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PII: S0223-5234(14)00458-9

DOI: 10.1016/j.ejmech.2014.05.042

Reference: EJMECH 7000

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 23 October 2013

Revised Date: 21 January 2014

Accepted Date: 14 May 2014

Please cite this article as: N. Devender, K.K.G. Ramakrishna, Hamidullah, S.K. Shukla, R. Konwar, R.P. Tripathi, Synthesis and anticancer activity of γ-(triazolyl ethylidene)butenolides and polyfunctional pyrrolinones, *European Journal of Medicinal Chemistry* (2014), doi: 10.1016/j.ejmech.2014.05.042.

Graphical Abstract

Synthesis and anticancer activity of γ-(triazolyl ethylidene)butenolides and polyfunctional pyrrolinones

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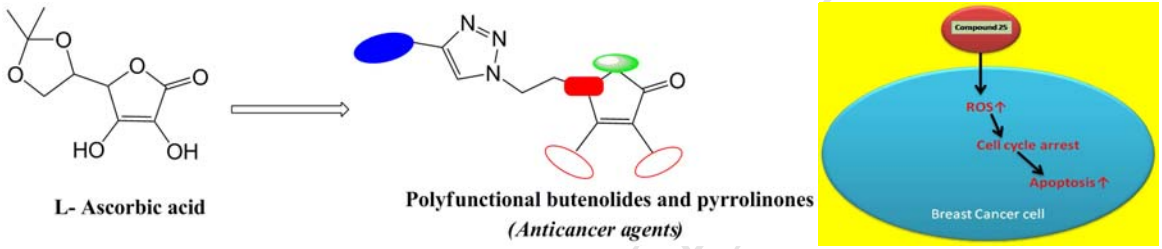
Synthesis and bioevaluation of a novel γ-(triazolyl ethylidene)butenolides and consequent pyrrolinones is reported. In which, compound **25** showed potent activity (IC_{50} = 11.3 µM) against MDA-MB-231 cells *in vitro*.



Synthesis and anticancer activity of γ-(triazolyl ethylidene)butenolides and polyfunctional pyrrolinones

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Abstract: A series of novel γ-(triazolyl ethylidene)butenolides (**4-23**) were prepared from commercially available L-ascorbic acid in good yields. These butenolides on reaction with ethanolic ammonia/amines led to formation of respective 5-hydroxy pyrrolinones (**24-33**). The two of these pyrrolinones on dehydration with *p*-toluenesulfonic acid, were transformed into γ-(triazolyl ethylidene)pyrrolinones (**34, 35**). Among all the newly synthesized hybrid molecules tested for anticancer activity *in vitro,* compounds **24, 25, 26, 27, 28, 30** and **32** showed significant activity against MCF-7, MDA-MB-231, PC-3 or U-937 cells. In particular compound **25** (IC_{50}=11.3 µM) exhibited most potent activity against breast cancer cells and preliminary studies revealed that potency of this compound is due to ROS generation, subsequent activation of p38, leading to apoptosis and inhibition of cancer cells.

Keywords: γ-ethylidenebutenolide, 5-hydroxy pyrrolinone**,** γ-ethylidenepyrrolinone, 1,2,3-triazole, anticancer activity.

1. Introduction

γ-Alkylidenebutenolides (γ-AIBs), an important class of naturally occurring molecules [1-4], are endowed with a wide range of biological activities such as antibacterial, anticancer, antibiotic, and phospholipase A2 inhibition etc [2,5-7]. Due to their biological significance, numerous synthetic strategies have been developed and reviewed by groups of Rao [3,4], Negishi and Kotora [8] and Bruckner [9]. The three extensive synthetic pathways known so far for γ-AIBs are: (1) alkylidenation of five membered heterocycles, such as 2-oxyfuran [10-14], γ-lactones [15-18], and maleic anhydrides [19,20]; (2) cyclization of γ-hydroxy and γ-oxoacids or their equivalents [21,22] such as γ-oxoacylpalladium complexes [23] and (3) lactonization of alk-4-ynoic and alk-4-enoic acids [24-26]. In this endeavour yet another route for γ-AIBs from commercially available L-ascorbic acid has been developed by our group and others [27,28]. Cochinolide (**Fig. 1, A**), naturally occuring γ-alkylidene bicyclic butenolide isolated from the root bark of *Homalium cochinchinensis* shows antiviral activity [29]. γ-AIBs can be transformed efficiently into pyrrolinone derivatives [30,31] and this class of compounds are wide ranging heterocycles as several of them are biologically active compounds, potent pharmaceuticals and natural products [32-34]. 3-Hydroxy-1*H*-pyrrol-2(5*H*)-one analogues possess various fascinating biological activities, such as enzyme inhibition, antiviral and antibacterial activities [35-37]. One of such pyrrolinone derivative ethyl-1-benzyl-4-hydroxy-5-oxo-2,5-dihydro-1*H*-pyrrole-3-carboxylate (**Fig. 1, B**) is a highly specific aldose reductase inhibitor [36]. Since the enhanced activity of aldose reductase is accomplice with increased resistance to chemotherapeutic drugs, it has been accounted that such compounds reinforce the cytotoxic effect of the anticancer agents, doxorubicin and cisplatin in HeLa cervical carcinoma cells [37]. Further, pyrrole-2(5*H*)-one series of compounds are known as blockers of S100 proteins, involving in regulation of the

localization and activity of other proteins by direct protein-protein interactions and have potential therapeutic relevance in cancer chemotherapy. One of such compound 5-(3,5-bis(trifluoromethyl)phenyl)-3-hydroxy-1-(2-hydroxypropyl)-4-(4-methylbenzoyl)-1*H*-pyrrol-2(5*H*)-one (**Fig. 1, C**) is shown to inhibit S100A10 proteins and displays anticancer activity [38].

< *Insert fig. 1 here* >

γ-Alkylidenepyrrolinones, where ring oxygen is replaced by nitrogen in γ-AIBs have distinct biological properties particularly among various other alkylidinepyrrolinones [39-44]. 1,2,3-Triazoles are privileged structures as they act as surrogate of peptide bond and offer intriguing biological activities. In particularly 1,4-disubstituted 1,2,3-triazoles can effectively mimic *trans*-amide bonds because of their similar size, planarity, H-bonding capabilities and dipole moment [45]. Keeping in view the above facts and our continuous efforts on the above mentioned prototypes, we were prompted to synthesize small libraries of novel hybrid molecules consisting of γ-AIBs or pyrrolinones as one pharmacophore and triazoles the other pharmacophore, and evaluate their biological activities. Thus, the present work depicts the synthesis of azido ethylidenebutenolides and their click reaction with different terminal alkynes to give respective triazolyl ethylidenebutenolides. The latter on reaction with ethanolic ammonia or different amines resulted in respective 5-hydroxy pyrrolinones in good yields. Finally, the obtained 5-hydroxy pyrrolinones were dehydrated to the respective triazolyl ethyliedenepyrrolinones by *p*-toluenesulfonic acid (*p*-TSA) catalyzed reaction at ambient temperature. All the newly synthesized hybrid molecules were screened for anticancer activity in the first instance. The ambient reaction conditions, efficient yields, environment friendly protocol and polyfunctional nature of the compounds prepared herein are noteworthy. In addition, these prospective hybrid molecules have inherent potential for other biological activities [46,47].

2. Results and Discussion:

2.1 Chemistry:

The synthesis of title compounds commenced from L-ascorbic acid (vitamin-C), a commercially available inexpensive starting material as shown in **Scheme 1**. Allyl alcohol derivatives (*Z*)-3,4-bis(benzyloxy)-5-(2-hydroxyethylidene)furan-2(5*H*)-one (**1a)** and (*Z*)-5-(2-hydroxyethylidene)-3,4-dimethoxyfuran-2(5*H*)-one (**1b)** were synthesized according to our previous reported procedure [27]. **1a** and **1b** were then converted into tosylated γ-ethylidenebutenolides *Z*-(2)-(3,4-bis(benzyloxy)-5-oxofuran-2(5*H*)-ylidine)ethyl 4-methylbenzenesulfonate (**2a**) and *Z*-(2)-(3,4-dimethoxy-5-oxofuran-2(5*H*)-ylidine)ethyl 4-methylbenzenesulfonate (**2b**) by reacting with *p*-toluenesulfonyl chloride in anhydrous CH_2Cl_2 by using pyridine as a base. Reaction of **2a** and **2b** with sodium azide in DMF at ambient temperature led to formation of the respective azido γ-ethylidenebutenolides (*Z*)-5-(2-azidoethylidene)-3,4-bis(benzyloxy)furan-2(5*H*)-one (**3a**) and (*Z*)-5-(2-azidoethylidene)-3,4-dimethoxyfuran-2(5*H*)-one (**3b**) in good yields. These intermediates (**2a, 2b, 3a** and **3b**) were characterized on the basis of their NMR (^{1}H and ^{13}C) and HRMS data. In IR spectrum, characteristic absorption peaks at 2105 cm^{-1} for **3a** and 2104 cm^{-1} for **3b** conform the formation of azido compounds and served as key intermediate for the synthesis of small library of novel triazolyl γ-ethylidenebutenolides. Azido compounds **3a** and **3b** on Copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction with different aliphatic, aromatic and heteroaromatic terminal alkynes led to 1,4-disubstituted regioselective formation of the respective (*Z*)-3,4-dimethoxy/benzyloxy-5-[2-(4-alkyl/aryl-1*H*-1,2,3-triazol-1-yl) ethylidene] furan-2(5*H*)-ones (**4-23**) in excellent yields (**scheme 1**). Regioisomeric nature of the 4-substituted triazole products were ascertained based on chemical shift value of triazole C-5 in the above compounds appear around δ 120-125 ppm [48].

<Insert Scheme 1 here>

In the next sequence of synthesis, some of the above synthesized triazolyl γ-ethylidine butenolides were reacted with ethanolic ammonia or different aliphatic amines in ethanol or THF as solvent at room temperature to obtain 3,4-dimethoxy/benzyloxy-5-[2-(1*H*-1,2,3-triazol-1-yl)ethyl]-5-hydroxy-1*H*-pyrrol-2(5*H*)-ones (**24-30**) and 3,4-dimethoxy/benzyloxy-5-[2-(1*H*-1,2,3-triazol-1-yl)ethyl]-5-hydroxy-1-alkyl/aralkyl-1*H*-pyrrol-2(5*H*)-ones (**31-33**) respectively in good yields. Reaction of the above 5-hydroxy pyrrolinones **31** and **32** with *p*-TSA in anhydrous CH_2Cl_2 at room temperature led to the formation of (*Z*)-3,4-bis(benzyloxy)-1-propyl-5-(2-(4-propyl-1*H*-1,2,3-triazol-1-yl)ethylidene)-1*H*-pyrrol-2(5*H*)-one (**34**) and (*Z*)-3,4-bis(benzyloxy)-1-octyl-5-(2-(4-(pyridin-2-yl)-1*H*-1,2,3-triazol-1-yl)ethylidene)-1*H*-pyrrol-2(5*H*)-one (**35**) respectively as dehydration products. All the newly synthesized compounds were fully characterized by their IR, NMR (^{1}H and ^{13}C) and HRMS data (experimental section). In NMR spectral data of the compounds, all proton and carbon signals were observed at their usual chemical shift values. Complete chemical shift (^{1}H and ^{13}C NMR) assignments and connectivity (given in supplementary data) of three different prototypes **4**, **31** and **34** (**4→31→34**) were carried out using COSY (correlation spectroscopy), HSQC (hetero nuclear single quantum coherence spectroscopy) and HMBC (hetero nuclear multiple bond correlation spectroscopy).

< Insert fig. 2 here >

All the important HMBC correlations of three ptototype compounds (**4**, **31** and **34**) are shown in **Fig. 2.** HMBC correlation of CH_2-9 to C-5 and CH_2-7 to C-4 in prototype **4** strongly established the formation of 1,4 disubstituted regioisomer in CuAAC reaction. COSY correlations established the four coupled systems H-3' to H-7', H-3" to H-7", CH_2-6 to CH_3-8 and CH_2-9 to CH_2-10 in compound **4,** five coupled systems H-3' to H-7', H-3" to H-7", CH_2-6 to CH_3-8, CH_2-

16 to CH_3-18 and CH_2-9 to CH_2-10 each in compounds **31** and **34.** HMBC correlation of CH_2-16 to C-11 and C-13 in prototype **31** conform the insertion of propyl amine into butenolide ring.

2.2 Biological evaluation

2.2.1 Anti-proliferative effect of compounds

All the newly synthesized compounds (**4-35)** were screened for anticancer activity against cancer cell lines MCF-7 and MDA-MB-231 (breast cancer), PC-3 (prostate cancer), HCT-116 (colorectal carcinoma), U-937 (histiocytic lymphoma) and human embryonic kidney (HEK-293) cells using MTT assay to assess cell proliferation, results are shown in **Table 1**. Of the analogues tested, none of the butenolides (**4-23**) found active which were synthesized initially considering $IC_{50} \leq 20\mu M$ as active. Due to this setback, we anticipated to elaborate synthetic sequence for modifying prototype structures in a way that, some of the butenolide derivatives were transformed into 5-hydroxy pyrrolinones (**24-33**). Among the 5-hydroxypyrrolinones tested for anticancer activity, compounds **25 (**IC_{50}= 11.3μM**)** and **27 (**IC_{50}= 13.1μM**)** showed promising activity specifically against aggressive breast cancer cell lines.

< *Insert table 1 here* >

Further, compounds **28, 30** and **32** exhibited potent cytotoxicity against MCF-7 cancer cell lines. Interestingly, compound **25** was also the most active compound in a series against PC-3 cells. Compounds **24** and **26** showed anticancer activity against majority of the cell line MCF-7, MDA-MB-231 and U-937, but lacks high potency to any specific cell line like compound **25**. Compound **24** was found to be most active against HCT-116, but none of the compounds of this series was found to be significantly active against this colorectal carcinoma cell line. Few compounds of this series was found to be active against lymphoma cell line, U-937 with compound **24** as most active in a series. Among all, compound **25** was the most active analogue

in this series with lowest IC_{50} against MDA-MB-231 cells. In addition, compound 25 was devoid of significant cytotoxicity against normal human embryonic kidney (HEK-293) cells suggesting that compound **25** was the most potent and specifically active in inhibiting growth of MDA-MB-231 cells.

*2.2.2 Compounds **24**, **25** and **26** induces cell cycle arrest.*

Data from MTT assay showed that compounds 24, 25 and 26 induces significant inhibition of MDA-MB-231, MCF-7, PC-3, U-937 and HCT 116 cells (**Fig. 3**). We tried to examine this unrevealed inhibition of cell growth by adopting cell cycle distribution analysis, treated with compounds **24**, **25** and **26**. Results demonstrated that all three compounds induced cell cycle arrest along with an increase of sub-G1 phase as compared to untreated vehicle control. Furthermore, sub-diploid population was increased consistently in all the cell lines, indicating apoptosis.

< Insert fig. 3 here >

*2.2.3 Compound **25** induces apoptosis*

To further confirmation, we explored whether this inhibition of cell growth and cell cycle arrest by these compounds is associated with physiological apoptosis or non-specific necrosis by Annexin-V-FITC and PI staining using flow cytometer. For this, we selected compound **25** as the most potent compound of the series with IC_{50} values of 11.3 μM against MDA-MB-231 cells. Data indicated that compound **25** significantly increased the percentage of apoptotic cells (Annexin-V-positive) in dose-dependent manner, and no significant change was observed in necrotic cells (only PI stained) as compared to untreated vehicle control at 24 h (**Fig. 4**)

< Insert fig. 4 here >

*2.2.4 Compounds **12, 24, 25, 26** and **27** induces reactive oxygen species generation*

Reactive oxygen species (ROS) is a known mediator of apoptosis and many anti-cancer compounds are well known to induce ROS. Therefore, we examined the production of ROS in MDA-MB-231 cells treated with compounds **12, 24, 25, 26** and **27** for 24 h. At the end of incubation, ROS generation was detected using ROS-sensitive probe DCFH-DA using flow cytometer. Data indicated that treatment of compounds **12, 24, 25, 26** and **27** increases ROS level in breast cancer cells (**Fig. 5**). Thus, ROS generation may be the viable explanation for apoptosis induction and inhibition of cell proliferation by these compounds.

< Insert fig. 5 here >

Our results corroborated with previous reports suggesting that ascorbic acid selectively kills cancer cells but not normal cells due to generation of reactive oxygen species [49]. Interestingly, aggressive and metastatic breast cancer model is more prone towards compound **25** mediated killing possibly due to difference in GSH concentration [50], while no cytotoxicity was observed in normal cells due to enrichment of antioxidant enzymes [51]. Since all the synthesised compounds are derivatives of ascorbic acid and majority of this induced ROS generation, probably due to ionization of compound which produces extracellular ROS as observed in ascorbic acid. The diffusion of extra cellular ROS might be involved in mitochondrial damage, depletion of ATP, thereby cell death [52].

*2.2.5 Compound **25** induced ROS mediate apoptosis*

To further validate, whether this ROS increase by these compounds is actually responsible for induction of apoptosis, we treated cancer cells with NAC (a well known ROS inhibitor), followed by treatment of compound **25** for 24 h and measured percent apoptotic cells using flowcytometry based Annexin-FITC and PI staining method. Results showed that ROS is critical for induction of apoptosis by compound **25** in MDA-MB-231 cells as inhibition of ROS by NAC

significantly reduced percent apoptotic cell as compared compound **25** alone (**Fig. 6**). This indicates that ROS generation induced by compound **25** play major role in cancer cells apoptosis.

<Insert fig. 6 here >

*2.2.6 Compound **25** induced ROS leads to p38 activation*

As ROS induced apoptosis commonly involves p38 activation [53], we performed western blot analysis of phospho-p38 in MDA-MB-231 cells after compound **25** treatment. It was found that activation of p38 was activated dose-dependently upon 24 h treatment of compound **25** (**Fig. 7A**). To confirm whether compound **25** induced ROS is leading to activation of p38, cells were pre-treated with NAC followed by compound **25** for 24 h. The activation of p38 was completely abolished in presence of NAC, indicating requirement of ROS for p38 activation (**Fig. 7B**).

<Insert fig. 7 here>

To explore the activity, we moved to transform two of the above 5-hydroxy pyrrolinones into γ-ethylidine pyrrolinones (**34, 35**) and screened for anticancer activity, results showed that none of these two compounds exhibited potent activity (IC_{50} = >46.2 µM). A closure look into the structure activity relationship indicates that unsubstituted 5-hydroxy pyrrolinones (NH free) showed significant activity, while N-substituted 5-hydroxy pyrrolinones inhibit the growth of MCF-7 cell lines at high concentrations (IC_{50} = >22.3 µM). This indicates the remarkable contribution of NH group to the activity by the unsubstituted 5-hydroxy pyrrolinones. All the γ-ethylidenebutenolides found inactive, while corresponding γ-ethylidenepyrrolinones inhibit the cell lines at very high concentrations (IC_{50} = >46.2 µM). Further, substitution on the triazole ring does not showing significant contribution to the activity irrespective of the alkyne used in synthesis.

3. Conclusion

In summary, a novel libraries of γ-(triazolyl ethylidene)butenolides, 5- hydroxy pyrrolinones and γ-(triazolyl ethylidene)pyrrolinones were synthesized and characterized. All these newly synthesized compounds were screened for anticancer activity *in vitro* against six cancer cell lines. Results demonstrated that 5-hydroxy pyrrolinone analogues **24**, **25**, **26**, **27**, **28**, **30** and **32** showed significant anticancer activity against MCF-7, MDA-MB-231, U-937 and PC-3 cell lines, in particular Compound **25** exhibited most potent cytotoxic activity (IC_{50} = 11.3 µM) against MDA-MB-231 cells. Environmentally benign, easily accessible synthesis and significant activity favours these 5-hydroxy pyrrolinones to become great potential in medicinal chemistry.

4. Experimental Section

4.1 Chemistry

Commercially available reagent grade chemicals and solvents were used as received. All reactions were monitored by TLC on E.Merck Kieselgel 60 F_{254}, with detection by UV light, spraying a 20% $KMnO_4$ aq. Solution or exposure to I_2 vapours. Column chromatography was performed on silica gel (60-120 mesh E.Merck). IR spectra were recorded as thin films or on KBr pellets with a PerkinElmer Spectrum RX-1 (4000-450 cm^{-1}) spectrophotometer. The ^{1}H (400 and 300 MHz) and ^{13}C NMR (100, 75 and 50 MHz) spectra were recorded on a Bruker Avance-400, Bruker DRX-300 and Bruker DRX-200 in $CDCl_3$ or DMSO-d_6. Chemical shift values were reported in ppm relative to TMS (tetramethylsilane) as internal reference, unless otherwise stated; s (singlet), d (doublet), t (triplet), dd (doublet of doublet), bs (broad singlet), m (multiplet); *J* in hertz. HRMS spectra were performed using a mass spectrometer Q-TOF. Optical rotation was recorded on Autopol III, S. No. 301, manufactured by Rudolph Research Analytical, USA.

*4.1.1 Typical procedure for the synthesis of intermediates **2a** and **2b***

To a magnetically stirred solution of the allylalcohol (**1a**, **1b**) (1.0 mmol), pyridine (1.5 mmol) in anhyd. CH_2Cl_2 at 0°C, *p*-toluenesulfonyl chloride (1.2 mmol) was added in small fractions. The resulting reaction mixture was stirred at ambient temperature for 12-15 h. After completion of reaction the solvent was evaporated under reduced pressure to give a crude mass which was extracted with ethylacetate and water.The organic layer was dried (anhyd. Na_2SO_4) , evaporated under reduced pressure to get the crude, which was chromatographed (SiO_2, 60-120 mesh) by using hexane: ethylacetate (9.6: 0.4) as eluent to give the desired intermediates.

*4.1.1.1 Z-(2)-(3,4-*bis*(benzyloxy)-5-oxofuran-2(5H)-ylidine)ethyl 4-methyl benzenesulfonate (**2a**)*

It was obtained by the reaction of allyl alcohol **1a** (0.5 g, 1.4 mmol), pyridine (0.18 mL, 2.1 mmol), *p*-toluenesulfonyl chloride (0.34 g, 1.7 mmol) in anhydrous CH_2Cl_2 (25 mL) in 62% yield (0.36 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3425, 3022, 2104, 1774, 1405, 1216; ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.96-7.94 (m, 5H, Ar), 7.44-7.37 (m, 9H, Ar), 5.50 (t, *J* = 8.28 Hz, 1H, C=CH), 5.26 (s, 2H, OCH_2), 5.21 (s, 2H, OCH_2), 4.31 (d, *J* = 8.28 Hz, 2H, CH_2), 2.51 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 163.9, 147.9, 143.9, 135.8, 135.4, 129.0, 128.9, 128.8, 127.8, 123.9, 103.9, 74.2, 73.5, 36.6, 29.8; ESI-HRMS calcd for $C_{27}H_{25}O_7S$ $[M + H]^+$ 493.1321 found 493.1316.

*4.1.1.2 Z-(2)-(3,4-dimethoxy-5-oxofuran-2(5H)-ylidine)ethyl 4-methylbenzenesulfonate (**2b**)*

It was obtained by the reaction of the allyl alcohol **1b** (0.5 g, 2.6 mmol), pyridine (0.32 mL, 4.0 mmol), *p*-toluenesulfonyl chloride (0.61 g, 3.2 mmol) in anhyd.CH_2Cl_2 (25 mL) in 59% yield as white solid (0.48 g); mp 110-112 °C; IR (KBr) ν_{max} cm^{-1}: 3432, 2931, 2400, 1648, 1332, 1049; ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.86 (d, *J* = 8.48 Hz, 2H, Ar), 7.35 (d, *J* = 8.52 Hz, 2H, Ar), 5.38 (t, *J* = 8.28 Hz, 1H, C=CH), 4.22 (d, *J* = 8.28 Hz, 2H, CH_2), 4.07 (s, 3H, OCH_3), 3.86 (s, 3H,

OCH_3), 2.42 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 163.7, 148.2, 143.8, 125.4, 103.8, 60.4, 59.5, 36.6; ESI-HRMS calcd for $C_{15}H_{17}O_7S$ $[M + H]^+$ 341.1656 found 341.1648.

*4.1.2 Typical procedure for the synthesis of intermediates **3a, 3b***

To a magnetically stirred solution of the above synthesized allyl tosylates (**2a, 2b**) (1.0 mmol) in DMF solvent, NaN_3 (1.0 mmol) was added. The resulting mixture was stirred at ambient temperature for 5-6 h. After completion, extracted the reaction mixture with ethylacetate and water. The organic layer was dried, evaporated under reduced pressure to get the crude, which was chromatographed (SiO_2, 60-120 mesh) by using hexane: ethylacetate (9.5: 0.5) as eluent to give the desired intermediates.

*4.1.2.1 (Z)-5-(2-Azidoethylidene)-3,4-bis(benzyloxy)furan-2(5H)-one (**3a**)*

It was obtained by the reaction of allyl tosylate **2a (**0.5 g, 1.0 mmol), NaN_3 (0.13 g, 2.0 mmol) in DMF (10 mL) in 68% yield (0.25 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3427, 2960, 2105, 1774, 1406, 1216; ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.36-7.33 (m, 8H, Ar), 7.24-7.20 (m, 2H, Ar), 5.35 (t, *J* = 7.65 Hz, 1H, C=CH), 5.24 (s, 2H, OCH_2), 5.19 (s, 2H, OCH_2), 4.03 (d, *J* = 7.56 Hz, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 164.0, 147.8, 144.5, 135.8, 135.5, 129.0, 128.9, 128.8, 127.8, 123.8, 101.6, 74.2, 73.5, 45.3; ESI-HRMS calcd for $C_{20}H_{18}N_3O_4$ $[M + H]^+$ 364.1297 found 364.1292.

*4.1.2.2 (Z)-5-(2-azidoethylidene)-3,4-dimethoxyfuran-2(5H)-one (**3b**)*

It was obtained by the reaction of allyl tosylate **2b** (0.5g, 1.4 mmol), NaN_3 (0.19 g, 2.9 mmol) in DMF (10 mL) as white solid in 71% yield (0.22 g); mp 108-110 °C; IR (KBr) ν_{max} cm^{-1}: 3431, 3021, 2104, 1660, 1216, 969; ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.30 (t, *J* = 7.68 Hz, 1H, C=CH), 4.08 (s, 3H, OCH_3), 3.97 (d, *J* = 7.65 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 50

MHz) δ: 163.7, 148.2, 143.8, 125.4, 103.8, 60.4, 59.5, 36.6; ESI-HRMS calcd for $C_8H_{10}N_3O_4$ [M + H]$^+$ 212.0671 found 212.0672.

