Original Article

Molecular Docking, ADME Study, Synthesis And Characterization Of New 4-Amino-5-Aryl-4H-1,2,4-Triazole-3-Thiol Derivatives

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Abstract

In this work, we discuss the pharmacological evaluation and synthesis of new heterocyclic molecules derived from some drug (indomethacin and mefenamic acid). 4-amino-5-aryl-4H-1,2,4-triazole-3-thiol (1a-c) were created by the interaction of drug has carboxylic group and thiocarbohydrazide, and the starting products 4-amino-5-aryl-4H-1,2,4-triazole-3-thiol) were treated with carbon disulfide in present NaOH to produce final products (2a-c). All derivatives (intermediate and final products) were characterized by FT-IR, ^{1}H-NMR, and Mass spectroscopy to confirm the structure of produced compounds. Afterward, assess each compound's toxicity to animals and in vivo anti-inflammatory activity (in vivo). In this present research,1.2.4-triazole derivatives were subjected to molecular docking to create safe and efficient molecules. To test each derivative's ability to bind to the enzyme's active site, it was docked into the active sites. To establish the topological polar surface area, bioavailability, and drug-likeness of the synthesized compound, an ADME investigation was conducted. The results showed that the tested compounds followed the Lipinski rule and were absorbed orally.

Keywords: 1,2,4-triazole, indomethacin, mefenamic acid, hippuric acid , Molecular docking, ADME study.

Introduction

Microbial pathogens have existed for a long time. **(1)(2)** As a result of the most advanced infectious agents, the emergence of antimicrobial resistance, and the extremely heavy use of antibiotics that lead to resistant antibacterial agents**.(3)** In the organic chemistry heterocyclic compounds represented an essential part due to the wide of their activity which making it different compounds from anther cyclic compounds and making it an important part in organic chemistry and drug design **(4).** 1,2,4-triazole derivatives have a wide variety of therapeutically important available in clinical therapy, Scientists have been paying considerable attention, in the last few decade to the synthesis of 1,2,4-triazole derivatives and study their pharmacological activities as an antifungal **(5,6)** antioxidant**(7),** antitubercular **(8)** , anti-inflammatory **(9)** , anticancer **(10)** , anticonvulsant **(11)** , analgesic **(12)** , antidiabetic **(13)** and anxiolytic. **(14)** amide derivatives of mefenamic acid were tested for their antibacterial and anticancer activities **(15)** it has been found induced significant reduction in the cytotoxic response as compared with controls.). In-vitro pharmacological assessment of derivatives were done for COX-1 and COX-2 enzymes for the determination of selectivity which show high activity **(16),** Also, Indomethacin is a nonselective inhibitor of (COX 1 & COX 2) enzymes that participate in production of prostaglandin from arachidonic acid **(17).**

Materials and general Methods

Commercial sources provided the synthetic chemicals employed in the synthesis as well as the solvents needed in the purification, recrystallization, and analysis of generated products (BDH-England, Himedia-India, CDH-India, HyperChem-China and Sigma Aldrich-Germany). At Mustansiriyah University/College of Science/Department of Chemistry/Iraq, the melting points of the produced compounds were measured using a (digital Stuart scientific SMP30) and were uncorrected. At Mustansiriyah University, College of Science, Department of Chemistry in Iraq, the infrared spectrum FTIR) was measured using a (Bruker FT-IR 8400S spectrophotometer) in the range (4000 – 600 cm^{-1}). At Sharif University of Technology in Iran, the ^1H -NMR spectra were recorded using a 500MHz Bruker DMX-500 NMR spectrophotometer and DMSO- d_6 solution with TMS (tetramethylsilane) as an internal standard reference. At Tehran University, Varrian 3900 and 5793 Network, Mass Selective Detector was used to record mass spectra.

