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Preparation of Some ١,٢,٤-Triazol Schiff Bases as Urease Inhibitors and Study Their Effect on Proteus Mirabilis Bacteria

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Abstract

The basic nucleus ٤-(amino)-٥-phenyl-l-٤H-١,٢,٤-triazole-٣-thiol was prepared by cyclisation of potassium dithiocarbazinate with hydrazine hydrate using ethanol as solvent under reflux condition for ٣-٤ hrs.. The compound which has been synthesized successfully was subjected to addition reaction with different aldehydes to synthesize Schiff bases. The compounds were confirmed by[physical parameters (solubility, melting point),^{١}H nuclear magnetic resonance (^{١}HNMR)and spectroscopic methods (FTIR)]. All the synthesized compounds were screened for their urease inhibition. Some compounds showed excellent urease inhibition activity.

Keywords: triazol ring, triazol Schiff base, urease inhibitors.

Introduction

Triazoles are five memberd heterocyclic compounds containing three nitrogen and two carbon atoms. The name triazole was first given to the carbon nitrogen ring system $C_٢N_٣H_٣$ by Bladin who described its derivatives in early ١٨٨٥, although the structures reported slightly incorrect [٨-١٠].

The ١,٢,٤-triazole is an ubiquitous feature of many pharmaceutical and agrochemical products. The substituted ١,٢,٤-triazole nucleas is particularly common, and can be found in marketed drugs such as fluconozole, terconazole, and rizatriptanalperazolam [١١].

Urease (urea amidohydrolase, E.C. ٣,٥,١,٥) is an enzyme that catalyzes the hydrolysis of urea to ammonia and carbamate, which is the final step of nitrogen metabolism in living organisms. Carbamate rapidly and spontaneously decomposes, yielding a second molecule of ammonia. These reactions may cause significant increase in pH and are responsible for negative effects of urease activity in human health and agriculture [١,٢]. Urease is produced by pathogenic or nonpathogenic bacteria [٣,٤].

All these bacteria produce urease that has a major role in urolithiasis by increasing the pH from ٥ to ٩ causing the mineral salts to precipitate in mucous material [٥], which is produced by the bacteria and entered in its cellular structure and acts as navies around which salts are precipitated to form stones. It also has been found that bacterial cells inside renal stones in proteins are treated with antibiotics [٦].This enzyme is high -specific, which means that the enzyme catalyzes the hydrolysis of urea only [٧].

Materials and Methods

All the reagents, starting materials as well as solvents were purchased commercially and used without any further purification. Melting points were measured by using (Gallen Kamp / England) melting point. F.T.IR-٨٣٠٠ Fourier transforms infrared spectrophotometer SHIMADZU the (٦٠٠-٤٠٠٠) $cm^{-١}$ spectral range. The spectra of ^{١}H NMR spectra were recorded on a Bruker Ultrasheild ٣٠٠ MHZ in Jordan, using deuterated DMSO-*d*٦ as the solvent.

Synthesis of benzoic acid hydrazide (compound ١) [١٢]

Methyl benzoate (٥ ml, ٠,٠٣٩mol) in ١٠ ml of ethanol is taken in a round bottom flask. To that hydrazine hydrate ٣ mladded and refluxed for ٤ hrs. . The mixture was filtered and recrystallization with ethanol. The precipitate is white crystal in color as a product .m.p (١١١-١١٣)°C, yield ٧١ %.

