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Nonclassical antifolates, Part 4. 5-(2-Aminothiazol-4-yl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols as a new class of DHFR inhibitors: Synthesis, biological evaluation and molecular modeling study

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PII: S0223-5234(13)00357-7

DOI: 10.1016/j.ejmech.2013.05.039

Reference: EJMECH 6217

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 28 February 2013

Revised Date: 19 May 2013

Accepted Date: 25 May 2013

Please cite this article as: G.S. Hassan, S.M. El-Messery, F.A.M. Al-Omary, S.T. Al-Rashood, M.I. Shabayek, Y.S. Abulfadl, E.-S.E. Habib, S.M. El-Hallouty, W. Fayad, K.M. Mohamed, B.S. El-Menshawi, H.I. El-Subbagh, Nonclassical antifolates, Part 4. 5-(2-Aminothiazol-4-yl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols as a new class of DHFR inhibitors: Synthesis, biological evaluation and molecular modeling study, *European Journal of Medicinal Chemistry* (2013), doi: 10.1016/j.ejmech.2013.05.039.

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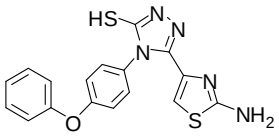
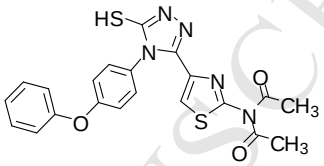
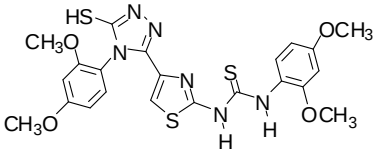


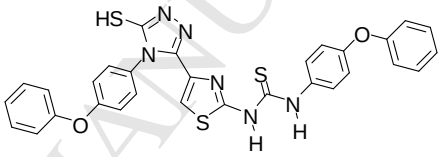
22 (Antifungal, antibacterial)



29 (Schistosomicidal)



34 (DHFR inhibitor, IC_{50} 0.03 μM)



36 (Antitumor)

Compounds **22, 29, 34** and **36** showed remarkable activities. Recognition with key amino acids Leu4 and Val1is essential for the new DHFR inhibitor **34** (IC_{50} 0.03 μM)

Nonclassical antifolates, Part 4. 5-(2-Aminothiazol-4-yl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols as a new class of DHFR inhibitors: Synthesis, biological evaluation and molecular modeling study. [#]

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Abstract

A new series of compounds possessing 5-(2-aminothiazol-4-yl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol skeleton was designed, synthesized, and evaluated for their *in vitro* DHFR inhibition, antimicrobial, antitumor and schistosomicidal activities. Four active compounds were allocated, the antibacterial **22** (comparable to gentamicin and ciprofloxacin), the schistosomicidal **29** (comparable to praziquantel), the DHFR inhibitor **34** (IC_{50} 0.03 μM, 2.7 fold more active than MTX), and the antitumor **36** (comparable to doxorubicin). Molecular modeling studies concluded that recognition with key amino acid Leu4 and Val1 is essential for DHFR binding. Flexible alignment and surface mapping revealed that the obtained model could be useful for the development of new class of DHFR inhibitors.

Key words: Synthesis, Substituted thiazoles, DHFR inhibitors, Molecular modeling study.

[#]For parts 1-3 see references 24-26.

1. Introduction

Dihydrofolate reductase (DHFR) is one of the key enzymes in the process of DNA replication, it catalyzes the transformation of 7,8-dihydrofolate into tetrahydrofolate [1]. The latter serves as a necessary cofactor in the important one-carbon transfer reactions involved in the pyrimidine, purine, and amino acid biosynthetic pathways. The lower levels of tetrahydrofolate result in decreased conversion of glycine to serine, reduced methionine synthesis, and lower thymidylate levels, with a subsequent arrest of DNA replication [2, 3]. DHFR has a proven record as a drug target in the treatment of bacterial, parasitic infections and cancer chemotherapy [4, 5]. Numerous studies showed that the structure of the classical DHFR inhibitor methotrexate (MTX) can be modified considerably while significant inhibitory potency still retained [6, 7]. Parasitic diseases are one of the causes of mortality and morbidity worldwide. The WHO estimates that one billion people are affected by parasitic infections, such as malaria, leishmaniasis, trypanosomiasis and schistosomiasis. The annual mortality associated with parasitic infections, is estimated 500,000 deaths [8]. The majority of the drugs used to treat these diseases is old and has several limitations, including high cost, poor efficacy, and toxicity [9]. Moreover, the development of drug resistance makes the search for new molecules able to act as selective and effective antiparasitic chemotherapeutic agents a very important task for medicinal chemists.

Thiazole heterocycle represents an important synthon which is responsible for numerous biological activities such as the antimicrobial agent Sulfatiazol [10], and the antineoplastic agents Tiazofurin (**A**), Netropsin **(B)**, Thia-netropsin **(C)**, Chart 1 [11-15]. On the other hand, 1,2,4-Triazole represents an important pharmacophore that plays a vital role as medicinal active agents exhibiting variety of biological potencies such as antimicrobial [16, 17], antifungal [18], antitubercular [19] and anticancer agents [20, 21]. Recently, hybrids of 4-phenyl-thiazole-1,3,5-triazines (**D**, Chart 1) are approved as effective parasitic and microbial dihydrofolate reductase (DHFR) inhibitors that selectively inhibit biochemical processes which are vital for parasite growth [22, 23].

In view of these facts, and in continuation to our previous efforts [24-33], the present study reports an efficient and reproducible synthesis of a new series of 2,4-substituted-thiazoles bearing 1,2,4-triazole-3-thione, acetylamino, and thioureido moieties known to contribute to biological potency [27-29], with anticipated DHFR inhibitory activity. The aim of this study is to

identify novel synthetic lead compound(s) tackling the folate pathway. Structure modification of MTX has been performed in this study by designing new compounds of different structure class and reduced molecular weight to define the structure requirements and features that enhance selectivity and specificity for the tight binding to DHFR. The synthesized derivatives were tested for their *in vitro* DHFR inhibition, and *in vitro* antimicrobial activity against a panel of standard strains of Gram-positive, Gram-negative bacteria and pathogenic fungi, in addition to the *in-vitro* antitumor activity using cell-based disease oriented approach [34-37**]** against Human hepatocellular carcinoma HepG2, caucasian breast adenocarcinoma MCF7, colon carcinoma HCT116, and lung carcinoma A549 cell lines. Furthermore, the synthesized compounds were screened for their antiparasitic activity using *schistosoma mansoni* as a model.

2. Results and Discussion

2.1 Chemistry

The synthesis of the target compounds is depicted in scheme 1. 2-Acetamido-thiazole-4-carboxyhydrazide (**1**) was prepared adopting reported procedures [38-41] and treated with a variety of phenylisothiocyanate derivatives (**2-8**) to give the *N*-{4-[2-(4-substituted-phenyl-carbamothioyl)hydrazinecarbonyl]thiazol-2-yl}acetamide derivatives (**9-15**) which were then cyclized and deacetylated using NaOH solution to produce the 5-(2-aminothiazol-4-yl)-4-substituted-phenyl-4*H*-1,2,4-triazole-3-thiols (**16-22**). The latter compounds were either acetylated by the aid of acetic anhydride to give compounds (**23-29**), or treated with the phenylisothiocyante derivatives (**2-8**) to produce the 1-[4-(5-mercapto-4-substituted-phenyl-4*H*-1,2,4-triazol-3-yl)thiazol-2-yl]-3-(4-substituted-phenyl)thiourea derivatives (**30-36**); (Scheme 1, Table 1). The ^{1}H NMR spectra of the synthesized intermediates **9-15** proved the presence of the phenylcarbamothioyl-hydrazinecarbonyl moiety in their structures by the appearance of aromatic protons signals appeared in the region 7.21–7.93 ppm which confirmed the structure of the molecule, while the signal for NH groups appeared as set of singlet at down field which could be attributed to the intra-molecular hydrogen-bonding. ^{1}H NMR spectra for compounds **23-29** proved the inclusion of diacetyl group into their structures by the appearance of two singlet peaks integrated for a couple of acetyl functions along with the absence of singlet peak of NH_2. The structures of compounds **30-36** were inferred by the presence of singlet peaks for two NH groups

at the expected range. The structures of the new compounds were verified by the aid of elemental analyses, mass spectrometry, ^{1}H, and ^{13}C-NMR spectra.

2.2 Dihydrofolate Reductase Inhibition

The synthesized compounds **16**-**19**, **21**-**27**, **29**, **31**-**36** were evaluated as inhibitors of bovine liver DHFR using reported procedure [42-44]. Results were reported as IC_{50} values (Table 2). All compounds showed weak to moderate DHFR inhibition with IC_{50} values range of 5.0-40.0 μM, except compound **34** (IC_{50}, 0.03 μM) which proved to be 2.7 fold more active than the positive control MTX (IC_{50}, 0.08 μM).

2.3 Antimicrobial Screening

The synthesized compounds **16-36** were tested for their *in vitro* antimicrobial activity against a panel of standard strains of the Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), the Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeuroginosa*), and the yeast-like pathogenic fungus *Candida albicans*. The primary screen was carried out using the agar disc-diffusion method using Müller-Hinton agar medium [45]. Results of the preliminary antimicrobial testing of the synthesized compounds, the antibacterial and the antifungal drugs are shown in Table 2. The results revealed that the majority of the synthesized compounds showed varying degrees of inhibition against the tested microorganisms. Gram-positive bacteria *B. subtilis* and Gram negative *E. coli* are considered the most sensitive among the tested microorganisms. Compounds **18, 22, 24, 25, 29, 32,** and **36** were moderate to strong active against the Gram-positive bacteria *S. aureus* and *B. subtilis*. The inhibitory activity against the tested Gram-negative bacteria *P. aeuroginosa* was rather lower than the other tested microorganisms. The minimal inhibitory concentration (MIC) for the most active compounds **18, 22, 24, 25, 29, 32,** and **36** against the same microorganism used in the primary screening was carried out using the micro-dilution susceptibility method in Müller-Hinton Broth as shown in Table 2 [46]. However, it could be concluded that the Gram-positive bacteria *B. subtilis* and to a certain extent *S. aureus* and the Gram-negative bacteria *E. coli* are sensitive to majority of the synthesized compounds. Compound **22**, **32**, and **36** showed remarkable antibacterial potency comparable to the known drugs gentamicin and ciprofloxacin antibiotics. None of the active compounds exert their activity through DHFR inhibition.

2.4 In vitro Antitumor Screening

The synthesized compounds **16-36** were subjected to *in-vitro* antitumor screening against human cancer cell lines using cell-based disease oriented approach [34-37**]**. Test compounds were used to evaluate their antitumor potency on four human tumor cell lines namely: hepatocellular carcinoma HepG2, caucasian breast adenocarcinoma MCF7, colon carcinoma HCT116, and lung carcinoma A549. Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] to purple Formosan [47,48]. A probit analysis was carried for LC_{50} determination using SPSS 11 program. The antitumor drug doxorubicin was used as positive control. In regard to the antitumor selectivity among the used tumor cell lines, compounds **23** and **26** proved to be selective toward the lung carcinoma A549 cell line with LC_{50} values of 33.2 and 35.4 μM, respectively; while compound **24** proved to be selective towards hepatocellular carcinoma HePG2 cell line with LC_{50} value of 29.1 μM. Compounds **28** and **36** exhibited antitumor potency comparable to the standard drug doxorubicin. Compound **28** showed LC_{50} values of 25.0, 18.9 and 23.8 μM against lung carcinoma A549, hepatocellular carcinoma HepG2, and caucasian breast adenocarcinoma MCF7 cell lines, respectively; while compound **36** exhibited LC_{50} values of 23.5 and 17.4 μM against colon carcinoma HCT116 and caucasian breast adenocarcinoma MCF7 cell lines, respectively.

2.5 Antischistosomal Screening

The synthesized compounds **16-36** were subjected to *in vitro* antischistosomal screening using reported procedures [49, 50**]**. The bio-screening results revealed that compounds **16**, **17**, **20**, **21**, **22**, **26**, and **29** possessed reproducible and confirmed *in vitro* antischistosomal activity with percentage mortality range of 5-100% at concentration of 50 μg/ml. Compound **27** proved to possess marginal activity manifested as worm deformation causing intensive contraction, bending and swelling of the worms, however, it did not cause mortality, compound **22** showed moderate activity of 55.6 % worm mortality; while **29** exihibted schistosomicidal potency comparable to praziquantel (the known antischistosomal drug) with 100% worm mortality.

3. Structure-Activity Correlation

Three different series of compounds were synthesized, namely: 5-(2-aminothiazol-4-yl)-4-substituted-phenyl-4*H*-1,2,4-triazole-3-thiols (**16-22**); *N*-acetyl-*N*-[4-(5-mercapto-4-substituted-phenyl-4*H*-1,2,4-triazol-3-yl)thiazol-2-yl]acetamides (**23-29**); and 1-[4-(5-mercapto-4-substituted-phenyl-4*H*-1,2,4-triazol-3-yl)thiazol-2-yl]-3-(4-substituted-phenyl)thiourea derivatives (**30-36**). The structure core skeleton of all of the synthesized compounds is 5-(2-aminothiazol-4-yl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol moiety, where it seemed to be that the type of substituent at the 2-amino group of the thiazole nucleus or at the 4-phenyl ring attached to the 1,2,4-triazole function manipulate the activity.

5-(2-Aminothiazol-4-yl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol (**16**) was tested and proved to be devoid of DHFR inhibition potency. Substitution at the 4-phenyl ring of the 1,2,4-triazole function of **16** either with electron withdrawing, (4-Cl, **17**) or electron donating functions, (2-CH_3O-, **18**; 4-CH_3O-, **19**; 4-CH_3-, **21**) brought the compounds into the activity side with IC_{50} range of 18-24 μM. Diacetylation of the 2-amino-thiazole function of **16** produced the moderate active compounds **24**, **26**, and **27** with IC_{50} range of 5-10 μM. The 2-amino function of **16** was used to synthesize the 3-phenyl-thioureido compounds with the production of the most active DHFR inhibitor in the present study, 1-(2,4-dimethoxyphenyl)-3-(4-(4-(2,4-dimethoxyphenyl)-5-mercapto-4H-1,2,4-triazol-3-yl)thiazol-2-yl)thiourea (**34**, IC_{50} value of 0.003 μM) which proved to be 2.7 fold more active than MTX. Compound **16** did not exert any antimicrobial activity. The introduction of electron withdrawing chlorine function to the 4-phenyl ring of the 1,2,4-triazole function produced **17** with antifungal activity. Replacing the chlorine atom of **17** with electron donating function as methoxy or methyl abolished the antimicrobial potency, but upon the introduction of phenoxy moiety to postion 4- of the phenyl ring of **16** produced **22** with a remarkable broad spectrum antimicrobial activity (comparable to gentamicin and ciprofloxacin). The 2-amino function of **16** was used to synthesize the 2-methoxy-phenyl-thioureido analogue **32** and the 4-phenoxy-phenyl-thioureido analogue **36** with a remarkable broad spectrum antibacterial activity. It looks that the antitumor potency is confined to the *N*-acetyl-acetamide series (**23-29**). The type of substituent on the 4-phenyl ring of the 1,2,4-triazole function of **23-29** manipulate the magnitude of activity. The tested compounds exhibited certain pattern of selectivity as shown in the un-substituted 4-phenyl analogue **23** proved toward lung carcinoma A549 cell line; the 4-(4-

chlorophenyl)- derivative **24** toward hepatocellular carcinoma HepG2; and the 4-(4-methoxyphenyl)- analogue **26** toward lung carcinoma A549. The introduction of 4-(4-phenoxyphenyl)- function produced **29** with abolished antitumor activity. Replacing the 2-diacetylamino function of **29** by 4-phenoxyphenyl-thioureido moiety produced **36** with broad spectrum antitumor activity comparable to doxorubicin. The antibacterial **22** showed moderate antischistosomal potency of 55.6 % worm mortality. Acetylation of the 2-amino function of **22** produced **29** with schistosomicidal activity comparable to praziquantel (100% worm mortality).

4. Molecular Modeling Study

The DHFR inhibitory activity of the new synthesized compounds **16**-**19**, **21**-**27**, **29**, **31**-**36** was experimentally determined showing that compound **34** (IC_{50}, 0.03 μM), in particular, is the most active derivative with 2.7 fold more active than the positive control MTX (IC_{50}, 0.08 μM). Molecular modeling study was essentially needed to understand and interpret the unusual DHFR inhibitory pattern of this new class of compounds. It was interesting to start a comparative modeling study of the most active DHFR inhibitor **34** and the least active compound **31** against MTX. The tertiary complex of human dihydrofolate reductase (hDHFR), NADPH and MTX were used as references for modeling and docking. The obtained results were quite interesting.

The binding mode of MTX to hDHFR is a complex interaction where the enzyme undergoes conformational changes resulting in tight binding; in addition to the ionic bonding of N1 and the 2-NH_2 group of MTX to Glu30, Figure 1a, [51-55]. The 3D binding mode and residues involved in the recognition of the most active compound **34** (IC_{50}, 0.03 μM) docked and minimized in the hDHFR binding pocket are shown in Figure 1b. The amino acid Leu4, which is not one of the key residues in the recognition of the parent ligand MTX, plays an essential role in the binding recognition of **34**, beside the involved hydrogen bonding network. Moreover, interaction to Val1 residue occurred in the hDHFR binding pocket seemed to be crucial for binding which explains the difference in the magnitude of activity of **31** and **34**. Compound **34** interacts with Val1 residue through the 1,2,4-triazole moiety, while compound **31** interacts surprisingly through the thiazole function with complete absence of binding to Leu4, and hence the loss of its DHFR inhibition potency (IC_{50}, 40.0 μM), Figure 1c. In addition, each of compound **34** or **31** has its

own unique pattern of binding profile, where the most active compound **34** is located deeply inside the pocket (Figure 2a), while the least active **31** is mostly exposed out of the binding pocket to the solvent surface (Figure 2b). Ligand-based active site alignment is a widely adopted technique for the structural analysis of protein–ligand complexes. A good alignment occurs if the strain energy of each molecule is small; molecules having a similar shape and their aromatic parts overlap [56]. The objective of computing a multiple ligands alignment is to maximize the similarity among those ligands, while keeping their sound conformations. So to probe similarity between the 3D structures of the most active compound **34** and MTX, flexible alignment was employed. The initial approach was to employ MOE/MMFF94 flexible alignment to automatically generate superposition of the compounds under investigation with minimal user bias [57]. 200 conformers of each compound were generated and minimized with a distance-dependant dielectric model. A low energy set of 100 was selected for further analysis. The top scoring alignment with the least strain energy is shown in Figure 3a; where a good alignment between compound **34** (IC_{50}, 0.03 μM) and MTX explains its activity. On the contrary, Figure 3b obviously indicated a different alignment profiles for compound **31** (IC_{50}, 40.0 μM) and MTX. These findings are in consistent with the obtained DHFR inhibition experimental data. In a further attempt to reveal the reasons behind the diminished DHFR inhibition of **31**, in comparison to the remarkable activity of **34**, hydrophobic surface mapping study was conducted. Compound **34**, showed more hydrophobic regions which could be attributed to the presence of the two methoxy groups which are responsible for the interaction with amino acid residues inside the enzyme active pocket**,** Figure 4a. On the other hand, the hydrophobic regions of the least active compound **31** are far less due to the presence of chlorine atoms, hence the required lipophilicity for the effective binding to DHFR is diminished, Figure 4b.

The performed molecular modeling studies and the proposed binding mode analysis for **34** revealed common features which highlight the importance of hydrophobicity, aromaticity and hydrogen bonding in such a crucial interaction with the active site. Recognition of **34** by Leu4 residue and the interaction of its 1,2,4-triazole moiety by Val1 residue of the DHFR active site are of primary importance for activity.

5. Conclusion

Compounds **16-22**, **23-29**, and **30-36** represent three different series of compounds designed and synthesized, with a structure core skeleton of 5-(2-aminothiazol-4-yl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol moiety. It seemed to be that the type of substituent at the 2-amino group of the thiazole nucleus or at the 4-phenyl ring attached to the 1,2,4-triazole function manipulate the type and magnitude of activity. Molecular modeling study performed and concluded that recognition with key amino acid Leu4 and Val1 is essential for binding and the DHFR inhibition activity. Flexible alignment with minimal user bias showed a good alignment between compound **34** (IC_{50}, 0.03 μM) and MTX explaining its activity. Four active compounds were allocated in the present study, the broad spectrum antibacterial **22** (comparable to gentamicin and ciprofloxacin), the schistosomicidal **29** (comparable to praziquantel), the DHFR inhibitor **34** (IC_{50}, 0.03 μM; 2.7 fold more active than MTX), and the antitumor **36** (comparable to doxorubicin); Figure 5. 5-(2-Aminothiazol-4-yl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol skeleton as a new class of DHFR inhibitors, could be used as a useful template for future development.

