P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2009,(3), (1) :59-65

Synthesis a Number of Triazene Compounds Derived From Purine and Studying their Biological Activity on Pathogenic Bacteria

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ARTICLE INFO

Received: 16 / 3 /2008
Accepted: 22 / 10 /2008
Available online: 14/6/2012
DOI: 10.37652/juaps.2009.15509

Keywords:
Synthesis ,
Triazene ,
Purine ,
Biological Activity ,
Bacteria.

ABSTRACT

In this research a number of compounds were prepared by coupling dizonium salts with purine bases. The structures of the prepared compounds were identified by ultra violet ,infra red spectra and Elemental (C.H.N) analysis .The biological activity of these compounds was investigated on five genera of pathogenic bacteria: S. aureus , Str. viridans , Ps. aeruginosa , E. coli and Sh. dysenteriae using Disc diffusion method. Also the minimum inhibitory concentration (MIC) was calculated .It was found that these compounds have medium biological effect against these genera of bacteria.

Introduction

Triazene compounds contain (-N=N-N-) group and migh be called Diazoamino compounds, but the most common name is Triazenates [1].Triazene compounds are generally prepared by coupling dizonium salts extracted from aromatic amines or aromatic hetrocyclic amines with other amines [2].

The biological activity of Dimethyl phenyl triazene derivatives was discovered in 1955. It was found that they work as Sarcom 180 in rats.

After this discovery, studies on this compounds contained. Danis and Rond Steved [3] were the firs to carr out a comprehensive study on phenyl triazene derivatives to test their biological activity as antitumor agents. Besides, some other researchers [4] have carried out many studies on 3-alkyl-1-pheyl-3-methyl triazene. They found that these compounds are biological active as antitumor agents.

On the other hand, Shealy et . al [5,6] succeeded in preparing 5(3,3-dimethyl-1-triazino)-imidazol-4-carboxyamid (DIC), by decomposing this compound to give carbonium ion which is characterized by a high activity and alkylate DNA , which results in inhibition of cell growth [7,8]. Shealy succeeded to prove that (DIC) inhibits the growth of tumor in rats. Due to the biological activity of (DIC), Skibbal, et .al [8,9] proved that (DIC) is active in inhibition of the growth of tumors in the human as well. In 1985 A. Mairuf [10] proved that Dimethyl triazino benzo thiazol derivatives have the ability to associate with melanine. This prove that such compounds are active in inhibiting tumors.

The cyclic system of purines is one of the most important systems in living creatures. Purines always exist as derivatives of great biological value. They are the most important types of hetrocyclic compounds [11]. Elion and Hitching were the first to synthesize and develop Purine Analogies that inhibit metabolic paths to produce DNA. Nowadays, these Analogies are useful in cancer chemotherapy [12]. In 1999, Chang et al succeeded in synthesizing a various series of Purine derivatives. They discovered that some of these derivatives had selective inhibition on cells derived from tumors [13]. Also, Kramata and Downey proved that Adenine derivative (Phosphonyl Methylether) and

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Guanine derivative Phospho. Methyether Guanine(PMEG) have an inhibition effect on DNA production through direct inhibition of DNA Poly.II enzymes in addition to its being used as virus antibiotics [14,15] morcover, studies have proved the derivatives of aryl purine show a standard activity in biotic medicine as antivirus, antibacterial and anticancer, besides they have the features of antihypertensive materials[16]

Experimental.

Synthesis of 1-(4 -Chloro phenyl) -3-(Guanine)-Triazene

4.45 m.mole of the aromatic amine was dissolved in 4 ml of concentrated HCl and 8 ml of distilled water .The mixture is cooled to 0C0 and 4 m.mole of sodium nitrite to added drop wise with continuous stirring. The solution left for 30 minutes to be stable after completing the addition then 5 m.mole of Guanine (dissolved in 20 ml NaoH) was added, a yellow precipitate was formed, filtered and recrystallised from (1:1) ethanol: water, compound(2-5),table (1) were prepared by following the same procedure.

Biological study.

Five genera of pathogenic bacteria were used in this study; S. aureus, Str. viridans, Ps. aeruginosa, E. coli and Sh. dysenteriae . These bacteria were isolated from patients and identified by the central Health Laboratory – Baghdad.

Bacterial sensitivity test toward the prepared compounds .

