وزارة التعليم العالي والبحث العلمي
جامعـــــة ديـــــــالـى
كليـــــــة العلـــــــوم
قســـــــم الكيميـــــاء

تحضير مشتقات جديدة من ١،٢،٤- ترايازول وتطبيقها على مرضى السكري من النوع الثاني

رسالة مقدمه الى

مجلس كلية العلوم - جامعة ديالى
كجزء من متطلبات نيل درجه الماجستير
في علوم الكيمياء

من قبل

سلام كاظم خلف

بكالوريوس علوم كيمياء ٢٠١٨

كلية العلوم - جامعة ديالى

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University of Diyala
College of Science
Department of Chemistry

Synthesis of New 1,2,4-Triazole Derivatives and Applied them in T2DM Patients

A Thesis

Submitted to Council of College of Science - the University of Diyala as a Partial Fulfillment of the Requirements for the Master's Degree of Sciences in Chemistry

By

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٢٠٢٤ A.D. **١٤٤٥ A.H.**

Summary

The new compounds of ١,٢,٤-triazole derivatives were synthesized. The chemical structures of the synthesized compounds S١-S١١ were determined by some spectroscopy techniques such as FTIR,^{1}HNMR. The reactions were followed by thin layer chromatography (TLC) to ensure that the synthesized of these compounds is complete and their purity , some physical properties of synthesized compounds were recorded such as melting points and colours.

The current study included some steps:

١. The Compounds (S١-S٥) were synthesized through the reaction of substituted benzoic acid [glycine, ٤-chlorobenzoic acid, ٤-aminobenzoic acid, ٤-hydroxybenzoic acid, ٣,٤-diaminobenzoic acid] with thiocarbohydrazide.

٢. The Compounds (S١,S٢,S٤,S٥) underwent condensation reaction with Chloroactylchloride to produce (S٦-S٩).

٣. The terephthalic acid was reacted with thiocarbohydrazide in the ratio (١:٢) to produce (S١٠).

٤. The Compounds (S١٠) was reacted with Chloroactyl chloride in the ratio (١:٢) to produce (S١١).

In addition, the study included (١٥٠) sample from serum of T٢DM patients and(٧٥) sample from serum sanples from people without diabetes were used as a control and determine level of biochemical parameters such as Glycated Hemoglobin,Glucose ,kidney function (urea creatinine, uric acid) and liver function; Alanine transaminase (ALT) Aspartate aminotransferase (AST), Alkaline phosphatase (ALP). There was also an aspect of the research that included partial purification of the ALP enzyme from serum of T٢DM patients people, and estimated the kinetics properties of the enzyme also this study was intended to determine .

Alkaline phosphatase Also this study included the effectiveness of the new synthesized compounds prepared (S_1, S_2, and S_3) on the activity of alkaline phosphatase enzyme (partly purified) in vitro and studied also the type of inhibition results of this study.

The Results showed that:

١. About age and BMI for the Studied Groups : The age among groups for patient and control shows no significant difference (p>٠,٠٥). The body mass index results were measurement showed no significant different (p>٠,٠٥) between the studied groups patients and control healthy group. When compare patient groups between themself showed the age among groups (good glycemic control, moderate glycemic control, and poor glycemic control) was found increase but no significant (p>٠,٠٥). The body mass index results showed no significant different (p>٠,٠٥) between the studied groups patients (good glycemic control, moderate glycemic control, and poor glycemic control).

٢. The present study showed statistically significant differences (p<٠,٠٠١) for Glucose, Glycated Hemoglobin,Urea and Uric acid in the T٢DM patients groups when compared with control group. Creatinine showed statistically significant differences between study groups (p< ٠,٠٥). Whilst, when we studied difference between T٢DM patients groups [Good Glycemic Control , Moderate Glycemic Control and Poor Glycemic Control]showed statistically significant differences (p<٠,٠٠١) for Glucose and Glycated Hemoglobin in the T٢DM patients groups, whilst Urea, Creatinine and Uric acid showed nonsignificant differences between study groups (p<٠,٠٠١).

٣. The present study showed statistically significant differences (p<٠,٠٠١) for GPT, GOT and ALP in the T٢DM patients groups when compared with control group. Whilst, when we were studied difference between T٢DM patients groups [Good Glycemic Control , Moderate Glycemic Control and Poor Glycemic Control] showed nonsignificant differences (p<٠,٠٠١) for GPT, GOT and ALP.