6. Experimental Part

Melting points (°C) were determined on Mettler FP80 melting point apparatus and are uncorrected. Microanalyses were performed on a Perkin-Elmer 240 elemental analyzer at the Central Research Laboratory, College of Pharmacy, King Saud University. All of the new compounds were analyzed for C, H and N and agreed with the proposed structures within ± 0.4% of the theoretical values. ^{1}H, ^{13}C-NMR spectra were recorded on a on Bruker 500 MHz FT spectrometer (the Central Research Laboratory, College of Pharmacy, King Saud University); chemical shifts are expressed in δ ppm with reference to TMS. Mass spectral (MS) data were obtained on a Perkin Elmer, Clarus 600 GC/MS and Joel JMS-AX 500 mass spectrometers. Thin layer chromatography was performed on precoated (0.25 mm) silica gel GF_{254} plates (E. Merck, Germany), compounds were detected with 254 nm UV lamp. Silica gel (60–230 mesh) was employed for routine column chromatography separations. DHFR inhibition activity experiments were performed at Pharmacology Department, Facult of Pharmacy; Future University in Egypt. Bovine liver DHFR enzyme and methotrexate (MTX) were used in the assay (Sigma Chemical Co, USA). Elisa reader SN208125 Bio Tek MQX200 and Software program GEN5 at wave

length 340 nm were used to measure the changes in absorbance. The *in vitro* antimicrobial testing was performed at Department of Microbiology, Faculty of Pharmacy, Mansoura University, Egypt. The agar disc-diffusion method and a panel of standard strains (*S. aureus* IFO 3060, *B. subtilis* IFO 3007, *M. luteus* IFO 3232, *E. coli* IFO 3301, and *P. aeuroginosa* IFO 3448) were employed**.** *In vitro* antitumor and schistosomicidal activities were conducted at Drug Bioassay-Cell Culture Laboratory, Pharmacognosy Department, Pharmaceutical and Drug Industries Division, National Research Center, Giza, Egypt. *Schistosoma mansoni* adult worms were obtained from the Schistosomes Biological Supply Center at Theodor Bilharz Research Institute, Cairo, Egypt. Concerning the molecular modeling study, all experiments were conducted with Hyperchem 8.0.5 package from Hypercube running on a PC computer. The docking of test compounds into DHFR pocket was performed with MOE software. Enzyme structure, starting coordinate of hDHFR enzyme in tertiary complex with reduced-nicotinamide adenine dinucleotide phosphate (NADPH) and MTX, code ID 3EIG, were obtained from the Protein Data Bank of Brookhaven National Laboratory [58].

6.1. Chemistry

*6.1.1. N-{4-[2-(4-Substituted-phenylcarbamothioyl)hydrazinecarbonyl]thiazol-2-yl}acetamide derivatives (**9-15**)*

A solution of the acid hydrazide **1** (0.5 g, 2.5 mmol), the appropriate phenylisothiocynate (**2-8,** 3.5 mmol) in ethanol was heated under reflux for 3 hrs, and then cooled. The separated solid was filtered, washed with aqueous ethanol and recrystallized from ethanol to yield the required products **9-15** (Table 1). **9:** ^{1}H-NMR (DMSO-d_6) δ 2.18 (s, 3H, CH_3), 7.15 (s, 1H, thiazole-H), 7.33 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.48 (d, 2H, *J* = 7 Hz, Ar-H), 7.94 (s, 1H, Ar-H), 9.81 (brs, 2H, 2 NH), 10.10 (s, 1H, NH), 12.34 (s, 1H, NH). **MS** *m/z* (%): 335 (2.5, M^+). **10:** ^{1}H-NMR (DMSO-d_6) δ 2.16 (s, 3H, CH_3), 7.39 (s, 1H, thiazole-H), 7.51 (d, 2H, *J* = 8 Hz, Ar-H), 7.93 (d, 2H, *J* = 7.5 Hz, Ar-H), 9.12 (brs, 1H, NH), 9.84 (s, 1H, NH), 10.12 (s, 1H, NH), 10.33 (s, 1H, NH). **MS** *m/z* (%): 370 (1.1, M^+). **11:** ^{1}H-NMR (DMSO-d_6) δ 2.18 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 6.91 (t, 2H, *J* = 13.5 Hz, Ar-H), 7.05 (s, 1H, thiazole-H), 7.14 (d, 2H, *J* = 7 Hz, Ar-H), 7.96 (s, 2H, 2NH), 10.27 (s, 1H, NH), 12.38 (s, 1H, NH). **MS** *m/z* (%): 365 (0.16, M^+). **12:** ^{1}H-NMR (DMSO-d_6) δ 2.19 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 6.89 (d, 2H, *J* = 6 Hz, Ar-H), 7.30 (d, 2H, *J* = 7 Hz, Ar-H), 7.94 (s, 1H, thiazole-H), 9.70 (s, 2H, 2NH), 10.10 (s, 1H, NH), 12.34 (s, 1H, NH).

^{13}C-NMR δ 22.5, 55.2, 113.3, 118.5, 127.2, 131.9, 132.6, 142.8, 149.3, 156.6, 157.7, 161.5, 169.1, 180.9. **MS** *m/z* (%): 365 (1.0, M^+). **13:** ^{1}H-NMR (DMSO-d_6) δ 2.19 (s, 3H, CH_3), 3.77 (s, 6H, OCH_3), 6.51 (d, 2H, *J* = 8 Hz, Ar-H), 6.60 (s, 1H, Ar-H), 7.62 (brs, 1H, NH), 7.94 (s, 1H, thiazole-H), 9.24 (s, 1H, NH), 10.15 (s, 1H, NH), 12.35 (s, 1H, NH). ^{13}C-NMR δ 22.5, 55.3, 55.7, 98.8, 104.0, 112.9, 113.2, 118.5, 122.3, 127.4, 132, 133.4, 148.7, 158, 169.1. **MS** *m/z* (%): 395 (4.8, M^+). **14:** ^{1}H-NMR (DMSO-d_6) δ 2.18 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 7.13 (d, 2H, *J* = 6.5 Hz, Ar-H), 7.33 (d, 2H, *J* = 6.9 Hz, Ar-H), 7.93 (s, 1H, thiazole-H), 9.71 (s, 2H, 2NH), 10.06 (s, 1H, NH), 12.32 (s, 1H, NH). ^{13}C-NMR δ 20.5, 22.4, 98.4, 110.2, 111.2, 112.8, 112.9, 113.5, 117.4, 118.5, 128.5, 133.9, 136.6, 169.1. **MS** *m/z* (%): 349 (5.2, M^+). **15:** ^{1}H-NMR (DMSO-d_6) δ 2.18 (s, 3H, CH_3), 7.10-7.14 (m, 5H, Ar-H), 7.41 (d, 4H, *J* = 25.5 Hz, Ar-H), 7.93 (s, 1H, thiazole-H), 9.73 (brs, 2H, 2NH), 10.10 (s, 1H, NH), 12.33 (s, 1H, NH). ^{13}C-NMR δ 22.5, 100.4, 101.5, 104.1, 111.0, 112.4, 113.8, 118.3, 118.6, 123.3, 127.4, 130.1, 131.0, 134.7, 156.9, 157.7, 169.1. **MS** *m/z* (%): 427 (3.3, M^+).

*6.1.2. 5-(2-Aminothiazol-4-yl)-4-substituted-phenyl-4H-1,2,4-triazole-3-thiols (**16-22**)*

The thiosemicarbazides **9-15** (10 mmol) were refluxed in NaOH solution (2N, 30 ml) for 3 hrs. The resultant solution was cooled, neutralized to pH 6 using dilute hydrochloric acid to give the crude form of the target compounds. This crude compounds were filtered, washed with water and crystallized from aqueous ethanol (Table 1). **16:** ^{1}H-NMR (DMSO-d_6) δ 6.27 (s, 1H, thiazole-H), 7.13 (s, 2H, NH_2), 7.34 (d, 2H, *J* = 3.5 Hz, Ar-H), 7.53 (s, 3H, Ar-H), 14.00 (s, 1H, SH).^{13}C-NMR δ 109.7, 128.5, 129.2, 129.4, 134.7, 136.2, 146.6, 168.2, 168.3. **MS** *m/z* (%): 275 (8.7, M^+). **17:** ^{1}H-NMR (DMSO-d_6) δ 6.02 (s, 1H, thiazole-H), 6.51 (s, 1H, SH), 7.11 (s, 2H, NH_2), 7.38 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.59 (d, 2H, *J* = 8.5 Hz, Ar-H). ^{13}C-NMR δ 110.0, 129.2, 130.4, 130.5, 131.9, 133.7, 136.1, 146.5, 151.8, 164.1, 168.3. **MS** *m/z* (%): 309 (2.2, M^+). **18:** ^{1}H-NMR (DMSO-d_6) δ 3.67 (s, 3H, OCH_3), 6.15 (s, 1H, thiazole-H), 7.11-7.13 (m, 3H, NH_2 and Ar-H), 7.22 (d, 1H, *J* = 8 Hz, Ar-H), 7.32 (d, 1H, *J* = 7 Hz, Ar-H), 7.52 (t, 1H, *J* = 14.5 Hz, Ar-H),13.91 (s, 1H, SH). ^{13}C-NMR δ 55.8, 108.3, 112.9, 120.6, 120.8, 123.3, 130.6, 131.3, 136.5, 146.9, 154.8, 168.1. **MS** *m/z* (%): 305 (7.1, M^+). **19:** ^{1}H-NMR (DMSO-d_6) δ 3.68 (s, 3H, OCH_3), 4.55 (s, 1H, SH), 6.77 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.95-7.00 (m, 3H, NH_2 and thiazole-H), 7.13 (d, 1H, *J* = 8 Hz, Ar-H), 7.26 (d, 1H, *J* = 8.5 Hz, Ar-H). ^{13}C-NMR δ 55.4, 106.6, 114.2, 114.5, 124.8, 128.1, 143.1, 149.3, 150.3, 150.6, 159.7, 170.5. **MS** *m/z* (%): 305 (5.0, M^+). **20:** ^{1}H-NMR (DMSO-d_6) δ 3.67 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.14 (s, 1H, thiazole-H), 6.69 (d, 1H, *J* =

8.5 Hz, Ar-H), 6.75 (s, 1H, Ar-H), 7.13 (s, 2H, NH_2), 7.21 (d, 1H, *J* = 8.5 Hz, Ar-H), 13.85 (s, 1H, SH). ^{13}C-NMR δ 55.5, 56.0, 99.7, 105.5, 108.3, 116.1, 130.7, 136.6, 147.1, 155.8, 161.4, 168.0, 168.8. **MS** *m/z* (%): 335 (9.2, M^+). **21:** ^{1}H-NMR (DMSO-d_6) δ 2.39 (s, 3H, CH_3), 6.24 (s, 1H, thiazole-H), 7.14 (s, 2H, NH_2), 7.22 (d, 2H, *J* = 8 Hz, Ar-H), 7.33 (d, 2H, *J* = 8 Hz, Ar-H), 13.96 (s, 1H, SH). ^{13}C-NMR δ 20.8, 100.5, 104.8, 109.7, 128.2, 129.8, 132.1, 136.2, 139, 146.7, 168.2, 168.3. **MS** *m/z* (%): 289 (11.4, M^+). **22:** ^{1}H-NMR (DMSO-d_6) δ 6.38 (s, 1H, thiazole-H), 7.09 (d, 4H, *J* = 9 Hz, Ar-H), 7.15 (s, 2H, NH_2), 7.23 (t, 1H, *J* = 6.5 Hz, Ar-H), 7.34 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.46 (t, 2H, *J* = 7.5 Hz, Ar-H), 13.99 (s, 1H, SH). ^{13}C-NMR δ 109.4, 118.3, 119.5, 124.2, 128.5, 130.2, 138.7, 144.5, 155.4, 156.0, 156.5, 157.3, 158.4, 168.1, 168.9. **MS** *m/z* (%): 367 (2.8, M^+).

*6.1.3. N-Acetyl-N-[4-(5-mercapto-4-substituted-phenyl-4H-1,2,4-triazol-3-yl)thiazol-2-yl]-acetamide derivatives (**23-29**)*

A solution of compounds **16-22** (2.5 mmol) in acetic anhydride (5.0 ml) were heated for 2 hours, after which the solution was poured in ice-water solution. The products formed were filtered, washed with excessive amount of water, and finally crystallized from ethanol to yield the target compounds **23-29** (Table 1). **23:** ^{1}H-NMR (DMSO-d_6) δ 2.13 (s, 3H, CH_3CO), 2.71 (s, 3H, CH_3CO), 6.63 (s, 1H, thiazole-H), 7.39 (d, 2H, Ar-H), 7.60 (t, 3H, Ar-H), 12.50 (s, 1H, SH). ^{13}C-NMR δ 22.4, 25.4, 60.3, 65.0, 117.8, 128.8, 130.2, 134.2, 145.4, 158.5, 167.8, 169.1, 171.4. **MS** *m/z* (%): 359 (9.4, M^+). **24:** ^{1}H-NMR (DMSO-d_6) δ 2.14 (s, 3H, CH_3CO), 2.70 (s, 3H, CH_3CO), 6.88 (s, 1H, thiazole-H), 7.50 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.67 (d, 2H, *J* = 8 Hz, Ar-H), 12.45 (s, 1H, SH). ^{13}C-NMR δ 22.4, 24.7, 62.9, 63.4, 118.4, 129.9, 130.8, 133.1, 134.0, 134.8, 141.0, 145.3, 158.6, 169.3, 172.0. **MS** *m/z* (%): 394 (12.5, M^+). **25:** ^{1}H-NMR (DMSO-d_6) δ 2.14 (s, 3H, CH_3CO), 2.70 (s, 3H, CH_3CO), 3.63 (s, 3H, OCH_3), 6.66 (s, 1H, thiazole-H), 7.18 (t, 1H, *J* = 14 Hz, Ar-H), 7.28 (d, 1H, *J* = 8.5 Hz, Ar-H), 7.44 (d, 1H, *J* = 7 Hz, Ar-H), 7.61 (t, 1H, *J* = 15 Hz, Ar-H), 12.51 (s, 1H, SH). ^{13}C-NMR δ 22.4, 24.7, 56.0, 62.3, 113.3, 116.8, 122.3, 130.3, 132.1, 134.2, 145.7, 154.6, 158.5, 167.8., 169.1, 171.9. **MS** *m/z* (%): 389 (7.2, M^+). **26:** ^{1}H-NMR (DMSO-d_6) δ 2.14 (s, 3H, CH_3CO), 2.70 (s, 3H, CH_3CO), 3.85 (s, 3H, OCH_3), 6.63 (s, 1H, thiazole-H), 7.13 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.35 (d, 2H, *J* = 8 Hz, Ar-H), 12.53 (s, 1H, SH). ^{13}C-NMR δ 21.1, 22.4, 24.7, 55.5, 61.8, 115.0, 117.9, 126.6, 130.1, 134.2, 145.7, 158.5, 160.1, 167.8, 169.1, 169.6. **MS** *m/z* (%): 389 (15.1, M^+). **27:** ^{1}H-NMR (DMSO-d_6) δ 2.14 (s, 3H, CH_3CO), 2.73 (s, 3H, CH_3CO), 3.60 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 6.69 (s, 1H, thiazole-

H), 6.73 (d, 1H, *J* = 9.5 Hz, Ar-H), 6.81 (s, 1H, Ar-H), 7.33 (d, 1H, *J* = 9 Hz, Ar-H), 12.55 (s, 1H, SH). ^{13}C-NMR δ 22.4, 24.7, 55.6, 56.1, 99.8, 105.6, 106.1, 114.4, 115.1, 130.9, 134.3, 145.9, 158.5, 162.0, 167.8, 169.0, 172.0. **MS** *m/z* (%): 419 (3.6, M^+). **28:** ^{1}H-NMR (DMSO-d_6) δ 2.14 (s, 3H, CH_3CO), 2.26 (s, 3H, CH_3), 2.71 (s, 3H, CH_3CO), 6.64 (s, 1H, thiazole-H), 7.31 (d, 2H, *J* = 7 Hz, Ar-H), 7.40 (d, 2H, *J* = 7 Hz, Ar-H), 12.52 (s, 1H, SH). ^{13}C-NMR δ 20.9, 22.4, 24.7, 55.9, 58.3, 117.9, 128.5, 130.3, 131.6, 134.2, 139.2, 145.5, 158.5, 167.9, 169.1, 169.4. **MS** *m/z* (%): 373 (4.1, M^+). **29:** ^{1}H-NMR (DMSO-d_6) δ 2.15 (s, 3H, CH_3CO), 2.70 (s, 3H, CH_3CO), 6.76 (s, 1H, thiazole-H), 7.17 (d, 4H, *J* = 6.5 Hz, Ar-H), 7.23 (t, 1H, *J* = 14 Hz, Ar-H), 7.43 (d, 2H, *J* = 8 Hz, Ar-H), 7.47 (t, 2H, *J* = 14 Hz, Ar-H), 12.53 (s, 1H, SH). ^{13}C-NMR δ 22.4, 24.7, 55.3, 56.9, 118.2, 118.9, 119.5, 124.4, 128.8, 130.3, 130.6, 134.1, 145.6, 155.6, 158.1, 158.5, 167.8, 169.1, 169.4. **MS** *m/z* (%): 451 (8.6, M^+).

*6.1.4. 1-[4-(5-Mercapto-4-substituted-phenyl-4H-1,2,4-triazol-3-yl)thiazol-2-yl]-3-(4-substituted-phenyl)thiourea derivatives (**30-36**)*

The 1,2,4-triazole derivatives (**16-22**, 2.5 mmol) were heated with phenylisothiocyanate derivatives (**2-8**, 3.5 mmol) in ethanol under reflux for 4 hrs. The separated solid obtained upon cooling was filtered, washed with water and crystallized from ethanol to yield the pure compounds **30-36** (Table 1). **30:** ^{1}H-NMR (DMSO-d_6) δ 3.37 (brs, 1H, NH), 3.44 (brs, 1H, NH), 6.26 (s, 1H, thiazole-H), 7.13 (s, 2H, Ar-H), 7.36 (s, 2H, Ar-H), 7.53-7.56 (m, 6H, Ar-H), 14.00 (s, 1H, SH). ^{13}C-NMR δ 99.9, 104.8, 109.7, 111.0, 114.2, 116.9, 118.7, 119.0, 128.5, 129.2, 129.4, 134.7, 136.2, 146.6, 168.2, 168.8. **MS** *m/z* (%): 410 (4.4, M^+). **31:** ^{1}H-NMR (DMSO-d_6) δ 3.71 (brs, 2H, 2NH), 6.02 (s, 1H, thiazole-H), 7.30 (d, 2H, *J* = 8 Hz, Ar-H), 7.39 (d, 2H, *J* = 8 Hz, Ar-H), 7.49 (d, 2H, *J* = 7 Hz, Ar-H), 7.62 (d, 2H, *J* = 8 Hz, Ar-H), 12.85 (s, 1H, SH). ^{13}C-NMR δ 102.3, 110.9, 112.5, 115.6, 117.1, 117.9, 119.3, 128.5, 129.4, 129.5, 130.5, 131.1, 131.9, 133.7, 147.9, 151.7, 164.1, 168.5. **MS** *m/z* (%): 479 (0.9, M^+). **32:** ^{1}H-NMR (DMSO-d_6) δ 3.79 (s, 6H, OCH_3), 6.92 (t, 2H, *J* = 14 Hz, Ar-H), 7.05 (d, 2H, *J* = 6.5 Hz, Ar-H), 7.14 (t, 2H, *J* = 14 Hz, Ar-H), 7.22 (brs, 2H, 2NH), 7.39 (s, 1H, thiazole-H), 8.13 (d, 2H, *J* = 6.5 Hz, Ar-H), 10.79 (s, 1H, SH). ^{13}C-NMR δ 55.5, 55.8, 100.0, 101.5. 106.0, 107.9, 111.3, 113.2, 114.2, 115.8, 119.8, 124.1, 125.4, 127.6, 138.1, 139.0, 143.3, 150.9, 164.8, 168.4. **MS** *m/z* (%): 470 (2.8, M^+). **33:** ^{1}H-NMR (DMSO-d_6) δ 3.82 (s, 6H, OCH_3), 6.09 (brs, 2H, 2NH), 7.03 (d, 4H, *J* = 8.5 Hz, Ar-H), 7.10 (s, 1H, thiazole-H), 7.20 (d, 4H, *J* = 8.5 Hz, Ar-H), 13.89 (brs, 1H, SH). ^{13}C-NMR δ 55.3, 55.8, 101.3, 104.8, 107.7, 108.5, 111.4, 114.2, 115.2, 117.5, 119.0, 129.6, 130.5, 135.4, 137.3,

139.0, 146.9, 159.2, 164.3, 138.1. **MS** *m/z* (%): 470 (5.4, M^+). **34:** ^{1}H-NMR (DMSO-d_6) δ 3.75 (s, 6H, OCH_3), 3.84 (s, 6H, OCH_3), 6.14 (s, 1H, thiazole-H), 6.64 (d, 2H, *J* = 8.5 Hz, Ar-H), 6.68 (s, 2H, Ar-H), 7.10 (d, 2H, *J* = 8.5 Hz, Ar-H), 12.63 (s, 2H, 2NH), 13.90 (s, 1H, SH). ^{13}C-NMR δ 55.2, 55.5, 55.8, 56.0, 99.5, 99.7, 105.3, 105.5, 108.4, 114.0, 116.0, 130.7, 131.2, 146.8, 152.5, 155.8, 156.2, 161.3, 161.5, 164.9, 168.2, 168.7. **MS** *m/z* (%): 530 (2.4, M^+). **35:** ^{1}H-NMR (DMSO-d_6) δ 2.38 (s, 6H, CH_3), 6.24 (s, 1H, thiazole-H), 4.15 (s, 2H, 2NH), 7.22 (d, 4H, *J* = 14 Hz, Ar-H), 7.32 (d, 4H, *J* = 14 Hz, Ar-H), 13.98 (s, 1H, SH). ^{13}C-NMR δ 20.8, 21.3, 55.5, 101.5. 107.9, 109.7, 124.1, 125.4, 127.6, 128.2, 129.8, 132.1, 136.2, 138.1, 139.0, 139.3, 143.3, 146.7, 168.2, 168.4. **MS** *m/z* (%): 438 (12.8, M^+). **36:** ^{1}H-NMR (DMSO-d_6) δ 6.37 (s, 1H, thiazole-H), 6.87-6.94 (m, 4H, Ar-H), 7.04 (t, 3H, *J* = 8.5 Hz, Ar-H), 7.22 (d, 4H, *J* = 8 Hz, Ar-H), 7.44 (d, 4H, *J* = 6.5 Hz, Ar-H),7.65 (t, 3H, *J* = 6.5 Hz, Ar-H), 12.81 (s, 2H, 2NH), 14.00 (s, 1H, SH). ^{13}C-NMR δ 109.9, 117.5, 118.6, 119.2, 119.5, 119.7, 122.7, 124.1, 124.2, 127.4, 130.2, 130.3, 136.1, 136.1, 146.8, 148.6, 150.3, 152.1, 155.7, 155.8, 156.0, 157.2, 157.5, 157.7, 164.2, 168.3. **MS** *m/z* (%): 594 (2.5, M^+).