Synthesis of ٤ [amino]-٥-phenyl-٤H-١,٢,٤-triazole-٣-Thiol (compound٢) [١٢]

Add to a solution of potassium hydroxide (٠,٠٣ mol, ١,٦٨ g)in absolute ethanol (١٥ml), benzoic acid hydrazide (٠,٠١ mol, ٢,٩٤g) and carbon disulphide(٠,٠٥ mol, ٥ ml) were added and the mixture was stirred for ١٦ hrs. To the resulting solution anhydrous ether (١٠ml) was

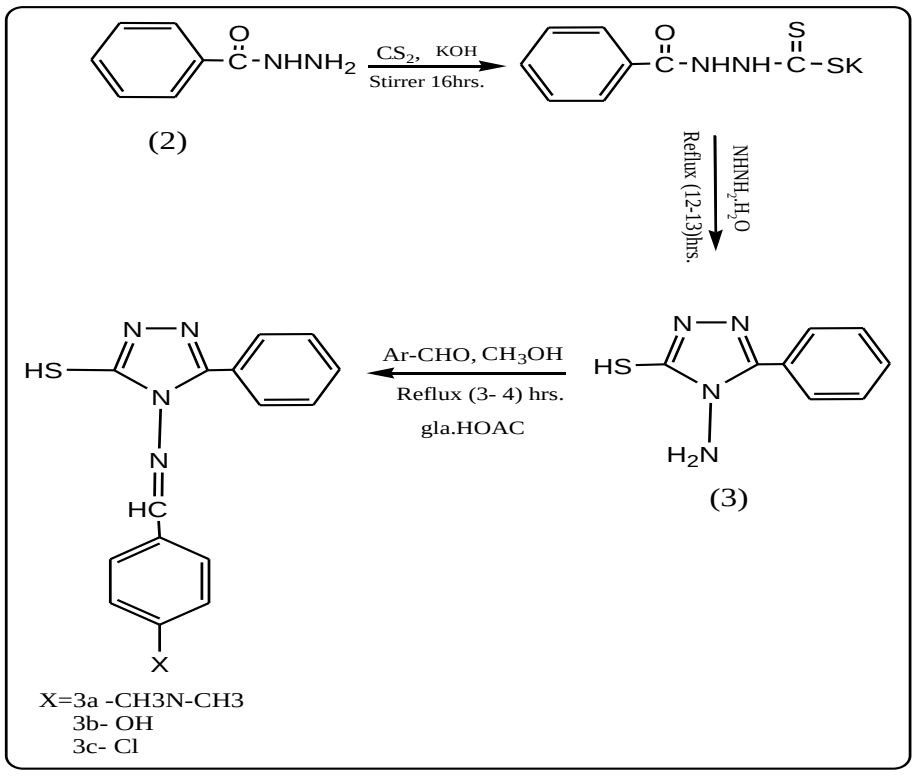
added and precipitated potassium dithiocarbazinate was collected by filtration, washed with diethyl ether and dried. The potassium salt obtained in quantitative yield was directly used without purification in the next stage. The precipitate (potassium salt) was added to an excess of hydrazine hydride (٢٠ ml), and was refluxed with stirring until the evaluation hydrogen sulfide; it was ceased by lead acetate paper. After cooling the reaction mixture was filtered, and then was acidified by Hydrochloric acid to yield the white precipitate. m.p (١٩٥-١٩٧)C°. Yield: ٦٢٪, color:white.

A mixture of ٤[amino]-٥-phenyl-٤H-١,٢,٤-triazole-٣-thiol (٠,٢٥g, ٠,٠٠١mol), with (٠,١٥g, ٠,٠٠١ mmol) from *p*-(N,N-dimethyl) aminobenzaldehyd (٣a), (٠,١٥g,٠,٠٠١mmol) from *p*-hydroxybenzaldehyd (٣b), (٠,١٨g, ٠,٠٠١mmol) from *p*-chlorobenzaldehyd (٣c), and ٢ drops of concentration glacial acetic acid in ethanol medium was refluxed for ٣ hrs. The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction, washed with cold ethanol and recrystallized with hot ethanol. m.p and physical properties are tabulated in Table (١).

Synthesis of Schiff bases (compound ٣a-c) [١٣]

Table (١)
Properties of the Prepared Compounds.

