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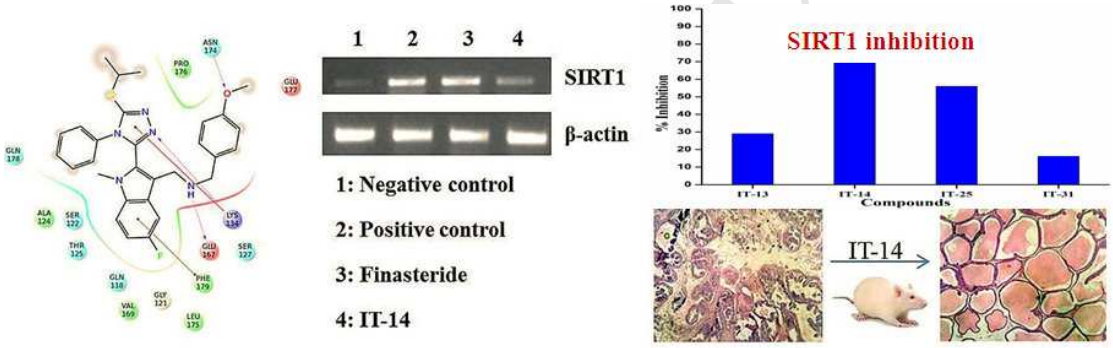
This protocol depicts the cytotoxicity and growth inhibitory activity of some indole derivatives, acting against benign prostatic hyperplasia through SIRT1 inhibition, in terms of *in vitro* and *in vivo* models.



Highlights

- 29 Molecules were screened for cytotoxicity evaluation.
- *In vivo* investigations were carried out to examine the activity of the molecule.
- Histopathology images and PCR proved the effectiveness of the molecule.
- Simple synthetic protocols were used to get a promising lead molecule.

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Abstract

In our pursuit to develop new potential anticancer leads, we designed a combination of structural units of indole and substituted triazole; and a library of 1-{1-methyl-2-[4-phenyl-5-(propan-2-ylsulfanyl)-4*H*-1,2,4-triazol-3-yl]-1*H*-indol-3-yl}methanamine derivatives were synthesized and characterized. Cytotoxic evaluations of these molecules over a panel of three human cancer cell lines were carried out. Few molecules exhibited potent growth inhibitory action against the treated cancer cell lines at lower micro molar concentration. An *in vitro* assay investigation of these active compounds using recombinant human SIRT1 enzyme showed that one of the compounds (IT-14) inhibited the deacetylation activity of the enzyme. The *in vivo* study of IT-14 exemplified its promising action by reducing the prostate weight to the body weight ratio in prostate hyperplasia animal models. A remarkable decrease in the disruption of histoarchitecture of the prostate tissues isolated from IT-14 treated animal compared to that of the positive control was observed. The molecular interactions with SIRT1 enzyme were also supported by molecular docking simulations. Hence this compound can act as a lead molecule to treat prostatic hyperplasia.

Key words: Indole, leukemia, breast cancer, prostate hyperplasia, human SIRT1.

1. Introduction

Cancer continues to be a major health problem all over the world. Apart from heart disease, cancer turns out to be one of the major killing diseases due to various worldwide factors. Numerous anticancer agents including taxol [1-3], vinblastine, vincristine, etoposide [4], camptothecin and its derivatives [5], mitoxantrone [6], 5-fluorouracil [7], indomethacin [8], cisplatin etc. are in clinical use throughout the world. However, these drugs resulted in adverse side effects like low blood pressure, bone marrow suppression, gastrointestinal toxicity, constipation, hair loss etc. Therefore, there is an urgent need for the scientific community to explore new chemical entities for an effective and safe cure of cancer.

Indole nucleus is constantly drawing interest for the development of newer drug moiety due to its wide range of pharmacological activities like antibacterial, antifungal [9], anti-malarial [10], anticonvulsant [11], anti-inflammatory [12**],** antivascular [13], ischemia/reperfusion injury [14], chronic diabetes [15], HIV inhibitors [16] and anticancer in particular. Since indole derivatives have shown quite good response as anticancer agents, structural modifications of the indole based pharmacophores, to scrutinize the pharmacological potency have been a core interesting strategy among researchers. The 3-substituted indole forms to be a main structural unit of many natural and pharmacologically active compounds, possessing various biological activities [17]. For instance, the naturally occurring molecule indole-3-carbinol (I3C) has been reported to exhibit promising anticancer activities against a number of human cancers acting through diverse mechanisms [18]. Go *et al.* have demonstrated the greater antiproliferative activity by structurally modifying cysmethynil by replacing the acetamide side chain with tertiary amino groups to give analogues, which are potent in inhibiting isoprenylcysteine carboxyl methyltransferase (Icmt) [17c]. In this direction, we were interested in the amalgamation of substituted 1,2,4-triazole-3-thioether unit to the indole nucleus pertaining to the anticancer properties of the former moiety through assorted mechanisms [19]. In view this, we were motivated to study the cytotoxicity of compounds based on indole and substituted 1,2,4-triazole units with the incorporation of different substituted methanamines at position-3 of the indole nucleus.

Silent mating type information regulation 2 homolog 1 (SIRT1), a NAD^+-dependent deacetylase enzyme, belonging to the family of class III histone deacetylase is involved in diverse cellular processes and has recently been emerged as a novel therapeutic target for

metabolic diseases [20]. Among the seven sirtuins, SIRT1 is the most extensively studied, and numerous groups have shown that it is involved in gene silencing, genomic stability, stress resistance, cell division and apoptosis. Over the past several years, many potent small molecules have been reported as SIRT1 inhibitors viz. Cambinol [21], Sirtinol [20], Splitomicin [22], Salermide [23], suramin [24] etc (Figure 1). Few indole based compounds (for example A and B in Figure 1) have also been reported to exhibit superior selectivity towards SIRT-1 over other deacetylases and NAD^+ possessing enzymes [25]. Since SIRT1 enzymes are involved in various physiological functions both in normal and diseased conditions, a strategy of designing small molecules to act on SIRT1 has been emerging out as a promising practice among medicinal chemists. Therefore in the present protocol, we present the synthesis of a library of novel indole derivatives and exploration of their SIRT1 inhibitory activity with respect to *in vitro* and *in vivo* models. The structural considerations of the designed molecules were further supported by the homology modeling and the docking study, with SIRT1 being the target enzyme.

Figure 1. Potent SIRT1 inhibitors.

2. Results and discussion

2.1. Chemistry

Most of the target compounds were designed based on the assumptions of Lipinski rule to fulfill the drug likeness properties of the molecules. The basic indole moiety was constructed by straightforward and efficient three-step synthesis based on Fischer indole chemistry protocol [26a, 26b] as given in Scheme 1. The synthesis involved the polyphosphoric acid (PPA) mediated cyclisation of the hydrazones **(3)**, obtained by the condensation of substituted phenyl hydrazine **(2)** and ethyl pyruvate. The triazole constituent was introduced by converting indole-2-carboxylate **(4)** into the hydrazide **(6)** followed by treating it with phenylisothiocyanate in alcoholic medium to afford the corresponding thiosemicarbazide **(7)** and then refluxing with aqueous KOH solution. The indole-triazole compound **(9)** was then formylated by the conventional Vilsmeier-Haack method to obtain the pre final intermediate (**10)**. Finally the target compounds were synthesized by executing the reductive amination protocol between intermediate (**10)** and different primary/secondary amines using sodium triacetoxyborohydride as the reducing agent [27] (Scheme 2). Our preliminary interest was focused in carrying out a study on the variation in cytotoxicity of the compounds by the assimilation of variety of

aliphatic/aromatic, primary and secondary amines at position-3 and presence of fluoro at position-5 of indole nucleus. Hence a library of 29 compounds were synthesized following the above mentioned multi step synthetic protocol. The structural details of the synthesized compounds are described in Table 1.

Scheme 1. Synthesis of the indole-triazole based scaffolds.

Scheme 2. Synthesis of the target molecules.

Table 1. Structural details of the compounds (the symbol '*' denotes the point of attachment to the position 3 of indole nucleus)

All the intermediate and final compounds were characterized using various spectroscopic techniques. One of the final compounds (IT-17) was subjected to single crystal X-Ray diffraction studies and the 3-dimentional structure of the molecule was confirmed. The ORTEP diagram of IT-17 is shown in Figure 2.

Figure 2. The ORTEP diagram of the compound IT-17 with the ellipsoids drawn at 50 % probability.

Crystal data: C25H28FN5OS, M =465.59, triclinic, a = 6.3811 (3), b = 13.0833 (9), c = 14.7067 (10) Å, α = 101.713 (6), β = 91.147 (5), γ = 93.891 (5), U = 1198.75 (13) Å^3, T = 293 K, space group P-1, Z = 2, reflections measured = 4225, unique (Rint = 0.083) which were used in all calculations.

2.2. *In vitro* anticancer activity

The synthesized 29 compounds were tested for cytotoxicity in three different cell lines: human chronic myeloid leukemia (K562), human metastatic breast cancer (MDA-MB 231) and human prostate (LNCaP) cancer cells using MTT assay method. All the compounds were screened at a concentration of 10 µM. Cytotoxic evaluation of the compounds demonstrated that the molecules IT-16 and IT-17**,** which consists of 4-methyl piperidine and morpholine entities respectively, to be potent enough to inhibit the cell growth to about 88 % against K562 cells. However, the compounds IT-03, IT-04, IT-05 and IT-09 were found to be active against the same cell lines (K562) with a cell growth inhibition to about more than 50 %. Compounds IT-04, IT-05, IT-14, IT-15, IT-22, IT-23, IT-26, IT-30 and IT-31 were found to inhibit the cell growth

to more than 50 % against MDA-MB 231 cells and IT-13, IT-14 and IT-31 showed their inhibitory activity against LNCaP cell growth to more than 50% at the tested concentration. Further study on non cancerous HEK293 cells, none of the compounds showed any significant inhibition suggesting the specificity towards cancer cells. The proliferation assay study results are presented in Figure 3. Among different amines introduced, 3,4,5-trimethoxy aniline, cyclopropyl amine, cyclopropylmethyl amine, 4-methyl piperidine, 3-methyl piperidine, 4-methoxy benzylamine, 3-methoxy benzylamine, morpholine and 2-methoxyethanamine were found to have an impact in enhancing the activity of the molecules against the tested cell lines. It is clear from the structural features and activity relation that presence of fluoro at position-5 of indole moiety also plays a key role in enhancing the activity.

Figure 3. Cell proliferation assay results of the tested compounds at a concentration of 10 µM against a) cancerous cells; K562, MDA-MB231 and LNCaP b) non cancerous HEK 293 cells.

It has been reported that SIRT1 inhibitors are best studied and used to control the benign hyperplasia of prostate cancer [28]. Therefore compounds (IT-13, IT-14, IT-25 and IT-31) showing good inhibitory activity on prostate cancer LNCaP cells were further investigated for *in vitro* SIRT1 inhibition using SIRT1 Fluorimetric Drug Discovery Kit. SIRT1 fluorimetric enzyme assay is based on unique SIRT1 substrate/developer combination. The substrate consists of 4 amino acids from 379-382 [(Arg-His-Lys-Lys (Ac)] of human p53, which was tagged with aminomethylcoumarin (AMC). The fluorescence signal is generated in proportional to the amount of deacetylation of lysine in the substrate. Among the four tested compounds at a concentration of 40 µM, IT-14 was found to exhibit close to 70 % enzyme inhibitory activity (Figure 4). Hence we took forward this compound to study its *in vivo* effects on prostate hyperplasia animal model.

Figure 4. SIRT1 inhibitory activity of the tested compounds at a concentration of 40 µM.

2.3. *In vivo* studies

Male wistar rats weighing 180–220 g were used for *in vivo* investigations, and the animals were divided into four groups. Group A served as negative control**,** Group B served as a positive control (received only testosterone propionate at 3 mg/kg of body weight dose). Group C and Group D received standard drug Finasteride (at a dosage of 5 mg/kg) and IT-14 (at a

dosage of 10 mg/kg) respectively along with testosterone propionate (at a dosage of 3 mg/kg) daily for 14 days to induce prostatic hyperplasia. Compound IT-14 was suspended in distilled water by using 5% methyl cellulose and administered intraperitoneally. Testosterone propionate was diluted with distilled water using Tween 80 as emulgent and injected subcutaneously. Animals were weighed before and after completion of dosage. After dosage on 15^{th} day, animals were sacrificed by light ether anesthesia, later prostate tissue and seminal vesicles were removed carefully and weighed immediately. Prostate weights to the body weight ratios were calculated. Percentage of inhibition was calculated as per the equation given below.

$$\%I = 100 - [(\text{treated group} - \text{negative control})/ (\text{positive control} - \text{negative control}) \times 100]$$

The results and the percentage inhibition details of the tested molecule (IT- 14) on the rats are tabulated in Table 2. A graphical representation of prostate gland weight and seminal vesicle weight of the control and treated rats is shown in Figure 5.

Table 2. Prostate weight to body weight ratio of the rats after treatments.

It is quite clear from the data (Table 2) that there is a marked deviation in the prostate weight to body weight ratio of Finasteride treated rat (group C) and the compound IT-14 treated rat (group D) when compared with that of the testosterone treated rat (group B). It is quite interesting to note that the ratio associated with group D is pretty approaching that of the control (group A) and is quite comparable with that of Finasteride treated group. These facts are also supported by the percentage inhibition calculation where in a growth inhibition of about 56 % and 70 % were observed with respect to an injection dose of Finasteride and IT-14 respectively. It can be seen that, the growth inhibitory activity of IT-14 is comparable with that of the known drug Finasteride, which implies IT-14 can be a lead molecule to act on prostatic hyperplasia.

Figure 5. Prostate gland weight and seminal vesicle weight of the control and treated rats.

2.3.1. Histopathology of prostate

The isolated prostate tissues were then fixed in bouins solution with haematoxylin and eosin stain and observed under light microscope. The histopathology images of the prostate tissues associated with the untreated rat, testosterone treated rat, Finasteride treated rat and IT-14 treated rat were recorded and are shown in Figure 6. The histoarchitecture of the isolated prostate

gland given in figure 6 (i) showed that the epithelium was cuboidal and regular in size. In the prostate tissues isolated from positive control rat (group B), there was some disruption in the histoarchitecture of the prostate tissue. Glandular hyperplasias with epithelial proliferation and nuclear stratification have been observed, whereas the drug and the compound treated ones (group C and group D respectively) showed significant reduction in histoarchitecture disruption when compared to that of the positive control. It can be seen that the cell morphology of the prostate tissues isolated from group C and group D is convincingly comparable with that of the prostate tissues isolated from the negative control (group A). The retention in the cell morphology confirms the inhibitory action of compound IT-14 on prostate hyperplasia.

Figure 6. Histopathological images of prostate tissues isolated from (i) group A, (ii) and (iii) group B, (iv) group C and (v) group D.

2.4. Reverse transcriptase PCR

In order to check the SIRT1 mRNA levels in treated and untreated prostate tissues with respect to β-actin as equal loading control, semi quantitative RT-PCR was performed. Prostate glands (50 mg) from treated and untreated animals were used for isolation of total mRNA with TRI reagent (Sigma) according to manufacturer's protocol. Reverse transcription of 5 µg of total RNA was conducted with AMV reverse transcriptase enzyme (Sigma). PCR amplification was carried out for 45 cycles, with each cycle consisting of denaturation for 15 sec at 95 ^{o}C, annealing for 1 min at 52-55 ^{o}C, and an extension for 1 min at 72 ^{o}C. The PCR products were analyzed by 1.5 % agarose gel electrophoresis and relative quantification was done by using Image lab analysis software. Primers used for PCR were: rat SIRT1 primer: sense 5′-CAGAGCAT CACACGCAAGC-3', antisense 5′-CAGGAAACAG AAACCCCAG C-3′; rat β-actin primer: sense 5′-GAGAGGGAAATCGTGCGTGAC-3', antisense 5′-TAGAGCCACCAATCCACACAGAG-3′; rat SIRT2 primer: sense 5′-AGCAAGGCACCACTAGCCACC-3′, antisense g5′-TGTTCCTCTTTCTCTTTGGTC-3′.

As shown in Figure 7, levels of SIRT1 are significantly decreasing in compound IT-14 treated animals when compared to testosterone induced animals. The mRNA levels of IT-14 treated group were almost similar to that of the negative control, which infers the activity of IT-14 best suits in treating benign prostate hyperplasia conditions.

Figure 7. Semi quantitative RT-PCR analysis of SIRT1, equal loading was confirmed by β-actin. Relative levels of SIRT1 were calculated by Image analysis software.

3. Molecular docking

The three dimensional model of hSIRT1 (uniprot code: Q96EB6, 244-498 amino acid residues) was developed by threading method using PRIME homology modeling program (Schrödinger L.L.C., USA). The multi-step Schrödinger's Protein preparation tool (PPrep) has been used for final preparation of receptor model. Hydrogen's were added to the model automatically *via* the Maestro interface. PPrep neutralizes side chains and residues which are not involving in salt bridges. This step is then followed by restrained minimization using the OPLS 2005 force field to RMSD of 0.3 Å. This model has 92.9 % residues in most favored regions and Prosa-Web Z score of -6.38. Active site pocket was identified by using SITEMAP, module of Schrödinger. The synthesized compound IT-14 was sketched by using chemdraw and prepared for docking using Ligprep, module of Schrödinger. GLIDE program was used for docking with grid coordinates of X:-12.9111; Y:-27.7633; Z:-33.29672.

Molecular docking simulation study to understand the interaction of IT-14 with the protein i.e. homology model of hSIRT1 (244-498 amino acid residues), indicated that Lys134 and Glu 167 involve in hydrogen bonding and strong π- π stacking with Phe 179 residues played key role with the docking score of -4.334. Docking pose and interacting amino acids within 5 A^0 distances are shown in Figure 8.

Figure 8. a) Ligand interaction diagram of IT-14. b) Docking pose of IT-14 in the catalytic core of hSIRT1. Pink dots denotes hydrogen bond and green dots denote hydrophobic interactions.

4. Conclusions

We have designed a set of indole based molecules and synthesized a library of indole derivatives that showed potent growth inhibitory activity against three cancer cell lines. Six molecules were found to be active against K562 cell lines with a cell growth inhibition to about more than 50 %, among which two molecules (IT-16 and IT-17) were found to inhibit the cell growth to about 88 %. Among the synthesized indole derivatives, nine molecules were found to inhibit the cell growth to more than 50% against MDA-MB-231 cells and three molecules showed inhibitory activity against LNCaP cell growth to more than 50 % at the tested

concentration. Further none of the compounds were found to be toxic against noncancerous HEK-293 cell line, signifying the specificity of the compounds towards cancer cells. In the present study we screened a sequence of molecules for SIRT1 inhibitory activity. We found that IT-14 prevents prostatic hyperplasia by inhibiting SIRT1. Compound IT-14 treatment significantly reduced the development of testosterone induced hyperplasia in rats. This was clearly evident with reduction of prostate weight, seminal vesicle weight and prostate weight to body weight ratio. Treatment of compound IT-14 did not show significant change in the body weight during the treatment, but the effect was significant on the prostate weight to body weight ratio. Moreover semi quantitative RT-PCR revealed decreased transcript levels of SIRT1 in prostate tissues treated with IT-14, indicating the activity of the molecule on SIRT1. Further the experimental studies were well supported by the computer simulation study where the electronic environment of the molecule was found to be quite favorable for the molecular interactions with the amino acids constituting the receptor enzyme. Hence from these studies, we conclude that IT-14 can be a good lead candidate to treat prostate hyperplasia.

5. Experimental

5.1. Materials and instruments

All chemicals and solvents were procured from Sigma Aldrich (Germany), Merck (India) and Spectrochem Chemicals Pvt.Ltd. All the solvents were distilled and dried before usage. The progress of the reactions was monitored by TLC using pre coated aluminum sheets with 60 F_{254} silica gel (Merck KGaA). Melting point of the synthesized compounds was recorded by a Stuart SMP3 melting point apparatus. ^{1}H NMR spectra of the intermediates and final compounds were recorded using Bruker 300 MHz, 400 MHz and 500 MHz NMR spectrometers using TMS as internal standard. ^{13}C NMR spectra of the compounds were recorded using Bruker 400 MHz and 500 MHz NMR spectrometers. Elemental analysis was done using a Thermo electron corporation EA-112 series C,H,N,S analyzer. LC-MS was recorded using an Agilent 1200 series mass spectrometer and HPLC purity of the compound was recorded using a Schimadzu Prominence HPLC instrument. Mass spectra were recorded using a Waters micro mass Q-Tof micro spectrometer with an ESI source.

5.2. Animal studies

Male Wistar albino rats weighing (180–220 g) were procured from the Institutional Animal Facility Centre. They were housed individually in clean and transparent polypropylene cages maintained at room temperature with 12-h light/dark cycle and had free access to food and water. After 3 days of acclimatization, they were randomly distributed into experimental groups. All the experimental procedures were carried out in accordance with CPCSEA (Committee for the purpose of control and supervision of experiments on animals) guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee.

5.3. Synthesis

5.3.1. General procedure for the synthesis of indole-2-carbohydrazide intermediates (**6a and 6b**):

The ethyl 1-methyl-1*H*-indole-2-carboxylate intermediate (**5**) was suspended in ethanol, to which excess (about 2 equivalents) hydrazine hydrate was added portion wise. The reaction mass was then refluxed for about 45 min. After the completion of reaction, the reaction mass was cooled and the solid obtained was filtered, washed with cold ethanol and dried under vacuum to obtain the hydrazide intermediates (**6**).

*5.3.1.1. 1-Methyl-1H-indole-2-carbohydrazide (**6a**):* Intermediate **6a** was prepared by following the above mentioned procedure for compound **5a** (9 g, 44.28 mmol) in ethanol (45 mL), to obtain the corresponding hydrazide (**6a**) as white solid (7.8 g, 94.3 %); mp 160-161 °C; **^{1}H NMR** (300 MHz, [D_6]DMSO): δ = 9.75 (s, 1H, NHCO), 7.00-7.62 (m, $5H_{aromatic}$), 4.49 (s, 2H, NH_2), 3.98 (s, 3H, N-CH_3); **^{13}C NMR** (100 MHz, [D_6]DMSO): δ = 160.25, 148.67, 145.20, 137.99, 124.22, 123.12, 120.39, 119.65, 112.86, 38.66.

*5.3.1.2. 5-Fluoro-1-methyl-1H-indole-2-carbohydrazide (**6b**):* Intermediate compound **6b** was prepared by following the above mentioned procedure for compound **5b** (8 g, 44.28 mmol) in ethanol (45 mL), to obtain the hydrazide (**6b**) as white solid (6.9 g, 92.1 %); mp 180-181 °C; **^{1}H NMR** (300 MHz, [D_6]DMSO): δ = 9.79 (s, 1H, NHCO), 6.92-7.65 (m, $4H_{aromatic}$), 4.48 (s, 2H, NH_2), 3.99 (s, 3H, N-CH_3); **^{13}C NMR** (100 MHz, [D_6]DMSO): δ = 162.56, 159.72, 144.11, 132.25, 122.79, 121.65, 120.09, 119.63, 118.63, 38.52.

5.3.2. General procedure for the synthesis of indole-2-thiosemicarbazide intermediate (**7a** and **7b**): A suspension of hydrazide **(6)** and phenylisothiocyanate in ethanol was heated under reflux in an oil bath for 3 h. After the completion of the reaction, the solvent was removed under reduced pressure and the residue thus obtained was taken in minimum quantity of ethanol, stirred for 10 min and filtered. The compound was recrystallized from ethanol to afford the phenylisothiosemicarbazide (**7**).

*5.3.2.1. 1-(1-Methyl-1H-indole-2-carbonyl)-4-phenylthiosemicarbazide (**7a**):* Compound **7a** was prepared by following the above procedure for the intermediate **6a** (7 g, 36.99 mmol) with phenylisothiocyanate (5.50 g, 40.69 mmol) in ethanol (70 mL) to afford the phenylisothiosemicarbazide (**7a**) as white crystalline solid (10.2 g, 85 %); mp 212-213 °C; **^{1}H NMR** (400 MHz, [D_6]DMSO) δ = 11.73 (s, 1H, CONH), 10.54 (s, 1H, NH-NH-CS), 9.87 (s, 1H, CS-NH), 7.03-7.66 (m, 10$H_{aromatic}$), 3.99 (s, 3H, N-CH_3); **^{13}C NMR** (100 MHz, [D_6]DMSO): δ = 162.92, 158.12, 139.76, 137.09, 130.18, 128.45, 127.41, 126.37, 125.50, 124.18, 122.17, 120.38, 112.79, 104.51, 31.33.

*5.3.2.2. 1-(5-Fluoro-1-methyl-1H-indole-2-carbonyl)-4-phenylthiosemicarbazide (**7b**):* Compound **7b** was prepared by following the above procedure for the intermediate **6b** (6.5 g, 31.40 mmol) with phenylisothiocyanate (4.70 g, 34.54 mmol) in ethanol (65 mL) to afford the phenylisothiosemicarbazide (**7b**) as white crystalline solid (9.2 g, 85.58 %); mp 180-181 °C; **^{1}H NMR** (400 MHz, [D_6]DMSO) δ = 10.56 (s, 1H, CONH), 9.87 (s, 1H, NH-NH-CS), 9.78 (s, 1H, CS-NH), 7.14-7.62 (m, 9$H_{aromatic}$), 4.00 (s, 3H, N-CH_3); **^{13}C NMR** (100 MHz, [D_6]DMSO): δ = 161.88, 159.04, 156.72, 139.69, 135.82, 132.09, 128.42, 126.40, 126.02, 125.49, 113.17, 112.47, 112.37, 106.58, 106.35, 105.89, 32.22.

5.3.3. General procedure for the synthesis of 5-(1-methyl-1H-indol-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol derivatives (**8a** and **8b**): A suspension of thiosemicarbazide (**7**) in aqueous potassium hydroxide was refluxed for 2 h. Then the reaction mixture was cooled to room temperature and was mixed with of water. The aqueous layer was washed with diethyl ether (2x100mL). Then the aqueous layer was cooled to 0 ºC, acidified to pH ≈ 4. The precipitate thus obtained was filtered and washed thoroughly with water and dried to obtain the desired product (**8**).

