL of distilled water with slight yellow solid precipitation. After standing for 20 minutes the slight yellow solid was filtered with suction, pressed to remove excess water, and allowed to dry in air. The dry residues were recrystallized with MeOH to give title compound **5i1** as yellow powder. Yield: 93 %, mp: 184.6-185.9 □. ^{1}H NMR (DMSO-d_6) δ 2.61 (s, 3H, C$\underline{H}_3$), 6.91 (d, J = 8.40 Hz, 1H, aromatic H), 7.07 (d, J = 3.00 Hz, 1H, aromatic H), 7.11 (dd, J = 8.40 Hz, 3.00 Hz, 1H, aromatic H), 7.88 (d, J = 9.00 Hz, 2H, aromatic H), 8.37 (d, J = 9.00 Hz, 2H, aromatic H), 10.29 (s, 1H, phenolic O$\underline{H}$), 10.55 (s, 1H, aromatic N$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 116.7, 117.5, 125.0, 127.6, 128.8, 129.0, 129.7, 144.8, 150.2, 156.0, 166.6, 167.0. ESI-MS *m/z* 425.3 $[M+H]^+$.

6.1.4.1.

*N-(4-hydroxy-3-((5-methyl-1,3,4-thiadiazol-2-yl)thio)phenyl)-4-nitrobenzamide (**5k1**)*: Compound **5k1** was synthesized following the procedure described above. Yield: 90 %, mp: 164.6-166.2 □. ^{1}H NMR (DMSO-d_6) δ 2.62 (s, 3H, -C$\underline{H}_3$), 6.93 (d, J = 9.00 Hz, 1H, aromatic H), 7.13-7.16 (m, 2H, aromatic H), 7.78-7.86 (m, 3H, aromatic H), 7.96 (d, J = 7.80 Hz, 1H, aromatic H), 10.40 (s, 1H, phenolic O$\underline{H}$), 10.56 (s,1H, aromatic N$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 15.7, 116.7, 117.5, 124.9, 127.7, 128.6, 129.7, 130.5, 131.3, 132.9, 135.0, 148.3, 156.0, 166.6, 167.1, 167.2. ESI-MS *m/z* 389.4 $[M+H]^+$.

*6.1.5. N-(3-((1,3,4-thiadiazol-2-yl)thio)-4-hydroxyphenyl)-4-nitrobenzenesulfonamide (**5i2**)*: To a solution of 1,3,4-thiadiazole-2-thiol (2.95 g, 25 mmol) in 10 mL of DMF,

was added 2.92 g (10 mmol) of compound **4i**, slowly [19]. After stirring at room temperature for 4 h, the solution was poured into 100 mL of distilled water with slight yellow solid precipitation. After standing for 20 minutes the slight yellow solid was filtered with suction, pressed to remove excess water, and allowed to dry in air. The dry residues were recrystallized with MeOH to give title compound **5i2** as white powder. Yield: 89 %, mp: 240.8-241.5 □. ^{1}H NMR (DMSO-d_6) δ 6.92 (d, J = 9.00 Hz, 1H, aromatic H), 7.09 (dd, J = 9.00 Hz, 3.00 Hz, 1H, aromatic H), 7.14 (d, J = 3.00 Hz, 1H, aromatic H), 7.89 (dd, J = 7.20 Hz, 1.80 Hz, 2H, aromatic H), 8.36 (dd, J = 6.60 Hz, 1.80 Hz, 2H, aromatic H), 9.42 (s, 1H, aromatic NH), 10.31 (s, 1H, phenolic OH), 10.59 (s, 1H, aromatic NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 116.5, 117.6, 125.0, 127.9, 128.8, 129.0, 130.0, 144.8, 150.2, 155.0, 156.2, 167.9. ESI-MS *m/z* 411.2 [M+H] $^{+}$.

*6.1.5.1. N-(3-((1,3,4-thiadiazol-2-yl)thio)-4-hydroxyphenyl)-4-nitrobenzamide (**5k2**)*: Compound **5k2** was synthesized following the procedure described above. Yield: 93 %, mp: 174.2-175.6 □. ^{1}H NMR (DMSO-d_6) δ 6.95 (d, J = 9.00 Hz, 1H, aromatic H), 7.15 (dd, J = 8.40 Hz, 3.00 Hz, 1H, aromatic H), 7.18 (d, J = 2.40 Hz, 1H, aromatic H), 7.79 (dd, J = 7.20 Hz, 1.80Hz, 1H, aromatic H), 7.84 (m, 2H, aromatic H), 7.95 (dd, J = 7.80 Hz, 0.60 Hz, 1H, aromatic H), 9.45 (s, 1H, aromatic NH), 10.40 (s, 1H, phenolic OH), 10.61 (s, 1H, aromatic NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 116.5, 117.6, 124.9, 128.0, 128.6, 130.0, 131.3, 132.8, 135.0, 148.3, 155.1, 156.2, 167.8. ESI-MS *m/z* 375.2 [M+H] $^{+}$.

*6.1.6. N-(4-hydroxy-3-((1-methyl-1H-tetrazol-5-yl)thio)phenyl)-4-nitrobenzenesulfonamide (**5i3**)*: To a solution of 1-methyl-1H-tetrazole-5-thiol (2.90 g, 25 mmol) in 10 mL of DMF, was added 2.92 g (10 mmol) of compound **4i**, slowly [19]. After stirring at room temperature for 4 h, the solution was poured into 100 mL of distilled water with slight yellow solid precipitation. After standing for 20 minutes the slight yellow solid was filtered with suction, pressed to remove excess water, and allowed to dry in air. The dry residues were recrystallized with MeOH to give title compound **5i3** as white powder. Yield: 89 %, mp: 177.7-179.7 □. ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 3H, CH_3), 6.76 (d, J = 2.40 Hz, 1H, aromatic H), 6.82 (d, J = 9.00 Hz, 1H, aromatic H), 6.97 (dd, J = 8.40 Hz, 3.00 Hz, 1H, aromatic H), 7.86 (d, J = 11.40 Hz, 2H, aromatic H), 8.35 (d, J = 11.40 Hz, 2H, aromatic H), 10.22 (s, 1H, phenolic OH), 10.48 (s, 1H, aromatic NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 34.5, 115.7, 116.9, 125.0, 125.9, 126.8, 128.7, 128.9, 144.8, 150.2, 151.4, 154.5. ESI-MS *m/z* 409.4 [M+H] $^{+}$.

*6.1.6.1. N-(4-hydroxy-3-((1-methyl-1H-tetrazol-5-yl)thio)phenyl)-4-nitrobenzamide (**5k3**)*: Compound **5k3** was synthesized following the procedure described above. Yield: 92 %, mp: 134.9-136.7 □. ^{1}H NMR (DMSO-d_6) δ 3.91 (s, 3H, CH_3), 6.83 (d, J = 9.00 Hz, 1H, aromatic H), 6.86 (s, 1H, aromatic H), 7.00 (d, J = 9.00 Hz, 1H, aromatic H), 7.77-7.86 (m, 3H, aromatic H), 7.95 (d, J = 7.80 Hz, 1H, aromatic H), 10.34(s, 1H, phenolic OH), 10.48 (s, 1H, aromatic NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 34.5, 115.7, 116.8, 124.9, 125.7, 126.7, 128.6, 130.4, 131.4, 132.8, 135.1, 148.2, 151.4, 154.5. ESI-MS *m/z* 373.2 [M+H] $^{+}$.

*6.1.7. N-(3-((7H-purin-6-yl)thio)-4-hydroxyphenyl)-4-nitrobenzenesulfonamide (**5i4**)*: To a solution of 7H-purine-6-thiol (3.80 g, 25 mmol) in 10 mL of DMF, was added 2.92 g (10 mmol) of compound **4i**, slowly [19]. After stirring at room temperature for 4 h, the solution was poured into 100 mL of distilled water with slight yellow solid precipitation. After standing for 20 minutes the slight yellow solid was filtered with suction, pressed to remove excess water, and allowed to dry in air. The dry residues were recrystallized with MeOH to give title compound **5i4** as white powder. Yield: 87 %, mp: 235.6-237.3 □. ^{1}H NMR (DMSO-d_6) δ 6.87 (d, J = 9.00 Hz, 1H, aromatic H), 7.05 (dd, J = 9.00 Hz, 1.8Hz, 1H, aromatic H), 7.13 (d, J = 2.40 Hz, 1H, aromatic H), 7.93 (d, J = 8.40 Hz, 2H, aromatic H), 8.36 (d, J = 9.00 Hz, 2H, aromatic H), 8.47 (m, 2H, aromatic H), 10.10 (s, 2H, phenolic OH), 13.50 (s, 1H, aromatic NH). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 113.6, 117.0, 124.9, 126.9, 128.4, 128.8, 132.0, 144.0, 145.2, 150.1, 151.8, 157.2. ESI-MS *m/z* 445.4 [M+H] $^{+}$.

*6.1.7.1. N-(3-((7H-purin-6-yl)thio)-4-hydroxyphenyl)-4-nitrobenzamide (**5k4**):* Compound **5k4** was synthesized following the procedure described above. Yield: 91 %, mp: 135.4-136.7 □. ^{1}H NMR (DMSO-d_6) δ 6.88 (d, J = 9.00 Hz, 1H, aromatic H), 7.10 (dd, J = 8.40 Hz, 3.00 Hz, 1H, aromatic H), 7.21 (d, J = 3.00 Hz, 1H, aromatic H), 7.78-7.85 (m, 2H, aromatic H), 7.89 (dd, J = 7.80 Hz, 1.20 Hz, 1H, aromatic H), 7.97 (dd, J = 8.40 Hz, 1.20 Hz, 1H, aromatic H), 8.38-8.46 (m, 2H, aromatic H), 10.06 (s, 1H, aromatic NH), 10.34 (s, 1H, phenolic OH), 13.53 (s, 1H, aromatic NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.6, 116.9, 124.9, 126.9, 128.0, 130.5, 131.6, 132.8, 134.9, 148.3, 151.9, 157.2, 162.7. ESI-MS *m/z* 409.2 [M+H] $^{+}$.

