Synthesis and Study the Biological Activity of New Heterocyclic Compounds Derived From Hydrazide Derivatives

Wasan M. Abd-AL Hameed[1], Jumbad H. Tomma[2], Rajaa K. Baker[3]

[1]Post graduate, Department of Chemistry, College of Education for Pure Science / Ibn Al-Haitham/ University of Baghdad Iraq, [2]Prof. Department of Chemistry, College of Education for Pure Science / Ibn Al-Haitham/ University of Baghdad Iraq, [3]Ass. Prof. Department of Chemistry, College of Education for Pure Science / Ibn Al-Haitham/ University of Baghdad Iraq

Abstract

The work involves synthesis of new compounds derived from 2-substituted-3-thio- acetohydrazide-1,2,4-triazin. The subsituted hydrazine thiocarbothioamide [IV] was obtained via reaction of hydrazide compound [III] with ammonium thiocyanate and HCl. The cyclization of compound [IV] in 4% NaOH led to formation 1,2,4-triazoles [V], which were refluxed with chloro-ethylacetate in ethanol and sodium acetate to give compound [VI]. Condensation of ester-compound [VI] with hydrazine (80%) led to formation new hydrazide compounds [VII]. Ring closure reaction of compound [VII] with 4-aminbenzoic acid in $POCl_3$ give 1,3,4-oxadiazoles [VIII]. Compound [VIII] was reacted with sub. benzaldehyde to form compounds of Schiff bases $[IX]_{a-d}$. The synthesized compounds were diagnosed via IR, Mass and ^{1}HNMR spectral data. Many of synthesized compounds were examined against activity of antibacterial: gram (+) (*Bacillus subtitis, staphylococcus aureus*) and *gram* (-): (*E.coli* bacteria) and candida tropicalis fungial. To ensure the safety of the synthesis compounds the toxicity was examined.

Keywords: *1,2,4-triazine, 1,2,4-triazoles, 1,3,4-oxadiazoles, Schiff bases, antibacterial activity.*

Introduction

Triazine is one type of heterocyclic compounds, with six-membered include three nitrogen atoms. There are various methods for the synthesis of triazine compounds. The first of them, reported by Bamberger[(1)]. On other hand, another general methods are reported in Literatures[(2,3)], including the dehydrogenation in potassium dichromate from dihydro-triazines . Also synthesized by the rig closure reaction of hydrazine compound with α-acylaminoketone[(4)]. Recently, Arshad et el. mentioned a method for prepared 1,2,4-triazine via cyclization of thiosemicarbazide with benzil in acetic acid[(5)].

Corresponding author:
Wasan M. Abd
alanewasan@gmail.com

1,2,4-Triazine derivatives are good type in many biologically fields, such as antimicrobial[(6)], antibacterial[(7)], antifungal[(8)] and anticancer[(9)]. Furthermore, 1,2,4-triazole ring systems have a wide spectrum from biological activities, Antibacterial[(10)] and antifungal[(11)]. Also the 2- mercapto-1,2,4-triazoles have good role in synthetic organic chemistry, for example, to formation other heterocyclic compounds in present of different reagents, undergo various types of reactions [(12)]. Heterocyclic having a five ring heterocyclic containing two nitrogen atoms at position 3, 4- and one oxygen atom at position 1 is called 1,3,4-oxadiazole[(13)] , which is have potent biologically are 1,3,4-oxadiazoles[(14)] . In addition to, 1,3,4-Oxadiazole is a thermally stable neutral aromatic molecule and its have widely applications[(15)]. On the other hand the aryl-oxadiazoles have been used for development of newly drugs, with attention of a special kind to their properties as antitubercular and antimicrobial agents. [(16)]. The synthetic methods for the formation of differently 1,3,4-oxadiazoles have been

reviewed [17].

Due to a number of biological activities of triazines, triazoles and oxadiazoles are reported in previous literature, we have synthesized new compounds containing triazole and oxadiazole starting from triazines by using simple and an efficient method besides to investigation of the biological activities of these compounds.

