J. Braz. Chem. Soc., Vol. 22, No. 7, 1379-1388, 2011.
Printed in Brazil - ©2011 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Short Report

Synthesis of Dihydropyrimidin-2-one/thione Library and Cytotoxic Activity against the Human U138-MG and Rat C6 Glioma Cell Lines

Rômulo F. S. Canto,[a] Andressa Bernardi,[b] Ana Maria O. Battastini,[b] Dennis Russowsky*,[c] and Vera Lucia Eifler-Lima*,[a]

[a]Laboratório de Síntese Orgânica Medicinal/LaSOM, Programa de Pós Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, 90610-00 Porto Alegre-RS, Brazil

[b]Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2660, 90035-003 Porto Alegre-RS, Brazil

[c]Instituto de Química, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, 91501-970 Porto Alegre-RS, Brazil

Duas séries de 4-aril-3,4-diidropirimidin-2(1*H*)-(tio)onas incluindo monastrol (**1a**), foram sintetizadas por uma metodologia não agressiva ao meio ambiente baseada no uso combinado de ácido cítrico ou ácido oxálico na presença de TEOF (*orto*formato de trietila). A atividade como inibidores da proliferação celular da quimioteca de compostos foi avaliada em duas linhagens de gliomas (U138-MG-humana e C6-rato). Os compostos derivados da tiouréia **1f** e **1d** mostraram atividade citotóxica maior do que a do monastrol. O composto derivado da uréia **2d** apresentou a maior atividade citotóxica dentre todos os compostos analisados.

Two series of 4-aryl-3,4-dihydropyrimidin-2(1*H*)-(thio)ones including monastrol (**1a**), have been synthesized by an environment-friendly methodology based on the combined use of citric acid or oxalic acid and TEOF (triethyl*ortho*formate). The library was evaluated as inhibitor of cell proliferation on two glioma cell lines (human-U138-MG and Rat-C6). The compounds derived from thiourea **1f** and **1d** were more cytotoxic than monastrol. The compound derived from urea **2d** showed the highest cytotoxic activity among the analyzed compounds.

Keywords: dihydropyrimidin-2(1*H*)-ones, Biginelli reaction, triethylorthoformate, TEOF, monastrol, cancer, glioma

Introduction

The 4-aryl-3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) are a class of compounds that has a huge interest in the medicinal chemistry community in recent years.

Using high throughput screening (HTS) Mayer *et al.*[1] have evaluated a library of 16,330 small molecules that vary in functional groups and charge. These findings have lead to discover a small molecule which they named monastrol, whose acts by inhibiting the motility of the mitotic kinesin Eg5, a motor protein required for spindle bipolarity. This revealed the potential of this compound as antitumor prototype and since that, some SAR (structure activity relationship) studies concerning this interaction have been performed.[2]

Moreover, this class of heterocycles revealed other pharmacological activities such as anti-inflammatory,[3] calcium channel modulators,[4] antifungal and antibacterial,[5] melanin concentrating hormone receptor (MCH1-R) antagonists,[6] chemical modulators of heat shock protein 70 (Hsp 70),[7] hepatitis B replication inhibitors,[8] and inhibitors of the fatty acid transporters.[9] This set of potentialities linked to the possibility of chemical modulation in all positions of the dihydropirimidinone/thione rings make DHPMs a privileged structure, justifying the great interest in their synthesis.

The original three component reaction of DHPMs consisted of a simple one-pot condensation of benzaldehyde **4b**, ethyl acetoacetate **5** and urea **6b** (Schemes 1 and 2), catalyzed by few drops of hydrochloric acid under refluxing

*e-mail: dennis@iq.ufrgs.br, veraeifler@ufrgs.br

ethanol.[10] However, these original Biginelli conditions suffer of poor yields despite the long reaction times and harsh conditions, and cannot also be applied when acid sensitive reactants are required. It can be found in the literature several reports of catalytic methods to improve the yields and scope of this reaction. The majority use Brønsted[11] or Lewis acid catalysis,[12] with methods based on metal salts with non-nucleophilic anions having pronounced catalytic activity.

The most effective ones involve reagents which have dehydrating properties in conjunction with protic or Lewis acidic behavior, for instance: ethyl polyphosphate,[13] TMSCl,[14] propane phosphonic acid anhydride[15] and TMSCl/NaI.[16] However, some of these methods with expensive catalysts have been reported with unsatisfactory yields. Besides, modern techniques employing microwave irradiation,[17] solid-phase organic synthesis,[18] ionic liquids,[19] and solvent-free organic reactions with different acids[20] have recently been reported to promote the DHPM ring formation.

In this context, we have reported the synthesis of a small library of DHPM, including monastrol (**1a**), using a Lewis acid as catalyst and this series was evaluated against seven human cancer cell lines.[21] The results showed that the oxo-monastrol analogue (**2a**) just shows cytostatic activity, while monastrol (**1a**) was cytotoxic against the seven cancer cell lines. We have also found that the 3,4-methylenodioxy derivative (piperastrol, **1c**) was approximately 30 times more potent than monastrol (**1a**) against five of the seven tested cancer cell lines, and it was also more potent than the positive control doxorrubicine against three of the tested cell lines (Figure 1).

These results have encouraged us to carry out new studies in order to investigate the *in vitro* antiproliferative activity of a DHPM library against the glioma cell lines. Gliomas are the main primary central nervous system (CNS) tumors in humans, accounting for almost 80% of brain malignances.[22] Glioblastoma multiforme (GBM), classified with a grade 4, represents the most aggressive of these tumors. Despite considerable progress in research regarding the molecular aspects of malignant gliomas, the prognosis of these tumors continues to be dismal.[23]

Multimodal combinations of target agents with radiation and chemotherapy may enhance treatment efficacy,[24] but despite these treatments, gliomas recur early due to their high proliferation, infiltrative and invasive behaviours.[25] Recently, Muller *et al.*[26] have observed that monastrol (**1a**), enastron (**3a**) and dimethylenastron (**3b**) exhibited antiproliferative activity against human glioblastoma cells (Figure 1). Their investigations revealed that these mitotic kinesin Eg5 inhibitors did not show cytotoxic effect on resting cells, in contrast with the antimicrotubular agent paclitaxel. This is an important result because it shows that these compounds should present less neurotoxic activity than the classical tubulin inhibitors.

The aim of the current study was to develop two series (oxo-serie and thio-serie) of DHPMs analogues employing an economical and environment-friendly methodology, in a rapid and efficient approach, to evaluate their cytotoxic effects against the human glioma cell line U138-MG and the rat glioma cell line C6. This work, to the best of our knowledge, is the first report on the study which the monocyclic analogues of monastrol with different pattern of substitution on aromatic ring are evaluated against glioma cells that justify our wish to report our results.

Results and Discussion

Chemistry

In connection with our early investigations regarding the antiproliferative activities of the DHPMs, we developed an alternative method to prepare this heterocyclic compounds in absence of metal salts as catalysts, due to the possible risk of trace contamination for biological assays. Taking in account that the cyclocondensations of an aldehyde (**4**), ethyl acetoacetate (**5**) and urea (**6b**) in the acid catalyzed Biginelli reaction produce 2 equivalents of water *per* equivalent of DHPM formed,[27] we envisaged that the use of a dehydrating agent could accelerate the reaction. The literature reports the use of dehydrating agents, however in the presence of metals, such as $FeCl_3$.[28] The orthoesters have been described as mild, non-toxic, non pollutant,

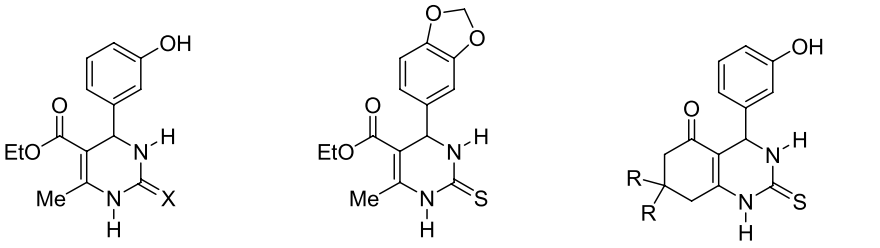
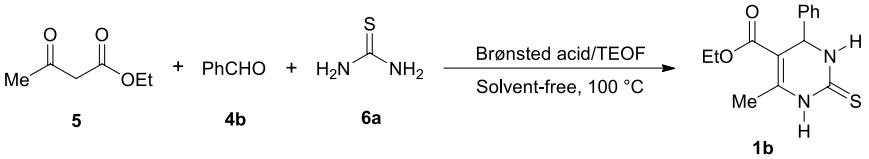


Figure 1. Chemical structures of some bioactive dihydropyrimidinones.



Scheme 1. Biginelli reaction catalyzed by Brønsted acid/TEOF system.

inexpensive, and effective dehydrating agents,[29] therefore, we considered the use of the triethylorthoformate (TEOF) as an adjuvant reagent in the dehydration steps during the DHPM synthesis.

First, we investigate the reaction between benzaldehyde (**4b**), ethyl acetoacetate (**5**), and thiourea (**6a**) in the presence of citric acid and oxalic acid to compare their reactivity with the classical method employing HCl as catalyst (Scheme 1).

In our hands, the use of 10 mol% of HCl as catalyst at 100 °C during 2 h, afforded the DHPM **1b** in 74% yield (Table 1, entry 1). To modulate the efficacy of citric acid, the reactions at three different temperatures were investigated. None product was produced at room temperature, even in 48 h (Entry 2), while the yield gives rise with the increase of temperature, from 50 °C and 100 °C, respectively during 2 h (entries 3 and 4, respectively). The use of oxalic acid in the same conditions, afforded the product **1b** in 65% yield (entry 5).

Table 1. Optimization of reaction conditions for synthesis of **1b**

Entry	Acid	TEOF[a]	time / h	T / (°C)	Yield / (%)
1	HCl	-	2	100	74
2	citric ac.	-	48	r.t	-
3	citric ac.	-	12	50	16
4	citric ac.	-	2	100	34
5	oxalic ac.	-	2	100	65
6	-	1	6	100	-
7	-	2	6	100	-
8	HCl	2	2	100	84
9	citric ac.	2	48	r.t.	-
10	citric ac.	1	2	100	81
11	citric ac.	2	2	100	92
12	oxalic ac.	2	2	100	93

[a]Number of equiv. of TEOF.

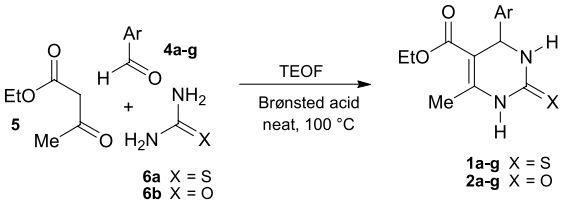
A second set of investigations was dedicated to evaluate the influence of TEOF in this reaction. Reactions performed in presence of 1 or 2 equiv. of TEOF for 6 h and in absence of Bronsted acids did not afford none product, evidencing the necessity of employment of Bronsted acid to activate the dehydrating form of TEOF (entries 6 and 7, respectively).[30] On the other hand, the reaction carried out in presence of HCl/TEOF during 2 h, afforded the desired DHPM **1b** in 84% yield (entry 8). We can compare this result with that in absence of TEOF (74%, cf. entry 1) to confirm the influence of TEOF. Although the use of citric acid/TEOF system at room temperature and 48 h does not has been effective to afford **1b**, the reactions which were performed in presence of 1 or 2 equiv. of TEOF at 100 °C during 2 h lead to the production of the desired product in 81% yield and 92% yield, respectively (entries 10 and 11, respectively). Similar result was observed for the oxalic acid/TEOF system (93%, entry 12). The effect of TEOF seems to be more evident with weak Bronsted acids as citric acid or oxalic acids related to the strong mineral acid HCl.

The comparison of these results with those obtained in absence of TEOF (entries 4 and 5) disclose the rule that the dehydrating agent plays in the efficiency of the process. We believe that TEOF is important for the loosing of water in the intermediate formation at weak acidic pH conditions.[31] The increase of the yields with 2 equiv. of TEOF can indicate their association with the loosing of a second equiv. of water in the last step.[27] While this work was running, the use of pure citric acid[32] and oxalic acid[33] as promoters of the Biginelli reaction was published. However, in our hands, the reported results were not reproducible. In any way, we demonstrate the importance of TEOF as dehydrating agent.

We extend the best condition highlighted above to the reactions of ethyl acetoacetate (**5**) with a series of aromatic aldehydes **4a**-**g** and thiourea (**6a**) or urea (**6b**) to afford the respective DHPMs **1a**-**g** and **2a**-**g** (Scheme 2). The results are presented in Table 2.

The ^{1}H NMR, ^{13}C NMR and IR spectroscopic data of compounds **1a**-**g** and **2a**-**g** were compatible with the proposed structures as well as the melting points were in accordance with those reported in the literature.

According to the Table 2, all aromatic aldehydes **4a-g** readily undergo reaction, with both urea (**6b**) and thiourea (**6a**) giving good-to-high yields of the corresponding DHPMs **1/2a-g** with times varying from 1 to 2 h.



Scheme 2. Synthesis of dihydropyrimidinones **1a**-**g** and **2a**-**g**.

Table 2. Synthesis of dihydropyrimidinones via Scheme 2

Entry	Ar	X	Product	Yield (%) / time (h)	
				Citric acid	Oxalic acid
1	3-HO-C_6H_4	S	**1a**	88 / 1.0	69 / 2.0
2	C_6H_5	S	**1b**	92 / 2.0	93 / 2.0
3	3,4(-OCH_2O-)-C_6H_3	S	**1c**	84 / 2.0	73 / 1.0
4	3-O_2N-C_6H_4	S	**1d**	97 / 2.0	82 / 2.0
5	4-NC-C_6H_4	S	**1e**	80 / 2.0	78 / 2.0
6	4-$(Me)_2N$-C_6H_4	S	**1f**	82 / 1.5	68 / 2.0
7	2-F-C_6H_4	S	**1g**	77 / 2.0	75 / 2.0
8	3-HO-C_6H_4	O	**2a**	87 / 1.0	77 / 1.0
9	C_6H_5	O	**2b**	93 / 1.0	67 / 1.0
10	3,4(-OCH_2O-)-C_6H_3	O	**2c**	81 / 1.0	84 / 2.0
11	3-O_2N-C_6H_4	O	**2d**	92 / 1.5	89 / 1.5
12	4-NC-C_6H_4	O	**2e**	86 / 1.0	68 / 1.0
13	4-$(Me)_2N$-C_6H_4	O	**2f**	75 / 1.5	66 / 1.0
14	2-F-C_6H_4	O	**2g**	74 / 2.0	78 / 2.0

Biology

To investigate the antiproliferative potential of monastrol (**1a**), oxo-monastrol (**2a**), sulfur (**1b**-**g**), and oxo (**2b**-**g**) analogues, initially we used the cell counting assay in glioma cell lines. Thus, the glioma cells were treated with several concentrations of monastrol (8.5-685 μmol L^{-1}) for 24, 48 or 72 h.

The time-course experiments revealed a significant decrease in the cell number of C6 and U138-MG treated with monastrol, which shows also concentration-dependent effect (data not shown). Thus, the cell cultures were treated with all compounds at 150 μmol L^{-1} for 48 h and to compare the effect with monastrol.

The results of the inhibitory effect of all compounds against the growth of both C6 and U138-MG glioma cell proliferation are presented in Figure 2. Figure 2 shows that all tested compounds were active in the described panels, including monastrol (**1a**), although their activities on U138-MG cells have been more pronounced. For both

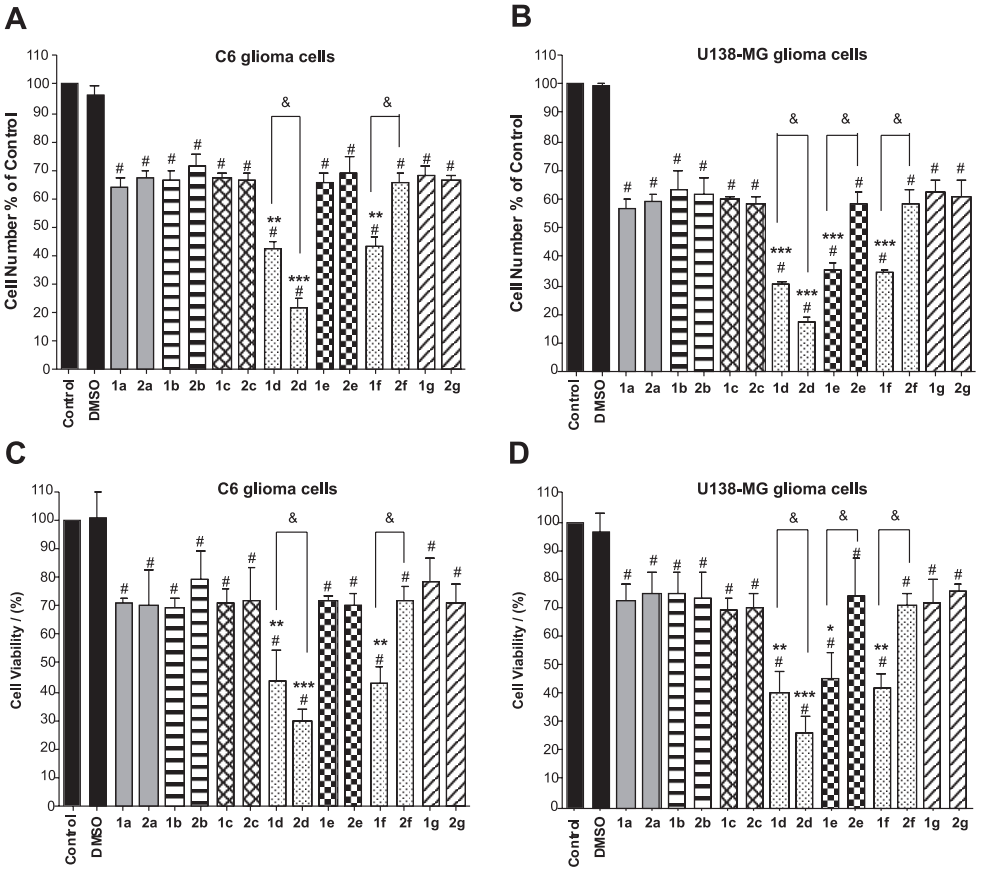


Figure 2. Effect of analogues of monastrol on cell proliferation (A and B) and on cell viability (C and D) of glioma cell lines. Semi-confluent cultures of C6 and U138-MG glioma cells were treated with 150 μmol L^{-1} of oxo or sulfur analogues of monastrol for 48 h. The cell number and the cell viability were represented in relation to the respective controls. The values are represented as means ± S.D. of three independent experiments made in duplicate. Data are analyzed by ANOVA followed by *post-hoc* comparisons (Tukey test). #Significantly different from the control and DMSO groups ($P < 0.01$); *Significantly different from the monastrol (**1a**) group ($P < 0.05$); **Significantly different from the monastrol (**1a**) group ($P < 0.01$); ***Significantly different from the monastrol (**1a**) group ($P < 0.001$); &significantly different between the two groups ($P < 0.01$).

cell lines cultures, the compounds **1d**, **1f** and **2d** displayed high inhibition of the cell proliferation, being the compound **2d** most effective (Figures 2A and 2B). Monastrol (**1a**) presents an effect in a range of 35-44% of inhibition for both glioma cell lines, while compound **1e** showed higher antiproliferative effect against the human U138-MG cells than the C6 glioma cells (34.3% and 64.9%, respectively).