*4.1.3 Typical procedure for the synthesis of compounds **4-23***

To a magnetically stirred solution of the above allyl azide (**3a, 3b)** (1.0 mmol) in *t*-BuOH: H_2O (1: 1 mL) as a solvent, at ambient temperature, $CuSO_4.5H_2O$ (7 mg for **3a**, 12 mg for **3b,** 2 mol%), sodium ascorbate (13 mg for **3a**, 23 mg for **3b**, 5mol%), and benzoic acid (8 mg for **3a**, 14 mg for **3b,** 5 mol%) were sequentially added followed by addition of terminal alkyne (1.0 mmol). The resulting mixture was stirred for 1 h, till the completion of reaction (TLC). After completion, reaction mixture was extracted with ethylacetate and H_2O, then organic layer was dried (anhydrous Na_2SO_4) and evaporated under reduced pressure to give the crude mass, which was chromatographed (SiO_2, 60-120 mesh) by using hexane: ethylaceate (7: 3) as eluent to give the desired products.

*4.1.3.1 (Z)-3,4-bis(benzyloxy)-5-(2-(4-ethyl-1H-1,2,3-triazol-1-yl)ethylidene)furan-2(5H)-one (**4**)*

It was obtained by the reaction of azide **3a** (0.5 g, 1.3 mmol), 1-pentyne (0.13 mL, 1.3 mmol) in 92 % yield (0.54 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3405, 3019, 2963, 1653, 1217, 1125; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.39-7.33 (m, 9H, 8 Ar-H+ 1 triazolyl-H), 7.21-7.19 (m, 2H, Ar), 5.53 (t, *J* = 7.64 Hz, 1H, C=CH), 5.23 (s, 2H, OCH_2), 5.21 (s, 2H, OCH_2), 5.16 (d, *J* = 7.56 Hz, 2H, CH_2), 2.67 (t, *J* = 7.56 Hz, 2H, CH_2), 1.70-1.65 (m, 2H, CH_2), 0.96 (t, *J* = 7.32 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 163.8, 148.8, 147.8, 144.5, 135.7, 135.2, 129.0, 129.0, 128.9, 128.8, 128.1, 127.8, 123.9, 120.8, 101.2, 74.2, 73.6, 44.5, 27.7, 22.7, 13.9; ESI-HRMS calcd for $C_{25}H_{27}N_3O_4$ [M + H]$^+$ 432.1923 found 432.1918.

*4.1.3.2 (Z)-3,4-bis(benzyloxy)-5-(2-(4-ethyl-1H-1,2,3-triazol-1-yl)ethylidene)furan-2(5H)-one (**5**)*

It was obtained by the reaction of azide **3a** (0.5 g, 1.3 mmol), 1-hexyne (0.16 mL, 1.3 mmol) in 94% yield (0.57 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3463, 2928, 1778, 1457, 1218, 1129; ^1H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 7.83 (s, 1H, triazolyl), 7.39-7.28 (m, 10H, Ar), 5.57 (t, *J* = 7.56 Hz, 1H, C=CH), 5.28 (s, 2H, OCH_2), 5.14-5.11 (m, 4H, OCH_2+ allylic CH_2), 2.54 (t, *J* = 7.44 Hz, 2H, CH_2), 1.56-1.46 (m, 2H, CH_2), 1.30-1.20 (m, 2H, CH_2), 0.83 (t, *J* = 7.29 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 163.5, 147.8, 143.0, 135.6, 135.3, 129.1, 128.7, 128.6, 128.5, 128.4, 127.8, 123.3, 121.8, 102.1, 73.9, 72.9, 43.8, 30.9, 24.5, 21.5, 13.5; ESI-HRMS calcd for $C_{26}H_{28}N_3O_4$ $[M + H]^+$ 446.2080 found 446.2075.

4.1.3.3 (Z)-3,4-bis(benzyloxy)-5-(2-(4-pentyl-1H-1,2,3-triazol-1-yl)ethylidene)furan-2(5H)-one ***(6)***

It was obtained by the reaction of azide **3a** (0.5 g, 1.3 mmol), 1-heptyne (0.18 mL, 1.3 mmol) in 92% yield (0.58 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3444, 2929, 1777, 1652, 1218, 1129; ^1H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 7.91 (s, 1H, triazolyl), 7.48-7.36 (m, 10H, Ar), 5.65 (t, *J* = 7.56 Hz, 1H, C=CH), 5.36 (s, 2H, OCH_2), 5.21-5.19 (m, 4H, OCH_2+ CH_2), 2.62 (t, *J* = 7.50 Hz, 2H, CH_2), 1.65-1.56 (m, 2H, CH_2), 1.33-1.26 (m, 4H, $2CH_2$), 0.89 (t, *J* = 6.72 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 163.9, 149.0, 147.8, 144.5, 135.7, 135.2, 130.2, 129.0, 129.0, 128.9, 128.9, 128.8, 127.9, 123.9, 120.8, 101.2, 74.2, 73.6, 44.6, 31.5, 29.8, 29.2, 25.7, 22.5, 14.1; ESI-HRMS calcd for $C_{27}H_{30}N_3O_4$ $[M + H]^+$ 460.2236 found 460.2212.

*4.1.3.4 (Z)-5-(2-(4-hexyl-1H-1,2,3-triazol-1-yl)ethylidene)-3,4-dimethoxyfuran-2(5H)-one (**7**)*

It was obtained by the reaction of azide **3b** (0.5 g, 2.3 mmol), 1-octyne (0.33 mL, 2.3 mmol) in 93% yield (0.70 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3421, 2857, 1642, 1395, 1215, 1107; ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.29 (s,1H, triazolyl), 5.51 (t, 1H, *J* = 7.56 Hz, C=CH), 5.16 (d, *J* = 7.56 Hz, 2H, CH_2), 4.12 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 2.69 (t, *J* = 7.47 Hz, 2H, CH_2),

1.64-1.62 (m, 2H, CH_2), 1.30-1.25 (m, 6H, $3CH_2$), 0.87 (t, *J* = 5.34 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 170.2, 163.3, 148.7, 147.8, 144.4, 133.1, 130.3, 130.1, 128.3, 125.2, 120.8, 100.7, 96.2, 60.1, 59.4, 44.7, 31.6, 29.4, 29.0, 25.6, 22.6, 14.2; ESI-HRMS calcd for $C_{16}H_{24}N_3O_4$ $[M + H]^+$ 322.1767 found 322.1761.

*4.1.3.5(Z)-5-(2-(4-(1-hydroxyhexyl)-1H-1,2,3-triazol-1-yl)ethylidene)-3,4-dimethoxyfuran-2(5H)-one (**8**)*

It was obtained by the reaction of azide **3b** (0.5 g, 2.3 mmol), 1-octyne-3-ol (0.33 mL, 2.3 mmol) in 92% yield (0.73 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3782, 2856, 1692, 1464, 1215, 995; ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 7.61 (s, 1H, triazolyl), 5.48 (t, *J* = 7.62 Hz, 1H, C=CH), 5.13 (d, *J* = 7.53 Hz, 2H, CH_2), 4.82 (bs, 1H, OH), 4.65 (t, *J* = 5.73, 1H, C**H**OH), 4.10 (s, 1H, OCH_3), 3.87 (s, 1H, OCH_3), 1.70 (t, *J* = 4.92 Hz, 2H, CH_2), 1.35-1.21 (m, 6H, $3CH_2$), 0.83 (t, *J* = 5.28 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 162.0, 151.8, 146.9, 142.8, 123.9, 119.9, 99.9, 94.9, 65.1, 59.1, 58.3, 43.1, 36.5, 30.5, 23.9, 21.4, 13.0; ESI-HRMS calcd for $C_{16}H_{24}N_3O_5$ $[M + H]^+$ 338.1716 found 338.1713.

*4.1.3.6 (Z)-5-(2-(4-(2-hydroxyethyl)-1H-1,2,3-triazol-1-yl)ethylidene)-3,4-dimethoxyfuran-2(5H)-one (**9**)*

It was obtained by the reaction of azide **3b** (0.5 g, 2.3 mmol), 5-hexyne-1-ol (0.25 mL, 2.3 mmol) in 94% yield (0.68 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3782, 2935, 1778, 1363, 1218, 1060; ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 7.23 (s, 1H, triazolyl), 5.35 (t, *J* = 7.47 Hz, 1H, C=CH), 5.00 (d, *J* = 7.47 Hz, 2H, CH_2), 3.99 (s, 1H, OCH_3), 3.79 (s,1H, OCH_3), 3.42 (t, *J* = 6.09 Hz, 2H, CH_2), 2.98 (bs, 1H, OH), 2.55 (t, *J* = 6.93 Hz, 2H, CH_2), 1.60-1.42 (m, 4H, $2CH_2$); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 162.8, 148.1, 147.5, 143.9, 124.9, 120.5, 100.5, 95.9,

61.3, 59.8, 59.1, 44.0, 31.9, 25.4, 25.1; ESI-HRMS calcd for $C_{14}H_{20}N_3O_5$ $[M + H]^+$ 310.1403 found 310.1396.

*4.1.3.7 (Z)-3,4-bis(benzyloxy)-5-(2-(4-(2-hydroxyethyl)-1H-1,2,3-triazol-1-yl)ethylidene)furan-2(5H)-one (**10**)*

It was obtained by the reaction of azide **3a** (0.5 g, 1.3 mmol), 5-hexyne-1-ol (0.15 mL, 1.3 mmol) in 95% yield (0.60 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3436, 2939, 1305, 1129, 1058; ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.38 (s, 1H, triazolyl), 5.33 (t, *J* = 7.62 Hz, 1H, C=CH), 5.23 (s, 2H, OCH_2), 5.21 (s, 2H, OCH_2), 5.16 (d, *J* = 5.67 Hz, 2H, CH_2), 3.66 (t, *J* = 4.77 Hz, 2H, CH_2OH), 2.75-2.69 (m, 2H, CH_2), 1.77-1.71 (m, 2H, CH_2), 1.66-1.59 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 163.9, 148.5, 147.8, 144.5, 135.6, 135.2, 129.0, 128.9, 128.9, 128.8, 127.8, 123.8, 121.0, 101.0, 74.2, 73.6, 62.3, 44.6, 32.1, 25.6, 25.3; ESI-HRMS calcd for $C_{26}H_{28}N_3O_5$ $[M + H]^+$ 462.2029 found 462.2023.

*4.1.3.8 (Z)-5-(2-(4-benzyl-1H-1,2,3-triazol-1-yl)ethylidene)-3,4-dimethoxyfuran-2(5H)-one (**11**)*

It was obtained by the reaction of azide **3b** (0.5 g, 2.3 mmol), 3-phenyl-1-propyne (0.28 mL, 2.3 mmol) in 96% yield (0.74 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3405, 3016, 2922, 1692, 1217, 1061; ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.29-7.20 (m, 6H, 5 Ar-H+ 1 triazolyl-H), 5.49 (t, *J* = 7.56 Hz, 1H, C=CH), 5.14 (d, *J* = 7.53 Hz, 2H, CH_2), 4.11 (s, 3H, OCH_3), 4.06 (s, 2H, CH_2Ph), 3.93 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 163.5, 148.0, 147.9, 144.3, 138.9, 128.7, 128.6, 126.5, 125.2, 121.4, 100.6, 60.2, 59.4, 44.5, 32.2; ESI-HRMS calcd for $C_{17}H_{18}N_3O_4$ $[M + H]^+$ 328.1297 found 328.1286.

*4.1.3.9 (Z)-5-(2-(4-benzyl-1H-1,2,3-triazol-1-yl)ethylidene)-3,4-bis(benzyloxy)furan-2(5H)-one (**12**)*

It was obtained by the reaction of azide **3a** (0.5 g, 1.3 mmol), 3-phenyl-1-propyne (0.17 mL, 1.3 mmol) in 95% yield (0.62 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3422, 3069, 1777, 1497, 1217, 990; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.38-7.17 (m, 16H, 15 Ar-H+ 1 triazolyl-H), 5.50 (t, *J* = 7.60 Hz, 1H, C=CH), 5.21 (s, 2H, OCH_2), 5.19 (s, 2H, OCH_2), 5.13 (d, *J* = 7.56 Hz, 2H, CH_2), 4.06 (s, 2H, CH_2Ph); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 163.6, 148.0, 147.6, 144.4, 138.9, 135.5, 135.1, 128.8, 128.7, 128.7, 128.6, 128.6, 127.7, 126.5, 123.8, 121.4, 100.8, 74.0, 73.4, 44.5, 32.2; ESI-HRMS calcd for $C_{29}H_{26}N_3O_4$ $[M + H]^+$ 480.1923 found 480.1918.

*4.1.3.10 (Z)-3,4-dimethoxy-5-(2-(4-phenyl-1H-1,2,3-triazol-1-yl)ethylidene)furan-2(5H)-one (**13**)*

It was obtained by the reaction of azide **3b** (0.5 g, 2.3 mmol), phenyl acetylene (0.25 mL, 2.3 mmol) in 94% yield (0.69 g) as white solid; mp 115-117 °C, IR (KBr) ν_{max} cm^{-1}: 3435, 2117, 1775, 1654, 1306, 1116; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.79-7.78 (m, 3H, 2 Ar-H+ 1 triazolyl-H), 7.41-7.28 (m, 3H, Ar), 5.55 (t, *J* = 7.71 Hz, 1H, C=CH), 5.23 (d, *J* = 7.62 Hz, 2H, CH_2), 4.12 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 163.3, 148.2, 147.9, 144.6, 130.5, 130.2, 128.9, 128.3, 125.8, 125.4, 119.7, 100.6, 96.3, 60.2, 59.5, 44.6; ESI-HRMS calcd for $C_{16}H_{16}N_3O_4$ $[M + H]^+$ 314.1141 found 314.1128.

*4.1.3.11 (Z)-3,4-bis(benzyloxy)-5-(2-(4-phenyl-1H-1,2,3-triazol-1-yl)ethylidene)furan-2(5H)-one (**14**)*

It was obtained by the reaction of azide **3a** (0.5 g, 1.3 mmol), phenyl acetylene (0.15 mL, 1.3 mmol) in 96% yield (0.61 g) as colourless oil; IR (neat) ν_{max} cm^{-1} 3438, 2086, 1776, 1305, 1220, 1125; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.82-7.78 (m, 3H, 2 Ar-H+1 triazolyl-H), 7.39-7.28 (m, 11H, Ar), 7.22-7.21 (m, 2H, Ar), 5.59 (t, *J* = 7.62 Hz, 1H, C= CH), 5.24-5.23 (m, 6H, 2×OCH_2+ CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 163.5, 148.3, 147.6, 144.8, 135.7, 135.2, 130.5, 130.3,

129.0, 128.9, 128.8, 128.5, 128.3, 127.9, 125.9, 123.9, 119.7, 100.8, 96.3, 74.1, 73.6, 44.7; ESI-HRMS calcd for $C_{28}H_{24}N_3O_4$ $[M + H]^+$ 466.1767 found 466.1754.

*4.1.3.12 (Z)-5-(2-(4-(4-tert-butylphenyl)-1H-1,2,3-triazol-1-yl)ethylidene)-3,4-dimethoxyfuran-2(5H)-one (**15**)*

It was obtained by the reaction of azide **3b** (0.5 g, 2.3 mmol), 4-*tert*-butyl phenylacetylene (0.41 mL, 2.3 mmol) in 95% yield (0.83 g) as white solid; mp 106-109 °C; IR (KBr) ν_{max} cm^{-1}: 3018, 1779, 1660, 1217, 1119, 968; ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.76-7.71 (m, 3H, 2 Ar-H+ 1 triazolyl-H), 7.48-7.40 (m, 2H, Ar), 5.57 (t, *J* = 7.47 Hz, 1H, C=CH), 5.24 (d, *J* = 7.59 Hz, 2H, CH_2), 4.14 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 1.35 (s, 9H, $3CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 163.6, 151.3, 148.3, 147.7, 144.8, 135.7, 135.3, 130.4, 129.1, 129.0, 128.9, 128.5, 127.9, 125.8, 125.7, 124.0, 119.4, 100.9, 96.3, 74.6, 74.1, 44.7, 34.9, 31.6, 29.9; ESI-HRMS calcd for $C_{20}H_{24}N_3O_4$ $[M + H]^+$ 370.1767 found 370.1760.

*4.1.3.13 (Z)-3,4-bis(benzyloxy)-5-(2-(4-(4-tert-butylphenyl)-1H-1,2,3-triazol-1yl)ethylidene) furan-2(5H)-one (**16**)*

It was obtained by the reaction of azide **3a** (0.5 g, 1.3 mmol), 4-*tert*-butyl phenylacetylene (0.25 mL, 1.3 mmol) in 96% yield (0.68 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 2959, 1777, 1457, 1218, 1123, 977; ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.75-7.73 (m, 3H, 2 Ar-H+ 1 triazolyl-H), 7.45-7.33 (m, 10H, Ar), 7.22-7.21 (m, 2H, Ar), 5.60 (t, *J* = 7.62 Hz, 1H, C=CH), 5.25-5.24 (m, 6H, $2\times OCH_2$+ CH_2), 1.37 (s, 9H, $3CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 163.2, 151.2, 148.2, 147.8, 144.6, 127.8, 125.8, 125.6, 119.4, 100.7, 96.3, 60.2, 59.5, 44.6, 34.8, 31.5; ESI-HRMS calcd for $C_{32}H_{32}N_3O_4$ $[M + H]^+$ 522.2393 found 522.2384.

*4.1.3.14 (Z)-3,4-dimethoxy-5-(2-(4-(pyridin-2-yl)-1H-1,2,3-triazol-1-yl)ethylidene)furan-2(5H)-one (**17**)*

It was obtained by the reaction of azide **3b** (0.5 g, 2.3 mmol), 2-ethynyl pyridine (0.23 mL, 2.3 mmol) in 92% yield (0.68 g) as white solid; mp 143-145 ^{o}C; IR (KBr) ν_{max} cm^{-1}: 3017, 2926, 1780, 1660, 1217, 1062; ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 8.62-8.60 (m, 2H, 1 Ar-H+ 1 triazolyl-H), 8.04-8.02 (m, 1H, Ar), 7.91-7.86 (m, 1H, Ar), 7.36-7.32 (m, 1H, Ar), 5.70 (t, *J* = 7.59 Hz, 1H, C=CH), 5.31 (d, *J* = 7.5 Hz, 2H, CH_2), 4.12 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 163.5, 150.2, 149.5, 148.8, 148.0, 145.0, 137.1, 125.5, 123.1, 122.1, 120.4, 100.1, 60.4, 59.6, 44.8, 29.8; ESI-HRMS calcd for $C_{15}H_{15}N_4O_4$ $[M + H]^+$ 315.1093 found 315.1089.

*4.1.3.15 (Z)-3,4-bis(benzyloxy)-5-(2-(4-(pyridin-2-yl)-1H-1,2,3-triazol-1-yl)ethylidene)furan-2(5H)-one (**18**)*

It was obtained by the reaction of azide **3a** (0.5 g, 1.3 mmol), 2-ethynyl pyridine (0.14 mL, 1.3 mmol) in 94% yield (0.60 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3436, 2925, 2141, 1776, 1125, 1058; ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 8.61 (s, 1H, triazolyl), 8.56-8.55 (m, 1H, Ar), 8.00-7.98 (m, 1H, Ar), 7.87-7.82 (m, 1H, Ar), 7.39-7.30 (m, 11H, Ar), 5.70 (t, *J* = 7.53 Hz, 1H, C=CH), 5.29-5.26 (m, 4H, OCH_2+ CH_2), 5.13 (s, 2H, OCH_2); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 163.5, 149.5, 147.9, 143.3, 137.1, 135.6, 135.3, 128.7, 128.6, 128.5, 128.5, 128.4, 127.8, 123.3, 122.9, 119.3, 101.8, 74.0, 73.0, 44.2; ESI-HRMS calcd for $C_{27}H_{23}N_4O_4$ $[M + H]^+$ 467.1719 found 467.1714.

*4.1.3.16 (Z)-5-(2-(4-cyclopropyl-1H-1,2,3-triazol-1-yl)ethylidene)-3,4-dimethoxyfuran-2(5H)-one (**19**)*

It was obtained by the reaction of azide **3b** (0.5 g, 2.3 mmol), cyclopropyl acetylene (0.19 mL, 2.3 mmol) in 95% yield (0.70 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3436, 2854, 2078, 1779, 1217, 1119; ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 7.84 (s, 1H, triazolyl), 5.58 (t, *J* = 7.56

Hz, 1H, C=CH), 5.14 (d, *J* = 7.53 Hz, 2H, CH_2), 4.12 (s, 1H, OCH_3), 3.85 (s, 1H, OCH_3), 1.97-1.88 (m, 1H, CH), 0.91-0.85 (m, 2H, CH_2), 0.72- 0.70 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 163.7, 150.8, 148.1, 144.4, 125.4, 119.8, 114.2, 100.9, 60.4, 59.5, 44.5, 29.8, 7.8, 6.7; ESI-HRMS calcd for $C_{13}H_{16}N_3O_4$ $[M + H]^+$ 278.1141 found 278.1145.

*4.1.3.17 (Z)-3,4-bis(benzyloxy)-5-(2-(4-cyclopropyl-1H-1,2,3-triazol-1-yl)ethylidene)furan-2(5H)-one (**20**)*

It was obtained by the reaction of azide **3a** (0.5 g, 1.3 mmol), cyclopropyl acetylene (0.12 mL, 1.3 mmol) in 96% yield (0.56 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3436, 3019, 2143, 1777, 1218, 1059; ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 7.83 (s, 1H, triazolyl), 7.42-7.33 (m, 10H, Ar), 5.59 (t, *J* = 7.44 Hz, 1H, C=CH), 5.32 (s, 2H, OCH_2), 5.16-5.12 (m, 4H, OCH_2+ allyl CH_2), 1.95-1.87 (m, 1H, CH), 0.88-0.86 (m, 2H, CH_2), 0.69-0.68 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 163.8, 150.7, 147.8, 144.5, 135.6, 135.2, 133.2, 130.1, 129.0, 128.9, 128.9, 128.8, 128.7, 128.4, 127.8, 123.8, 119.9, 101.1, 74.2, 73.5, 44.5, 29.7, 7.8, 6.7; ESI-HRMS calcd for $C_{25}H_{23}N_3O_4$ $[M + H]^+$ 430.1767 found 430.1750.

*4.1.3.18 (Z)-5-(2-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)ethylidene)-3,4-dimethoxyfuran- 2-(5H)-one (**21**)*

It was obtained by the reaction of azide **3b** (0.5 g, 2.3 mmol), propargyl alcohol (0.12 mL, 2.3 mmol) in 90% yield (0.57 g) as white solid; mp 133-136 ^oC; IR (KBr) ν_{max} cm^{-1}: 3395, 2922, 1769, 1308, 1119, 992; ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 7.63 (s, 1H, triazolyl), 5.51 (t, *J* = 7.62 Hz, 1H, C=CH), 5.19 (d, *J* = 7.62 Hz, 2H, CH_2), 4.68 (s, 2H, CH_2OH), 4.14 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 163.1, 148.5, 147.7, 143.9, 124.8, 121.5, 100.3, 59.9, 59.1, 55.7, 44.0; ESI-HRMS calcd for $C_{11}H_{14}N_3O_5$ $[M + H]^+$ 268.0933 found 268.0928.

*4.1.3.19 (Z)-3,4-bis(benzyloxy)-5-(2-(4-p-tolyl-1H-1,2,3-triazol-1-yl)ethylidene)furan-2(5H)-one (**22**)*

It was obtained by the reaction of azide **3a** (0.5 g, 1.3 mmol), 4-ethynyl toluene (0.17 mL, 1.3 mmol) in 92% yield (0.60 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3401, 3018, 1778, 1366, 1215, 848; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.68-7.64 (m, 3H, 2 Ar-H+ 1 triazolyl-H), 7.36-7.29 (m, 9H, Ar), 7.18-7.16 (m, 4H, Ar), 5.54 (t, *J* = 5.73 Hz, 1H, C=CH), 5.20-5.17 (m, 6H, 2×OCH_2+ CH_2), 2.35 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 163.5, 148.4, 147.6, 144.8, 137.9, 135.7, 135.2, 129.6, 129.1, 129.0, 128.9, 128.8, 127.9, 125.8, 124.0, 119.3, 100.8, 96.3, 74.1, 73.6, 44.6, 21.5; ESI-HRMS calcd for $C_{29}H_{26}N_3O_4$ $[M + H]^+$ 480.1923 found 480.1918.

*4.1.3.20 (Z)-3,4-bis(benzyloxy)-5-(2-(4-cyclohexenyl-1H-1,2,3-triazol-1-yl)ethylidene)furan-2(5H)-one (**23**)*

It was obtained by the reaction of azide **3a** (0.5 g, 1.3 mmol), 1-ethynyl cyclohexene (0.16 mL, 1.3 mmol) in 95% yield (0.61 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3386, 2933, 1777, 1216, 1057, 922; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.37-7.30 (m, 9H, 8 Ar-H+ 1 triazolyl-H), 7.18-7.17 (m, 2H, Ar), 6.46 (t, *J* = 6.3 Hz, 1H), 5.51 (t, *J* = 7.64 Hz, 3H, C=CH), 5.21 (s, 2H, OCH_2), 5.20 (s, 2H, OCH_2), 5.16 (d, *J* = 7.60 Hz, 2H, CH_2), 2.36-2.34 (m, 2H, CH_2), 2.18-2.17 (m, 2H, CH_2), 1.78-1.62 (m, 4H, $2CH_2$), ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 163.3, 147.4, 144.4, 135.5, 135.1, 133.2, 128.8, 128.7, 128.6, 128.3, 127.7, 123.7, 100.8, 96.1, 73.9, 73.4, 44.5, 26.2, 25.3, 22.4, 22.2; ESI-HRMS calcd for $C_{28}H_{28}N_3O_4$ $[M + H]^+$ 470.2080 found 470.2074.

*4.1.4 Typical procedure for the synthesis of compounds **24-30***

A solution of the above synthesized triazole compound (1.0 mmol) in ethanolic ammonia (10-15 mL) was magnetically stirred at ambient temperature for 6-10 h in a sealed vessel. The excess of ammonia and solvent were evaporated under reduced pressure, then extracted the crude with

ethylacetate, H_2O. The organic layer was dried (anhydrous Na_2SO_4), evaporated under reduced pressure, then chromatographed (SiO_2, 60-120 mesh) by using hexane: ethylacetate (6: 4) as eluent to give the desired products.