Compound No.	*Molecular weight*	*Molecular formula.*	*M.P.*	*Yield %.*	*Color.*	*Solvent system.*
٢	١٩٢,٢٥	$C_8H_8N_4S$	١٩٩-٢٠١	٦٢٪	white	Ethanol
٣a	٣٢٣,٤٢	$C_{17}H_{17}N_5S$	١٨٠-١٨٢	٦٠٪	red	DMSO
٣b	٢٩٦,٣٥	$C_{10}H_{12}N_4OS$	٢٥٠-٢٥٣	٥٥٪	Pale yellow	DMSO
٣c	٣١٤,٧٩	$C_{10}H_{11}ClN_4S$	٢١١-٢١٣	٦٣٪	yellow	DMSO



Scheme (١) chemical steps for synthesis of compounds.

Pharmacology Urease inhibition bioassay [١,١٤]

The synthesized compounds were screened for their urease inhibition activity, which is shown in Table (٣). The compounds were found inhibiting the urease in variable concentrations. The urease activity was determined by measuring the amount of ammonia being produced using the indophenol method described by Weatherburn. The assay mixture, containing ١٠µL of enzyme and ١٠µL of test compound in ٢٠٥µL buffer (٠,٣g in ١٠ml distilled water urea, ٠,١٧٤g K_2HPO_4, ٠,٠١٨٥g EDTA in ٥٠ ml distilled water pH ٧,٥), were incubated for ٣٠ min at ٣٧ºC in water bath. Briefly, ٥ml each of phenol reagents (٥ gphenol and ٠,٠٢٥g sodium nitroprusside) and ٥ml of alkali reagent (٢,٥ sodium hydroxide and ٤,٢ ml sodium hypochlorite) were added to each tube. The absorbance at ٦٢٥ nm it was read by spectrophotometer. Percentage inhibition was calculated by using the following equation.

$$\text{Inhibition (\%)} = 100 - \frac{\text{Abs}_{\text{test well}}}{\text{Abs}_{\text{control}}} 100$$

In *vitro* antibacterial assay [١٥]

The synthesized compounds (٢,٣a-c) were tested for their anti-bacterial activity against Gram negative proteus (mirabilis) bacterial strains adapting the agar disc diffusion method. Prepared agar and petridishes were sterilized by autoclaving for ١٥ min at ١٢١C°. The agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium suitably spaced apart holes were made all ٥ mm in diameter. These holes were filled with ٠,١ ml of the prepared compounds, four concentrations for each compound was prepared, (١٠٠, ٥٠, ٢٥ and ١٠ µg/ml), tetracycline was used as references antibiotic drugs. DMSO was used as a solvent. One of these holes were filled with DMSO as control, to see the effect of solvent, These plates were incubated at ٣٧C° for ٢٤hrs.

Results and Discussion

All the synthesized final compounds were first purified by successive recrystallization using appropriate solvents. Triazole was soluble in ethanol, DMSO, DMF, dioxane. All synthesized Schiff base derivatives were soluble in DMSO at room temperature and in methanol, DMF, dioxane on heating. Physical measurements and analytical data of the all compounds are given in Table (١) and (٢).

Spectroscopic characterization

The FTIR spectrum of compound (١) shows appearance of two stretching bands of NH_2 asymmetric and symmetric at (٣٣٠١ and ٣٢١٤cm^{-1}), carbonyl of Amide group was also seen at ١٦٦١ cm^{-1}. The FTIR spectrum of compound (٢) showed some characteristic stretching bands at: ٣٢٤٤ and ٣١٠٠, ٢٦٩٦, ١٦٢٠ and ٦٦٣ cm^{-1} assigned to NH_2, S-H, C = N of triazole ring, and the last one is for stretching of C-S bond, respectively. The nucleus ٤-[amino]-٥-phenyl- ١,٢,٤-triazole-٣-thiol was used to synthesise Schiff bases [٣a-c]. The Schiff bases are confirmed by the disappearance of NH_2 stretching band of compound [٢] at (٣٢٤٤cm^{-1} and ٣١٠٠ cm^{-1}). A weak band due to =CH stretching appeared at (٣١٠٥ cm^{-1},٣١٦٧ cm^{-1} and ٣١٠٠cm^{-1}) Assigned to (٣a,٣b and ٣c) respectively. The major FTIR bands are given in Table (٢):

Characterization of prepared compound by Nuclear magnetic resonance

The data of ^{1}H NMR ٤[amino]-٥-phenyl-٤H-١,٢,٤-triazole-٣-Thiol and its Schiff base displayed good solubility in DMSO.