The cytotoxic effect of monastrol **1a** and its analogues were confirmed by MTT assay where, in agreement to cell counting results, the same compounds exhibited higher cytotoxicity in comparison to monastrol (Figure 2C and 2D).

Even if the structure-activity relationship can not be formulate because the target is unknown and the chemical variations were performed only in the aromatic ring of the DHPMs, some considerations can be made. The library was designed to cover a wide range of functional groups, leading to different electronic and conformational effects in the compounds. As disclosed in previous studies,[21] the introduction of electron donor methylenedioxy group (**1c**) led to a highest activity against several cancer cell lines as the template monastrol and doxorubicin. In contrast with the present work, the most effective were the compounds **1d** and **2d**, containing electron withdrawing groups at 3-position of aromatic ring. This leads us to think, in a first moment, that these DHPMs act by a different mechanism of action in the glioma cell lines, supported by the fact of these compounds show different activities.

For the thio-series, the removal of the 3-hydroxy group of monastrol (**1a**) to generate **1b** does not affect the activity what indicates that this functional group is not essential for the cytotoxic activity on the gliomas. On the other hand, the introduction of substituents at *meta*- and *para*-positions improves this cytotoxicity. In comparison with monastrol, the replacement of *meta*-OH by a *meta*-NO_2 group (compound **1d**) results in a marked increase of antiproliferative effect in U138-MG glioma cells. This effect is also observed when the *meta*-OH group of **1a** is substituted either by a *para*-$N(CH_3)_2$ group in compound **1f** or by a *para*-CN group in compound **1e**.

The compound 5-ethoxycarbonyl-6-methyl-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-one (**2d**) has two fold higher activity against both the C6 and U138-MG glioma cells compared to its analogue monastrol (**1a**). In the oxo-series, other substitutions such as *meta*-OH, *para*-$N(CH_3)_2$ or *para*-CN, *ortho*-F, 3,4-(-OCH_2O-) or even no substitution at the benzene nucleus results in compounds with similar or lower effect than **1a**. Besides, the oxo-analogue **2d** is more active than its thio counterpart **1d**.

The Figure 3 below, shows representative pictures of U138-MG glioma cells treated with 150 μmol L^{-1} of monastrol (**1a**) and its analogues **1d**, **1e**, **1f** and **2d** for 48 h. Note the decrease of total number of cells with monastrol treatment when compared to DMSO (negative control cultures). As observed in Figure 3, when the cells were treated with compounds **1d**, **1e**, **1f** and **2d**, this reduction is more evident, confirming the results with cell counting assay.

Among the fourteen DHPMs synthesized, the three more active against both glioma cells lines were the compounds **1d** and **1f** from thio-series and the compound **2d** from the oxo-series (Figure 4).

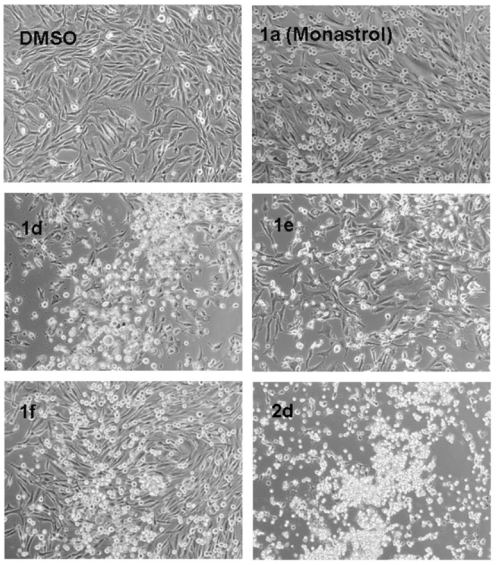


Figure 3. Representative pictures of U138-MG cell cultures treated with monastrol and analogues. After 48 h of treatment with DMSO (non-treated cultures), 150 μmol L^{-1} of monastrol (**1a**) or analogues of monastrol, the cells were visualized using a Nikon inverted microscope. Panels correspond to contrast phase photomicrographs. The pictures are representative of three different experiments.

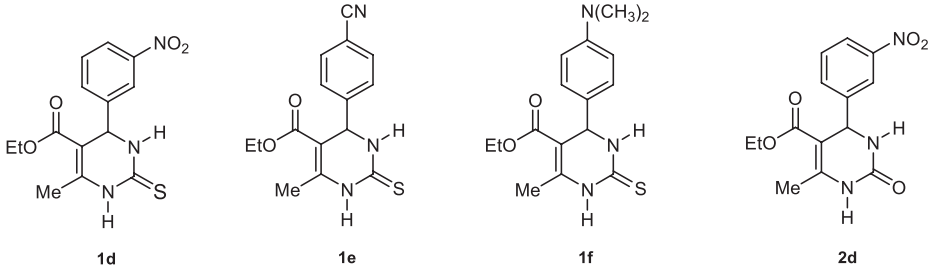


Figure 4. The active DHPMs **1d**, **1e**, **1f** and **2d** against the proliferation of glioma cell lines.

Conclusions

The synthetic studies showed that triethylorthoformate (TEOF), associated with citric acid or oxalic acid, acts as an efficient promoter system of the Biginelli reaction yielding dihydropyrimidinones in good to high yields. The use of TEOF as dehydrating agent enhances drastically the yields obtained when weak acids are employed. This method offers a simple, inexpensive, versatile and environmental-friendly free of metals approach to synthesize a library of 3,4-dihydropyrimidin-2(1*H*)-(thio)ones, addressed to biological assays.

The results of the biological assays on U138-MG glioma cell line and C6 glioma cell line showed that monastrol presents cytotoxicity against both cell lines. In addition, four other analogues (**1f**, **1d**, **1e** and **2d**) presented higher cytotoxic effect on the same cell lines, where compound **2d** was the most effective (two fold higher than monastrol).

The overall profile of **2d** makes it suitable candidate to extend the pharmacological investigations. The anti-tumor effects of the dihydropyrimidinones/thiones analogues of monastrol on U138-MG and C6 gliomas cells have not been reported previously. Other biological and medicinal studies are currently underway in our research group aiming to identify the cytotoxic mechanism of action found here.

Experimental

Chemical analysis

All chemicals are research grade and were used as obtained. Nuclear magnetic resonance spectra (^{1}HNMR and ^{13}C NMR) were recorded in an Varian INOVA-300 spectrometer or Brucker Avance DPX-250 NMR spectrometer with standard pulse sequences operating at 300 MHz or 250 MHz for ^{1}H NMR and 75 MHz or 62.5 MHz for ^{13}C NMR, respectively, using DMSO-*d6* as solvent. Chemical shifts are reported as δ values (ppm) relative to TMS (0.0 ppm). The NMR multiplicities br s, s, d, t, q, and m stand for broad singlet, singlet, doublet, triplet, quartet and multiplet, respectively. FT-IR spectra were recorded in a Perkin Elmer Spectrometer BXII using an ATR probe. TLC analyses were performed on Merck´s silica plates 60 F254. Melting-points (mp) were determined on a System Kofler type WME apparatus and are uncorrected. The term room temperature means 20-30 °C. All products were identified through their spectroscopic data and the melting-points which were confirmed by comparison with those reported in the literature.

*General procedure for the synthesis of compounds **1a**-**g** and **2a**-**g** under citric acid/TEOF system and oxalic acid/TEOF system*

A mixture of ethyl acetoacetate (**5**, 2.5 mmol, 325 mg), aromatic aldehyde (**4a**-**g**, 2.5 mmol), thiourea (**6a**, 5.0 mmol, 360 mg) or urea (**6b**, 5.0 mmol, 300 mg), citric acid (0.25 mmol, 48 mg, 10 mol%) or oxalic acid (0.25 mmol, 22.51 mg, 10 mol%) and triethylorthoformate (5.0 mmol, 741 mg) were placed in a 50 mL round bottom flask and heated under stirring in a pre-heated oil batch (100 °C) for the time indicated in the Table 1. The reaction was stopped by addition of 5 mL of water and the crude mixture was cooled in an ice-batch under vigorous stirrer. The solid formed was filtered, washed with small portions of cold ethanol and then, dried under vacuum to afford the desired product with good purity grade.

The NMR and IR data of compounds **1a**-**g** and **2a**-**g** are presented in the items bellow.

*5-Ethoxycarbonyl-6-methyl-4-(3-hydroxyphenyl)-3,4-dihydropyrimidin-2-(1H)-thione (**1a**)*[35]

mp 184-187 ºC. ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.14 (t, 3H, *J* 7.1 Hz), 2.30 (s, 3H), 4.04 (q, 2H, *J* 7.1 Hz), 5.11 (d, 1H, *J* 3.7 Hz), 6.65-6.69 (m, 3H), 7.10-7.18 (m, 1H), 9.46 (s, 1H, OH), 9.62 (br s, 1H, NH), 10.31 (br s, 1H, NH). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 14.1, 17.2, 53.9, 59.6, 100.8, 113.2, 114.6, 117.0, 129.5, 144.8, 144.9, 157.5, 165.2, 174.2. IR (neat) ν_{max}/cm^{-1}: 3299, 3180, 2984, 1663, 1573, 1474, 1445, 1370, 1282, 1188, 1153, 1113, 1024, 788, 752, 700.

*5-Ethoxycarbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2-(1H)-thione (**1b**)*[34]

mp 204-207 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09 (t, 3H, *J* 7.0 Hz), 2.29 (s, 3H), 4.00 (q, 2H, *J* 7.0 Hz), 5.17 (d, 1H, *J* 3.7 Hz), 7.23-7.37 (m, 5H), 9.65 (d, 1H, *J* 1.8 Hz, NH), 10.34 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.1, 17.2, 54.1, 59.9, 100.7, 126.4, 127.7, 128.6, 143.5, 145.1, 165.1, 174.2. IR (neat) ν_{max}/cm^{-1}: 3324, 3170, 2980, 1666, 1572, 1464, 1370, 1326, 1283, 1192, 1175, 1116, 1028, 1002, 758, 722, 691, 651.

*5-Ethoxycarbonyl-6-methyl-4-(3,4-methylenedioxyphenyl)-3,4-dihydropyrimidin-2-(1H)-thione (**1c**)*[36]

mp 156-159 ºC. ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.11 (t, 3H, *J* 7.0 Hz), 2.28 (s, 3H), 3.99 (q, 2H, *J* 7.0 Hz), 5.08 (d, 1H, *J* 3.8 Hz), 5.99 (s, 2H), 6.64-6.72 (m, 1H), 6.87 (m, 2H), 9.61 (br s, 1H, NH), 10.33 (br s, 1H, NH). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 14.1, 17.2, 53.6, 59.6, 100.6,

101.0, 106.7, 108.1, 119.6, 137.4, 145.0, 146.7, 147.3, 165.0, 173.9. IR (neat) ν_{max}/cm^{-1}: 3312, 3176, 2981, 1662, 1574, 1484, 1445, 1371, 1336, 1266, 1235, 1189, 1110, 1038, 938, 917, 815, 748, 657.

*5-Ethoxycarbonyl-6-methyl-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2-(1H)-thione (**1d**)*[38]

mp 206-209 ºC. ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.13 (t, 3H, *J* 7.1 Hz), 2.34 (s, 3H), 4.05 (q, 2H, *J* 7.1 Hz), 5.36 (d, 1H, *J* 3.6 Hz), 7.70-7.72 (m, 2H), 8.10-8.11 (m, 1H), 8.17-8.20 (m, 1H), 9.81 (br s, 1H, NH), 10.55 (br s, 1H, NH). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 14.0, 17.3, 53.5, 59.8, 99.8, 121.2, 122.8,130.5, 133.0, 145.5, 146.0, 147.8, 164.9, 174.5. IR (neat) ν_{max}/cm^{-1}: 3175, 2990, 1707, 1659, 1593, 1529, 1472, 1343, 1278, 1184, 1100, 893, 727, 688.

*5-Ethoxycarbonyl-6-methyl-4-(4-cyanophenyl)-3,4-dihydropyrimidin-2-(1H)-thione (**1e**)*[39]

mp 130-133 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09 (t, 3H, *J* 7.0 Hz), 2.30 (s, 3H), 4.00 (q, 2H, *J* 7.0 Hz), 5.24 (d, 1H, *J* 3.5 Hz), 7.40 (d, 2H, *J* 8.3 Hz), 7.83 (d, 2H, *J* 8.3 Hz), 9.74 (br s, 1H, NH), 10.47 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 14.0, 17.2, 53.8, 59.8, 99.8, 127.5, 129.9, 132.7, 133.2, 138.8, 145.9, 148.5, 164.9, 174.5. IR (neat) ν_{max}/cm^{-1}: 3299, 3274, 2984, 2239, 1651, 1557, 1456, 1370, 1320, 1282, 1198, 1167, 1108, 1032, 1003, 842, 758, 611.

*5-Ethoxycarbonyl-6-methyl-4-(4-N,N-dimethylaminophenyl)-3,4-dihydropyrimidin-2-(1H)-thione (**1f**)*[37]

mp 206-208 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.11 (t, 3H, *J* 7.0 Hz), 2.28 (s, 3H), 2.85 (s, 6H), 3.97 (q, 2H, *J* 7.0 Hz), 5.04 (d, 1H, *J* 3.2 Hz), 6.66 (d, 2H, *J* 8.5 Hz), 7.01 (d, 2H, *J* 8.5 Hz), 9.55 (br s, 1H, NH), 10.24 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.1, 17.1, 40.1, 53.5, 59.5, 101.3, 112.2, 127.1, 131.2, 144.3, 150.0, 165.3, 173.8. IR (neat) ν_{max}/cm^{-1}: 3322, 3169, 2982, 1666, 1576, 1523, 1462, 1364, 1327, 1284, 1182, 1116, 1023, 804, 755, 650.

*5-Ethoxycarbonyl-6-methyl-4-(2-fluorphenyl)-3,4-dihydropyrimidin-2-(1H)-thione (**1g**)*[41]

mp 140-143 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (t, 3H, *J* 7.0 Hz), 2.30 (s, 3H), 3.93 (q, 2H, *J* 7.0 Hz), 5.45 (d, 1H, *J* 3.1 Hz), 7.13-7.34 (m, 4H), 9.59 (s, 1H, NH), 10.37 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.5, 17.8, 49.4, 60.2, 100.0, 116.1 , 116.4, 125.3, 129.9, 130.5, 131.27, 146.1, 165.5, 174.7. IR (neat) ν_{max}/cm^{-1}: 3184, 3007, 1716, 1653, 1583, 1479, 1380, 1318, 1264, 1185, 1102, 846, 760, 744, 646.

*5-Ethoxycarbonyl-6-methyl-4-(3-hidroxyphenyl)-3,4-dihydropyrimidin-2-(1H)-one (**2a**)*[5]

mp 163-166 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.11 (t, 3H, *J* 7.0 Hz), 2.22 (s, 3H), 3.98 (q, 2H, *J* 7.0 Hz), 5.04 (s, 1H), 6.64-7.09 (m, 4H), 7.64 (s, 1H, NH), 9.15 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.2, 17.6, 52.9, 59.2, 100.1, 113.2, 114.9, 116.7, 129.4, 147.0, 148.5, 152.2, 157.3, 165.9. IR (neat) ν_{max}/cm^{-1}: 3513, 3341, 3237, 3116, 1723, 1675, 1633, 1599, 1452, 1296, 1218, 1089, 1026, 872, 775, 701.

*5-Ethoxycarbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2-(1H)-one (**2b**)*[34]

mp 210-212 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09 (t, 3H, *J* 7.1 Hz), 2.25 (s, 3H), 3.98 (q, 2H, *J* 7.1 Hz), 5.15 (d, 1H, *J* 2.9 Hz), 7.22-7.32 (m, 5H), 7.74 (br s, 1H, NH), 9.20 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.2, 16.0, 52.1, 57.3, 97.4, 124.4, 125.3, 126.4, 143.1, 145.2, 150.2, 163.3. IR (neat) ν_{max}/cm^{-1}: 3237, 3113, 2975, 1722, 1697, 1643, 1454, 1419, 1313, 1290, 1217, 1086, 879, 772, 756, 697, 660.