6.2. Biological assay

6.2.1. In vitro kinase activity

The kinase selectivity of compounds (at 10 μM) was profiled by screening as follows: The test compounds, reference compound or water (control) were mixed with the appropriate enzyme in a buffer containing Hepes/Tris (pH 7.4), EGTA/Tris, $MgCl_2$, DTT and 0.008 % Tween 20. Thereafter, the reaction was initiated by adding the substrate and ATP, and the mixture was incubated for 30 min at room temperature. For control basal measurements, the enzyme was omitted from the reaction mixture. Following incubation, the reaction was stopped by adding EDTA. After 5 min, the anti-phopho-PT66 antibody labeled with europium chelate was added. After 60 more min, the fluorescence transfer was measured using a microplate reader (Envision, Perkin Elmer). The results were expressed as a percent inhibition of the control enzyme activity. The standard inhibitory reference compound was staurosporine. The experiment was performed in duplicate.

6.2.2. In vitro HUVECs tuber formation assay

Forty eight-well slide chambers were coated with 100 μL of Matrigel (BD Biosciences, NJ) and allowed to gel at 37 °C and 5 % CO_2 for 30 min. HUVECs were then seeded at 40,000 cells/well in M199 (5 % FBS) containing either the vehicle (0.5 % DMSO), pazopanib or synthesized compounds and incubated at 37 °C and 5 % CO_2 for 6 h. The morphological changes of the cells and HUVEC tubes formation

were observed under a phase-contrast microscope and photographed at × 100 magnification. Experiments were repeated three times.

6.2.3. Rat thoracic aorta rings (TARs) assay

Forty eight-well tissue culture plates were coated with 100 μL of Matrigel (BD Biosciences, NJ) and allowed to gel for 30 min at 37 °C and 5 % CO_2. Thoracic aortas were excised from 8- to 10-week-old male Sprague Dawley rats. After careful removal of fibroadipose tissues, the aortas were cut into 1-mm-long cross-sections, placed on Matrigel-coated wells, and covered with an additional 100 μL of Matrigel. After the second layer of Matrigel set, the rings were incubated for 30 min at 37 °C and 5 % CO_2. Aorta rings were treated every other day with either the vehicle (0.5 % DMSO), Pazopanib, or synthesized compounds for 6 days and photographed on the 7^{th} day at × 200 magnification. Experiments were repeated three times.

6.2.4. Docking study

Compound **5i** was docked to the active site of the modeled AKT, ABL, ALK and EGFR, respectively. Surflex dock program [26,27] in Sybyl 8.0 software was used, ten poses was generated for each ligand. The protomol was generated based on the ligand in the crystal structure. Other parameter was set referring the default values.

Acknowledgments

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Figure captions

Fig. 1. Strategy for the design of 4-amino-2-(thio)phenol derivatives.

Fig. 2. Representative images of the tube formation assay depicting the formation of a HUVEC capillary-like tubular network by treatment with synthesized compounds, Pazopanib (positive control) and 0.5 % DMSO (vehicle control).

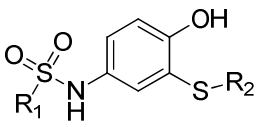
Fig. 3. Representative images of rat aortic rings treated with synthesized compounds, Pazopanib (positive control) and 0.5 % DMSO (vehicle control).

Fig. 4. Western blots showing protein expression of VEGFR-2 after treatment with Pazopanib, compounds **5i** and **5k4** at different concentrations (1 and 5 μg/mL).

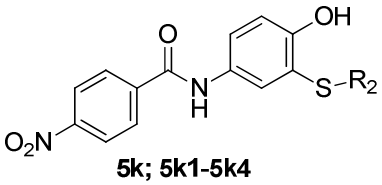
Fig. 5. Calculated binding modes of compound **5i** in the ATP-binding pocket of the AKT (A), ABL (B), ALK (C) and VEGFR-2 (D) kinase domains. The structural framework of compound **5i** was colored according to the atomic coloring scheme (S in yellow, C in green, O in red and N in blue). Hydrogen bonds were indicated by dash lines.

Table 1

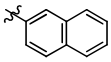
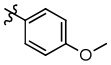
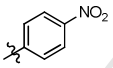
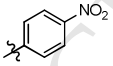
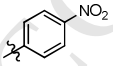
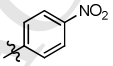
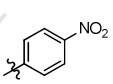
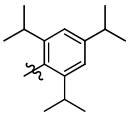
In vitro inhibitory activity against AKT, ABL ALK and EGFR



5a-5j; 5i1-5i4



5k; 5k1-5k4

Compd	R_1	R_2	Protein kinase inhibition (%) at 10 μM			
			AKT	ABL	ALK	EGFR
5a			NA[a]	13.05	NA[a]	NA[a]
5b			12.10	51.15	29.50	48.71
5c			29.79	31.39	15.33	21.26
5d			20.37	32.28	10.02	24.84
5e			56.39	62.60	NA[a]	13.02
5f			48.38	10.33	10.32	23.00
5g			19.66	14.49	17.98	15.46
5h			14.30	NA[a]	15.86	NA[a]
5i			100 (1.26 μM)[b]	97.42 (1.50 μM)[b]	49.43	55.64
5i1			14.25	NA[a]	21.81	13.37
5i2			14.87	13.35	11.37	17.36
5i3			11.48	NA[a]	NA[a]	16.70
5i4			37.45	20.68	NA[a]	26.78
5j			14.51	NA[a]	17.12	44.93

5k		50.67	36.18	NA[a]	37.84
5k1		12.35	NA[a]	13.98	17.52
5k2		NA[a]	15.92	NA[a]	19.46
5k3		10.05	19.57	NA[a]	23.74
5k4		64.63	68.17	21.91	23.50
Staurosporine		96.52	94.87	97.89	97.60

[a] Not active.

[b] IC_{50} values are indicated in the parenthesis.

