

# Accepted Manuscript

Synthesis and biological evaluation of novel 4,5-disubstituted 2H-1,2,3-triazoles as *cis*-constrained analogues of combretastatin A-4

Nikhil R. Madadi, Narsimha R. Penthala, Kevin Howk, Amit Ketkar, Robert L. Eoff, Michael J. Borrelli, Peter A. Crooks



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## Graphical Abstract

**Synthesis and biological evaluation of novel 4,5-disubstituted 2*H*-1,2,3-triazoles as *cis*-constrained analogues of combretastatin A-4**

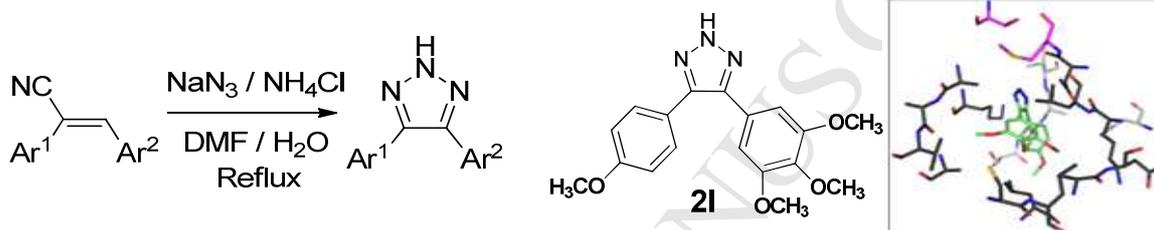
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Nikhil R. Madadi<sup>a</sup>, Narsimha R. Penthala<sup>a</sup>, Kevin Howk<sup>ab</sup>, Amit Ketkar<sup>c</sup>, Robert L. Eoff<sup>c</sup>, Michael J. Borrelli<sup>b</sup> and Peter A. Crooks<sup>a\*</sup>

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**2i**:  $\text{GI}_{50} = < 10.0$  nM (against 95% of NCI 60-cell panel)

**2d**:  $\text{Ar}^1 = 3,4,5$ -trimethoxyphenyl;  $\text{Ar}^2 = 2$ -quinolyl;  $\text{GI}_{50} = < 10$  nM (MDA-MB-435, melanoma)

**2e**:  $\text{Ar}^1 = 3,4,5$ -trimethoxyphenyl;  $\text{Ar}^2 = 3$ -methoxy-4-hydroxyphenyl;  $\text{LD}_{50} = 7.5$  nM (9LSF rat gliosarcoma cells)

**2m**:  $\text{Ar}^1 = 4$ -methoxyphenyl;  $\text{Ar}^2 = 3,5$ -dimethoxyphenyl;  $\text{GI}_{50} = < 10$  nM (K-562, leukemia; HCT-116, HCT-15, colon cancer; M14, MDA-MB-435, melanoma; NCI/ADR-RES ovarian cancer; A498, renal cancer)

**2h**:  $\text{Ar}^1 = 3,4,5$ -trimethoxyphenyl;  $\text{Ar}^2 = 3,4$ -ethylenedioxyphenyl;  $\text{GI}_{50} = < 10$  nM (against 36% of NCI 60-cell panel)



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## Original article

## Synthesis and biological evaluation of novel 4,5-disubstituted 2*H*-1,2,3-triazoles as *cis*-constrained analogues of combretastatin A-4

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A series of combretastatin A-4 (CA-4) analogues have been prepared from (*Z*)-substituted diarylacrylonitriles (**1a-1p**) obtained in a two-step synthesis from appropriate arylaldehydes and acrylonitriles. The resulting 4,5-disubstituted 2*H*-1,2,3-triazoles were evaluated for their anti-cancer activities against a panel of 60 human cancer cell lines. The diarylacrylonitrile analogue **2l** exhibited the most potent anti-cancer activity in the screening studies, with GI<sub>50</sub> values of <10 nM against almost all the cell lines in the human cancer cell panel and TGI values of <10nM against cancer cell lines SF-539, MDA-MB-435, OVCAR-3 and A498. Furthermore, *in silico* docking studies of compounds **2l**, **2e** and **2h** within the active site of tubulin were carried out in order to rationalize the mechanism of anti-cancer properties of these compounds. From the *in silico* studies, compound **2e** was predicted to have better affinity for the colchicine binding site on tubulin compared to compounds **2l** and **2h**. Analogue **2e** was also evaluated for its anti-cancer activity by colony formation assay against 9LSF rat gliosarcoma cells and afforded an LD<sub>50</sub> of 7.5 nM. A cell cycle redistribution assay using analogue **2e** was conducted to further understand the mechanism of action of these CA-4 analogues. From this study, analogues **2e** and **2l** were the most potent anti-cancer agents in this structural class, and were considered lead compounds for further development as anti-cancer drugs.

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### 1. Introduction

Cancer is the second most life threatening disease after cardiovascular disease, affecting more than six million people per year worldwide. Although, significant research has done to date to treat cancer, there is still a lack of effective chemotherapeutic treatment to cure it completely with minimal side effects. Also, considerable effort has been put into identifying molecules with anti-cancer properties from both natural and synthetic sources. More than 60% of the anti-cancer drugs currently available are from natural sources [1]. The search for potent semi synthetically derived anti-cancer agents from the parent natural products continues to be an important part of drug discovery process. Anti-mitotic agents are a major class of cytotoxic drugs for the treatment of cancer and drugs that target microtubule/tubulin dynamics are widely used in cancer chemotherapy [2].

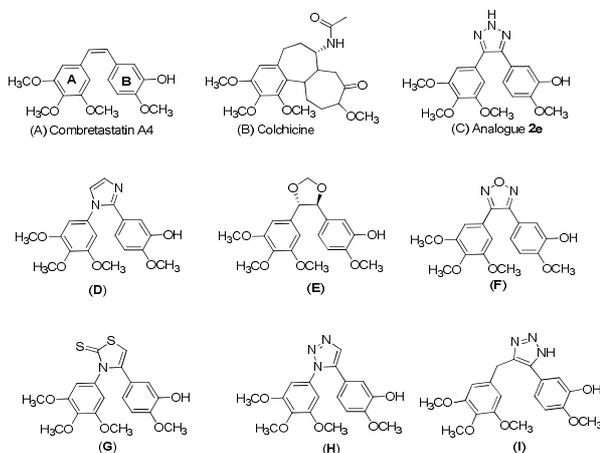
There are three major binding sites for tubulin; i.e. the vinca, taxane and colchicine domains. Vinca alkaloids, such as vincristine and vinblastine, bind to the vinca domain inhibiting the assembly of microtubule structures and arresting mitosis [3]. Paclitaxel acts at the taxane domain stabilizing microtubules and interfering with the normal breakdown of microtubules during mitosis [4]. Our area of interest focused on the colchicine binding site. Colchicine binds to tubulin and inhibits microtubule polymerization. Anti-mitotic agents such as combretastatin A-4 (CA-4) bind at the colchicine domain of tubulin, and have received much attention in recent years; CA-4P, the water soluble phosphate salt of CA-4 is currently in phase III clinical trial for anaplastic thyroid cancer, and is also in phase II trials for polypoidal choroidal vasculopathy and neovascular age-related macular degeneration [5, 6].

CA-4 is classified as a *cis*-stilbene originating from the South African willow tree *combretum caffrum*. CA-4 functions as a microtubule targeting agent interfering with microtubule dynamics and perturbs the mitotic cycle [7]. When compared to colchicine, the vascular disrupting effects of CA-4 are well below the maximum tolerable dose with fewer side effects *in vivo* [8].

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However, CA-4 suffers from stability issues because of its tendency to undergo *cis-trans* double bond isomerism in solution. CA-4 is a *cis*-configured stilbene which is readily converted to the thermodynamically more stable, but less potent *trans*-isomer [9]. Extensive studies have been conducted in attempts to stabilize the *cis* configuration by replacing the olefinic double bond with heterocyclic ring systems such as  $\beta$ -lactam, azetidone, thiazole, tetrazole, imidazole, pyrazole, oxazolone, triazole, furanone moieties [10-15].



**Fig. 1.** Structures of combretastatin A-4 (CA-4, **A**), colchicine (**B**), triazole analogue (**2e**, **C**) and reported anti-tubulin compounds (**D-I**).

In the work described herein, we report on the synthesis of series of novel *cis*-constrained 4,5-disubstituted 2*H*-1,2,3-triazole analogues of CA-4. The 2*H*-1,2,3-triazole ring system was designed to not only halt *cis-trans* isomerization, but also improve the drug likeness of the resulting CA-4 analogue, along with providing scope for further structural diversification. Evaluation of these novel triazole analogues of CA-4 against a panel of 60 human tumor cell lines has been performed, along with cell cycle redistribution assays. The molecular mechanism responsible for the anti-cancer activity of the three most potent molecules, **2e**, **2h** and **2i**, has been investigated by performing molecular docking studies with the target molecule, tubulin.

## 2. Results and discussion

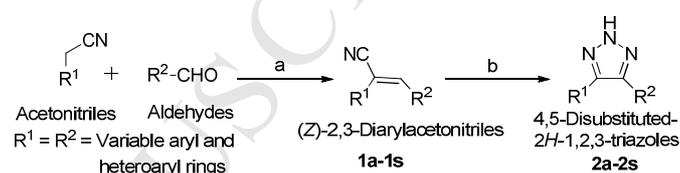
### 2.1. Drug synthesis

The general procedure for the synthesis of the 4,5-disubstituted 2*H*-1,2,3-triazole CA-4 analogues is illustrated in Scheme 1. In the first step, a series of (*Z*)-substituted diarylacrylonitrile analogues were synthesized by reacting substituted benzyl carbaldehydes with their corresponding substituted phenylacetonitriles in 5% NaOMe in methanol. The reaction mixture was stirred at room temperature for 2-3 hours, during which time the desired product precipitated out of the solution. The resulting product was then filtered, washed with water, and dried to yield the final compound; yields ranged from 70-95 % (Scheme 1) [16].

In the second step, 4,5-disubstituted-2*H*-1,2,3-triazoles were obtained by refluxing a mixture of the (*Z*)-2,3-diarylacrylonitrile (**1a-1s**) from step 1 with NaN<sub>3</sub>, and NH<sub>4</sub>Cl in a mole ratio of 1:3:3 in 10:1 volumes of DMF/H<sub>2</sub>O for 5–12 h. The reaction mixture was monitored by TLC, and when the starting material

had completely disappeared, cold water was added and the mixture stirred over 10–15 min, during which the final product precipitated out and was filtered off, washed with water and dried. In cases where there was an absence of a precipitate, the product was extracted into ethyl acetate, the organic extract washed with copious amounts of water, and the resulting organic liquor evaporated to dryness on a rotary evaporator. The residue obtained was purified by flash column chromatography to afford the corresponding triazole (Scheme 1) [17]. The structure and purity of the triazole derivatives were verified by <sup>1</sup>H, <sup>13</sup>C-NMR spectroscopy, high resolution mass spectroscopy and X-ray crystallography [17-20].

Based on previous SAR studies on *cis* constrained CA-4 analogues, triazole analogues initially chosen for synthesis contained the 3,4,5-trimethoxyphenyl moiety (Ring A) and a variably substituted aryl or heteroaryl moiety (Ring B) [7, 21]. In later structural modifications we introduced halogeno, nitro and hydroxyl functionalities ring A.



**Scheme 1.** Synthesis of 4,5-disubstituted 2*H*-1,2,3-triazoles; reagents and conditions: (a) 5% NaOMe, MeOH, reflux. (b) NaN<sub>3</sub>, NH<sub>4</sub>Cl, DMF/H<sub>2</sub>O.

### 2.2. Biological Evaluation

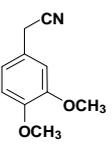
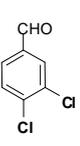
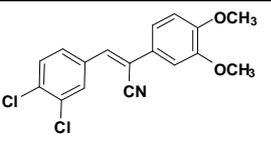
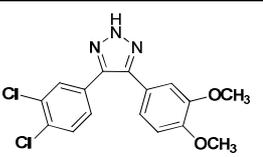
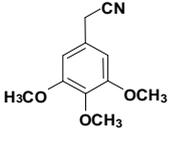
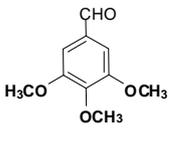
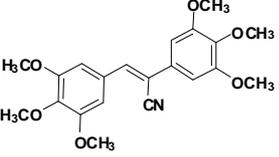
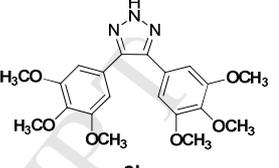
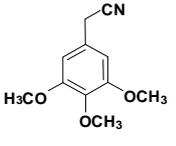
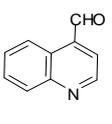
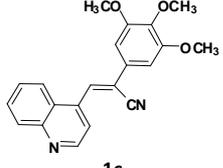
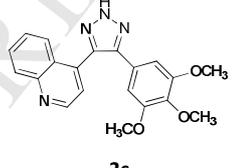
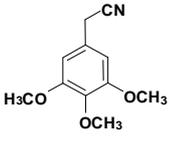
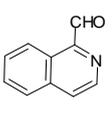
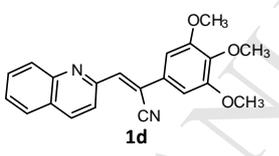
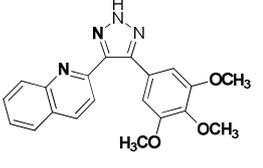
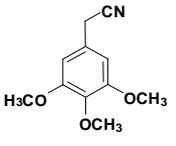
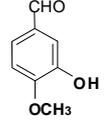
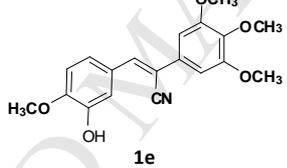
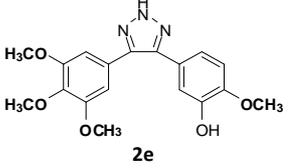
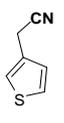
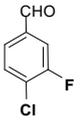
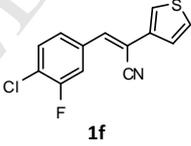
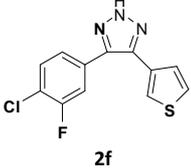
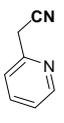
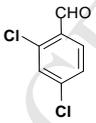
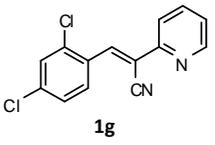
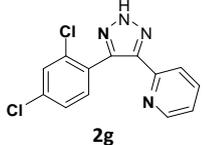
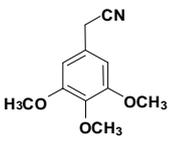
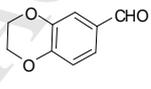
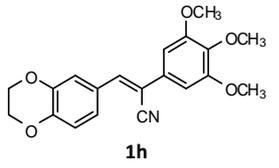
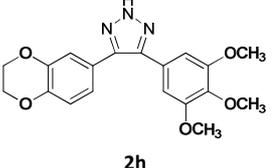
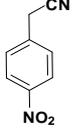
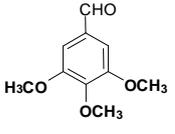
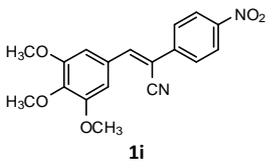
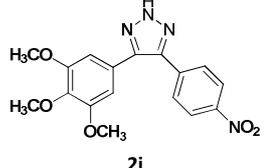
#### 2.2.1. Anti-cancer activity against a panel of NCI 60 human cancer cells

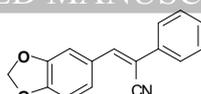
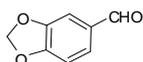
The sulforhodamine B (SRB) assay procedure described by Rubinstein et al. was used to screen the CA-4 analogues **2a-2s** against a panel of 60 human tumor cell lines [22]. Growth inhibitory or cytotoxic effects were measured by percentage growth (PG), which is proportional to optical density (OD) [23, 24]. OD measurements of SRB-derived color prior to and 48 hrs after exposure of cells to the test compound or vehicle control were recorded. Ten compounds (**2c-2e**, **2g**, **2j**, **2l-2n**, **2r**, and **2s**) were initially identified as “hits” after screening at all the analogues at 10<sup>-5</sup> M concentration. These single concentration results are presented in the supplementary data section. The screening protocol utilized to identify a hit was 60% growth inhibition at 10<sup>-5</sup> M in at least eight cell lines from the panel of 60 cell lines. Compounds that met these criteria were then selected for a complete dose response study at five different concentrations, viz. 10<sup>-4</sup> M, 10<sup>-5</sup> M, 10<sup>-6</sup> M, 10<sup>-7</sup> M and 10<sup>-8</sup> M. Six of the preliminary hits (**2d**, **2g**, **2h**, and **2l-2n**) were evaluated in the full dose-response studies had effective GI<sub>50</sub> and TGI (Total growth inhibition) values against a variety of human tumor cell lines (Table 2). All the compounds have LD<sub>50</sub> values >100  $\mu$ M against most of the human cancer cell lines, indicating that the compounds are anti-proliferative.

Analogue **2i** had impressive GI<sub>50</sub> values of less than 10 nM against almost all of the 60 cancer cell lines in the panel, except for melanoma cancer cell line UACC-62, and colon cancer cell lines COLO 205 and HCC-2998. Compound **2i** also showed potent anti-proliferative activity with TGI values of <10 nM against CNS cancer cell line SF-539, melanoma cell MDA-MB-435, ovarian cancer cell line OVCAR-3, and renal cancer cell line A498.

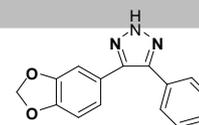
Table 1.

Synthesized (*Z*)-2,3-diarylacrylonitriles and 4,5-disubstituted-2*H*-1,2,3-triazoles from their corresponding acetonitrile and aldehyde precursors.

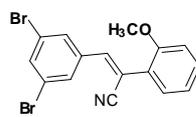
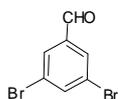
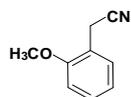
Acetonitrile	Aldehyde	( <i>Z</i> )-2,3-Diarylacetonitriles ( <b>1a-1s</b> )	4,5-Disubstituted 2 <i>H</i> -1,2,3-triazoles ( <b>2a-2s</b> )
			
			
			
			
			
			
			
			
			



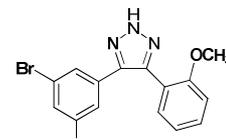
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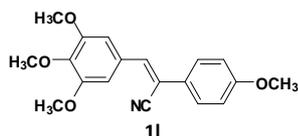
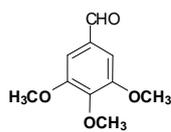
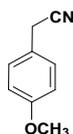
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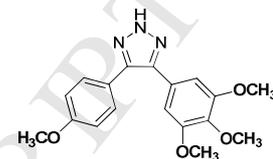
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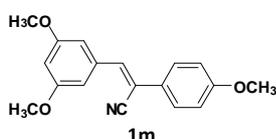
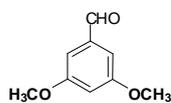
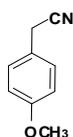
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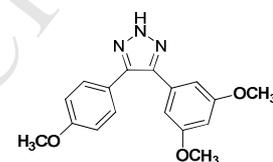
1l



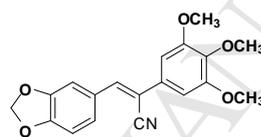
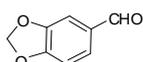
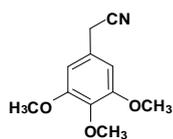
2l



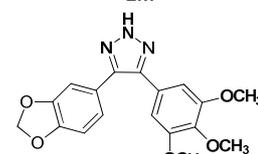
1m



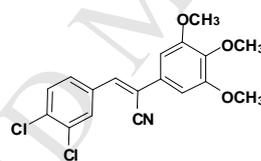
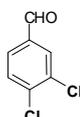
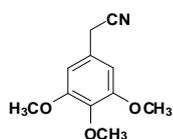
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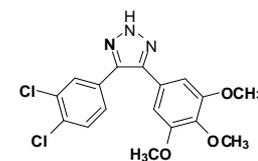
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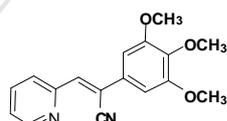
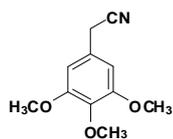
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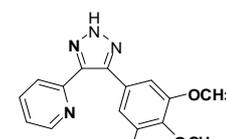
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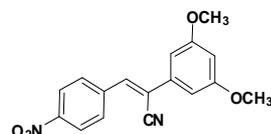
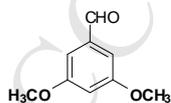
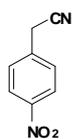
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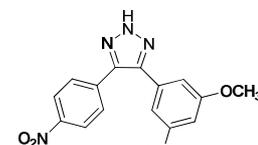
1p



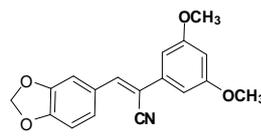
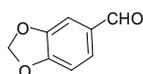
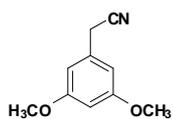
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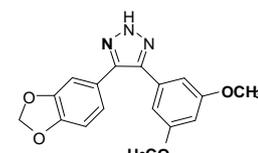
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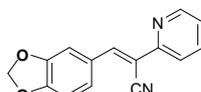
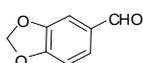
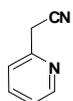
2q



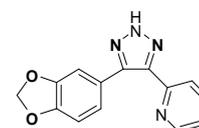
1r



2r



1s



2s

**Table 2.**

Growth inhibition ( $GI_{50}/\mu M$ )<sup>a</sup> data of **2d**, **2m**, **2l**, **2h** and **2g** against a panel of 60 human cancer cells.

Panel/cell line	<b>2d</b> ( $\mu M$ )	<b>2m</b> ( $\mu M$ )	<b>2l</b> ( $\mu M$ )	<b>2h</b> ( $\mu M$ )	<b>2n</b> ( $\mu M$ )	<b>2g</b> ( $\mu M$ )
<i>Leukemia</i>						
CCRF-CEM	0.037	0.032	<0.01	0.034	0.28	3.38
HL-60(TB)	0.030	0.023	<0.01	0.019	0.20	5.75
K-562	0.026	<0.01	<0.01	<0.01	0.05	5.50
MOLT-4	0.068	0.048	0.012	0.040	0.57	4.23
RPMI-8226	0.037	0.037	<0.01	0.033	0.33	10.7
SR	0.021	<0.01	<0.01	<0.01	0.04	5.43
<i>Lung Cancer</i>						
A549/ATCC	0.054	0.037	<0.01	0.023	0.29	4.85
HOP-62	0.047	0.025	<0.01	0.018	0.34	5.32
HOP-92	0.099	<0.01	<0.01	<0.01	0.12	3.28
NCI-H23	0.065	0.038	<0.01	0.048	0.77	9.13
NCI-H460	0.038	0.034	<0.01	0.025	0.34	3.67
<i>Colon Cancer</i>						
COLO 205	0.228	0.149	0.029	0.027	0.31	2.89
HCC-2998	0.230	0.046	0.024	0.039	>100	9.16
HCT-116	0.040	<0.01	<0.01	<0.01	0.18	2.89
HCT-15	0.037	<0.01	<0.01	<0.01	0.13	2.93
HT29	0.214	0.048	<0.01	0.025	0.39	4.56
KM12	0.035	0.030	<0.01	<0.01	0.07	6.87
SW-620	0.041	0.030	<0.01	<0.01	0.11	4.40
<i>CNS Cancer</i>						
SF-268	0.503	0.177	<0.01	0.069	>100	7.36
SF-295	0.014	0.011	<0.01	<0.01	0.10	3.01
SF-539	0.020	0.014	<0.01	<0.01	0.18	na
SNB-75	0.016	0.014	<0.01	<0.01	0.08	1.58
U251	0.037	0.038	<0.01	0.020	0.30	5.35
<i>Melanoma</i>						
LOX IMVI	0.065	0.076	<0.01	0.018	0.55	4.83
M14	0.022	<0.01	<0.01	<0.01	na	14.3
MDA-MB-435	<0.01	<0.01	<0.01	<0.01	0.09	2.20
SK-MEL-2	0.027	0.056	<0.01	<0.01	0.02	6.52
SK-MEL-28	>100	na	na	>100	0.24	5.39
SK-MEL-5	0.013	0.027	<0.01	<0.01	>100	7.89
UACC-62	0.157	<0.01	nd	>100	>100	3.35
<i>Ovarian Cancer</i>						
IGROV1	0.065	0.051	<0.01	0.033	>100	5.94
OVCAR-3	0.011	0.024	<0.01	<0.01	0.43	3.38
OVCAR-4	0.077	na	<0.01	0.034	0.08	3.04
NCI/ADR-RES	0.023	<0.01	<0.01	<0.01	0.08	3.92
SK-OV-3	0.075	0.046	<0.01	<0.01	0.49	2.72
<i>Renal Cancer</i>						
786-0	0.043	0.015	<0.01	<0.01	0.62	2.75
A498	0.033	<0.01	<0.01	0.010	0.34	3.18
ACHN	0.081	0.145	<0.01	<0.01	0.71	1.75
CAKI-1	0.043	0.050	<0.01	<0.01	0.32	8.42
UO-31	0.092	0.020	<0.01	<0.01	0.66	6.84
<i>Prostate Cancer</i>						
PC-3	0.046	0.042	<0.01	0.018	0.26	4.50
DU-145	0.027	0.045	<0.01	0.025	0.35	1.41
<i>Breast Cancer</i>						
MCF7	0.027	0.025	<0.01	<0.01	0.08	2.58
MDA-MB-231/ATCC	0.094	0.046	<0.01	0.044	0.53	5.89
HS 578T	na	0.041	<0.01	0.668	0.44	11.6
MDA-MB-468	0.035	0.023	<0.01	0.015	0.23	3.20

na: Not analyzed, nd; not determined. <sup>a</sup> $GI_{50}$ : 50% growth inhibition, concentration of drug resulting in a 50% reduction in net cell growth as compared to cell numbers on day 0.

Analogue **2m** exhibited potent growth inhibitory activity with  $GI_{50} < 10$  nM against leukemia cancer cell lines K-562 and SR, non-small cell lung cancer cell line HOP-92, colon cancer cell lines HCT-116 and HCT-15, melanoma cancer lines M14 and UACC-62, ovarian cancer cell line NCI/ADR-RES, and renal cancer cell line A498. Melanoma cancer cell line MDA-MB-435

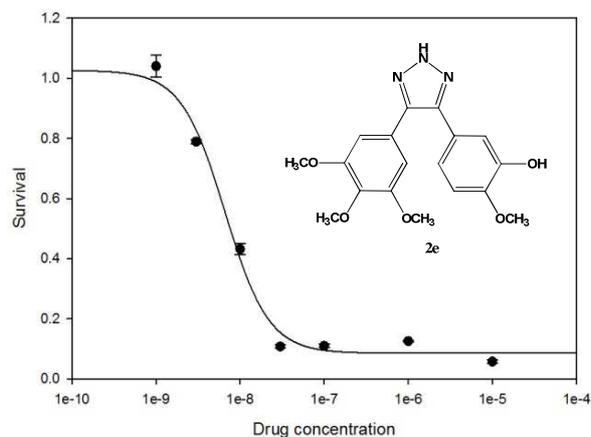
appeared to be the most sensitive to the growth inhibitory effects of **2m**, exhibiting a TGI value of 10 nM.

Analogue **2h** also showed potential growth inhibitory properties with  $GI_{50} < 10$  nM against leukemia cancer cell lines K-562 and SR, non-small cell lung cancer cell line HOP-92, colon cancer cell lines HCT-116, HCT-15, KM-12, and SW-620, CNS cancer cell lines SF-295, SF-539, and SNB-75, melanoma cancer lines M14, MDA-MB-435, and SK-MEL-2, ovarian cancer cell lines NCI/ADR-RES, and SK-OV-3, renal cancer cell lines A498, ACHN, CAKI-1, and UO-31, and breast cancer cell line MCF-7.