*5-Ethoxycarbonyl-6-methyl-4-(3,4-methylenedioxyphenyl)-3,4-dihydropyrimidin-2-(1H)-one (**2c**)*[37]

mp 188-190 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.11 (t, 3H, *J* 7.0 Hz), 2.25 (s, 3H), 3.99 (q, 2H, *J* 7.0 Hz), 5.08 (d, 1H, *J* 2.6 Hz), 5.98 (s, 2H), 6.69-6.75 (m, 2H), 6.84-6.86 (m, 1H), 7.71 (br s, 1H, NH), 9.20 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.1, 17.8, 53.7, 59.2, 99.3, 101.0, 106.7, 108.0, 119.4, 138.9, 146.4, 147.3, 148.3, 152.1, 165.4. IR (neat) ν_{max}/cm^{-1}: 3354, 3221, 3104, 2965, 1688, 1637, 1488, 1446, 1373, 1295, 1242, 1223, 1167, 1090, 1039, 928, 810, 794, 674.

*5-Ethoxycarbonyl-6-methyl-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2-(1H)-one (**2d**)*[38]

mp 225-227 ºC. ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.08 (t, 3H, *J* 6.9 Hz), 2.26 (s, 3H), 3.98 (q, 2H, *J* 6.9 Hz), 5.29 (s, 1H), 7.64-8.10 (m, 4H), 8.90 (s, 1H, NH), 9.37 (s, 1H, NH); ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 14.0, 17.9, 53.6, 59.4, 98.4, 121.2, 122.3, 130.0, 133.0, 147.0, 147.8, 149.3, 151.9, 165.0. IR (neat) ν_{max}/cm^{-1}: 3326, 3090, 2963, 1706, 1686, 1626, 1523, 1456, 1345.1, 1310, 1266, 1221, 1086, 900, 816, 794, 739, 685.

*5-Ethoxycarbonyl-6-methyl-4-(4-cyanophenyl)-3,4-dihydropyrimidin-2-(1H)-one (**2e**)*[12f]

mp 130-133 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.07 (t, 3H, *J* 7.1 Hz), 2.25 (s, 3H), 3.97 (q, 2H, *J* 7.1 Hz), 5.21 (s, 1H), 7.42 (d, 2H, *J* 8.1 Hz), 7.80 (d, 2H, *J* 8.1 Hz), 7.88 (s, 1H, NH), 9.33 (s, 1H, NH); ^{13}C NMR (75 MHz,

DMSO-d_6) δ 14.5, 18.3, 54.3, 59.8, 98.7, 110.5, 119.2, 127.8, 133.0, 149.8, 150.5, 152.3, 165.6. IR (neat) ν_{max}/cm^{-1}: 3299, 2980, 2230, 1700, 1652, 1543, 1385, 1363, 1251, 1200, 1075, 1019, 935, 825, 756.

*5-Ethoxycarbonyl-6-methyl-4-(4-N,N-dimethyl-aminophenyl)-3,4-dihydropyrimi- din-2-(1H)-one (**2f**)*[40]

mp 257-259 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.05 (t, 3H, *J* 7.0 Hz), 2.23 (s, 3H), 2.84 (s, 6H), 4.00 (q, 2H, *J* 7.0 Hz), 5.03 (d, 1H, *J* 3.1 Hz), 6.64 (d, 2H, *J* 8.5 Hz), 7.04 (d, 2H, *J* 8.5 Hz), 7.59 (br s, 1H, NH), 9.09 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.2, 17.7, 40.2, 53.3, 59.1, 99.9, 112.2, 126.9, 132.7, 147.6, 149.8, 152.3, 165.5. IR (neat) ν_{max}/cm^{-1}: 3241, 3115, 2973, 1720, 1700, 1647, 1525.9, 1456, 1365, 1290, 1219, 1088, 1047, 880, 784, 659.

*5-Ethoxycarbonyl-6-methyl-4-(2-fluorphenyl)-3,4-dihydropyrimidin-2-(1H)-one (**2g**)*[12]

mp 235-237 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (t, 3H, *J* 7.1 Hz), 2.44 (s, 3H), 4.07 (q, 2H, *J* 7.1 Hz), 5.62 (s, 1H), 7.28-7.48 (m, 4H), 7.87 (s, 1H, NH), 9.43 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.8, 17.5, 54.5, 61.1, 115.2, 122.4, 124.0, 127.8, 129.3, 135.8, 154.7, 157.2, 158.3, 160.3. IR (neat) ν_{max}/cm^{-1}: 3229, 3158, 2973, 1747, 1699, 1616, 1539, 1436, 1385, 1319, 1229, 1199, 1144, 1075, 933, 755.

Cytotoxicity assays

Maintenance of cell lines

The human glioblastoma cell line U138-MG and the rat glioma cell line C6 were obtained from American Type Culture Collection (ATCC) (Rockville, Maryland, USA). The cells were grown and maintained in Dulbecco's modified Eagle's medium (DMEM) containing antibiotics penicillin/streptomycin 0.5 U mL^{-1}, and supplemented with 5% (C6) or 15% (U138-MG) (v/v) fetal bovine serum (FBS). Cells were kept at a temperature of 37 ºC, a minimum relative humidity of 95%, and an atmosphere of 5% CO_2 in air. All the experiments throughout this study were conducted in serum supplemented DMEM.

Assessment of glioma cell viability

The method MTT provides a quantitative measure of the number cells with metabolically active mitochondria and it is based on the mitochondrial reduction of a tetrazolium bromide salt, MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]), to a chromophore, formazan product, whose absorbance can be determined by spectrophotometric measurement.

Glioma cells were plated in a 96-well plate at 10^3 *per* well and, after reaching semi-confluence, the cultures were treated with monastrol **1a** for 24, 48 or 72 h or its analogues **1b**-**g**, **2b**-**g** for 48 h. After the end of treatment, each culture medium containing the drug was removed and the cells were washed twice with 100 µL of PBS. After removing the PBS, 90 µL of culture medium and 10 µL of MTT were added to each of the wells. The cells were incubated for 3 h and the solution was then removed from the precipitate. A total of 100 µL of DMSO were added to the wells and the level of absorbance was read by an ELISA plate reader at 490 nm. This absorbance was linearly proportional to the number of live cells with active mitochondria. The cell viability was calculated using the equation **1** bellow, where Abs_s is the absorbance of cells treated with different formulations and $Abs_{control}$ is the absorbance of negative control cells (incubated with cell culture medium only, equation 1).

$$\text{Cell viability (\%)} = (Abs_s/Abs_{control})100 \quad (1)$$

Cell counting

The human glioma cells (U138-MG) were seeded at 1×10^4 cells *per* well in DMEM/15% FBS in 24-well plates, and allowed to grown. After reaching semi-confluence, the glioma cells were treated with monastrol (**1a**) for 24, 48 or 72 h. The same procedure was repeated for the compounds **1b**-**g**, **2b**-**g** for 48 h. At the end of the treatment, the medium was removed. The cells were washed with phosphate buffered saline (PBS) and 200 µL of 0.25% trypsin/EDTA solution was added to detach the cells, which were counted immediately in a hemocytometer. The procedure was the same for the rat glioma cells (C6) except that they were seeded at 5×10^3 cells *per* well in DMEM/5% FBS.

Statistical analysis

Data are expressed as mean ± S.D. and analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by *post-hoc* for multiple comparisons (Tukey test) using a GraphPad Prism Software. Differences between mean values were considered significant when $P < 0.05$.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

The authors are thankful to CNPq/MCT (VLEL-Universal No. 477657/2008-7, PQ10/2008, 566201/2008 postgraduate, INCT-if (Instituto Nacional de Ciência e Tecnologia para Inovação Farmacêutica) and PRONEX/FAPERGS/CNPq and DR-Universal No. 484615/2007-6), for support the program on the antitumoral agents.

References

1. Mayer, T. U.; Kappor, T. M.; Haggarty, S. J.; King, R. W.; Schreiber, S. L.; Mitchison, T. J.; *Science* **1999**, *286*, 971.
2. Maliga, Z.; Mitchison, T. J.; *BMC Chem. Biol*. **2006**, *6*, 2; Liu, F.; You, Q. D.; Chen, Y. D.; *Bioorg. Med. Chem. Lett*. **2007**, *17*, 722; Klein, E.; DeBonis, S.; Thiede, B.; Skoufias, D. A.; Kozielski, F.; Lebeau, L.; *Bioorg. Med. Chem*. **2007**, *15*, 6474.
3. Bahekar, S. S.; Shinde, D. B.; *Bioorg. Med. Chem. Lett*. **2004**, *14*, 1733.
4. Sujatha, K.; Shanmugam, P.; Perumal, P. T.; Muralidharan, D.; Rajendran, M.; *Bioorg. Med. Chem. Lett*. **2006**, *16*, 4893.
5. Ashok, M.; Holla, B. S.; Kumari, N. S.; *Eur. J. Med. Chem*. **2007**, *42*, 380.
6. Basso, A. M.; Bratcher, N. A.; Gallagher, K. B.; Cowart, M. D.; Zhao, C.; Sun, M.; Esbenshade, T. A.; Brune, M. E.; Fox, G. B.; Schmidt, M.; Collins, C. A.; Souers, A. J.; Iyengar, R.; Vasudevan, A.; Kym, P. R.; Hancock, A. A.; Rueter, L. E.; *Eur. J. Pharmacol*. **2006**, *540*, 115.
7. Wisen, S.; Androsavich, J.; Evans, C. G.; Chang, L.; Gestwicki, J. E.; *Bioorg. Med. Chem. Lett*. **2008**, *18*, 60.
8. Deres, K.; Schröder, C. H.; Paessens, A.; Goldmann, S.; Hacker, H. J.; Weber, O.; Krämer, T.; Niewöhner, U.; Pleiss, U.; Stoltefuss, J.; Graef, E.; Koletzki, D.; Masantschek, R. N. A.; Reimann, A.; Jaeger, R.; Groß, R.; Beckermann, B.; Schlemmer, K.-H.; Haebich, D.; Rübsamen-Wagmann, H.; *Science* **2003**, *299*, 893.
9. Blackburn, C.; Guan, B.; Brown, J.; Cullis, C.; Condon, S. M.; Jenkins, T. J.; Peluso, S.; Ye, Y.; Gimeno, R. E.; Punreddy, S.; Sun, Y.; Wu, H.; Hubbard, B.; Kaushik, V.; Tummino, P.; Sanchetti, P.; Sun, D. Y.; Daniels, T.; Tozzo, E.; Balani, S. K.; Raman, P.; *Bioorg. Med. Chem. Lett*. **2006**, *16*, 3504.
10. Bigineli, P.; *Gazz. Chim. Ital.* **1893**, *23*, 360.
11. Debache, A.; Boumoud, B.; Amimour, M.; Belfaitah, A.; Rhouati, S.; Carboni B.; *Tetrahedron Lett.* **2006**, *47*, 5697; Yadav, J. S.; Kumar, S. P.; Kondaji, G.; Rao, R. S.; Nagaiah K.; *Chem. Lett.* **2004**, *33*, 1168; Tu, S.; Fang, F.; Miao, C.; Jiang, H.; Feng, Y.; Shi, D.; Wang, X.; *Tetrahedron Lett.* **2003**, *44*, 6153.
12. Russowsky, D.; Lopes, F. A.; Silva, V. S. S.; Canto, K. F. S.; D'Oca, M. G. M.; Godoi, M. N.; *J. Braz. Chem. Soc.* **2004**, *15*, 165; Godoi, M. N.; Costenaro, H. S.; Kramer, E.; Machado, P. S.; D'Oca, M. G. M.; Russowsky D.; *Quim. Nova* **2005**, *28*, 1010; Dondoni, A.; Massi, A.; Minghini, E.; Sabbatini, S.; Bertolasi, V.; *J. Org. Chem.* **2003**, *68*, 6172; Ma, Y.; Qian, C.; Wang, L.; Yang, M.; *J. Org. Chem.* **2000**, *65*, 3864; Maiti, G.; Kundu, P.; Guin, C.; *Tetrahedron Lett.* **2003**, *44*, 2757; Fu, N.-Y.; Yuan, Y.-F.; Cao, Z.; Wang, S.-W.; Wang, J.-T.; Peppe, C.; *Tetrahedron* **2002**, *58*, 4801; Paraskar, A. S.; DewKar, G. K.; Sudalai, A.; *Tetrahedron Lett.* **2003**, *44*, 3305.
13. Kappe, C. O.; Falsone, S. F.; *Synlett* **1998**, 718.
14. Zhu, Y.; Pan, Y.; Huang, S.; *Synth. Commun.* **2004**, *34*, 3167.
15. Zumpe, F. L.; Flüβ, M.; Schmitz, K.; Lender, A.; *Tetrahedron Lett.* **2007**, *48*, 1421.
16. Sabitha, G.; Reddy, G. S. K. K.; Reddy, C. S.; Yadav, J. S.; *Synlett* **2003**, 858.
17. Misra, A. K.; Agnihotri, G.; Madhusudan, S. K.; *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **2004**, *43*, 2018; Dallinger, D.; Kappe, C. O.; *Nat. Protoc.* **2007**, *2*, 317.
18. Studer, A.; Jeger, P.; Wipf, P.; Curran, D. P.; *J. Org. Chem*. **1997**, *62*, 2917; Studer, A.; Hadida, S.; Ferrito, R.; Kim, S. –Y.; Jeger, P.; Wipf, P.; Curran, D. P.; *Science* **1997**, *275*, 823; Kappe, C. O.; *Bioorg. Med. Chem. Lett*. **2000**, *10*, 49; Valverde, M. G.; Dallinger, D.; Kappe, C. O.; *Synlett* **2001**, 741; Strohmeier, G. A.; Kappe, C. O.; *Angew. Chem., Int. Ed*. **2004**, *43*, 621; Comas, H.; Buisson, D. A.; Najman, R.; Kozielski, F.; Rousseau, B.; Lopez, R.; *Synlett* **2009**, *11*, 1737.
19. Peng, J.; Deng, Y.; *Tetrahedron Lett.* **2001**, *42*, 5917; Legeay, J. C.; Vanden Eynde, J. J.; Bazureau, J. P.; *Tetrahedron* **2005**, *61*, 12386; Legeay, J. C.; Vanden Eynde, J. J.; Toupet, L.; Bazureau, J. P.; *ARKIVOC* **2007**.
20. Yu, Y.; Liu, D.; Liu, C.; Luo, G.; *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3508; Jiang, C.; You, Q. D.; *Chin. Chem. Lett.* **2007**, *18*, 647; Shaabani, A.; Bazgir, A.; Teimouri, F.; *Tetrahedron Lett.* **2003**, *44*, 857; Stefani, H. A.; Oliveira, C. B.; Almeida, R. B.; Pereira, C. M. P.; Braga, R. C.; Cella, R.; Borges, V. C.; Savegnago, L.; Nogueira, C. W.; *Eur. J. Med. Chem.* **2006**, *41*, 513; Amini, M. M.; Shaabani, A.; Bazgir, A.; *Catal. Commun.* **2006**, *7*, 843; Mishra, B. G.; Kumar, D.; Rao, V. S.; *Catal. Commun.* **2006**, *7*, 457; Nandurkar, N. S.; Bhanushali, M. J.; Bhor, M. D.; Bhanage, B. M.; *J. Mol. Catal. A: Chem.* **2007**, *271*, 14; Kamal, A.; Krishnaji, T.; Azhar, M. A.; *Catal. Commun.* **2007**, *8*, 1929; Banik, B. K.; Reddy, A. T.; Datta, A.; Mukhopadhyay, C.; *Tetrahedron Lett.* **2007**, *48*, 7392; Ahmed, N.; VanLier, J. E.; *Tetrahedron Lett.* **2007**, *48*, 5407; Chen, W.; Qin, S.; Jin, J.; *Catal. Commun.* **2007**, *8*, 123; Domínguez, J. C. R.; Bernardi, D.; Kirsch, G.; *Tetrahedron Lett.* **2007**, *48*, 5777; Adibi, H.; Samimi, H. A.; Beygzadeh, M.; *Catal. Commun.* **2007**, *8*, 2119; Su, W.; Li, J.; Zheng, Z.; Shen, Y.; *Tetrahedron Lett.* **2005**, *46*, 6037; Bose, D. S.; Fatima, L.; Mereyala, H. B.; *J. Org. Chem.* **2003**, *68*, 587; Ma, Y.; Qian, C.; Wang, L.; Yang, M.; *J. Org. Chem.* **2000**, *65*, 3864; Kalita, H. R.; Phukan, P.; *Catal. Commun.* **2007**, *8*, 179.