Figure 1

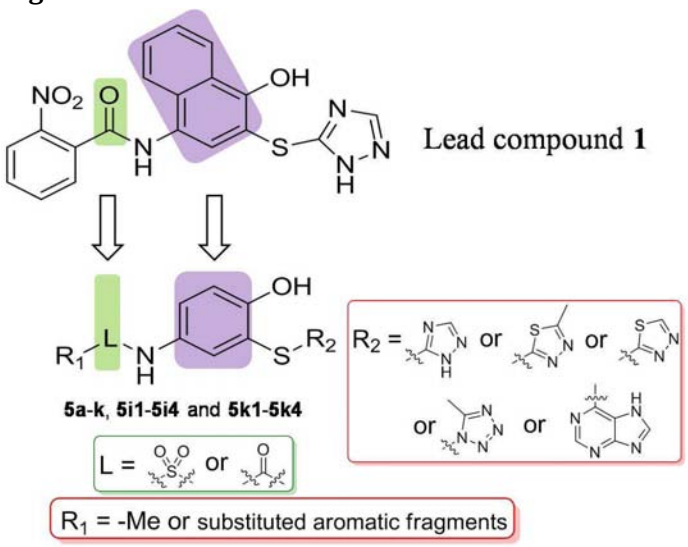


Figure 2

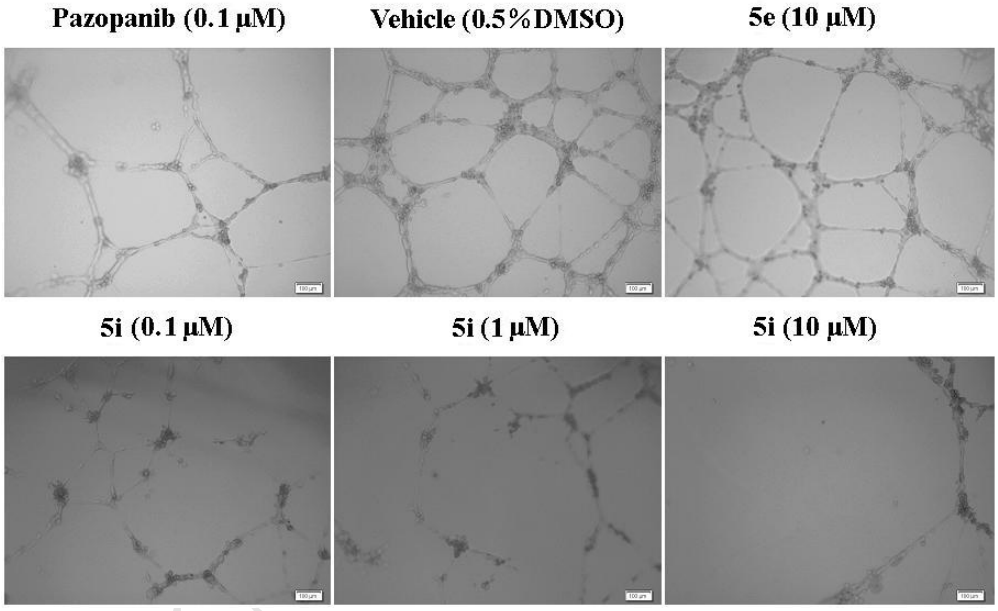


Figure 3

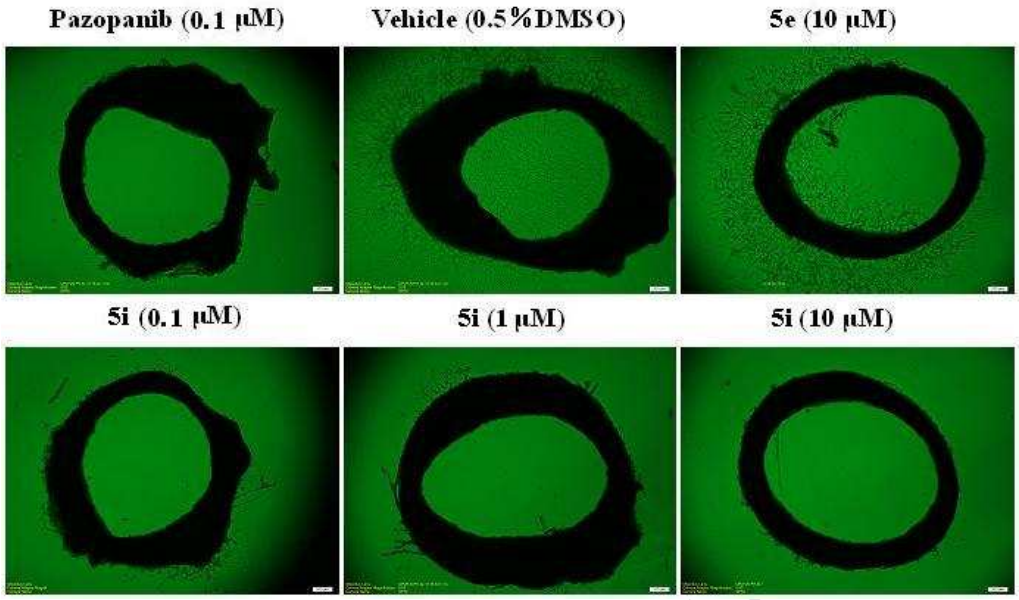


Figure 4

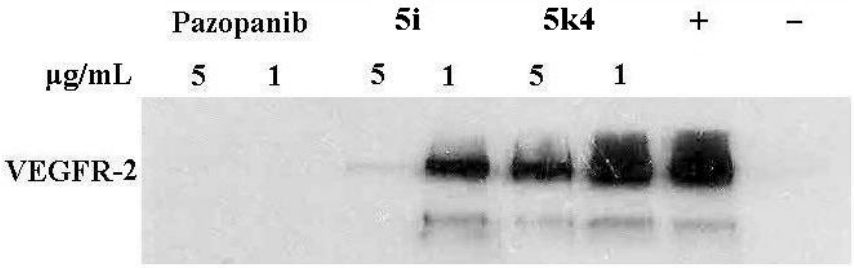
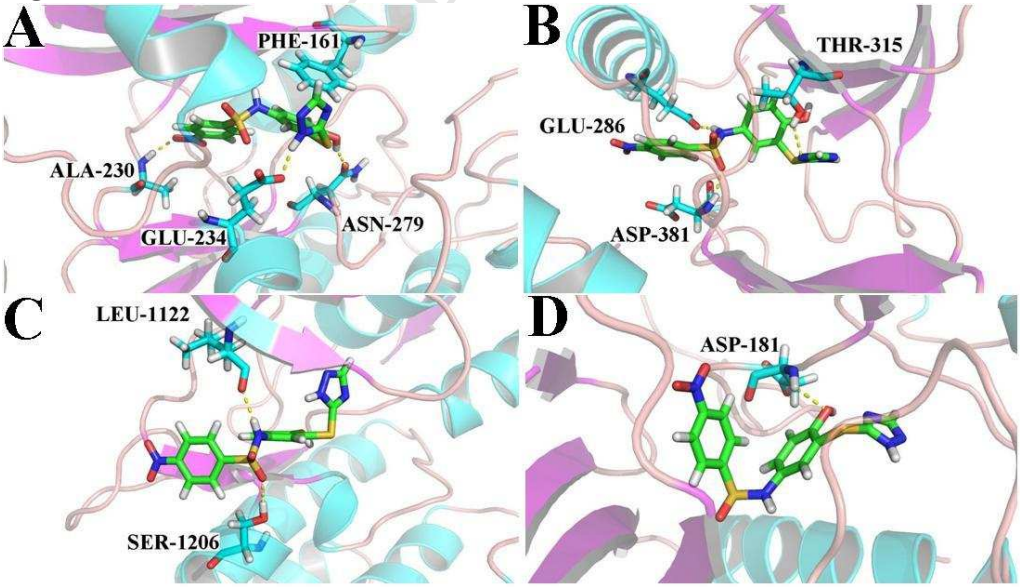
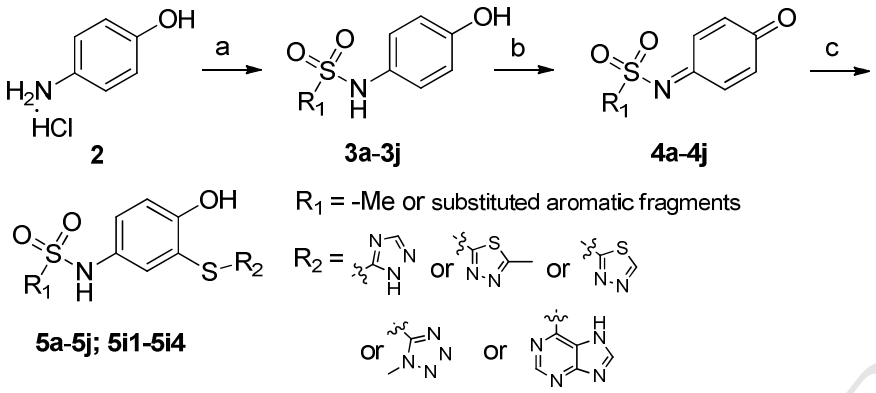
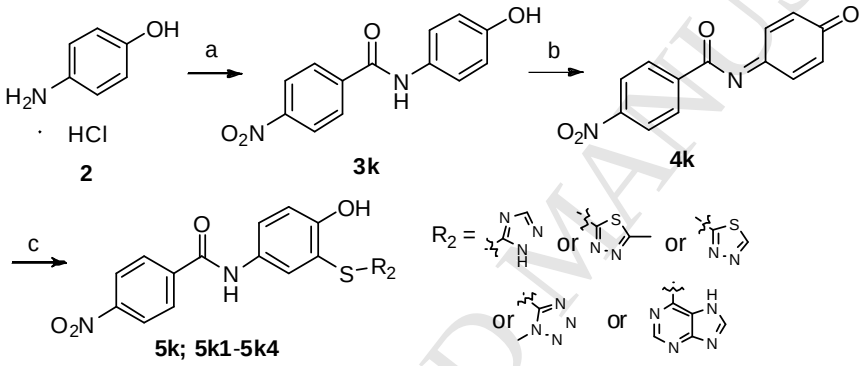