5.3.3.1. *5-(1-Methyl-1H-indol-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol (**8a**):* The thiosemicarbazide compound **7a** (11 g, 33.91 mmol) was treated with aqueous potassium hydroxide (50 mL, 4 mmol) as per the above mentioned procedure to afford the intermediate product **8a** as white fluffy solid (8.4 g, 80.85 %); mp 238-239 °C; **^{1}H NMR** (400 MHz, [D_6]DMSO) δ = 7.00-7.68 (m, 9$H_{aromatic}$), 6.06 (s, 1H, indole-CH), 4.02 (s, 1H, SH), 3.87 (s, 3H, -NCH_3); **^{13}C NMR** (100 MHz, [D_6]DMSO): δ = 159.12, 157.26, 145.33, 134.96, 132.12, 130.11, 129.99, 129.14, 126.62, 126.44, 122.12, 119.86, 113.22, 113.18, 107.55, 105.86, 32.41.

*5.3.3.2. 5-(5-Fluoro-1-methyl-1H-indol-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol (**8b**):* The intermediate **8b** was prepared by treating the thiosemicarbazide intermediate **7b** (9 g, 27.74 mmol) with aqueous potassium hydroxide (50 mL, 4 mmol) as per the above mentioned procedure to obtain **8b** as white solid (7.1 g, 83.53 %); mp 287-288 °C; **^{1}H NMR** (400 MHz, [D_6]DMSO) δ = 7.08-7.56 (m, 8$H_{aromatic}$), 6.07 (s, 1H, indole-CH), 4.03 (s, 1H, SH), 3.88 (s, 3H, -NCH_3); **^{13}C NMR** (100 MHz, [D_6]DMSO): δ = 168.88, 158.98, 156.66, 144.67, 134.93, 130.10, 129.81, 129.15, 126.50, 126.15, 118.26, 112.63, 112.40, 112.30, 106.08, 105.85, 32.41.

*5.3.4. General procedure for the synthesis of 1-methyl-2-[4-phenyl-5-(propan-2-ylsulfanyl)-4H-1,2,4-triazol-3-yl]-1H-indole intermediates (**9a** and **9b**):* To a solution of intermediate **8** in DMF, calculated amount of K_2CO_3 was added and stirred at RT for about 30 min. Excess isopropylbromide (5 equivalents) was then charged in to the reaction mass and allowed to stir overnight at RT. After the completion of the reaction, the reaction mass was quenched with ice cold water and kept stirring for about an hour to precipitate the crude product. The solid mass was then filtered, washed with water, dried and recrystalised from ethanol/water mixture (60 %) to afford pure product (**9**).

*5.3.4.1. 2-[5-(Isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indole (**9a**):* The intermediate **9a** was prepared by carrying out the reaction for the compound **8a** (8 g, 26.11 mmol) in DMF with K_2CO_3 (9 g, 65.28 mmol) and isopropylbromide (16.06 g, 130.57 mmol) according to the procedure mentioned above to obtain **9a** as white crystalline solid (8.2 g, 90.11 %); mp 177-178 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.03-7.55 (m, 9$H_{aromatic}$), 5.94 (s, 1H, indole-CH), 4.13 (s, 3H, N-CH_3), 4.04 (m, 1H, S-CH), 1.47 (d, 6H, *J* = 6.8 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 152.71, 148.88, 138.24, 134.29, 130.09, 129.93, 127.68, 126.75, 125.25, 123.33, 121.27, 120.06, 109.87, 104.81, 38.56, 32.25, 23.52.

*5.3.4.2. 5-Fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indole (**9b**):* The intermediate **9b** was prepared by carrying out the reaction for the compound **8b** (7 g, 21.58 mmol) in DMF with K_2CO_3 (7.45 g, 53.95 mmol) and isopropylbromide (13.27 g, 107.9 mmol) according to the procedure mentioned above to obtain **9b** as white crystalline solid (7.1 g, 89.76 %); mp 192-193 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.97-7.55 (m, $8H_{aromatic}$), 5.88 (s, 1H, indole-CH), 4.11 (s, 3H, N-CH_3), 4.00-4.07 (m, 1H, S-CH), 1.47 (d, 6H, *J* = 6.4 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 159.13, 156.78, 152.91, 148.57, 134.91, 134.14, 130.22, 120.00, 127.61, 126.82, 126.67, 112.11, 111.85, 110.72, 105.80, 104.46, 38.58, 32.50, 23.51.

*5.3.5. General procedure for the synthesis of 2-[5-(Isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indole-3-carbaldehydes (**10a** and **10b**):* The iminium cation was generated by adding $POCl_3$ to a round bottomed flask (RBF) containing DMF, at very slow rate maintaining the internal temperature below 0 °C. After the formation of iminium cation, a solution of intermediate (**9**) in DMF was introduced into the RBF and the mass was heated to 55 °C for 1 h. Reaction was monitored using TLC and after the starting material was completely consumed, the temperature was brought back to RT and 50 mL of ice cold water was added and kept stirring for 30 min. The precipitated solid was filtered, washed and dried to obtain the formylated product (**10**).

5.3.5.1. *2-[5-(Isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indole-3-carbaldehyde **(10a)**:* The intermediate compound **10a** was synthesized by carrying out above reaction for **9a** (7 g, 20.08 mmol) with DMF (24.11 mmol) and $POCl_3$ (24.11 mmol) to afford the product as white solid (6.2 g, 82.01 %); mp 179-180 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 9.81 (s, 1H, CHO), 8.27 (d, $1H_{aromatic}$), 7.11-7.39 (m, $8H_{aromatic}$), 4.09-4.16 (m, 1H, S-CH), 3.68 (s, 3H, N-CH_3), 1.51 (d, 6H, *J* = 6.8 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 184.55, 154.07, 145.69, 137.80, 133.46, 132.81, 130.00, 126.29, 125.08, 124.85, 123.65, 122.39, 118.10, 110.14, 38.94, 31.48, 23.54.

*5.3.5.2. 5-Fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indole-3-carbaldehyde **(10b)**:* The intermediate compound **10b** was synthesized by carrying out above reaction for **9b** (7 g, 19.10 mmol) with DMF (22.92 mmol) and $POCl_3$ (22.92 mmol) to afford the product as white solid (6.6 g, 83.44 %); mp 165-166 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 9.76 (s, 1H, CHO), 7.96 (d, $1H_{aromatic}$), 7.09-7.44 (m, $7H_{aromatic}$), 4.12-4.15 (m, 1H, S-CH), 3.68

(s, 3H, N-CH_3), 1.52 (d, 6H, *J* = 6.4 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 184.24, 161.31, 158.92, 154.23, 145.39, 134.46, 132.72, 130.17, 130.05, 126.22, 125.43, 117.93, 113.94, 111.20, 111.11, 107.83, 107.59, 38.96, 31.74, 23.52.

5.3.6. General procedure for the synthesis of final molecules: The prefinal aldehyde compound (**10a – 10b)** (100 mg) was dissolved in 1,2-dichloroethane (for reaction with primary amines) or THF (for reaction with secondary amines), to which calculated quantity of amine was added followed by a drop of glacial acetic acid. After stirring for about an hour, sodium triacetoxyborohydride (1.5 equivalents) was added and the reaction mass was further stirred at RT for 4 hr. After the completion of reaction, the reaction mass was washed with 10 % sodium bicarbonate solution, the organic layer was then washed with water three times, dried over Na_2SO_4 and evaporated under vacuum. The crude product was purified using column chromatography with dichloromethane/methanol as eluent to obtain the pure compounds.

5.4. Characterization of the final molecules

The characterization data of the final compounds are listed below.

5.4.1. N-{{5-fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}-3,4,5-trimethoxybenzenamine ***(IT-03).***

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.99-7.06 (m, 3H), 7.19-7.38 (m, 5H), 5.74 (s, 2H), 4.12 (s, 2H), 4.05 (m, 1H), 3.72-3.80 (m, 9H), 3.55 (s, 3H), 1.48 (d, 6H, *J* = 6.8 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 158.13, 155.78, 152.86, 146.34, 143.76, 132.21, 129.24, 128.79, 128.45, 125.76, 125.01, 123.84, 114.58, 111.45, 111.18, 109.82, 103.76, 103.53, 97.17, 89.63, 60.08, 55.22, 38.57, 37.76, 22.50; **ESI-MS** (m/z) 562.2 $(M+H)^+$; Anal. calculated for $C_{30}H_{32}FN_5O_3S$; C, 64.15; H, 5.74; N, 12.47; O, 8.55; S, 5.71. Found: C, 64.28; H, 5.68; N, 12.42; O, 8.55; S, 5.68.

5.4.2. N-{{5-fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}cyclopropanamine ***(IT-04).***

^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.99-7.43 (m, 8H), 4.06-4.15 (m, 1H), 3.400 (s, 3H), 2.12-2.16 (m, 1H), 1.52 (d, 6H, *J* = 6.6 Hz), 0.60 (m, 2H), 0.51 (m, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 158.99, 157.10, 152.95, 147.54, 134.29, 133.31, 129.78, 127.51, 126.07, 125.08, 116.41, 112.36, 110.61, 104.86, 43.16, 38.89, 31.33, 30.04, 23.51, 5.98; Anal. calculated for $C_{24}H_{26}FN_5S$; C, 66.18; H, 6.02; N, 16.08; S, 7.36. Found C, 66.16; H, 5.92; N, 15.97; S, 7.29.

*5.4.3. Cyclopropyl-N-{{5-fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}methanamine **(IT-05)**.*

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.06-7.28 (m, 4H), 7.40-7.51 (m, 4H), 4.29 (s, 2H), 4.08-4.15 (m, 1H), 3.13 (s, 3H), 2.80 (d, 2H, *J* = 7.5 Hz), 1.54 (d, 6H, *J* = 6.6 Hz), 1.43-1.53 (m, 1H), 0.65-0.72 (m, 2H), 0.34-0.38 (m, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 159.37, 157.48, 153.66, 147.38, 134.40, 133.12, 129.95, 127.51, 127.43, 126.26, 113.00, 110.99, 104.70, 58.53, 52.28, 43.50, 42.21, 38.99, 38.90, 31.67, 23.56; **ESI-MS** (m/z) 450.2 $(M+H)^+$, 472.2 $(M+Na)^+$; Anal. calculated for $C_{25}H_{28}FN_5S$; C, 66.79; H, 6.28; N, 15.58; S, 7.13. Found: C, 66.70; H, 6.21; N, 15.49; S, 7.18.

*5.4.4. N-{{5-fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}-2-morpholinoethan-1-amine **(IT-06)**.*

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.04-7.17 (m, 4H), 7.40-7.56 (m, 4H), 4.21 (s, 2H), 4.07 (m, 1H), 3.70 (t, 4H, *J* = 4.5 Hz), 3.17 (s, 3H), 3.03 (t, 2H, *J* = 5.5 Hz), 2.74 (t, 2H, *J* = 5.5 Hz), 2.44 (broad peak, 4H), 1.56 (d, 6H, *J* = 7 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 158.72, 154.62, 147.11, 130.71, 129.02, 126.75, 126.29, 124.60, 122.18, 120.84, 115.12, 113.22, 111.31, 110.01, 67.13, 54.8, 54.4, 47.22, 42.50, 36.50, 23.82; Anal. calculated for $C_{27}H_{33}FN_6OS$; C, 63.75; H, 6.54; N, 16.52; S, 6.30. Found: C, 63.65; H, 6.45; N, 16.43; S, 6.21.

*5.4.5. N-{{5-fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}-3-morpholinopropan-1-amine **(IT-07)**.*

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.06-7.18 (m, 4H), 7.42-7.63 (m, 4H), 4.25 (s, 2H), 4.07-4.11 (m, 1H), 3.54-3.57 (m, 4H), 3.14-3.19 (m, 5H), 2.53-2.57 (m, 6H), 2.03-2.07 (m, 2H), 1.52 (d, 6H, *J* = 6.6 Hz); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 159.62, 157.72, 154.04, 146.72, 134.11, 132.97, 130.09, 127.28, 127.11, 125.81, 113.50, 113.28, 111.21, 110.37, 104.77, 104.57, 66.63, 57.45, 53.43, 47.32, 41.67, 39.00, 31.79, 23.50, 22.15; Anal. calculated for $C_{28}H_{35}FN_6OS$; C, 64.34; H, 6.75; N, 16.08; O, 3.06; S, 6.13. Found: C, 64.45; H, 6.86; N, 16.20; O, 3.09; S, 6.18.

*5.4.6. N-{{2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}cyclopropanamine **(IT-08)**.*

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.20-7.80 (m, 9H), 4.70 (s, 2H), 3.10-3.21 (m, 1H), 3.35 (s, 3H), 2.35-2.55 (m, 1H), 1.80 (d, 4H), 1.55 (d, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ = 158.21, 156.54, 152.99, 148.52, 136.31, 133.81, 130.09, 128.91, 125.27, 124.97, 115.71, 113.79, 110.52,

104.25, 39.82, 36.52, 28.68, 22.50, 6.20 ; Anal. calculated for $C_{24}H_{27}N_5S$; C, 69.03; H, 6.52; N, 16.77; S, 7.68. Found: C, 69.11; H, 6.48; N, 16.69; S, 7.72.

5.4.7. Cyclopropyl-N-{{2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}methanamine ***(IT-09)***.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.16-7.79 (m, 9H), 4.43 (s, 2H), 4.07-4.16 (m, 1H), 3.08 (s, 3H), 2.82 (d, 2H, *J* = 7.2 Hz), 1.53 (d, 6H, *J* = 6.9 Hz), 1.33-1.41 (m, 1H), 0.66-0.70 (m, 2H), 0.34-0.39 (m, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ = 154.34, 147.48, 137.52, 132.99, 130.14, 127.28, 125.92, 124.64, 121.84, 119.25, 110.25, 109.04, 50.52, 41.01, 39.06, 31.77, 23.53, 8.02; **ESI-MS** (m/z) 432.2 $(M+H)^+$, 454.2 $(M+Na)^+$; Anal. calculated for $C_{25}H_{29}N_5S$; C, 69.57; H, 6.77; N, 16.23; S, 7.43. Found: C, 69.51; H, 6.70; N, 16.14; S, 7.65.

5.4.8. N-{{2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}-2-morpholinoethan-1-amine ***(IT-10).***

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.10-7.8 (m, 9H), 4.35 (s, 2H), 4.06-4.09 (m, 1H), 3.66 (t, 4H), 3.14 (s, 3H), 3.10 (t, 2H, *J* = 5.5 Hz), 2.75 (t, 2H, *J* = 5.5 Hz), 2.40 (t, 4H), 1.55 (d, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 153.10, 146.03, 136.56, 132.10, 129.15, 129.02, 128.83, 126.02, 125.40, 124.93, 123.72, 122.76, 120.91, 118.53, 109.19, 108.09, 65.56, 52.47, 51.89, 44.51, 41.15, 40.30, 38.09, 30.75, 22.53; Anal. calculated for $C_{27}H_{34}N_6OS$;C, 66.09; H, 6.98; N, 17.13; S, 6.54. Found: C, 66.01; H, 6.89; N, 17.18; S, 6.49.

5.4.9. N-{{2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}-3-morpholinopropan-1-amine ***(IT-11)***.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.13-7.45 (m, 8H), 7.92 (m, 1H), 4.28 (s, 2H), 4.06-4.09 (m, 1H), 3.48-3.49 (m, 4H), 3.21 (s, 3H), 3.16 (t, 2H, *J* = 7 Hz), 2.48-2.53 (m, 6H), 1.99-2.01 (m, 2H), 1.51 (d, 6H, *J* = 8.5 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 159.86, 156.98, 154.12, 146.77, 135.97, 131.82, 130.11, 127.35, 127.56, 124.22, 112.10, 112.99, 110.11, 110.84, 103.45, 102.87, 66.55, 53.06, 52.11, 46.32, 43.59, 36.58, 35.14, 28.29, 22.55; Anal. calculated for $C_{28}H_{36}N_6OS$; C, 66.63; H, 7.19; N, 16.65; S, 6.35. Found: C, 66.59; H, 7.24; N, 16.71; S, 6.32.

*5.4.10. N-{{5-fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}-2-(pyrrolidin-1-yl)ethan-1-amine **(IT-12)**.*

^{1}H NMR (500 MHz, $CDCl_3$) δ = 6.98-7.41 (m, 8H), 4.21 (s, 2H), 4.02-4.07 (m, 1H), 3.43 (s, 3H), 2.98 (broad peak, 4H), 2.72-2.77 (m, 4H), 1.84-1.88 (m, 4H), 1.49 (d, 6H, *J* = 6.5 Hz); **^{13}C NMR** (125 MHz, $CDCl_3$) δ = 159.12, 156.77, 152.88, 147.49, 134.27, 133.21, 129.69, 127.12, 126.09, 125.04, 116.09, 112.33, 110.58, 104.94, 54.88, 53.99, 46.05, 43.39, 38.81, 31.22, 23.48; Anal. calculated for $C_{27}H_{33}FN_6S$; C, 65.82; H, 6.75; N, 17.06; S, 6.51. Found: C, 65.79; H, 6.68; N, 17.12; S, 6.48.

*5.4.11. N-{{5-fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}-2-methoxyethan-1-amine **(IT-13)**.*

^{1}H NMR (500 MHz, $CDCl_3$) δ = 6.91-7.31 (m, 8H), 3.98-4.01 (m, 1H), 3.71 (s, 1H), 3.39 (t, 2H, *J* = 6.5 Hz), 3.35 (s, 3H), 3.25 (s, 3H), 2.68 (t, 2H, *J* = 6.5 Hz), 1.41 (d, 6H, *J* = 8.5 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 158.44, 156.08, 152.27, 146.37, 133.24, 132.22, 128.84, 128.74, 125.03, 111.72, 111.46, 109.83, 109.73, 103.79, 103.55, 69.15, 57.93, 46.29, 41.90, 37.92, 30.54, 22.51; Anal. calculated for $C_{24}H_{28}FN_5OS$; C, 63.55; H, 6.22; N, 15.44; S, 7.07. Found: C, 63.45; H, 6.18; N, 15.42; S, 7.15.

*5.4.12. N-{{5-fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}(4-methoxyphenyl)methanamine **(IT-14)**.*

^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.80-7.55 (m, 12H), 4.35 (s, 2H), 4.21 (s, 2H), 4.01-4.10 (m, 1H), 3.86 (s, 3H), 3.72 (s,3 H), 1.50 (d, 6H, *J* = 6.6 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 159.90, 157.52, 153.86, 146.75, 134.16, 133.02, 131.69, 130.72, 129.98, 127.67, 125.81, 120.56, 119.14, 113.45, 111.11, 110.24, 104.93, 104.69, 67.08, 55.36, 46.55, 41.32, 39.12, 31.85, 23.00; Anal. calculated for $C_{29}H_{30}FN_5OS$; C, 67.55; H, 5.86; F, 3.68; N, 13.58; O, 3.10; S, 6.22. Found: C, 67.52; H, 5.80; N, 13.62; S, 6.18; **LC-MS:** Compound molecular weight = 515.64, Obtained mass = 516.6 $(M+H)^+$; **HPLC Purity:** > 98 %.

*5.4.13. N-{{5-fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}(3-methoxyphenyl)methanamine **(IT-15)**.*

^{1}H NMR (500 MHz, $CDCl_3$) δ = 6.78-7.36 (m, 12H), 4.40 (s, 2H), 4.02-4.09 (m, 1H), 3.81 (s, 3H), 3.79 (s, 2H), 3.46 (s, 3H), 1.48 (d, 6H, *J* = 8.5 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 161.21, 158.57, 148.56, 142.97, 138.91, 133.38, 131.55, 130.62, 130.78, 129.11, 127.27, 126.94,

123.36, 121.57, 120.44, 120.09, 117.55, 114.61, 114.08, 113.46, 110.69, 55.36, 54.84, 42.82, 38.22, 31.80, 23.51; Anal. calculated for $C_{29}H_{30}FN_5OS$; C, 67.55; H, 5.86; N, 13.58; S, 6.22. Found: C, 67.57; H, 5.81; N, 13.51; S, 6.29.

5.4.14. 2-[5-(Isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-3-[(4-methylpiperidin-1-yl)methyl]-1H-indole ***(IT-16)***.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.09-7.39 (m, 8H), 7.72-7.74 (m, 1H), 4.01-4.05 (m, 1H), 3.54 (s, 3H), 2.76-2.79 (m, 2H). 2.02-2.16 (m, 4H), 1.48-1.54 (m, 5H), 1.46 (d, 6H, *J* = 6.8 Hz), 0.86 (d, 3H, *J* = 6.4 Hz); **^{13}C NMR** (100 MHz; $CDCl_3$) δ =151.99, 148.06, 137.44, 133.47, 129.73, 129.56, 129.34, 127.40, 126.38, 123.79, 123.12, 120.46, 119.83, 109.71, 55.52, 53.57, 52.68, 38.78, 34.55, 31.06, 23.45; Anal. calculated for $C_{27}H_{33}N_5S$; C, 70.55; H, 7.24; N, 15.24; S, 6.98. Found: C, 70.51; H, 7.28; N, 15.19; S, 7.02.

5.4.15. 5-Fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-3-(morpholinomethyl)-1H-indole ***(IT-17).***

^{1}H NMR (500 MHz, [D_6]DMSO) δ = 7.06-7.10 (m, 1H), 7.30-7033 (m, 2H), 7.42-7.49 (m, 5H), 3.76-3.82 (m, 1H), 3.54 (s, 3H), 3043 (broad peak, 4H), 2.18 (broad peak, 4H), 1.36 (d, 6H, 5 Hz); **^{13}C NMR** (100 MHz; $CDCl_3$) δ = 158.92, 156.53, 152.25, 147.70, 134.27, 133.43, 129.43, 127.38, 126.18, 125.07, 112.07, 111.80, 110.42, 110.33, 105.43, 67.04, 53.58, 53.37, 38.84, 31.14, 23.52; **ESI-MS** (m/z) 466.2 $(M+H)^+$, 488.2 $(M+Na)^+$; Anal. calculated for $C_{25}H_{28}FN_5OS$; C, 64.49; H, 6.06; N, 15.04; S, 6.89. Found: C, 64.46; H, 6.09; N, 15.08; S, 6.81.

5.4.16. 2-[5-(Isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-3-(morpholinomethyl)-1H-indole ***(IT-18)***.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.09-7.35 (m, 8H), 7.72-7.74 (m, 1H), 4.02-4.09 (m, 1H), 3.51-3.62 (m, 7H), 3.38 (broad peak, 2H), 2.31 (broad peak, 4H), 1.47 (d, 6H, *J* = 6.8 Hz); **^{13}C NMR** (125 MHz, $CDCl_3$) δ = 152.03, 148.01, 137.56, 133.51, 129.37, 127.21, 126.21, 123.55, 123.19, 120.42, 119.68, 114.79, 109.58, 66.99, 53.46, 53.14, 38.78, 31.87, 29.64, 23.46; Anal. calculated for $C_{25}H_{29}N_5OS$; C, 67.08; H, 6.53; N, 15.65; S, 7.16. Found: C, 67.15; H, 6.49; N, 15.58; S, 7.11.

5.4.17. 3-[(2,6-Dimethylmorpholino)methyl]-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indole ***(IT-19)***.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.09-7.28 (m, 8H), 7.67-7.69 (m, 1H), 3.94-3.97 (m, 1H), 3.79-3.83 (m, 2H), 3.5 (s, 3H), 3.43 (s, 2H), 2.49-2.52 (m, 4H), 1.39 (d, 6H, *J* = 6.8 Hz), 1.04 (d, 3H, *J* = 6.4 Hz), 0.99 (d, 3H, 6 Hz); **^{13}C NMR** (125 MHz, $CDCl_3$) δ = 153.07, 148.22, 136.76, 135.24, 132.87, 130.99, 129.47, 128.98, 125.96, 123.21, 123.01, 121.47, 120.03, 115.19, 110.52, 70.22, 60.31, 49.19, 37.56, 32.01, 23.45, 20.85; Anal. calculated for $C_{27}H_{33}N_5OS$; C, 68.18; H, 6.99; N, 14.72; S, 6.74. Found: C, 68.27; H, 6.91; N, 14.63; S, 6.76.

5.4.18. N-{{2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}-2-(pyrrolidin-1-yl)ethan-1-amine ***(IT-20)***.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.05-7.31 (m, 8H), 7.63-7.65 (m, 1H), 3.96-3.99 (m, 1H), 3.75 (s, 2H), 3.35 (s, 3H), 2.73 (t, 2H, *J* = 6.8 Hz), 2.61 (t, 2H, J = 6.4 Hz), 2.52-2.55 (m, 4H), 1.69-1.73 (m, 4H), 1.41 (d, 6H, *J* = 6.8 Hz); **^{13}C NMR** (125 MHz, $CDCl_3$) δ = 152.72, 147.74, 137.63, 133.31, 129.64, 129.48, 126.89, 126.14, 123.59, 120.12, 119.89, 116.24, 109.69, 54.87, 53.93, 46.44, 43.30, 38.78, 30.98, 29.63, 23.47; **ESI-MS** (m/z) 475.2 $(M+H)^+$; Anal. calculated for $C_{27}H_{34}N_6S$; C, 68.32; H, 7.22; N, 17.71; S, 6.76. Found: C, 68.38; H, 7.29; N, 17.69; S, 6.70.

5.4.19. 2-[5-(Isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-3-[(2-methylpiperidin-1-yl)methyl]-1H-indole ***(IT-21).***

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.13-7.93 (m, 9H), 4.48 (s, 2H), 4.04-4.18 (m, 1H), 3.47 (s, 3H), 3.18 (broad peak, 2H), 2.76 (broad peak, 1H), 1.52 (d, 6H, J = 6.6 Hz), 1.27-1.35 (m, 9H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 151.92, 148.16, 137.38, 133.59, 129.46, 129.32, 127.78, 126.44, 123.67, 122.90, 120.76, 119.46, 117.23, 109.49, 56.87, 51.46, 47.70, 38.81, 34.74, 30.87, 25.99, 23.52; Anal. calculated for $C_{27}H_{33}N_5S$; C, 70.55; H, 7.24; N, 15.24; S, 6.98. Found C, 70.51; H, 7.29; N, 15.14; S, 7.06.