From the above screening studies, analogue **2l** was considered to be a lead candidate, since it exhibited very promising  $GI_{50}$  and TGI values against a wide variety of both hematological and solid tumor cell types. However, *in silico* tubulin docking studies carried out with the triazole analogues revealed slightly better tubulin binding affinity for compound **2e** compared to **2l**. Unfortunately, analogue **2e**, which is the triazole-derived form of the CA4 molecule, was not selected for the 60 human cancer cell screen. We therefore evaluated **2e** for its anti-cancer activity by colony formation assay against 9LSF rat gliosarcoma cells.

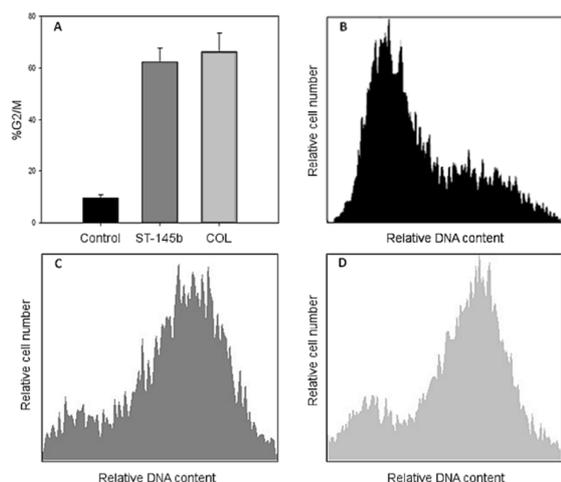
### 2.2.2 Colony formation assay results for **2e** utilizing 9LSF rat gliosarcoma cells

9LSF cells were acquired from the laboratory of Dennis Deen, Ph.D. (Brain Tumor Research Center, University of California, San Francisco). Cells were exposed to **2e** at concentrations of 1 nM, 3 nM, 10 nM, 30 nM, 100 nM, 1  $\mu M$ , or 10  $\mu M$  for 24 hours prior to seeding for the colony formation assay. Cells were then trypsinized, counted, seeded into 25 cm<sup>2</sup> flasks, and incubated at 37°C to form colonies [25]. All conditions were seeded in triplicate. Flasks that were plated with less than 50,000 cells were previously plated with 50,000 lethally irradiated A549 cells to serve as feeders [26]. Flasks were removed from incubation when colonies were large enough to count (>50 cells). Colonies were then fixed and stained with crystal violet, rinsed, allowed to dry, and counted. The protocol was run in triplicate, and results averaged, with error representing SEM. The  $LD_{50}$  value for **2e** was determined to be 7.5 nM using SigmaPlot 11.



**Fig. 2.** Colony formation assay, **2e**-treated 9LSF cells. Cells were exposed to **2e** for 24 hours. Curve was fitted as four-parameter logistic curve ( $f1 = \min + (\max - \min) / (1 + (x/EC50)^{-Hillslope})$ ). Values represent mean  $\pm$  SEM of 3 independent colony formation experiments.

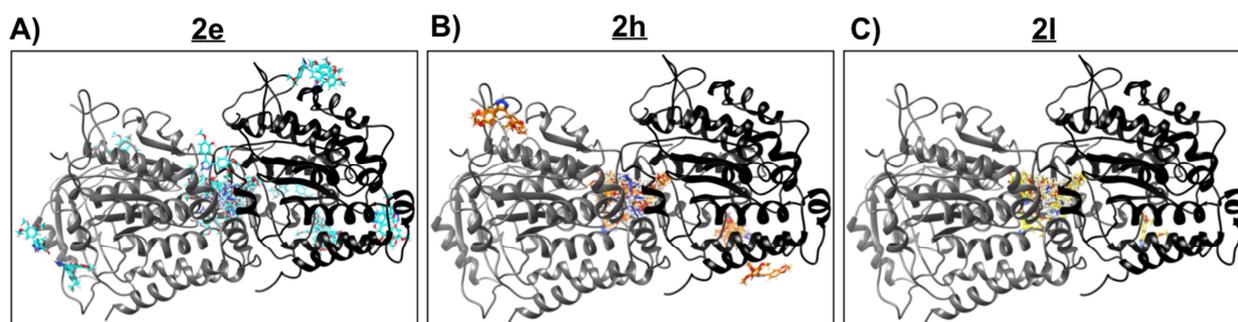
9LSF cells were exposed to 50 nM **2e** or 50 nM colchicine (COL) for 24 hrs *in vitro*, along with controls. Cells were then trypsinized, counted, rinsed with PBS, and fixed using ice-cold 70% EtOH in PBS at  $10^6$  cells/mL. Samples were stored at 4°C prior to flow cytometry analysis;  $10^6$  cells from each condition were then pelleted and rinsed thrice with PBS. Samples were resuspended in 1 mL PBS, to which 1  $\mu$ L propidium iodide (PI, 12.5 mg/mL in DMSO) was added. Samples were exposed to PI for 2 minutes at room temperature, then immediately analyzed by flow cytometry (Cell Lab Quanta SC, Beckman Coulter, Inc., Brea, CA.) The protocol was run in triplicate. Cell cycle distributions were analyzed using FCS Express 3 (De Novo Software, Glendale, CA).



**Fig 3.** Cell cycle redistribution effects of **2e** on 9L cells. (A) Percentage of cell population in G2/M phase in control cells versus those exposed to 50  $\mu$ M **2e** or 50  $\mu$ M COL for 24 hrs. (B) Representative distribution of control cells. (C) Representative distribution of **2e**-treated cells. (D) Representative distribution of COL-treated cells.

Two analogues (**2h** and **2l**) from the 60 human cancer cell panel screens, and analogue **2e**,  $GI_{50} < 10$  nM) were chosen for molecular docking studies utilizing the available crystal structure of tubulin, in order to understand the possible mechanism of their action as inhibitors of tubulin polymerization. Atomic coordinates for tubulin (PDB 1SA0) were downloaded from the protein structure database ([www.rcsb.org/pdb](http://www.rcsb.org/pdb)). Colchicine was removed from the coordinate file and the coordinates of only chains A and B, corresponding to a  $\alpha,\beta$ -tubulin heterodimer were used for the docking studies. Atomic coordinates for all compounds were generated using MarvinSketch (ChemAxon), and both the ligand and target protein coordinate files were prepared for docking using the Dock Prep module in the UCSF-Chimera package (<http://www.cgl.ucsf.edu/chimera>). Docking was performed using SwissDock (<http://www.swissdock.ch/>), based on the docking algorithm EADock DSS [27]. Docking was performed using protocols established in our previous studies [16]. Use of the most exhaustive and unbiased option in SwissDock ensured the sampling of the maximum number of binding modes for each molecule. The best hits based on the SwissDock FullFitness scoring function (FF) from three repeated docking runs were considered further.

All three compounds docked almost exclusively to the colchicine-binding pocket on the  $\alpha,\beta$ -tubulin heterodimer. This indicates that the mode of inhibition of tubulin polymerization by these three molecules is similar to that of colchicine and CA-4, and that the introduction of a triazole ring in place of the *cis*-olefinic bond still retains potent tubulin binding properties. Among the three molecules examined, **2e** had the most number of 'outlier' poses that docked in non-colchicine binding, surface-exposed pockets. However, 86% of the docked poses were still localized at the colchicine-pocket. In the case of **2h** and **2l**, this number was 95% and 99%, respectively (Fig. 4).



**Fig. 4.** Docking poses of molecules **2e**, **2h** and **2l** bound to tubulin. Subunits  $\alpha$  and  $\beta$  of the tubulin heterodimer are shown as gray and black cartoons respectively. All the docking poses generated by Swissdock for molecules **2e**, **2h** and **2l** are shown in panels A), B) and C) respectively. As seen clearly, majority of the poses bind to the 'colchicine-binding' pocket located at the interface of the two tubulin subunits for all three molecules. **2e** shows the most, while **2l** shows the least number of 'outlier' poses.

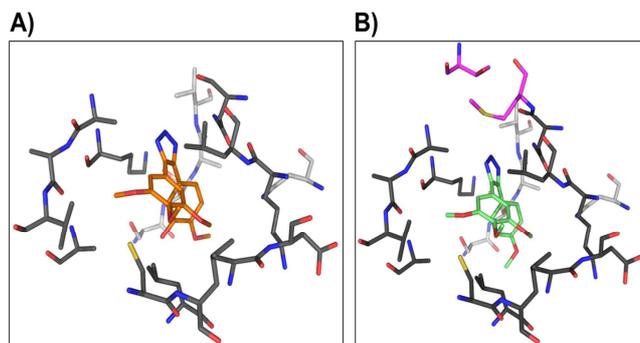
All three molecules were primarily stabilized through several van der Waals' contacts with residues from the  $\alpha$ - and  $\beta$ -subunits of tubulin. Compound **2e** was found to interact with 13  $\beta$  residues and 5  $\alpha$  residues of tubulin (Fig 5). In contrast, both molecules **2h** and **2l**, besides sharing all the contact residues of **2e**, made additional contacts with Met259 and Thr314 of the  $\beta$ - subunit of tubulin (Fig 5B). This is reflected in the FF and  $\Delta G$  scores of the three compounds (Table 3), with the FF score and free energy values for **2l** and **2h** being

higher than that for **2e**. Both scores followed the order: **2e** < **2h** < **2l**.

**Table 3.**

SwissDock statistics for the docking runs with compounds **2e**, **2h** and **2l** (scores shown are averages from 3 docking runs).

Comp	FF score (kcal/mol)	$\Delta G$ (kcal/mol)
<b>2l</b>	-4224.7	-8.4
<b>2h</b>	-4216.5	-8.2
<b>2e</b>	-4211.4	-7.9



**Fig. 5.** Atomic contacts between tubulin and molecules **2e** and **2l**. A) **2e** is shown as orange sticks, while residues of the  $\alpha$  and  $\beta$  subunit of tubulin are in light and dark gray, respectively. B) **2l** (green sticks) is shown bound at the colchicine-binding pocket of tubulin. The color scheme is the same as in A, while the residues of  $\beta$ -tubulin unique to **2l** pocket are shown in magenta.

#### 4. Conclusions

A series of 4,5-disubstituted 2H-1,2,3-triazoles designed as CA4 analogues were synthesized from corresponding (Z)-substituted diarylacrylonitriles (**1a-1s**). The synthesis represents a facile and efficient reaction procedure for the preparation of 4,5-diaryl-2H-1,2,3-triazoles in modest to good yields. The resulting 4,5-disubstituted 2H-1,2,3-triazoles were evaluated for their anticancer properties against a panel of 60 human cancer cell lines. The diarylacrylonitrile analogue **2l** exhibited the most potent anticancer activity in the cancer cell screening studies, with  $GI_{50}$  values of  $<10$  nM against almost all the cell lines in the panel. Analogue **2l** also exhibited promising TGI values of  $<10$  nM against CNS cancer cell line SF-539, melanoma cell MDA-MB-435, ovarian cancer cell line OVCAR-3 and renal cancer cell line A498. Analogues **2m** and **2h** exhibited  $GI_{50}$  values  $<10$  nM against leukemia cell lines K-562, SR, non-small lung cancer cell line HOP-92, colon cancer cell lines HCT-116, and HCT-15, melanoma cancer lines M14, and UACC-62, ovarian cancer cell line NCI/ADR-RES and renal cancer cell line A498. Analogue **2e** was evaluated for anti-cancer activity by colony formation assay against 9LSF rat gliosarcoma cells, and afforded an  $LD_{50}$  value of 7.5 nM. A cell cycle redistribution assay with **2e** was also conducted to further understand the mechanism of action of this CA-4 analogue. *In silico* docking studies with compounds **2l**, **2e** and **2h** within the active site of tubulin were carried out in order to rationalize the anticancer properties of these compounds. From these studies, compound **2e** exhibited higher affinity for the colchicine binding site on tubulin compared to **2l** and **2h**. From this study, analogues **2e** and **2l** were considered lead compounds in this new structural class, and worthy of further development as anti-cancer agents.

#### 5.0 Experimental Section

##### 5.1. Chemistry

TLC experiments were carried out on pre-coated silica gel plates (F 254 Merck).  $^1H$  and  $^{13}C$  NMR spectra were recorded on a Varian 400 MHz spectrometer equipped with a Linux workstation running on vNMRj software. All spectra were phased, baseline was corrected where necessary, and solvent signals ( $CDCl_3$ ) were used as reference for both  $^1H$  and  $^{13}C$  spectra. HRMS data was obtained on an Agilent 6210 LCTOF instrument operated in multimode.

In the first synthetic step, a series of (Z)-substituted diarylacrylonitrile analogues were synthesized by reacting substituted benzyl carbaldehydes with their corresponding substituted phenylacetonitriles in 5% NaOMe in methanol. The reaction mixture was stirred at room temperature for 2-3 hours for the reaction to complete and the final product to crash out of the solution. The precipitate is filtered, washed with water and dried to yield the final compounds with yields ranging from 70-95 % (Scheme 1) [16].

In the second synthetic step the 4,5-disubstituted-2H-1,2,3-triazoles were synthesized by refluxing a mixture of the (Z)-2,3-diarylacrylonitrile (**1a-1s**),  $NaN_3$ , and  $NH_4Cl$  in a mole ratio of 1:3:3 in 10:1 volumes of DMF/ $H_2O$  for 5–12 h. The reaction was monitored by TLC. When the starting material had completely disappeared, cold water was added and the mixture was stirred over 10–15 min, during which time the final product precipitated out and was filtered off. In the absence of a precipitate, the product was extracted into ethyl acetate, the organic extract washed with copious amounts of water, and the resulting organic liquor evaporated to dryness on a rotavaporator. The residue obtained was purified by flash column chromatography to afford the corresponding triazole analogue of CA-4.

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##### 5.3. Analytical data of 4,5-disubstituted-2H-1,2,3-triazoles

###### 5.3.1. 4-(3,4-dichlorophenyl)-5-(3,4-dimethoxyphenyl)-2H-1,2,3-triazole (**2a**)

$^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  3.82 (s, 3H,  $-OCH_3$ ),  $\delta$  3.93 (s, 3H,  $-OCH_3$ ), 6.88 (d,  $J = 8$  Hz, 1H, ArH), 7.06 (d,  $J = 12$  Hz, 2H, ArH), 7.42 (s, 2H, ArH), 7.78 (s, 1H, ArH), 12.49 (bs, 1H, NH) ppm.  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  55.87, 55.96, 111.19, 121.31, 127.31, 129.80, 129.89, 130.54, 132.60, 132.86, 149.15, 149.73 ppm. HRMS (ESI):  $m/z$  calcd for  $C_{16}H_{14}Cl_2N_3O_2$   $[M+H]^+$ : 350.0463; found 350.0465.

###### 5.3.2. 4,5-bis(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (**2b**)

$^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  3.76 (s, 12H,  $-OCH_3$ ), 3.88 (s, 6H,  $-OCH_2$ ), 6.84 (s, 4H, ArH), 12.50 (bs, 1H, NH) ppm.  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  56.08, 60.96, 105.57, 125.58, 138.28, 153.27 ppm. HRMS (ESI):  $m/z$  calcd for  $C_{20}H_{24}N_3O_6$   $[M+H]^+$ : 402.1665; found 402.1668.

###### 5.3.3. 4-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)quinolone (**2c**)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.47 (s, 6H, -OCH<sub>3</sub>), 3.81 (s, 3H, -OCH<sub>3</sub>), 6.65 (s, 2H, ArH), 7.50-7.52 (t, *J* = 8 Hz, 1H, ArH), 7.55 (d, *J* = 4.4 Hz, 1H, ArH), 7.77 (t, *J* = 1.6 Hz, 1H, ArH), 7.83 (d, *J* = 8 Hz, 1H, ArH), 8.28 (d, *J* = 8.8 Hz, 1H, ArH), 9.03 (d, *J* = 4.4 Hz, 1H, ArH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 55.70, 60.85, 104.47, 122.68, 122.76, 124.69, 125.95, 126.66, 127.52, 129.42, 130.21, 138.21, 138.45, 148.15, 149.63, 149.69, 153.24 ppm. HRMS (ESI): *m/z* calcd for C<sub>20</sub>H<sub>19</sub>N<sub>4</sub>O<sub>3</sub> [M+H]<sup>+</sup>: 363.1457; found 363.1460.

5.3.4. 2-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)quinolone (2d)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.78 (s, 6H, -OCH<sub>3</sub>), 3.91 (s, 3H, -OCH<sub>3</sub>), 7.08 (s, 2H, ArH), 7.56 (t, *J* = 14.8 Hz, 1H, ArH), 7.72 (t, *J* = 14.8 Hz, 1H, ArH), 7.83 (d, *J* = 6.4 Hz, 2H, ArH), 8.09 (d, *J* = 8.8 Hz, 1H, ArH), 8.17 (d, *J* = 8.4 Hz, 1H, ArH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 56.08, 60.92, 106.21, 120.70, 125.57, 127.12, 127.56, 127.73, 127.68, 129.04, 130.20, 136.83, 138.50, 147.76, 153.19 ppm. HRMS (ESI): *m/z* calcd for C<sub>20</sub>H<sub>19</sub>N<sub>4</sub>O<sub>3</sub> [M+H]<sup>+</sup>: 363.1457; found 363.1456.

5.3.5. 2-methoxy-5-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)phenol (2e)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.73 (s, 6H, -OCH<sub>3</sub>), 3.88 (s, 6H, -OCH<sub>3</sub>), 6.82 (d, *J* = 2 Hz, 3H, ArH), 7.04 (d, *J* = 8.4 Hz, 1H, ArH), 7.20 (s, 1H, ArH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 55.92, 56.98, 56.07, 60.91, 60.98, 105.23, 110.63, 114.72, 120.61, 123.01, 125.74, 138.09, 145.66, 147.10, 153.23 ppm. HRMS (ESI): *m/z* calcd for C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>O<sub>5</sub> [M+H]<sup>+</sup>: 358.1403; found 358.1408.

5.3.6. 4-(4-chloro-3-fluorophenyl)-5-(thiophen-3-yl)-2H-1,2,3-triazole (2f)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.22 (d, *J* = 4.8 Hz, 1H, ArH), 7.32 (d, *J* = 8.4 Hz, 1H, ArH), 7.38-7.41 (m, 3H, ArH), 7.50 (d, *J* = 1.2 Hz, 1H, ArH), 10.5 (bs, 1H, NH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 116.17, 121.32, 124.56, 126.73, 127.01, 129.50, 130.75, 138.32, 141.30, 156.84, 159.31 ppm. HRMS (ESI): *m/z* calcd for C<sub>12</sub>H<sub>8</sub>N<sub>3</sub>FSCl [M+H]<sup>+</sup>: 280.0111; found 280.0118.

5.3.7. 2-(5-(2,4-dichlorophenyl)-2H-1,2,3-triazol-4-yl)pyridine (2g)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.26 (s, 1H, ArH), 7.39 (d, *J* = 8 Hz, 2H, ArH), 7.49-7.54 (m, 2H, ArH), 7.67 (d, *J* = 6.8 Hz, 1H, ArH), 8.63 (s, 1H, ArH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 121.68, 123.21, 127.27, 129.64, 132.76, 134.74, 135.52, 136.94, 149.45 ppm. HRMS (ESI): *m/z* calcd for C<sub>13</sub>H<sub>9</sub>N<sub>4</sub>Cl<sub>2</sub> [M+H]<sup>+</sup>: 291.0204; found 291.0201.

5.3.8. 4-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2h)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.77 (s, 6H, 2xOCH<sub>3</sub>), 3.89 (s, 3H, OCH<sub>3</sub>), 4.26-4.29 (m, 2xCH<sub>2</sub>), 6.84 (s, 2H, ArH), 6.88 (d, 1H, *J* = 8 Hz, Ar-H), 7.04-7.07 (dd, 1H, *J* = 2 and 8 Hz, 1H, Ar-H), 7.15 (d, 1H, Ar-H), 12.2 (bs, 1H, NH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 55.99, 60.99, 64.27, 105.24, 105.28, 117.42, 121.80, 138.18, 143.59, 144.09, 153.26 ppm. HRMS (ESI): *m/z* calcd for C<sub>19</sub>H<sub>20</sub>N<sub>3</sub>O<sub>5</sub> [M+H]<sup>+</sup>: 370.1403; found 370.1398.

5.3.9. 4-(4-nitrophenyl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2i)

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 3.70-3.72 (d, 9H, -OCH<sub>3</sub>), 6.80 (s, 2H, ArH), 7.86 (s, 2H, ArH), 8.30 (d, *J* = 8.4 Hz, 2H, ArH) ppm. <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ 56.23, 61.06, 105.75, 123.91, 128.76, 137.57, 138.73, 147.50, 153.62 ppm. HRMS (ESI): *m/z* calcd for C<sub>17</sub>H<sub>17</sub>N<sub>4</sub>O<sub>5</sub> [M+H]<sup>+</sup>: 357.1199; found 357.1199.

5.3.10. 4-(benzo[*d*][1,3]dioxol-5-yl)-5-phenyl-2H-1,2,3-triazole (2j)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 6.00 (s, 2H, -CH<sub>2</sub>), 6.81 (d, *J* = 8.8 Hz, 1H, ArH), 7.04 (d, *J* = 6.8 Hz, 2H, ArH), 7.39 (t, *J* = 5.2 Hz, 3H, ArH), 7.57 (m, *J* = 9.6 Hz, 1H, ArH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 101.43, 108.77, 108.91, 122.46, 123.96, 128.41, 128.73, 128.88, 130.25, 142.29, 148.01, 148.11 ppm. HRMS (ESI): *m/z* calcd for C<sub>15</sub>H<sub>12</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 266.0930; found 266.0928.

5.3.11. 4-(3,5-dibromophenyl)-5-(2-methoxyphenyl)-2H-1,2,3-triazole (2k)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.76 (s, 3H, -OCH<sub>3</sub>), 7.04 (d, *J* = 4.8 Hz, 2H, ArH), 7.39-7.47 (m, 2H, ArH), 7.63 (d, *J* = 1.6 Hz, 1H, ArH), 7.71 (s, 2H, ArH), 12.2 (bs, 1H, NH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 55.51, 111.54, 121.21, 122.83, 129.17, 130.48, 131.29, 133.34, 133.42, 134.96, 156.51 ppm. HRMS (ESI): *m/z* calcd for C<sub>15</sub>H<sub>12</sub>N<sub>3</sub>O Br<sub>2</sub> [M+H]<sup>+</sup>: 407.9347; found 407.9342.

5.3.12. 4-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2l)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.74 (s, 6H, -OCH<sub>3</sub>), 3.84 (s, 3H, -OCH<sub>3</sub>), 3.89 (s, 3H, -OCH<sub>3</sub>), 6.83 (s, 2H, ArH), 6.92 (d, *J* = 8.4 Hz, 2H, ArH), 7.52 (d, *J* = 8.0 Hz, 2H, ArH), 12.2 (bs, 1H, NH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 55.27, 55.96, 60.90, 105.14, 105.18, 114.03, 125.86, 129.79, 138.07, 153.27, 159.97 ppm. HRMS (ESI): *m/z* calcd for C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>O<sub>4</sub> [M+H]<sup>+</sup>: 342.1454; found 342.1448.

5.3.13. 4-(3,5-dimethoxyphenyl)-5-(4-methoxyphenyl)-2H-1,2,3-triazole (2m)

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 3.68 (s, 6H, -OCH<sub>3</sub>), 3.78 (s, 3H, -OCH<sub>3</sub>), 6.5 (s, 1H, Ar-H), 6.64 (2H, Ar-H), 7.01 (d, 2H, *J* = 8 Hz, ArH), 7.43 (d, 2H, *J* = 8.8 Hz, Ar-H), HRMS (ESI): *m/z* calcd for C<sub>17</sub>H<sub>18</sub>N<sub>3</sub>O<sub>3</sub> [M+H]<sup>+</sup>: 312.1348; found 312.1344.

5.3.14. 4-(benzo[*d*][1,3]dioxol-5-yl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2n)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.77 (s, 6H, -OCH<sub>3</sub>), 3.89 (s, 3H, -OCH<sub>3</sub>), 4.26-4.29 (q, *J* = 12.4 Hz, 4H, ArH), 6.84 (s, 2H, ArH), 6.88 (d, *J* = 8 Hz, 1H, ArH), 7.04-7.07 (dd, *J* = 2 Hz, 8 Hz, 1H, ArH), 7.16 (d, *J* = 1.6 Hz, 1H, ArH), 7.26 (s, 1H, ArH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 55.99, 56.08, 60.90, 60.99, 64.27, 64.47, 105.24, 105.28, 117.42, 121.80, 138.18, 143.59, 144.09, 153.26 ppm. HRMS (ESI): *m/z* calcd for C<sub>18</sub>H<sub>18</sub>N<sub>3</sub>O<sub>5</sub> [M+H]<sup>+</sup>: 356.1246; found 356.1248.

5.3.15. 4-(3,4-dichlorophenyl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2o)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.79 (s, 6H, -OCH<sub>3</sub>), 3.91 (s, 3H, -OCH<sub>3</sub>), 6.77 (s, 2H, ArH), 7.46 (s, 2H, ArH), 7.82 (s, 1H, ArH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 56.14, 60.98,

105.49, 127.51, 129.92, 130.57, 132.51, 132.77, 138.46, 153.47 ppm. HRMS (ESI):  $m/z$  calcd for  $C_{17}H_{16}N_3O_3$  [M+H]<sup>+</sup>: 380.0569; found 380.0564.

5.3.16. 2-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)pyridine (**2p**)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.81 (s, 6H, -OCH<sub>3</sub>), 3.90 (s, 3H, -OCH<sub>3</sub>), 6.9 (s, 2H, ArH), 7.28 (d,  $J$  = 12.8 Hz, 2H, ArH), 7.70 (s, 2H, ArH), 8.67 (s, 1H, ArH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 56.10, 60.94, 106.04, 123.22, 123.46, 125.66, 137.06, 138.37, 149.36, 153.26 ppm. HRMS (ESI):  $m/z$  calcd for  $C_{16}H_{17}N_4O_3$  [M+H]<sup>+</sup>: 313.1301; found 313.1298.

5.3.17. 4-(3,5-dimethoxyphenyl)-5-(4-nitrophenyl)-2H-1,2,3-triazole (**2q**)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.74 (s, 6H, -OCH<sub>3</sub>), 6.51 (d,  $J$  = 1.2 Hz, 1H, ArH), 6.62 (s, 2H, ArH), 7.8 (d,  $J$  = 8.4 Hz, 2H, ArH), 8.19 (d,  $J$  = 8.4 Hz, 2H, ArH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 55.43, 101.28, 106.53, 123.85, 128.67, 137.08, 147.49, 161.13 ppm. HRMS (ESI):  $m/z$  calcd for  $C_{16}H_{15}N_4O_4$  [M+H]<sup>+</sup>: 327.1093; found 327.1084.

5.3.18. 4-(benzo[d][1,3]dioxol-5-yl)-5-(3,5-dimethoxyphenyl)-2H-1,2,3-triazole (**2r**)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.75 (s, 6H, -OCH<sub>3</sub>), 5.99 (s, 2H, ArH), 6.48 (s, 1H, ArH), 6.73 (d,  $J$  = 2.4 Hz, 2H, ArH), 6.82 (d,  $J$  = 8.8 Hz, 1H, ArH), 7.06 (d,  $J$  = 6.4 Hz, 2H, ArH), 12.1 (bs, 1H, NH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 55.34, 55.51, 101.05, 101.25, 106.12, 108.51, 108.80, 122.38, 131.91, 147.81, 147.98, 160.87 ppm. HRMS (ESI):  $m/z$  calcd for  $C_{17}H_{16}N_3O_4$  [M+H]<sup>+</sup>: 326.1141; found 326.1133.