Materials and Methods

Materials: The chemicals were equipped from Merck , Fluka ,GCC and Aldrich chemicals company.

Techniques: FTIR spectra (using KBr disc) were determined by on a Shimadzo (8300), ^{1}HNMR spectra were determined by: Bruker company (model: ultra-shield 400 MHz), origin: Switzerland (in solvent DMSO), ppm(δ), uses internal standard (TMS). Mass spectra were recorded on MS model: 5973(Manufacture Company: Agilent Technology HP). Gallen Kamp melting point apparatus was used for determined uncorrected melting points. The TLC were done on aluminum plates, supplied from Merck co, using ((7:3), n-hexan/ethyl acetat).

General Experimental Procedures

The reaction sequence leading to the formation of new compounds [IX]-[X] are outlined (**Scheme not shown contact corresponding author**).

1,2,4-triazine-3-thiol derivative [I], their ester[II] and hydrazide [III]: These compounds were prepared according to the let. [18].

2-(2-((5,6-di-phenyl-1,2,4-triazin-3-yl)-thio)-acetyl)-hydrazine-1-carbo-thioamide [IV]

Heating a solution of ammonium thiocyanate (6gm, 0.078 mol) and acid hydrazide [II] (0.013 mol) in ethanol (60 mL) and HCl (12 mL) via reflux for (22 hrs.). Then, evaporated the solvent and the mixture of reaction was added to crushed ice with stirring, the precipeted was filtered off [19], dried and recrystallized from ethanol.

1,2,4- triazoles [V]

Refluxed for 22 hrs a mixture of compound [IV] (0.01mol) and (10mL) from 4% aqueous NaOH. Then, filtered the mixture, the filtrate was acidified with dilute HCl. Afterwards, filtered the powder which precipitate [20], washed it for several times with water, recrystallized from ethanol.

Ester compound [VI]

The compound [V] (0.001 mol) was heated with chloroethyl acetate (0.001 mol) and sodium acetate (0.003 mol) in ethanol was for 5hrs. Then, cool the mixture and added it onto ice water, the solid resulting was filtered, dried. Recrystallization by used ethanol to give ester compound [VI].

hydrazide compound [VII]

A solution of compound [VI] (0.06 mol) and(15 mL) hydrazine(80%) in ethanol (25 mL) was heating for 4hrs. Afterwards, cooling the mixture of reaction (to room temperature), the solid compound was filtered, then, recrystallized from ethanol.

Synthesis of 1,3,4-oxadiazole compound [VIII]

4- Aminobenzoic acid (1,37g, 0.01 mol) was mixed with thiosemicarbazide [VII] (0.01mol) in phosphorus oxy-chloride (5 mL), afterward the mixture was refluxed softly for 6 hrs. After cooling, its poured onto ice water (50 mL) with stirring. The precipitate was filtered, washed with solution of $NaHCO_3$, then water, dried then recrystallized from ethanol.

Synthesis of Schiff bases $[IX]_{a-d}$

Reflux of a mixture of aromatic aldehydes (0.001 mol), 2-amino-1,3,4-oxadiazoles [V] (0.001 mol), glacial acetic acid (3 drops) in (4 mL) ethanol for 4hrs. Recrystallized the obtained solid from ethanol after evaporating the solvent. The physical properties, nomenclature, structure of the synthesized compounds $[IV]_{a,b}$ are listed in Table (not shown contact corresponding author)

Biological activity

Adult male mice, weighting about (23-46) gm were housed in plastic cages under standard condition. the animal were divided into five groups each group consisting of five mic, including the control group, the mice were injected with the synthesized compounds with a concentration of (10^{-2}). After two weeks toxicity

was measured as the method [21].