5.4.20. 2-[5-(Isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-3-[(3-methylpiperidin-1-yl)methyl]-1H-indole ***(IT-22)***.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.14-7.36 (m, 8H), 7.75-7.77 (m, 1H), 4.04-4.07 (m, 1H), 3.55 (s, 3H), 2.86-2.94 (m, 2H), 2.03-2.17 (m, 4H), 1.61-1.83 (m, 5H), 1.48 (d, 6H, *J* = 6.8 Hz), 0.82 (d, 3H, *J* = 8Hz); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 149.25, 147.23, 138.58, 133.89, 130.17, 129.98, 129.01, 128.08, 127.86, 125.98, 123.94, 123.11, 121.09, 199.52, 118.03, 110.08, 56.28,

54.64, 48.12, 37.51, 35.86, 34.09, 30.16, 23.55, 23.20, 15.91; Anal. calculated for $C_{27}H_{33}N_5S$; C, 70.55; H, 7.24; N, 15.24; S, 6.98. Found: C, 70.51; H, 7.28; N, 15.32; S, 6.89.

5.4.21. N-{{2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}(3-methoxyphenyl)methanamine ***(IT-23)***.

^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.82-7.38 (m, 12H), 7.69-7.72 (m, 1H), 4.06-4.13 (m, 1H), 3.91 (s, 2H), 3.85 (s, 2H), 3.84 (s, 3H), 3.43 (s, 3H), 1.51 (d, 6H, *J* = 6.6 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 159.69, 152.57, 147.84, 140.95, 137.72, 133.38, 129.71, 129.56, 129.39, 129.26, 127.02, 126.13, 123.45, 120.57, 120.48, 120.02, 116.55, 113.43, 113.38, 113.00, 109.69, 55.194, 52.73, 43.68, 38.79, 31.88, 29.65, 23.50.

5.4.22. 2-[5-(Isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-3-{[4-(pyrrolidin-1-yl)piperidin-1-yl]methyl}-1H-indole ***(IT-24)***.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.01-7.71 (m, 9H), 3.91-3.94 (m, 1H), 3.44 (s, 3H), 2.69 (broad peak, 2H), 2.49 (broad peak, 4H), 2.09 (m, 1H), 1.61-1.82 (m, 4H), 1.44 (d, 6H, *J* = 6.4 Hz).1.01-1.39 (m, 8H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 153.02, 144.65, 136.76, 132.41, 131.77, 129.03, 128.96, 125.26, 124.04, 123.81, 122.61, 121.36, 117.06, 109.09, 37.91, 37.70, 30.46, 28.71, 28.68, 28.64, 22.51, 21.67, 13.10; Anal. calculated for $C_{30}H_{38}N_6S$; C, 70.00; H, 7.44; N, 16.33; S, 6.23. Found: C, 70.11; H, 7.39; N, 16.29; S, 6.21.

5.4.23. N-{{2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}-2-methoxyethan-1-amine ***(IT-25)***.

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.16-7.77 (0M, 9H), 4.23 (s, 2H), 4.05-4.16 (m, 1H), 3.64 (t, 2H, *J* = 4.5 Hz), 3.38 (s, 3H), 3.23 (s, 3H), 3.01 (t, 2H, *J* = 4.5 Hz), 1.51 (d, 6H, J = 6.9 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 153.15, 152.29, 147.79, 137.59, 133.34, 129.79, 127.35, 127.15, 126.26, 126.06, 124.54, 123.66, 121.27, 120.91, 119.59, 109.85, 70.96, 58.87, 47.07, 42.68, 38.88, 31.27, 23.49; Anal. calculated for $C_{24}H_{29}N_5OS$; C, 66.18; H, 6.71; N, 16.08; S, 7.36. Found: C, 66.22; H, 6.78; N, 16.18; S, 7.45.

5.4.24. N-{{2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}(4-methoxyphenyl)methanamine ***(IT-26)***.

^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.3-7.35 (m, 12H), 7.84-7.87 (m, 1H), 3.98-4.08 (m, 1H), 3.83 (s, 2H), 3.68 (s, 3H), 3.32 (s, 3H), 3.09 (s, 2H), 1.49 (d, 6H, *J* = 6.6 Hz); **^{13}C NMR** (100 MHz,

$CDCl_3$) δ = 158.58, 150.12, 149.92, 146.71, 135.18, 134.45, 132.99, 131.08, 130.11, 128.17, 121.68, 119.08, 113.68, 112.89, 110.54, 104.69, 63.13, 56.85, 43.65, 38.14, 32.66, 23.28; Anal. calculated for $C_{29}H_{31}N_5OS$; C, 69.99; H, 6.28; N, 14.07; S, 6.44. Found: C, 69.91; H, 6.39; N, 14.14; S, 6.34.

5.4.25. N-{{2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}-3,4,5-trimethoxybenzenamine ***(IT-27)***.

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.10-7.42 (m, 9 H), 5.78 (s, 2H), 4.18 (s, 2H), 4.01-4.14 (m, 1H), 3.74-3.82 (m, 9H), 3.52 (s, 3H), 1.47 (d, 6H, *J* = 6.8 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 153.88, 152.86, 147.66, 144.95, 137.79, 133.32, 130.10, 129.60, 129.39, 126.56, 126.34, 123.65, 123.37, 120.14, 119.84, 115.76, 109.94, 92.70, 90.52, 61.11, 55.96, 39.57, 38.76, 31.07, 23.53; **ESI-MS** (m/z) 544.2 $(M+H)^+$, 566.2 $(M+Na)^+$; Anal. calculated for $C_{30}H_{33}N_5O_3S$; C, 66.27; H, 6.12; N, 12.88; S, 5.90. Found: C, 66.15; H, 6.18; N, 12.94; S, 5.81.

5.4.26. 5-Fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-3-[(3-methylpiperidin-1-yl)methyl]-1H-indole ***(IT-30)***.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.00-7.46 (m, 8H), 4.03-4.09 (m, 1H), 3.56 (s, 3H), 3.32 (s, 2H), 2.65 (broad peak, 4H), 1.55-1.65 (m, 5H), 1.48 (d, 6H, *J* = 7 Hz), 0.80 (d, 3H, *J* = 7 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 161.08, 156.58, 152.16, 147.83, 134.20, 133.49, 129.43, 129.36, 127.64, 126.28, 111.93, 111.67, 110.28, 110.19, 105.60, 105.37, 61.66, 53.75, 53.23, 38.86, 32.85, 31.12, 29.71, 25.32, 23.48, 19.66; Anal. calculated for $C_{27}H_{32}FN_5S$; C, 67.89; H, 6.75; N, 14.66; S, 6.71. Found: C, 67.92; H, 6.65; N, 14.72; S, 6.63.

5.4.27. 5-Fluoro-2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-3-[(4-methylpiperidin-1-yl)methyl]-1H-indole ***(IT-31)***.

^{1}H NMR (400 MHz. $CDCl_3$) δ = 6.97-7.41 (m, 8H), 4.00-4.07 (m, 1H), 3.53 (s, 3H), 3.35 (broad peak, 2H), 2.69-2.72 (m, 2H), 2.17 (s, 2H), 1.46-1.53 (m, 9H), 1.26 (broad peak, 2H), 0.86 (d, 3H, J = 8 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 158.92, 156.58, 152.13, 147.86, 134.17, 133.48, 129.42, 127.62, 127.52, 126.28, 111.93, 111.66, 110.31, 110.21, 105.49, 105.25, 53.73, 53.03, 38.85, 34.23, 31.11, 30.66, 23.52, 21.83; Anal. calculated for $C_{27}H_{32}FN_5S$ C, 67.89; H, 6.75; 14.66; S, 6.71. Found: C, 67.95; H, 6.71; 14.55; S, 6.76.

*5.4.28. N-{{2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}-1H-benzo[d]imidazol-2-amine **(IT-35)**.*

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.18-7.78 (m, 13 H), 4.68 (s, 2H), 4.05-4.14 (m, 1H), 3.37 (s, 3H), 1.51 (d, 6H, *J* = 8 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 152.12, 147.56, 141.52, 139.68, 138.14, 130.35, 129.93, 127.96, 126.06, 124,38, 124.01, 122.22, 121.59, 120.85, 119.09, 115.37, 113.16, 112.55, 38.56, 35.19, 30.46, 23.59; Anal. calculated for $C_{28}H_{27}N_7S$; C, 68.13; H, 5.51; N, 19.86; S, 6.50. Found: C, 68.06; H, 5.59; N, 19.78; S, 6.57.

*5.4.29. N-{{2-[5-(isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl]-1-methyl-1H-indol-3-yl}methyl}pyrazin-2-amine **(IT-36)**.*

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.14-7.38 (m, 9H), 7.72-7.95 (m, 3H), 4.65 (s, 2H), 4.03-4.10 (m, 1H), 3.36 (s, 3H), 1.48 (d, 6H, *J* = 6.8 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$) δ = 154.55, 152.84, 147.81, 141.87, 137.74, 134.00, 133.33, 132.63, 129.70, 129.54, 126.36, 126.18, 123.59, 123.57, 120.26, 119.84, 119.26, 109.71, 55.51, 38.79, 31.02, 23.46; **ESI-MS** (m/z) 456.2 $(M+H)^+$; Anal. calculated for $C_{25}H_{25}N_7S$; C, 65.91; H, 5.53; N, 21.52; S, 7.04. Found: C, 65.98; H, 5.43; N, 21.63; S, 6.96.

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List of contents

Table 1. Structural details of the compounds (the symbol '*' denotes the point of attachment to the position 3 of indole nucleus)

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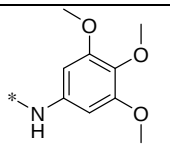
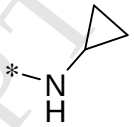
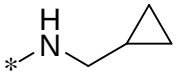
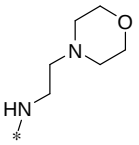
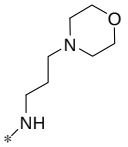
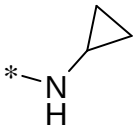
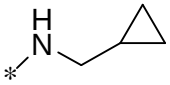
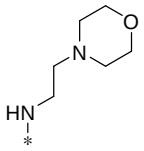
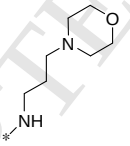
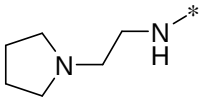
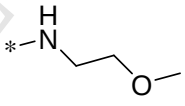
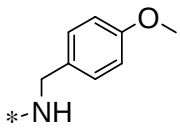
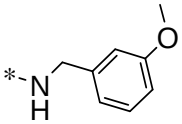
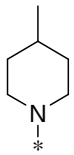
1	IT-03	F		2	IT-04	F	
3	IT-05	F		4	IT-06	F	
5	IT-07	F		6	IT-08	H	
7	IT-09	H		8	IT-10	H	
9	IT-11	H		10	IT-12	F	
11	IT-13	F		12	IT-14	F	
13	IT-15	F		14	IT-16	H	

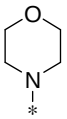
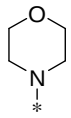
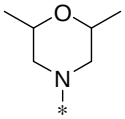
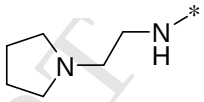
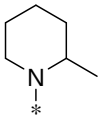
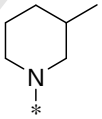
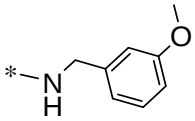
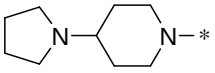
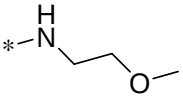
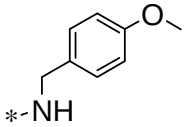
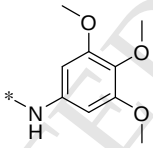
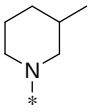
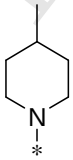
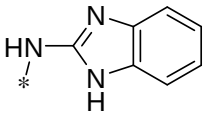
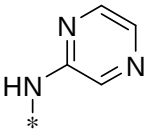
15	IT-17	F		16	IT-18	H	
17	IT-19	H		18	IT-20	H	
19	IT-21	H		20	IT-22	H	
21	IT-23	H		22	IT-24	H	
23	IT-25	H		24	IT-26	H	
25	IT-27	H		26	IT-30	F	
27	IT-31	F		28	IT-35	H	
29	IT-36	H					

Table 2. Prostate weight to body weight ratio of the rats after treatments.

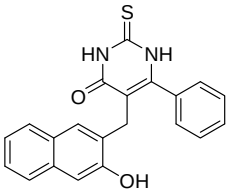
Animal group	PWt[a] (g)	SVWt[b] (g)	PWt/BWt ratio (10^{-3})	%Inhibition	BWt Initial (g)	BWt Final (g)
Group A[c]	0.519± 0.027	0.717±0.0195	1.937±0.0937	-	268±1.1547	270.66±2.9060
Group B[d]	0.838±0.0293[g]	1.6071±0.0821[g]	3.1634±0.1264[g]	-	265±1.7321	269.66±1.7638
Group C[e]	0.556±0.0213[i]	0.9051±0.0440[i]	2.471±0.0731[h]	56.46	225±2.8868	224.66±4.9104
Group D[f]	0.537±0.0181[i]	0.619±0.0150[i]	2.298±0.0681[h]	70.51	233.66±1.2018	234±1.5275

[a] PWt: prostate weight, [b] SVWt: seminal vesicle weight, BWt: body weight, [c] Group A: negative control (untreated rat), [d] Group B: positive control (only testosterone treated rat), [e] Group C: Finasteride (5 mg/kg) treated rat, [f] Group D: IT-14 (10 mg/kg) treated rat. Values are expressed as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Bonferroni's multiple comparison tests.

[g] $P<0.001$ when compared with normal control.

[h] $P<0.01$ when compared with testosterone treatment.

[i] $P<0.001$ when compared with testosterone treatment.



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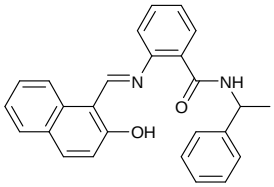

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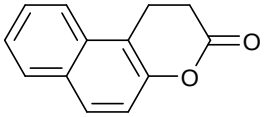

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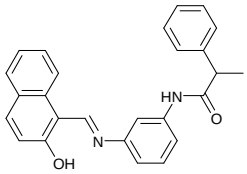

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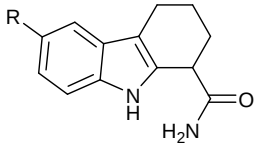

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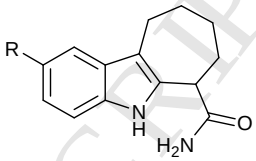

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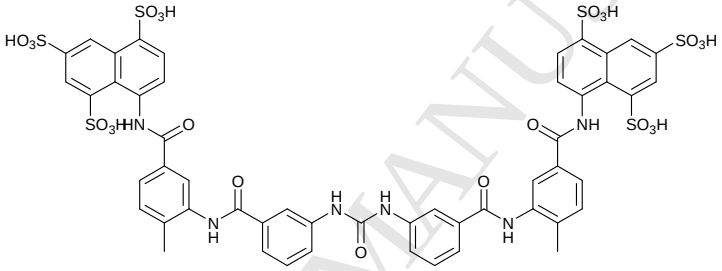
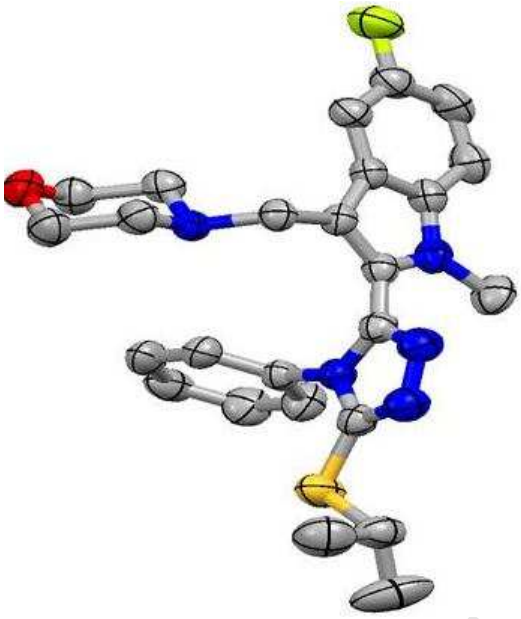


Suramin

Figure 1



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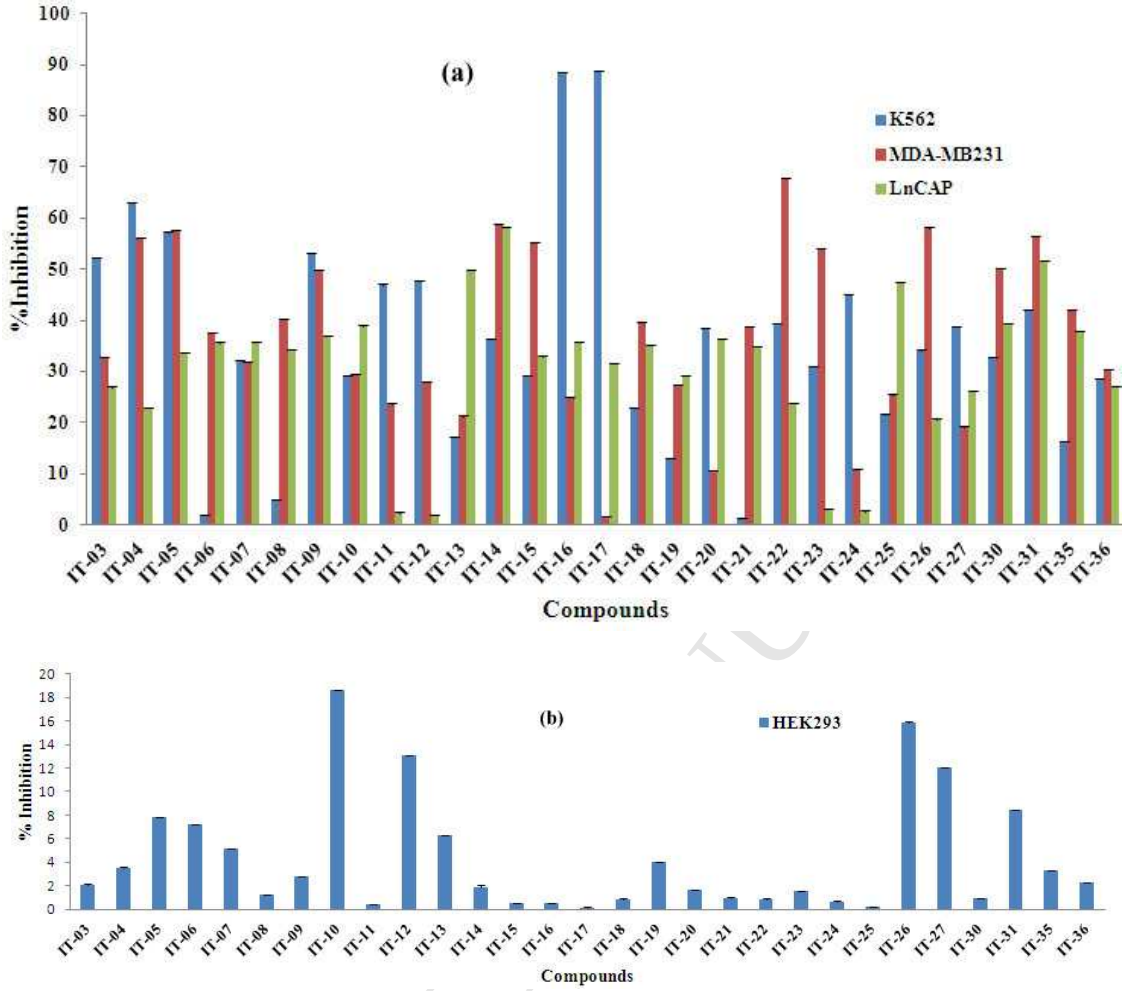

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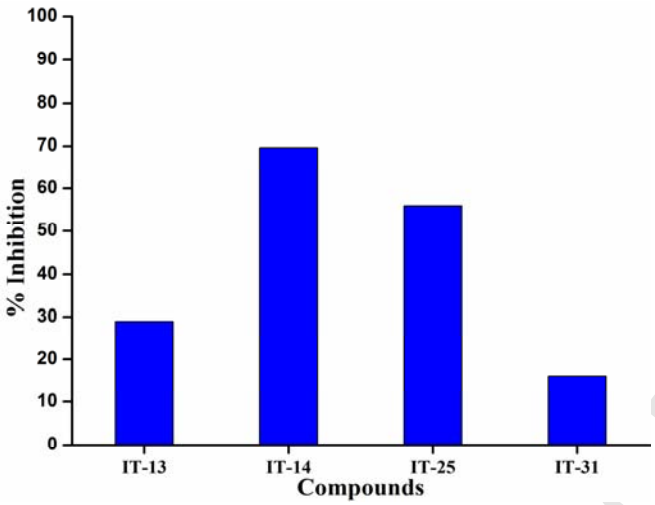

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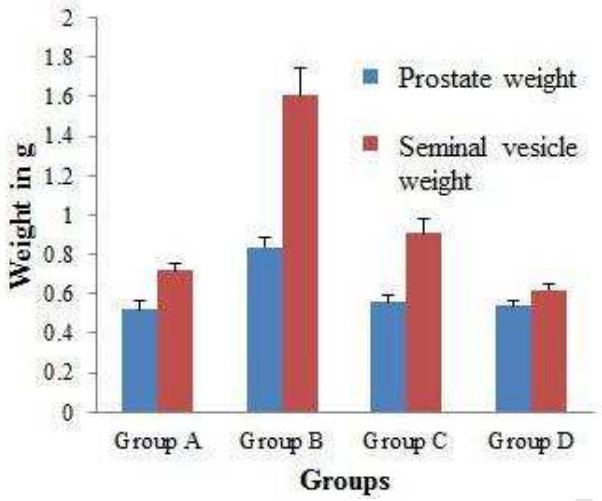

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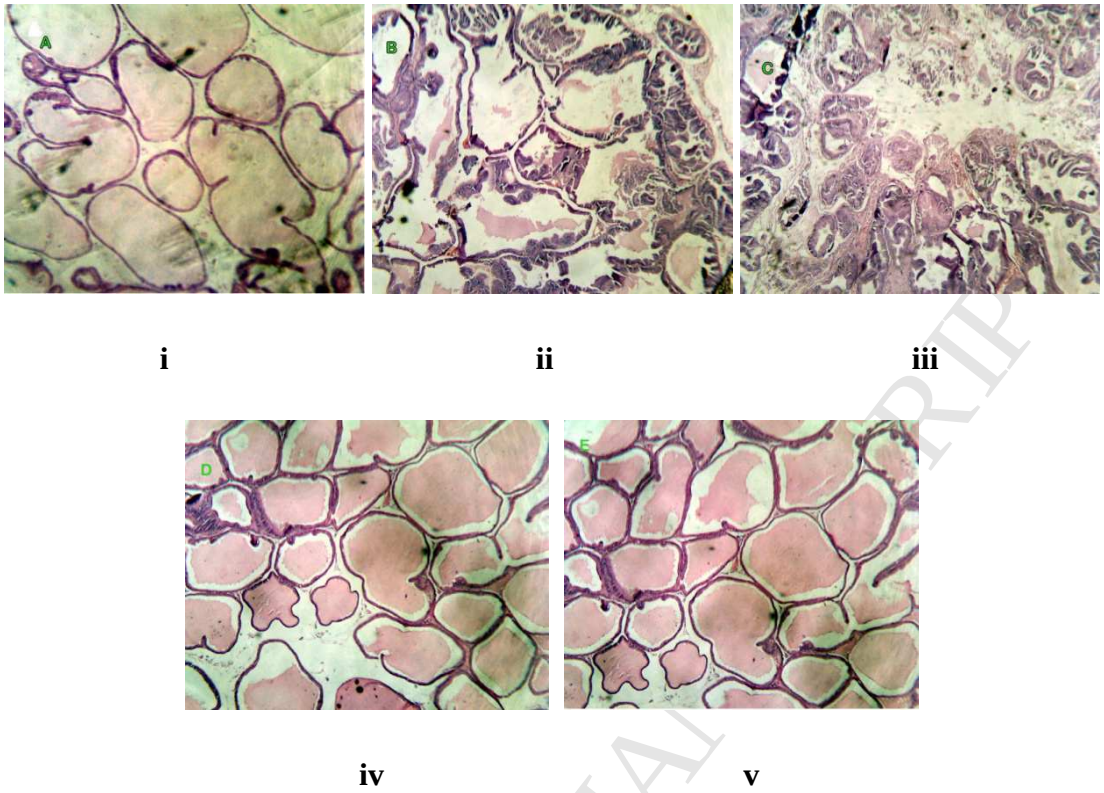

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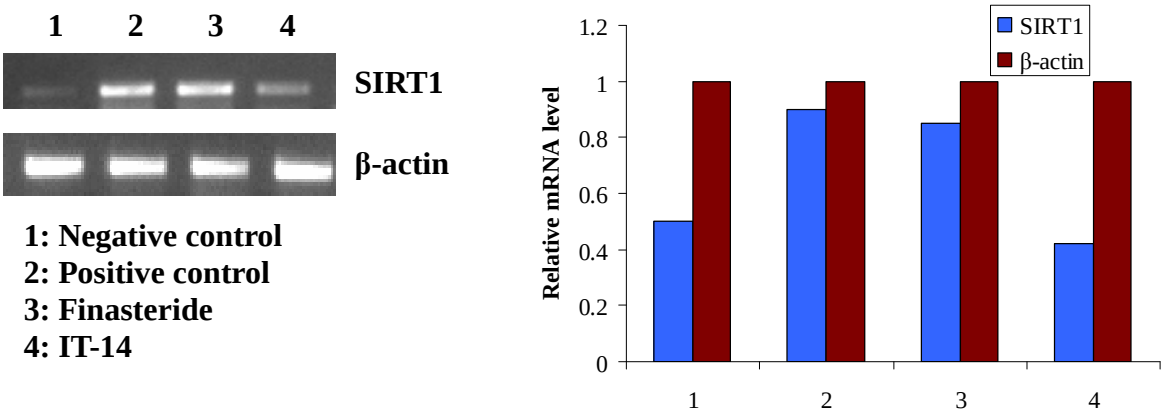
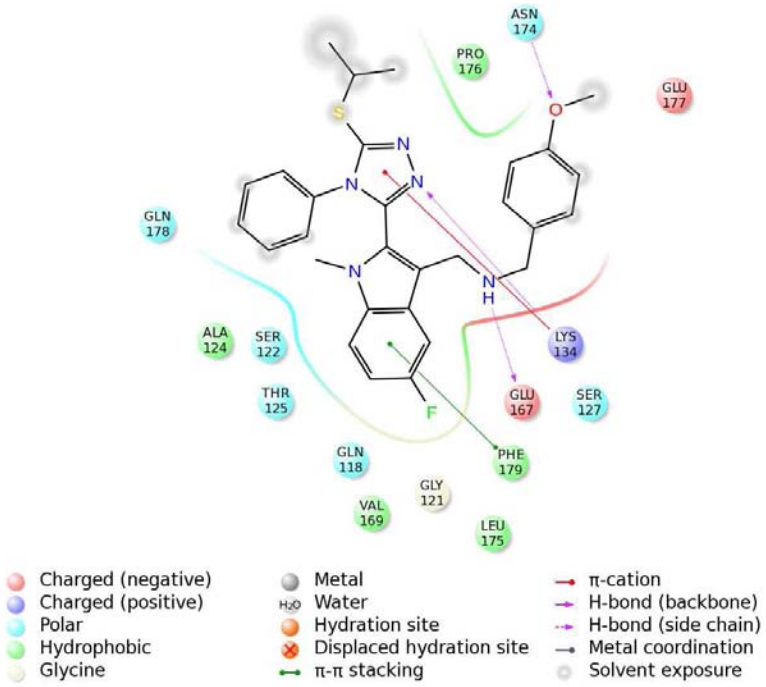