5.3.19. 2-(5-(benzo[d][1,3]dioxol-5-yl)-2H-1,2,3-triazol-4-yl)pyridine (**2s**)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 5.99 (s, 2H, -CH<sub>2</sub>), 6.83 (d,  $J$  = 8.4 Hz, 1H, ArH), 7.14 (d,  $J$  = 6 Hz, 2H, ArH), 7.27 (d,  $J$  = 8.4 Hz, 1H, ArH), 7.70 (d,  $J$  = 7.2 Hz, 2H, ArH), 8.69 (s, 1H, ArH) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 101.15, 101.25, 101.35, 108.52, 109.31, 109.34, 122.78, 123.30, 124.04, 137.02, 147.77, 148.06, 149.56 ppm. HRMS (ESI):  $m/z$  calcd for  $C_{14}H_{11}N_4O_2$  [M+H]<sup>+</sup>: 267.0882; found 267.0879.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/...>

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**Fig. 1.** Structures of combretastatin A-4 (CA-4, **A**), colchicine (**B**), triazole analogue (**2e**, **C**) and reported anti-tubulin compounds (**D-I**).

**Scheme 1.** Synthesis of 4,5-disubstituted 2*H*-1,2,3-triazoles; reagents and conditions: (a) 5% NaOMe, MeOH, reflux. (b) NaN<sub>3</sub>, NH<sub>4</sub>Cl, DMF/H<sub>2</sub>O.

**Table 1.** Synthesized (*Z*)-2,3-diarylacrylonitriles and 4,5-disubstituted-2*H*-1,2,3-triazoles from their corresponding acetonitrile and aldehyde precursors.

**Table 2.** Growth inhibition (GI<sub>50</sub>/μM)<sup>a</sup> data of **2d**, **2m**, **2l**, **2n** and **2g** against a panel of 60 human cancer cells.

**Fig. 2.** Colony formation assay, **2e**-treated 9LSF cells. Cells were exposed to **2e** for 24 hours. Curve was fitted as four-parameter logistic curve ( $f1 = \min + (\max - \min) / (1 + (x/EC50)^{(-Hillslope)})$ ). Values represent mean ± SEM of 3 independent colony formation experiments.

**Fig. 3.** Cell cycle redistribution effects of **2e** on 9L cells. (A) Percentage of cell population in G2/M phase in control cells versus those exposed to 50 μM **2e** or 50 μM COL for 24 hrs. (B) Representative distribution of control cells. (C) Representative distribution of **2e**-treated cells. (D) Representative distribution of COL-treated cells.

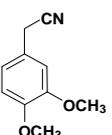
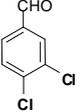
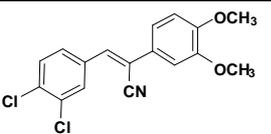
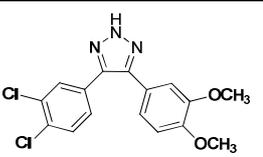
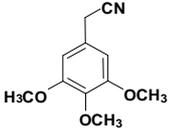
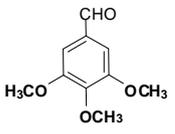
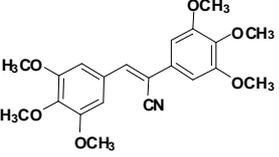
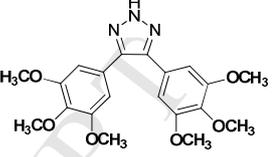
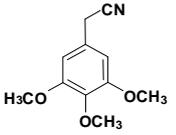
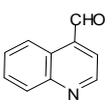
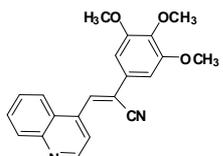
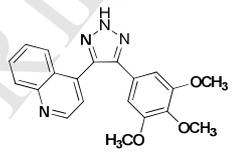
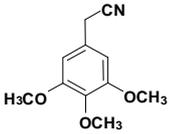
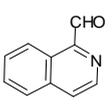
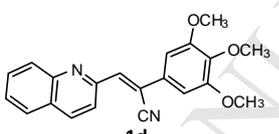
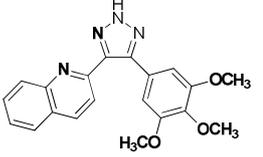
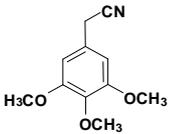
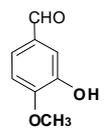
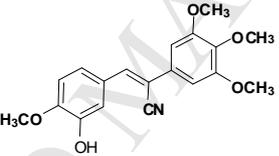
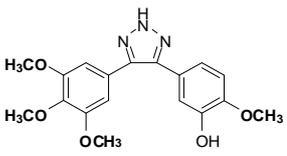
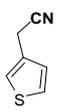
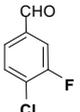
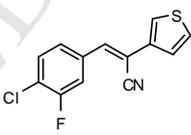
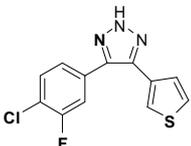
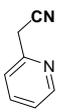
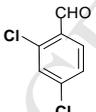
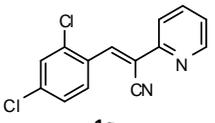
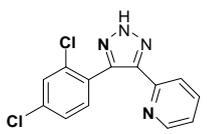
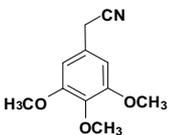
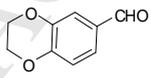
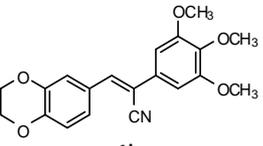
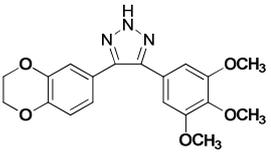
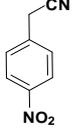
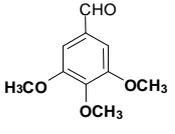
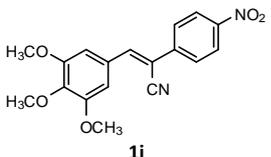
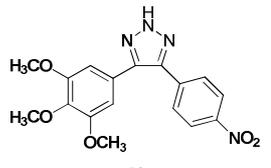
**Fig. 4.** Docking poses of molecules **2e**, **2h** and **2l** bound to tubulin. Subunits α and β of the tubulin heterodimer are shown as *gray* and *black* cartoons respectively. All the docking poses generated by Swissdock for molecules **2e**, **2h** and **2l** are shown in panels **A**, **B** and **C** respectively. As seen clearly, majority of the poses bind to the 'colchicine-binding' pocket located at the interface of the two tubulin subunits for all three molecules. **2e** shows the most, while **2l** shows the least number of 'outlier' poses.

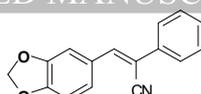
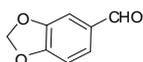
**Table 3.** SwissDock statistics for the docking runs with compounds **2e**, **2h** and **2l** (scores shown are averages from 3 docking runs).

**Fig. 5.** Atomic contacts between tubulin and molecules **2e** and **2l**. A) **2e** is shown as *orange* sticks, while residues of the α and β subunit of tubulin are in *light* and *dark gray*, respectively. B) **2l** (*green* sticks) is shown bound at the colchicine-binding pocket of tubulin. The color scheme is the same as in A, while the residues of β-tubulin unique to **2l** pocket are shown in *magenta*.

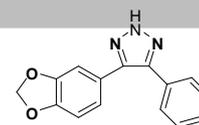
Table 1.

Synthesized (*Z*)-2,3-diarylacrylonitriles and 4,5-disubstituted-2*H*-1,2,3-triazoles from their corresponding acetonitrile and aldehyde precursors.

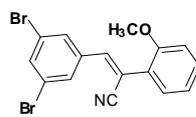
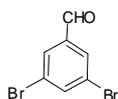
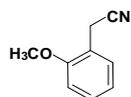
Acetonitrile	Aldehyde	( <i>Z</i> )-2,3-Diarylacetonitriles ( <b>1a-1s</b> )	4,5-Disubstituted 2 <i>H</i> -1,2,3-triazoles ( <b>2a-2s</b> )
			
			
			
			
			
			
			
			
			



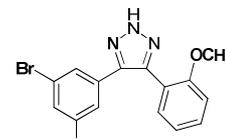
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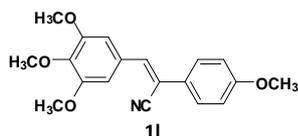
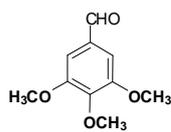
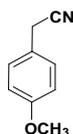
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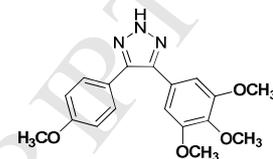
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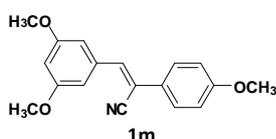
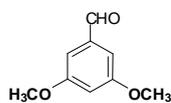
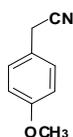
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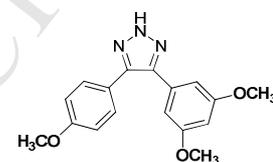
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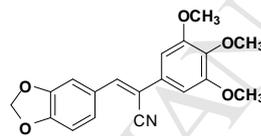
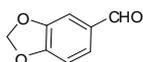
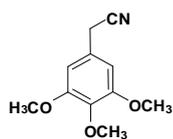
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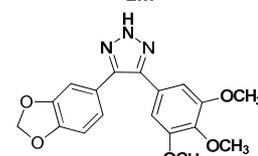
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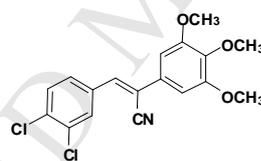
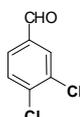
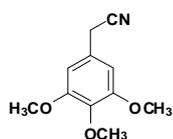
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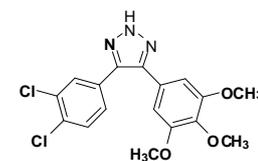
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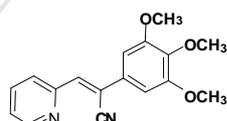
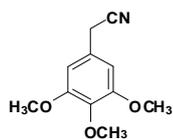
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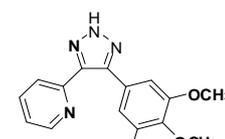
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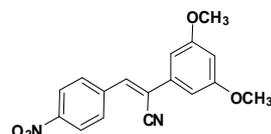
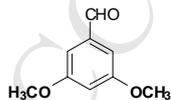
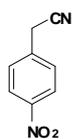
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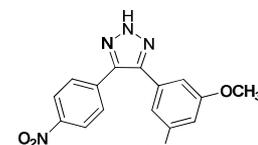
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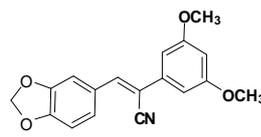
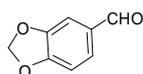
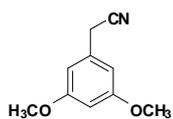
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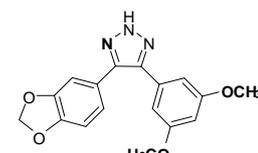
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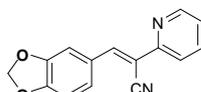
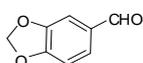
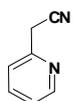
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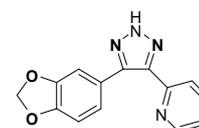
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2r



1s



2s

**Table 2.**

Growth inhibition ( $GI_{50}/\mu M$ )<sup>a</sup> data of **2d**, **2m**, **2l**, **2n** and **2g** against a panel of 60 human cancer cells.

Panel/cell line	<b>2d</b> ( $\mu M$ )	<b>2m</b> ( $\mu M$ )	<b>2l</b> ( $\mu M$ )	<b>2h</b> ( $\mu M$ )	<b>2n</b> ( $\mu M$ )	<b>2g</b> ( $\mu M$ )
<i>Leukemia</i>						
CCRF-CEM	0.037	0.032	<0.01	0.034	0.28	3.38
HL-60(TB)	0.030	0.023	<0.01	0.019	0.20	5.75
K-562	0.026	<0.01	<0.01	<0.01	0.05	5.50
MOLT-4	0.068	0.048	0.012	0.040	0.57	4.23
RPMI-8226	0.037	0.037	<0.01	0.033	0.33	10.7
SR	0.021	<0.01	<0.01	<0.01	0.04	5.43
<i>Lung Cancer</i>						
A549/ATCC	0.054	0.037	<0.01	0.023	0.29	4.85
HOP-62	0.047	0.025	<0.01	0.018	0.34	5.32
HOP-92	0.099	<0.01	<0.01	<0.01	0.12	3.28
NCI-H23	0.065	0.038	<0.01	0.048	0.77	9.13
NCI-H460	0.038	0.034	<0.01	0.025	0.34	3.67
<i>Colon Cancer</i>						
COLO 205	0.228	0.149	0.029	0.027	0.31	2.89
HCC-2998	0.230	0.046	0.024	0.039	>10	9.16
HCT-116	0.040	<0.01	<0.01	<0.01	0.18	2.89
HCT-15	0.037	<0.01	<0.01	<0.01	0.13	2.93
HT29	0.214	0.048	<0.01	0.025	0.39	4.56
KM12	0.035	0.030	<0.01	<0.01	0.07	6.87
SW-620	0.041	0.030	<0.01	<0.01	0.11	4.40
<i>CNS Cancer</i>						
SF-268	0.503	0.177	<0.01	0.069	0	7.36
SF-295	0.014	0.011	<0.01	<0.01	0.10	3.01
SF-539	0.020	0.014	<0.01	<0.01	0.18	na
SNB-75	0.016	0.014	<0.01	<0.01	0.08	1.58
U251	0.037	0.038	<0.01	0.020	0.30	5.35
<i>Melanoma</i>						
LOX IMVI	0.065	0.076	<0.01	0.018	0.55	4.83
M14	0.022	<0.01	<0.01	<0.01	na	14.3
MDA-MB-435	<0.01	<0.01	<0.01	<0.01	0.09	2.20
SK-MEL-2	0.027	0.056	<0.01	<0.01	0.02	6.52
SK-MEL-28	>100	na	na	>100	0.24	5.39
SK-MEL-5	0.013	0.027	<0.01	<0.01	>10	7.89
UACC-62	0.157	<0.01	nd	>100	>10	3.35
<i>Ovarian Cancer</i>						
IGROV1	0.065	0.051	<0.01	0.033	>10	5.94
OVCAR-3	0.011	0.024	<0.01	<0.01	0.43	3.38
OVCAR-4	0.077	na	<0.01	0.034	0.08	3.04
NCI/ADR-RES	0.023	<0.01	<0.01	<0.01	0.08	3.92
SK-OV-3	0.075	0.046	<0.01	<0.01	0.49	2.72
<i>Renal Cancer</i>						
786-0	0.043	0.015	<0.01	<0.01	0.62	2.75
A498	0.033	<0.01	<0.01	0.010	0.34	3.18
ACHN	0.081	0.145	<0.01	<0.01	0.71	1.75
CAKI-1	0.043	0.050	<0.01	<0.01	0.32	8.42
UO-31	0.092	0.020	<0.01	<0.01	0.66	6.84
<i>Prostate Cancer</i>						
PC-3	0.046	0.042	<0.01	0.018	0.26	4.50
DU-145	0.027	0.045	<0.01	0.025	0.35	1.41
<i>Breast Cancer</i>						
MCF7	0.027	0.025	<0.01	<0.01	0.08	2.58
MDA-MB-231/ATCC	0.094	0.046	<0.01	0.044	0.53	5.89
HS 578T	na	0.041	<0.01	0.668	0.44	11.6
MDA-MB-468	0.035	0.023	<0.01	0.015	0.23	3.20

na: Not analyzed, nd; not determined. <sup>a</sup> $GI_{50}$ : 50% growth inhibition, concentration of drug resulting in a 50% reduction in net cell growth as compared to cell numbers on day 0.

**Table 3.**SwissDock statistics for the docking runs with compounds **2e**, **2h** and **2l** (scores shown are averages from 3 docking runs).

Comp	FF score (kcal/mol)	$\Delta G$ (kcal/mol)
<b>2l</b>	-4224.7	-8.4
<b>2h</b>	-4216.5	-8.2
<b>2e</b>	-4211.4	-7.9

## Highlights

- A variety of novel 4,5-disubstituted 2H-1,2,3-triazoles were synthesized from their corresponding (Z)-substituted diacylacrylonitriles.
- The synthesized analogs were evaluated for their anti-cancer activity against NCI 60 human cancer cell lines.
- Conducted *in silico* docking studies to rationalize the anti-cancer properties of the synthesized compounds.
- Cell cycle redistribution assay was conducted to further understand the mechanism of action of the synthesized CA-4 analogues.
- One of the analogues **2e** showed promising anticancer activity with an LD<sub>50</sub> value of 7.5 nM against 9LSF rat gliosarcoma cells.

## Supporting Information

### Synthesis and biological evaluation of novel 4,5-disubstituted 2*H*-1,2,3-triazoles as *cis*-constrained analogues of combretastatin A-4

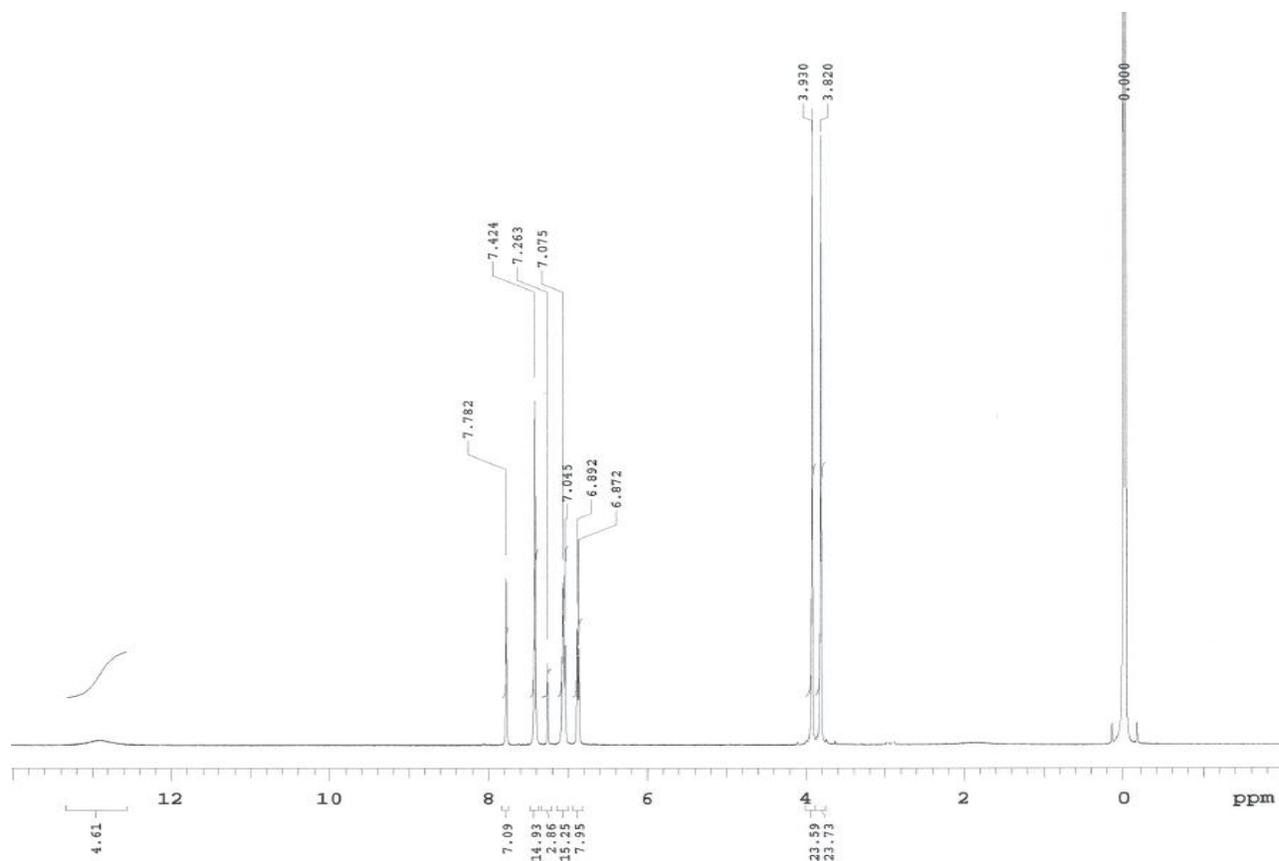
Nikhil R. Madadi<sup>a</sup>, Narsimha R. Penthala<sup>a</sup>, Kevin Howk<sup>ab</sup>, Amit Ketkar<sup>c</sup>, Robert L. Eoff<sup>c</sup>, Michael J. Borrelli<sup>b</sup> and Peter A. Crooks<sup>a\*</sup>

<sup>a</sup> Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR 72205-7199, USA

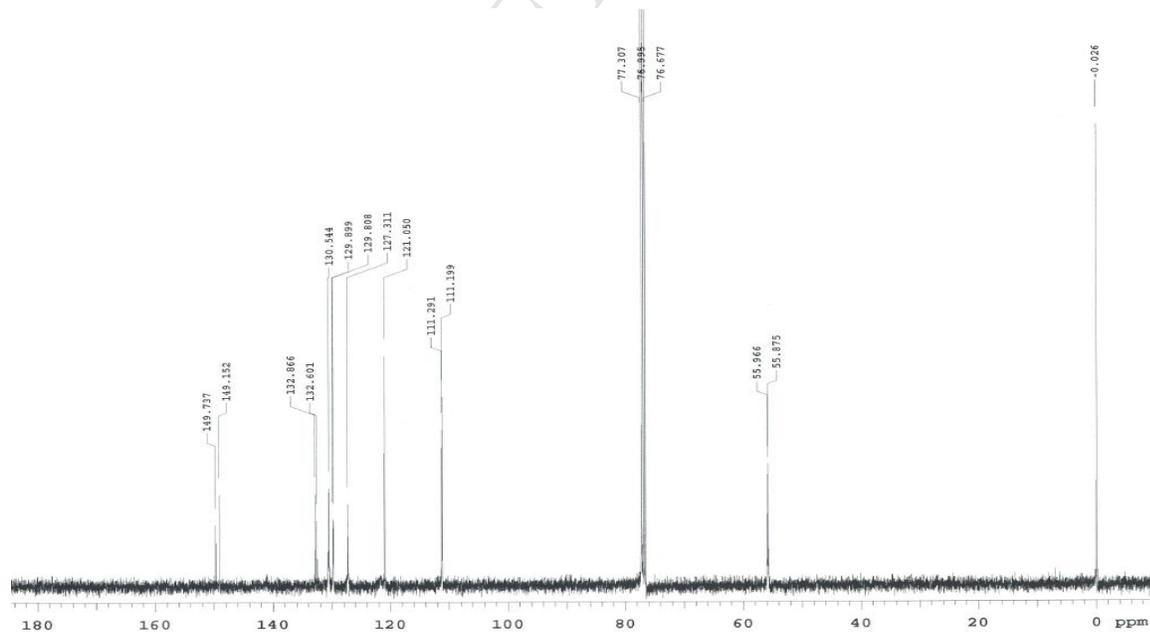
<sup>b</sup> Department of Radiology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205-7199, USA

<sup>c</sup> Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR 72205-7199, U.S.A.

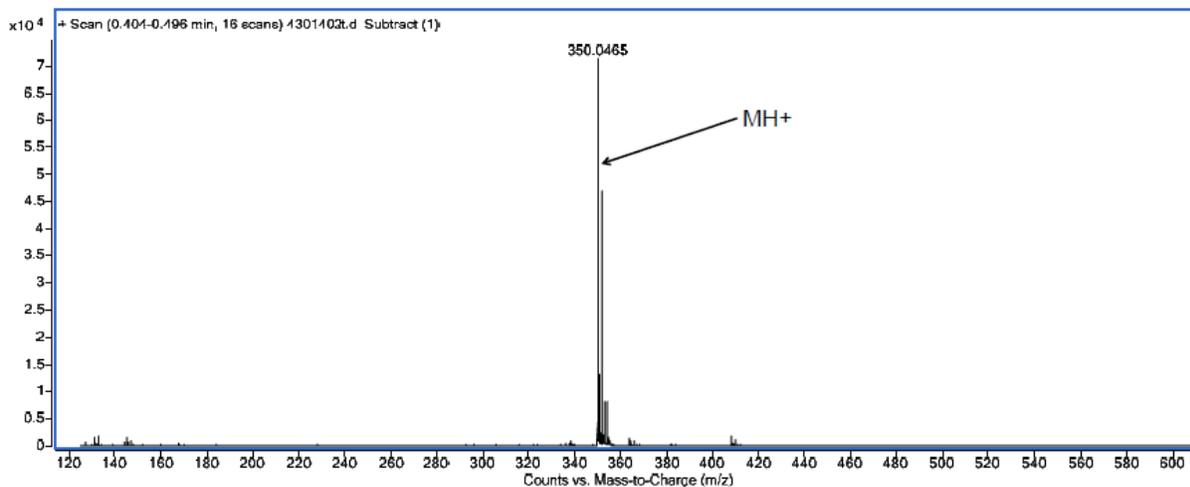
\*Corresponding author: Tel.: +1-501-686-6495; fax: +1-501-686-6057; E-mail: [pacrooks@uams.edu](mailto:pacrooks@uams.edu).



