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Graphical Abstract:

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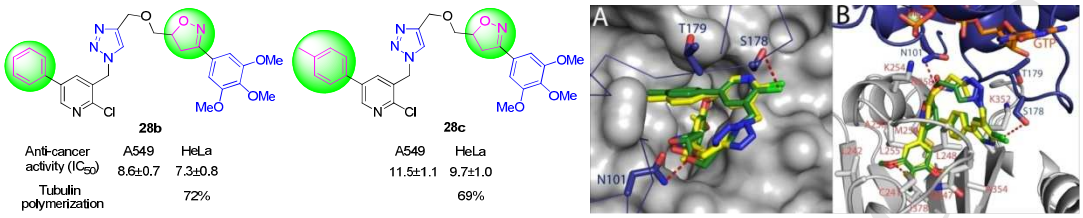

Three series of compounds were prepared and among them, pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles (**28b** and **28c)** with TMP was found to be potent anti-cancer agents and tubulin inhibitors.

Synthesis and structure-activity relationships of pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles as potent inhibitors of tubulin polymerization

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ABSTRACT: Three series of compounds; pyridinyl-1*H*-1,2,3-triazoles, pyridinyl-1*H*-1,2,3-triazolylisoxazoles and pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles with TMP moiety were designed, synthesized and screened for their anti-cancer and anti-tubulin properties. By sequentially designing three series of compounds comprising of dihydroisoxazole in the linker, a small substituent like chlorine on one side (R^1) and aromatic group (R) on the pyridine ring, we have optimized the anti-cancer as well as anti-tubulin activity. Pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles **28b** and **28c** were found to be potent anti-cancer agents against all the cell lines tested with a concomitant accumulation of cells in the G2/M phase of the cell cycle. Molecular modeling suggests that the trimethoxyphenyl ring in **28b** and **28c** occupies the cholchicine binding domain of β-tubulin, whereas, the dihydroisoxazole extends towards the interface of α,β-tubulin.

Keywords: 2-Chloronicotinaldehyes, Pyridinyl-1*H*-1,2,3-triazoles, Triazolylisoxazoles, Triazolyldihydroisoxazoles, Anti-cancer activity, Anti-Tubulin activity, Molecular modeling.

1. Introduction

Cancer is the second major cause of death in U.S. and rest of the world [1]. Hormones, immune conditions, inherited genes, viruses, chemicals and radiation are some of the responsible factors for the development of various cancers [2]. The uncontrolled growth and spreading of abnormal cells result in the disruption of tissues that leads to death in cancer patients. Tumor requires the development of vasculature to gather oxygen and nutrients; therefore, inhibition of tumor growth by targeting vasculature could be one of the efficient ways to fight against cancer [3]. Microtubules are involved in cellular process and suppression of microtubule polymerization blocks cell division at mitosis, leading to cell death and also the disruption of tumor vasculature. Therefore, microtubules are proven targets for the development of anti-cancer agents. Drugs that disrupt microtubule/tubulin dynamics are widely used in cancer therapy. The majority of these molecules act by binding to tubulin, an α,β-heterodimer that forms the core of the microtubule. Microtubule targeting agents (MTA) perturb the mitosis and arrest the cell cycle during interphase [4]. MTAs are known to interact with tubulin in any of the four sites namely laulimalide, taxane/epothilone, vinca alkaloid, and colchicines binding pockets [5]. Because of its effective inhibition of mitosis, colchicine (**1**, Fig. 1) is identified as effective anti-cancer agent. Colchicine based molecules disrupt the vasculature agents which are under investigation for cancer therapy [6].

Combretastatins are natural products that bind to tubulin and inhibit its polymerization [7]. Combretastatin A-4 (**2**, CA-4, Fig. 1) is the potent anti-cancer drug, binds to the colchicine binding site of β-tubulin. CA-4 exhibits strong growth inhibition at nanomolar concentrations against a wide variety of human cancers including multidrug resistant (MDR) cancer [8]. Low water solubility of CA-4 limits its efficacy *in vivo*. Hence, water soluble combretastatin analogs have been developed [9]. Combretastatin A-4P (**3**, fosbretabulin disodium, Fig. 1), and its amino acid derivative AVE-8062 (**4**, Ombrabulin, Fig. 1) act as vascular-disrupting

agents (VDA) by rapidly depolymerizing microtubules. Structure-activity relationship (SAR) studies have shown that 3,4,5-trimethoxyphenyl ring (TMP, **5-6**, Fig. 1) [10] and 4-methoxysubstituted phenyl ring by a double bond with *cis* geometry are important for cytotoxic activity of the combretastatins [11]. Therefore, various five-membered heterocyclic compounds such as thiazoles, imidazolones, pyrazoles, and triazoles (**7**, Fig. 1) with *cis* restricted geometry were reported as tubulin inhibitors [12]. Based on these results, the TMP moiety was found to be essential for anti-tubulin activity. In a recent study, Shetty *et al* synthesized nicotinyl based 1*H*-indolyl-methoxyphenylsulfonamide derivatives as potent antimitotic agents [13].

2-Chloronicotinaldehydes based heterocyclic compounds, such as, Imidacloprid analogues, 2-chloro-5-methylpyridine-3-olefin derivatives, 1,8-naphthyridines and Baylis-Hillman (BH) adducts were synthesized. BH adducts displayed potent anti-malarial activity; quinolines and olefins derivatives displayed good anti-microbial activity [14]. Further, 2-chloronicotinaldehyde based *Knoevenagel* derivatives, (*E*)-α,β-unsaturated esters/ketones and 1*H*-1,2,3-triazolylbenzohydrazides displayed promising *anti-mycobacterial* and anti-cancer activities [15]. As part of our research interest in the design and synthesis of biologically important heterocyclic compounds [16], we focused on 2-chloronicotinaldehydes in combination with TMP. Based on our design, we presume that TMP will act as a pharmacophore in directing the molecules into the colchicine binding pocket of tubulin while the variable region provides affinity. In this study, we report synthesis of 2-chloronicotinaldehydes based 1*H*-1,2,3-triazoles, 1*H*-1,2,3-triazolylisoxazoles and 1*H*-1,2,3-triazolyldihydroisoxazoles with TMP and evaluation of their anti-tubulin and anti-proliferative activity.

2. Chemistry

Three series of compounds such as pyridinyl-1*H*-1,2,3-triazoles **13a-j**, pyridinyl-1*H*-1,2,3-triazolylisoxazoles **25a-j** and pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles **28a-j** with trimethoxyphenyl moiety were prepared starting from 2-chloronicotinaldehydes (Scheme 1-6). Vilsmeier reaction of enamides provided 2-chloronicotinaldehydes **8a-d** [17]. Sodium borohydride ($NaBH_4$) reduction of 2-chloronicotinaldehydes **8a-d** afforded corresponding alcohols **9a-d** and subsequent azidation with diphenylphosphoryl azide (DPPA) in presence of DBU at room temperature [11a] provided azides **10a-d** (Scheme 1). Cycloaddition reaction (Click) of azide **10a** with propargyl alcohol in presence of copper sulphate and sodium ascorbate in aqueous *tert*-butanol at room temperature afforded pyridinyl-1*H*-1,2,3-triazolylmethanol **11** [18]. Initially, we have chosen the Williamson reaction to prepare pyridinyl-1*H*-1,2,3-triazole **13a**. Triazolyl alcohol **11** and trimethoxy benzyl bromide **12** in presence of K_2CO_3 and KI in acetone under reflux conditions provided pyridinyl-1*H*-1,2,3-triazole **13a** in moderate yield (21%, Scheme 2). A parallel etherification reaction with NaH in dry DMF also did not improve the yield. Hence, we have developed an alternate method to prepare pyridinyl-1*H*-1,2,3-triazoles **13a-d** with improved yields as depicted in Scheme 3.

The synthetic strategy for the preparation of pyridinyl-1*H*-1,2,3-triazoles **13a-d** has been accomplished by employing click reaction of 3-(azidomethyl)-2-chloropyridines **10a-d** with 1,2,3-trimethoxy-5-propynyloxy-methylbenzene **16**. $NaBH_4$ reduction of 3,4,5-trimethoxy-benzaldehyde **14** provided alcohol **15** and subsequent propargylation of **15** with propargyl bromide in presence of NaH in dry THF afforded trimethoxy-5-propynyloxy-methylbenzene **16** [19]. Click reaction of azides **10a-d** with alkyne **16** in presence of copper sulphate and sodium ascorbate in aqueous *tert*-butanol provided triazoles **13a-d** (Scheme 3). Having achieved the synthesis of pyridinyl-1*H*-1,2,3-triazoles **13a-d**, in the next step, we have prepared morpholine and thiomorpholine substituted pyridinyl compounds **13e-j**.

Nucleophilic substitution reaction of 2-chloronicotinaldehydes **8a-c** with morpholine/thiomorpholine **17a-b** in presence of K_2CO_3 in dry DMF at 110 °C furnished the corresponding nicotinaldehydes **18a-f**. Nicotinaldehydes **18a-f** upon reduction, azidation and click reaction provided corresponding pyridinyl-1*H*-1,2,3-triazoles **13e-j** (Scheme 4). All compounds **13a-j** reported in this manuscript are new and hence are characterized by various spectroscopic methods.

After successful preparation of **13a-j**, the present protocol was extended to prepare pyridinyl-1*H*-1,2,3-triazolylisoxazoles **25a-j** and dihydroisoxazoles **28a-j**. Oxime **22** was prepared from 3,4,5-trimethoxybenzaldehyde **21** with hydroxylamine hydrochloride in MeOH-H_2O. 1,3-Dipolar cycloaddition of oxime **22** with propargyl alcohol furnished trimethoxyphenyl-isoxazolyl-methanol **23** [20]. Propargylation of **23** with propargyl bromide provided corresponding alkyne derivative **24**. Cycloaddition of substituted 3-azidomethylpyridines **10a-d** and **20a-f** with alkyne **24** furnished pyridinyl-1*H*-1,2,3-triazolylisoxazoles **25a-j** (Scheme 5). Next, 1,3-dipolar cycloaddition of oxime **22** with allyl alcohol produced trimethoxyphenyl-dihydroisoxazolyl-methanol **26** and upon propargylation of **26** with propargyl bromide furnished corresponding alkyne **27**. Cycloaddition of substituted azides **10a-d** and **20a-f** with alkyne **27** provided dihydroisoxazoles **28a-j** (Scheme 6). **25a-j** and **28a-j** have been characterized by spectroscopic methods.

3. Biology

Three set of compounds i) pyridinyl-1*H*-1,2,3-triazoles, ii) pyridinyl-1*H*-1,2,3-triazolylisoxazoles and iii) pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles with trimethoxyphenyl (TMP) were synthesized. Anti-cancer activity was performed on four cancer cell lines. In addition, the ability of compounds in inhibiting tubulin polymerization was studied. The interactions of inhibitor within the colchicine binding pocket of tubulin was

also analyzed employing molecular modeling studies in order to identify the mode of inhibition of the most active compounds.

4. Results and discussion

The pyridinyl-1*H*-1,2,3-triazoles **13a-j**, pyridinyl-1*H*-1,2,3-triazolylisoxazoles **25a-j** and pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles **28a-j** were screened for anti-cancer activity on four cancer cell lines (lung adenocarcinoma (A549), prostate cancer (DU-145), human epithetical cervical cancer (HeLa) and human breast cancer (MCF7) by MTT assay [21] followed by anti-tubulin activity and compared the efficacy with colchicine [22].

4.1. Anti-cancer activity of trimethoxybenzyloxy-1H-1,2,3-triazolylpyridines

The IC_{50} values of the pyridinyl-1*H*-1,2,3-triazoles **13a-j** were tabulated in Table 1; based on the obtained IC_{50} values against the four cancer cell lines and percentage inhibition of tubulin polymerization, moderate activity was observed. There was no specific pattern among the compound structures and their inhibition potential suggesting that the variable region (triazolylpyridine derivatives) is not able to dock in the enzyme pocket that also affects their anti-cancer activity. It is important to note that the TMP in the combretastatin is linked to a *cis*-stilbene which is a rigid juncture. Since a flexible linker in the **13a-j** series connects the TMP, to bring some rigidity near the TMP group we decided to add an isoxazole moiety. Based on this we have synthesized next series of compounds **25a-j**.

4.2. Anti-cancer activity of 1H-1,2,3-triazolyl-3,4,5-trimethoxyphenylisoxazoles

The IC_{50} values of the pyridinyl-1*H*-1,2,3-triazolylisoxazoles **25a-j** were presented in Table 2. Based on the observed IC_{50} values against four cancer lines and extent of anti-tubulin activity, some changes in the potency have been observed as compared to the **13a-j** series. Compounds **25c** and **25i** displayed improved activity against tubulin polymerization as compared to their counterparts (**13c** and **13i** respectively). However, an opposite trend is

noticed in the case of **25e**, **25h** and **25j**. We believe that our design from **13** to **25** series is in the right direction. However, based on modeling studies we realized that converting the isoxazole to dihydroisoxazole might provide increase in flexibility to the linker that may bring better affinity for the molecules to bind to the tubulin. Therefore, we have designed and synthesized dihydroisoxazoles **28a-j** and their activity was measured against four cancer cell lines and their anti-tubulin effects.

4.3. Anti-cancer activity of 1H-1,2,3-triazolyl-3,4,5-trimethoxyphenyl-4,5-dihydroisoxazoles

The IC_{50} values of the pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles **28a-j** are presented in Table 3. As inferred from the modeling studies, there has been a drastic improvement in the affinity of molecules to bind tubulin and thereby exhibited better anti-cancer activity. Five compounds **28a-d** and **28h** displayed improved activity. There is clear correlation with respect to anti-tubulin activity and cytotoxicity. For instance, **28b** and **28c** inhibited the tubulin polymerization to about 70% at 10 μM concentration and these compounds exhibited cytotoxicity in low micromolar concentrations against all the four cell lines tested. Since there is a direct correlation between tubulin polymerization and observed cytotoxicity, we rationalized their behavior using structure-activity relationship. Among the **28a-j**, the compounds **28a-d**, one of the substituents on the pyridine ring was common (chlorine atom at 2^{nd} position) while substituent at 5^{th} position is variable. The best activity was observed when a phenyl **28b** or *p*-tolyl **28c** group occupied this position. However, when the *p*-flurophenyl group **28d** replaces this position, a slight decrease in the activity was observed. The compound with a small substituent like methyl **28a** also displayed better activity. On the other hand, when morpholine or thiomorpholine group replaces the chlorine atom at 2^{nd} position, invariably, almost all molecules except for **28h** lost their potential against tubulin polymerization as well as anti-cancer activity. To confirm that the anti-cancer activity by **28b**

and **28c** is indeed through their anti-tubulin effects (Table 4), we have carried out series of experiments described below.

*4.4. Anti-mitotic effects of compounds **28b-c***

Previous reports suggest that the pharmacological inhibition of tubulin assembly leads to cell cycle arrest at G2/M phase [22]. To examine the anti-mitotic effects of pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazole derivatives, we performed cell cycle analysis of most active compounds **28b** and **28c** by flow cytometry. Cell cycle analysis revealed that treatment with **28b** and **28c** exhibited 87% and 78% accumulation of cells in G2/M phase respectively (Fig. 2).

*4.5. Effects of **28b** and **28c** on cellular microtubules network and nuclear morphology*

To examine the anti-tubulin effects of compounds **28b** and **28c** at cellular level, we treated HeLa cells at 10 µM concentrations for 24 h and analyzed the cellular microtubule network by immunohistochemistry followed by nuclear staining with 4',6-diamidino-2-phenylindole (DAPI). Results demonstrate that cells treated with **28b** and **28c** exhibited severely disrupted microtubule organization, as compared to the controls (Fig. 3). The planar arrangement of chromosomes in **28b** and **28c** treated cells in conjunction with distinct microtubule organization centers clearly indicates mitotic-arrest, whereas, control cells exhibited a normal microtubule organization (Fig. 3).

4.6. Cell cycle related expression of Cyclin B1

Cyclin B1/CDc2 complex regulates the progression of cell cycle at G2/M phase. Maximum expression of cyclin B1 expression will be seen during metaphase [23]. Immunoblot analysis of cyclin B1 following treatment of cells with **28b** and **28c** (10 µM for 24 h) resulted in an increased expression of cyclin B1 as compared to DMSO treated controls [24]. Nocodazole, a known anti-tubulin agent (2 µM) also increased cyclin B1 expression under similar

experimental conditions (Fig. 4A). Therefore, the increased protein levels of cyclin B1 by **28b** and **28c** confirms that these compounds acts as anti-tubulin agents and block the cells at mitotic phase.

4.7. Analysis of soluble versus polymerized tubulin in cells

A dynamic equilibrium exists between the intracellular pool of α,β-tubulin heterodimers and the microtubule polymer. Microtubule disrupting agents target this dynamic equilibrium [22]. Microtubule stabilizing (Paclitaxel) drugs promote polymerization, whereas, tubulin depolymerization agents (Nocodazole and Colchicin) inhibit polymerization [25]. To further analyze whether the block in cell cycle at G2/M by **28b** and **28c** can be reflected even in the cellular levels of soluble and polymerized tubulin (microtubules), we treated HeLa cells with **28b** and **28c** at 10 µM concentration for 24 h and nocodazole (2 µM) was employed as a positive control. Following treatments, intracellular levels of soluble (free tubulin) and polymerized (tubulin from microtubules) fractions of tubulin were analyzed by immunoblotting. Results indicate that while DMSO treated cells (controls) exhibited nearly equal distribution of tubulin in soluble and insoluble fraction, cells treated with nocodazole demonstrated complete shift into soluble fraction. Similar to nocodazole, cells treated with **28b** and **28c** showed higher amounts of tubulin in the soluble fraction as compared to the polymerized fraction (Fig. 4B).

4.8. Docking study

AutoDock version 4.2 was used to dock pyridinyl-1*H*-1,2,3-triazolylisoxazoles **25b-c** and pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles **28b-c** into the colchicine-binding site of β-tubulin [26]. Results suggest that the docking position of the trimethoxyphenyl ring binds well in the colchicine-binding pocket with extensive hydrophobic contacts within the binding pocket of the β-chain (Fig. 5). TMP binds in the pocket where the A ring of colchicine normally binds and surrounded by amino acids Cys241, Leu242, Ala250, Leu255, Val318,

and Ile378 of β-chain. Presence of isoxazole (**25b** and **25c)** and 4,5-dihydroisoxazole (**28b** and **28c)** affects the activity. Due to the presence of 4,5-dihydroisoxazole in compounds **28b** and **28c**, triazole moiety bends toward Asn101, Ser178 and Thr179 of α-chain and participates in a series of hydrogen bonds. In the case of planar isoxazole (**25b** and **25c**), triazole moves away from these amino acids. Extensive interactions of **28b** and **28c** with the tubulin core when compared to **25b** and **25c** may be responsible for their enhanced anti-tubulin effects. A similar explanation can be provided for pyridinyl-1*H*-1,2,3-triazole series **13a-j** that lack the 4,5-dihydroisoxazole moiety for their poor activity profile. Chlorine atom of 2-chloro-5-phenylpyridine **28b** is involved in hydrogen bond with side chain of Ser178. This also suggests that the space in this region is limited and bulkier substituent's like morpholine and thiomorpholine **28e-j** cannot occupy this position providing the basis for poor activity of compounds with these substitutions.

5. Conclusion

In conclusion, three series of compounds such as pyridinyl-1*H*-1,2,3-triazoles, pyridinyl-1*H*-1,2,3-triazolylisoxazoles and pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles were prepared and screened for their anti-proliferative and anti-tubulin activity. By sequential designing of three series of compounds comprising dihydroisoxazole in the linker, a small substituent like chlorine on one side (R^1) and aromatic group (R) on the other on the pyridine ring, we have optimized the anti-tubulin as well as anti-cancer activity. Pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles **28b** and **28c** were found to be potent anti-cancer agents against all the cell lines tested with accumulation of cells in the G2/M phase of the cell cycle. Molecular modeling suggests that the trimethoxyphenyl ring in **28b** and **28c** occupy the cholchicine binding domain of β-tubulin, whereas, the dihydroisoxazole extends towards the interface of α,β-tubulin. Thus, these results suggest that pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles,

particularly **28b** and **28c** have potential to be developed as new a class of tubulin polymerization inhibitors.

6. Experimental section

6.1. General

All chemicals and reagents were purchased from Aldrich (Sigma-Aldrich, USA), AVRA Chemicals Pvt. Ltd (Hyderabad, India) and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F_{254} (mesh); spots were visualized under UV light. Column chromatographic separations were carried out on silica gel (60-120 mesh). Melting points were determined on a *Mettler-Temp* apparatus and are uncorrected. An IR spectrum was recorded with a *Thermo Nicolet Nexus* 670 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on *Bruker Avance* 300 and 500 MHz spectrometers. Chemical shifts (δ) are quoted in parts per million and are referenced to tetramethylsilane (TMS) as internal standard. ESIMS obtained on *7070H spectrometer* operating at 70 eV using a direct inlet system. HRMS were carried out on Agilent 6510 Q-TOF LC/MS instrument.

*6.2. General procedure for the synthesis of 2-chloronicotinaldehydes (**8a-d**)*

The compounds **8a-d** were prepared from its corresponding enamides as per our previous reported method. Ref. [17]

*6.3. General procedure for the synthesis of 2-morpholine/thiomorpholinenicotinaldehydes (**18a-f**)*

Morpholine **17a** (1.2 mmol) was added to a stirred solution of 2-chloro-5-methylnicotinaldehyde **8a** (1 mmol) in *N*,*N*-dimethylformamide and K_2CO_3 (1.5 mmol) at room temperature. The reaction mixture was heated at 110 ºC for 5 h. After completion of the reaction (TLC), the mixture was quenched with ice water and solid was filtered off. The solid was recrystallized (EtOAc/hexane) gave 5-methyl-2-morpholinonicotinaldehyde **18a** as

yellow color solid. Similarly **18b-f** was prepared from corresponding substituted 2-chloronicotinaldehydes **8a-c**.

*6.3.1. 5-Methyl-2-morpholinonicotinaldehyde (**18a**)*

Yield: 86%; yellow solid; m.p: 90-92 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.32 (s, 3H), 3.38 (t, J = 4.53 Hz, 4H), 3.88 (t, J = 4.53 Hz, 4H), 7.84 (s, 1H), 8.25 (s, 1H), 10.09 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.21, 51.75, 66.77, 119.60, 126.106, 140.33, 152.86, 160.42, 190.00; ESI-MS: *m/z*, 207 $[M+H]^+$.

*6.3.2. 5-Methyl-2-thiomorpholinonicotinaldehyde (**18b**)*

Yield: 83%; yellow solid; m.p: 85-87 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.30 (s, 3H), 2.80 (t, J = 5.03 Hz, 4H), 3.66 (t, J = 5.03 Hz, 4H), 7.84 (s, 1H), 8.23 (s, 1H), 10.04 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.22, 27.53, 53.97, 119.75, 126.08, 139.99, 152.79, 161.18, 189.99; ESI-MS: *m/z*, 223 $[M+H]^+$.

*6.3.3. 2-Morpholino-5-phenylnicotinaldehyde (**18c**)*

Yield: 88%; yellow solid; m.p: 110-112 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.52 (t, J = 4.14 Hz, 4H), 3.89 (t, J = 4.14 Hz, 4H), 7.38 (t, J = 6.77 Hz, 1H), 7.47 (t, J = 7.15 Hz, 2H), 7.56 (d, J = 7.52 Hz, 2H), 8.22 (s, 1H), 8.64 (s, 1H), 10.10 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 51.34, 66.78, 119.04, 126.29, 127.67, 129.03, 129.34, 136.54, 139.09, 150.64, 160.38, 189.62; ESI-MS: *m/z*, 269 $[M+H]^+$.

*6.3.4. 5-Phenyl-2-thiomorpholinonicotinaldehyde (**18d**)*

Yield: 85%; yellow solid; m.p: 104-106 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.80-2.88 (m, 4H), 3.75-3.83 (m, 4H), 7.37 (t, J = 7.17 Hz, 1H), 7.47 (t, J = 7.17 Hz, 2H), 7.56 (d, J = 8.12 Hz, 2H), 8.22 (s, 1H), 8.64 (s, 1H), 10.06 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 27.51, 53.64, 119.24, 126.33, 127.70, 129.06, 129.37, 136.58, 138.60, 150.55, 161.11, 189.61; ESI-MS: *m/z*, 285 $[M+H]^+$.

*6.3.5. 2-Morpholino-5-p-tolylnicotinaldehyde (**18e**)*

Yield: 81%; yellow solid; m.p: 114-116 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.39 (s, 3H), 3.50 (t, J = 4.36 Hz, 4H), 3.86 (t, J = 4.36 Hz, 4H), 7.27 (d, J = 7.55 Hz, 2H), 7.46 (d, J = 7.74 Hz, 2H), 8.19 (s, 1H), 8.63 (s, 1H), 10.10 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.43, 51.80, 67.17, 119.53, 126.52, 129.80, 130.14, 134.01, 137.96, 139.04, 150.89, 160.73, 190.11; ESI-MS: *m/z*, 283 $[M+H]^+$.

*6.3.6. 2-Thiomorpholino-5-p-tolylnicotinaldehyde (**18f**)*

Yield: 85%; yellow solid; m.p: 134-136 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.41 (s, 3H), 2.81-2.89 (m, 4H), 3.75-3.81 (m, 4H), 7.28 (d, J = 6.79 Hz, 2H), 7.45 (d, J = 8.30 Hz, 2H), 8.19 (s, 1H), 8.62 (s, 1H), 10.07 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.08, 27.53, 53.71, 114.20, 119.35, 126.18, 129.78, 135.65, 137.63, 138.27, 150.43, 161.09, 189.75; ESI-MS: *m/z*, 299 $[M+H]^+$.

*6.4. General procedure for the synthesis of pyridinylmethanols (**9a-d** & **19a-f**)*

Sodium borohydride (4.2 mmol) was added portion wise to a stirred solution of nicotinaldehyde **8a** (3.5 mmol) in methanol (10 mL) at 0 ºC. The reaction mixture was stirred for 1 h at room temperature. Methanol was removed under reduced pressure; water (5 mL) was added and extracted with ethyl acetate (2 x 10 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and solvent was removed under reduced pressure. The residue was purified by column chromatography using silica gel (ethyl acetate/hexane) afforded (2-chloro-5-methylpyridin-3-yl)methanol **9a** as color less solid. Similarly compounds **9b-d** & **19a-f** was synthesized from corresponding **8b-d** & **18a-f**.

*6.4.1. (2-Chloro-5-methylpyridin-3-yl)methanol (**9a**)*

Yield: 89%; colorless solid; m.p: 84-86 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.31 (s, 3H), 4.72 (s, 2H), 7.73 (s, 1H), 8.06 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.64, 60.94, 132.69, 134.48, 137.63, 145.92, 147.74; ESI-MS: *m/z*, 158 $[M+H]^+$.

*6.4.2. (2-Chloro-5-phenylpyridin-3-yl)methanol (**9b**)*

Yield: 85%; colorless solid; m.p: 138-140 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.85 (s, 2H), 7.42 (t, *J* = 7.32 Hz, 1H), 7.48 (t, *J* = 7.78 Hz, 2H), 7.52 (d, *J* =7.17 Hz, 2H), 8.10 (s, 1H), 8.52 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 61.32, 127.02, 128.40, 129.11, 134.48, 135.20, 136.13, 136.44, 146.04, 147.71; ESI-MS: *m/z*, 220 $[M+H]^+$.

*6.4.3. (2-Chloro-5-p-tolylpyridin-3-yl)methanol (**9c**)*

Yield: 87%; colorless solid; m.p: 103-105 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.32 (t, *J* = 5.28 Hz, 1H), 2.42 (s, 3H), 4.84 (d, *J* = 5.28 Hz, 2H), 7.27 (d, *J* = 7.55 Hz, 2H), 7.47 (d, *J* = 8.30 Hz, 2H), 8.07 (s, 1H), 8.50 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.16, 61.62, 126.88, 129.86, 133.53, 134.48, 135.06, 135.19, 136.10, 138.47, 146.12; ESI-MS: *m/z*, 234 $[M+H]^+$.

*6.4.4. (2-Chloro-5-(4-fluorophenyl)pyridin-3-yl)methanol (**9d**)*

Yield: 89%; colorless solid; m.p: 108-110 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.84 (s, 2H), 7.16 (t, *J* = 8.54 Hz, 2H), 7.50-7.55 (dd, *J* = 5.16, 8.69 Hz, 2H), 8.06 (s, 1H), 8.45 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 61.32, 116.03, 116.31, 128.71, 128.82, 132.54, 134.48, 135.06, 145.88, 147.74, 161.38, 164.68; ESI-MS: *m/z*, 238 $[M+H]^+$.

*6.4.5. (5-Methyl-2-morpholinopyridin-3-yl)methanol (**19a**)*

Yield: 84%; colorless solid; m.p: 102-104 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.28 (s, 3H), 3.11 (t, *J* = 4.73 Hz, 4H), 3.85 (t, *J* = 4.73 Hz, 4H), 4.71 (s, 2H), 7.44 (s, 1H), 8.08 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): 17.58, 50.72, 62.01, 67.10, 127.81, 128.84, 137.92, 146.86, 158.04; ESI-MS: *m/z*, 209 $[M+H]^+$.

*6.4.6. (5-Methyl-2-thiomorpholinopyridin-3-yl)methanol (**19b**)*

Yield: 86%; colorless solid; m.p: 90-92 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.22 (s, 3H), 2.75 (t, *J* = 4.53 Hz, 4H), 3.28 (t, *J* = 4.53 Hz, 4H), 4.63 (s, 2H), 7.41(s, 1H), 8.02 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.50, 28.03, 52.76, 61.61, 128.04, 128.79, 137.79, 146.66, 158.94; ESI-MS: *m/z*, 225 $[M+H]^+$.

*6.4.7. (2-Morpholino-5-phenylpyridin-3-yl)methanol (**19c**)*

Yield: 84%; colorless solid; m.p: 124-126 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.21 (brs, 4H), 3.88 (brs, 4H), 4.79 (s, 2H), 7.39 (t, *J* = 6.98 Hz, 1H), 7.46 (t, *J* = 7.55 Hz, 2H), 7.54 (d, *J* = 7.17 Hz, 2H), 7.84 (s, 1H), 8.49 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 50.64, 62.18, 67.11, 126.72, 127.65, 127.78, 128.98, 132.22, 135.80, 137.43, 145.05, 159.20; ESI-MS: *m/z*, 271 $[M+H]^+$.

*6.4.8. (5-Phenyl-2-thiomorpholinopyridin-3-yl)methanol (**19d**)*

Yield: 87%; colorless solid; m.p: 122-124 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.81-2.87 (m, 4H), 3.41-3.48 (m, 4H), 4.78 (s, 2H), 7.38 (t, *J* = 6.79 Hz, 1H), 7.46 (t, *J* = 7.55 Hz, 2H), 7.54 (d, *J* = 6.79 Hz, 2H), 7.85 (s, 1H), 8.49 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 28.09, 52.83, 62.12, 126.78, 127.71, 128.16, 129.05, 137.48, 144.92, 145.06, 160.18. ESI-MS: *m/z*, 287 $[M+H]^+$.

*6.4.9. (2-Morpholino-5-p-tolylpyridin-3-yl)methanol (**19e**)*

Yield: 88%; colorless solid; m.p: 100-102 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.39 (s, 3H), 3.19 (t, *J* = 4.57 Hz, 4H), 3.88 (t, *J* = 4.73 Hz, 4H), 4.79 (s, 2H), 7.25 (d, *J* = 7.78 Hz, 2H), 7.43 (d, *J* = 8.08 Hz, 2H), 7.80 (s, 1H), 8.48 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.05, 50.71, 62.11, 67.11, 126.52, 127.81, 129.67, 132.17, 134.54, 135.58, 137.49, 144.86, 159.02; ESI-MS: *m/z*, 285 $[M+H]^+$.

*6.4.10. (2-Thiomorpholino-5-p-tolylpyridin-3-yl)methanol (**19f**)*

Yield: 86%; colorless solid; m.p: 140-142 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.32 (s, 3H), 2.84 (t, *J* = 5.03 Hz, 4H), 3.44 (t, *J* = 5.03 Hz, 4H), 4.76 (s, 2H), 7.26 (d, *J* = 8.08 Hz, 2H), 7.43 (d, *J* = 7.93 Hz, 2H), 7.81 (s, 1H), 8.47 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.15, 28.16, 52.86, 62.25, 126.63, 128.12, 129.77, 132.42, 134.57, 135.59, 137.63, 144.92, 160.03; ESI-MS: *m/z*, 301 $[M+H]^+$.

*6.5. General procedure for the synthesis of azidomethylpyridines (**10a-d** & **20a-f**)*

DBU (8.4 mmol) was added to a stirred solution of (2-chloro-5-methylpyridin-3-yl)methanol **9a** (5.6 mmol) in dry toluene (15 mL) at room temperature, and followed by diphenyl phosphoryl azide (6.7 mmol). The reaction mixture was stirred for additional 3 h at room temperature. Reaction was quenched with aqueous NH_4Cl solution (8 mL) and extracted with ethyl acetate (2 x 10 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and solvent was removed under reduced pressure. The residue was purified by column chromatography using silica gel (ethyl acetate/hexane) provided 3-(azidomethyl)-2-chloro-5-methylpyridine **10a** as color less solid. Similarly, compounds **10b-d** & **20a-f** was synthesized from corresponding **9b-d** & **19a-f**.

*6.5.1. 3-(Azidomethyl)-2-chloro-5-methylpyridine (**10a**)*

Yield: 79%; colorless solid; m.p: 120-122 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.35 (s, 3H), 4.50 (s, 2H), 7.57 (s, 1H), 8.18 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.53, 51.31, 129.32, 132.76, 138.68, 14.19, 149.00; ESI-MS: *m/z*, 183 $[M+H]^+$.

*6.5.2. 3-(Azidomethyl)-2-chloro-5-phenylpyridine (**10b**)*

Yield: 82%; colorless solid; m.p: 143-145 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.57 (s, 2H), 7.38-7.58 (m, 5H), 7.92 (s, 1H), 8.54 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 51.20, 126.75, 128.38, 128.96, 129.83, 135.65, 135.94, 146.70, 148.51, 146.70; ESI-MS: *m/z*, 245 $[M+H]^+$.

*6.5.3. 3-(Azidomethyl)-2-chloro-5-p-tolylpyridine (**10c**)*

Yield: 84%; colorless solid; m.p: 114-116 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.40 (s, 3H), 4.59 (s, 2H), 7.30 (d, J = 8.30 Hz, 2H), 7.47 (d, J = 7.55 Hz, 2H), 7.92 (s, 1H), 8.54 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.20, 51.54, 120.25, 125.08, 126.94, 129.66, 130.01, 133.03, 136.23, 138.84, 146.82, 148.39; ESI-MS: *m/z*, 259 $[M+H]^+$.

*6.5.4. 3-(Azidomethyl)-2-chloro-5-(4-fluorophenyl)pyridine (**10d**)*

Yield: 87%; colorless solid; m.p: 116-118 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.62 (s, 2H), 7.19 (t, J = 8.30 Hz, 2H), 7.52-7.59 (dd, J = 5.28, 9.06 Hz, 2H), 7.90 (s, 1H), 8.53 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 51.49, 116.18, 116.48, 128.96, 130.21, 135.42, 136.11, 146.90, 161.54, 164.48; ESI-MS: *m/z*, 263 $[M+H]^+$.

*6.5.5. 4-(3-(Azidomethyl)-5-methylpyridin-2-yl)morpholine (**20a**)*

Yield: 82%; colorless solid; m.p: 120-122 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.32 (Ss, 3H), 3.08 (t, J = 4.70 Hz, 4H), 3.86 (t, J = 4.70 Hz, 4H), 4.40 (s, 2H), 7.46 (d, J = 1.81 Hz, 1H), 8.14 (d, J = 1.81 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.45, 50.25, 51.10, 66.87, 122.98, 128.28, 139.36, 147.43, 158.84; ESI-MS: *m/z*, 234 $[M+H]^+$.

*6.5.6. 4-(3-(Azidomethyl)-5-methylpyridin-2-yl)thiomorpholine (**20b**)*

Yield: 79%; colorless solid; m.p: 110-112 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.28 (s, 3H), 2.77-2.88 (m, 4H), 3.32-3.56 (m, 4H), 4.37 (s, 2H), 7.46 (s, 1H), 8.14 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.55, 27.90, 50.38, 53.20, 129.74, 129.93, 139.05, 147.80, 160.12; ESI-MS: *m/z*, 250 $[M+H]^+$.

*6.5.7. 4-(3-(Azidomethyl)-5-phenylpyridin-2-yl)morpholine (**20c**)*

Yield: 85%; colorless solid; m.p: 132-134 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.21 (t, J = 4.53 Hz, 4H), 3.87 (t, J = 4.72 Hz, 4H), 4.48 (s, 2H), 7.38 (t, J = 7.17 Hz, 1H), 7.48 (t, J = 7.74 Hz, 2H), 7.56 (d, J = 7.17 Hz, 2H), 7.84 (s, 1H), 8.54 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 50.59, 51.01, 66.94, 122.82, 126.68, 127.66, 128.97, 131.72, 137.01, 137.19, 145.75, 160.02; ESI-MS: *m/z*, 296 $[M+H]^+$.

*6.5.8. 4-(3-(Azidomethyl)-5-phenylpyridin-2-yl)thiomorpholine (**20d**)*

Yield: 84%; colorless solid; m.p: 122-124 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.77-2.89 (m, 4H), 3.40-3.52 (m, 4H), 4.46 (s, 2H), 7.38 (t, J = 7.17 Hz, 1H), 7.47 (t, J = 7.55 Hz, 2H), 7.56 (d, J = 6.98 Hz, 2H), 7.86 (s, 1H), 8.52 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 27.45,

50.56, 52.99, 123.36, 126.65, 127.77, 128.97, 131.81, 136.85, 137.42, 145.07, 160.37; ESI-MS: *m/z*, 312 $[M+H]^+$.

*6.5.9. 4-(3-(Azidomethyl)-5-p-tolylpyridin-2-yl)morpholine (**20e**)*

Yield: 88%; colorless solid; m.p: 100-102 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.41 (s, 3H), 3.20 (t, *J* = 4.51 Hz, 4H), 3.88 (t, *J* = 4.51 Hz, 4H), 4.47 (s, 2H), 7.27 (d, *J* = 7.90 Hz, 2H), 7.45 (d, *J* = 7.90 Hz, 2H), 7.82 (s, 1H), 8.52 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.05, 50.61, 51.05, 66.95, 122.85, 126.51, 129.69, 131.75, 134.27, 136.83, 137.56, 145.61, 159.84; ESI-MS: *m/z*, 310 $[M+H]^+$.

*6.5.10. 4-(3-(Azidomethyl)-5-p-tolylpyridin-2-yl)thiomorpholine (**20f**)*

Yield: 85%; colorless solid; m.p: 128-130 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.40 (s, 3H), 2.80-2.86 (m, 4H), 3.42-3.48 (m, 4H), 4.45 (s, 2H), 7.28 (d, *J* = 7.55 Hz, 2H), 7.48 (d, *J* = 8.30 Hz, 2H), 7.82 (s, 1H), 8.52 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.06, 27.85, 50.67, 53.11, 120.153, 123.16, 126.54, 129.70, 129.96, 136.75, 137.58, 145.64, 160.80; ESI-MS: *m/z*, 326 $[M+H]^+$.

*6.6. Synthesis of (1-((2-chloro-5-methylpyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl) methanol (**11**)*

Propargyl alcohol (0.75 mmol) was added to a stirred solution of 3-(azidomethyl)-2-chloro-5-methylpyridine **10a** (0.83 mmol) in *tert*-butanol (0.7 mL) and H_2O (0.7 mL) at room temperature, followed by $CuSO_4 \cdot 5H_2O$ (0.04 mmol) and sodium ascorbate (0.11 mmol). The reaction mixture was stirred for 12-16 h at room temperature and after completion of the reaction (TLC), the reaction mixture was extracted with ethyl acetate (2 x 5 mL). The organic extract was washed with H_2O and dried over anhydrous Na_2SO_4. The residue was purified by column chromatography using silica gel (ethyl acetate/hexane) furnished (1-((2-chloro-5-methylpyridin-3-yl)methyl)-1*H*-1,2,3-triazol-4-yl) methanol **11**.

*6.6.1. (1-((2-Chloro-5-methylpyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl) methanol (**11**)*

Yield: 82%; colorless solid; m.p.: 120-122 ºC. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.29 (s, 3H), 4.80 (s, 2H), 5.65 (s, 2H), 7.33 (s, 1H), 7.68 (s, 1H), 8.21 (s, 1H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 17.48, 49.34, 56.02, 122.50, 126.12, 132.69, 137.63, 145.92, 147.15, 147.74. ESI-MS: *m/z*, 239 $[M+H]^+$.

*6.7. Synthesis of 2-chloro-5-methyl-3-((4-((3,4,5-trimethoxybenzyloxy)methyl)-1H-1,2,3-triazol-1-yl) methyl)pyridine (**13a**)*

Potassium carbonate (1.5 mmol) and potassium iodide (0.2 mmol) were added to a stirred solution of (1-((2-chloro-5-methylpyridin-3-yl)methyl)-1*H*-1,2,3-triazol-4-yl) methanol **11** (1 mmol) in acetone at room temperature and followed by 3,4,5-trimethoxybenzyl bromide (1.2 mmol). The reaction mixture was heated to reflux for 6 h and solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (2 x 5 mL) and washed with cold water. The organic extract was dried over Na_2SO_4, and solvent was removed under reduced pressure. The residue was subjected to column chromatography purification to provide 2-chloro-5-methyl-3-((4-((3,4,5-trimethoxybenzyloxy)methyl)-1*H*-1,2,3-triazol-1-yl) methyl)pyridine **13a**.

*6.7.1. 2-Chloro-5-methyl-3-((4-((3,4,5-trimethoxybenzyloxy)methyl)-1H-1,2,3-triazol-1-yl) methyl)pyridine (**13a**)*

Yield: 21%; colorless solid; m.p.: 145-147 ºC; IR ν_{max} (cm^{-1}): 3136, 2854, 2938, 1592, 1459, 1428, 1332, 1236, 1127, 1078, 1048; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.29 (s, 3H), 3.83 (s, 3H), 3.85 (s, 6H), 4.54 (s, 2H), 4.69 (s, 2H), 5.62 (s, 2H), 6.58 (s, 2H), 7.35 (s, 1H), 7.67 (s, 1H), 8.20 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.51, 50.72, 55.99, 60.72, 63.37, 72.80, 104.75, 123.00, 128.43, 133.16, 133.34, 137.35, 139.52, 145.53, 147.13, 150.11, 153.17; ESI-MS: *m/z*, 419 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{20}H_{23}ClN_4O_4$ $[M+H]^+$ 419.1474, found 419.1480.

*6.8. Synthesis of (3,4,5-trimethoxyphenyl)methanol (**15**)*

$NaBH_4$ (6.1 mmol) was added portion wise to a stirred solution of 3,4,5-trimethoxy benzaldehyde **14** (5.1 mmol) in methanol (10 mL) at 0 ºC. The reaction mixture was stirred for 1 h at room temperature. Methanol was removed under reduced pressure; water (5 mL) was added and extracted with ethyl acetate (2 x 10 mL). The organic extract was washed with brine, dried over Na_2SO_4, and solvent was removed under reduced pressure. The residue was purified by column chromatograph using silica gel (ethyl acetate/hexane 1:4) afforded **15** as colorless liquid in 92% yield.

*6.9. Synthesis of 1,2,3-trimethoxy-5-((prop-2-yn-1-yloxy)methyl)benzene (**16**)*

The compound (3,4,5-trimethoxyphenyl)-methanol **15** (4.5 mmol) was dissolved in THF (15 mL) and added to a stirred suspension of NaH (60%, 5.4 mmol) in THF (5 mL) at 0 ºC. The reaction mixture was stirred for 30 min at the same temperature. Propargyl bromide (4.9 mmol) was added to the reaction mixture at the same temperature and stirring was continued for an additional 5 h at room temperature. After completion the reaction (TLC), the reaction mixture was quenched with ice water and extract with ethyl acetate (2 x 20 mL). The organic extract was washed with brine, dried over Na_2SO_4, and solvent removed under reduced pressure. The residue was subjected to silica gel column chromatography purification (ethyl acetate/hexane 1:10) to afford 1,2,3-trimethoxy-5-((prop-2-yn-1-yloxy)methyl)benzene **16**.

*6.9.1. 1,2,3-Trimethoxy-5-((prop-2-yn-1-yloxy)methyl)benzene (**16**)*

Yield: 85%; cream brown liquid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.48 (s, J = 2.44 Hz, 1H), 3.83 (s, 3H), 3.87 (s, 6H), 4.19 (d, J = 2.28 Hz, 2H), 4.54 (s, 2H), 6.59 (s, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 55.97, 57.01, 60.73, 71.65, 74.69, 79.47, 104.85, 132.82, 137.42, 153.16; ESI-MS: *m/z*, 237 $[M+H]^+$.

*6. 10. Synthesis of 3,4,5-trimethoxybenzaldehyde oxime (**22**)*

$NaHCO_3$ (5.6 mmol) was added portion wise at 0 ºC to a stirred solution of hydroxylamine hydrochloride (3.7 mmol) in water (7 mL) at room temperature and the mixture was stirred for 30 min. The 3,4,5-trimethoxybenzaldehyde **14** (3.1 mmol) dissolved in methanol (5 mL) and added to the above reaction mixture and stirring was continued for an additional 6 h. Methanol was removed under reduced pressure and the residue was extracted with diethyl ether. The organic extract was washed with brine, dried over Na_2SO_4 and solvent removed under reduced pressure gave **22** as colorless solid in 85% yield.

*6.11. (3-(3,4,5-Trimethoxyphenyl)isoxazol-5-yl)methanol (**23**)*

N-Chlorosuccinimide (1.3 mmol) and pyridine (2 drops) was added to a stirred solution of oxime **22** (1.3 mmol) in anhydrous $CHCl_3$ (15 mL) at room temperature. The reaction mixture was stirred for 1 h at 50-60 ºC. Propargyl alcohol (1.4 mmol) was added to the reaction mixture and followed by triethylamine (1.95 mmol) in $CHCl_3$ (5 mL). The reaction mixture was stirred at 25 ºC for 2 h and water was added (10 mL). The organic layer was separated, washed with 2.5% HCl (15 mL) and followed by water (15 mL) and with brine solution (15 mL). The solvent was removed under reduced pressure and the residue was purified by column chromatography using silica gel (ethyl acetate/hexane 1:3) afforded (3-(3,4,5-Trimethoxyphenyl)isoxazol-5-yl)methanol **23**.

*6.11.1. (3-(3,4,5-Trimethoxyphenyl)isoxazol-5-yl)methanol (**23**)*

Yield: 75%; Colorless liquid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.89 (s, 3H), 3.91 (s, 6H), 4.81 (s, 2H), 6.50 (s, 1H), 6.99 (s, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 56.25, 56.60, 60.92, 99.90, 104.09, 124.26, 139.62, 153.56, 162.25, 172.03; ESI-MS: *m/z*, 266 $[M+H]^+$.

*6.12. 5-((Prop-2-ynyloxy)methyl)-3-(3,4,5-trimethoxyphenyl)isoxazole (**24**)*

The compound **24** was prepared according to the method described as per section 6.9 by employing (3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl)methanol **23** (3.4 mmol) with propargyl bromide (3.8 mmol).

*6.12.1. 5-((Prop-2-ynyloxy)methyl)-3-(3,4,5-trimethoxyphenyl)isoxazole (**24**)*

Yield: 84%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.54 (t, J = 2.54 Hz, 1H), 3.89 (s, 3H), 3.92 (s, 6H), 4.28 (d, J = 2.44 Hz, 2H), 4.75 (s, 2H), 6.58 (s, 1H), 7.02 (s, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 56.19, 57.94, 60.89, 62.03, 75.72, 78.39, 101.38, 103.92, 124.15, 139.54, 153.53, 162.24, 168.98; ESI-MS: *m/z*, 304 $[M+H]^+$.

*6.13. Synthesis of (3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)methanol (**26**)*

The compound **26** was prepared according to the method described as per section 6.11. by employing oxime **22** (1.6 mmol) and allyl alcohol (1.6 mmol).

*6.13.1. (3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)methanol (**26**)*

Yield: 80%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.24-3.32 (dd, J = 7.93, 16.42 Hz, 1H), 3.37-3.43 (dd, J = 10.38, 16.42 Hz, 1H), 3.66-3.72 (dd, J = 4.53, 12.27 Hz, 1H), 3.84-3.86 (dd, J = 3.39, 10.95 Hz, 1H), 3.87 (s, 3H), 3.88 (s, 6H), 4.82-4.91 (m, 1H), 5.30 (s, 2H), 6.89 (s, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 39.53, 40.67, 56.00, 62.91, 81.62, 101.49, 131.81, 146.00, 149.93, 151.19, 156.45; ESI-MS: *m/z*, 268 $[M+H]^+$.

*6.14. Synthesis of 5-((prop-2-ynyloxy)methyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro isoxazole (**27**)*

The compound **27** was prepared according to the method described as per section 6.9 by employing compound **26** (3.4 mmol) and propargyl bromide (3.8 mmol).

*6.14.1. 5-((prop-2-ynyloxy)methyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro isoxazole (**27**)*

Yield: 87%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.46 (t, J = 2.44 Hz, 1H), 3.25-3.30 (dd, J = 7.47, 16.63 Hz, 1H), 3.37-3.43 (dd, J = 10.83, 16.47 Hz, 1H), 3.68-3.70 (dd, J = 5.18, 10.37

Hz, 1H), 3.74-3.77 (dd, *J* = 5.03, 10.37 Hz, 1H), 3.88 (s, 3H), 3.89 (s, 6H), 4.24 (d, *J* = 2.28 Hz, 2H), 4.91-4.97 (m, 1H), 6.91 (s, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 37.28, 56.13, 58.65, 60.80, 70.30, 74.93, 79.14, 79.49, 103.92, 124.75, 139.67, 153.16, 156.17; ESI-MS: *m/z*, 306 $[M+H]^+$.

*6.15. General procedure for the synthesis of pyridinyl-1H-1,2,3-triazoles, triazolylisoxazoles, triazolyldihydroisoxazoles (**13a-j, 25a-j** and **28a-j**)*

1,2,3-Trimethoxy-5-((prop-2-yn-1-yloxy)methyl)benzene **16** (0.75 mmol) was added to a stirred solution of 3-(azidomethyl)-2-chloro-5-methylpyridine **10a** (0.83 mmol) in *tert*-butanol (0.7 mL) and H_2O (0.7 mL) at room temperature, followed by $CuSO_4 \cdot 5H_2O$ (0.04 mmol) and sodium ascorbate (0.11 mmol). The reaction mixture was stirred for 13 h and extracted with ethyl acetate (2 x 5 mL), the organic extract was washed with H_2O, dried over Na_2SO_4. The residue was purified by column chromatography using silica gel (ethyl acetate/hexane) furnished *2*-chloro-5-methyl-3-((4-((3,4,5-trimethoxybenzyloxy)methyl)-1*H*-1,2,3-triazol-1-yl) methyl)pyridine **13a** as colorless solid. Yield: 78%. Similarly compounds **13b-j**, **25a-j** and **28a-j** were prepared from the corresponding azides **10b-d**, **20a-f** and alkynes **16**, **24** and **27**.

*6.15.1. 2-Chloro-5-phenyl-3-((4-((3,4,5-trimethoxybenzyloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)pyridine (**13b**)*

Yield: 78%; colorless solid; m.p.: 141-143 ºC; IR ν_{max} (cm^{-1}): 3135, 2938, 2841, 1591, 1458, 1429, 1235, 1126, 1084, 764; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.82 (s, 3H), 3.84 (s, 6H), 4.53 (s, 2H), 4.69 (s, 2H), 5.72 (s, 2H), 6.57(s, 2H), 7.41-7.47 (m, 5H), 7.72 (s, 2H), 8.60 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 50.86, 56.03, 60.76, 63.41, 72.80, 104.80, 123.05, 127.01, 128.83, 129.08, 129.24, 133.19, 135.42, 136.75, 137.27, 145.71, 148.05, 148.70, 153.21; ESI-MS: *m/z*, 481 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{25}H_{26}ClN_4O_4$ $[M+H]^+$ 481.1630, found 481.1637.

*6.15.2. 2-Chloro-5-(p-tolyl)-3-((4-(((3,4,5-trimethoxybenzyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)pyridine (**13c**)*

Yield: 75%; colorless solid; m.p.: 143-145 ºC; IR ν_{max} (cm^{-1}): 2930, 2851, 1591, 1459, 1430, 1331, 1227, 1126, 772; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.39 (s, 3H), 3.80 (s, 3H), 3.83 (s, 6H), 4.52 (s, 2H), 4.69 (s, 2H), 5.72 (s, 2H), 6.58 (s, 2H), 7.25 (d, *J* = 8.12 Hz, 2H), 7.38 (d, *J* = 7.91 Hz, 2H), 7.70 (s, 1H), 7.72 (s, 1H), 8.58 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.11, 50.87, 55.99, 60.75, 63.38, 72.77, 104.75, 123.03, 126.79, 128.96, 129.93, 132.44, 133.18, 136.65, 137.02, 138.94, 145.65, 147.87, 148.32, 153.18; ESI-MS: *m/z*, 495 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{26}H_{28}O_4N_4Cl$ $[M+H]^+$ 495.1782, found 495.1793.

*6.15.3. 2-Chloro-5-(4-fluorophenyl)-3-((4-(((3,4,5-trimethoxybenzyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)pyridine (**13d**)*

Yield: 79%; colorless solid; m.p.: 123-125 ºC; IR ν_{max} (cm^{-1}): 2939, 2841, 1592, 1510, 1459, 1435, 1422, 1231, 1126, 1084, 772; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.83 (s, 3H), 3.85 (s, 6H), 4.51 (s, 2H), 4.69 (s, 2H), 5.71 (s, 2H), 6.58 (s, 2H), 7.15 (t, *J* = 8.68 Hz, 2H), 7.42-7.49 (dd, *J* = 5.09, 8.68 Hz, 2H), 7.68 (s, 1H), 7.73 (s, 1H), 8.55 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 50.67, 55.88, 60.61, 63.28, 72.67, 104.67, 116.02, 116.30, 123.74, 128.06, 128.63, 129.08, 131.42, 133.09, 135.59, 137.00, 145.52, 147.66, 148.60, 153.08, 161.38, 164.68; ESI-MS: *m/z*, 499 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{25}H_{25}O_4N_4ClF$ $[M+H]^+$ 499.1536, found 499.1542.

*6.15.4. 4-(5-Methyl-3-((4-(((3,4,5-trimethoxybenzyl)oxy)methyl)-1H-1,2,3-triazol-1-yl) methyl)pyridin-2-yl)morpholine (**13e**)*

Yield: 81%; colorless solid; m.p.: 109-111 ºC; IR ν_{max} (cm^{-1}): 3473, 3135, 2957, 2927, 2853, 1592, 1506, 1457, 1331, 1238, 1123, 1048; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.24 (s, 3H), 3.04 (m, 4H), 3.79-3.92 (m, 13H), 4.54 (s, 2H), 4.69 (s, 2H), 5.60 (s, 2H), 6.59 (s, 2H), 7.15 (s, 1H), 7.56 (s, 1H), 8.15 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.57, 49.34, 51.31,

56.03, 60.74, 63.47, 66.97, 72.72, 104.48, 114.24, 122.76, 122.93, 129.11, 133.32, 138.36, 145.48, 148.45, 153.22, 158.75; ESI-MS: *m/z*, 470 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{24}H_{32}O_5N_5$ $[M+H]^+$ 470.2381, found 470.2398.

*6.15.5. 4-(5-Methyl-3-((4-(((3,4,5-trimethoxybenzyl)oxy)methyl)-1H-1,2,3-triazol-1-yl) methyl)pyridin-2-yl)thiomorpholine (**13f**)*

Yield: 76%; colorless solid; m.p.: 131-133 ºC; IR ν_{max} (cm^{-1}): 2924, 2845, 1591, 1456, 1456, 1421, 1232, 1126, 773; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.21 (s, 3H), 2.79-2.81 (m, 4H), 3.27-3.31 (m, 4H), 3.82 (s, 3H), 3.85 (s, 6H), 4.53 (s, 2H), 4.69 (s, 2H), 5.54 (s, 2H), 6.58 (s, 2H), 7.13 (s, 1H), 7.54 (s, 1H), 8.14 (s, 1H); ^{3}C NMR ($CDCl_3$, 75 MHz): δ 17.44, 27.82, 49.21, 53.21, 55.87, 60.60, 63.34, 72,61, 104.64, 122.69, 129.03, 133.11, 137.22, 138.22, 145.32, 148.27, 153.03, 159.58; ESI-MS: *m/z*, 486 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{24}H_{32}O_4N_5S$ $[M+H]^+$ 486.2153, found 486.2169.

*6.15.6. 4-(5-Phenyl-3-((4-((3,4,5-trimethoxybenzyloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl) pyridin-2-yl)morpholine (**13g**)*

Yield: 73%; colorless solid; m.p.: 101-103 ºC; IR ν_{max} (cm^{-1}): 2938, 2851, 1592, 1453, 1238, 1123, 768; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.16 (t, J = 4.57 Hz, 4H), 3.82 (s, 9H), 3.88 (t, J = 4.57 Hz, 4H), 4.51 (s, 2H), 4.68 (s, 2H), 5.66 (s, 2H), 6.55 (s, 2H), 7.35-745 (m, 5H), 7.48 (s, 1H), 7.56 (s, 1H), 8.55 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 49.37, 51.02, 55.85, 60.59, 63.33, 66.75, 72.54, 104.61, 122.55, 122.74, 126.49, 127.72, 128.87, 132.25, 133.11, 135.96, 136.62, 145.51, 146.20, 153.03, 159.55; ESI-MS: *m/z*, 532 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{29}H_{34}O_5N_5$ $[M+H]^+$ 532.2541, found 532.2554.

*6.15.7. 4-(5-Phenyl-3-((4-((3,4,5-trimethoxybenzyloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl) pyridin-2-yl)thiomorpholine (**13h**)*

Yield: 77%; colorless solid; m.p.: 131-133 ºC; IR ν_{max} (cm^{-1}): 3135, 2931, 2841, 1593, 1505, 1457, 1421, 1370, 1331, 1234, 1126, 819, 771; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.82-

2.85 (m, 4H), 3.39-3.43 (m, 4H), 3.82 (s, 9H), 4.51 (s, 2H), 4.68 (s, 2H), 5.62 (s, 2H), 6.55 (s, 2H), 7.35-744 (m, 5H), 7.46 (s, 1H), 7.55 (s, 1H), 8.55 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 27.84, 49.53, 53.24, 56.01, 60.71, 63.49, 72.69, 104.85, 122.72, 122.85, 126.63, 127.85, 128.98, 132.48, 133.20, 136.13, 136.74, 137.51, 145.68, 146.36, 153.19, 160.66; ESI-MS: *m/z*, 548 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{29}H_{33}N_5O_4S$ $[M+H]^+$, 549.1543, found 549.1549.

*6.15.8. 4-(5-p-Tolyl-3-((4-((3,4,5-trimethoxybenzyloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl) pyridin-2-yl)morpholine (**13i**)*

Yield: 79%; colorless solid; m.p.: 116-118 ºC; IR ν_{max} (cm^{-1}): 3136, 3006, 2959, 2852, 1593, 1505, 1454, 1365, 1331, 1239, 1122, 819, 755; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.37 (s, 3H), 3.14 (t, *J* = 4.57 Hz, 4H), 3.82 (s, 9H), 3.87 (t, *J* = 4.73 Hz, 4H), 4.52 (s, 2H), 4.67 (s, 2H), 5.65 (s, 2H), 6.55 (s, 2H), 7.20 (d, *J* = 7.92 Hz, 2H), 7.33 (d, *J* = 8.08 Hz, 2H), 7.47 (s, 1H), 7.55 (s, 1H), 8.54 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.01, 49.52, 51.19, 56.00, 60.73, 63.46, 66.90, 72.68, 104.81, 122.65, 122.76, 126.46, 129.71, 132.45, 133.30, 133.81, 135.92, 137.45, 137.82, 145.66, 146.24, 153.18, 159.50; ESI-MS: *m/z*, 546 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{30}H_{36}O_5N_5$ $[M+H]^+$ 546.2703, found 546.2712.