21. Russowsky, D.; Canto, R. F. S.; Sanches, S. A. A.; D'Oca, M. G. M.; Fátima, A.; Pilli, R. A.; Kohn, L. K.; Antônio, M. A.; Carvalho, J. E.; *Bioorg. Chem.* **2006**, *34*, 173.
22. Schwartzbaum, J. A.; Fisher, J. L.; Aldape, K. D.; Wrensch, M.; *Nature* **2006**, *2*, 494.
23. Holland, E. C.; *Nature* **2001**, *2*, 120.
24. Sathornsumetee, S.; Reardon, D.; Desjardins, A.; Quinn, J.; Vredenburgh, J. J.; Rich, J. N.; *Cancer* **2007**, *110*, 13.
25. Schwartzbaum, J. A.; Fisher, J.L.; Aldape, K. D.; Wrensch, M.; *Nature Clin. Pract. Neur.* **2006**, *2*, 494.
26. Müller, C.; Gross, D.; Sarli, V.; Gartner, M.; Giannis, A.; Bernhardt, G.; Buschauer, A.; *Cancer Chemother. Pharmacol.* **2007**, *59*, 157.
27. Kappe, C. O.; *J. Org. Chem.* **1997**, *62*, 7201.
28. Cepanec, I.; Litvic, M.; Bartolinc, A.; Lovric, M.; *Tetrahedron* **2005**, *61*, 4275.
29. Look, G. C.; Murphy, M. M.; Campbell, D. A.; Gallop, M. A.; *Tetrahedron Lett.* **1995**, *36*, 2937.
30. DeWolfe, R. H.; Jensen, J. L.; *J. Am. Chem. Soc.* **1963**, *85*, 3264.
31. Cordes, E. H.; Jencks, W. P.; *J. Am. Chem. Soc.* **1962**, *84*, 832.
32. Ramu, E.; Kotra, V.; Bansal, N.; Varala, R.; Adapa, S. R.; *Rasāyan J. Chem.* **2008**, *1*, 188.
33. Sanghetti, J. N.; Sinde, D. B.; Kokare, N. D.; *J. Heterocycl. Chem.* **2009**, *45*, 1191.
34. Angeles-Beltran, D.; Lomas-Romero, L.; Lara-Corona, V. H.; Gonzales-Zamora, E.; Negron-Silva, G.; *Molecules* **2006**, *11*, 731.
35. Dwivedi, N.; Mishra, R. C.; Tripathi, R. P.; *Lett. Org. Chem.* **2005**, *2*, 450.
36. Kolb, S.; Mondesert, O.; Goddard, M. L.; Jullien, D.; Villoutreix, B. O.; Ducommun, B.; Garbay, C.; Braud, E.; *Chem. Med. Chem.* **2009**, *4*, 633.
37. Ghosh, R.; Maiti, S.; Chakraborty, A.; *J. Mol. Catal. A: Chem.* **2004**, *217*, 47.
38. Karade, H. N.; Sathe, M.; Kaushik, M. P.; *Molecules* **2007**, *12,* 1341.
39. Wu, Y. Y.; Chai, Z.; Liu, X. Y.; Zhao, G.; Wang, S. W.; *Eur. J. Org. Chem.* **2009**, *6*, 904.
40. Attaby, F. A.; Ramla, M. M.; Harukuni, T.; *Phosphorus, Sulfur Silicon Relat. Elem.* **2008**, *183*, 2956.
41. Gholap, A. R.; Venkatesan, K.; Daniel, T.; Lahoti, R. J.; Srinivasan, K. V.; *Green Chem.* **2004**, *6*, 147.

Submitted: November 3, 2010
Published online: March 15, 2011

J. Braz. Chem. Soc., Vol. 22, No. 7, S1-S15, 2011.
Printed in Brazil - ©2011 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Supplementary Information

Synthesis of Dihydropyrimidin-2-one/thione Library and Cytotoxic Activity against the Human U138-MG and Rat C6 Glioma Cell Lines

Rômulo F. S. Canto,[a] Andressa Bernardi,[b] Ana Maria O. Battastini,[b] Dennis Russowsky*,[c] and Vera Lucia Eifler-Lima*,[a]

[a]Laboratório de Síntese Orgânica Medicinal/LaSOM, Programa de Pós Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, 90610-00 Porto Alegre-RS, Brazil

[b]Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2660, 90035-003 Porto Alegre-RS, Brazil

[c]Instituto de Química, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, 91501-970 Porto Alegre-RS, Brazil

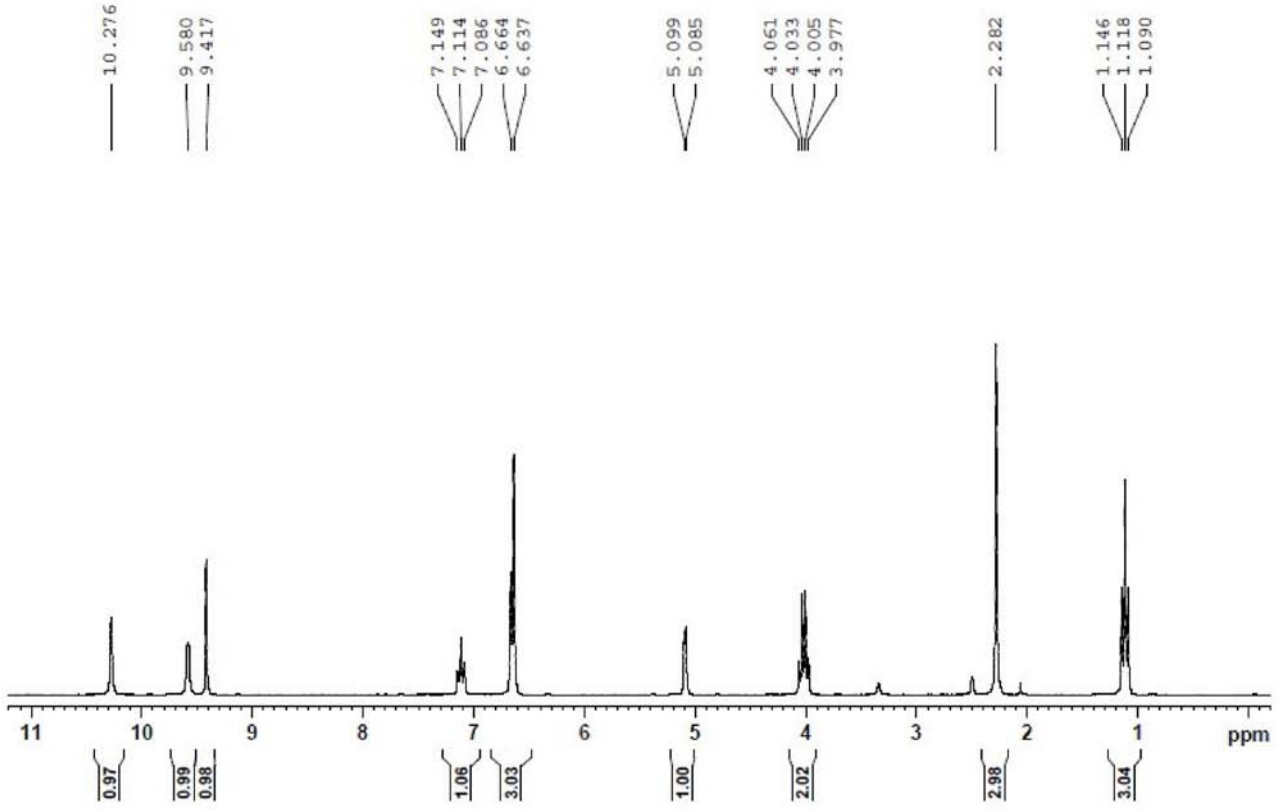


Figure S1. ^{1}H NMR (250 MHz, DMSO-d_6) of compound **1a** (monastrol).

*e-mail: dennis@iq.ufrgs.br, veraeifler@ufrgs.br

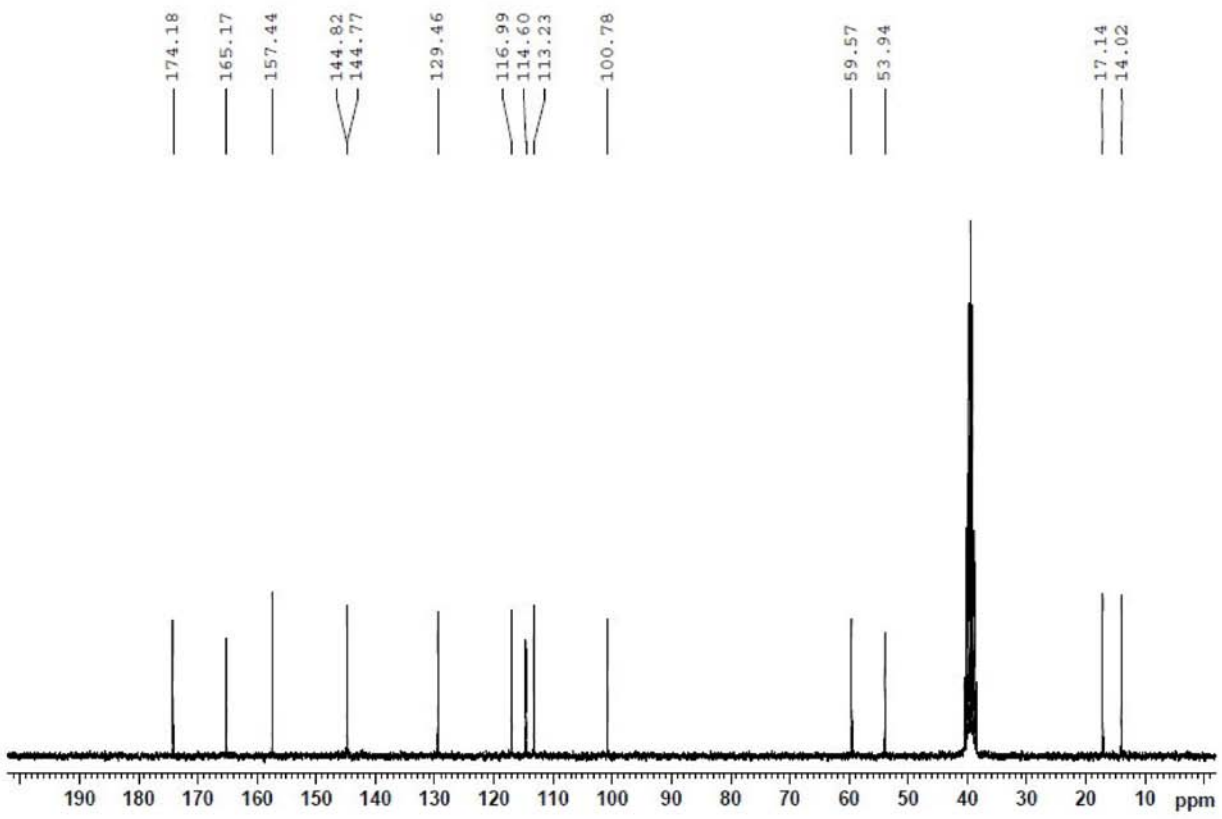


Figure S2. ^{13}C NMR (62.5 MHz, DMSO-d_6) of compound **1a** (monastrol).

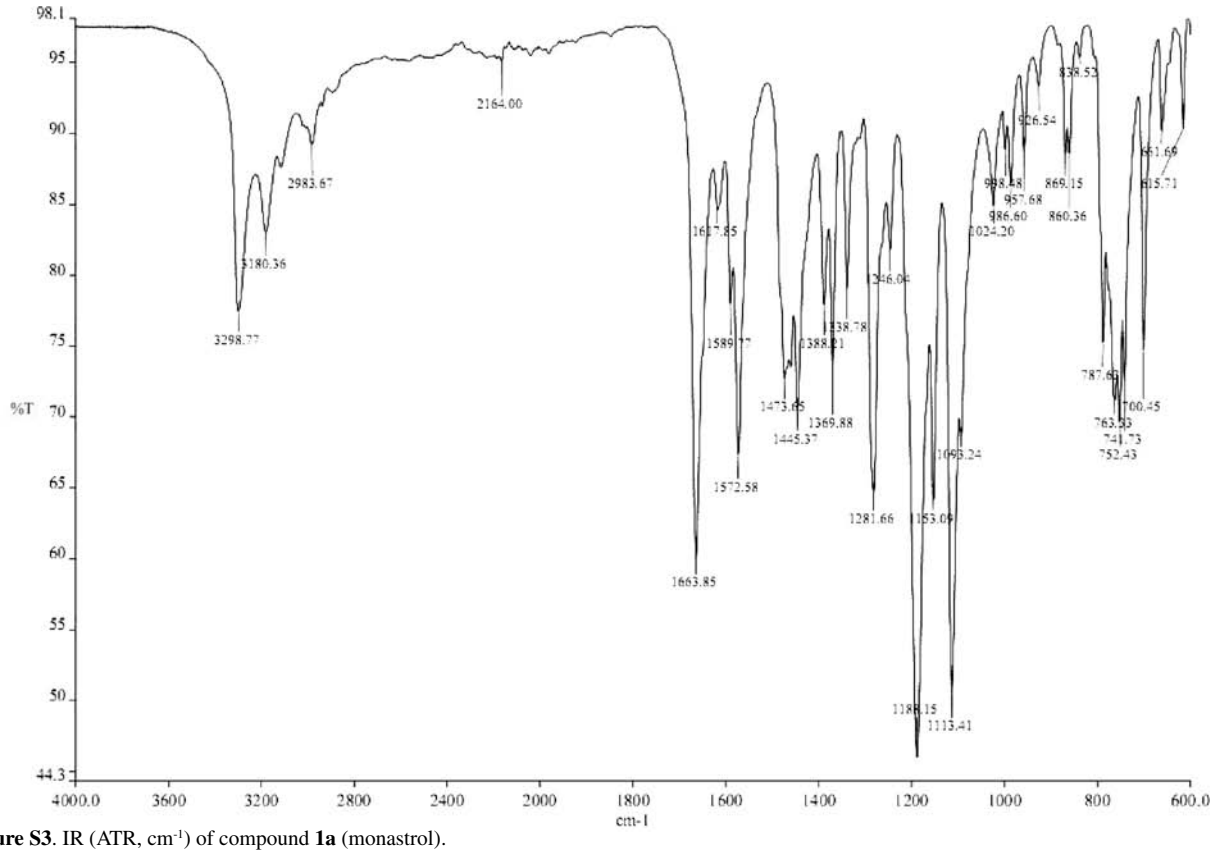


Figure S3. IR (ATR, cm^{-1}) of compound **1a** (monastrol).

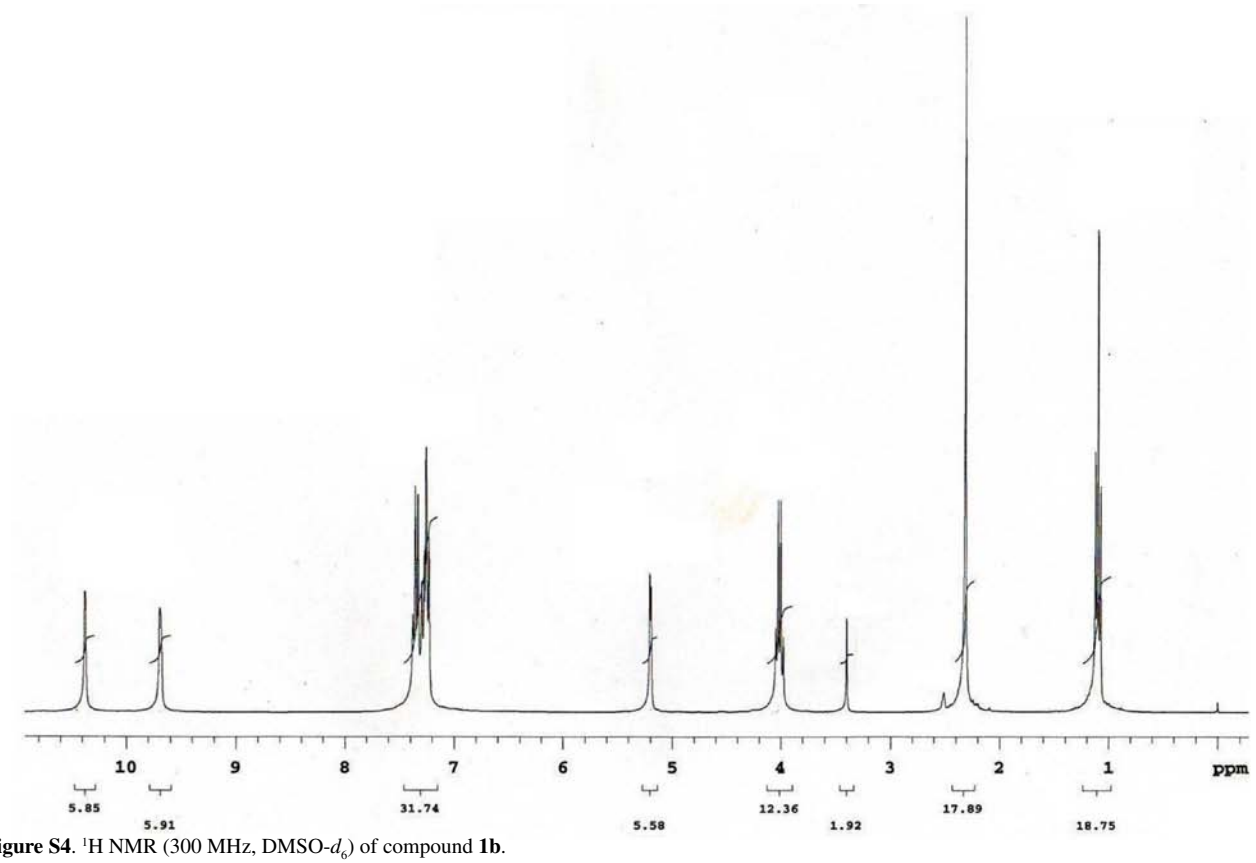


Figure S4. [1]H NMR (300 MHz, DMSO-d_6) of compound **1b**.