To study the impact of compounds on bacteria, Disc diffusion method [17] was applied. Where a series of concentrations in DMSO were prepared 0.1 mg/ml, 1 mg/ml, 10 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml and for each concentration 100 filter paper discs in glass tubes, autoclaved for fifteen minutes then 1 ml of the prepared solutions were added and tubes were shacked and the discs were dried at 40 C0 for 48 hours. A control sample for DMSO solvent was prepared by adding 1 ml to 100 sterilised discs.

Test method.

Muller Hinton agar was prepared by dissolving 37 g of agar in one liter of distilled water, sterilised then distributed in petridishes. The bacterial species were grown up on nutrient broth for 24 hours at 37C0 then 0.1 ml of bacterial suspension was transferred to the agar in each dish , left for half an hour and a disc for each concentration was left in the dish beside the control (DMSO) sample . The samples were autoclaved for 18 hours at 37 C0.The diameter of inhibition zone was measured using a ruler.

Determination of minimum inhibitory concentration MIC

Eight concentration have been prepared for each compound as follows: 1mg/ml, 5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml, 25mg/ml, 50mg/ml, 100mg/ml in DMSO 0.1ml of each solution from the prepared concentration was added to test tubs containg 5ml of the nutrient broth Tow test tubs were left one with out addition and to the other tube, DMSO was added only as control, the bacterial suspension was diluted and 1ml of the diluted suspension to the tubes including the control. The solution and bacterial suspension was mixed throughly and incubated at 37C0 for 18 hours and the minimum inhibitory concentration (MIC) was detected for each bacterial genus. Table (6).

Results and Discussion.

Compounds were identified by ultra violet (Table 2) and infra red spectroscopy (Table 3) in addition to (C,H,N) analysis (Table 4) and the results were in accord with the structural formula .

Ultra violet spectra for triazene compounds in ethanol of (10M) appeared with in the range (410 - 475 nm) which are attributed to (N - N =N-) group,the presence of(-JI JI*) transition at (205 – 220 nm) is due to aromatic cycles [18 ,19].Besides an absorption band at (245-265 nm) due to the absorption of Purine [20] .

Infra red spectra for the prepared triazene compounds revealed the presence of wide strong bands between (3600-3000) cm-1 due to the stretching vibration V(O-H) and V(N-H) in accord with the results in reference[21]. Sirroki [22]found it difficult to get reasonable resolution between bands due to OH and NH, hence abroad band between (3500 - 2500) cm-1 is definitely due to OH and NH stretching.

Infra red spectra also show absorption bands between (1590-1400) cm-1 with in the range of vibrational frequencies for V(C=C) and V(N=N) [23].

The infrared spectra show a distinctive band (1645-1625) cm-1 due to (C=N) stretch, besides the carbonyl band at (1730-1680) cm-1 .The usual frequency for this band is (1700-1400) cm-1 Hadzi[24]explained the abnormal position due to the formation of hydrogen bonds in the tautomeric formula for these compounds and the bands between(730-590) cm-1 due to the stretching vibration V(Ar-x)[21].

Biological Investigation of triazine compounds

The impact of the prepared compounds was studied on five genera of pathogenic bacteria: S. aureus , Str. viridans , Ps. aeruginosa , E. coli and Sh. dysenteriae .The sensitivity of bacteria toward the prepared compounds was tested by application of Kirby-Bauer technique [17] where six concentrations in DMSO of the compound have been tried.

These compounds show positive penetration in the bacterial cell with a considerable antibacterial potency. It looks from table (5) that compound (4) show the highest activity at concentration 100mg/ml against S. aureus where the inhibitory zone is (15 mm) and compound (3) show the minimum inhibitory zone of (10 mm) at the same concentration.

Compounds (2) looks the most effective against Str. viridans with in inhibitory zone of (14 mm) at the highest concentration 100 mg/ml. Compound (5) show the lowest activity with an inhibitory zone of (10.5 mm) at the same concentration. Compound (3) has the minimum potency toward Ps. aeruginosa where the inhibition zone diameter was (8 mm) at concentration 100mg/ml and compound (2) seems to be the most active with an inhibition zone of (10 mm) at the same concentration. Compound (4) looks the most effective against E. coli with in inhibitory zone of (13 mm) at the highest concentration 100 mg/ml. Compound (1) show the lowest activity with an inhibitory zone of (10 mm) at the same concentration. Compound (4) show the highest activity at concentration 100 mg/ml against Sh. dysenteriae where the inhibitory zone is (12 mm) and compound (1) show the lowest activity with an inhibitory zone of (9 mm) at the same concentration. These compounds might have chelating properties which build coordination complexes with metal ions in the bacterial cell like K+, Ca++, Mg++ ,Zn++ ,Fe++ ,Cu+ , which play vital role in the cell . Or the ability of these compounds to make hydrogen bonds with water inside the cell, which impair the biological activity of the bacterial cell. These compounds have a considerable activity toward different genera of pathogenic bacteria, which makes them of fairly potent

antibacterial agents then to play a distinguished role in chemotherapy.

