ISSN: 1991-8941

SYNTHESIS A NUMBER OF AZO COMPOUNDS DERIVED FROM GUANINE AND STUDYING THEIR BIOLOGICAL ACTIVITY ON PATHOGENIC BACTERIA

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***Received*:20/1/2008** ***Accepted*: 20/11/2008**

ABSTRACT: A series of compounds were prepared by coupling dizonium salts with guanine. 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 bacteri

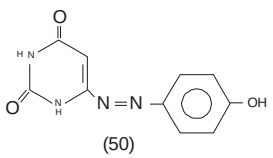
Key words : Synthesis , Azo Compounds , Guanine and , Biological Activity , Bacteria

Introduction

Azo ligands of heterocyclic compounds have received a special interest in biological fields, due to the use some of these compounds as biological stains [1]. These compounds were under intese investigation and their activity as neoplastic and antibacterial agents worth extra attention[2,3].

Pyridium (2,6-Diamino-3- phenyl azo) Pyridine Monohydrochloride is used as an anticeptic in urinary tract infection [4]

Other examples of azo compounds in therapeutic displines are the application of 6-(p-Hydroxy phenyl azo) uracil (6) and 6–(p-Hydroxy phenyl azo) cytosine (7) as antibacterial drugs.



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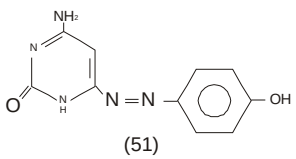

(51)

Where these compounds inhibit the DNA synthesis in gram positive bacteria after reduction of azo compounds to the hydrazo analogues [5]in the bacterial cell by the enzyme azo reductase making the compound more potent[6].

4-Dimethyl amino –4-nitro azo benzene has been prepared in 1994 and the in vitro effect of this compound on four different Salmonella strains. It was found that the compound causes genotoxic effect in all Salmonella strains. A study on the effect of this compound on the genetic material in rat liver show there is no harm on the DNA synthesis[7].

In 1994 A. Kalski [8]et-al studied the toxic effect of azo compounds derived from thiadiazole with ß-napthol and p-chlorophenol. The study included in vivo effect of these derivatives on bone marrow of rats and in vitro effect of these products on human blood cells. The study indicated no toxic impact of these derivatives, hence the possibility of safe application of these compounds in medical therapy.

Substituted purine compounds have wide medical value as medications [9]. In 1945, 8-azaguanine was synthesized as the first derivative for guanine with antitoumer activity [10]. . Also, (6-TG) was synthesized as anticancer, especially leukemia ([11].Acyclovir (ACV) was synthesized as a derivative for guanine, and used to inhibit the growth of cancer cells[12]. It was also used as anti virus for the treatment of Hypris and DNA viruses [13]. Hocek succeeded in synthesizing a series of modified purine derivatives carrying a substitute of carbon atom No. (12,8) through coupling , and studying their activity as anticellulars ([14]. Also, they were used as anti virus, antibacterial and anticancer substances as well as anti hypertesive[15].

Experimental.

2-Amino-8-(4-chloro-phenylazo)-1,9-dihydro-purin-6-one

4 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 was left for 30 minutes to be stable after completing the addition 4.5 m.mole of guanine (dissolved in 40 ml HCl %50) was added, a brown 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 [16] 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 thoroughly and incubated at 37C0 for 18 hours and the minimum inhibitory concentration (MIC) was detected for each bacterial genus. Table (6).

Results and Discussions.

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 azo compounds in ethanol revealed clearly the presence of (n-Л*) transfer group

at (335 - 350 nm) [17,18] due to (ph - N =N-) besides an absorption band at (240 – 265 nm) due to the absorption of purine [18] .

Infra red spectra for the prepared azo compounds revealed the presence of wide strong bands between (3600-2900) cm-1 due to the stretching vibration V(O-H) and V(N-H) in accord with the results in reference[19]. Sirroki [20]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 (1570-1410) cm-1 with in the range of vibrational frequencies for V(C=C) and V(N=N). The position of azo group V(N=N) absorption band was mentioned in the literature[21], and depends upon the groups attached to it. The assignment of this band seems easier by Raman spectroscopy than by IR [22]. In aliphatic azo compounds, the band is within the range (1575-1550) cm-1. , while in aromatic azo compounds, it appears in the range (1510-1400) cm-1 [23]

The infrared spectra show a distinctive band (1645-1610) cm-1 due to (C=N) stretch besides the carbonyl band at(1870-1680) 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(730-570) cm-1 due to the stretching vibration V(Ar-x) [19] .