*6.15.9. 4-(5-p-Tolyl-3-((4-((3,4,5-trimethoxybenzyloxy)methyl)-1H-1,2,3-triazol-1-yl) methyl)pyridin-2-yl)thiomorpholine (**13j**)*

Yield: 76%; colorless solid; m.p: 135-137 ºC; IR ν_{max} (cm^{-1}): 2923, 2851, 1592, 1505, 1505, 1453, 1370, 1331, 1219, 1126, 772; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.37 (s, 3H), 2.81-2.85 (m, 4H), 3.38-3.42 (m, 4H), 3.82 (s, 9H), 4.51 (s, 2H), 4.68 (s, 2H), 5.61 (s, 2H), 6.55 (s, 2H), 7.20 (d, *J* = 7.93 Hz, 2H), 7.33 (d, *J* = 8.12 Hz, 2H), 7.45 (s, 1H), 7.54 (s, 1H), 8.52 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.05, 27.89, 49.55, 53.29, 56.03, 60.76, 63.51, 72.72, 104.83, 122.73, 122.91, 126.58, 129.74, 132.52, 133.24, 133.81, 135.99, 137.48, 137.87, 145.71, 146.18, 153.21, 160.44; ESI-MS: *m/z*, 562 $[M+H]^+$.

*6.15.10. 5-(((1-((2-Chloro-5-methylpyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy) methyl)-3-(3,4,5-trimethoxyphenyl)isoxazole (**25a**)*

Yield: 83%; colorless solid; m.p.: 80-82 ºC; IR ν_{max} (cm^{-1}): 3132, 2935, 1585, 1466, 1424, 1238, 1127, 1078; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.29 (s, 3H), 3.89 (s, 3H), 3.91 (s, 6H), 4.73 (s, 2H), 4.80 (s, 2H), 5.62 (s, 2H), 6.60 (s, 1H), 7.00 (s, 2H), 7.35 (s, 1H), 7.73 (s, 1H), 8.21(s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.46, 50.75, 56.15, 60.80, 62.87, 63.95, 101.42, 103.93, 123.28, 124.05, 128.31, 133.31, 139.44, 139.53, 144.57, 147.12, 150.09, 153.46, 162.15, 169.08; ESI-MS: *m/z*, 486 $[M+H]^+$; ESI-HRMS: *m/z*, calcd for $C_{23}H_{25}ClN_5O_5$ $[M+H]^+$ 486.1525, found 486.1538.

*6.15.11. 5-(((1-((2-Chloro-5-phenylpyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy) methyl)-3-(3,4,5-trimethoxyphenyl)isoxazole (**25b**)*

Yield: 85%; colorless solid; m.p.: 96-98 ºC; IR ν_{max} (cm^{-1}): 2933, 2855, 1571, 1508, 1454, 1431, 1371, 1237, 1127, 1084, 766; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.77 (s, 9H), 4.60 (s, 2H), 4.64 (s, 2H), 5.58 (s, 2H), 6.48 (s, 1H), 6.87 (s, 2H), 7.19-7.39 (m, 5H), 7.58 (s, 1H), 7.71 (s, 1H), 8.44 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 50.68, 55.95, 60.64, 62.67, 63.75, 101.29, 103.69, 123.37, 123.90, 126.74, 128.58, 128.85, 129.00, 129.42, 135.47, 136.39, 137.00, 139.27, 144.41, 147.71, 148.49, 153.28, 161.98, 168.95; ESI-MS: *m/z*, 548 $[M+H]^+$; ESI-HRMS: *m/z*, calcd for $C_{28}H_{27}ClN_5O_5$ $[M+H]^+$ 548.1690, found 548.1695.

*6.15.12. 5-(((1-((2-Chloro-5-p-tolylpyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy) methyl)-3-(3,4,5-trimethoxyphenyl)isoxazole (**25c**)*

Yield: 83%; colorless solid; m.p.: 106-108 ºC; IR ν_{max} (cm^{-1}): 3133, 3005, 2937, 1586, 1467, 1424, 1238, 1127, 818, 756; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.36 (s, 3H), 3.89 (s, 3H), 3.91 (s, 6H), 4.72 (s, 2H), 4.77 (s, 2H), 5.71 (s, 2H), 6.58 (s, 1H), 7.01 (s, 2H), 7.25 (d, J = 7.78 Hz, 2H), 7.38 (d, J = 7.78 Hz, 2H), 7.70 (s, 1H), 7.76 (s, 1H), 8.57 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.07, 50.91, 56.16, 60.84, 62.89, 63.96, 101.43, 103.89, 123.33,

124.09, 126.76, 128.84, 129.09, 132.40, 136.60, 136.97,138.92, 139.49, 144.68, 147.85, 148.32, 153.48, 162.19, 169.10; ESI-MS: *m/z,* 562 $[M+H]^+$; ESI-HRMS: *m/z,* calcd for $C_{29}H_{29}ClN_5O_5$ $[M+H]^+$ 562.1845, found 562.1850.

*6.15.13. 5-(((1-((2-Chloro-5-(4-fluorophenyl)pyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)-3-(3,4,5-trimethoxyphenyl)isoxazole (**25d**)*

Yield: 85%; colorless solid; m.p.: 111-113 ºC; IR ν_{max} (cm^{-1}): 3516, 3138, 3007, 2940, 1601, 1572, 1512, 1436, 1416, 1372, 1236, 1128, 1085, 838; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.86 (s, 3H), 3.87 (s, 6H), 4.69 (s, 2H), 4.75 (s, 2H), 5.68 (s, 2H), 6.50 (s, 1H), 6.96 (s, 2H), 7.11 (s, 2H), 7.41-7.43 (s, 2H), 7.64 (s, 1H), 7.75 (s, 1H), 8.51 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 50.83, 56.16, 60.83, 62.91, 63.99, 101.44, 103.93, 116.18, 116.35, 123.36, 124.05, 128.73, 128.80, 129.01, 131.52, 135.71, 137.05, 139.56, 144.72, 147.82, 148.70, 153.49, 162.19, 164.13, 169.05; ESI-MS: *m/z,* 566 $[M+H]^+$; ESI-HRMS: *m/z,* calcd for $C_{28}H_{26}ClFN_5O_5$ $[M+H]^+$ 566.1602, found 566.1601.

*6.15.14. 4-(5-Methyl-3-((4-(((3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)pyridin-2-yl)morpholine (**25e**)*

Yield: 87%; colorless solid; m.p.: 118-120 ºC; IR ν_{max} (cm^{-1}): 2929, 2852, 1570, 1454, 1414, 1370, 1238, 1124, 770; ^1H NMR ($CDCl_3$, 300 MHz): δ 2.22 (s, 3H), 3.03-3.06 (t, J = 4.53 Hz, 4H), 3.83- 3.36 (t, J = 4.72 Hz, 4H), 3.89 (s, 3H), 3.91 (s, 6H), 4.70 (s, H), 4.77 (s, 2H), 5.58 (s, 2H), 6.58 (s, 1H), 7.01 (s, 2H), 7.13 (s, 1H), 7.58 (s, 1H), 8.15 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.53, 49.34, 51.27, 56.14, 60.83, 62.80, 64.02, 66.91, 101.45, 103.86, 122.51, 123.05, 124.05, 129.10, 138.25, 139.49, 144.56, 148.49, 153.48, 158.69, 162.19, 169.08; ESI-MS: *m/z*, 537 $[M+H]^+$; ESI-HRMS: *m/z*, calcd for $C_{27}H_{33}N_6O_6$ $[M+H]^+$ 537.2449, found 537.2456.

*6.15.15. 5-(((1-((5-Methyl-2-thiomorpholinopyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)-3-(3,4,5-trimethoxyphenyl)isoxazole (**25f**)*

Yield: 86%; colorless solid; m.p.: 130-133 ºC; IR ν_{max} (cm^{-1}): 3136, 2931, 2844, 1570, 1455, 1414, 1371, 1234, 1126, 756. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.21 (s, 3H), 2.79-2.81 (m, 4H), 3.28-3.30 (m, 4H), 3.89 (s, 3H), 3.92 (s, 6H), 4.72 (s, 2H), 4.77 (s, 2H), 5.55 (s, 2H), 6.59 (s, 1H), 7.01 (s, 2H), 7.12 (s, 1H), 7.57 (s, 1H), 8.14 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.54, 27.93, 49.41, 53.32, 56.17, 60.84, 62.83, 64.03, 101.46, 103.90, 122.70, 123.03, 124.06, 129.21, 129.67, 138.30, 139.50, 144.55, 148.48, 153.48, 159.72, 162.20, 169.07. ESI-MS: *m/z*, 553 $[M+H]^+$. ESI-HRMS: *m/z*, calcd for $C_{27}H_{33}N_6O_5S$ $[M+H]^+$ 553.2215, found 553.2227.

*6.15.16. 4-(5-Phenyl-3-((4-(((3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)pyridin-2-yl)morpholine (**25g**)*

Yield: 83%; colorless solid; m.p.: 96-98 ºC; IR ν_{max} (cm^{-1}): 2946, 2852, 1600, 1571, 1508, 1414, 1450, 1370, 1124, 762; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.16 (t, J = 4.57 Hz, 4H), 3.87 (t, J = 4.57 Hz, 4H), 3.90 (s, 3H), 3.92 (s, 6H), 4.70 (s, 2H), 4.76 (s, 2H), 5.66 (s, 2H), 6.56 (s, 1H), 7.00 (s, 2H), 7.34 (t, J = 7.01 Hz, 1H), 7.39 (t, J = 7.78 Hz, 2H), 7.43 (d, J = 6.86 Hz, 2H), 7.48 (s, 1H), 7.61 (s, 1H), 8.55 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 49.54, 51.13, 56.15, 60.85, 62.80, 64.01, 66.86, 101.43, 103.87, 122.51, 123.08, 124.07, 126.63, 127.85, 128.82, 128.99, 132.42, 136.07, 136.71, 139.49, 144.73, 146.41, 153.48, 159.66, 162.19, 169.07; ESI-MS: *m/z*, 599 $[M+H]^+$; ESI-HRMS: *m/z*, calcd for $C_{32}H_{35}N_6O_6$ $[M+H]^+$ 599.2619, found 599.2612.

*6.15.17. 5-(((1-((5-Phenyl-2-thiomorpholinopyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-3-(3,4,5-trimethoxyphenyl)isoxazole (**25h**)*

Yield: 86%; colorless solid; m.p.: 80-82 ºC; IR ν_{max} (cm^{-1}): 2925, 2846, 1601, 1572, 1451, 1371, 1235, 1126, 761; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.81-2.83 (m, 4H), 3.40-3.41 (m, 4H),

3.88 (s, 3H), 3.90 (s, 6H), 4.70 (s, 2H), 4.76 (s, 2H), 5.62 (s, 2H), 6.56 (s, 1H), 7.00 (s, 2H), 7.33 (t, *J* = 7.17 Hz, 1H), 7.40 (t, *J* = 7.62 Hz, 2H), 7.43 (d, *J* = 7.01 Hz, 2H), 7.47 (s, 1H), 7.60 (s, 1H), 8.54 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 27.77, 49.51, 53.16, 56.11, 60.80, 62.76, 63.97, 101.39, 103.81, 122.70, 123.05, 124.01, 126.58, 127.80, 128.94, 132.40, 136.03, 136.66, 139.42, 144.65, 146.32, 153.42, 160.57, 162.13, 169.02; ESI-MS: *m/z*, 615 $[M+H]^+$; ESI-HRMS: *m/z*, calcd for $C_{32}H_{35}N_6O_6S$ $[M+H]^+$ 615.2394, found 615.2384.

6.15.18. 4-(5-p-Tolyl-3-((4-(((3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)pyridin-2-yl)morpholine ***(25i)***

Yield: 84%; colorless solid; m.p.: 141-143 ºC; IR ν_{max} (cm^{-1}): 3503, 2957, 2853, 1454, 1417, 1370, 1241, 1121, 818, 757; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.35 (s, 3H), 3.14 (t, *J* = 4.57 Hz, 4H), 3.87 (t, *J* = 4.57 Hz, 4H), 3.88 (s, 3H), 3.90 (s, 6H), 4.70 (s, 2H), 4.76 (s, 2H), 5.66 (s, 2H), 6.56 (s, 1H), 7.00 (s, 2H), 7.21 (d, *J* = 7.93 Hz, 2H), 7.33 (d, *J* = 8.08 Hz, 2H), 7.46 (s, 1H), 7.60 (s, 1H), 8.53 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.99, 49.58, 51.18, 56.17, 60.83, 62.82, 64.02, 66.88, 101.40, 103.97, 122.53, 123.05, 124.08, 126.48, 129.70, 132.45, 133.81, 135.90, 137.80, 139.58, 144.72, 146.29, 153.50, 159.49, 162.19, 169.10; ESI-MS: *m/z*, 613 $[M+H]^+$; ESI-HRMS: *m/z*, calcd for $C_{33}H_{37}N_6O_6$ $[M+H]^+$ 613.2768, found 613.2769.

6.15.19. 5-(((1-((2-Thiomorpholino-5-p-tolylpyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)-3-(3,4,5-trimethoxyphenyl)isoxazole ***(25j)***

Yield: 83%; colorless solid; m.p.: 135-137 ºC; IR ν_{max} (cm^{-1}): 3472, 2930, 2845, 1455, 1415, 1371, 1235, 1127, 819, 755; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.35 (s, 3H), 2.81-2.83 (m, 4H), 3.38-3.40 (m, 4H), 3.88 (s, 3H), 3.90 (s, 6H), 4.70 (s, 2H), 4.76 (s, 2H), 5.61 (s, 2H), 6.56 (s, 1H), 7.00 (s, 2H), 7.21 (d, *J* = 7.93 Hz, 2H), 7.33 (d, *J* = 8.08 Hz, 2H), 7.45 (s, 1H), 7.59 (s, 1H), 8.52 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.00, 27.84, 49.57, 53.23, 56.16, 60.85, 62.82, 64.02, 101.41, 103.88, 122.74, 123.03, 124.07, 126.46, 129.70, 132.47, 133.78,

135.88, 137.82, 139.49, 144.70, 146.23, 153.48, 160.43, 162.18, 169.09; ESI-MS: *m/z*, 629 $[M+H]^+$; ESI-HRMS: *m/z*, calcd for $C_{33}H_{37}N_6O_5S$ $[M+H]^+$ 629.2550, found 629.2540.

*6.15.20. 5-(((1-((2-Chloro-5-methylpyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy) methyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (**28a**)*

Yield: 81%; colorless solid; m.p.: 102-104 ºC; IR ν_{max} (cm^{-1}): 2937, 1570, 1508, 1431, 1415, 1372, 1235, 1127; ^1H NMR ($CDCl_3$, 300 MHz): δ 2.28 (s, 3H), 3.21-3.25 (dd, *J* = 7.32, 16.32 Hz, 1H), 3.34-3.39 (dd, *J* = 10.83, 15.86 Hz, 1H), 3.68-3.79 (m, 2H) 3.88 (s, 9H), 4.74 (s, 2H), 4.91 (brs, 1H), 5.60 (s, 2H), 6.88 (s, 2H), 7.31(s, 1H), 7.66 (s, 1H), 8.20 (s, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 17.47, 37.16, 50.68, 56.14, 60.81, 64.74, 71.18, 79.70, 103.90, 123.15, 124.69, 128.42, 133.27, 139.33, 139.68, 145.115, 147.06, 150.01, 153.15, 156.18; ESI-MS: *m/z*, 488 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{23}H_{27}O_5N_5Cl$ $[M+H]^+$ 488.1683, found 488.1695.

*6.15.21. 5-(((1-((2-Chloro-5-phenylpyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy) methyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (**28b**)*

Yield: 79%; colorless solid; m.p.: 120-122 ºC; IR ν_{max} (cm^{-1}): 3133, 3004, 2936, 2862, 1585, 1467, 1426, 1238, 1127, 1085, 763; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.22 (brs, 1H), 3.34 (brs, 1H), 3.71 (brs, 1H), 3.86-3.89 (brs, 10H), 4.74 (s, 2H), 4.90 (brs, 1H), 5.69 (s, 2H), 6.86 (s, 2H), 7.40-7.46 (m, 5H), 7.69 (s, 1H), 7.73 (s, 1H), 8.53 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 37.31, 51.05, 56.26, 60.84, 64.88, 71.30, 79.76, 104.00, 124.69, 127.01, 128.77, 129.01, 129.20, 135.40, 136.67, 137.28, 139.74, 147.98, 148.65, 153.18, 156.21; ESI-MS: *m/z*, 550 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{28}H_{29}O_5N_5Cl$ $[M+H]^+$ 550.1847, found 550.1851.

*6.15.22. 5-(((1-((2-Chloro-5-p-tolylpyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy) methyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (**28c**)*

Yield: 82%; colorless solid; m.p.: 143-145 ºC; IR ν_{max} (cm^{-1}): 3470, 2936, 1434, 1415, 1372, 1237, 1127, 818, 757; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.38 (s, 3H), 3.20-3.24 (dd, *J* = 7.47, 16.74 Hz, 1H), 3.32-3.38 (dd, *J* = 10.68, 16.47 Hz, 1H), 3.67-3.70 (dd, *J* = 4.73, 10.52 Hz, 1H), 3.71-3.74 (dd, *J* = 5.18, 10.52 Hz, 1H), 3.87 (s, 9H), 4.74 (s, 2H), 4.87-4.93 (m, 1H), 5.69 (s, 2H), 6.88 (s, 2H), 7.25 (d, *J* = 6.40 Hz, 2H), 7.38 (d, *J* = 8.08 Hz, 2H), 7.68 (s, 1H), 7.71 (s, 1H), 8.57 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.05, 37.15, 50.81, 56.11, 60.81, 64.71, 71.13, 79.68, 103.84, 123.21, 124.68, 126.75, 128.92, 129.86, 132.42, 136.54, 136.89, 138.85, 139.61, 145.18, 147.74, 148.25, 153.13, 156.20; ESI-MS: *m/z*, 564 $[M+H]^+$; ESI-HRMS: *m/z*, calcd for $C_{29}H_{31}O_5N_5Cl$ $[M+H]^+$ 564.1999, found 564.2000.

*6.15.23. 5-(((1-((2-Chloro-5-(4-fluorophenyl)pyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazolecarbaldehyde (**28d**)*

Yield: 84%; colorless solid; m.p.: 111-113 ºC; IR ν_{max} (cm^{-1}): 3516, 3138, 3007, 2940, 1601, 1572, 1512, 1436, 1416, 1372, 1236, 1128, 1085, 838; ^{1}H NMR ($CDCl_3$, 300 MHz); δ 3.19-3.25 (dd, *J* = 7.62, 16.74 Hz, 1H), 3.32-3.39 (dd, *J* = 10.83, 16.44 Hz, 1H), 3.67-3.71 (dd, *J* = 4.57, 11.52 Hz, 1H), 3.72-3.75 (dd, *J* = 5.18, 10.52 Hz, 1H), 3.87 (s, 6H), 3.88 (s, 3H), 4.74 (s, 2H), 4.87-4.94 (m, 1H), 5.69 (s, 2H), 6.87 (s, 2H), 7.15 (t, *J* = 8.54 Hz, 2H), 7.43-7.47 (dd, *J* = 5.08, 8.69 Hz, 2H), 7.65 (s, 1H), 7.74 (s, 1H), 8.54 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 37.15, 50.75, 56.15, 60.82, 64.77, 71.22, 79.73, 103.93, 116.16, 116.33, 123.24, 124.69, 128.74, 128.81, 129.13, 134.54, 135.61, 136.95, 139.74, 145.30, 147.72, 148.65, 153.19, 156.19, 162.14, 164.12; ESI-MS: *m/z*, 568 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{28}H_{28}O_5N_5ClF$ $[M+H]^+$ 568.1757, found 568.1755.

*6.15.24. 4-(5-methyl-3-((4-(((3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)methoxy) methyl)-1H-1,2,3-triazol-1-yl)methyl)pyridin-2-yl)morpholine (**28e**)*

Yield: 81%; colorless solid; m.p.: 106-108 ºC; IR ν_{max} (cm^{-1}): 2929, 2852, 1570, 1454, 1414, 1370, 1238, 1124, 770; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.19 (s, 3H), 3.04 (brs, 4H), 3.16-3.26 (dd, *J* = 7.62, 16.74 Hz, 1H), 3.32-3.42 (dd, *J* = 10.83, 16.44 Hz, 1H), 3.66-3.75 (br, 1H), 3.80-3.92 (m, 13H), 4.70 (s, 2H), 4.88 (br, 1H), 5.57 (s, 2H), 6.87 (s, 2H), 7.10 (s, 1H), 7.56 (s, 1H), 8.14 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.40, 37.08, 49.25, 51.20, 56.10, 60.71, 64.74, 66.84, 71.07, 79.68, 103.99, 122.62, 122.84, 124.64, 128.96, 138.16, 139.69, 145.08, 148.36, 153.15, 156.09, 158.64. ESI-MS: *m/z*, 539 $[M+H]^+$. ESI-HRMS: *m/z* calcd for $C_{27}H_{35}O_6N_6$ $[M+H]^+$ 539.2602, found 539.2612.

*6.15.25. 5-(((1-((5-Methyl-2-thiomorpholinopyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (**28f**)*

Yield: 83%; colorless solid; m.p.: 92-95 ºC; IR ν_{max} (cm^{-1}): 3136, 2931, 2844, 1570, 1455, 1414, 1371, 1234, 1126, 756; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.20 (s, 3H), 2.78-2.81 (m, 4H), 3.18-3.26 (dd, *J* = 7.55, 16.61 Hz, 1H), 3.27-3.29 (m, 4H), 3.31-3.36 (dd, *J* = 10.73, 16.61 Hz, 1H), 3.69-3.72 (m, 1H), 3.88 (s, 9H), 4.73 (s, 2H), 4.86-4.96 (m, 1H), 5.53 (s, 2H), 6.88 (s, 2H), 7.09 (s, 1H), 7.53 (s, 1H), 8.14 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.53, 27.93, 37.16, 49.35, 53.30, 56.16, 60.85, 64.80, 71.13, 79.72, 103.86, 122.90, 124.69, 129.17, 138.20, 145.10, 148.41, 153.18, 156.21, 159.68; ESI-MS: *m/z*, 555 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{27}H_{35}O_5N_6S$ $[M+H]^+$ 555.2370, found 555.2384.

*6.15.26. 4-(5-Phenyl-3-((4-(((3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)methoxy) methyl)-1H-1,2,3-triazol-1-yl)methyl)pyridin-2-yl)morpholine (**28g**)*

Yield: 79%; colorless solid; m.p.: 114-116 ºC; IR ν_{max} (cm^{-1}): 2946, 2852, 1600, 1571, 1508, 1414, 1450, 1370, 1124, 762; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.15 (t, *J* = 4.57 Hz, 4H), 3.17-3.22 (dd, *J* = 7.62, 16.63 Hz, 1H), 3.30-3.35 (dd, *J* = 10.83, 16.47 Hz, 1H), 3.65-3.68

(dd, *J* = 4.57, 10.52 Hz, 1H), 3.69-3.72 (dd, *J* = 5.18, 10.52 Hz, 1H), 3.83-3.91 (m, 13H), 4.72 (s, 2H), 4.85-4.90 (m, 1H), 5.64 (s, 2H), 6.86 (s, 2H), 7.24 (t, *J* = 7.93 Hz, 1H), 7.33 (t, *J* = 7.47 Hz, 2H), 7.44 (d, *J* = 6.71 Hz, 2H), 7.45 (s, 1H), 7.57 (s, 1H), 8.54 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 37.18, 49.51, 51.15, 56.18, 60.88, 64.83, 66.90, 71.12, 79.73, 103.90, 122.66, 122.96, 124.73, 126.68, 127.86, 129.01, 132.42, 136.01, 136.80, 145.34, 146.38, 153.21, 156.22, 159.66; ESI-MS: *m/z*, 601 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{32}H_{37}O_6N_6$ $[M+H]^+$ 601.2770, found 601.2769.

*6.15.27. 5-(((1-((5-Phenyl-2-thiomorpholinopyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (**28h**)*

Yield: 82%; colorless solid; m.p.: 128-130 ºC; IR ν_{max} (cm^{-1}): 2925, 2846, 1601, 1572, 1451, 1371, 1235, 1126, 761; ^{1}H NMR ($CDCl_3$, 300 MHz); δ 2.82-2.84 (m, 4H), 3.15-3.23 (dd, *J* = 7.62, 16.47 Hz, 1H), 3.31-3.36 (dd, *J* = 10.68, 16.47 Hz, 1H), 3.40-3.42 (m, 4H), 3.65-3.68 (dd, *J* = 4.57, 10.37 Hz, 1H), 3.69-3.72 (dd, *J* = 5.05, 10.37 Hz, 1H), 3.86 (s, 6H), 3.87 (s, 3H), 4.73 (s, 2H), 4.85-4.91 (m, 1H), 5.61 (s, 2H), 6.86 (s, 2H), 7.34-7.44 (m, 6H), 7.57 (s, 1H), 8.54 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 27.85, 37.17, 49.55, 53.22, 56.19, 60.86, 64.83, 71.12, 79.74, 103.98, 122.86, 122.92, 124.72, 126.67, 127.84, 128.99, 132.47, 136.05, 136.79, 139.78, 145.31, 146.36, 153.21, 156.19, 160.63. ESI-MS: *m/z*, 617 $[M+H]^+$. ESI-HRMS: *m/z*, calcd for $C_{32}H_{36}O_5N_6S$ $[M+H]^+$ 617.2539, found 617.2540.

*6.15.28. 4-(5-p-Tolyl-3-((4-(((3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)methoxy) methyl)-1H-1,2,3-triazol-1-yl)methyl)pyridin-2-yl)morpholine (**28i**)*

Yield: 85%; colorless solid; m.p.: 120-122 ºC; IR ν_{max} (cm^{-1}): 3503, 2957, 2853, 1454, 1417, 1370, 1241, 1121, 818, 757; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.36 (s, 3H), 3.14 (brs, 4H), 3.17-3.22 (dd, *J* = 7.47, 16.47 Hz, 1H), 3.30-3.36 (dd, *J* = 10.83, 16.47 Hz, 1H), 3.65-3.68 (dd, *J* = 4.88, 10.68 Hz, 1H), 3.69-3.72 (dd, *J* = 5.18, 10.52 Hz, 1H), 3.86-3.90 (m, 13H), 4.72 (s, 2H), 4.88 (brs, 1H), 5.64 (s, 2H), 6.86 (s, 2H), 7.21 (d, *J* = 7.93 Hz, 2H), 7.33

(d, *J* = 7.47 Hz, 2H), 7.44 (s, 1H), 7.57 (s, 1H), 8.53 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.10, 21.00, 37.16, 49.52, 51.16, 56.15, 60.29, 60.83, 64.79, 66.89, 71.09, 79.72, 103.94, 122.66, 124.71, 126.48, 129.68, 132.42, 133.85, 135.83, 137.77, 139.73, 146.21, 153.19, 156.19, 159.46; ESI-MS: *m/z*, 615 $[M+H]^+$; ESI-HRMS: *m/z* calcd for $C_{33}H_{39}O_6N_6$ $[M+H]^+$ 615.2921, found 615.2956.

*6.15.29. 5-(((1-((2-Thiomorpholino-5-p-tolylpyridin-3-yl)methyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (**28j**)*

Yield: 82%; colorless solid; m.p.: 160-162 ºC; IR ν_{max} (cm^{-1}): 3472, 2930, 2845, 1455, 1415, 1371, 1235, 1127, 819, 755; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.36 (s, 3H), 2.81-2.83 (m, 4H), 3.17-3.22 (dd, *J* = 7.62, 16.47 Hz, 1H), 3.30-3.36 (dd, *J* = 10.83, 16.47 Hz, 1H), 3.38-3.40 (m, 4H), 3.65-3.68 (dd, *J* = 4.73, 10.52 Hz, 1H), 3.69-3.72 (dd, *J* = 5.32, 10.52 Hz, 1H), 3.86 (s, 6H), 3.87 (s, 3H), 4.72 (s, 2H), 4.85-4.88 (m, 1H), 5.60 (s, 2H), 6.86 (s, 2H), 7.21 (d, *J* = 8.08 Hz, 2H), 7.33 (d, *J* = 8.08 Hz, 2H), 7.43 (s, 1H), 7.57 (s, 1H), 8.52 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.00, 27.83, 37.14, 49.50, 53.20, 56.12, 60.82, 64.76, 71.06, 79.68, 103.84, 122.89, 124.68, 126.46, 129.66, 132.41, 133.80, 135.79, 137.76, 139.63, 145.21, 146.14, 153.14, 156.16, 160.38; ESI-MS: *m/z*, 631 $[M+H]^+$; ESI-HRMS: *m/z*, calcd for $C_{33}H_{39}O_5N_6S$ $[M+H]^+$ 631.2618, found 631.2575.

6.16. Biology

6.16.1. Cell Cultures, maintenance and evaluation of cytotoxicity

All the cell lines used in this study were purchased from the American Type Culture Collection (ATCC, United States). A549, HeLa and MCF7 were grown in Dulbecco's modified Eagle's medium (containing 10% FBS in a humidified atmosphere of 5% CO_2 at 37 ^{0}C). DU145 cells were cultured in Eagle's minimal essential medium (MEM) containing non-essential amino acids, 1 mM sodium pyruvate, 10 mg/mL bovine insulin, and 10% FBS. Cells were trypsinized when sub-confluent from T25 flasks/60 mm dishes and seeded in 96-

well plates. The synthesized test compounds were evaluated for their *in vitro* anti-proliferative activity in the above mentioned cell lines. A protocol of 48 h continuous drug exposure was used, and a MTT cell proliferation assay was employed to estimate cell viability. The cell lines were grown in their respective media containing 10% fetal bovine serum and were seeded into 96-well microtiter plates in 200 μL aliquots at plating densities depending on the doubling time of individual cell lines. The microtiter plates were incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs. Aliquots of 2 μL of the test compounds were added to the wells already containing 198 μL of cells, resulting in the required final concentrations of test compounds. For each compound, four concentrations (1, 10, 25 and 100 μM) were evaluated, and each was done in triplicate wells. Plates were incubated further for 48 h, and the experiment was terminated by the addition of 10 μL of 5% MTT and incubated further for 60 min at 37 ^{0}C. Later, the plates were washed and air-dried and bound stain was subsequently dissolved using 100 μL of DMSO. The absorbance was monitored on a multimode plate reader (Tecan M 200) at a wavelength of 560 nm. Percent growth was calculated on a plate by plate basis for test wells relative to control wells. The above determinations were repeated thrice. The growth inhibitory effects of the compounds were analyzed by generating dose response curves as a plot of the percentage surviving cells versus compound concentration. The sensitivity of the cancer cells to the test compound was expressed in terms of IC_{50}, the concentration of compound that produced 50% reduction as compared to the control absorbance. IC_{50} values are indicated as means ± SD of three independent experiments.

6.16.2. In vitro tubulin polymerization assay

An *in vitro* assay for monitoring the time-dependent polymerization of tubulin to microtubules was performed employing a fluorescence-based tubulin polymerization assay kit according to the manufacturer's protocol (BK011, Cytoskeleton, Inc.). The reaction

mixture in a final volume of 10 µL in PEM buffer (80 mM PIPES, 0.5 mM EGTA, 2 mM $MgCl_2$, pH 6.9) contained 2 mg/mL bovine brain tubulin, 10 µM fluorescent reporter, 1 mM GTP in the presence or absence of test compounds (3 µM final concentration) at 37 ^{0}C. Tubulin polymerization was followed by monitoring the fluorescence enhancement due to the incorporation of a fluorescence reporter into microtubules as polymerization proceeds. Fluorescence emission at 420 nm (excitation wavelength is 360 nm) was measured for 1 h at 1-min intervals in a multimode plate reader (Tecan M 200,). Colchicine was used as positive control in parallel with the test compounds. To determine the IC_{50} values of the compounds against tubulin polymerization, the compounds were pre-incubated with tubulin at varying concentrations (5, 10, 25 and 50 µM). Assays were performed under similar conditions as employed for polymerization assays as described earlier [22b].

6.16.3. Analysis of cell cycle

HeLa cells in 60 mm dishes were incubated for 24 h in the presence or absence of test compounds (10 µM). Cells were harvested with Trypsin-EDTA, fixed with ice-cold 70% ethanol at 4 ^{0}C for 30 min. Ethanol was removed by centrifugation and cells were stained with 1 ml of DNA staining solution (containing 50 µg of Propidium Iodide (PI), and 0.1 mg RNase A) for 30 min as described earlier [22]. The DNA contents of 20,000 events were measured by flow cytometer (MoFlo) and histograms were analyzed using FCS express 4 plus.

6.16.4. Western blot analysis

Cells were seeded in 12-well plates at 1×10^5 cells per well in complete growth medium. Following treatment with respective compounds (10 µM) for 24 h, cells were washed with PBS and subsequently soluble and insoluble tubulin fractions were collected. To collect the soluble tubulin fractions, cells were permeablized with 200 µL of pre-warmed lysis buffer [80 mM Pipes-KOH (p^H 6.8), 1 mM $MgCl_2$, 1 mM EGTA, 0.2% Triton X-100, 10% glycerol,

0.1% protease inhibitor cocktail (Sigma-Aldrich)] and incubated for 3 min at 30 ^{0}C. Lysis buffer was gently removed, and mixed with 100 µL of 3×Laemmli's sample buffer (180 mM Tris-Cl p^H 6.8, 6% SDS, 15% glycerol, 7.5% β-mercaptoethanol and 0.01% bromophenol blue). Samples were immediately heated to 95 ^{0}C for 3 min. To collect the insoluble tubulin fraction, 300 µL of 1×Laemmli's sample buffer was added to the remaining pellet in each tube and the samples were heated to 95 ^{0}C for 3 min. Equal volumes of samples were run on an SDS-10 % polyacrylamide gel and were transferred to a nitrocellulose membrane by semidry transfer at 50 mA for 1 h. Blots were probed with mouse anti-α-tubulin antibody (Sigma) and stained with rabbit anti-mouse secondary antibody coupled with horseradish peroxidase, (Sigma). Bands were visualized using an enhanced Chemiluminescence detection (Amersham) [24].

6.16.5. Immunohistochemistry

HeLa cells were seeded on glass cover slips, incubated for 24 h in the presence or absence of test compounds (10 µM). Following treatments, cover slips were fixed in 3.5% formaldehyde in phosphate-buffered saline (PBS) pH 7.4 for 10 minutes at room temperature. Cells were permeabilized for 6 minutes in PBS containing 0.5% Triton X-100 (Sigma) and 0.05% Tween-20 (Sigma). The permeabilized cells were blocked with 2% BSA (Sigma) in PBS for 1h. Later, the cells were incubated with primary anti-tubulin antibody (sigma) (1:200) diluted in blocking solution for 4 h at room temperature. Subsequently, cells were washed thrice with PBS and then incubated with FITC labeled anti-mouse secondary antibody (1:500) for 1h at room temperature. Finally, cells were washed thrice with PBS and mounted in medium containing DAPI. Images were captured using the Olympus confocal microscope and analyzed with Provision software [22a].

6.16.6. Molecular modeling

Docking of selected compounds was performed on colchicine binding site of tubulin with version 4.0 of the program AutoDock. During docking, colchicine-binding site of tubulin was kept rigid, but all the torsional bonds in ligand were set free to perform flexible docking. Polar hydrogens were added by using the hydrogen module in AutoDock tools for tubulin; after that, Kollman charges were assigned. Coordinates of each of the compound were generated using Chemdraw11 followed by MM2 energy minimization. Grid map in Autodock that defines the interaction of protein and ligands in binding pocket was defined. The grid map was used with 60 points in each x, y, and z direction, equally spaced at 0.375 Å. Docking of ligand was carried out using the empirical free energy function and the genetic algorithm, applying a standard protocol with an initial population of 100 randomly placed individuals, a maximum number of 2.0×100 energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80, and an elitism value of 1, and local search rate of 0.06. Simulations were performed with a maximum of 1.5 million energy evaluations and a maximum of 50,000 generations. Final docked conformations were clustered using a tolerance of 1.0 Å root mean square deviation. The best model was picked based on the best stabilization energy.

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Captions

Tables:

Figures:

Schemes:

Table 1

Compound	Cytotoxicity[a] IC_{50}[b] (μM)				Tubulin polymerization (% inhibition)[c]
	A549	DU145	HeLa	MCF7	
13a	41.9±1.9	34.8±1.5	264.2±5.9	67.4±2.7	24
13b	62.6±2.5	45.5±1.9	41.9±2.4	86.7±2.5	18
13c	150.4±2.8	37.0±2.4	28.6±1.7	145.9±3.9	35
13d	50.6±2.1	38.8±2.5	49.6±2.6	96.6±2.1	41
13e	64.9±1.6	66.7±3.1	35.6±2.7	48.5±1.8	45
13f	71.5±2.7	48.6±2.4	61.8±3.1	82.1±1.9	27
13g	84±3.2	70.3±3.4	85.1±2.7	97.6±2.6	16
13h	45.2±1.8	48.9±2.4	31.5±1.8	59.0±1.6	39
13i	38.5±1.7	34.8±1.9	35.8±2.4	51.7±1.3	48
13j	33.9±2.3	41.9±2.3	352.4±5.4	67.8±2.4	21
Colchicine	0.12±0.09	0.69±0.02	0.54±0.09	0.07±0.002	87

[a]Cell lines were treated with different concentrations of compounds for 48 h as described under materials and methods. Cell viability was measured employing MTT assay. [b]IC_{50} values are indicated as the mean +/- SD of three independent experiments. [c]Compounds were preincubated with tubulin (2 mg/ml) at a final concentration of 10 μM.

Table 2

Compound	Cytotoxicity[a] IC_{50}[b] (μM)				Tubulin polymerization (% inhibition)[c]
	A549	DU145	HeLa	MCF7	
25a	255.8±3.8	39.8±1.6	57.7±2.6	194.2±3.8	22
25b	112.1±2.7	78.5±2.7	43.1±2.6	68.5±2.6	16
25c	30.6±1.8	37.3±1.4	35.3±2.3	58.4±1.8	59
25d	40.6±1.5	31.9±1.6	37.3±2.5	76.4±2.1	46
25e	39.5±2.3	54.7±2.4	127.7±3.9	195.3±3.1	19
25f	40.0±2.5	73.9±2.3	53.4±1.9	82.9±2.4	32
25g	39.2±1.6	54.0±1.8	39.4±1.9	63.4±2.3	27
25h	52.6±2.7	210.1±4.2	174.8±4.6	212.8±4.2	11
25i	45.3±2.4	52.1±1.7	36.1±1.4	73.4±2.2	64
25j	61.0±3.5	94.3±2.5	52.6±2.3	64.8±1.9	15
Colchicine	0.12±0.09	0.69±0.02	0.54±0.09	0.07±0.002	87

[a]Cell lines were treated with different concentrations of compounds for 48 h as described under Materials and Methods. Cell viability was measured employing MTT assay. [b]IC_{50} values are indicated as the mean +/- SD of three independent experiments. [c]Compounds were preincubated with tubulin (2 mg/ml) at a final concentration of 10 μM.

Table 3

Compound	Cytotoxicity[a] IC_{50}[b] (μM)				Tubulin polymerization (% inhibition)[c]
	A549	DU145	HeLa	MCF7	
28a	37.7±2.7	58.9±1.8	50.5±2.0	85.5±2.7	53
28b	8.6±0.7	11.4±1.1	7.3±0.8	14.6±2.1	72
28c	11.5±1.1	13.1±0.9	9.7±1.0	12.9±1.3	69
28d	25.2±0.9	32.8±1.8	27.2±1.9	45.7±2.4	51
28e	45.4±2.8	138.5±4.1	37.3±1.8	146.5±3.8	27
28f	45.6±2.6	29.4±0.9	41.0±0.9	45.1±1.2	38
28g	39.2±1.8	44.8±1.8	42.6±1.6	68.8±2.3	46
28h	31.5±1.9	34.5±1.7	29.2±1.8	45.0±1.5	50
28i	39.6±2.1	49.7±2.3	38.7±1.5	59.6±2.7	27
28j	43.2±2.8	87.3±3.8	39.2±2.1	71.1±2.8	22
Colchicine	0.12±0.09	0.69±0.02	0.54±0.09	0.07±0.002	87

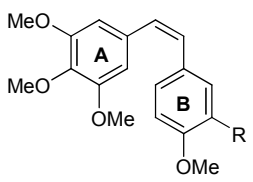
[a]Cell lines were treated with different concentrations of compounds for 48 h as described under Materials and Methods. Cell viability was measured employing MTT assay. [b]IC_{50} values are indicated as the mean +/- SD of three independent experiments. [c]Compounds were preincubated with tubulin (2 mg/ml) at a final concentration of 10 μM.

Table 4

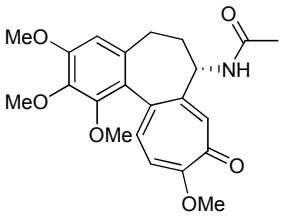
Compound	IC_{50} (μM)[a]
Colchicine	1.7±0.8
28b	6.8±1.5
28c	8.1±1.2

[a]Effect of congeners on tubulin polymerization. IC_{50} values for **28b**, **28c** and colchicine were determined from the tubulin polymerization assays

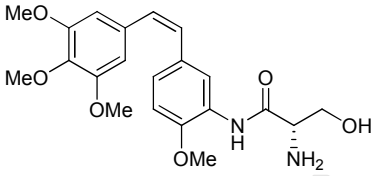
Fig. 1



1 Combretastatin A-4: R = OH
2 Combretastatin A-4P: R = OPO_3Na_2



3 Colchicine



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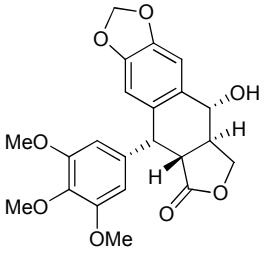
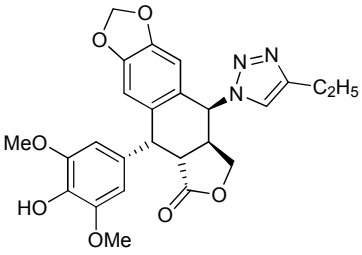
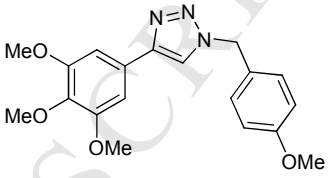


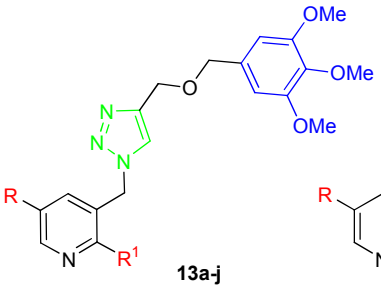
5 Podophyllotoxin



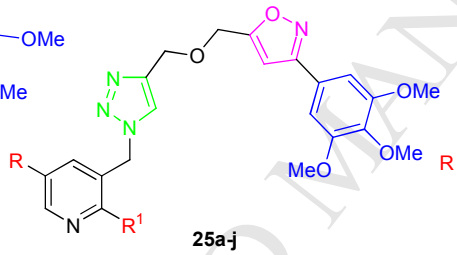
6 Podophyllotoxin derivative



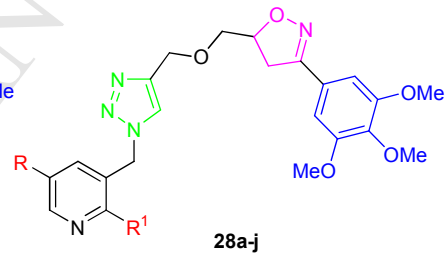
7



13a-j

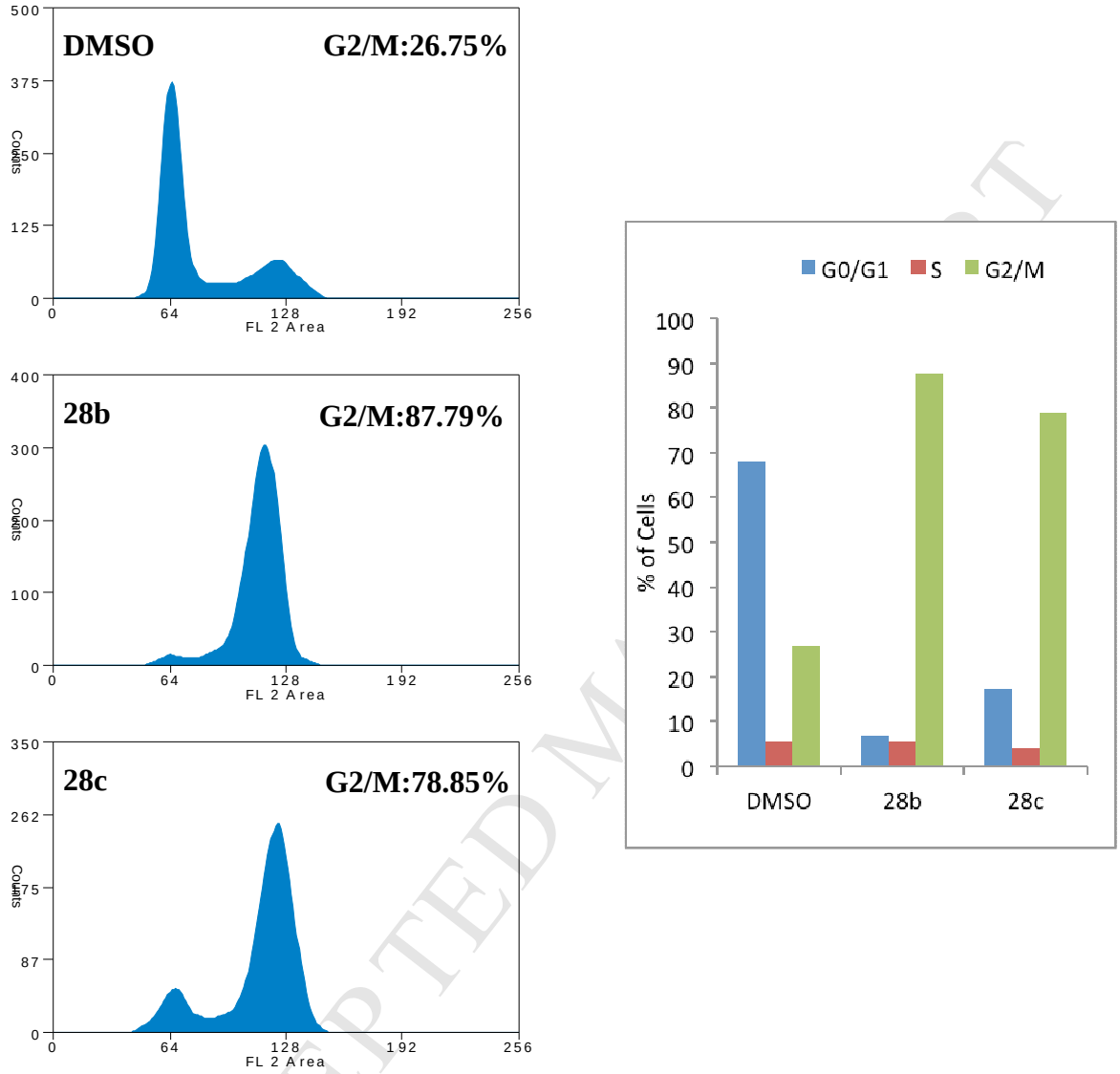


25a-j



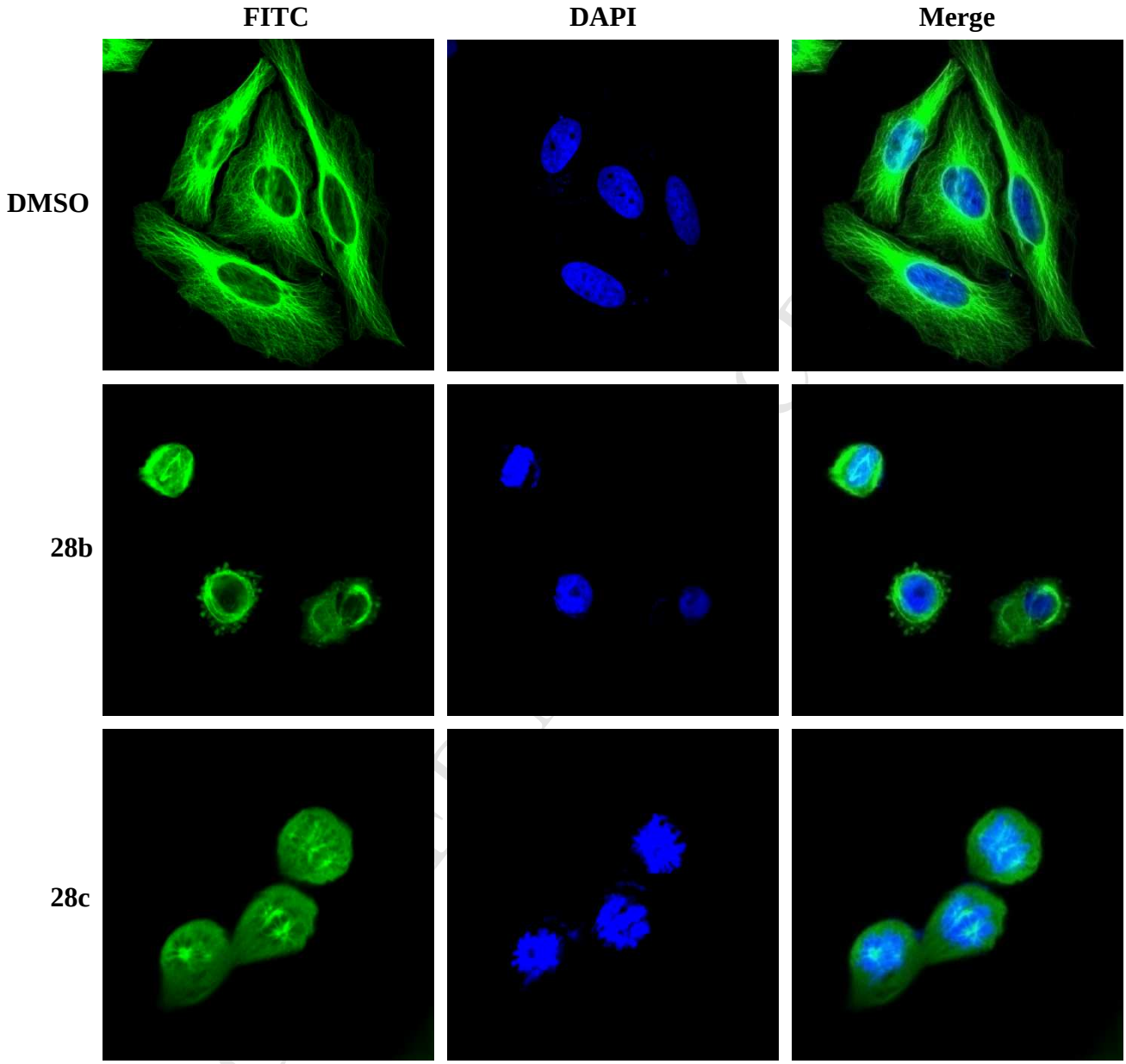
28a-j

Fig. 2



Panel A: Cell cycle effects of compounds **28b** and **28c**. HeLa cells were harvested after treatment with compounds at 10 μM for 24 h. Untreated cells and DMSO treated cells served as controls. The percentage of cells in each phase of cell cycle was quantified by flow cytometry. *Panel B*: Distribution of cells at G0/G1, S and G2/M phase of cell cycle following treatment with **28b** and **28c** in HeLa cells.

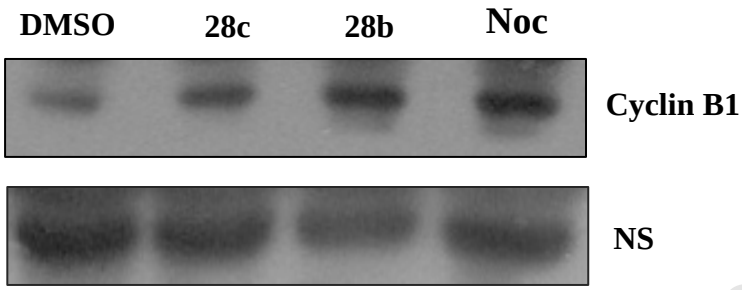
Fig. 3



HeLa cells were independently treated with **28b** and **28c** at 10 μM concentrations for 24h. Following the termination of experiment, cells were fixed and stained for tubulin. DAPI was used as counter stain. The merged images of cells stained for tubulin and DAPI are represented. The photographs were taken using Olympus confocal microscope equipped with FITC and DAPI filter settings. Data is the representative of five different fields of view.

Fig. 4

A



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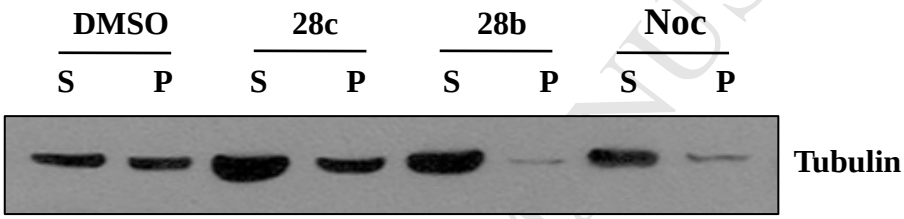
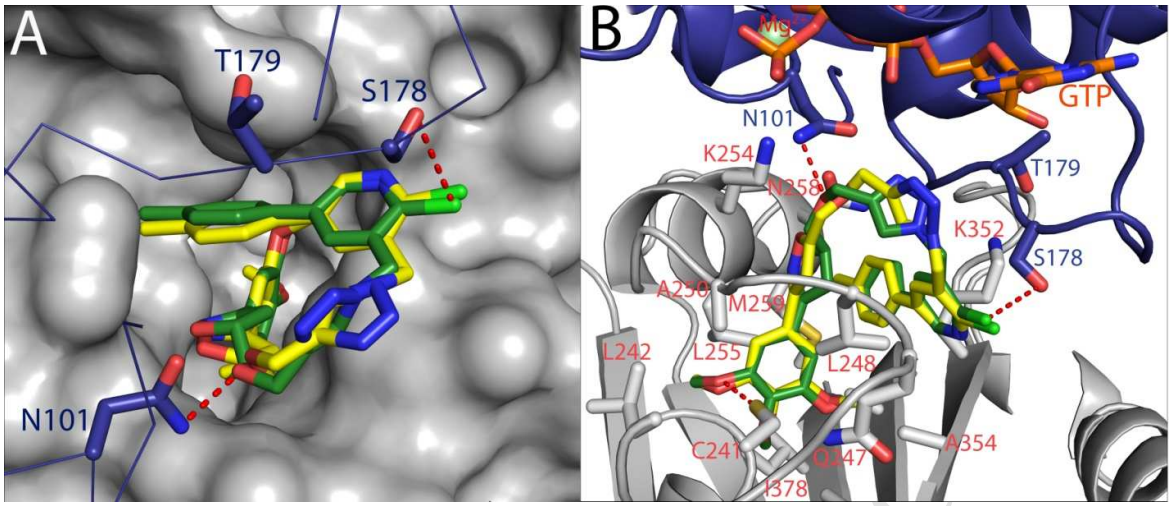


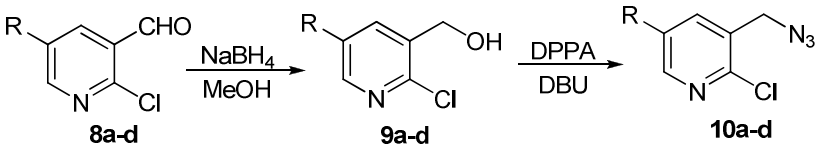
Panel A: HeLa cells were treated with 10 μM concentration of compound **28b** and **28c** for 24h. Nocodazole was treated at 2 μM concentrations for 24h. Subsequently, whole lysates were prepared and analyzed for cyclin-B1. A non-specific (NS) band at 90kDa was used as loading control. *Panel B:* HeLa cells were treated with 10 μM of **28b** and **28c** for 24h. Nocodazole was utilized at 2 μM concentration for 24h treatments. The fractions containing soluble and polymerized tubulin were collected and resolved on SDS-PAGE. Tubulin was detected by immunoblot analysis.

Fig. 5



Panel A: Docking pose of **25c** (yellow) and **28c** (green). The gray surface represents β-chain while the blue line diagram indicates the α-chain of tubulin. The trimethoxyphenyl moiety of compounds is buried in hydrophobic pocket of colchicine binding domain. *Panel B:* Stick representation of selected amino acids of α and β-subunits of tubulin interacting with compound **25c**, and **28c**. Red dot line represents hydrogen bonds.