6.2. Dihydrofolate Reductase (DHFR) Inhibition Assay

The assay mixture contained 0.5 mol/l Tris buffer (A), pH 7.5 and 0.05 mol/l Tris buffer (B), pH 7.5. Stock solutions of FH_2 (25 mg in 1.5 ml of 2-mercaptoethanol and 6.0 ml of buffer A in 0.25 ml aliquots), NADPH (50 mg in 10 ml of buffer (A) in 0.4 ml aliquots), and DHFR (2.1 U in 10 ml of buffer (B) in 0.5 ml aliquots) stored at −70 °C. 130 µl FH_2 reaction solutions, 0.25 ml aliquot of FH_2 stock solution in 8.0 ml of buffer B, yielding a final working concentration of 0.104 g/l, were added to each well of the 96-well flat-bottom microplate reader. 20 µl MTX calibrators or unknown samples and blank of different concentrations 10^{-11} to 10^{-5} (adjusted by DMSO) were added into duplicate wells. The microplate was shaken in a shaking incubator for 1 min, after which 50 µl NADPH/DHFR reaction solution, 0.4-ml aliquot of NADPH stock solution and 0.5 ml aliquot of DHFR stock solution in 6.0 ml of buffer B, yielding a final working concentration of 0.29 g NADPH/l and 15u DHFR/l were added to each well, and the microplate was again shaken in a shaking incubator for 1 min. The absorbance of each well was read in the microplate reader at room temperature at wavelengths of 340 nm [42-44], using the kinetic mode with a reading interval of 1 min for duration of 10 min. The changes in absorbance were downloaded directly into an ELISA reader SN208125 Bio Tek MQX200 computer and

analyzed with software Gen5 wave length 340 nm. Results were reported as % inhibition of enzymatic activity calculated using the following formula:

$$\% \text{ Inhibition} = \left(1 - \frac{{}_{\Delta}\text{A/min}_{\text{test}}}{{}_{\Delta}\text{A/min}_{\text{DMSO}}}\right) \times 100$$

The linear decrease of absorbance (ΔA/min) between 0 and 10 min for each MTX or sample was calculated and presented by % of inhibition then plotted against their concentrations (log scale) to obtain a calibration curve [44]. The 50% inhibitory concentration (IC_{50}) of each compound was obtained.

6.3. Determination of in vitro Antimicrobial Activity

The primary screen was carried out using the agar disc-diffusion method [45] using Müller-Hinton agar medium. Sterile filter paper discs (8 mm diameter) were moistened with the compound solution in dimethylsulphoxide of specific concentration 200 µg/disc, the antibacterial antibiotic gentamicin, ciprofloxacin, (200 µg/disc) and the antifungal drug clotrimazole (200 µg /disc) were carefully placed on the agar cultures plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37 ^{o}C, and the diameter of the growth inhibition zones was measured after 24 hours in case of bacteria and at 25 ^{o}C for 48 hours in case of *C. albicans*. The minimal inhibitory concentrations (MIC) for the compounds against the same microorganisms used in the primary screening were carried out using the microdilution susceptibility method in Müller-Hinton Broth [46]. The compounds, gentamicin, and ciprofloxacin were dissolved in dimethylsulphoxide at concentration of 64 µg /mL. The twofold dilutions of the solution were prepared (64, 32, …, 0.5, 0.25 and 0.125 µg/ml). The microorganism suspensions at 10^6 CFU/ml (colony forming unit/ml) concentrations were inoculated to the corresponding wells. The plates were incubated at 37 ^{o}C for 24 hours. The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganism as detected by unaided eye.

6.4. Antitumor Screening

The *in vitro* Antitumor screening was performed adopting previously reported procedures [37,47,48]. Cells were suspended in RPMI 1640 medium for HepG2, MCF7 and HCT116 and DMEM for A549, 1% antibiotic-antimycotic mixture (10,000 u/ml potassium penicillin, 10,000

µg/ml streptomycin sulfate and 25 µg/ml amphotericin B) and 1% L-glutamine at 37°C, under 5% CO_2 and 95% humidity. Cells were seeded at concentration of 10 x 10^3 cells/well in fresh complete growth medium in 96-well microtiter plates for 24 hrs. Media was aspirated, fresh medium (without serum) was added and cells were incubated with different concentrations of sample to give a final concentration of (100-50-25-12.5-6.25-3.125- 0.78 and 1.56 µM). 0.5% DMSO was used as negative control and 100 µg/ml of doxorubicin was used as positive control. MTT assay was used for assessment of cytotoxicity [37,47,48]. After 48 h of incubation, medium was aspirated, 40 µl MTT salt (2.5 µg/ml) were added to each well and incubated for further 4 h. To stop the reaction and dissolving the formed crystals, 200 µl of 10% sodium dodecyl sulphate (SDS) in deionized water were added to each well and incubated overnight at 37°C. The absorbance was then measured at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. The % cytotoxicity was calculated according to the formula:

$$[1-(\text{OD compound} / \text{OD negative control})] \times 100$$

A probit analysis was carried for LC_{50} determination using SPSS 11 program.

6.5 Antischistosomal Screening

The bioassay was performed as previously described [49,50], where the experiment was performed in 24-well sterile culture plates. Six worms (3 males and 3 females) were introduced into each well in triplicates. The compounds were dissolved in DMSO and were tested at 50 µg/ml. The respective amount of DMSO (0.5%) was used as negative control. The culture plates were incubated at 37°C for 24 h. Worms were examined for the toxic effect under a dissecting microscope. Worms that did not show any movement through at least a minute especially associated with coiling and becoming much darker in colour, were considered dead and counted. The activity of each of the test compounds was measured by calculating the % of dead worms per well. Praziquantel was used as the positive control at 0.1 µg/ml. Five different experiments at five different dates, each in triplicate, were done to calculate different validation parameters.

6.6 Docking and Molecular Modeling Study

The three-dimensional structures of the 2,4-substituted thiazole derivatives, in their neutral forms, were constructed using the MOE of Chemical Computing Group Inc software. Lowest energy conformer of each of the new analogue 'global-minima' was docked into the hDHFR enzyme-binding domain. All the hydrogens were added and enzyme structure was subjected to a refinement protocol in which the constraints on the enzyme were gradually removed and minimized until the rms gradient was 0.01 kcal/mol A^o. The energy minimization was carried out using the molecular mechanics force field 'AMBER.' The energy-minimized structure was used for molecular dynamics studies. For each of the thiazole analogues, energy minimizations (EM) were performed using 1000 steps of steepest descent, followed by conjugate gradient minimization to a RMS energy gradient of 0.01 Kcal/mol Å. The active site of the enzyme was defined using a radius of 10.0 A^o around MTX. Energy of binding was calculated as the difference between the energy of the complex and individual energies of the enzyme and ligand [59-62].

6.7 Flexible Alignment

The investigated compounds were subjected to flexible alignment experiment using 'Molecular Operating Environment' software (MOE of Chemical Computing Group Inc., on a Core 2 duo 2.3 GHz workstation). The molecules were built using the Builder module of MOE. Their geometry was optimized by using the MMFF94 forcefield followed by a flexible alignment using systematic conformational search. . Lowest energy aligned conformation(s) were identified [59-62].

Acknowledgment

This research project was supported by a grant from the "Research Center of the Center for Female Scientific and Medical Colleges", Deanship of Scientific Research, King Saud University.

7. References

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Captions

Table 1: Physicochemical properties of the newly synthesized compounds **9-36**

Table 2: DHFR inhibition (IC_{50}, μM), and the antimicrobial activity results of compounds **16-19**, **21-29**, **31-36**

Table 3: Median lethal concentration (LC_{50}, μM) of the most active antitumor compounds **23**, **24**, **26**, **28** and **36**

Table 4: Antischistosomal testing results of compounds **16**, **17**, **20-22**, **26**, **27** and **29** at concentration of 50 μg/ml

Chart 1: Structures of Tiazofurin (**A**), Netropsin (**B**), Thia-netropsin (**C**) and 4-Phenyl-thiazole-1,3,5-triazines (**D**).

Figure 1: (**a**) 2D binding mode and residues involved in the recognition for MTX; (**b**) 3D binding mode and residues involved in the recognition for the most active compound **34** (IC_{50} 0.03 μM); (**c**) 2D binding mode and residues involved in the recognition and the least active compound **31** (IC_{50} 40.0 μM) docked and minimized in the DHFR binding pocket.

Figure 2: The aligned conformation of (**a**) the most active compound **34** (red, IC_{50} 0.03 μM) occupying the DHFR binding pocket; (**b**) the most inactive compound **31** (cyan, IC_{50} 40.0 μM) exposing out of the DHFR binding pocket surface map.

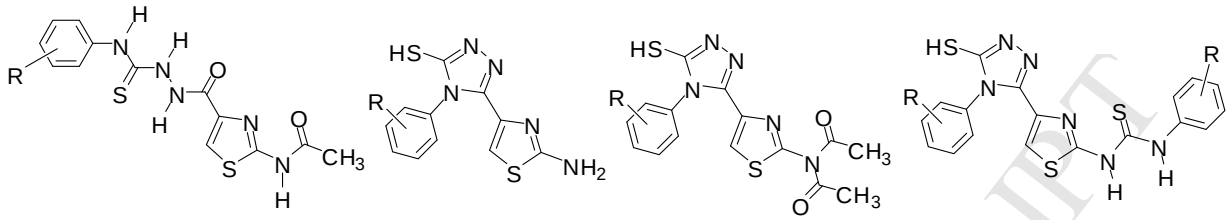
Figure 3: Flexible alignment of (**a**) the most active compound **34** (red); (**b**) the least active compound **31** (cyan); and MTX (gray).

Figure 4: Hydrophobic surface map for (**a**) the most active compound **34** (red); (**b**) the least active compound **31** (cyan) in pocket side. Pink: hydrogen bond; blue: mild polar; green: hydrophobic area.

Figure 5: Structures of the broad spectrum antibacterial **22** (comparable to gentamicin and ciprofloxacin), the schistosomicidal **29** (comparable to praziquantel), the DHFR inhibitor **34** (IC_{50} 0.03 μM, 2.7 fold more active than MTX), and the antitumor **36** (comparable to doxorubicin).

Scheme 1: Synthesis of the target compounds **16-22**, **23-29**, and **30-36.**

Table 1: Physicochemical properties of the newly synthesized compounds **9-36**



9-15 **16-22** **23-29** **30-36**

Compound	R	Solvent	Yield %	Mp ^{0}C	Molecular Formulae
9	H	EtOH	65	123-5	$C_{13}H_{13}N_5O_2S_2$
10	4-Cl	EtOH	70	138-40	$C_{13}H_{12}ClN_5O_2S_2$
11	2-CH_3O	EtOH	73	124-6	$C_{14}H_{15}N_5O_3S_2$
12	4-CH_3O	EtOH/H_2O	69	141-3	$C_{14}H_{15}N_5O_3S_2$
13	2,4-$(CH_3O)_2$	EtOH/H_2O	54	150-2	$C_{15}H_{17}N_5O_4S_2$
14	4-CH_3	EtOH/H_2O	55	144-6	$C_{14}H_{15}N_5O_2S_2$
15	4-C_6H_5O	EtOH	90	169-72	$C_{19}H_{17}N_5O_3S_2$
16	H	EtOH/H_2O	48	149-52	$C_{11}H_9N_5S_2$
17	4-Cl	EtOH/H_2O	82	151-3	$C_{11}H_8ClN_5S_2$
18	2-CH_3O	EtOH	46	142-5	$C_{12}H_{11}N_5OS_2$
19	4-CH_3O	EtOH	33	163-7	$C_{12}H_{11}N_5OS_2$
20	2,4-$(CH_3O)_2$	EtOH/H_2O	52	165-8	$C_{13}H_{13}N_5O_2S_2$
21	4-CH_3	EtOH/H_2O	67	121-3	$C_{12}H_{11}N_5S_2$
22	4-C_6H_5O	EtOH	69	135-7	$C_{17}H_{13}N_5OS_2$
23	H	EtOH/H_2O	66	98-100	$C_{15}H_{13}N_5O_2S_2$
24	4-Cl	EtOH	51	121-4	$C_{15}H_{12}ClN_5O_2S_2$
25	2-CH_3O	EtOH/H_2O	52	115-7	$C_{16}H_{15}N_5O_3S_2$
26	4-CH_3O	EtOH	49	139-42	$C_{16}H_{15}N_5O_3S_2$
27	2,4-$(CH_3O)_2$	EtOH	59	129-32	$C_{17}H_{17}N_5O_4S_2$
28	4-CH_3	EtOH/H_2O	63	158-60	$C_{16}H_{15}N_5O_2S_2$
29	4-C_6H_5O	EtOH/H_2O	75	144-7	$C_{21}H_{17}N_5O_3S_2$
30	H	EtOH	50	152-5	$C_{18}H_{14}N_6S_3$
31	4-Cl	EtOH	54	143-5	$C_{18}H_{12}Cl_2N_6S_3$
32	2-CH_3O	EtOH	63	161-3	$C_{20}H_{18}N_6O_2S_3$
33	4-CH_3O	EtOH/H_2O	66	145-7	$C_{20}H_{18}N_6O_2S_3$
34	2,4-$(CH_3O)_2$	EtOH	67	149-51	$C_{22}H_{22}N_6O_4S_3$
35	4-CH_3	EtOH/H_2O	58	163-5	$C_{20}H_{18}N_6S_3$
36	4-C_6H_5O	EtOH	49	158-60	$C_{30}H_{22}N_6O_2S_3$

Table 2: DHFR inhibition (IC_{50}, μM), and the antimicrobial activity results of compounds **16-19**, **21-29**, **31-36**

Compound	DHFR inhibition	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeuroginosa*	*C. albicans*
16	-	-	-	-	-	-
17	18.0	-	12	-	-	12
18	24.0	12	16 (4.0)	15 (8.0)	-	-
19	21.0	-	12	-	-	-
21	24.0	-	-	-	-	-
22	-	28 (0.5)	32 (0.5)	20 (0.5)	16 (1.0)	14
23	35.0	-	-	-	-	-
24	10.0	16 (4.0)	21 (4.0)	-	-	-
25	-	-	20 (4.0)	16 (0.8)	-	-
26	5.0	12	14	-	-	-
27	8.0	-	-	-	-	-
28	-	-	-	-	-	-
29	15.0	18 (4.0)	20 (2.0)	-	-	-
31	40.0	-	14	-	-	-
32	-	16	18	18 (0.8)	-	-
33	30.0	-	-	-	-	-
34	0.03	-	-	-	-	-
35	10.0	-	-	-	-	-
36	35.0	16 (4.0)	18 (2.0)	14	-	-
Gentamicin	-	26.5 (2.0)	25 (2.0)	20.8 (0.5)	19 (1.0)	Nd
Ciprofloxacin	-	32 (0.5)	35 (0.5)	38 (0.25)	36 (1.0)	Nd
Clotrimazole	-	Nd	Nd	Nd	Nd	21
Methotrexate	0.08	-	-	-	-	-

Inhibition Zone (mm): (-) Not active (8 mm), Weak activity (8-12 mm), Moderate activity (12-15 mm), Strong activity (> 15 mm). Solvent: DMSO (8 mm). MICs showed in parentheses. Nd, not determined.

Table 3: Median lethal concentration (LC_{50}, µM) of the most active antitumor compounds **23**, **24**, **26**, **28** and **36**

Compound.	**A549**	**HCT116**	**HepG2**	**MCF7**
23	33.2	-	-	-
24	-	-	29.1	-
26	35.4	-	-	-
28	25.0	-	18.9	23.8
36	-	23.5	-	17.4
Doxorubicin	28.3	37.7	21.4	26.1

Table 4: Antischistosomal testing results of compounds **16**, **17**, **20**-**22**, **26**, **27** and **29** at concentration of 50 µg/ml.

Compound	Results[a]	% Mortality
16	3/18	16.6
17	1/18	5.0
20	2/18	11.1
21	2/19	10.5
22	10/18	55.6
26	1/16	6.0
27	0/18 (deformation)[b]	0.0
29	18/18	100
DMSO	0/18	0.0
Untreated	0/18	0.0
Praziquantel	18/18	100

[a] Number of dead worms/Total number of worms.

[b] Worm morphological deformities (intensive contraction, bending and swelling).

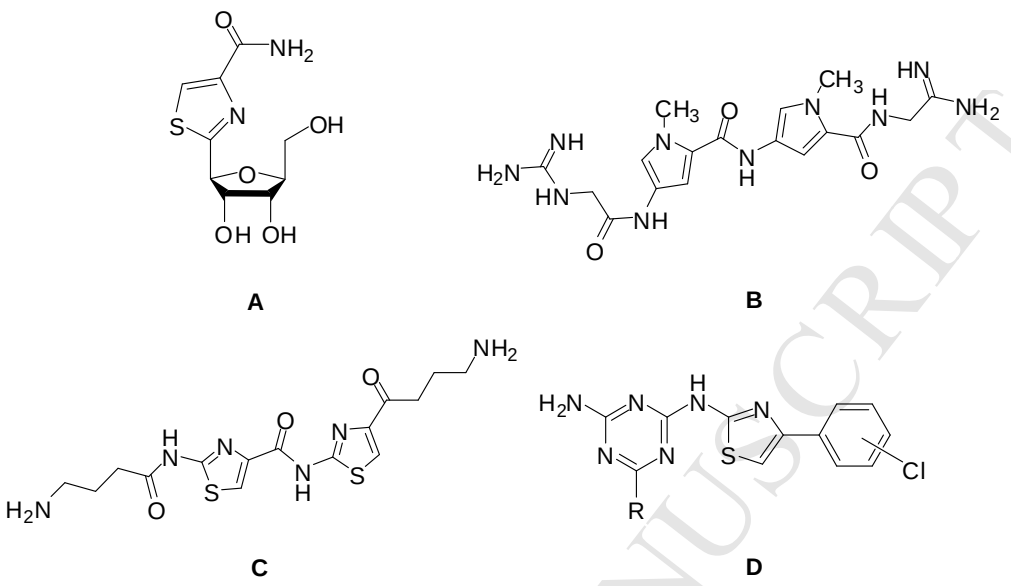


Chart 1: Structures of Tiazofurin (**A**), Netropsin (**B**), Thia-netropsin (**C**) and 4-Phenyl-thiazole-1,3,5-triazines (**D**).