*4.1.4.1 5-hydroxy-3,4-dimethoxy-5-(2-(4-phenyl-1H-1,2,3-triazol-1-yl)ethyl)-1H-pyrrol-2(5H)-one (**24**)*

It was obtained by using the compound **13** (0.5 g, 1.59 mmol) in 92% yield (0.48 g), as white solid; mp 145-147 °C; IR (KBr) ν_{max} cm^{-1}: 3360, 2856, 1766, 1642, 1250, 1035; $[\alpha]^{25}_D$ 4.00° (c 0.05, CH_3OH); ^1H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 8.56 (s, 1H, NH), 8.14 (s, 1H, triazolyl), 7.83-7.81 (m, 2H, Ar), 7.46-7.31 (m, 3H, Ar), 6.24 (s, 1H, OH), 4.40 (t, *J* = 6.78 Hz 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 2.31-2.28 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 167.9, 153.6, 146.3, 130.8, 128.9, 127.8, 125.1, 124.8, 121.5, 81.4, 60.2, 58.5, 45.2, 36.9; ESI-HRMS calcd for $C_{16}H_{19}N_4O_4$ $[M + H]^+$ 331.1406 found 331.1401.

*4.1.4.2 5-hydroxy-3,4-dimethoxy-5-(2-(4-p-tolyl-1H-1,2,3-triazol-1-yl)ethyl)-1H-pyrrol-2(5H)-one (**25**)*

It was obtained by using compound **15** (0.4 g, 1.08 mmol) in 95% yield (0.39 g) as colourless oil; IR (neat) ν_{max} cm^{-1}: 3362, 2959, 1680, 1216, 1085, 761; $[\alpha]^{25}_D$ 8.38° (c 0.05, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.83 (s, 1H, triazolyl), 7.70-7.68 (m, 2H, Ar), 7.50 (bs, NH), 7.40-7.37 (m, 2H, Ar), 4.51 (t, *J* = 7.08 Hz, 2H, CH_2), 3.93 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 2.59-2.43 (m, 2H, CH_2), 1.30 (s, 9H, $3CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 168.5, 153.5, 150.3, 146.7, 127.4, 125.1, 124.8, 124.7, 119.6, 95.5, 81.4, 60.0, 58.4, 45.1, 36.8, 34.1, 30.8, 29.1; ESI-HRMS calcd for $C_{20}H_{27}N_4O_4$ $[M + H]^+$ 387.2032 found 387.2027.

*4.1.4.3 3,4-bis(benzyloxy)-5-hydroxy-5-(2-(4-phenyl-1H-1,2,3-triazol-1-yl)ethyl)-1H-pyrrol-2(5H)-one (**26**)*

It was obtained by using compound **14** (0.5 g, 1.07 mmol) in 90% yield (0.46 g) as white solid; mp 151-153 °C; IR (KBr) ν_{max} cm^{-1}: 3429, 3019, 2400, 1706, 1424, 1215; $[\alpha]^{25}_{D}$ 8.34° (c 0.05, CH_3OH); ^1H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 8.10 (s, 1H, triazolyl), 7.75-7.29 (m, 2H, Ar), 7.35-7.21 (m, 13H, Ar), 6.23 (bs, 1H, NH), 5.12-4.97 (m, 4H, 2×OCH_2), 4.36-4.31 (m, 2H, NCH_2), 2.40-2.21 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 167.4, 152.1, 145.7, 135.5, 135.1, 129.6, 127.5, 127.4, 127.2, 127.0, 127.0, 126.6, 126.4, 124.2, 122.3, 119.6, 94.7, 80.7, 72.4, 71.3, 44.3, 36.2; ESI-HRMS calcd for $C_{28}H_{27}N_4O_4$ $[M + H]^+$ 483.2032 found 483.2026.

*4.1.4.4 3,4-bis(benzyloxy)-5-(2-(4-cyclohexenyl-1H-1,2,3-triazol-1-yl)ethyl)-5-hydroxy-1H-pyrrol-2(5H)-one (**27**)*

It was obtained by using compound **23** (0.4 g, 0.85 mmol) in 92% yield (0.38 g) as colourless oil. IR (neat) ν_{max} cm^{-1}: 3401, 2926, 1677, 1454, 1216, 1155; $[\alpha]^{25}_{D}$ 8.32° (c 0.05, CH_3OH); ^1H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 7.30-7.17 (m, 11H, 10 Ar-H+ 1 triazolyl-H), 6.71 (bs, 1H, NH), 6.37 (bs, 1H, OH), 5.61 (t, J = 6.9 Hz, 1H), 5.09-4.98 (m, 4H, 2×OCH_2), 4.24-4.11 (m, 2H, NCH_2), 2.28-2.24 (m, 2H, CH_2), 2.12-2.08 (m, 2H, CH_2), 1.67-1.57 (m, 4H, 2×CH_2), 0.80-0.75 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 168.9, 153.4, 136.5, 136.1, 128.7, 128.4, 128.3, 128.2, 127.6, 124.7, 118.7, 82.0, 73.8, 72.8, 45.2, 37.3, 29.5, 26.2, 25.1, 22.3, 22.1; ESI-HRMS calcd for $C_{28}H_{31}N_4O_4$ $[M + H]^+$ 487.2345 found 487.2340.

*4.1.4.5 3,4-bis(benzyloxy)-5-hydroxy-5-(2-(4-p-tolyl-1H-1,2,3-triazol-1-yl)ethyl)-1H-pyrrol-2(5H)-one (**28**)*

It was obtained by using compound **22** (0.3 g, 0.62 mmol) in 95% yield (0.29 g) as white solid; mp 159-161 °C; IR (KBr) ν_{max} cm^{-1}: 3407, 3020, 1684, 1405, 1215, 924; $[\alpha]^{25}_{D}$ 8.90° (c 0.05, CH_3OH); ^1H NMR ($CDCl_3$+ DMSO-d_6, 300 MHz) δ: 8.29-8.22 (m, 2H, 1 triazolyl-H+ 1 Ar-H),

7.70-7.67 (m, 2H, Ar), 7.37-7.18 (m, 11H, Ar), 6.27 (s, 1H, NH), 5.06-5.04 (m, 4H, 2×CH_2), 4.37-4.34 (m, 2H, CH_2), 2.33-2.31 (m, 5H, CH_2+ CH_3); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 166.5, 151.4, 144.9, 135.3, 135.1, 134.7, 127.6, 126.8, 126.6, 126.4, 125.9, 123.5, 121.8, 119.1, 80.1, 71.7, 70.5, 43.6, 35.5, 19.3; ESI-HRMS calcd for $C_{29}H_{29}N_4O_4$ $[M + H]^+$ 497.2189 found 497.2183.

*4.1.4.6 5-(2-(4-benzyl-1H-1,2,3-triazol-1-yl)ethyl)-3,4-bis(benzyloxy)-5-hydroxy-1H-pyrrol-2(5H)-one (**29**)*

It was obtained by using compound **12** (0.5 g, 1.0 mmol) in 93% yield (0.48 g) as white solid; mp 121-123 °C; IR (KBr) ν_{max} cm^{-1}: 3290, 3018, 1679, 1388, 1216, 1095; $[\alpha]^{25}_D$ -2.94° (c 0.05, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.32-7.17 (m, 16H, 15 Ar-H+ 1 triazolyl), 7.06 (s, 1H, NH), 7.01(s, 1H, OH), 5.14-4.99 (m, 4H, 2×OCH_2), 4.26-4.12 (m, 2H, NCH_2), 3.97 (s, 2H, CH_2Ph), 2.45-2.23 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 169.8, 154.1, 147.6, 139.0, 136.4, 136.1, 129.0, 128.8, 128.7, 128.7, 128.6, 128.5, 127.8, 126.6, 123.6, 122.2, 82.7, 74.3, 73.1, 45.4, 36.9, 32.2; ESI-HRMS calcd for $C_{29}H_{29}N_4O_4$ $[M + H]^+$ 497.2189 found 497.2183.

*4.1.4.7 3,4-bis(benzyloxy)-5-(2-(4-(4-tert-butylphenyl)-1H-1,2,3-triazol-1-yl)ethyl)-5-hydroxy-1H-pyrrol-2(5H)-one (**30**)*

It was obtained by using compound **16** (0.4 g, 0.76 mmol) in 94% yield (0.39 g) as white solid; mp 147-149 °C; IR (KBr) ν_{max} cm^{-1}: 3366, 2973, 1672, 1441, 1212, 1114; $[\alpha]^{25}_D$ -5.36° (c 0.05, CH_3OH); ^{1}H NMR ($CDCl_3$+ DMSO-d_6, 400 MHz) δ: 7.83 (s, 1H, triazolyl), 7.71-7.64 (m, 3H, Ar), 7.42-7.26 (m, 11H, Ar), 6.15 (bs, 1H, NH), 5.15-5.11 (m, 4H, 2×OCH_2), 4.40-4.39 (m, 2H, NCH_2), 2.45-2.38 (m, 2H, CH_2), 1.34 (s, 9H, 3CH_3); ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 50 MHz) δ: 167.8, 152.4, 149.8, 146.0, 135.7, 135.3, 127.6, 127.4, 127.2, 127.2, 126.7, 124.6, 124.2, 119.4,

95.0, 81.05, 72.7, 71.6, 44.5, 36.3, 33.5, 30.3; ESI-HRMS calcd for $C_{32}H_{35}N_4O_4$ $[M + H]^+$ 539.2658 found 539.2652.

*4.1.5 Typical procedure for the synthesis of compounds **31-33***

To a magnetically stirred solution of triazole compound (1.0 mmol) in ethanol or THF as a solvent at ambient temperature, alkyl amine (1.0 mmol) was added. The resulting mixture was stirred for overnight at same temperature until completion of starting material (TLC). After completion, solvent was evaporated under reduced pressure, then extracted with ethylacetate, H_2O. The organic layer was dried (anhyd.Na_2SO_4), evaporated under reduced pressure to get the crude, which was chromatographed (SiO_2, 60-120 mesh) by using hexane: ethylacetate (6: 4) as eluent to give the desired products.

*4.1.5.1 (Z)-3,4-bis(benzyloxy)-1-propyl-5-(2-(4-propyl-1H-1,2,3-triazol-1-yl)ethylidene)-1H-pyrrol-2(5H)-one (**31**)*

It was obtained by the reaction of compound **4** (0.2 g, 0.46 mmol), n-propylamine (0.19 mL, 0.46 mmol) in ethanol (10 mL) in 94% yield (0.21 g) as colourless oil: IR (neat) ν_{max} cm^{-1}: 3368, 3017, 2875, 1683, 1550, 1216; $[\alpha]^{25}_D$ 3.03° (c 0.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.40-7.24 (m, 10H, 10 Ar), 6.95 (s, 1H, triazolyl), 5.29 (d, *J* = 11.2 Hz, 1H, OCH_2), 5.18 (s, 2H, OCH_2), 5.09 (d, *J* = 11.2 Hz, 2H, OCH_2), 3.93-3.75 (m, 2H, NCH_2), 3.26-3.08 (m, 2H, NCH_2 of propylamine), 2.60 (t, *J* = 7.48 Hz, 2H, CH_2), 2.50-2.42 (m, 2H, CH_2), 1.65-1.60 (m, 4H, $2CH_2$), 0.93 (t, *J* = 7.32 Hz, 3H, CH_3 of propylamine), 0.88 (t, *J* = 7.36 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 167.6, 151.0, 148.0, 136.6, 136.3, 129.2, 128.7, 128.6, 128.5, 127.9, 124.2, 121.0, 85.5, 73.8, 73.1, 45.1, 39.9, 35.0, 27.6, 22.9, 22.7, 13.8, 11.8; ESI-HRMS calcd for $C_{28}H_{35}N_4O_4$ $[M + H]^+$ 491.2658 found 491.2653.

*4.1.5.2 (Z)-3,4-bis(benzyloxy)-1-propyl-5-(2-(4-(pyridin-2-yl)-1H-1,2,3-triazol-1-yl)ethylidene)-1H-pyrrol-2(5H)-one (**32**)*

It was obtained by the reaction of compound **18** (0.4 g, 0.85 mmol), n-octylamine (0.14 mL, 0.85 mmol) in ethanol (15 mL) in 92% yield (0.46 g) as colourless oil: IR (neat) ν_{max} cm^{-1}: 3407, 3018, 1682, 1312, 1112, 993; $[\alpha]^{25}_{D}$ 6.33° (c 0.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.45-8.44 (m, 1H, Ar), 8.22-8.18 (m, 2H, 1Ar-H+ 1 triazolyl-H), 7.83-7.79 (m, 1H, Ar), 7.35-7.24 (m, 9H, Ar), 7.07-7.05 (m, 2H, Ar), 5.30 (d, *J* = 11.16 Hz, 1H, OCH_2), 5.08-5.03 (m, 2H, OCH_2), 4.95 (d, *J* = 11.52 Hz, 1H, OCH_2), 4.04-3.73 (m, 2H, CH_2), 3.37-3.14 (m, 2H, CH_2), 2.38-2.04 (m, 2H, CH_2), 1.68-1.63 (m, 2H, CH_2), 1.32-1.25 (m, 10H, $5CH_2$), 0.86 (t, *J* = 7.04 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 167.4, 151.0, 150.0, 148.9, 147.7, 137.6, 136.5, 135.8, 129.3, 128.7, 128.6, 128.2, 124.0, 123.2, 122.8, 120.6, 96.2, 85.2, 73.8, 73.3, 45.4, 38.4, 35.2, 31.9, 29.9, 29.4, 29.4, 27.6, 22.7, 14.2; ESI-HRMS calcd for $C_{35}H_{42}N_5O_4$ $[M + H]^+$ 596.3237 found 596.3231.

*4.1.5.3 (Z)-3,4-bis(benzyloxy)-5-(2-(4-(4-tert-butylphenyl)-1H-1,2,3-triazol-1-yl)ethylidene)-1-phenethyl-1H-pyrrol-2(5H)-one (**33**)*

It was obtained by the reaction of compound **16** (0.5 g, 0.95 mmol), phenethylamine (0.12 mL, 0.95 mmol) in ethanol (20 mL) in 93% yield (0.57 g) as white solid; mp 131-133 °C; IR (KBr) ν_{max} cm^{-1}: 3316, 3065, 1695, 1356, 1250, 1018; $[\alpha]^{25}_{D}$ 9.75° (c 0.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.69-7.68 (m, 2H, Ar), 7.46-7.10 (m, 18H, 17 Ar-H+ 1 triazolyl-H), 5.29-5.28 (m, 2H, OCH_2), 5.22 (s, 1H, OCH_2), 5.10 (s, 1H, OCH_2), 3.92-3.83 (m, 1H, CH_2), 3.74 (t, *J* = 7.12 Hz, 1H, CH_2), 3.60-3.34 (m, 1H, CH_2), 3.04-2.98 (m, 1H, CH_2), 2.83-2.79 (t, *J* = 7.08 Hz, 2H, CH_2), 2.47-2.26 (m, 2H, CH_2), 1.33 (s, 9H, $3CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 164.6, 151.6, 147.7, 143.6, 138.3, 136.4, 135.9, 135.1, 129.0, 128.9, 128.7, 128.7, 128.6, 128.2, 126.7, 125.8,

125.6, 119.4, 103.9, 74.2, 74.0, 46.1, 40.2, 34.9, 34.8, 31.4; ESI-HRMS calcd for $C_{40}H_{43}N_4O_4$ $[M + H]^+$ 643.3284 found 643.3275.

*4.1.6 Typical procedure for the synthesis of compounds **34, 35***

To a magnetically stirred solution of compound (**31, 32**) (1.0 mmol) in anhyd.CH_2Cl_2 as a solvent, *p*-toluenesulfonic acid (1.0 mmol) was added at ambient temperature. The resulted mixture was stirred at same temperature for 30 min to 1 h. After completion, reaction mixture was neutralized (pH 7.0) with solid $NaHCO_3$, filtered and filtrate was concentrated to give a crude mass. The latter was dissolved in ethyl acetate (75 mL), washed with water (2 x 25 mL). The organic layer was dried (anhydrous Na_2SO_4) and evaporated under reduced pressure to give gummy mass, which was chromatographed (SiO_2, 60-120 mesh) by using hexane: ethylacetate (7: 3) as eluent to give the desired products.

*4.1.6.1 (Z)-3,4-bis(benzyloxy)-1-propyl-5-(2-(4-propyl-1H-1,2,3-triazol-1-yl)ethylidene)-1H-pyrrol-2(5H)-one (**34**)*

It was obtained by the reaction of compound **31** (0.4 g, 0.81 mmol), *p*-toluenesulfonic acid (0.14 g, 0.81 mmol) in anhydrous CH_2Cl_2 (25 mL) in 84% yield (0.32 g) as white solid; mp 144-146 °C; IR (KBr) ν_{max} cm^{-1}: 3432, 3021, 2401, 1699, 1215, 928; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.34-7.26 (m, 8H, Ar), 7.19-7.15 (m, 2H, Ar), 6.90 (s, 1H, triazolyl), 5.32-5.28 (m, 1H, C=CH), 5.24-5.22 (m, 4H, OCH_2+ CH_2), 5.16 (s, 2H, OCH_2), 3.40 (t, *J* = 7.40 Hz, 2H, NCH_2), 2.56 (t, *J* = 7.60 Hz, 2H, CH_2), 1.59-1.53 (m, 2H, CH_2), 1.47-1.42 (m, 2H, CH_2), 0.87 (t, *J* = 7.36 Hz, 3H, CH_3 of propylamine), 0.80 (t, *J* = 7.36 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 164.8, 148.4, 143.5, 136.5, 136.0, 134.8, 128.8, 128.8, 128.7, 128.6, 128.1, 120.5, 104.3, 74.1, 73.9, 45.8, 40.1, 27.9, 22.7, 21.8, 13.9, 11.3; ESI-HRMS calcd for $C_{28}H_{33}N_4O_3$ $[M + H]^+$ 473.2553 found 473.2544.

*4.1.6.2 (Z)-3,4-bis(benzyloxy)-1-octyl-5-(2-(4-(pyridin-2-yl)-1H-1,2,3-triazol-1-yl)ethylidene)-1H-pyrrol-2(5H)-one (**35**)*

It was obtained by the reaction of compound **32** (0.4 g, 0.67 mmol), *p*-toluenesulfonic acid (0.11 g, 0.67 mmol) in anhydrous CH_2Cl_2 (25 mL) in 82% yield (0.31 g) as white solid; mp 133-135 °C; IR (KBr) ν_{max} cm^{-1}: 3431, 2969, 2401, 1701, 1423, 1215; ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.52-8.51 (m, 1H, Ar), 8.12-7.71 (m, 1H, Ar), 7.70 (s, 1H, triazolyl), 7.70-7.68 (m, 1H, Ar), 7.34-7.16 (m, 11H, Ar), 5.38-5.29 (m, 3H, C=CH+ CH_2), 5.23 (s, 2H, OCH_2), 5.17 (s, 2H, OCH_2), 3.42 (t, *J* = 7.2 Hz, 2H, NCH_2), 1.35-1.34 (m, 2H, CH_2), 1.21-1.15 (m, 10H, $5CH_2$), 0.79 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 164.8, 149.5, 143.5, 137.1, 136.5, 136.0, 135.4, 129.0, 128.9, 128.8, 128.8, 128.7, 128.1, 123.0, 122.0, 120.4, 103.4, 74.2, 74.1, 46.3, 38.7, 32.0, 31.9, 28.6, 27.0, 22.8, 14.2; ESI-HRMS calcd for $C_{35}H_{40}N_4O_3$ $[M + H]^+$ 578.3131 found 578.3125.

4.1.7 2D NMR studies.

The **2D** NMR Study of compounds **4, 31 and 34** were evaluated on a Bruker Avance- 400 MHz (300K) in $CDCl_3$ solvent with TMS as an internal standard. ^{13}C NMR Spectra were recorded with complete proton decoupling. The assignment was carried out with the help of DEPT-135, DEPT-90, COSY, HSQC and HMBC experiments. All the spectra were recorded in phase sensitive mode except for COSY which is in magnitude mode. The spectra were acquired with 2*256 FID for HMBC, 256 Fids for HSQC and 128 Fids for COSY containing 4-16 transients for all the experiments with a recycle delay of 1.5 sec.

4.2 Biology

4.2.1 Cell lines and culture conditions

MCF-7 and MDA-MB-231 (breast cancer cell line), PC-3 (prostate cancer cell line), HCT-116 (colorectal cancer cell line) and U-937 (histiocytic lymphoma cell line) originally obtained from American type of cell culture collection (ATCC), USA and stock was maintained in laboratory. HEK-293 cells were obtained from institutional cell repository of animal tissue culture facility (CSIR-CDRI). Cells were grown in tissue culture flask in DMEM (Dulbecco modified eagle medium, Sigma) supplemented with 10% fetal bovine serum with 1X antibiotic-antimycotic solution (Sigma) in a CO_2 incubator (Sanyo, Japan) at 37 ^oC with 5% CO_2 and 90% relative humidity.

4.2.2 Anti-proliferative assay

The cytotoxic activity of the compounds was determined using MTT assay. 1×10^4 cells/well were seeded in 96-well micro culture plates in 200 µL DMEM, supplemented with 10% FBS and 1×stabilized antibiotic-antimycotic solution (Sigma) and incubated for 24 h at 37 ^oC in CO_2 incubator. Compounds were diluted to the desired concentrations in culture medium DMEM without phenol red, supplemented with 2% FBS, and were added to the wells with respective vehicle control. After 24 h of incubation, 20 µL (5 mg/mL) MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoliumbromide)(Sigma) was added to each well and the plates were further incubated for 3 h. Then the supernatant from each well was carefully removed and were dissolved in 200 µL of dimethyl sulfoxide (DMSO) using plate shaker (Biosan) and absorbance at 570 nm wavelength was recorded in a microplate reader (Microquant; BioTek).

4.2.3 Cell cycle analysis

To determine the effect of compound **24**, **25** and **26** on cell cycle distribution pattern, MDA-MB-231, MCF-7, PC-3, U-937 and HCT-116 cells (1X 10^6) were seeded in six-well plates and treated for 24 h. After 24 h, both floating and trypsinized adherent cells were collected and fixed

with 70% ethanol. After fixation cells were washed with PBS and stained with 30 μg/mL propidium iodide in PBS containing 10 mg/mL RNase A for 30 min at room temperature in dark. The DNA content of the cells was measured using a FACS Calibur flow cytometer (Becton-Dickinson, San Jose, CA, USA) and Cell Quest software.

4.2.4 Apoptosis assay

MDA-MB-231 cells ($1X10^6$) were seeded in six well plates and allowed to grow overnight. Cells were treated with compound **25** for 24 h. In another experiment, the effect of NAC on compound **25** induced apoptosis was studied on MDA-MB-231 cells. For this, cells were pre-treated with 10 mM NAC for 1h followed by compound 25 treatment for 24 h. At the end of incubation, cells were harvested by trypsinization, washed with PBS and stained with Annexin-V-FITC and propidium iodide using the Annexin-V-PI apoptosis detection kit (Sigma). Results were acquired by flow cytometry using a FACScan (Becton Dickinson).

4.2.5 Reactive oxygen species detection assay

MDA-MB-231 cells ($1X10^6$) were seeded in six-well plate for 24 h. Cells were treated with compound **12**, **24**, **25**, **26** and **27** for 24 h, and harvested for flow cytometry analysis. Cells were collected, washed with PBS, fixed in absolute methanol, resuspended in PBS containing 15 μg/mL DCFH-DA. After 30 min of incubation in dark, cells were centrifuged, resuspended in 300 μL PBS and ROS level was measured using FACS Calibur flow cytometer (Becton-Dickinson, San Jose, CA, USA).

4.2.6 Western blot analysis

MDA-MB-231 cells ($1X10^6$) were seeded in six well plates for 24 h. Cells were treated with compound **25** for 24 h. At the end of incubation, cells were scraped in ice cold RIPA lysis buffer, followed by quantitation using Bradford method. 40 μg protein was resolved by SDS-PAGE gel

electrophoresis. Proteins were transferred to PVDF membrane, blocked with 1% BSA after that incubated with p-p38 and p38 primary antibodies (Cell signal technology, USA) overnight at 4^0C, and then secondary antibodies conjugated with horseradish peroxidase for 2 h at room temperature. Protein bands were visualized by Doc Imaging System (Biorad, CA, USA).

Acknowledgments

ND and GRK thank CSIR-New Delhi for SRF. We are grateful to CSIR-New Delhi for Financial assistance in the form of NETWORK Project and THUNDER. SAIF-CDRI Lucknow is also acknowledged for providing spectroscopic data of the compounds. We also thank Mrs Shashi Rastogi for her technical assistance during course of this work.

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Figure captions:

Figure 1: Naturally occurring γ-alkylidene bicyclic butenolide and 3-Hydroxy-1*H*-pyrrol-2(5*H*)-one derivatives.

Figure 2: Important HMBC correlation of compounds **4, 31 and 34**.

Figure 3: Effect of compounds on cell cycle progression in MDA-MB-231, MCF-7, PC-3, U-937 and HCT 116. Cells were treated with compounds at their respective IC_{50} for 24 h, stained with Propidium iodide and cell cycle distribution was observed by flow cytometer.

Figure 4: Effect of compound **25** on apoptosis in MDA-MB-231cells. Apoptosis was measured by Annexin V/PI staining by flow cytometry. MDA-MB-231 cells were treated with 11 and 22 μM of compound for 24 h and stained with Annexin V FITC/PI and then acquired by flow cytometry.

Figure 5: Effect of compound on reactive oxygen species generation in MDA-MB-231. Cells were treated with compounds for 24 h and stained with DCFH-DA, acquired by flow cytometry

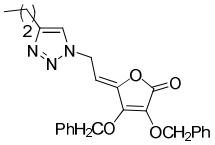
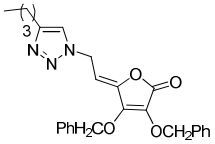
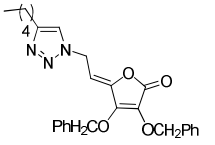
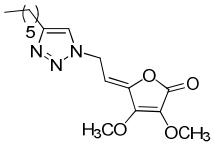
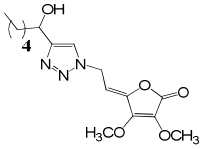
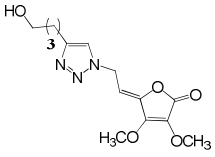
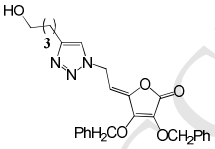
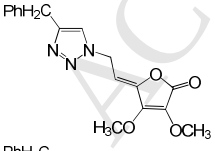
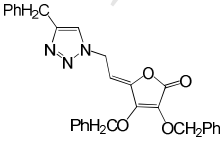
Figure 6: Effect of compound **25** induced apoptosis in presence of NAC in MDA-MB-231 cells. Cells were pre-treated with 10 mM NAC for 1h followed by treatment of compound **25** for 24 h. Cells were harvested, stained with Annexin V/PI and then acquired by flow cytometry.