Figure 7

(a)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GLU 177
GLN 178
ALA 124
SER 122
THR 125
LYS 134
GLU 167
SER 127
PHE 179
GLN 118
GLY 121
VAL 169
LEU 175
Charged (negative)
Charged (positive)
Polar
Hydrophobic
Glycine
Metal
Water
Hydration site
Displaced hydration site
π-π stacking
π-cation
H-bond (backbone)
H-bond (side chain)
Metal coordination
Solvent exposure

(b)

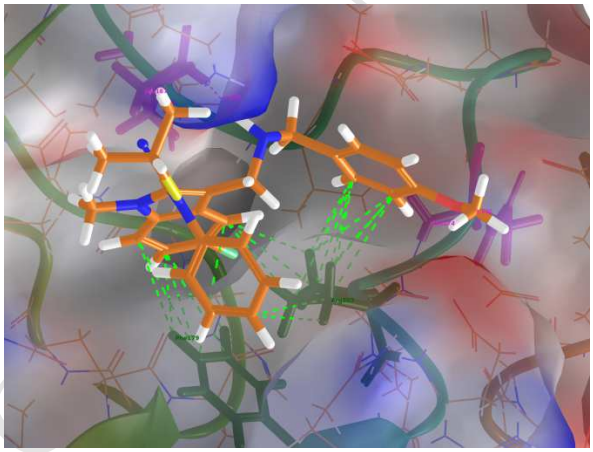
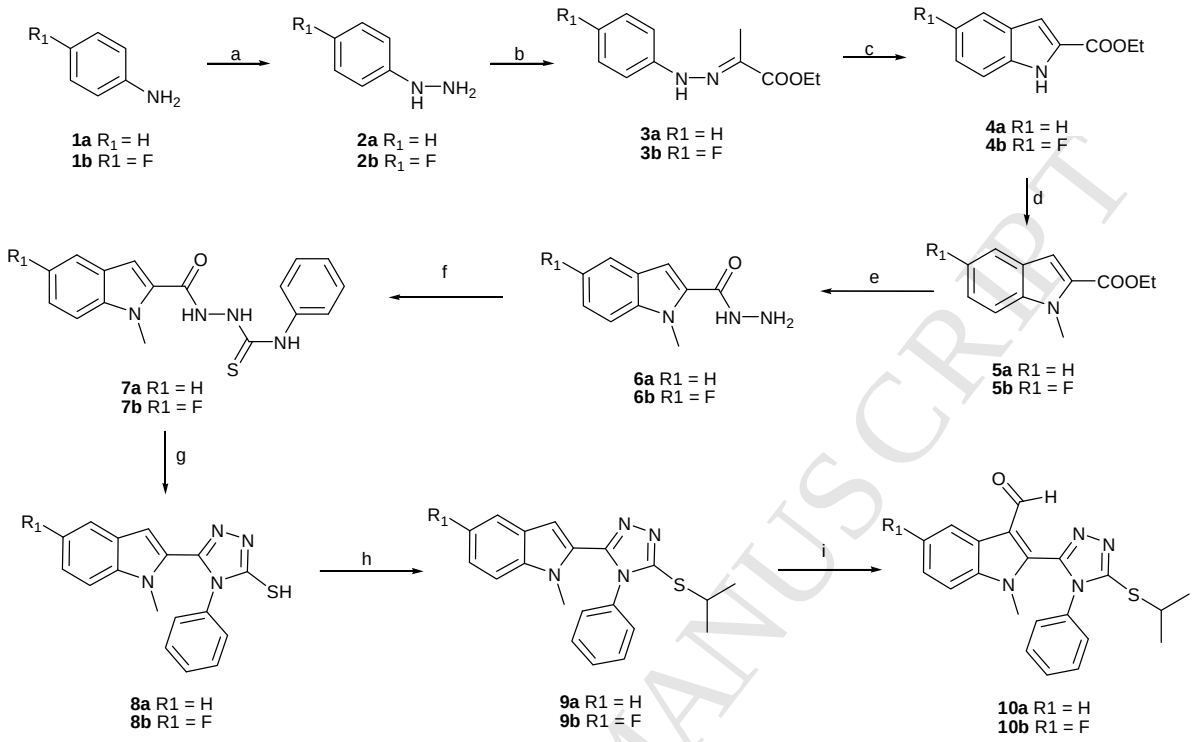
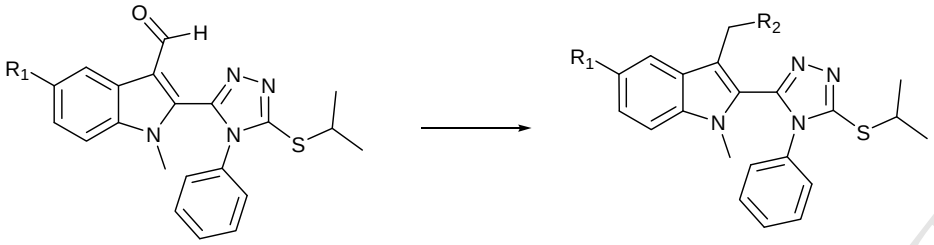


Figure 8



Regents and conditions: a) HCl, $NaNO_2$, $SnCl_2$, -5 °C to room temperature (RT), overnight (O/N); b) Ethyl pyruvate, cat. HOAc, EtOH, 80 °C 1 h; c) PPA, Toluene, 100 °C, 5 h;d) NaH, THF, MeI, 0 °C, 40 min; e) $N_2H_4.H_2O$, EtOH, 80 °C, 45 min; f) C_6H_5SCN, EtOH, 80 °C, 3 h ; g) aq.KOH, 110 °C, 2 h; h) K_2CO_3, DMF, isopropyl bromide, RT O/N; i) DMF, $POCl_3$, -5 to 55 °C, 1 h, H_2O.

Scheme 1



10a R1 = H
10b R1 = F

Reagents and conditions: Primary or secondary amines, cat. AcOH, EDC or THF, $NaBH(OAc)_3$, RT, 4h.

Scheme 2

Identification and characterization of novel indole based small molecules as anticancer agents through SIRT1 inhibition

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[b] Medicinal Chemistry and Drug Discovery Research Laboratory, Pharmacy Group, Birla Institute of Technology and Science-Pilani, Hyderabad Campus, Jawahar Nagar, Andhra Pradesh – 500078, India.

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CONTENTS

Table S1. Physical properties of the compounds.

Entry	Compound	Nature of compound	Melting point (°C)	Log P	Entry	Compound	Nature of compound	Melting point (°C)	Log P
1	IT-03	White semi solid	---	5.96	17	IT-19	White semi solid	---	5.13
2	IT-04	White solid	174-175	4.97	18	IT-20	Brown thick mass	---	4.83
3	IT-05	Off white solid	230-231	5.4	19	IT-21	Yellow thick mass	---	5.95
4	IT-06	White semi solid	---	4.28	20	IT-22	Yellow semi solid	---	6.03
5	IT-07	White solid	126-127	4.38	21	IT-23	White semi solid	---	6.27
6	IT-08	Off white solid	118-119	4.81	22	IT-24	White semi solid	---	5.16
7	IT-09	White solid	232-233	5.24	23	IT-25	Yellow semi solid	---	4.36
8	IT-10	Yellow semi solid	---	4.12	24	IT-26	White solid	99-100	6.27
9	IT-11	Brown viscous liquid	---	4.22	25	IT-27	Brown viscous liquid	---	5.8
10	IT-12	Yellow thick mass	---	4.99	26	IT-30	White solid	145-146	6.18
11	IT-13	Yellow semi solid	---	4.52	27	IT-31	Yellow thick mass	---	6.12
12	IT-14	White solid	146-147	6.42	28	IT-35	Yellow solid	178-179	5.94
13	IT-15	Yellow thick mass	---	6.42	29	IT-36	Yellow semi solid	---	4.22
14	IT-16	White solid	111-112	5.96					
15	IT-17	White crystalline solid	160-161	4.65					
16	IT-18	Brown semi solid	---	4.5					

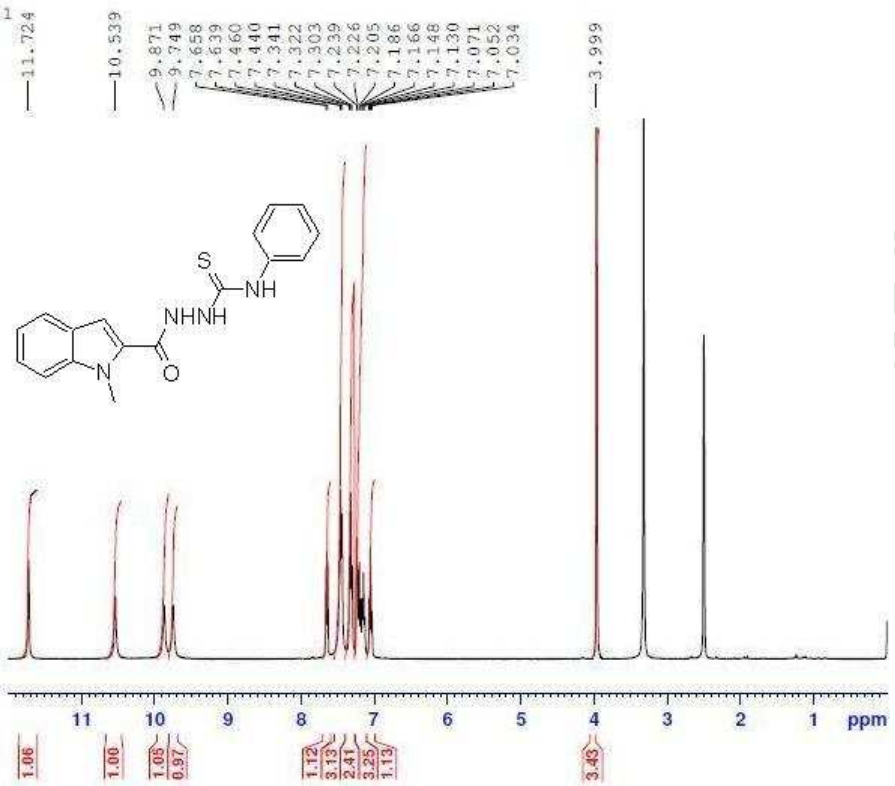


Figure S1. ^{1}H NMR spectrum of intermediate 7a (400 MHz, DMSO-d_6)

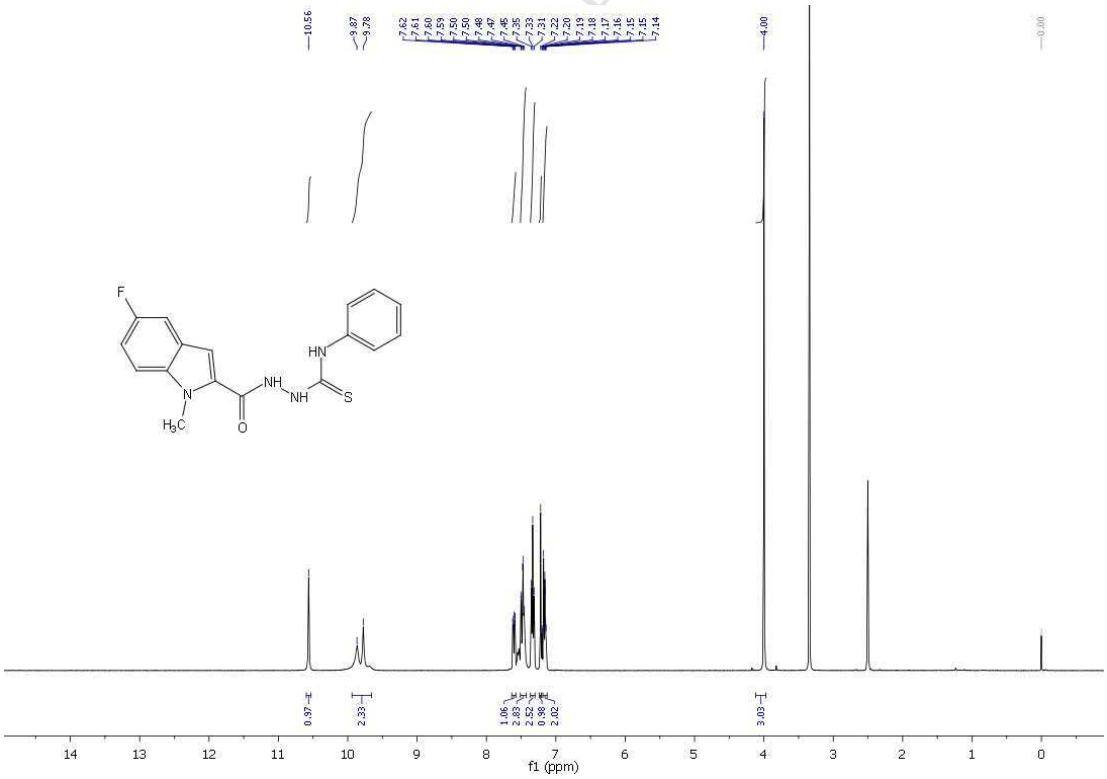


Figure S2. ^{1}H NMR spectrum of intermediate 7b (400 MHz, DMSO-d_6).

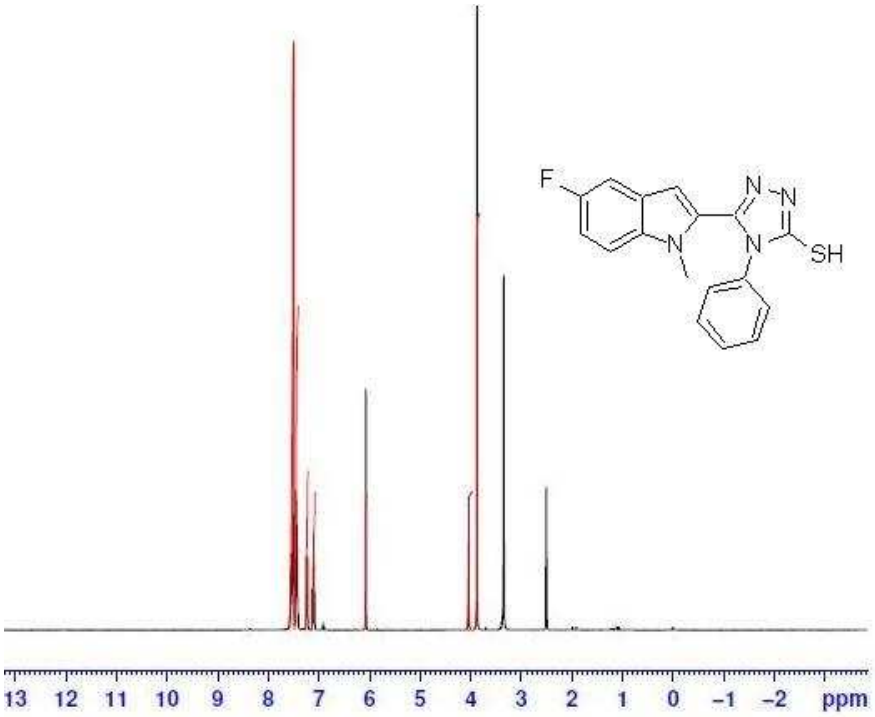


Figure S3. ^{1}H NMR spectrum of intermediate 8b (400 MHz, DMSO-d_6).

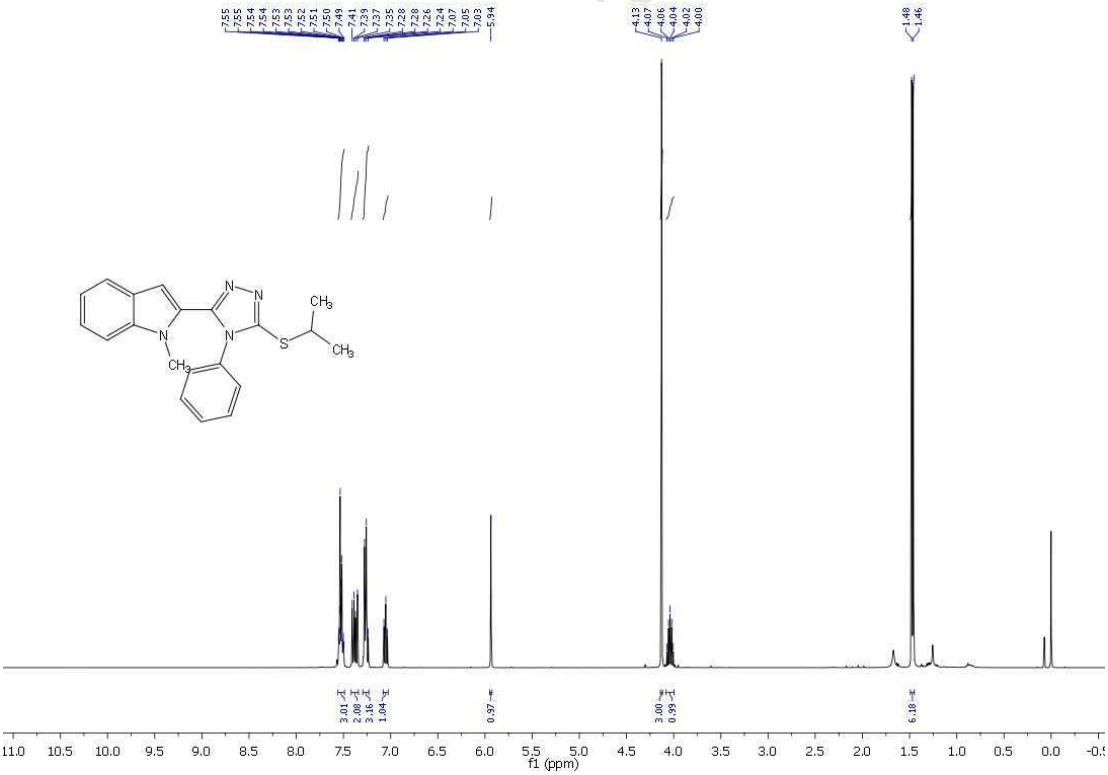


Figure S4. ^{1}H NMR spectrum of intermediate 9a (400 MHz, $CDCl_3$).

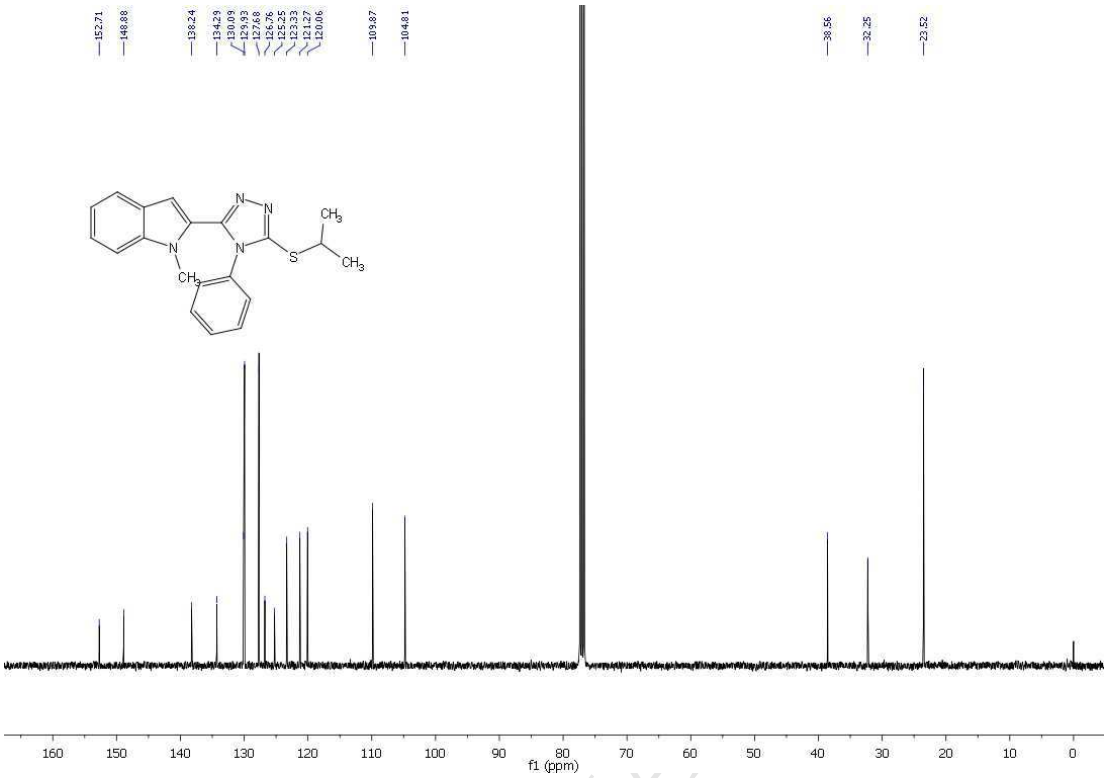


Figure S5. ^{13}C NMR spectrum of intermediate 9a (100 MHz, $CDCl_3$).

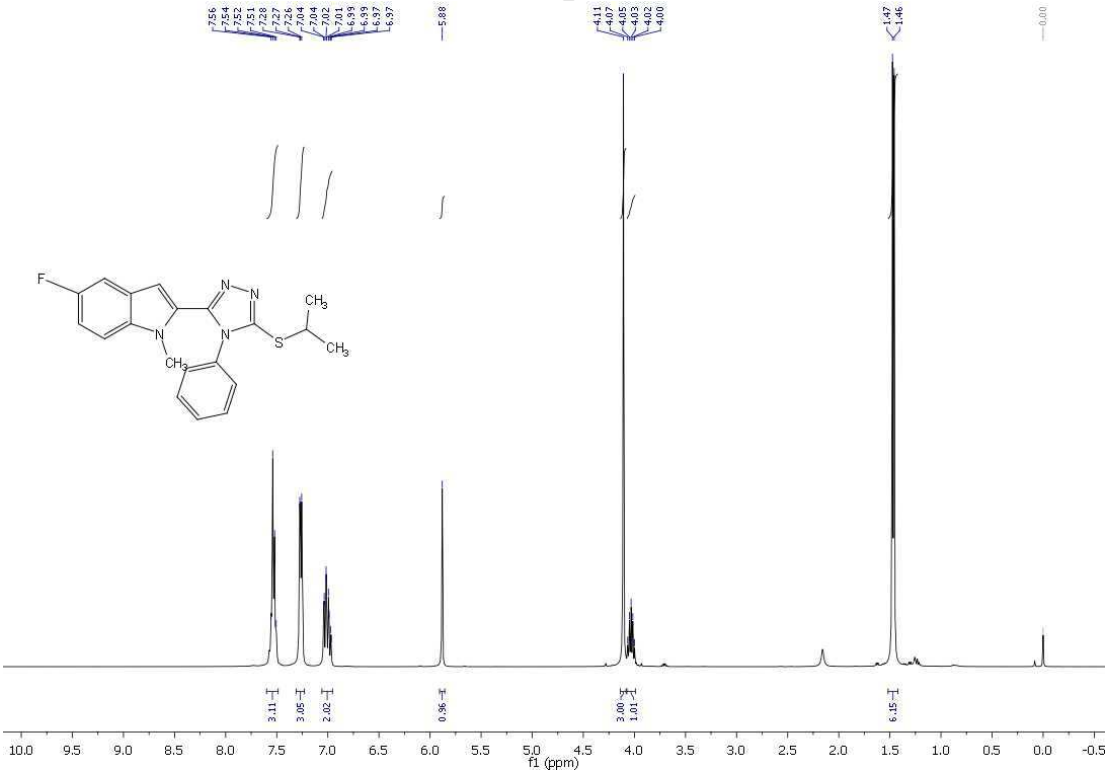


Figure S6. ^{1}H NMR spectrum of intermediate 9b (400 MHz, $CDCl_3$).