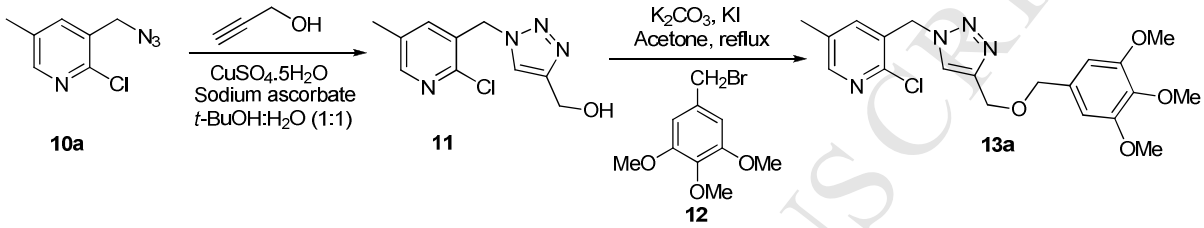
Scheme 1



8a-d **9a-d** **10a-d**

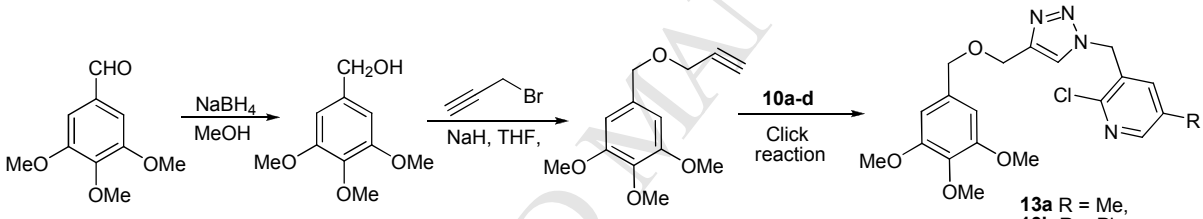
10a R = Me
10b R = Ph
10c R = *p*-Me-Ph
10d R = *p*-F-Ph

Scheme 2



10a **11** **12** **13a**

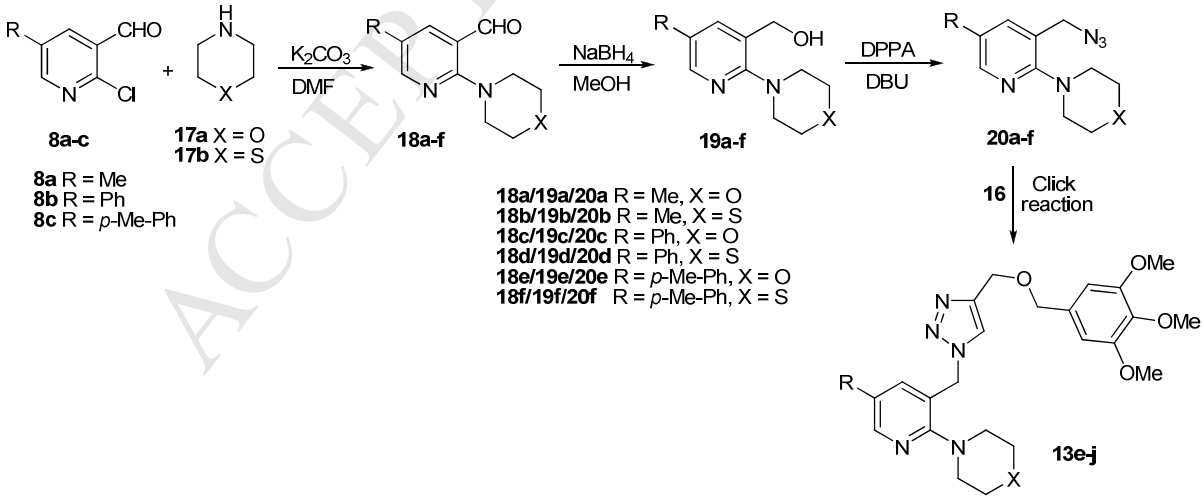
Scheme 3



14 **15** **16** **13a-d**

13a R = Me,
13b R = Ph,
13c R = *p*-Me-Ph,
13d R = *p*-F-Ph

Scheme 4



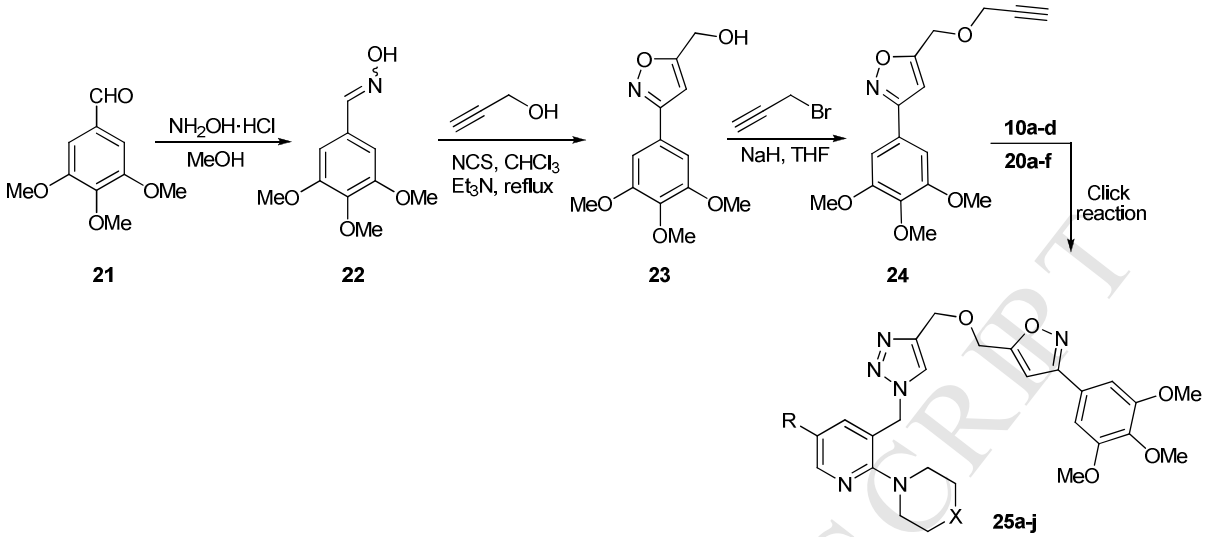
8a-c **17a** X = O **17b** X = S **18a-f** **19a-f** **20a-f**

8a R = Me
8b R = Ph
8c R = *p*-Me-Ph

18a/19a/20a R = Me, X = O
18b/19b/20b R = Me, X = S
18c/19c/20c R = Ph, X = O
18d/19d/20d R = Ph, X = S
18e/19e/20e R = *p*-Me-Ph, X = O
18f/19f/20f R = *p*-Me-Ph, X = S

13e-j

Scheme 5



Scheme 6

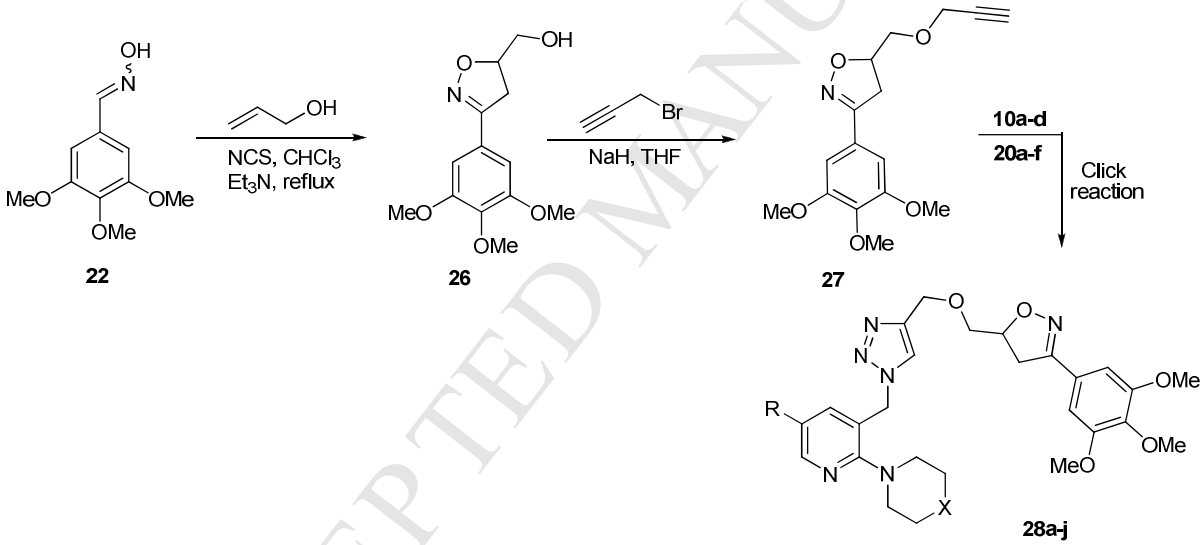


Table 1

Compound	Cytotoxicity[a] IC_{50}[b] (μM)				Tubulin polymerization (% inhibition)[c]
	A549	DU145	HeLa	MCF7	
13a	41.9±1.9	34.8±1.5	264.2±5.9	67.4±2.7	24
13b	62.6±2.5	45.5±1.9	41.9±2.4	86.7±2.5	18
13c	150.4±2.8	37.0±2.4	28.6±1.7	145.9±3.9	35
13d	50.6±2.1	38.8±2.5	49.6±2.6	96.6±2.1	41
13e	64.9±1.6	66.7±3.1	35.6±2.7	48.5±1.8	45
13f	71.5±2.7	48.6±2.4	61.8±3.1	82.1±1.9	27
13g	84±3.2	70.3±3.4	85.1±2.7	97.6±2.6	16
13h	45.2±1.8	48.9±2.4	31.5±1.8	59.0±1.6	39
13i	38.5±1.7	34.8±1.9	35.8±2.4	51.7±1.3	48
13j	33.9±2.3	41.9±2.3	352.4±5.4	67.8±2.4	21
Colchicine	0.12±0.09	0.69±0.02	0.54±0.09	0.07±0.002	87

[a]Cell lines were treated with different concentrations of compounds for 48 h as described under materials and methods. Cell viability was measured employing MTT assay. [b]IC_{50} values are indicated as the mean +/- SD of three independent experiments. [c]Compounds were preincubated with tubulin (2 mg/ml) at a final concentration of 10 μM.

Table 2

Compound	Cytotoxicity[a] IC_{50}[b] (μM)				Tubulin polymerization (% inhibition)[c]
	A549	DU145	HeLa	MCF7	
25a	255.8±3.8	39.8±1.6	57.7±2.6	194.2±3.8	22
25b	112.1±2.7	78.5±2.7	43.1±2.6	68.5±2.6	16
25c	30.6±1.8	37.3±1.4	35.3±2.3	58.4±1.8	59
25d	40.6±1.5	31.9±1.6	37.3±2.5	76.4±2.1	46
25e	39.5±2.3	54.7±2.4	127.7±3.9	195.3±3.1	19
25f	40.0±2.5	73.9±2.3	53.4±1.9	82.9±2.4	32
25g	39.2±1.6	54.0±1.8	39.4±1.9	63.4±2.3	27
25h	52.6±2.7	210.1±4.2	174.8±4.6	212.8±4.2	11
25i	45.3±2.4	52.1±1.7	36.1±1.4	73.4±2.2	64
25j	61.0±3.5	94.3±2.5	52.6±2.3	64.8±1.9	15
Colchicine	0.12±0.09	0.69±0.02	0.54±0.09	0.07±0.002	87

[a]Cell lines were treated with different concentrations of compounds for 48 h as described under Materials and Methods. Cell viability was measured employing MTT assay. [b]IC_{50} values are indicated as the mean +/- SD of three independent experiments. [c]Compounds were preincubated with tubulin (2 mg/ml) at a final concentration of 10 μM.

Table 3

Compound	Cytotoxicity[a] IC_{50}[b] (μM)				Tubulin polymerization (% inhibition)[c]
	A549	DU145	HeLa	MCF7	
28a	37.7±2.7	58.9±1.8	50.5±2.0	85.5±2.7	53
28b	8.6±0.7	11.4±1.1	7.3±0.8	14.6±2.1	72
28c	11.5±1.1	13.1±0.9	9.7±1.0	12.9±1.3	69
28d	25.2±0.9	32.8±1.8	27.2±1.9	45.7±2.4	51
28e	45.4±2.8	138.5±4.1	37.3±1.8	146.5±3.8	27
28f	45.6±2.6	29.4±0.9	41.0±0.9	45.1±1.2	38
28g	39.2±1.8	44.8±1.8	42.6±1.6	68.8±2.3	46
28h	31.5±1.9	34.5±1.7	29.2±1.8	45.0±1.5	50
28i	39.6±2.1	49.7±2.3	38.7±1.5	59.6±2.7	27
28j	43.2±2.8	87.3±3.8	39.2±2.1	71.1±2.8	22
Colchicine	0.12±0.09	0.69±0.02	0.54±0.09	0.07±0.002	87

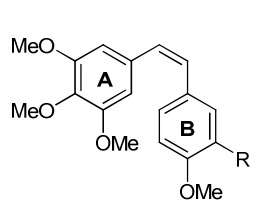
[a]Cell lines were treated with different concentrations of compounds for 48 h as described under Materials and Methods. Cell viability was measured employing MTT assay. [b]IC_{50} values are indicated as the mean +/- SD of three independent experiments. [c]Compounds were preincubated with tubulin (2 mg/ml) at a final concentration of 10 μM.

Table 4

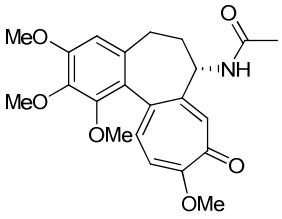
Compound	IC_{50} (μM)[a]
Colchicine	1.7±0.8
28b	6.8±1.5
28c	8.1±1.2

[a]Effect of congeners on tubulin polymerization. IC_{50} values for **28b**, **28c** and colchicine were determined from the tubulin polymerization assays

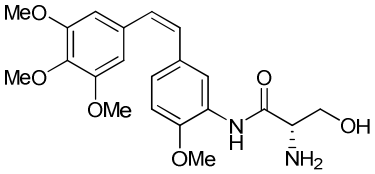
Fig. 1



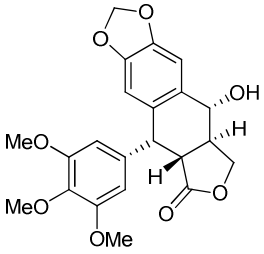
1 Combretastatin A-4: R = OH
2 Combretastatin A-4P: R = OPO_3Na_2



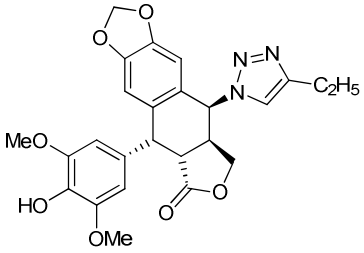
3 Colchicine



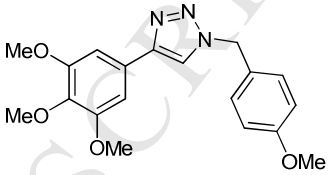
4 AVE8062



5 Podophyllotoxin



6 Podophyllotoxin derivative



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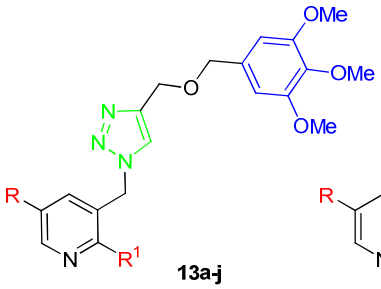
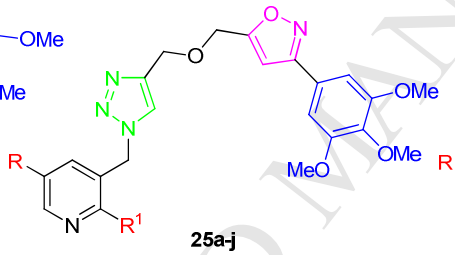
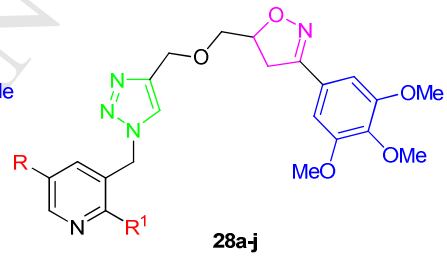


13a-j

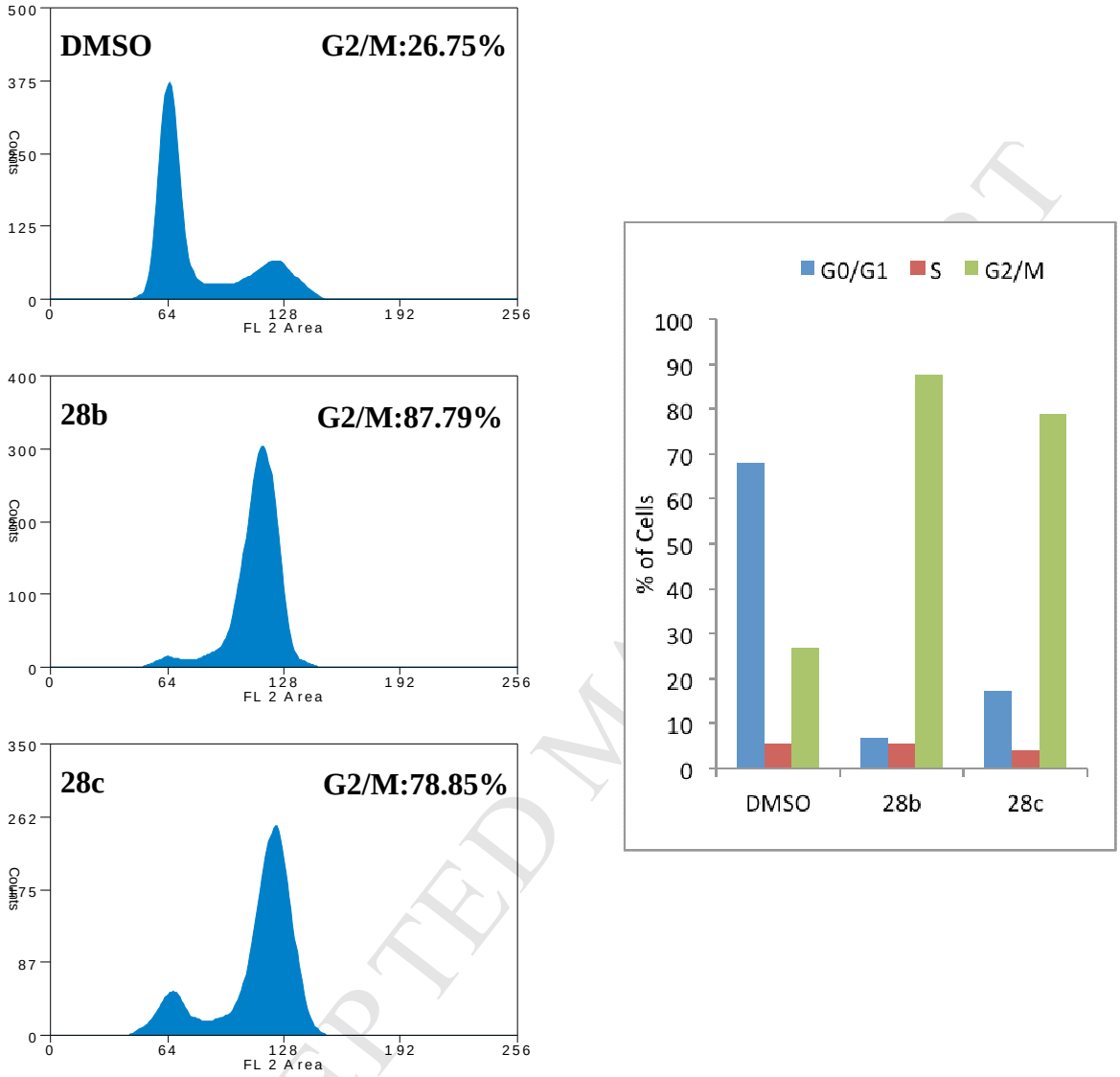


25a-j



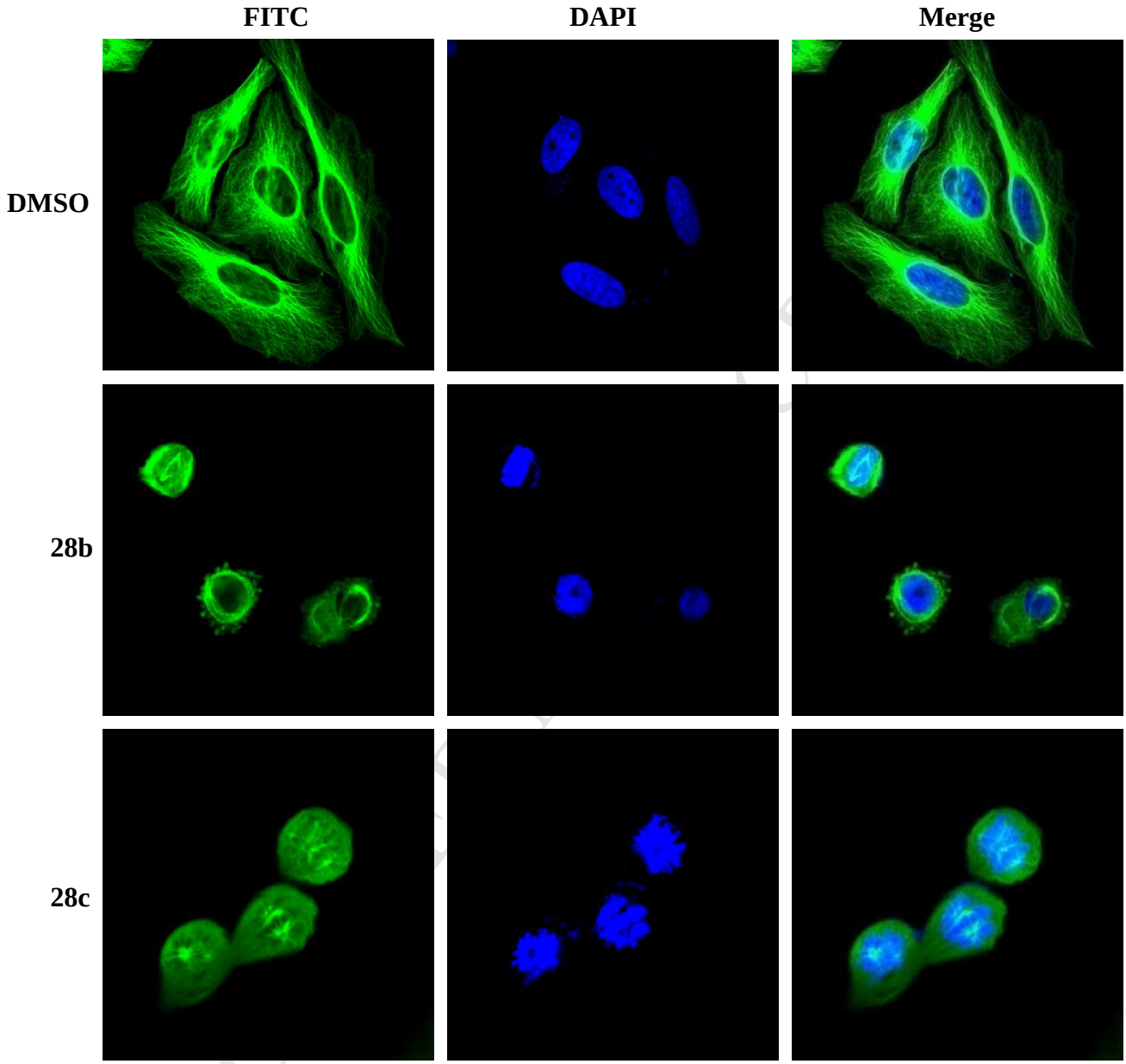
28a-j

Fig. 2



Panel A: Cell cycle effects of compounds **28b** and **28c**. HeLa cells were harvested after treatment with compounds at 10 μM for 24 h. Untreated cells and DMSO treated cells served as controls. The percentage of cells in each phase of cell cycle was quantified by flow cytometry. *Panel B*: Distribution of cells at G0/G1, S and G2/M phase of cell cycle following treatment with **28b** and **28c** in HeLa cells.

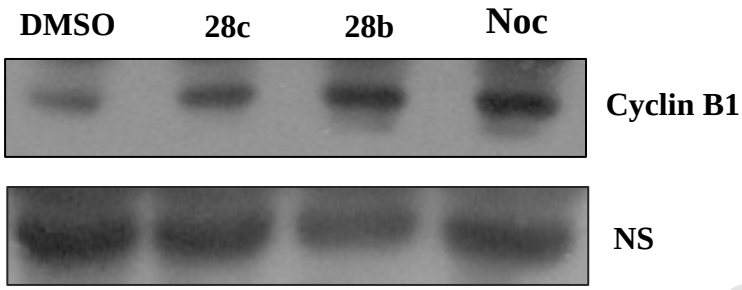
Fig. 3



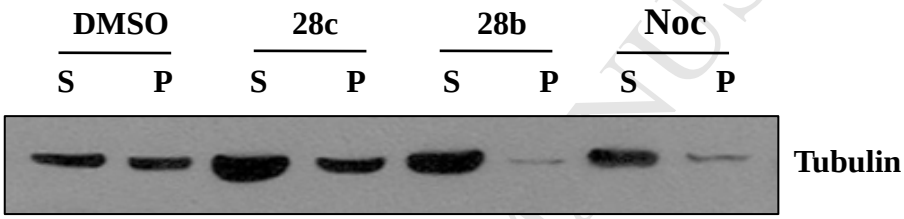
HeLa cells were independently treated with **28b** and **28c** at 10 μM concentrations for 24h. Following the termination of experiment, cells were fixed and stained for tubulin. DAPI was used as counter stain. The merged images of cells stained for tubulin and DAPI are represented. The photographs were taken using Olympus confocal microscope equipped with FITC and DAPI filter settings. Data is the representative of five different fields of view.

Fig. 4

A

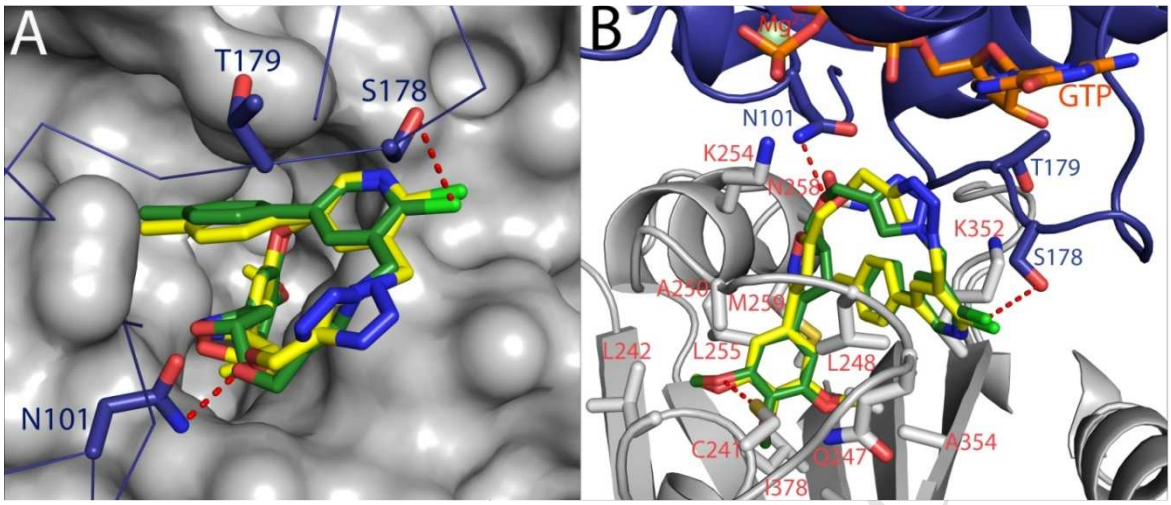


B



Panel A: HeLa cells were treated with 10 μM concentration of compound **28b** and **28c** for 24h. Nocodazole was treated at 2 μM concentrations for 24h. Subsequently, whole lysates were prepared and analyzed for cyclin-B1. A non-specific (NS) band at 90kDa was used as loading control. *Panel B:* HeLa cells were treated with 10 μM of **28b** and **28c** for 24h. Nocodazole was utilized at 2 μM concentration for 24h treatments. The fractions containing soluble and polymerized tubulin were collected and resolved on SDS-PAGE. Tubulin was detected by immunoblot analysis.

Fig. 5



Panel A: Docking pose of **25c** (yellow) and **28c** (green). The gray surface represents β-chain while the blue line diagram indicates the α-chain of tubulin. The trimethoxyphenyl moiety of compounds is buried in hydrophobic pocket of colchicine binding domain. *Panel B:* Stick representation of selected amino acids of α and β-subunits of tubulin interacting with compound **25c**, and **28c**. Red dot line represents hydrogen bonds.

Research highlights

- Novel pyridinyl-1*H*-1,2,3-triazoles, triazolylisoxazoles, triazolyldihydroisoxazoles were designed as anti-cancer agents.
- Triazolyldihydroisoxazoles **28b** and **28c** shown potent anti-cancer activity and inhibited tubulin polymerization.
- FACS analysis was performed on HeLa cell lines for compounds **28b** and **28c**.
- Molecular modelling studies revealed that the compounds **28b** and **28c** binds well in the colchicine binding site of α,β-tubulin.

Synthesis and structure-activity relationships of pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles as potent inhibitors of tubulin polymerization

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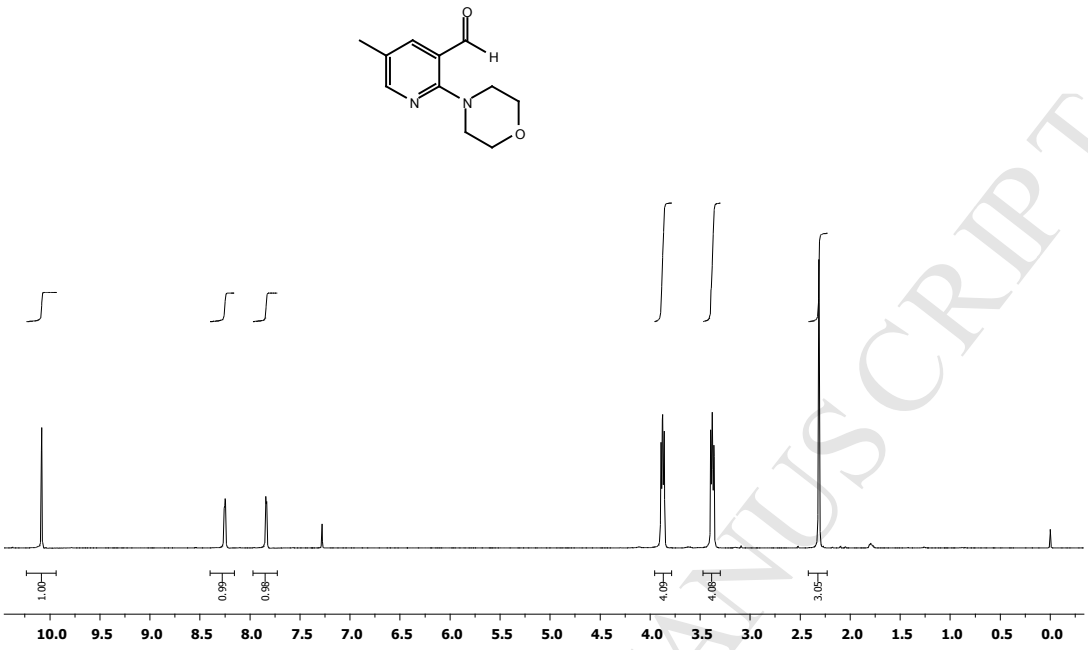
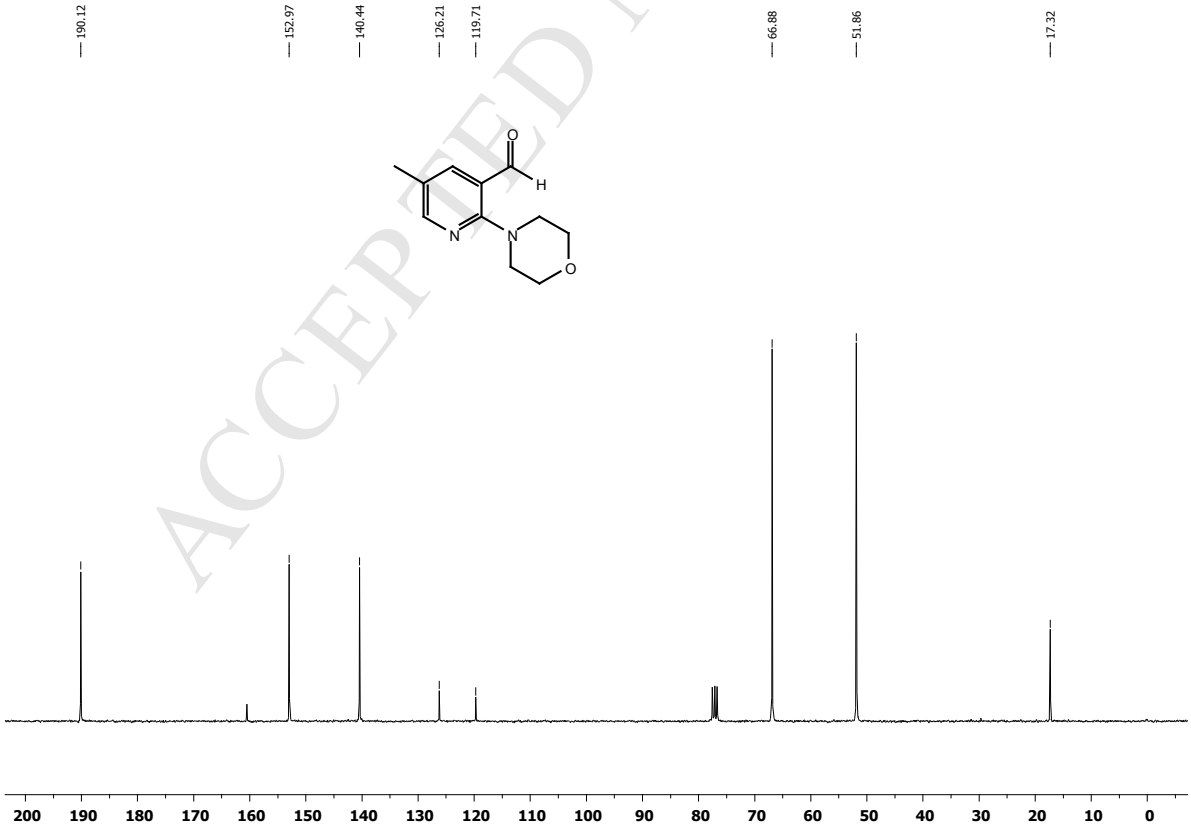
General

All chemicals and reagents were purchased from Aldrich (Sigma-Aldrich, USA), AVRA Chemicals Pvt. Ltd (Hyderabad, India) and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F_{254} (mesh); spots were visualized under UV light. Column chromatographic separations were carried out on silica gel (60-120 mesh). Melting points were determined on a *Mettler-Temp* apparatus and are uncorrected. An IR spectrum was recorded with a *Thermo Nicolet Nexus* 670 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a *Bruker Avance 300* and 500 MHz spectrometers. Chemical shifts (δ) are quoted in parts per million and are referenced to tetramethylsilane (TMS) as internal standard. ESIMS obtained on *7070H spectrometer* operating at 70 eV using a direct inlet system. HRMS were carried out on Agilent 6510 Q-TOF LC/MS instrument.