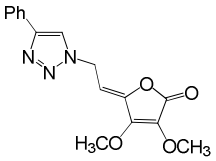
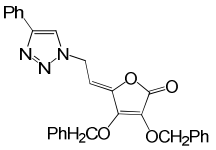
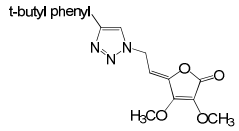
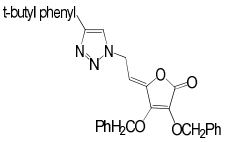
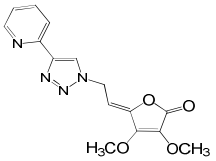
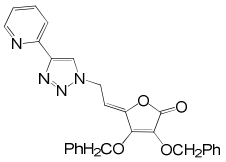
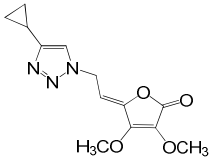
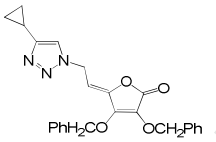
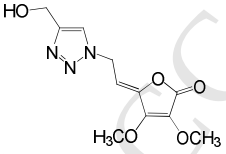
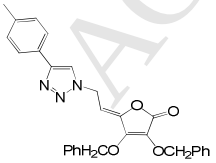
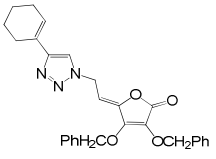
Figure 7: Effect of compound **25** on p38 activation. **A**: MDA-MB-231 cells were treated with compound **25** (11μM and 22 μM) and immunoblotting was performed to detect p-P38 and p38 expression in the lysate of MDA-MB-231 cells. **B**. Cells were pre treated with NAC followed by compound **25** (11μM) and activation of p38 was checked by immunoblot.

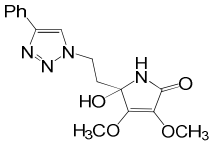
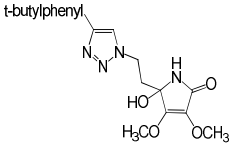
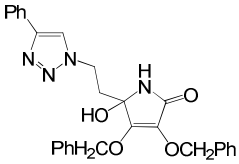
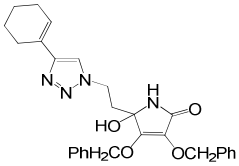
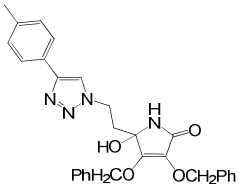
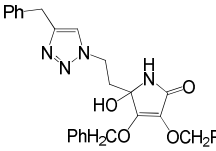
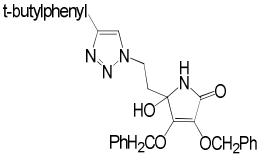
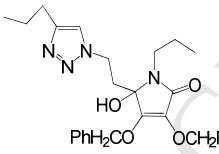
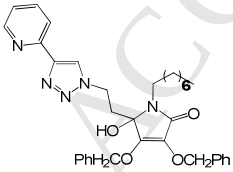
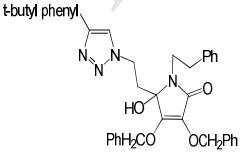
Table 1: *In vitro* anticancer activity results of compounds **4-35.**

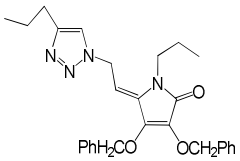
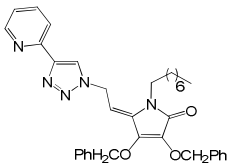
Scheme 1: Synthesis of γ-(triazolyl ethylidine)butenolides, 5-hydroxy pyrrolinones and γ-(triazolyl ethylidine)pyrrolinones.

Table 1: *In vitro* anticancer activity results of compounds **4-35.**

Comp. code	structure	IC_{50} (μM)[a]±SEM					
		MCF-7	MDA-MB-231	PC-3	HCT-116	U-937	HEK-293
4		>100	>100	>100	>100	>100	>100
5		>100	>100	>100	64.3±0.19	>100	>100
6		>100	>100	>100	>100	>100	>100
7		>100	74.7±0.09	>100	>100	>100	>100
8		>100	35.5±0.02	>100	>100	>100	80±0.12
9		35±0.09	87.5±0.04	>100	>100	>100	>100
10		53.6±0.04	>100	>100	>100	>100	>100
11		>100	>100	>100	>100	>100	>100
12		40±0.13	29.2±0.25	71.3±0.01	83.1±0.43	>100	70±0.59

13		>100	>100	>100	>100	>100	>100
14		>100	44±0.14	>100	>100	>100	>100
15		>100	>100	>100	>100	30±0.15	>100
16		>100	51.2±0.13	>100	>100	>100	>100
17		>100	>100	>100	>100	>100	>100
18		>100	>100	>100	>100	70.2±0.09	>100
19		>100	>100	>100	>100	>100	>100
20		>100	>100	>100	>100	>100	>100
21		35±0.01	>100	>100	>100	>100	>100
22		>100	>100	>100	>100	>100	>100
23		>100	>100	55.6±0.13	>100	>100	>100

24		22.6±1.2	18.3±0.18	35.3±0.16	46.3±0.16	20.3±0.17	62±0.37
25		>100	11.3±0.06	30.4±0.32	50.3±0.12	>100	62±0.02
26		20.5±0.98	21.1±0.19	>100	53.4±0.18	21.5±0.38	>100
27		>100	13.1±0.07	67.2±0.24	>100	>100	70±0.05
28		24±0.01	82.2±0.05	84.2±0.14	74.3±0.26	54±0.17	45±0.12
29		39.2±0.03	>100	>100	51.2±0.16	>100	86±0.05
30		25.1±0.04	>100	43±0.34	>100	56.5±0.12	>100
31		>100	>100	35±0.64	>100	>100	90±0.02
32		24.3±0.09	>100	80.3±0.21	>100	84.2±0.18	>100
33		32±0.02	>100	65.5±0.13	>100	60.2±0.06	71±0.03

34		66.8±0.02	>100	>100	>100	80.5±0.26	82±0.07
35		46.2±0.02	>100	>100	>100	90.2±0.14	83±0.13
	Tamoxifen	6±0.04	14±0.12	7±0.18	ND	ND	50±0.09

[a] IC_{50} is the drug concentration effective in inhibiting 50% of the cell growth measured by the MTT assay.

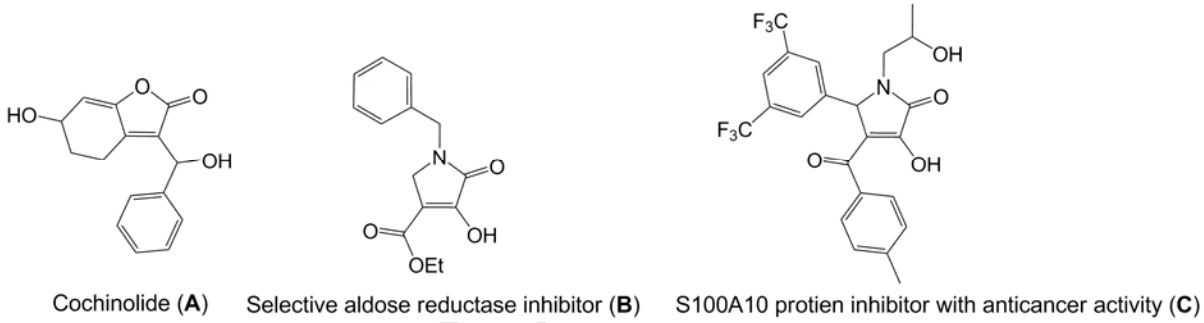


Figure 1. Naturally occurring γ-alkylidene bicyclic butenolide and 3-Hydroxy-1*H*-pyrrol-2(5*H*)-one derivatives.

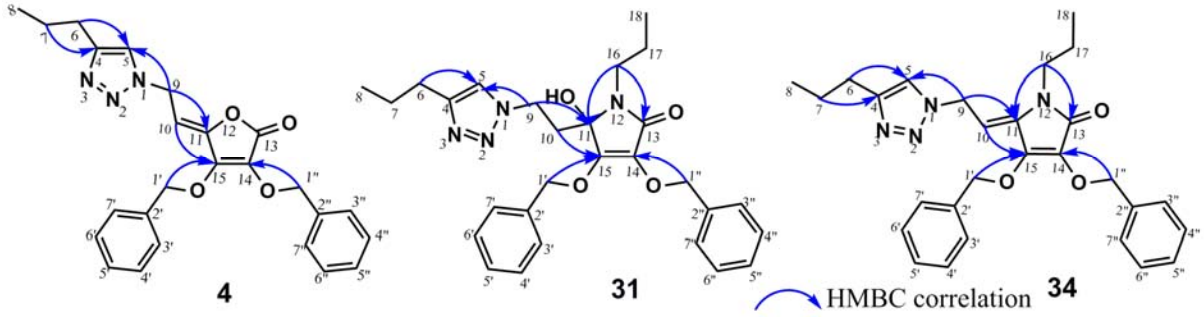


Figure 2. Important HMBC correlation of compounds **4, 31 and 34**.

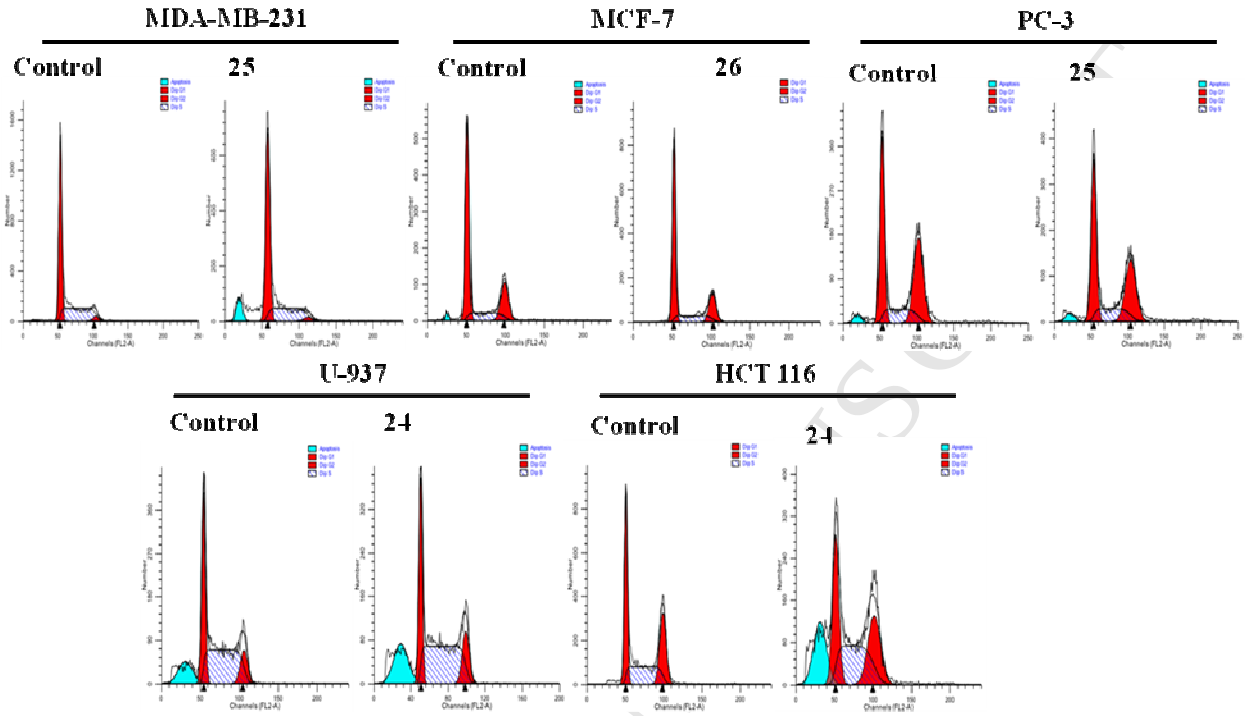


Figure 3. Effect of compounds on cell cycle progression in MDA-MB-231, MCF-7, PC-3, U-937 and HCT-116. Cells were treated with compounds at their respective IC_{50} for 24 h, stained with Propidium iodide and cell cycle distribution was observed by flow cytometer.

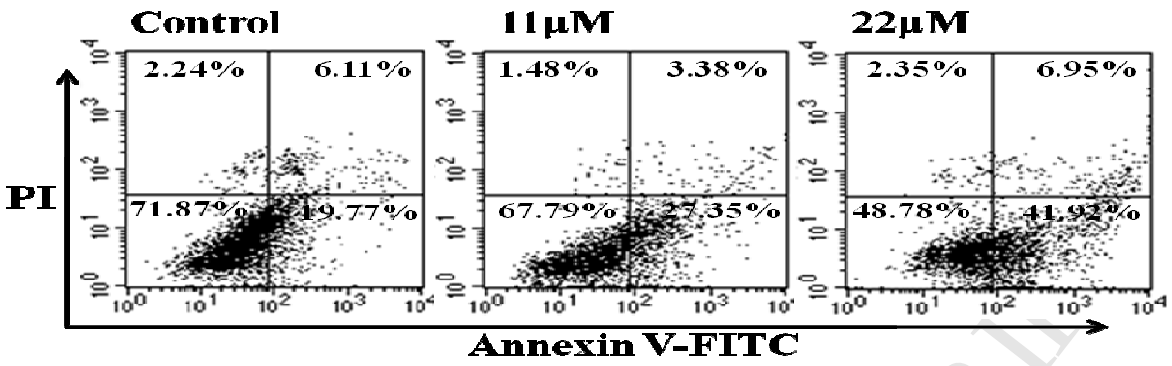


Figure 4. Effect of compound **25** on apoptosis in MDA-MB-231cells. Apoptosis was measured by Annexin V/PI staining by flow cytometry. MDA-MB-231 cells were treated with 11 and 22 μM of compound for 24 h and stained with Annexin V FITC/PI and then acquired by flow cytometry.

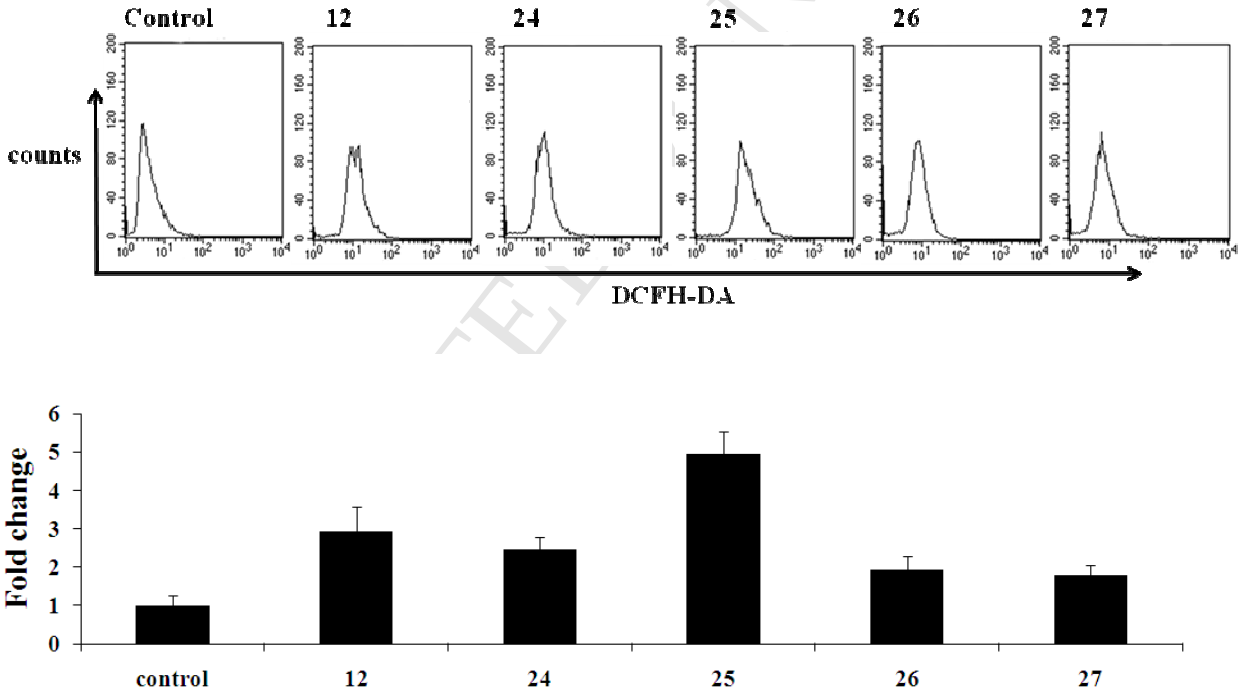


Figure 5. Effect of compounds on reactive oxygen species generation in MDA-MB-231. Cells were treated with compounds for 24 h and stained with DCFH-DA, acquired by flow cytometry.

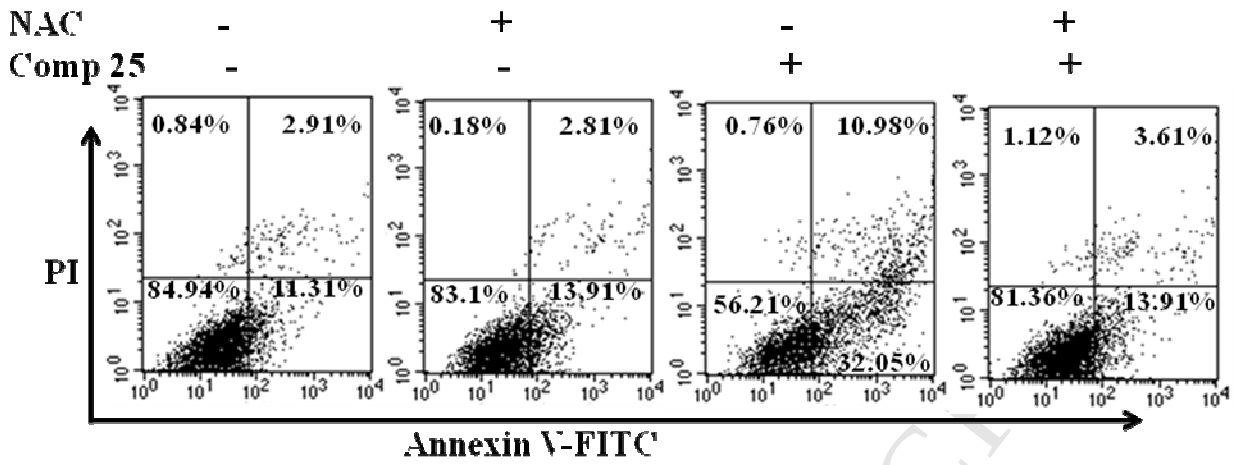


Figure 6. Effect of compound **25** induced apoptosis in presence of NAC in MDA-MB-231 cells. Cells were pre treated with 10 mM NAC for 1h followed by treatment of compound **25** for 24 h. Cells were harvested, stained with Annexin V/PI and then acquired by flow cytometry.

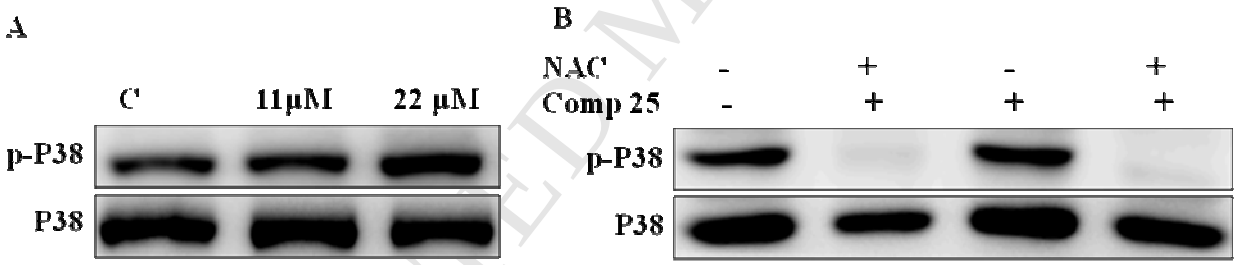
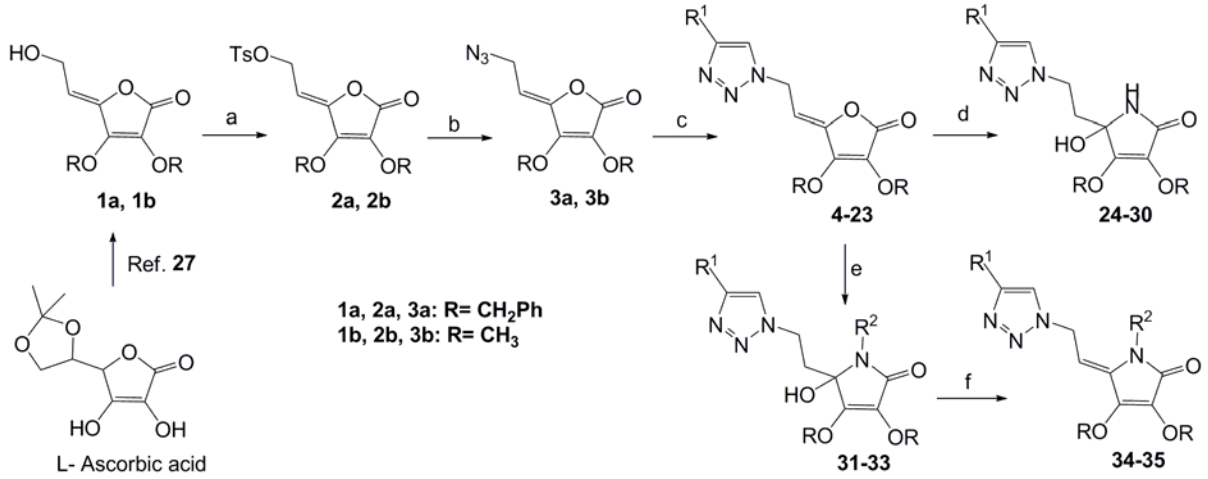


Figure 7. Effect of compound **25** on p38 activation. **A**. MDA-MB-231 cells were treated with compound **25** (11μM and 22 μM) and immunoblotting was performed to detect p-P38 and p38 expression in the lysate of MDA-MB-231 cells. **B**. Cells were pre treated with NAC followed by compound **25** (11μM) and activation of p38 was checked by immunoblot.

Scheme 1. Synthesis of γ-(triazolyl ethylidine)butenolides, 5-hydroxy pyrrolinones and γ-(triazolyl ethylidine)pyrrolinones.



Reagent and conditions: (a) tosyl chloride, pyridine, anhydrous CH_2Cl_2, 0^{o}C- RT,12-15 h; (b) NaN_3, DMF, RT, 10-12 h; (c) $R^1C{\equiv}CH$, $CuSO_4.5H_2O$ (2 mol%), Na-ascorbate (5 mol%), PhCOOH (5 mol%), *t*-BuOH: H_2O (1:1), 1-2 h; (d) ethanolic NH_3, RT, 10-15 h; (e) R^2 NH_2, ethanol or THF, RT, 15-20 h; (f) *p*-TSA (10 mol%), anhydrous CH_2Cl_2, RT, 30 min-1 h.

Research highlights

- Access of novel polyfunctional butenolides and pyrrolinones of high therapeutic value
- One of the compounds with IC_{50} = 11.3 μM against MDA-MB-231 cancer cell lines
- Compounds act via ROS production and apoptosis induction

Supplementary data

Synthesis and anticancer activity of γ-(triazolyl ethylidene)butenolides and polyfunctional pyrrolinones

N. Devender[a,1], K. K. G. Ramakrishna[a], Hamidullah[b,1], Sanjeev K. Shukla[c,d], Rituraj Konwar[b,d]*, Rama P. Tripathi[a,d]*

[a]Medicinal and Process Chemistry Division, [b]Endocrinology Division, [c]Sophisticated Analytical Instrumental Facility, CSIR-Central Drug Research Institute, Lucknow-226031, India. [d]Academy of Scientific and Innovative Research, New Delhi-110001, India.