٤. This study included partially purification ALP enzyme from serum of T٢DM patients using Sephadex G ١٠٠. The activity of purified ALP enzyme reached (٠,٠٦٧ IU/L), and the specific activity of ALP enzyme was enhanced to (٠,٠٠٥٥ IU/mg) by (١,٥٧) folds and enzyme yields (١٢,٤١٪) . Kinetics studies were carried out which values of Km and Vmax of ALP were (١٠ mM) for a substrate, optimum temperature was (٢٧°C), optimum pH was (١٠,٤) and optimum time was (١ min).

٥. When examining the effect of these compounds with a different concentration on Purified enzyme (ALP) in serum of T٢DM patients, it was found that the compounds ($S_{١}$,$S_{٢}$,$S_{٦}$) acts as an inhibitor for enzyme, and this was demonstrated by the decrease in readings resulting from the addition of this compounds and increased inhibition with increased concentration of the compounds. The all compounds acts as an inhibitor of ALP . The results shown in the model indicated that the type of inhibition of compounds ($S_{١}$,$S_{٢}$, and $S_{٦}$),, was non-competitive

Chapter One

(Preface & Literature review)

١,١. Preface

Heterocyclic compounds are compounds that possess a complex toroidal component containing atoms in addition to carbon atoms. The most common heterogeneous atoms are nitrogen, oxygen and sulphur. The importance of these compounds are involved in the installation of chlorophyll in the plant, and the Haemoglobin contains four rings of Pyrrole that is a widespread compound in Nature [١,٢]. These compounds acquire more importance in recent years due to the pharmacological activities. Nitrogen, sulphur and oxygen containing five/six member heterocyclic compounds have occupied enormous significance in the field of medicinal chemistry [٣]. These compounds played an important role in regulating biological activities [٤].

A wide variety of heterocyclic systems have been explored in order to develop pharmaceutically important molecules. Nitrogen-containing heterocycles are found in many medicines. Derivatives of triazole have particularly interesting therapeutic properties [٥].

The ١,٢,٤-triazole parent consists of a five-membered aromatic ring containing three atoms of nitrogen, two of which are adjacent for stability, water-soluble solid. It is possible to envisage two tautomeric forms, ١ H-tautomer ١٣ and ٤ H- tautomer ١٤ Figure ١,١. Theoretical and empirical approaches suggest that the preferred structure is the ١H-tautomer. Two nitrogen atoms are connected to any carbon atom in ١,٢,٤-triazole, and thus, this ring structure is deficient in electrons. For electrophilic attack, the ring is deactivated so that nitration and other carbon reactions typical of aromatic chemistry do not occur to the parent compound. However, in the literature, electrophilic attacks on nitrogen are found in abundance. There is a pKa of ١٠,٢٦ in the parent compound, so alkali metal salts form easily at the N١ position. The protonated species pKa is ٢,١٩ and the weakly basic nature enables electrophilic attack in ١-substituted-١,٢,٤-triazoles at the N٤ position [٦].

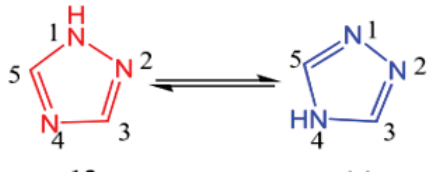
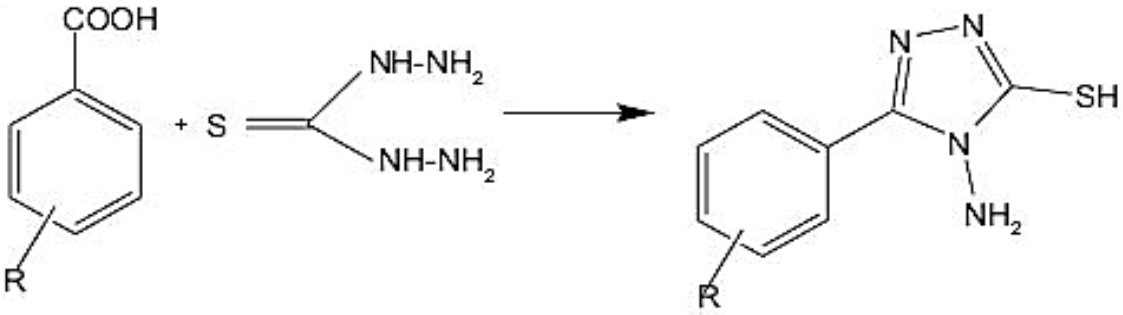