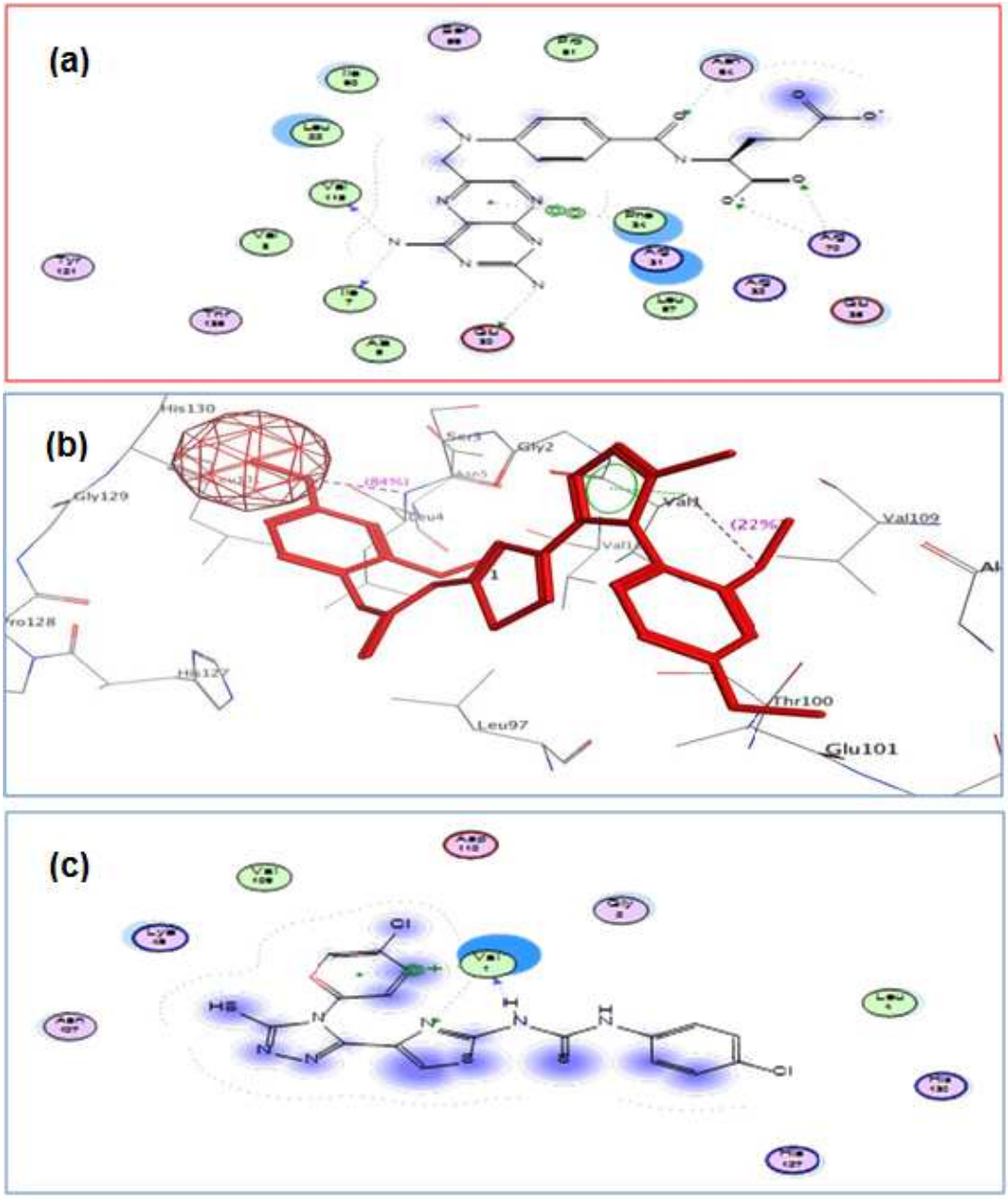


Figure 1: (**a**) 2D binding mode and residues involved in the recognition for MTX; (**b**) 3D binding mode and residues involved in the recognition for the most active compound **34** (IC_{50} 0.03 μM); (**c**) 2D binding mode and residues involved in the recognition and the least active compound **31** (IC_{50} 40.0 μM) docked and minimized in the DHFR binding pocket.

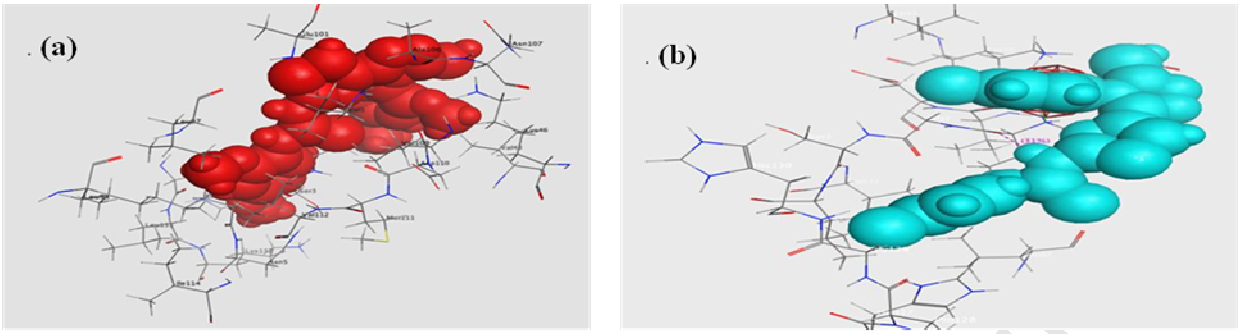


Figure 2: The aligned conformation of (**a**) the most active compound **34** (red, IC_{50} 0.03 μM) occupying the DHFR binding pocket; (**b**) the most inactive compound **31** (cyan, IC_{50} 40.0 μM) exposing out of the DHFR binding pocket surface map.

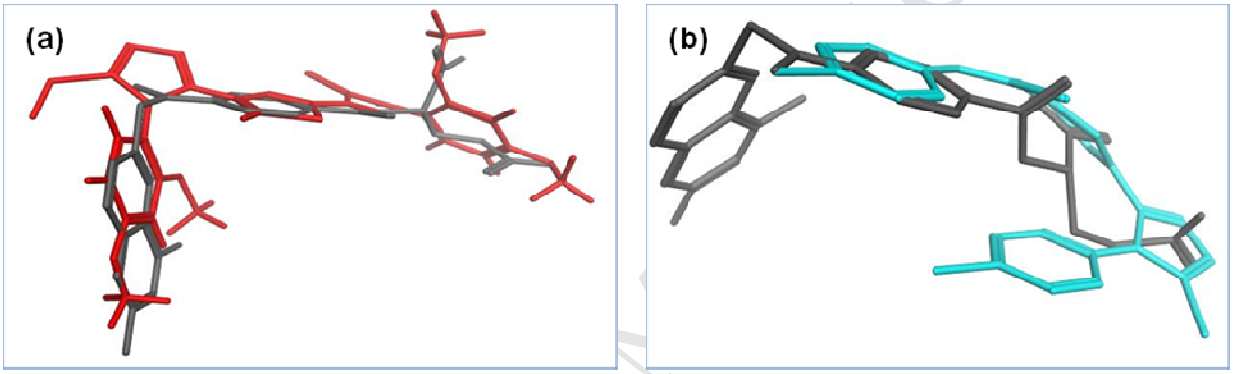


Figure 3: Flexible alignment of (**a**) the most active compound **34** (red); (**b**) the least active compound **31** (cyan); and MTX (gray).

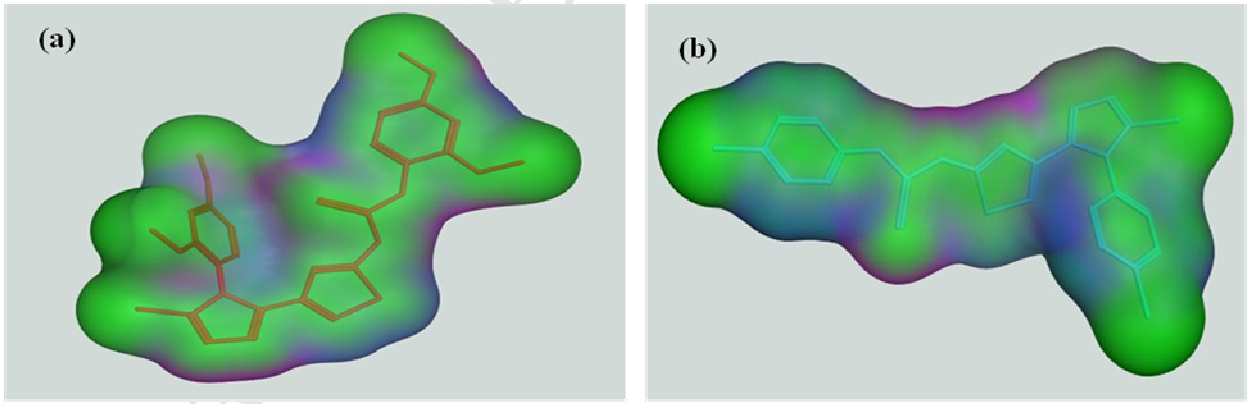


Figure 4: Hydrophobic surface map for (**a**) the most active compound **34** (red); (**b**) the least active compound **31** (cyan) in pocket side. Pink: hydrogen bond; blue: mild polar; green: hydrophobic area.

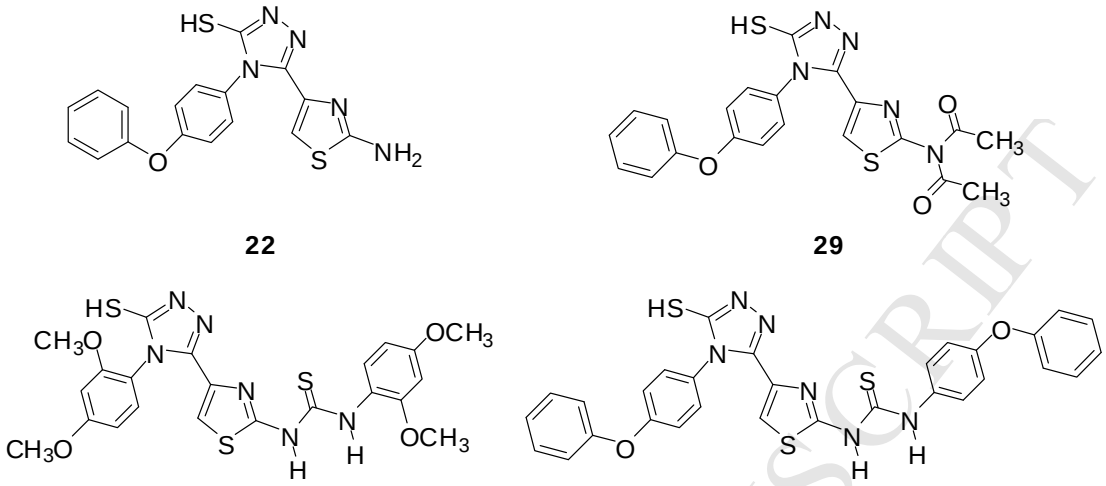
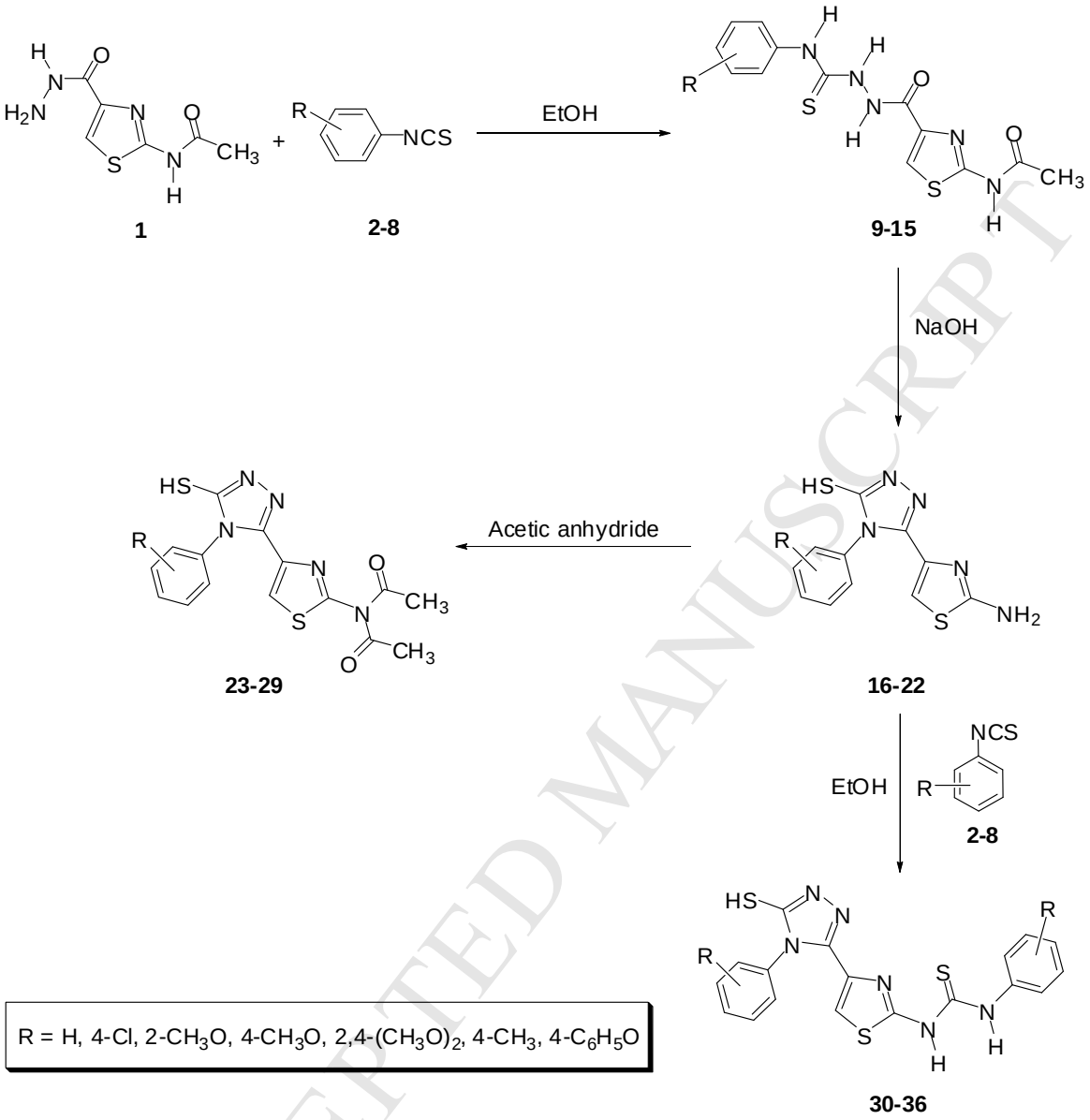


Figure 5: Structures of the broad spectrum antibacterial **22** (comparable to gentamicin and ciprofloxacin), the schistosomicidal **29** (comparable to praziquantel), the DHFR inhibitor **34** (IC_{50} 0.03 μM, 2.7 fold more active than MTX), and the antitumor **36** (comparable to doxorubicin).

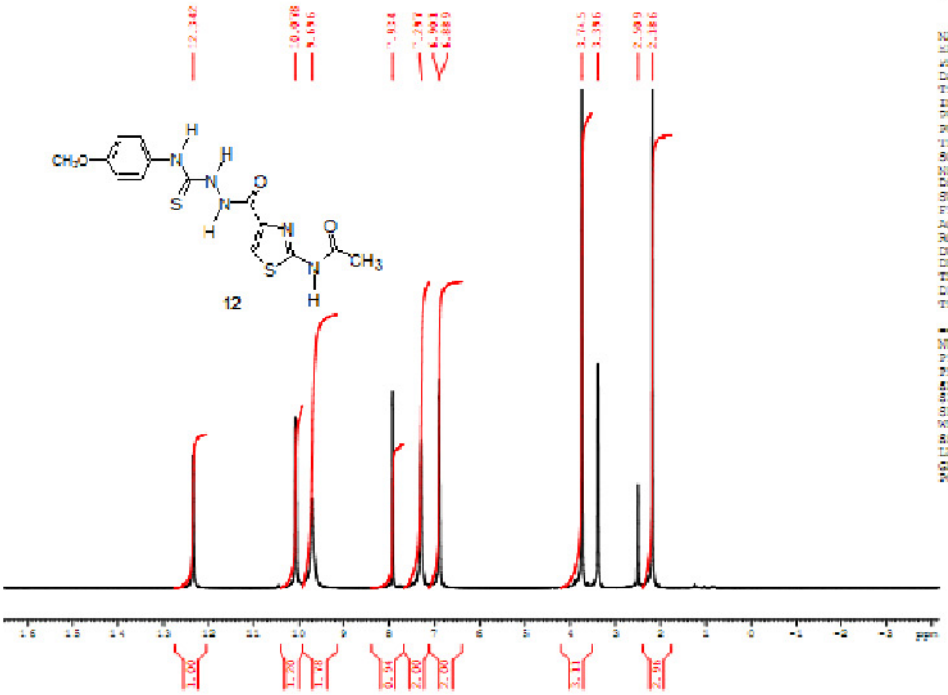


1 **2-8** **9-15** **16-22** **23-29** **30-36**

Scheme 1: Synthesis of the target compounds **16**-**22**, **23**-**29**, and **30**-**36**.

Research Highlights

- Synthesis of 5-(2-aminothiazol-4-yl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols.
- Compound **22**, antibacterial comparable to Gentamicin and Ciprofloxacin.
- Compound **29**, schistosomicidal comparable to Praziquantel.
- Compound **34**, DHFR inhibitor, IC_{50} 0.03 μM, 2.7 fold more active than MTX.
- Compound **36**, antitumor comparable to Doxorubicin.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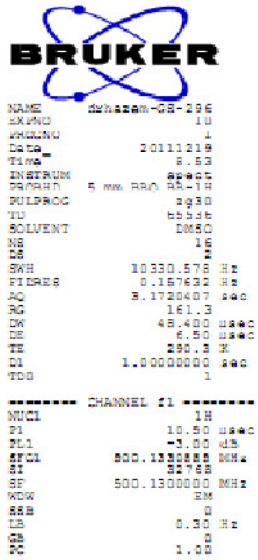
7.934
7.297
6.901
6.889
3.745
3.396
2.509
2.186
12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SOLVENT DMSO
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FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 161.3
DW 48.400 usec
DE 6.50 usec
TE 290.3 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
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SI 32768
SF 500.1300000 MHz
WDW EM
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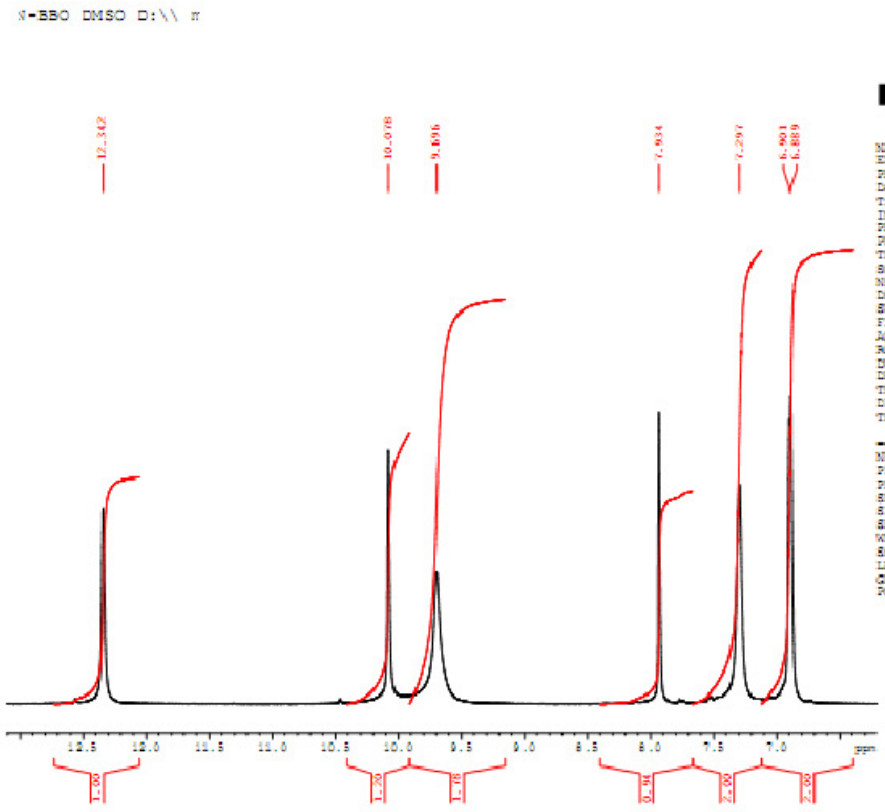
PC 1.00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10.078
9.896
7.934
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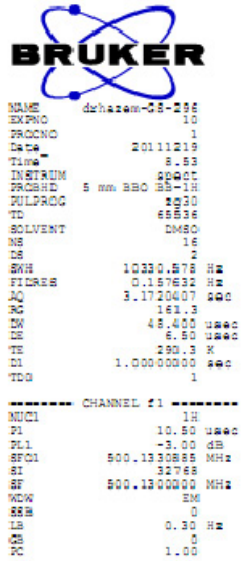
6.901
6.889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PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
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FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 161.3
DW 48.400 usec
DE 6.50 usec
TE 290.3 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
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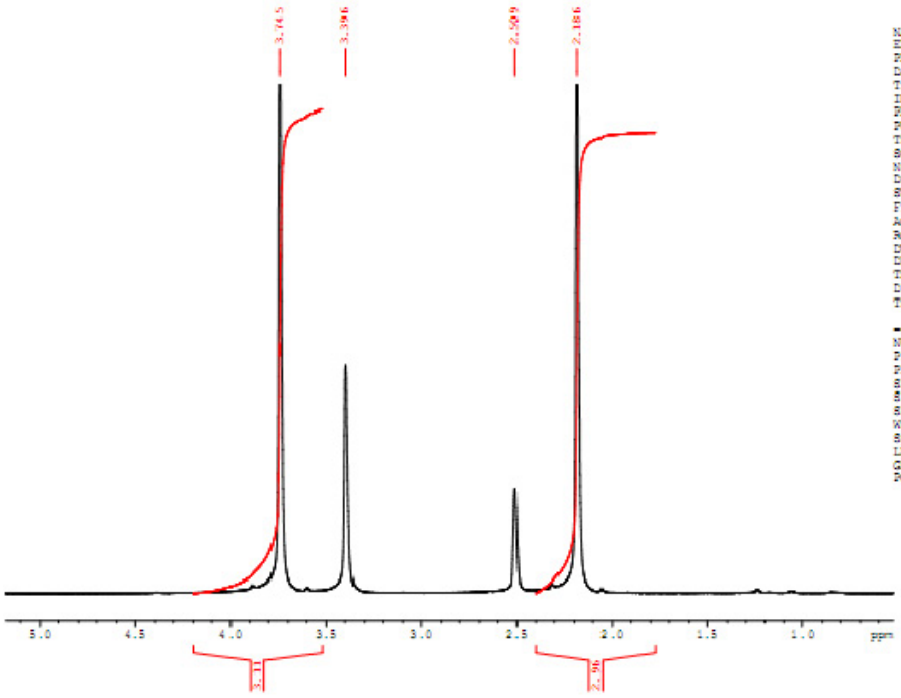