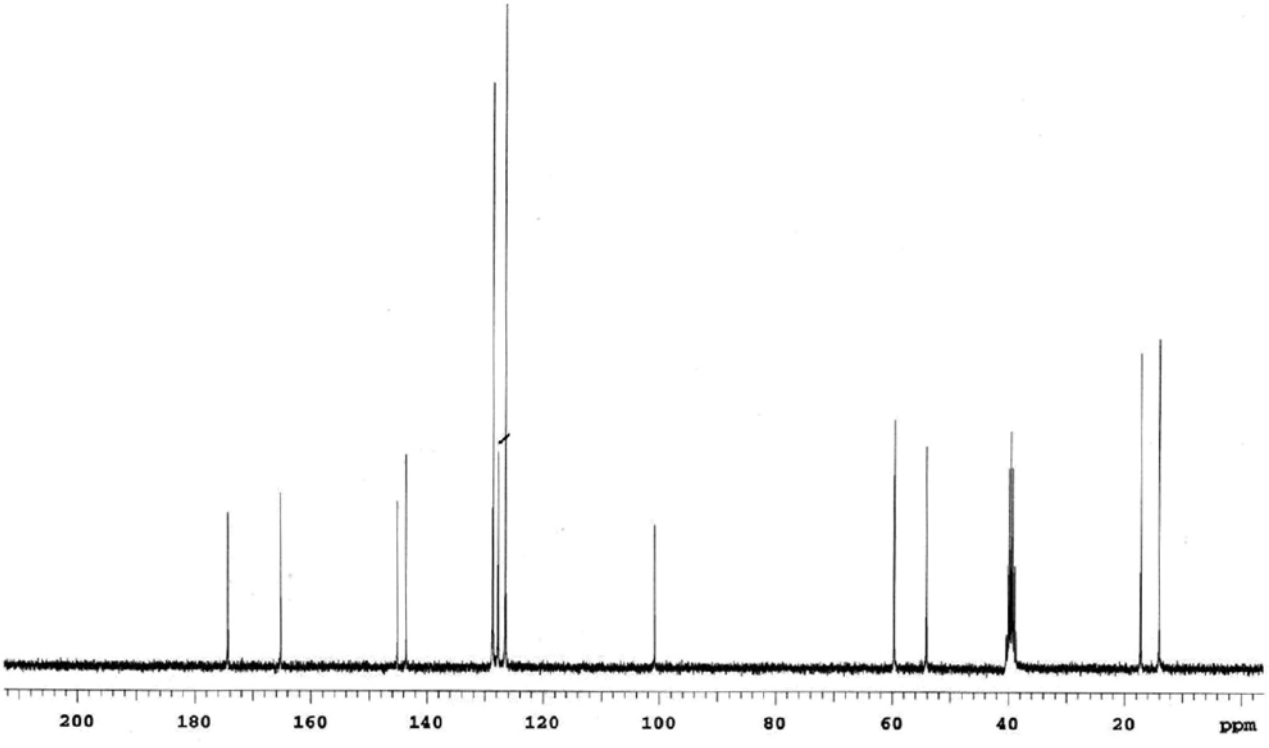


Figure S5. [13]C NMR (75 MHz, DMSO-d_6) of compound **1b**.

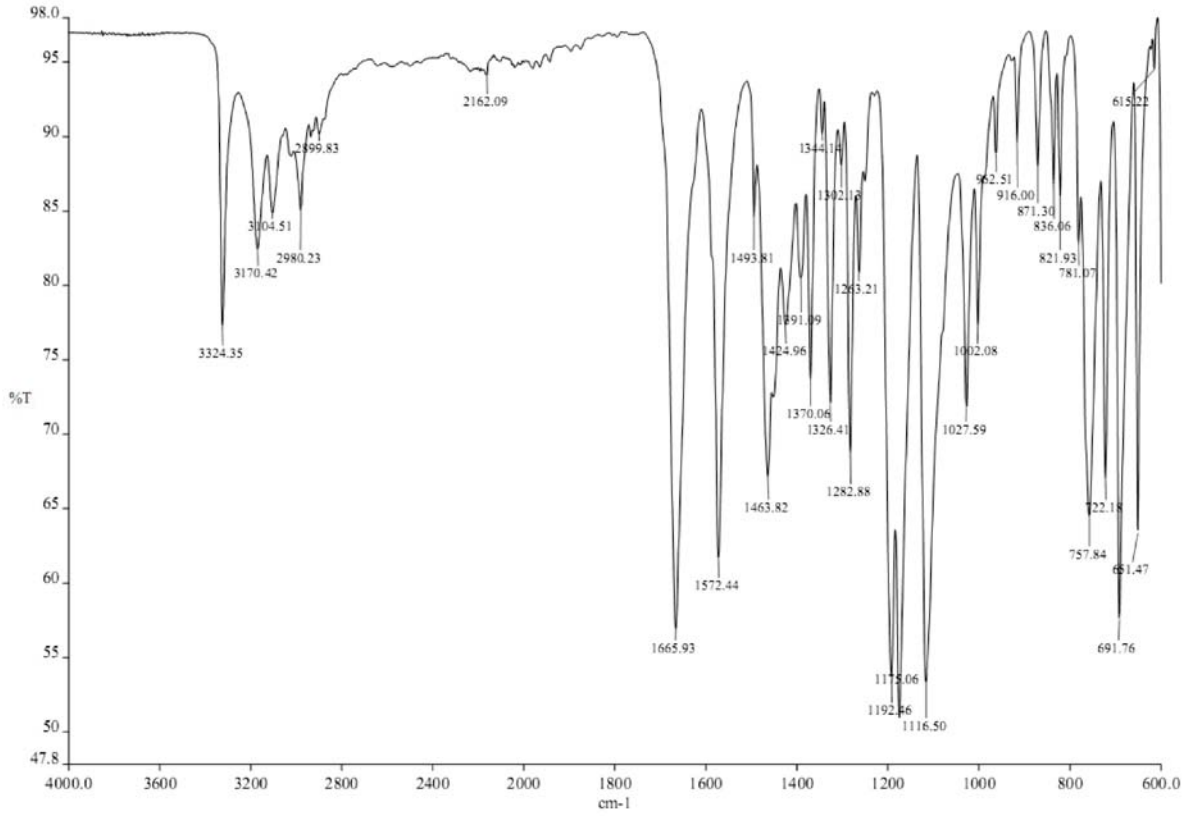


Figure S6. IR (ATR, cm^{-1}) of compound **1b**.

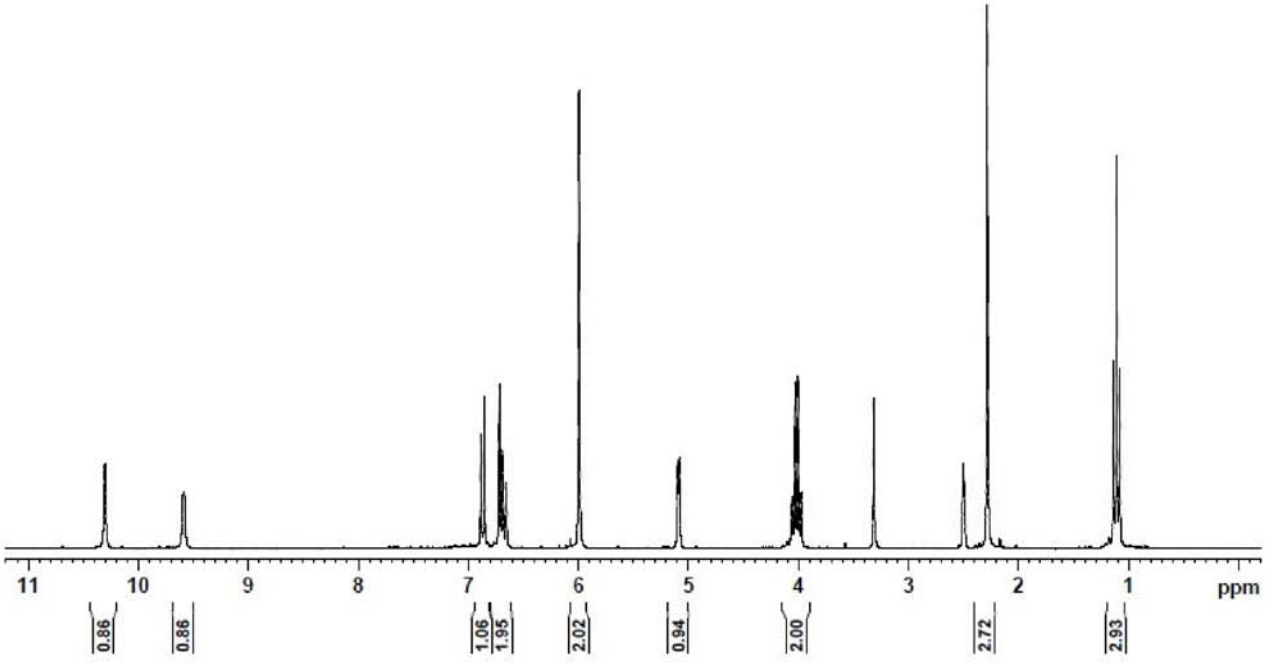


Figure S7. ^1H NMR (250 MHz, DMSO-d_6) of compound **1c**.

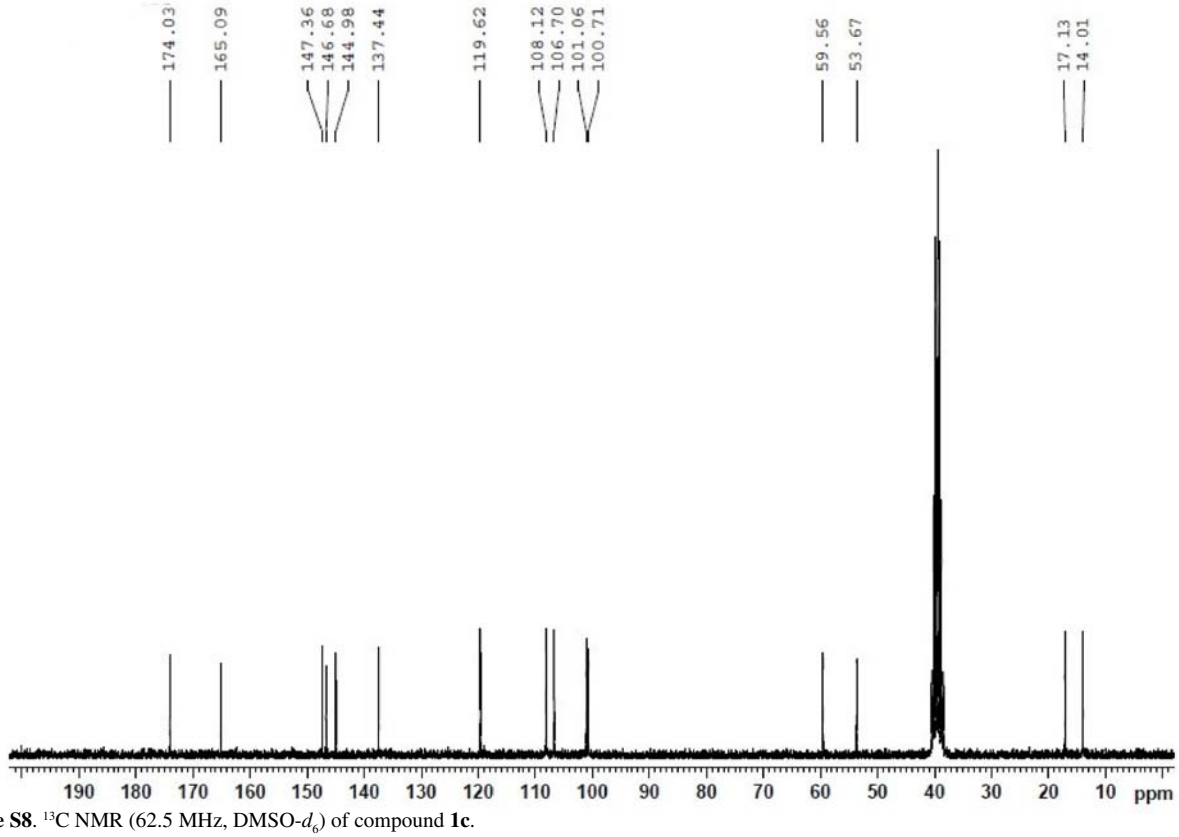


Figure S8. ^{13}C NMR (62.5 MHz, DMSO-d_6) of compound **1c**.

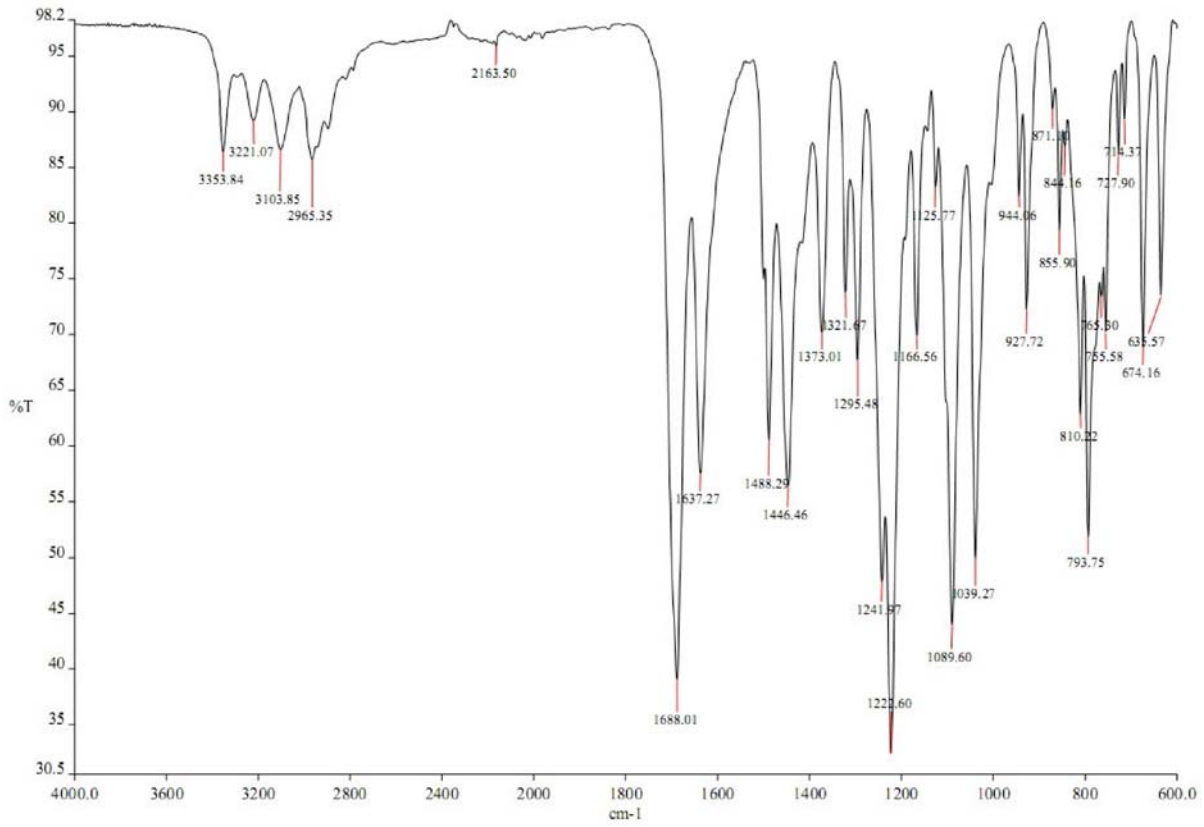


Figure S9. IR (ATR, cm^{-1}) of compound **1c**.

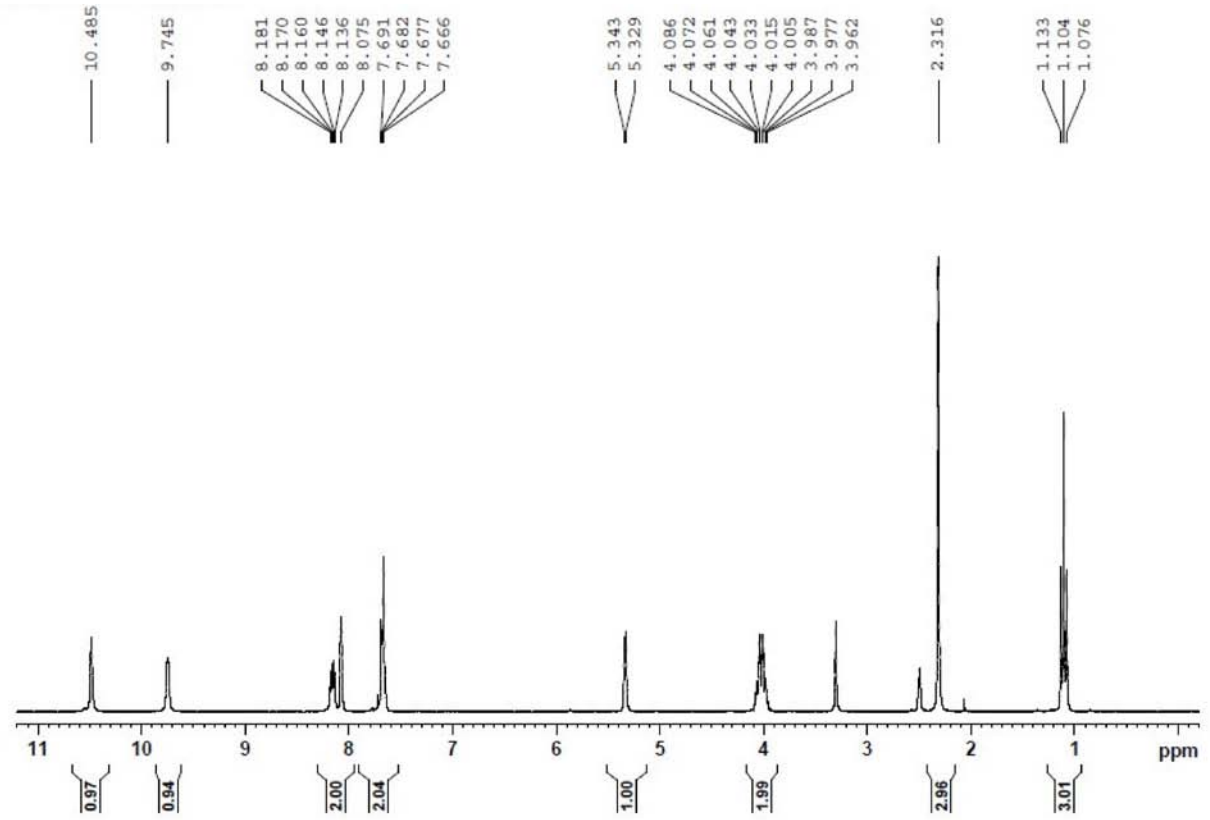


Figure S10. ^{1}H NMR (250 MHz, DMSO-d_6) of compound **1d**.