Compound ٢

^{1}H NMR data (ppm), δ_H(٣٠٠ MHz, DMSO-d_6): signals at ٥,٢٧٦ (٢H, s, NH_2),٧,٥٦٧-٧,٨٢٥ (٥H, m, CH aromatic ring) and ١٢,٨٥٢ (١H, s, SH).

Compound ٣b

^{1}H NMR data (ppm), δ_H(٣٠٠ MHz, DMSO-d_6): signals at ٩,٢٩٢ (١H, s, NH_2), ٧,٤٩٢-٨,١٩٢ (٩H, m, CH aromatic ring), ١٣,٨٩١ (١H, s, SH)and ١٤,٧٤٥(١H, s,OH).

Table (٢)
FTIR Spectral Data of the Prepared Compound.

Compounds No.	*NH٢*	*-S-H*	*=CH*	*C=N*	*C-S*
٢	٣٢٤٤-٣١٠٠	٢٦٩٦	-	١٦٢٠	٦٦٣
٣a	-	٢٦٠٠	٣١٠٥	١٦٠٠	٦٨٢
٣b	-	٢٩٦٢	٣١٦٧	١٦٠٨	٦٤٤
٣c	-	٢٦٠٣	٣١٠٠	١٦٠٠	٦١٧

Urease inhibition bioassay

The compounds (٢,٣a-c) were tested for their potential to inhibit urease and the results are tabulated in Table٤. Compounds **٣a** and **٣b** exhibited very good urease inhibition activity with the $IC_{٥٠}$ values of ٤٠,٩ and ٤١,٨μM, respectively, whereas the activity of compounds ٢ and **٣c** was only moderate ($IC_{٥٠}$ = ١٠٨,٧ and ١١٢,٧μM, respectively).

It is noticed from the results that ٣a a Schiff base, is considered as the strongest inhibitor used in this study as it's ($IC_{٥٠}$=٤٠,٩ μM) and this is because of (C=N) bond presence in its structure which is characteristic of Schiff base and has high activity on enzyme itself. That the aryl part of the test compounds with its electronic effects is playing a significant role in manipulation of the activity. The most active compound, **٣a**, have a N,N-dimethyl aminas a substituent on the aryl part, which when compared to hydroxyl group **٣b** and chloro **٣c** group has a lower electronegativity and donates its electrons more effectively to the phenyl ring and thus, onto the triazole nucleus. This probably positively affects the binding of the molecules to the active site of the enzyme rendering this compound more potent than the standard drugs.

Antibacterial activity

The inhibition zones caused by the various compounds were examined. (١٠,٢٥,٥٠, ١٠٠) μg/ml concentration for all of these compounds). The results are listed in Table (٣). tetracycline was used as standard drug. The compounds (٣a and ٣b) have higher biological activity as antibacterial agent than tetracycline. Compounds (٢,٣c) have a biological activity a little less than Tetracycline zone inhibition in mm. The presence of –N-N-C- moiety along with mercapto group imparts activities.

Table (٣)
Antibacterial activity of synthesize compound Zone of inhibition (mm).

Compounds	١٠٠μg	٥٠μg	٢٥μg	١٠μg
٢	١٧	٧	٠	٠
٣a	٢٠	١٧	١٧	٠
٣b	١٩	١٧	١٥	٠
٣c	١٦	٠	٠	٠
tetracycline	١٩	١٧	١٤	١٠

Table (٤)
Inhibition Rate% and IC٥٠ of the synthesize compound.