BRUKER

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EXPNO                     10
PROCNO                     1
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PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                        16
DS                         2
SWH             10330.578 Hz
FIDRES          0.157632 Hz
AQ              3.1720407 sec
RG                     161.3
DW                 48.400 usec
DE                  6.50 usec
TE                  290.3 K
D1              1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                       1H
P1                    10.50 usec
PL1                   -3.00 dB
SFO1          500.1330885 MHz
SI                      32768
SF            500.1300000 MHz
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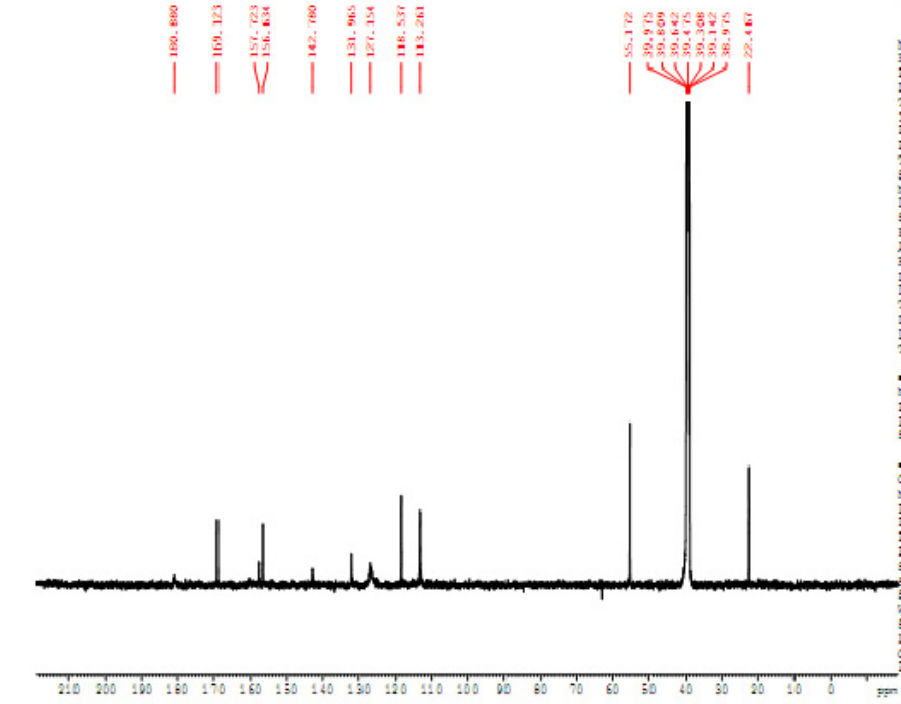

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RG                    5160.6
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DE                  6.50 usec
TE                  296.9 K
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D11             0.03000000 sec
TD0                        1

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PL1                   -2.00 dB
SFO1          125.7703643 MHz

======== CHANNEL f2 ========
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NUC2                       1H
PCPD2                 80.00 usec
PL2                   -3.00 dB
PL12                  14.64 dB
PL13                  17.64 dB
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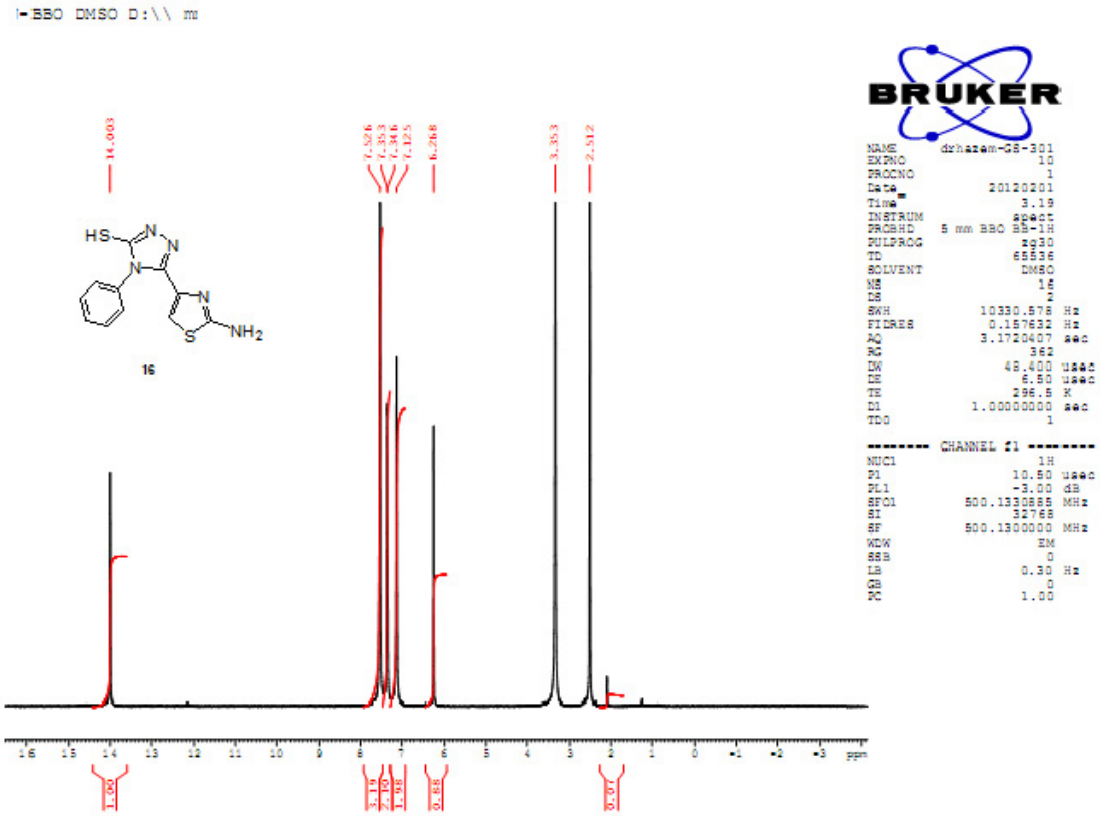

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FIDRES	0.157632 Hz
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RG	362
DW	48.400 usec
DE	6.50 usec
TE	296.5 K
D1	1.00000000 sec
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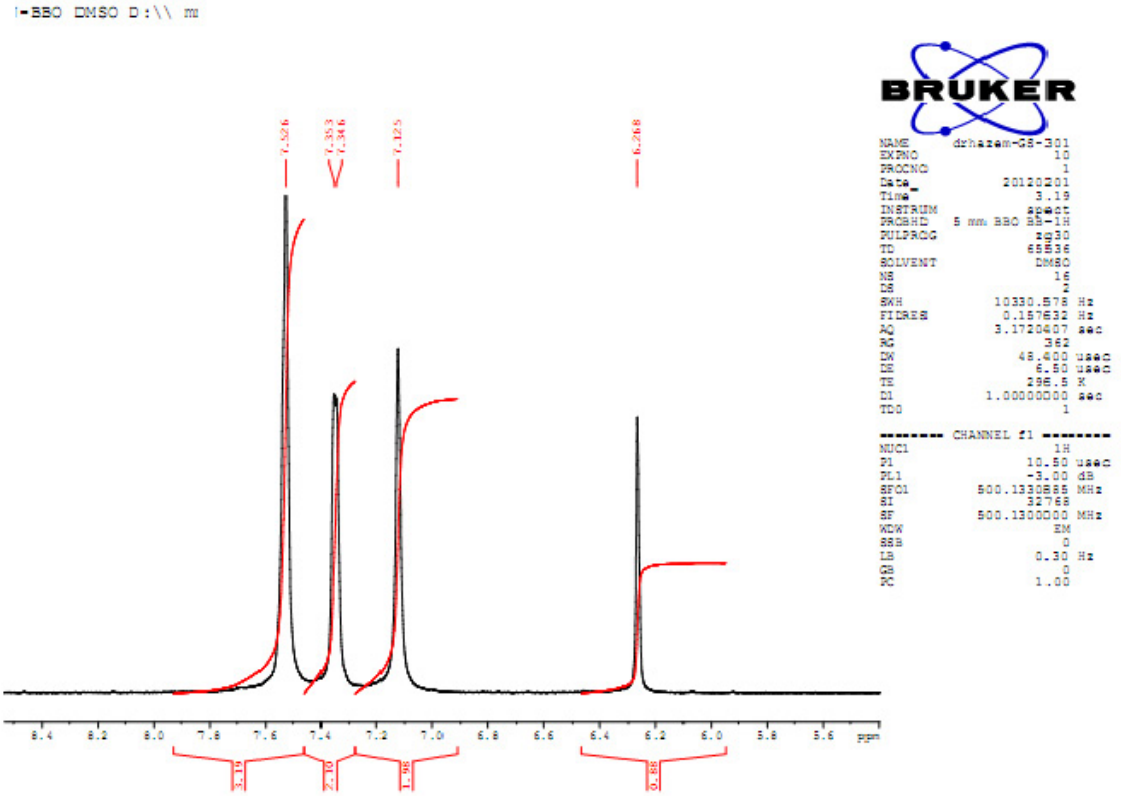

BRUKER

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INSTRUM	spect
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PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	362
DW	48.400 usec
DE	6.50 usec
TE	296.5 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
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P1	10.50 usec
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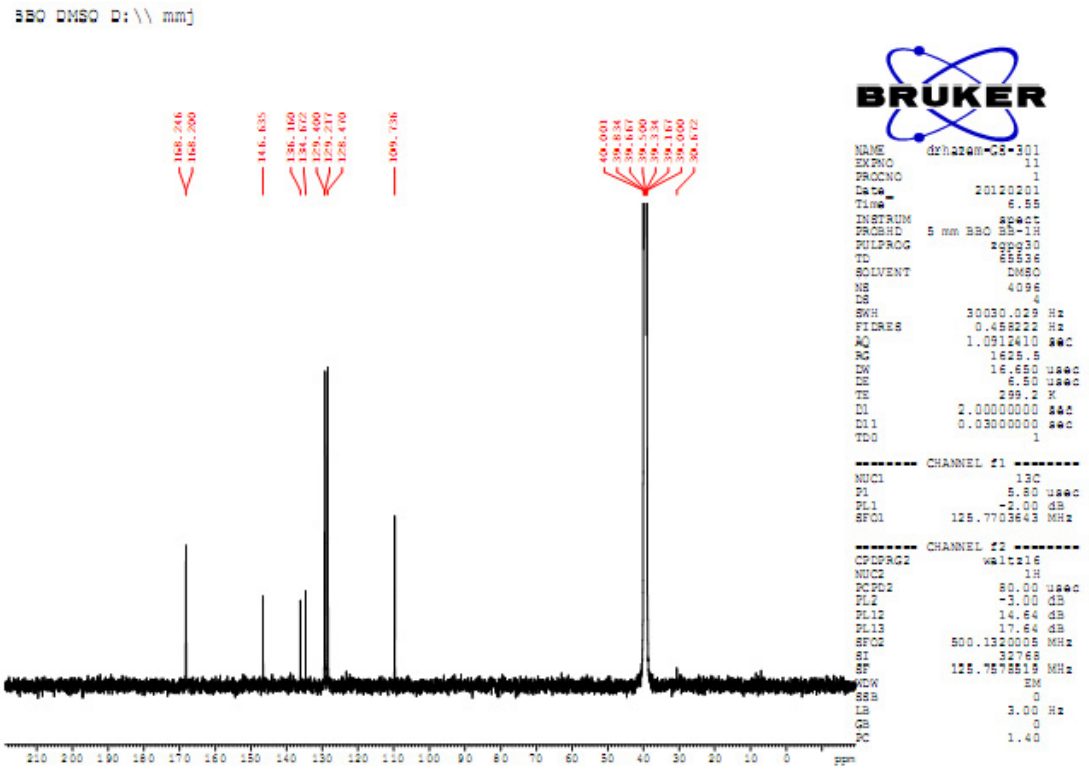
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39.167
39.000
38.672
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EXPNO 11
PROCNO 1
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FIDRES 0.458222 Hz
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RG 1625.5
DW 16.650 usec
DE 6.50 usec
TE 298.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
NUC1 13C
P1 5.80 usec
PL1 -2.00 dB
SFO1 125.7703643 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -3.00 dB
PL12 14.64 dB
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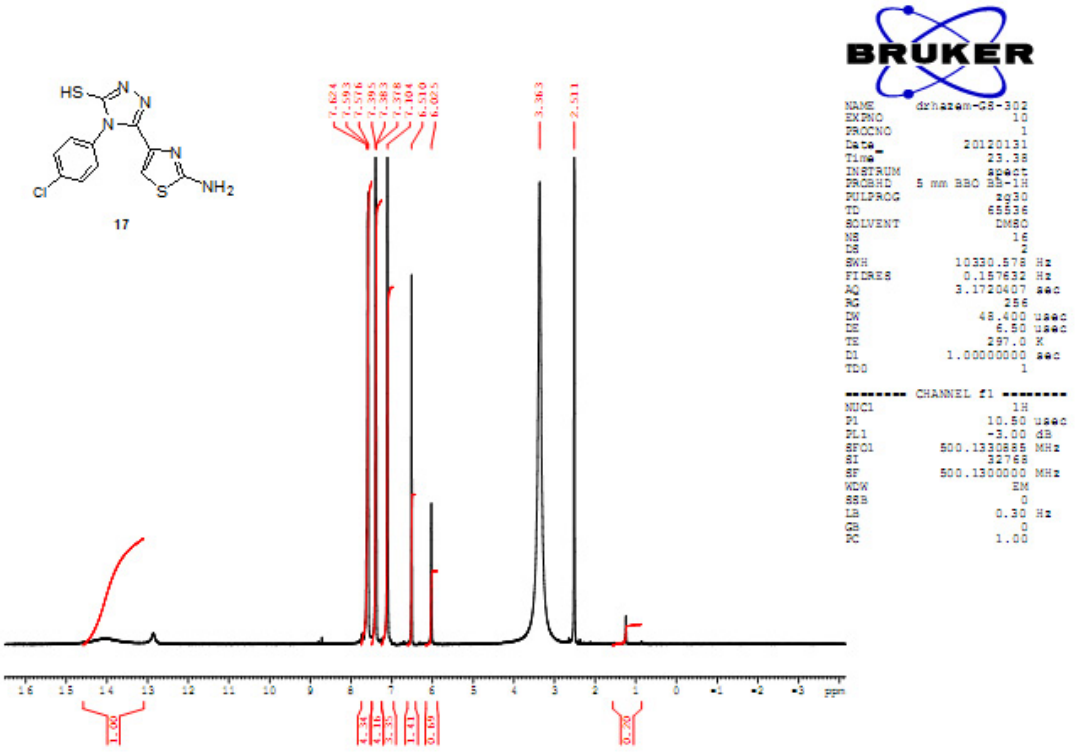
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N
N
N
N
S
NH2
Cl
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7.576
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7.378
7.364
7.359
6.825
3.363
2.511
1.00
1.34
1.16
3.35
1.11
0.89
0.20
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EXPNO 10
PROCNO 1
Date_ 20120131
Time 23.38
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PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 256
DW 48.400 usec
DE 6.50 usec
TE 297.0 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
SFO1 500.1330885 MHz
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SF 500.1300000 MHz
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GB 0
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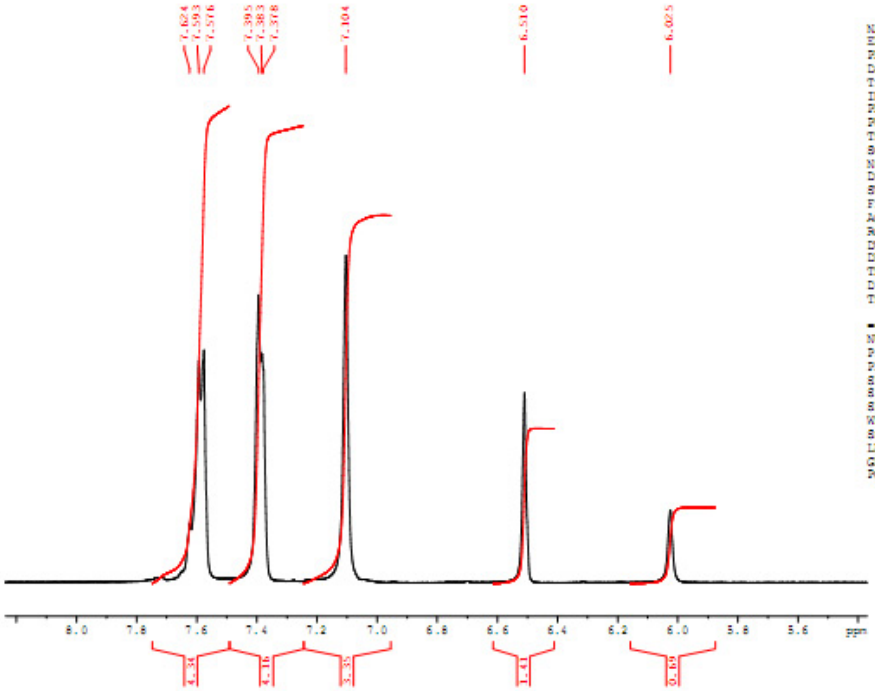
1-BBO DMSO D:\\ mu



BRUKER

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TD                    65536
SOLVENT                DMSO
NS                       16
DS                        2
SWH           10330.578 Hz
FIDRES        0.157632 Hz
AQ            3.1720407 sec
RG                      256
DW              48.400 usec
DE               6.50 usec
TE                297.0 K
D1            1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                    1H
P1                 10.50 usec
PL1                -3.00 dB
SFO1          500.1330885 MHz
SI                    32768
SF            500.1300000 MHz
WDW                     EM
SSB                      0
LB                  0.30 Hz
GB                       0
PC                    1.00
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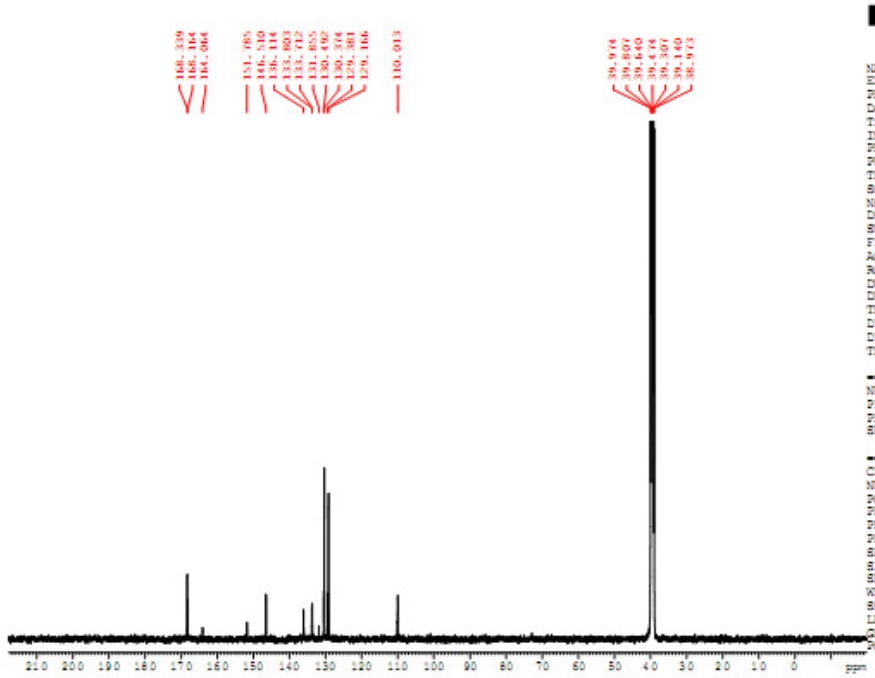
BBO DMSO D:\\ mmj



BRUKER

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TD                    65536
SOLVENT                DMSO
NS                     4096
DS                        4
SWH           30030.029 Hz
FIDRES        0.458222 Hz
AQ            1.0912410 sec
RG                    1625.5
DW              16.650 usec
DE               6.50 usec
TE                297.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                   13C
P1                 5.80 usec
PL1                -2.00 dB
SFO1        125.7703643 MHz

