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Synthesis, Characterization and Study Biological Activity of Substituted 4-Amino -3,5-Bis (2,4-dichloro phenoxy)-1,2,4-Triazole

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ABSTRACT

In this paper, the ester (2) was prepared by reacting 2,4-dichlorophenoxy acetic acid with methyl alcohol and using concentrated sulfuric acid as a catalyst in this reaction, which, when reacted with hydrazine hydrate, obtained acid hydrazide. (3), under reflux conditions with dimethyl sulfoxide and at solvent temperature, the compound 4-amino-3,5-bis-(2,4-dichlorophenoxy)-1,2,4-triazole was obtained (4). It was obtained and used to prepare a series of new Schiff bases (5a-e) through their reaction with different substitutes of benzaldehyde. The completeness and purity of all the prepared compounds were confirmed by thin-layer chromatography (TLC) and showed some physical and spectroscopic properties, namely (FT -IR 1H-NMR and 13C-NMR). In addition, the biological activity of the prepared compounds was studied, and their effectiveness against different types of bacteria such as *Bacillus*, *Escherichia coli*, and *Staphylococcus aureus* was discussed. Studies of mainly fungi and bacteria have shown good efficacy against the mentioned species using disc diffusion technology.

Keywords: 2,4-dichlorophenoxyacetic acid, hydrazide, amino triazole, Schiff bases, biological activity.

INTRODUCTION

The Chemistry of five-membered heterocyclic compounds containing nitrogen atoms (mostly triazoles and their derivatives) has great and continuing importance. The structural components and molecules physiologically active are five-membered N-heterocyclic compounds (Rebaz *et al*., 2023). Substituted -4-amino -1,2,4-triazole was investigated for its inhibition action on the corrosion composite in sodium hydroxide solution (Reena *et al.*, 2011).

The biological actions of 1,2,4-triazole derivatives, such as their antibacterial, analgesic and anticancer effects, have drawn much interest to their synthesis (Wahi and Singh, 2011; Neslihan *et al.*, 2005; Demirba *et al.*, 2005) while some of the 1,2,4- triazole derivatives have antimicrobial activity (Sahar *et al.*, 2011; Fedotov *et al.*, 2023; Hamida *et al*.,1999; Xin-Ping Hui *et al.*, 1999), and growth regulatory activities (Chang-Hu-Chu *et al.*, 2002), anticancer activities (Hipara *et al.*, 2003), some of the triazole compounds evaluated for antioxidant (Suresh *et al.*, 2010). Because 1,2,4-triazole substitution is advantageous in synthesizing organic chemistry, several techniques have been used and documented in the literature (Maysaa *et al.*, 2022; Bentiss *et al.*, 2002; Cheng *et al.*, 2007; Sudeep *et al*., 2010).

the aim to create alternative 1,2,4-triazole derivatives and implement our previous research on bioactive molecules. This would involve creating new 1,2,4-triazoles that would contain the substituted 1,2,4-triazole moiety through the reaction of 4-Amino - 3,5-bis (2,4-dichlorophenoxy)-1,2,4-Triazole with a variety of compounds.

EXPERIMENTAL

Melting points have been measured uncorrected using an electrothermal -9300 melting point instrument. Using KBr-disk, FT-I.R. spectroscopy was captured on a Bruker optics (FT-IR) spectrometer company. On a Bruker 300-MHz spectrometer, 1H- and 13C-NMR spectra were obtained using DMSO-d6 as a solvent and Tetramethylsilane as an internal standard.

Preparation of 2,4-dichloro phenoxy acetate (2) (Ronald *et al*., 1974)

Dissolved (0.01 moles) of 2,4-dichlorobenzoic acid (1) in (20ml) of methanol, (1ml) of conc. sulfuric acid was added, this mixture was reflexed for (4 hours). Then was poured into an ice water, the white precipitate was filtered, washed with water, dried and the product was recrystallized from ethanol to afford the as oily product.

Preparation of 2,4-dichloro phenoxy acetic acid hydrazide (3)

Dissolved (0.001moles) of 2,4-dichlorophenoxy acetate (2) in absolute ethanol (20ml), with (0.001moles) of hydrazine hydrate (85%) was added. This mixture was refluxed for (5hrs.), and the solvent was evaporated. The product was filtered and dried to give a pure compound with a clear yellow colour, m.p. (155-158^{o}C), and yield (82%).

Preparation of 4-Amino -3,5-Bis (2,4-dichlorophenoxy)-1,2,4-Triazole (4) (Badie *et al*., 2014).