Synthesis of 4-amino-5-aryl-4H-1,2,4-triazole-3-thiol derivatives 1a-c (18)

An equimolar mixture of NSAID (indomethacin or hippuric acid or mefenamic acid) (0.01 mole) and thiocarbohydrazide (0.01 mole) put in round bottom flask and continuous heating on an oil bath until melting all the contented. Then persistent stirring and kept the heat at (165-175°**C)** for additional 15 min. The product that obtained was permitted to cool then treating by dilute sodium bicarbonate solution; to remove any unreacted acid lift. then solid was filtering, washing with water, drying, recrystallizing by ethanol for getting the purred triazoles (1a-c)

1a: N-[(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]benzamide

Yield 78 %, mp.165-168°C, FTIR v (cm^{-1}),), peak of the (NH_2) at (3163- 3132) Cm^{-1} (Asym. and Sym.), peak at (3199) for NH (amide) , (C=O) of amide at (1632 Cm^{-1}), (2952, 2828) return to the (CH_2) and three peaks of the (C=C ar) at (1579, 1500, 1479) Cm^{-1}) returning to the aromatic ring ^1H -NMR(DMSO- d_6,500MHz)(ppm), signal at (11.31 ppm) (singlet, 1H,) due to SH group, (10.31 ppm) (singlet, 1H, NH) of amide group signal at (7.25-7.30 ppm) (multi. , 5H) related to the phenyl group (4.82 ppm) (singlet, 2H,) due to the (NH_2) group , and (4.22 ppm) (singlet, 2H) due to (CH_2) group. Mass, molecular ion (m/z , 249.00) calculated Mwt (249.29 g/mol).

1b: 4-amino-5-{2-[(2,3-dimethylphenyl)amino]benzyl}-4H-1,2,4-triazole-3-thiol

Yield 85 %, mp.58-60°C, FTIR v (cm^{-1}), (NH_2) at (3186- 3164) Cm^{-1} (Asym. and Sym.), peak at (3355) for NH (secondary amine) (3064 Cm^{-1}) for CH aromatic ring . (2921, 2860) return to the (CH_2) and three peaks at (1587, 1535, 1503, Cm^{-1}) returning to the (C=C ar) of aromatic ring .^1H -NMR(DMSO- d_6,500MHz)(ppm) signal at (11.33 ppm) (singlet, 1H,) due to SH group, signal at (7.69 - 6.93) ppm) (multi. , 7H) related to the aryl group (4.82 ppm) (singlet, 2H,) due to the (NH_2) group , and (4.74 ppm) (singlet, 1H) due to (NH) of amine group. Signal at (2.93 ppm , 3H , singlet) for CH_3 at m-position of NH, signal at (2.74 ppm) , (3H , singlet due to CH_3 at o-position of NH Mass, molecular ion (m/z , 311.00) calculated Mwt (311.25 g/mol).

1c:{3-[(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-5-methoxy-2-methyl-1H-indol-1-yl}(4-chlorophenyl)methanone

Yield 71 %, mp.148-150°C, FTIR v (cm^{-1}), (NH_2) at (3282- 3153) Cm^{-1} (Asym. and Sym.), peak at (3052 Cm^{-1}) for CH aromatic ring , (2926, 2851) return to the (CH) aliphatic , strong peak at (1642 Cm^{-1}) for carbonyl of amide group and three peaks at (1599, 1562, 1485, Cm^{-1}) returning to the (C=C ar) of aromatic ring . ^1H -NMR(DMSO- d_6,500MHz)(ppm), signal at (11.31 ppm) (singlet, 1H,) due to SH group, signal at (8.19 – 7.13) ppm) (multi. , 7H) related to the aryl group (4.82 ppm) (singlet, 2H,) due to the (NH_2) group , and (4.22 ppm) (singlet, 3H) due to (CH_3O), Signal at (2.82 ppm , 2H , singlet) for CH_2 , while signal at (2.26 ppm) , (3H , singlet due to CH_3, Mass, molecular ion (m/z , 427.00) calculated Mwt (427.12 g/mol).

General procedure for synthesis of 3-Aryl[1,2,4]triazolo[3,4-b] [1,3,4]thiad iazole-6(5H)-thione 2a-c (19,20)

Solution of 1a-c (0.001mol) in (20ml) %10 ethanolic solution of KOH was stirring at R.T. for 30min. then CS_2 (5 mL) was added dropwise and the mixture was refluxed for 8hrs.After that the reaction mixture was purred in cool water and neutralized with HCl until PH 7 to obtain the precipitate which filtered off and wish with distilled water several time then dried and recrystallized from ethanol.