======== CHANNEL f2 ========
CPDPRG2             waltz16
NUC2                    1H
PCPD2              80.00 usec
PL2                -3.00 dB
PL12               14.64 dB
PL13               17.64 dB
SFO2          500.1320005 MHz
SI                    32768
SF            125.7578519 MHz
WDW                     EM
SSB                      0
LB                  3.00 Hz
GB                       0
PC                    1.40
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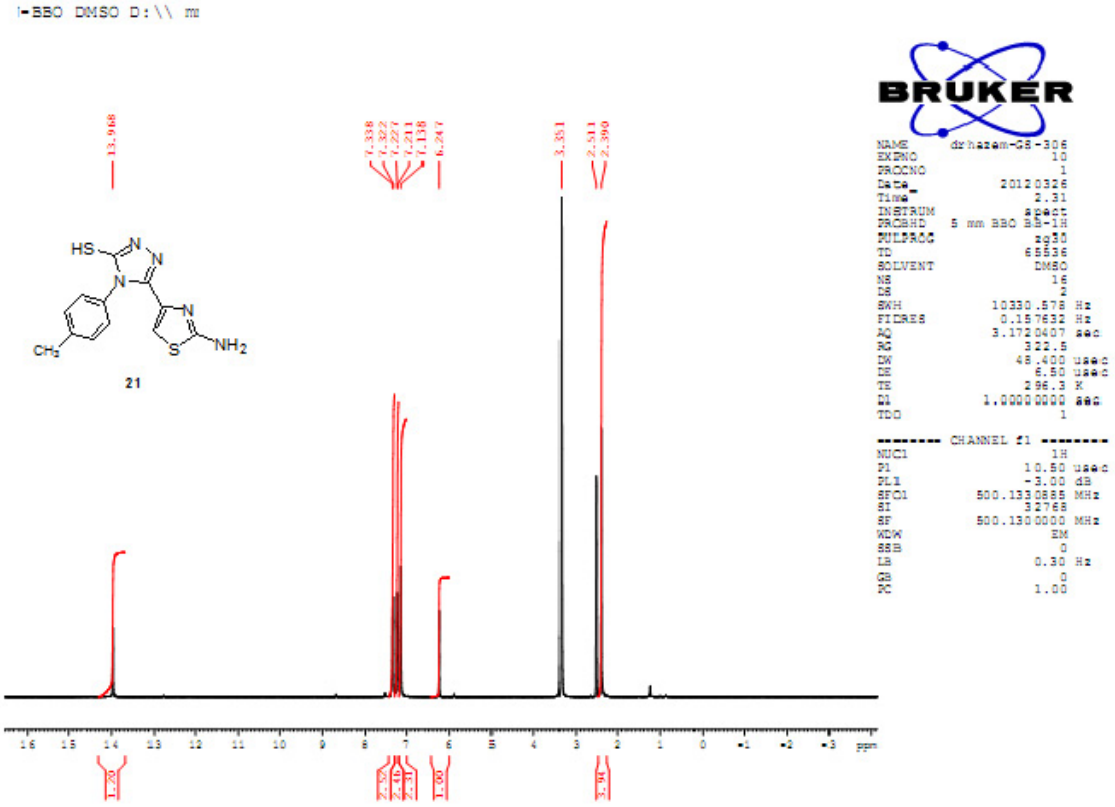
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7.322
7.277
7.211
7.138
6.247
3.351
2.511
2.390
HS
N
N
N
N
CH2
S
NH2
21
1.20
2.30
2.46
2.31
1.00
3.94
ppm
BRUKER
NAME dr hazem-GS-306
EXPNO 10
PROCNO 1
Date_ 20120326
Time 2.31
INSTRUM spect
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PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 322.5
DW 48.400 usec
DE 6.50 usec
TE 296.3 K
D1 1.00000000 sec
TD0 1
-------- CHANNEL f1 --------
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
SFO1 500.1330885 MHz
SI 32768
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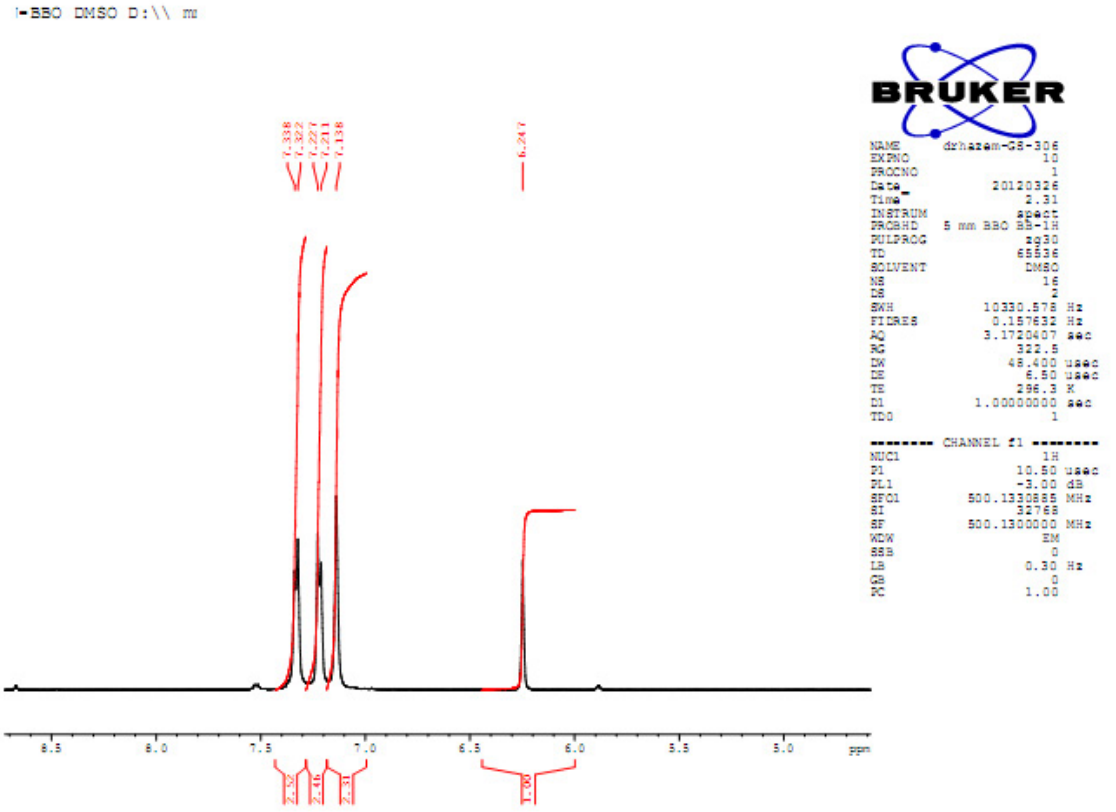
GB 0
PC 1.00


1-BBO DMSO D:\\ m
7.338
7.322
7.277
7.211
7.138
6.247
2.30
2.46
2.31
1.00
ppm
BRUKER
NAME dr hazem-GS-306
EXPNO 10
PROCNO 1
Date_ 20120326
Time 2.31
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 322.5
DW 48.400 usec
DE 6.50 usec
TE 296.3 K
D1 1.00000000 sec
TD0 1
-------- CHANNEL f1 --------
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
SFO1 500.1330885 MHz
SI 32768
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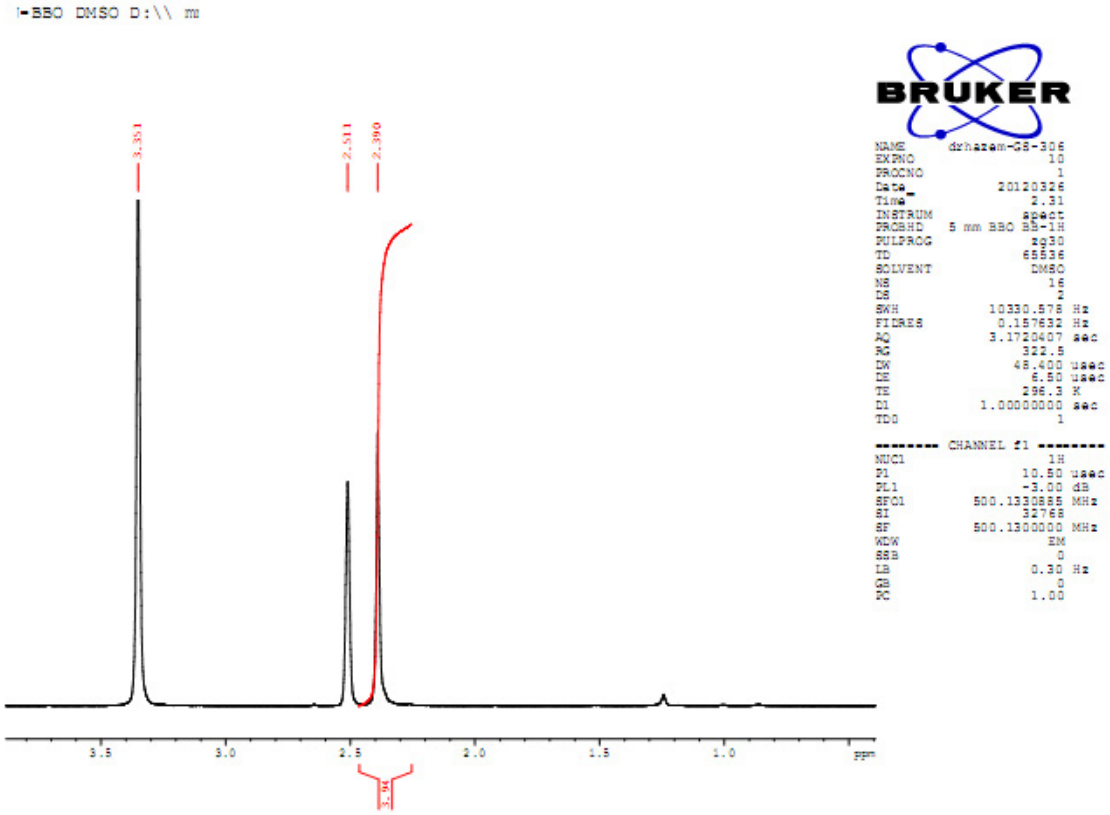
LB 0.30 Hz
GB 0
PC 1.00


1-BBO DMSO D:\\ m
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2.511
2.390
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3.5
3.0
2.5
2.0
1.5
1.0
ppm
BRUKER
NAME drhazam-GS-306
EXPNO 10
PROCNO 1
Date_ 20120326
Time 2.31
INSTRUM spect
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PULPROG zg30
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SOLVENT DMSO
NS 16
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FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 322.5
DW 48.400 usec
DE 6.50 usec
TE 296.3 K
D1 1.00000000 sec
TD0 1
-------- CHANNEL f1 --------
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
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SSB 0
LB 0.30 Hz
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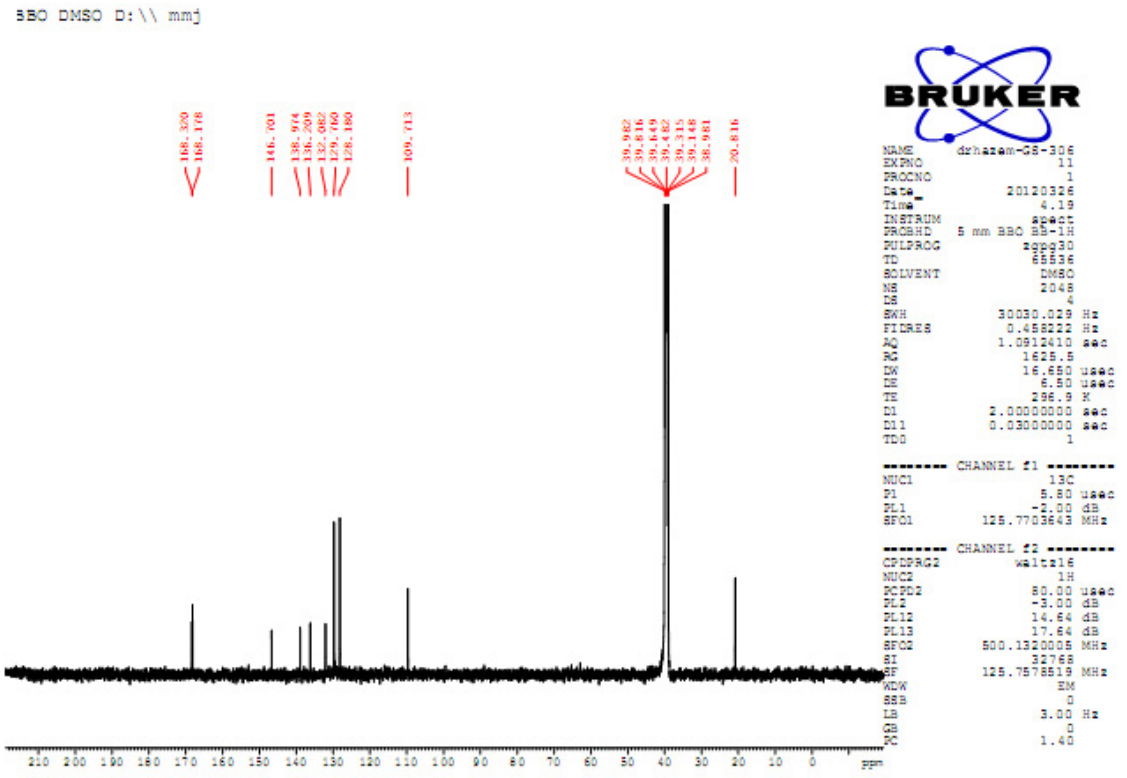
PC 1.00


BBO DMSO D:\\ mmj
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168.178
146.701
138.974
136.209
132.082
129.160
128.180
109.713
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39.816
39.649
39.482
39.315
39.148
38.981
20.816
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm
BRUKER
NAME drhazam-GS-306
EXPNO 11
PROCNO 1
Date_ 20120326
Time 4.19
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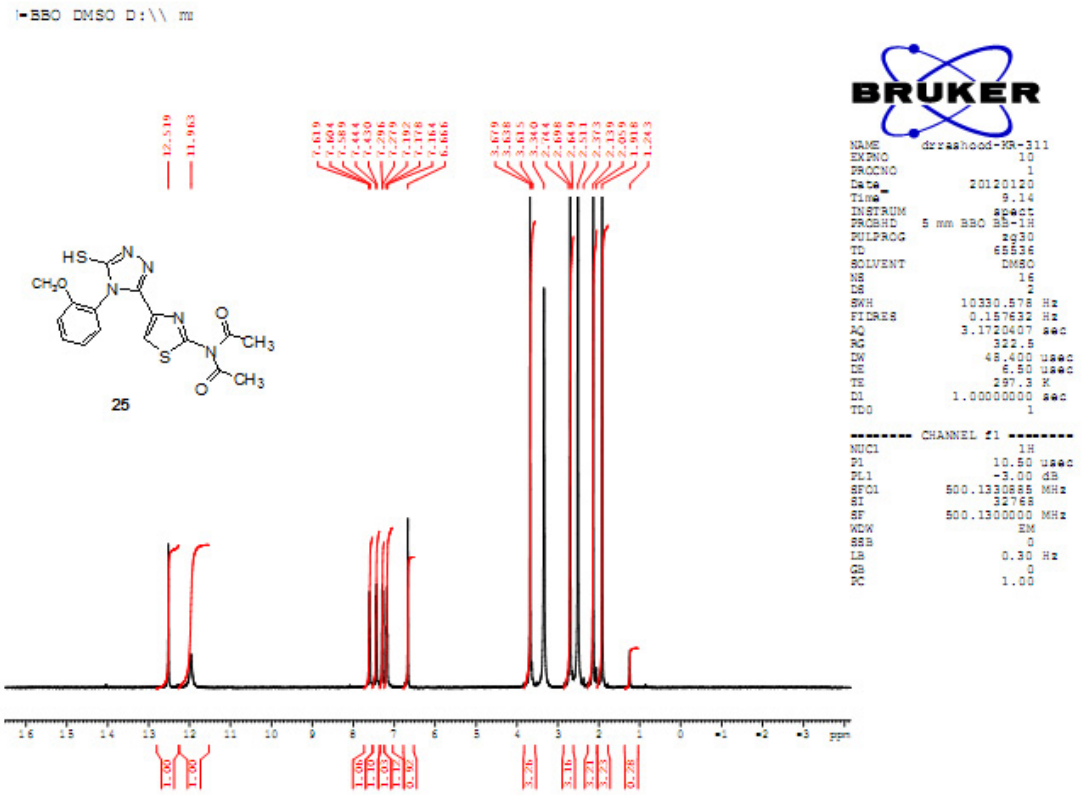
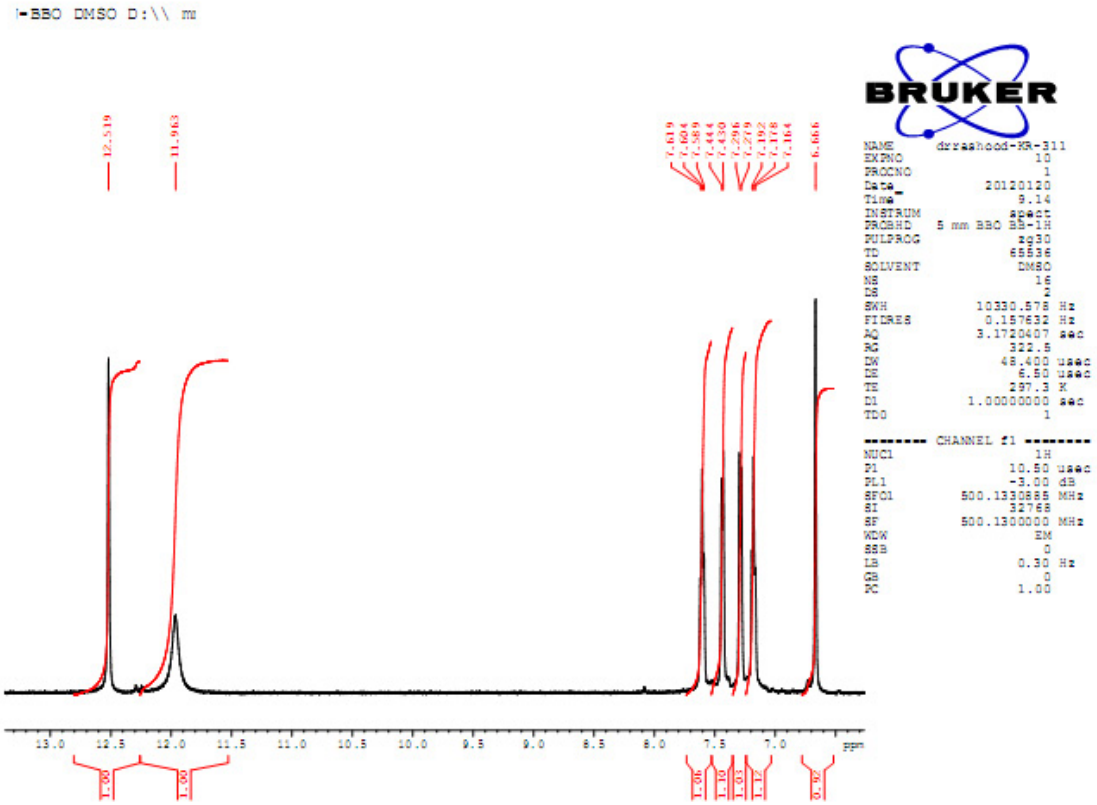
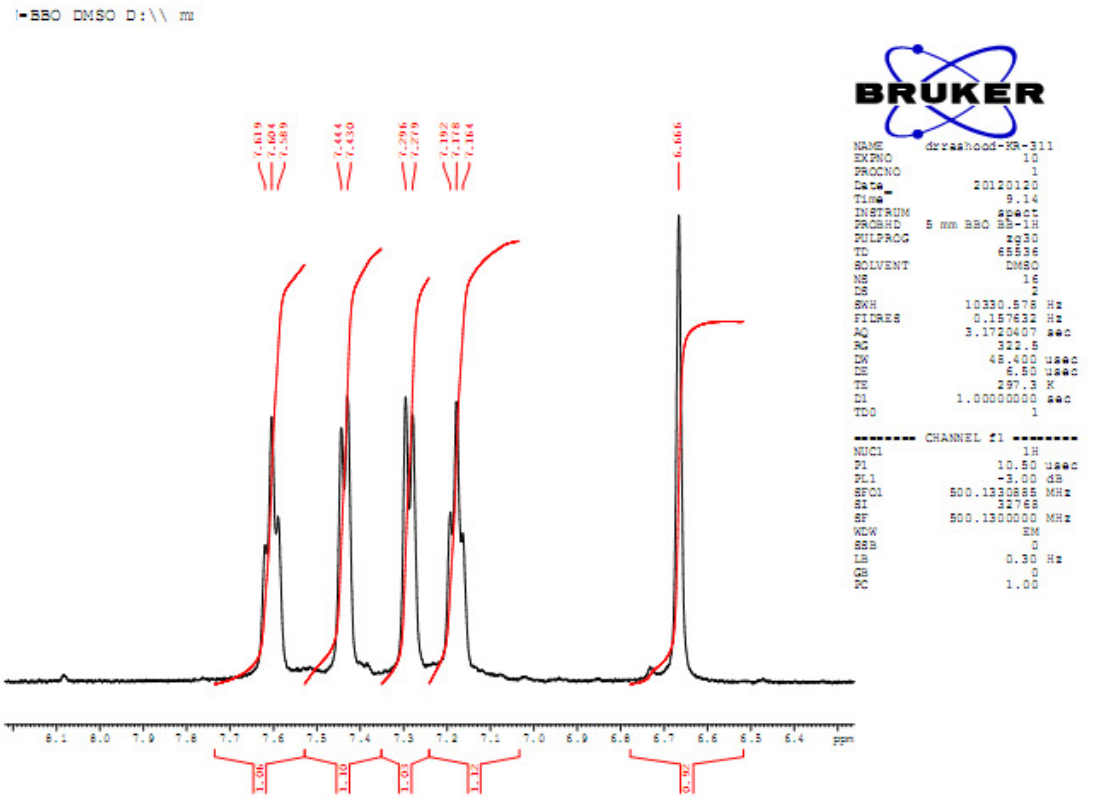
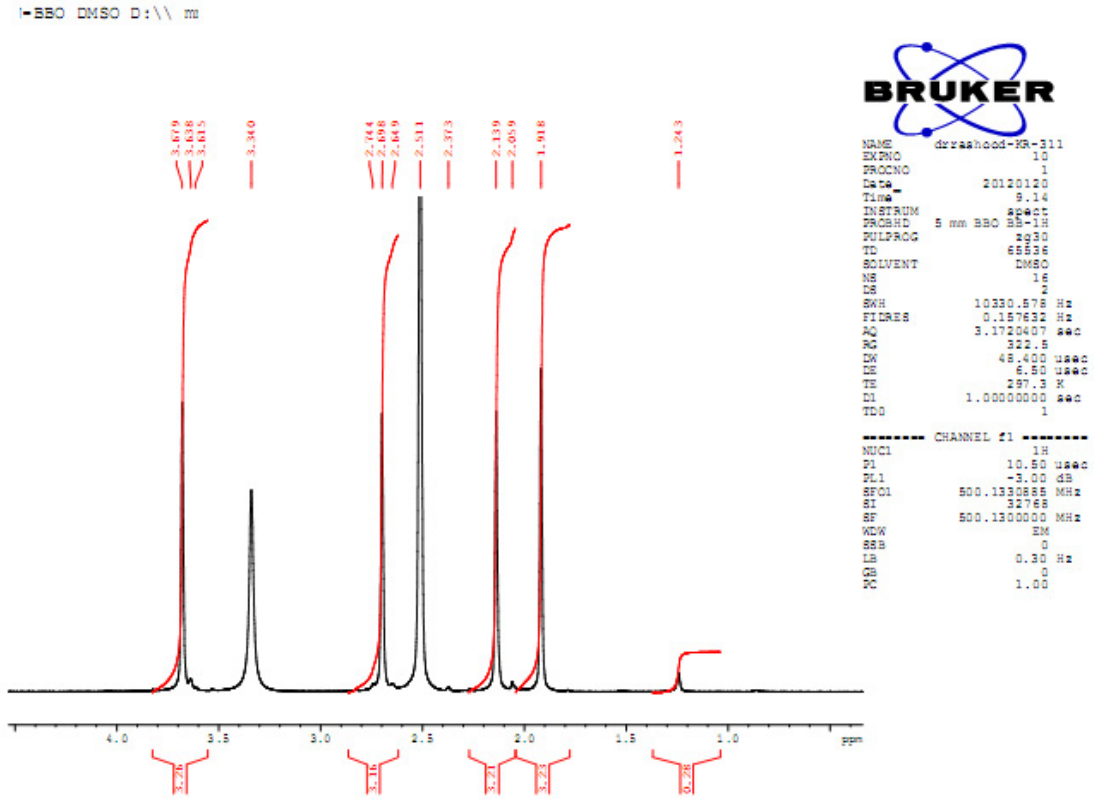
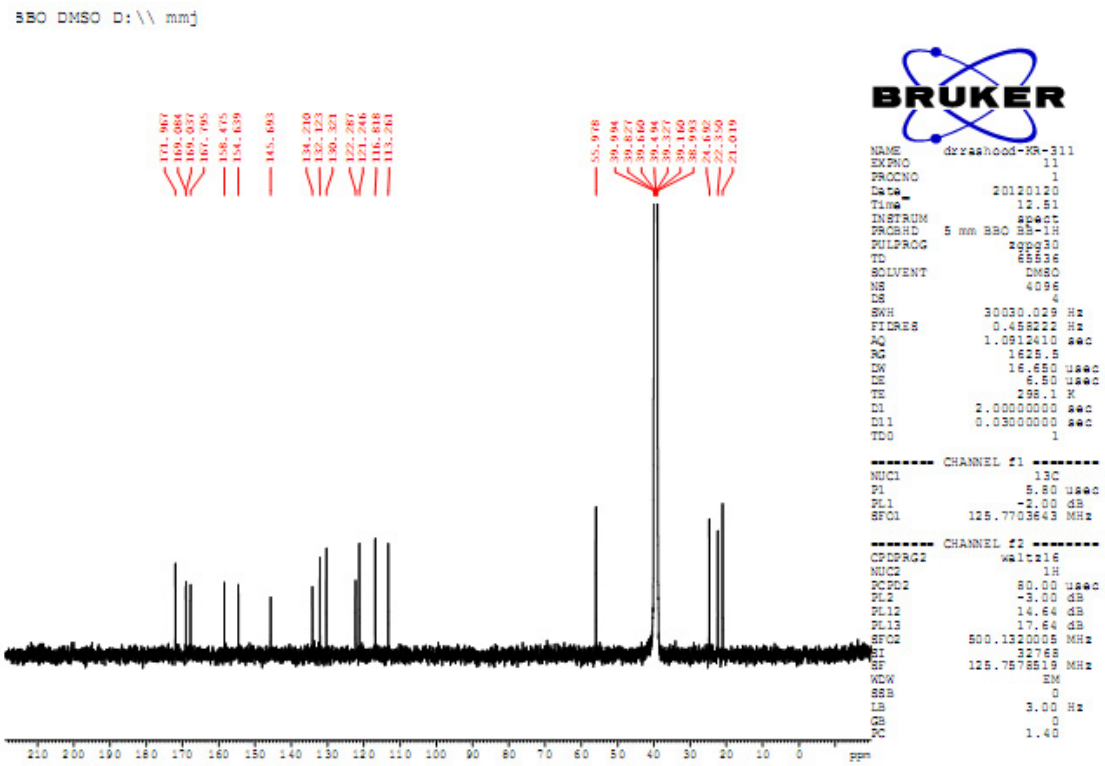
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 2048
DS 4
SWH 30030.029 Hz
FIDRES 0.458222 Hz
AQ 1.0912410 sec
RG 1625.5
DW 16.650 usec
DE 6.50 usec
TE 296.9 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
-------- CHANNEL f1 --------
NUC1 13C
P1 5.80 usec
PL1 -2.00 dB
SFO1 125.7703643 MHz
-------- CHANNEL f2 --------
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -3.00 dB
PL12 14.64 dB
PL13 17.64 dB
SFO2 500.1320005 MHz
SI 32768
SF 125.7578519 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.40