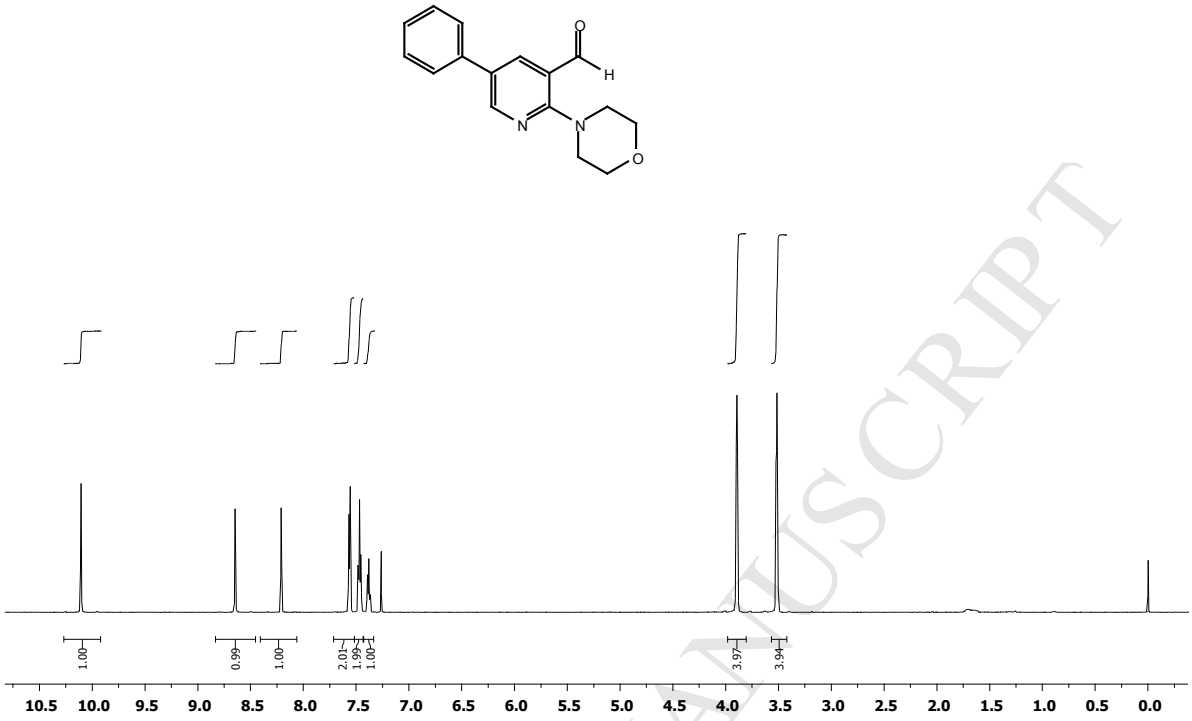
^{1}H-NMR and ^{13}C-NMR Spectra



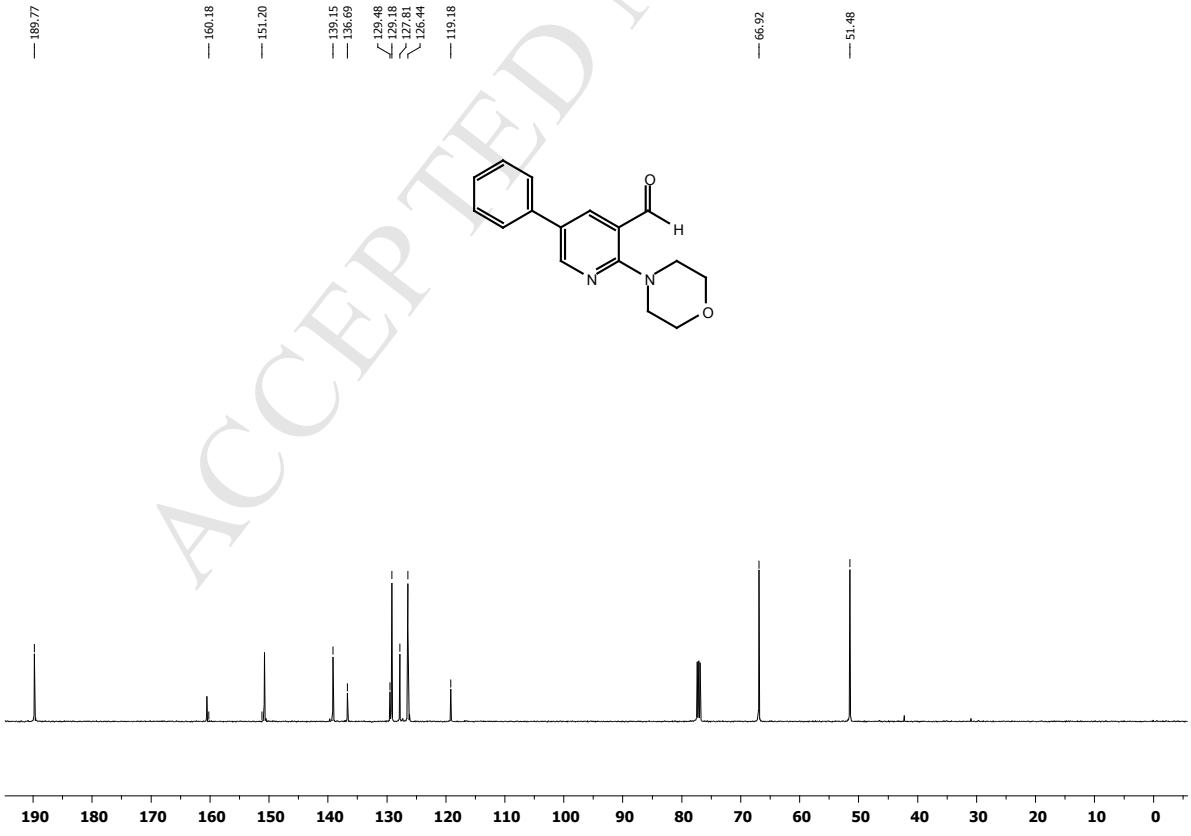
^{1}H-NMR OF COMPOUND **18a**



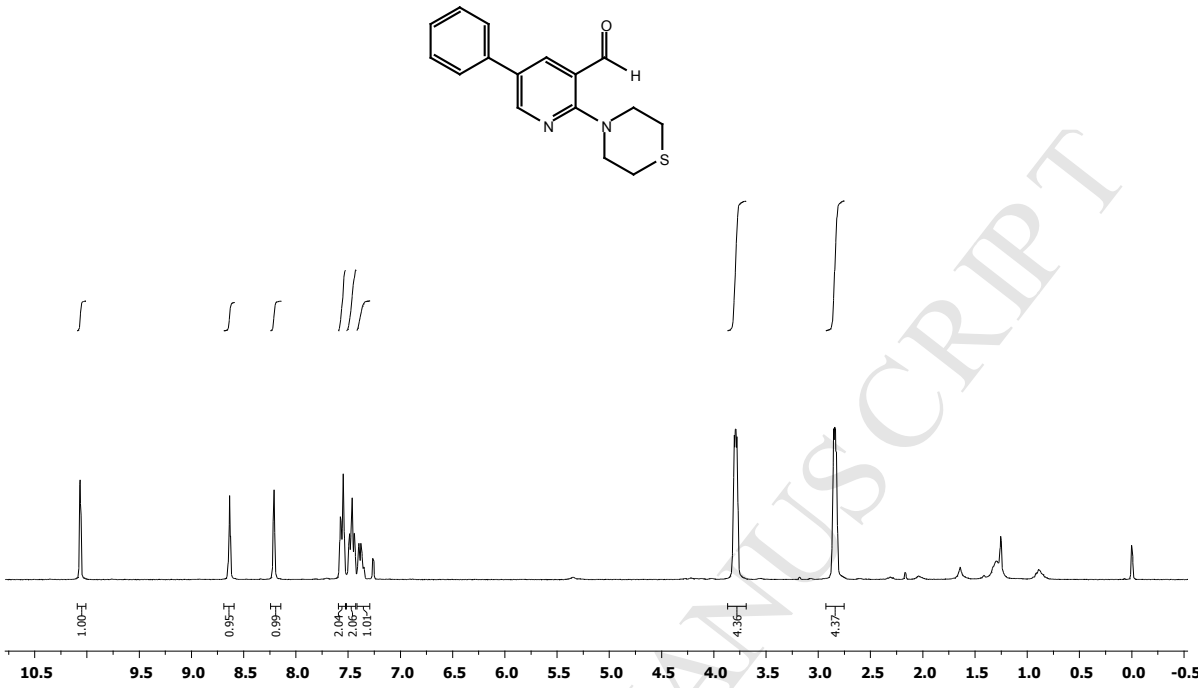
^{13}C-NMR OF COMPOUND **18a**



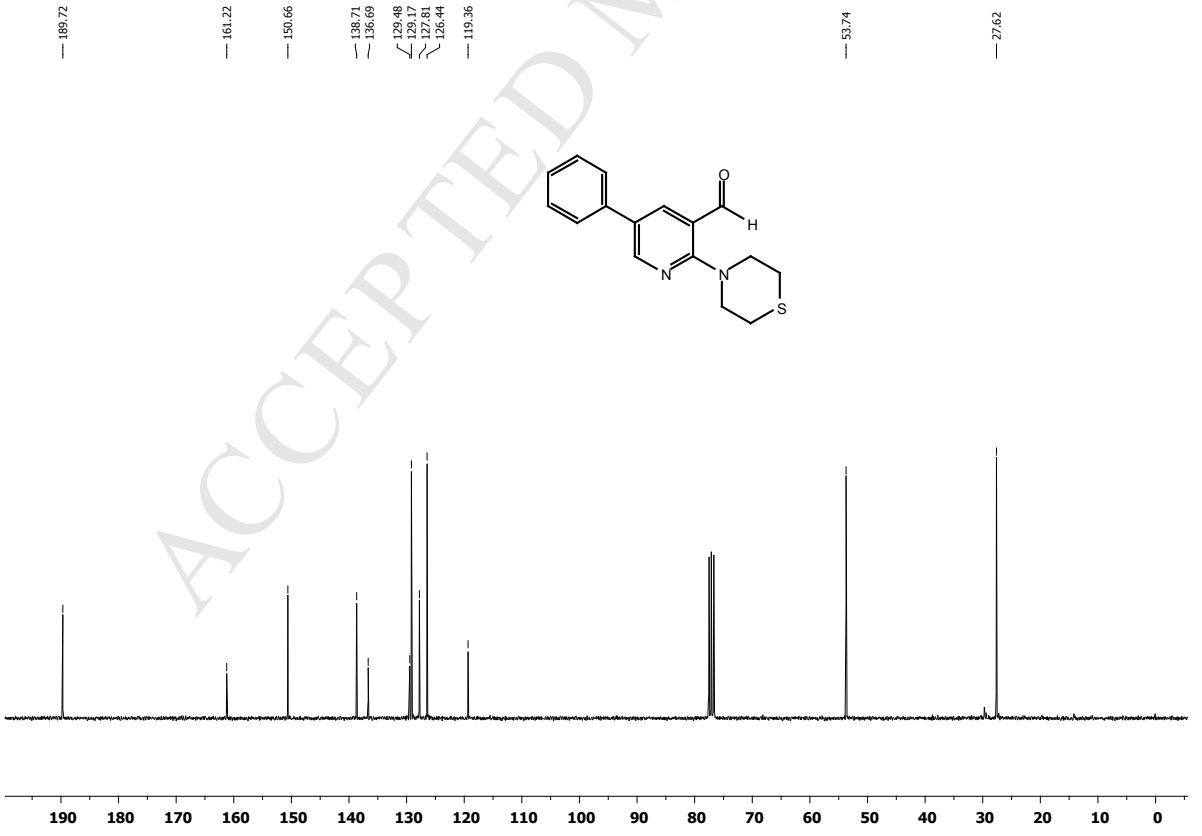
^{1}H-NMR OF COMPOUND **18c**



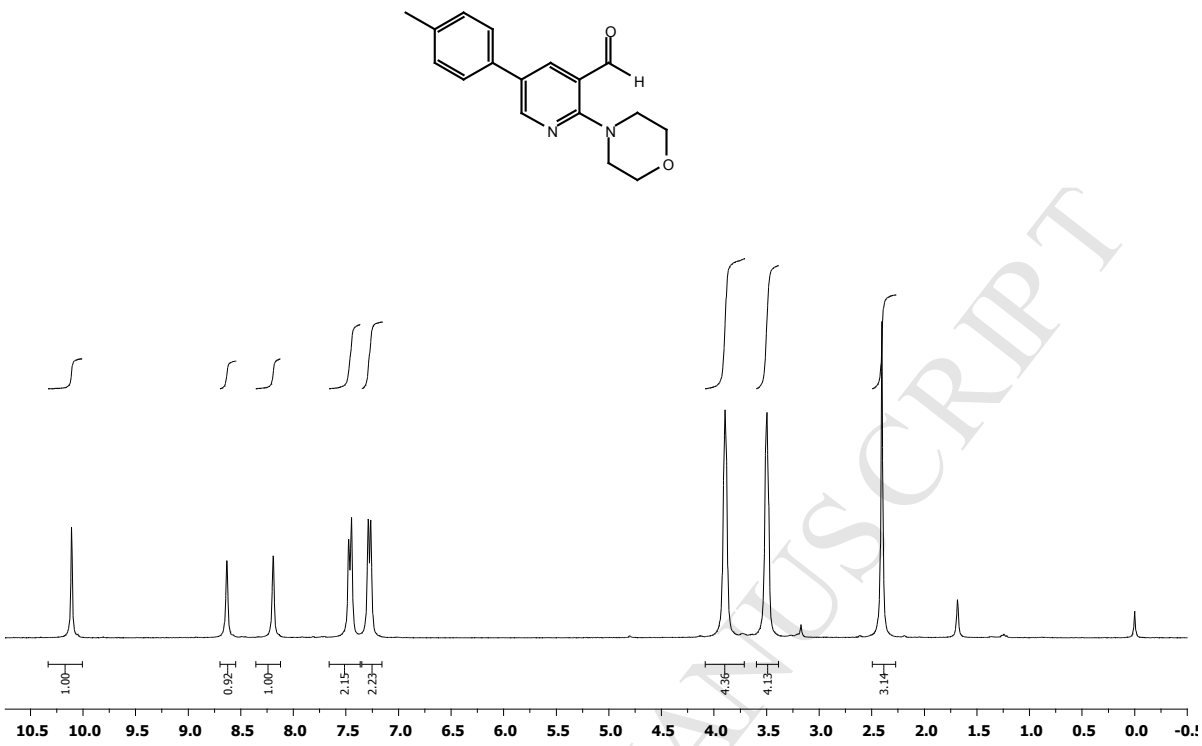
^{13}C-NMR OF COMPOUND **18c**



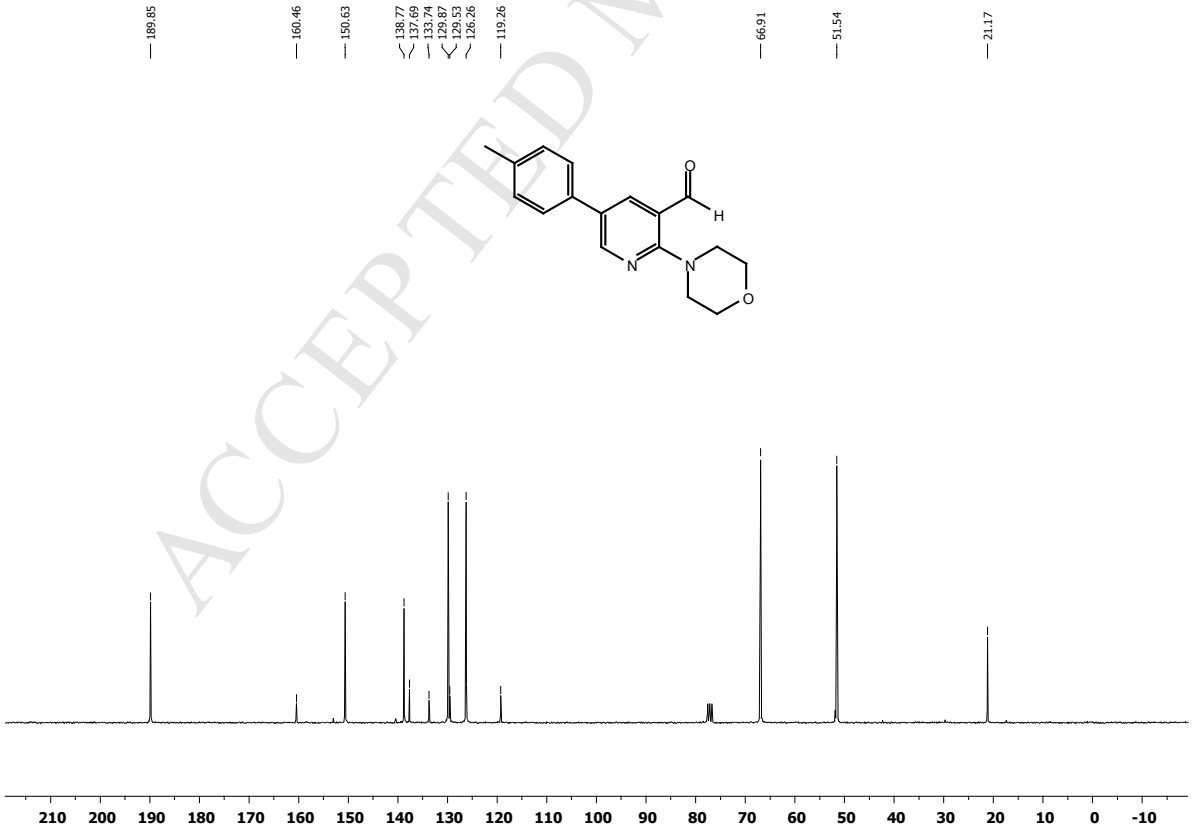
^{1}H-NMR OF COMPOUND **18d**



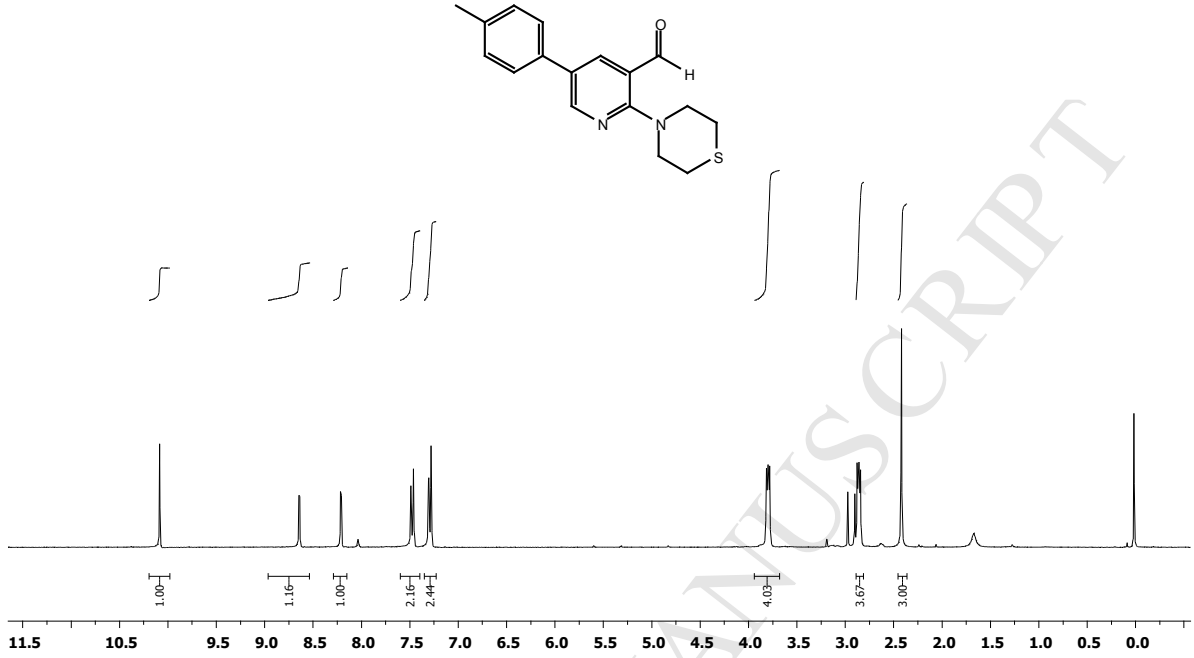
^{13}C-NMR OF COMPOUND **18d**



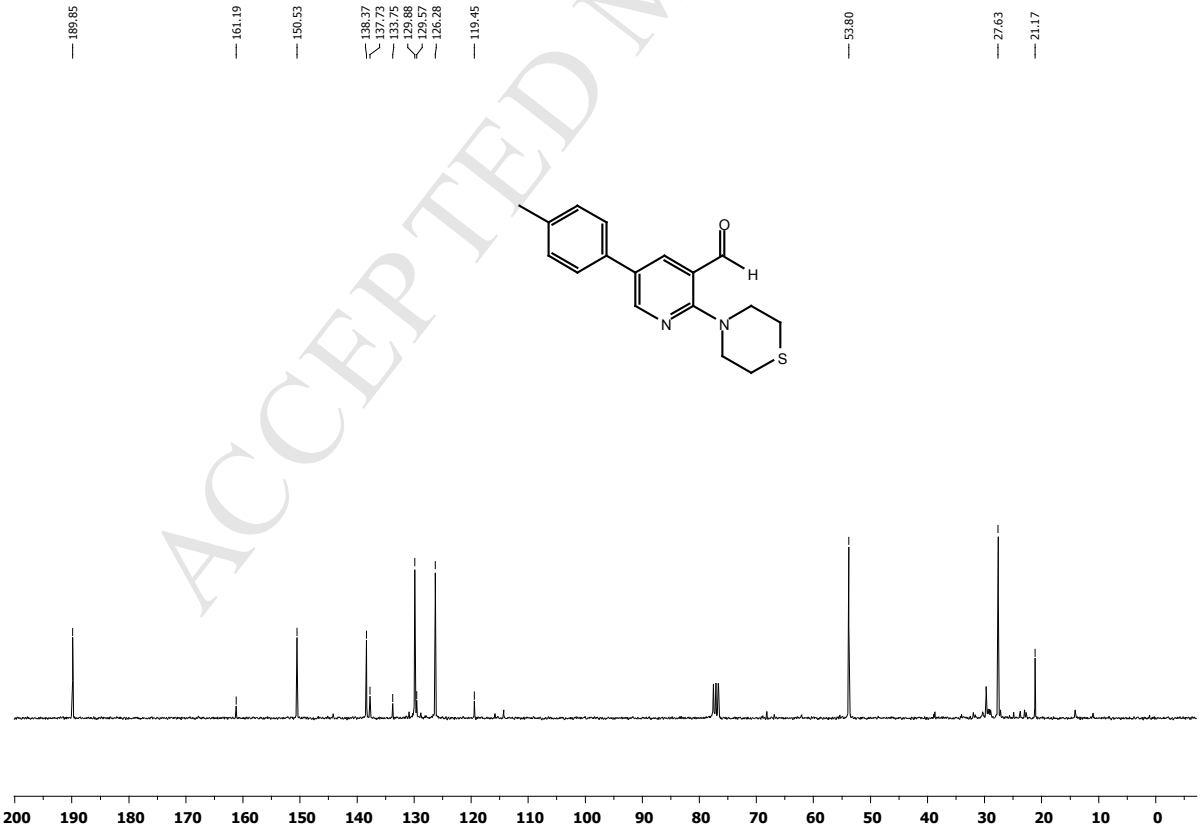
^{1}H-NMR OF COMPOUND **18e**



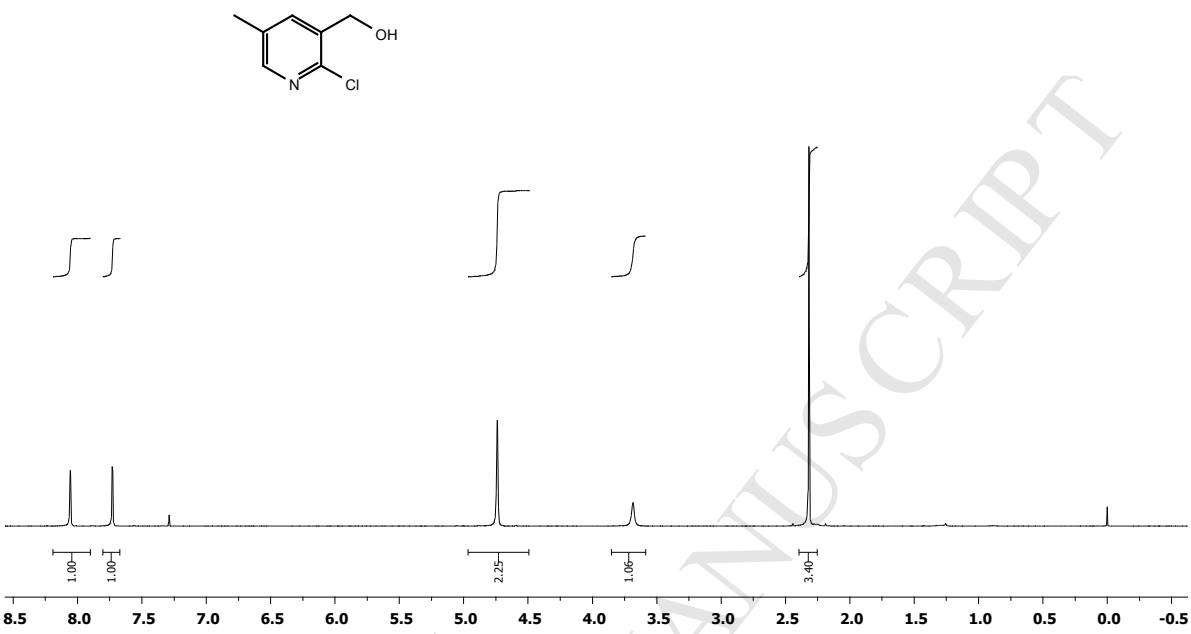
^{13}C-NMR OF COMPOUND **18e**



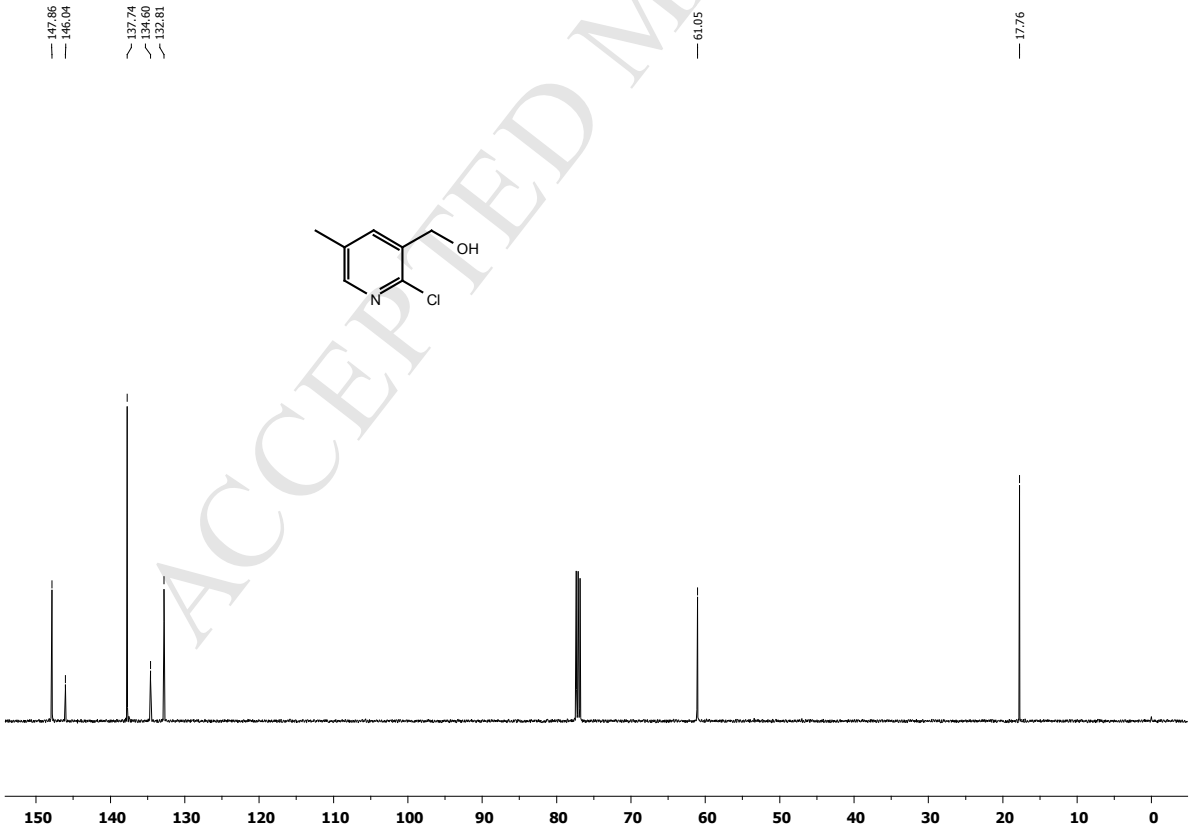
^{1}H-NMR OF COMPOUND **18f**



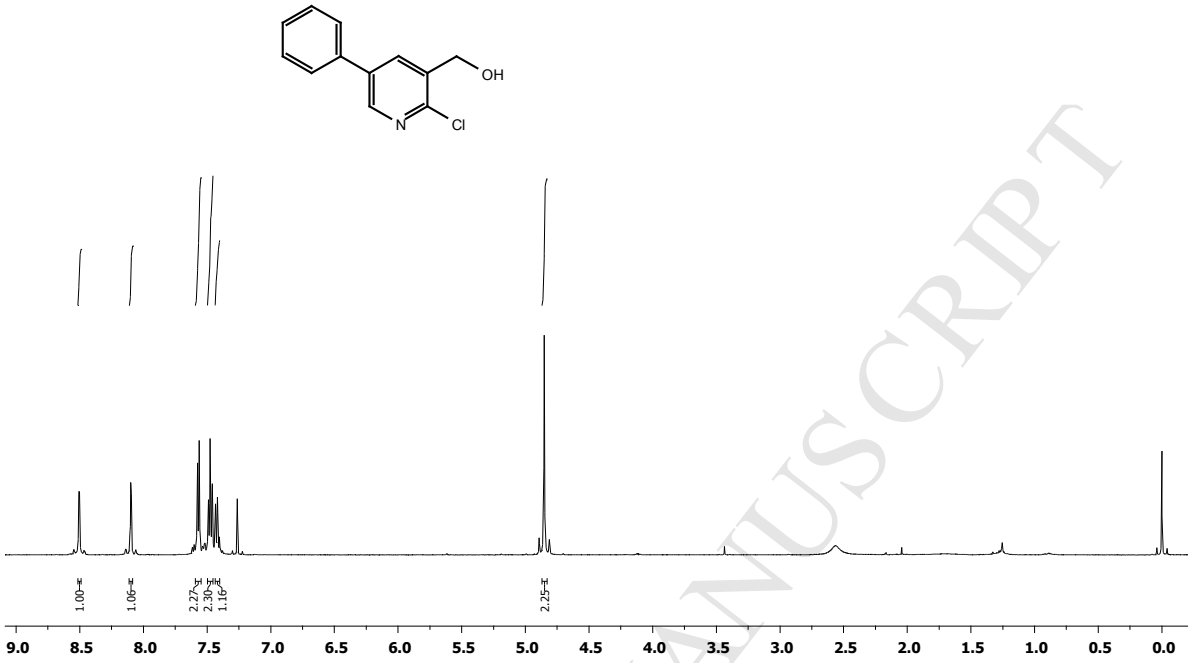
^{13}C-NMR OF COMPOUND **18f**



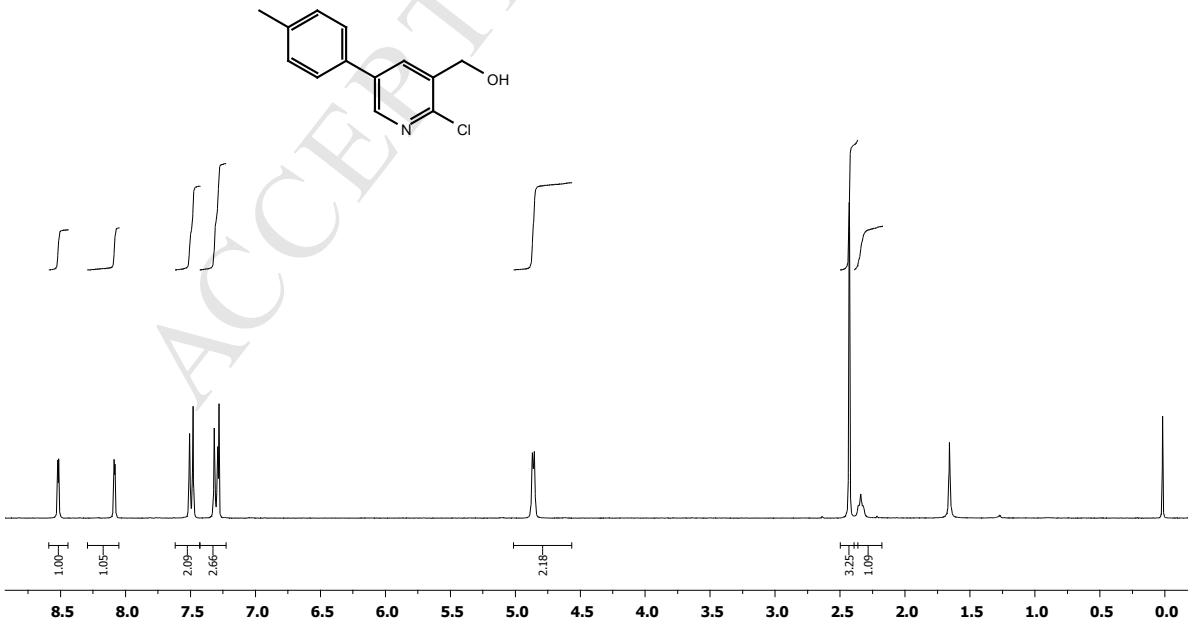
^{1}H-NMR OF COMPOUND **9a**



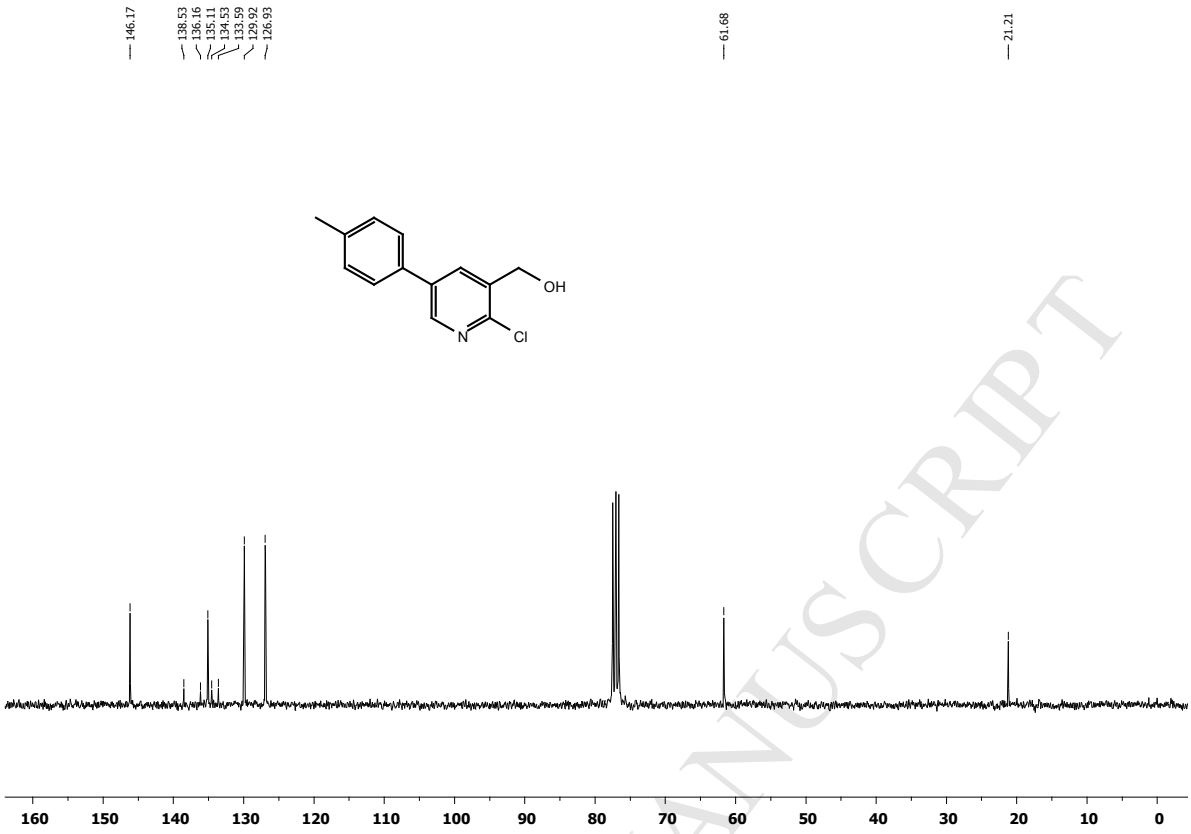
^{13}C-NMR OF COMPOUND **9a**



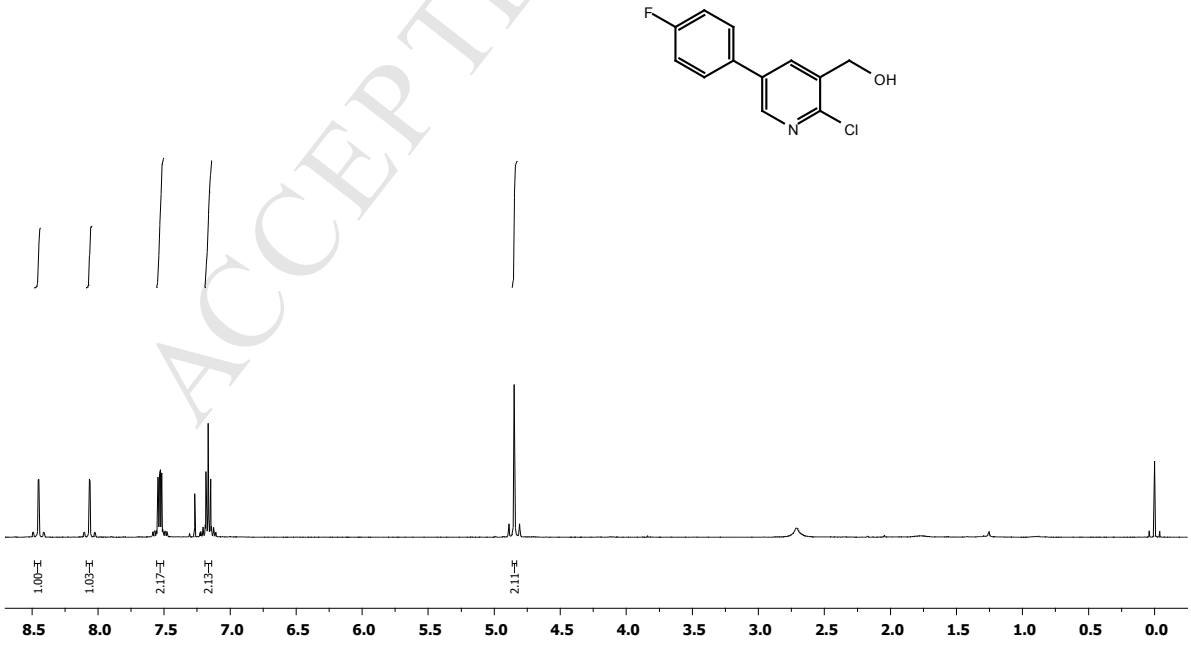
^{1}H-NMR OF COMPOUND **9b**



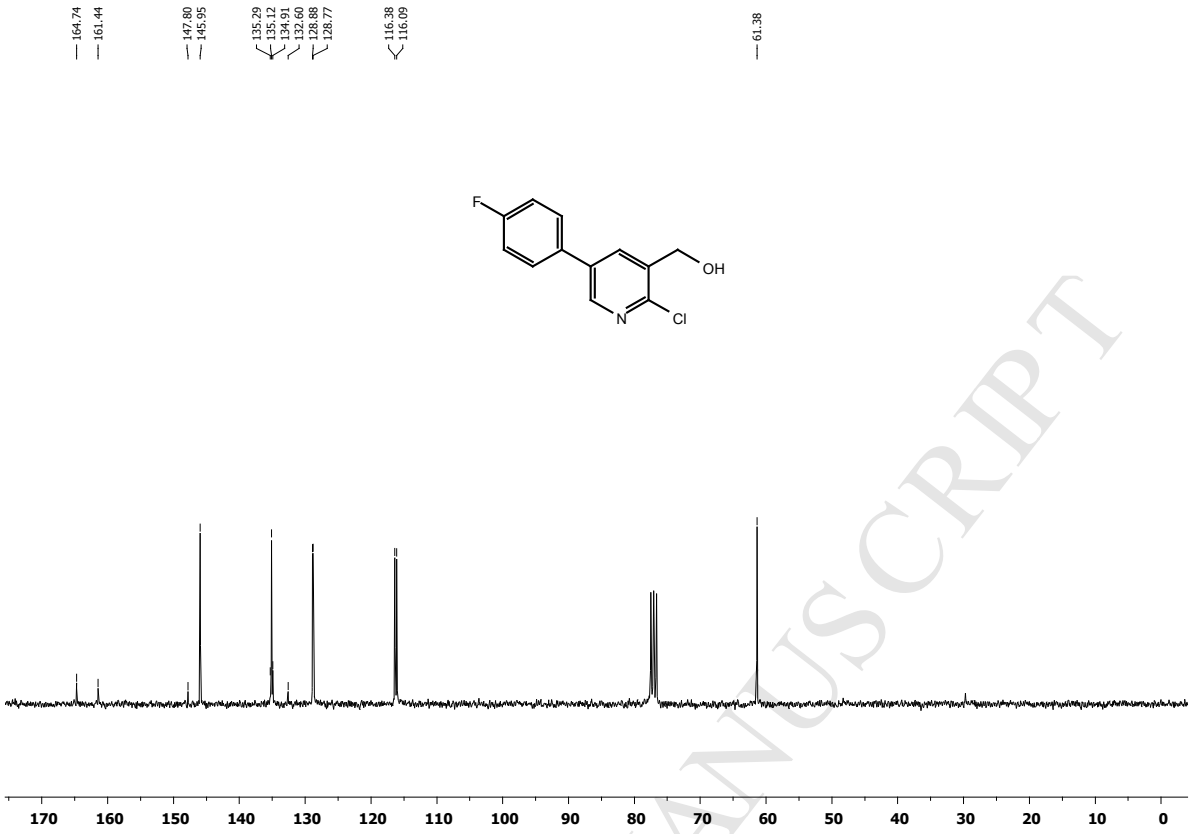
^{1}H-NMR OF COMPOUND **9c**



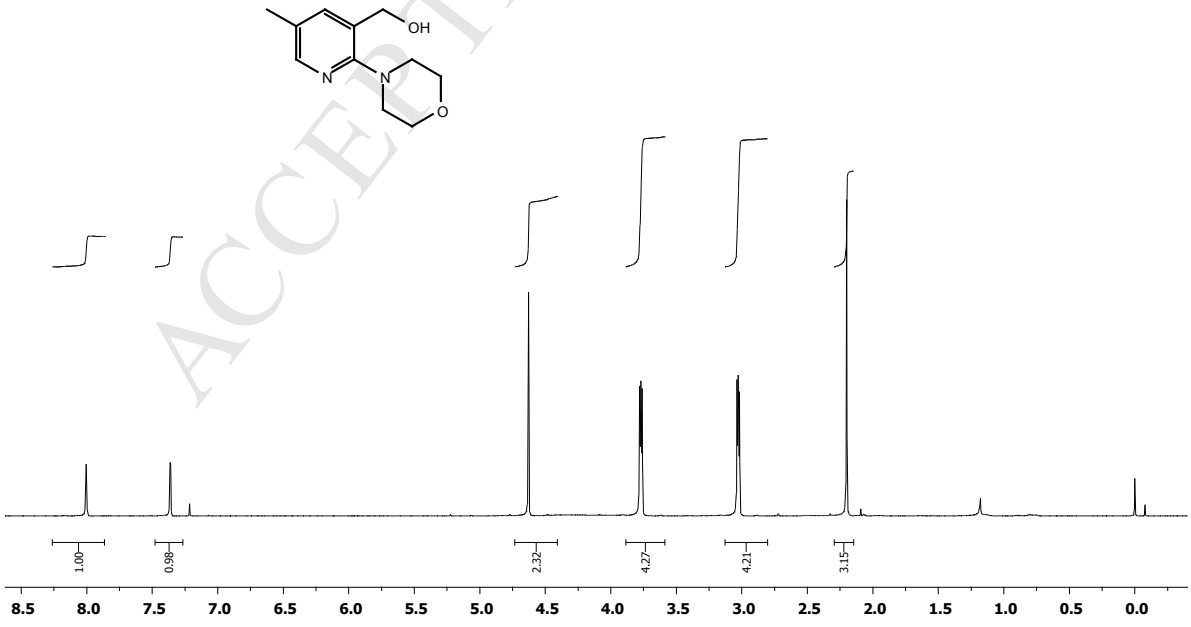
^{13}C-NMR OF COMPOUND **9c**



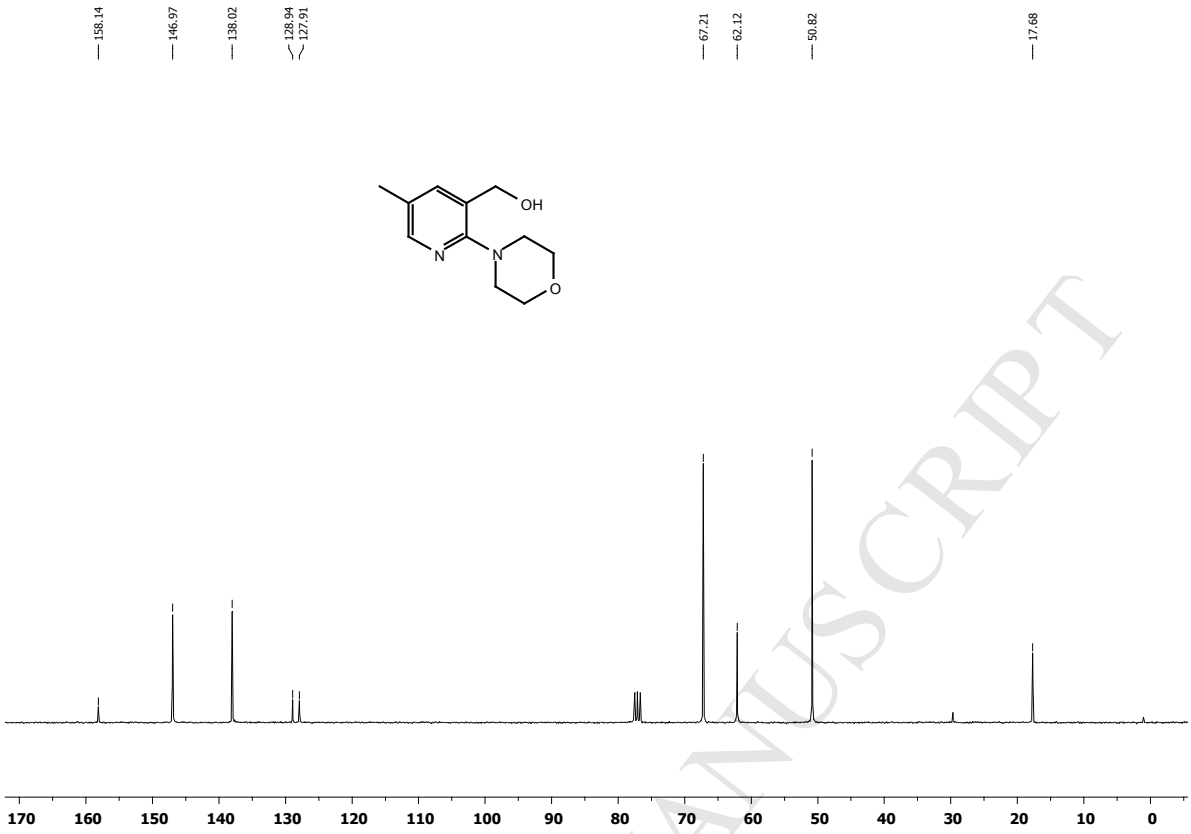
^{1}H-NMR OF COMPOUND **9d**



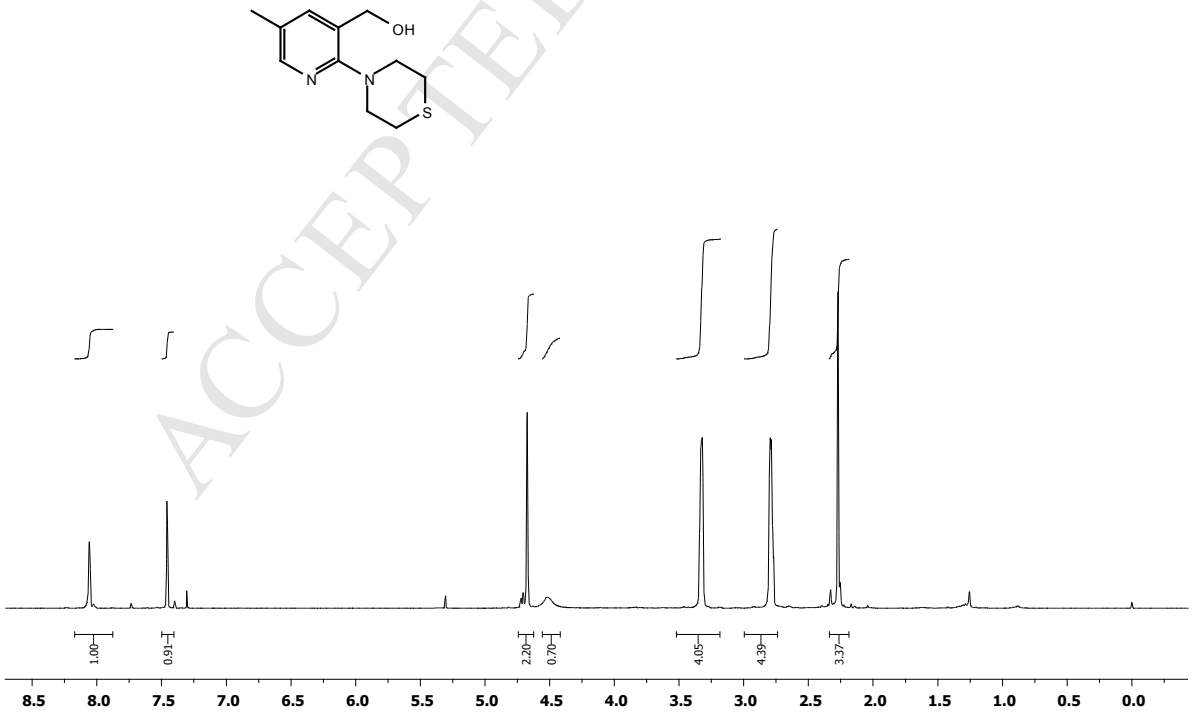
^{13}C-NMR OF COMPOUND **9d**



^{1}H-NMR OF COMPOUND **19a**

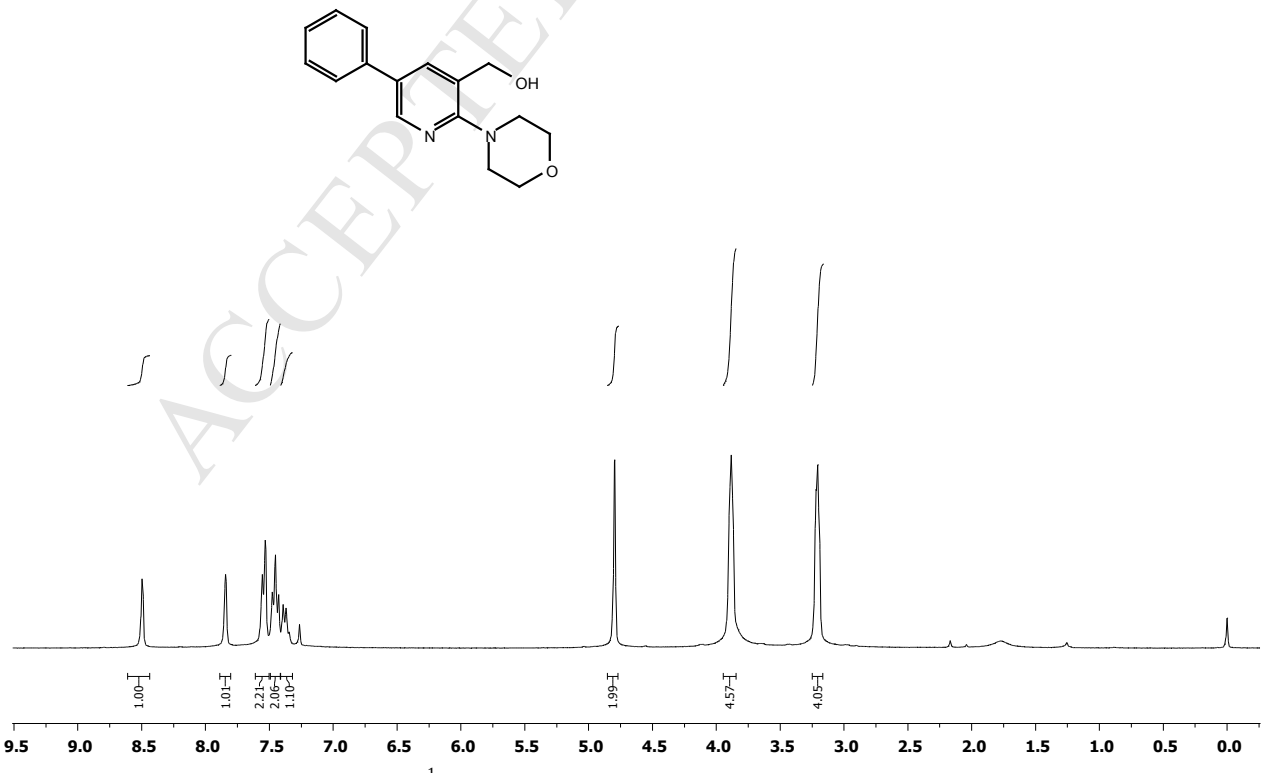


^{13}C-NMR OF COMPOUND **19a**

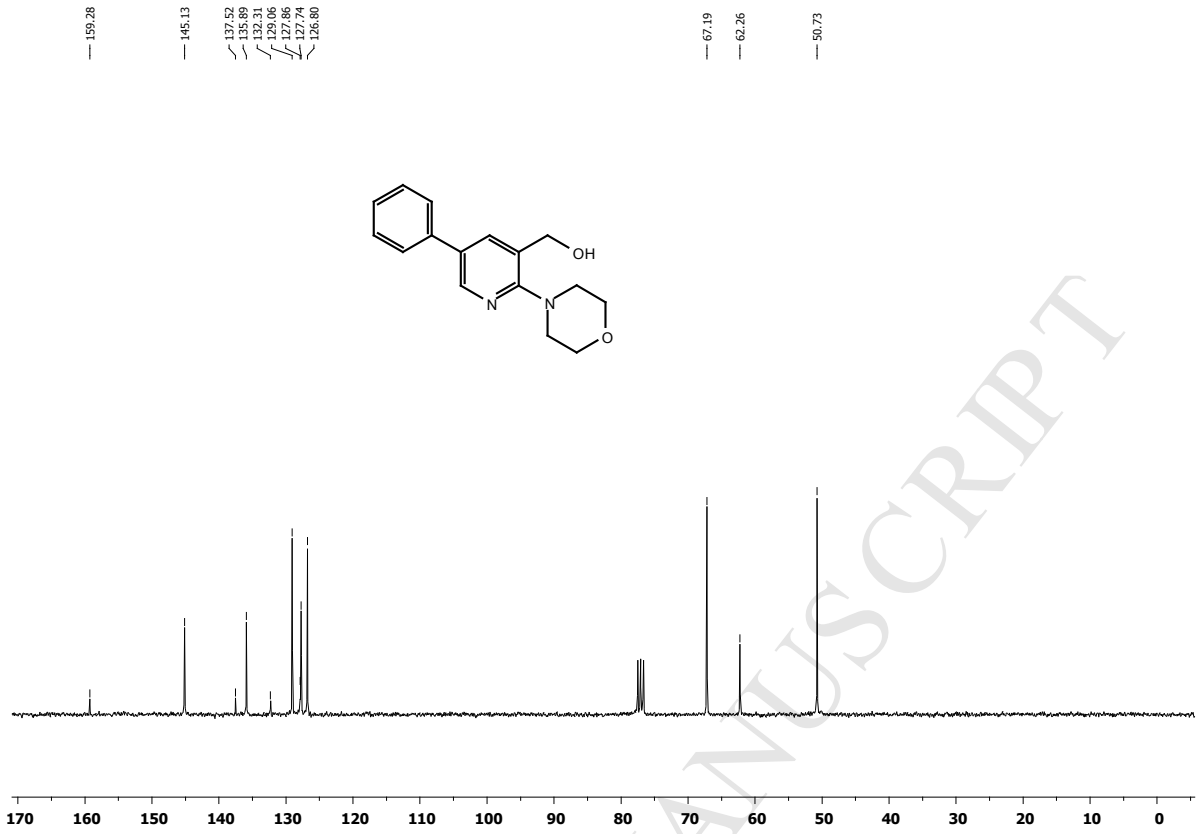


^{1}H-NMR OF COMPOUND **19b**

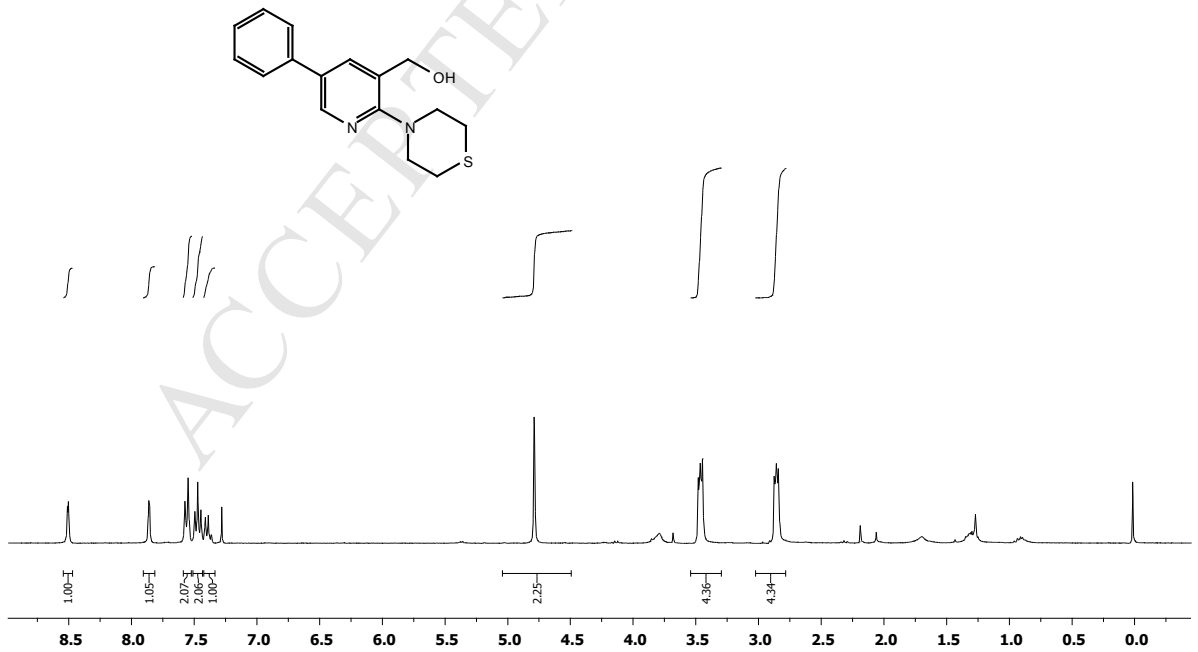
^{13}C-NMR OF COMPOUND **19b**



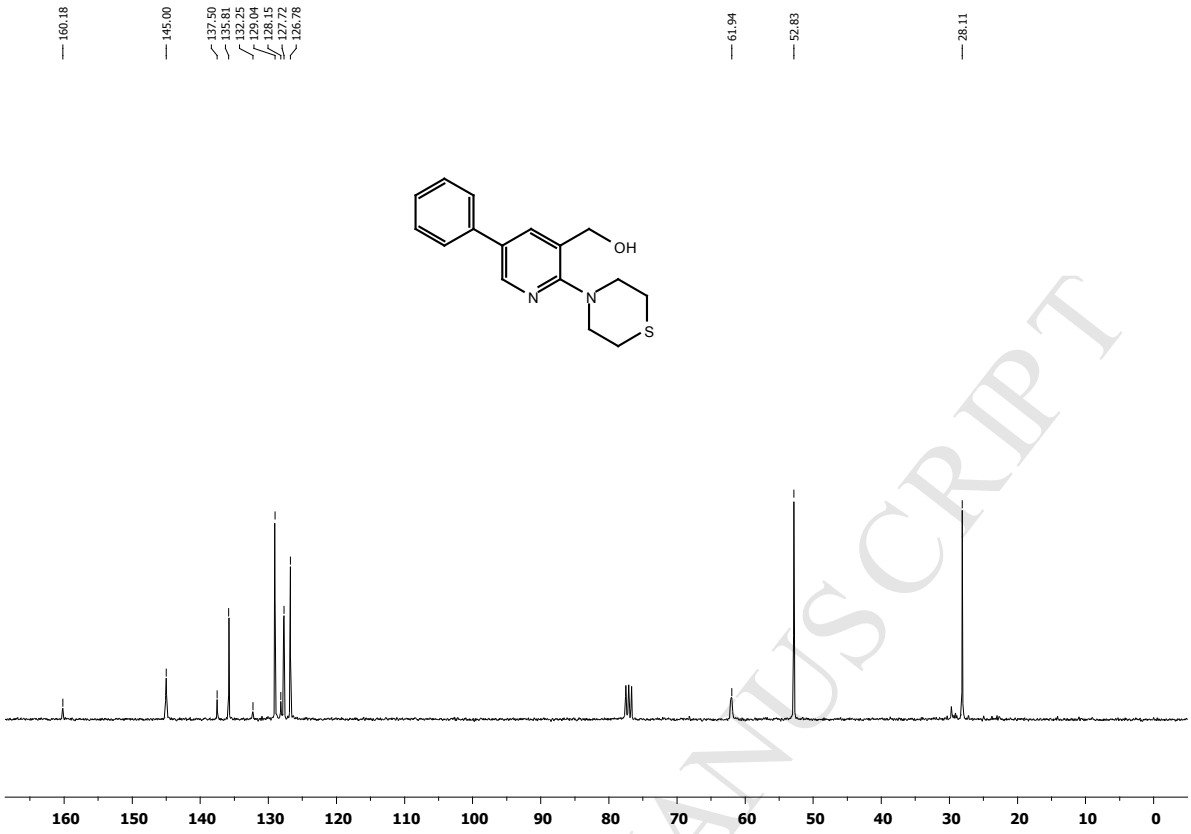
