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Synthesis and Antimicrobial Activity of 2-Substituted-7-Chloro-4, 5-Dioxopyrano [3,4-e] [1,3]-Oxazine-4, 5-Dione

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ABSTRACT. 2-N-Alanylacetate (4a), 2-N-Tyrosyl ethyl acetate (4b), 2-N-Ethylcarbazate (3), 2-N-Piprazino (4c) and 2-N-(2-aminothiazole) (4d)-7-chloro-4, 5-dioxopyrano [3,4-e] [1-3]-oxazine-4, 5-dione were synthesized and tested for their antimicrobial activity against three bacteria *E. coli*, *P. acruginosa* and *S. aureus*, and two types of fungi. *C. albican*, and *A. spinosa*. Compounds number 3,4a, and 4b gave significant results toward tested microorganism.

The continuous interest in the chemistry of 1,3-oxazines manfested by numerous papers and patents may be attributed to further evidence of the biological activity according to Ozaki (1972). Oxazinomycin and Indolmycin showed antibictic activity according to Haneishi *et al.* (1971) and Schach *et al.* (1961) respectively. Many patents described the preparation of 1,3-oxazine derivatives as antibacterials and antifungals (Ozaki 1972, and Meyer and Chia-Cheng 1974). 1,3-oxazine was isolated as a metabolic of *penicillium gilmanii* and that of tetrahydro 1,3-oxazine-2-one also showed anti leukemic macrolide antibiotic (Kupchan *et al.* 1972, and Kupchan *et al.* 1972a). Furthermore sulphur containing nitrogen compounds found to have a biological activities, Thiadiazole derivatives (Abdul-Hafidh *et al.* 1987) and 1,3,4-thiadiazole oxides have been found to have antimicrobial activities (Ghattas *et al.* 1982).

In this work two new compounds were synthesised. In addition to three other compounds were previously synthesised. All compounds have been tested for their

antimicrobial activities against three gram positive and gram negative bacteria and two fungi.

Materials and Methods

All melting points were uncorrected. I.R. spectra were taken in liquid cell and KBr disc. The spectra were performed using pye-unicam SP 2000 spectrophotometer. The ^{1}H.N.M.R. spectra were measured using Bruker WH90DS (The elemental analysis were perform in Kazakh state University, USSR).

Methyl thiocyanate and malonyl chloride were prepared according to previously reported method (Walden 1970, and Raha 1963). 2-Amino thiazole was prepared from thiouria and cholroacetaldehyde dimethyl acetol according to the published procedure (Robert *et al.* 1979).

2-Methyl, 2-Benzyl thio-7-chloropyrano [3,4-e] [1,3]-oxazine-4, 5-dione were prepared from the reaction of malonyl chloride with methyl and benzyl thiocyanate respectively (see Scheme 1), following the same published procedure (Al-Rawi and Elvidge 1973) with the yield of about 85% and m.p. of 160-162 °C, 152-145 °C respectively. The purity and the structure of the above prepared compounds were checked by I.R., ^{1}H.N.M.R. and were identical with that previously reported. 2-N-Alanyl, 2-N-Tyrosyl ethylester and 2-N-piprazino, 7-chloro-4, 5-dioxopyrano [3,4-e][1,3] -oxazine-4,5-dione (4a-4e) were prepared following the reported procedure (Al-Rawi and Al-Ajiely 1989 and Al-Rawi *et al.* 1989). In 100 ml round bottomed flask fitted with reflux condenser and dropping funnel, was placed (0.01 mol.) of compound (1a) in 40 ml of dichloromethane. The reaction was carried by dropwise addition of the amino acid ester (0.01 mol.) in 20 ml dichloromethane *via* the dropping funnel with continuous stirring. After the addition has been completed, the reaction mixture was refluxed for 4 hours, cooled and the solvent was then evaporated under reduced pressure. The solid crude product was then recrystallized. The physical constants together with their I.R. and ^{1}H.N.M.R. were identical with that reported previously. The new 2-N-Ethyl carbazato-7-chloro-4,5-dioxopyrano [3,4-e][1,3]-oxazine-4, 5-dione (3) was prepared following the above procedure using (0.01 mol.) of compound (1b) and (0.01 mol.) of ethyl carbazate, the reflux time was 6 hours and the solvent of crystallization was toluene, while crystals was obtained m.p. 171-173°C. The infrared spectrum using KBr disc characterized by the following absorption V max (cm-1) 1590 (S), 1740 (sh), 1750 (sh), 1510 (sh) which were assigned to C=N, 4-C=O, 5-C=O, C=C aromatic respectively and 1680 assigned to the carbonyl carbazate moiety together with N-H stretch within the range 3500-3600 cm-1. The ^{1}H.N.M.R. spectrum showed the following signals S (d6. DMSO) 6-3 (s) for the