2a:N-[(6-thioxo-5,6-dihydro[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl]benzamide

Yield 65 %, mp.207-209°C, FTIR v (cm^{-1}), (NH) at (3216) Cm^{-1}), secondary thioamide, (3162) for NH (amide) , (3035 Cm^{-1}) for CH aromatic ring, (2968, 2816) return to the (CH) aliphatic , (C=O) amide (1638 Cm^{-1}). (C=C ar) at (1591, 1579, 1513) Cm^{-1}). ^1H -NMR(DMSO- d_6,500MHz)(ppm), signal at (11.31 ppm) (singlet, 1H,) for SH group and at (4.82 ppm) (singlet, 2H,) for the (NH_2) group, signal at (10, 20 ppm) (singlet, 1H, NH) amide group, (11.63 ppm) (singlet, 1H, NH) thioamide group, (7.72-7.68 ppm) (multi. , 5H) phenyl group, , and (4.28 ppm) (singlet, 2H) (CH_2) group. Mass, molecular ion (m/z , 291.00) calculated Mwt (291.35 g/mol).

2b:3-{2-[(2,3-dimethylphenyl)amino]benzyl}[1,2,4]triazolo[3,4-b][1,3,4]thiadi- -azole-6(5H)-thione.

Yield 62 %, mp.170-172°C, FTIR v (cm^{-1}), (NH_2) at (3186- 3164) Cm^{-1} (Asym. and Sym.), (NH) at (3314) Cm^{-1}), secondary thioamide (3166) NH (secondary amine) (3064 Cm^{-1}) CH aromatic ring, (2974, 2920) (CH_2) and (1599, 1532, 1507, Cm^{-1}) (C=C ar). ^1H -NMR(DMSO- d_6,500MHz)(ppm),(11.33 ppm) (singlet, 1H,) SH group, (11.71 ppm) , (singlet, 1H) NH thioamide , (7.72 – 7.46) ppm) (multi. , 7H) aryl group, (4.78 ppm) (singlet, 1H,) due to (NH) of amine group, (3,04 ppm , 3H , singlet) CH_3 at m-position of NH, (2.66 ppm), (3H , singlet CH_3 at o-position of NH, Mass, molecular ion (m/z , 353.00) calculated Mwt (353.19 g/mol).

2c:(4-chlorophenyl){5-methoxy-2-methyl-3-[(6-thioxo-5,6-dihydro[1,2,4]tria-zolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl]-1H-indol-1-yl}methanone.

Yield 69 %, mp.210-212°C, FTIR v (cm^{-1}) , (NH_2) at (3282-3153) Cm^{-1} (Asym. and Sym.), (NH) at (3342) Cm^{-1}), secondary thioamide, (3040 Cm^{-1}) CH aromatic ring . (2945, 2888) (CH) aliphatic, (1652 Cm^{-1}) carbonyl of amide, (1610, 1570, 1509, Cm^{-1}) (C=C ar) of aromatic ring . ^1H -NMR(DMSO- d_6,500MHz)(ppm), signal at (11.31 ppm) (singlet, 1H,) SH group, (4.82 ppm) (singlet, 2H,) (NH_2) group, (10.29 ppm) , (singlet, 1H) NH thioamide, (8.18 – 7.24) ppm)(multi. ,7H) aryl group , (4.26 ppm) (singlet, 3H) (CH_3O), (2.84 ppm , 2H , singlet) CH_2 , (2.23 ppm), (3H , singlet (CH_3), Mass, molecular ion (m/z , 469.00) calculated Mwt (469.41 g/mol).

Molecular docking studies

Drug discovery and development has become a serious issue in recent years due to the cost and effort required to synthesize novel compounds with adequate pharmacological properties. Due to the toxicity and lack of action of many medications during phase II and phase III of clinical trials, this expense has partially increased.(21)(22). Since they help researchers save time, money, and effort, computational tools are quickly gaining popularity and taking on a more significant role in the discovery and development of medicines. The docking process is one of these strategies for foretelling the conformation and orientation of the ligand within the binding site of the target. The molecular docking was examined by utilizing GlideTM, (version 5.7, Schrödinger, LLC, New York, NY, 2011). The most active compounds were docked on the active sites of enzyme. The crystal structure form of the enzymes in complex with anti-inflammatory drug Indomethacin, mefenamic acid and hippuric acid, whose PDB ID were 1NNI, 3G7E, 4HL2, 2W9S, and 4RKX. The molecules of hetero atoms and water were eliminated from enzymes behind 5Å radius of reference ligand (Indomethacin, mefenamic acid and hippuric acid).The Protein Preparation Wizard™ application employed the OPLS-2005 force field to minimize protein structure, and the Receptor Grid Generation program was used to create the grid of enzyme. The Ligand Preparation™ algorithm then optimized each ligand to create the lowest energy state using the OPLS-2005 force field. After five poses for each ligand were generated using docking simulations, the best pose (with the highest score) for each molecule was displayed.