Figure 5



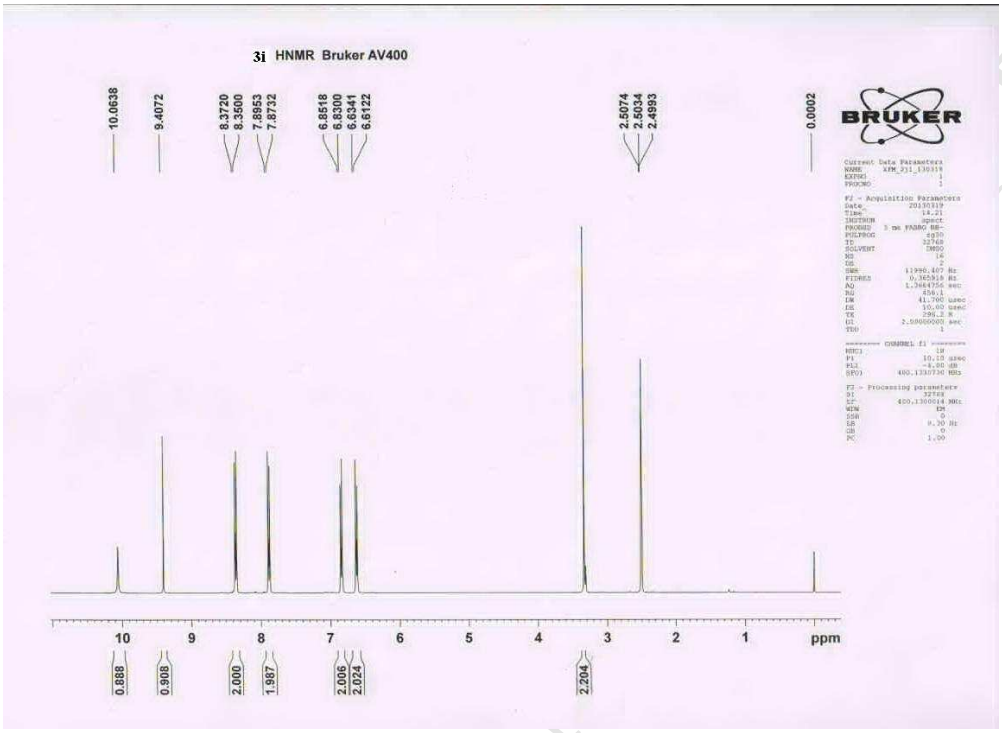


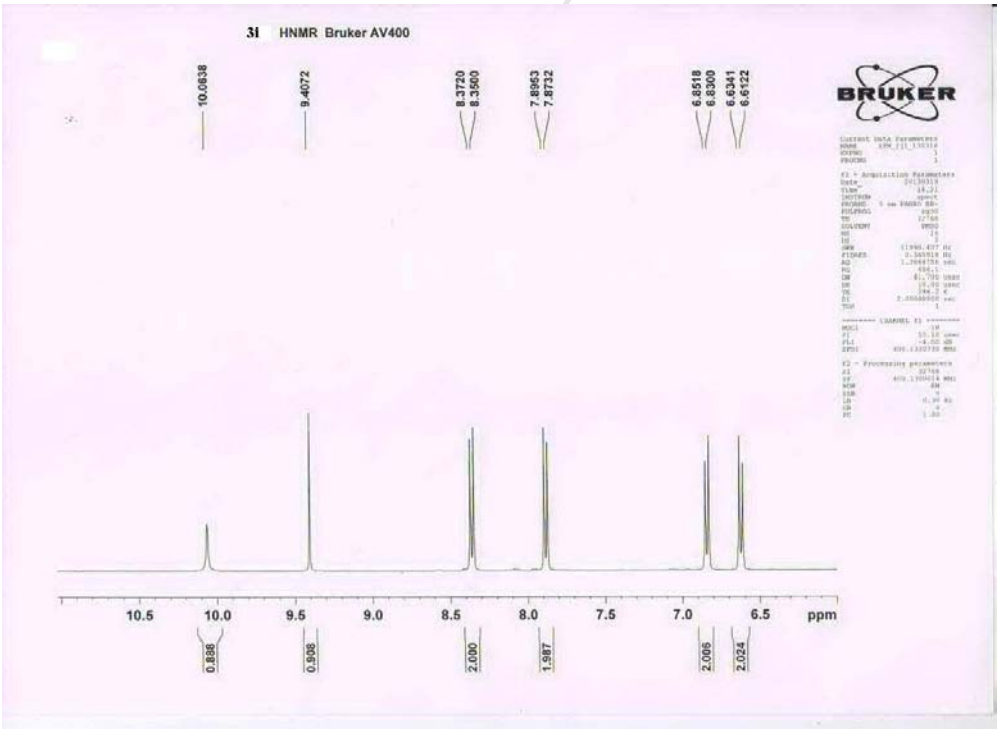
Scheme 1. Reagents and conditions: (a) Pyridine, 0□; (b) $NaIO_4/SiO_2$, DCM; (c) DMF, R_2SH.



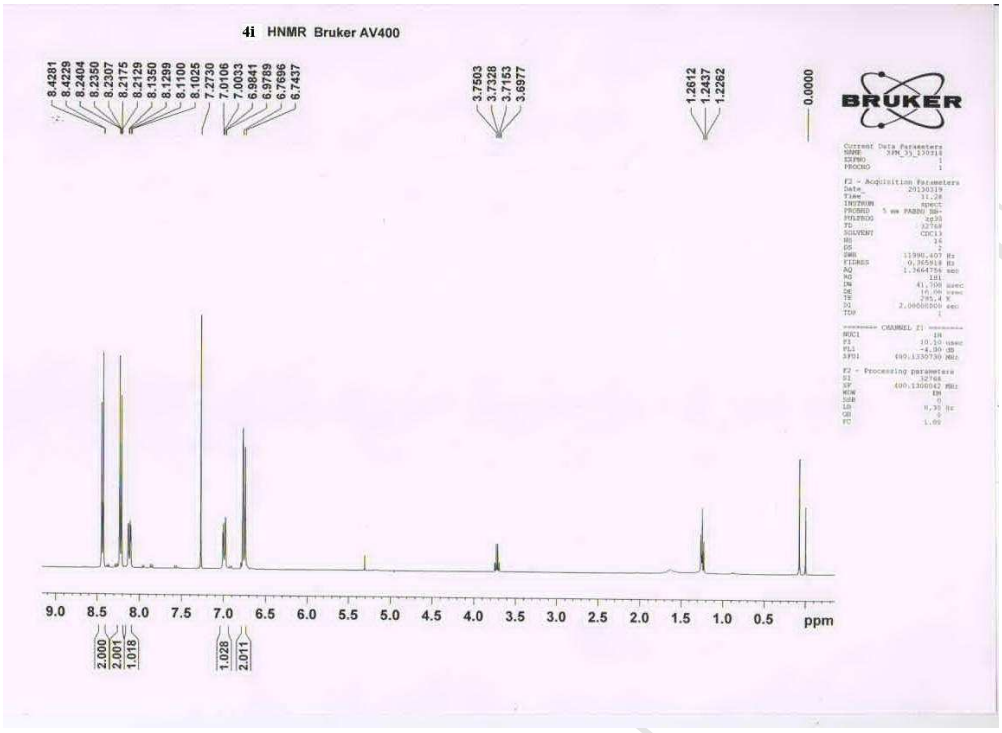
Scheme 2. Reagents and conditions: (a) THF/H_2O (50/1), $NaHCO_3$, 0□; (b) $NaIO_4/SiO_2$, DCM; (c) DMF, R_2SH.

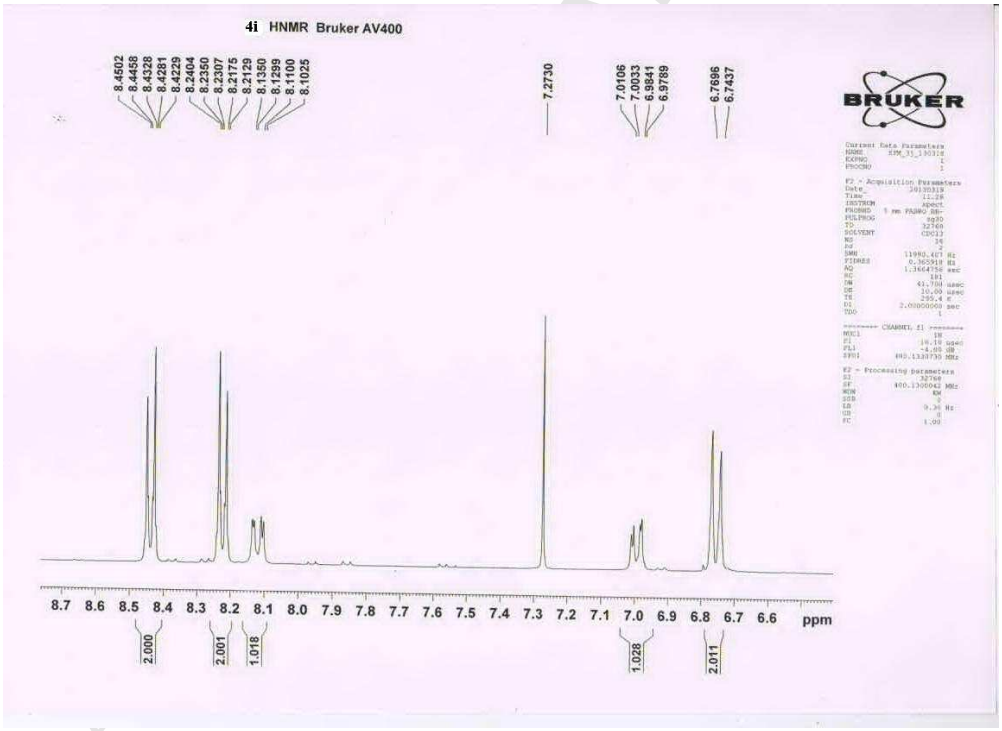
Supplementary material for publication online with representative ^{1}H and ^{13}C NMR spectra