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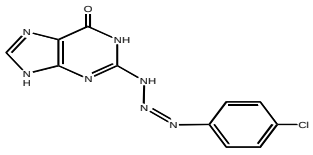
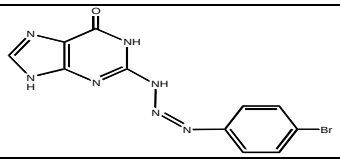
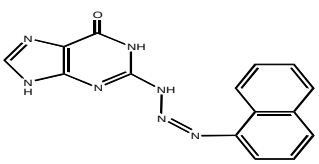
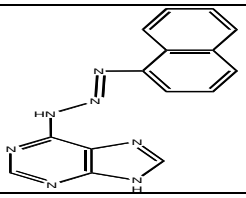
Table (1) shows the names and structures for the prepared compounds

Cpd. No.	Structure	Name
1		1-(4-Chloro phenyl)-3-(Guanine)-Triazene
2		1-(4-Bromo phenyl)-3-(Guanine)-Triazene
3		1-(Naphthalen-1-yl)-3-(Guanine)-Triazene
4		1-(Naphthalen-1-yl)-3-(Adenine)-Triazene

P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2009,(3), (1) :59-65

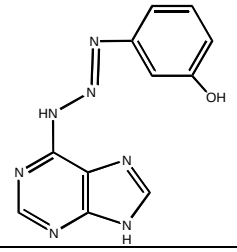
5		1-(3-Hydroxy phenyl)-3-(Adenine)-Triazene

Table (2) U.V absorption bands of triazine Compounds

CPD	λ Max(nm)
1	215 , 245, 425
2	205 , 245, 415
3	220 , 265 , 475
4	215 , 260 , 410
5	215 , 260 , 440

Table (3) I.R absorption bands of triazine Compounds

CPD No.	V(O-H) +V(N-H)	V(C=0)	V(C=N)	V(C=C) +V(N=N)	V(C-N)	Ar-Br
1	(3600-3000) (Sb)	1730(s) 1680(s)	1625(s)	1510(s) 1450(s) 1430(w) 1400(w)	1120(s) 1280(s)	Ar-Cl 590(w) 700(s)
2	(3600-3000) (Sb)	1720(s)	1625(m)	1510(s) 1460(m) 1430(w) 1410(w)	1040(m)	Ar-Br 710(w) 730(m)
3	(3600-3000) (Sb)	1700(s)	1645(sb)	1590(w) 1460(w) 1440(w) 1410(w)	1080(m) 1120(m)	
4	(3500-3000) (Sb)		1645(s)	1580(s) 1540(w) 1470(m) 1440(w)	1080(sb) 1220(sb)	
5	(3500-2800) (sb)		1625(s)	1590(m) 1560(s) 1470(w) 1420(s)	1080(s) 1220(sb)	

s=strong , m=medium , w=weak , b=broad

Table(4) C.H.N Elemental analysis and melting points for the prepared compound

CPD No.	mol Formula	M.Wt g/mol	mp°c	Analysis % calc.(found)		
				C	H	N
1	$C_{11}H_8N_7ClO$	٢٨٩.٥٥	340	45.62 (46.76)	2.76 (3.92)	33.84 (32.10)
2	$C_{11}H_4N_7Br O$	334	325	39.55 (40.42)	2.39 (3.55)	29.34 (30.45)
3	$C_{15}H_{11}N_7O$	٣٠٥.١٤	335	59.03 (60.12)	3.6 (4.22)	32.11 (31.14)
4	$C_{15}H_{11}N_7$	289.15	318	62.3 (63.64)	3.45 (4.86)	33.89 (34.25)
5	$C_{11}H_9N_7$	255.10	311	51.78 (50.14)	3.52 (4.44)	38.41 (37.05)

Table (5) Diameter of inhibition zones for triazine compounds against some of bacteria genera