Procedures of ADME

Chem Create Sketch (v. 19) was used to sketch the entire set of compounds (2a-c), and the Swiss ADME tool software changed these ligands into the name SMILE in order to forecast their physical, chemical, and pharmacokinetic characteristics. We could ascertain the polarity and lipophilicity of the tiny compounds using BOILED EGG.(23)

Study the safety of new derivatives

The degree of lethality of compounds were detected by determining the lethal dose 50% (LD50)**(24) .**Mice were injected intraperitoneally with concentration (500 mg/kg 2a,b hippuric acid and mefenamic acid) and (50 mg/kg 2c and indomethacin) of derivatives in 0.2ml Dimethyl sulphaoxide DMSO. All animals survive, the result shown below table (1)

Table (1) % of death in of new derivative 2a , drugs and DMSO administered intraperitoneally in mice

Groups n=6	**Mortality(x/N)**	**% of death**	**symptom**
2a	0/6	0%	Nil
2b	0/6	0%	Nil
2c	0/6	0%	Nil
Hippuric acid	0/6	0%	nil
indomethacin	0/6	0%	nil
Mefenamic acid	0/6	0%	nil
DMSO 0.2ml	0/6	0%	Nil

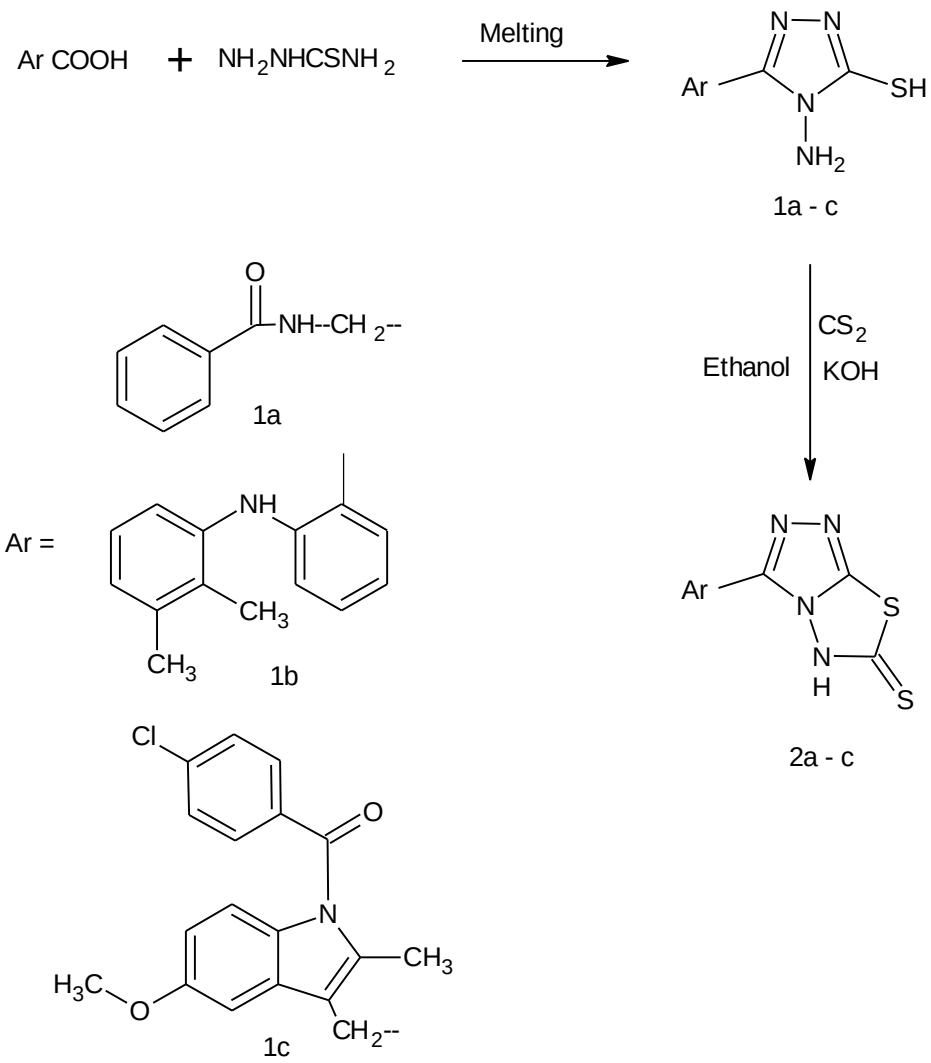
Table (2) Effects of different concentrations of new imidazole derivative(compound 3) and compared with metronidazole and diclofenac sodium on granuloma formation in cotton pellet-induced granuloma in mice