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^{1}H & ^{13}C NMR of Intermediate **2b**.....S3

^{1}H & ^{13}C NMR of Intermediate **3b**.....S4

^{1}H & ^{13}C NMR of Compound **4**.....S5

^{1}H & ^{13}C NMR of Compound **8**.....S6

^{1}H & ^{13}C NMR of Compound **9**.....S7

^{1}H & ^{13}C NMR of Compound **11**.....S8

^{1}H & ^{13}C NMR of Compound **12**.....S9

^{1}H & ^{13}C NMR of Compound **13**.....S10

^{1}H & ^{13}C NMR of Compound **15**.....S11

^{1}H & ^{13}C NMR of Compound **17**.....S12

^{1}H & ^{13}C NMR of Compound **19**.....S13

^{1}H & ^{13}C NMR of Compound **21**.....S14

^{1}H & ^{13}C NMR of Compound **22**.....S15

^{1}H & ^{13}C NMR of Compound **24**.....S16

^{1}H & ^{13}C NMR of Compound **26**.....S17

^{1}H & ^{13}C NMR of Compound **27**.....S18

^{1}H & ^{13}C NMR of Compound **28**.....S19

^{1}H & ^{13}C NMR of Compound **29**.....S20

^{1}H & ^{13}C NMR of Compound **32**.....S21

^{1}H & ^{13}C NMR of Compound **33**.....S22

^{1}H & ^{13}C NMR of Compound **34**.....S23

^{1}H & ^{13}C NMR of Compound **35**.....S24

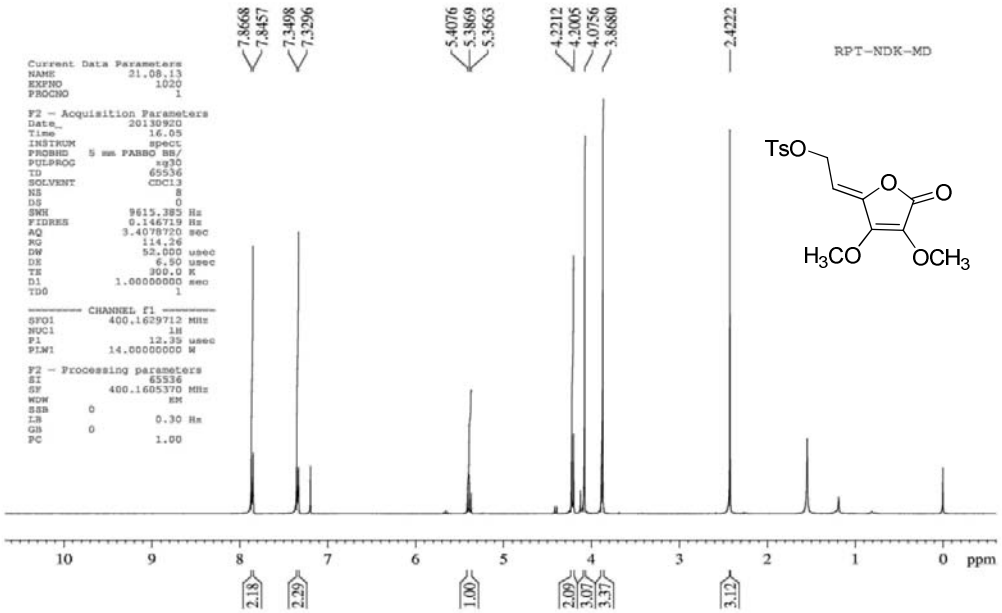
Complete chemical shift values of compound **4**.....S25

COSY, HSQC and HMBC spectra of compound **4**.....S26

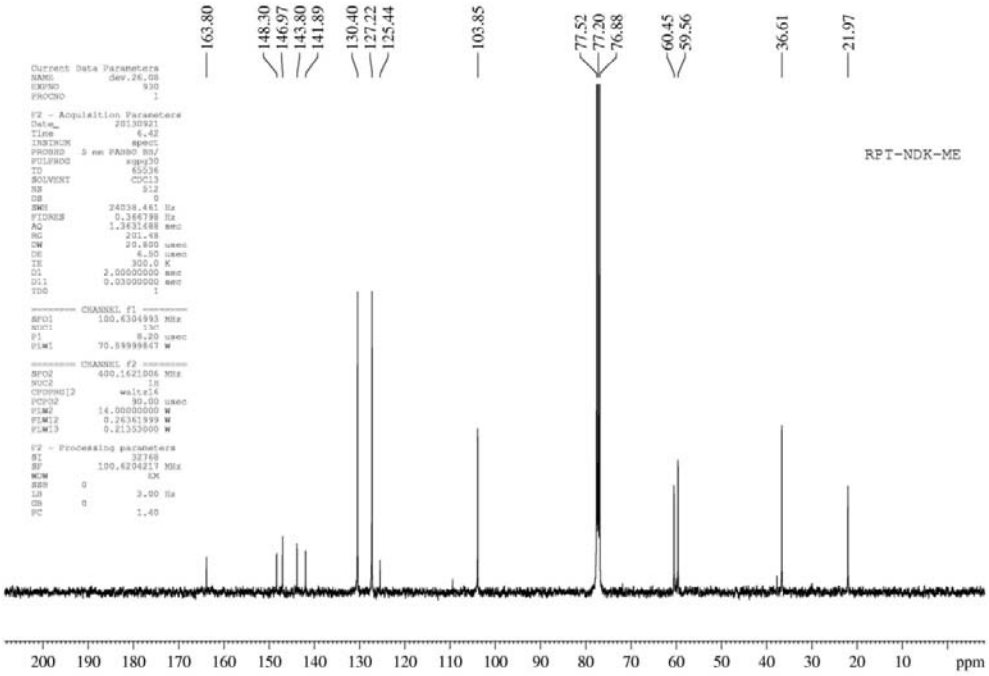
Complete chemical shift values of compound **31** S27