Figure ١-١: Tautomerism of ١,٢,٤-triazoles

Inhibitors of the azole family such as triazole and benzotriazole derivatives are considered as good corrosion inhibitors and some research even shown that the inhibition efficiency of these families of azole is much greater than that for other heterocyclic compounds . Additionally, it has been confirmed that triazole derivatives play a very important role in the medical field as a new anti-agent and exhibit various biological activities [٧] . Diabetes mellitus (DM) is chronic endocrine and metabolic condition characterized by persistent hyperglycemia and changes in carbohydrate, lipid, and protein metabolism caused by insulin insufficiency production or by defect in insulin action, or both [٨]. It is a major global health issue that is linked to a significant Various consequences of diabetes mellitus, such as diabetic macro vascular disease and diabetic peripheral vascular disease, have also increased in frequency, as well as considerable morbidity and death from nephropathy, neuropathy and retinopathy[٩] .

١,٢. Literature review

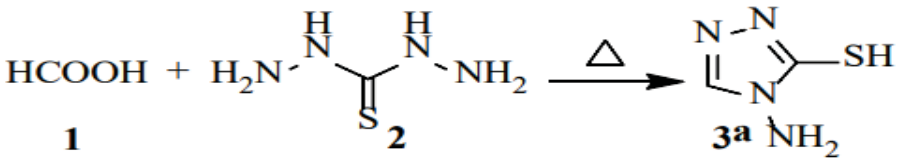
Gupta, D., et. al.(2015). Published a novel series of Schiff bases based on of ٤-(benzylideneamino)-٥-phenyl-٤H-١,٢,٤-triazole-٣-thiol scaffold was prepared by heating thiocarbohydrazide and substituted benzoic acid and subsequently, treating with substituted benzaldehydes. Seventeen derivatives were synthesized and how been studied for its biologically against antifungal and antibacterial activity [١٠]. ٢NH٢ , CL ,

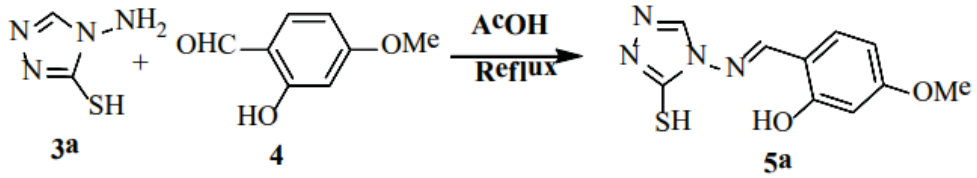


R=CH٢NH٢, Cl, OH, NH٢, NH

Equation ١-١: Synthesis of ٤-amino-٥-phenyl-٤H-١,٢,٤-triazole-٣-thiol.

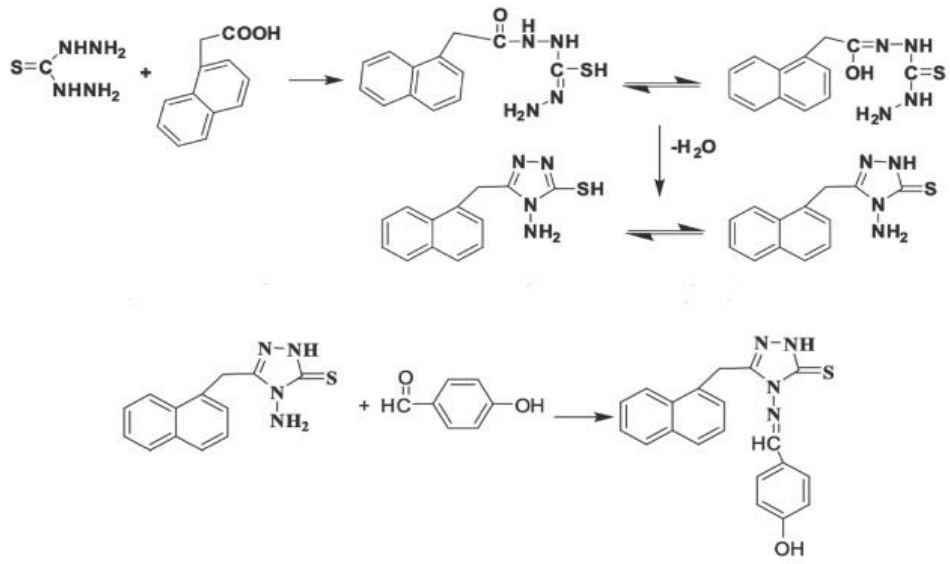
Bhale, S. P. et al. (2020). Reported a novel nitrogen containing ٤-[(٢-hydroxy-٤-methoxyphenyl)methyleneamino]-٢,٤-dihydro-٣H-١,٢,٤-triazole-٣-thione ligand (H_2L) was synthesized using an equimolar ratio of ٤-amino-١,٢,٤-triazole-٣-thione and ٢-hydroxy-٤-methoxybenzaldehyde. A series of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes were synthesized using the ligand. The synthesized ligand and transition metal complexes were characterized by using IR, ^{1}H NMR, ^{13}C NMR, Mass spectrometry, UV, XRD and TGA investigation methods [١١].