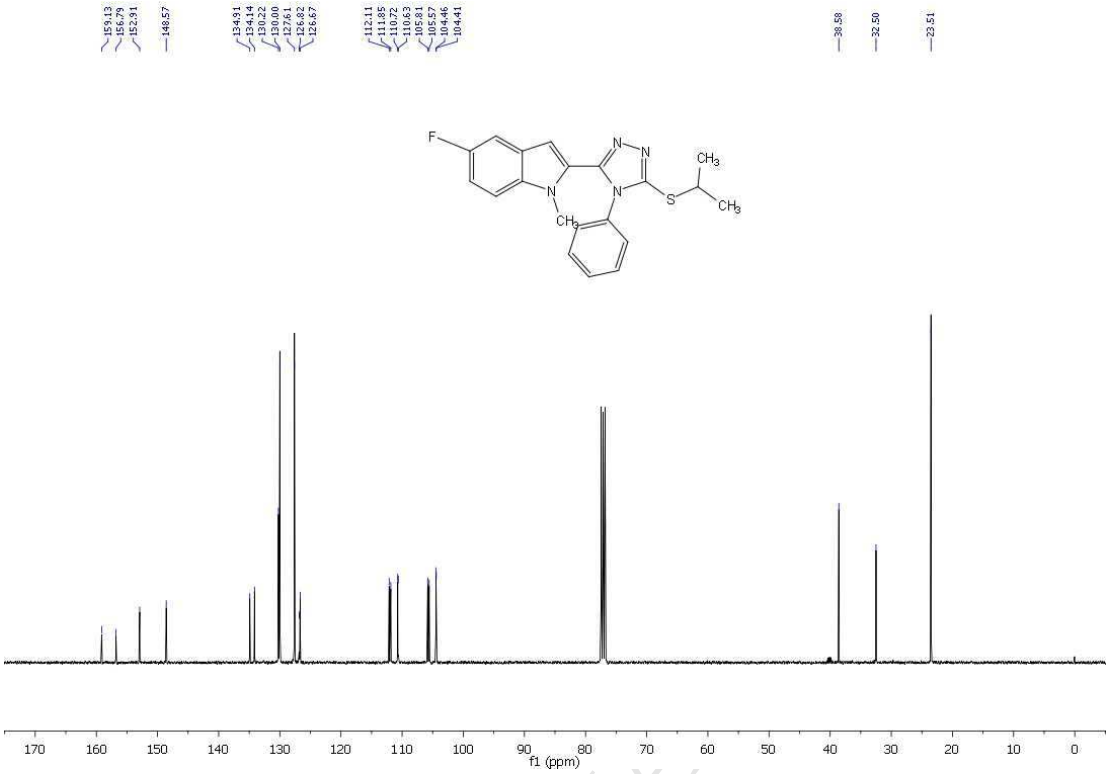


Figure S7. ^{13}C NMR spectrum of intermediate 9b (100 MHz, $CDCl_3$).

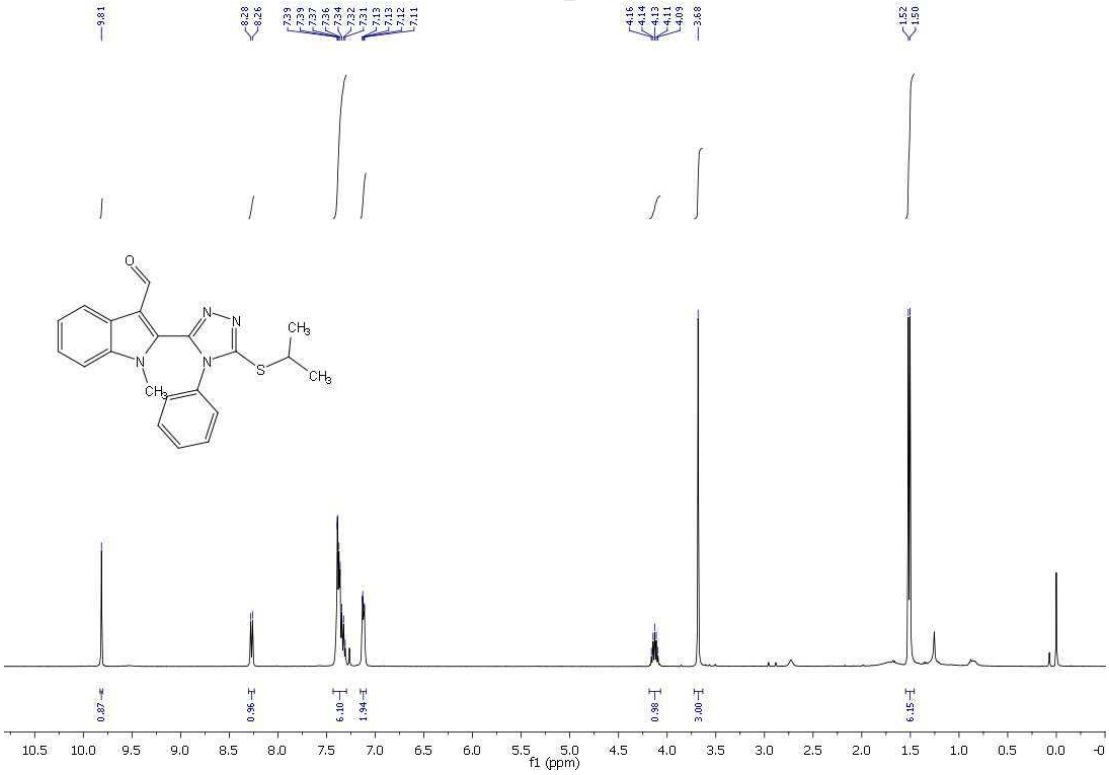


Figure S8. ^{1}H NMR spectrum of intermediate 10a (100 MHz, $CDCl_3$).

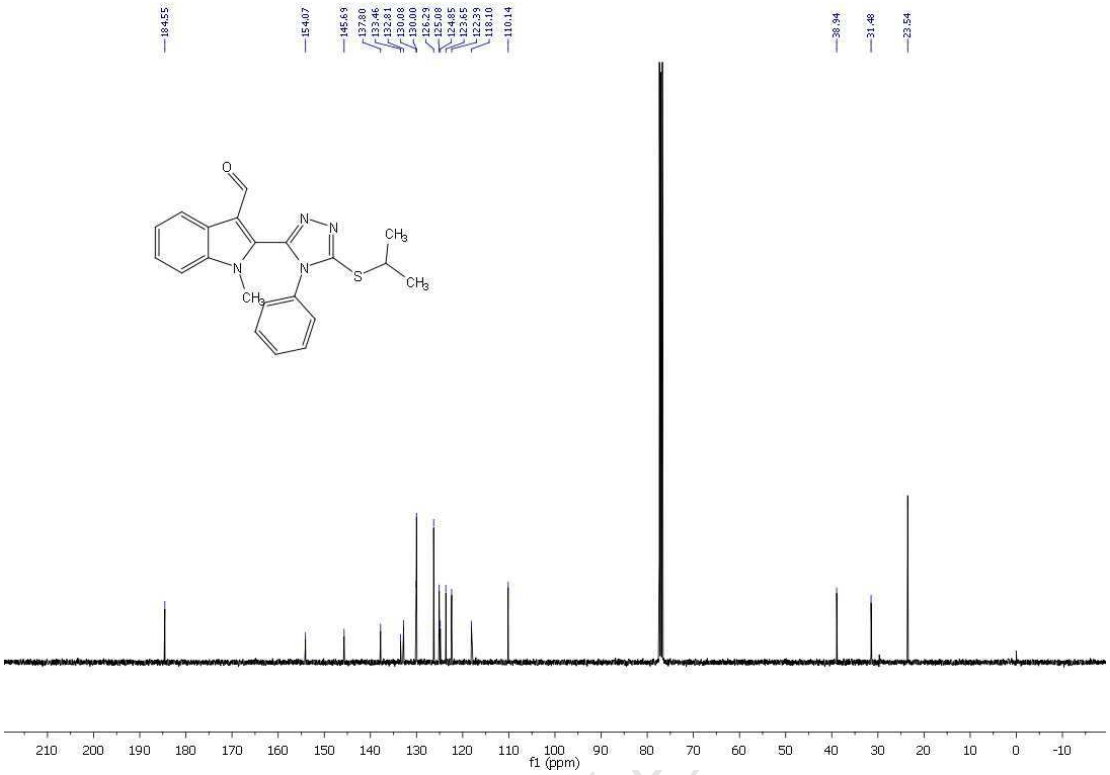


Figure S9. ^{13}C NMR spectrum of intermediate 10a (100 MHz, $CDCl_3$).

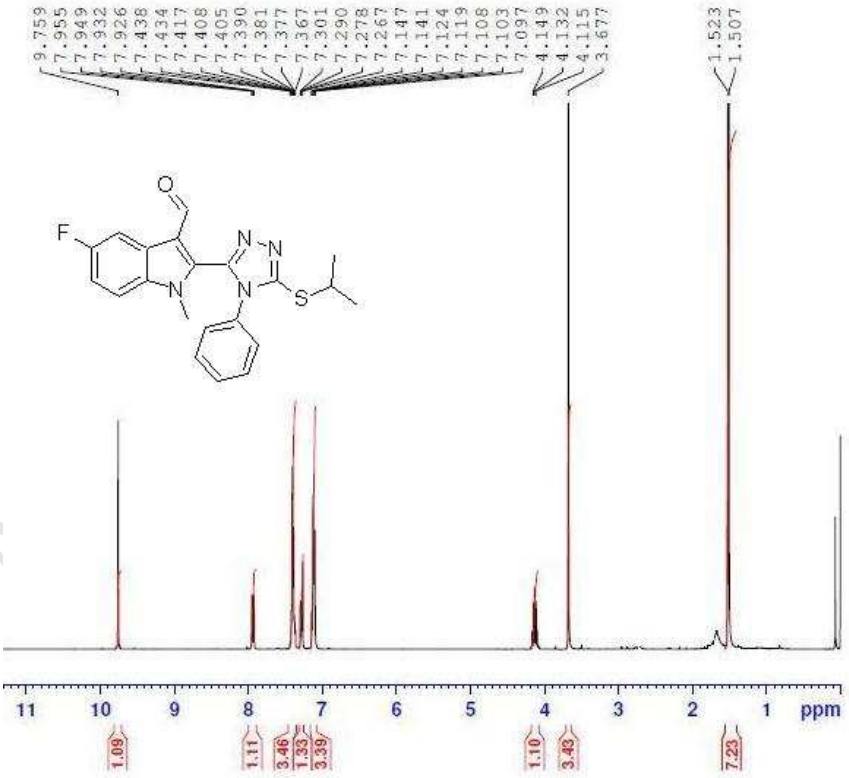


Figure S10. ^{1}H NMR spectrum of intermediate 10b (400 MHz, $CDCl_3$).

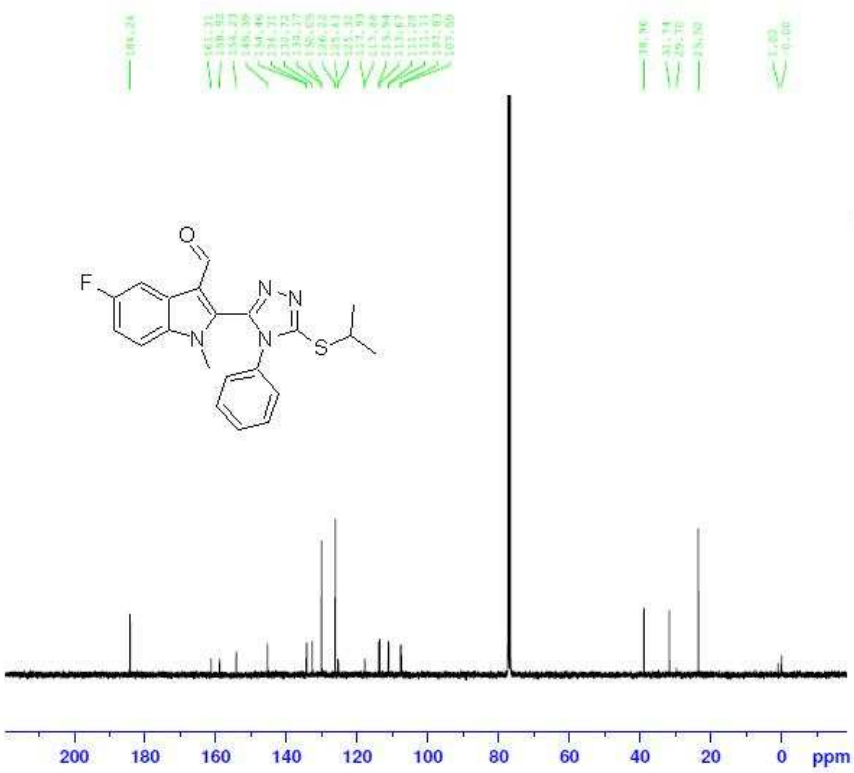


Figure S11. ^{13}C NMR spectrum of intermediate 10b (100 MHz, $CDCl_3$).

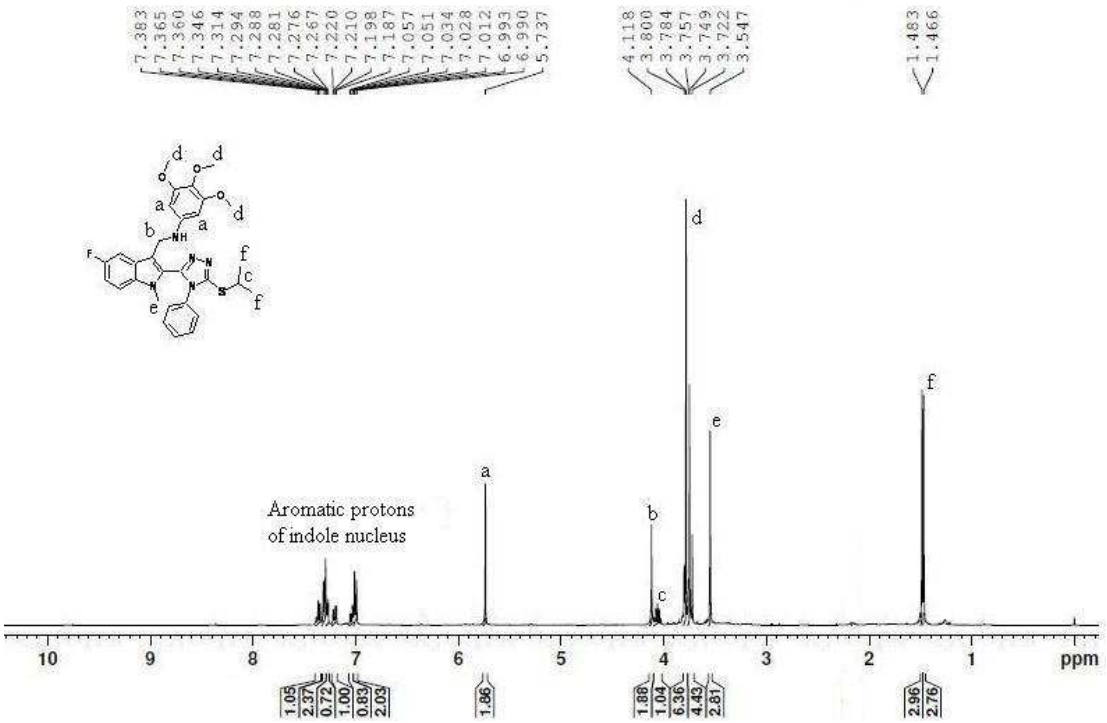


Figure S12. ^{1}H NMR spectrum of IT-03 (400 MHz, $CDCl_3$)

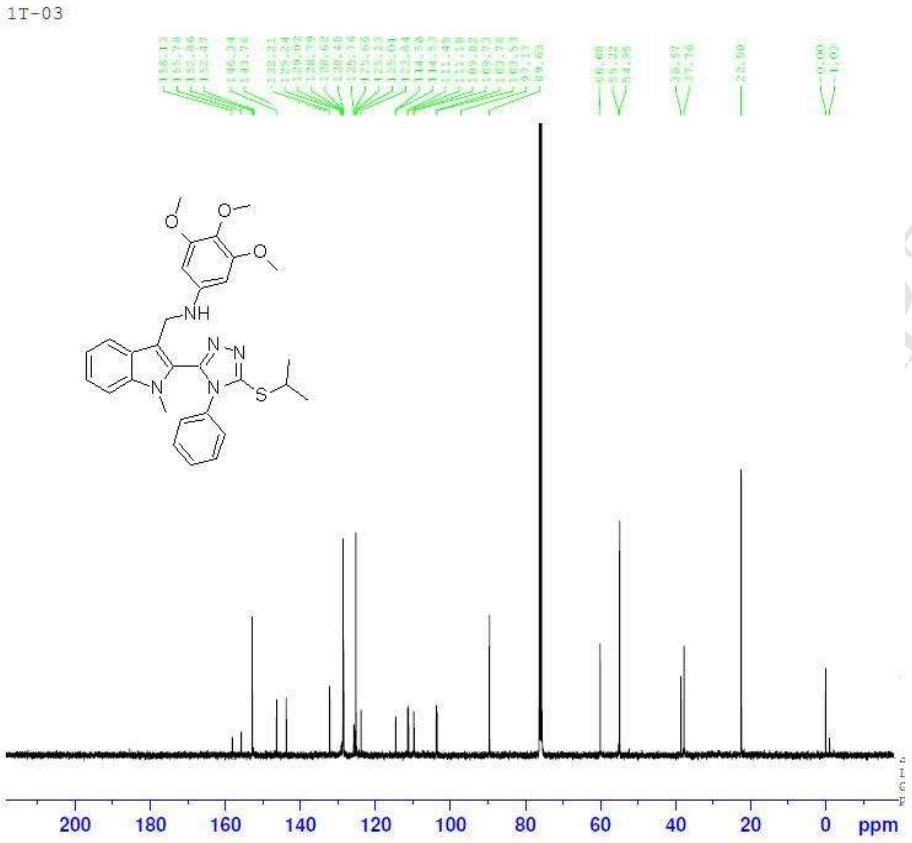


Figure S13. ^{13}C NMR spectrum of IT-03 (100 MHZ, $CDCl_3$)

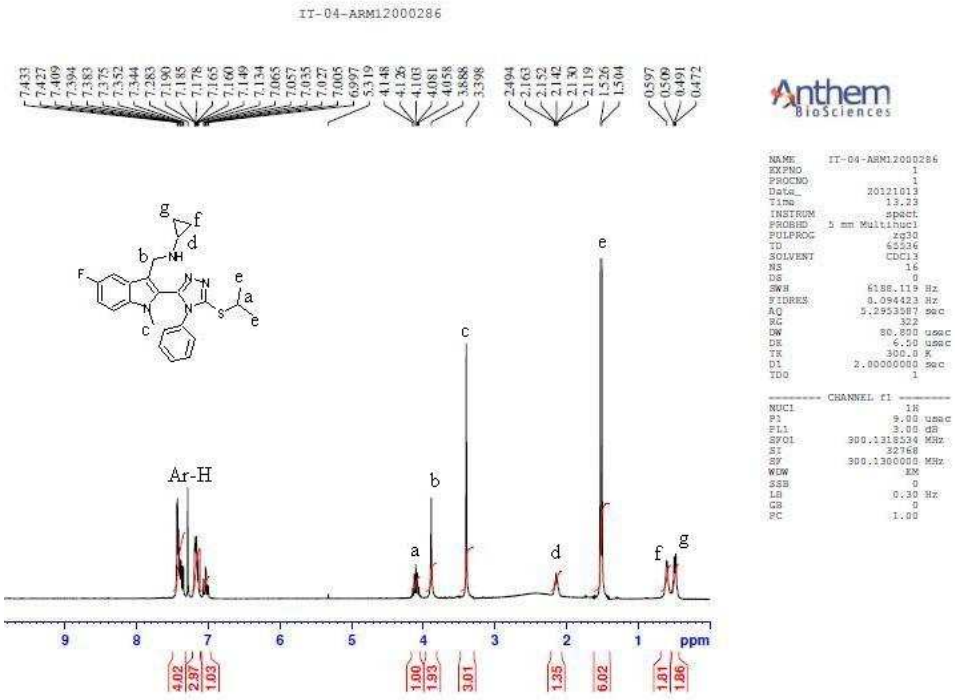


Figure S14. ^{1}H NMR spectrum of IT-04 (300 MHz, $CDCl_3$)

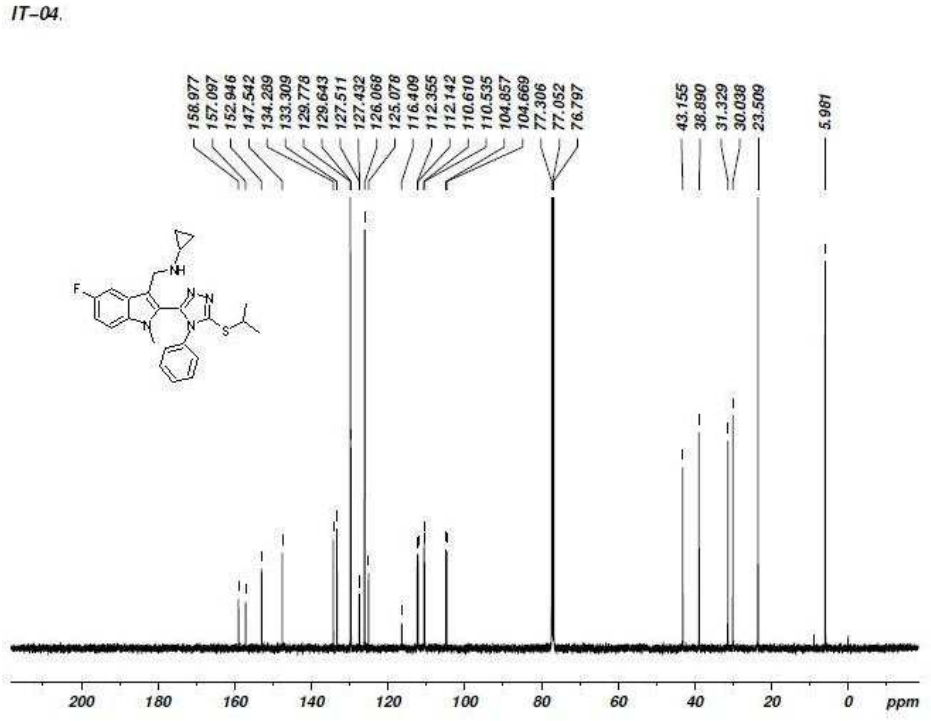


Figure S15. ^{13}C NMR spectrum of IT-04 (125 MHz, $CDCl_3$)

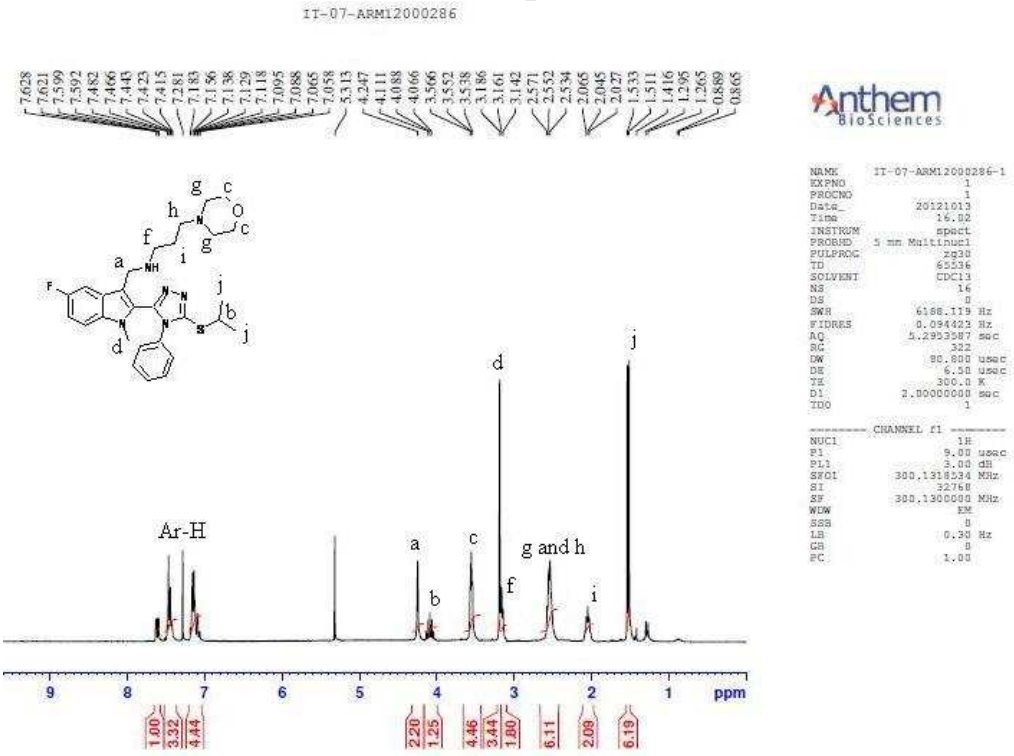


Figure S16. ^{1}H NMR spectrum of IT-07 (300 MHz, $CDCl_3$)

IT-07.