COSY, HSQC and HMBC spectra of compound **31**.....S28

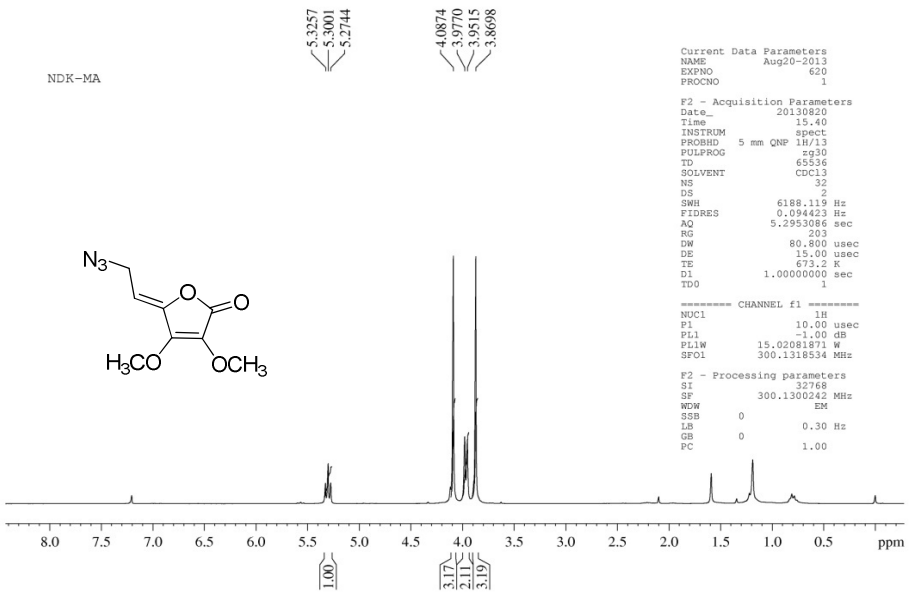
^{1}H NMR of Intermediate **2b**



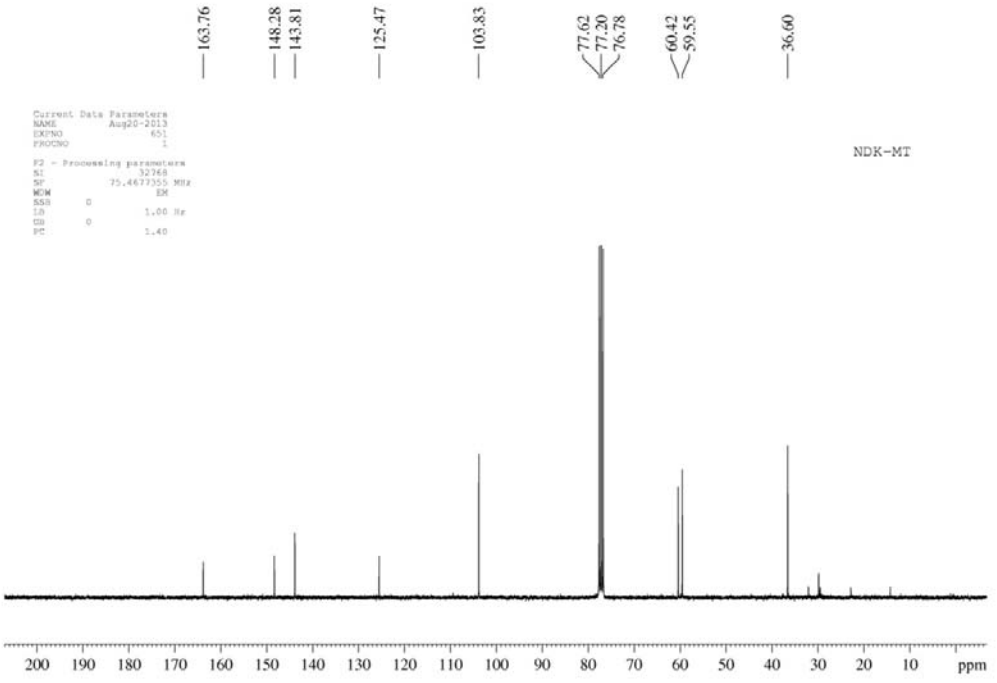
^{13}C NMR of Intermediate **2b**



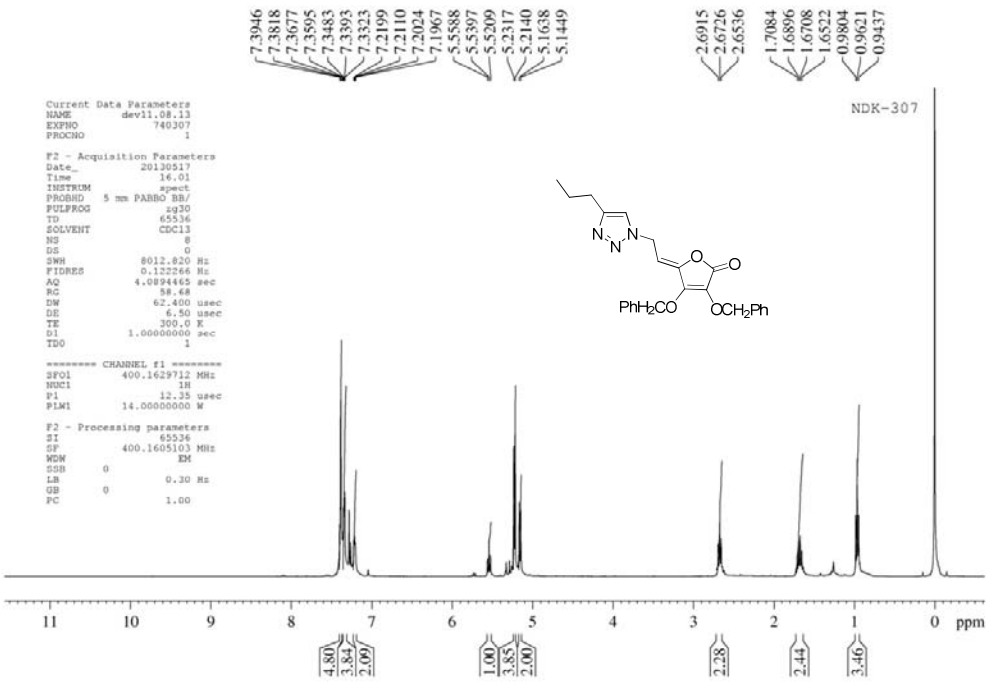
^{1}H NMR of Intermediate **3b**



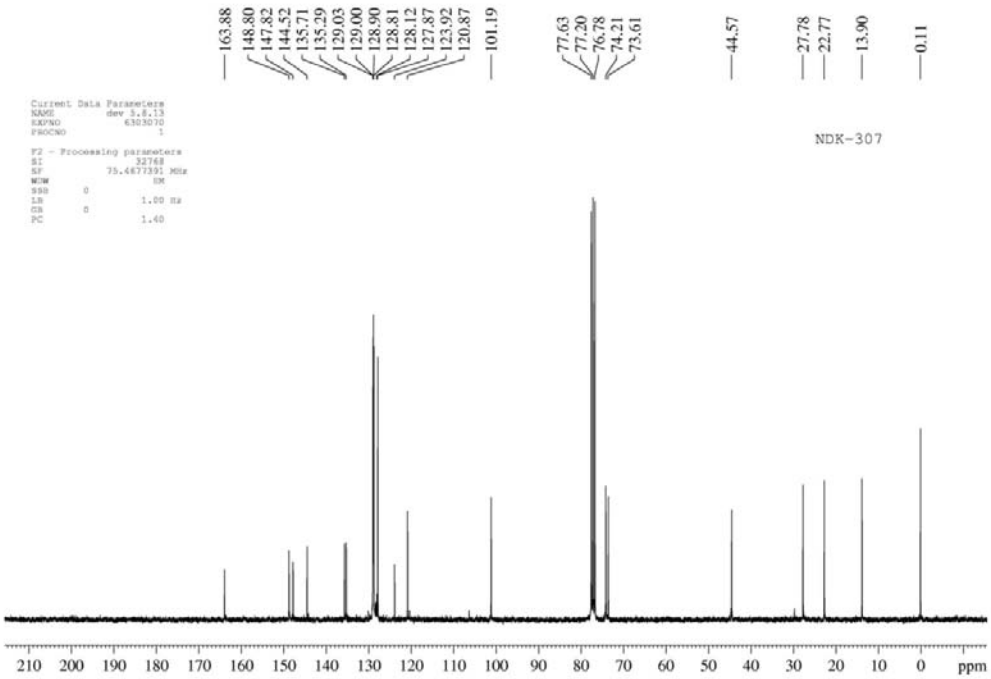
^{13}C NMR of Intermediate **3b**



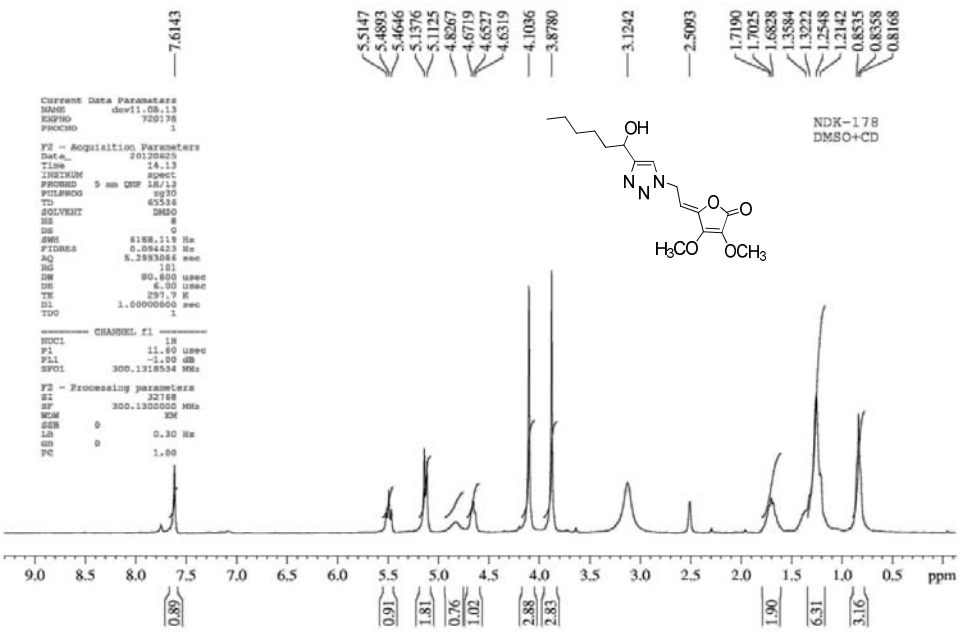
^{1}H NMR of Compound **4**



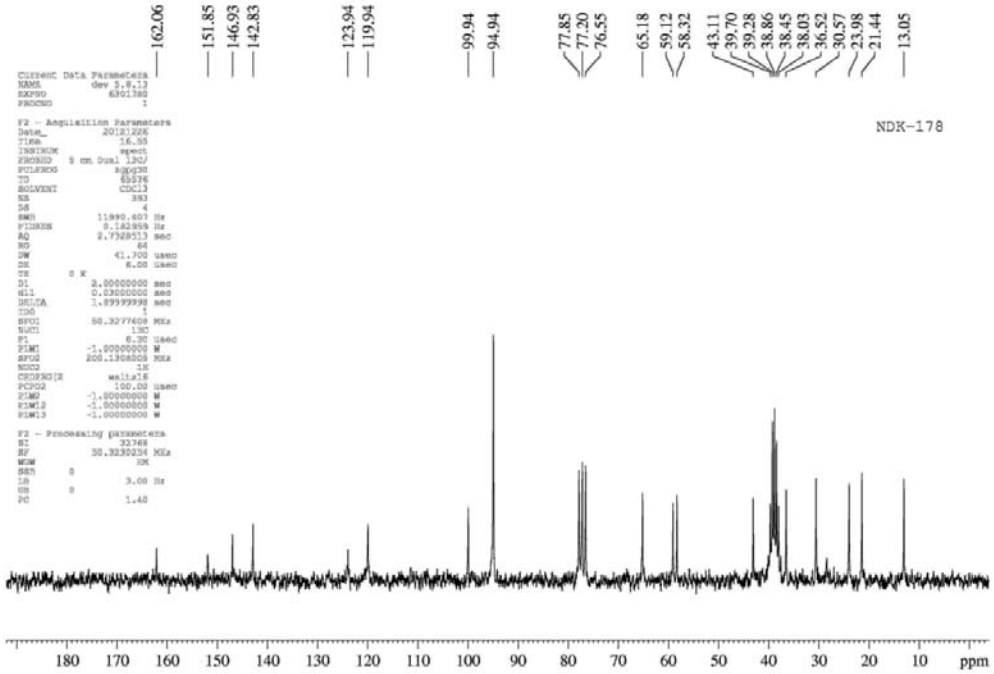
^{13}C NMR of Compound **4**



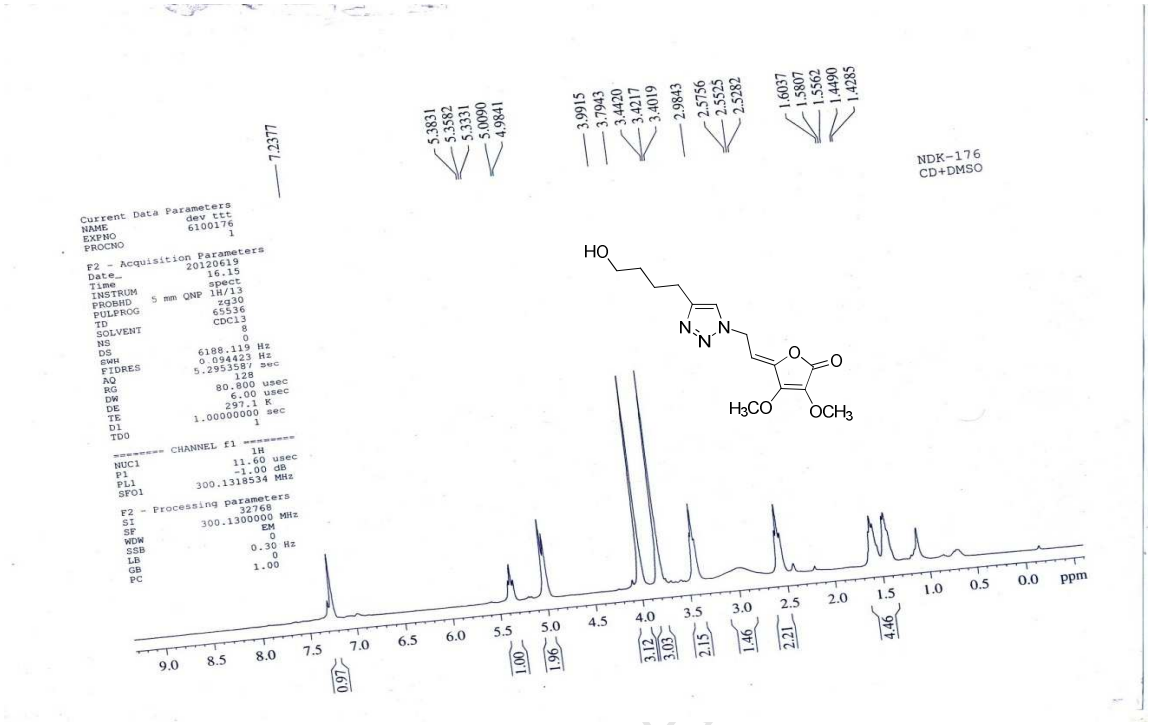
^{1}H NMR of Compound **8**



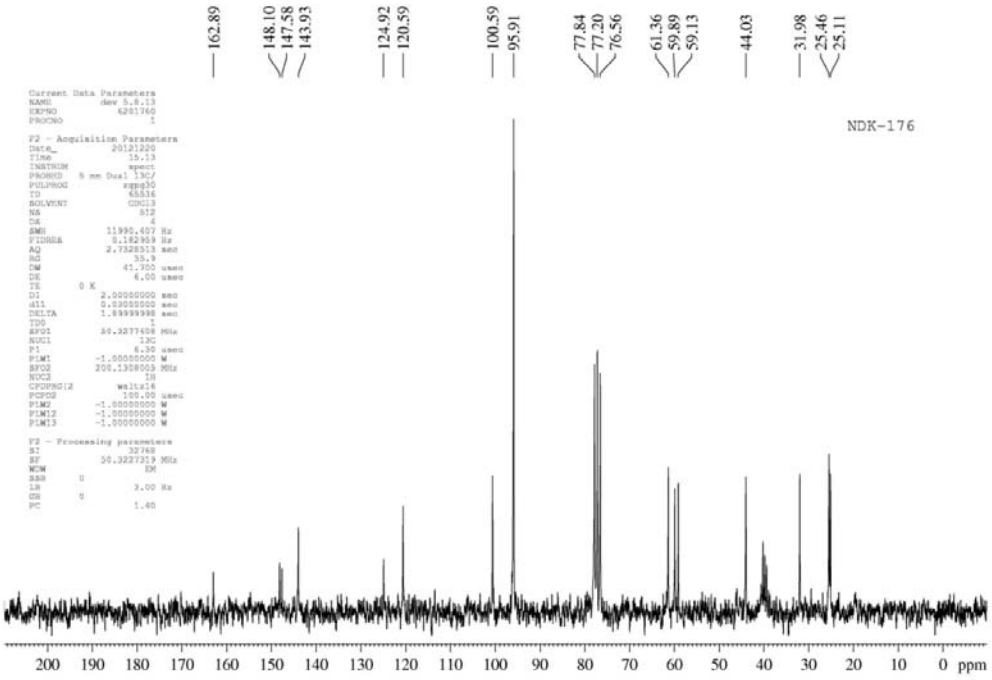
^{13}C NMR of Compound **8**



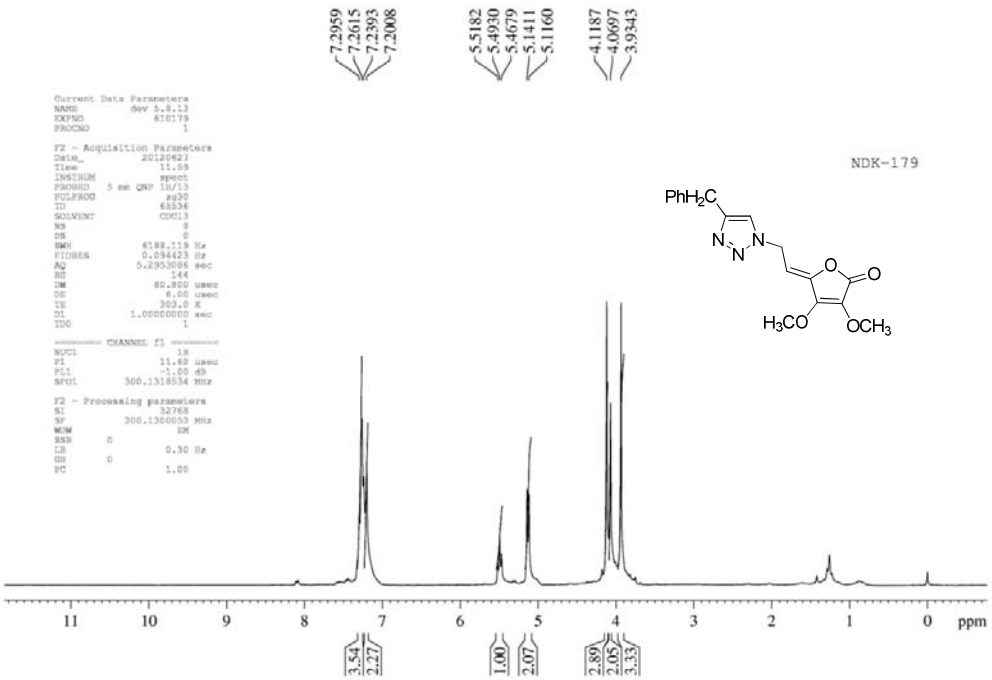
^{1}H NMR of Compound **9**



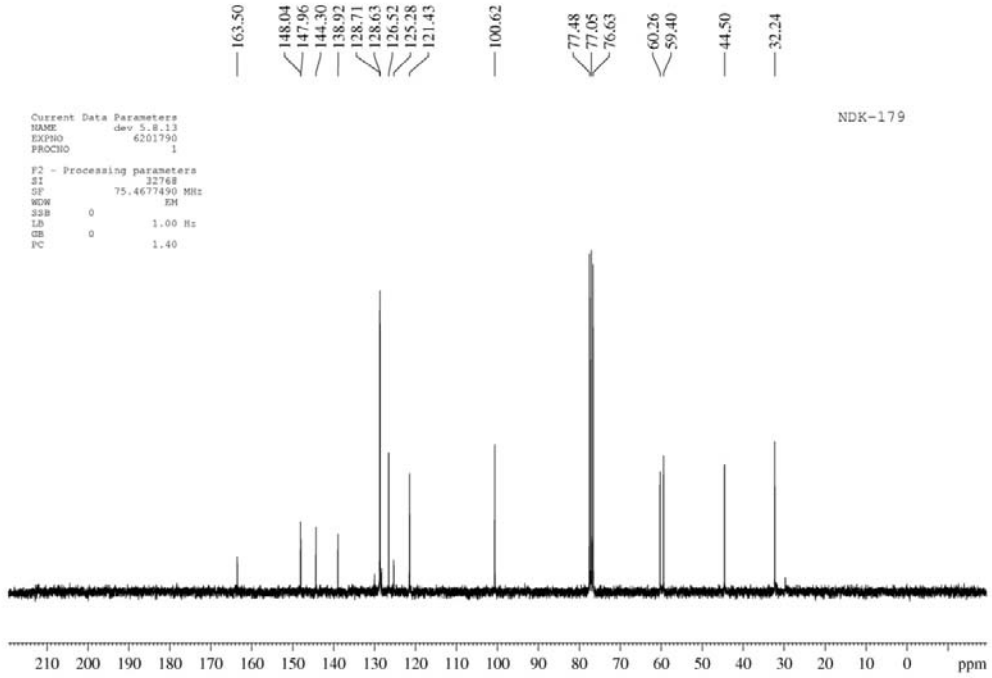
^{13}C NMR of Compound **9**



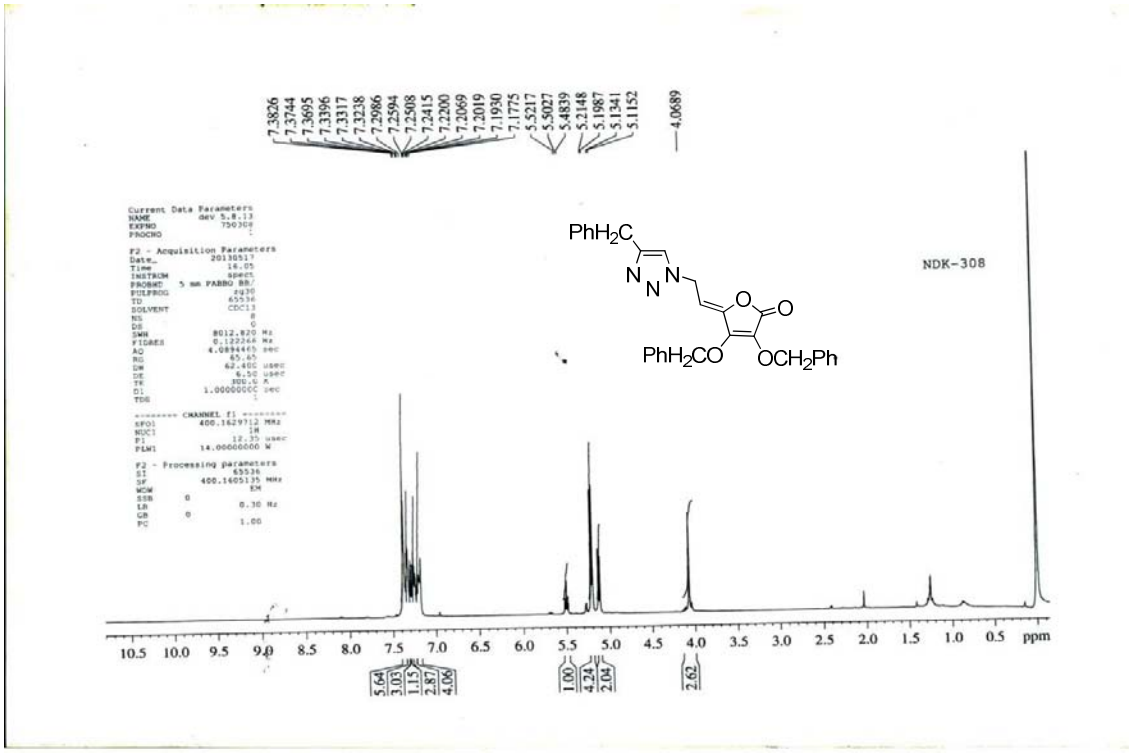
^{1}H NMR of Compound **11**



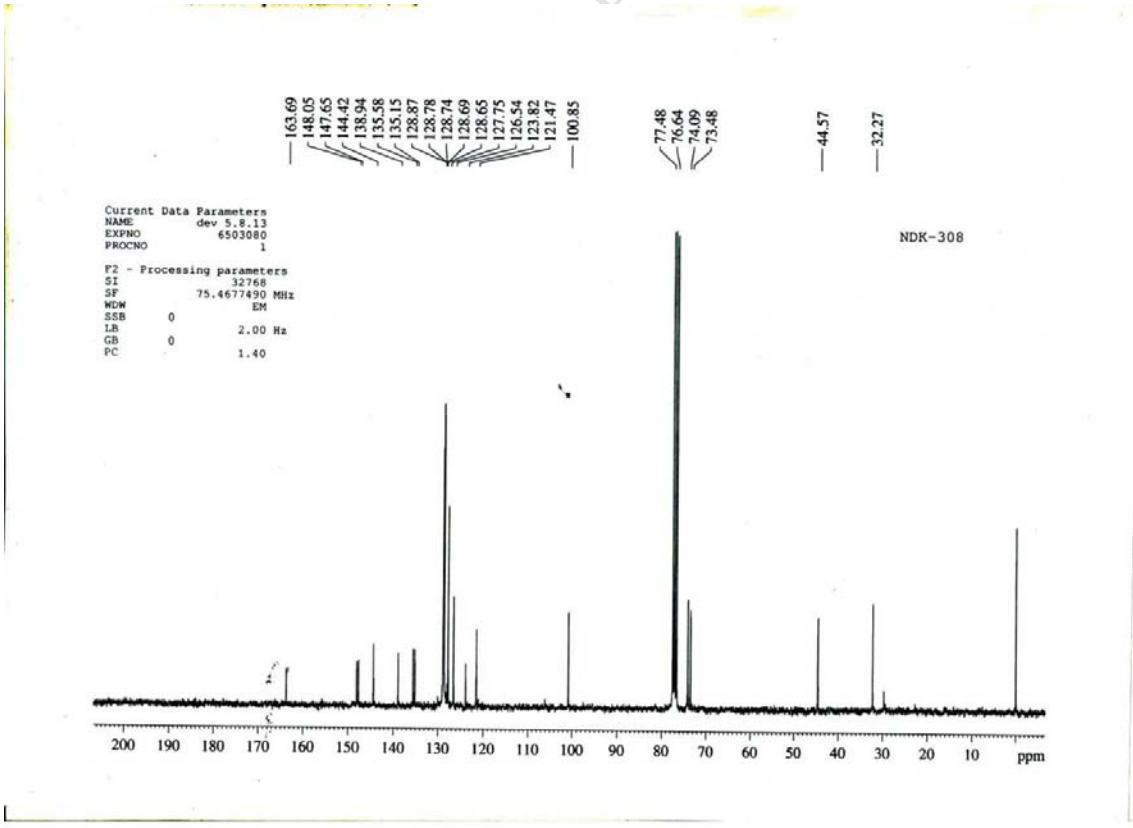
^{13}C NMR of Compound **11**



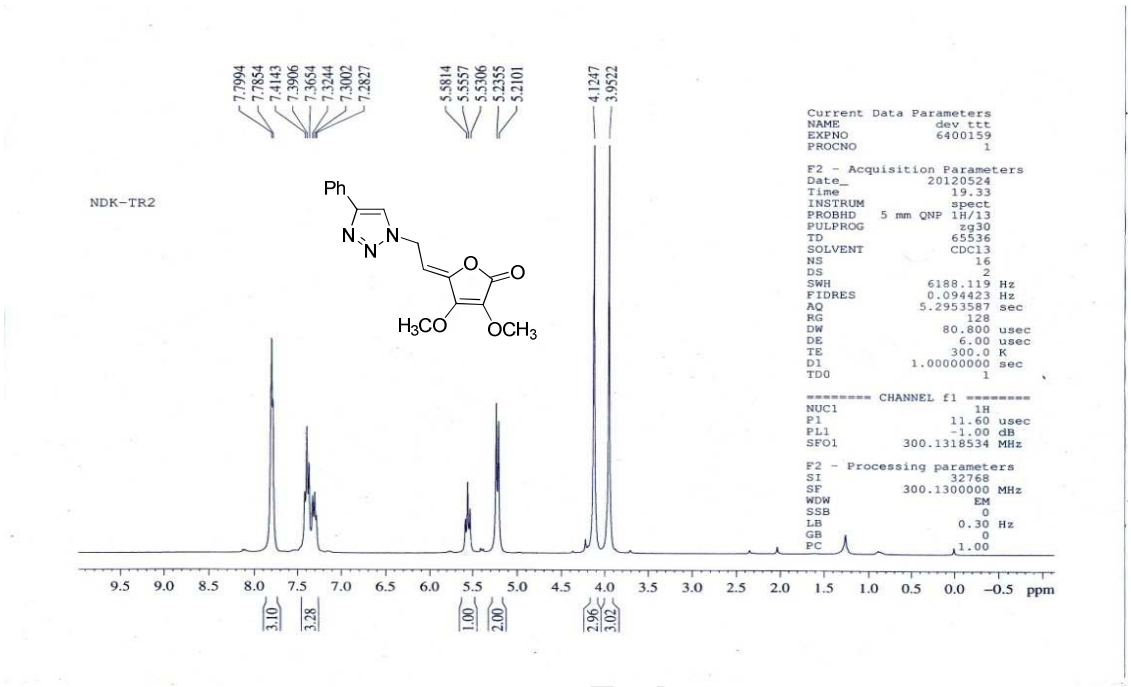
^{1}H NMR of Compound **12**



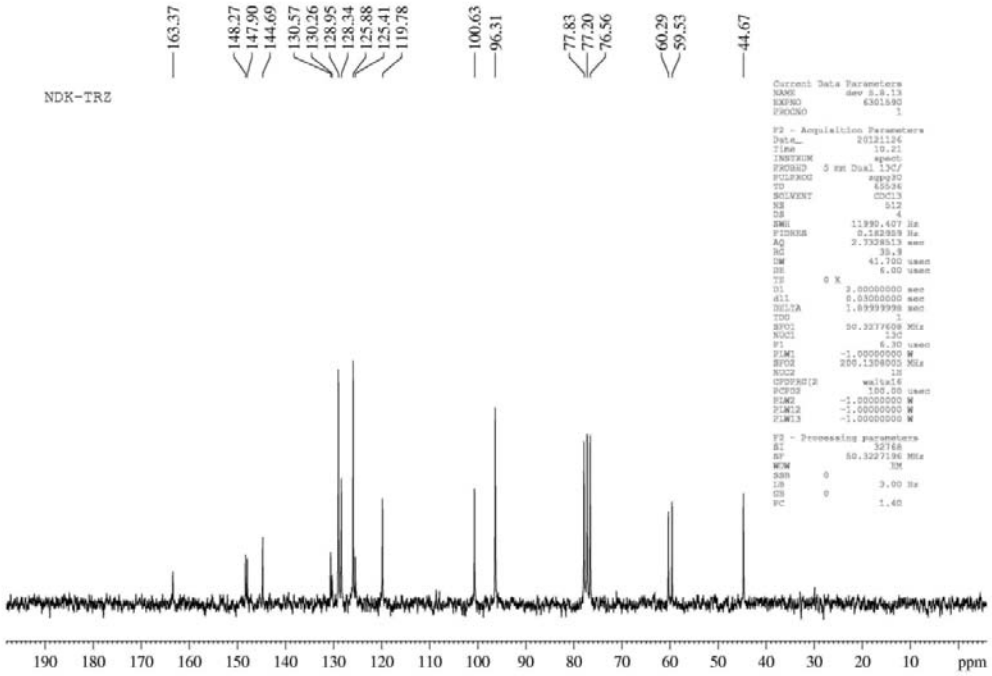
^{13}C NMR of Compound **12**



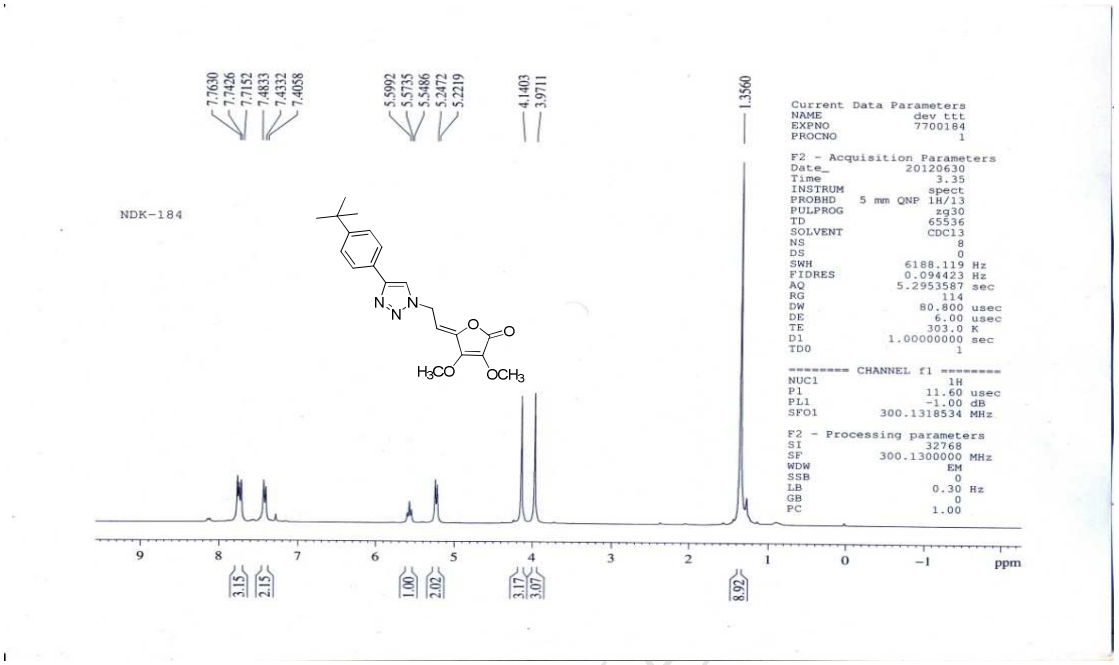
^{1}H NMR of Compound **13**



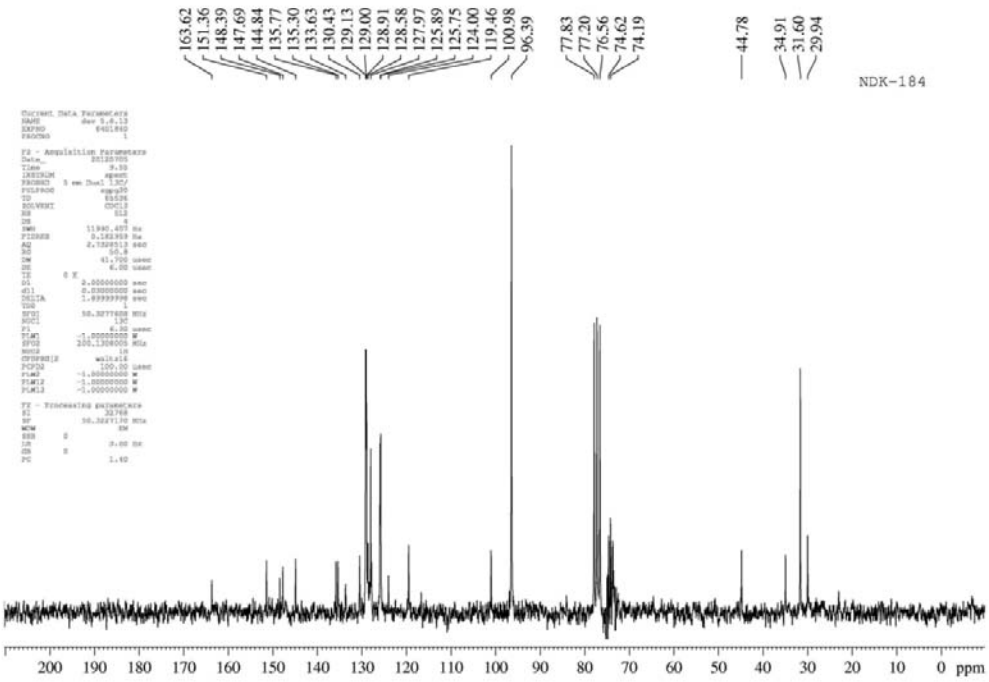
^{13}C NMR of Compound **13**



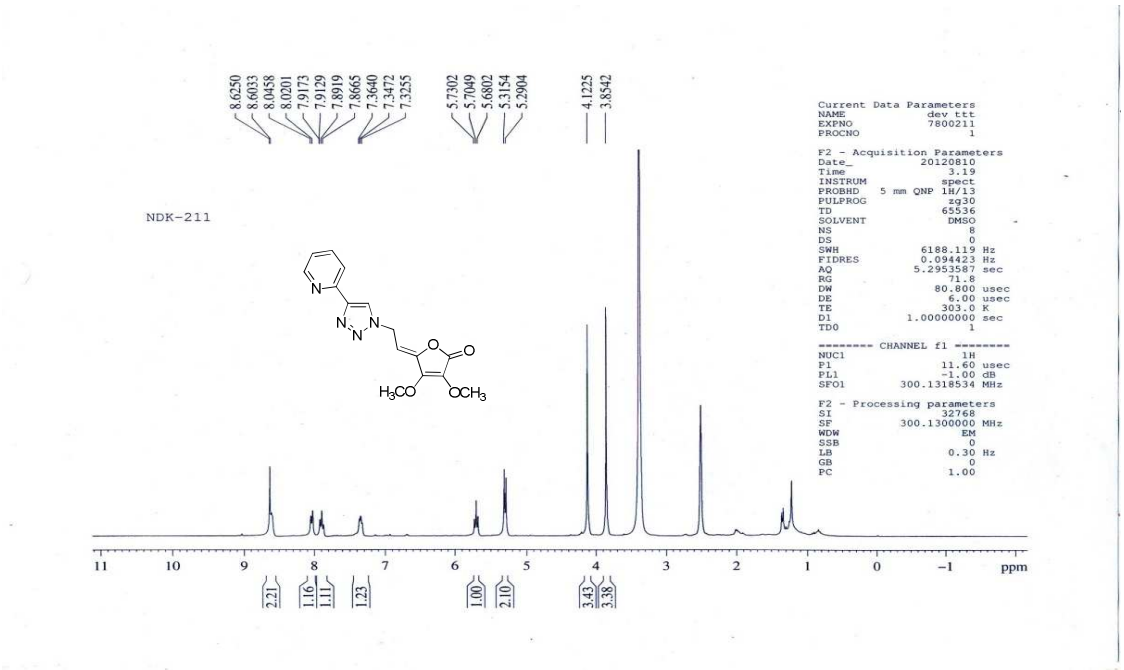
^{1}H NMR of Compound **15**



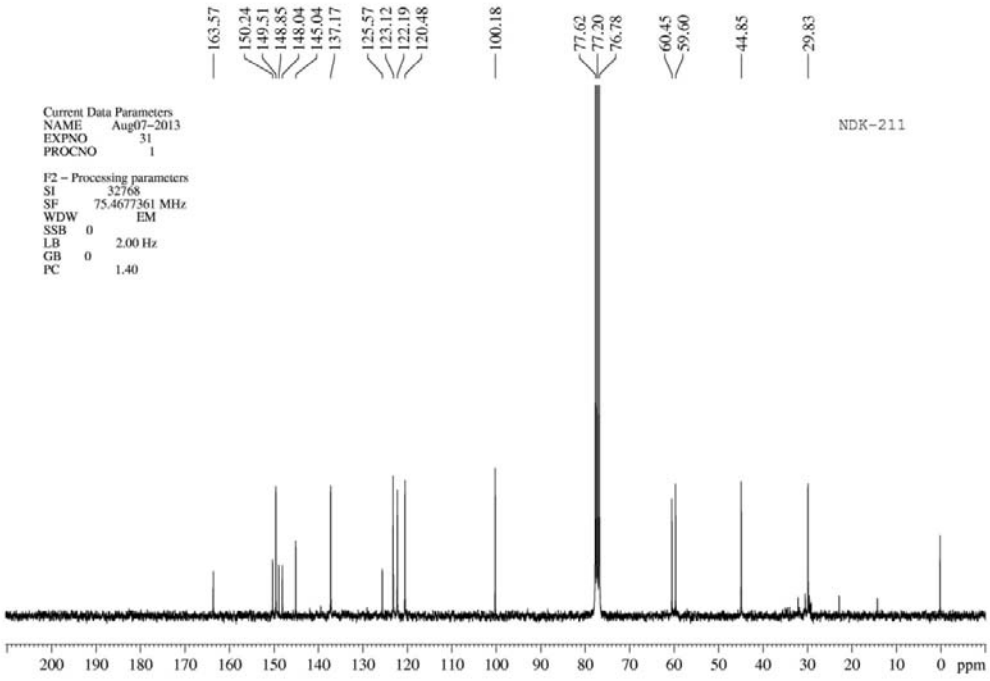
^{13}C NMR of Compound **15**



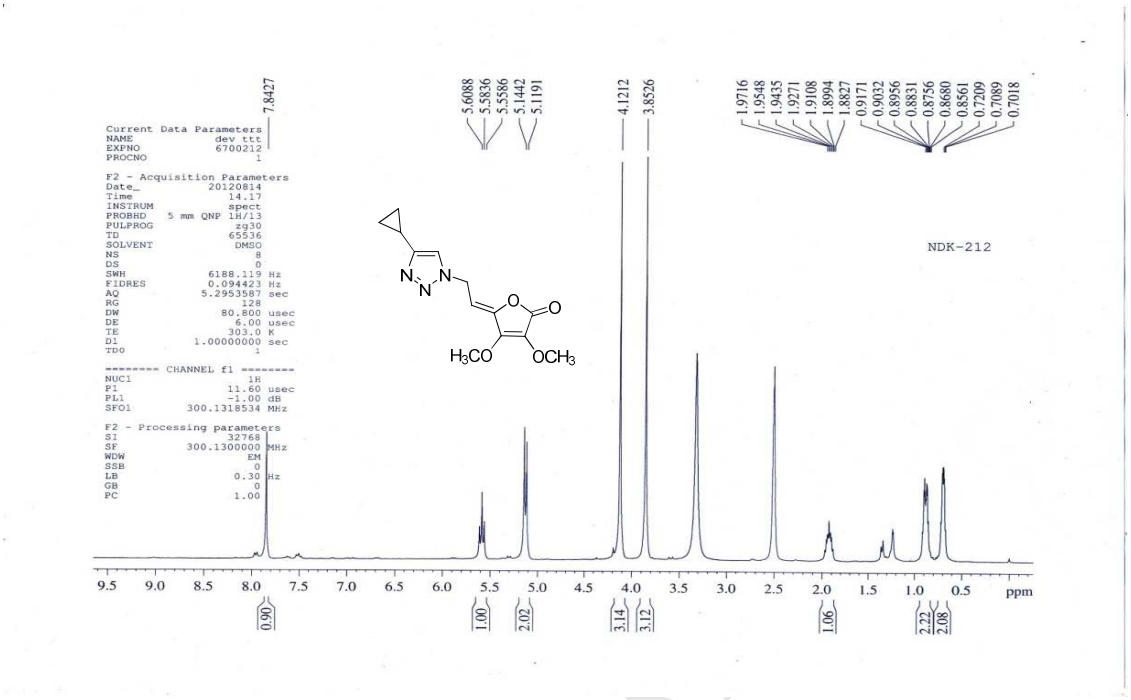
^{1}H NMR of Compound **17**



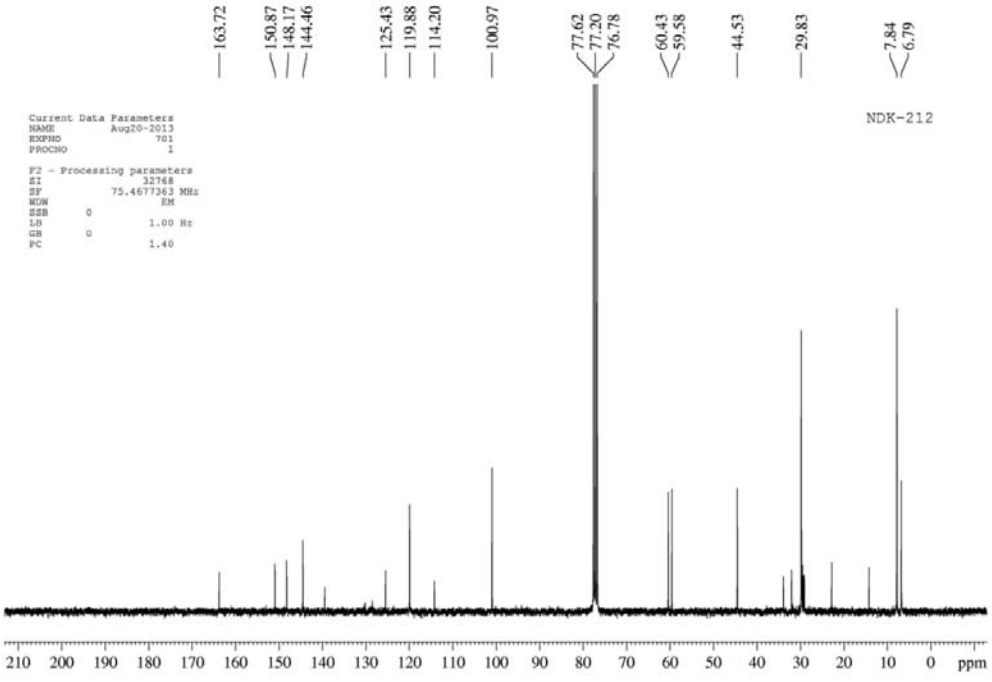
^{13}C NMR of Compound **17**



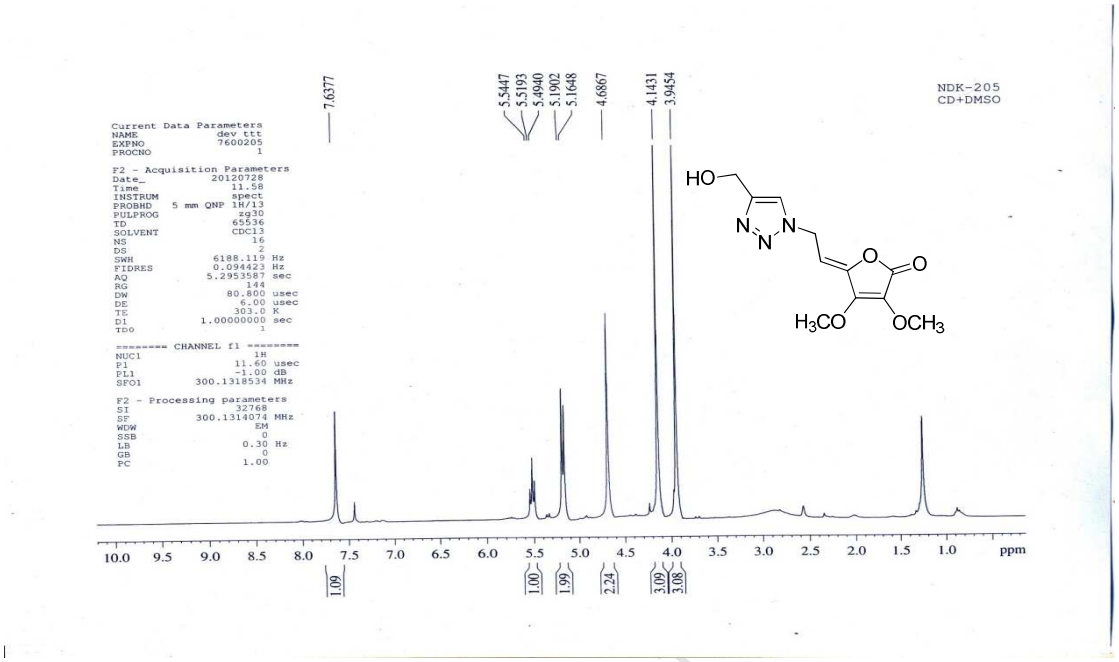
^{1}H NMR of Compound **19**



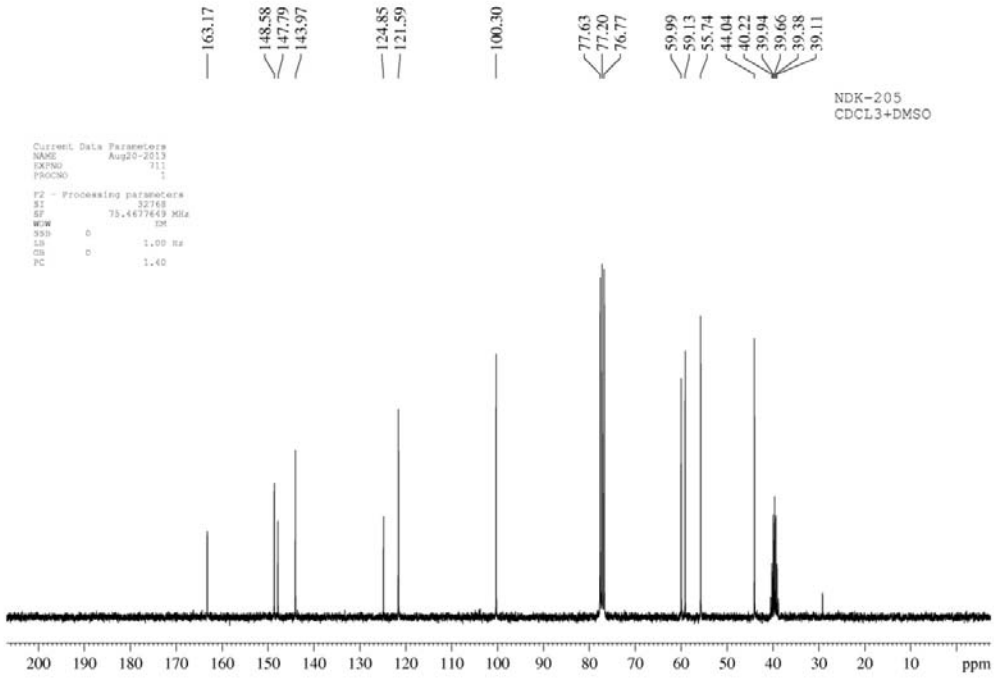