Representative <sup>1</sup>H NMR spectra of compound 4-(3,4-dichlorophenyl)-5-(3,4-dimethoxyphenyl)-2H-1,2,3-triazole (2a)



Representative <sup>13</sup>C NMR spectra of compound 4-(3,4-dichlorophenyl)-5-(3,4-dimethoxyphenyl)-2H-1,2,3-triazole (2a)

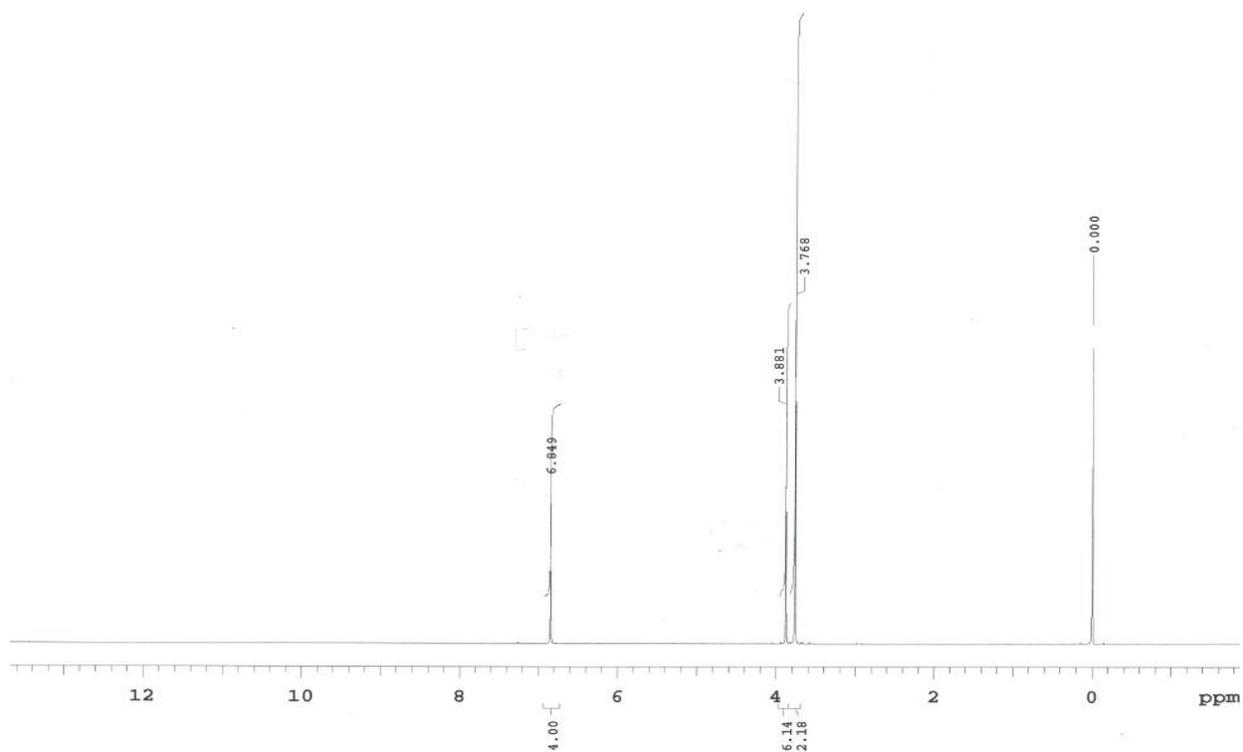


Measured Mass 350.0465

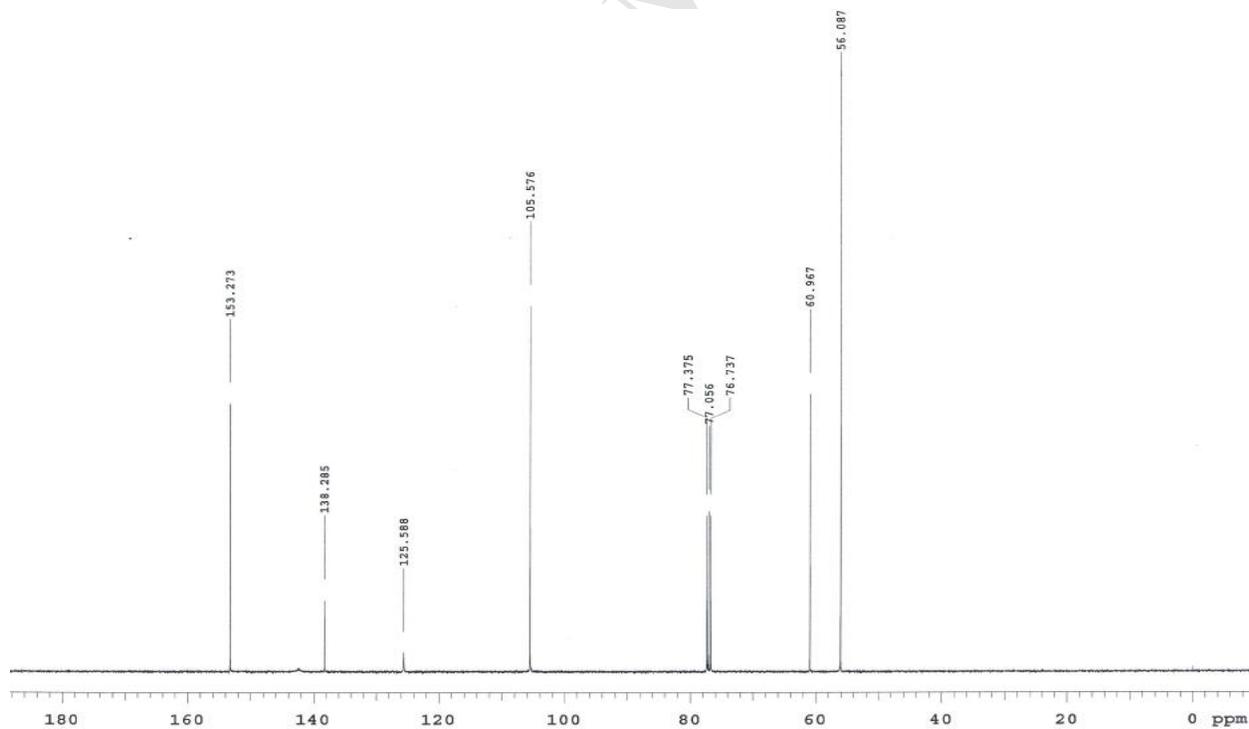
Element	Low Limit	High Limit
C	5	25
H	5	25
N	0	5
O	0	7
Cl	0	2

Formula	Calculated Mass	mDaError	ppmError	RDB
C <sub>18</sub> H <sub>16</sub> O <sub>3</sub> Cl <sub>2</sub>	350.0471	-0.6	-1.7	10
C <sub>16</sub> H <sub>14</sub> N <sub>3</sub> O <sub>2</sub> Cl <sub>2</sub>	350.0458	0.7	2.1	10.5
C <sub>25</sub> H <sub>6</sub> N <sub>2</sub> O	350.0475	-1.0	-2.8	24
C <sub>19</sub> H <sub>11</sub> N <sub>2</sub> O <sub>3</sub> Cl	350.0453	1.2	3.5	15
C <sub>22</sub> H <sub>9</sub> N <sub>3</sub> Cl	350.0480	-1.5	-4.1	19.5
C <sub>22</sub> H <sub>8</sub> N <sub>4</sub> O	350.0448	1.7	4.9	19.5

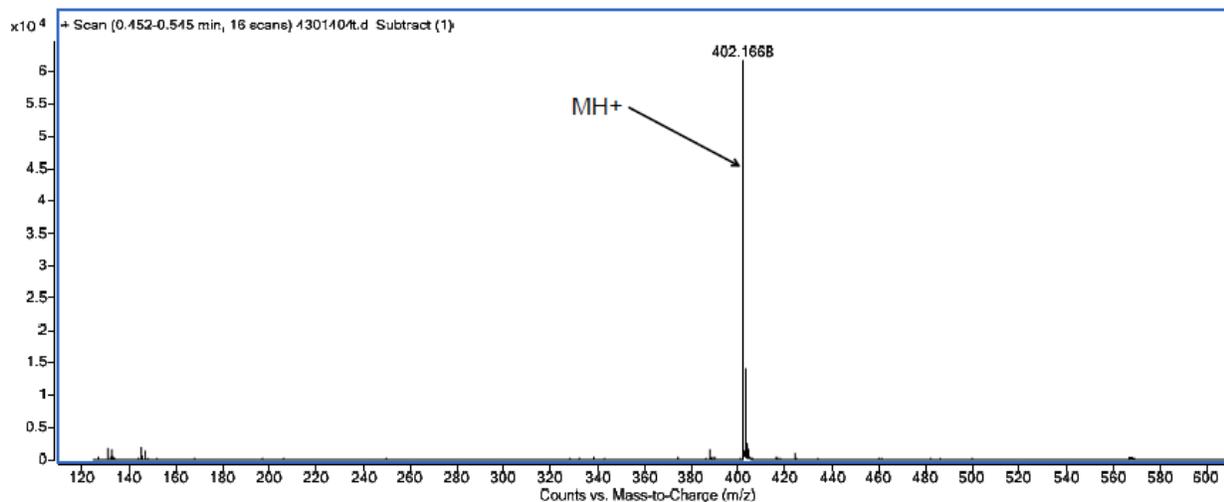
*Representative HRMS spectra of compound 4-(3,4-dichlorophenyl)-5-(3,4-dimethoxy phenyl)-2H-1,2,3-triazole (2a)*



Representative  $^1\text{H}$  NMR spectra of compound 4,5-bis(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2b)



Representative  $^{13}\text{C}$  NMR spectra of compound 4,5-bis(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2b)

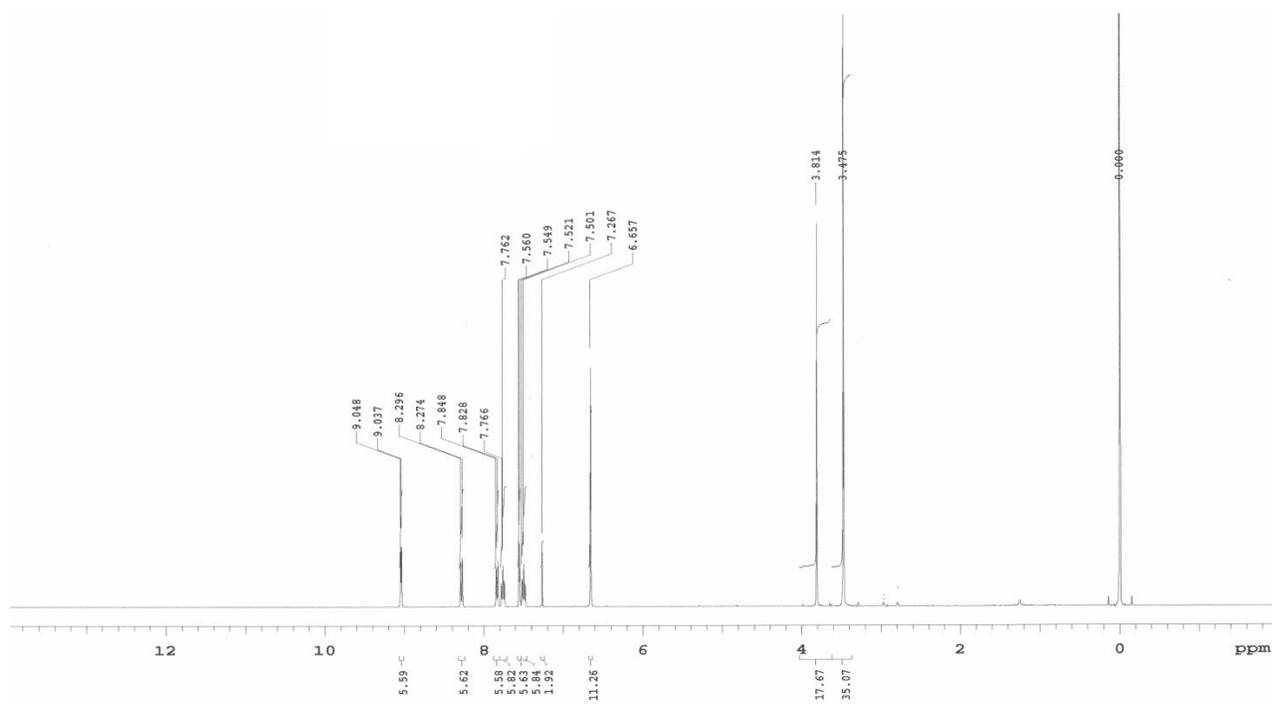
Measured Mass

402.1668

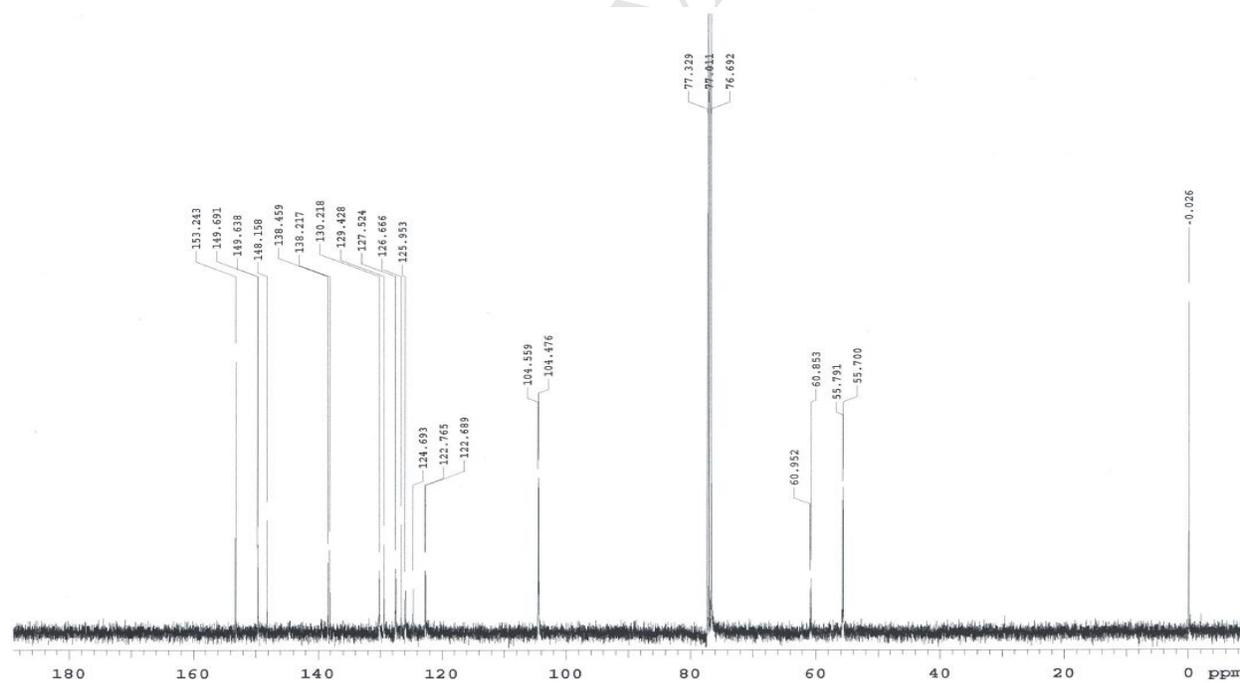
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	5	25
H	5	25
N	0	5
O	0	7
Cl	0	2

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>20</sub> H <sub>24</sub> N <sub>3</sub> O <sub>6</sub>	402.1660	0.8	2.1	10.5
C <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub>	402.1686	-1.8	-4.6	15

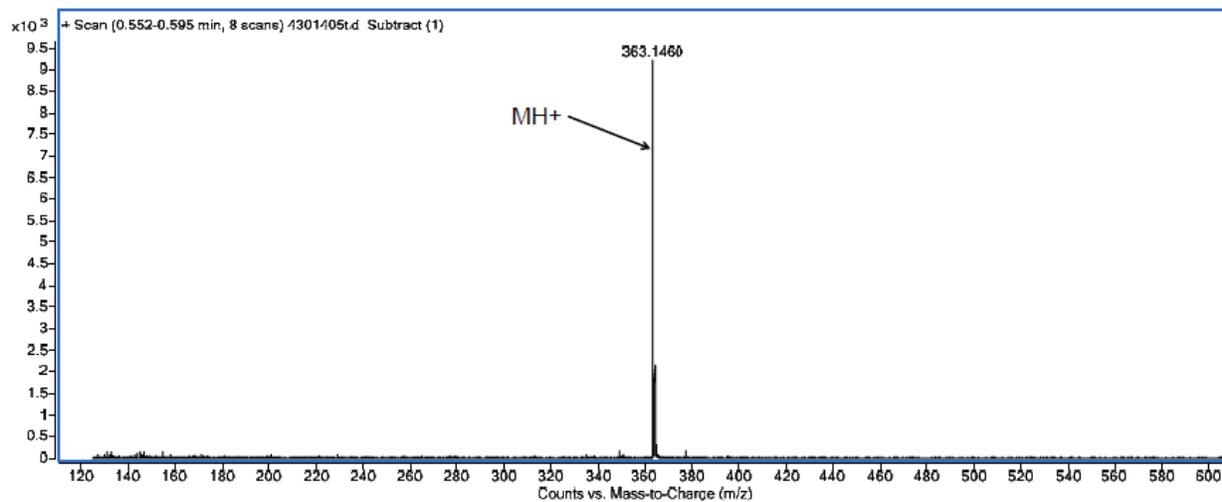
*Representative HRMS spectra of compound 4,5-bis(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2b)*



Representative  $^1\text{H}$  NMR spectra of compound 4-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)quinolone (2c)



Representative  $^{13}\text{C}$  NMR spectra of compound 4-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)quinolone (2c)

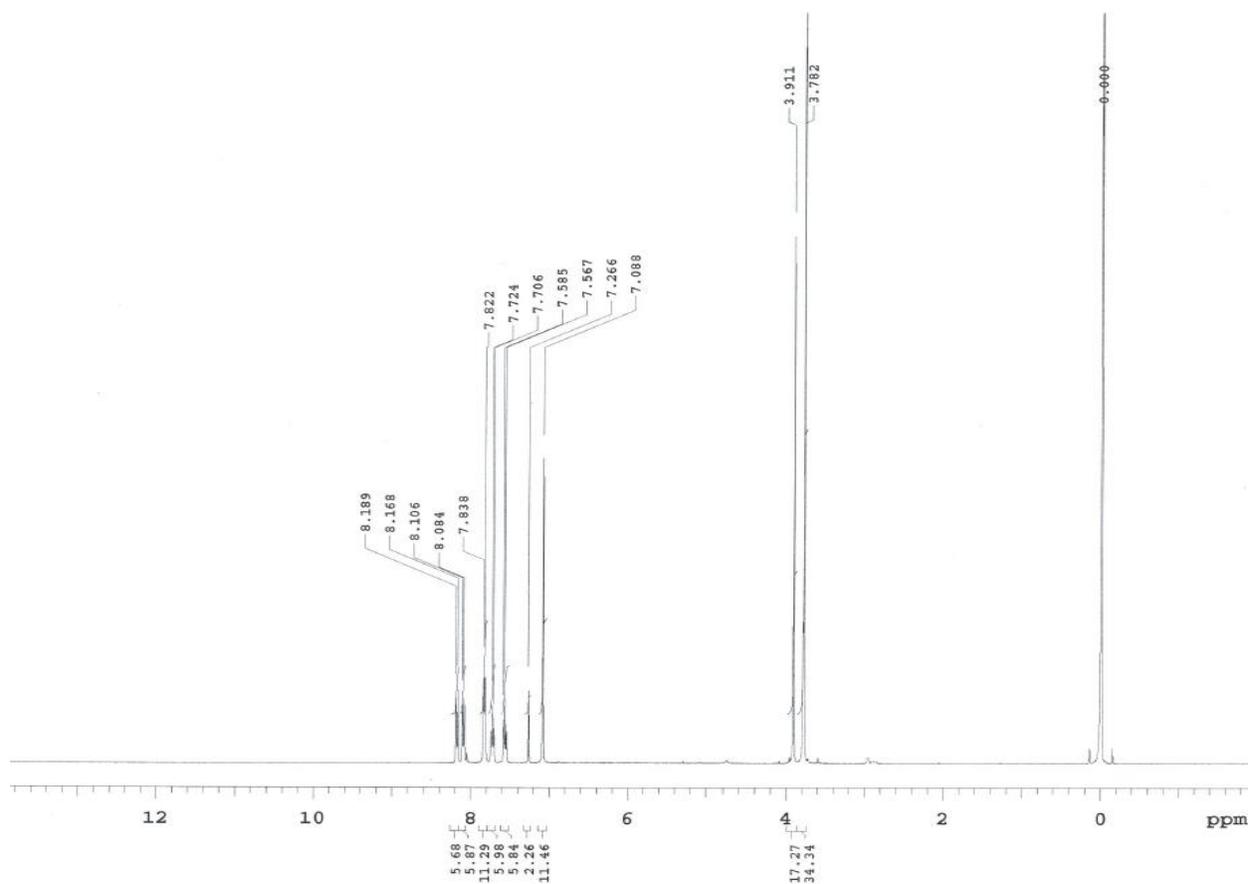
Measured Mass

363.146

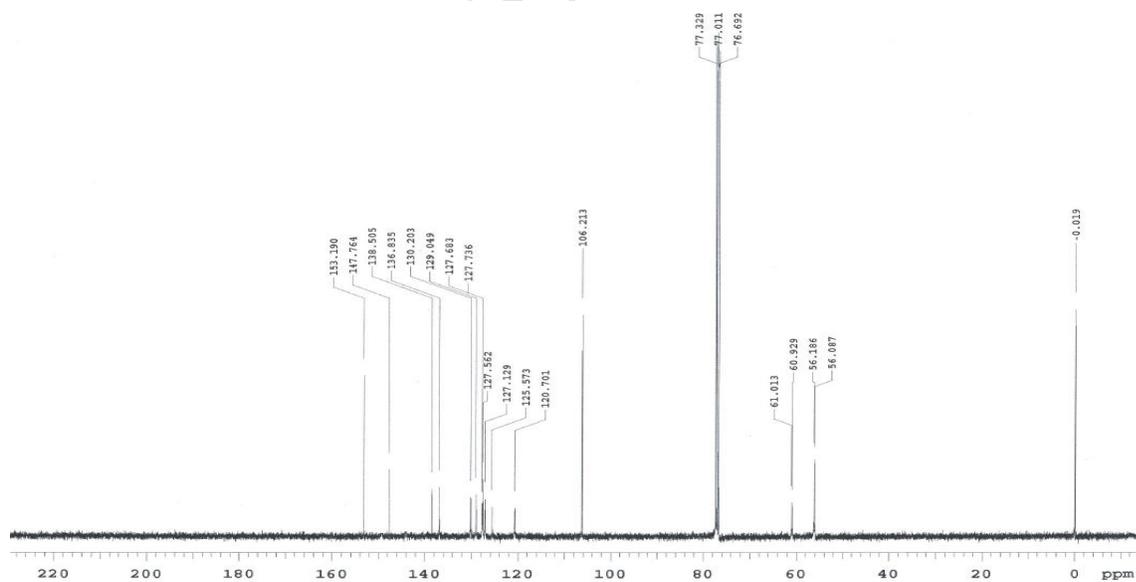
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	5	25
H	5	25
N	0	5
O	0	7

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C22 H21 N O4	363.1465	-0.5	-1.4	13
C20 H19 N4 O3	363.1452	0.8	2.3	13.5

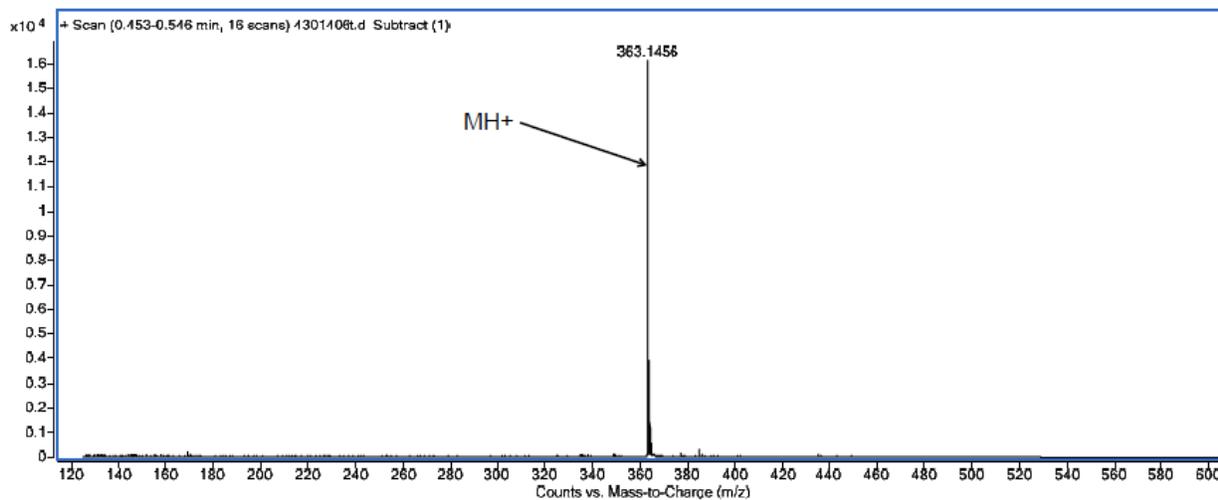
*Representative HRMS spectra of compound 4-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)quinolone (2c)*



Representative  $^1\text{H}$  NMR spectra of compound 2-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)quinolone (2d)



Representative  $^{13}\text{C}$  NMR spectra of compound 2-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)quinolone (2d)

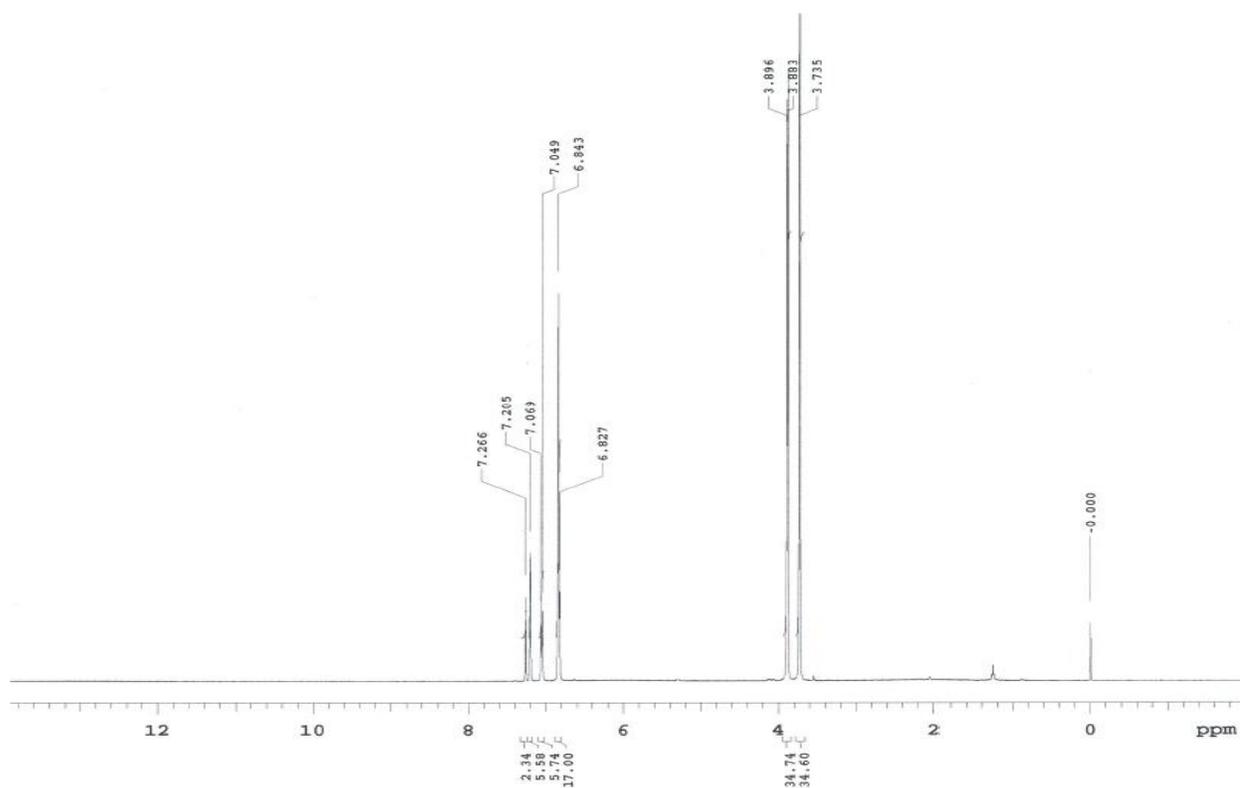
Measured Mass

363.1456

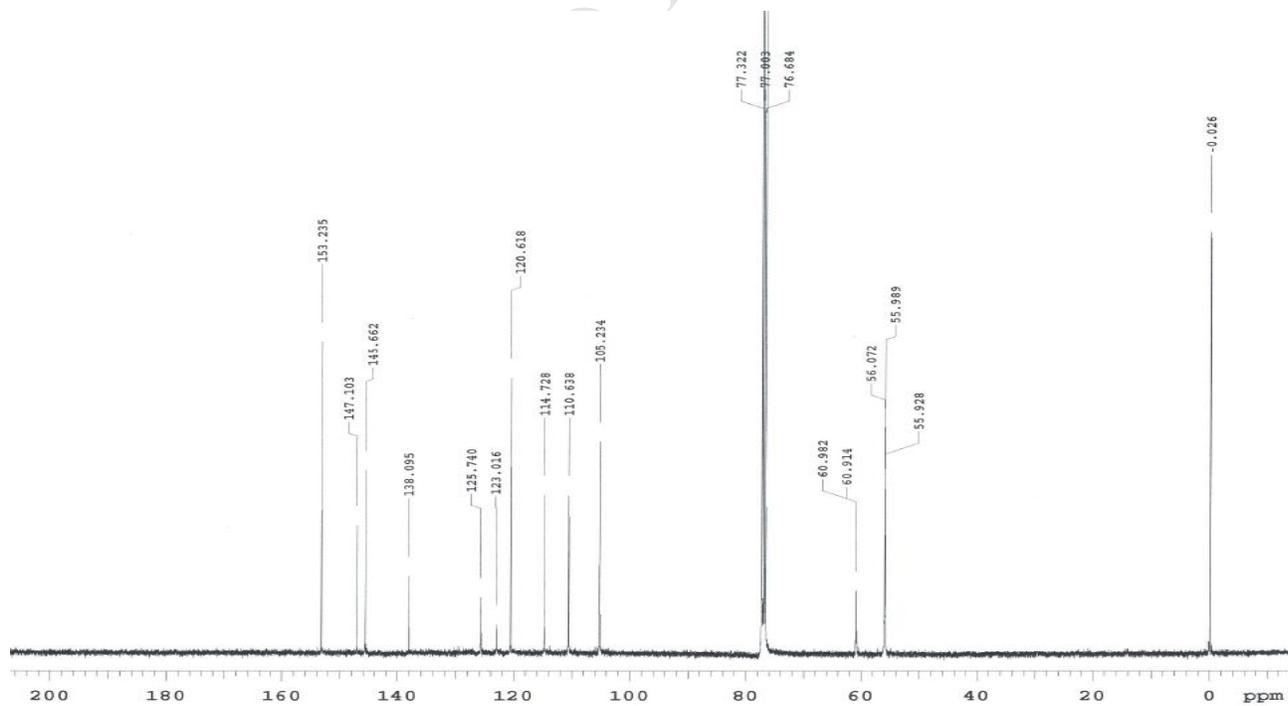
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	5	25
H	5	25
N	0	5
O	0	7