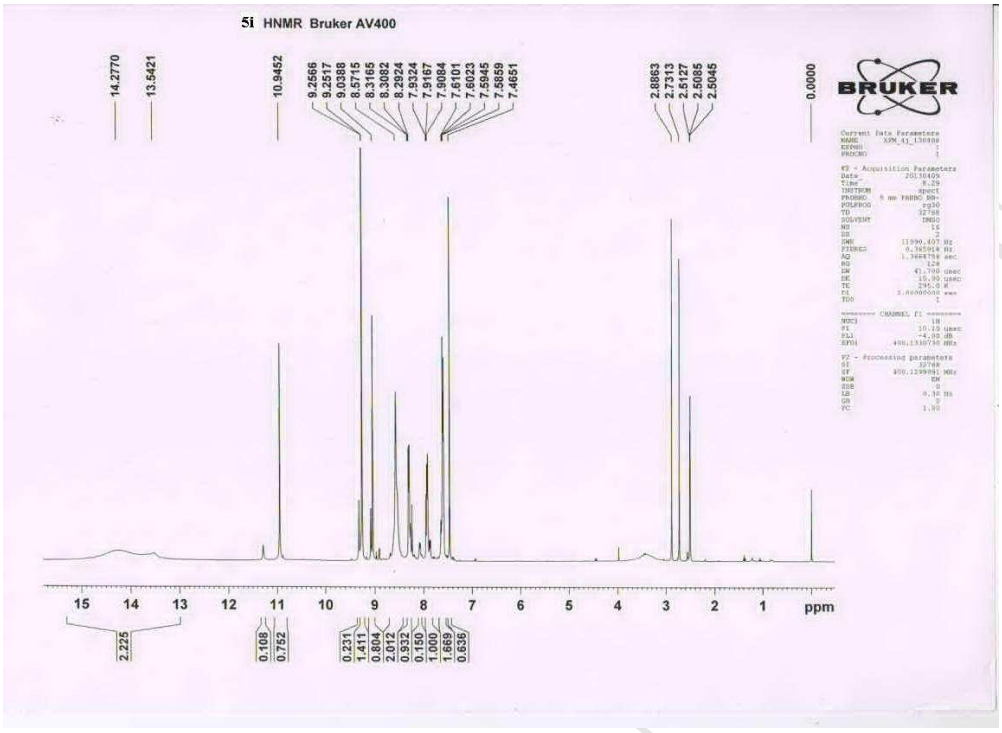


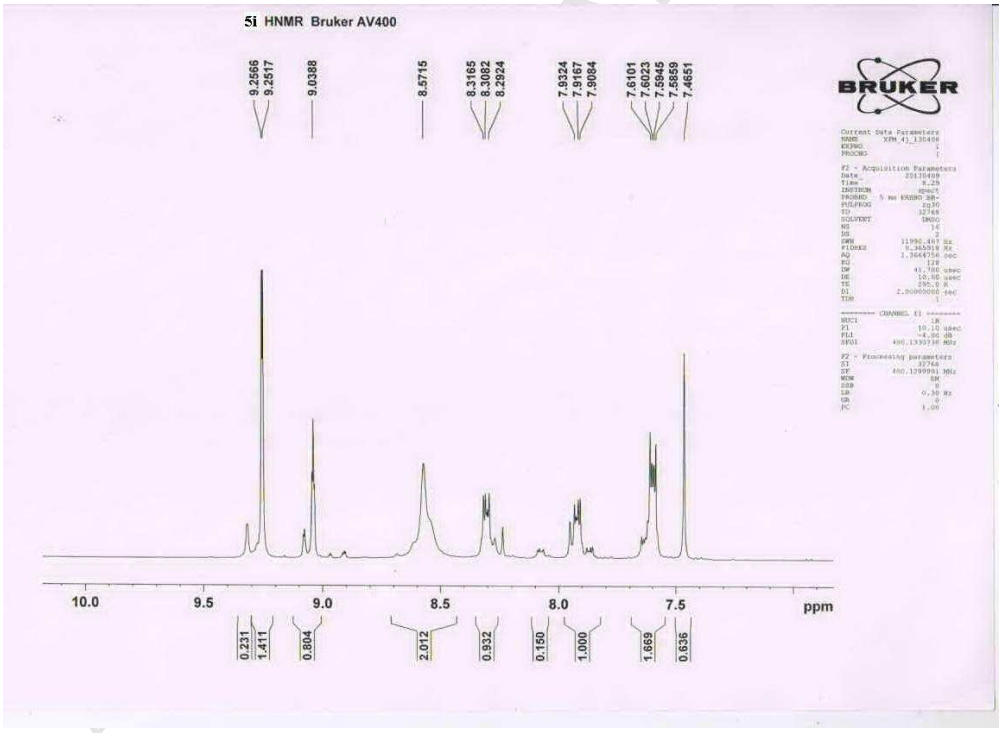
^{1}H NMR spectra of compound **3i**



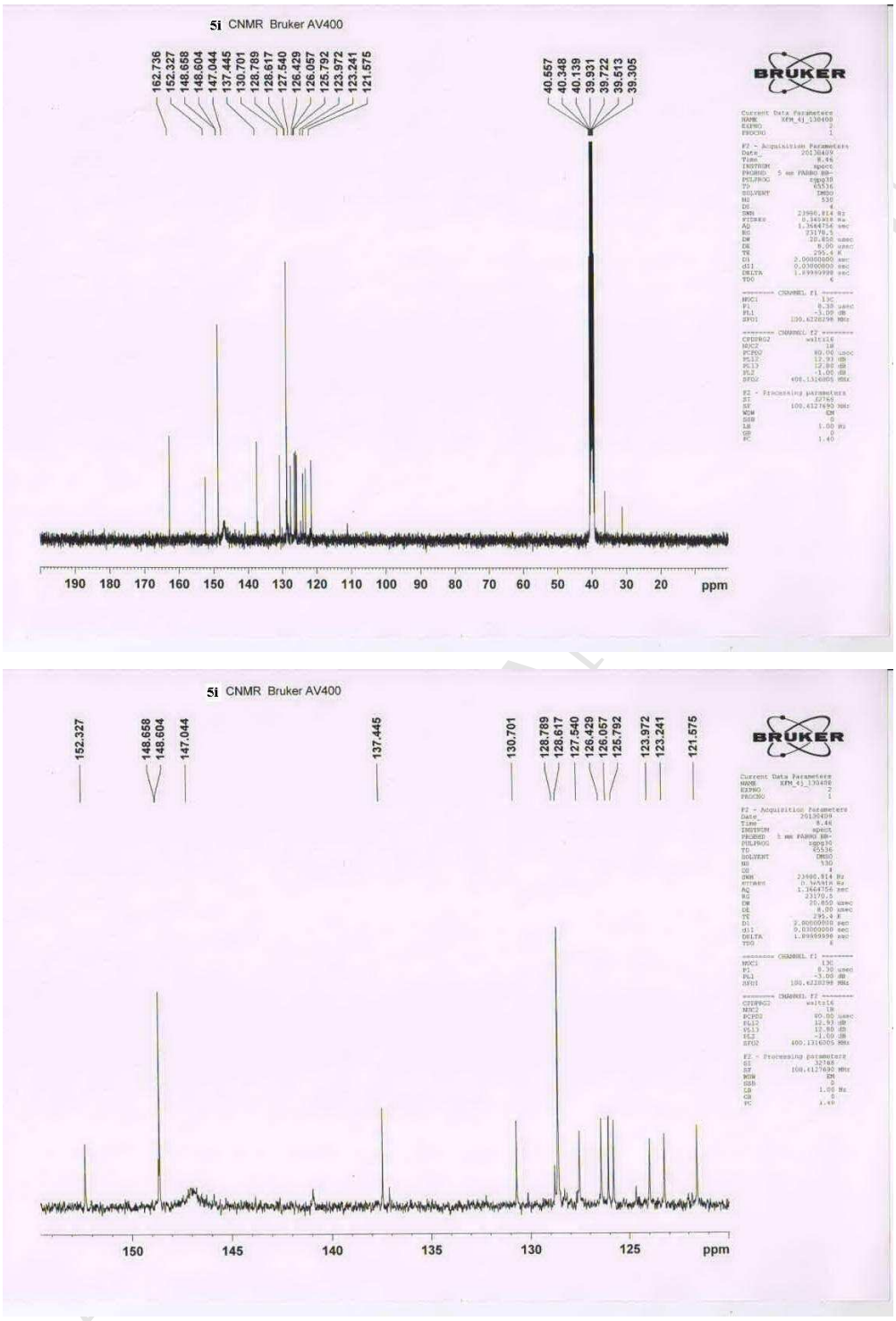


^{1}H NMR spectra of compound **4i**

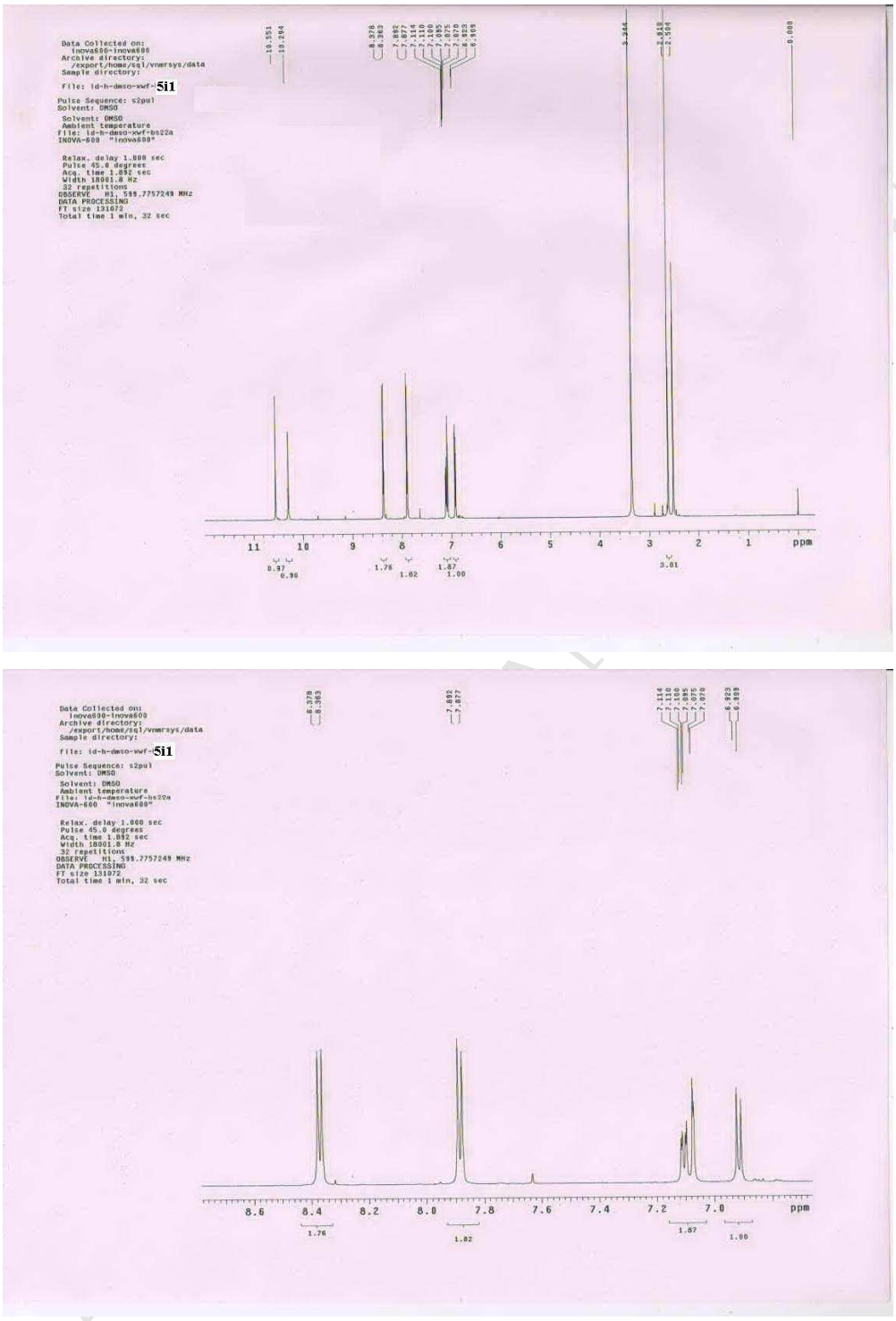




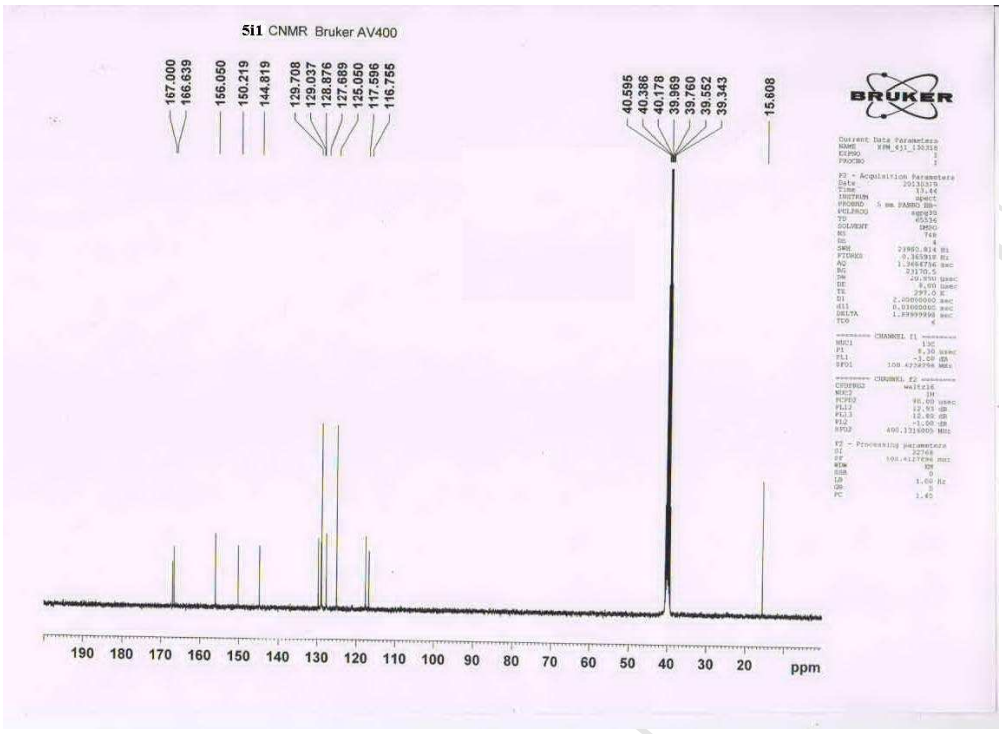
^{1}H NMR spectra of compound **5i**