Biological Investigation of azo 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 [9] 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 (2) show the highest activity at concentration 100mg/ml against S. aureus where the inhibitory zone is (14 mm) and compound (5) show the minimum inhibitory zone of (9 mm) at the same concentration.

Compound (4) looks the most effective against Str. viridans with in inhibitory zone of (15 mm) at the highest concentration 100 mg/ml. Compound (1) show the lowest activity with an inhibitory zone of (9 mm) at the same concentration. Compound (5) has the minimum potency toward Ps. aeruginosa where the inhibition zone diameter was (7 mm) at concentration 100mg/ml and compound (2) seems to be the most active with an inhibition zone of (9 mm) at the same concentration. Compound (2) looks the most effective against E. coli with in inhibitory zone of (13 mm) at the highest concentration 100 mg/ml. Compound (5) show the lowest activity with an inhibitory zone of (9 mm) at the same concentration. Compound (4) show the highest activity at concentration 100 mg/ml against Sh. dysenteriae where the inhibitory zone is (11 mm) and compound (1) show the lowest activity with an inhibitory zone of (9 mm) at the same concentration. 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.

Mechanism of azo compounds action as antibacterial agents.

Biological activity of azo compounds is attributed to the ability of these compounds to inhibit DNA synthesis. To full fill this task the azo compound must be reduced to the hydrazo analogue with in the bacterial cell then the reduced azo compound will act to inhibit the DNA polymerase III, the enzyme which is responsible for DNA

multiplication. These compounds create an abnormal base pairing in the DNA .A complex will be created between the compound and the primer strand, which increases the stability of the complex.

Further more the compound interacts with the uncoupled pyrimidine in the template strand to make unusual base pairing (figure 1)

The aromatic part of the compound is thought to precipitate with the aromatic amino acids the DNA polymerase III through hydrophobic interaction[5].

Other explainations for the mechanism of antibacterial activity of these compounds look feasible. 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. The exact mechanism of action is still a waiting for further investigation. From pure chemical thoughts these compounds seem to have marvelous antibacterial properties to be used for external use.

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

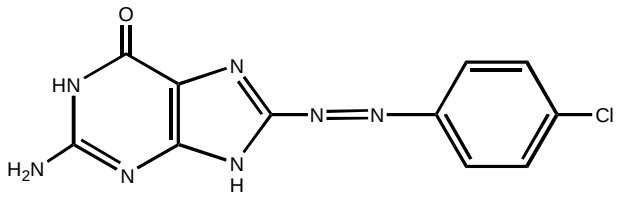
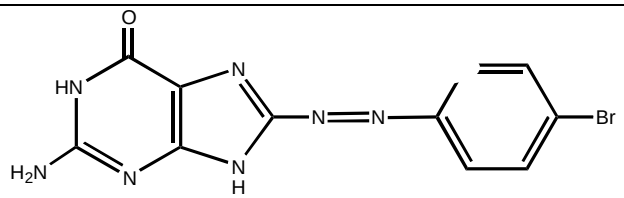
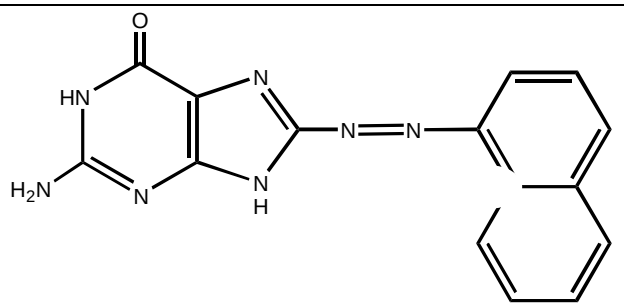
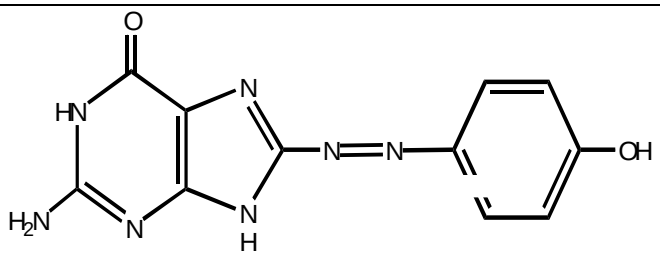
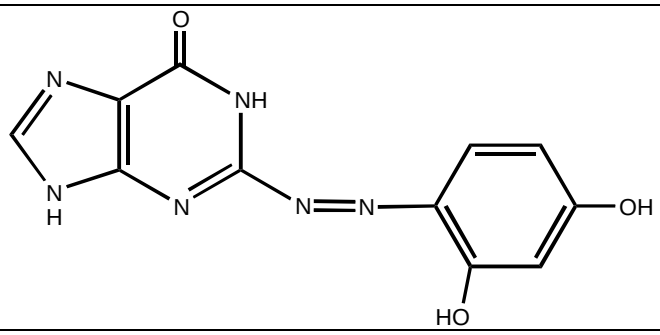
Cpd. No.	Structure	Name
1		2-Amino-8-(4-chloro-phenylazo)-1,9-dihydro-purin-6-one
2		2-Amino-8-(4-bromo-phenylazo)-1,9-dihydro-purin-6-one
3		2-Amino-8-(naphthalen-1-ylazo)-1,9-dihydro-purin-6-one
4		2-Amino-8-(4-hydroxy-phenylazo)-1,9-dihydro-purin-6-one
5		2-(2,4-Dihydroxy-phenylazo)-1,9-dihydro-purin-6-one