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>20</sub> H <sub>19</sub> N <sub>4</sub> O <sub>3</sub>	363.1452	0.4	1.2	13.5
C <sub>22</sub> H <sub>21</sub> N <sub>4</sub> O <sub>4</sub>	363.1465	-0.9	-2.5	13
C <sub>19</sub> H <sub>23</sub> O <sub>7</sub>	363.1438	1.8	4.9	8.5

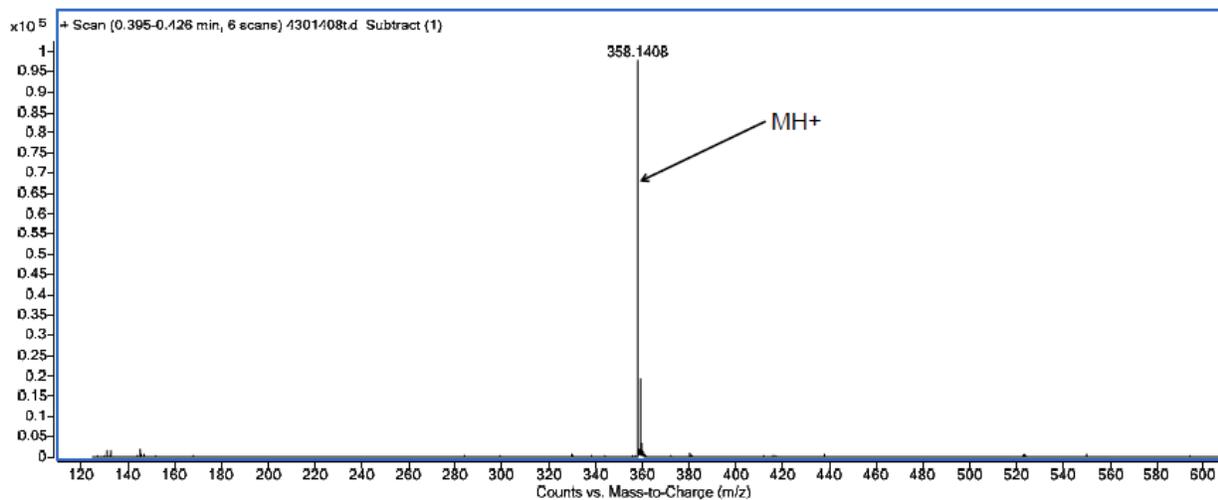
*Representative HRMS spectra of compound 2-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)quinolone (2d)*



Representative <sup>1</sup>H NMR spectra of compound 2-methoxy-5-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)phenol (2e)



Representative <sup>13</sup>C NMR spectra of compound 2-methoxy-5-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)phenol (2e)

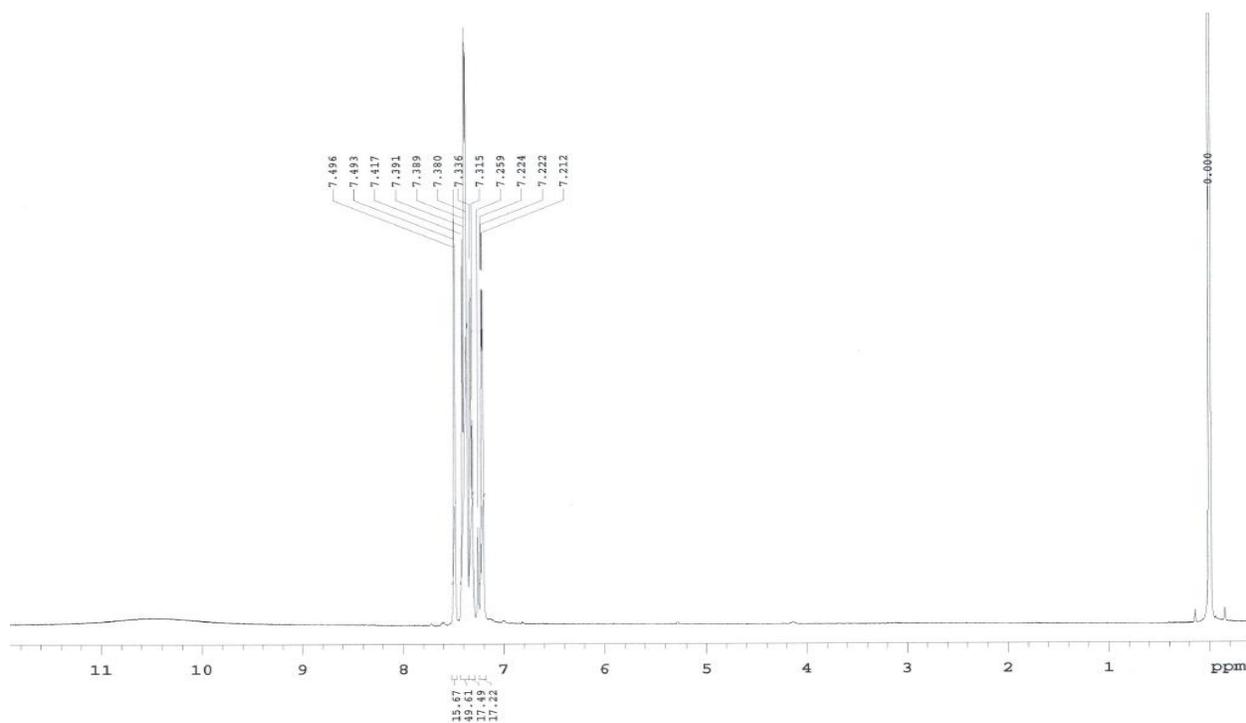
Measured Mass

358.1408

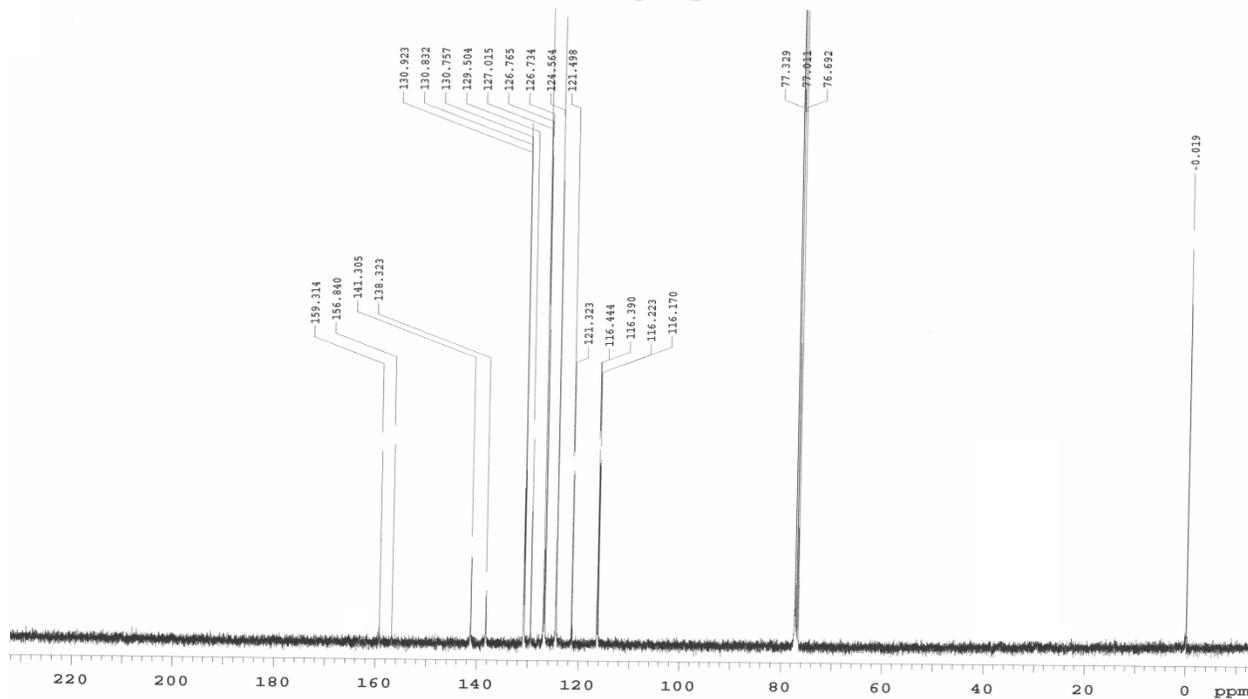
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	5	25
H	5	25
N	0	5
O	0	7

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>20</sub> H <sub>22</sub> O <sub>6</sub>	358.1411	-0.3	-0.8	10
<b>C<sub>18</sub> H<sub>20</sub> N<sub>3</sub> O<sub>5</sub></b>	<b>358.1397</b>	<b>1.1</b>	<b>2.9</b>	<b>10.5</b>
C <sub>21</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub>	358.1424	-1.6	-4.5	15

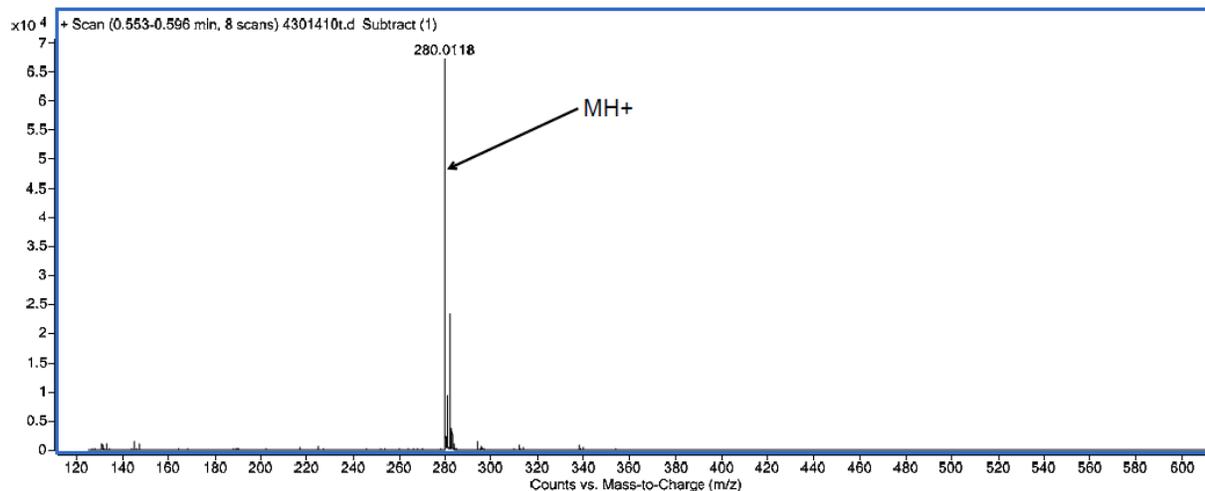
*Representative HRMS spectra of compound 2-methoxy-5-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)phenol (2e)*



Representative <sup>1</sup>H NMR spectra of compound 4-(4-chloro-3-fluorophenyl)-5-(thiophen-3-yl)-2H-1,2,3-triazole (2f)



Representative <sup>13</sup>C NMR spectra of compound 4-(4-chloro-3-fluorophenyl)-5-(thiophen-3-yl)-2H-1,2,3-triazole (2f)

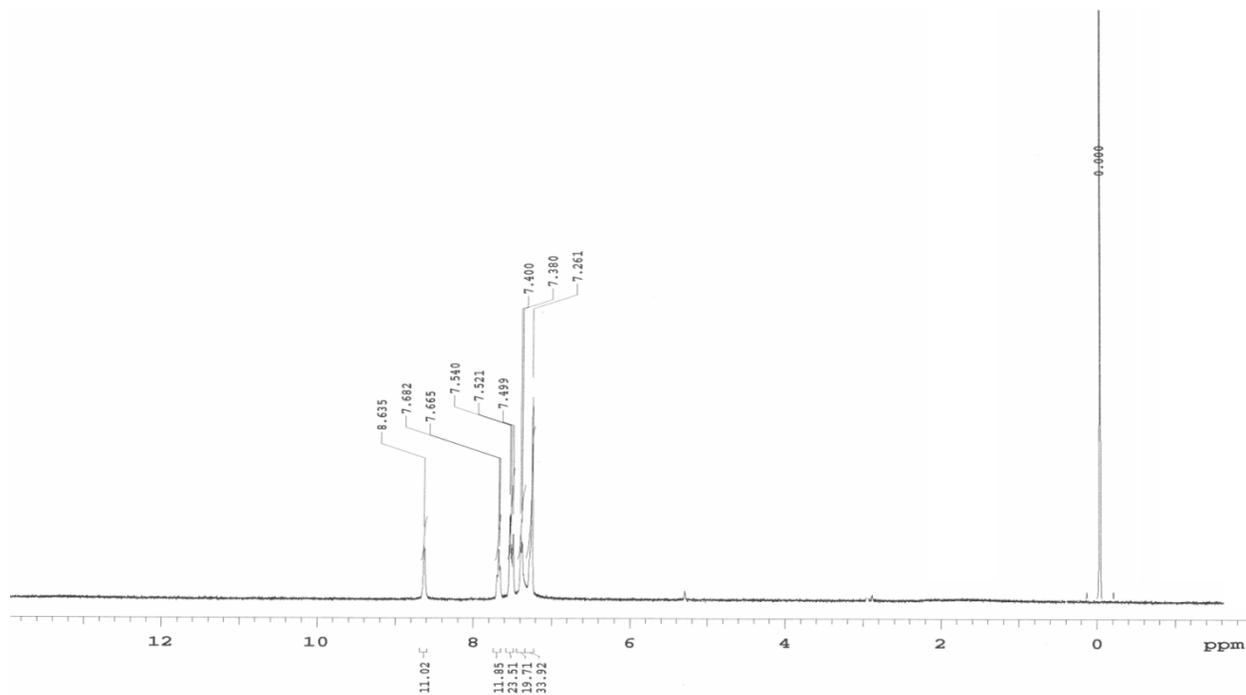
Measured Mass

280.0118

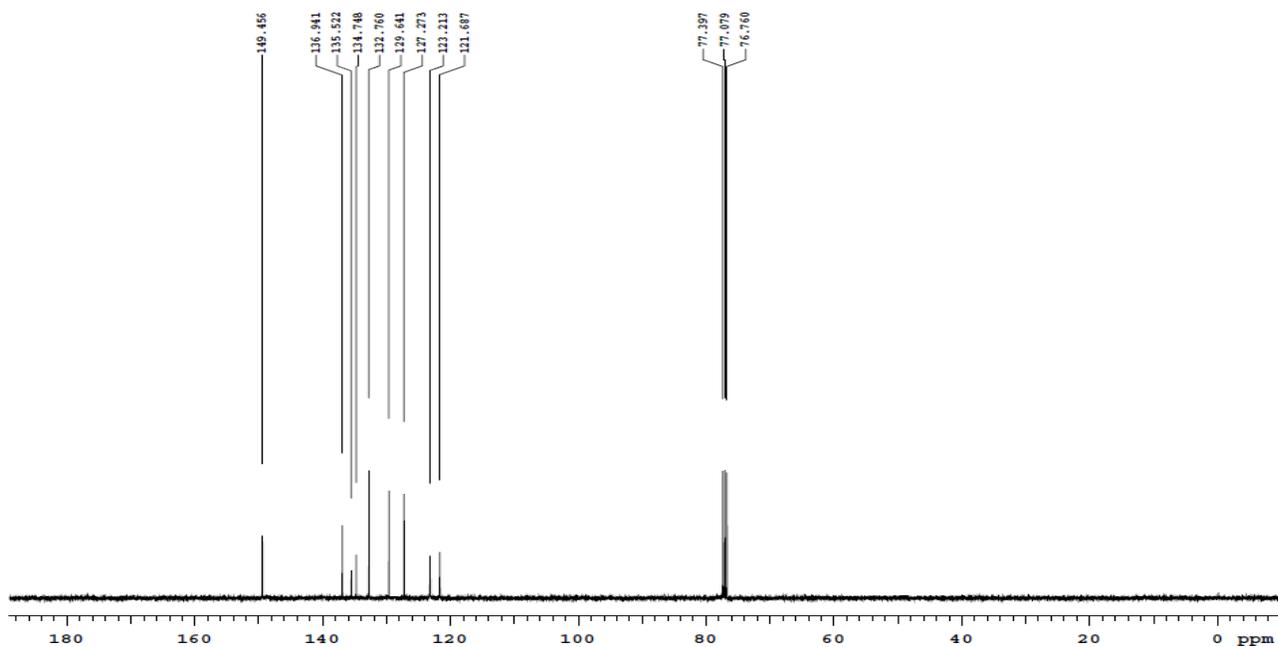
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	5	25
H	5	25
S	0	2
N	0	5
Cl	0	1
F	0	1

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>10</sub> H <sub>7</sub> N <sub>5</sub> F S <sub>2</sub>	280.0121	-0.3	-1.2	9.5
C <sub>15</sub> H <sub>8</sub> N <sub>2</sub> S <sub>2</sub>	280.0123	-0.5	-1.9	13
C <sub>17</sub> H <sub>9</sub> S Cl	280.0108	1.0	3.6	13
<b>C<sub>12</sub> H<sub>8</sub> N<sub>3</sub> F S Cl</b>	<b>280.0106</b>	<b>1.2</b>	<b>4.3</b>	<b>9.5</b>

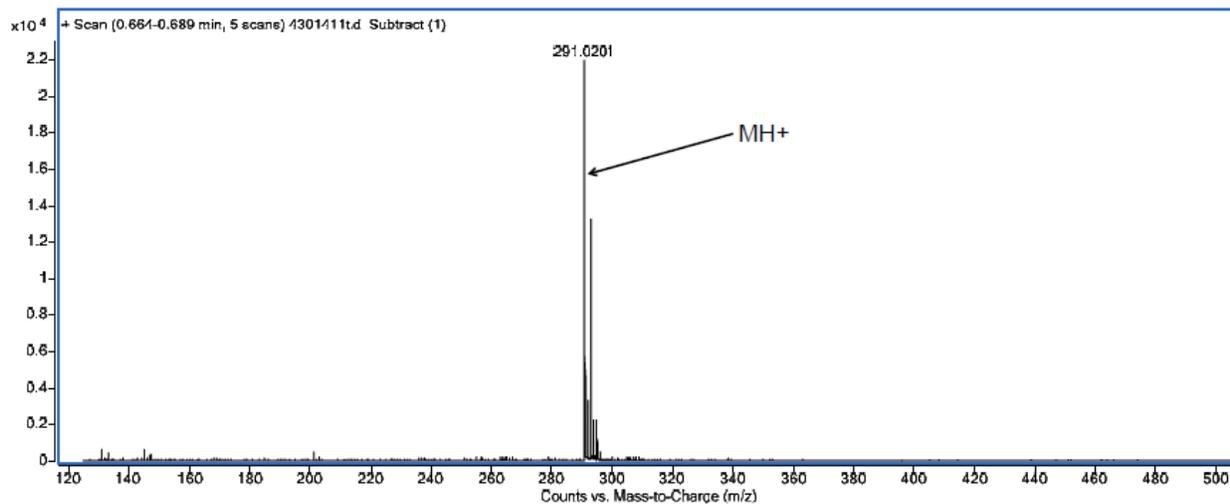
*Representative HRMS spectra of compound 4-(4-chloro-3-fluorophenyl)-5-(thiophen-3-yl)-2H-1,2,3-triazole (2f)*



*Representative  $^1\text{H}$  NMR spectra of compound 2-(5-(2,4-dichlorophenyl)-2H-1,2,3-triazol-4-yl)pyridine (2g)*



*Representative  $^{13}\text{C}$  NMR spectra of compound 2-(5-(2,4-dichlorophenyl)-2H-1,2,3-triazol-4-yl)pyridine (2g)*

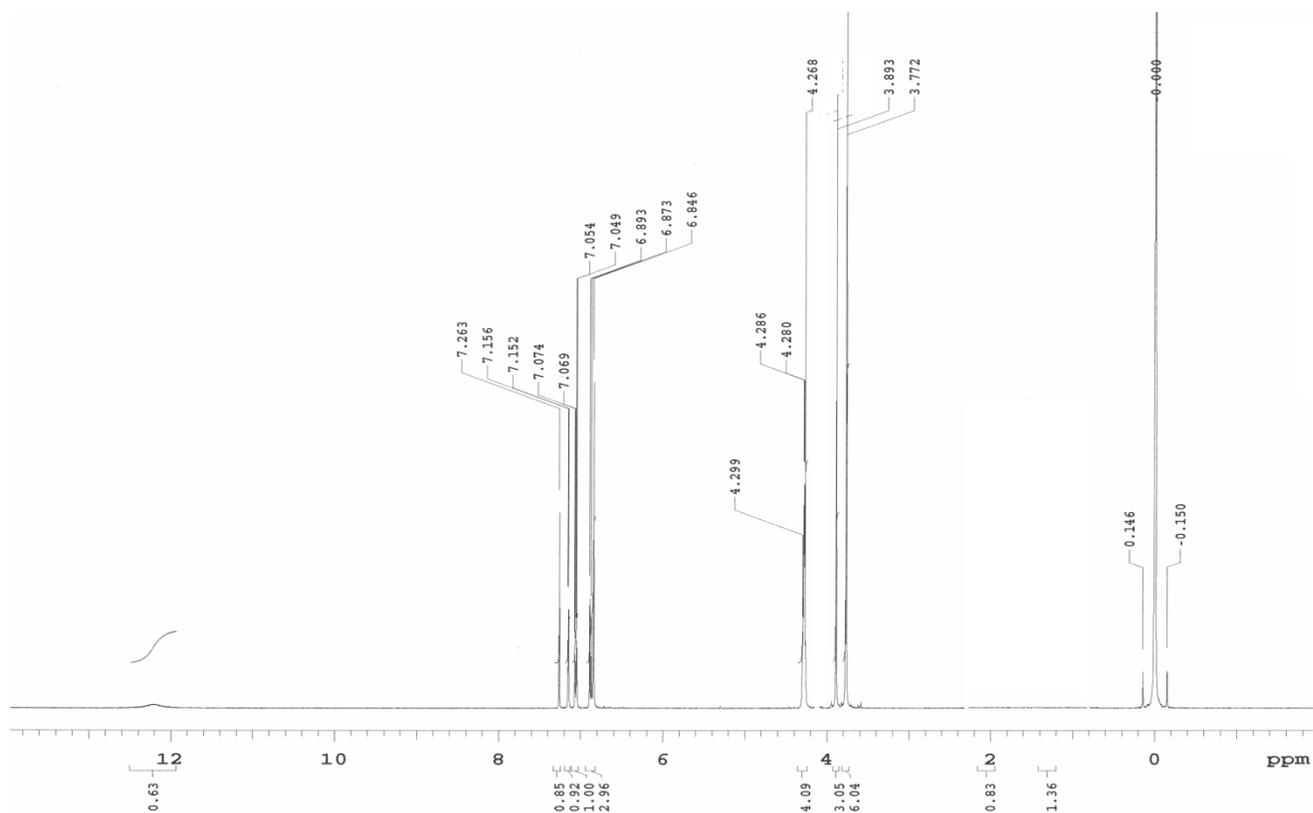
Measured Mass

291.0201

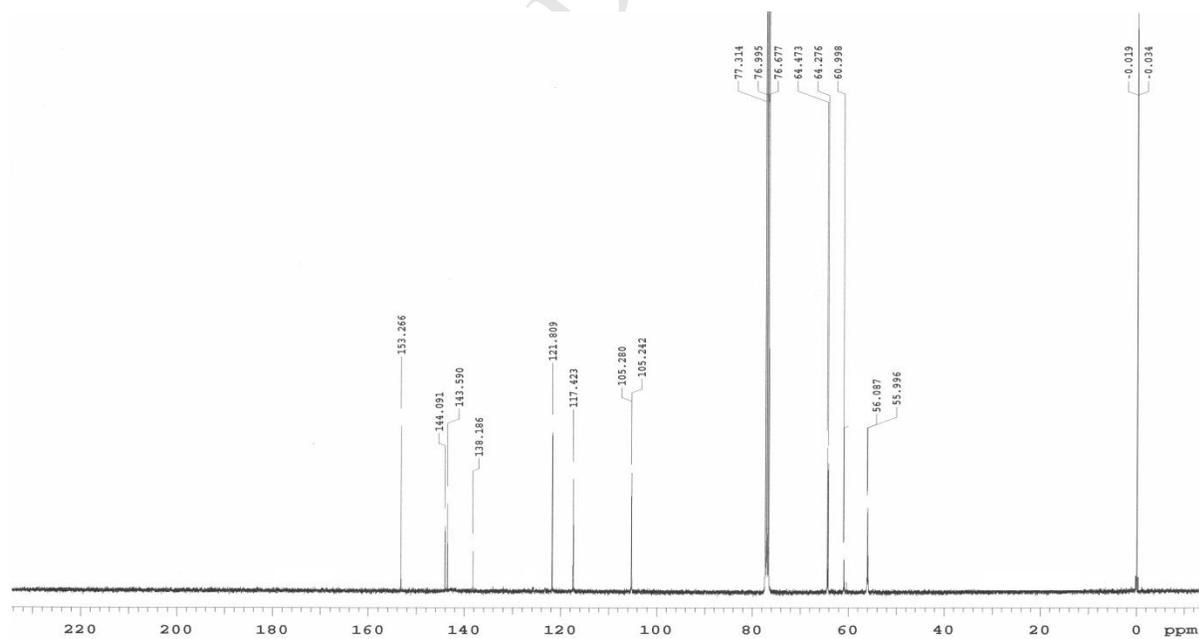
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	5	25
H	5	25
N	0	5
Cl	0	2

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>13</sub> H <sub>9</sub> N <sub>4</sub> Cl <sub>2</sub>	291.0199	0.2	0.8	10.5

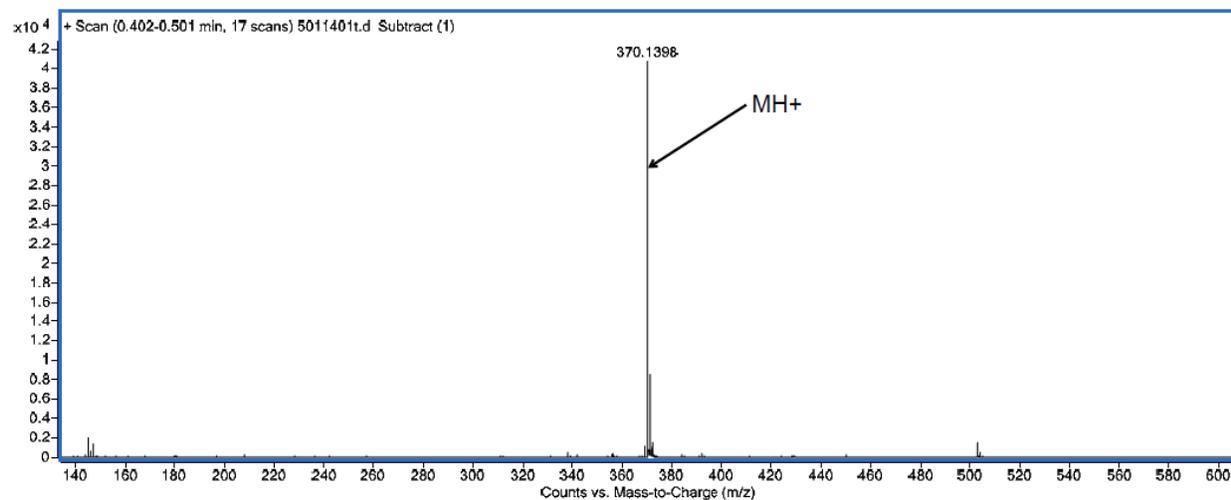
*Representative HRMS spectra of compound 2-(5-(2,4-dichlorophenyl)-2H-1,2,3-triazol-4-yl)pyridine (2g)*