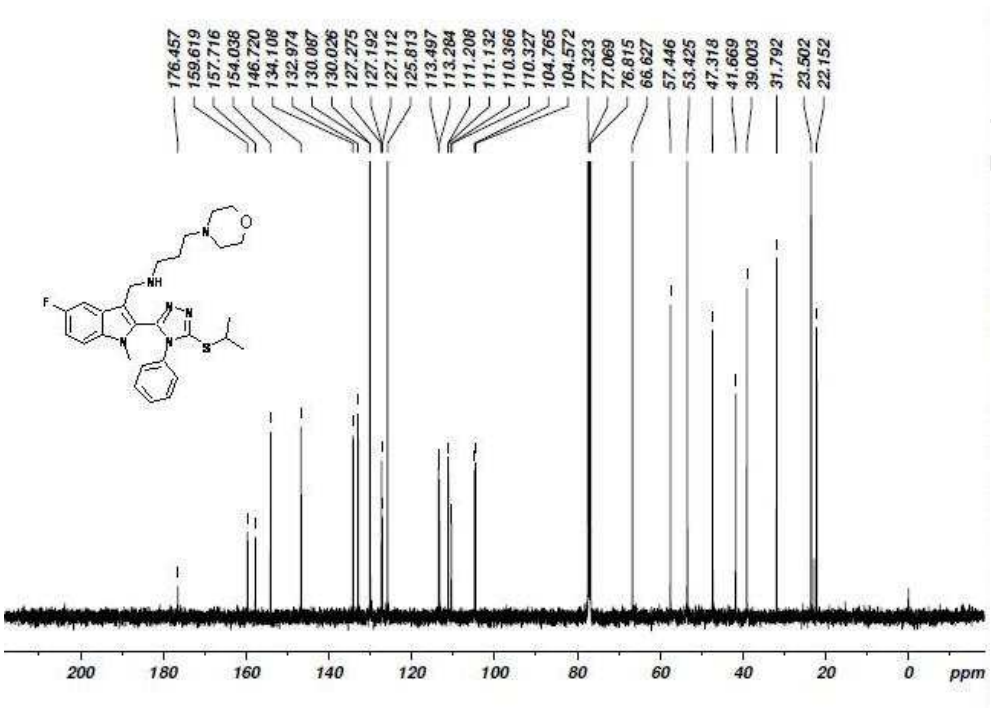


Figure S17. ^{13}C NMR spectrum of IT-07 (125 MHz, $CDCl_3$)

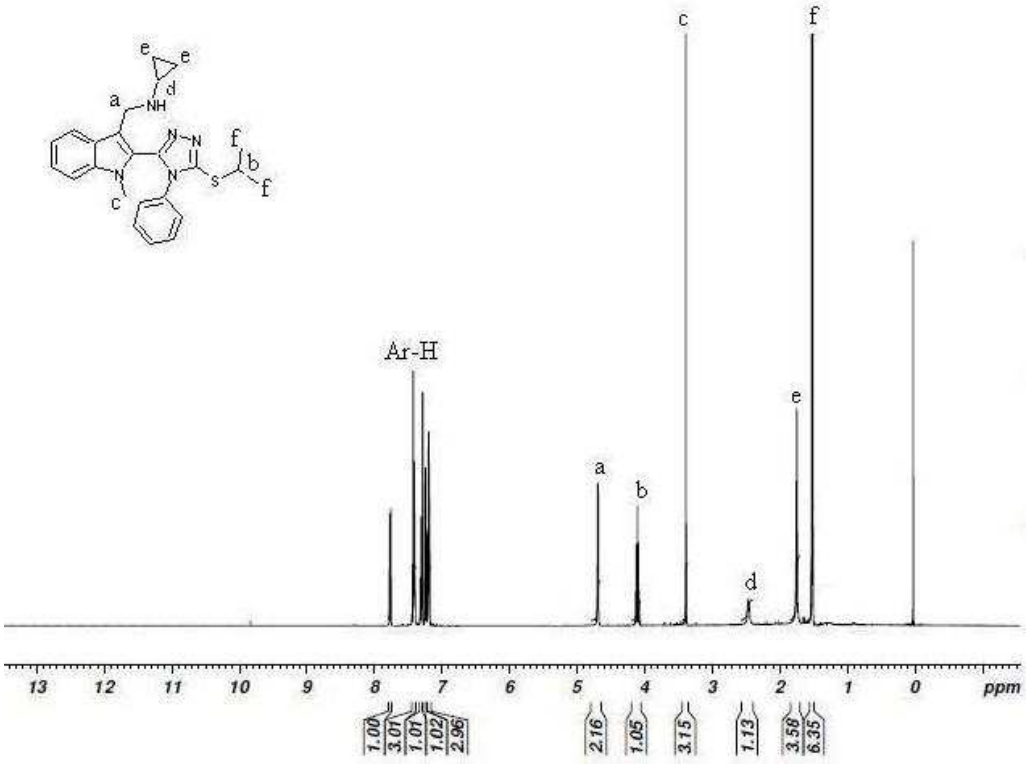


Figure S18. ^{1}H NMR spectrum of IT-08 (500 MHz, $CDCl_3$)

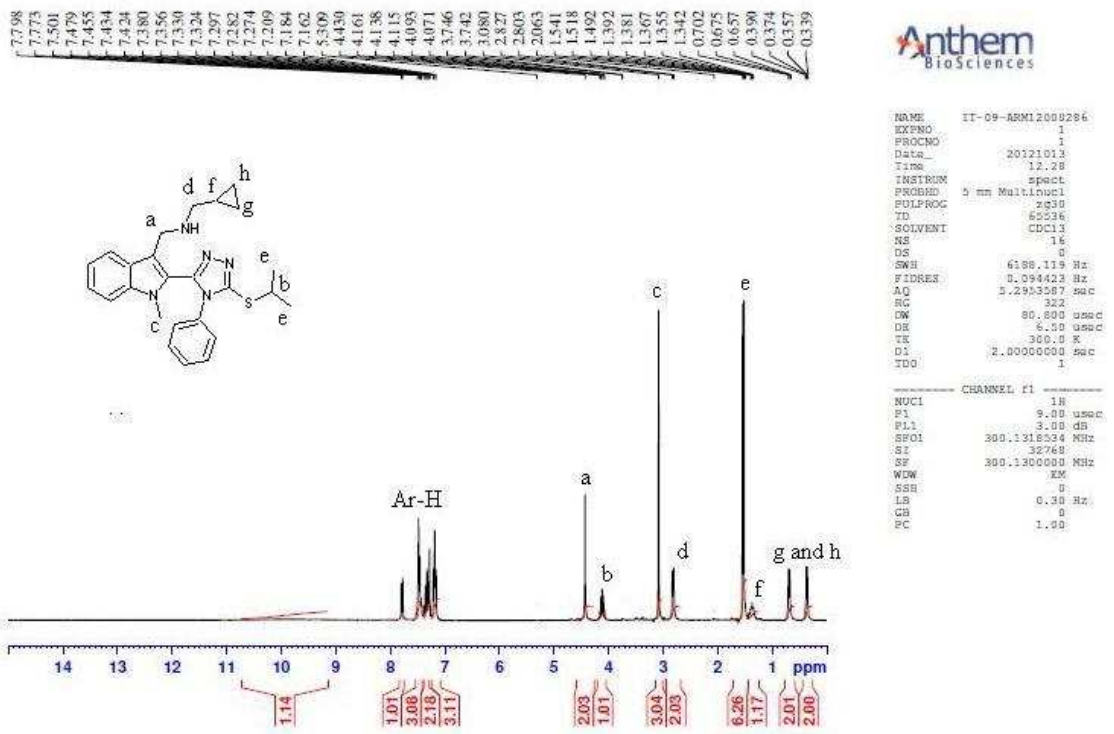


Figure S19. ^{1}H NMR spectrum of IT-09 (300 MHz, $CDCl_3$)

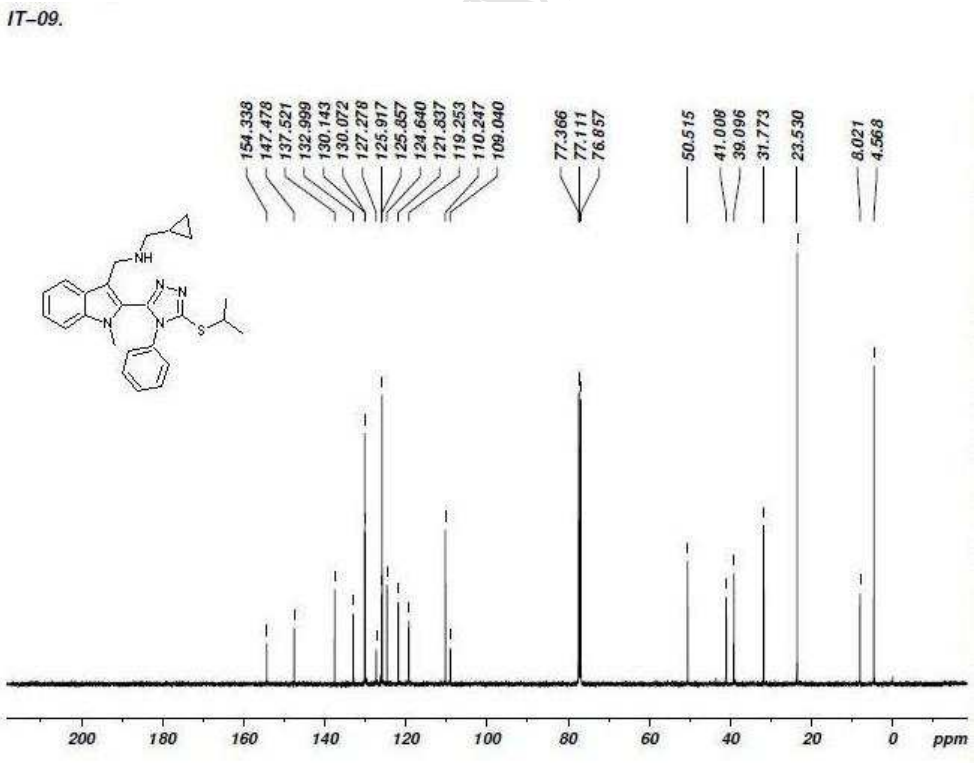


Figure S20. ^{13}C NMR spectrum of IT-09 (125 MHz, $CDCl_3$)

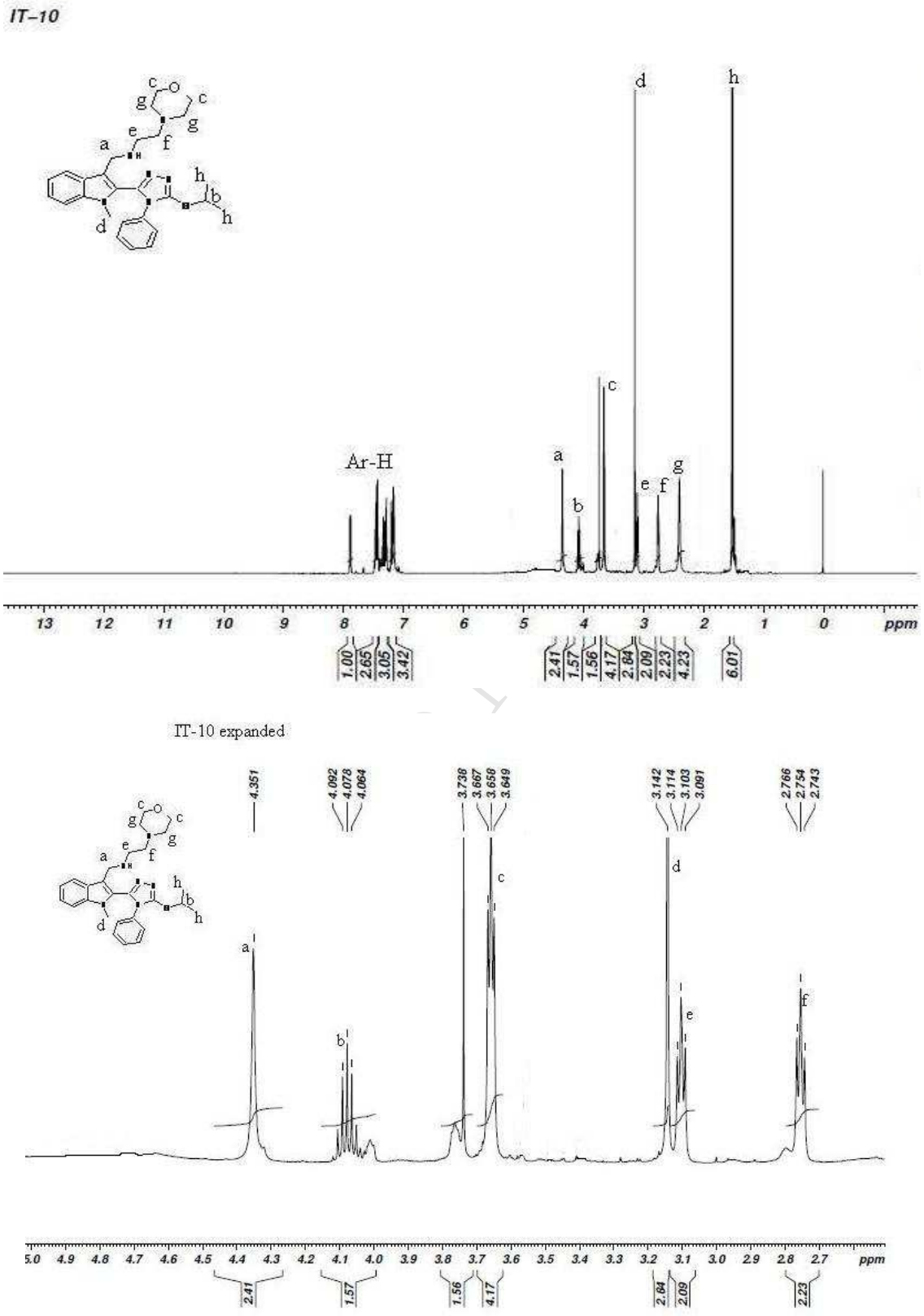


Figure S21. ^{1}H NMR spectrum of IT-10 (500 MHz, $CDCl_3$)

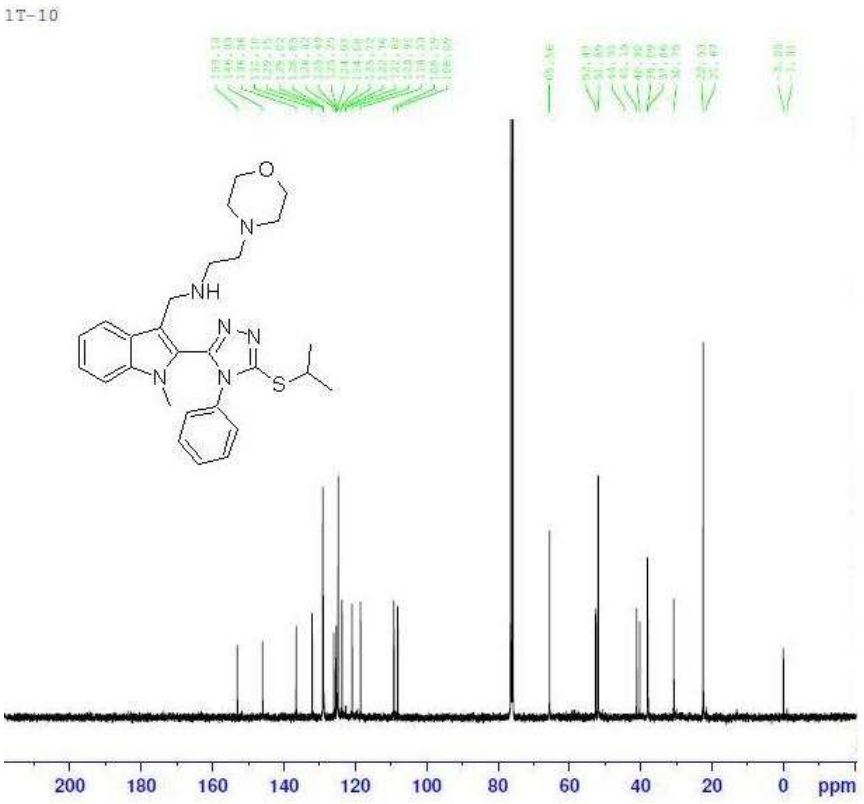
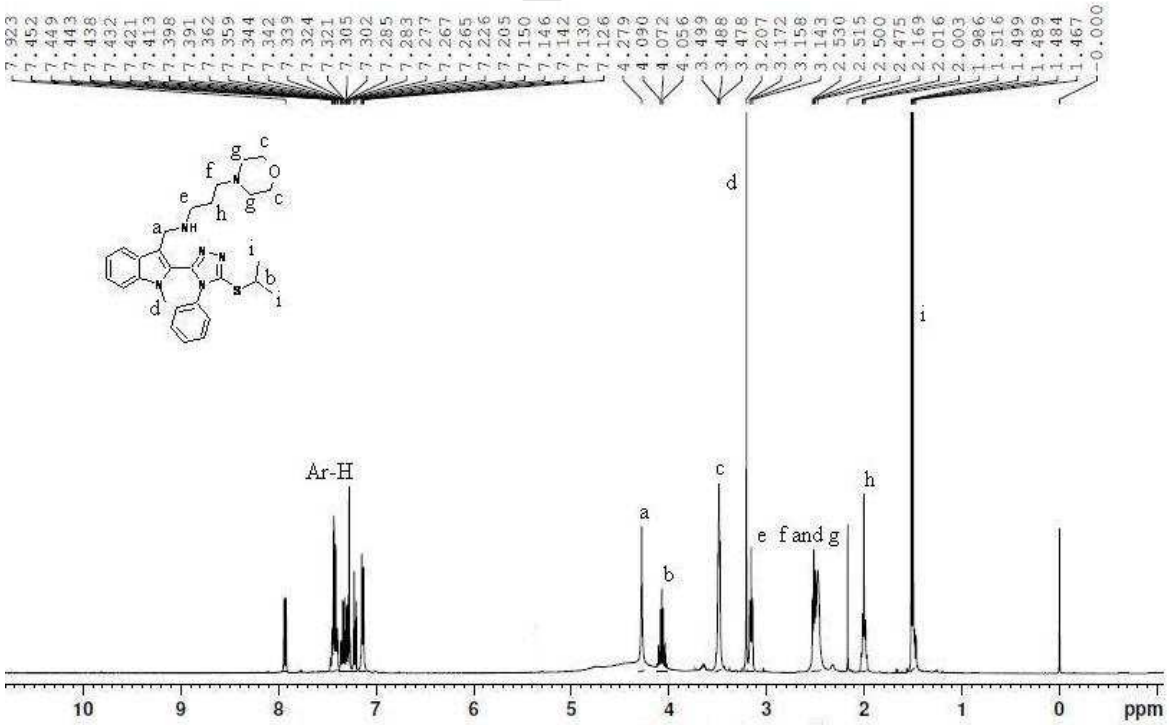


Figure S22. ^{13}C NMR spectrum of IT-10 (100 MHz, $CDCl_3$).



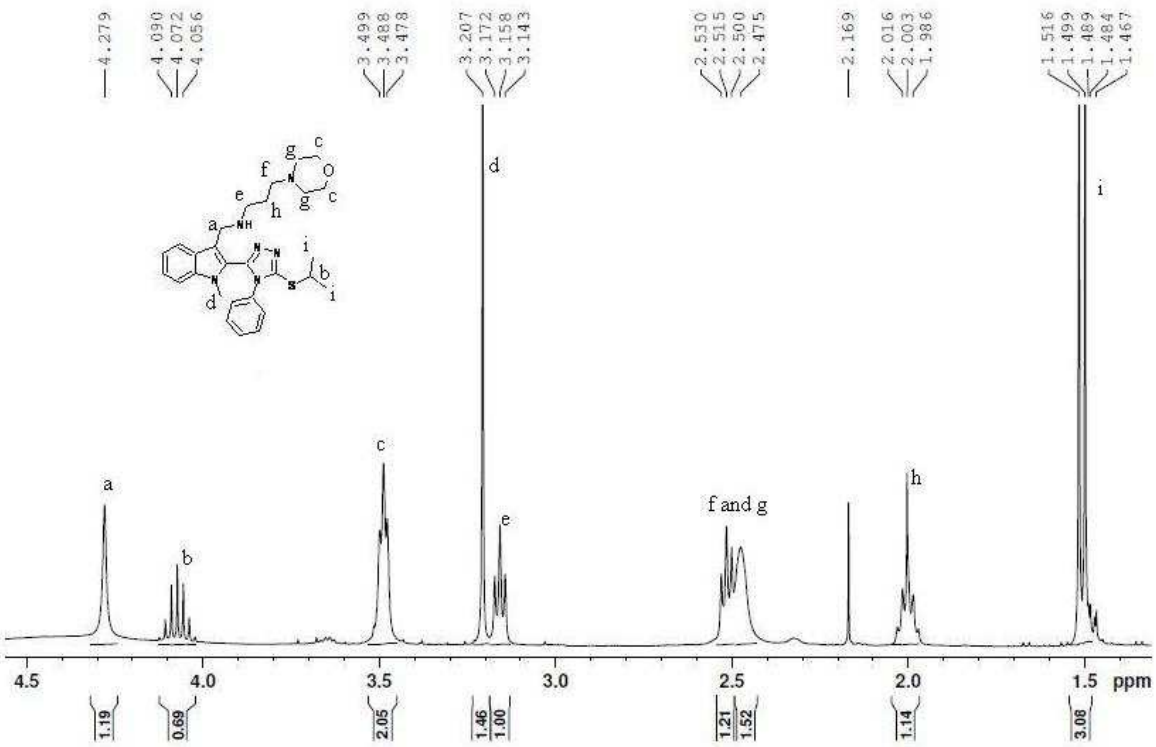


Figure S23. ^{1}H NMR spectrum of IT-11 (400 MHz, $CDCl_3$)

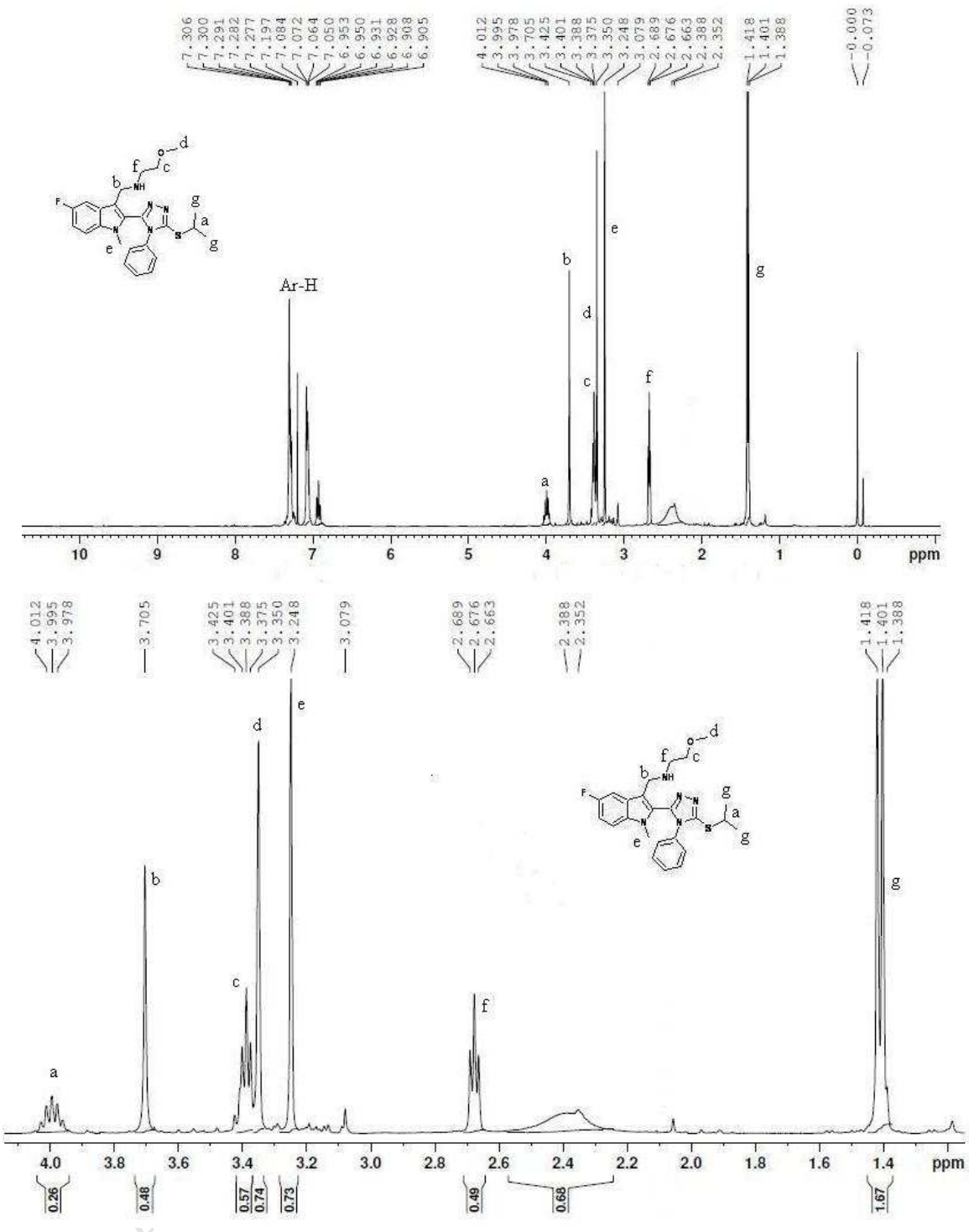


Figure S24. ^{1}H NMR spectrum of IT-13 (400 MHz, $CDCl_3$)

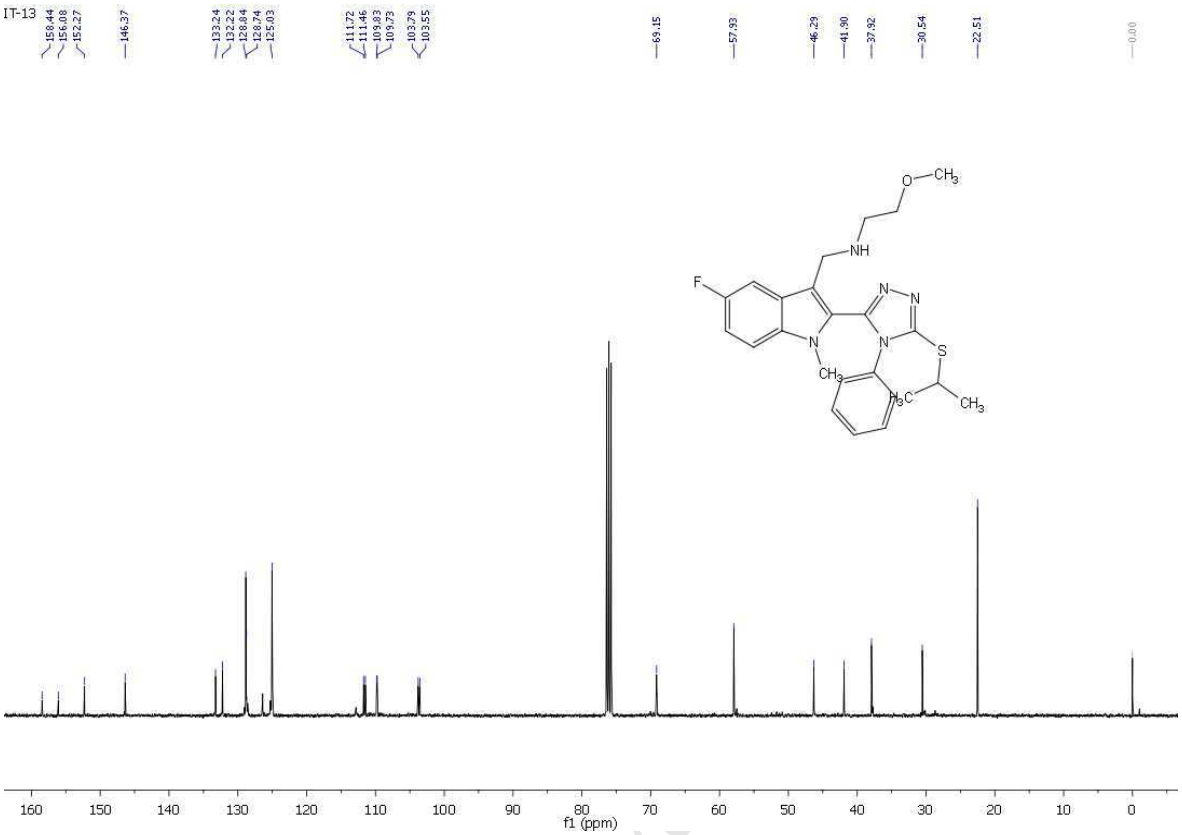


Figure S25. ^{13}C spectrum of IT-13 (100 MHz, $CDCl_3$).