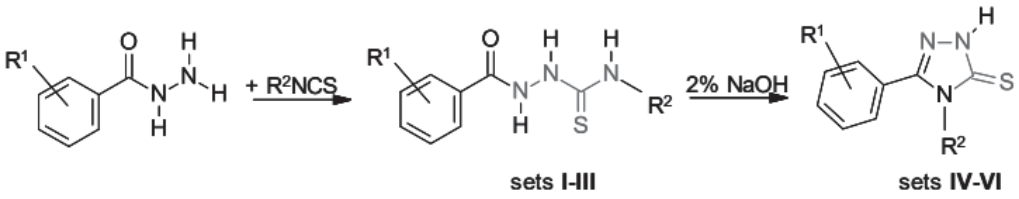
Scheme ١-١: Synthesis of ٤-amino-١,٢,٤-triazole-٣-thione, Schiff base ligand H٢L and metal complexes (٦a-٦f).

Ali, A. et al. (2020). A novel micro and nanoparticle complexes of Ag(I), Ni(II), and Pd(II) ion with new asymmetrical Schiff base triazole ligand (٤-(((٣-mercapto-٥-(naphthalen-١-ylmethyl)-٤H-١,٢,٤-triazol-٤-yl) imino)methyl)phenol) were prepared. The Schiff base micro complexes were identified using Fouriertransform infrared spectroscopy (FTIR), Ultraviolet–visible spectroscopy (UV-Vis), flame atomic absorption, elemental analysis C.H.N.S, conductivity measurements and magnetic susceptibility. New nanoparticles Schiff base triazole ligand (٤-(((٣-mercapto-٥-(naphthalen-١-ylmethyl)-٤H-١,٢,٤- triazol-٤-yl)imino)methyl)phenol) ligand and Ag(I), Ni(II) and Pd(II) complexes were synthesized as a novel compounds by using sonication method, and were fully characterized by using FTIR, atomic force microscopy (AFM), scanning electron microscope (SEM), and X-ray powder diffraction (XRD). The antioxidant activity of tested compounds was tested using DPPH assay. The effect of synthetic novel compounds on cancer cell line MCF-٧ proliferation was measured by MTT assay [١٢].



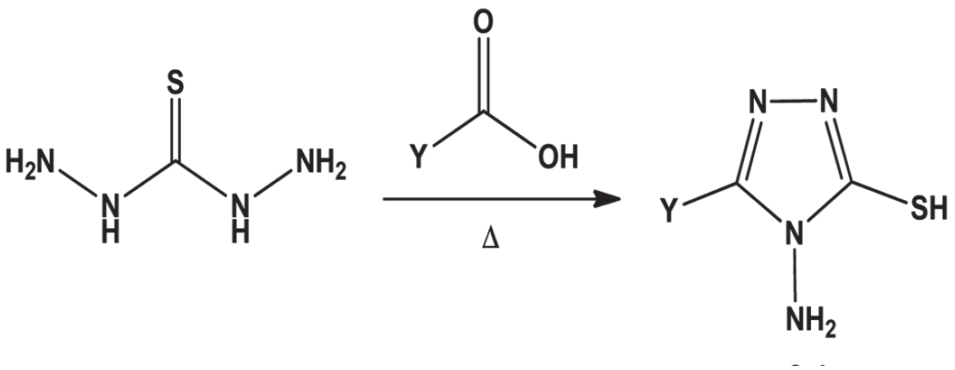
Scheme ١-٢: Synthesis of ٤-amino-٥-(naphthalen-١-ylmethyl)-٤H-١,٢,٤-triazole-٣-thiol and triazole Schiff base L١.

Kosikowska, U., et al. (2020). ortho-, meta-, and parafluorobenzoylthiosemicarbazides, and their cyclic analogues with ١,٢,٤-triazole scaffold, was created and tested for antibacterial activity against Gram-positive bacteria strains. While all tested ١,٢,٤- triazoles were devoid of potent activity, the antibacterial response of the thiosemicarbazides was highly dependent on substitution pattern at the N٤ aryl position [١٣].