Results and Discussion

The equimolar of benzil and thiosmecarbazid were reacted under ring closure reaction in glacial acetic acid to formed triazine compound. Ester compound [II] which prepared from the reaction of triazine with chloro acetic acid in basic medium and ethanol was diagnosed via FTIR spectroscopy. The FTIR spectral data showed two bands at 1732 cm^{-1} and 1253 cm^{-1} due to (υ C=O) and (υ C-O) related to ester moiety respectively, with disappearance (υ C=S). The condensation hydrazine (80%) with ester [II] in ethanol give a corresponding hydrazide type[III]. The FTIR of compound [III] exhibited new bands at (3329-3207) cm^{-1} due to υ NH_2 and υ NH (asym., sym.) bands and (υ C=O) vibration band for of amide group at 1668 cm^{-1}.

Nucleophilic addition reaction for acid hydrazide [III] with ammonium thiocyanate in ethanol using HCl as a catalyst to give hydrazine carbothioamide derivatives [IV]. The structure of this compound was identified by IR and ^{1}H-NMR spectroscopy. IR spectrum indicated υ C=S absorption band at 1280 cm^{-1}, in addition to new bands which related to (asymmetric, symmetric) vibration of υ NH_2 and υ N-H groups appear at (3388-3168) cm^{-1}. Also a shifting on υ C═O (amid) group to 1672 cm^{-1}. The spectrum of ^{1}HNMR for compound [IV] showed a singlet signal of SCH_2 groups at δ 4.97 ppm and a signal at δ5.57 ppm due to NH_2 protons of group .Signals due to aromatic (ten) protons for benzene rings at δ (7.06-7.90) ppm. Also two signals for protons of two NH group of (CO-NH-NH-CS) moiety at δ12.78 ppm and δ13.56 ppm .

1,2,4-triazole-3-thiol [V] was obtained by cyclization of hydrazine carbothioamide compound [IV] in a solution of 4% NaOH followed by neutralized with dil. HCl to yielded this compound. The triazoles were diagnosed via FTIR and NMR spectroscopy. The FTIR spectral data showed a good peak at (3394-3153) cm^{-1} for stretching band of NH, also show the disappearance of the characteristic bands of starting materials [IV], in addition to new peak around 1284 cm^{-} for C=S stretching. The ^{1}H-NMR spectrum (in DMSO) showed signals as in the following: a signal type singlet at δ10.09 ppm for NH proton of triazole ring. Aromatic protons appeared at δ (7.26-7.53)ppm as many signals, signal appeared at δ 9.8 ppm for NH proton at C_2 of triazole ring (which is toutomerism with SH). Also exhibited a signal type singlet at δ 5.57ppm for CH_2 protons. The spectrum of mass exhibited parent ion [M^+] at 378.

The ester compound [VI] which prepared from the condensation of compound [V] with chloro acetic acid in CH_3COONa medium and ethanol was diagnosed via spectroscopy (IR and ^{1}HNMR). The IR spectral data showed: υ C=O and υ C-O for ester group and 1211 cm^{-1}, respectively. at 1735 cm^{-1}. Disappearance band of (υ C=S). The reaction of ester [VI] with hydrazine hydrate in ethanol give a corresponding acid hydrazide [VII]. The FTIR spectral data exhibited asym., sym. bands at 3199 cm^{-1} of υ NH and υ NH_2 groups, and (υ C=O) vibration band at 1670 cm^{-1} for amide group.

The reaction of compound [VII] with thiosemicarbazide in $POCl_3$ yielded a new compounof 1,3,4-oxadiazoles [VIII]. The oxadiazole compound was diagnosed via IR and ^{1}HNMR spectra. The IR spectral data showed disappearance bands characteristic for hydrazide compounds and the appearance of a new two bands due to (υ NH_2) between (3442-3213) cm^{-1} beside to a band around 1670 cm^{-1} for υ C=N. While the spectrum of ^{1}HNMR of [VIII] showed: a signal type singlet at δ 9.8 ppm for of NH proton. The protons type aromatic appeared as many signals at δ (6.9-7.6) ppm, also a singlet signal due to SCH_2 protons appearance at δ 4.0 ppm. Finally, a signal type singlet appeared at δ 6.6ppm for NH_2protons. The mass spectrum exhibited parent ion [M^+] at 551.