$$\text{Granuloma inhibition (\%)} = 1 - \frac{\text{Weight of granuloma in mg of treated group of mice}}{\text{Weight of granuloma in mg of control group of mice}} \times 100$$

Groups of mice N=6	**Mean dry weight of granuloma (mg) ±SE**	**Granuloma inhibition (%)**
Cotton weight (before)	0.010 ±0.00	------
Positive control	0.041±0.002	------
2a	0.011 ±0.001	98.4%
2b	0.012 ±0.001	83.3%
2c	0.012 ±0.001	83.3%
Hippuric acid	0.040 ±0.001 [a]	2.8%
indomethacin	0.028 ±0.0009 [b]	41%
Mefenamic acid	0.024 ±0.0004 [c]	53.9%

Results and Discussion

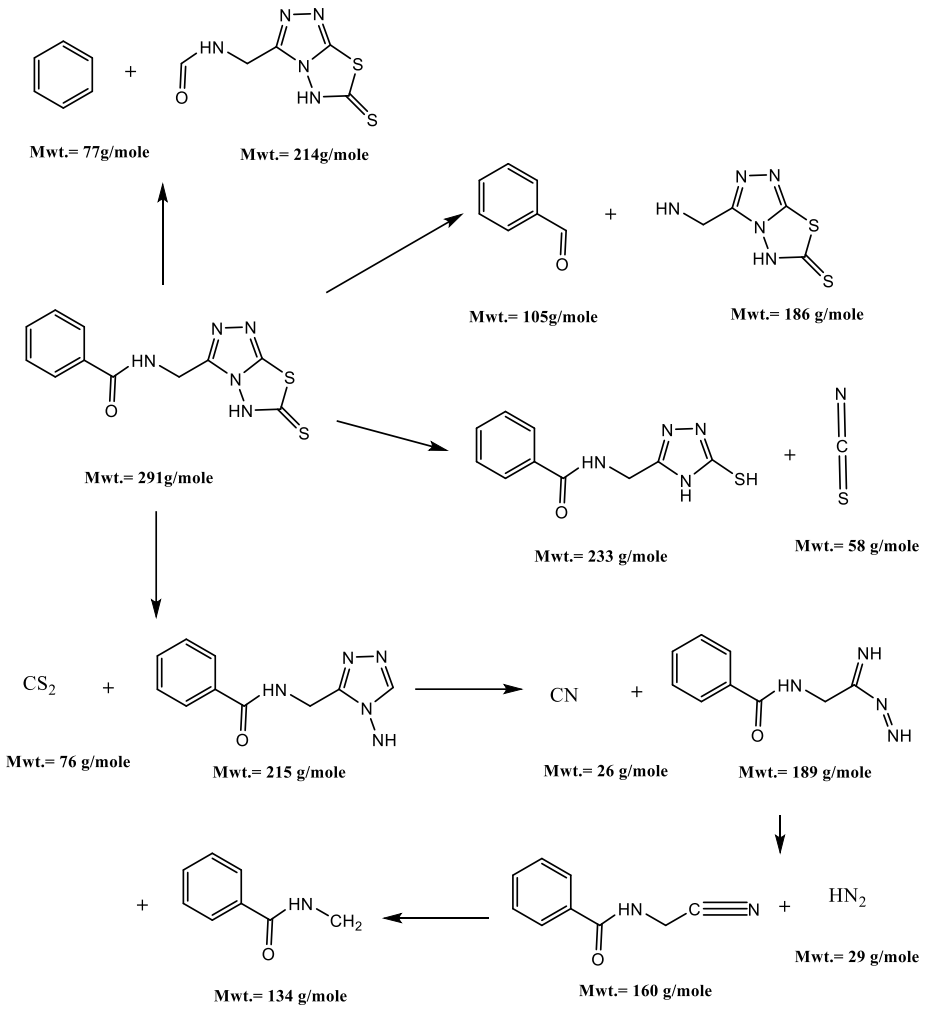
The sequences of reactions used in Scheme (1) to create the desired novel indomethacin , mefenamic acid and hippuric acid derivatives with different moieties. FTIR, H-NMR and mass spectra were used to confirm the structure of (2a – c).