Bacteria		Diameter of inhibition Zone (mm) ± Standard Errors					
	Conc.						
	Mg/ml	1	2	3	4	5	DMSO
S. aureus	100	11 ± 0.0	12 ± 0.2	10 ± 0.5	15 ± 0.0	11 ± 0.0	-----
	50	9 ± 0.1	10 ± 1.0	8 ± 0.0	12 ± 0.2	9 ± 0.0	-----
	25	8.5 ± 0.1	8 ± 0.2	7 ± 0.5	10 ± 0.0	8 ± 0.0	-----
	10	7 ± 0.0	7 ± 0.2	7 ± 0.0	7.5 ± 0.5	7 ± 0.5	-----
	1	6.5 ± 0.0	6.5 ± 0.0	-----	6.5 ± 0.0	-----	-----
	0.1	-----	-----	-----	-----	-----	-----
Str. Viridans	100	13 ± 0.0	14 ± 0.1	12 ± 0.5	13 ± 0.0	10.5 ± 0.0	-----
	50	12 ± 0.2	13 ± 0.5	10 ± 0.5	10 ± 0.0	10 ± 0.2	-----
	25	10 ± 0.2	11 ± 0.2	8.5 ± 0.0	9 ± 0.0	9 ± 0.2	-----

P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
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Sh.dysenteriae					*E. coli*					*Ps. aeruginosa*				
0.1	1	10	25	50	100	0.1	1	10	25	50	100	0.1	1	10
----	---	------	7 ± 0.0	8 ± 0.5	9 ± 0.5	---	-----	6.5 ± 0.0	7 ± 0.2	٧.٥ ± 0.0	10 ± 0.5	-----	-----	8 ± 0.0
----	---	6.5 ± 0.0	7.5 ± 0.5	9 ± 0.0	10 ± 0.5	---	-----	6.5 ± 0.0	9 ± 0.2	10 ± 0.0	11 ± 0.5	-----	-----	8 ± 0.0
---	---	-----	٧.٥ ± 0.0	9 ± 0.0	٩.٥ ± 0.5	---	-----	6.5 ± 0.5	8 ± 0.5	8.5 ± 0.5	10.5 ± 0.5	------	-----	7 ± 0.0
---	---	6.5 ± 0.0	٨ ± 0.0	١٠ ± 0.5	١٢ ± 0.0	---	6.5 ± 0.2	7.5 ± 0.5	٩ ± 0.0	١١.٥ ± 0.0	13 ± 0.5	-----	-----	8 ± 0.0
--	---	-----	6.5 ± 0.5	9 ± 0.2	10 ± 0.0	---	------	6.5 ± 0.1	7 ± 0.0	10 ± 0.2	11 ± 0.0	----	6.5	-----
----	---	-----	-----	-----	-----	---	-----	-----	-----	-----	-----	-----	-----	-----

(-----) = No activity

Table (6) MIC values for triazine compounds against some of bacteria genera

CBD NO.	MIC values (mg/ml)				
	S. aureus	E. coli	p. aeruginosa	S. styphi	K. pneumoniae
1	50	٢٥	١٥	١٠	١٥
2	٥٠	٢٠	٢٠	15	15
3	١٠	١٥	١٠	١٠	١
4	٢٥	١٠	٢٥	١٠	10
5	20	١٥	20	١٠	١

P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
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تحضير عدد من مركبات الترايازين المشتقة من البيورينات ودراسة فعاليتها البايولوجية على البكتريا المرضية

يوسف هندي خلف حميد حسين علي احمد مشعل محمد

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الخلاصة:

تضمن هذا البحث تحضير عدد من مركبات الترايازين بطريقة ازدواج أملاح الديازونيوم مع قواعد البيورينات .وتم تشخيص المركبات المحضرة بدراسة أطياف الاشعه فوق البنفسجية (U.V) وأطياف الاشعه تحت الحمراء (I.R) و التحليل الدقيق لعناصر الكربون،الهيدروجين والنتروجين(C.H.N) .وتضمن البحث ايضا دراسة الفعالية البايولوجيه لهذه المركبات الجديدة على خمسة أجناس من البكتريا المرضية: *S. aureus , Str. viridans , Ps. aeruginosa , E. coli and Sh. dysenteriae*

وباستخدام طريقة نشر الأقراص وكذلك تم حساب أدنى تركيز مثبط للنمو (MIC)، ووجد ان لهذه المركبات تأثير متوسط في تثبيط هذه الأجناس من البكتيريا.