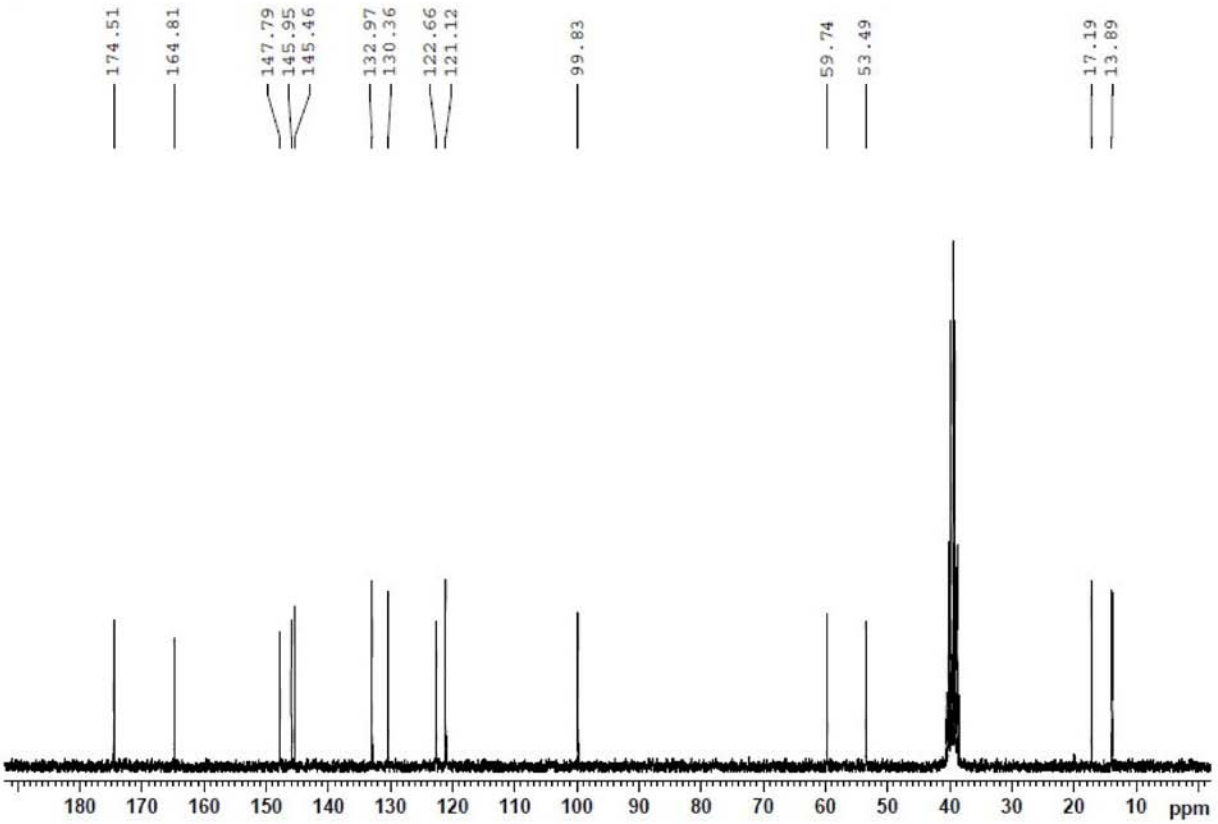


Figure S11. ^{13}C NMR (62.5 MHz, DMSO-d_6) of compound **1d**.

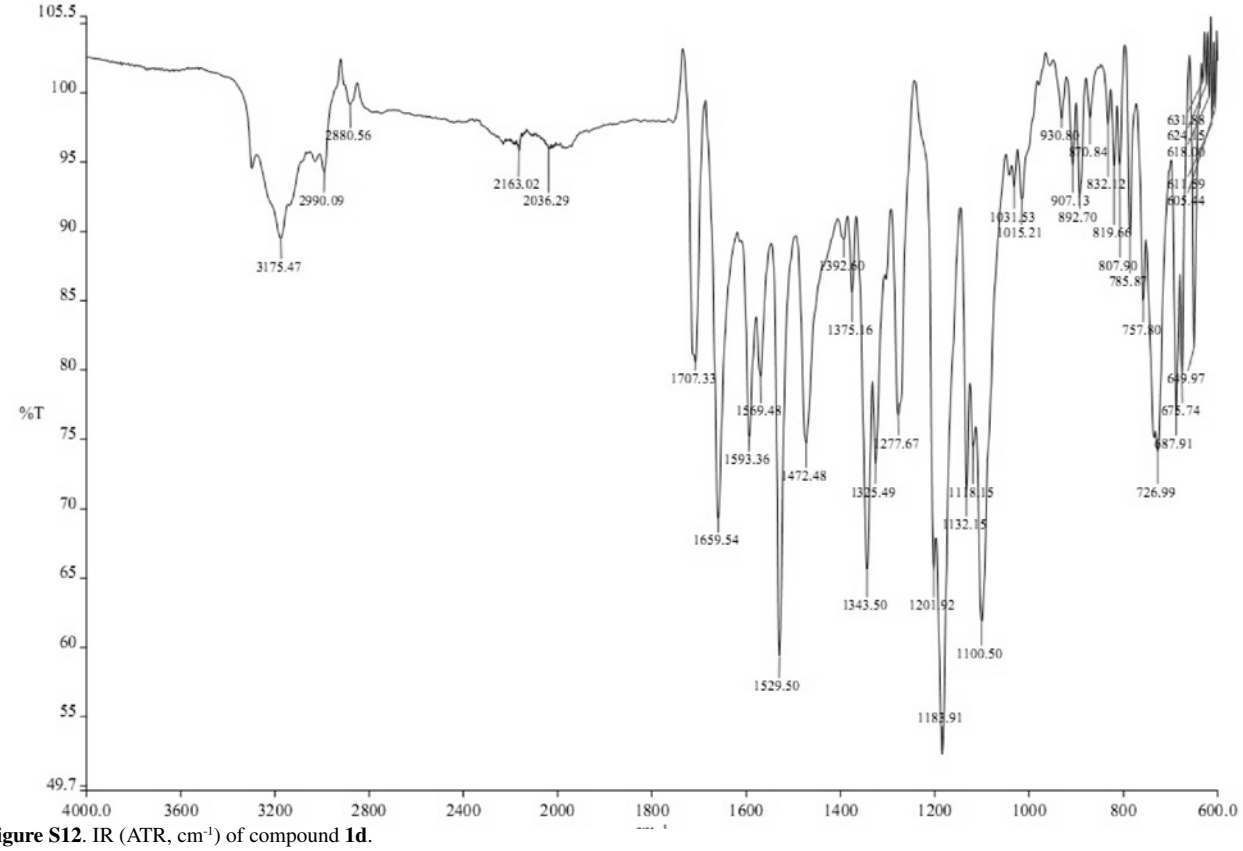


Figure S12. IR (ATR, cm^{-1}) of compound **1d**.

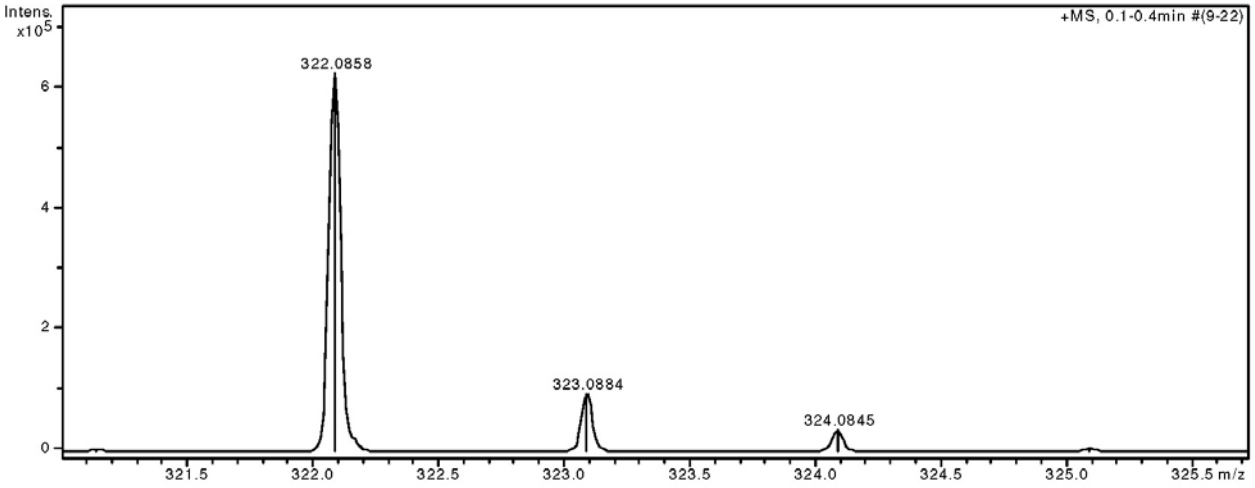


Figure S13. HRMS of compound **1d**.

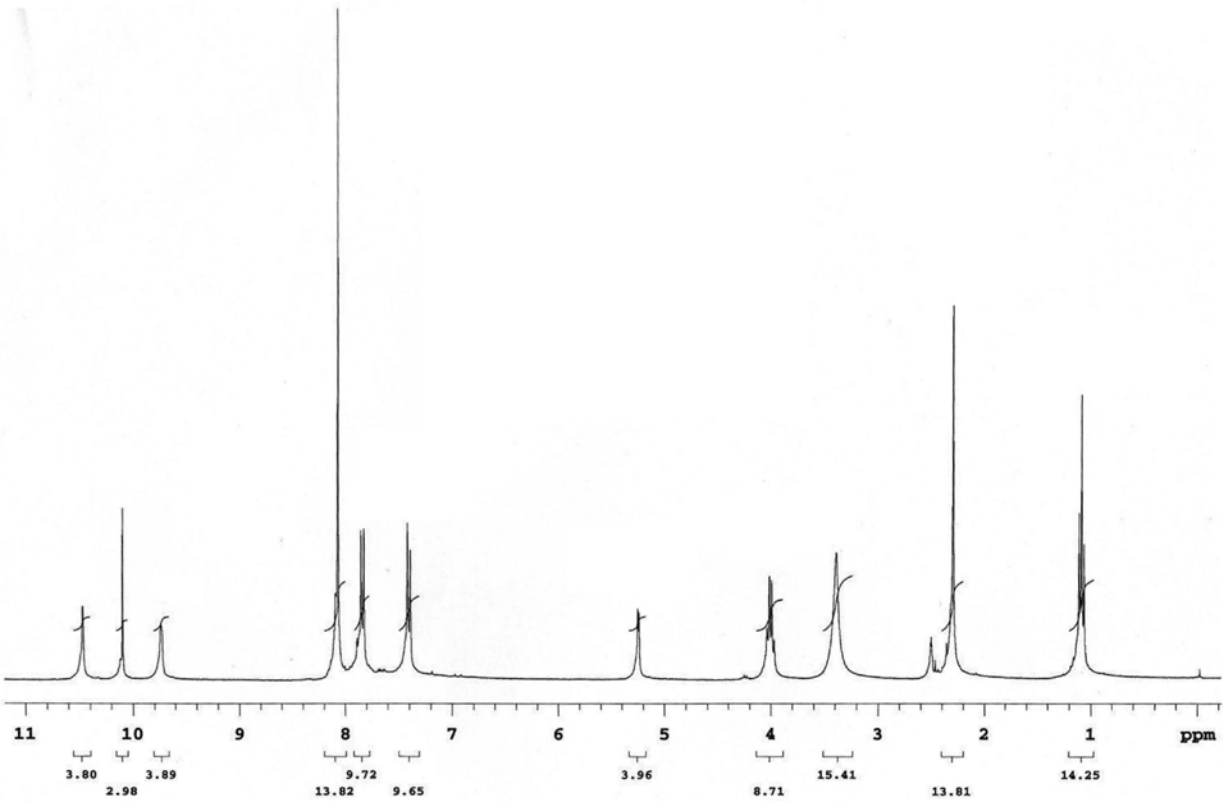


Figure S14. ^{1}H NMR (300 MHz, DMSO-d_6) of compound **1e**.

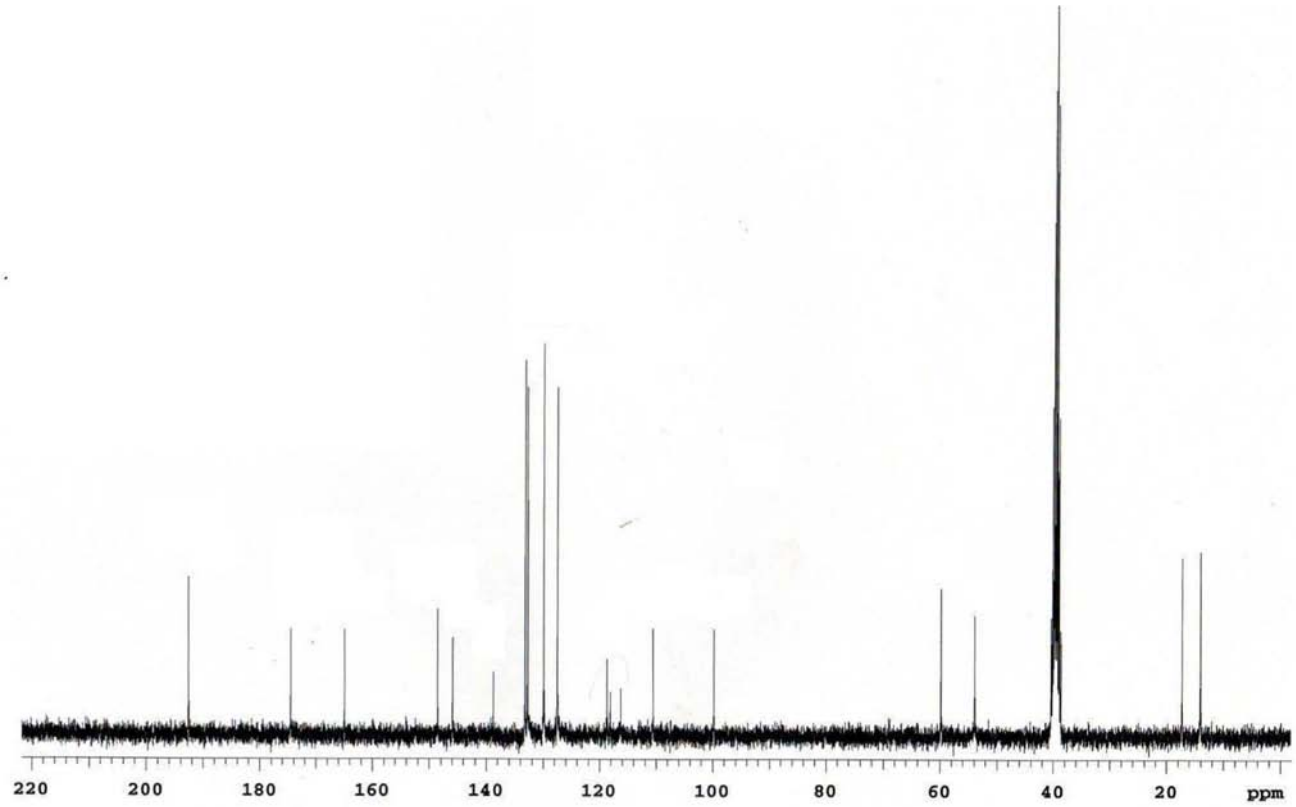


Figure S15. ^{13}C NMR (75 MHz, DMSO-d_6) of compound **1e**.

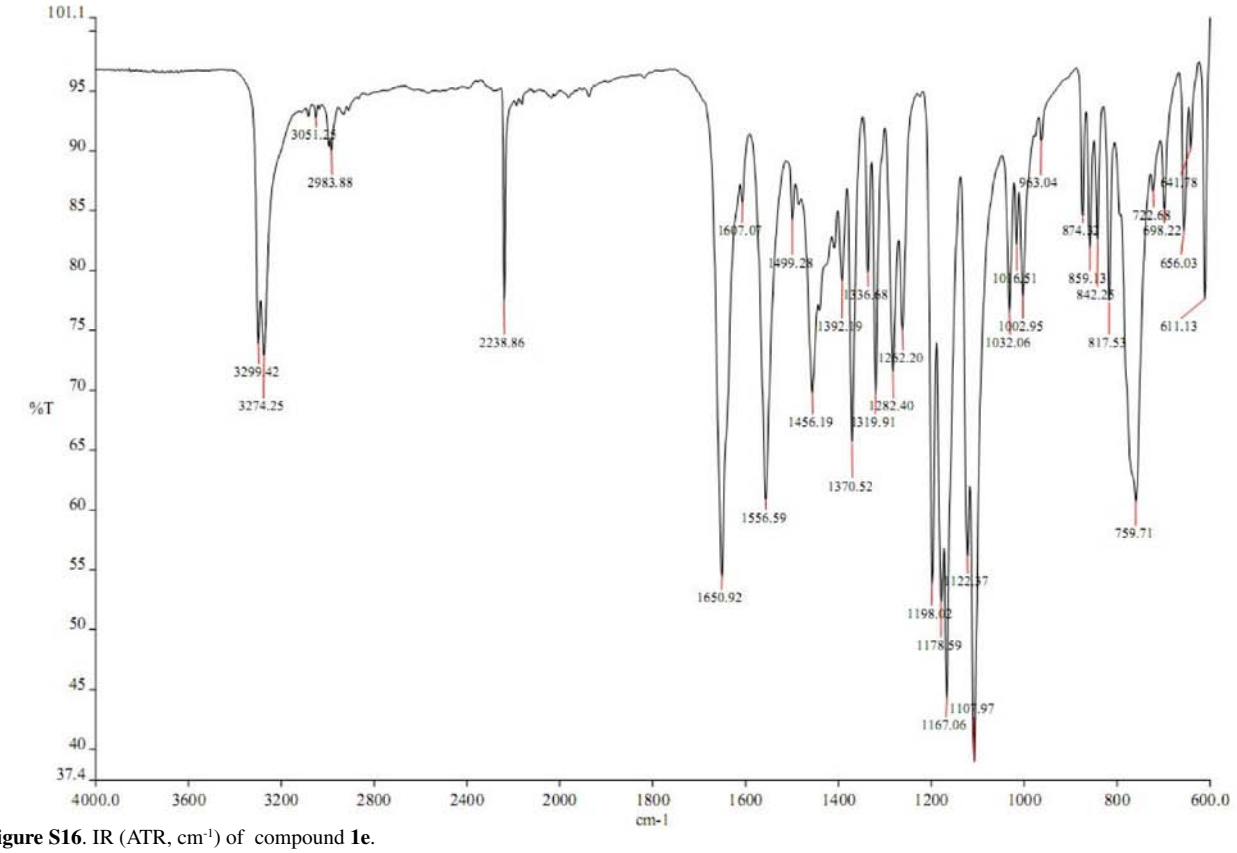


Figure S16. IR (ATR, cm^{-1}) of compound **1e**.