Scheme (1): synthesis of the intermediates and target products

The FTIR spectrum of compound 2a displays an appearance band of the (NH) at (3216) Cm^{-1}), secondary thioamide, peak at (3162) for NH (amide) , peak at (3035 Cm^{-1}) for CH aromatic ring, the spectrum has two peaks at (2968, 2816) return to the (CH) aliphatic , (C=O) group in amide appeared at (1638 Cm^{-1}). and three peaks of the (C=C ar) at (1591, 1579, 1513) Cm^{-1}) returning to the aromatic ring . ^{1}H-NMR spectrum of compound (2a) showed the disappearance signal at (11.31 ppm) (singlet, 1H,) for SH group and at (4.82 ppm) (singlet, 2H,) for the (NH_2) group while appearance signal at (10. 20 ppm) (singlet, 1H, NH) of amide group and at (11.63 ppm) (singlet, 1H, NH) of thioamide group, signal at (7.72-7.68 ppm) (multi. , 5H) related to the phenyl group, , and (4.28 ppm) (singlet, 2H) due to (CH_2) group. While the MS spectrum of the derivative (2a) gave a signal at (291 m/z) representing the molecular ion and this corresponds to the calculated molecular weight of the derivative (2a).

The mechanism fragmentations for compound(2a) as shown



As a result, the derivative (2b)'s infrared spectrum exhibits the disappearance peak of the (NH2) at (3186-3164) Cm-1 (Asym. and Sym.) and the appearance peak of the (NH) at (3314) Cm-1. In addition, the secondary thioamide peak for the NH (secondary amine) is located at (3166), while the peak for the CH aromatic ring is located at (3064 Cm -1). The spectrum has three peaks at (1599, 1532, 1507, Cm-1) returning to the aromatic ring's (C=C ar) and two peaks at (2974, 2920) returning to the (CH2). Compound (2b) 1H-NMR spectrum revealed the disappearance signal at (11.33 ppm) (singlet, 1H), which was caused by the SH group, and the appearance signal at (11.71 ppm) (singlet, 1H), which was caused by the NH thioamide signals, which were related to the aryl group signal at (4.78 ppm) (singlet, 1H,) due to (NH) of amine group. Signal at (3,04 ppm , 3H , singlet) for CH_3 at m-position of NH while signal at (2.66 ppm) , (3H , singlet due to CH_3 at o-position of NH. So, MS spectrum of the derivative (2b) gave a signal at (353 m/z) representing the molecular ion and this corresponds to the calculated molecular weight of the derivative (2a). Infrared spectrum of the derivative (2c) shows the disappearance peak of the (NH_2) at (3282-3153) Cm^{-1} (Asym. and Sym.), and appearance peak of the (NH) at (3342) Cm^{-1}), secondary thioamide peak at (3040 Cm^{-1}) for CH aromatic ring . The spectrum has two peaks at (2945, 2888) return to the (CH) aliphatic, strong peak at (1652 Cm^{-1}) for carbonyl of amide group and three peaks at (1610, 1570, 1509, Cm^{-1}) returning to the (C=C ar) of aromatic ring . The compound (2c) ^{1}H-NMR spectrum revealed the signal at (11.31 ppm) (singlet, 1H), at (4.82 ppm) (singlet, 2H), due to the SH group, disappearing, and the appearance of signal at (10.29 ppm) (singlet, 1H), related to the NH thioamide, signal at (8.18 - 7.24) ppm) (multi., 7H), related to the aryl group , and (4.26 ppm) (singlet, 3H) due to (CH_3O),