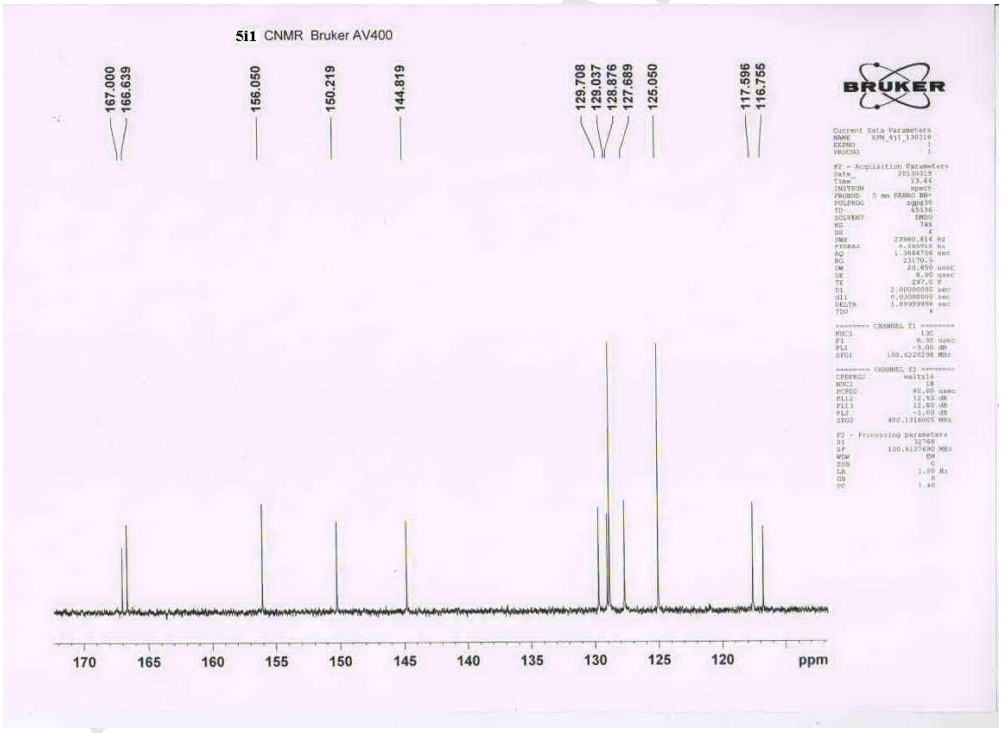


^{13}C NMR spectra of compound **5i**

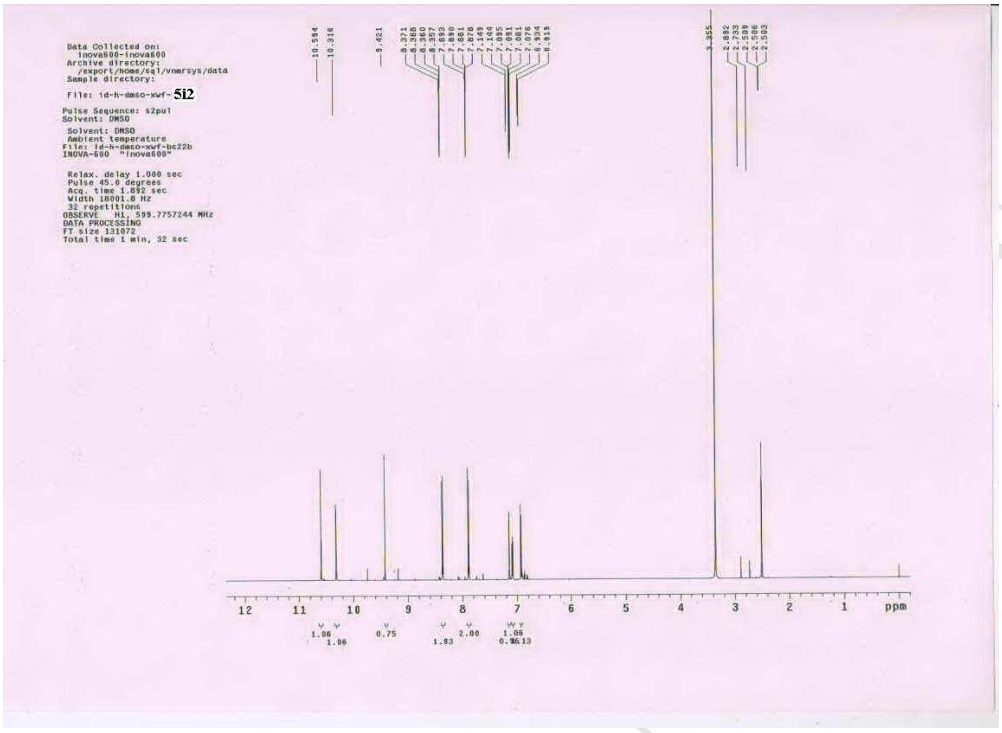


^{1}H NMR spectra of compound **5i1**

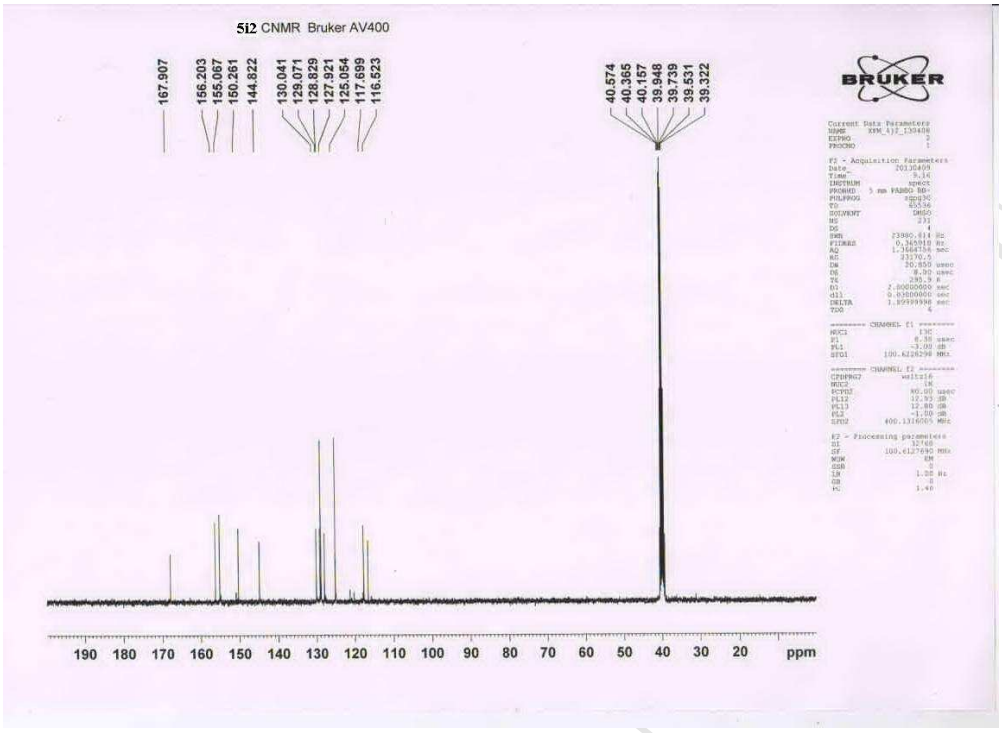


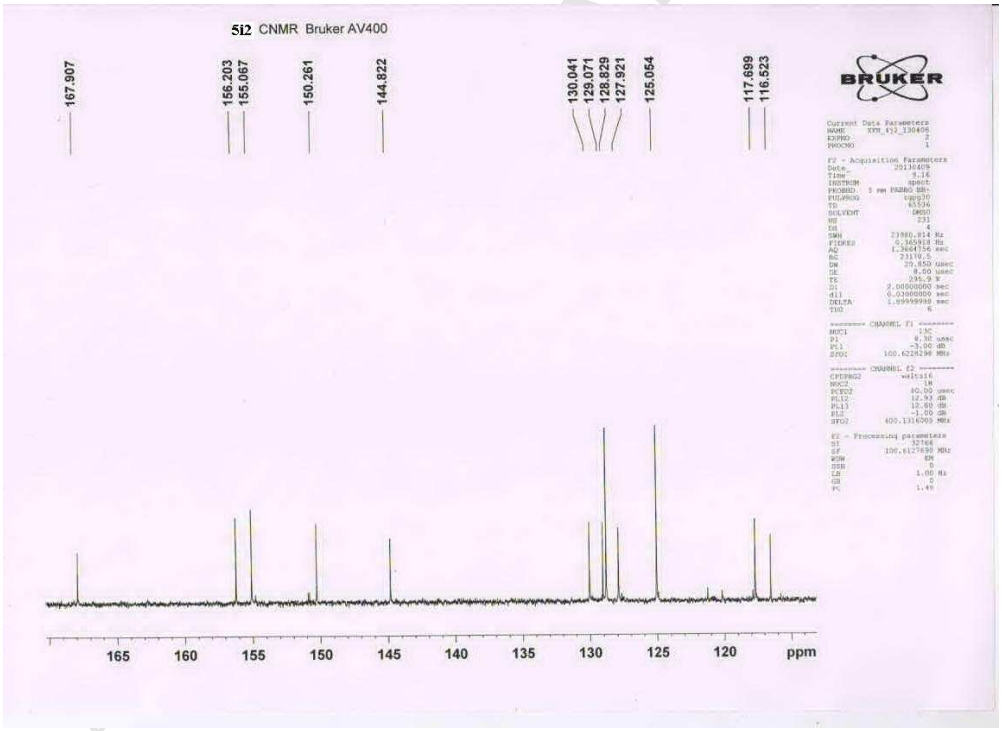


^{13}C NMR spectra of compound **5i1**

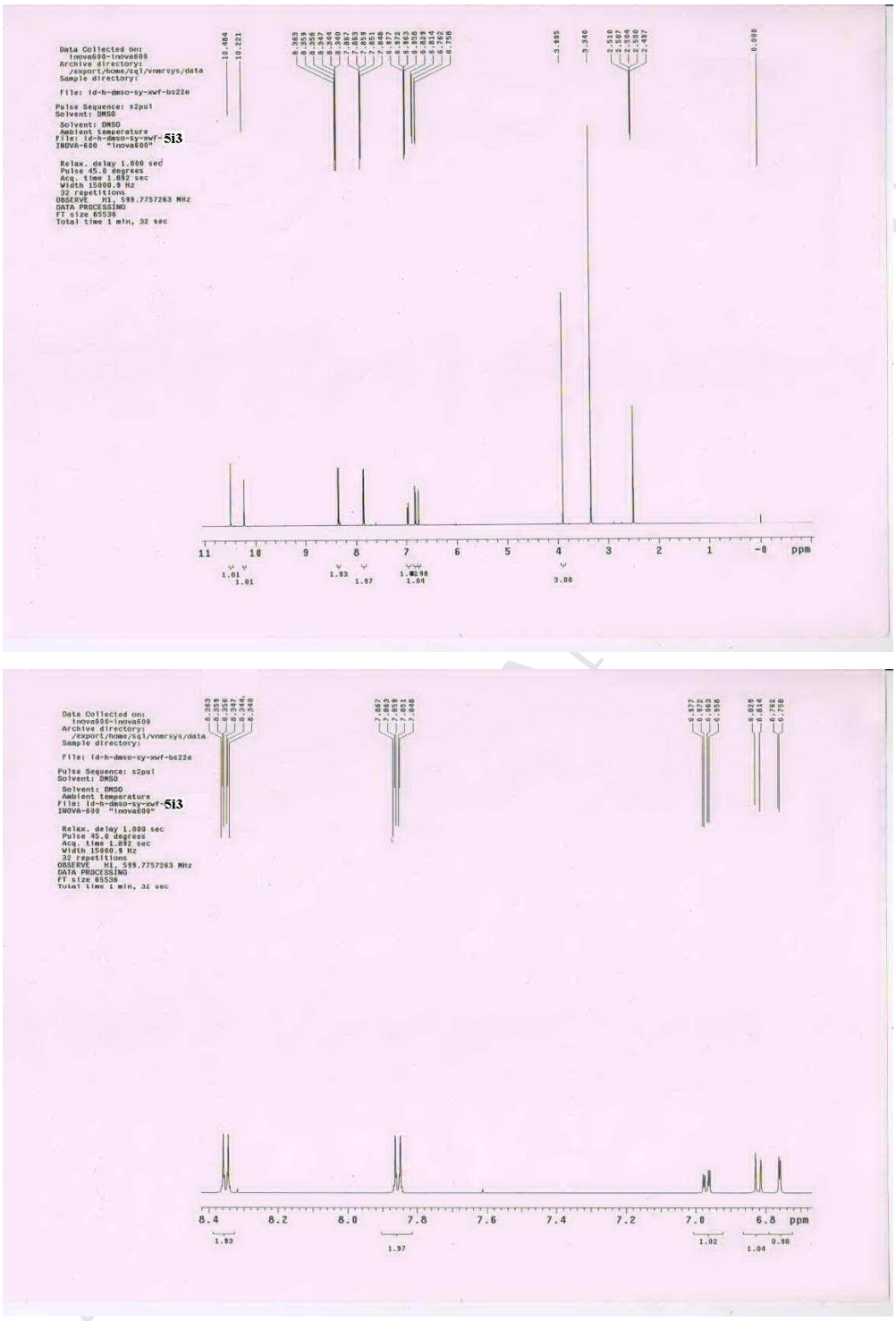


^{1}H NMR spectra of compound **5i2**