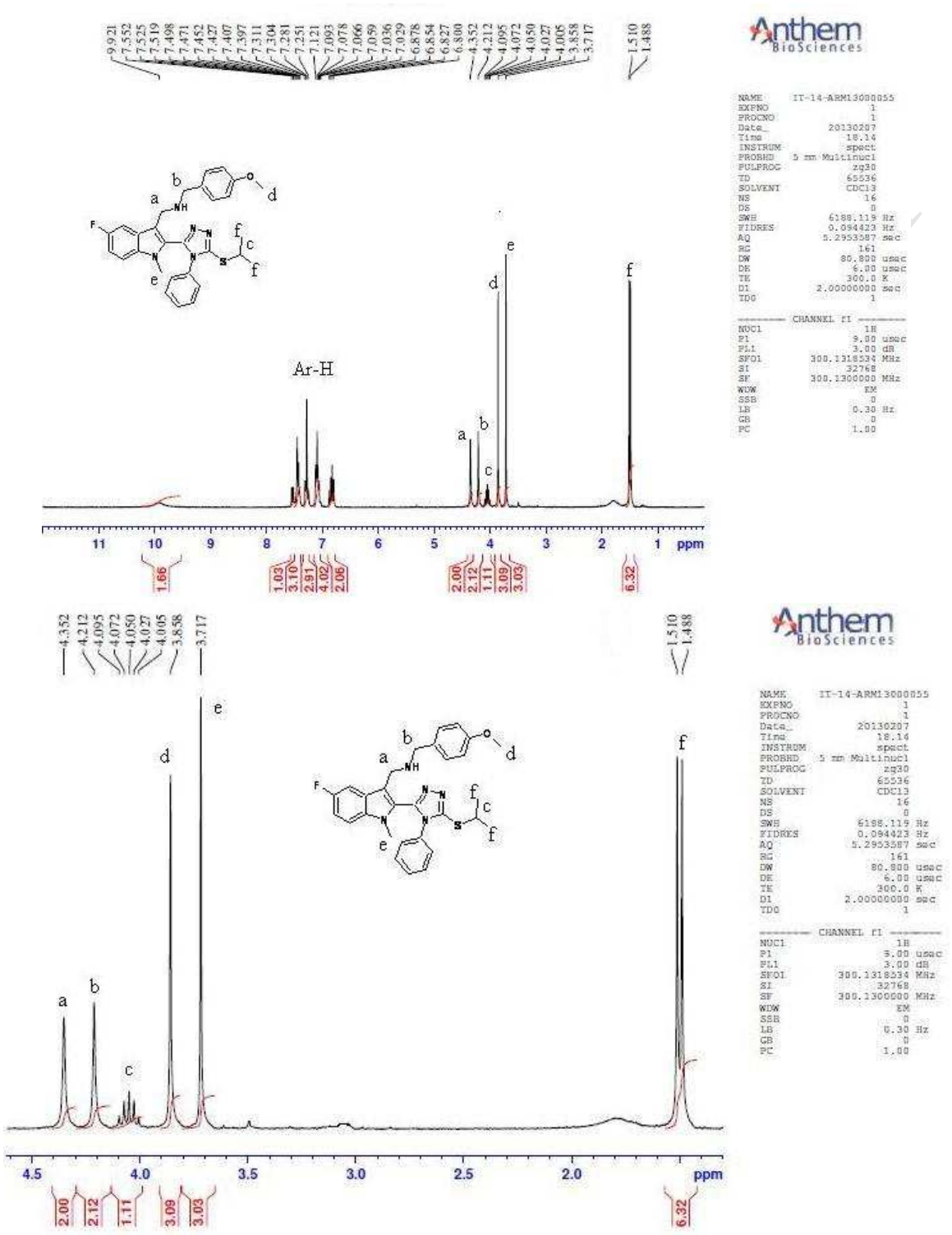


Figure S26. ^{1}H NMR spectrum of IT-14 (300 MHz, $CDCl_3$)

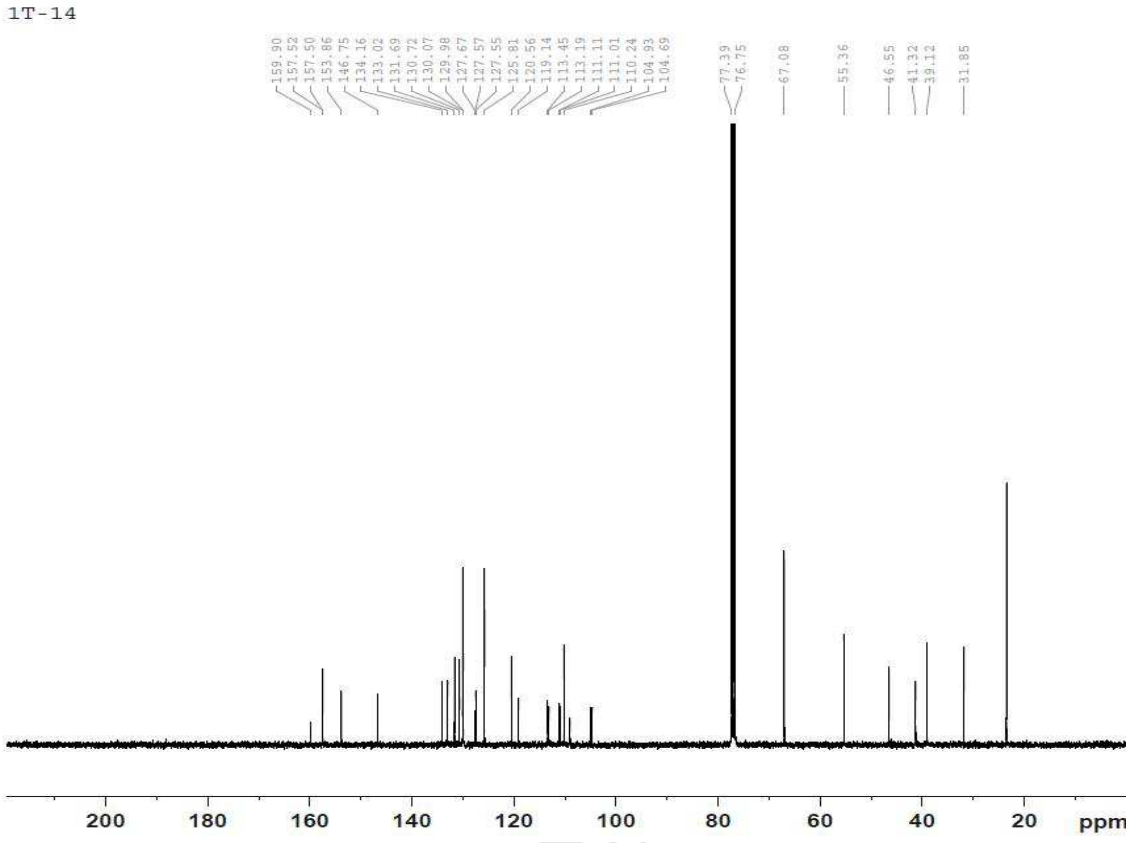


Figure S27. ^{13}C NMR spectrum of IT-14 (100 MHz, $CDCl_3$)

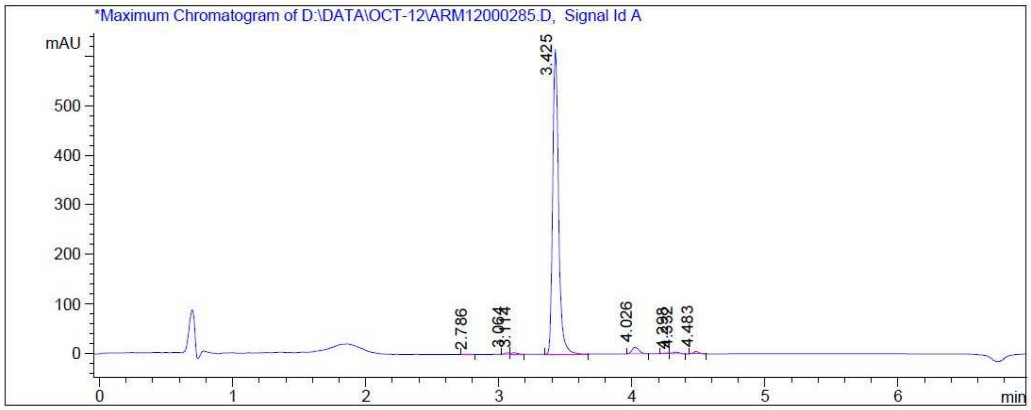
LC/MS REPORT

Data file :D:\DATA\OCT-12\ARM12000285.D Vail No. : Vial 11

Injection Date: 13-10-2012 Injection Vol: 3.00 µL

Sample Name :IT-14 Operator : POORNACHANDRA.T

Acq Method : XT_095FA.M



#	Meas. Ret	Area	Area %
1	2.786	3.324	0.168
2	3.064	6.300	0.318
3	3.114	9.205	0.464
4	3.425	1.888e3	95.229
5	4.026	48.529	2.448
6	4.298	3.229	0.163
7	4.332	10.508	0.530
8	4.483	13.496	0.681

MASS REPORT ANTHEM BIOSCIENCES

Data File: ARM12000285.D **Instrument** LC-MSD-Trap-XCT_Plus

Method: XT_095FA.M **Sample Name** IT-14

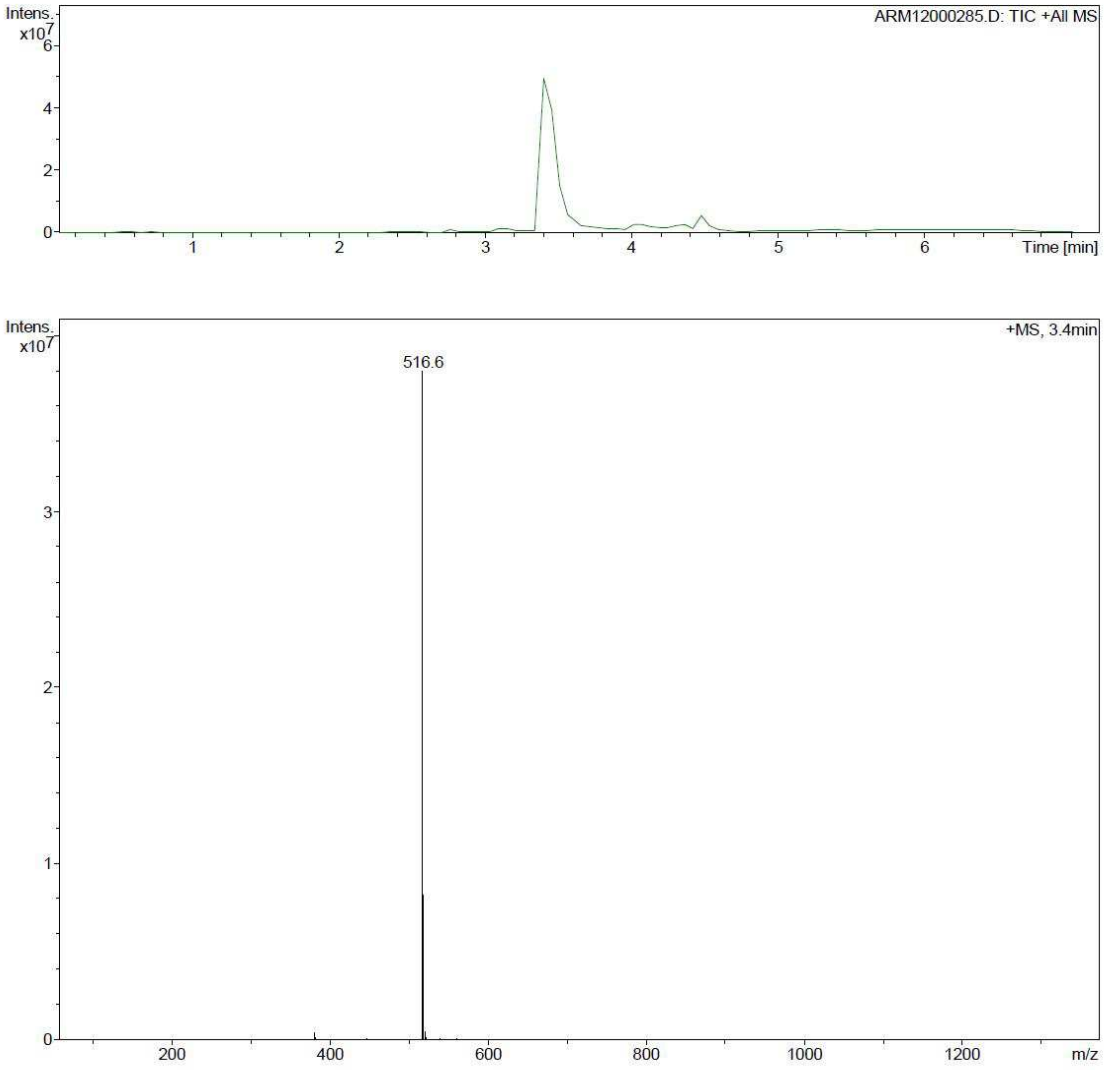


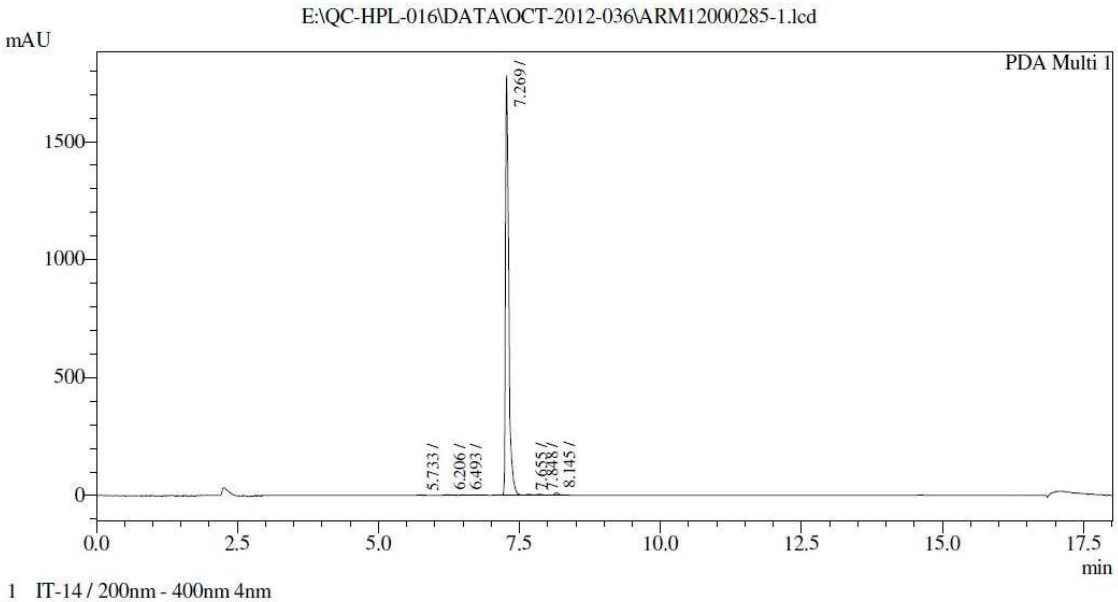
Figure S28. LCMS Report of IT-14

HPLC REPORT

Sample Name	:IT-14	Operator	:Manasa
Location	: 63	Injection Date	:13-10-2012
Injection Volume	: 5 uL	Injection Time	:12:30:33 PM
Acq Method	: POLAR.lcm		
Data File	: E:\QC-HPL-016\DATA\OCT-2012-036\ARM12000285-1.lcd		

Column :Zorbax SB-CN,150mm X 4.6 mm, 5µM, Flow:1.0 ml/minute,
MPA- 0.1% TFA in Water, MPB- Acetonitrile,
Column Temp-30°C,

Time (in min)	0	7	12	14	18
%B	0	95	95	0	0



1 IT-14 / 200nm - 400nm 4nm

PDA Ch1 200nm - 400nm 4nm

Peak#	Ret. Time	Area	Area %
1	5.733	2221	0.030
2	6.206	14126	0.194
3	6.493	8072	0.111
4	7.269	7178252	98.493
5	7.655	17893	0.246
6	7.848	17860	0.245
7	8.145	49676	0.682
Total		7288101	100.000

Figure S29. HPLC Purity of IT-14

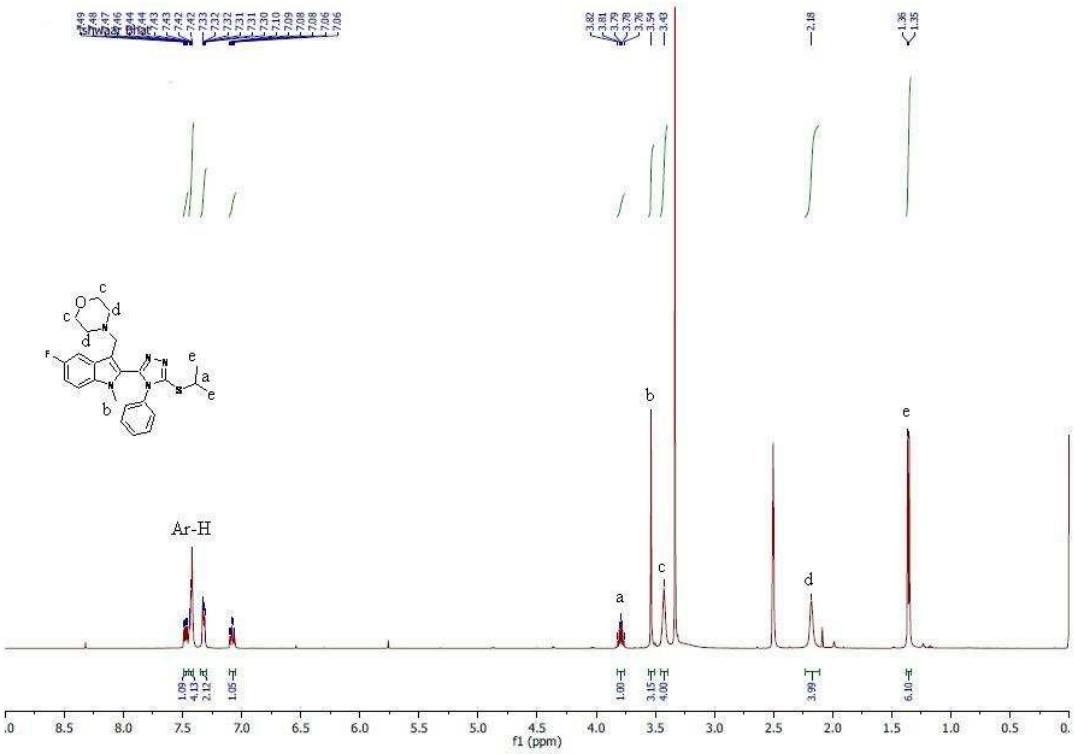


Figure S30. ^{1}H NMR spectrum of IT-17 (500 MHz, DMSO-D_6)

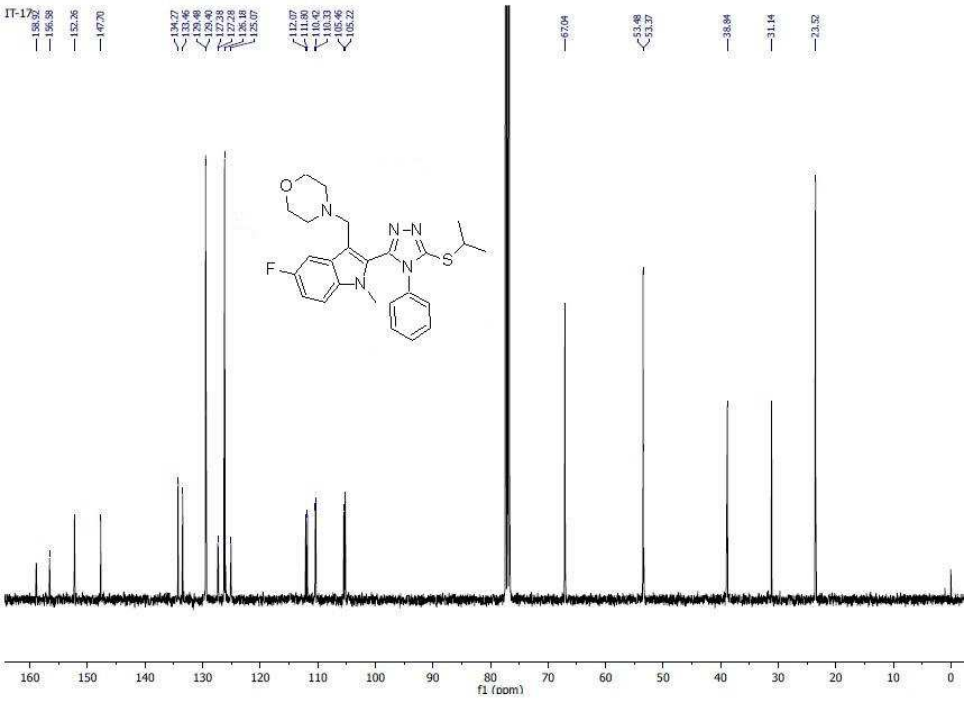


Figure 31. ^{13}C spectrum of IT-31 (100 MHz, $CDCl_3$).

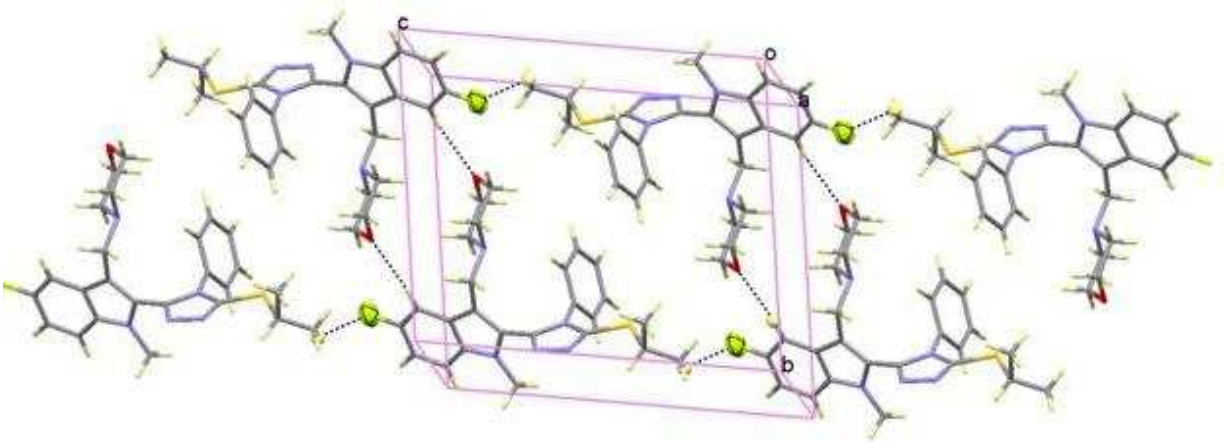


Figure S32. The packing diagram shows the C-H…F and C-H…O interactions.