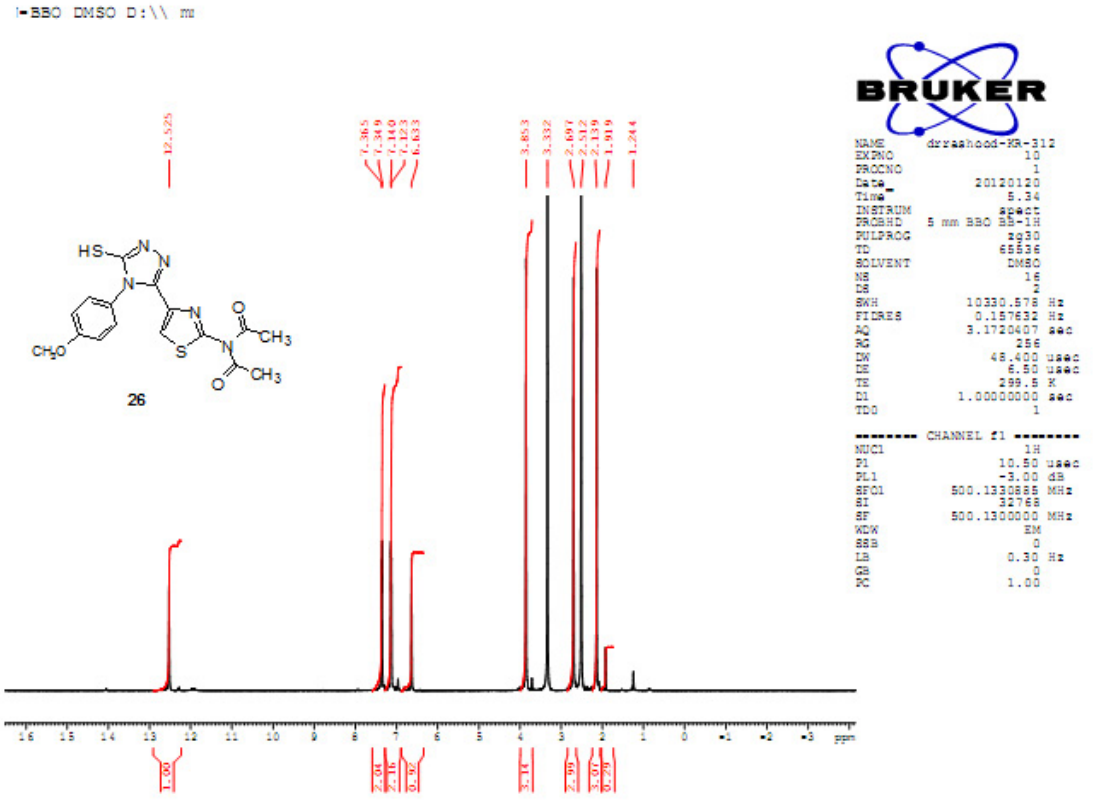

1-BBO DMSO D:\\ m
12.519
11.963
7.619
7.604
7.589
7.444
7.430
7.296
7.279
7.192
7.178
7.164
6.666
3.679
3.638
3.615
3.340
2.744
2.698
2.649
2.511
2.373
2.139
2.059
1.918
1.243
HS
N
N
N
CH2O
N
O
CH3
S
N
O
CH3
25
BRUKER
NAME drrashood-KR-311
EXPNO 10
PROCNO 1
Date_ 20120120
Time_ 9.14
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 322.5
DW 48.400 usec
DE 6.50 usec
TE 297.3 K
D1 1.00000000 sec
TD0 1
-------- CHANNEL f1 --------
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
SFO1 500.1330885 MHz
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm
1.00
1.00
1.06
1.10
1.03
1.17
0.96
3.26
3.16
3.23
3.23
0.28

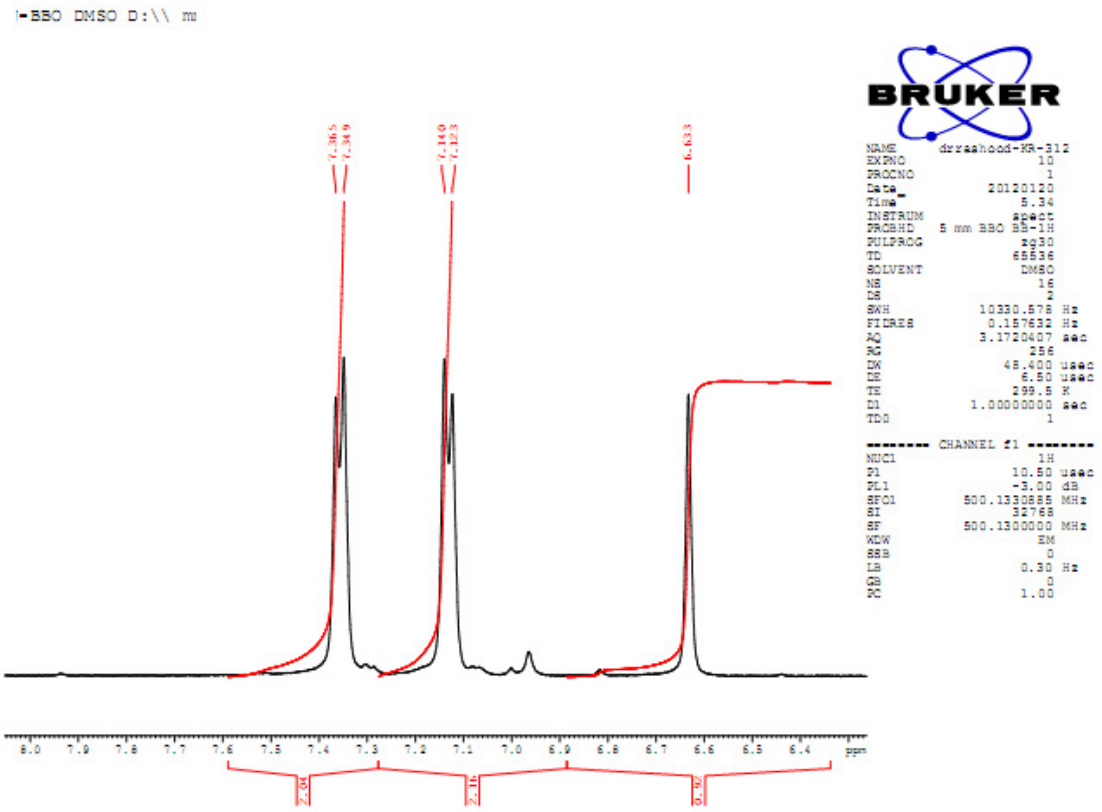

1-BBO DMSO D:\\ m
12.519
11.963
7.619
7.604
7.589
7.444
7.430
7.296
7.279
7.192
7.178
7.164
6.666
BRUKER
NAME drrashood-KR-311
EXPNO 10
PROCNO 1
Date_ 20120120
Time_ 9.14
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 322.5
DW 48.400 usec
DE 6.50 usec
TE 297.3 K
D1 1.00000000 sec
TD0 1
-------- CHANNEL f1 --------
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
SFO1 500.1330885 MHz
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm
1.00
1.00
1.06
1.10
1.03
1.17
0.96


I-BBO DMSO D:\\ m
7.619
7.604
7.589
7.444
7.430
7.296
7.279
7.192
7.178
7.164
6.666
8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 ppm
1.06
1.16
1.03
1.12
0.92
BRUKER
NAME drrsahood-KR-311
EXPNO 10
PROCNO 1
Date_ 20120120
Time 9.14
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 322.5
DW 48.400 usec
DE 6.50 usec
TE 297.3 K
D1 1.00000000 sec
TD0 1
-------- CHANNEL f1 --------
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
SFO1 500.1330885 MHz
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

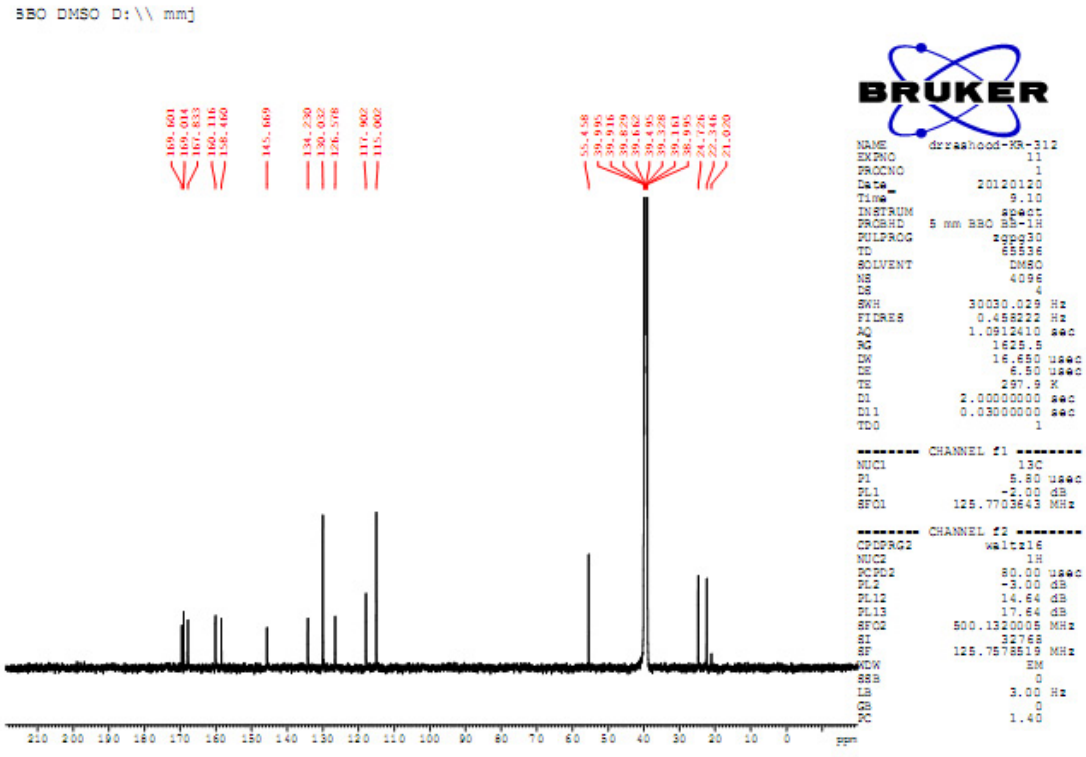

I-BBO DMSO D:\\ m
3.679
3.638
3.615
3.340
2.744
2.698
2.649
2.511
2.373
2.139
2.059
1.918
1.243
4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
3.26
3.16
3.21
3.23
0.28
BRUKER
NAME drrsahood-KR-311
EXPNO 10
PROCNO 1
Date_ 20120120
Time 9.14
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 322.5
DW 48.400 usec
DE 6.50 usec
TE 297.3 K
D1 1.00000000 sec
TD0 1
-------- CHANNEL f1 --------
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
SFO1 500.1330885 MHz
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


5BO DMSO D:\\ mmj
171.967
169.084
169.037
167.795
158.475
154.639
145.693
134.219
132.123
130.321
122.287
121.246
116.818
113.261
55.978
39.994
39.827
39.660
39.494
39.327
39.160
38.993
24.692
22.350
21.019
BRUKER
NAME drrsahood-KR-311
EXPNO 11
PROCNO 1
Date_ 20120120
Time 12.51
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 4096
DS 4
SWH 30030.029 Hz
FIDRES 0.458222 Hz
AQ 1.0912410 sec
RG 1625.5
DW 16.650 usec
DE 6.50 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
NUC1 13C
P1 5.80 usec
PL1 -2.00 dB
SFO1 125.7703643 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -3.00 dB
PL12 14.64 dB
PL13 17.64 dB
SFO2 500.1320005 MHz
SI 32768
SF 125.7578519 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.40

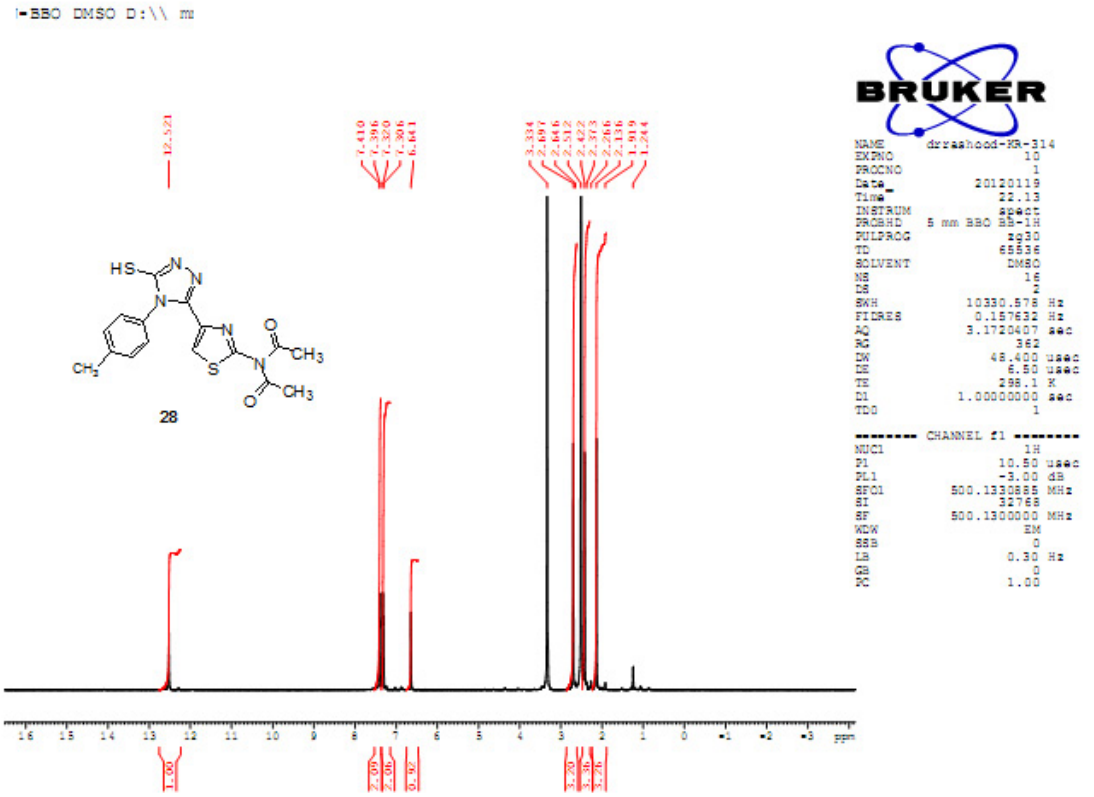

1-BBO DMSO D:\\ mm
12.525
7.365
7.349
7.140
7.123
6.833
3.853
3.332
2.697
2.512
2.338
1.919
1.244
HS
N
N
N
S
O
CH3
N
O
CH3
CH3O
26
1.00
2.04
2.16
1.07
0.79
3.14
2.99
3.07
0.29
BRUKER
NAME drrsahood-KR-312
EXPNO 10
PROCNO 1
Date_ 20120120
Time 5.34
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 256
DW 48.400 usec
DE 6.50 usec
TE 299.5 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
SFO1 500.1330885 MHz
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