Dissolved 2,4-dichloro phenoxyacetic acid hydrazide (3) (0.004 mole) in 10 ml DMSO, the liquid was refluxed for 18 hours, distilled under reduces pressure, allowed to cool, and then poured into ice water. The mixture was then stirred for 12 hours at room temperature, and the product is filtered, dried, and crystallized with water- ethanol to yield the corresponding chemical (4) as a light-yellow powder (65%, m.p. 141-143 C).

Reaction of 4-Amino -3,5-bis (2,4-dichloro phenoxy)-1,2,4-Triazole with substituted benzaldehyde (General method)

Dissolved 4- Amino-3,5-Bis(2-Dichlorophenoxy)1,2,4-triazole (4) (0.001mol) in absolute ethanol, 5 drops of glacial acetic acid, and (0.001mol) of substituted benzaldehyde, for four hours, the reaction of the mixture was refluxed. Reduced pressure evaporated the solvent, and the result

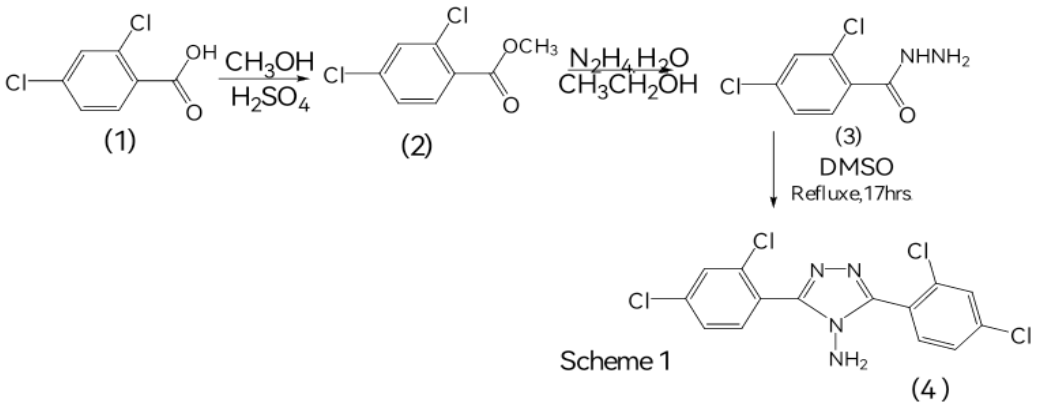
was filtered out. then recrystallized in an appropriate solvent to provide the products (5a–e). Physical data for these substances are included in (Table I).

Table 1: Physical Properties for compounds (5a-e)

Compd. No.	X	m.p ^{o}C	Yield %	Color
5a	p-NH_2	113-115	97	Dark brown
5b	p-CH_3	196-198	91	Brown
5c	o-OH	212-214	57	Light brown
5d	m-OCH_3	207-209	55	Light brown
5e	p-NO_2	183-185	65	Pale yellow

RESULTS AND DISCUSSION

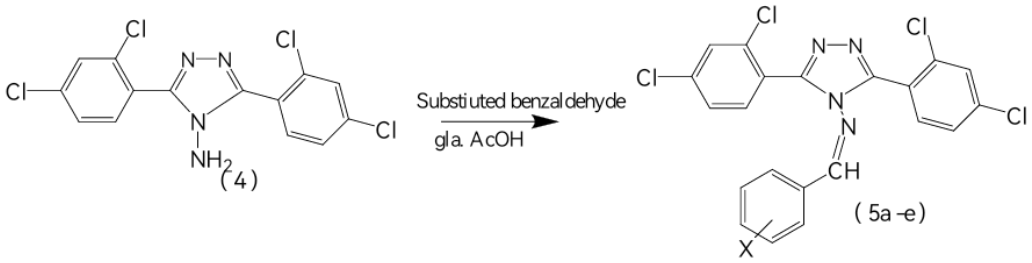
In the current study, a reaction with methanol converted 2,4-dichlorophenoxyacetic acid (1) into ester (2). 2,4-dichlorophenoxyacetic acid hydrazide (3) was obtained by treating this ester with hydrazine hydrate in ethanol. When this hydrazide (3) dissolves in dimethyl sulfoxide under refluxing conditions, 4-Amino -3,5-Bis (2,4-dichloro phenoxy)-1,2,4-Triazole (4) was produced, as indicated in schema (1).