Representative  $^1\text{H}$  NMR spectra of compound 4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-(3,4,5-trimethoxy phenyl)-2H-1,2,3-triazole (2h):



Representative  $^{13}\text{C}$  NMR spectra of compound 4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-(3,4,5-trimethoxy phenyl)-2H-1,2,3-triazole (2h):

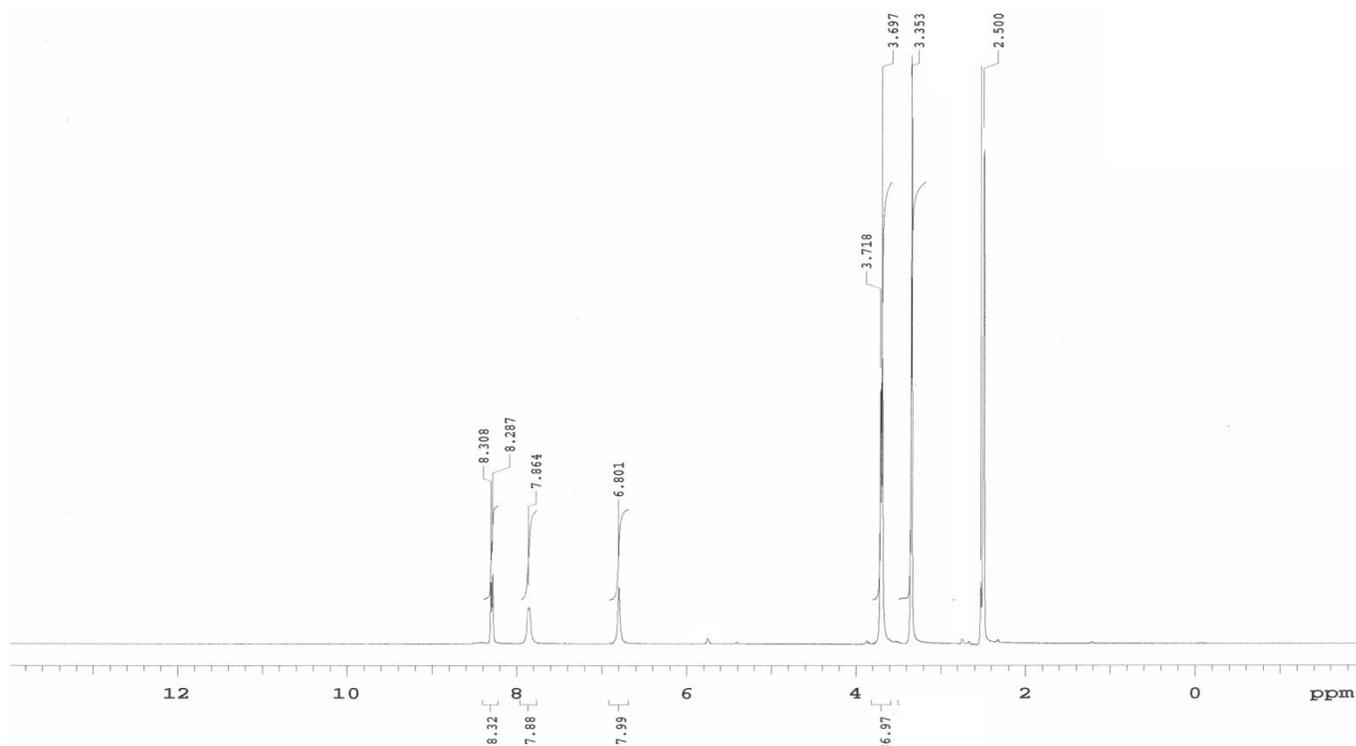
Measured Mass

370.1398

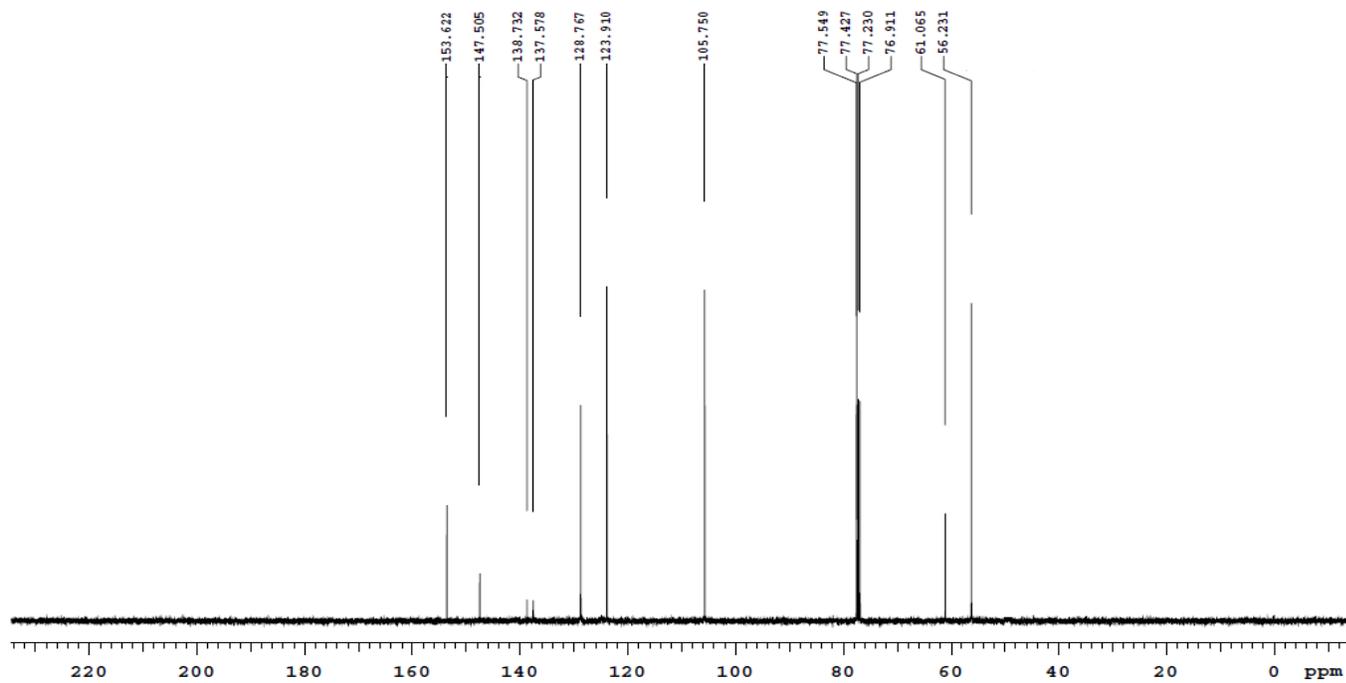
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	10	25
H	5	25
N	0	5
O	0	6

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>19</sub> H <sub>20</sub> N <sub>3</sub> O <sub>5</sub>	370.1397	0.1	0.1	11.5
C <sub>21</sub> H <sub>22</sub> O <sub>6</sub>	370.1411	-1.3	-3.5	11

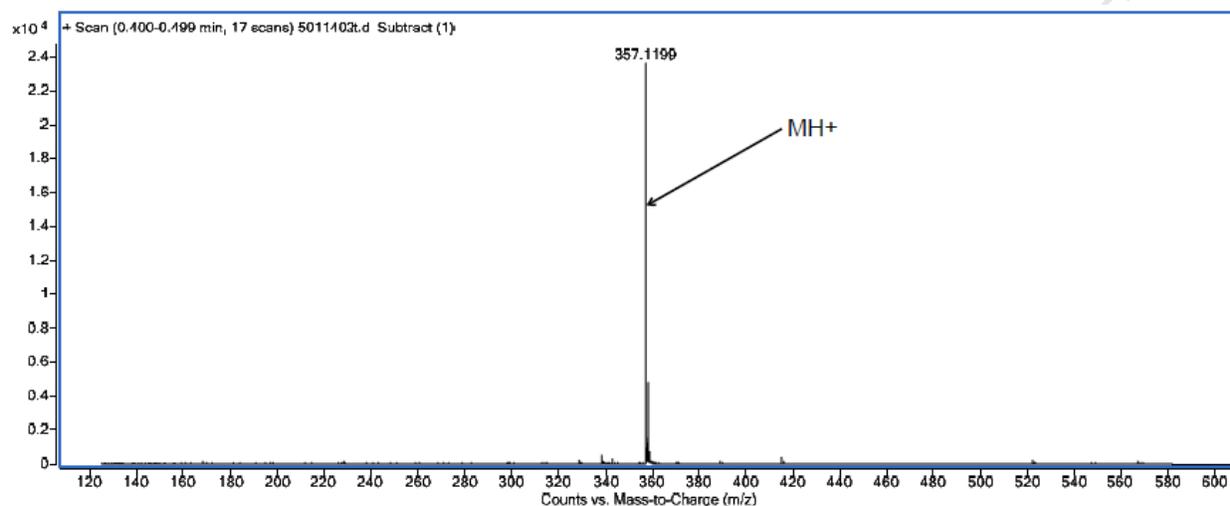
**Representative HRMS spectra of compound 4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-(3,4,5-trimethoxy phenyl)-2H-1,2,3-triazole (2h):**



Representative  $^1\text{H}$  NMR spectra of compound 4-(4-nitrophenyl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2i)



Representative  $^{13}\text{C}$  NMR spectra of compound 4-(4-nitrophenyl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2i)

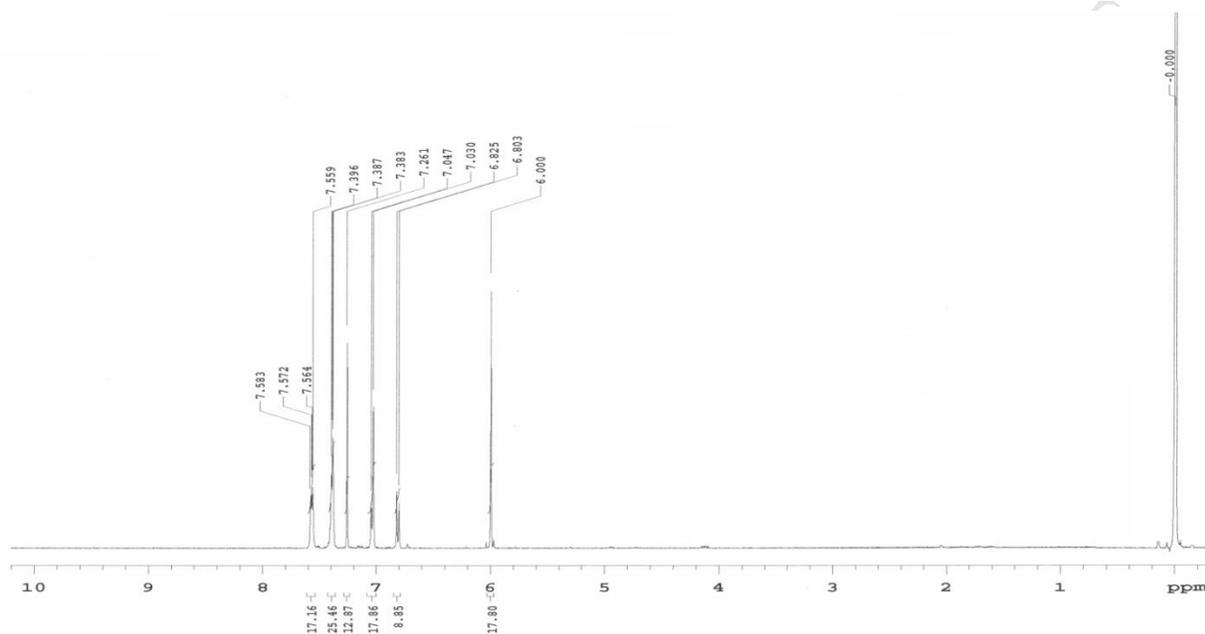
Measured Mass

357.1199

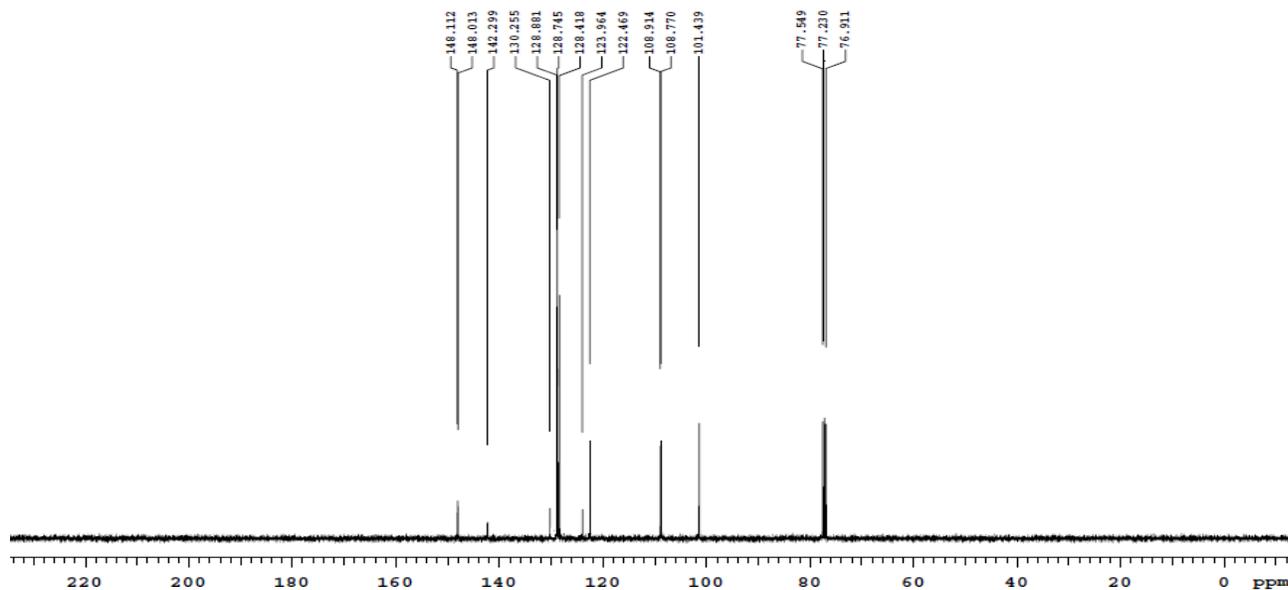
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	10	25
H	5	25
N	0	5
O	0	6

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>17</sub> H <sub>17</sub> N <sub>4</sub> O <sub>5</sub>	357.1193	0.6	1.6	11.5
C <sub>19</sub> H <sub>19</sub> N O <sub>6</sub>	357.1207	-0.8	-2.2	11

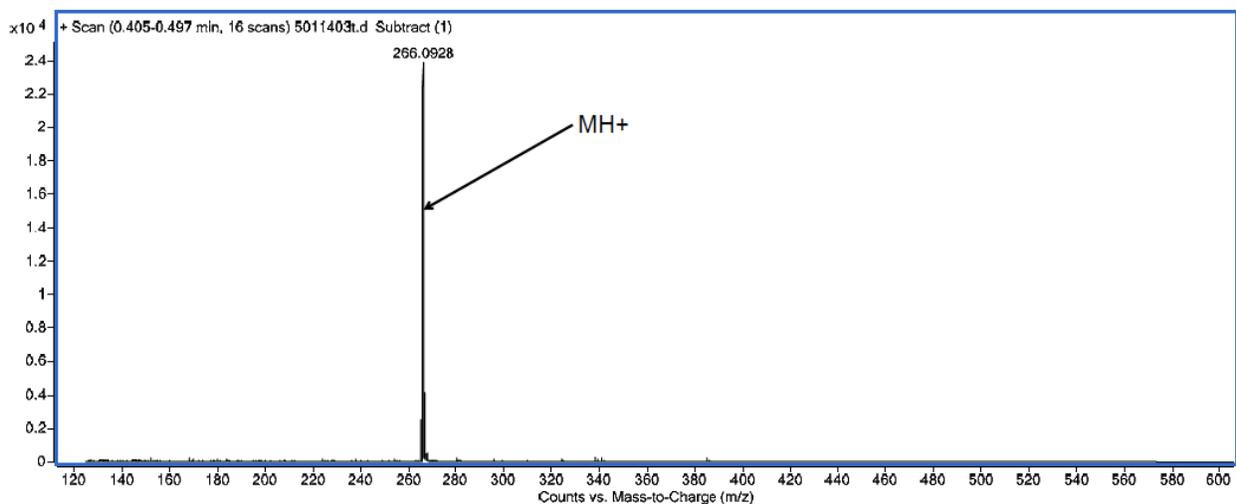
*Representative HRMS spectra of compound 4-(4-nitrophenyl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2i)*



Representative <sup>1</sup>H NMR spectra of compound 4-(benzo[d][1,3]dioxol-5-yl)-5-phenyl-2H-1,2,3-triazole (2j)



Representative <sup>13</sup>C NMR spectra of compound 4-(benzo[d][1,3]dioxol-5-yl)-5-phenyl-2H-1,2,3-triazole (2j)

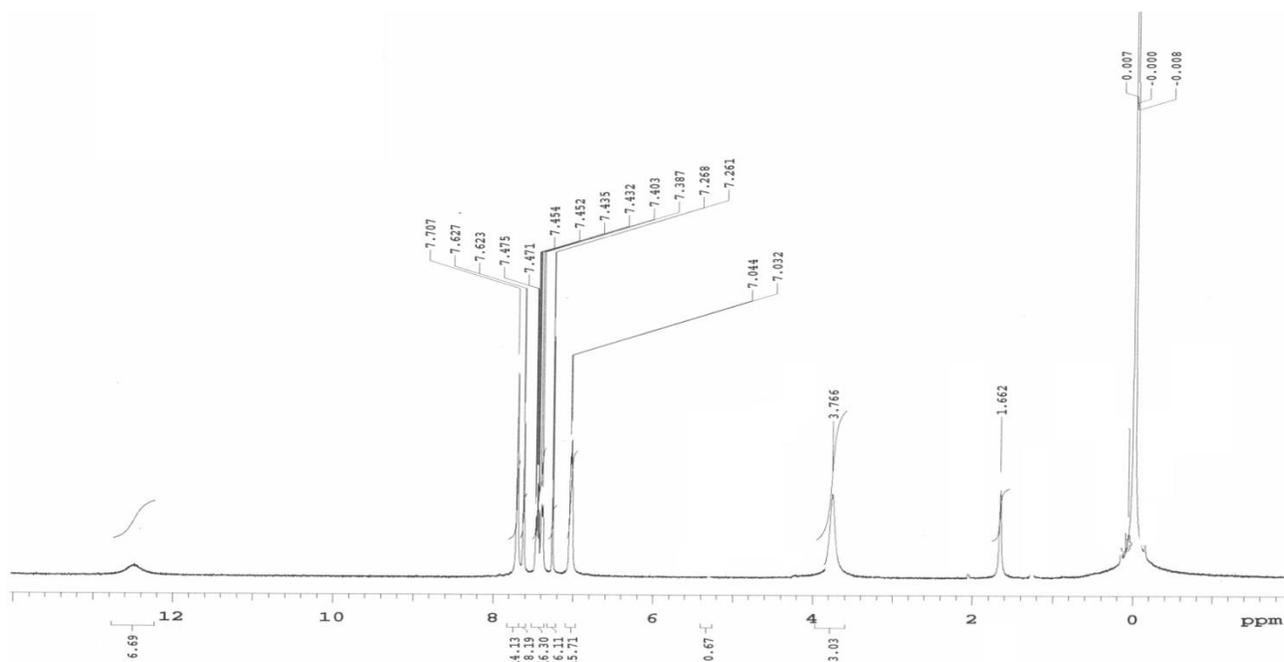
Measured Mass

266.0928

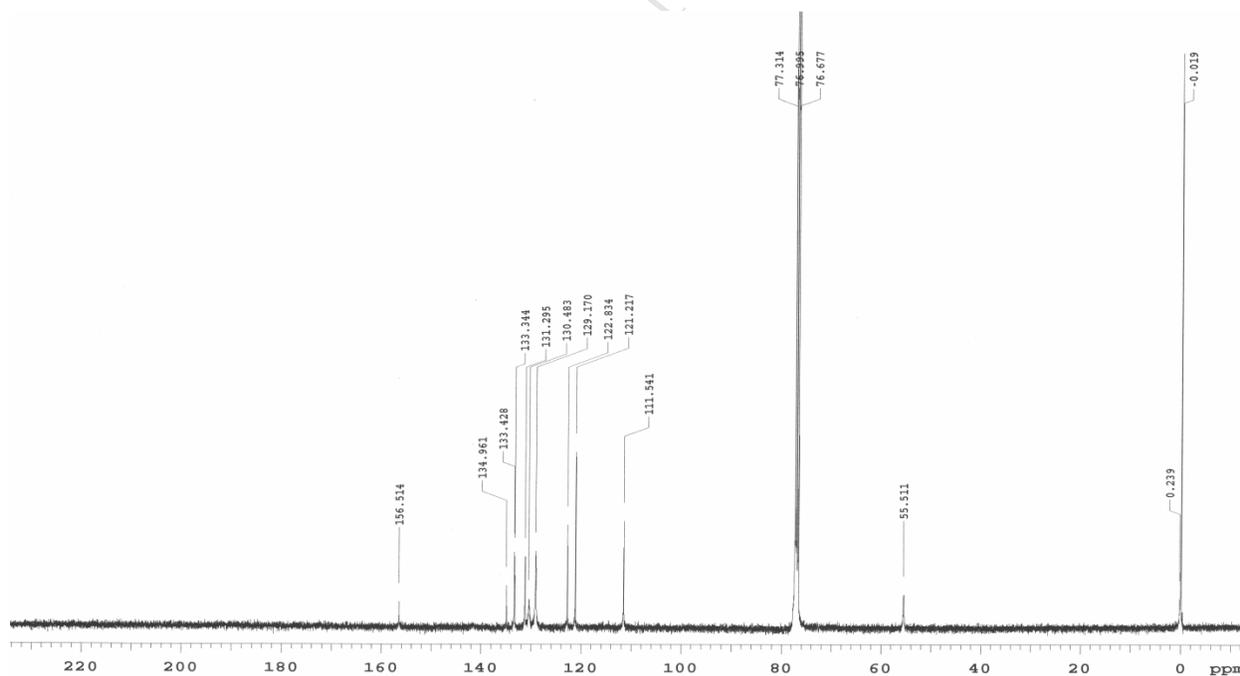
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	10	25
H	5	25
N	0	5
O	0	6

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>15</sub> H <sub>12</sub> N <sub>3</sub> O <sub>2</sub>	266.0924	0.4	1.5	11.5
C <sub>17</sub> H <sub>14</sub> O <sub>3</sub>	266.0937	-0.9	-3.6	11

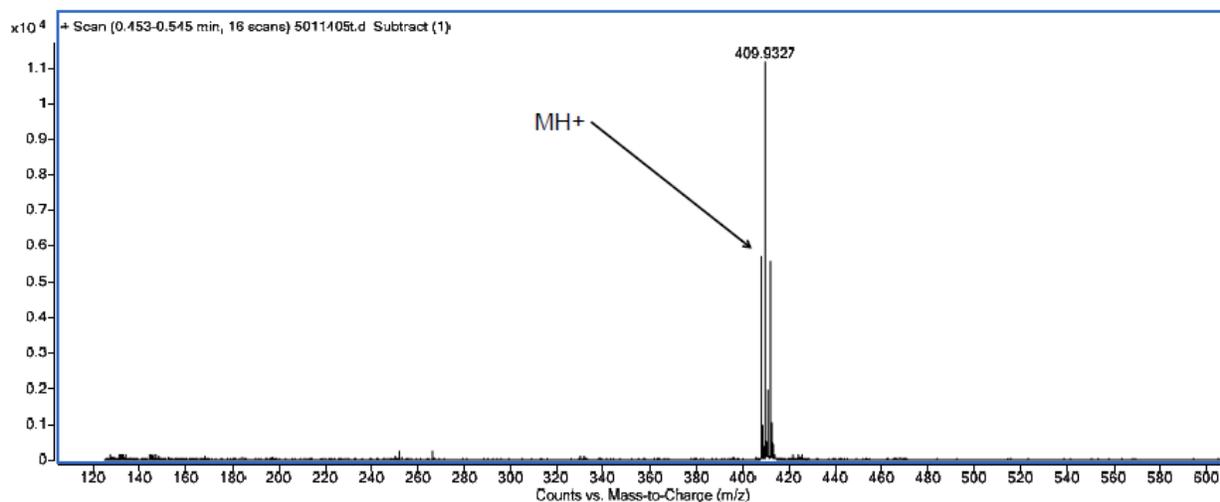
*Representative HRMS spectra of compound 4-(benzo[d][1,3]dioxol-5-yl)-5-phenyl-2H-1,2,3-triazole (2j)*



Representative <sup>1</sup>H NMR spectra of compound 4-(3,5-dibromophenyl)-5-(2-methoxyphenyl)-2H-1,2,3-triazole (2k)



Representative <sup>13</sup>C NMR spectra of compound 4-(3,5-dibromophenyl)-5-(2-methoxyphenyl)-2H-1,2,3-triazole (2k)