^{13}C NMR of Compound **19**



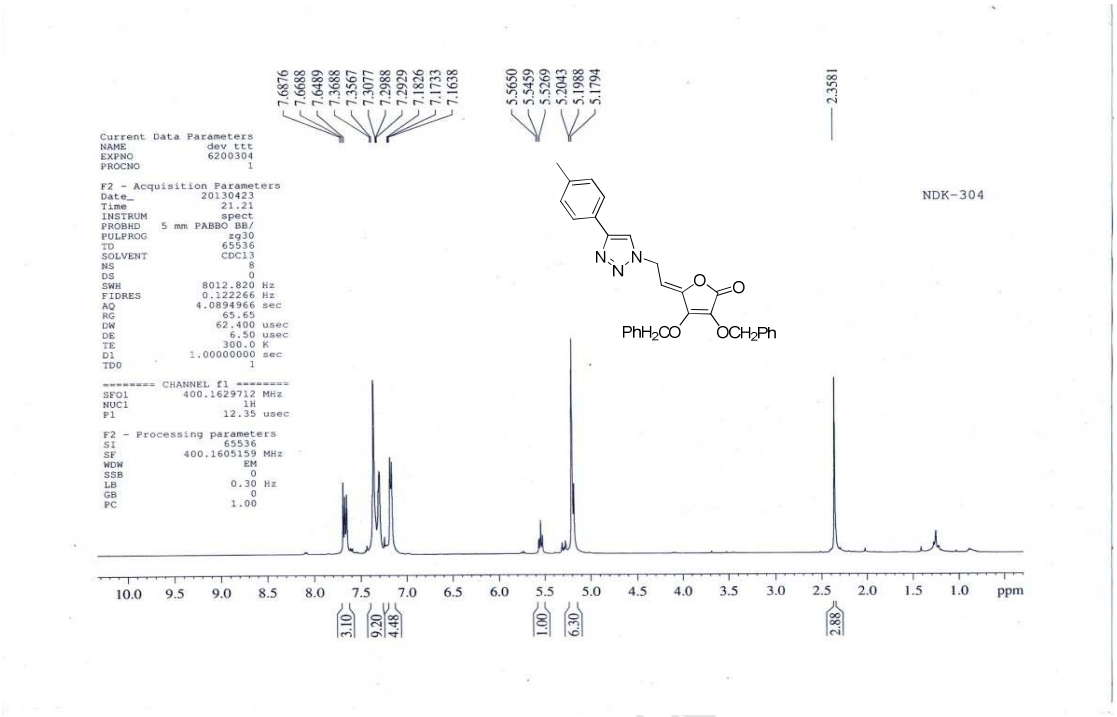
^{1}H NMR of Compound **21**



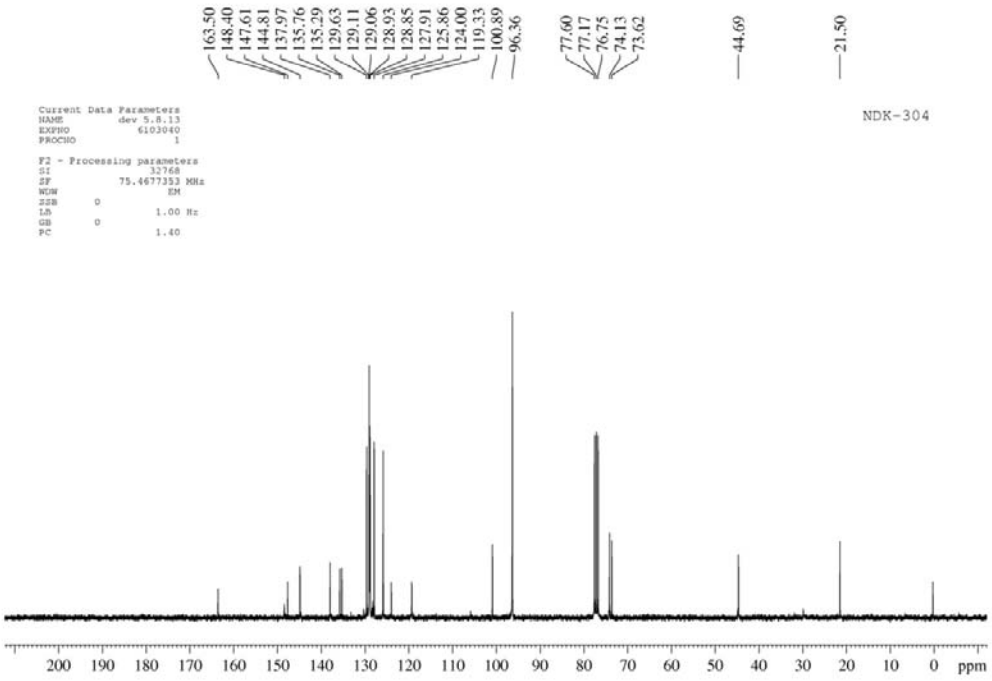
^{13}C NMR of Compound **21**



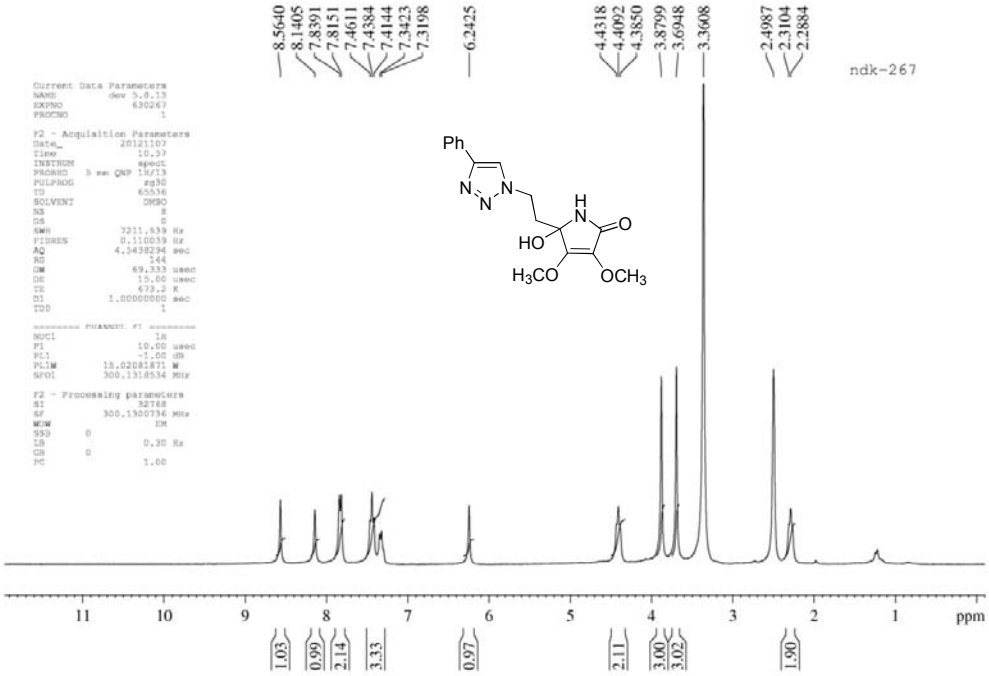
^{1}H NMR of Compound **22**



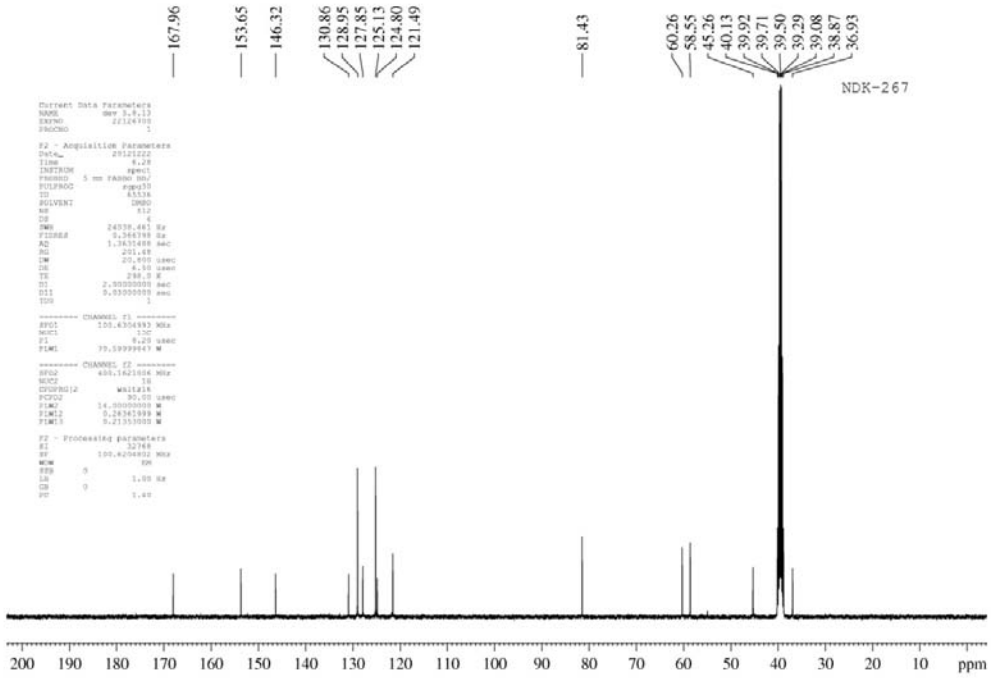
^{13}C NMR of Compound **22**



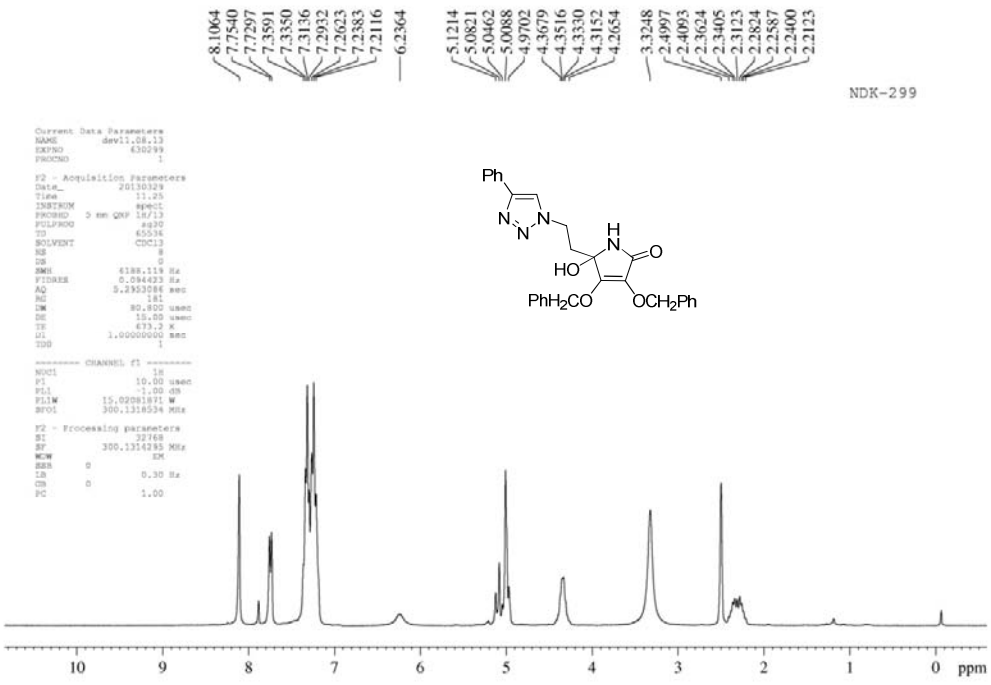
^{1}H NMR of Compound **24**



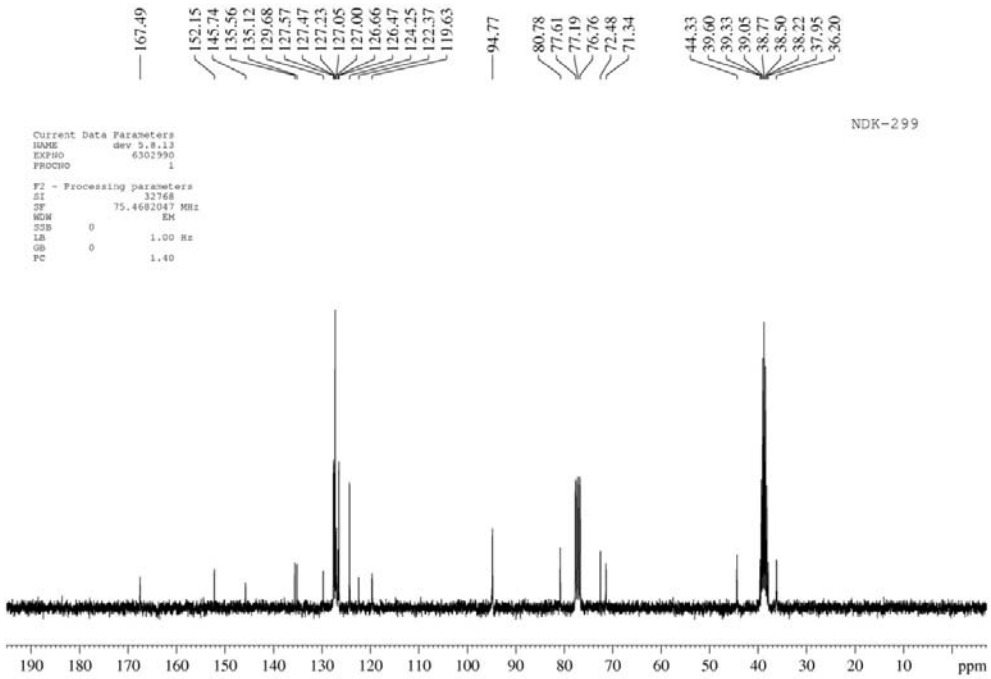
^{13}C NMR of Compound **24**



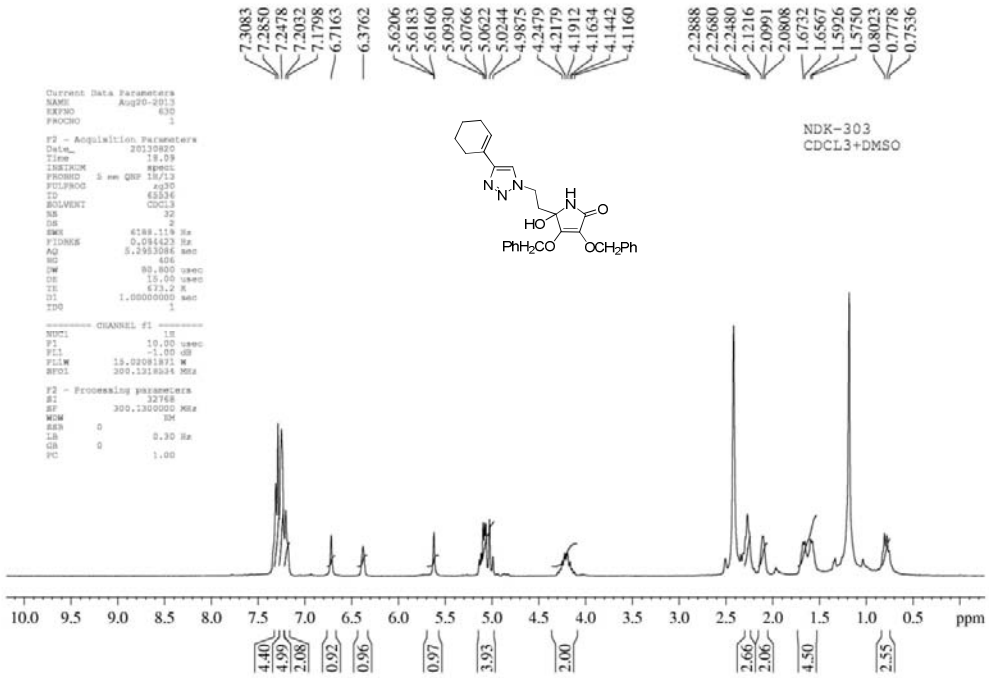
^{1}H NMR of Compound **26**



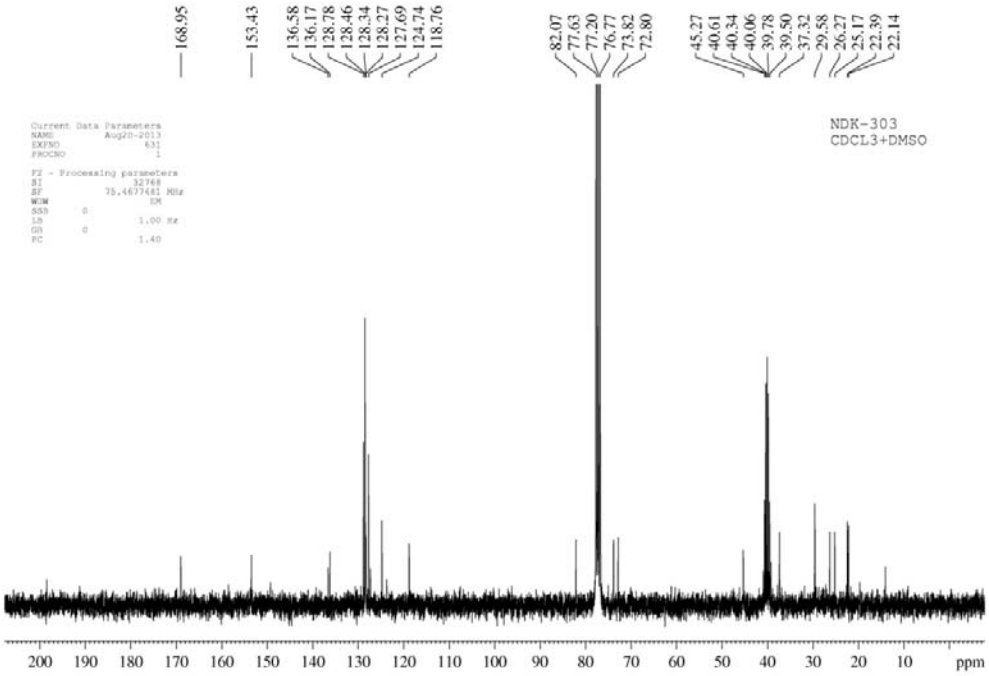
^{13}C NMR of Compound **26**



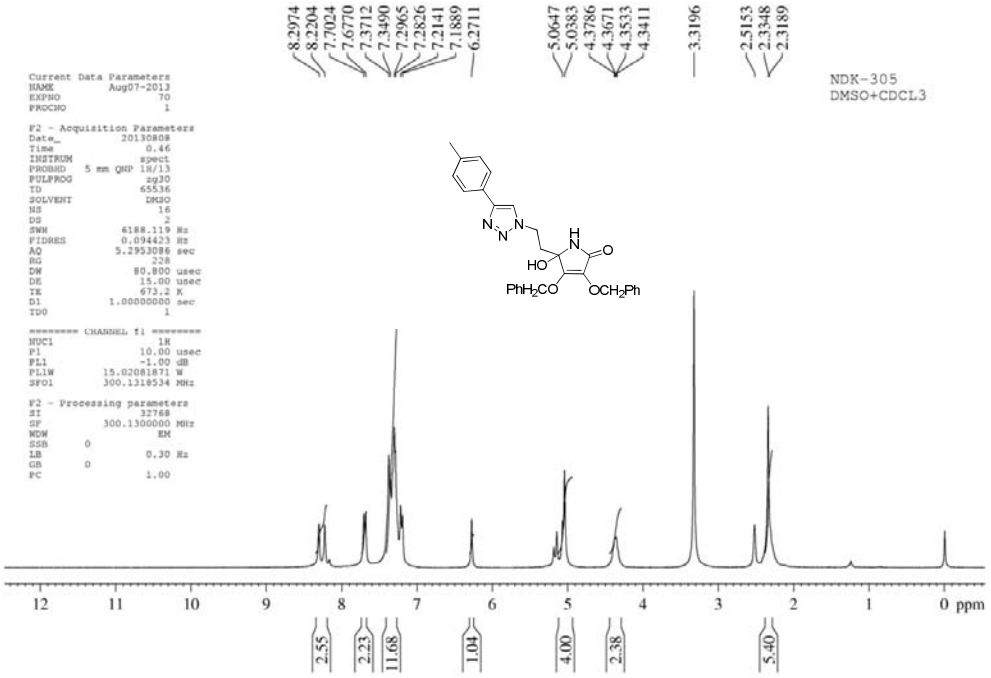
^{1}H NMR of Compound **27**



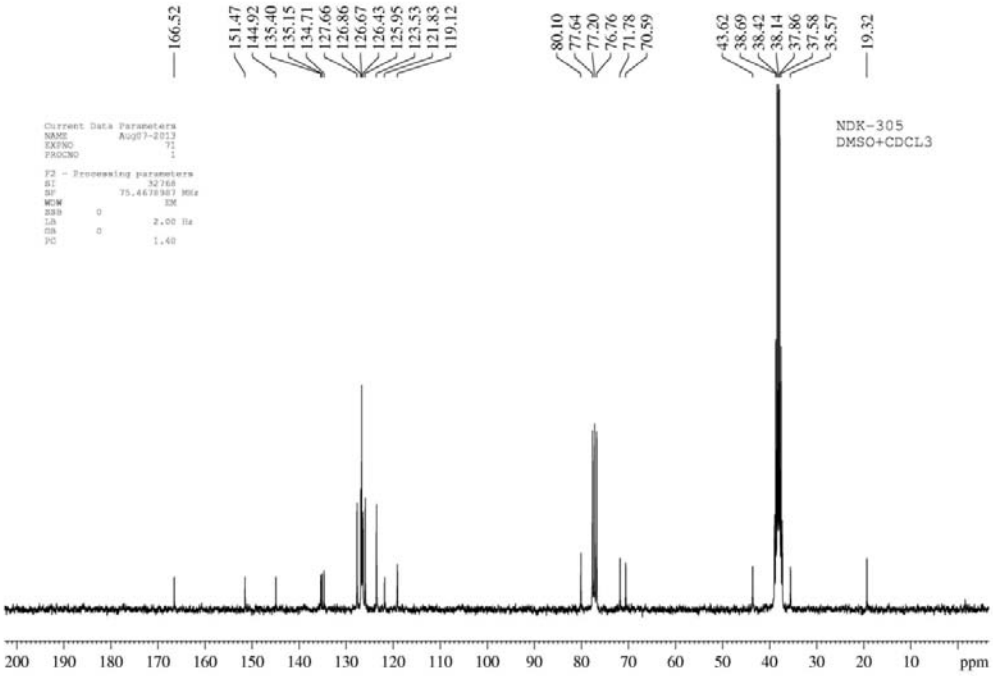
^{13}C NMR of Compound **27**



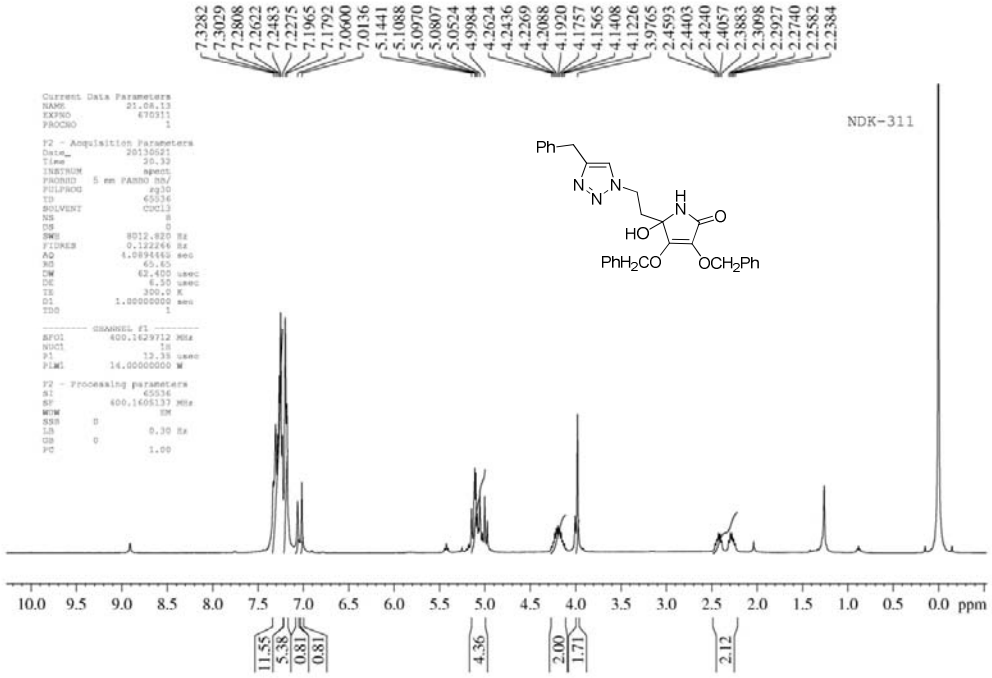
^{1}H NMR of Compound **28**



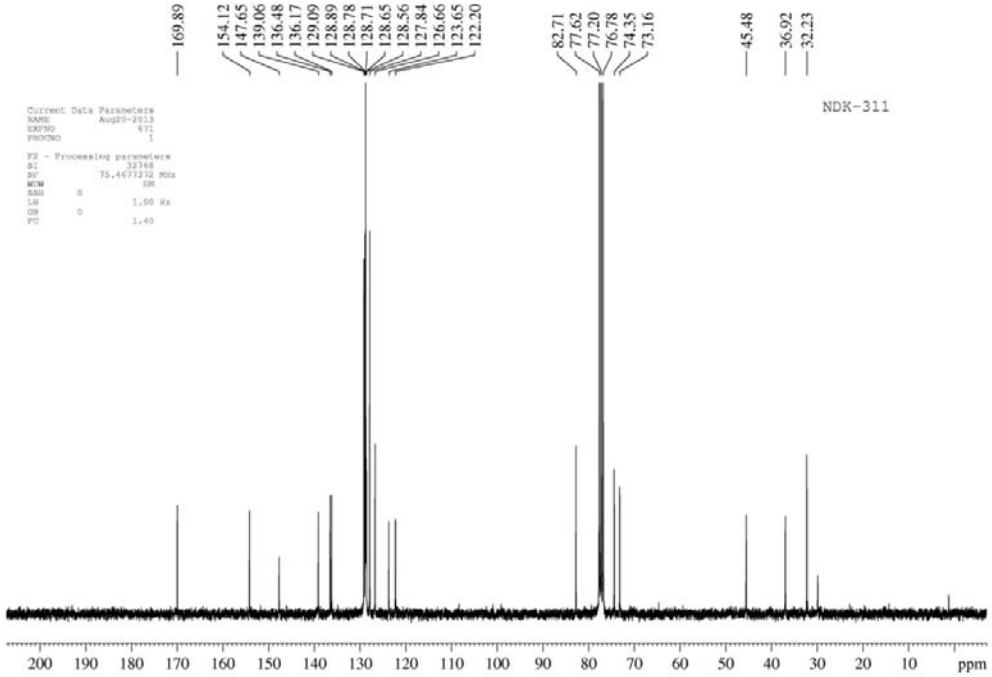
^{13}C NMR of Compound **28**



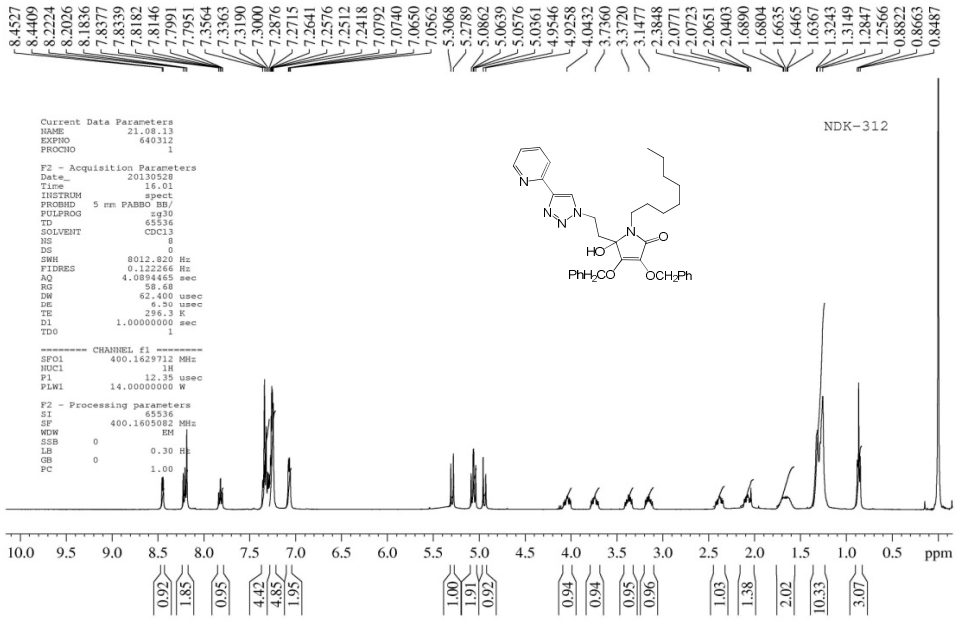
^{1}H NMR of Compound **29**



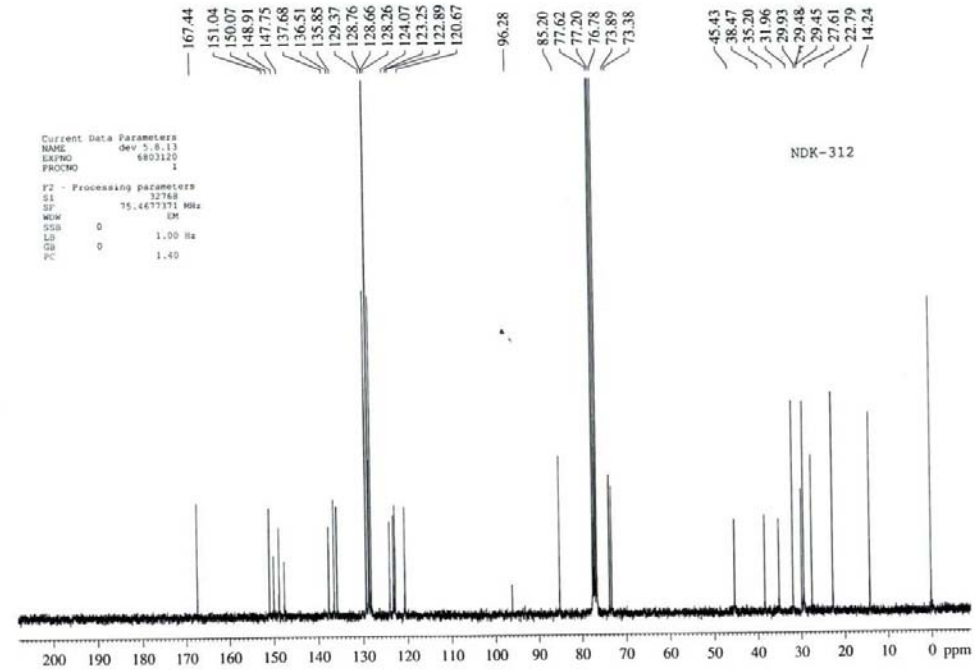
^{13}C NMR of Compound **29**



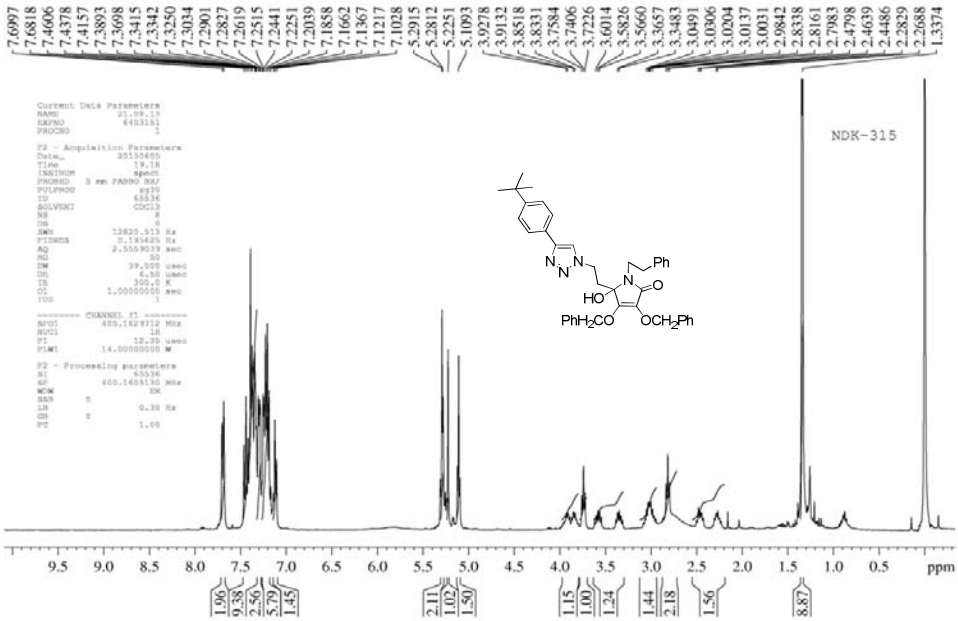
^{1}H NMR of Compound **32**



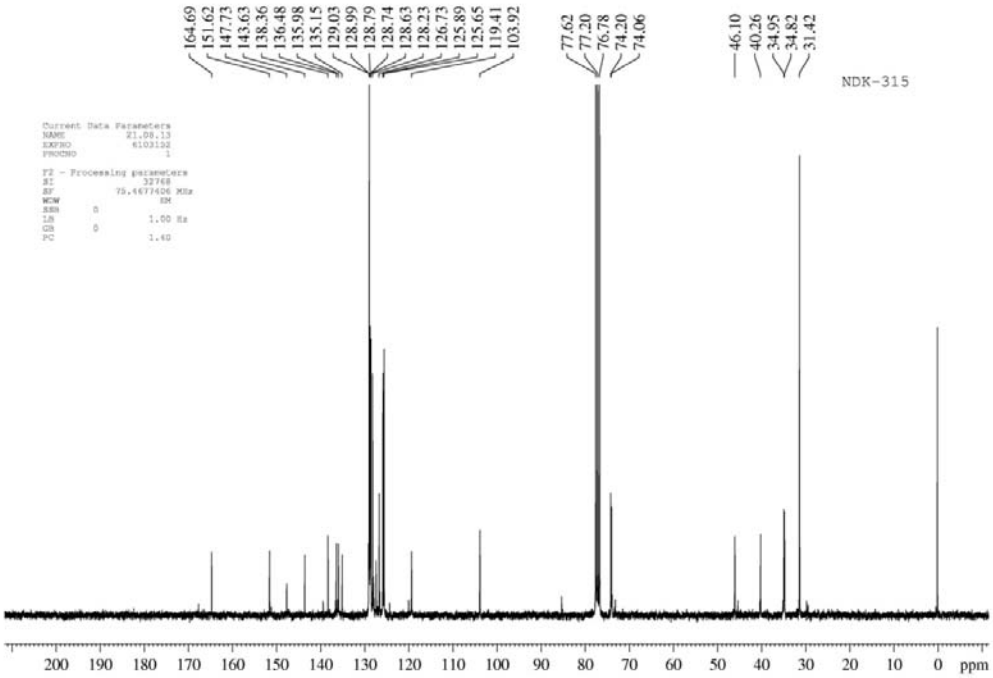
^{13}C NMR of Compound **32**



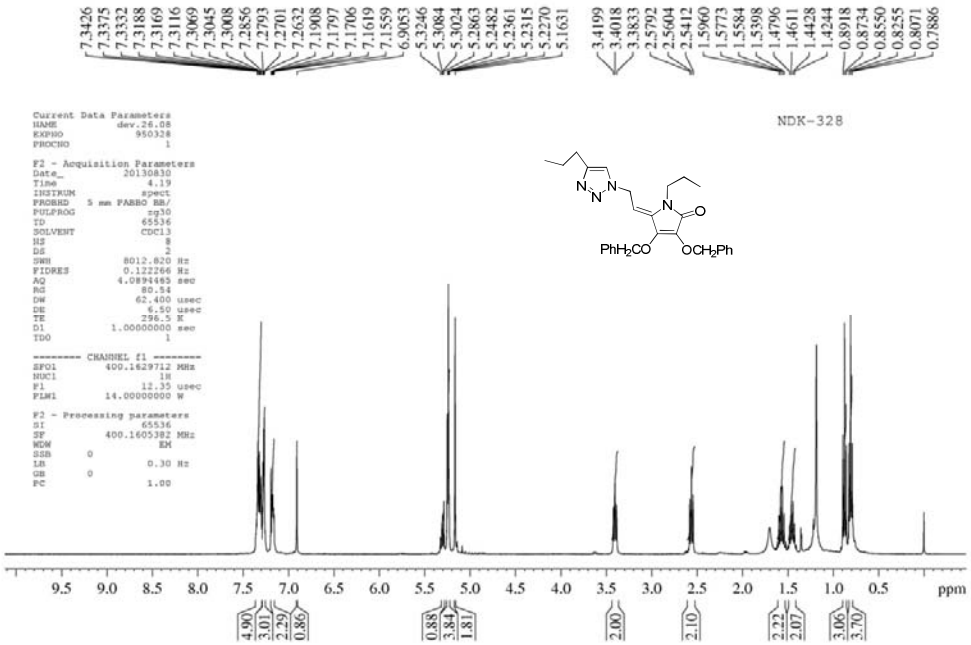
^{1}H NMR of Compound **33**



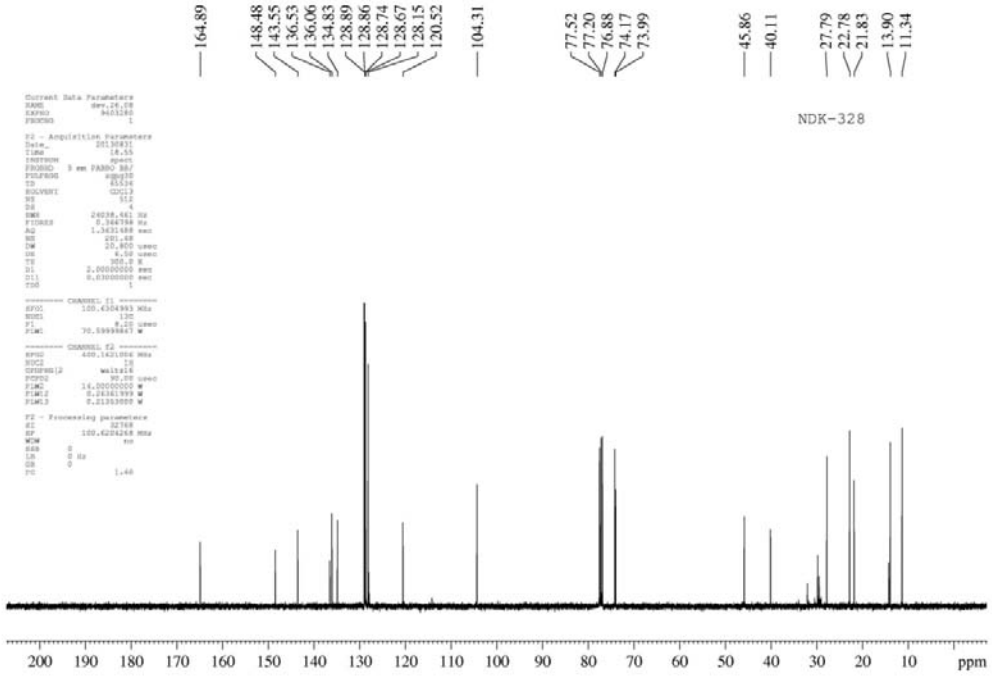
^{13}C NMR of Compound **33**



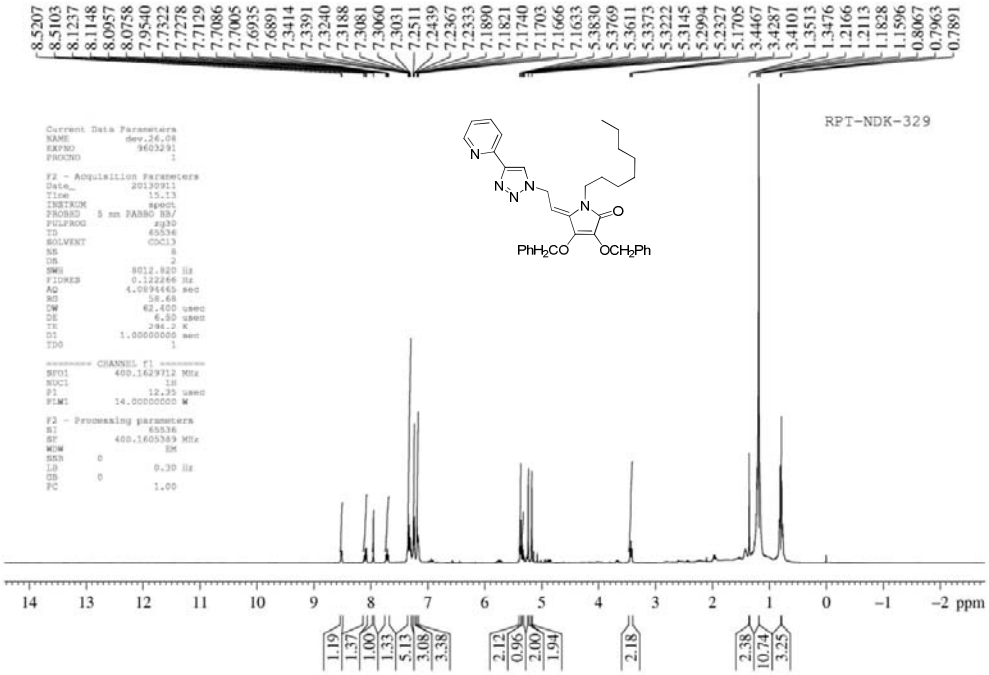
^{1}H NMR of Compound **34**



^{13}C NMR of Compound **34**



^{1}H NMR of Compound **35**



^{13}C NMR of Compound **35**

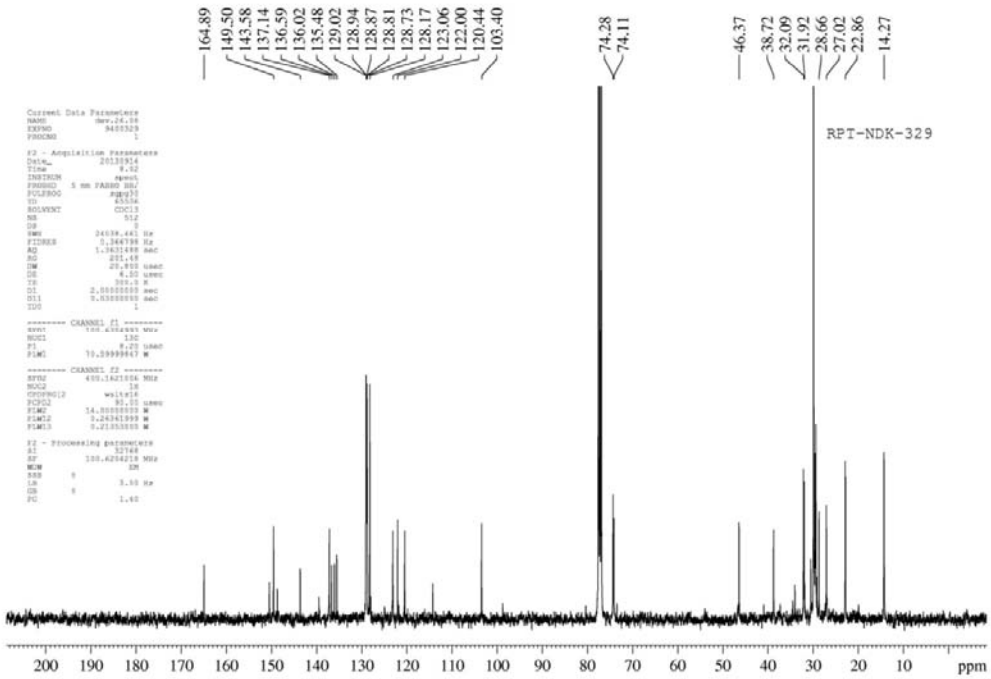
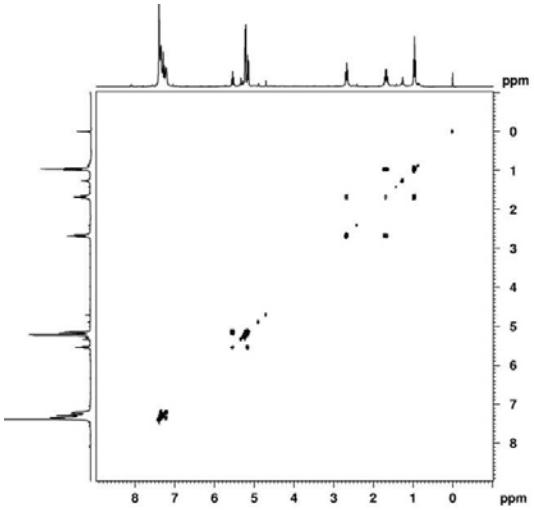


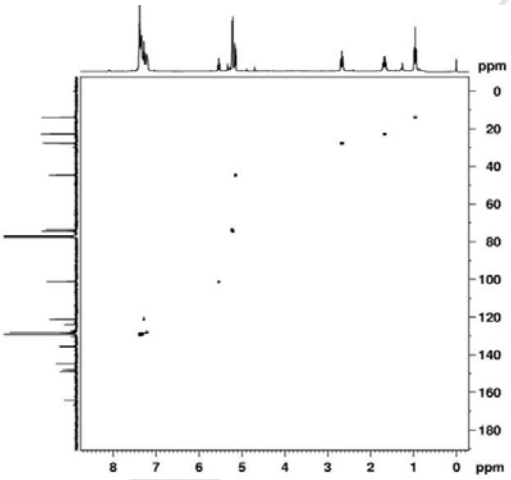