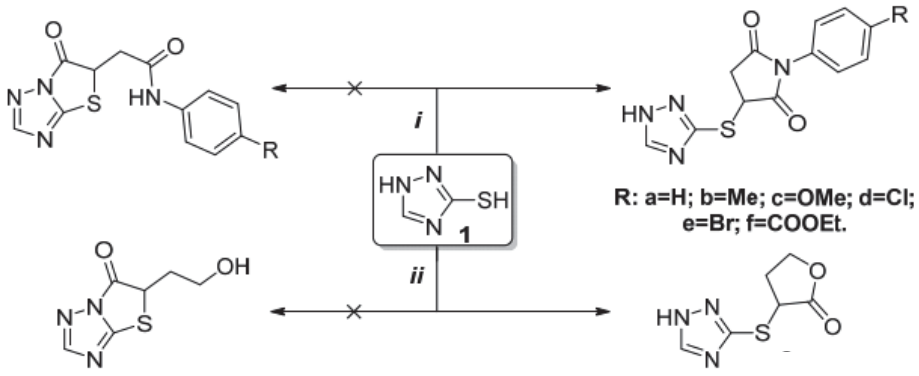
Scheme ١-٣: Synthesis of ١,٢,٤-triazole derivatives

Riyadh, S. M., et al. (2020). Have proven that ٤-Amino-٥-mercapto[١,٢,٤]triazole and its ٣-substituted derivatives to be of biological interest and provide access to a new class of biologically active heterocyclic compounds for biomedical applications. This study was explains all the methods for the preparation of ٣-substituted-٤-amino-٥-mercapto[١,٢,٤]triazoles with aliphatic, aromatic, and heterocyclic moieties [١٤].



Equation ١-٢: Synthesis of triazoles ٢–٤.

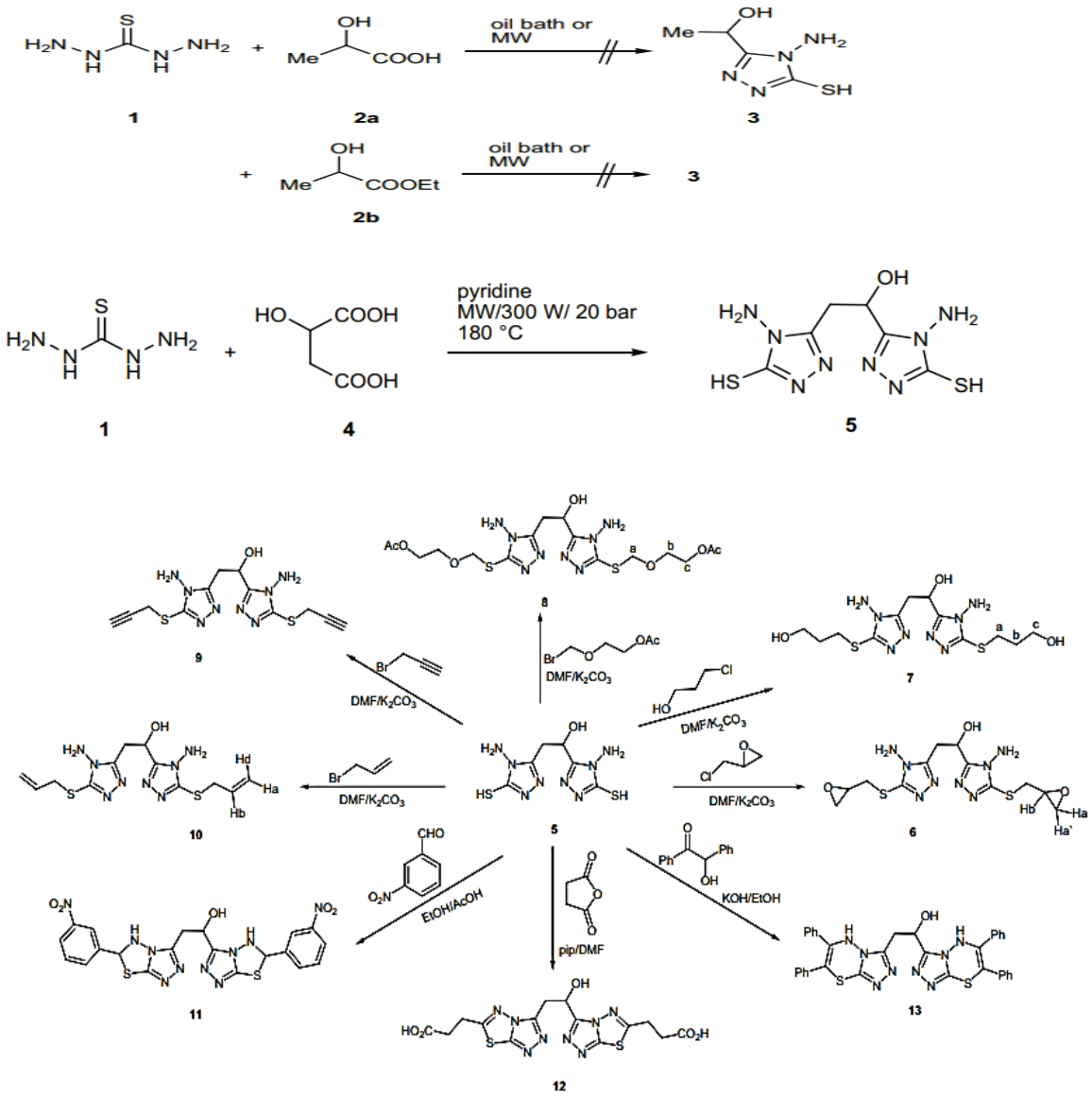
Holota, S. et al. (2020). Reported a novel of ١-phenyl-١H-pyrrole-٢,٥-dione derivatives (N-arylmaleimides), ٣-bromodihydrofuran-٢(٣H)-one (α-bromo-γ-butyrolactone) and novel ٥-substituted thiazolo[٣,٢-b][١,٢,٤]triazole-٦(٥H)-ones. The structure of compounds was studied using ^{١}H, ^{١٣}C NMR spectroscopy, LC-MS spectrometry, and X-ray analysis. The prescreening of antimicrobial activity for synthesized compounds was performed against Gram-positive and Gram-negative bacteria, as well as yeasts [١٥].



