Molecular docking, Synthesis and Characterization of New Indomethacin and Mefenamic Acid Analogues as Potential Anti-inflammatory Agents

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Abstract:

In this work the pharmacological study and synthesis of new thiadiazine bearing on triazole which obtained from hippuric acid , indomethacin and mefenamic acid that have carboxylic acid moiety, Drugs with carboxylic groups and thiocarbohydrazide interacted to produce the 4-amino-5-aryl-4H-1,2,4-triazole-3-thiol (1a-c). and the starting products 4-amino-5-aryl-4*H*-1,2,4-triazole-3-thiol) were treated with chloroacetyl chloride to produce final products (2a-c). To confirm the structure of the generated compounds, FT-IR, 1H-NMR, and mass spectroscopy were used to characterize all derivatives (intermediate and final products). The in vivo anti-inflammatory efficacy of some derivatives and thier toxicity to animals (in vivo) were evaluated. And then 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 determine the synthetic compound's topological polar surface area, bioavailability, and drug-likeness, An investigation of absorption, distribution, metabolism and elimination was performed. According to the findings, the tested derivatives adhered to the Lipinski rule and were ingested.

Keywords: Indomethacin, mefenamic acid1,2,4-triazole, Molecular docking, Anti-inflammatory

دراسة النمذجة وتخليق وتشخيص مشتقات جديدة لعقارالاندوميثاسين وحامض الميفينامك كأدوية محتملة مضادة للالتهاب

مصطفى طه عبد الله، منذر فيصل مهدي، اياد كريم خان

قسم الكيمياء الصيدلانية، كلية الصيدلة، الجامعة المستنصرية، بغداد، العراق

الخلاصة:

في هذا العمل تم تحضير ودراسة دوائية لمركبات الثاياديازين المشتقة من عقار الاندوميثاسين وحامض الميفينامك. تمت مفاعلة الادوية التي تحتوي على مجموعة كاربوكسيل مع مركب الثايوكارباهيدرازايد للحصول على ٤-امينو -٥- اريل - ١,٢,٤-ترايازول (١١-ج) ثم مفاعلة الناتج مع كلورو خلات الكلوريد ليعطي الناتج النهائي (١٢-ج) تم اثبات تراكيب المركبات المحضرة باستخدام أطياف الأشعة تحت الحمراء وطيف الرنين المغناطيسي وطيف الكتلة , كما تم دراسة الفعالية لهذه المشتقات كمضادات للالتهابات وكذلك دراسة سمية هذه المشتقات على فئران المختبر كما درست النمذجة الجزيئية لهذه المشتقات لإنتاج مركبات آمنة وفعالة. تم تثبيت كل مشتق في المواقع النشطة للانزيم لمعرفة ما إذا كان يمكن أن يرتبط بالموقع النشط للإنزيم. تم إجراء تحقيق في الحركة الدوائيه من أجل تقييم مساحة سطح القطب القطبية للمواد الكيميائية الاصطناعية، والتوافر البيولوجي، وشبه الدوائية. وفقًا للنتائج المستحصلة من الدراسة، فإن المشتقات المختبرة يمكن امتصاصها عن طريق الجهاز الهظمي وأنها مستوفية لشروط قاعدة لبنسكي.

الكلمات المفتاحية: الاندوميثاسين , حامض الميفينامك , ترايازول , النمذجة الجزيئية , مضادات الالتهابات