^{1}H-NMR OF COMPOUND **19c**



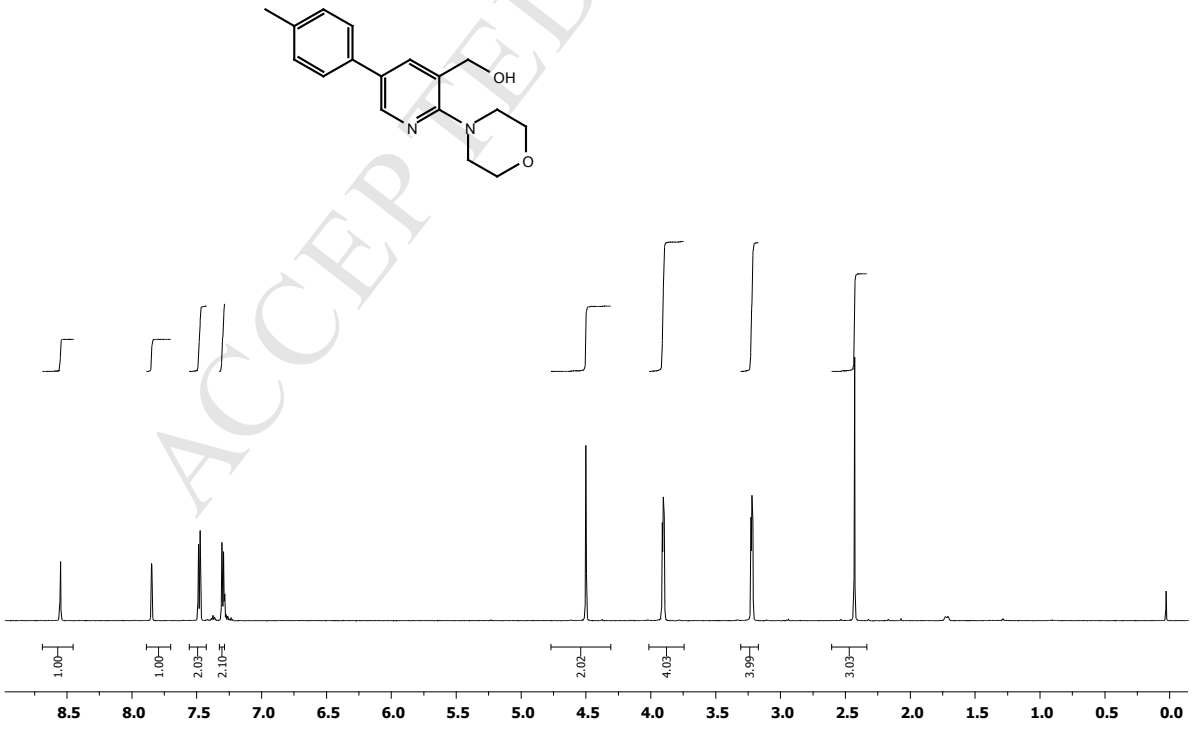
^{13}C-NMR OF COMPOUND **19c**



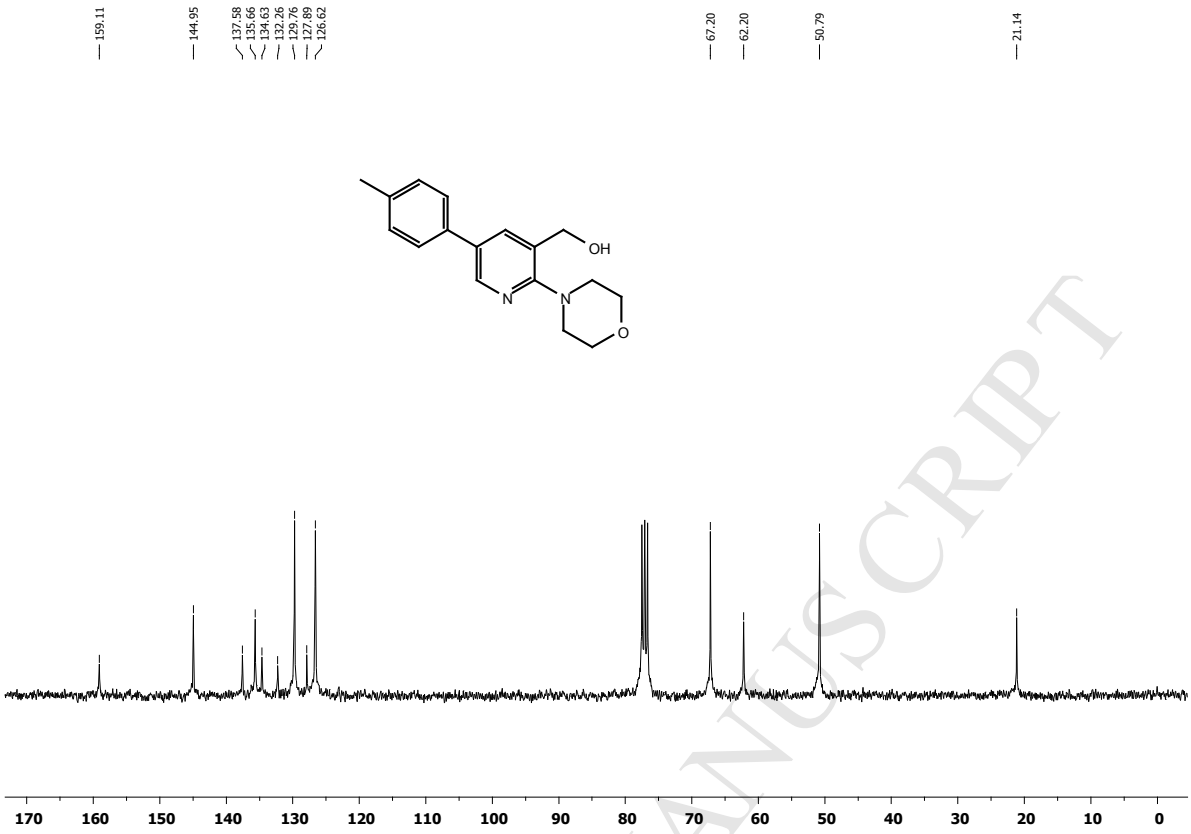
^{1}H-NMR OF COMPOUND **19d**



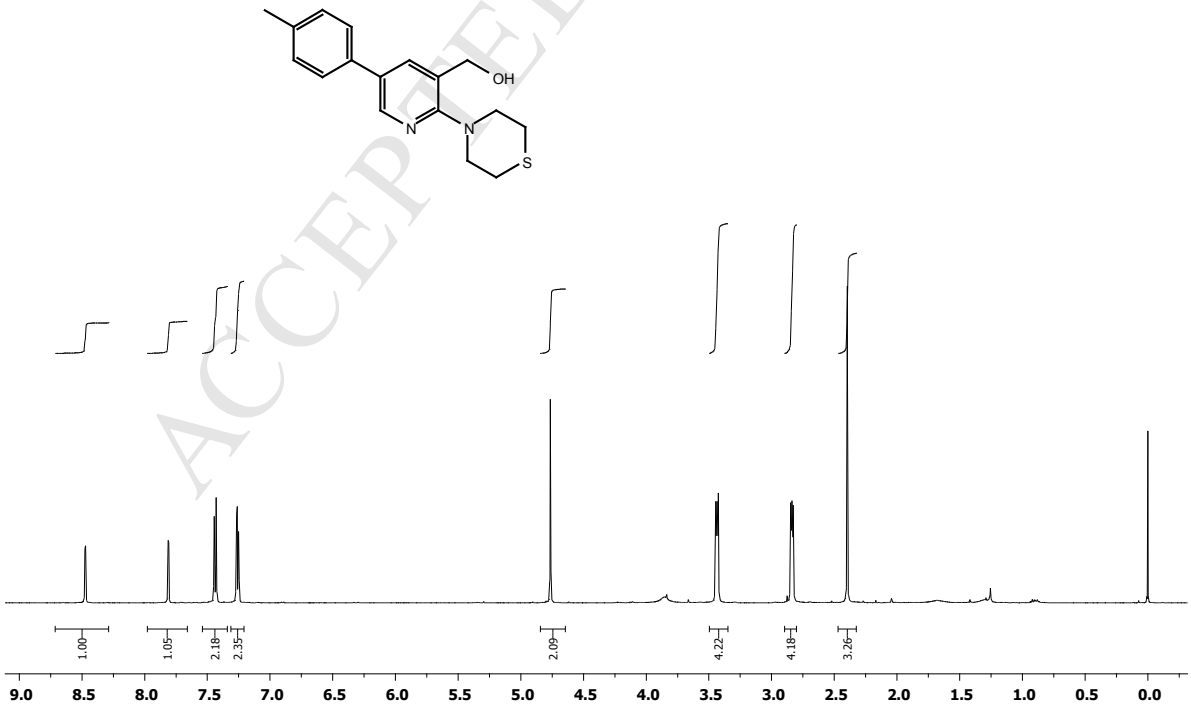
^{13}C-NMR OF COMPOUND **19d**



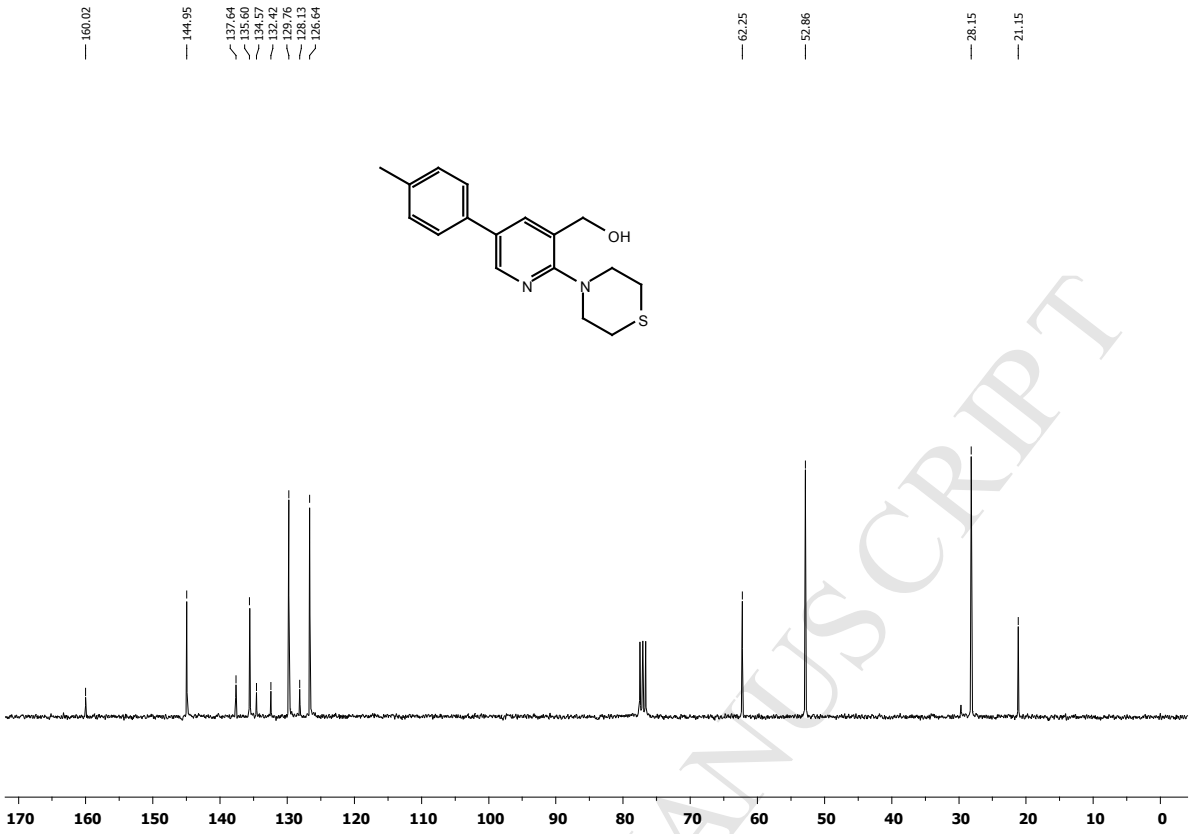
^{1}H-NMR OF COMPOUND **19e**



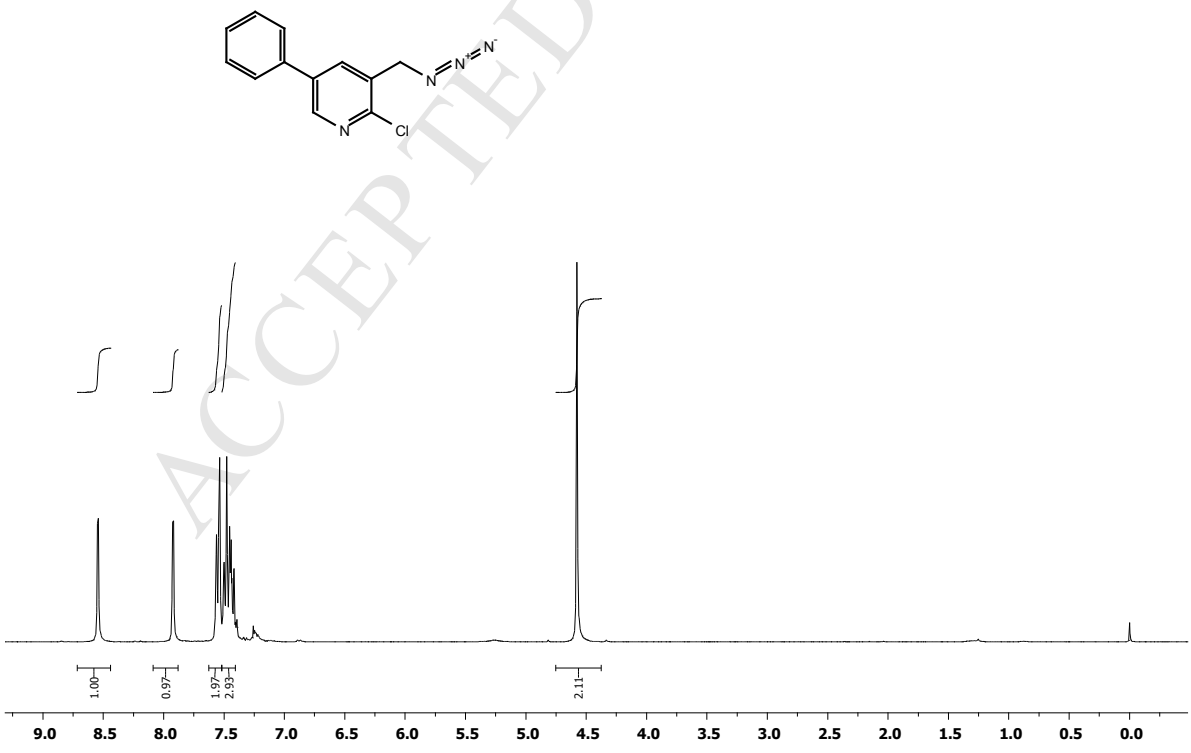
^{13}C-NMR OF COMPOUND **19e**



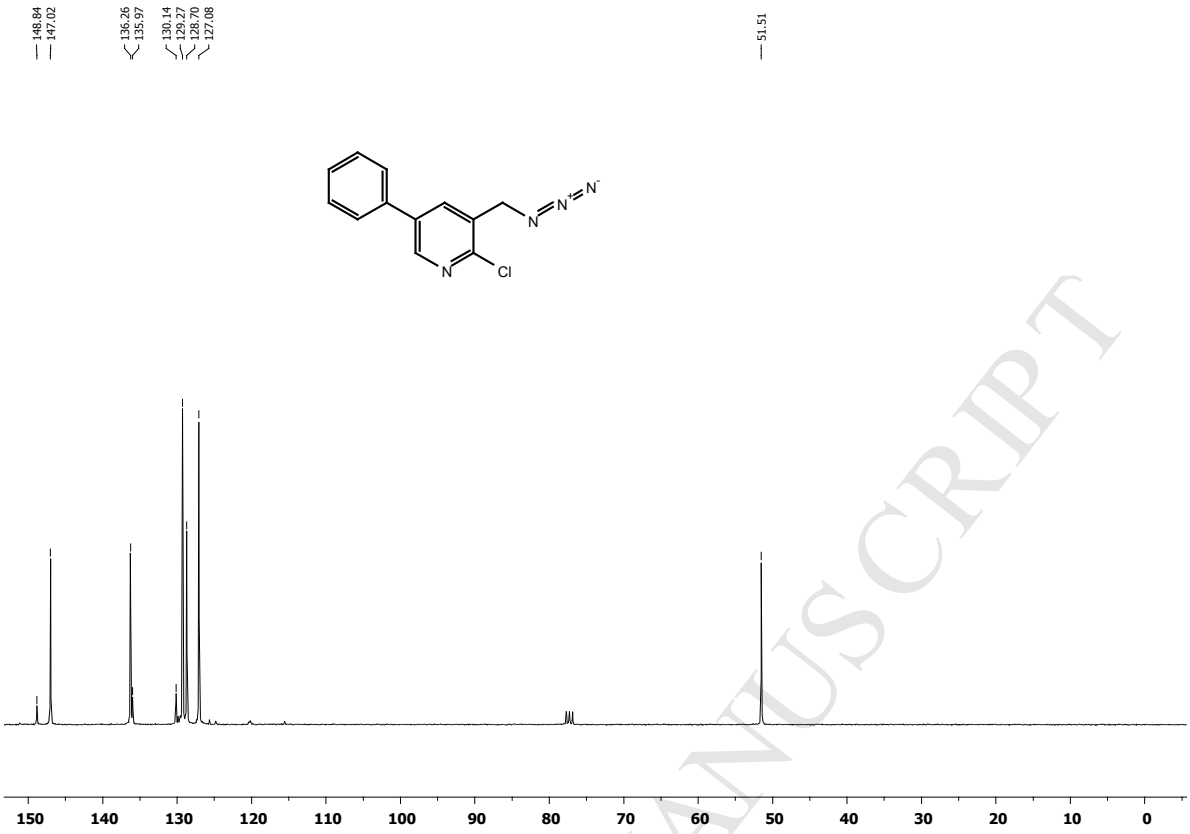
^{1}H-NMR OF COMPOUND **19f**



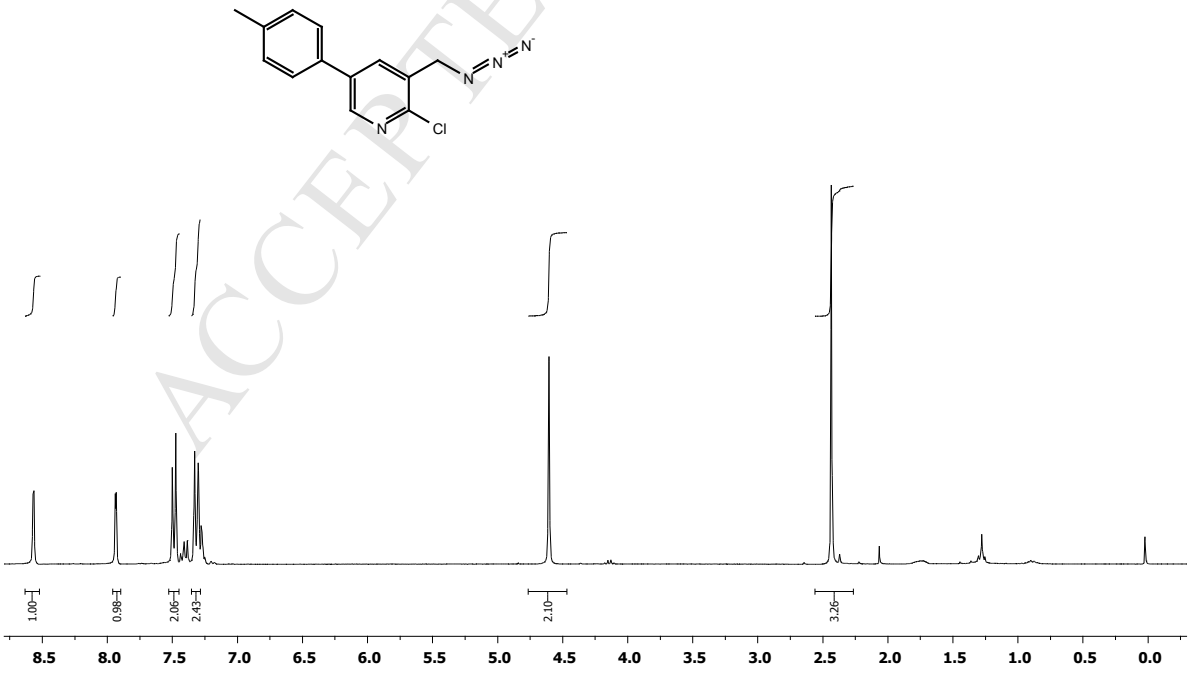
^{13}C-NMR OF COMPOUND **19f**



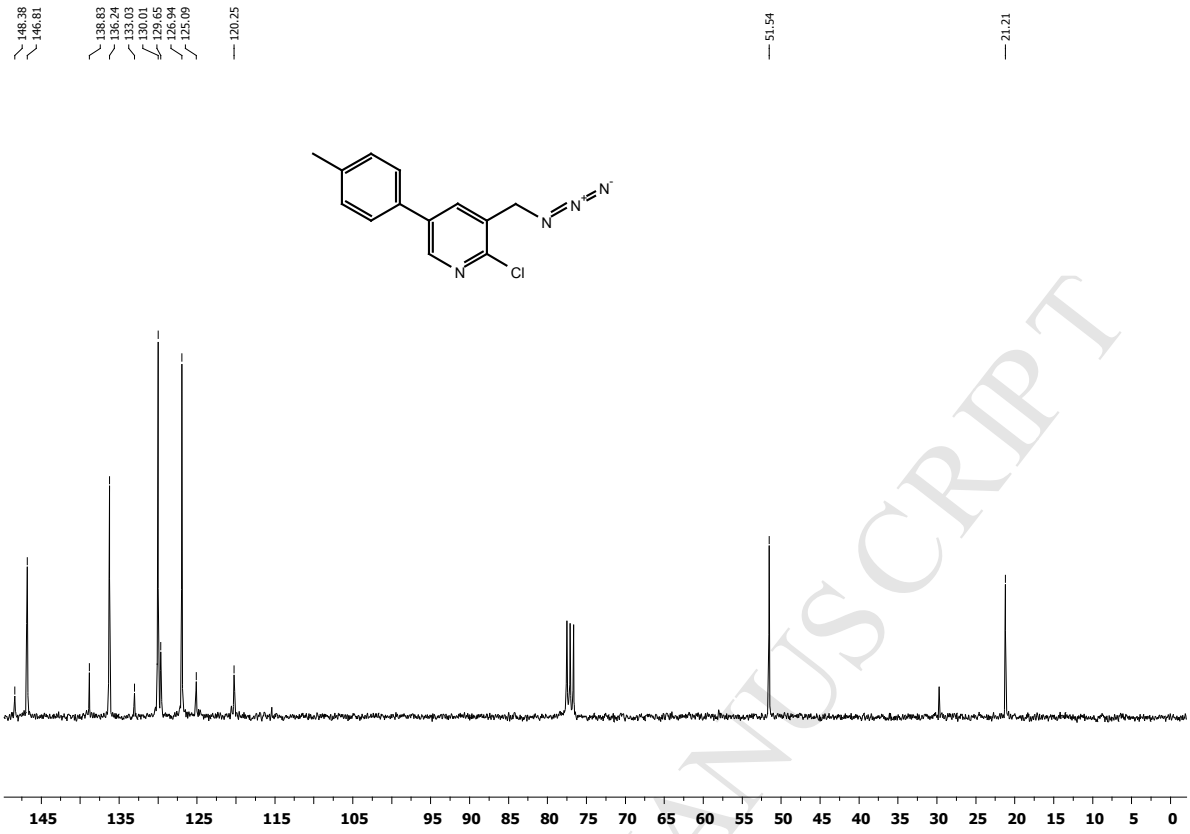
^{1}H-NMR OF COMPOUND **10b**



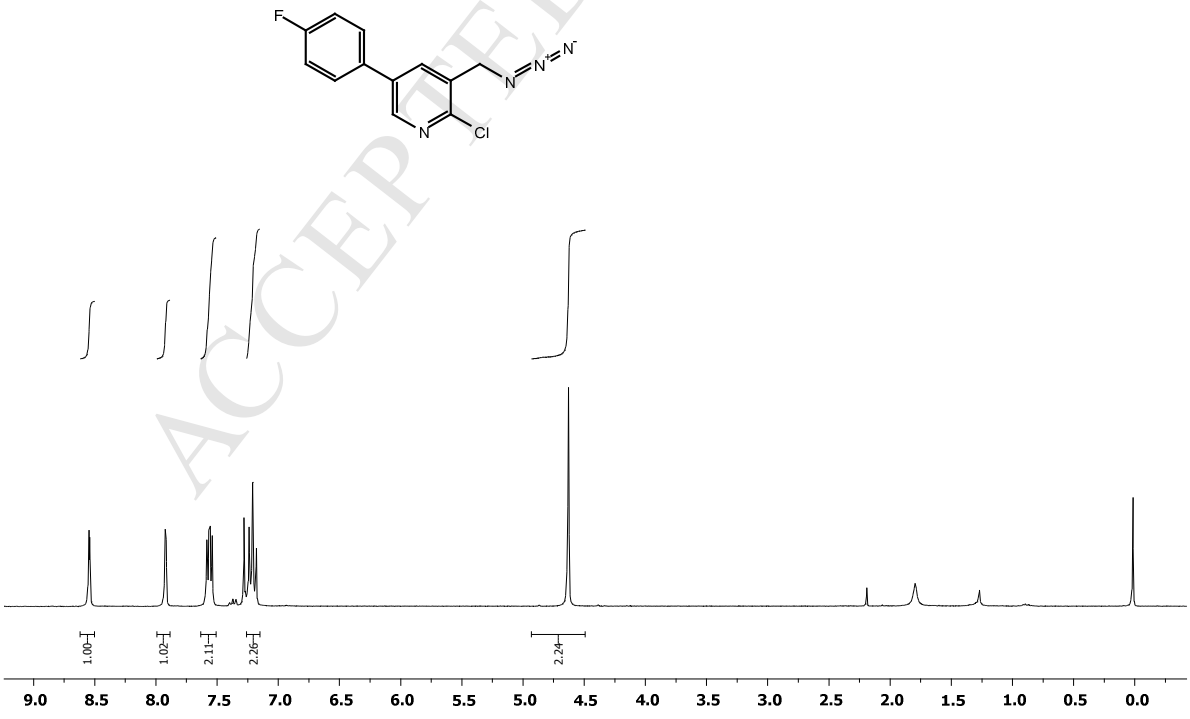
^{13}C-NMR OF COMPOUND **10b**



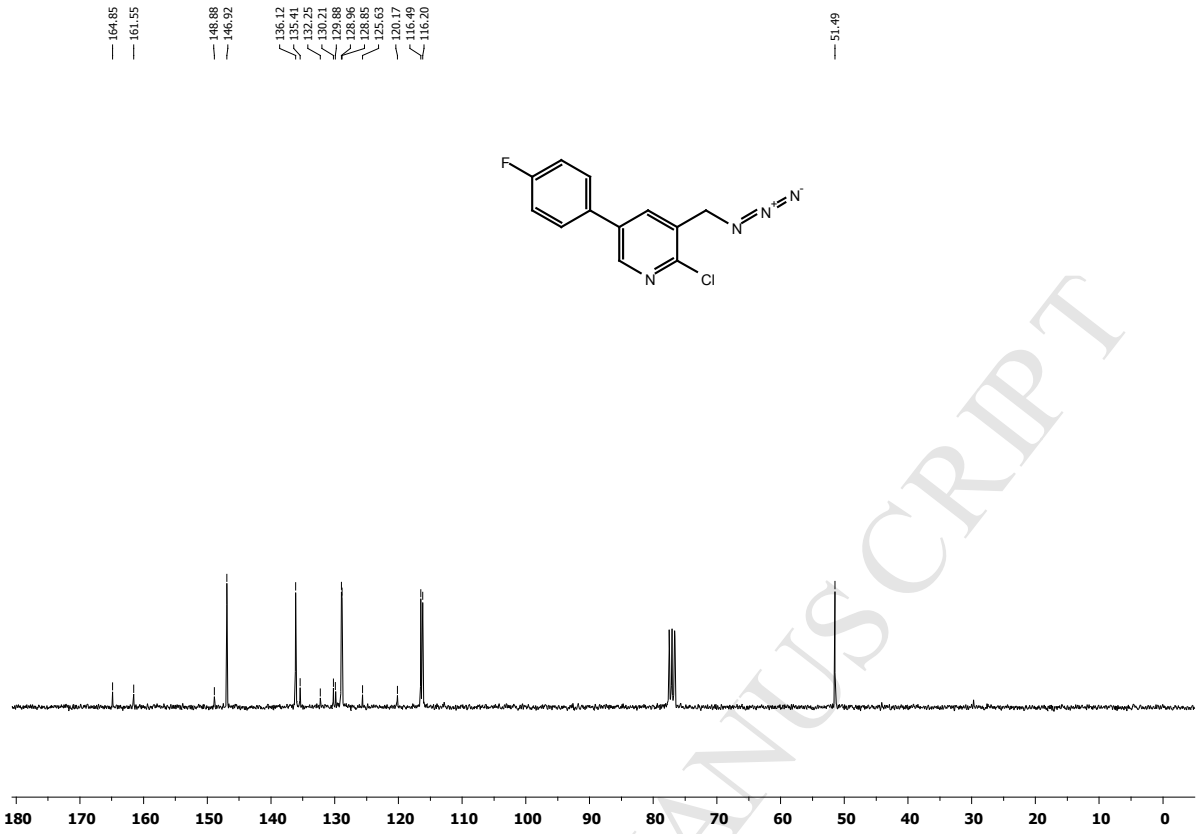
^{1}H-NMR OF COMPOUND **10c**



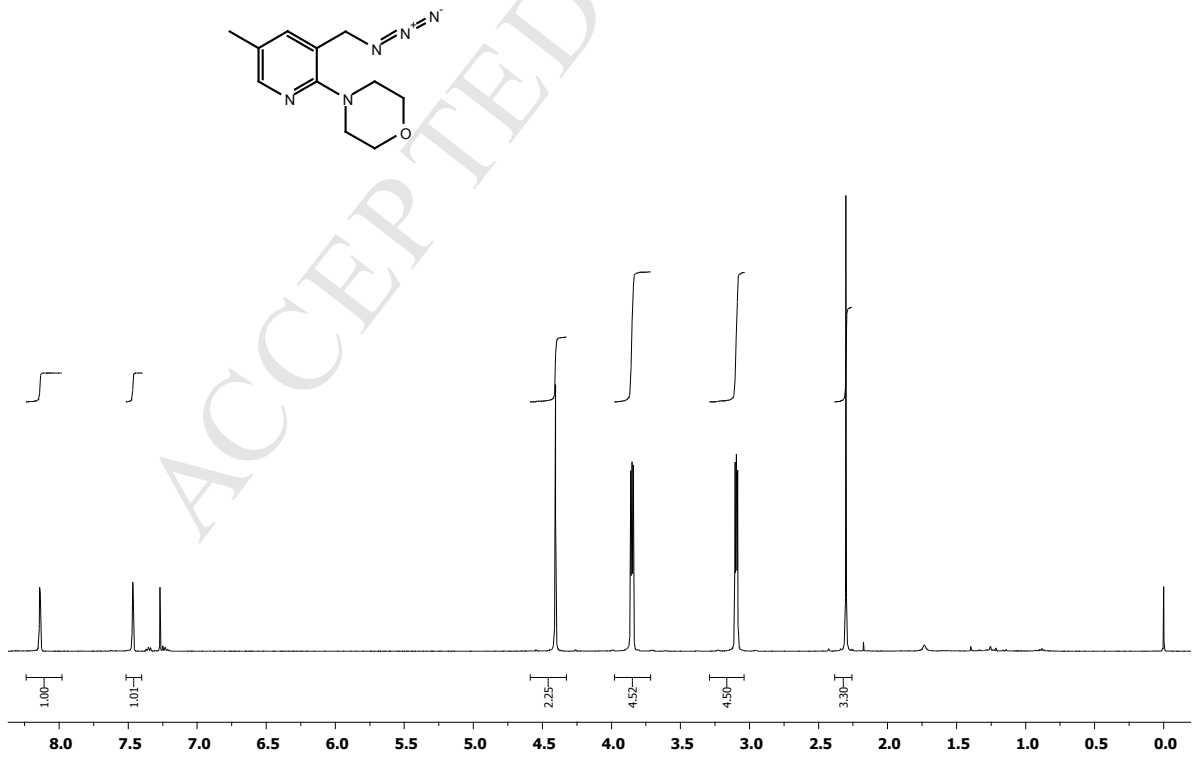
^{13}C-NMR OF COMPOUND **10c**



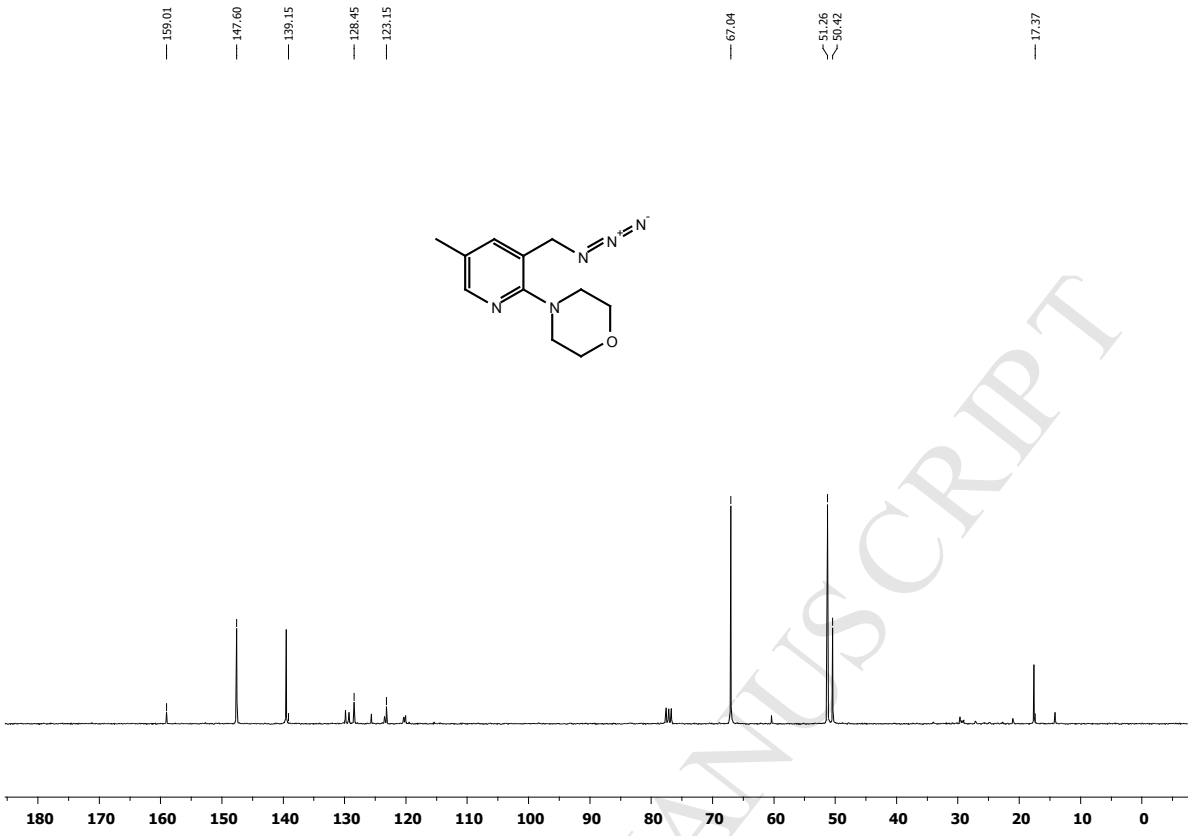
^{1}H-NMR OF COMPOUND **10d**



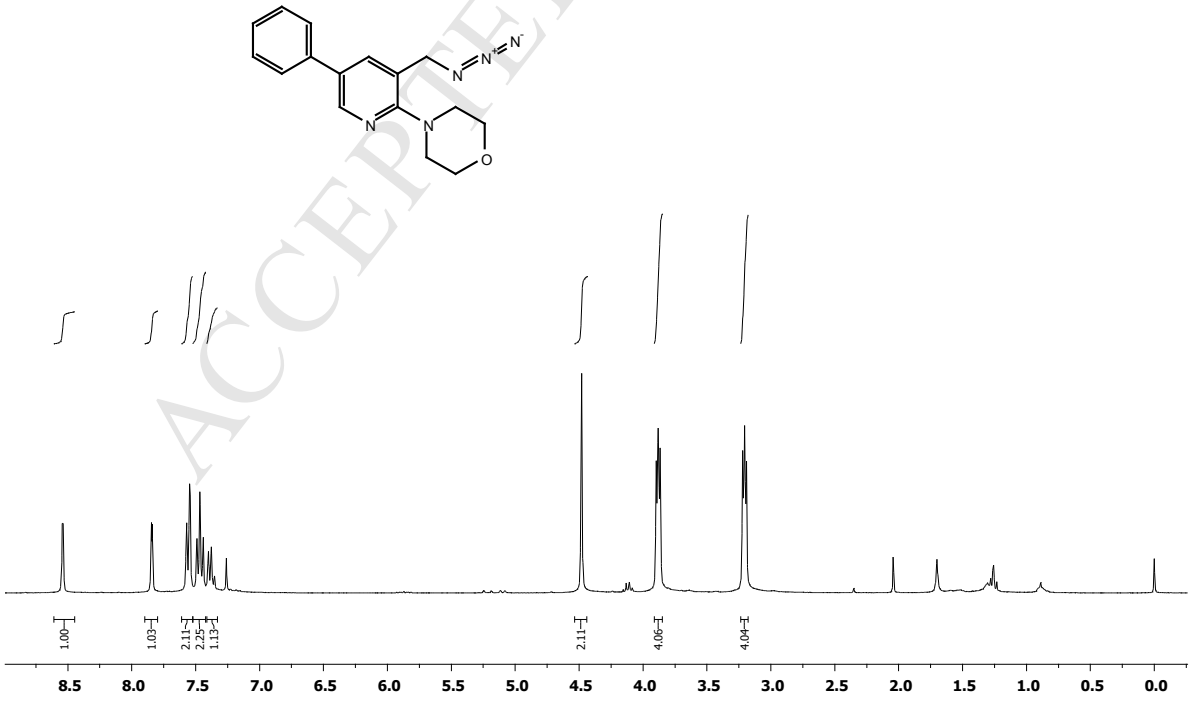
^{13}C-NMR OF COMPOUND **10d**



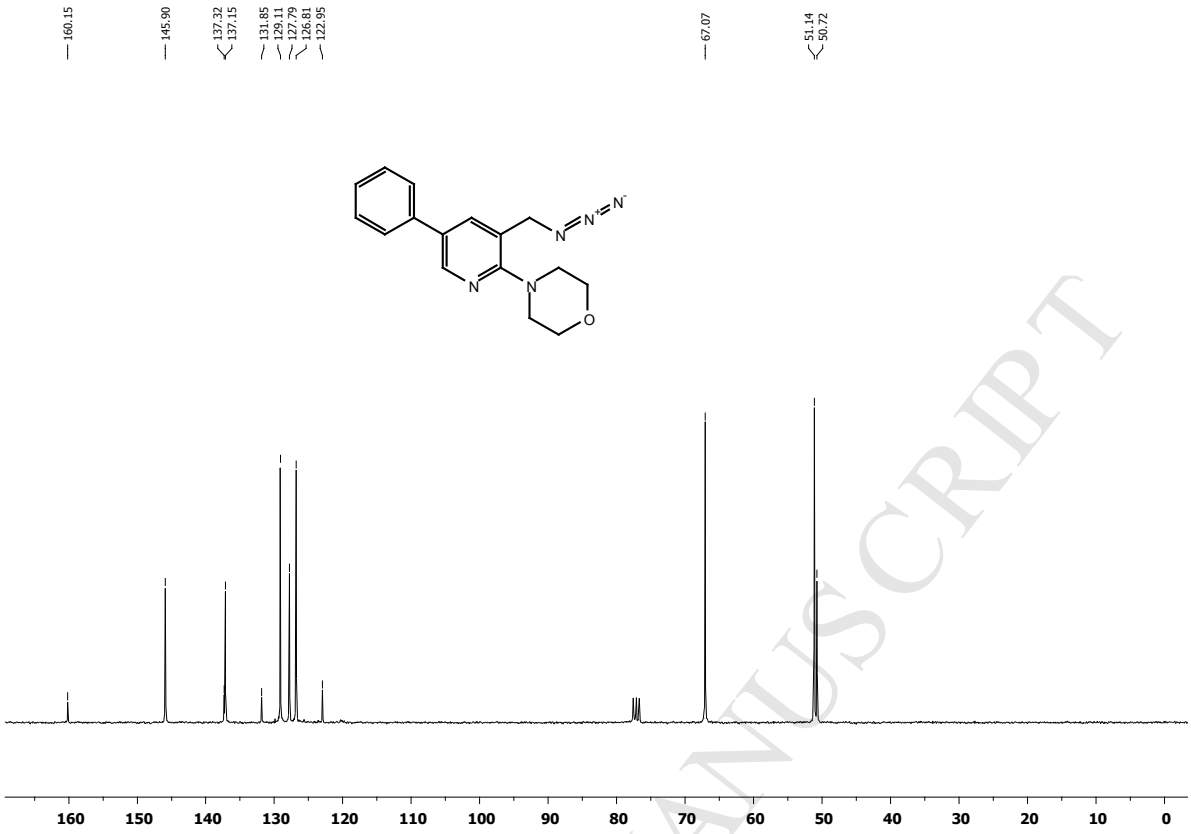
^{1}H-NMR OF COMPOUND **20a**



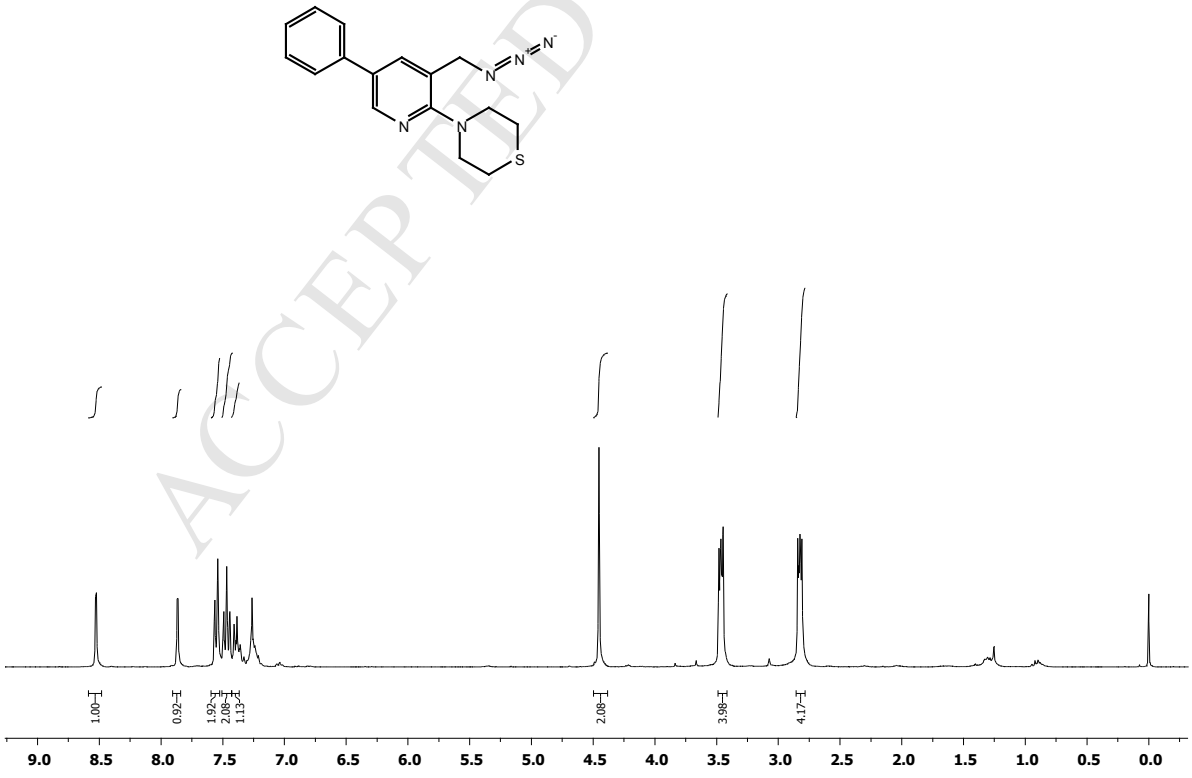
^{13}C-NMR OF COMPOUND **20a**



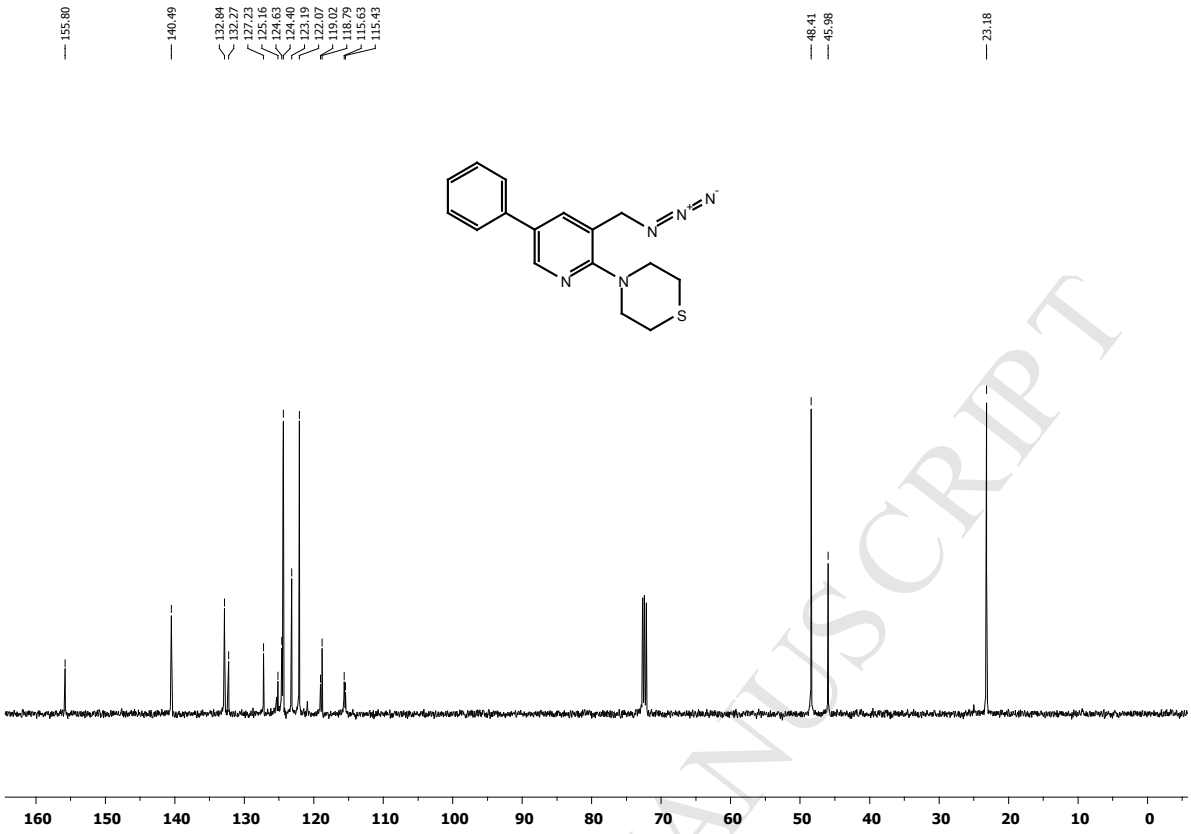
^{1}H-NMR OF COMPOUND **20c**



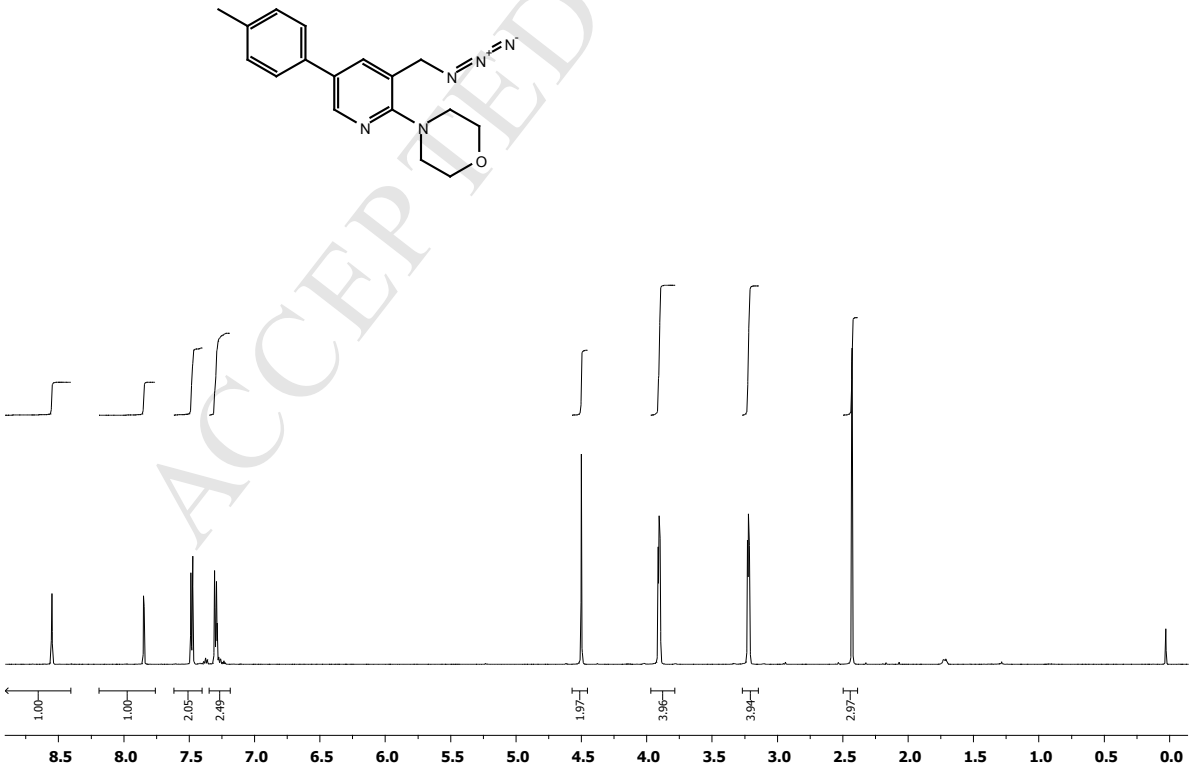
^{13}C-NMR OF COMPOUND **20c**



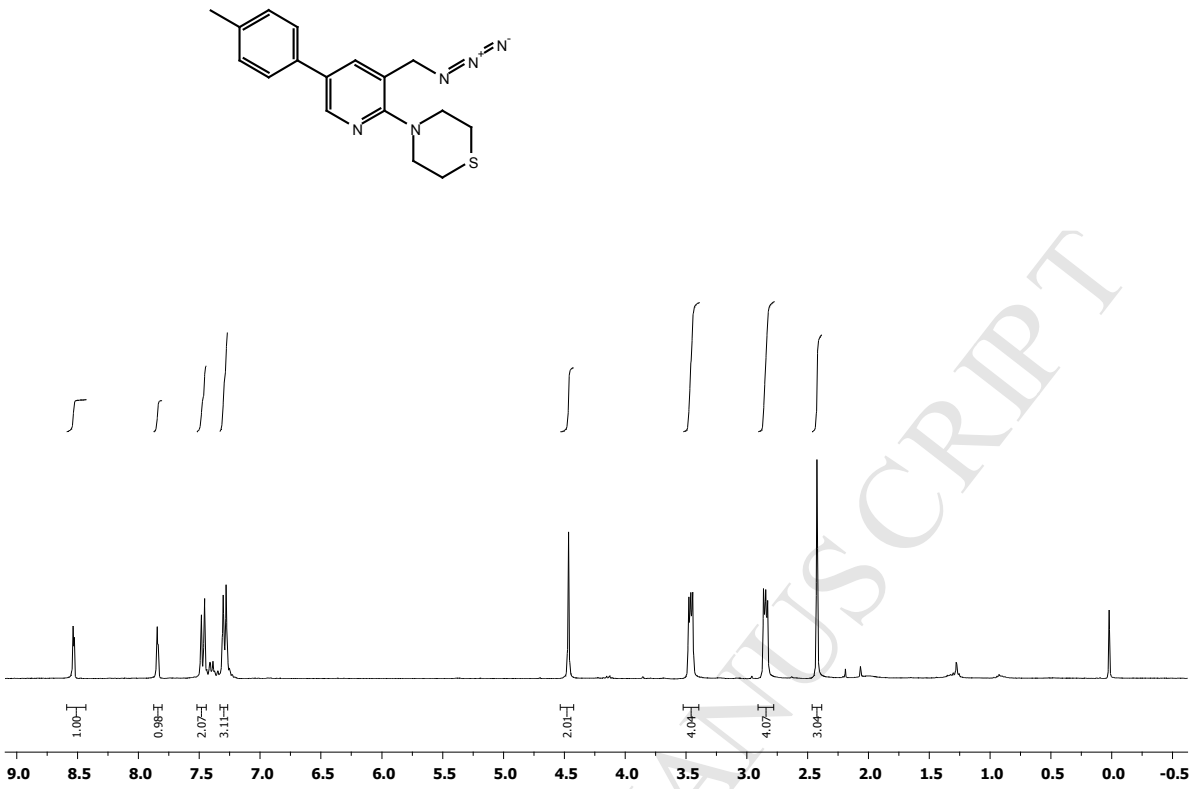
^{1}H-NMR OF COMPOUND **20d**



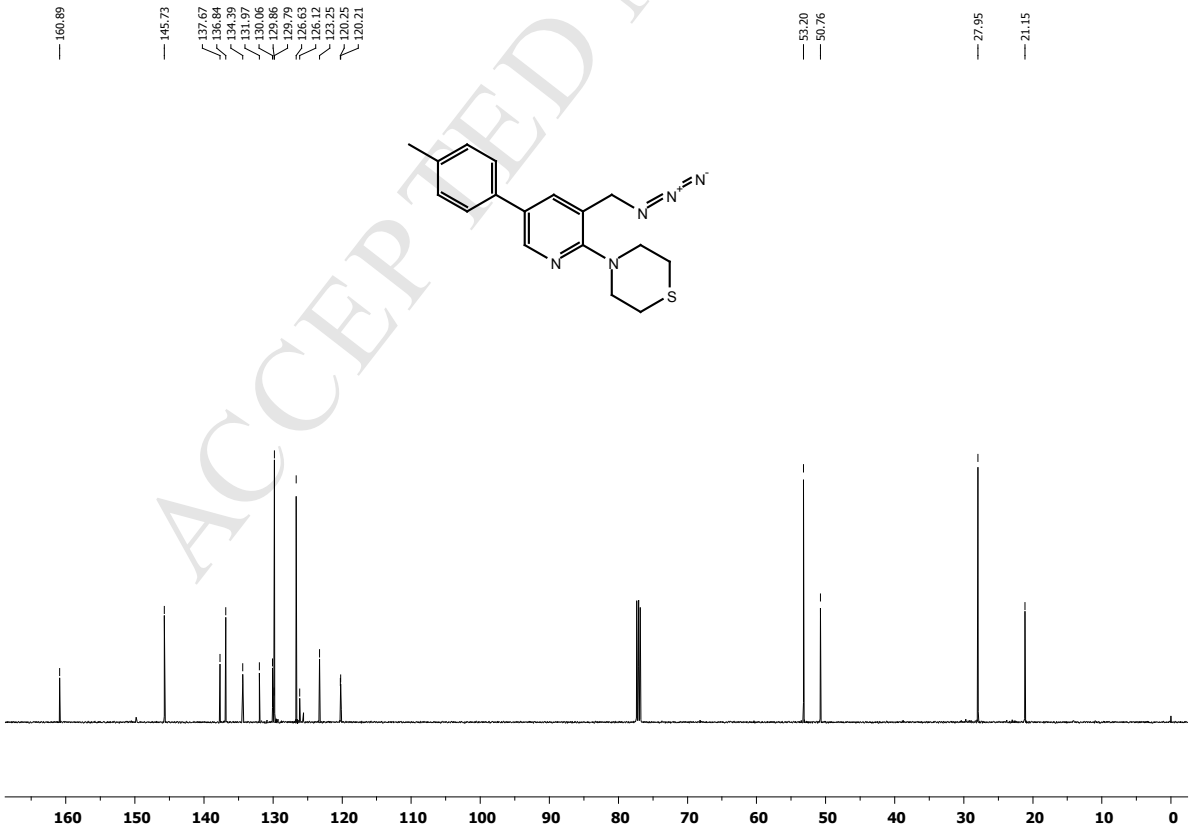
^{13}C-NMR OF COMPOUND **20d**



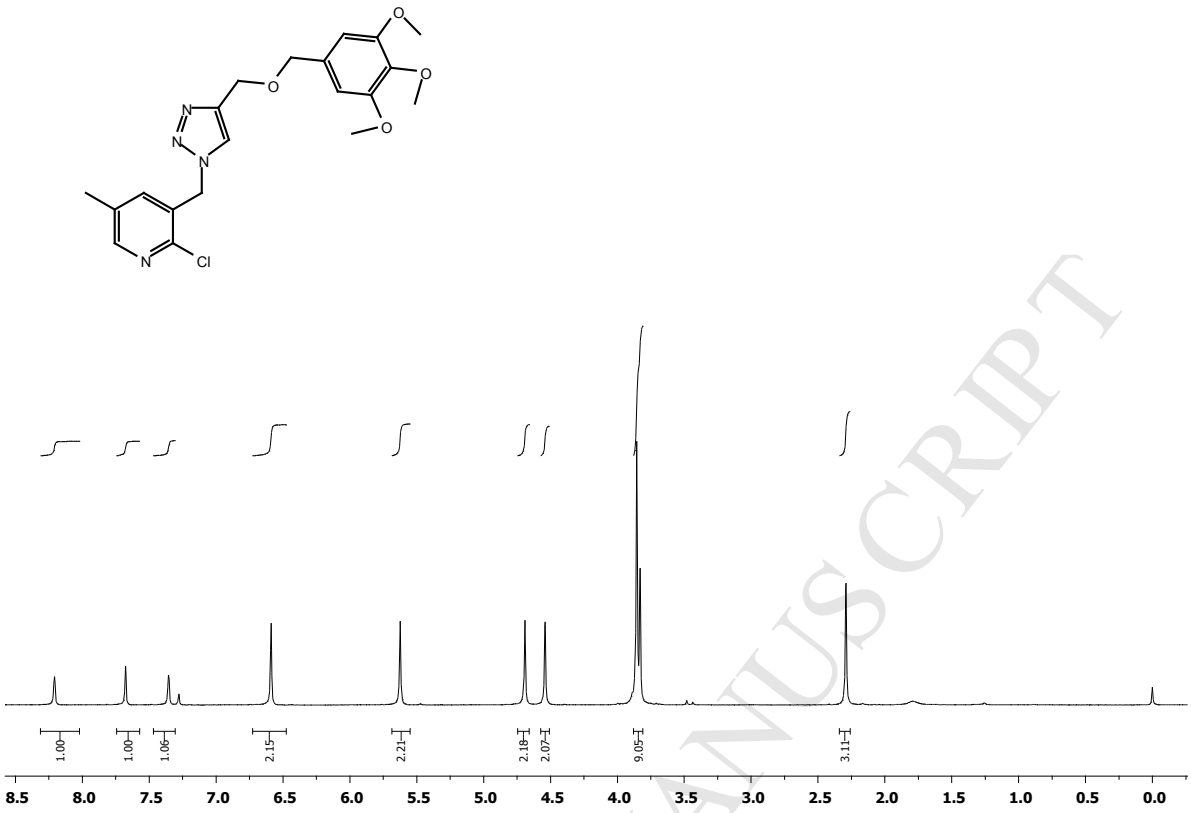
^{1}H-NMR OF COMPOUND **20e**



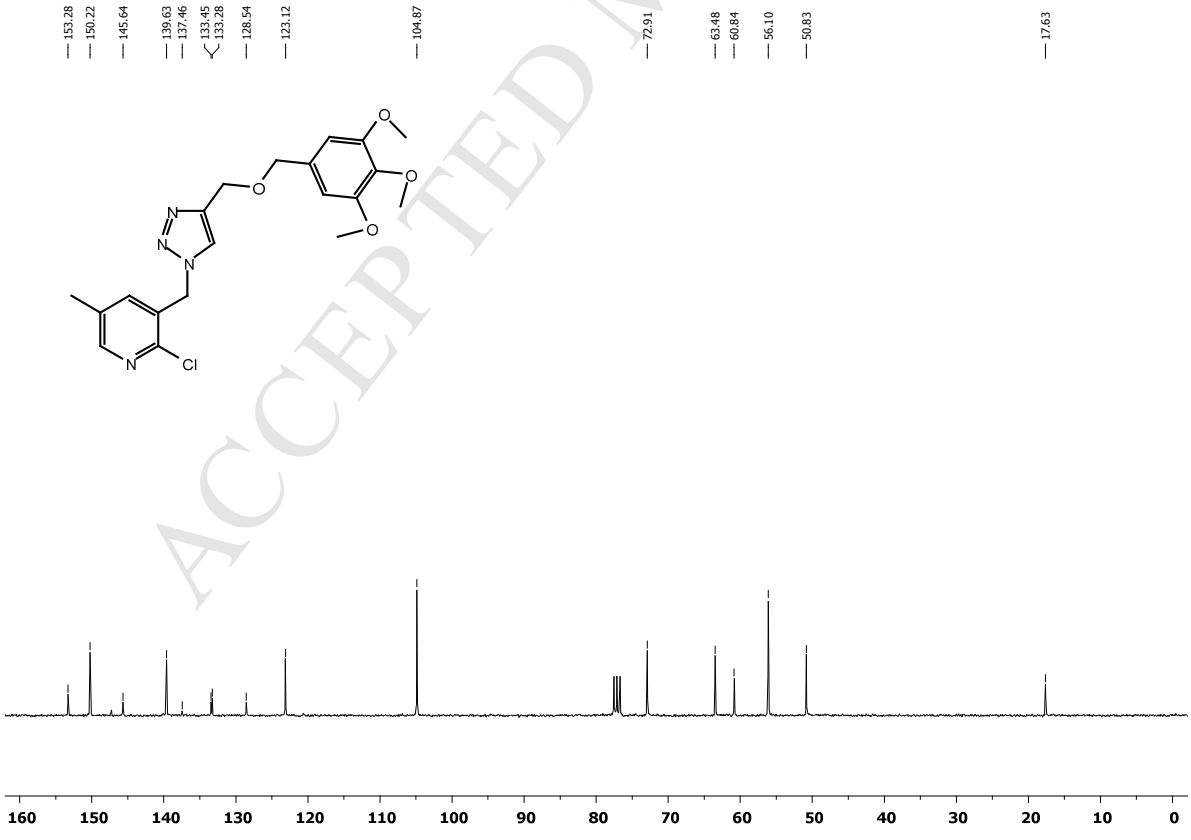
^{1}H-NMR OF COMPOUND **20f**



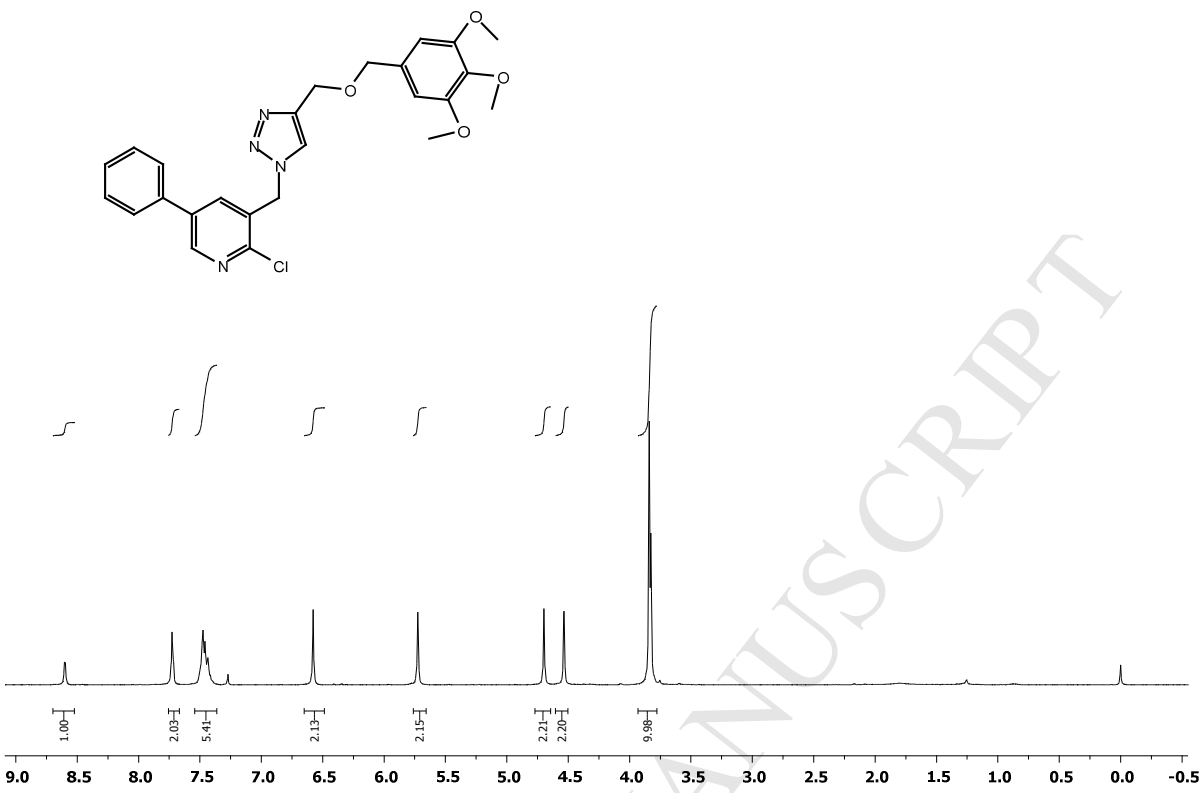
^{13}C-NMR OF COMPOUND **20f**