pyrano ring proton, 4.2 (9) for $-CH_2-$, 1.7 (t) for $-CH_3$ of the carbazato moiety. The elemental analysis was as follows:

	found	**calculated**
C %	39.818	40.621
H %	2.673	2.582
N %	13.930	13.900

Preparation of new 2-N-(2-aminothiazole)-7-chloro-4, 5-dioxopyrano [3,4-e] [1,3]-oxazine-4, 5-dione (4d). This compound was prepared following the above procedure using CH_2CL_2 as solvent to the reaction of 2-amino thiazole (0.01 mol.) with (1a), (0.01 mol.) in unhydrous condition gave crude product. Recrystallization from acetic acid gave yellowish crystals m.p. 217-220 °C with the yield of 70%. The I.R. spectrum using KBr disc showed the following absorption γ max (cm−1), 1760, 1750, 1600, 1590 and abroad band at 1500 related to 5-C=O, 4-C=O, C=C aromatic, C=N, C=C aromatic respectively, The ^{1}H.N.M.R. spectrum showed δ (d6-DMSO) 7.3(d), 7.0(d) related to the thiazole protons and 6.1(s) related to the pyrano ring proton. The elemental analysis was as follows:

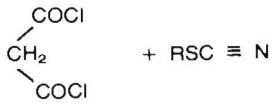
	found	**calculated**
C %	40.349	40.575
H %	1.354	1.026
N %	14.116	14.100

Microorganism

The antimicrobial activity of the studied compounds have been determined by agar diffusion method using three kinds of bacterria, gram negative (*Escherichia coli* ATCC 25922), and (*Pseudomonas aerogenosa* ATCC 27853), and gram positive bacteria (*Staphila aureus* ATCC 25923) supplied kindly from stock culture of Rashid Arm Hospital. The other microorganisms were two kinds of fungi, *Candida albicans* and *Absidia spinosa* supplied kindly from stock culture of the Department of Biology, College of Science, University of Baghdad.

Culture condition

An overnight culture of bacteria grown at 37°C in nutrient broth (oxoid) were used to inoculate triplicate plates of nutrient agar. One hour was allowed between inoculation and the biological activity of the chemicals. Fungi overnight cultures in Czapek Dox liquid media grown at 25°C were used to inoculate malt agar (oxoid) triplicate plates.