Introduction

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), which have anti-inflammatory characteristics, are the most often utilized medicinal substances worldwide [(1)] and NSAIDs are frequently used to reduce pain and inflammation [(2)] **,** Since their initial release, numerous antibiotics have been developed to treat resistant forms of organisms that were previously sensitive to other antibiotics. [(3)] Due to the extensive range of activities that heterocyclic compounds exhibit and played a crucial role and represented an essential part in organic chemistry [(4)]**.** Microbial pathogens have existed for a long time. [(5)(6)]. Due to the most complex viral illnesses, the emergence of antimicrobial resistance, and the overuse of antibiotics that leads to the emergence of antibacterial agents with resistance (7). Many 1,2,4-triazole compounds with therapeutic value are employed in clinical therapy. Over the past few decades, scientists have paid close attention to synthesis of 1,2,4-triazole derivatives and the study their pharmacological activity. As an antioxidant [(8)], anti-inflammatory [(9)], antitubercular[(10)], anticancer[(11)], anticonvulsant [(12)] , analgesic [(13)] , antidiabetic[(14)] anxiolytic.[(15)] **and** antifungal [(16,17)]. Mefenamic acid's antibacterial and anticancer properties were investigated [(18)]**,** it has been found induced significant reduction in the cytotoxic response as compared with controls. For the purpose of determine selectivity of enzymes with high activity like COX-1 and COX-2,an *in-vitro* pharmacological evaluation of derivatives was carried out. [(19)]**.** Also, A nonselective inhibitor of (COX 1 & COX 2) enzymes involved in the generation of prostaglandin from arachidonic acid is indomethacin [(20)]**.**

Materials and general Methods

Chemicals that used in synthesis, purification and recrystallization, were obtained from commercial sources **(BDH-England; Himedia-India, CDH-India; HyperChem-China; Germany; Sigma Aldrich).**

FTIR spectra were measured with a spectrophotometer (Bruker FT-IR 8400) over the Range between 4000 to 600 cm^{-1}. The ^{1}H-NMR spectra were performed at the Sharif University of Technology in Iran using a 500 MHz Bruker DMX-500 NMR spectrophotometer, DMSO-d6 solution, and TMS (tetramethylsilane) as an internal standard reference. At Tehran University, mass spectra were carried out using Varian 3900 and 5793 Network, Mass Selective Detector.

Synthesis of 4-amino-5-aryl-4*H*-1,2,4-triazole-3-thiol derivatives 1a-c (21)

A mixture of 0.01 mole of NSAID (indomethacin, hippuric acid, or mefenamic acid) and thiocarbohydrazide (0.01 mole) were combined in a round bottom flask and heated continuously over an oil bath until all the contents melted. The heat was then maintained at (165–175)°C for an additional 15 minutes only with constant stirring . After cooling the final product, it was treated with dilute sodium bicarbonate solution to abolish remaining unreacted acid. The solids were then clarified, washed with water, dried and recrystallized from ethanol to give the purified triazoles (1a-c).

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

Compound (1a) was obtained as white tan, Yield 78 %, mp.165-168°C, FTIR *vmax* (cm^{-1}),), (3199) for NH (amide stretching , (3163- 3132) Cm^{-1} (Asym. and Sym.), NH_2 stretching), (1632 Cm^{-1}) (C=O amide stretching), (2952, 2828), (CH_2 stretching), (1579, 1500, 1479 Cm^{-1}) (C=C ar. stretching). ^{1}H -NMR *(DMSO-d_6*,500MHz)(ppm^{1}H –NMR (*DMSO- d_6*, 500 MHz) δ, ppm: 11.31 (S, 1H,SH), 10.31 (S, 1H, NH, amide),7.25-7.30 (M , 5H)phenyl group, 4.82 (S , 2H, NH_2) , and 4.22 (S , 2H, CH_2) . MS *m/z* : (M^+, 249), calculated M.wt. (249.29 g/mol).

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

Compound (1b) was obtained as white grey powder, Yield 85 %, mp.58-60°C, FTIR *vmax* (cm^{-1} (3355) for NH amine stretching , ((3186- 3164)) Cm^{-1} (Asym. and Sym.), NH_2 stretching), (2921, 2860), (CH_2 stretching), (1587, 1535, 1503Cm^{-1}) (C=C ar. stretching). ^1H -NMR *(DMSO- d_6*,500MHz)(ppm^1H –NMR (*DMSO- d_6*, 500 MHz) δ, ppm: 11.33 (S, 1H,SH 7.69 - 6.93 (M , 5H)Aryl group, 4.82 (S , 2H, NH_2) , and 4.74 (S , 1H, NH) 2.93 , 3H , S) f CH_3 at m-position of NH, signal (2.74 , 3H , S), CH_3 at o-. MS *m/z* : (M^+, 311), calculated M.wt. (311.25 g/mol).