Signal at (2.84 ppm , 2H , singlet) for CH_2 , while signal at (2.23 ppm) , (3H , singlet due to CH_3. So, MS. spectrum of the derivative (2c) figure gave a signal at (469 m/z) representing the molecular ion and this corresponds to the calculated molecular weight of the derivative (2c)

References:

1- Morse SS. Factors in the emergence of infectious diseases. Plagues Polit. 2001;8–26.
2- Jwaid MM, Ali KF, Abd-alwahab MH. Synthesis, Antibacterial study and ADME Evaluation of Novel Isonicotinoyl Hydrazide Derivative Containing 1, 3, 4-Oxadiazole Moiety. Al Mustansiriyah J Pharm Sci. 2020;20(4):113–21

3- Mölstad S, Löfmark S, Carlin K, Erntell M, Aspevall O, Blad L, et al. Lessons learnt during 20 years of the Swedish strategic programme against antibiotic resistance. Bull World Health Organ. 2017;95(11):764.

4- Abbas Washeel Salman, Haitham K RAl- Sharifi and SalihAM Kadim (2017) Antibacterial activity of new benzylbenzimidazole complexes against Grampositive (+ve) and-negative (-ve) bacterial species isolated from surgical wounds of Iraqi patients. Int. J. Multidiscipl. Curr. Res. 5, 1450-1453.