Compound no.	Concentration μM	Inhibition Rate %	IC٥٠
٢	١٠٠	٥٠,٧٥	١٠٨,٧٨
	٥٠	٥,٠٢	
	٢٥	٤,٠١	
	١٠	٢,٥١	
٣a	١٠٠	٧٨,٣٩	٤٠,٩
	٥٠	٦٦,٨٣	
	٢٥	٥٥,٧٧	
	١٠	١٢,٠٦	
٣b	١٠٠	٧٦,٨	٤١,٨
	٥٠	٦٥,٨٢	
	٢٥	٥١,٧٥	
	١٠	٩,٥٤	
٣c	١٠٠	٤٨,٧٢	١١٢,٧
	٥٠	٤,٠٢	
	٢٥	٣,٥١	
	١٠	٢,٠١	

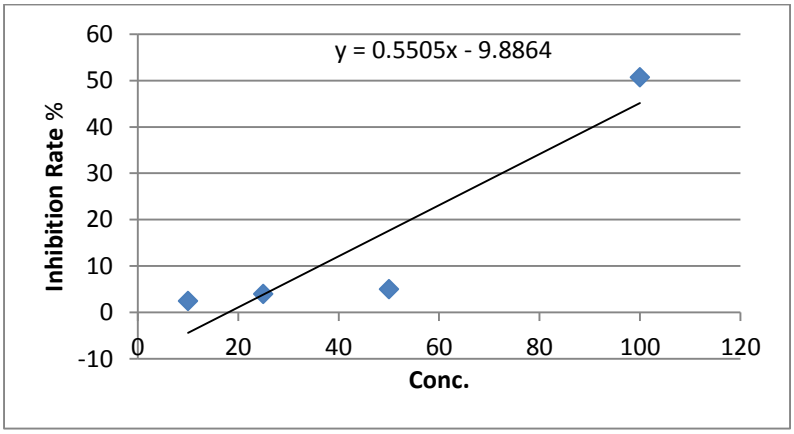


Fig. (٣) Linear relation between inhibition Rate and concentration of Compound ٣.

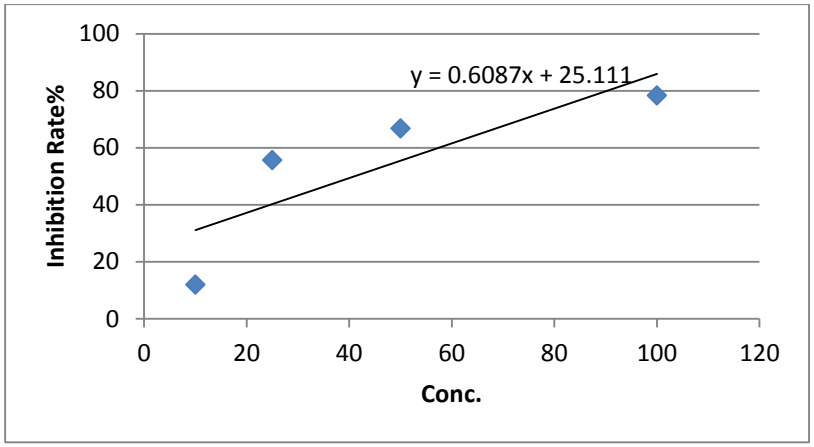


Fig. (٤) Linear relation between inhibition Rate and concentration of Compound ٣a.