Scheme ١-٤: Synthesis of ١,٢,٤-triazole derivatives

Haggam, R. A. (2021). Efcient synthesis of a series of some novel derivatives for ١,٢-bis-(٤-amino-٥-mercapto-١,٢,٤-triazol-٣-yl)-ethan-١-ol ٥–٢١ using dl-malic acid under microwave (MW) irradiation. Reaction with several alkylating agents such as epichlorohydrin, ٣-chloropropanol, (٢-acetoxyethoxy) methyl bromide, propargyl bromide and chloroacetamide was studied. Structures of the realized products have been established on the basis of their ^{١} H/^{١٣}C NMR, IR, elemental analysis and correlation experiments. It is well known that ١,٢,٤-triazole ring systems are famous for their

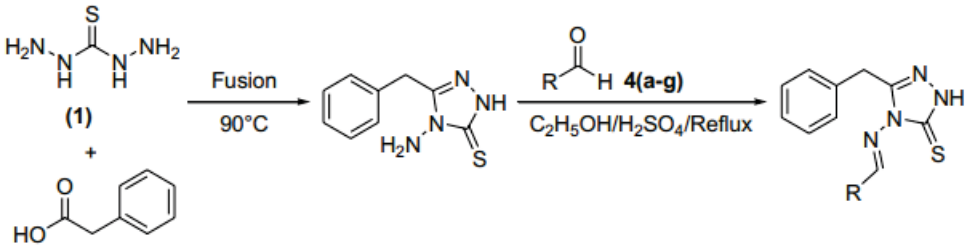
antifungal activities, so the synthesized compounds were screened for their antifungal activities [١٦].



Scheme ١,٥: Reaction of aminomercaptotriazole ٥ with diferent alkylating or condensing agents under thermal and microwave conditions