5- Karaca Gençer, H.; Acar Çevik, U.; Levent, S.; Sağlık, B.N.; Korkut, B.; Özkay, Y.; Ilgın, S.; Öztürk, Y. New Benzimidazole-1,2,4-Triazole Hybrid Compounds: Synthesis, Anticandidal Activity and Cytotoxicity Evaluation. Molecules 2017, 22, 507.
6-Appna, N.R.; Nagiri, R.K.; Korupolu, R.B.; Kanugala, S.; Chityal, G.K.; Thipparapu, G.; Banda, N. Design and Synthesis of Novel 4-Hydrazone Functionalized/1,2,4-Triazole Fused Pyrido[2,3-d]Pyrimidine Derivatives, Their Evaluation for Antifungal Activity and Docking Studies. Med. Chem. Res. 2019, 28, 1509–1528.
7- Peng, Z.; Wang, G.; Zeng, Q.H.; Li, Y.; Wu, Y.; Liu, H.; Wang, J.J.; Zhao, Y. Synthesis, Antioxidant and Anti-Tyrosinase Activity of 1,2,4-Triazole Hydrazones as Antibrowning Agents. Food Chem. **2021**, 341, 128265
8- Rode, N.D.; Sonawane, A.D.; Nawale, L.; Khedkar, V.M.; Joshi, R.A.; Likhite, A.P.; Sarkar, D.; Joshi, R.R. Synthesis, Biological Evaluation, and Molecular Docking Studies of Novel 3-Aryl-5-(Alkyl-Thio)-1H-1,2,4-Triazoles Derivatives Targeting Mycobacterium Tuberculosis. Chem. Biol. Drug Des. **2017**, 90, 1206–1214.
9- Li, S.M.; Tsai, S.E.; Chiang, C.Y.; Chung, C.Y.; Chuang, T.J.; Tseng, C.C.; Jiang, W.P.; Huang, G.J.; Lin, C.Y.; Yang, Y.C.; et al. New Methyl 5-(Halomethyl)-1-Aryl-1H-1,2,4-Triazole-3-Carboxylates as Selective COX-2 Inhibitors and Anti-Inflammatory Agents: Design, Synthesis, Biological Evaluation, and Docking Study. Bioorg. Chem. **2020**, 104, 104333.
10- Grytsai, O.; Valiashko, O.; Penco-Campillo, M.; Dufies, M.; Hagege, A.; Demange, L.; Martial, S.; Pagès, G.; Ronco, C.; Benhida, R. Synthesis and Biological Evaluation of 3-Amino-1,2,4-Triazole Derivatives as Potential Anticancer Compounds. Bioorg. Chem. 2020, 104, 104271.
11- Kaproń, B.; Łuszczki, J.J.; Siwek, A.; Karcz, T.; Nowak, G.; Zagaja, M.; Andres-Mach, M.; Stasiłowicz, A.; Cielecka-Piontek, J.; Kocki, J.; et al. Preclinical Evaluation of 1,2,4-Triazole-Based Compounds Targeting Voltage-Gated Sodium Channels (VGSCs) as Promising Anticonvulsant Drug Candidates. Bioorg. Chem. **2020**, 94, 103355.
12- Khanage, S.G.; Raju, A.; Mohite, P.B.; Pandhare, R.B. Analgesic Activity of Some 1,2,4-Triazole Heterocycles Clubbed with Pyrazole, Tetrazole, Isoxazole and Pyrimidine. Adv. Pharm. Bull. **2013**, 3, 13–18.
13-Hichri, F.; Omri, A.; Hossan, A.S.M.; Ben Jannet, H. Alpha-Glucosidase and Amylase Inhibitory Effects of Eruca Vesicaria Subsp. Longirostris Essential Oils: Synthesis of New 1,2,4-Triazole-Thiol Derivatives and 1,3,4-Thiadiazole with Potential Inhibitory Activity. Pharm. Biol. **2019**, 57, 564–570.
14- Navidpour, L.; Shabani, S.; Heidari, A.; Bashiri, M.; Ebrahim-Habibi, A.; Shahhosseini, S.; Shafaroodi, H.; Abbas Tabatabai, S.; Toolabi, M. 5-[Aryloxypyridyl (or Nitrophenyl)]-4H-1,2,4-Triazoles as Novel Flexible Benzodiazepine Analogues: Synthesis, Receptor Binding Affinity and Lipophilicity-Dependent Anti-Seizure Onset of Action. Bioorg. Chem. **2021**, 106, 104504.
15-Iman Assim Mohammed , Mohanad Mousa Kareem Synthesis, Characterization and Study of some of New Mefenamic Acid Derivatives as cytotoxic Agents , Journal of Physics: Conference Series 1664 (2020) 012081 IOP Publishing doi:10.1088/1742 -6596/1664/1/012081.
16- Jignasa K.Savjani, SujaMulamkattil , BhaveshVariya , SnehalPatel , Molecular docking, synthesis and biological screening of mefenamic acid derivatives as anti-inflammatory agents, European Journal of Pharmacology,Volume 801, 2017, Pages 28-34
17- Miyandoab SA, Babazadeh M. Indomethacin macromolecular prodrugs: Synthesis, characterization and in vitro evaluation. Der Pharma Chem. 2015;7(1):148-155.
18- -Iman Assim Mohammed , Mohanad Mousa Kareem Synthesis, Characterization and Study of some of New Mefenamic Acid Derivatives as cytotoxic Agents , Journal of Physics: Conference Series 1664 (2020) 012081 IOP Publishing doi:10.1088/1742 -6596/1664/1/012081
19- Altintop, M. D.; Ciftci, H. I.; Radwan, M. O.; Sever, B.; Kaplancikli, Z. A.; Ali, T. F. S.; Koga, R.; Fujita, M.; Otsuka, M.; Özdemir, A. Design, synthesis, and biological evaluation of novel 1,3,4-thiadiazole derivatives as potential antitumor agents against chronic myelogenous leukemia: Striking effect of nitrothiazole moiety. Molecules 2018, 23, 59.

20- Barbosa, G. A. D.; de Aguiar, A. P.*., Synthesis of 1,3,4-Thiadiazole Derivatives and Microbiological Activities , Rev. Virtual Quim., 2019, 11 (3), 806-848.

21- Omran SM, Abd Razik BM, Mahdi MF. Density Functional Theory and Molecular Modeling Studies of New 4-(Furan-2-yl) Thiazol-2-Amine

Derivatives as Cyclooxygenase Inhibitors. Egypt J Chem. 2021;64(9):4833–41.
22- Cobanoglu MC, Liu C, Hu F, Oltvai ZN, Bahar I. Predicting drug–target interactions using probabilistic matrix factorization. J Chem Inf Model. 2013;53(12):3399–409.
23- Daina A, Zoete V. A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. ChemMedChem. 2016;11(11):1117–21.
24-) Patil KR, Patil CR. Anti-inflammatory activity of bartogenic acid containing fraction of fruits of Barringtonia racemosa Roxb. in acute and chronic animal models of inflammation. Journal of traditional and complementary medicine. 2017 Jan 1;7(1):86-93