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

Compound (1b) was obtained as white grey powder, Yield 71 %, mp.148-150°C, FTIR *vmax* (cm^{-1}), (3282- 3153) Cm^{-1} (Asym. and Sym.), NH_2 stretching) , (2926, 2851), (CH_2 stretching), 1642 (C=O amide) , (1599, 1562, 1485Cm^{-1}) (C=C ar. stretching). ^1H -NMR*(DMSO- d_6*,500MHz)(ppm^1H –NMR (*DMSO- d_6*, 500 MHz) δ, ppm: 11.31 (S, 1H,SH 8.19 – 7.13 (M , 7H) Aryl group, 4.82 (S , 2H, NH_2) , and 4.22 (S , 3H, OCH_3) 2.82, 2H , S) f CH_2, signal (2.26, 3H , S). MS *m/z* : (M^+, 427), calculated M.wt. (427.12 g/mol).

General procedure for synthesis of(3-Aryl-5,6-dihydro-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-one derivatives) (22,23)

To a solution of (1a-c) (0.001 moles) in (20ml) of 1,4-dioxane chloro acetyl chloride (0.56 g, 0.005 moles) was added dropwise, the reaction mixture was refluxed for (8hrs), after that the mixture cooled to R.T., purred in cool water solids precipitate was formed filtered off and recrystallized from ethanol: Dioxan (8:2).

2a: *N*-[(7-oxo-6,7-dihydro-5*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl]benzamide

Compound (2a) was obtained as yellow powder, Yield 85 %, mp.244-248°C, FTIR *vmax* (cm^{-1}), (3433) Cm^{-1}, NH thiadiazinone stretching) , 3124) NH amide , (2991, 2833), (CH_2 stretching), 1706 (C=O thioester), 1645 (C=O amide) , (1591, 1581, 1489 Cm^{-1}) (C=C ar. stretching). ^1H -NMR *(DMSO- d_6*,500MHz)(ppm^1H –NMR (*DMSO- d_6*, 500 MHz) δ, ppm: 10, 75 ppm) (S , 1H, NH), 7.55-7.24 (M , 5H) phenyl group, 4.72 (S , 1H) (N-HN, and 4.69 ppm), (S , 2H) (CH_2), 4.22) (S , 2H) due to (N-CH_2). MS *m/z* : (M^+, 429), calculated M.wt. (429.34 g/mol).

2b:3-{2-[(2,3-dimethylphenyl) amino]benzyl}-5,6-dihydro-7*H*-[1,2,4]triazole [3,4-*b*][1,3,4]thiadiazin-7-one

Compound (2b) was obtained as brawn powder, Yield 88 %, mp.244-248°C, FTIR *vmax* (cm^{-1}), (3492) Cm^{-1}, NH thiadiazinone stretching), (3364) NH amide, (2919, 2880), (CH_2 stretching), 1716 (C=O thioester), (1600, 1572, 1516Cm^{-1}) (C=C ar. stretching). ^1H -NMR*(DMSO- d_6*,500MHz)(ppm^1H –NMR (*DMSO- d_6*, 500 MHz) δ, ppm: 10, 75 ppm) (S , 1H, NH), 7.70 – 7.15 (M , 7H) Aryl group, 4.84 (S , 1H) (N-HN, and 4.43 ppm), (S , 2H) ((N-CH_2). MS *m/z* : (M^+, 351), calculated M.wt. (351.21 g/mol).