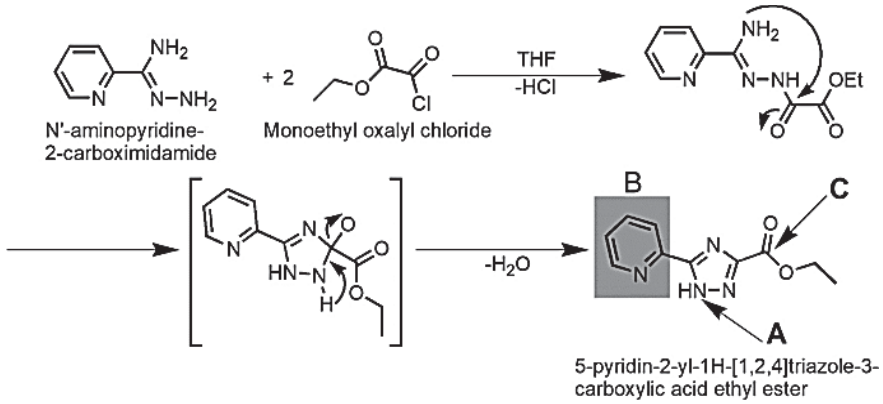
Aziz, H., et al. (2021). synthesis, characterization, in vitro antioxidant, cytotoxic and α-glucosidase inhibitory potential of ٤-amino-٥-benzyl-٢H-١,٢,٤-triazol-٣(٤H)-thione (٣) and ٥-benzyl-٤-(benzylideneamino)-٢H-١,٢,٤-triazole-٣(٤H)-thiones ٥(a–g). the Percent DPPH free radical scavenging ability The antioxidant derivatives were screened

in brine shrimp lethality as well as protein kinase inhibitory assay to unveil their toxic nature. Similarly, in protein kinase inhibitory assay, maximum inhibitory potential .Likewise, in α-glucosidase inhibition assay . Molecular docking studies of the screened derivatives were performed in order to assess their binding potential and mechanism of their binding with α-glucosidase, α-kinase and β-kinase enzymes [١٧].



Scheme ١،٦: Synthetic scheme adopted for the synthesis of ٥-benzyl-٤-(benzylideneamino)-٢H-١،٢،٤-triazole-٣(٤H)-thiones ٥(a–g)

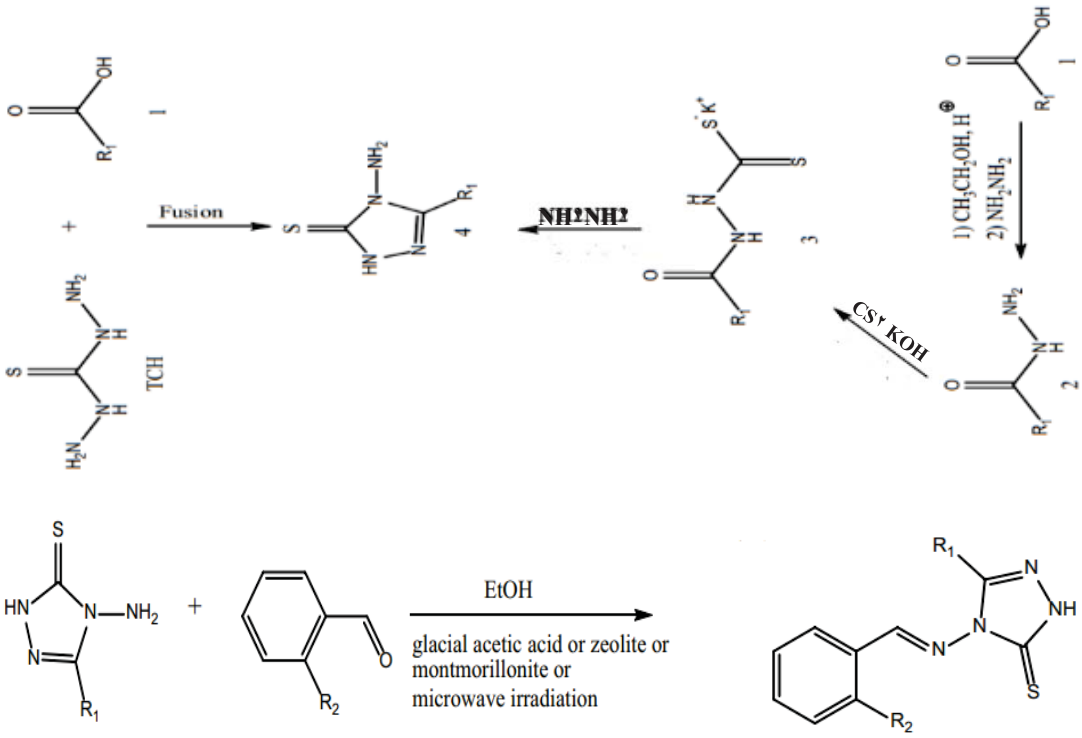
Eya'ane Meva, F., et al. (2021). A novel ١،٢،٤-triazole intermediate ٥-pyridin-٢-yl-١H-[١،٢،٤]triazole-٣-carboxylic acid ethyl ester was prepared by the reaction of N'-aminopiridyne-٢-carboximidamine and an excess monoethyl oxalyl chloride and screened for biological activities. The compound was structurally characterized by nuclear magnetic resonance spectroscopy, elemental analysis, infrared spectroscopy, and single-crystal X-ray diffraction [١٨].