Scheme 1

FT-IR spectrum for traiazole compound(4) showed a broad bands of the (OH) group at (3309cm^{-1}), the (NH_2) at (3360cm^{-1}), and the (C=N) group at (1647cm^{-1}). While the 1H-NMR spectrum data, which revealed a wide band at 12.8 ppm indicating two protons of the (NH_2) group in this molecule and another band at δ (7.15-7.88 ppm) for (6H) aromatic protons, further validated the structure of the compound.

Compounds of 1,2,4-triazole exhibit a range of biological functions (Vishnoi, 1982). And for our ongoing fascination with 2,4-dichlorophenoxyacetic acid chemistry. As seen in Scheme 2, we discovered that 4-Amino -3,5-Bis (2,4-dichlorophenoxy)-1,2,4-Triazole (4) may be prepared by reacting with different benzaldehyde substituents to give Schiff bases (5a-e).



Scheme 2

X= p-NH_2, p-CH_3, o-OH, m-OCH_3, p-NO_2

The structures of the target Schiff base (5a-e) were confirmed using (FT-IR, ^{1}H-NMR, and ^{13}C-NMR). Also, the FT-IR spectra for these compounds showed the following stretching bands;

(1620-1681cm^{-1}) to the (C=N) bond for Schiff bases. The 1H-NMR spectra for compounds (5a-e) in (DMSO-d6) representation for these Schiff bases showed the peaks of one proton for CH=N at the range (7.53-7.87ppm) and at the range (6.81-6.88ppm) and at (7.61-7.64 ppm) for aromatic protons and other groups in these compounds is in full agreement with the proposed structures which confirmed in (Table 2). While ^{13}C-NMR for compounds (5a-e) in (DMSO-d_6) representation for these Schiff bases showed the signal for all carbon atoms in these compounds are listed and as shown in (Table 2) also.

Table 2: Spectral data of compounds (5a-e)

Compd. No.	C=N	^{1}H-NMR, ppm), DMSO-d_6	^{13}C-NMR, (ppm), DMSO-d_6
5a	1642	9.09(s,1H, HC=N), 7.59 -7.83(m,6H, ArH), 7.63(d,2H, ArH), 6.82(d,2H, ArH), 5.43(bs,2H, NH_2).	113.9,122.6,126.95,128.4,130.7, 130.9,135.2,136.9,151.22.
5b	1626	9.07(s,1H, HC=N), 7.62-7.87(m,6H, ArH), 7.63(d,2H, ArH), 6.88(d,2H, ArH), 2.39(s,3H, CH_3).	21.6, 112.2,122.3,126.6,127.1,128.4, 130.7,130.9,135.2,136.9,140.3,151.22.154 .1
5c	1644	9.73(s,1H, OH), 9.15(s,1H, HC=N), 7.53 -7.79(m,6H, ArH), 6.85(d,2H, ArH).	112.8,116.5,122.1,124.3,126.9,128.4,130. 7,130.9,135.2,136.9,151.22, 154.7,160.1
5d	1633	9.11(s,1H, HC=N), 7.61 7.87(m,6H,ArH),7.61(d,2H, ArH), 6.87(d,2H, ArH), 3.79(s,3H, OCH_3).	112.2,114.5,123.3,124.6,126.2,127.3128.4 ,130.7,130.9,135.2,136.9,151.22, 154.4,160.4
5e	1638	9.15(s,1H, HC=N), 7.58- 7.83(m,6H, ArH), 7.64(d,2H, ArH), 6.81(d,2H, ArH).	113.1,123.3,124.2,126.7,126.8,128.5 ,130.1,130.7,132.6,136.3,138.6,138.9, 140.3,154.7,161.1

BIOLOGICAL ACTIVITY

Antibacterial and antifungal studies

This study measured the antifungal properties and antibacterial activity of all the produced compounds using the disc diffusion technique. *Microsporum destortum, Microsporum* gypseum, and *Trichophytonrubrum* were utilized as microorganisms for the antifungals. Micrococcus, Pseudomonas, Bacillus 11, Bacillus 12, Escherichia coli, and Staphylococcus aureus were the microorganisms employed in the antibacterial research. Meanwhile, the lowest inhibitory concentration was used to assess these substances. Compounds (5a and 5d) exhibit excellent efficacy against staphylococci and Escherichia coli bacteria. At doses of 1 mg/ml and 2 mg/ml, compound (5c) demonstrates almost substantial action against all tested fungi. Conversely, the residual compounds exhibited modest action against the other examined bacteria and fungi.as shown (Tables 3 and 4).