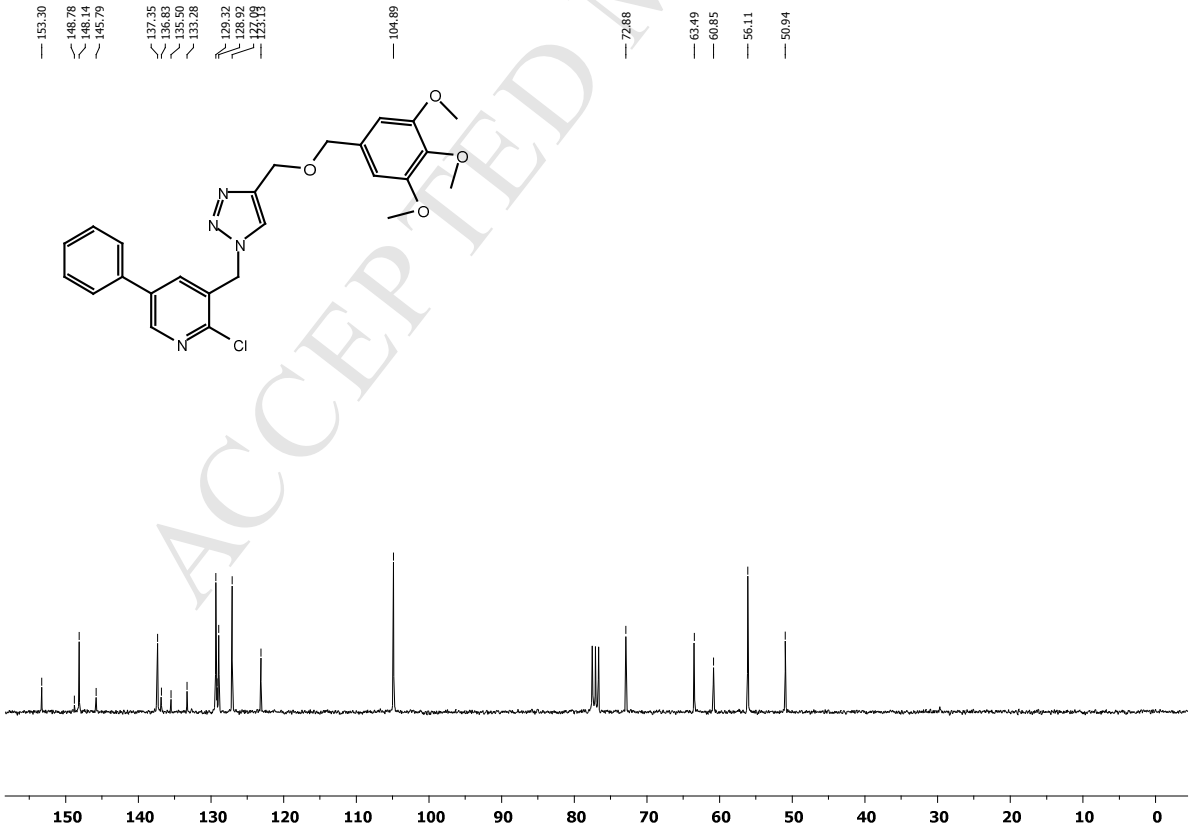
^{1}H-NMR OF COMPOUND **13a**



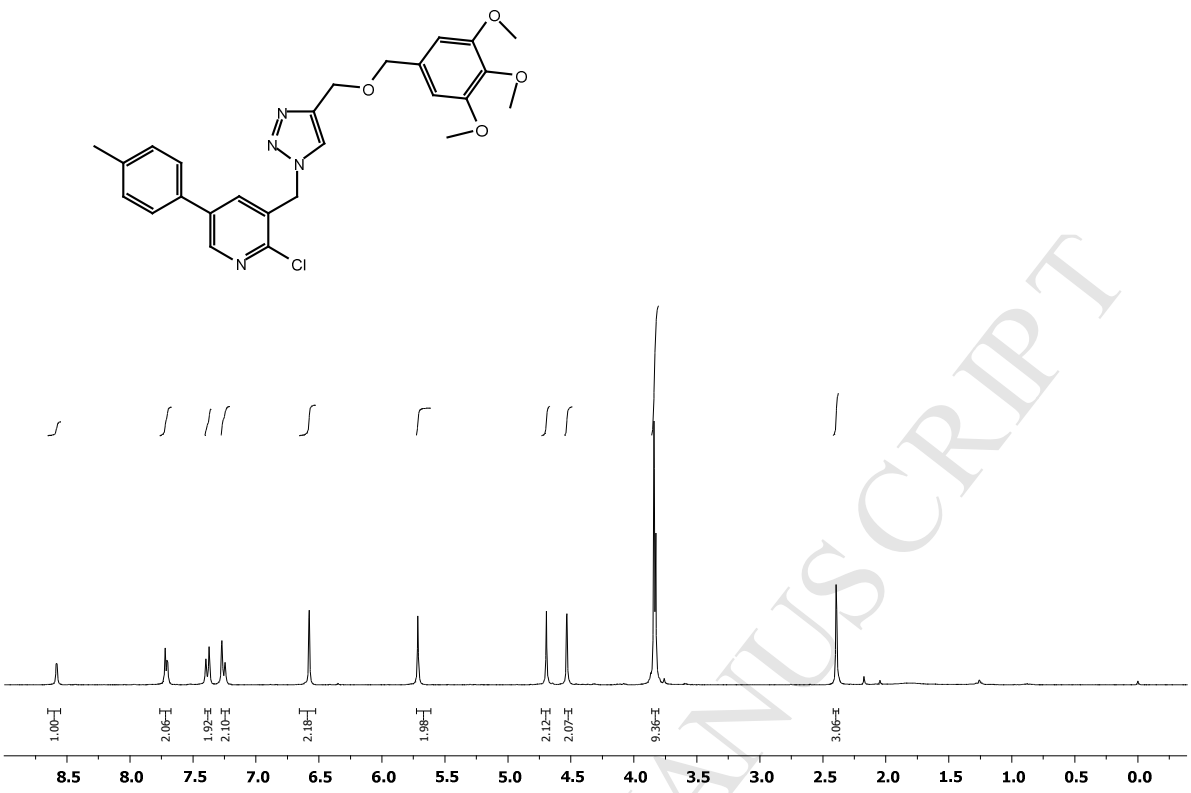
^{13}C-NMR OF COMPOUND **13a**



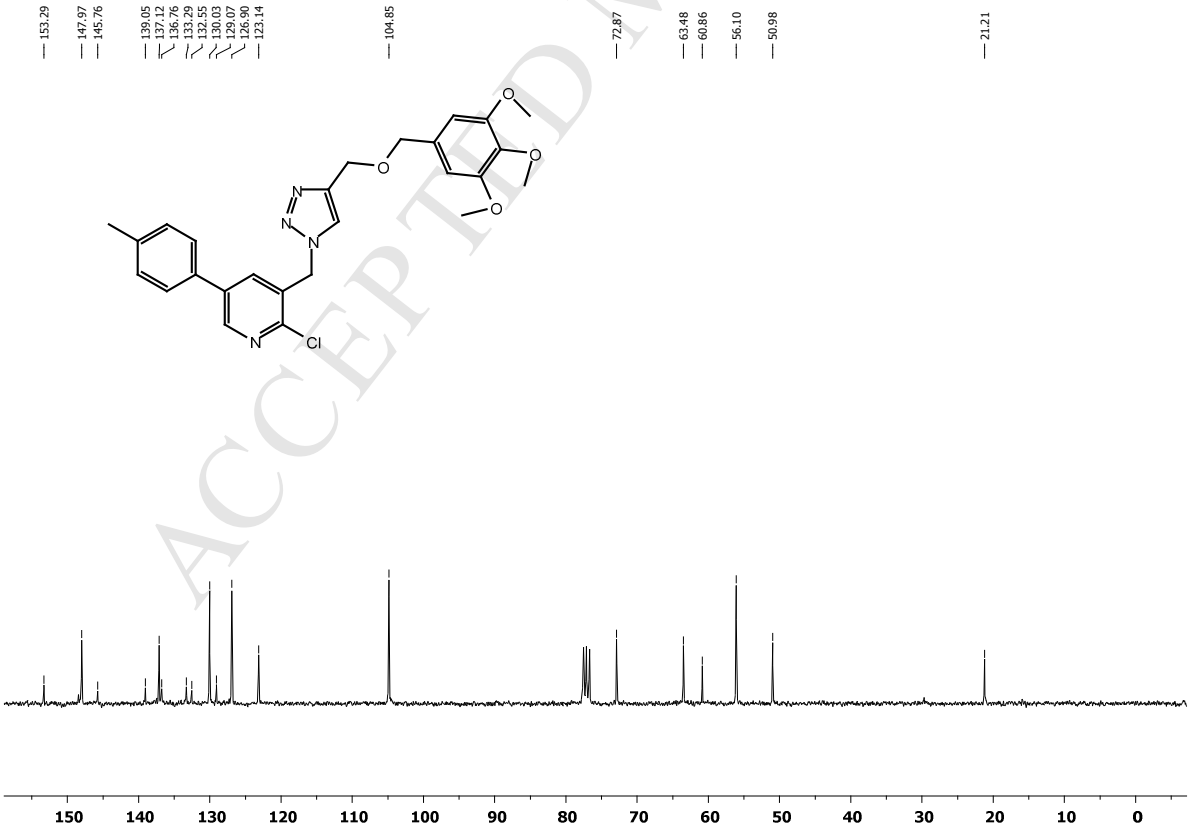
^{1}H-NMR OF COMPOUND **13b**



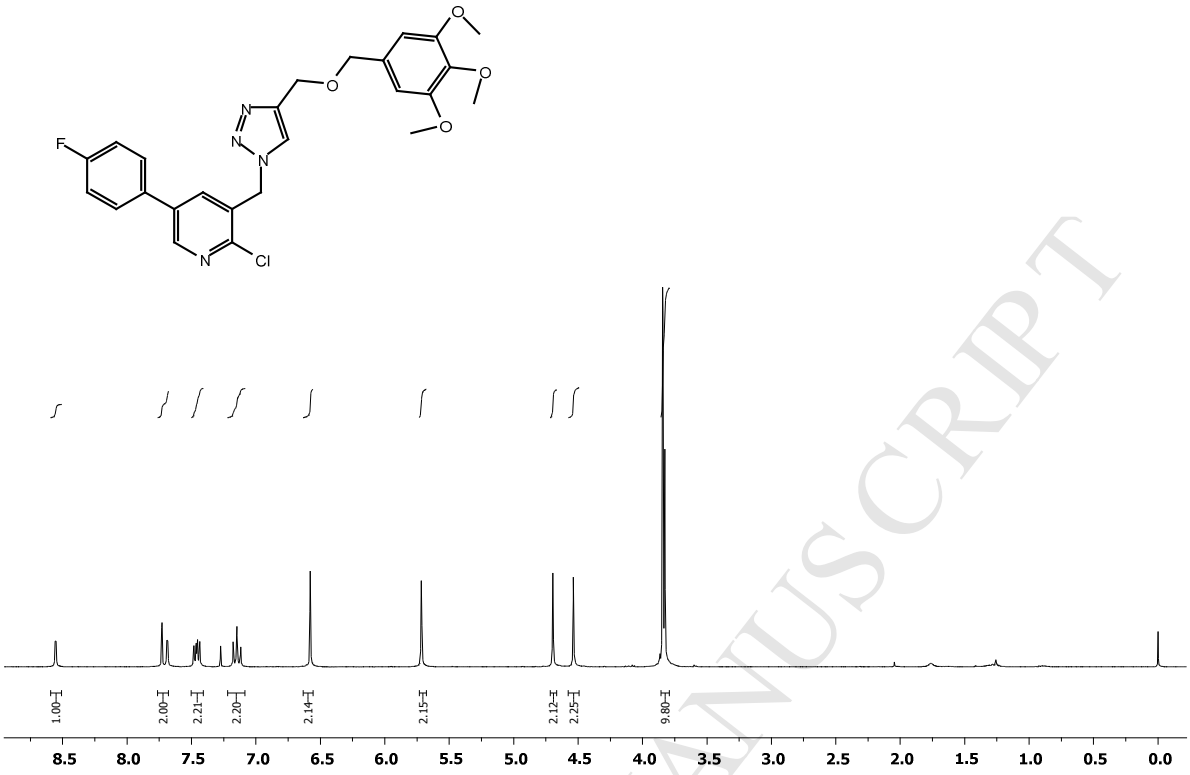
^{1}H-NMR OF COMPOUND **13b**



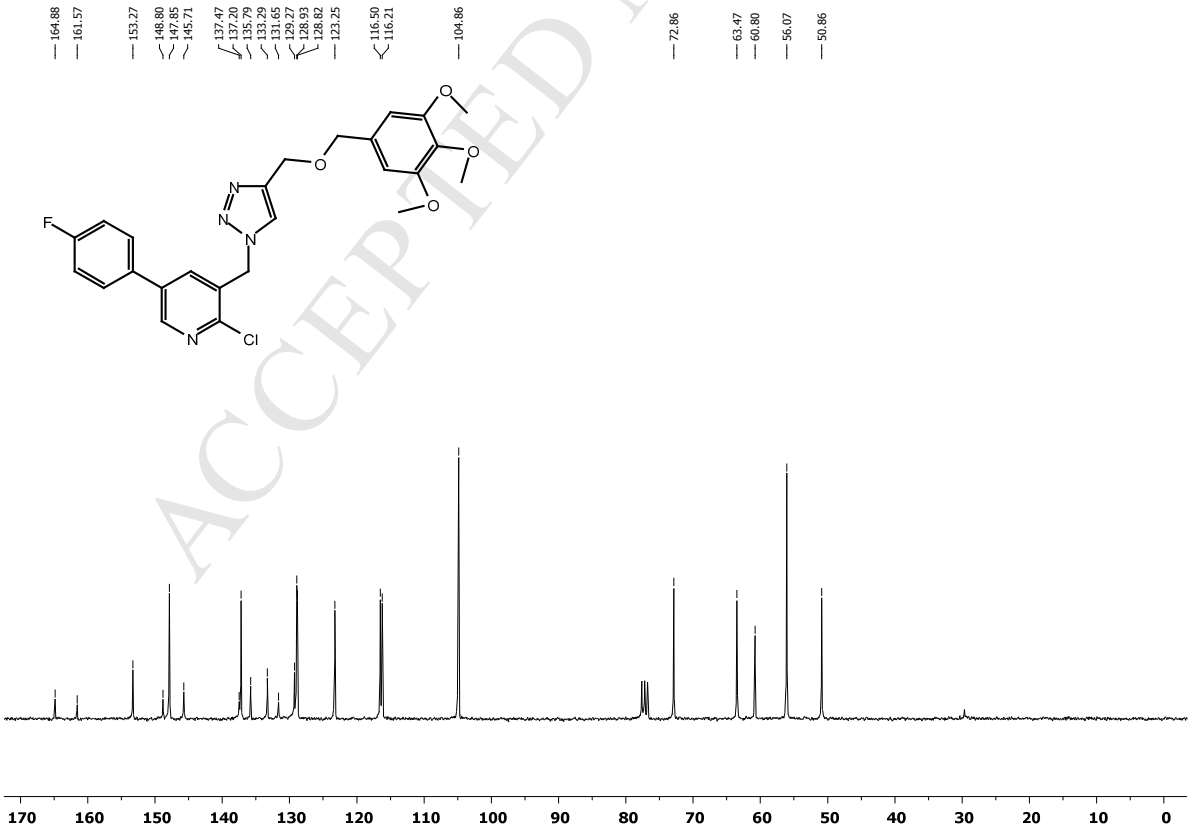
^{1}H-NMR OF COMPOUND **13c**



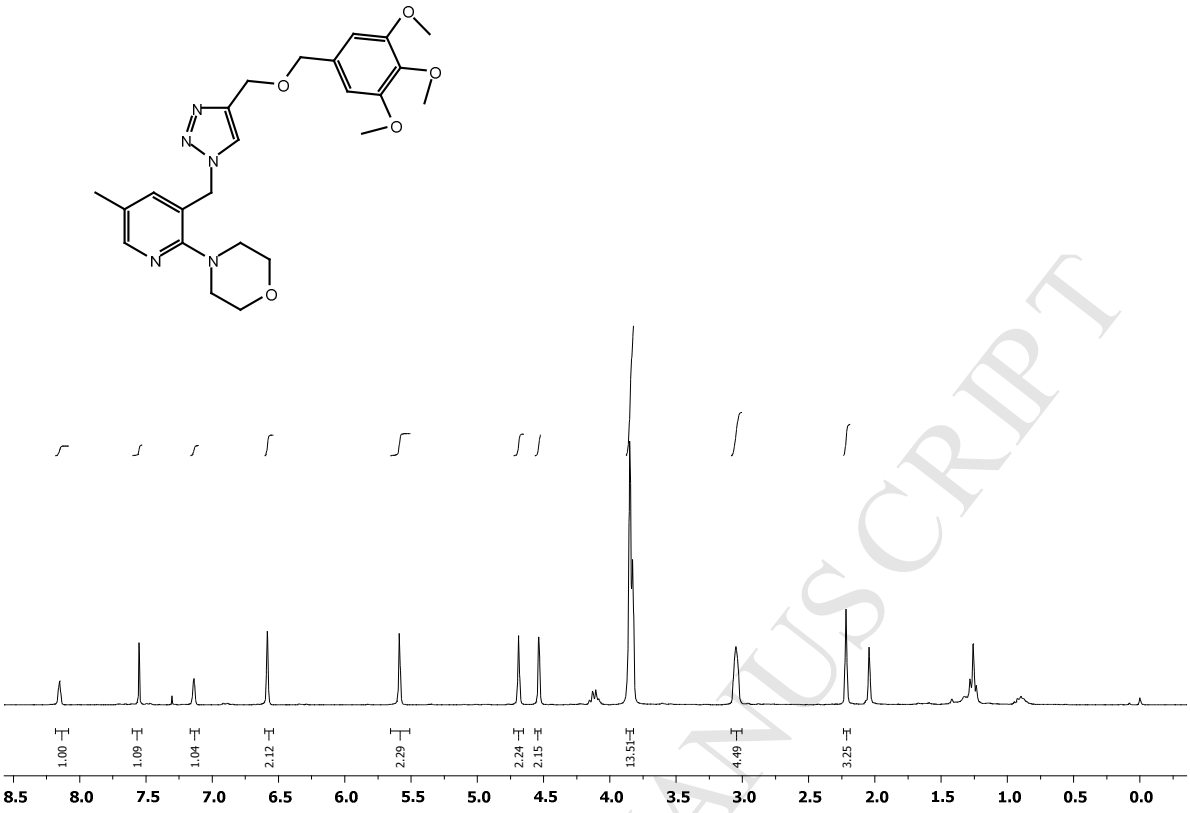
^{13}C-NMR OF COMPOUND **13c**



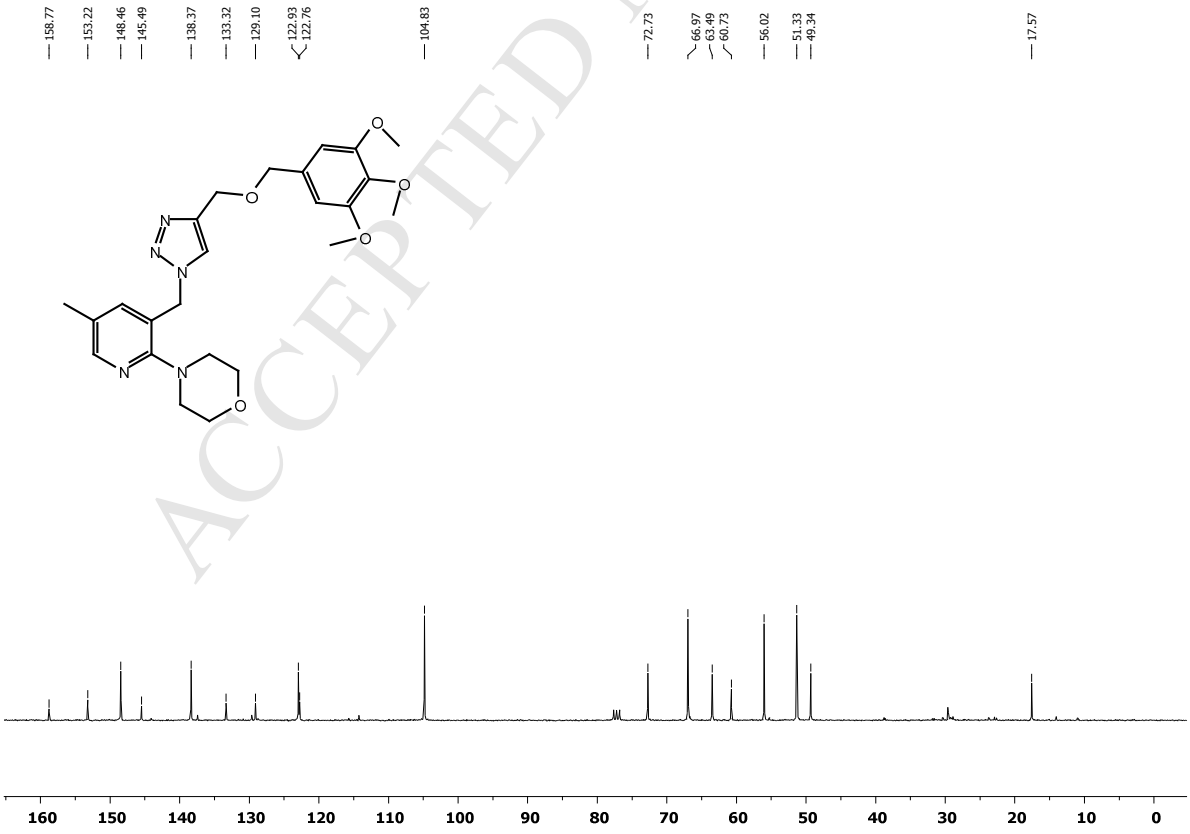
^{1}H-NMR OF COMPOUND **13d**



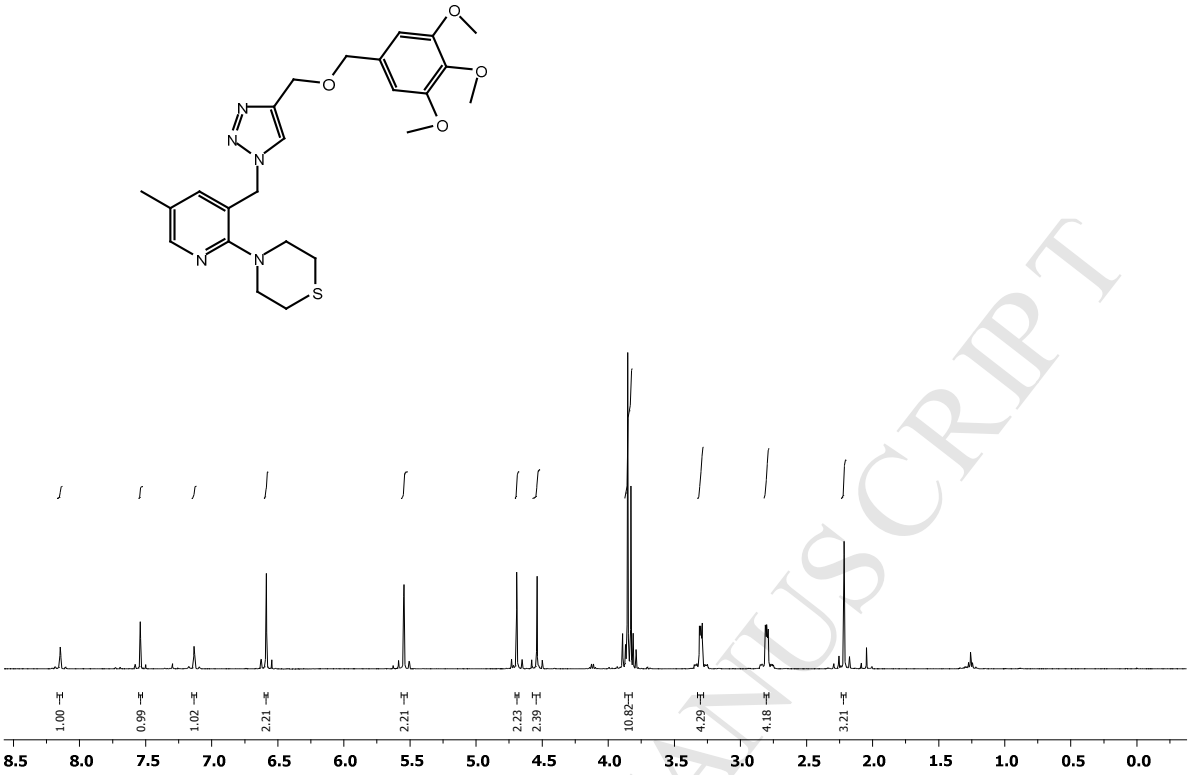
^{13}C-NMR OF COMPOUND **13d**



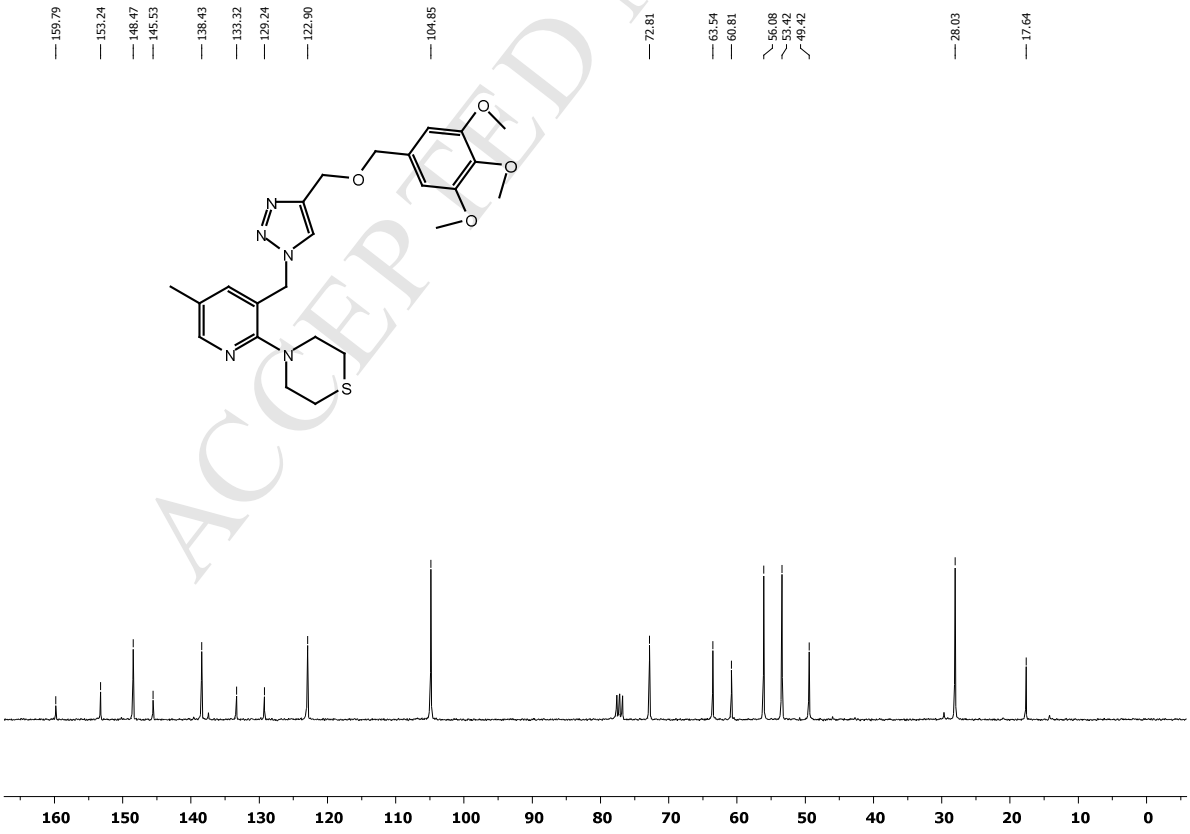
^{1}H-NMR OF COMPOUND **13e**



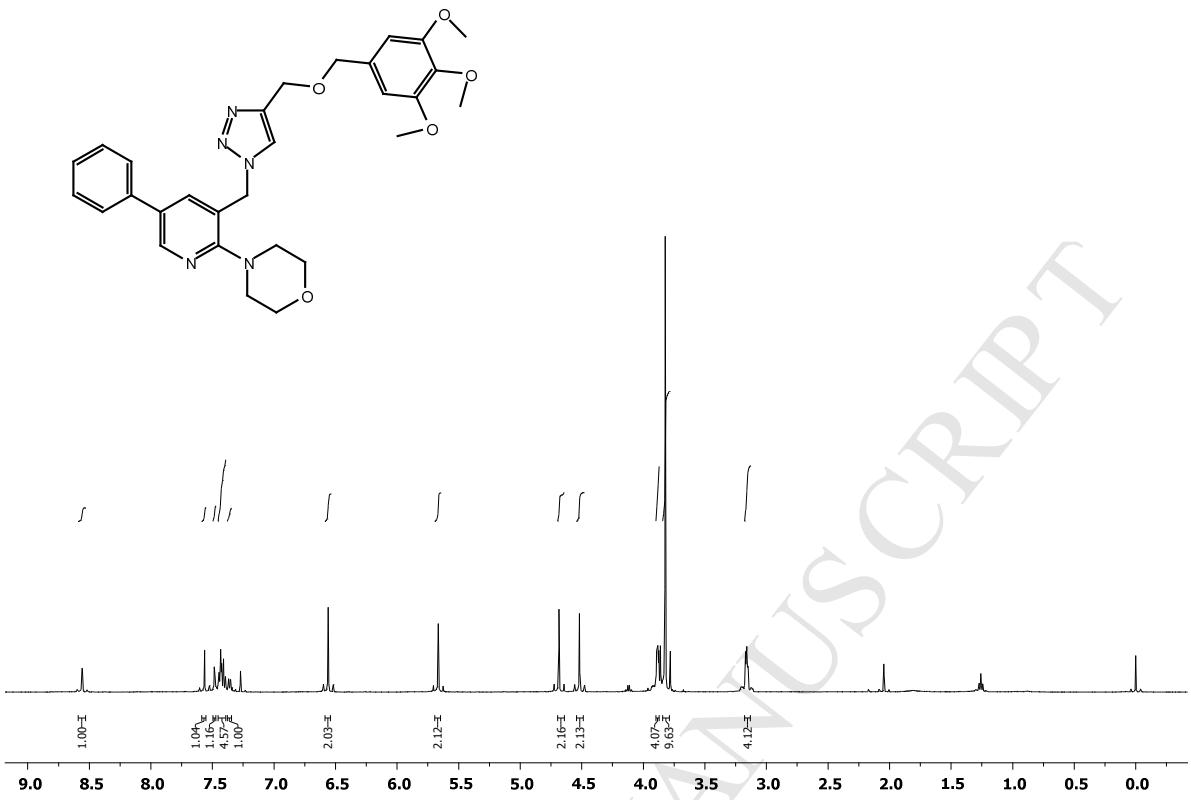
^{13}C-NMR OF COMPOUND **13e**



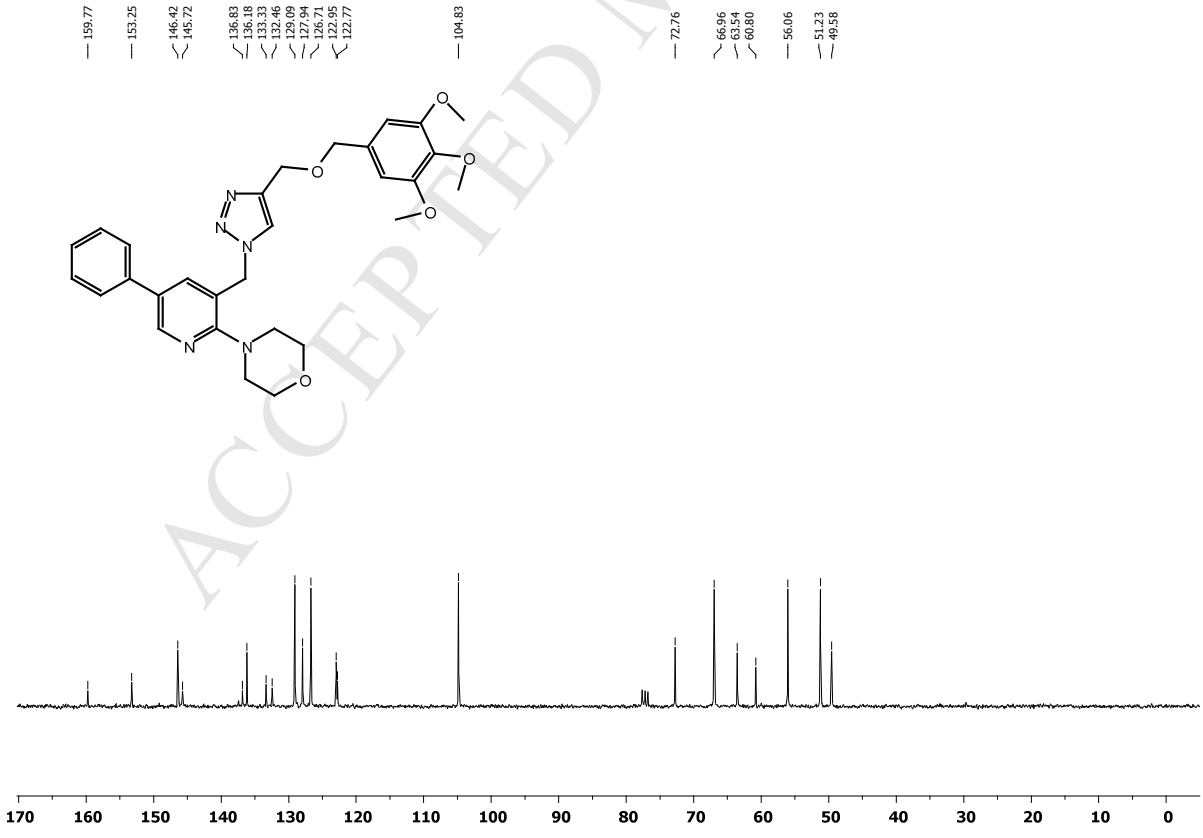
^{1}H-NMR OF COMPOUND **13f**



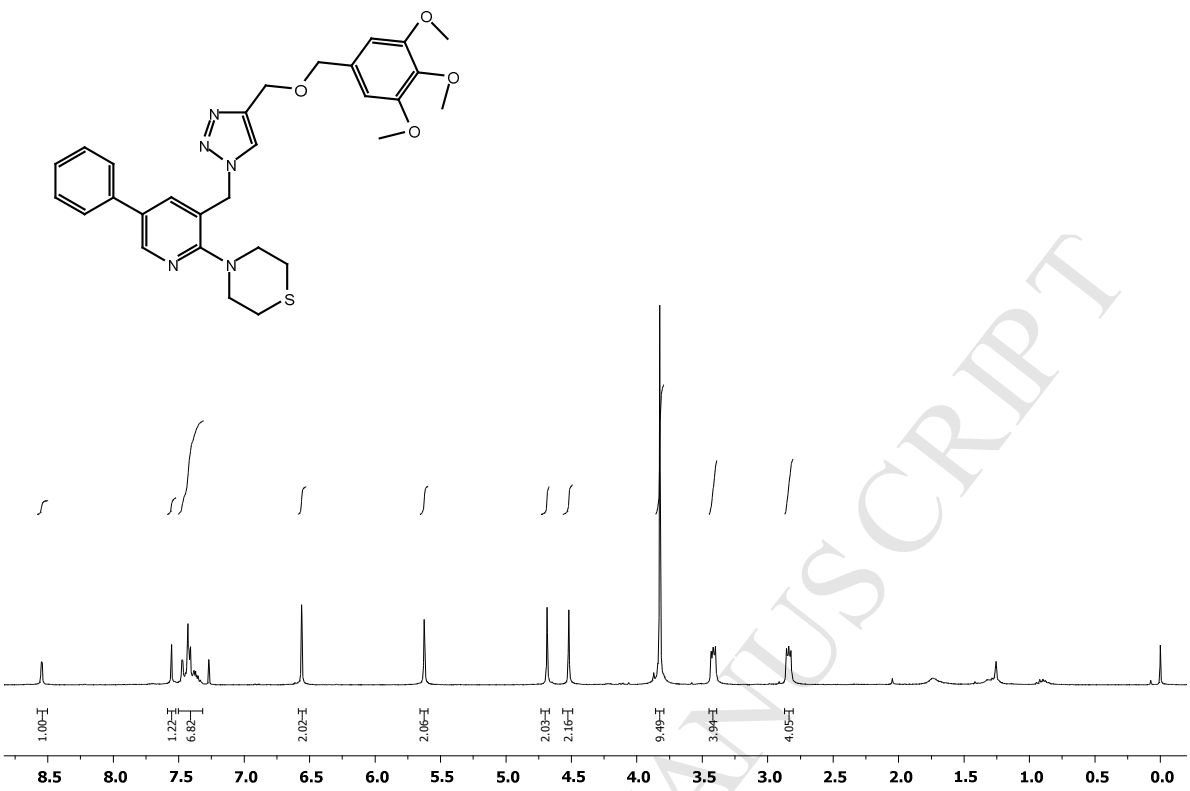
^{13}C-NMR OF COMPOUND **13f**



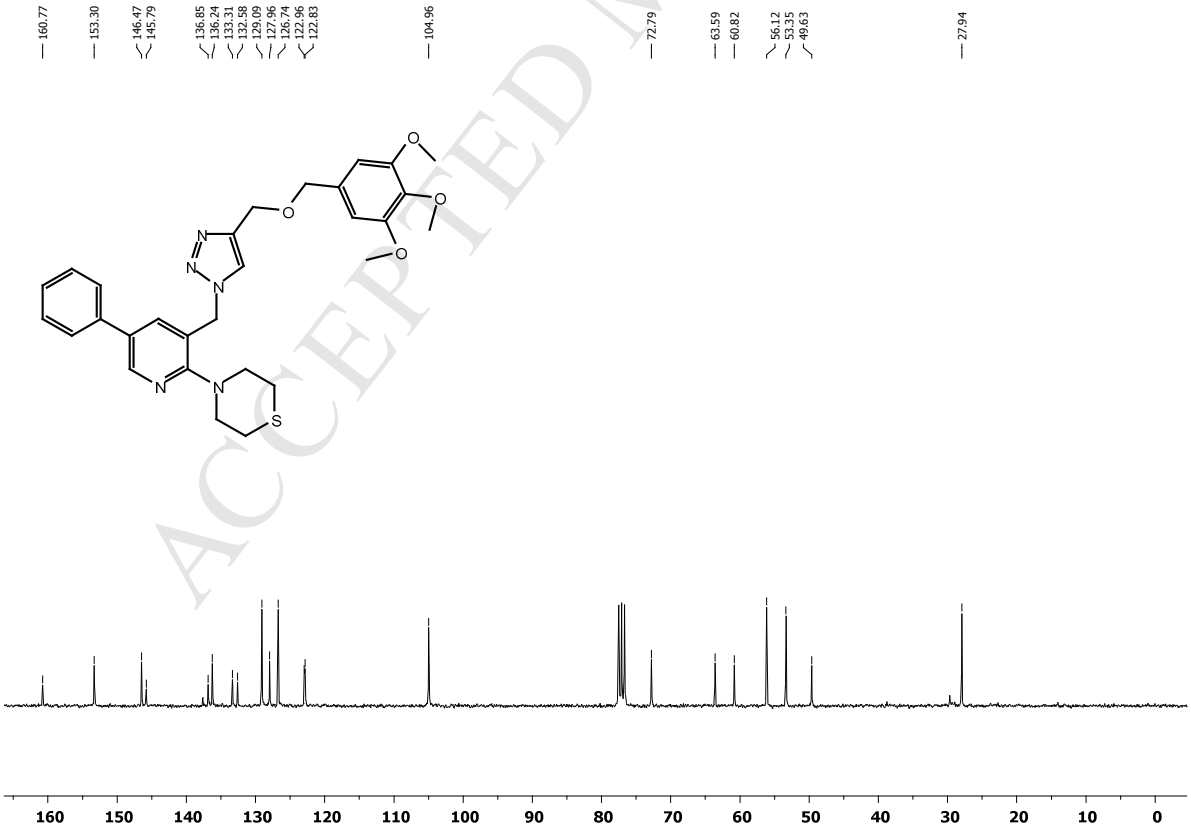
^{1}H-NMR OF COMPOUND **13g**



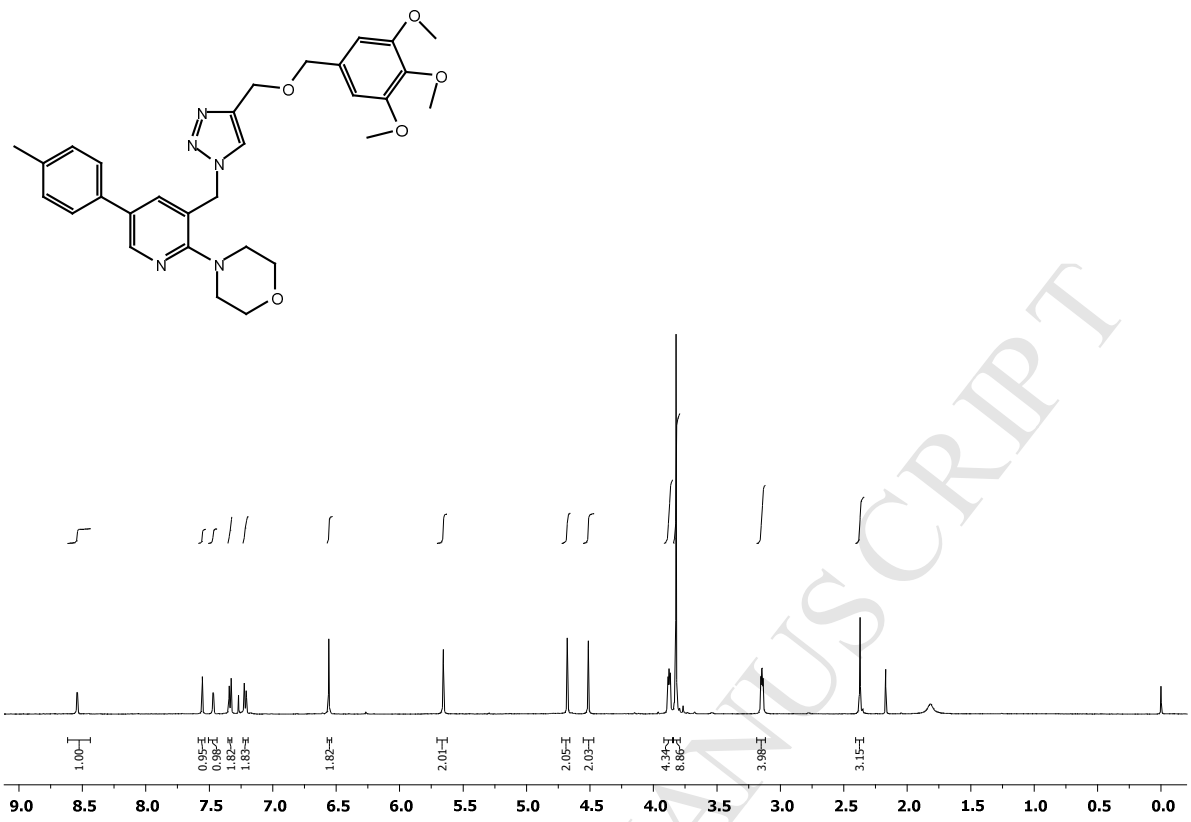
^{13}C-NMR OF COMPOUND **13g**



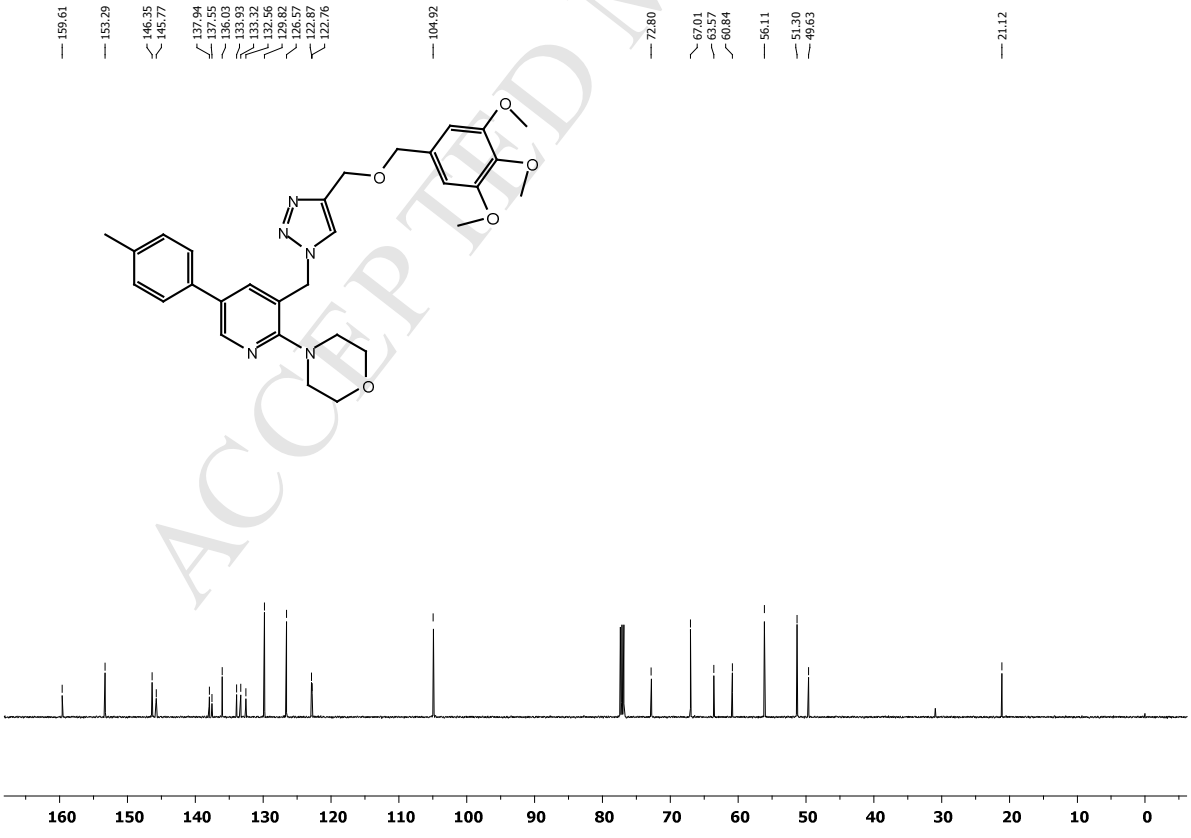
^{1}H-NMR OF COMPOUND **13h**



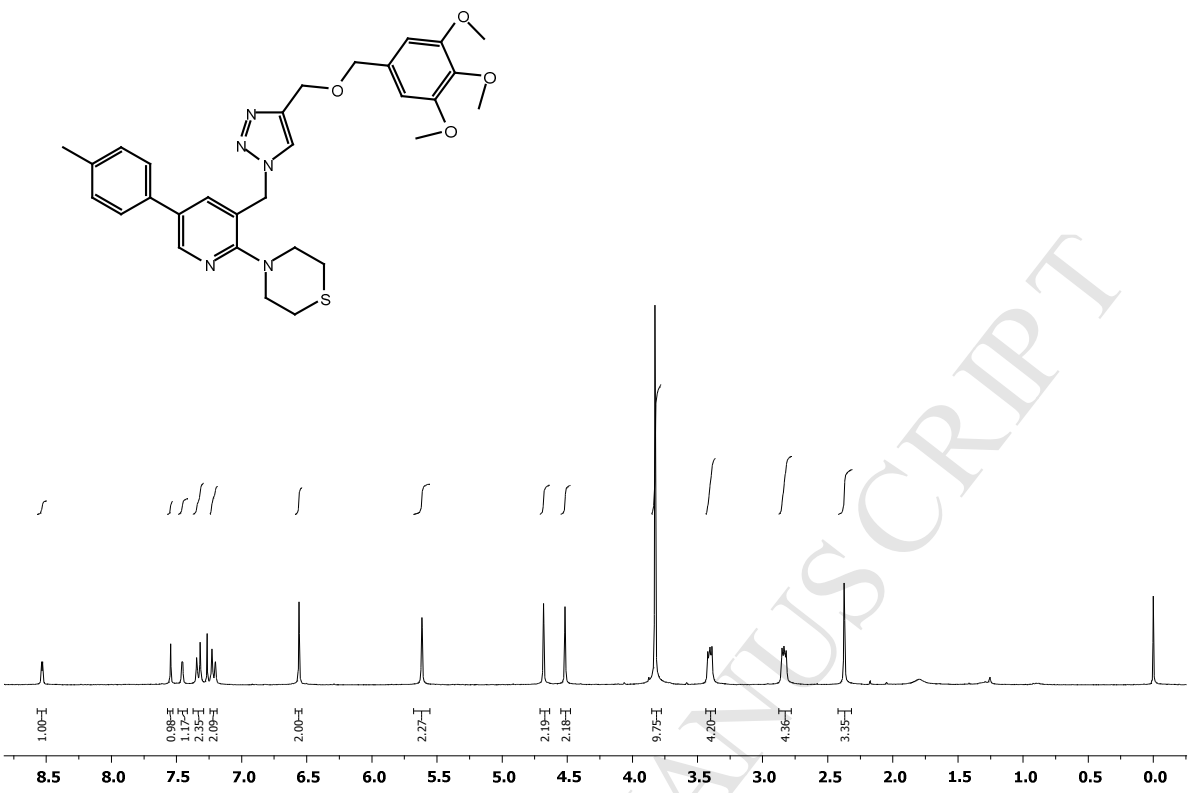
^{13}C-NMR OF COMPOUND **13h**



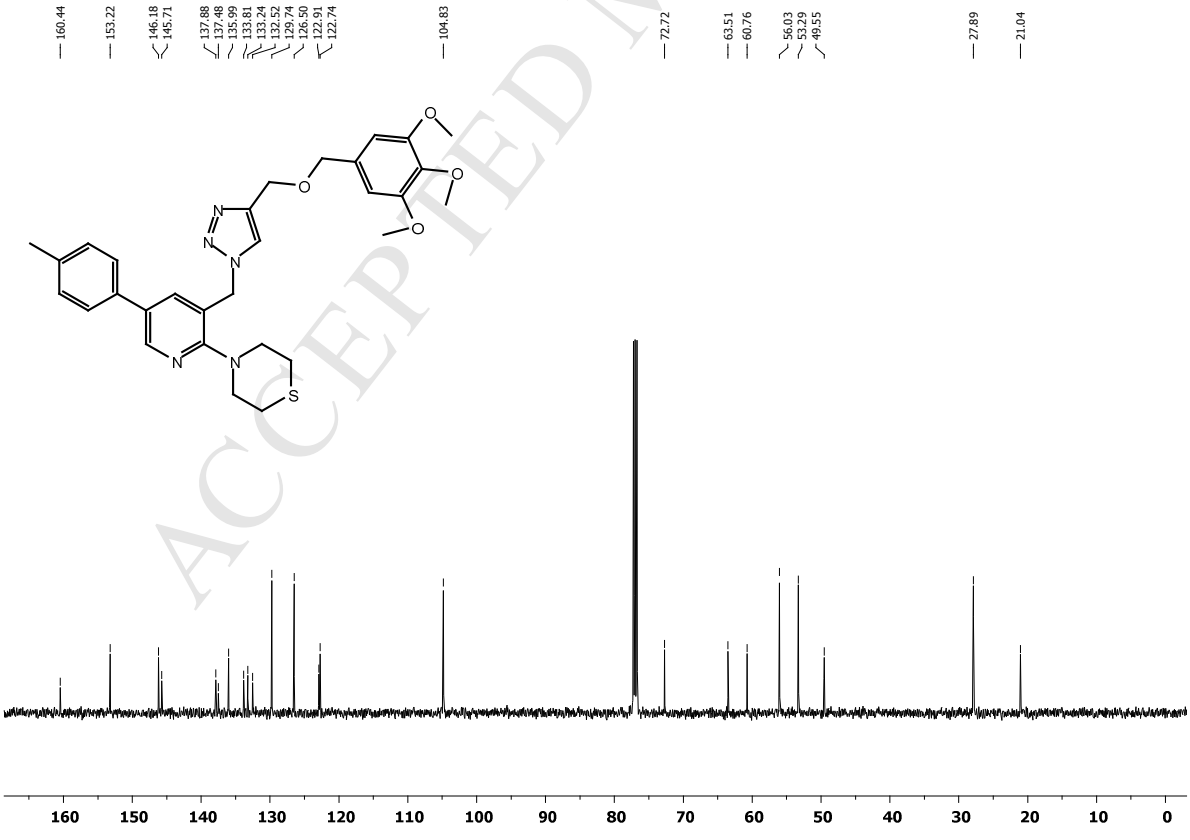
^{1}H-NMR OF COMPOUND **13i**



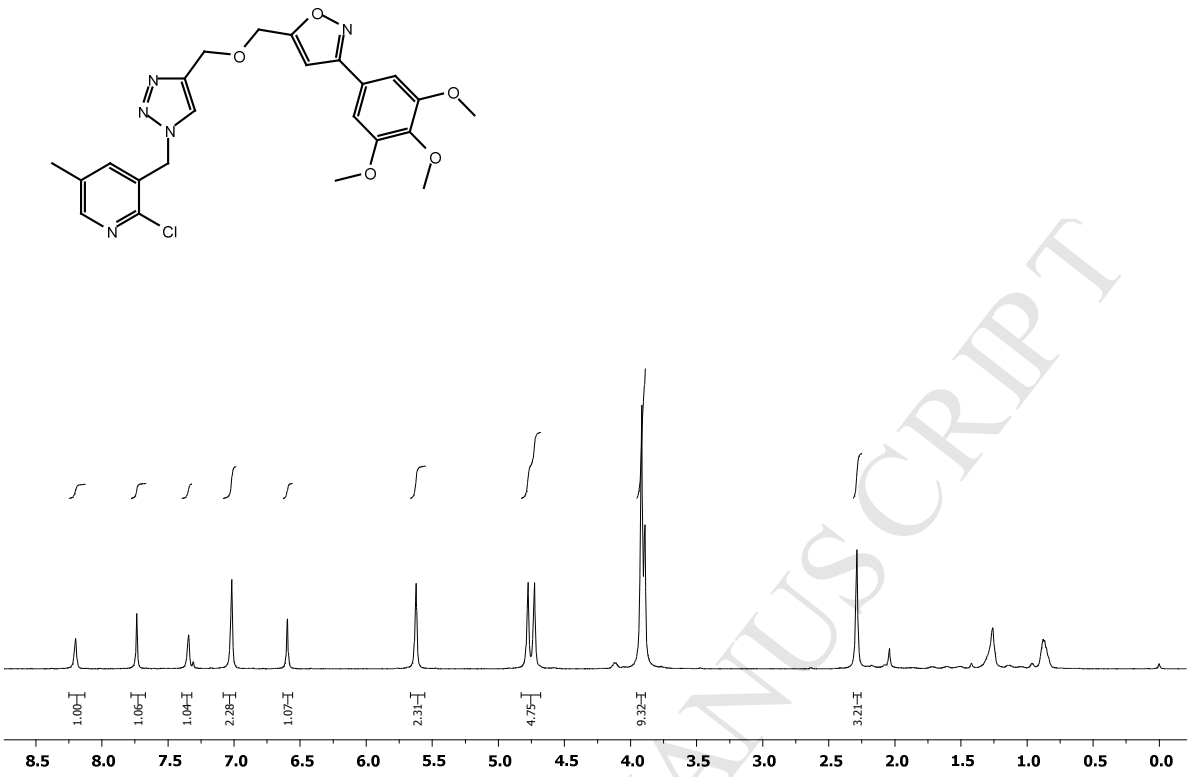
^{13}C-NMR OF COMPOUND **13i**



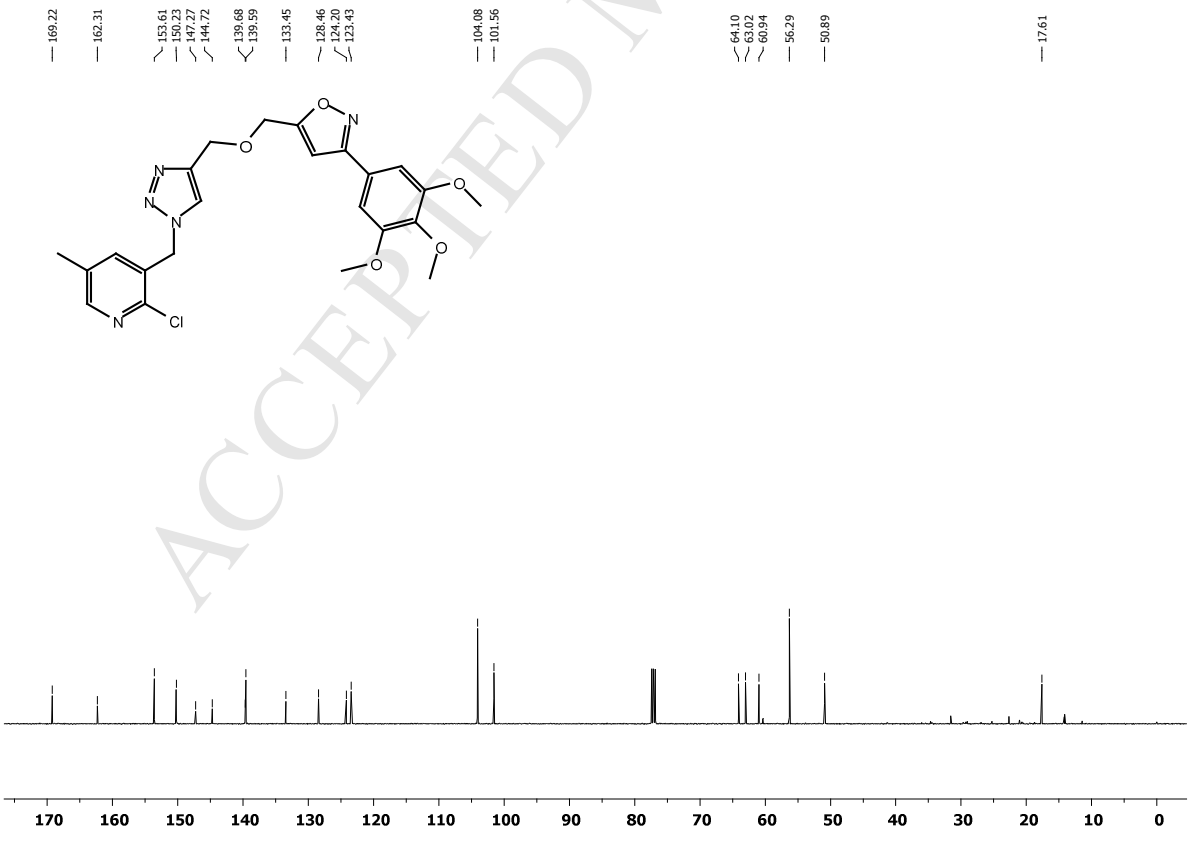
^{1}H-NMR OF COMPOUND **13j**



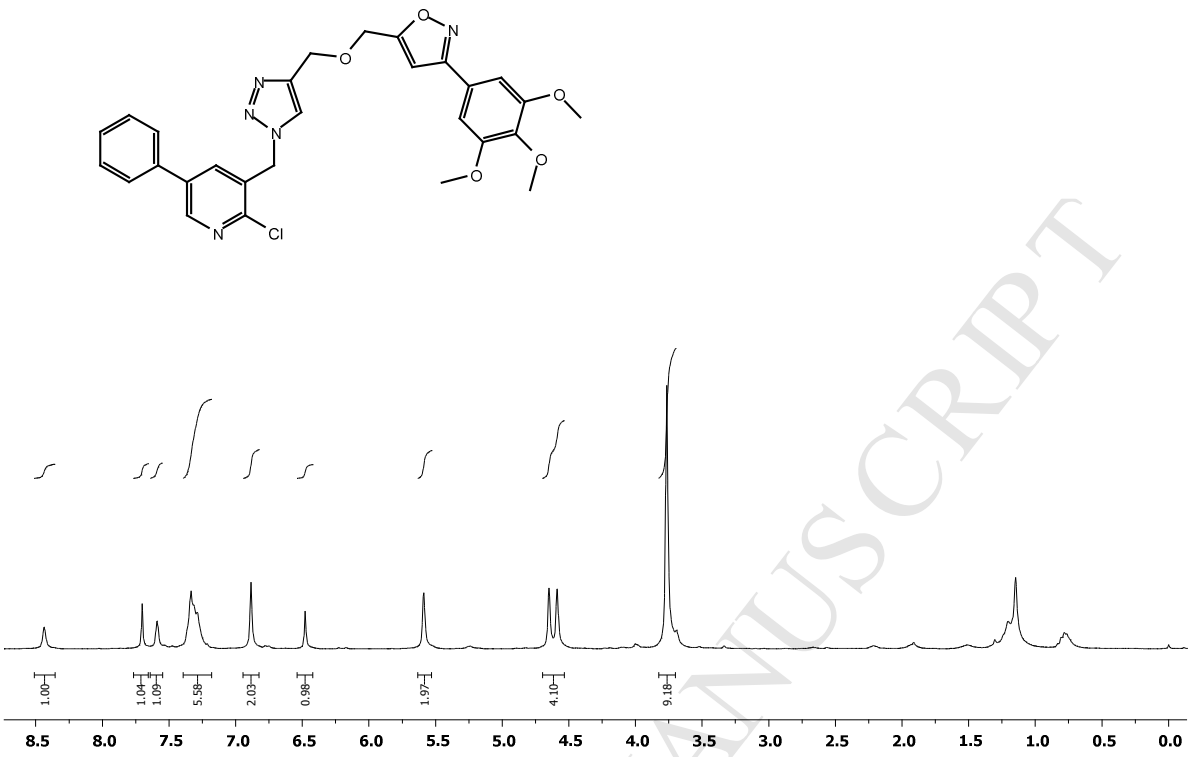
^{13}C-NMR OF COMPOUND **13j**



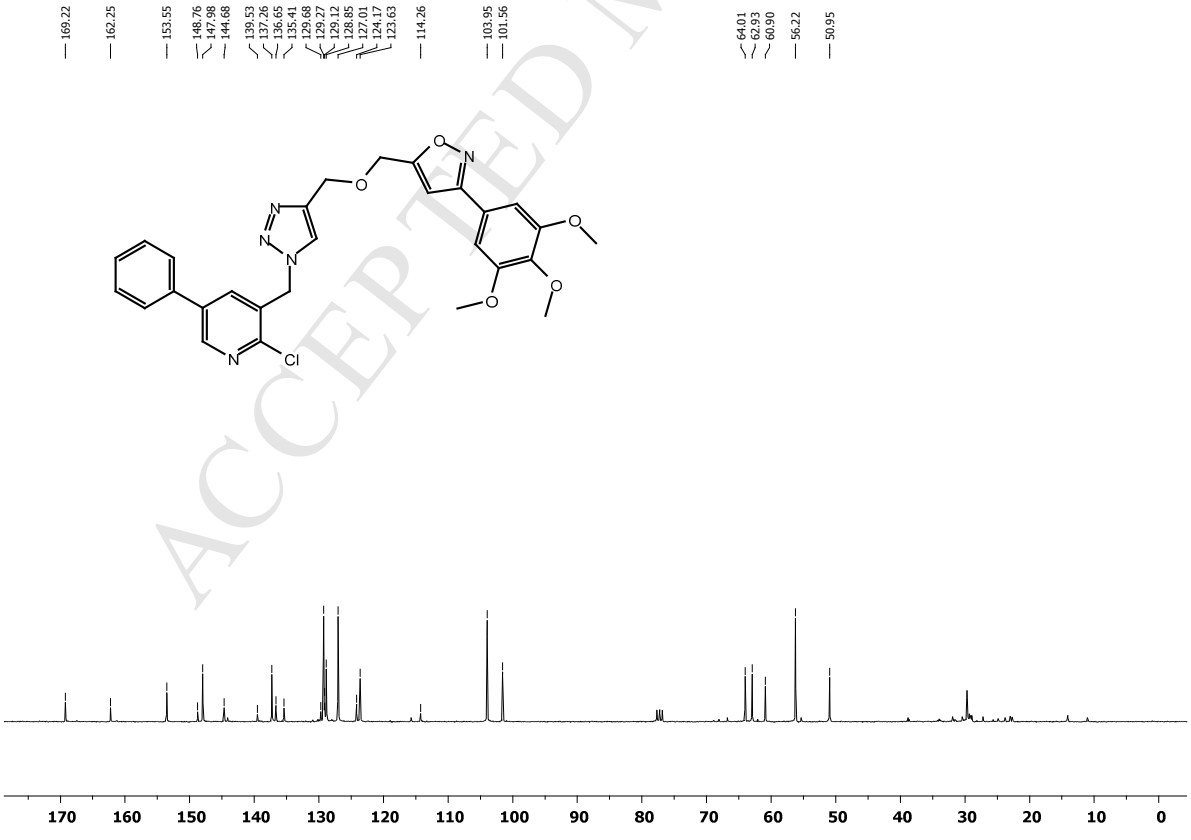
^{1}H-NMR OF COMPOUND **25a**



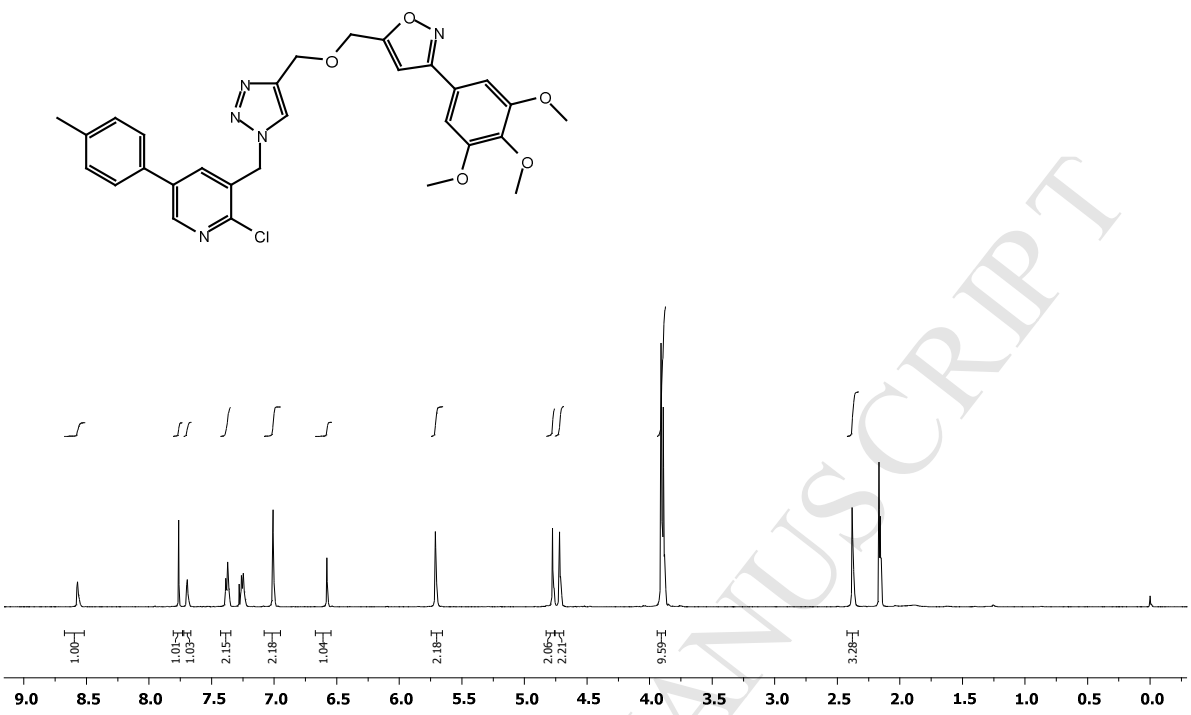
^{13}C-NMR OF COMPOUND **25a**