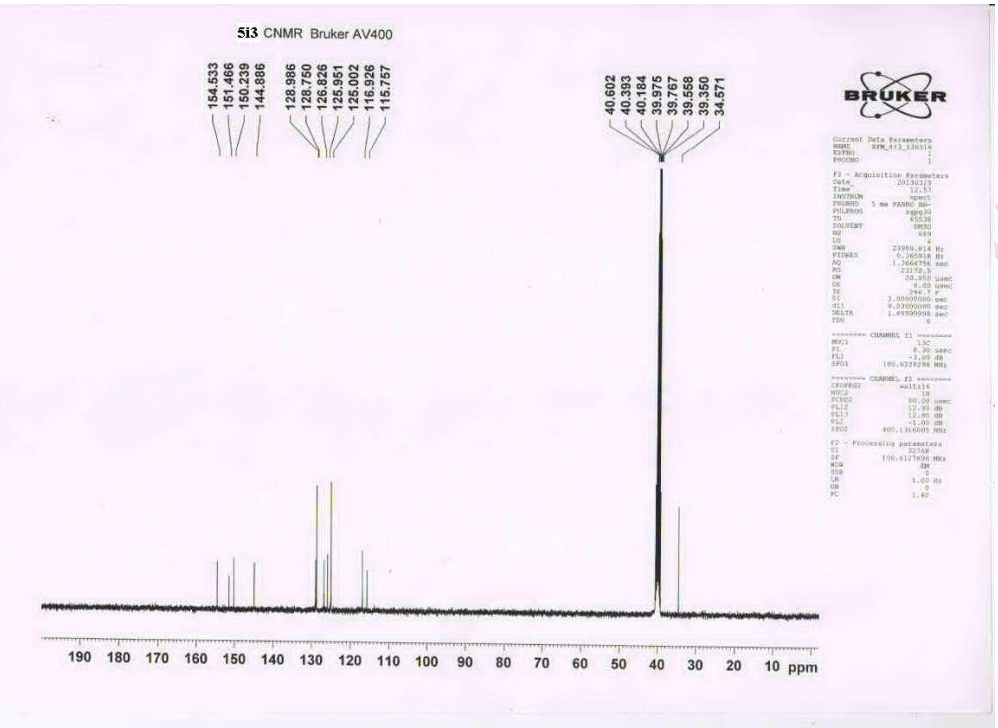




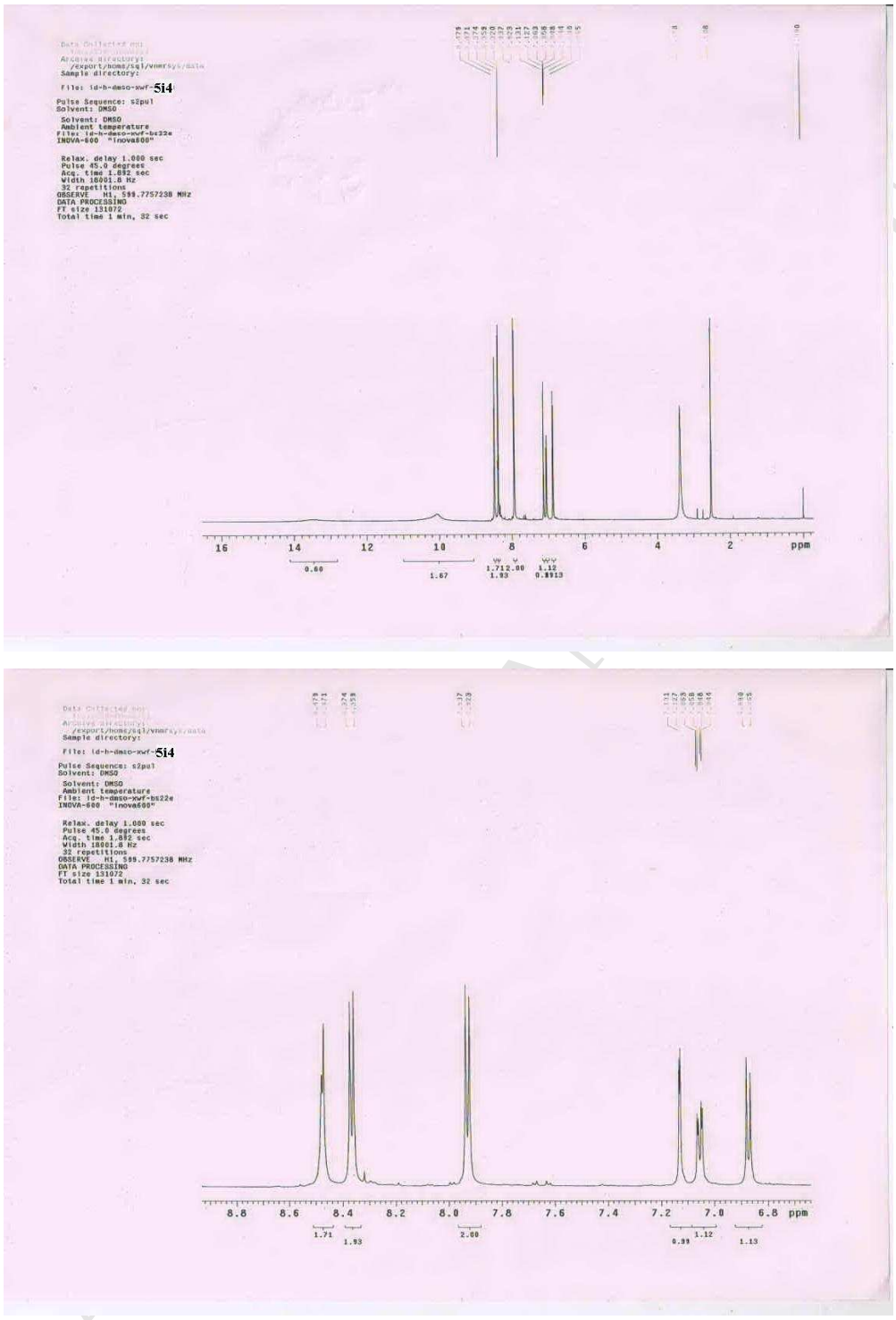
^{13}C NMR spectra of compound **5i2**



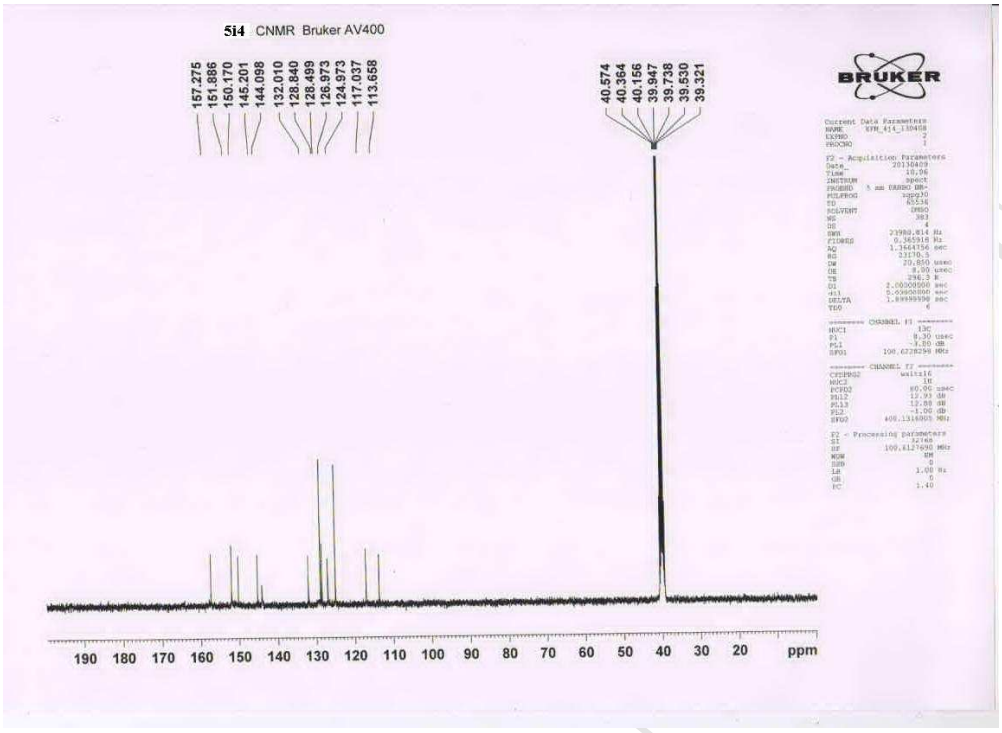
^{1}H NMR spectra of compound **5i3**

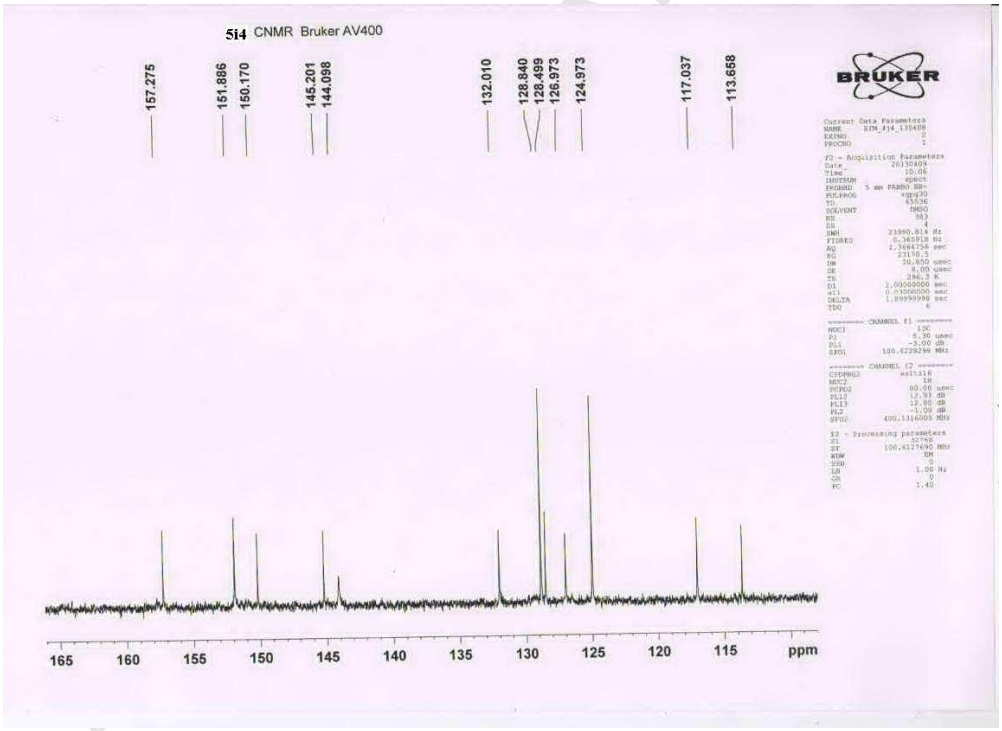


^{13}C NMR spectra of compound **5i3**



^{1}H NMR spectra of compound **5i4**





^{13}C NMR spectra of compound **5i4**

> A series of 4-amino-2-(thio)phenol derivatives were synthesized.
> The compounds were evaluated as inhibitors of protein kinase and angiogenesis.
> Some of them exhibited remarkably *in vitro* protein kinase inhibition.
> Anti-angiogenic activity of **5i** was similar to clinically used drug pazopanib.