Table 3. Antibacterial activity of compounds 5a-e.

Comp. No.	*E. coli*	*Staphylococcus aurous*	*Micrococcus*	*Pseudomonas*	*Bacillius 11*	*Bacillius12*
5a	**12**	**9**	------	6	------	------
5b	**8**	**9**	------	------	------	------
5c	------	------	**9**	**6**	**6**	**6**
5d	**6**	**14**	------	------	------	------
5e	------	------	**6**	**6**	**6**	**6**

Inhibition zone diameter (mm) (% inhibition): 6-10 (27-45%); 10-14 (45-64%); 14-18 (64-82%); 18-22 (82-100%).

Table 4: Antifungal activity of some selected compounds 5a-e

Comp. No.	*Microsporumgypseum* (cm)		*Microsporumdestortum* (cm)		*Trichophytonrubrum* (cm)	
	1mg/ml	2mg/ml	1mg/ml	2mg/ml	1mg/ml	2mg/ml
5a	0.25	zero	1.25	zero	0.25	zero
5b	1.25	0.35	1.45	0.15	1.35	zero
5c	1.51	zero	2.55	zero	2.5	zero
5d	1.75	0.35	3.15	0.25	2.25	zero
5e	0.45	zero	1.15	zero	0.35	zero

+Inhibition zone diameter (cm) (% inhibition): 5.5-3.3 (0-40%); 3.3-2.2 (40-60%); 2.2-1.1 (60-80%); 1.1- zero (80-100%).

From the obtained data, it is evident that compounds (5a and 5d) possess a very good activity against bacteria Strains like E. coli and Staphylococcus. And the compounds (5b and 5d) possess almost a significant activity against all fungi tested at 1 mg/ml and 2 mg/ml. The remaining compounds showed a moderate activity against other bacteria and fungi tested.

CONCLUSION

In this research, 2,4-dichlorophenoxyacetic acid was used to prepare a series of Schiff base compounds. The compound bistriazole was prepared by using the aforementioned acid. Triazole was also used to prepare the target compounds by reacting with different substitutes for benzaldehyde. All the prepared compounds were characterized using Various spectroscopic methods. A study was conducted on these compounds to evaluate their biological effectiveness against different types of bacteria and fungi. This study revealed high effectiveness for some of these compounds.

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تحضير، تشخيص ودراسة الفعالية البايولوجية لمعوضات 4-امينو -5,3-بس (4,2- ثنائي كلورو فينوكسي)-4,2,1- ترايازول

عمر محمد يحيى

فرع الكيمياء الحياتية/ كلية الطب/ جامعة الموصل

الملخص

في هذا البحث، تم تحضير الإستر (2) من خلال تفاعل 4,2-ثنائي كلوروفينوكسي حامض الخليك مع كحول الميثيل واستخدام حامض الكبريتيك المركز كعامل مساعد في هذا التفاعل، والذي عند تفاعله مع الهيدرازين المائي، يتم الحصول على هيدرازيد الحامض المقابل (3)، تحت ظروف التصعيد مع ثنائي ميثيل سلفوكسيد وعند درجة حرارة المذيب، تم الحصول على المركب 4-أمينو-5،3-بس-(4،2-ثنائي كلوروفينوكسي)-4،2،1-ترايازول (4). ثم استخدم لتحضير سلسلة من قواعد شيف الجديدة المقابلة (e-a5) من خلال تفاعلها مع بدائل البنزالديهايد المختلفة. تم التأكد من نقاوة جميع المركبات المحضرة بواسطة تحليل كروماتوغرافيا الطبقة الرقيقة (TLC) وأظهرت بعض الخصائص الفيزيائية والطيفية وهي (^{1}H-NMR FT -IR و ^{13}C-NMR). بالإضافة إلى ذلك تمت دراسة النشاط البيولوجي للمركبات المحضرة ومناقشة فعاليتها ضد أنواع مختلفة من البكتيريا مثل *Bacillus* و*Escherichia coli* و*Staphylococcus aureus*. أظهرت الدراسات التي أجريت على الفطريات والبكتيريا بشكل رئيسي فعالية جيدة ضد الأنواع المذكورة باستخدام تقنية الانتشار بالقرص.

الكلمات الدالة: 2و4-ثنائي كلورو فينوكسي حامض الخليك، هيدرازايد، امينو ترايازول، شيف بس، فعالية بيولوجية.