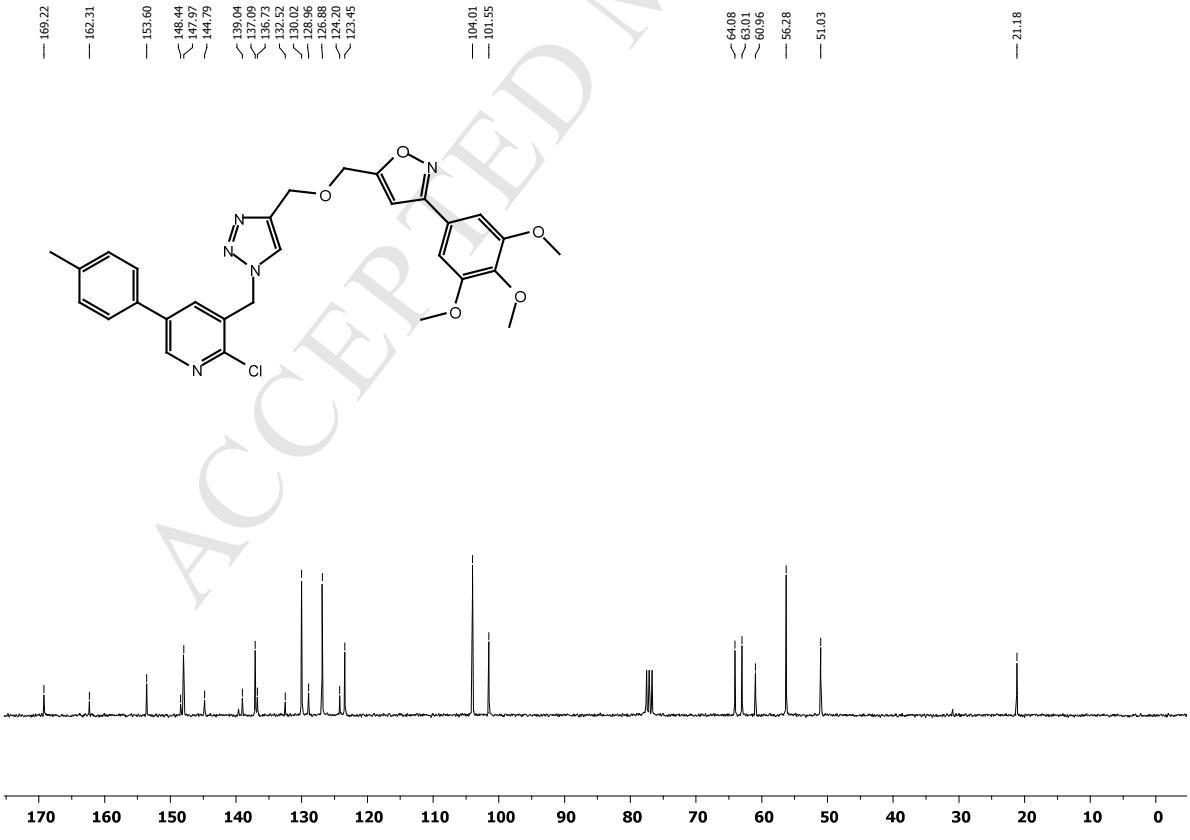
^{1}H-NMR OF COMPOUND **25b**



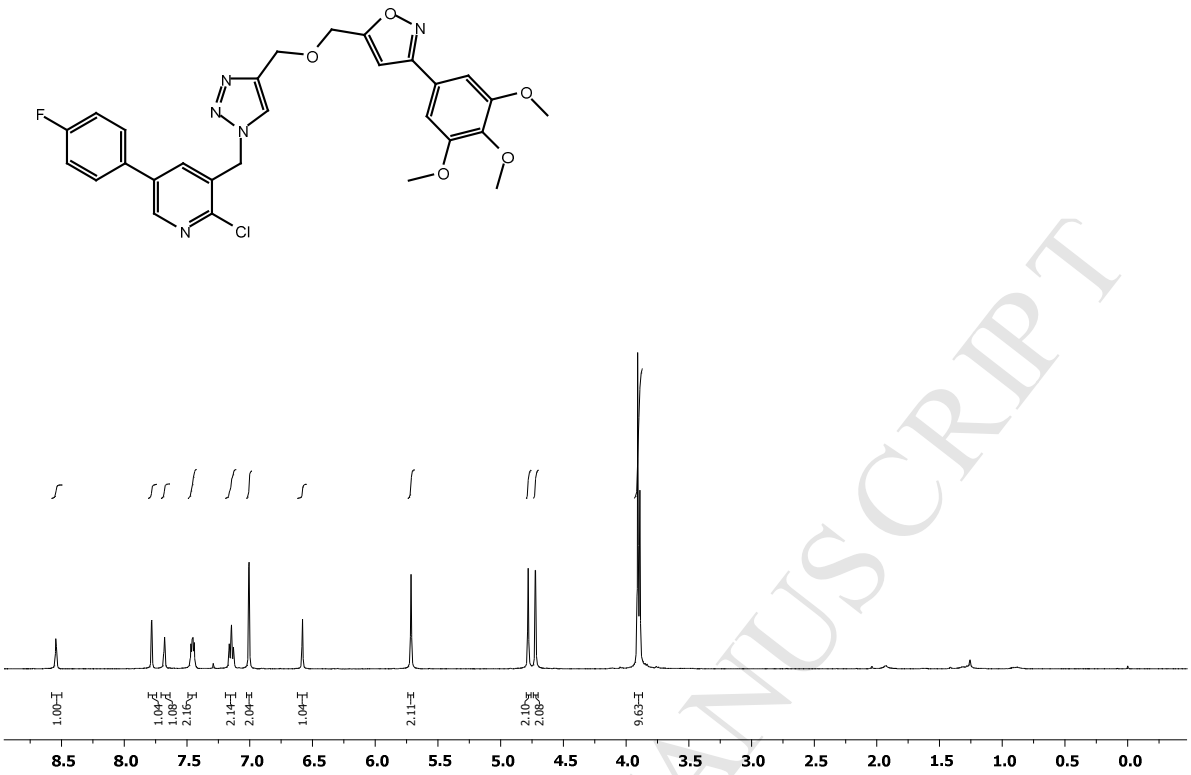
^{13}C-NMR OF COMPOUND **25b**



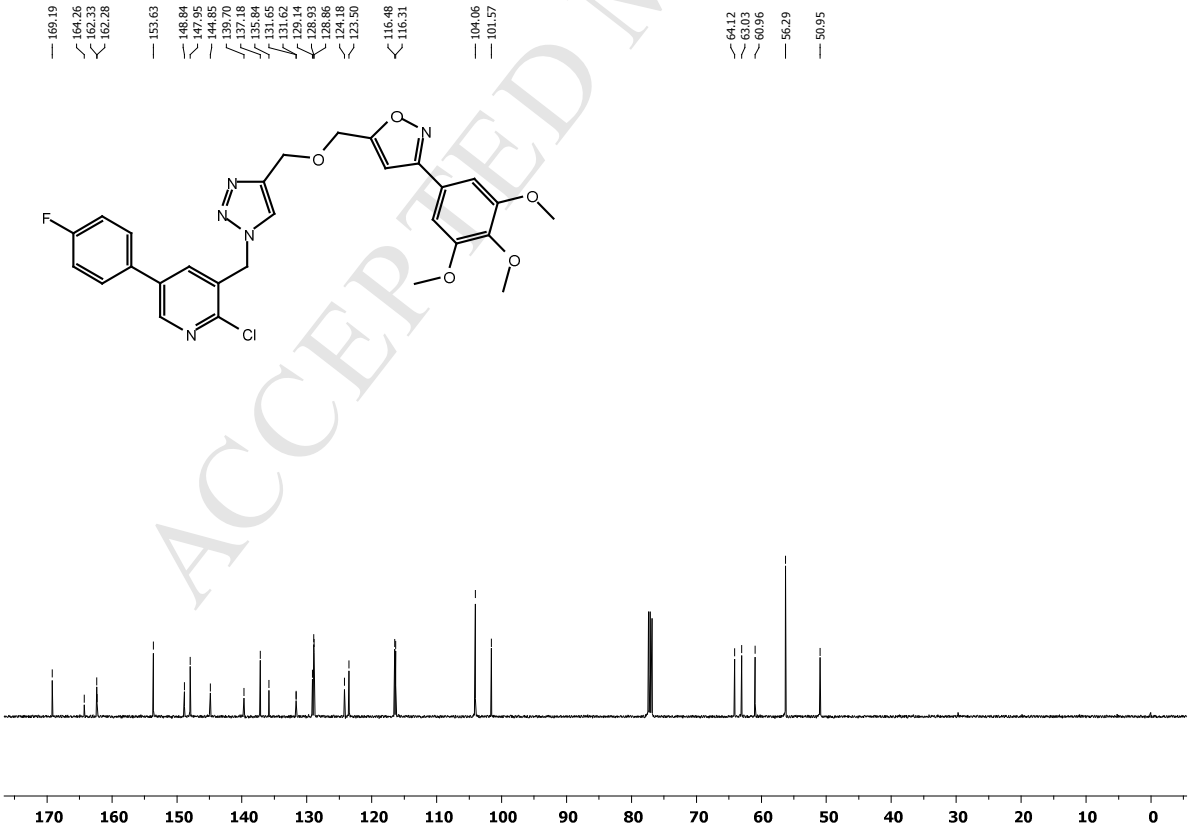
^{1}H-NMR OF COMPOUND **25c**



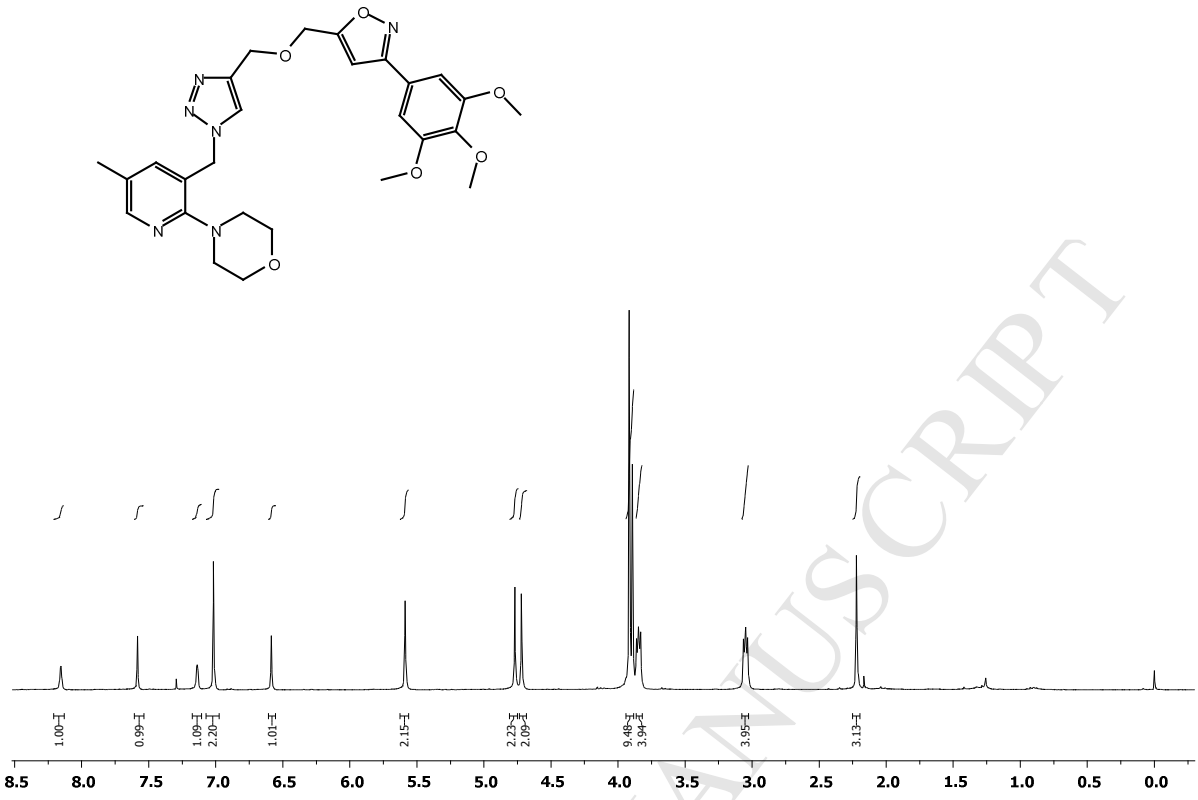
^{13}C-NMR OF COMPOUND **25c**



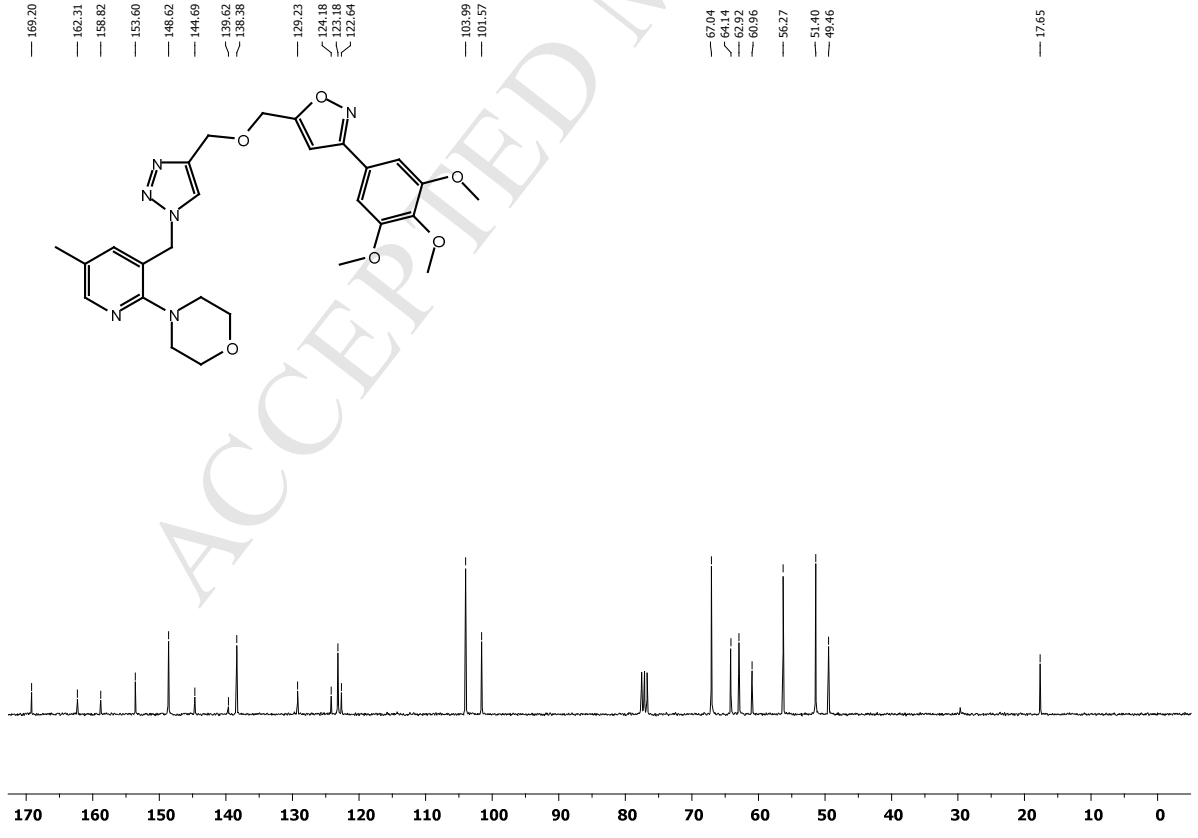
^{1}H-NMR OF COMPOUND **25d**



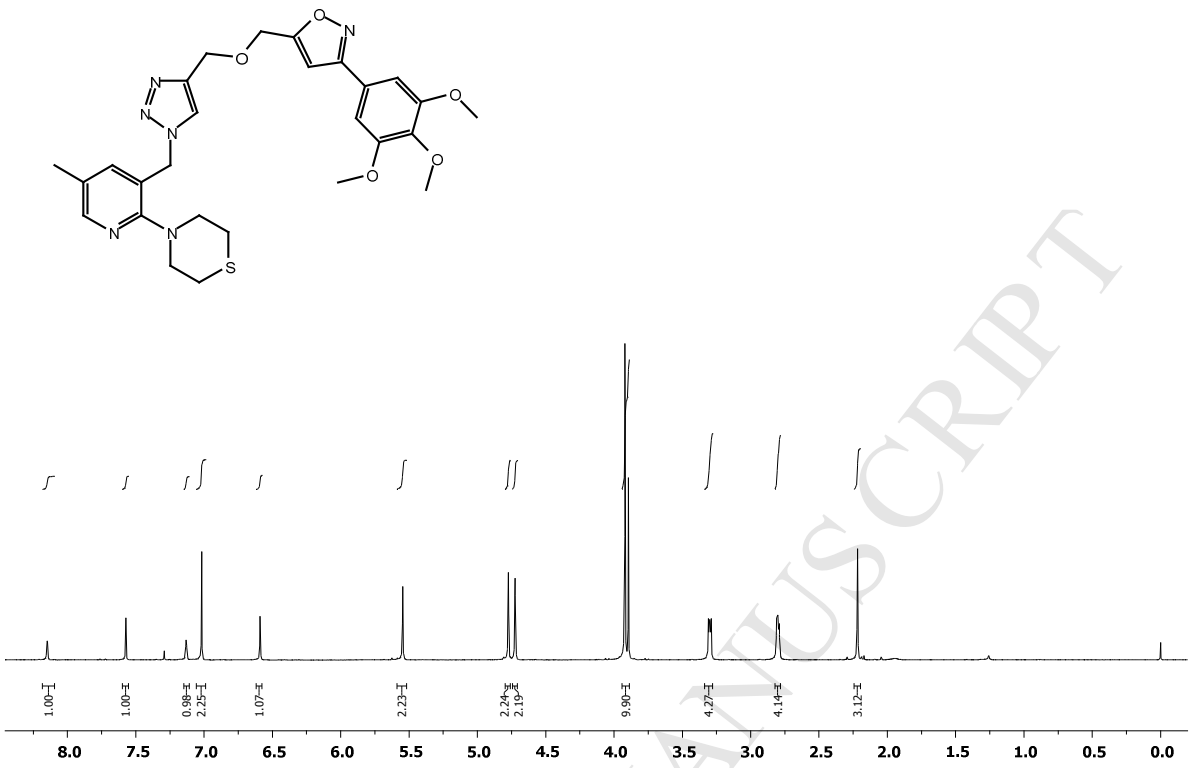
^{13}C-NMR OF COMPOUND **25d**



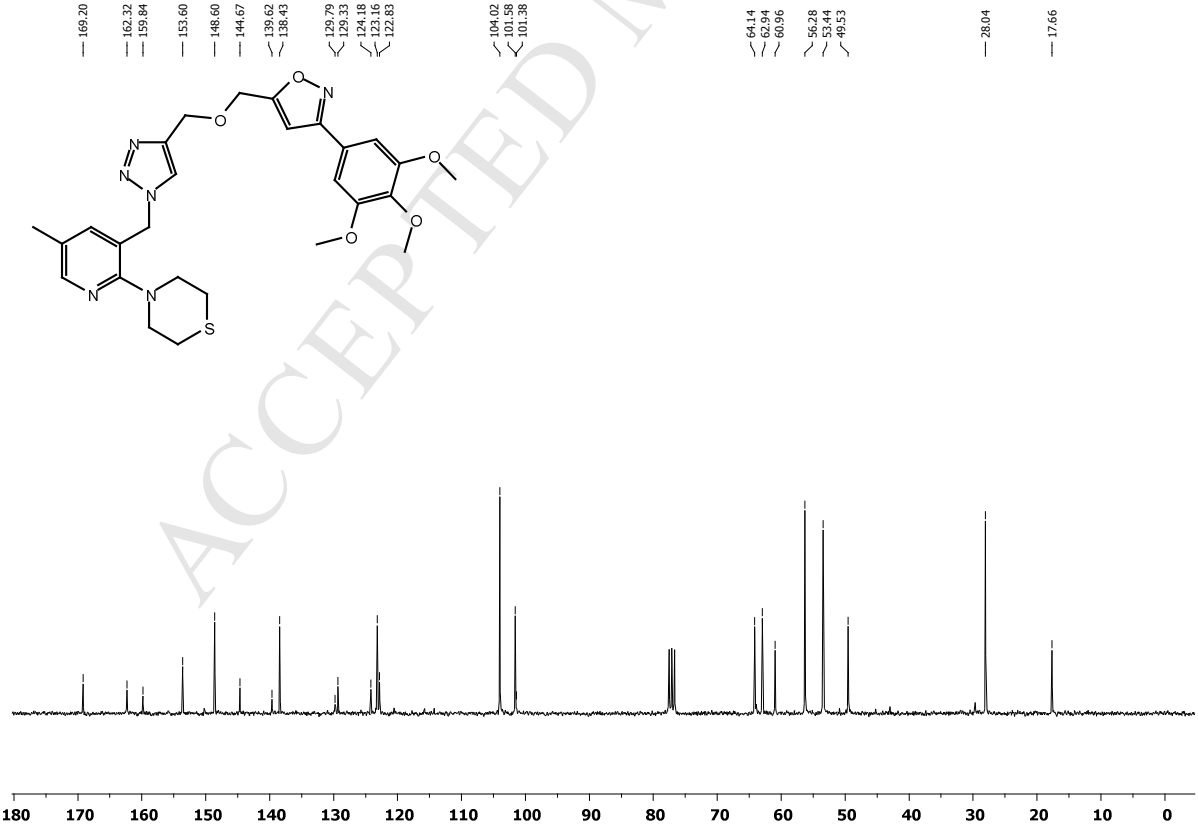
^{1}H-NMR OF COMPOUND **25e**



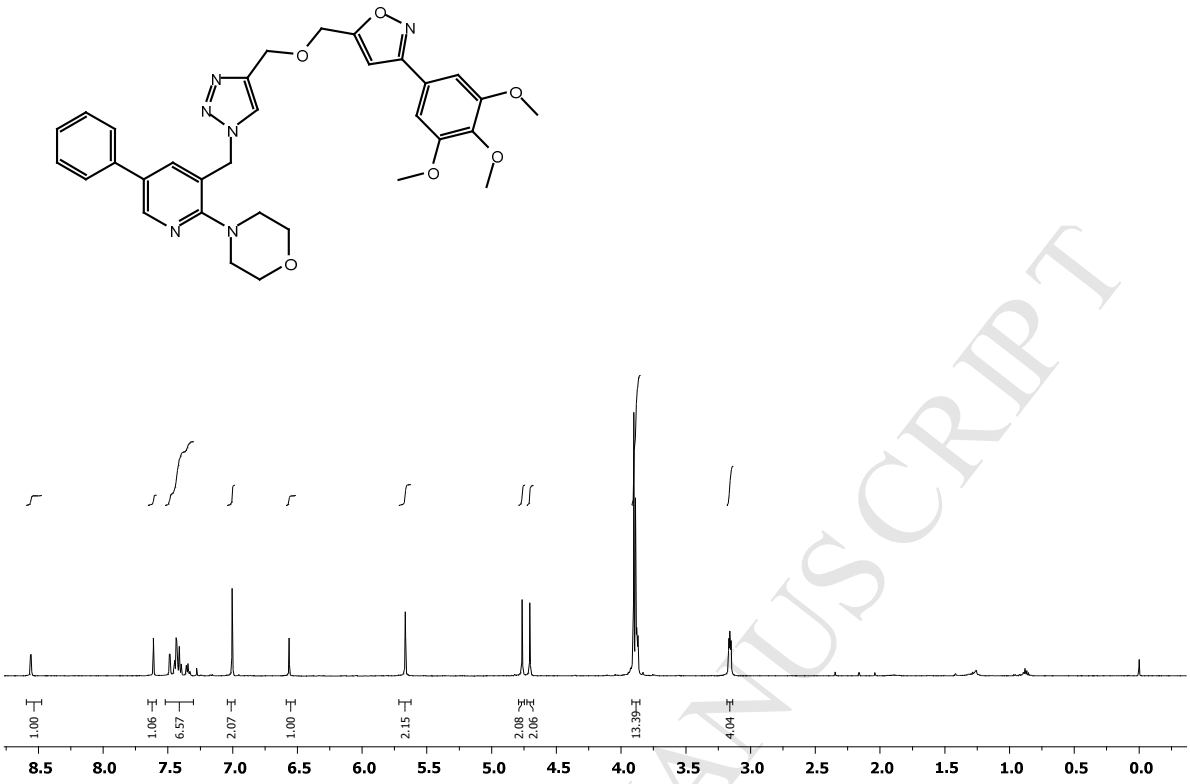
^{13}C-NMR OF COMPOUND **25e**



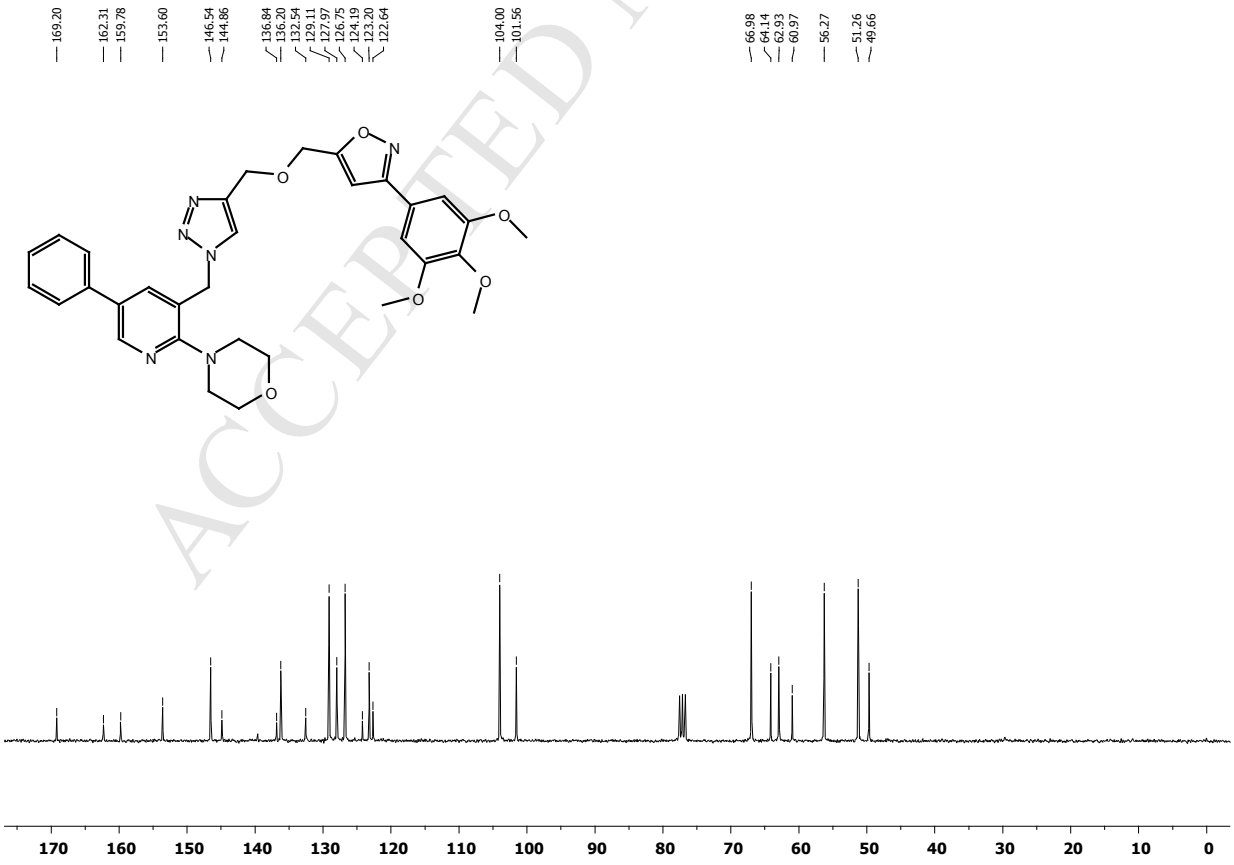
^{1}H-NMR OF COMPOUND **25f**



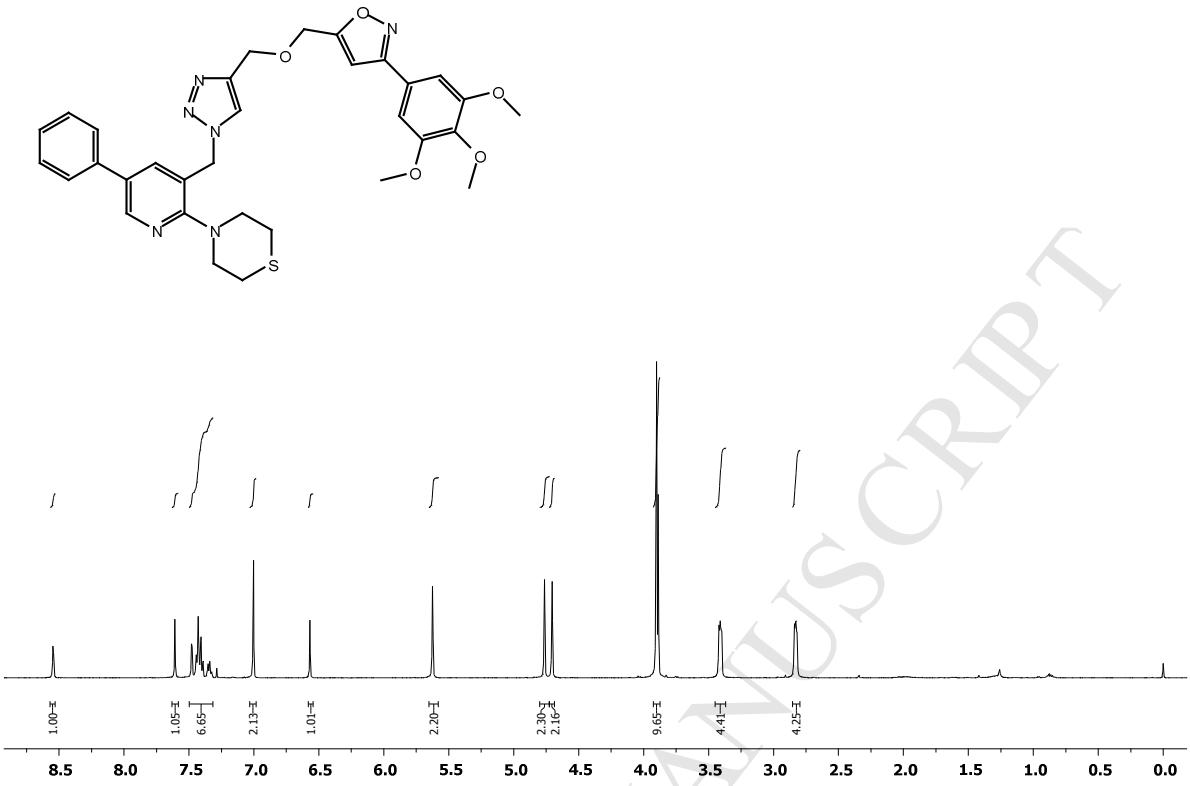
^{13}C-NMR OF COMPOUND **25f**



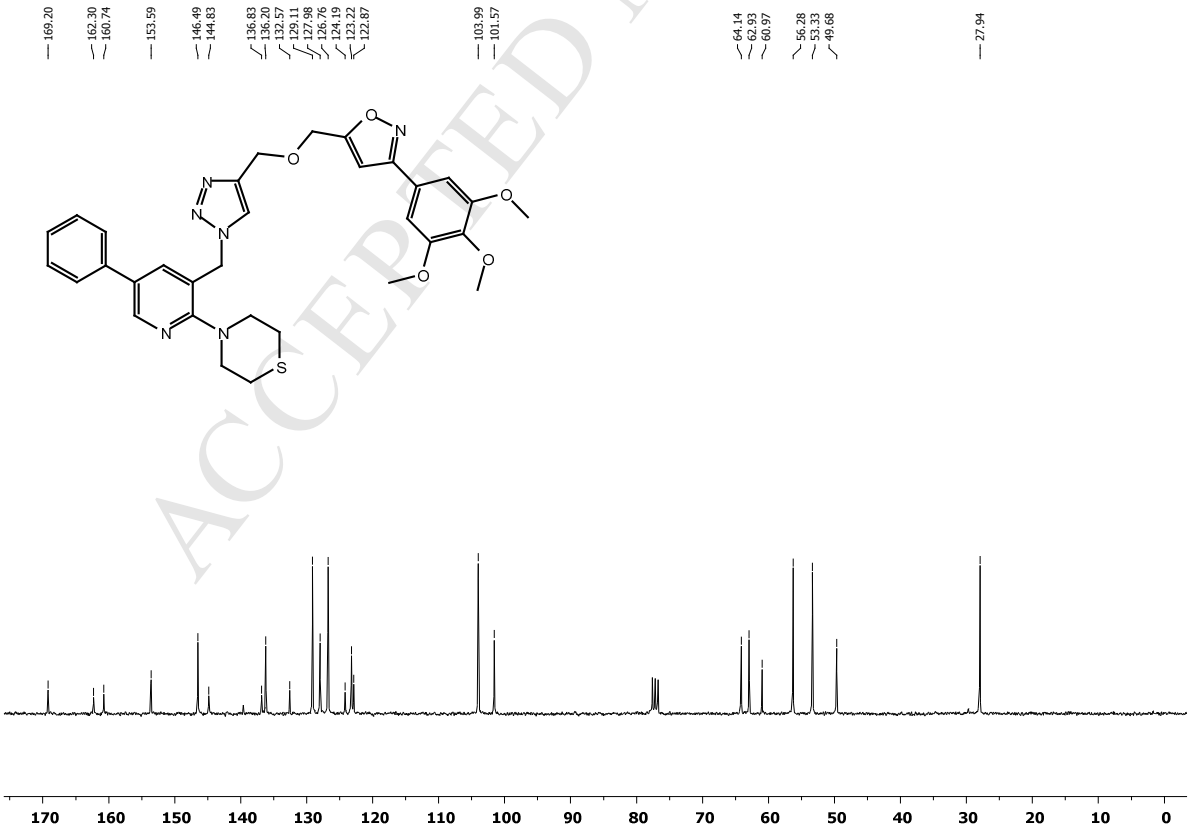
^{1}H-NMR OF COMPOUND **25g**



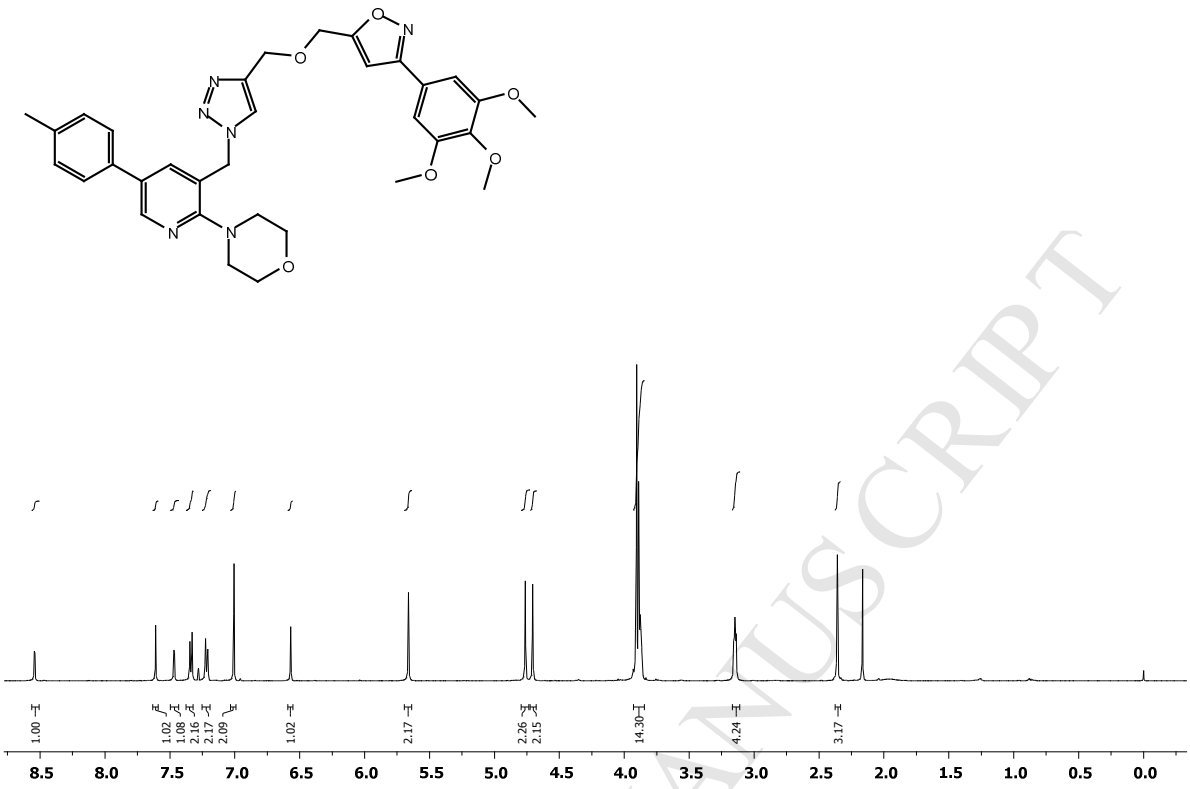
^{13}C-NMR OF COMPOUND **25g**



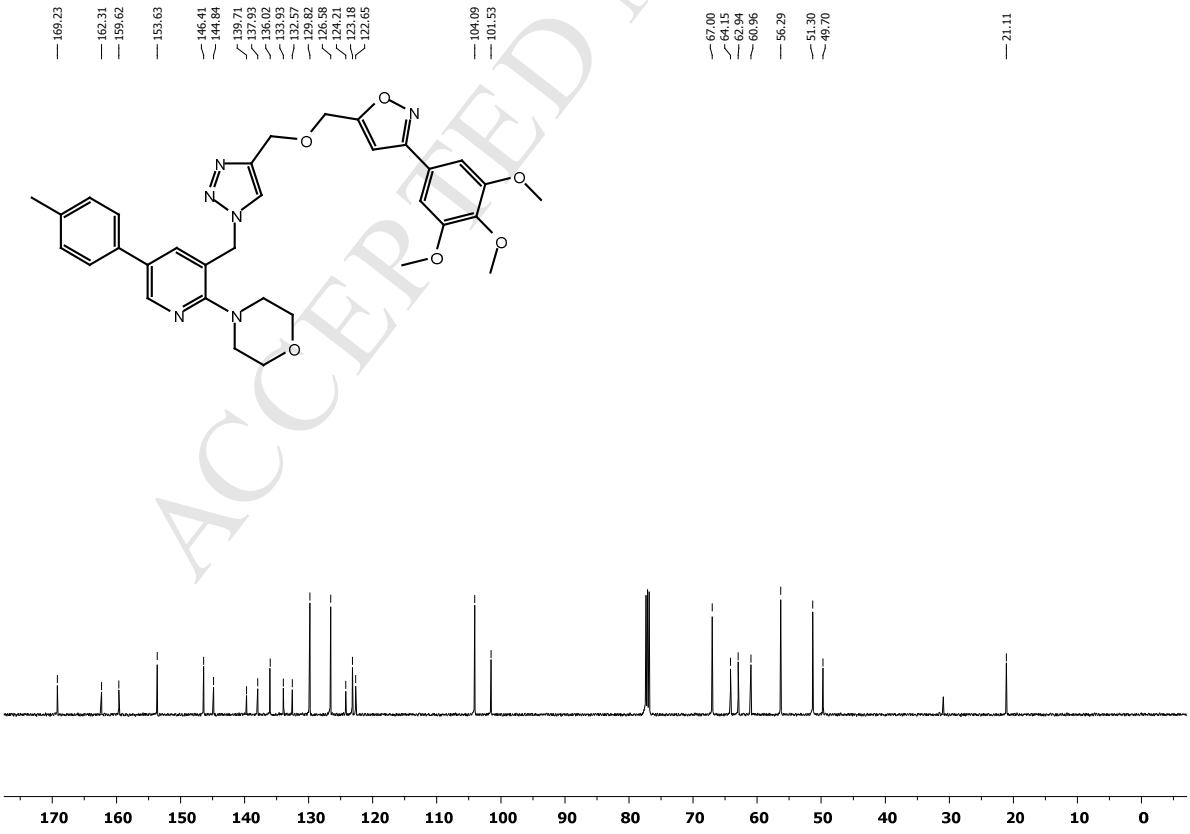
^{1}H-NMR OF COMPOUND **25h**



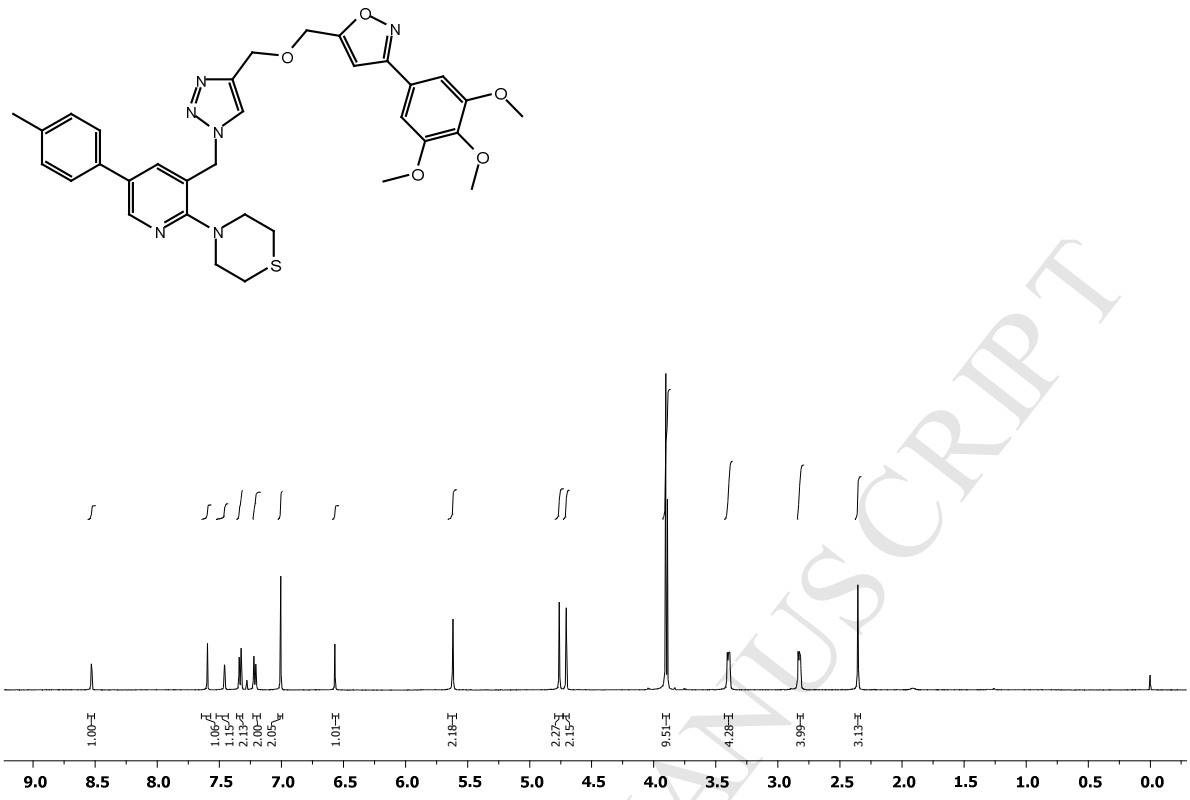
^{13}C-NMR OF COMPOUND **25h**



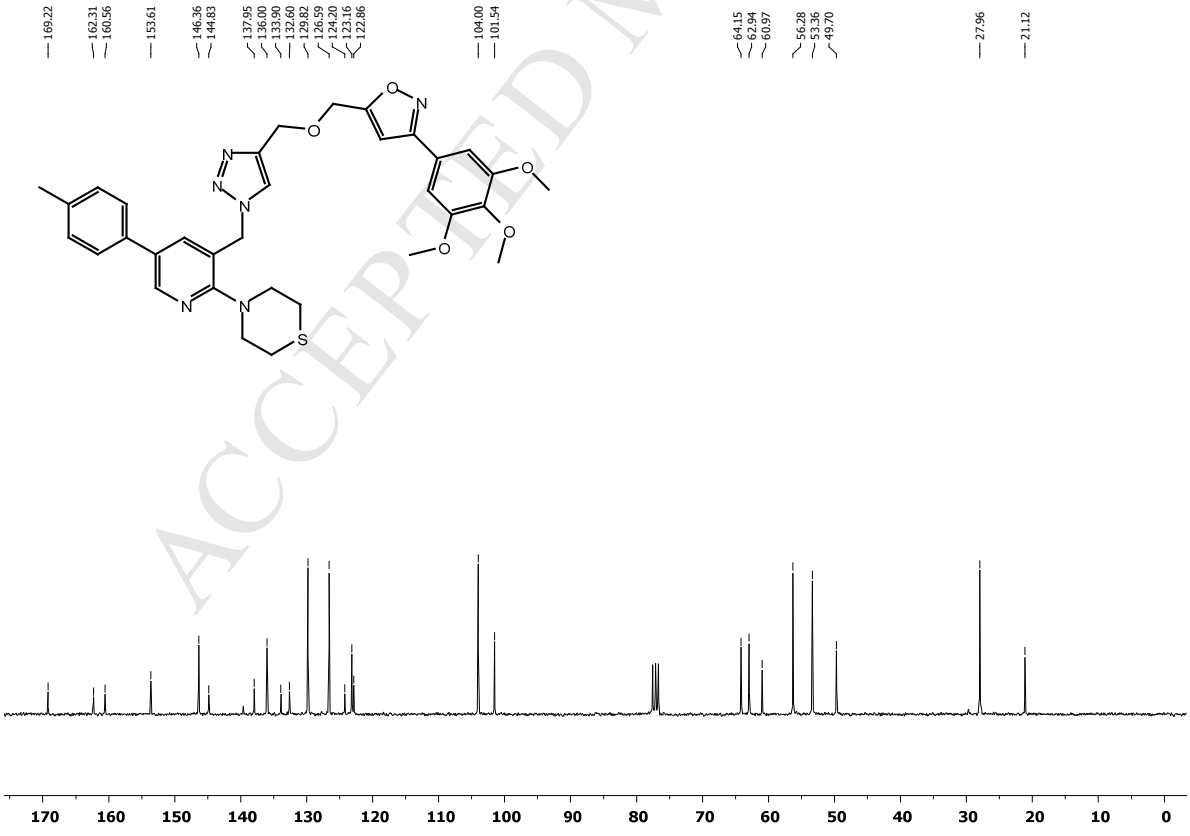
^{1}H-NMR OF COMPOUND **25i**



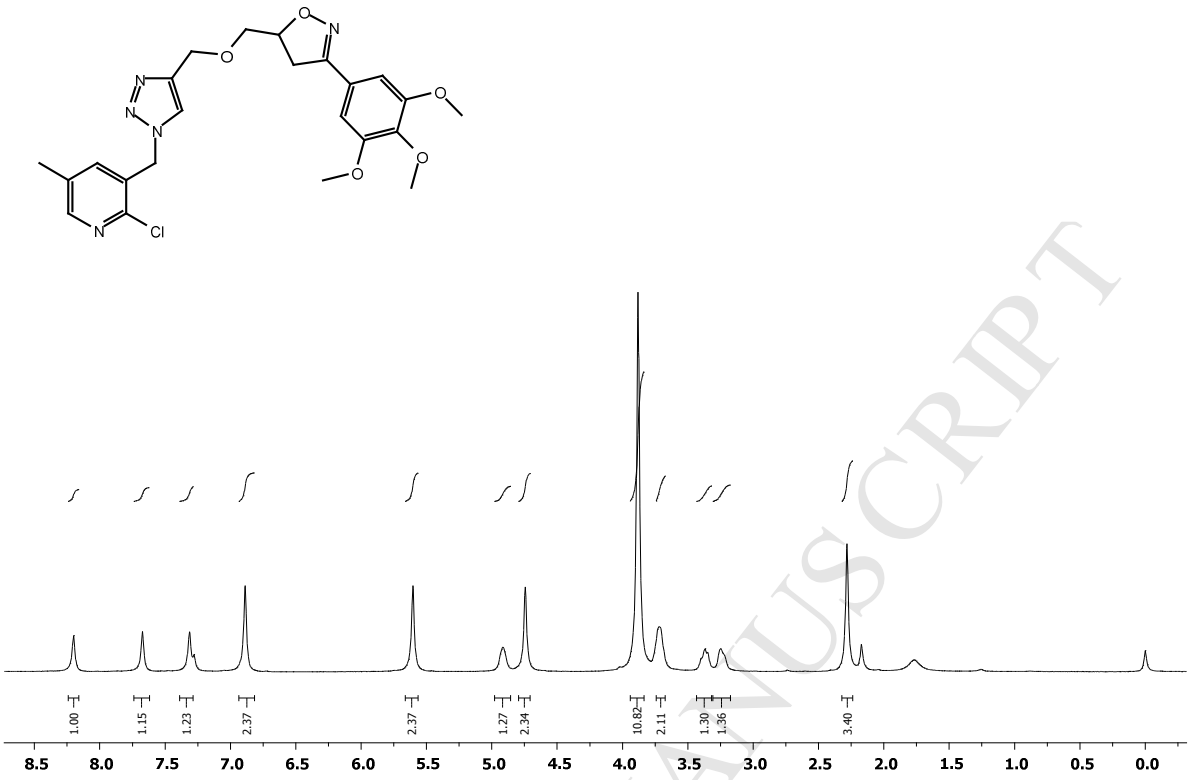
^{13}C-NMR OF COMPOUND **25i**



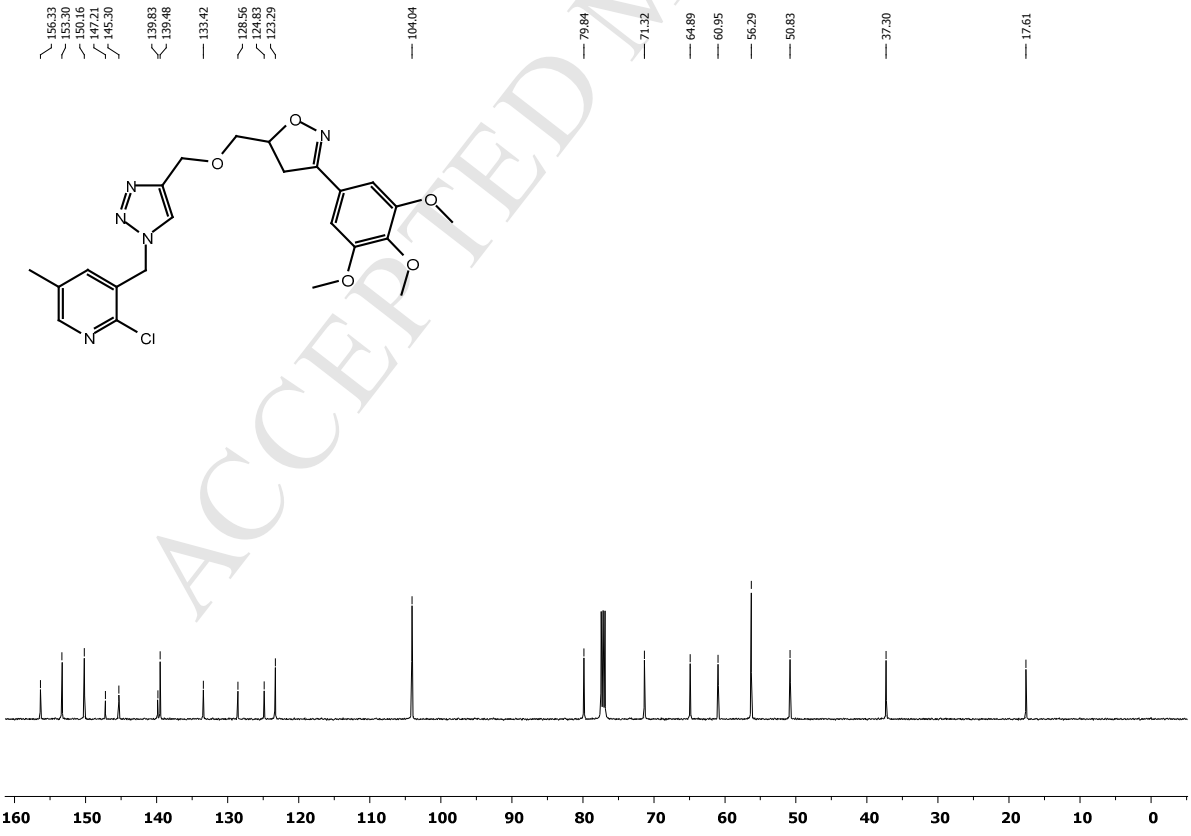
^{1}H-NMR OF COMPOUND **25j**



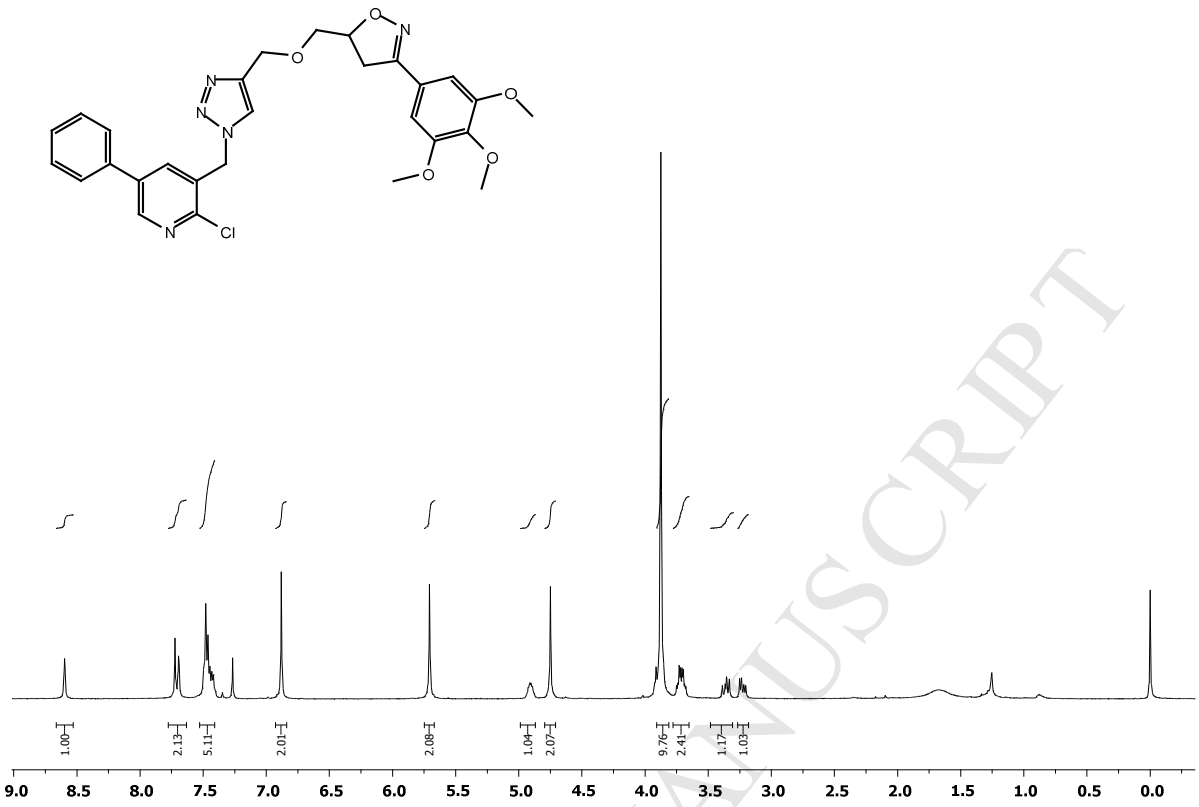
^{13}C-NMR OF COMPOUND **25j**



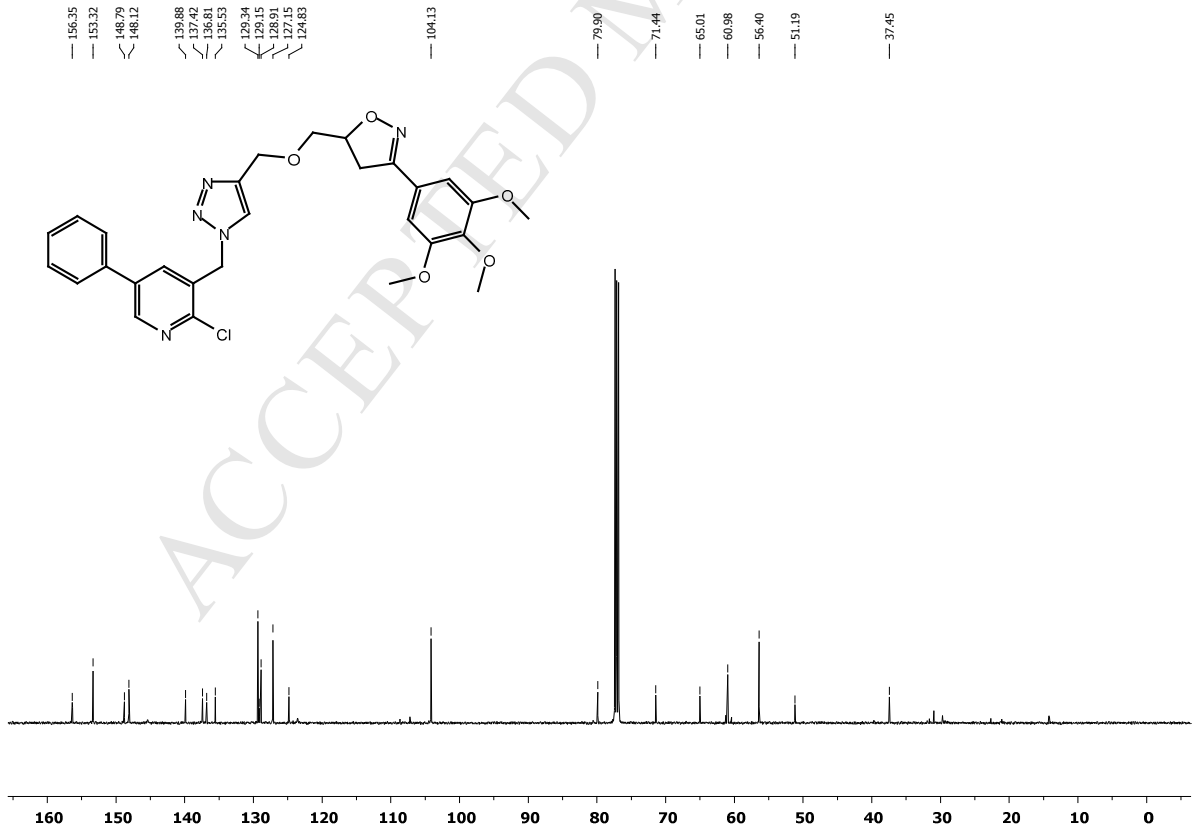
^{1}H-NMR OF COMPOUND **28a**



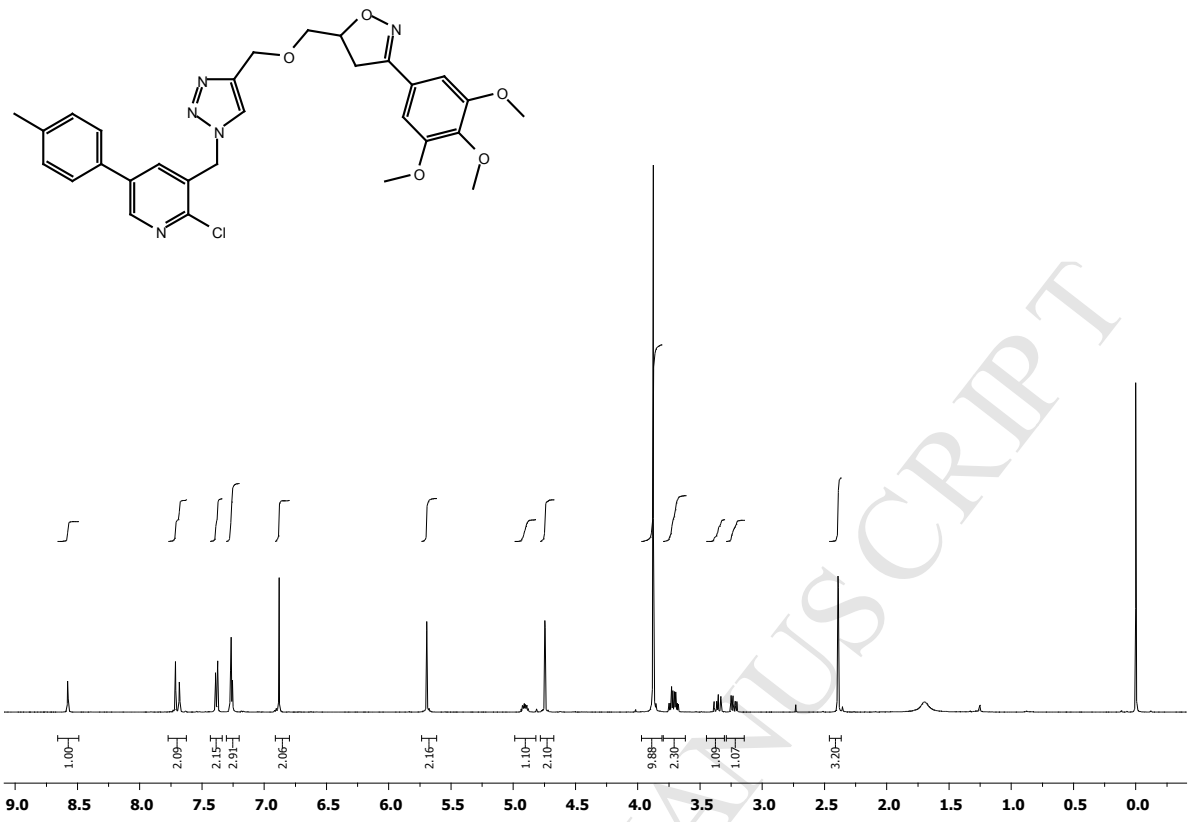
^{13}C-NMR OF COMPOUND **28a**



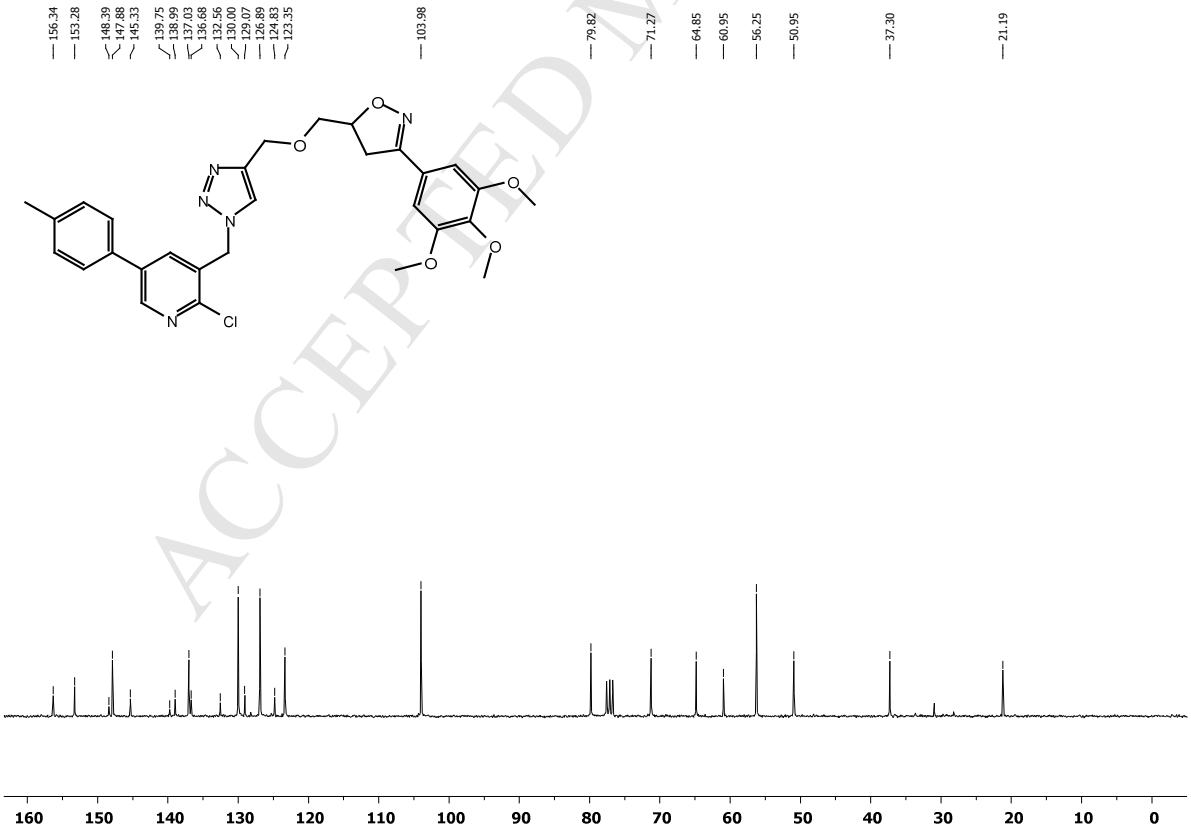
^{1}H-NMR OF COMPOUND **28b**



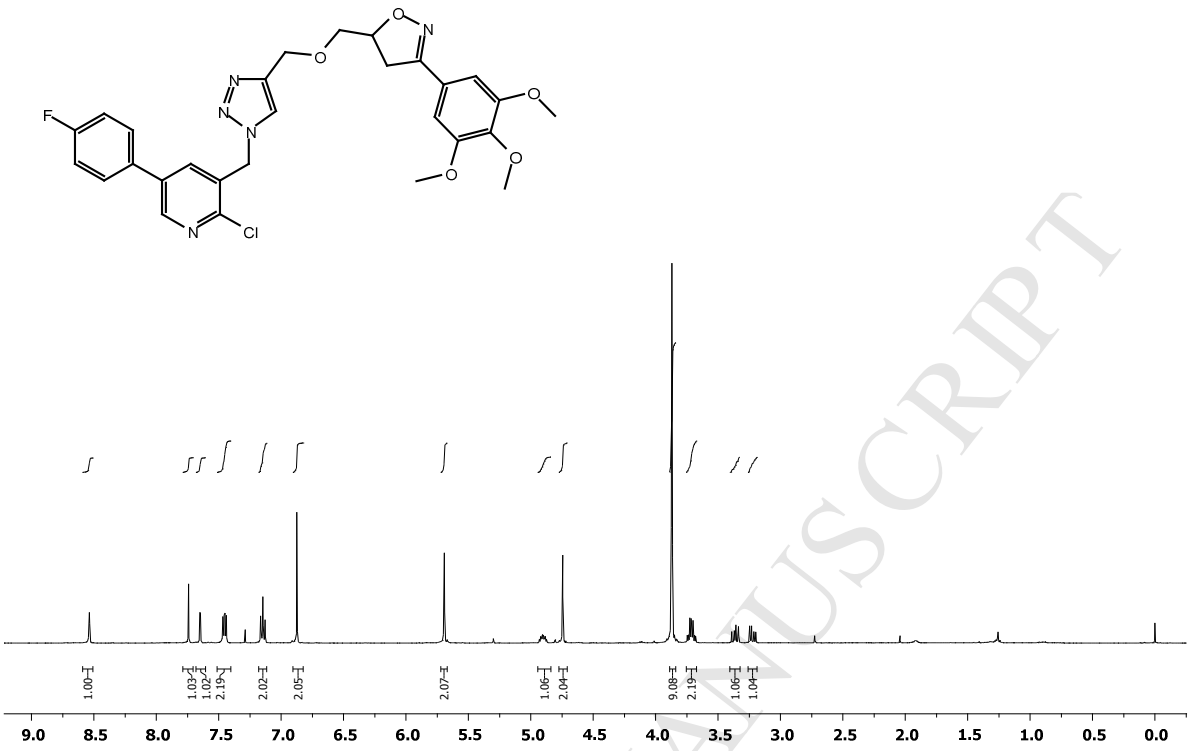
^{13}C-NMR OF COMPOUND **28b**