Scheme ١،٧: Synthesis of ٥-pyridin-٢-yl-١H-[١،٢،٤]triazole-٣-carboxylic acid ethyl ester with indications of substitution possibilities of further pharmacophoric groups at positions A–C

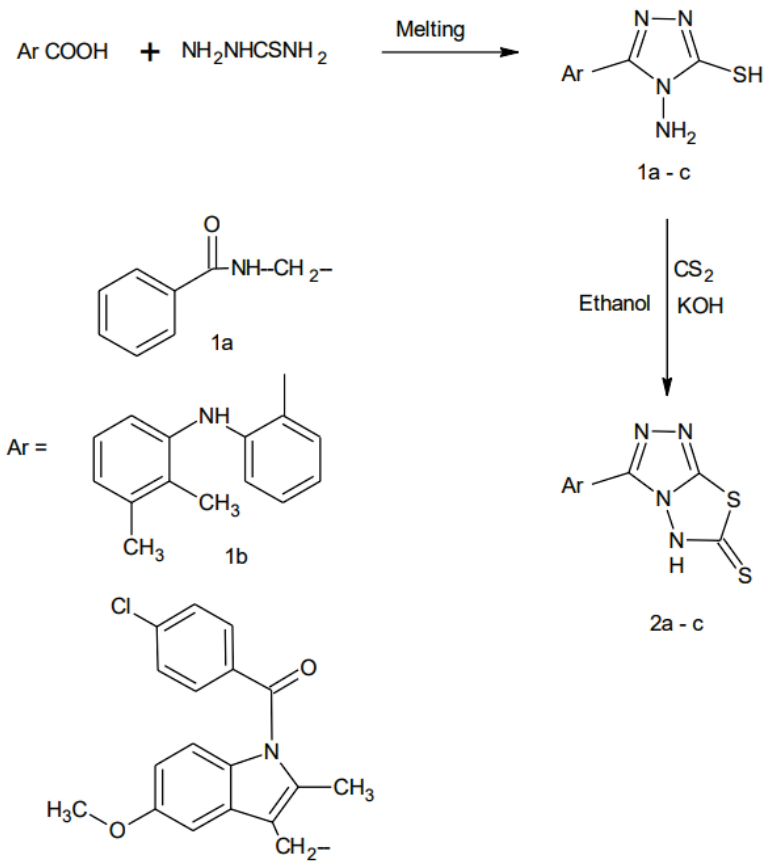
Shirmohammadi, M.,et al. (2021). Published Novel compounds based on the ١،٢،٤-

triazole skeleton . A class of ٤-amino-٥-alkyl-٤H-١,٢,٤-triazole-٣- thione created by reaction of thiocarbohydrazide with long-chain aliphatic carboxylic acids, and then the Schiff bases were obtained in the media of heat and microwave waves, in the presence and the absence of a catalyst. Their chemical structures were assayed by elemental analysis, also device spectroscopic methods [١٩].



Scheme ١,٨: Synthesis of ١,٢,٤-triazole amines ٤a-f and Schiff base ٥a-l.

Mahdi, M. F. (2022). ٤-amino-٥-aryl-٤H-١,٢,٤-triazole-٣-thiol (١a-c) were created by the interaction of drug has carboxylic group and thiocarbohydrazide, and the starting products ٤-amino-٥-aryl-٤H-١,٢,٤-triazole-٣-thiol) were treated with carbon disulfide in present NaOH to produce final products (٢a-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) [٢٠].



Scheme ١,٩: synthesis of the intermediates and target products

The aim of the study

The aim of the present study can be summarized in:

١. The new compounds of ١,٢,٤-triazole derivatives were synthesized. The chemical structures of the prepared compounds S١-S١١ were determined by some spectroscopy techniques such as FTIR,^{1}HNMR.

٢. Determine level of biochemical parameters such as Glycated Hemoglobin,Glucose ,kidney function (urea creatinine, uric acid) and liver function; Alanine transaminase (ALT) Aspartate aminotransferase (AST), Alkaline phosphatase (ALP). There was also an aspect of the research that included partial purification of the ALP enzyme from serum of T٢DM patients

people, and estimated the kinetics properties of the enzyme also this study was intended to determine .

٣. Study the effectiveness of the new compounds prepared (S_1, S_2, S_3) on the activity of alkaline phosphatase enzyme (partly purified) in vitro and studied also the type of inhibition results of this study.