Table 1: Complete chemical shift values of compound **4**

Carbon position	^{13}C Chemical shift (ppm)	^{1}H Chemical shift(ppm)
C-4	148.7	-
C-5	120.6	6.90 (s)
C-6	27.9	2.65 (t, *J* = 7.20 Hz)
C-7	22.9	1.65 (m)
C-8	14.0	0.95 (t, *J* = 7.20 Hz)
C-9	46.0	5.31 (m)
C-10	104.3	5.35 (m)
C-11	134.8	-
C-13	164.4	-
C-14	134.8	-
C-15	143.6	-
C-16	40.1	3.48 (t, *J* = 7.20 Hz)
C-17	21.9	1.54 (m)
C-18	11.4	0.88 (t, *J* = 7.20 Hz)
C-1′	74.0	5.23 (s)
C-2′	136.0	-
C-3′, C-7′′	128.8	7.31 (m)
C-4′, C-6′′	129.0	7.28 (m)
C-5′	128.7	7.17 (m)
C-1′′	74.4	5.31 (s)
C-2′′	136.5	-
C-3′′, C-7′′	128.9	7.33 (m)
C-4′′, C-6′′	128.2	7.26 (m)
C-5′′	128.9	7.15 (m)

COSY spectrum of compound **4**



HSQC spectrum of compound **4**



HMBC spectrum of compound **4**

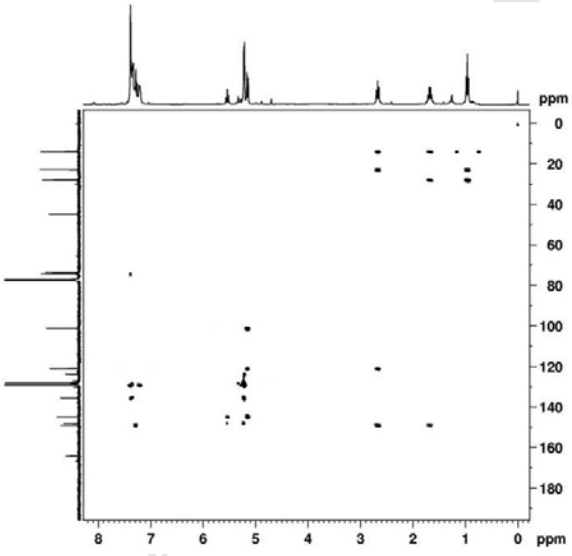
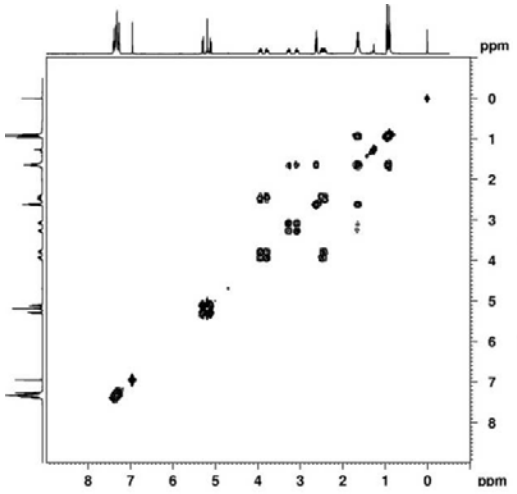


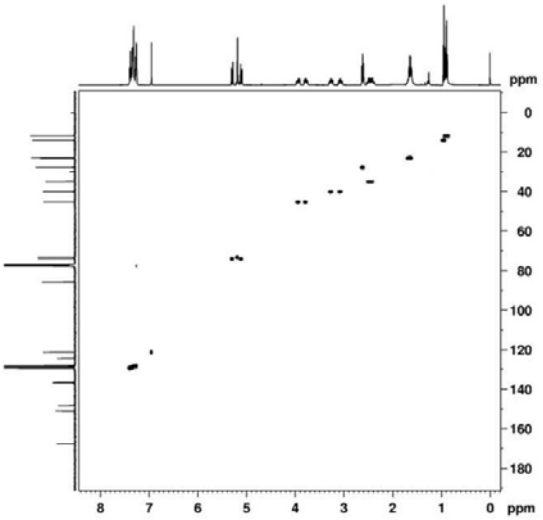
Table 2: Complete chemical shift values of compound **31**

Carbon position	^{13}C Chemical shift (ppm)	^{1}H Chemical shift (ppm)
C-4	148.2	-
C-5	121.1	6.95 (s)
C-6	27.7	2.61 (t, *J* = 7.20 Hz)
C-7	22.8	1.63 (m)
C-8	11.8	0.89 (t, *J* = 7.20 Hz)
C-9	45.1	3.95 (m), 3.79 (m)
C-10	34.9	2.46 (m)
C-11	85.6	-
C-11-OH	-	1.91 (brs)
C-13	167.4	-
C-14	124.3	-
C-15	150.8	-
C-16	40.0	3.27 (m), 3.07 (m)
C-17	23.0	1.61 (m)
C-18	13.9	0.94 (t, *J* = 7.20 Hz)
C-1′	73.2	5.18 (s)
C-2′	136.3	-
C-3′, C-7′′	128.0	7.40 (m)
C-4′, C-6′′	128.7	7.36 (m)
C-5′	128.8	7.27 (m)
C-1′′	73.9	5.29 (d, *J* = 11.20 Hz)
C-2′′	136.6	5.11 (d, *J* = 11.20 Hz)
C-3′′, C-7′′	129.3	7.38 (m)
C-4′′, C-6′′	128.7	7.33 (m)
C-5′′	128.7	7.26 (m)

COSY spectrum of compound **31**



HSQC spectrum of compound **31**



HMBC spectrum of compound **31**