The Schiff bases type $[IX]_{a-d}$ were produced from the refluxing of amino compound [VIII] with aromatic sub. aldehydes in glacial acetic acid (3 drops) with ethanol. The FTIR spectral data (Table 2) of these compounds showed appearance new υ C=N absorption band. The spectrum of ^{1}HNMR for Schiff base $[IX]_a$ showed a signal type singlet at δ 9.86 ppm for NH proton and δ (6.92-7.76) ppm for protons type aromatic, CH=N proton at δ 8.67 ppm appeared as a singlet signal. Also the spectrum showed a singlet signal at δ 4.86 ppm for SCH_2 protons. Finally a signal type singlet appeared at δ 2.3 ppm refer to CH_3 protons. The ^{1}HNMR spectrum of Schiff base $[IX]_d$ showed aromatic protons at δ 9.68 ppm for NH proton and δ (6.7-7.95) ppm protons type aromatic appeared at δ 8.6 ppm and a signal type singlet

for one CH=N proton. Finally a signal type singlet at δ 3.06 ppm for $(CH_3)_2$ protons.

Biological activity

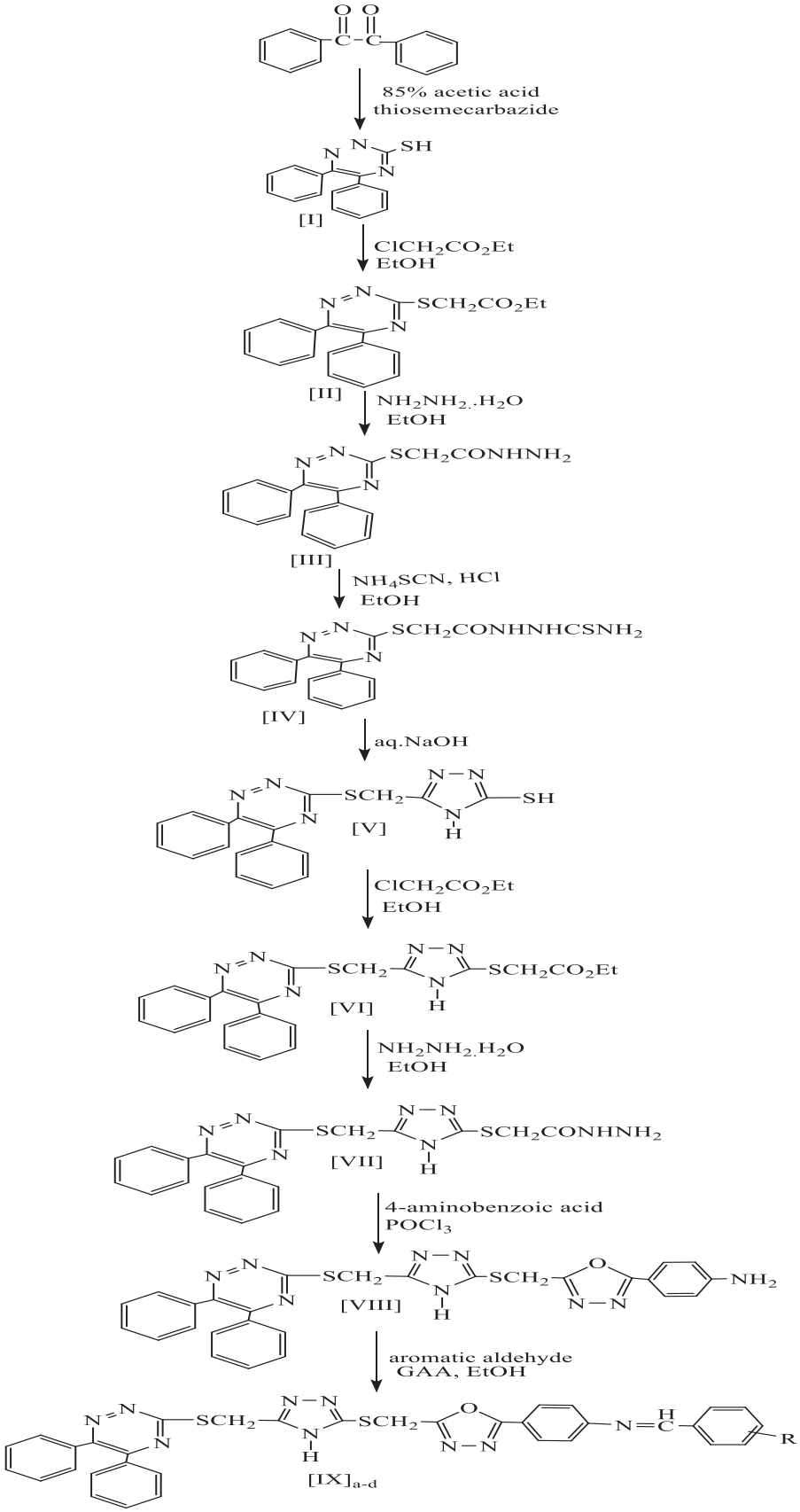
The agar diffusion [22] was used for examined the activity of antibacterial and anti-fungial of some of the synthesized compounds, using three types of bacteria; (G-) *Escherichia coli*, (G+) *Bacillus subtilties* and *staphylo coccus aureus*, and Candida tropical as fungal. The prepared (agar medium and petri-dishes) were sterilized via autoclaving for (20 min) at (121ºC). The agar was surface inoculated uniformly from the both cultures of the tested micro-organisms, the fungal and three types of bacterial were activated in dextrose agar medium and nutrient agar medium for 24 hrs at 37 ºC, respectively. Every one of examined compounds was dissolved in DMSO (used as a control and a solvent) to give two type of concentration (10^{-2} M and 10^{-3} M). The inhibition zones which formed were measured in millimeter. The antibacterial activities and anti-fungal data of the examined compounds were given in Table (3). The administrated does of Compounds [V] and[VIII] did not result in death of any of mice except $[IX]_b$ in toxicity test.