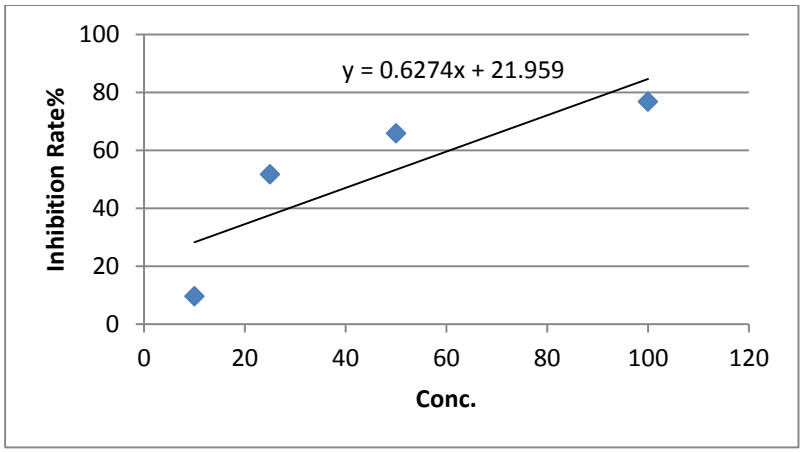


Fig. (٥) Linear relation between inhibition Rate and concentration of Compound ٣b.

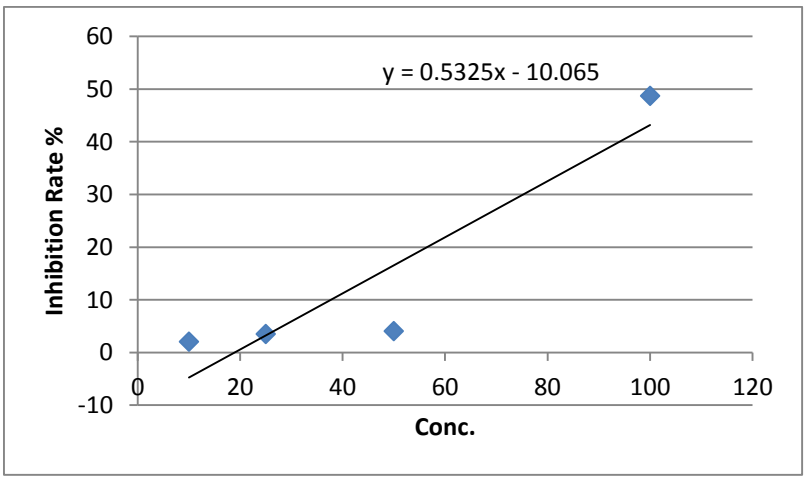


Fig. (٦) Linear relation between inhibition Rate and concentration of Compound ٣c.

Conclusion

A series of triazole-٣-thiol (**٣a-c**) was synthesized. The urease inhibition ability of these compounds was evaluated. Some of the compounds were found to be excellent inhibitors. The compounds ٢ and **٣a** showed most urease inhibition activity. Therefore, the discovered inhibitors should be further investigated for the control of diseases whose tangible and beneficial alternatives are still insufficient.

All the synthesized compounds were further screened for antibacterial activities, demonstrating that some compounds in the series are most promising. The identified compounds can be utilized for further optimization of bioactivity using structural variations in the parent skeleton.

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الخلاصة

في هذا البحث تم تحضير ٤–امين –٥– فنيل –١،٢،٤ ترايزول –٣– ثايول بواسطه الغلق الحلقي للبوتاسيوم ثنائي ثايو كاربوزينيت مع الهيدرازين بالتقطير لمده ٣–٤ ساعات باستعمال الايثانول كمذيب. حيت تم تحضير مركب الترايزول بنجاح وبعده تم اضافه الديهيدات مختلفه لتحضير قواعد شف. تم تشخيص هذه المركبات المحضرة بواسطة الطرق الطيفية (طيف الاشعة تحت الحمراء F.T.IR) وبواسطة

كمثبطات لانزيم اليوريزوقد اظهرت بعض المركبات فعاليه عاليه لتثبيط انزيم اليوريز.

جهاز الرنين النووي المغناطيسي (HNMR) و قياس درجة الانصهار لهذه المركبات. وتم فحص المركبات المحظرة