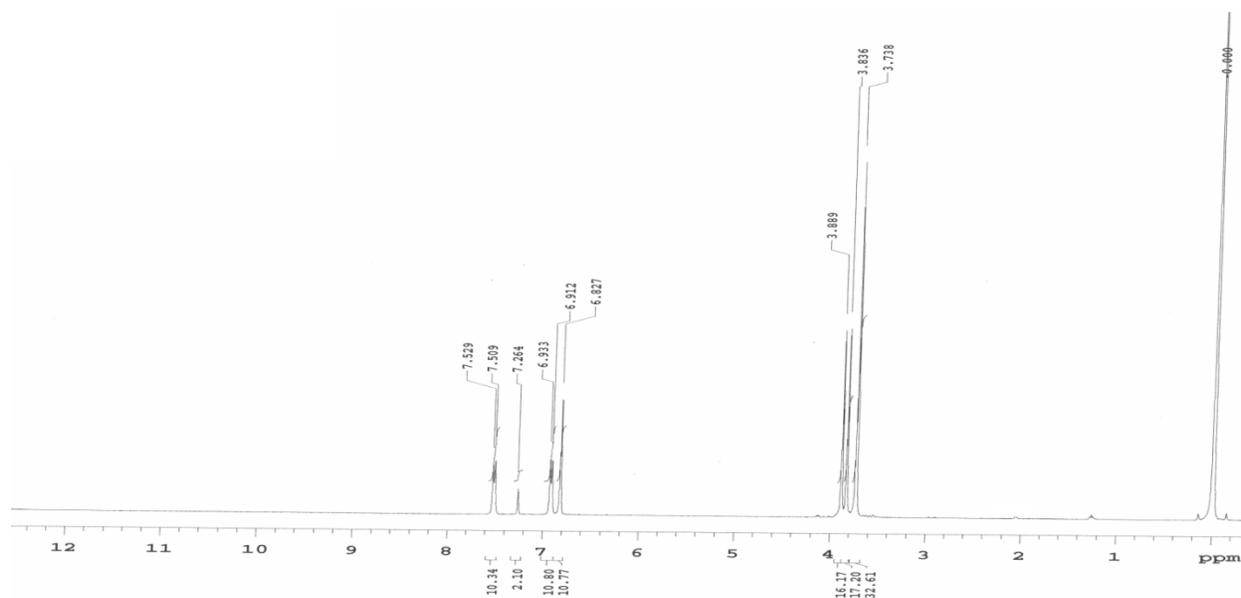
Measured Mass

407.9342

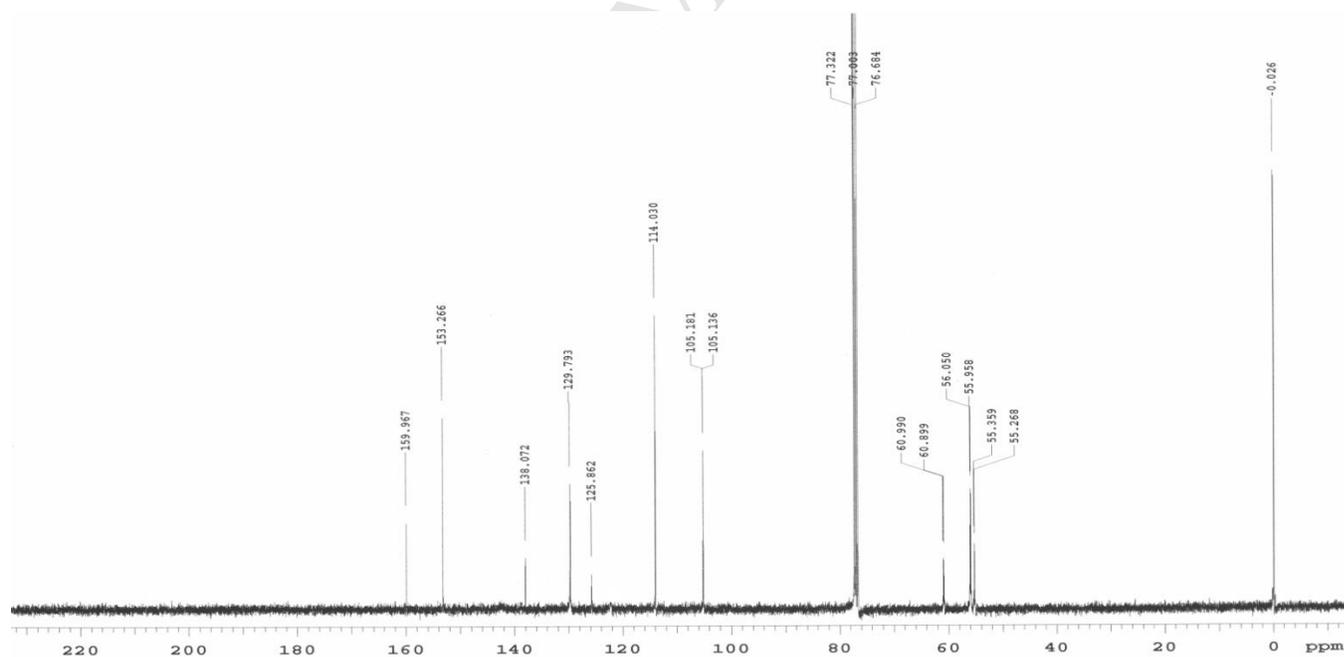
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
Br	0	2
C	10	25
H	5	25
N	0	5
O	0	6

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>15</sub> H <sub>12</sub> N <sub>3</sub> O Br <sub>2</sub>	407.9342	0.0	0.1	10.5
C <sub>17</sub> H <sub>14</sub> O <sub>2</sub> Br <sub>2</sub>	407.9355	-1.3	-3.2	10

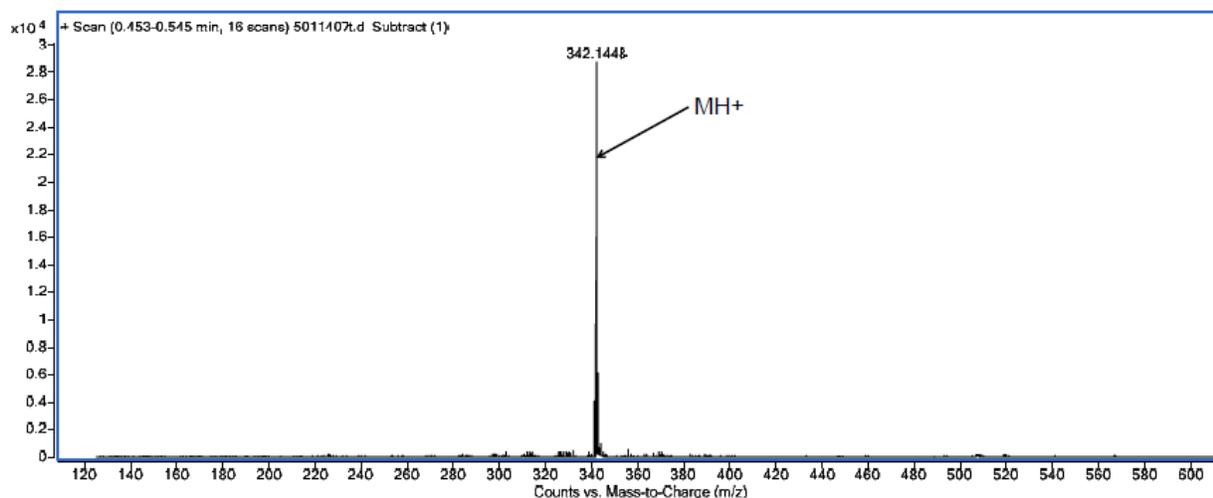
*Representative HRMS spectra of compound 4-(3,5-dibromophenyl)-5-(2-methoxyphenyl)-2H-1,2,3-triazole (2k)*



Representative <sup>1</sup>H NMR spectra of compound 4-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2l)



Representative <sup>13</sup>C NMR spectra of compound 4-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2l)

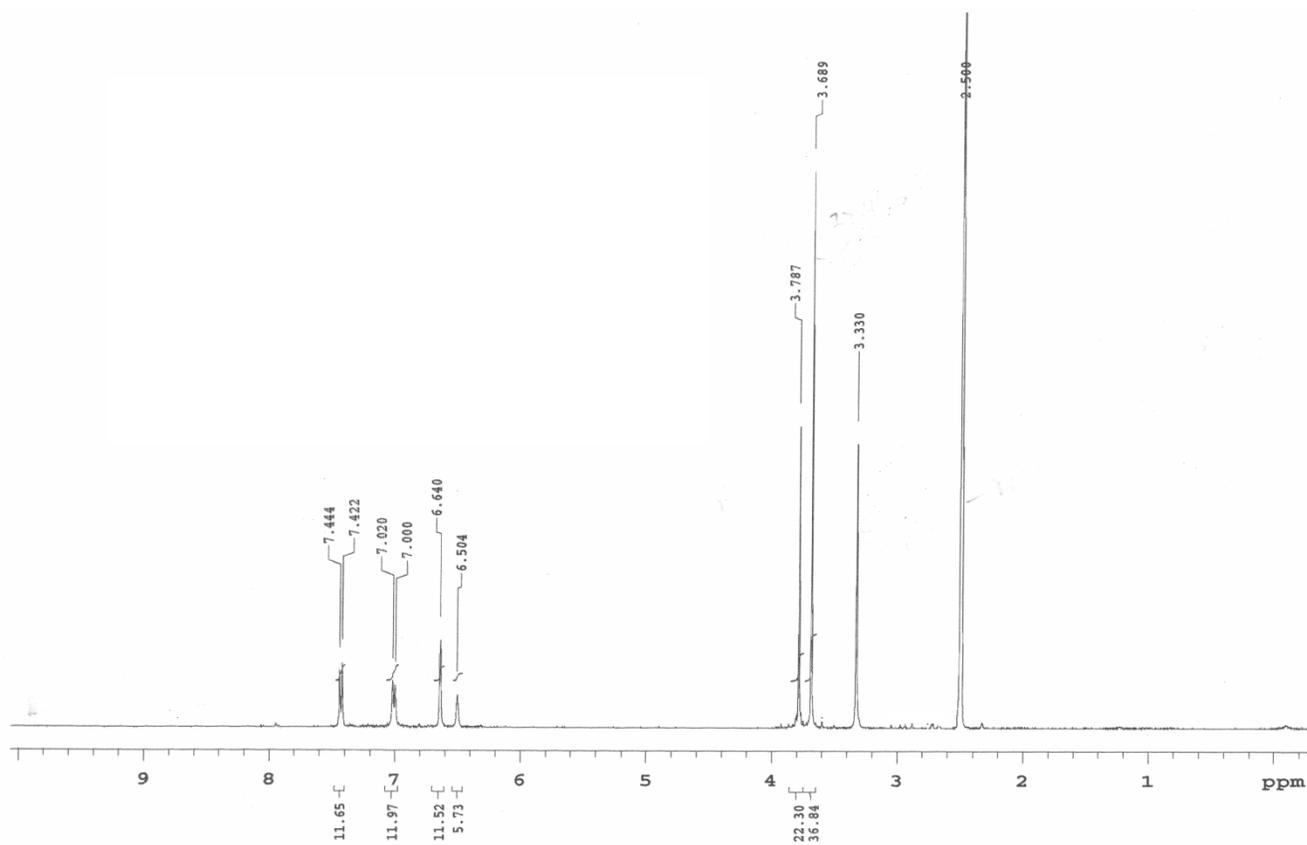
Measured Mass

342.1448

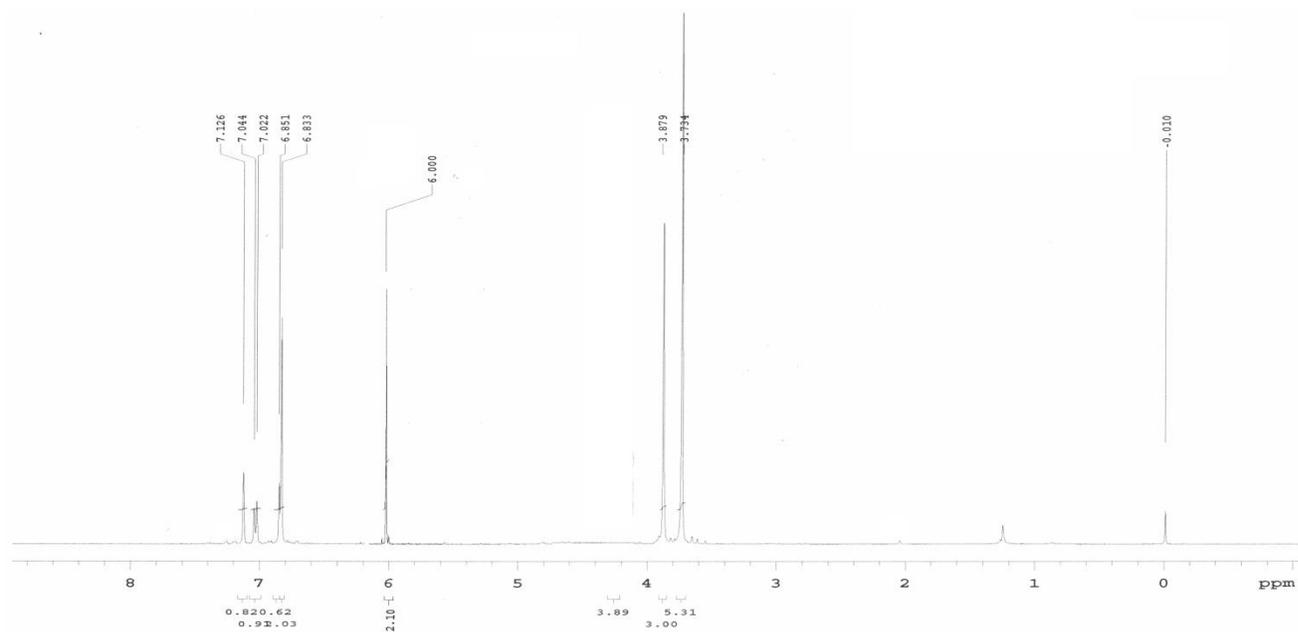
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	10	25
H	5	25
N	0	5
O	0	6

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>18</sub> H <sub>20</sub> N <sub>3</sub> O <sub>4</sub>	342.1448	0.0	-0.1	10.5
C <sub>20</sub> H <sub>22</sub> O <sub>5</sub>	342.1462	-1.4	-4.0	10

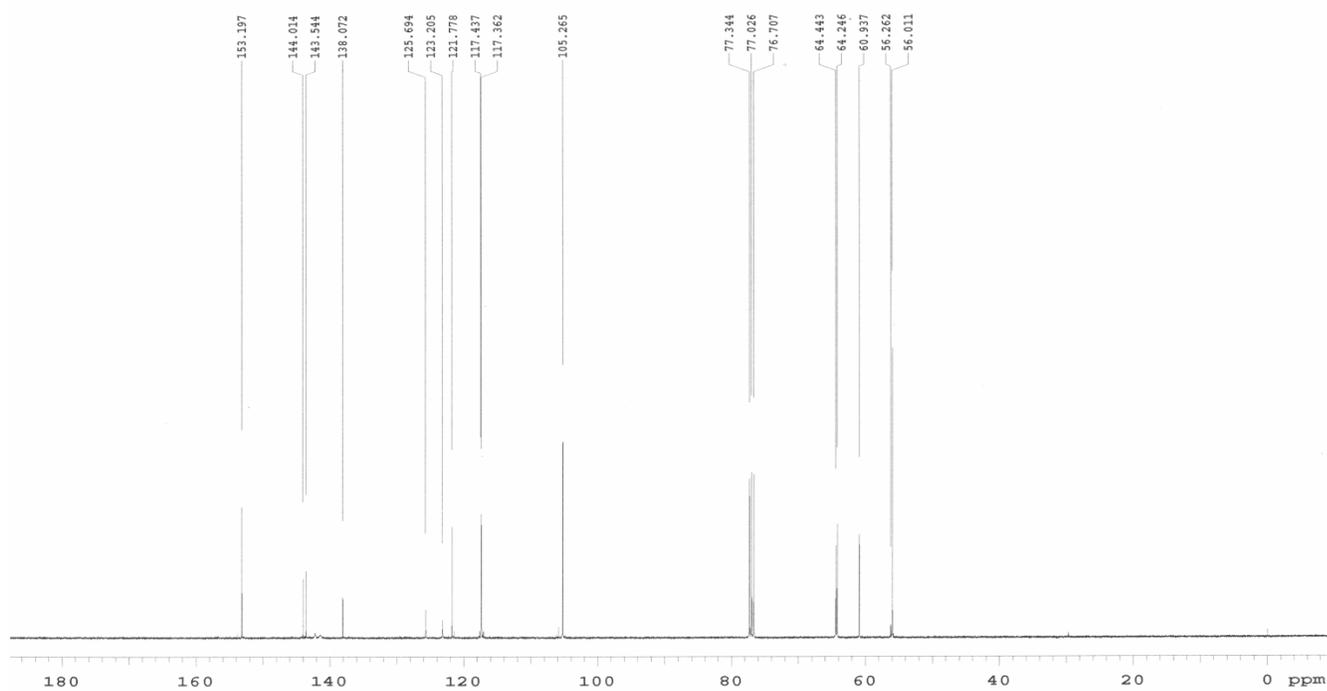
*Representative HRMS spectra of compound 4-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2l)*



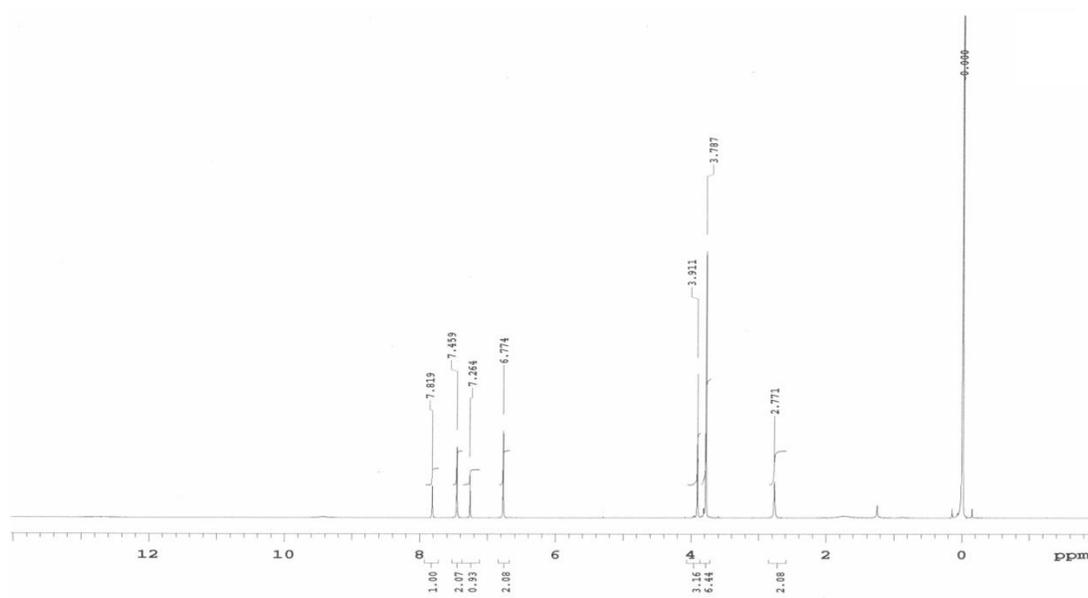
**Representative  $^1\text{H}$  NMR spectra of compound 4-(3,5-dimethoxyphenyl)-5-(4-methoxyphenyl)-2H-1,2,3-triazole (2m):**



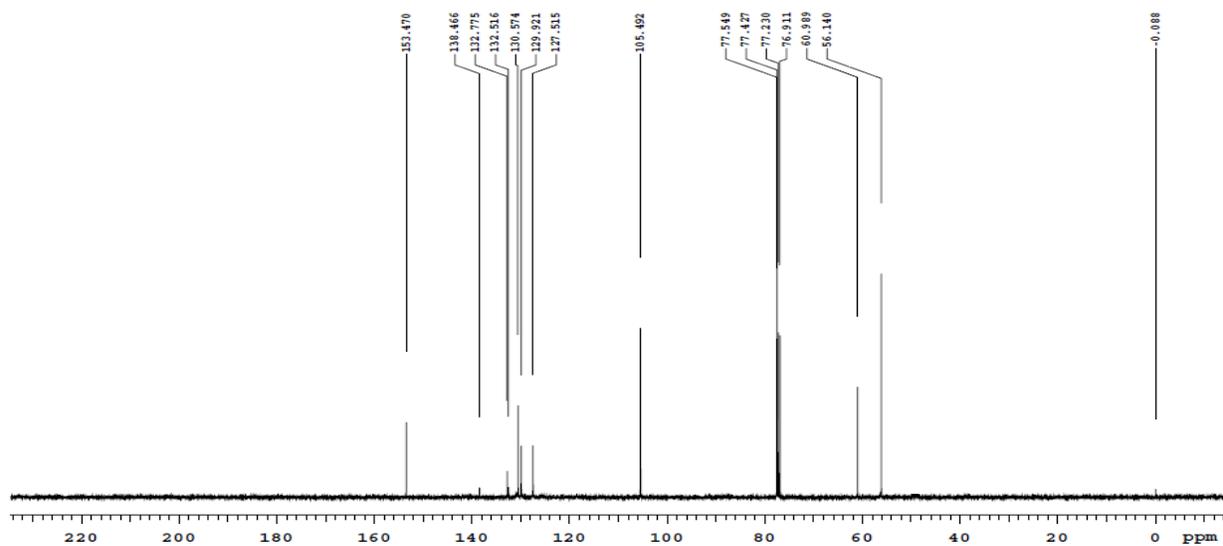
**Representative  $^1\text{H}$  NMR spectra of 4-(benzo[d][1,3]dioxol-5-yl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2n)**



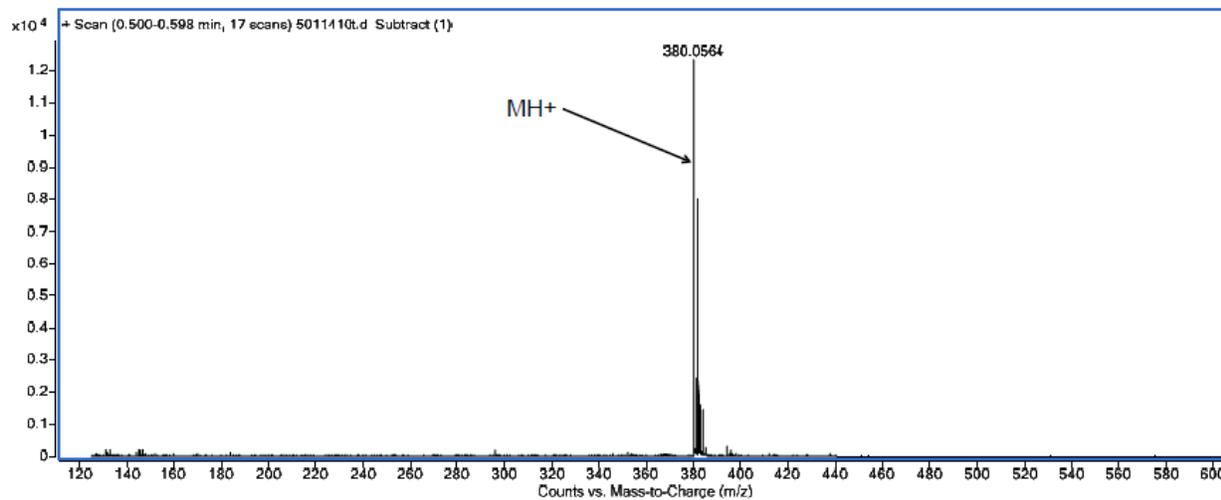
**Representative  $^{13}\text{C}$  NMR spectra of compound 4-(benzo[d][1,3]dioxol-5-yl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2n)**



**Representative <sup>1</sup>H NMR spectra of compound 4-(3,4-dichlorophenyl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2o)**



**Representative <sup>13</sup>C NMR spectra of compound 4-(3,4-dichlorophenyl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2o)**

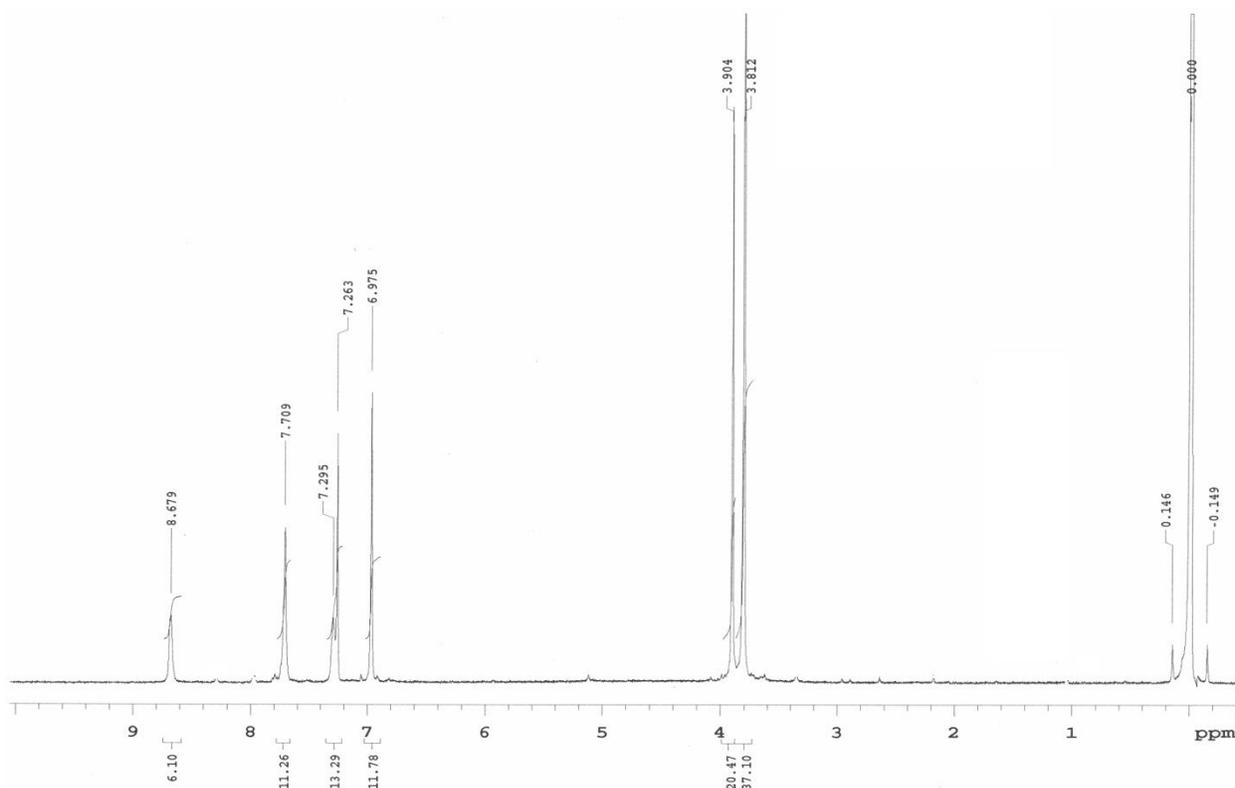


Measured Mass 380.0564

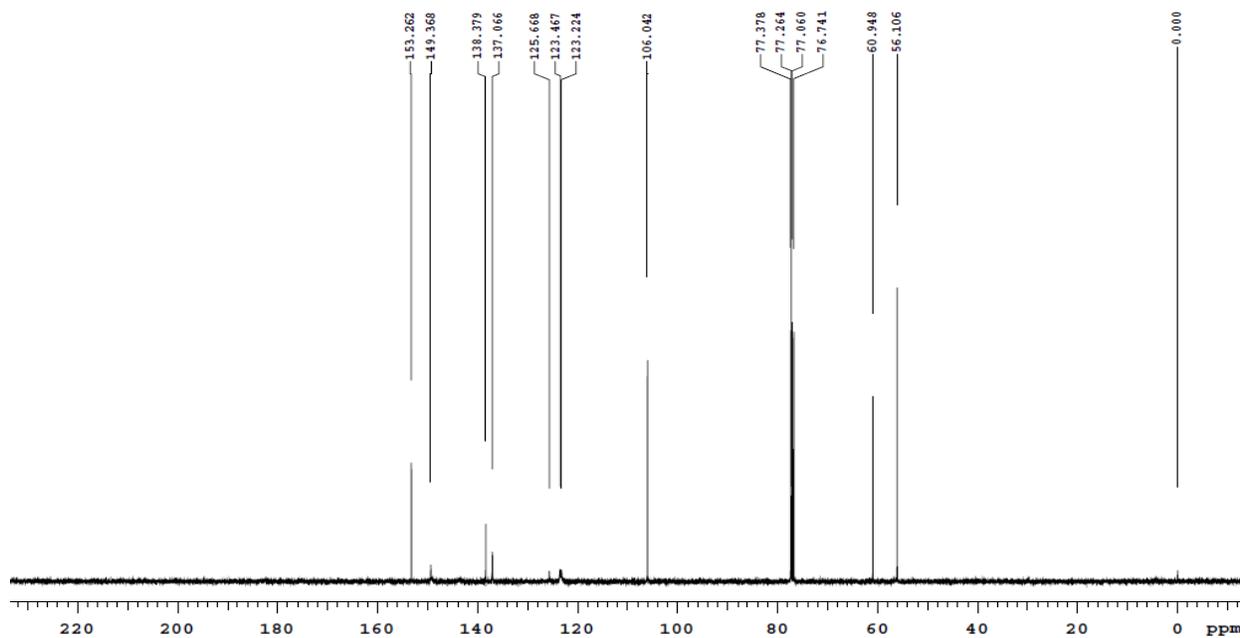
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	10	25
H	5	25
N	0	5
O	0	6
Cl	0	2

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C17 H16 N3 O3 Cl2	380.0563	0.1	0.2	10.5
C24 H6 N5 O	380.0567	-0.3	-0.8	24.5
C20 H13 N2 O4 Cl	380.0558	0.6	1.5	15
C23 H10 N O5	380.0553	1.1	2.8	19.5
C19 H18 O4 Cl2	380.0577	-1.3	-3.3	10