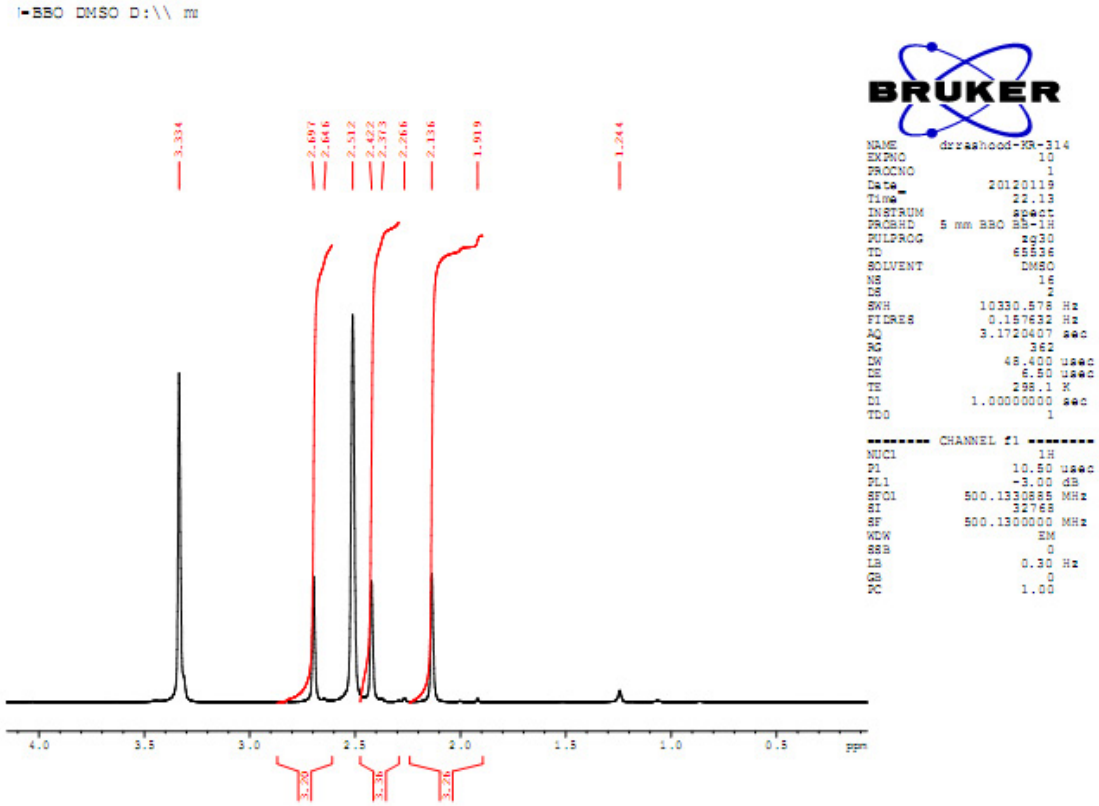



```
BRUKER
NAME            drraahood-KR-312
EXPNO                        10
PROCNO                        1
Date_               20120120
Time                      5.34
INSTRUM                   spect
PROBHD     5 mm BBO BB-1H
PULPROG                   zg30
TD                       65536
SOLVENT                   DMSO
NS                          16
DS                           2
SWH                 10330.578 Hz
FIDRES               0.157632 Hz
AQ                   3.1720407 sec
RG                         256
DW                    48.400 usec
DE                     6.50 usec
TE                     299.5 K
D1                1.00000000 sec
TD0                          1

======== CHANNEL f1 ========
NUC1                        1H
P1                    10.50 usec
PL1                    -3.00 dB
SFO1               500.1330885 MHz
SI                       32768
SF                 500.1300000 MHz
WDW                         EM
SSB                          0
LB                     0.30 Hz
GB                           0
PC                        1.00
```

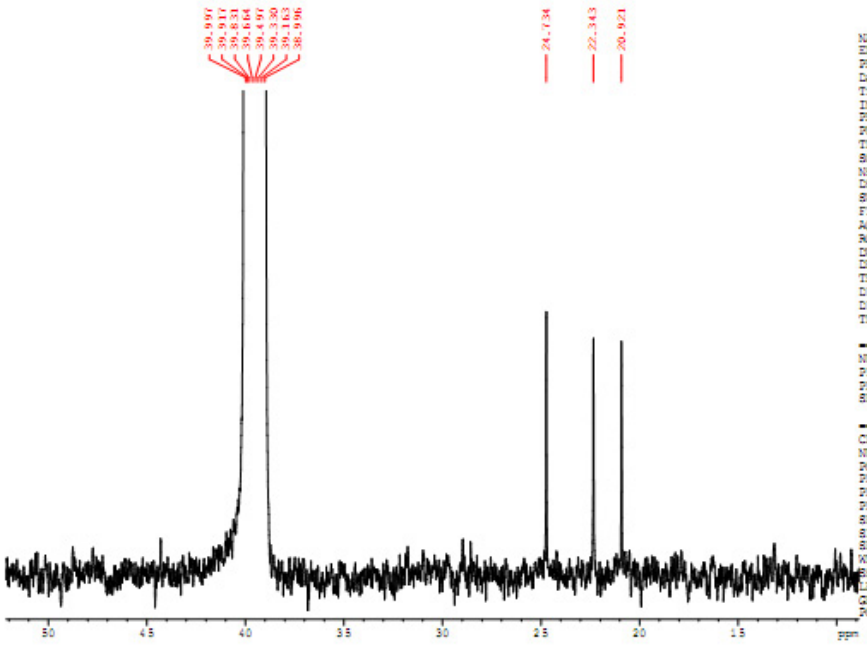



```
BRUKER
NAME            drraahood-KR-312
EXPNO                        11
PROCNO                        1
Date_               20120120
Time                      9.10
INSTRUM                   spect
PROBHD     5 mm BBO BB-1H
PULPROG                zgpg30
TD                       65536
SOLVENT                   DMSO
NS                         4096
DS                           4
SWH                 30030.029 Hz
FIDRES               0.458222 Hz
AQ                   1.0912410 sec
RG                        1625.5
DW                    16.650 usec
DE                     6.50 usec
TE                     297.9 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                          1

======== CHANNEL f1 ========
NUC1                       13C
P1                     5.80 usec
PL1                    -2.00 dB
SFO1               125.7703643 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                        1H
PCPD2                 80.00 usec
PL2                    -3.00 dB
PL12                   14.64 dB
PL13                   17.64 dB
SFO2               500.1320005 MHz
SI                       32768
SF                 125.7578519 MHz
WDW                         EM
SSB                          0
LB                     3.00 Hz
GB                           0
PC                        1.40
```


1-BBO DMSO D:\\ m
12.521
7.419
7.396
7.320
7.306
6.641
3.334
2.697
2.646
2.512
2.422
2.373
2.266
2.136
1.919
1.244
HS
N
N
N
O
CH3
S
N
O
CH3
CH2
28
1.00
2.09
2.06
0.92
3.09
3.36
3.24
ppm
BRUKER
NAME drraahood-KR-314
EXPNO 10
PROCNO 1
Date_ 20120119
Time 22.13
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 362
DW 48.400 usec
DE 6.50 usec
TE 298.1 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
SFO1 500.1330885 MHz
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

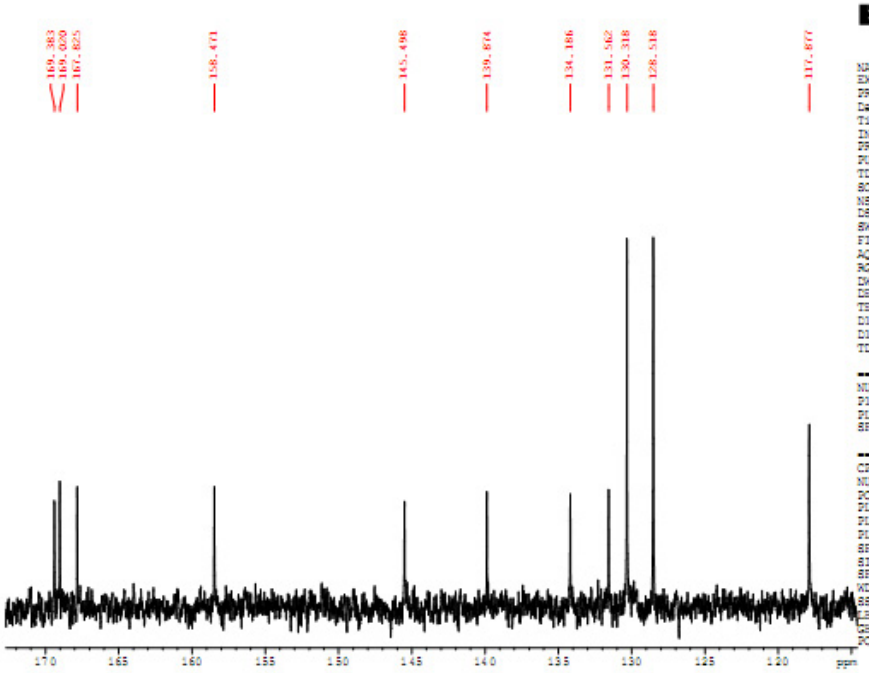

1-BBO DMSO D:\\ m
3.334
2.697
2.646
2.512
2.422
2.373
2.266
2.136
1.919
1.244
3.09
3.36
3.24
ppm
BRUKER
NAME drraahood-KR-314
EXPNO 10
PROCNO 1
Date_ 20120119
Time 22.13
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 362
DW 48.400 usec
DE 6.50 usec
TE 298.1 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
SFO1 500.1330885 MHz
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

BBO DMSO D:\\ mmj

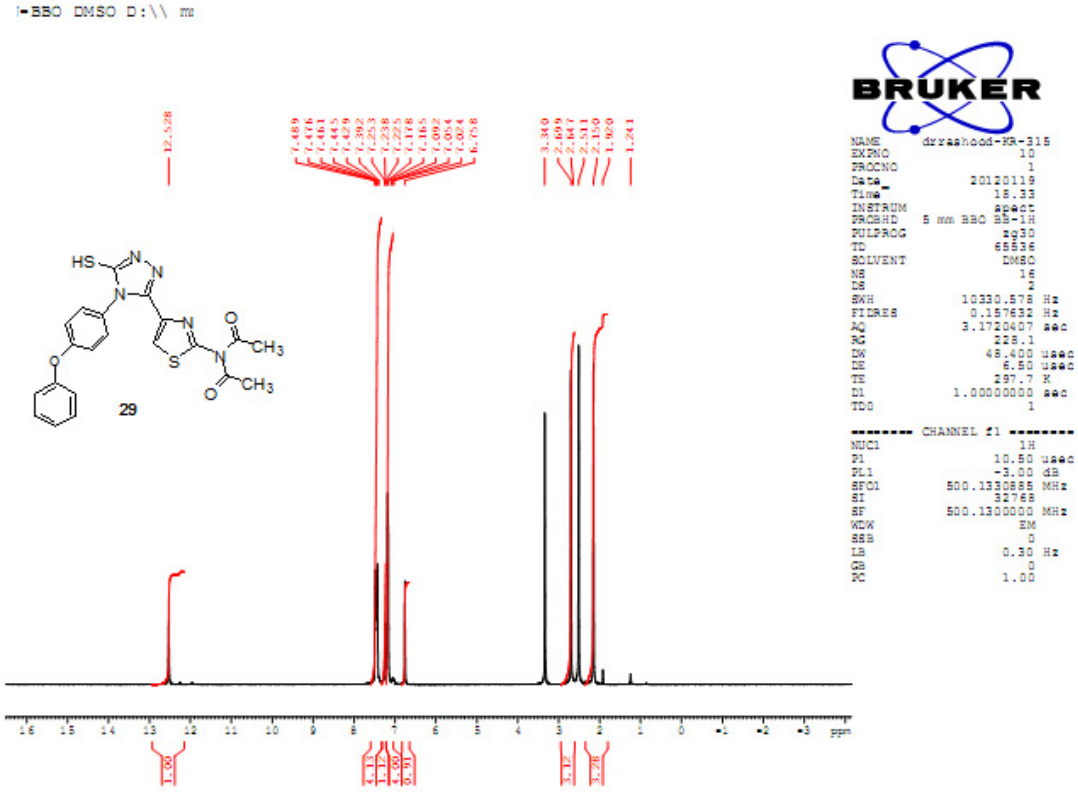


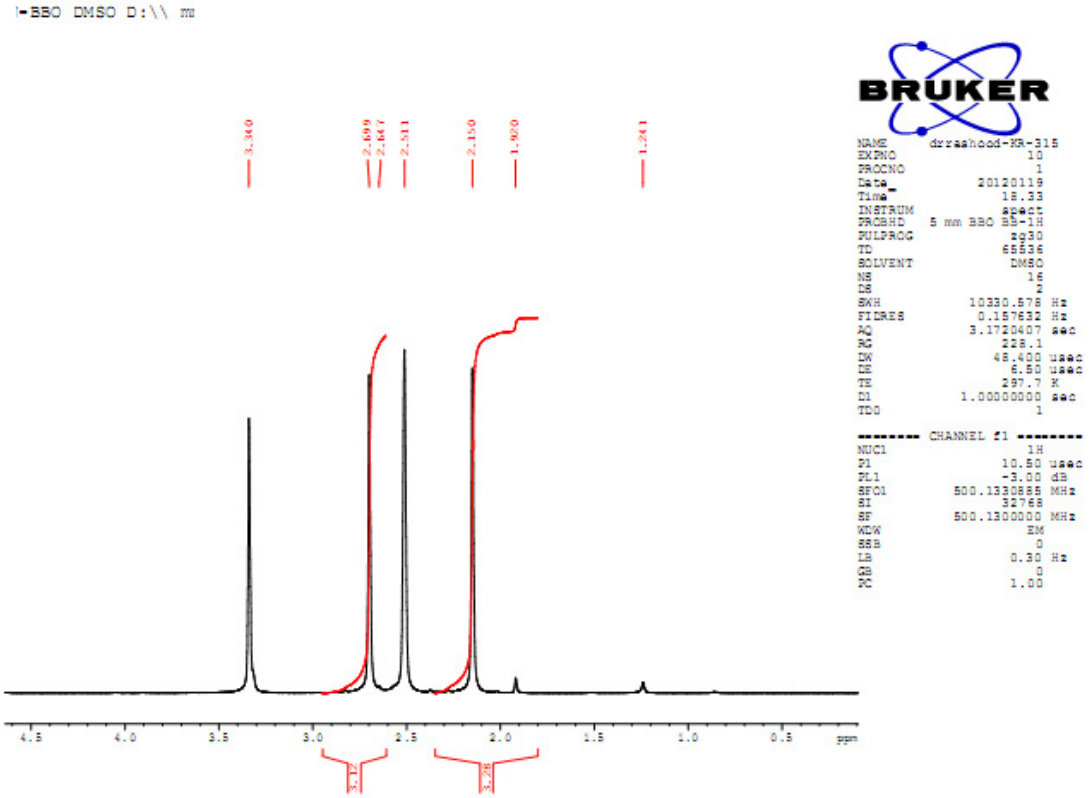

```
NAME          drxashood-KR-314
EXPNO                      11
PROCNO                      1
Date_              20120120
Time                    1.49
INSTRUM                 spect
PROBHD     5 mm BBO BB-1H
PULPROG             zgpg30
TD                      65536
SOLVENT                 DMSO
NS                      4096
DS                         4
SWH              30030.029 Hz
FIDRES            0.458222 Hz
AQ                1.0912410 sec
RG                    1625.5
DW                  16.650 usec
DE                   6.50 usec
TE                   297.9 K
D1               2.00000000 sec
D11              0.03000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                     13C
P1                    5.80 usec
PL1                  -2.00 dB
SFO1           125.7703643 MHz

======== CHANNEL f2 ========
CPDPRG2              waltz16
NUC2                     1H
PCPD2                80.00 usec
PL2                  -3.00 dB
PL12                 14.64 dB
PL13                 17.64 dB
SFO2           500.1320005 MHz
SI                    32768
SF             125.7578519 MHz
WDW                      EM
SSB                       0
LB                     3.00 Hz
GB                        0
PC                     1.40
```

BBO DMSO D:\\ mmj

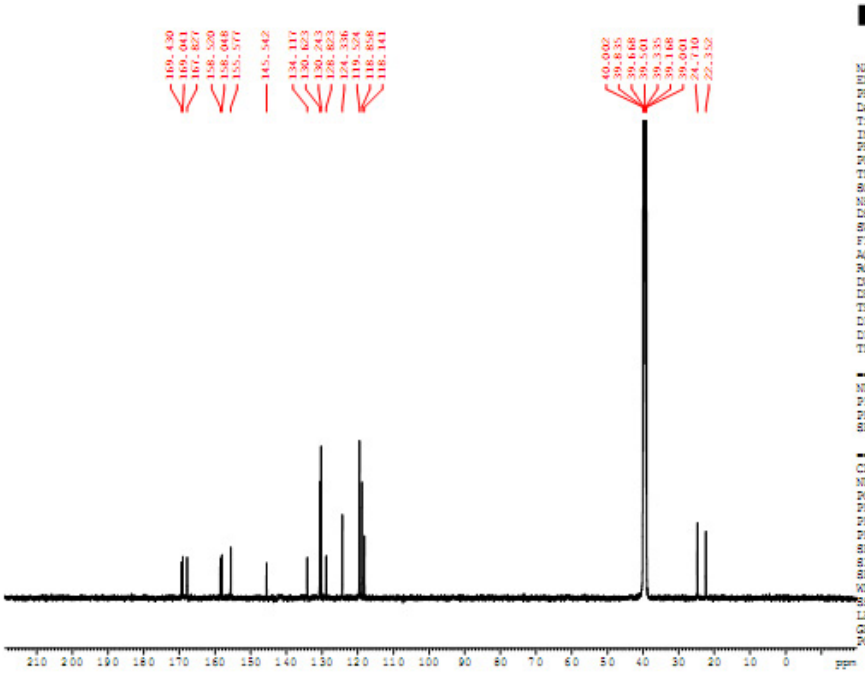



```
NAME          drxashood-KR-314
EXPNO                      11
PROCNO                      1
Date_              20120120
Time                    1.49
INSTRUM                 spect
PROBHD     5 mm BBO BB-1H
PULPROG             zgpg30
TD                      65536
SOLVENT                 DMSO
NS                      4096
DS                         4
SWH              30030.029 Hz
FIDRES            0.458222 Hz
AQ                1.0912410 sec
RG                    1625.5
DW                  16.650 usec
DE                   6.50 usec
TE                   297.9 K
D1               2.00000000 sec
D11              0.03000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                     13C
P1                    5.80 usec
PL1                  -2.00 dB
SFO1           125.7703643 MHz

======== CHANNEL f2 ========
CPDPRG2              waltz16
NUC2                     1H
PCPD2                80.00 usec
PL2                  -3.00 dB
PL12                 14.64 dB
PL13                 17.64 dB
SFO2           500.1320005 MHz
SI                    32768
SF             125.7578519 MHz
WDW                      EM
SSB                       0
LB                     3.00 Hz
GB                        0
PC                     1.40
```


1-BBO DMSO D:\\ m
12.528
7.489
7.476
7.461
7.445
7.439
7.392
7.253
7.238
7.225
7.178
7.165
7.092
7.054
7.024
6.758
3.340
2.699
2.647
2.511
2.150
1.920
1.241
HS
N
N
N
S
O
CH3
O
CH3
O
29
BRUKER
NAME drrashood-KR-315
EXPNO 10
PROCNO 1
Date_ 20120119
Time 18.33
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 228.1
DW 48.400 usec
DE 6.50 usec
TE 297.7 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
SFO1 500.1330885 MHz
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm
1.00
4.13
1.13
1.00
0.91
3.12
3.28


1-BBO DMSO D:\\ m
3.340
2.699
2.647
2.511
2.150
1.920
1.241
BRUKER
NAME drrashood-KR-315
EXPNO 10
PROCNO 1
Date_ 20120119
Time 18.33
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 228.1
DW 48.400 usec
DE 6.50 usec
TE 297.7 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 10.50 usec
PL1 -3.00 dB
SFO1 500.1330885 MHz
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm
3.12
3.28

BBO DMSO D:\\ mmj



BRUKER

NAME	drxashood-KR-315
EXPNO	11
PROCNO	1
Date_	20120119
Time	22.09
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	4096
DS	4
SWH	30030.029 Hz
FIDRES	0.458222 Hz
AQ	1.0912410 sec
RG	1625.5
DW	16.650 usec
DE	6.50 usec
TE	298.3 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	5.80 usec
PL1	-2.00 dB
SFO1	125.7703643 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-3.00 dB
PL12	14.64 dB
PL13	17.64 dB
SFO2	500.1320005 MHz
SI	32768
SF	125.7578519 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.40