2c:3-{[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]methyl}-5,6-dihydro-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-one

Compound (2c) was obtained as white powder, Yield 75 %, mp.255-257°C, FTIR *vmax* (cm^{-1}), (3210) Cm^{-1}, NH thiadiazinone stretching) , (2942, 2858), (CH_2 stretching), 1715 (C=O thioester), (1602, 1571, 1521 Cm^{-1}) (C=C ar. stretching). ^1H -NMR*(DMSO- d_6*,500MHz)(ppm^1H –NMR (*DMSO- d_6*, 500 MHz) δ, ppm:, 8.16 – 7.19 (M , 7H)

Aryl group, 4.66 (S , 1H) (N-HN, and 4.59 ppm), (S , 2H) ((N-CH_2), 4.25 ppm) (S , 3H) (CH_3O), 2.78 , 2H , S), (CH_2) (2.22 ppm) , (3H , CH3) , MS *m/z* : (M^+, 467), calculated M.wt. (467.29 g/mol).

Molecular docking studies

Due to the expense and effort needed to create novel compounds with sufficient pharmacological properties, drug discovery and development has recently become a serious problem. This cost has increased to some extent as a result of the toxicity and ineffectiveness of many medications during phase II and phase III of clinical trials. (21)(22). Computational tools are increasingly gaining popularity and playing a bigger role in the discovery and development of medications since they save researchers time, money, and effort. One of these methods for predicting the conformation and orientation of the ligand within the binding site of the target is the docking procedure. Using GlideTM (version 5.7, Schrödinger, LLC, New York, NY, 2011), the molecular docking was evaluated. The most active compounds were docked on the active sites of enzyme. The PDB ID of the enzymes' crystal structure in complex with the anti-inflammatory drug Indomethacin, mefenamic acid, and hippuric acid was 1NNI. 3G7E, 4HL2, 2W9S, and 4RKX. Enzymes were cleaned of the water and hetero atom molecules behind 5A reference ligand radius. The Receptor Grid Generation program was used to create the grid of enzyme receptors, and the Protein Preparation Wizard ^{TM} application used the OPLS-2005 force field to minimize protein structure. Then, using the OPLS-2005 force field, the Ligand Preparation^{TM} algorithm optimized each ligand to produce the lowest energy state. The best pose (with the highest score) for each molecule was displayed following the generation of five poses for each ligand using docking simulations.

Procedures of ADME

The entire set of compounds (2a-c) were sketched using Chem Create Sketch (v. 19), and the Swiss ADME tool software renamed these ligands as SMILE in order to predict their physical, chemical, and pharmacokinetic properties. Using BOILED EGG, we could determine the polarity and lipophilicity of the small compounds**.** [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 , hippuric acid and mefenamic acid) and (50 mg/kg indomethacin mefenamic acid) 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