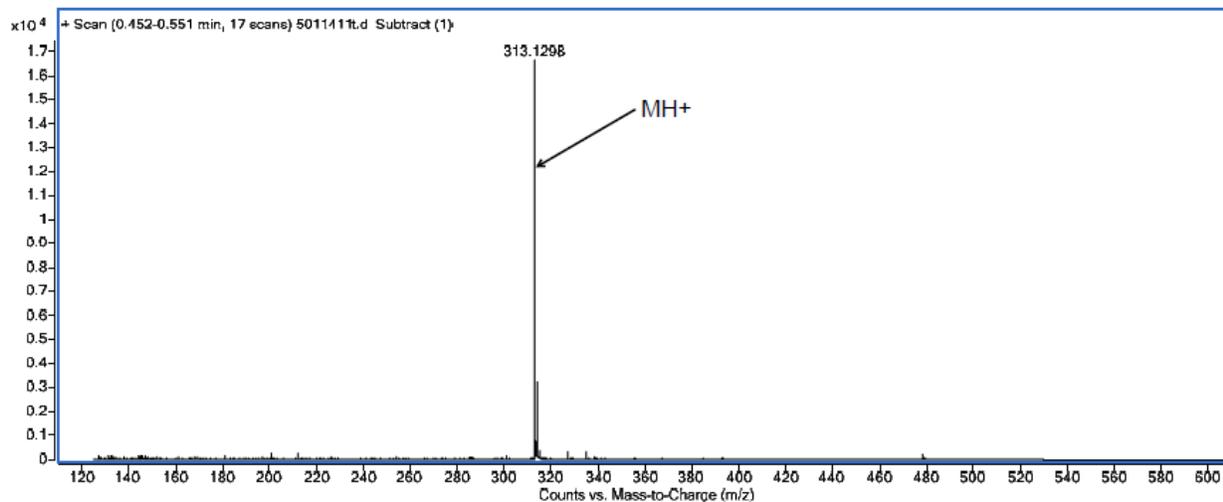
*Representative HRMS spectra of compound 4-(3,4-dichlorophenyl)-5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazole (2o)*



Representative  $^1\text{H}$  NMR spectra of compound 2-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)pyridine (2p)



Representative  $^{13}\text{C}$  NMR spectra of compound 2-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)pyridine (2p)

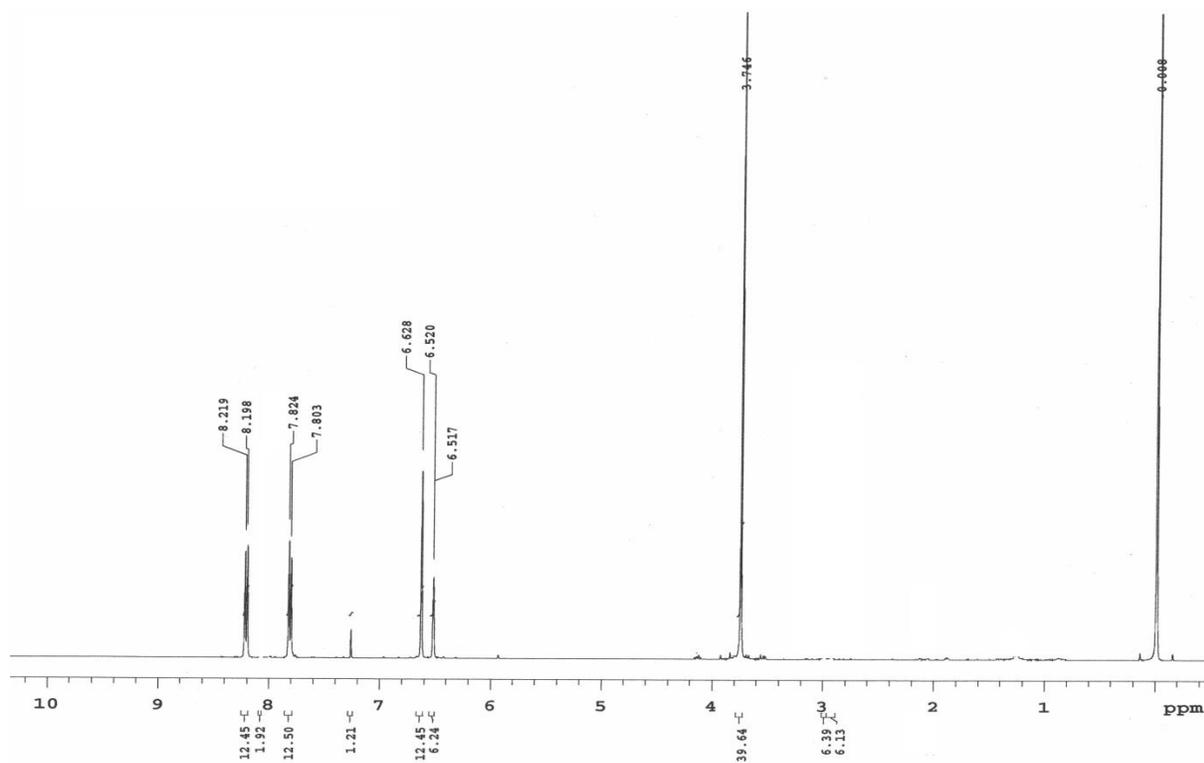
Measured Mass

313.1298

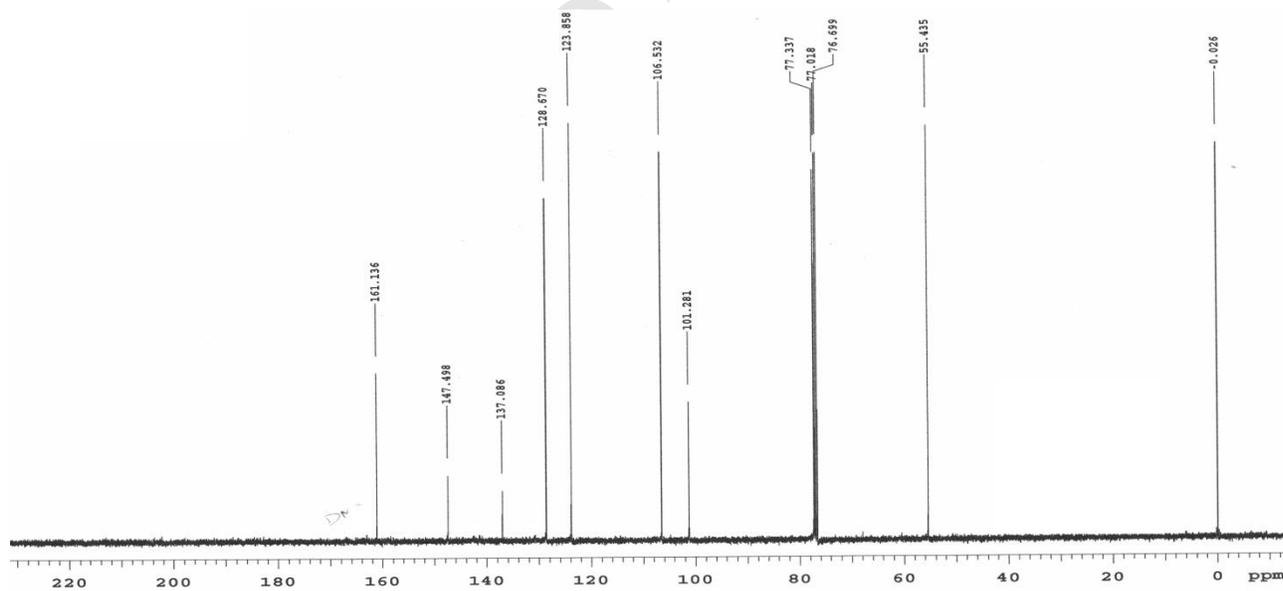
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	10	25
H	5	25
N	0	5
O	0	6

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>16</sub> H <sub>17</sub> N <sub>4</sub> O <sub>3</sub>	313.1295	0.3	0.9	10.5
C <sub>18</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub>	313.1309	-1.1	-3.4	10

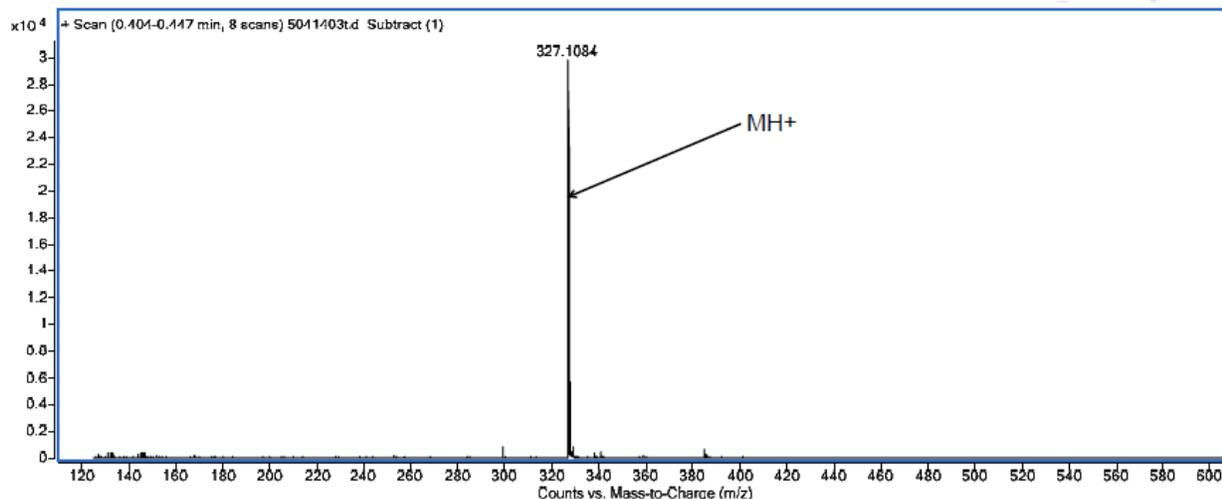
*Representative HRMS spectra of compound 2-(5-(3,4,5-trimethoxyphenyl)-2H-1,2,3-triazol-4-yl)pyridine (2p)*



Representative <sup>1</sup>H NMR spectra of compound 4-(3,5-dimethoxyphenyl)-5-(4-nitrophenyl)-2H-1,2,3-triazole (2q)



Representative <sup>13</sup>C NMR spectra of compound 4-(3,5-dimethoxyphenyl)-5-(4-nitrophenyl)-2H-1,2,3-triazole (2q)

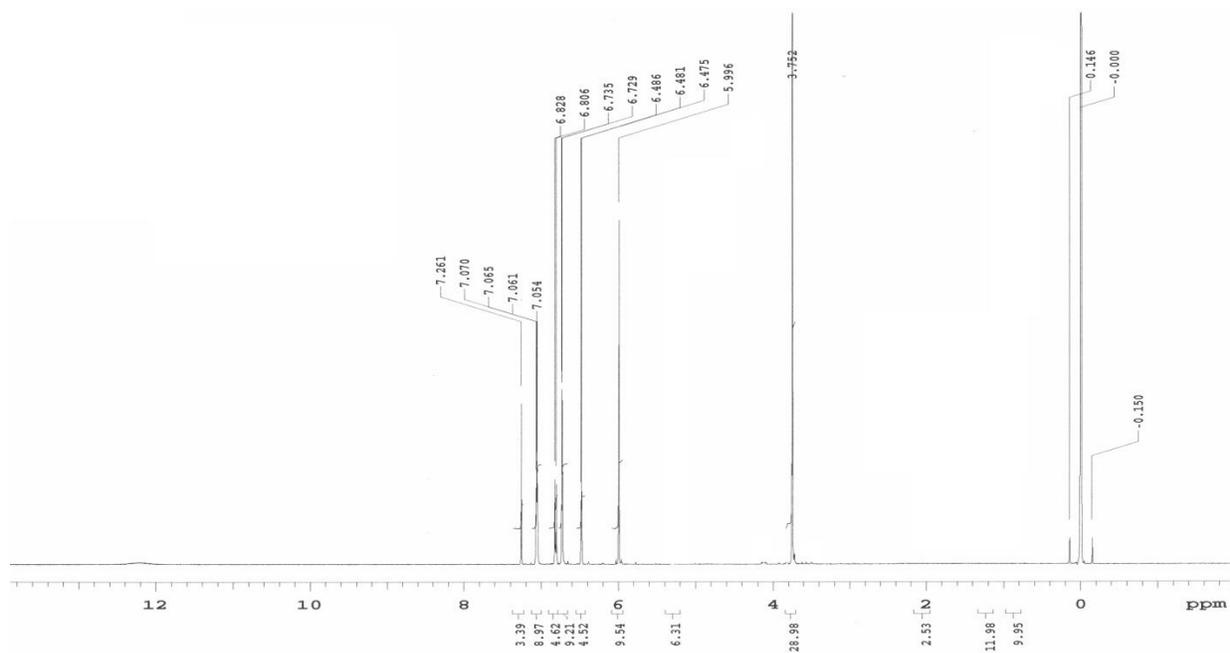
Measured Mass

327.1084

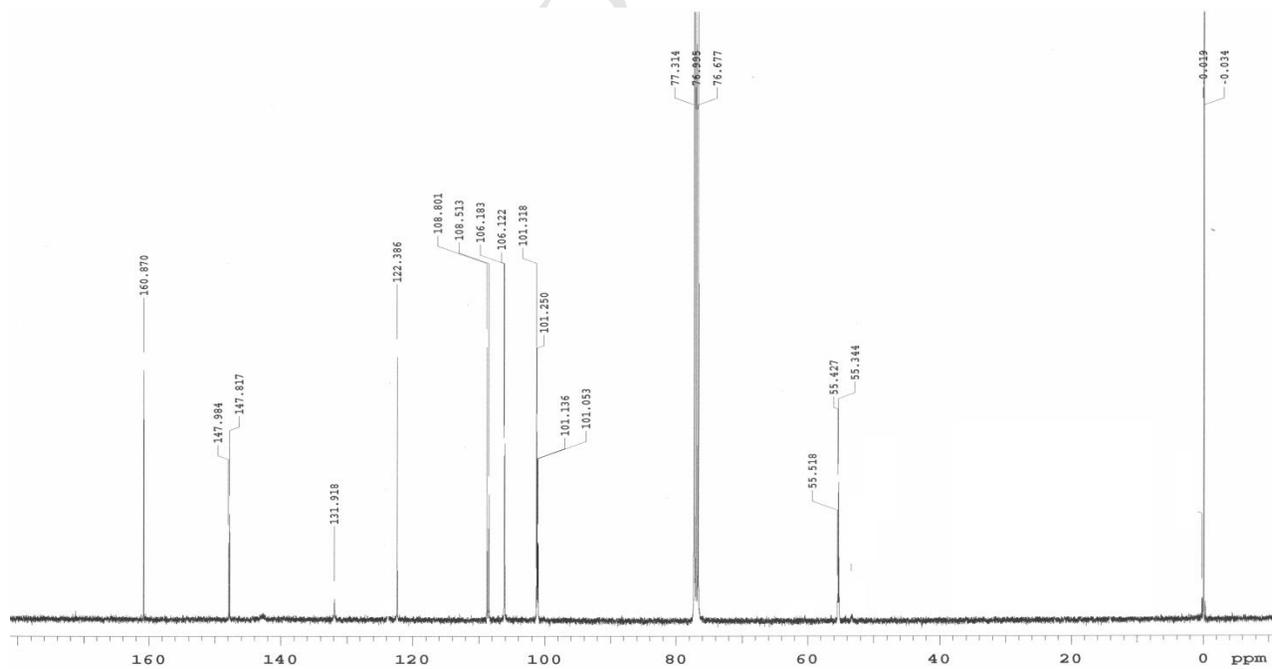
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	10	25
H	5	25
N	0	5
O	0	6

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>16</sub> H <sub>15</sub> N <sub>4</sub> O <sub>4</sub>	327.1088	-0.4	-1.2	11.5

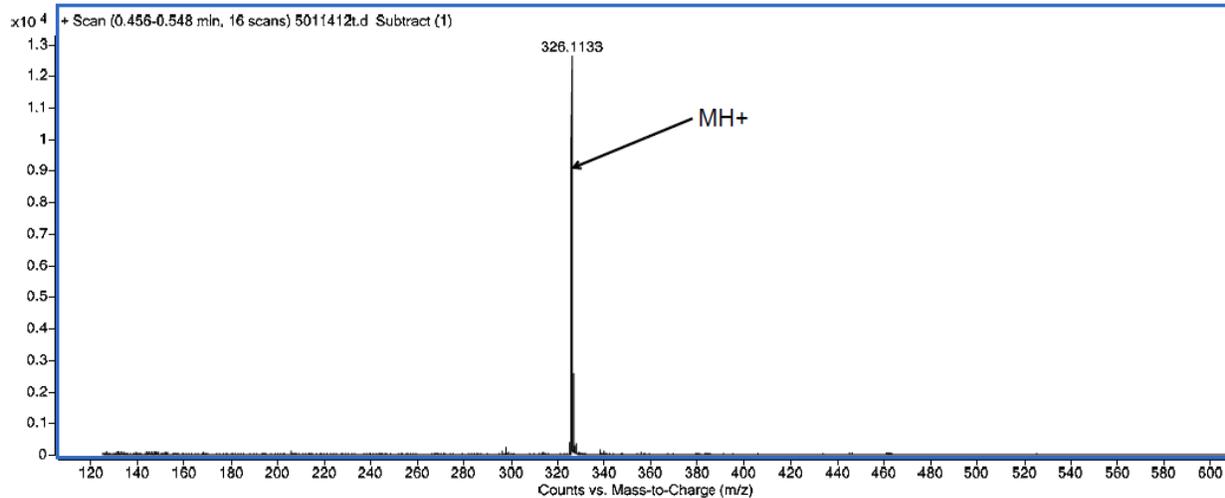
*Representative HRMS spectra of compound 4-(3,5-dimethoxyphenyl)-5-(4-nitrophenyl)-2H-1,2,3-triazole (2q)*



Representative  $^1\text{H}$  NMR spectra of compound 4-(benzo[d][1,3]dioxol-5-yl)-5-(3,5-dimethoxyphenyl)-2H-1,2,3-triazole (2r)



Representative  $^{13}\text{C}$  NMR spectra of compound 4-(benzo[d][1,3]dioxol-5-yl)-5-(3,5-dimethoxyphenyl)-2H-1,2,3-triazole (2r)

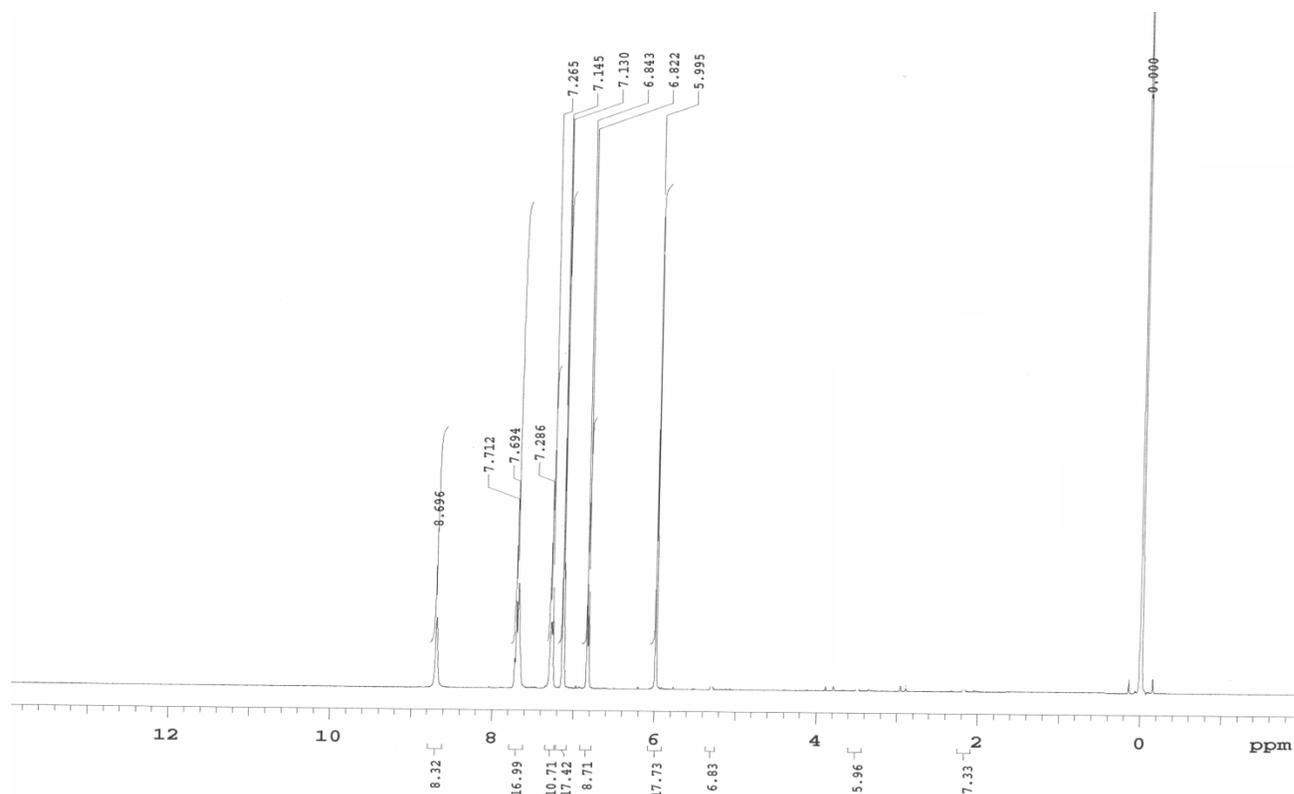
Measured Mass

326.1133

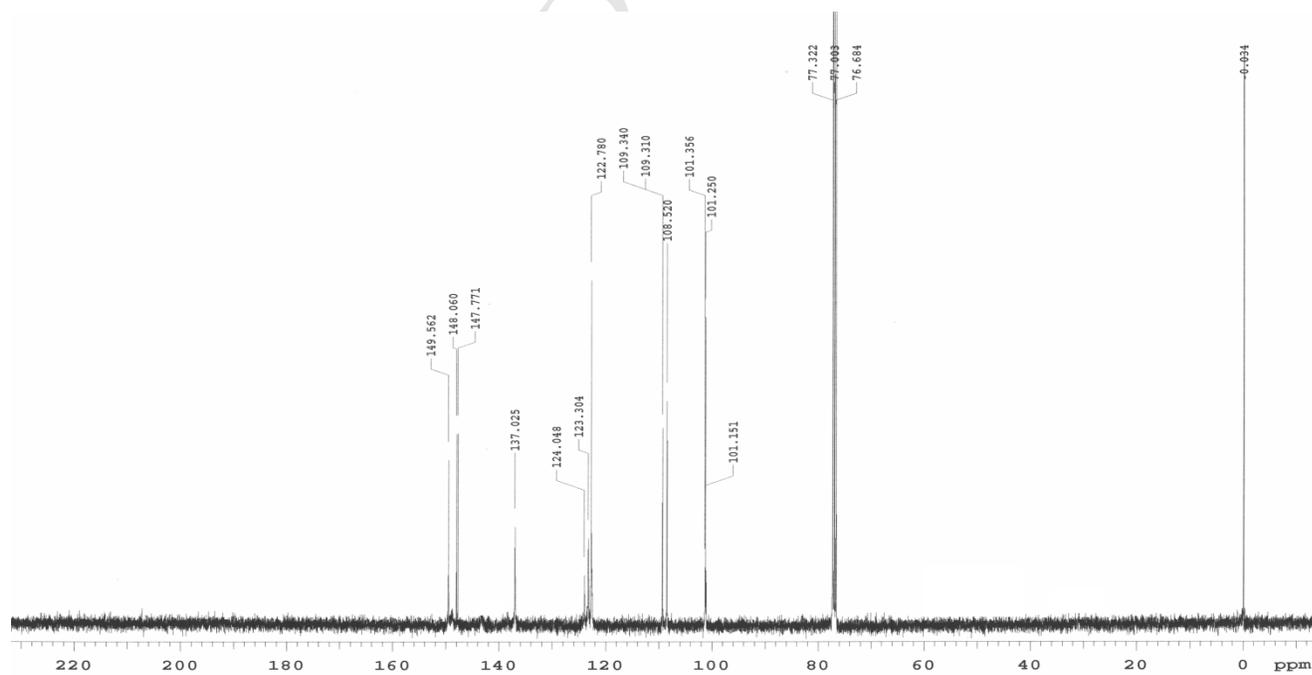
<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	10	25
H	5	25
N	0	5
O	0	6

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>17</sub> H <sub>16</sub> N <sub>3</sub> O <sub>4</sub>	326.1135	-0.2	-0.7	11.5
C <sub>19</sub> H <sub>18</sub> O <sub>5</sub>	326.1149	-1.6	-4.8	11

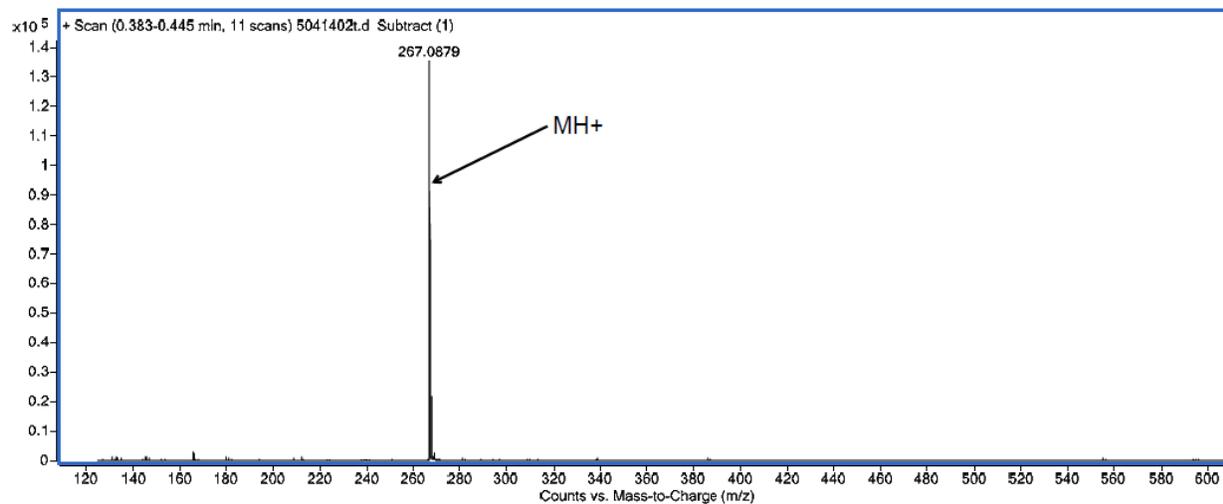
*Representative HRMS spectra of compound 4-(benzo[d][1,3]dioxol-5-yl)-5-(3,5-dimethoxyphenyl)-2H-1,2,3-triazole (2r)*



Representative  $^1\text{H}$  NMR spectra of compound 2-(5-(benzo[d][1,3]dioxol-5-yl)-2H-1,2,3-triazol-4-yl)pyridine (2s)



Representative  $^{13}\text{C}$  NMR spectra of compound 2-(5-(benzo[d][1,3]dioxol-5-yl)-2H-1,2,3-triazol-4-yl)pyridine (2s)

Measured Mass

267.0879

<u>Element</u>	<u>Low Limit</u>	<u>High Limit</u>
C	10	25
H	5	25
N	0	5
O	0	6

<u>Formula</u>	<u>Calculated Mass</u>	<u>mDaError</u>	<u>ppmError</u>	<u>RDB</u>
C <sub>14</sub> H <sub>11</sub> N <sub>4</sub> O <sub>2</sub>	267.0877	0.2	0.9	11.5
C <sub>16</sub> H <sub>13</sub> N O <sub>3</sub>	267.0890	-1.1	-4.1	11

*Representative HRMS spectra of compound 2-(5-(benzo[d][1,3]dioxol-5-yl)-2H-1,2,3-triazol-4-yl)pyridine (2s)*