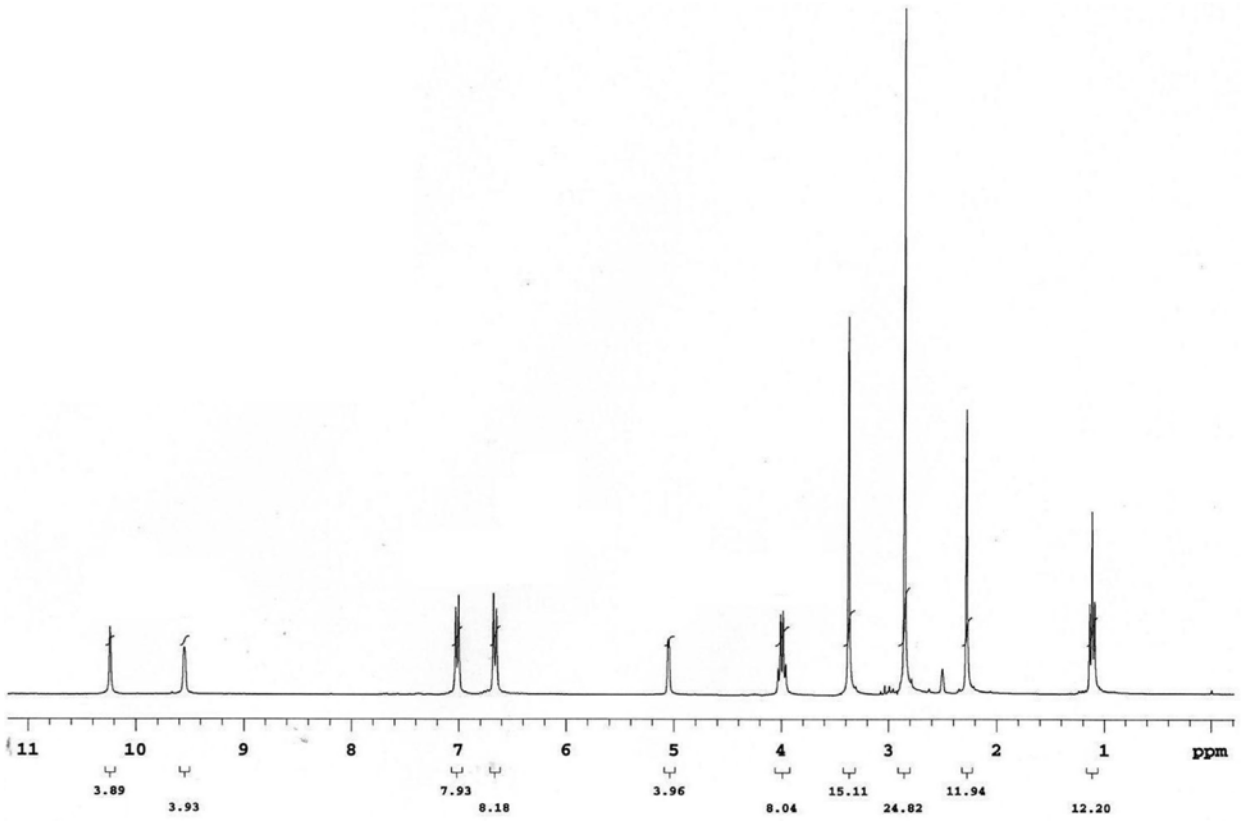


Figure S17. ^{1}H NMR (300 MHz, DMSO-d_6) of compound **1f**.

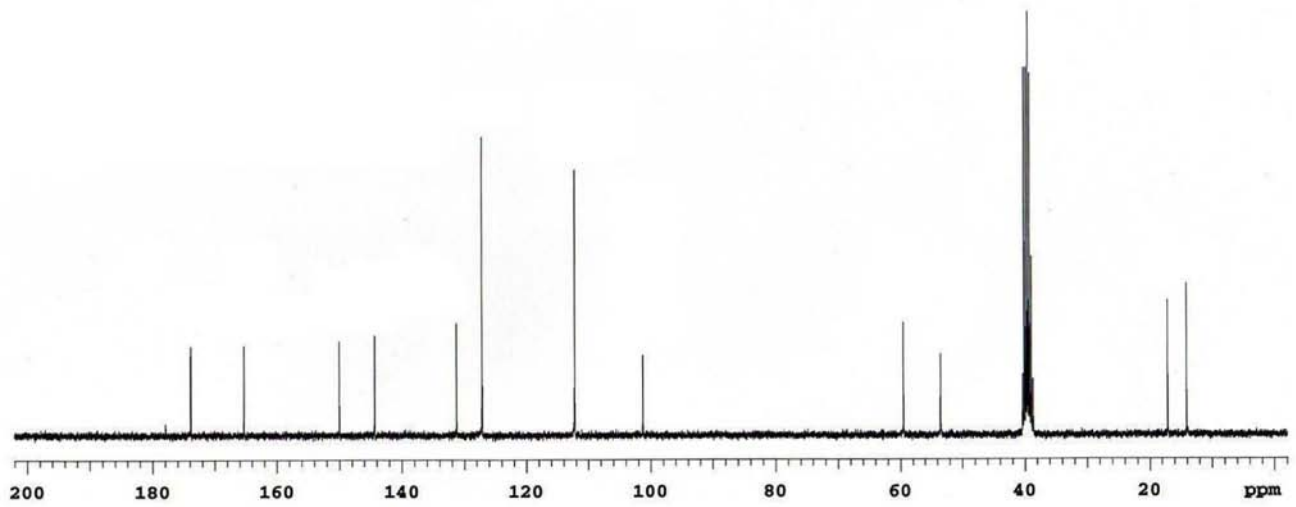


Figure S18. ^{13}C NMR (75 MHz, DMSO-d_6) of compound **1f**.

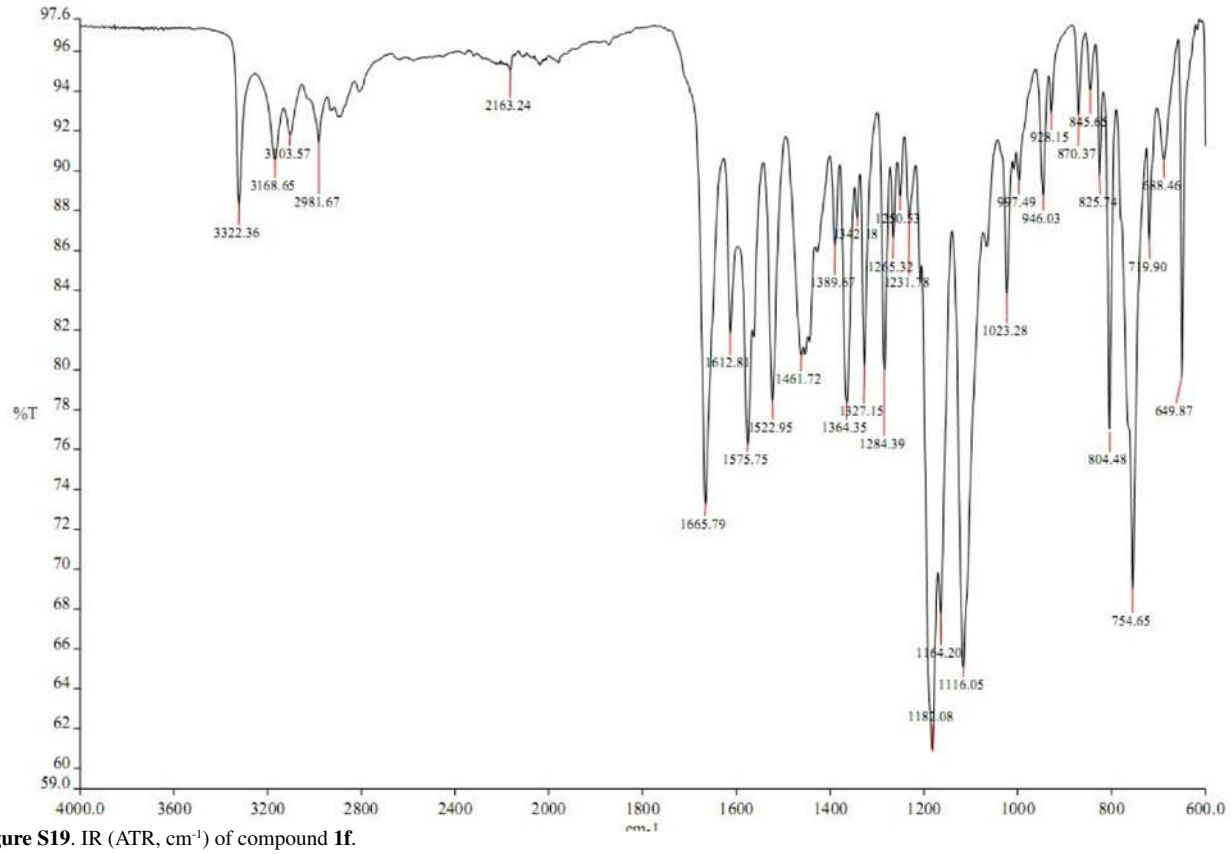


Figure S19. IR (ATR, cm^{-1}) of compound **1f**.

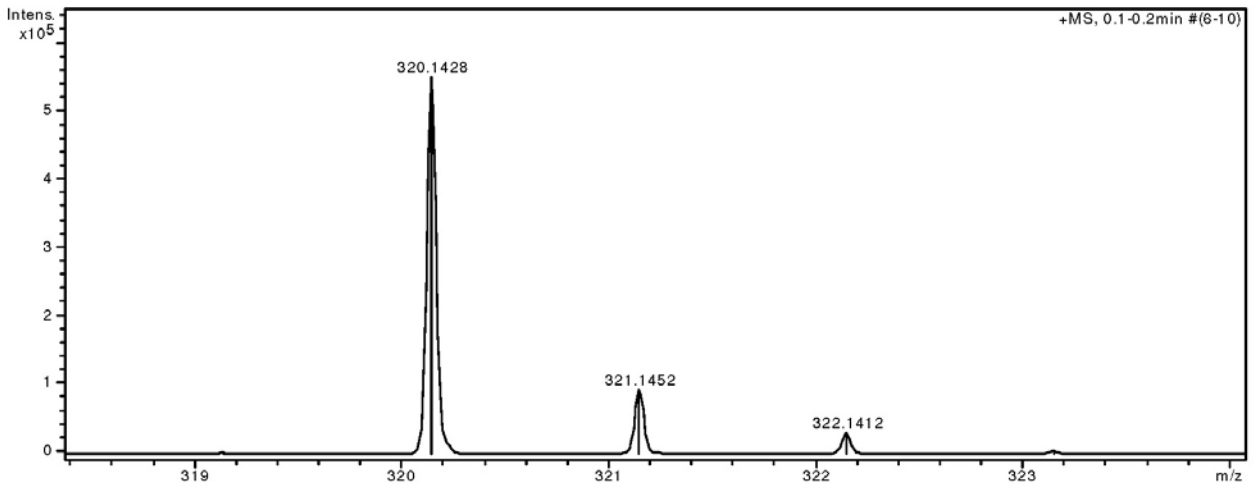


Figure S20. HRMS of compound **1f**.

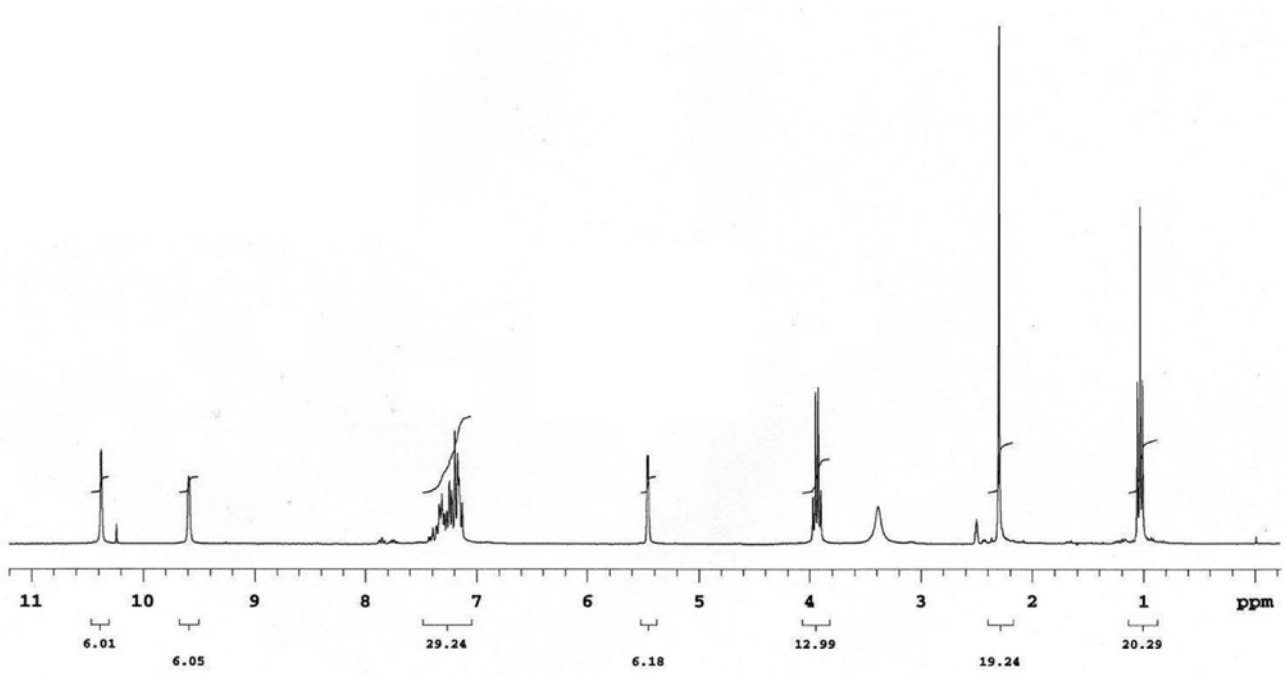


Figure S21. ^{1}H NMR (300 MHz, DMSO-d_6) of compound **1g**.

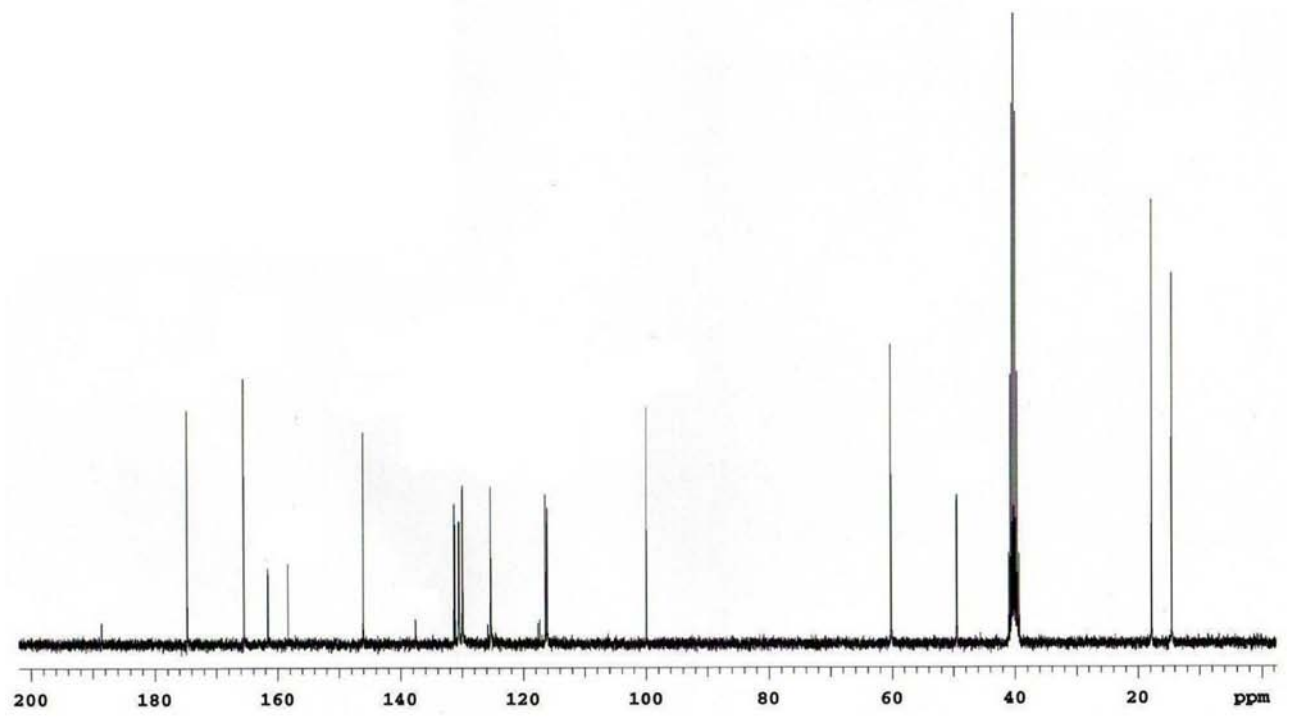


Figure S22. ^{13}C NMR (75 MHz, DMSO-d_6) of compound **1g**.