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

CPD	λ Max(nm)
1	210 , 240 , 335
2	215 , 255 , 340
3	220 , 265 , 338
4	210 , 250 , 350
5	205 , 255 , 345

Table (3) I.R absorption bands of azo 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-X
1	(3400-2900) (Sb)	1680(s)	1610(m)	1530(s) 1430(w) 1410(w)	1030(s) 1180(s)	Ar-Cl 570(m) 730(s)
2	(3400-3000) (Sb)	1770(s) 1870(s)	1625(s)	1550(w) 1520(s) 1450(s) 1410(s)	1020(s) 1120(s)	Ar-Br 590(s) 630(s)
3	(3600-3000) (Sb)	1720(sb)	1610(m)	1500(s) 1460(m) 1420(s)	1060(m)	
4	(3500-3080) (Sb)	1730(s) 1680(s)	1625(s)	1570(w) 1540(w) 1420(s)	1040(m) 1100(s)	
5	(3600-3300) (sb)	1690(sb)	1645(sb)	1570(w) 1490(w) 1420(s)	1000(s) 1080(s)	

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

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

CPD No.	mol Formula	M.Wt g/mol	mp°c	Analysis % calc.(found)		
				C	H	N
1	$C_{11}H_8N_7ClO$	289.55	322	45.62 (46.62)	2.76 (3.90)	33.84 (34.25)
2	$C_{11}H_8N_7BrO$	334	310	39.55 (38.05)	2.39 (3.35)	29.34 (28.68)
3	$C_{15}H_{11}N_7O$	305.14	351	59.03 (60.66)	3.6 (4.03)	32.11 (33.14)
4	$C_{11}H_9N_7O_2$	271.09	315	48.73 (49.48)	3.31 (4.93)	36.15 (37.52)
5	$C_{11}H_8N_6O_2$	256.09	327	51.58 (52.31)	3.12 (2.29)	32.8 (33.95)

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

CBD NO.	MIC (mg/ml)				
	S. aureus	*E. coli*	*p. aeruginosa*	*S. styphi*	*K. pneumoniae*
1	50	25	25	20	20
2	20	10	20	10	10
3	50	50	20	20	15
4	25	20	20	25	20
5	25	20	15	15	15