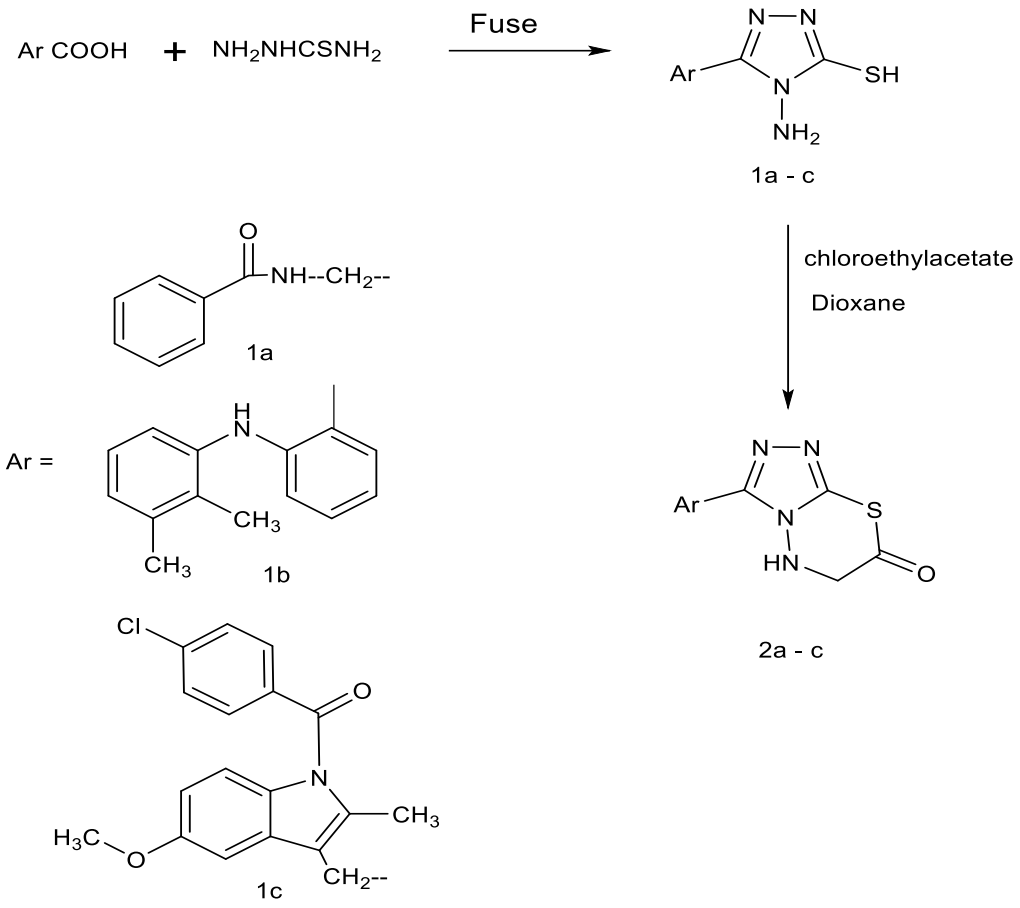
Groups n=6	Mortality(x/N)	% of death	symptom
2a	0/6	0%	nil
Hippuric acid	0/6	0%	nil
indomethacin	0/6	0%	nil
Mefenamic acid	0/6	0%	nil
DMSO 0.2ml	0/6	0%	nil

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-2induced granuloma in mice.

Granuloma inhibition (%) = 1- (weight of granuloma in mg of treated group of mice) / (weight of granuloma in mg of control group of mice) × 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%
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%

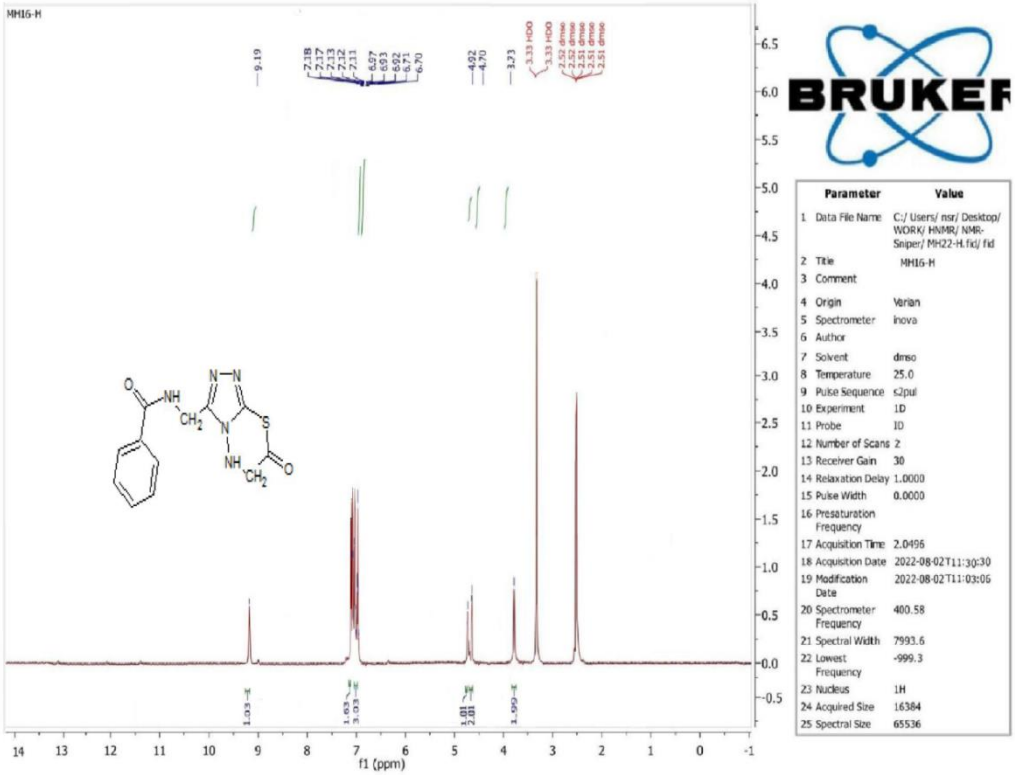
Results and Discussion:

The series of reactions used in Scheme (1) to produce the desired novel derivatives of hippuric acid, mefenamic acid, and indomethacin with various moieties. The structure of was confirmed using FTIR, H-NMR, and mass spectra.

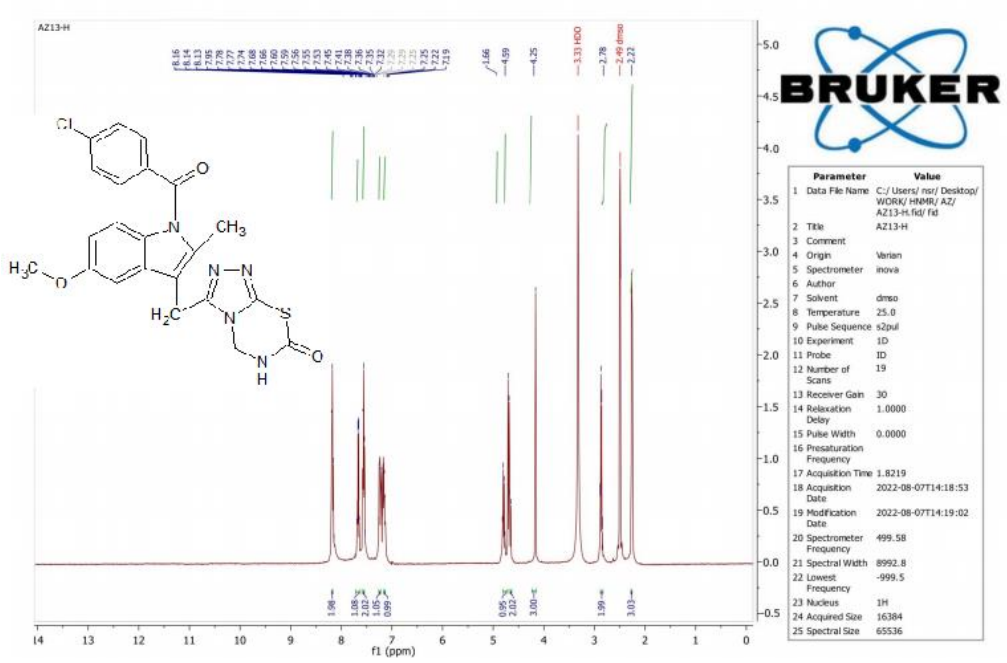


Scheme (1): synthesis of the intermediates and target products

The appearance peak of the (NH) at (3433) Cm^{-1}, secondary amine of thiadiazinone, peak at (3124) for NH (amide), peak at (3061 Cm^{-1}), and two peaks at (2991, 2833) return to the (CH) aliphatic ring are all visible in the infrared spectrum of the derivative (2a), peak of (C=O) group of thioester appeared at 1706 Cm^{-1} while peak at (1645 Cm^{-1}) due to C=O of amide and three peaks of the (C=C ar) at (1591, 1581, 1489 Cm^{-1}) returning to the aromatic ring . The 1H-NMR spectrum of compound (2a) revealed disappearance signals for the SH group and the (NH2) group at (11.31 ppm and 4.82 ppm, respectively, and an appearance signal for the amide group at (10.75 ppm) (S , 1H, NH), respectively. and at, signal at (7.55-7.24 ppm) (M , 5H) related to the phenyl group, at (4.72 ppm) (S , 1H) due to (N-HN-) group while signal at (4.69 ppm), (S , 2H) for (CH_2 –C=O) cyclic amide and (4.22 ppm) (S , 2H) due to (N-CH_2) group of acyclic amide. While the MS spectrum of the derivative (2a) gave a signal at (289 m/z) representing the molecular ion and this corresponds to the calculated molecular weight of the derivative. infrared spectrum of the derivative (2b) shows the disappearance peak of the (NH_2) at (3186- 3164) Cm^{-1} (Asym. and Sym.) and appearance peak of the (NH) at (3492) Cm^{-1}), secondary amine of thiadiazinone and at (3364 Cm^{-1}) for secondary amine, while peak at (3086 Cm^{-1}) for CH aromatic ring. The spectrum has two peaks at (2919, 2880) return to the (CH) aliphatic, peak of (C=O) group of thioester appeared at 1716 Cm^{-1} and three peaks at (1600, 1572, 1516, Cm^{-1}) returning to the (C=C Ar) of aromatic ring . 