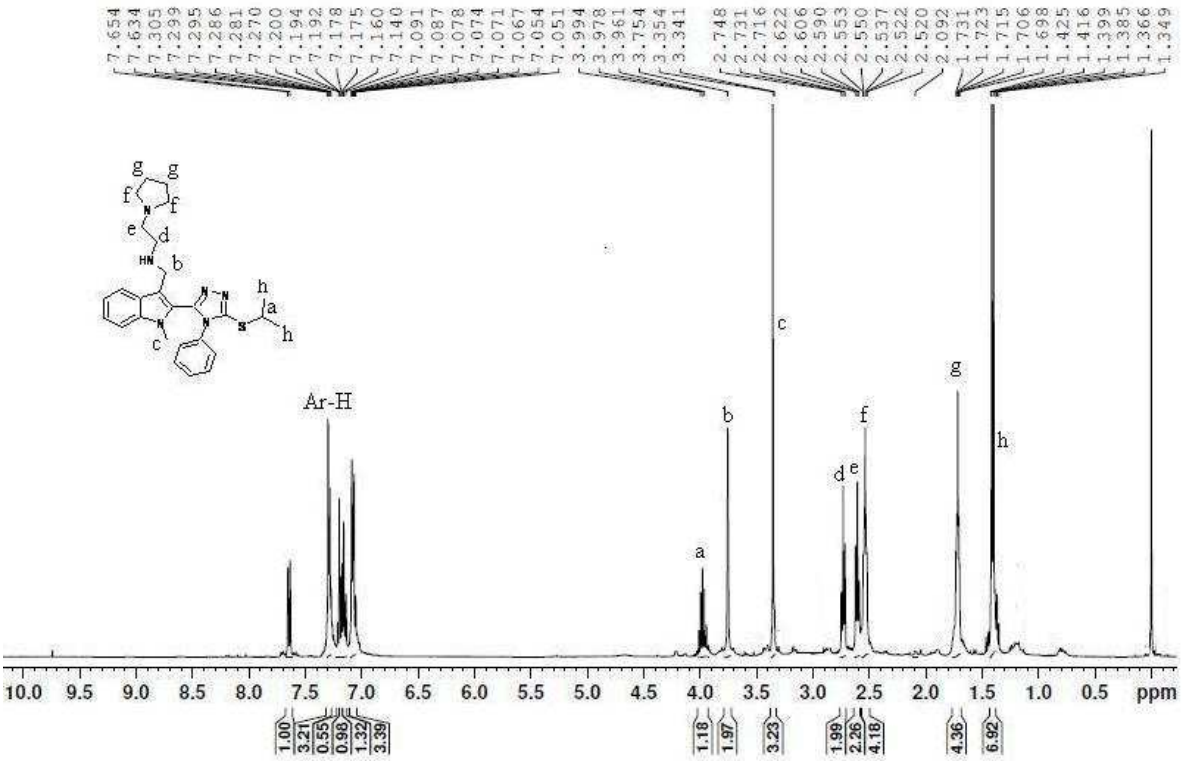


Figure S33. ^{1}H NMR spectrum of IT-20 (400 MHz, $CDCl_3$)

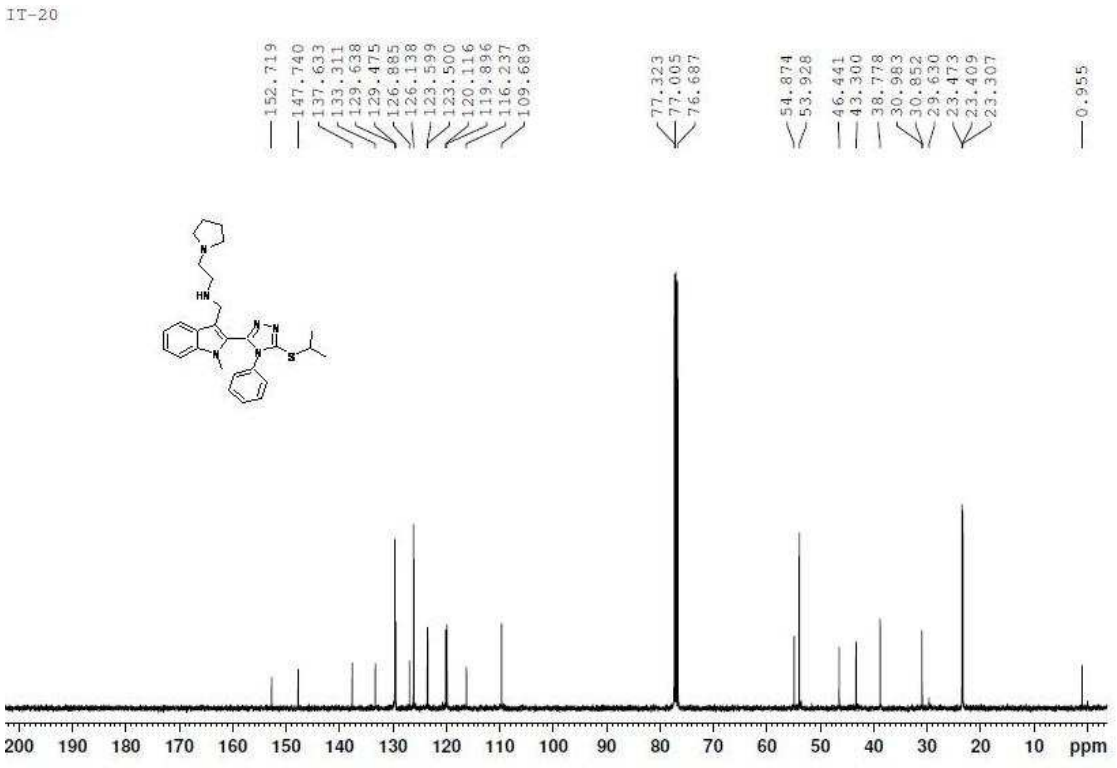


Figure S34. ^{13}C NMR spectrum of IT-20 (125 MHz, $CDCl_3$)

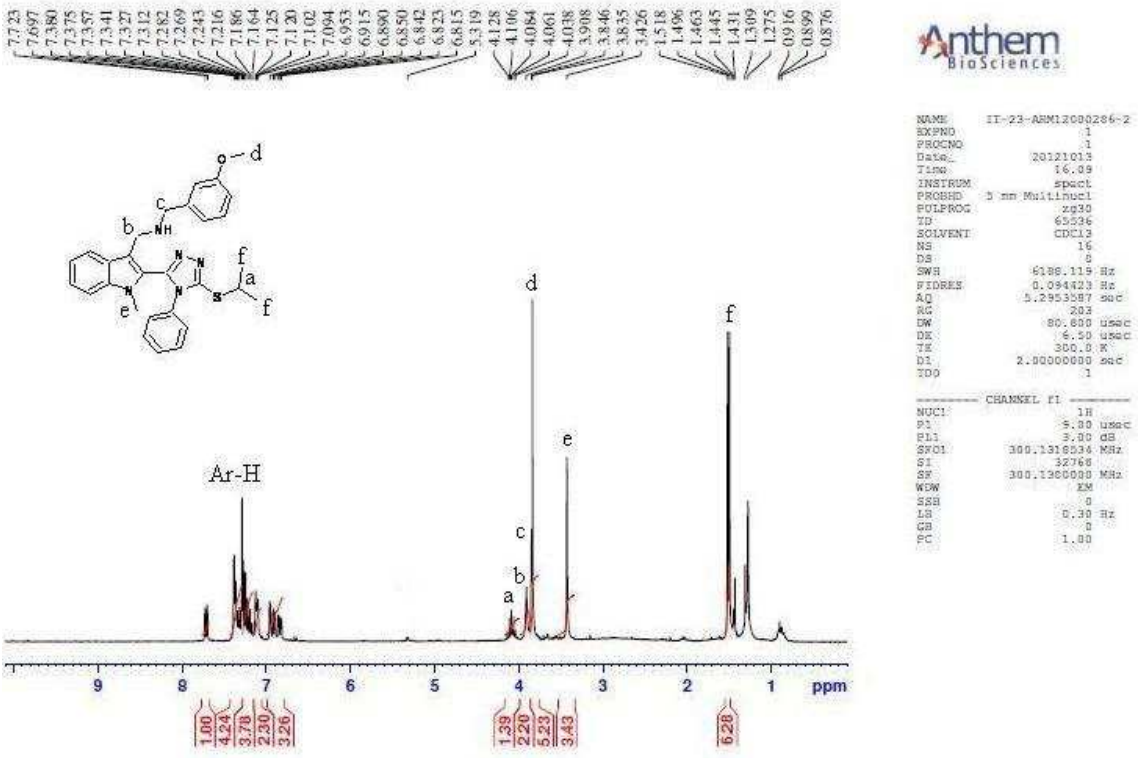


Figure S35. ^{1}H NMR spectrum of IT-23 (300 MHz, $CDCl_3$)

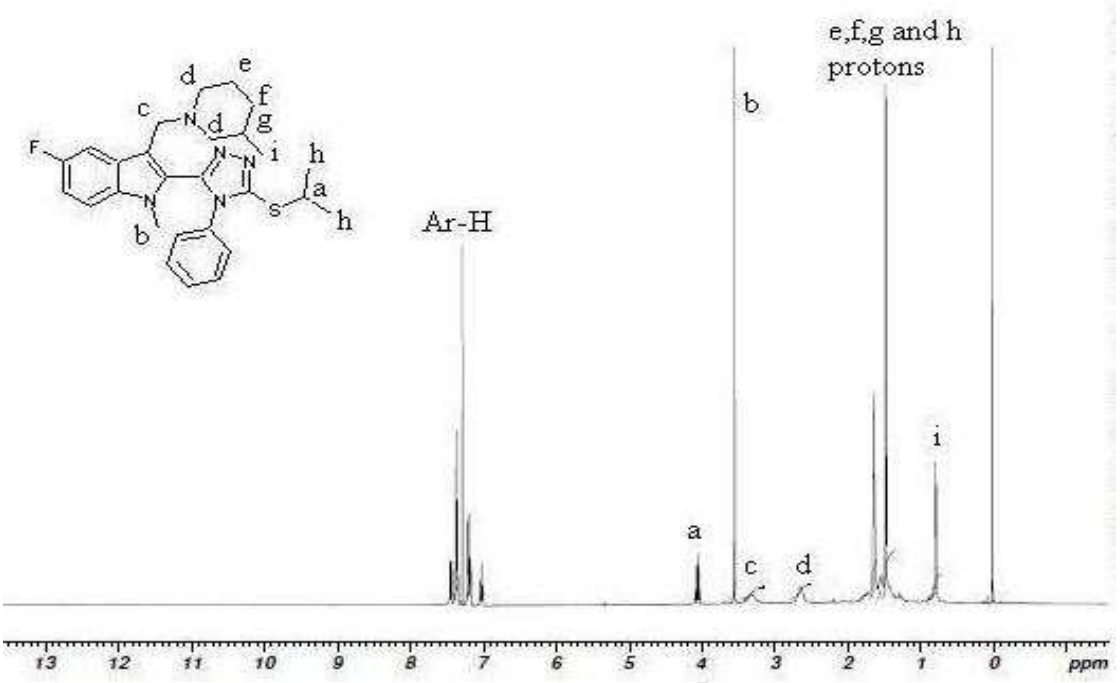


Figure S36. ^{1}H NMR spectrum of IT-30 (500 MHz, $CDCl_3$)

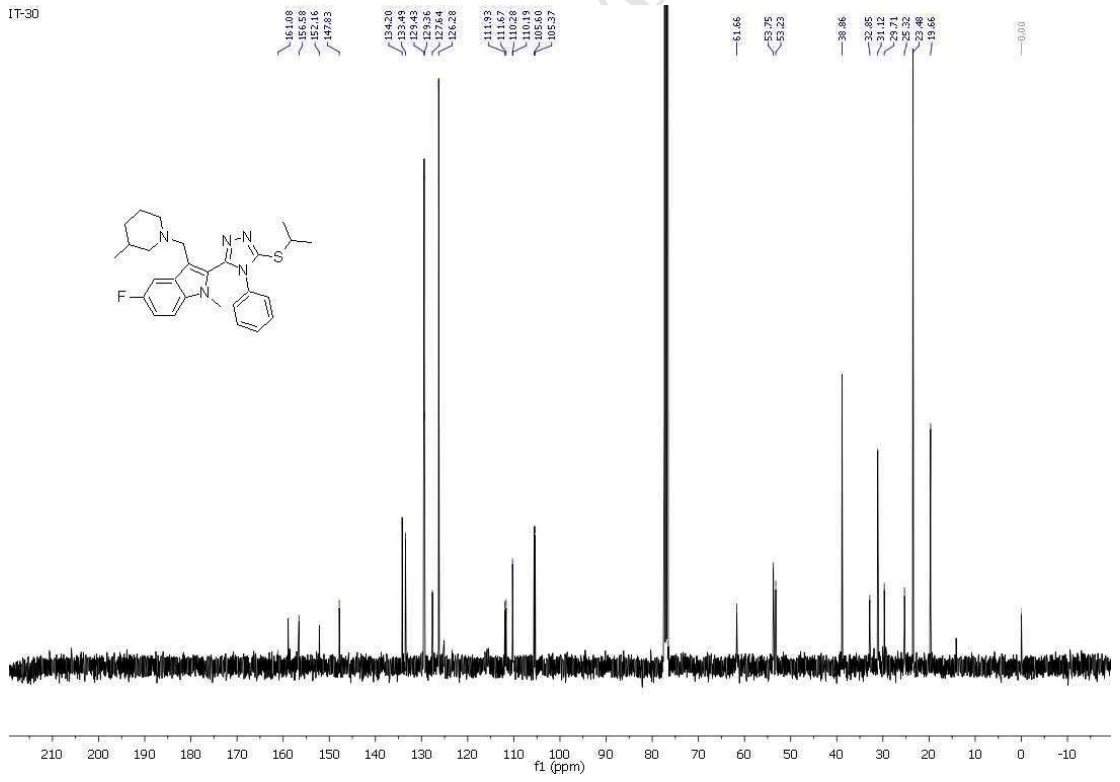


Figure S37. ^{13}C NMR spectrum of IT-30 (100 MHz, $CDCl_3$).

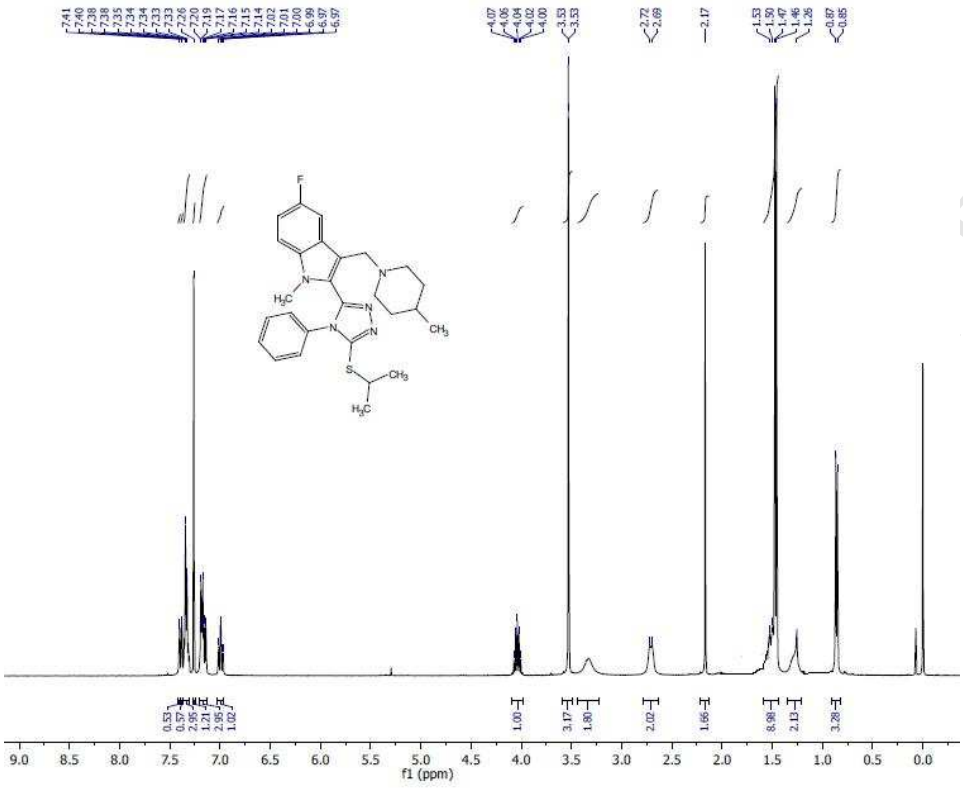


Figure S38. ^{1}H NMR spectrum of IT-31 (500 MHz, $CDCl_3$)

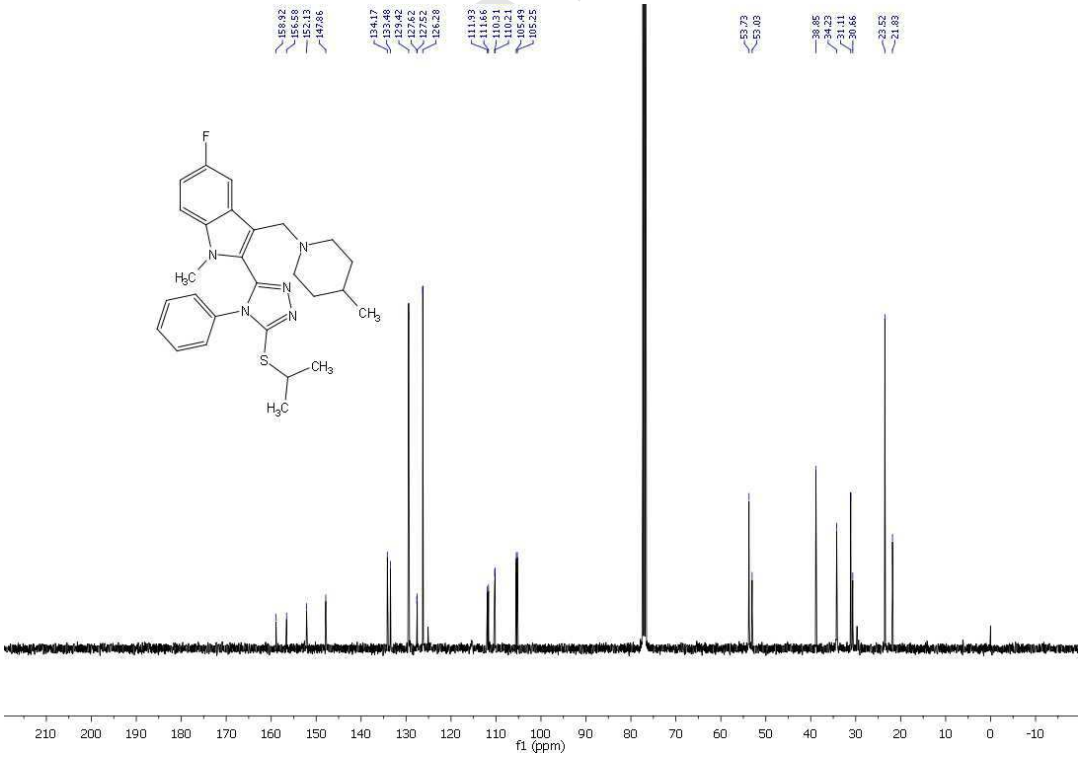


Figure 39. ^{13}C NMR spectrum of IT-31 (100 MHz, $CDCl_3$)

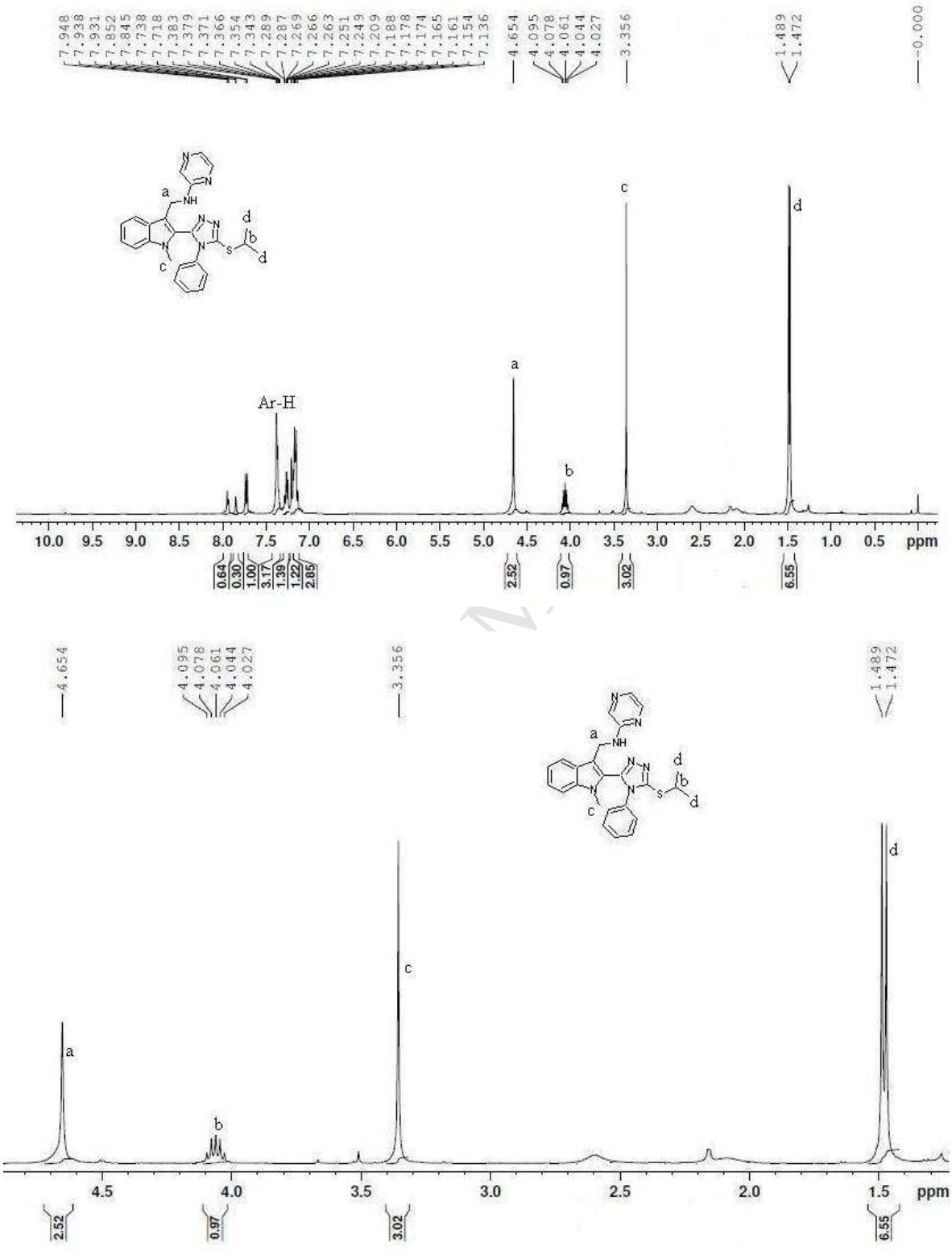


Figure S40. ^{1}H NMR spectrum of IT-36 (400 MHz, $CDCl_3$)

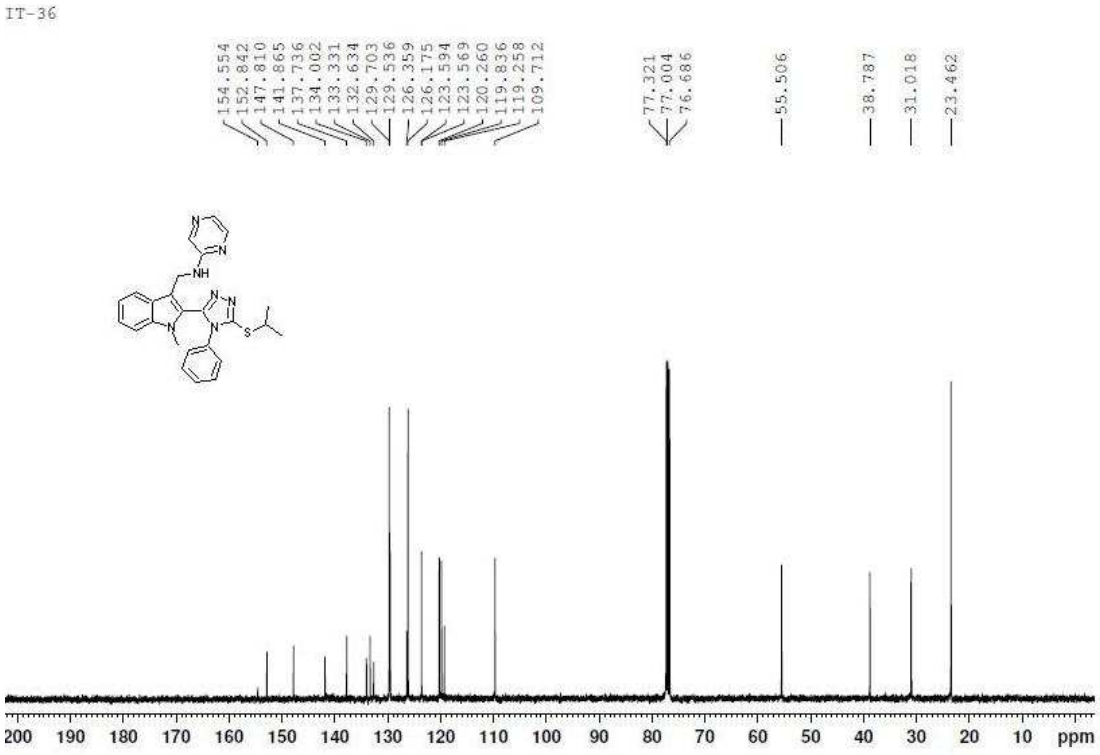


Figure S41. ^{13}C NMR spectrum of IT-36 (125 MHz, $CDCl_3$)

Cell proliferation assay study

Cell lines and culture conditions:

Human metastatic breast cancer cells (MDA-MB 231), human chronic myeloid leukemia cells (K562) and the prostate cancer cells (LnCAP) were procured from National Center for Cell Sciences, Pune, India. All cells were grown in RPMI-1640 supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM-glutamine. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 °C. The cells were sub cultured twice each week, seeding at a density of about 2×10^3 cells/ml.

MTT Assay:

Cell viability was determined by (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells (5×10^3 cells/well) were seeded to 96-well culture plate and cultured with or without compounds at 10 µM concentration for 24 h in a final volume of 200 µL. After treatment, the medium was removed and 20 µl of MTT (5 mg/ml in PBS) was added to the fresh medium. After 2 h incubation at 37 °C, 100 µl of DMSO was added to each well and plates were

agitated for 1 min. Absorbance was read at 570 nm on a multi-well plate reader (VICTOR3, Perkin Emler). Percent inhibition of proliferation was calculated as a fraction of control (without compound).

SIRT1 assay method

The enzyme inhibition studies was performed using SIRT1 Fluorimetric Drug Discovery Kit (AK-555, Biomol, Plymouth Meeting, PA). Assay is based on unique SIRT1 substrate/Developer combination. The substrate usually consist of 4 aminoacids from 379-382 ((Arg-His-Lys-Lys (Ac))of human p53, which was tagged with aminomethylcoumarin (AMC). The fluorescence signal is generated in proportional to the amount of deacetylation of lysine in the substrate (known SIRT1substrate *in vivo* target). In general the assay procedures include two steps. First the substrate containing p53 sequence was incubated with recombinant SIRT1 enzyme along with the cosubstrate NAD^+. While in the second step developer was incubated to produce fluorophore. As per the supplier protocol all the reactions were carried in a reaction buffer consist of 50mM Tis/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1mM $MgCl_2$, 1 mg/cl BSA in the presence of 2 % DMSO. Initially compounds were diluted in the reaction buffer with varying concentrations and it is followed by the addition of enzyme to the total volume of 25 µL. The reaction was then initiated by the addition of 25 µL of 2X substrate solution containing 25 µM substrate and 500 µM of NAD^+ (co substrate). The whole 50 µL reaction mixture was incubated for 45 minutes at 37^oC, later 50 µL of developer containing 2mM nicotinamide was then added to terminate the reaction. Fluorescence reading was taken using PerkinElmer VICTOR 1420 Multilabel Plate Reader with excitation set at 355 nm and emission measured at 460 nm. Percentage inhibition was calculated for the inhibited wells in relative to the control wells.

Administration and dosage: Fasted rats were divided into four groups. Group A served as control**,** Group B served as a positive control (received only testosterone propionate at 3 mg/kg dose), Group C received standard drug Finasteride (5 mg/kg) and Group D received IT-14 (10 mg/kg) along with testosterone propionate (3 mg/kg) daily for 14 days to induce prostatic hyperplasia. Compound IT-14 was suspended in distilled water by using 5% methyl cellulose and administered intraperitoneally. Testosterone propionate was diluted with distilled water using Tween 80 as emulgent and injected subcutaneously.

Histopathological investigations: The Dorsolateral and Ventral prostate glands were isolated and placed in cassettes, fixed in 10% formalin (neutral buffered pH), dehydrated in a series of alcohol dilutions, fixed in xylene, embedded in paraffin wax, sliced into 3 micron sections. Sections were stained with hematoxylin and eosin. The sections were evaluated and blinded to the treatment groups for the incidence and degree of pathology within the tissue samples.