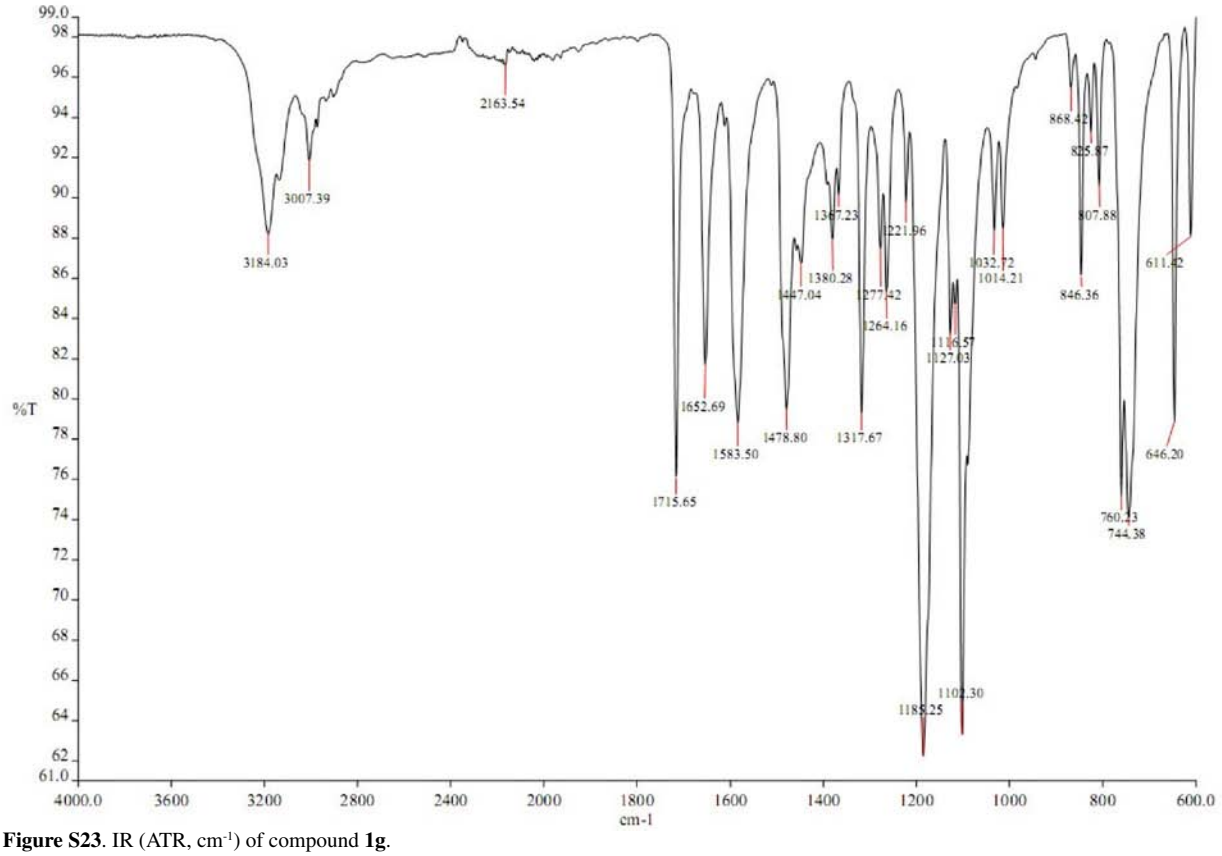


Figure S23. IR (ATR, cm^{-1}) of compound **1g**.

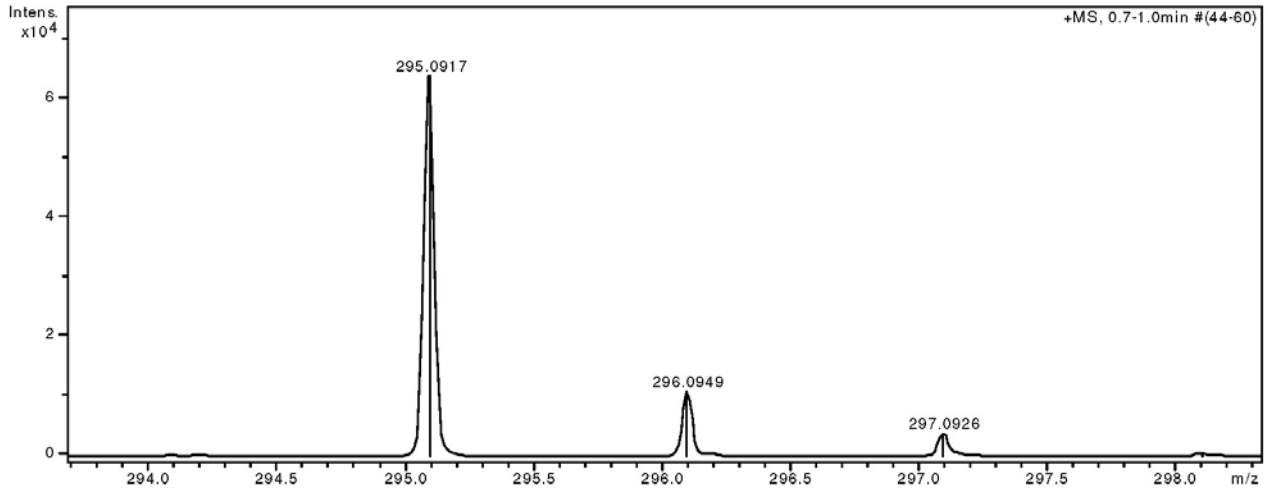


Figure S24. HRMS of compound **1g**.

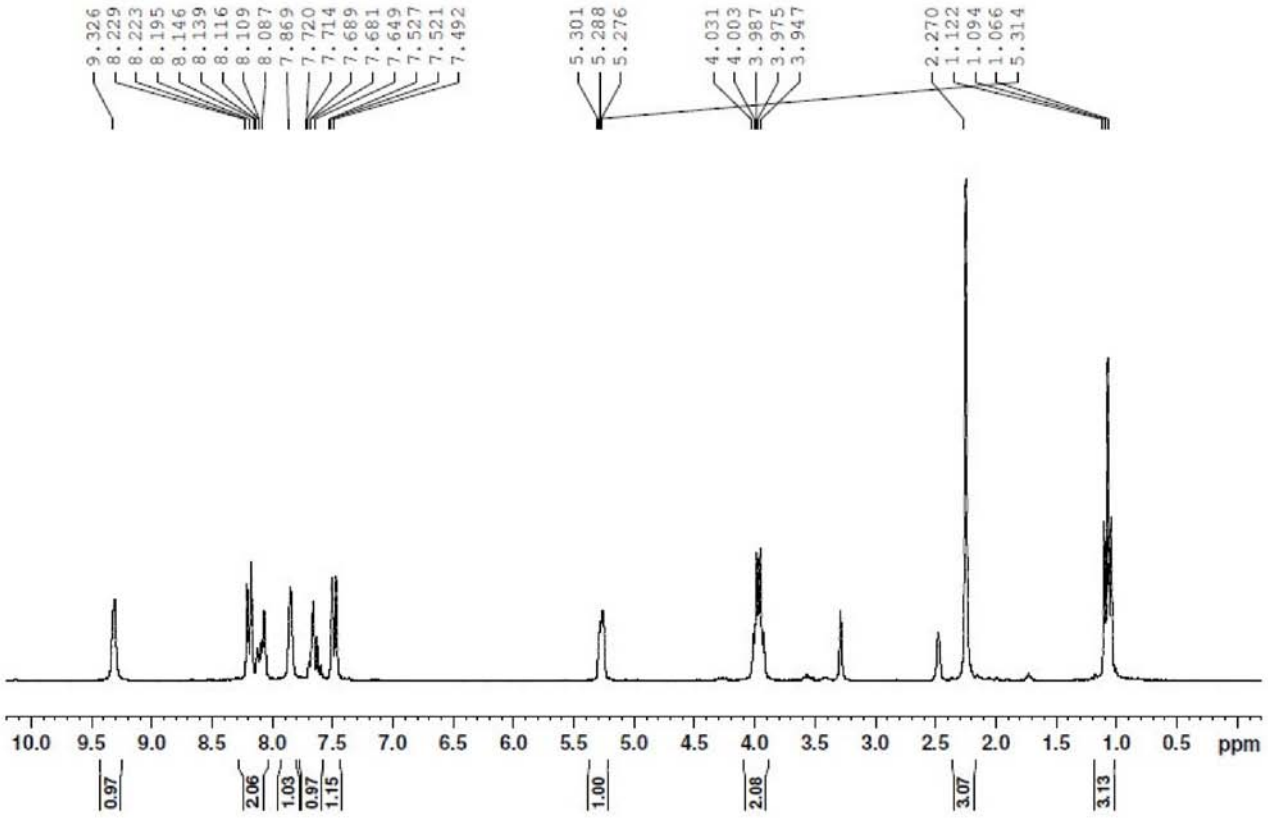


Figure S25. ^{1}H NMR (250 MHz, DMSO-d_6) of compound **2d**.

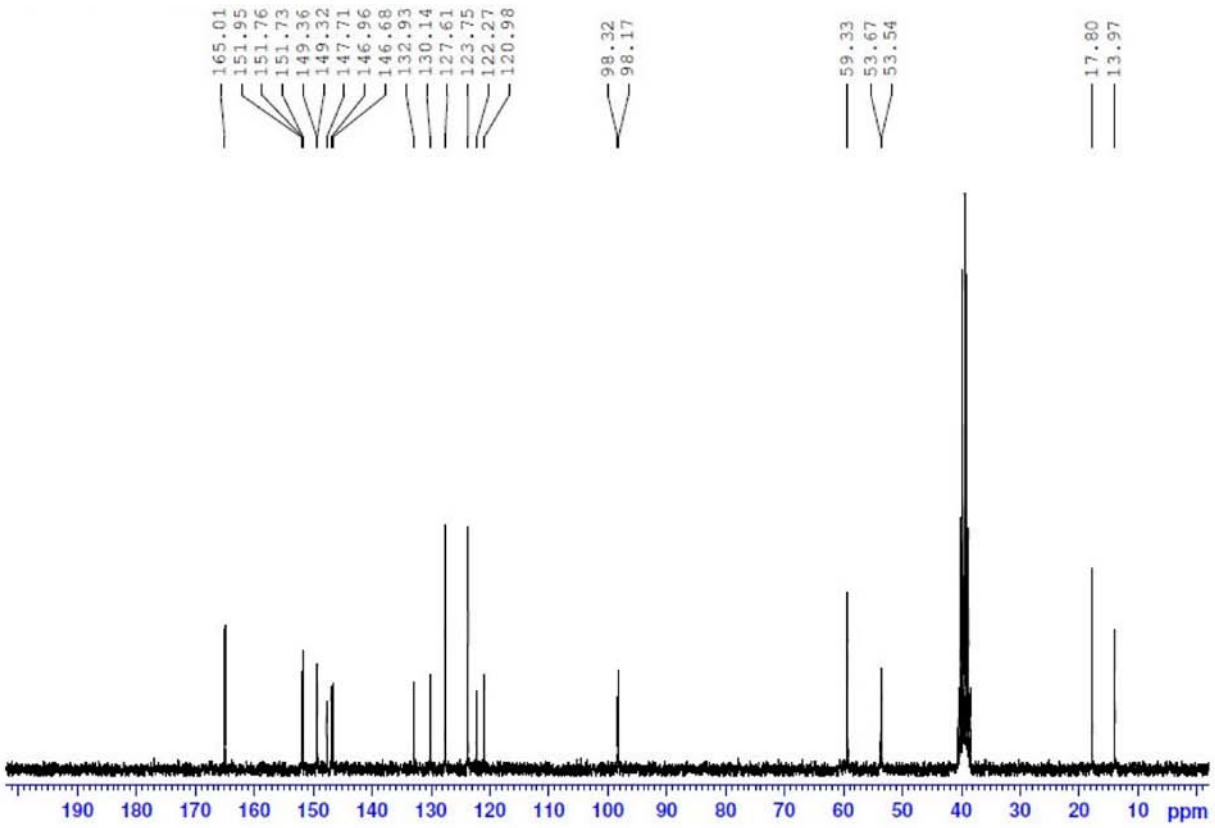


Figure S26. ^{13}C NMR (62.5 MHz, DMSO-d_6) of compound **2d**.

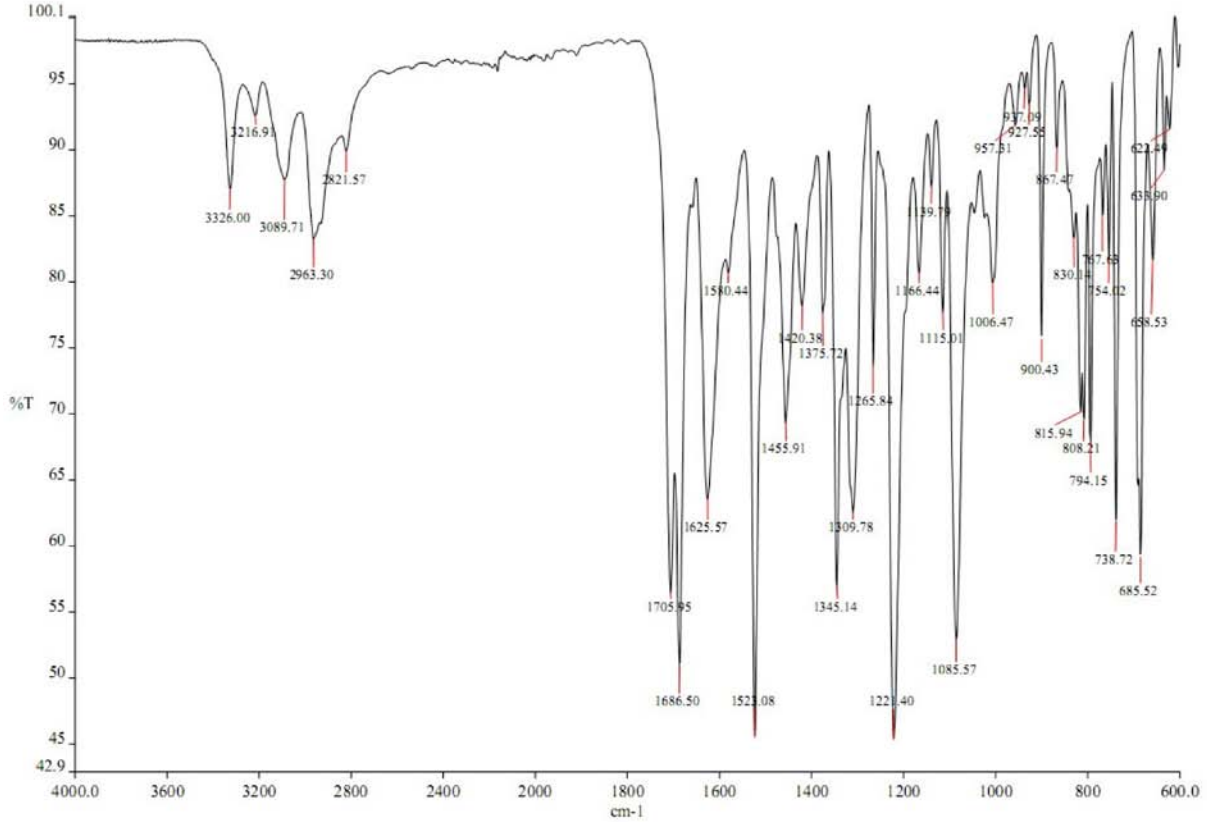


Figure S27. IR (ATR, cm^{-1}) of compound **2d**.

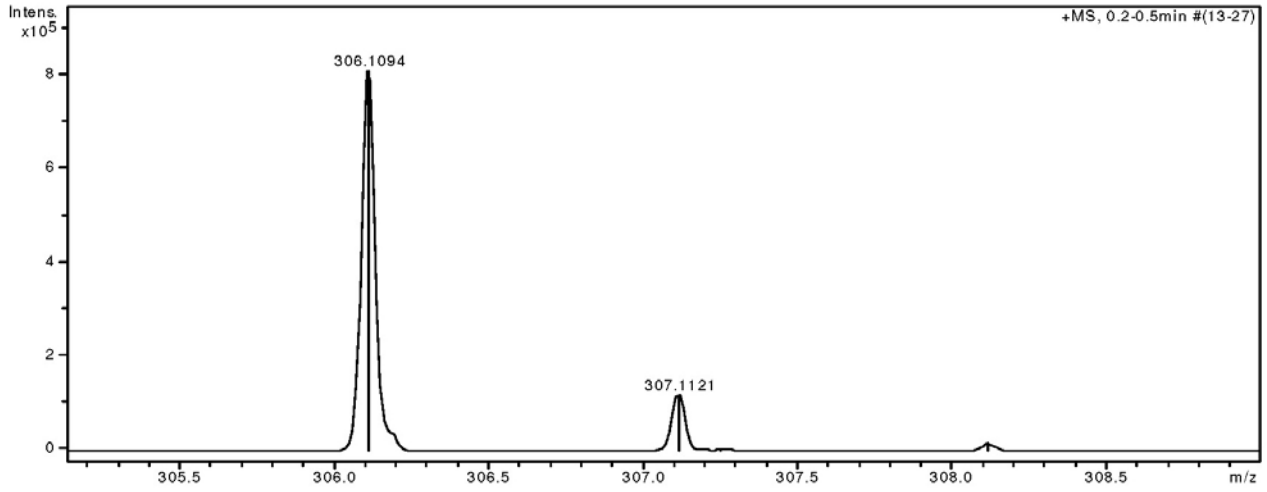


Figure S28. HRMS of compound **2d**.