1H-NMR spectrum of compound (2b) showed the disappearance signal at (11.33 ppm) (singlet, 1H,) due to SH group and appearance signal at (7.70 – 7.15) ppm) (M , 7H) related to the aryl group, signal at (4.84 ppm) (S , 1H,) due to (NH) thiadiazinone and signal at (4.66 ppm) (S , 1H,) due to (NH) of acyclic amine, signal at (4.43 ppm) (S , 2H,) due to (CH_2) thiadiazinone and Signal at (3,06 ppm , 3H , singlet) for CH_3 at m-position of NH while signal at (2.86 ppm) , (3H , singlet due to CH_3 at o-position of NH, So, The derivative (2b) (MS)'s spectrum produced a signal at a wavelength of 351 m/z, which matches to the The derivative (2c) shows infrared spectrum which displays the secondary amine of thiadiazinone peak at (3040 Cm -1) for CH aromatic ring, disappearance peak of the (NH2) at (3282-3153) Cm-1 (Asym. and Sym.), and appearance peak of the (NH) at (3210) Cm-1. The spectrum has two peaks at the return to the (CH) aliphatic region at (2942, 2858), strong peak of (C=O) group of thioester appeared at 1715 Cm^{-1} and peak at (1651 Cm^{-1}) for carbonyl of amide group and three peaks at (1602, 1571, 1521, Cm^{-1}) returning to the (C=C ar) of aromatic ring . The compound (2c)'s.1H-NMR spectrum revealed the signal dissipating at (11.31 ppm) (S , 1H) due to the SH group and at (4.82 ppm) (S , 2H), due to the (NH2) group.signal at (8.16 – 7.19) ppm) (M , 7H) related to the aryl group , and at (4.66 ppm) , (S , 1H) due to NH of thiadiazinone , signal at (4.25 ppm) (S , 3H) due to (CH_3O), signal at (4.59 ppm) (S , 2H) due to (CH_2) of thiadiazinone Signal at (2.78 ppm , 2H , singlet) for CH_2 ,while signal at (2.22ppm) (3H , singlet due to CH_3) So, the derivative (2c) was analyzed using MS, a signal at (467 m/z) that represented a molecular ion appeared. This signal matches the derivative's
calculated molecular weight.



^{1}H-NMR spectrum of compound (2a)



^{1}H-NMR spectrum of compound (2c)

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