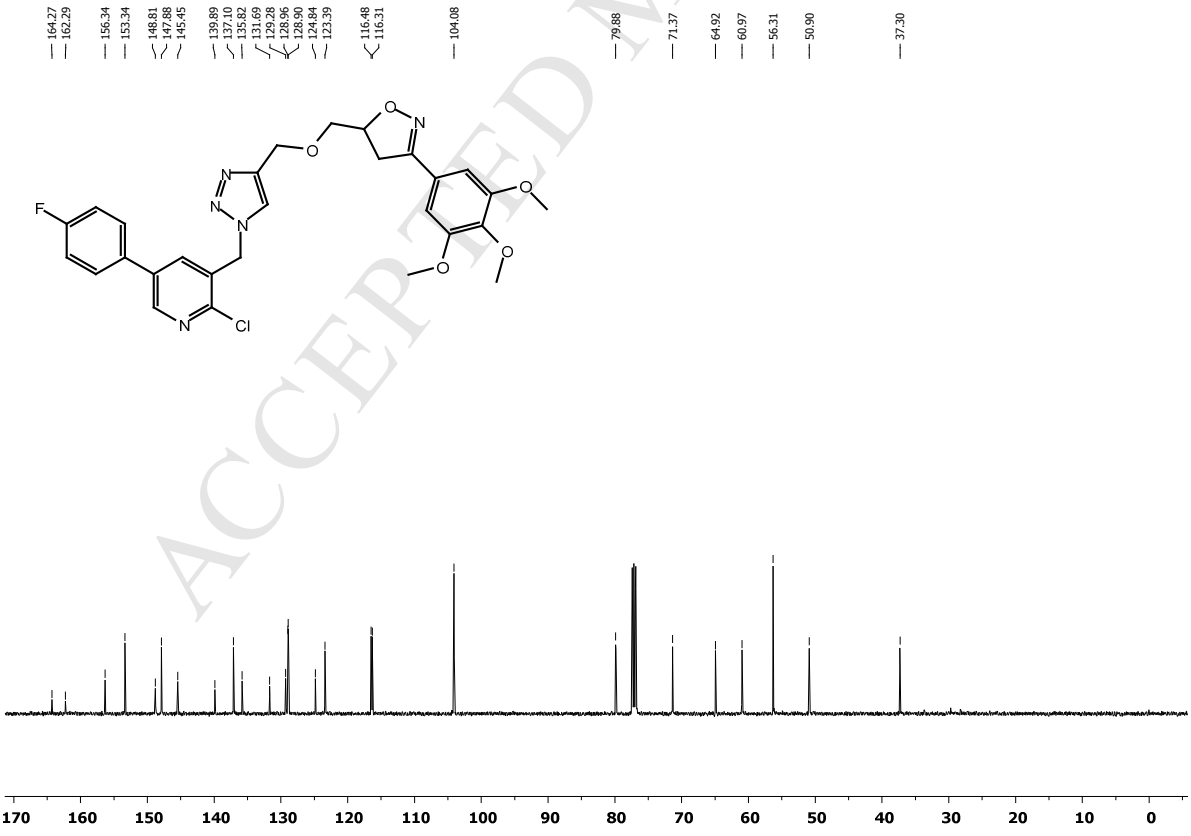
^{1}H-NMR OF COMPOUND **28c**



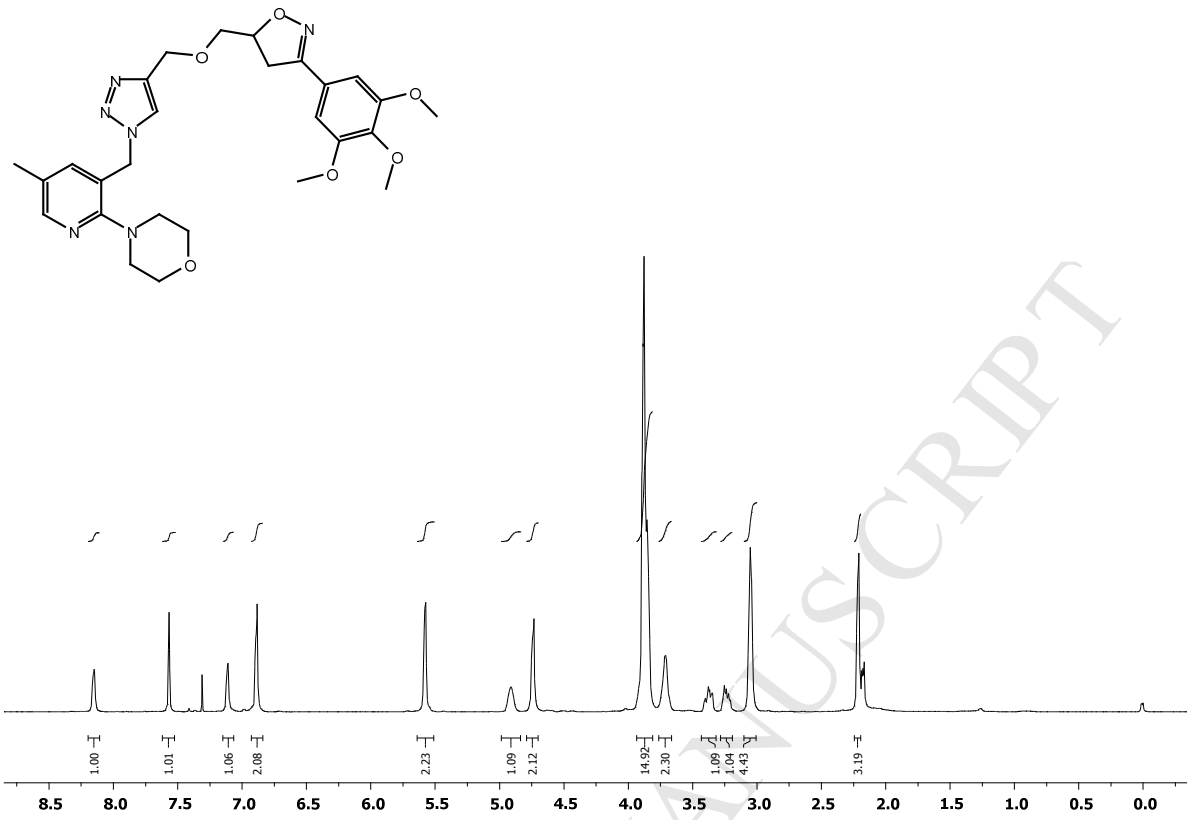
^{13}C-NMR OF COMPOUND **28c**



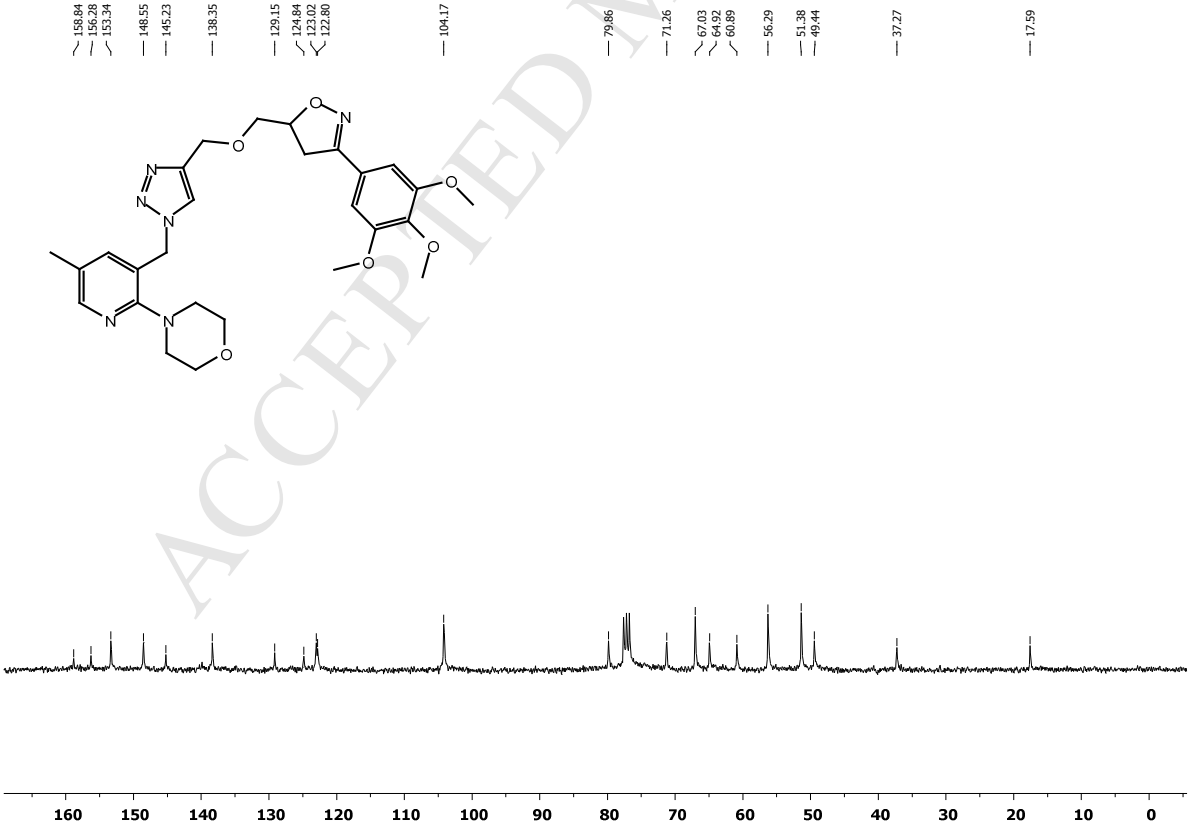
^{1}H-NMR OF COMPOUND **28d**



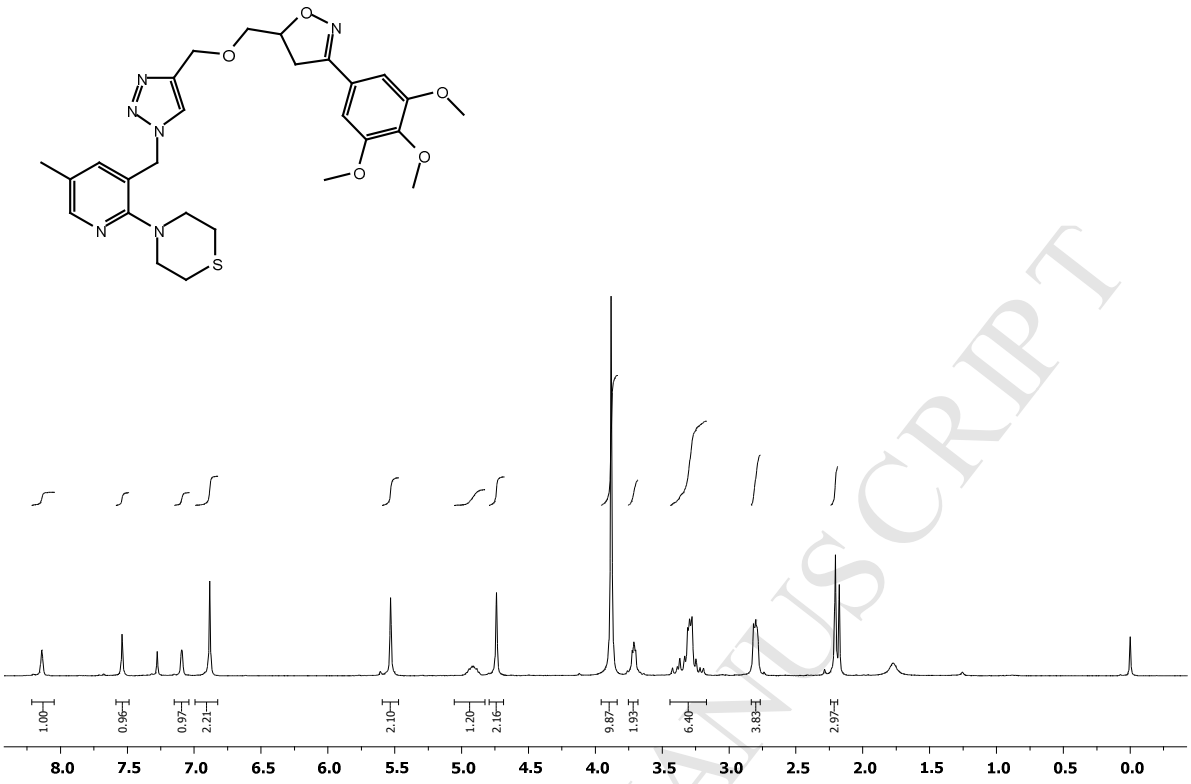
^{13}C-NMR OF COMPOUND **28d**



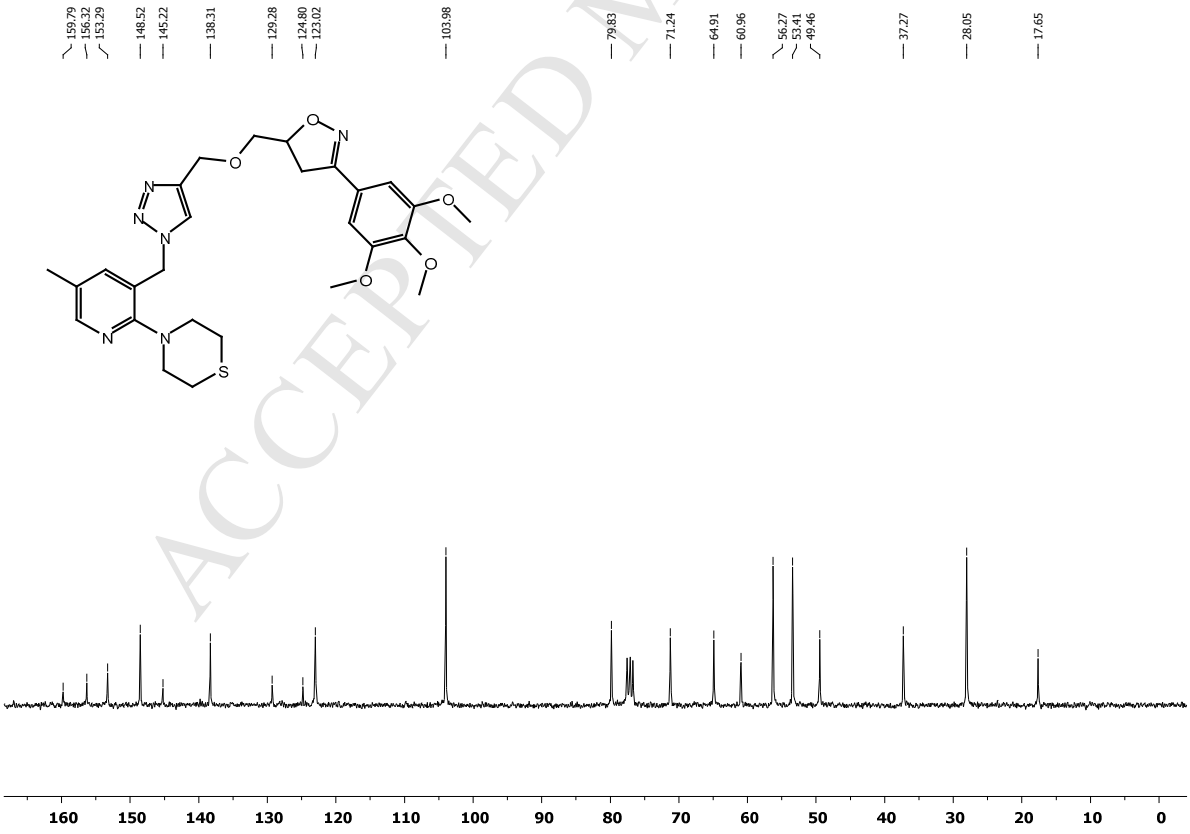
^{1}H-NMR OF COMPOUND **28e**



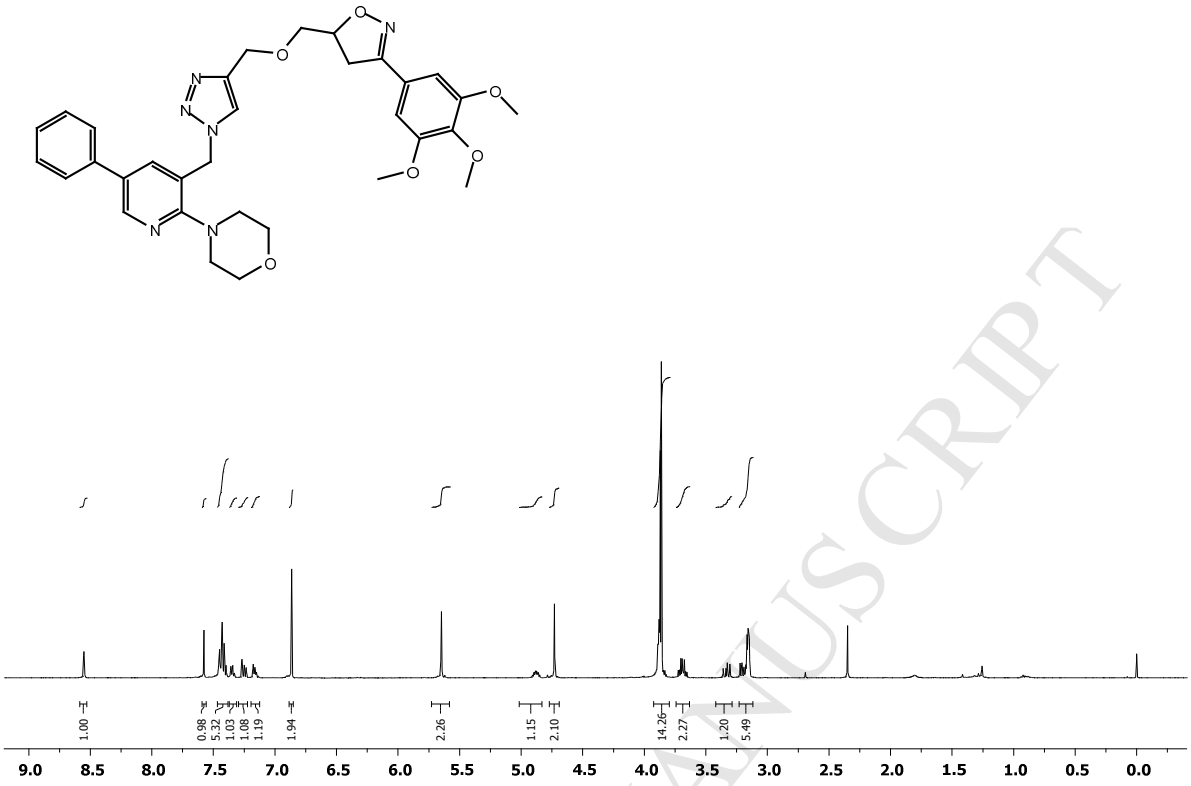
^{13}C-NMR OF COMPOUND **28e**



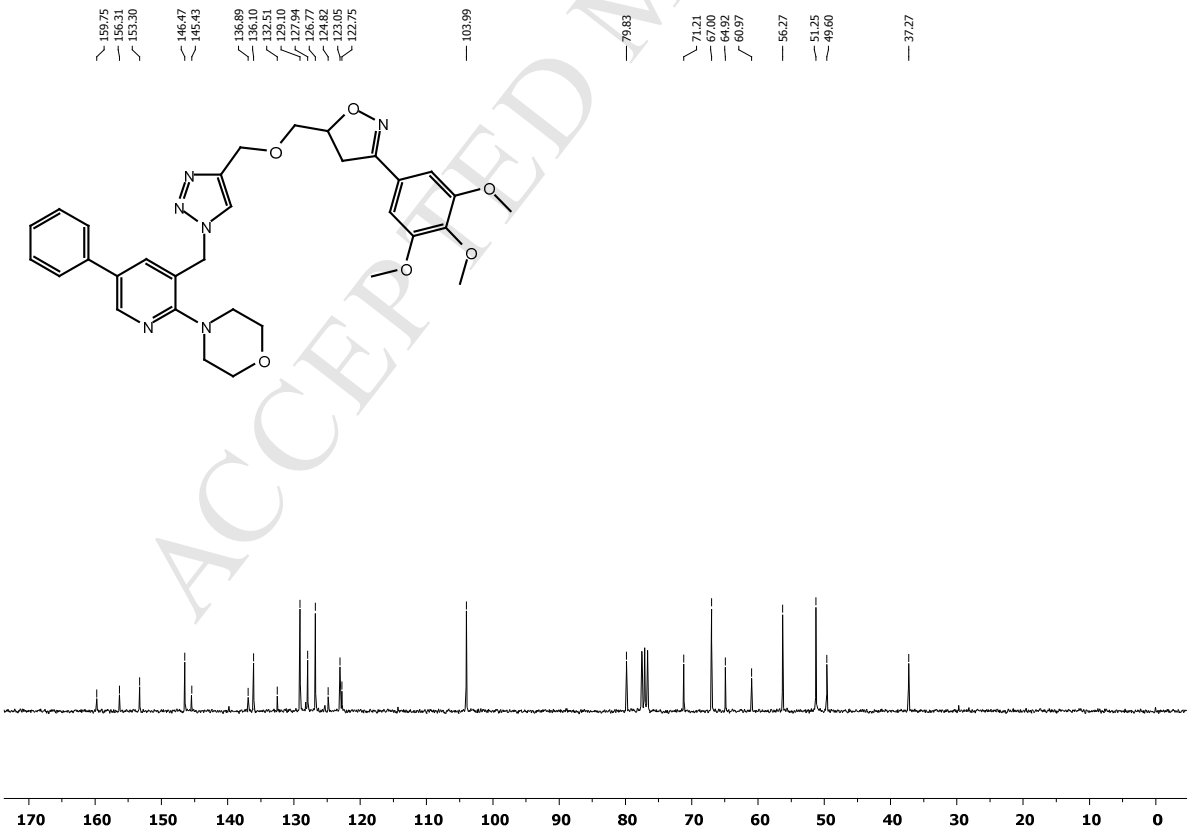
^{1}H-NMR OF COMPOUND **28f**



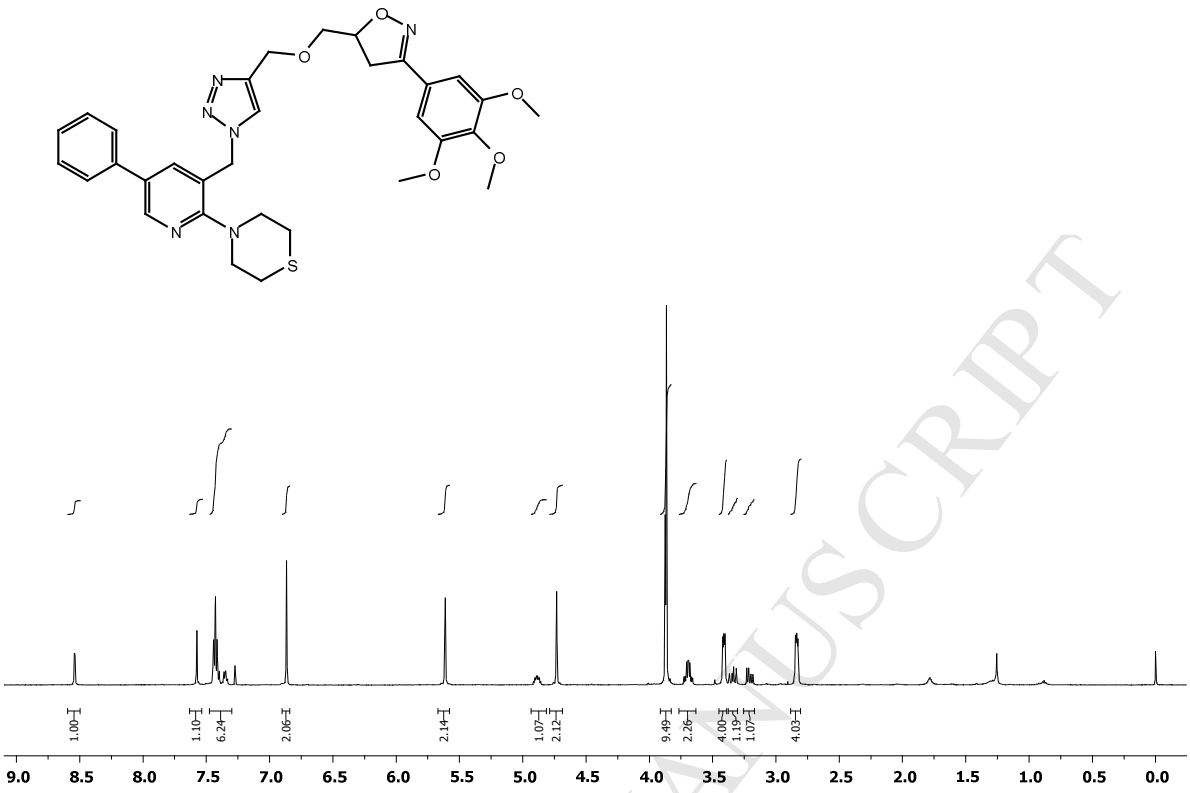
^{13}C-NMR OF COMPOUND **28f**



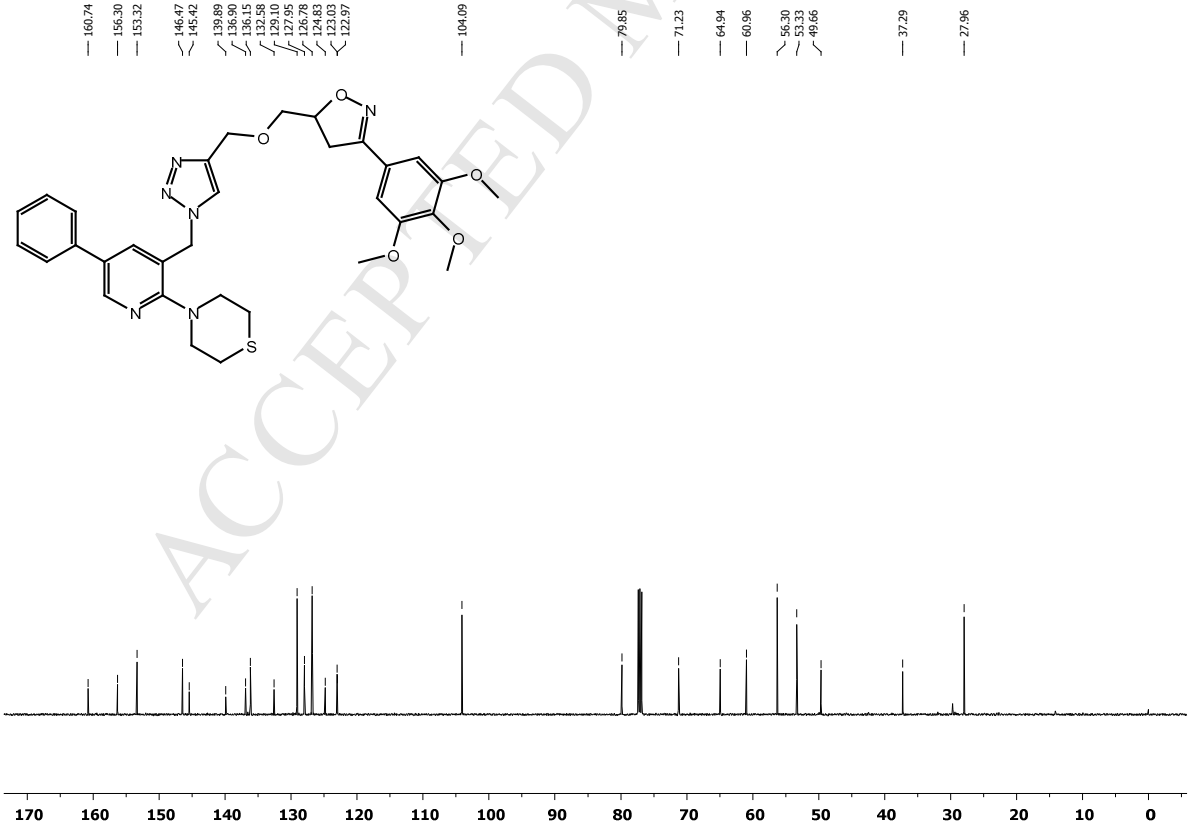
^{1}H-NMR OF COMPOUND **28g**



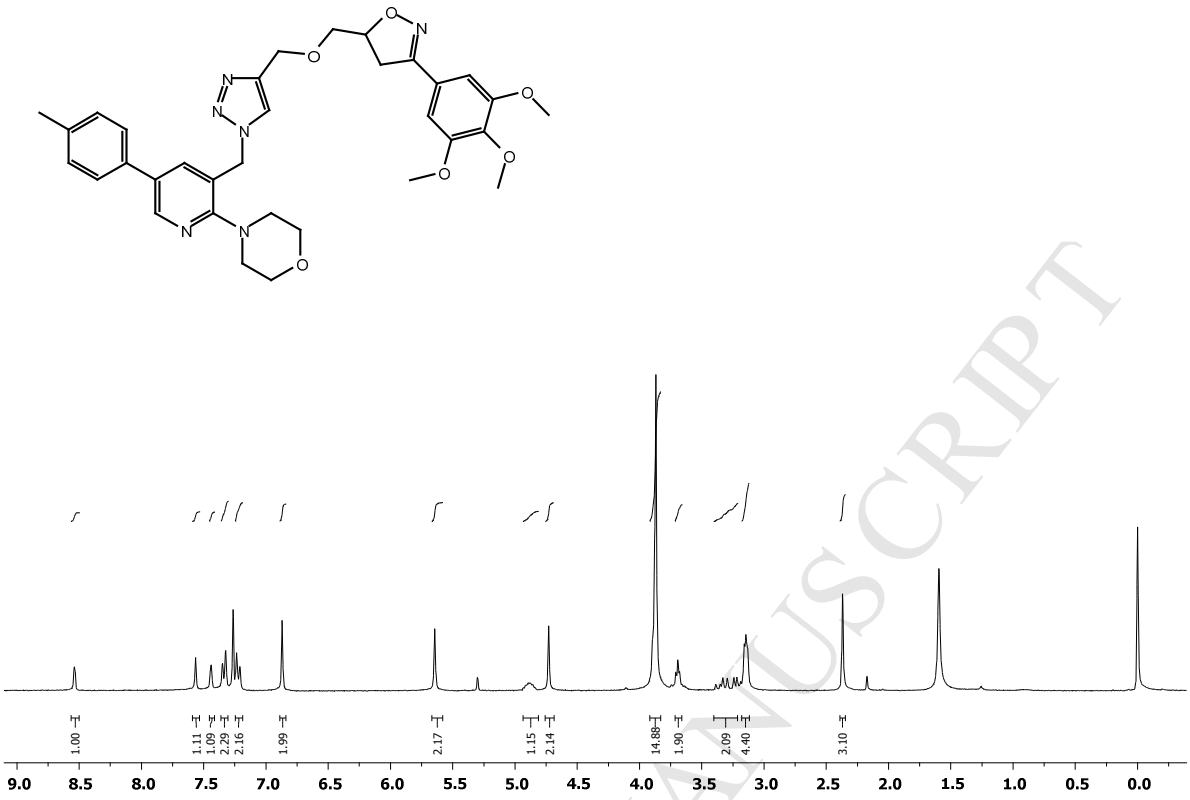
^{13}C-NMR OF COMPOUND **28g**



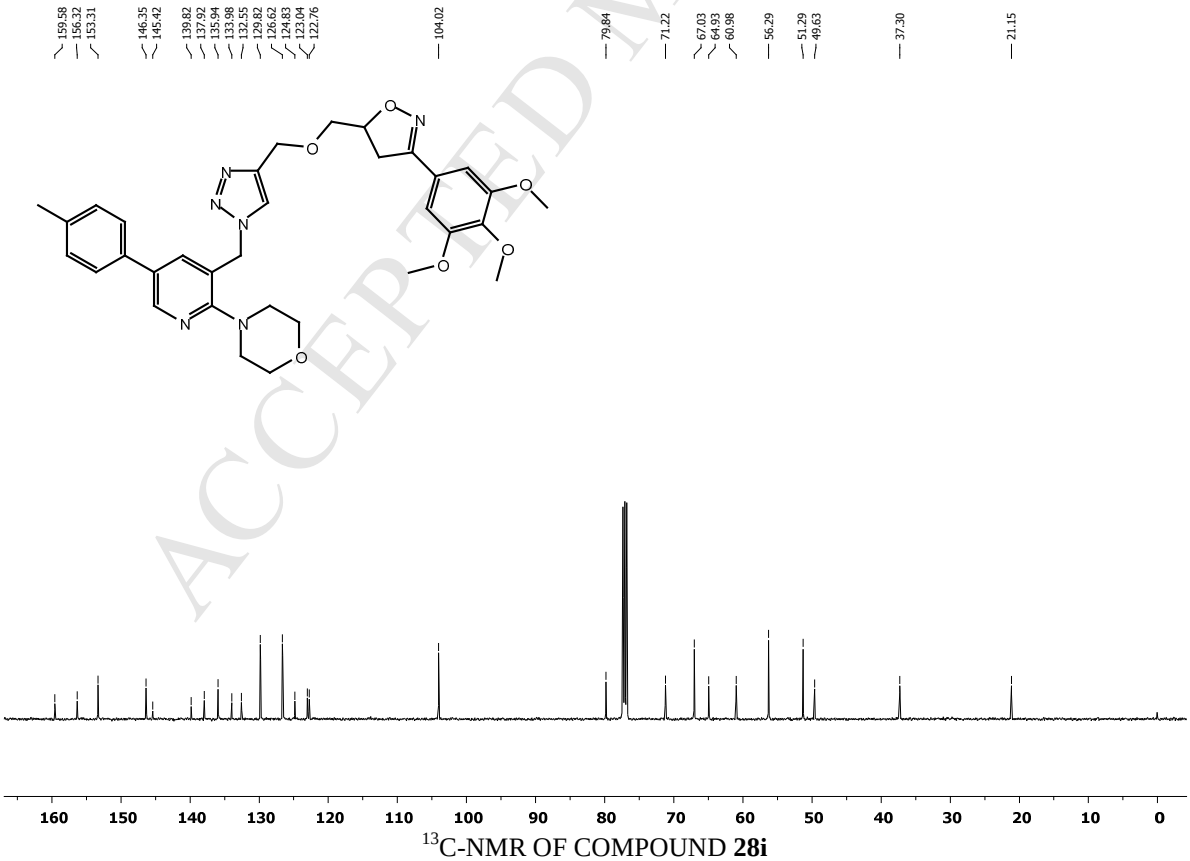
^{1}H-NMR OF COMPOUND **28h**



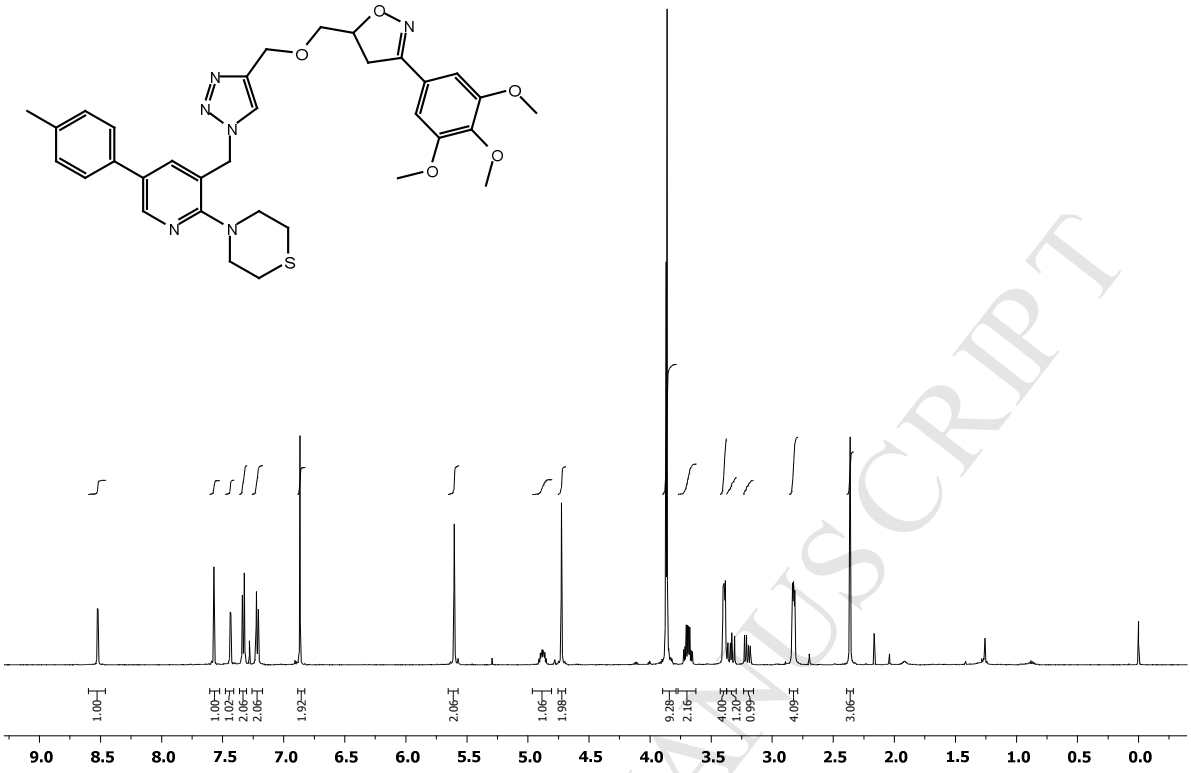
^{13}C-NMR OF COMPOUND **28h**



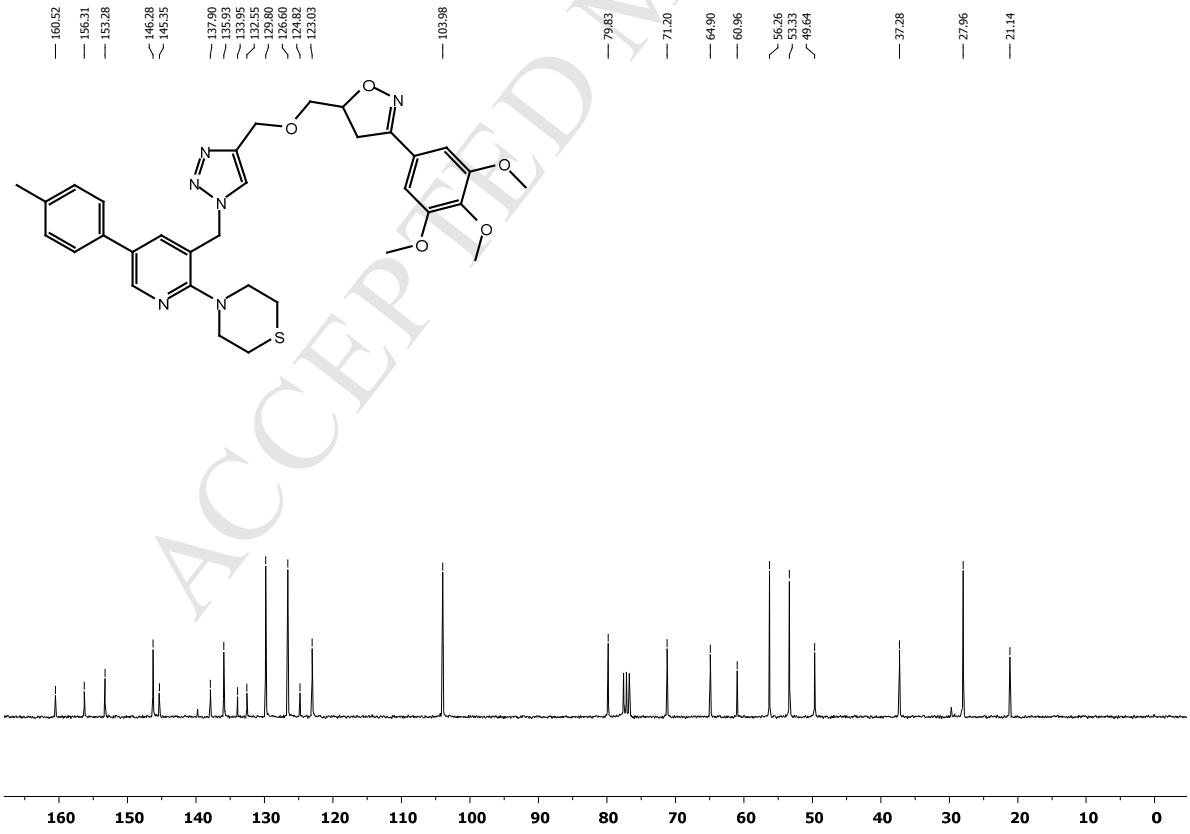
^{1}H-NMR OF COMPOUND **28i**



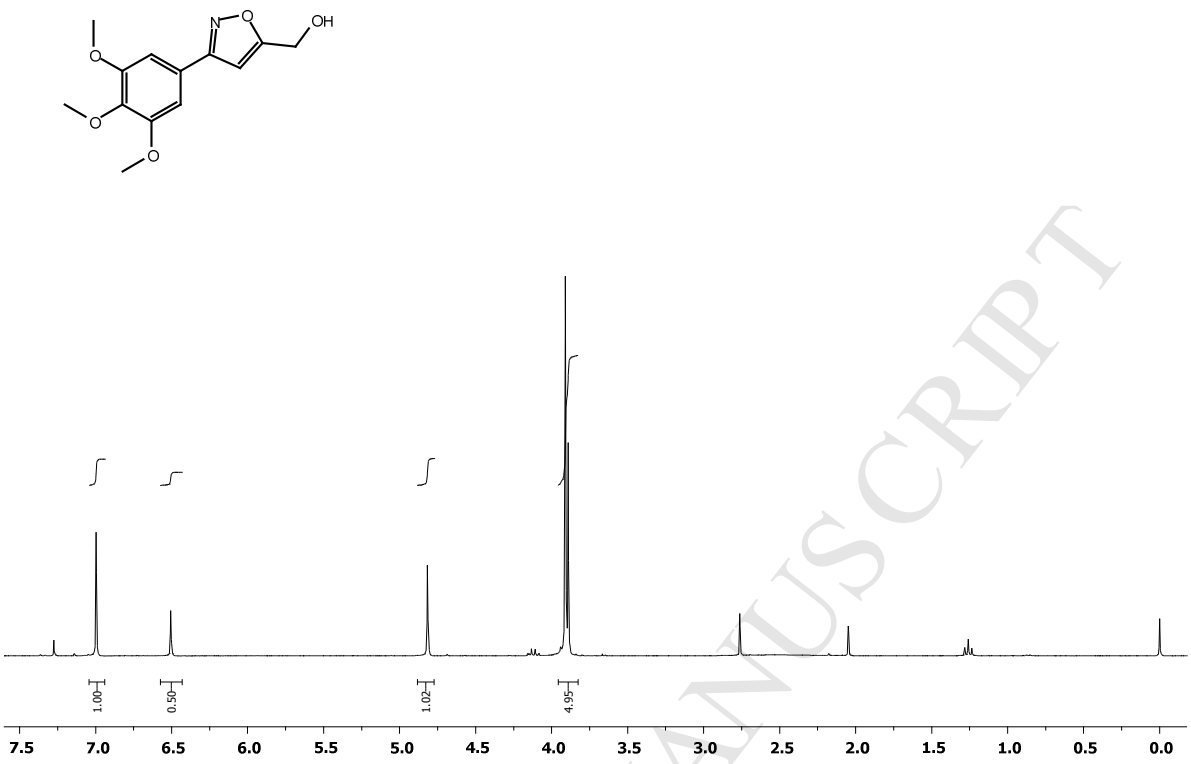
^{13}C-NMR OF COMPOUND **28i**



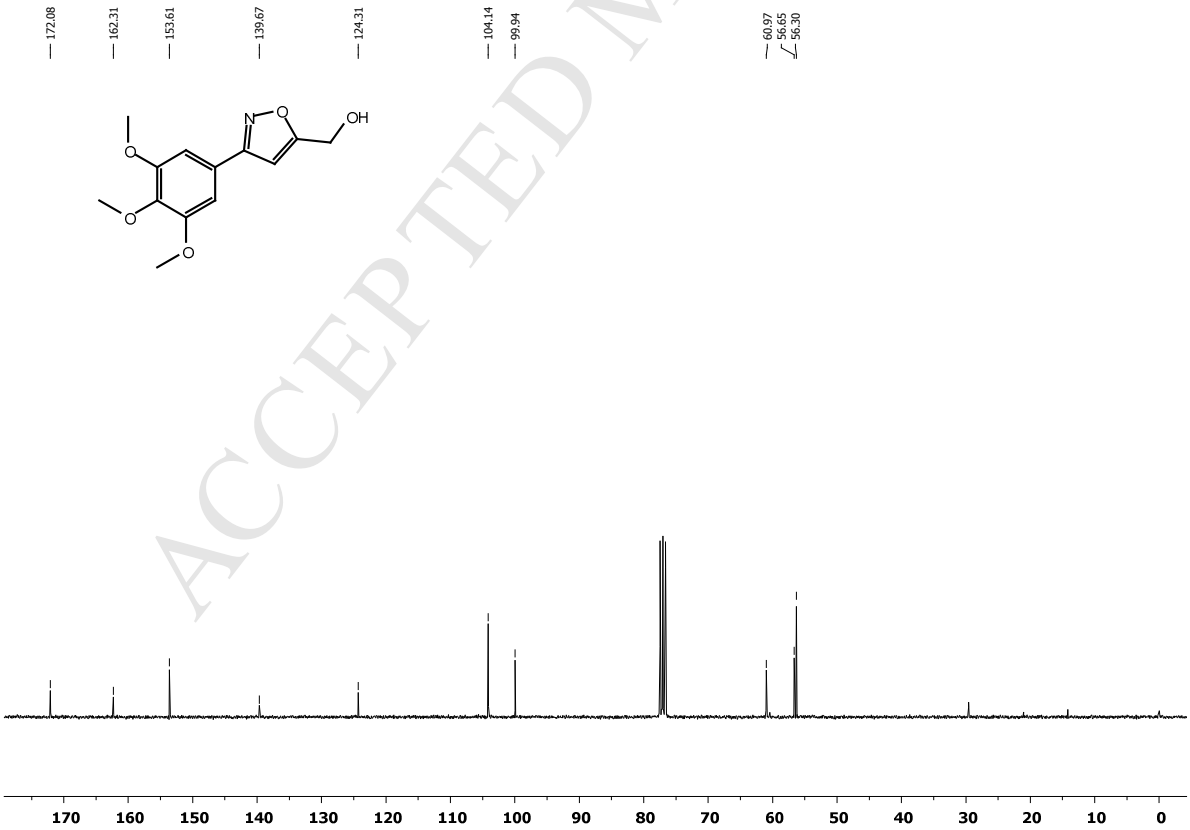
^{1}H-NMR OF COMPOUND **28j**



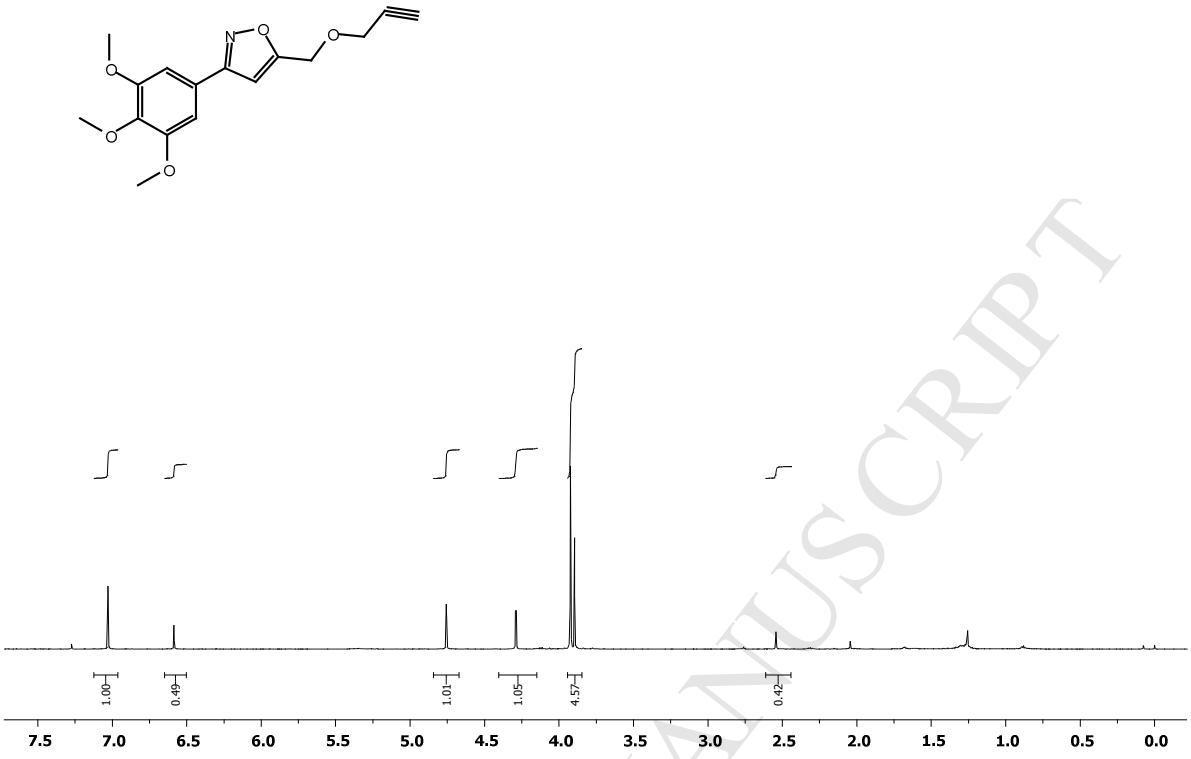
^{13}C-NMR OF COMPOUND **28j**



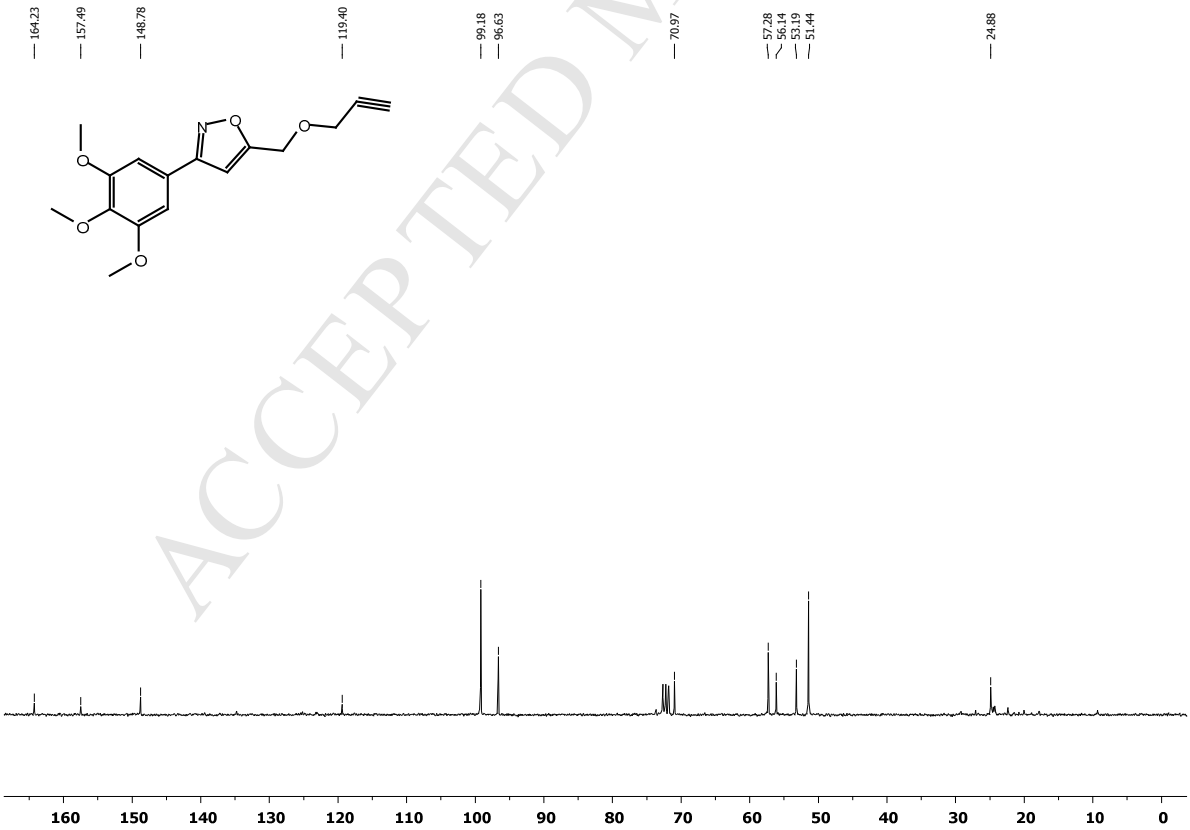
^{1}H-NMR OF COMPOUND **23**



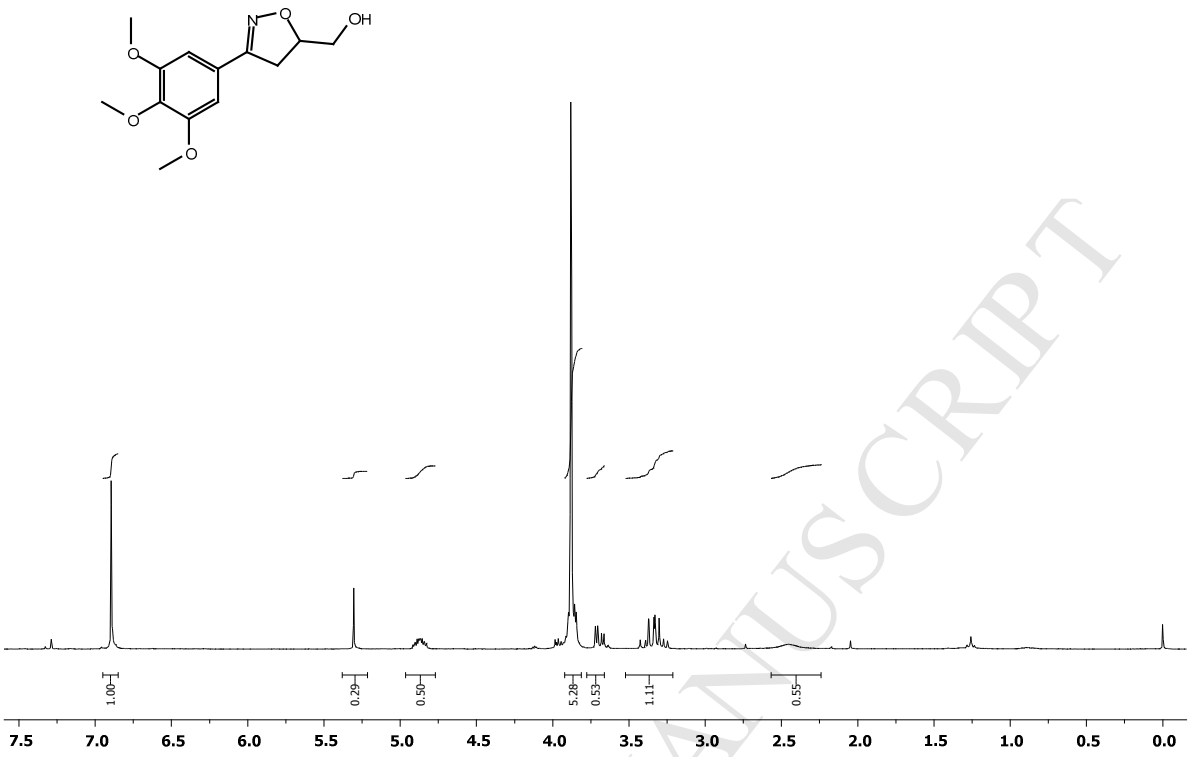
^{13}C-NMR OF COMPOUND **23**



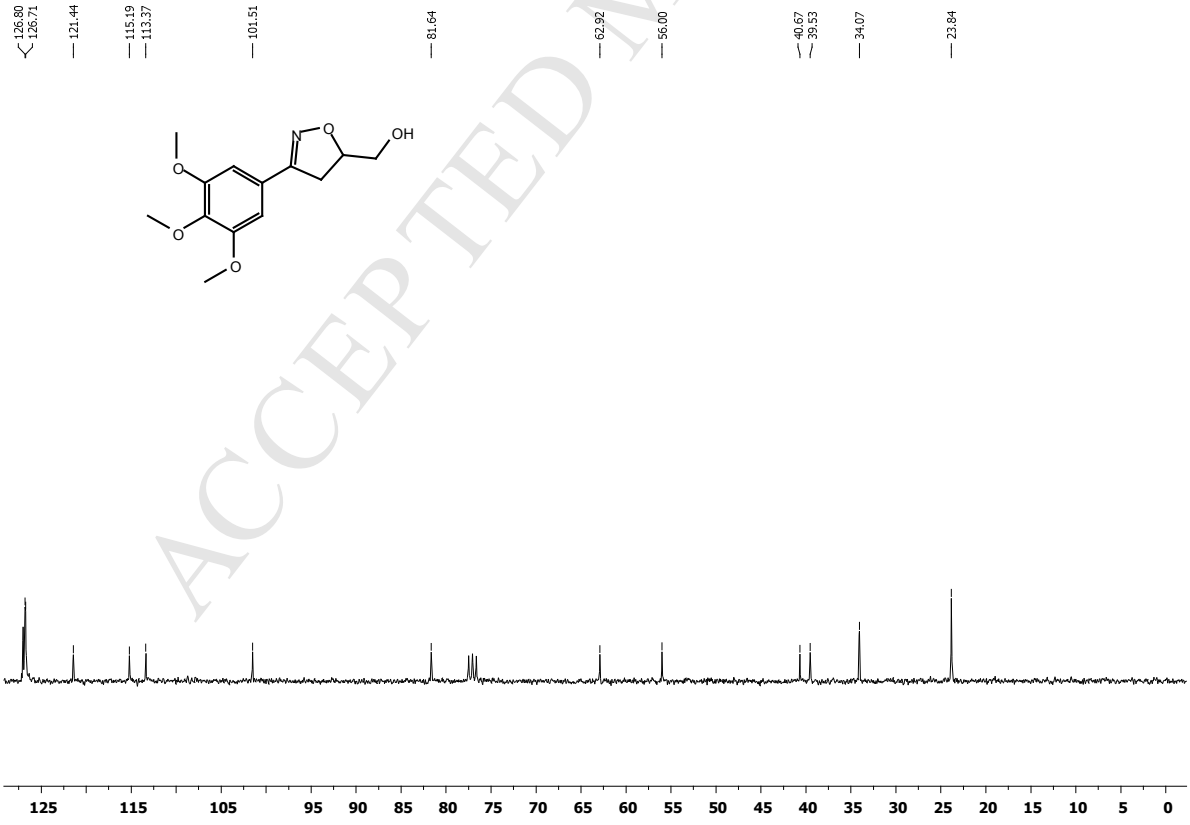
^{1}H-NMR OF COMPOUND **24**



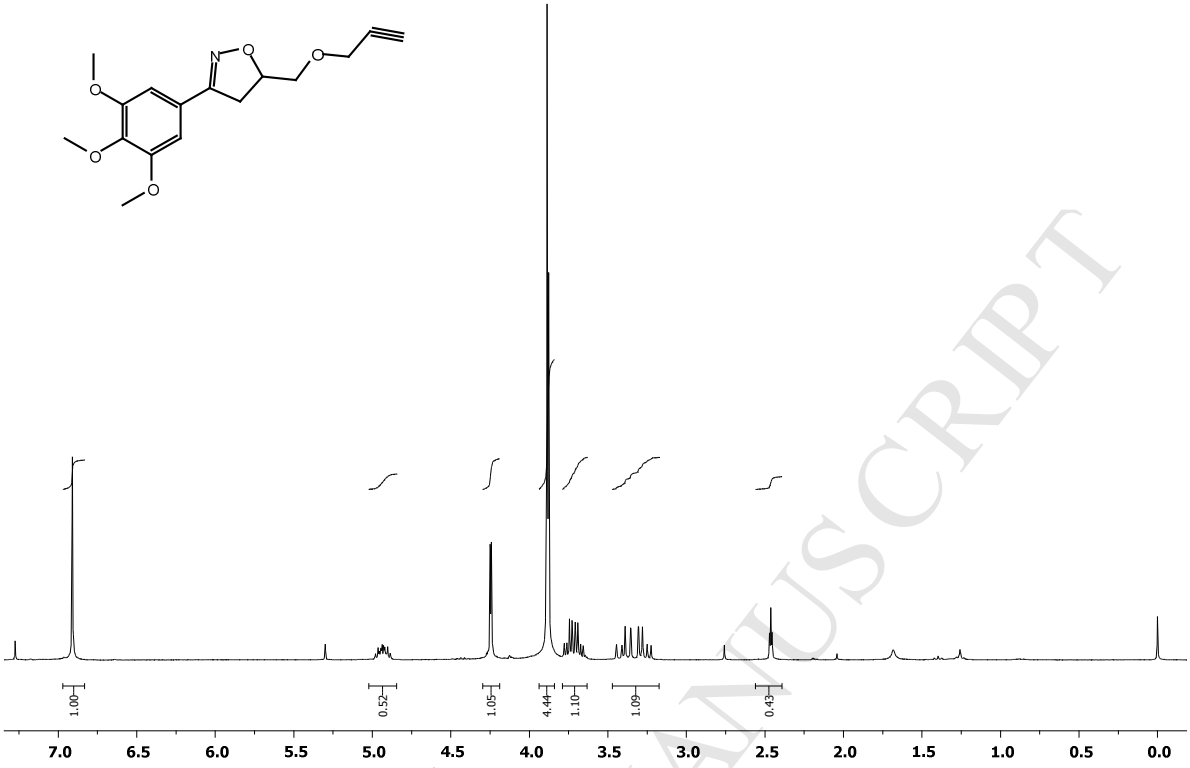
^{13}C-NMR OF COMPOUND **24**



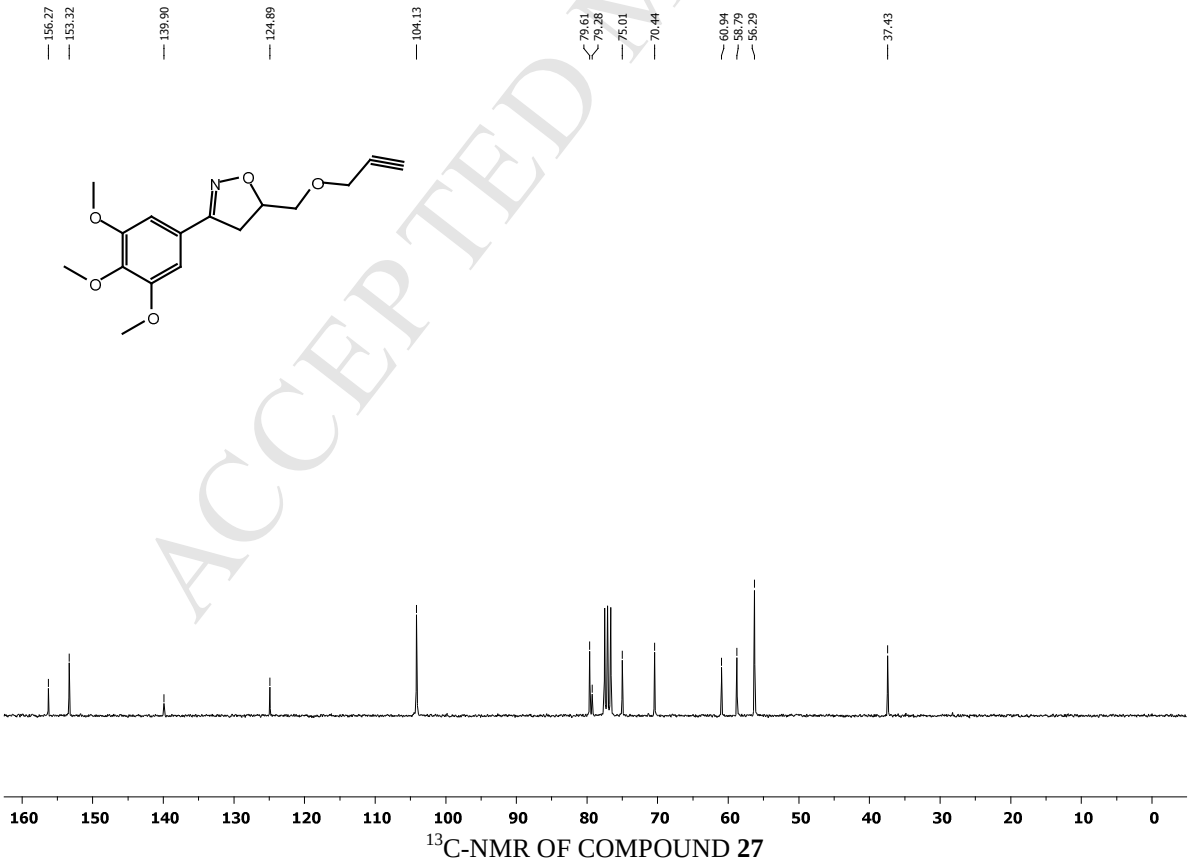
^{1}H-NMR OF COMPOUND **26**



^{13}C-NMR OF COMPOUND **26**



^{1}H-NMR OF COMPOUND **27**



^{13}C-NMR OF COMPOUND **27**