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

Bacteria	Conc.	Diameter of inhibition Zone (mm) ± Standard Errors					
	Mg/ml	1	2	3	4	5	DMSO
S. aureus	100	10 ± 0.0	14 ± 0.2	10 ± 0.5	10.5 ± 0.0	9 ± 0.0	-----
	50	9 ± 0.1	12 ± 1.0	8 ± 0.0	8.5 ± 0.2	8.5 ± 0.0	-----
	25	10 ± 0.1	11 ± 0.2	7.5 ± 0.5	7.5 ± 0.0	8 ± 0.0	-----
	10	8.5 ± 0.0	9 ± 0.2	7 ± 0.0	7 ± 0.5	7 ± 0.5	-----
	1	6.5 ± 0.0	6.5 ± 0.0	------	6.5 ± 0.0	6.5 ± 0.0	-----
	0.1	------	------	------	------	------	-----
Str. Viridans	100	9 ± 0.0	12 ± 0.1	10 ± 0.5	15 ± 0.0	11 ± 0.0	-----
	50	8 ± 0.2	10.5 ± 0.5	9 ± 0.5	14 ± 0.0	10 ± 0.2	-----
	25	7.5 ± 0.2	9.5 ± 0.2	8 ± 0.0	10.5 ± 0.0	8.5 ± 0.2	-----
	10	6.5 ± 0.0	8 ± 0.0	7.5 ± 0.0	8.5 ± 0.0	8 ± 0.0	-----
	1	------	6.5 ± 0.0	------	7 ± 0.5	6.5 ± 0.0	-----
	0.1	------	-------	---------	-----	-------	-----
Ps. aeruginosa	100	8 ± 0.0	9 ± 0.2	8 ± 0.0	8.5 ± 0.5	7.5 ± 0.0	-----
	50	7 ± 0.0	8 ± 0.0	7.5 ± 0.2	8 ± 0.0	7 ± 0.0	-----
	25	6.5 ± 0.0	7.5 ± 0.0	7 ± 0.0	7.5 ± 0.0	6.5 ± 0.0	-----
	10	6.5 ± 0.0	6.5 ± 0.0	---------	6.5 ± 0.0	------	-----
	1	------	------	--------	------	------	-----
	0.1	------	------	--------	------	-----	-----
E. coli	100	11 ± 0.5	13 ± 0.5	10 ± 0.5	9.5 ± 0.5	9 ± 0.0	-----
	50	9 ± 0.0	11.5 ± 0.0	8.5 ± 0.5	8.5 ± 0.0	8 ± 0.2	-----
	25	7.5 ± 0.2	10 ± 0.2	8 ± 0.5	7 ± 0.0	7.5 ± 0.0	-----
	10	7 ± 0.0	8 ± 0.0	7.5 ± 0.5	6.5 ± 0.5	6.5 ± 0.1	-----
	1	------	6.5 ± 0.0	6.5 ± 0.0	------	-------	-----
	0.1	-------	-------	------	------	------	-----
Sh.dysenteriae	100	9 ± 0.5	9.5 ± 0.5	10.5 ± 0.5	11 ± 0.0	9.5 ± 0.0	-----
	50	7.5 ± 0.5	8.5 ± 0.0	9.5 ± 0.0	9 ± 0.5	8.5 ± 0.2	-----
	25	7 ± 0.0	7.5 ± 0.5	8 ± 0.0	8 ± 0.0	7 ± 0.5	-----
	10	6.5 ± 0.0	6.5 ± 0.0	6.5 ± 0.0	6.5 ± 0.0	6.5 ± 0.0	-----
	1	-----	----------	----------	----------	------	-----
	0.1	-------	-------	----------	----------	-------	-----

(-----) = No activity

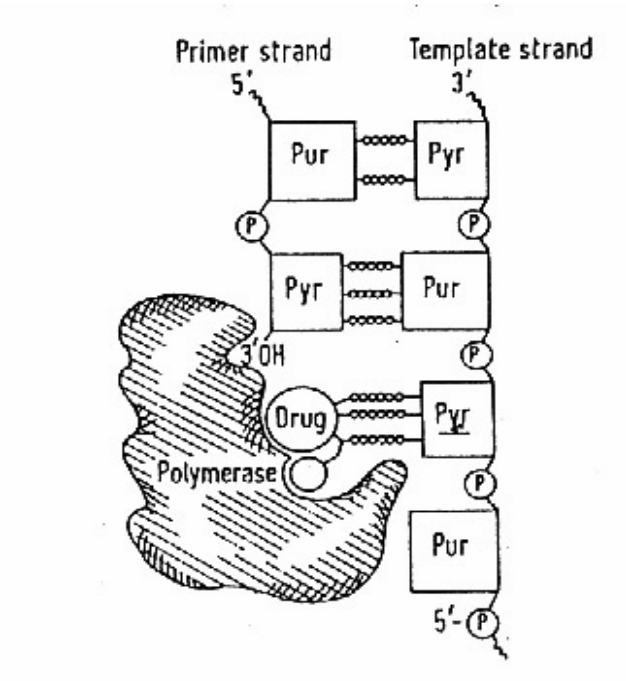


Figure (1): A proposed mechanism of action for the hydroxy phenyl azo pyrimidines, showing the drug binding to the DNA template and preventing the Polymerase Action

تحضير عدد من مركبات الآزو المشتقة من الكوانين ودراسة فعاليتها البايولوجية على البكتريا المرضية

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الخلاصة:

يشتمل هذا البحث على تحضير سلسله من المركبات بطريقة ازدواج أمـــلاح الـــديازونيوم مـــع الكوانين .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.