Table 2 IR spectral data of compounds types [IX]a-d

Comp No	υ NH	υ CH arom	υ CH aliph.	υ C=N	υ C=C arom	Other
[IX]a	3400	3057	2931,2868	1680,1604	1556	δ CH3:1370
[IX]b	3305	3059	2935,2871	1678,1601	1527	υNo2 asy:1527 sy: 1348
[IX]C	3410	3057	2935,2866	1680,1601	1556	υ No2 asy:1522 sy:1344
[IX]d	3194	3055	2937,2868	1676,1597	1523	υ NMe2:1367,1169

Table 3 Inhibition Zones of Titled Compounds

Compound No.	Zone of Inhibition (mm.)							
	Bacillus Subtilis		Staphylo cocas aureus		E. Coli		Candida tropicalis	
	Gram Positive(+)		Gram Positive (+)		Gram negative (-)			
	Con. 10^{-3} 10-3	Con. 10^{-2}	Con. 10^{-3}	Con . 10^{-2}	Con . 10^{-3}	Con . 10^{-2} 10^{-2}	Con . 10^{-3}	Con . 10^{-2}
[V]	16	18	15	18	-	-	-	-
[VIII]	-	16	12	15	-	-	-	-
$[IX]_b$	-	17	-	-	-	-	-	10



R= CH_3; $[IX]_a$, 3-NO_2; $[IX]_b$, 4-NO_2; $[IX]_c$, N-Me_2; $[IX]_d$

Scheme I :synthetic route of compounds $[IX]_{a-d}$

Conclusions

In this work new heterocyclic compounds derived from 5,6-diphenyl-1,2,4-triazine-3-thiol were synthesized in good yields via simple methods. Some compounds give good biological activity that may be related to the functional groups for the examined compounds.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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