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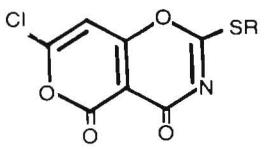
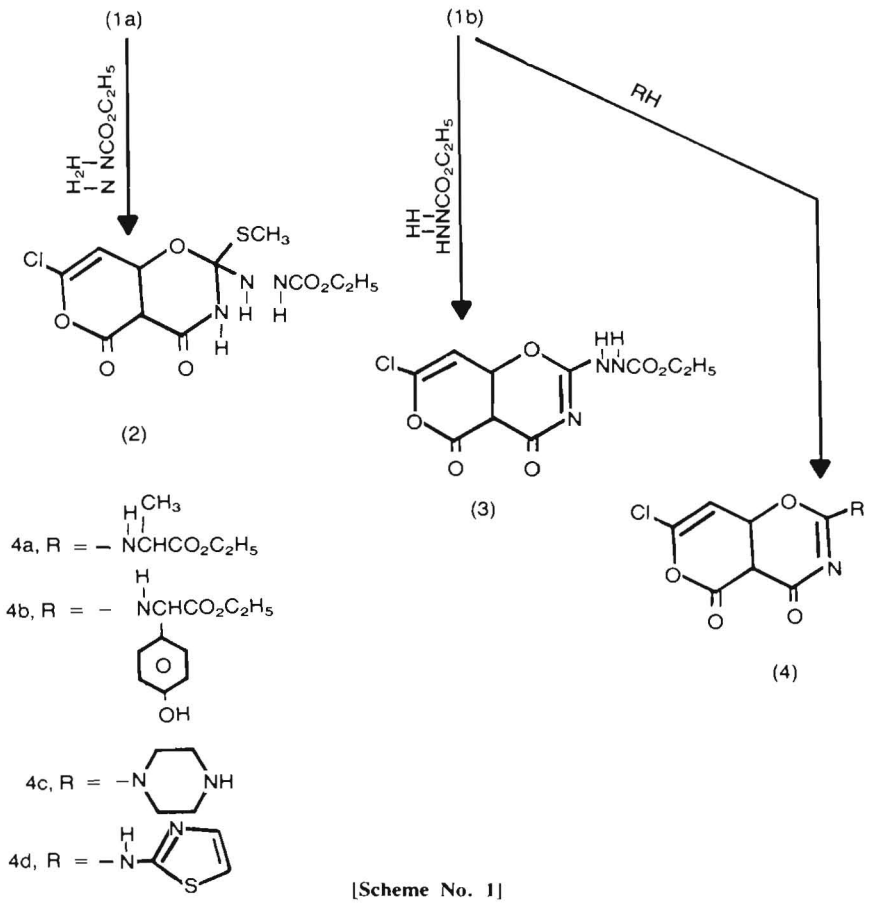


(1)

1a, R = $-CH_3$

1b, R = $-CH_2ph$



[Scheme No. 1]

Biological activity

Compounds number 3,4a, and 4b were dissolved in chloroform to make 30000, 15000, 5000, 1000, and 500 μg/ml concentration. The same concentrations were prepared for compounds number 4c, and 4d using DMSO (Dimethyl sulfoxide) as a solvent.

Watman filter paper # 1 were cut into discs of 0.6 mm diameter. Each disc was saturated with 0.01 ml of each of the above concentration. Discs were placed in oven at 60°C untill dried, and placed in nutrient agar plate which were inoculated with the above organisms.

A control discs saturated with each of the solvent were also prepared. Antibiotic discs of the same size were also tested for their activities against the same bacteria. The antibiotics used are: Amoxillin 250 μg, Chloramphenicol 30 μg, and Penicillin G 10 μg. For fungi Mycostatin 100000 unit/ml and Phenol 2% were used as known antifungal. The plates of bacteria were inoculated for 24 hr at 37°C, and that of fungi were inoculated for 24 hr at 25°C. Results were recorded as diameter of growth inhibition zones.

Results and Discussion

From the infrared spectrum of compound 4d it appeared the oxazino carbonyl groups together with the thiazole ring absorption bands. The ^{1}H.N.M.R spectrum also support the structure of compound 4d together with the elemental analysis. Compound 3 showed different reaction pathway from that of compound 2 (Rashan *et al.* 1988). This difference was attributed to the sterric factor in which the 2-methyl thio was not so bulky substituent as compared with the benzyl. Therefore compound 3 resulted by the substitution of the carbazato moiety while in compound 2 there was an addition to C=N band.

Table 1 recorded the observed inhibition zones of five compounds against three bacteria strains and two fungi. Table 2 revealed the effects of commercial antibiotics against the same microorganism. No effects of solvents used on the tested organism has been observed.

Three of the tested compounds, 3, 4a, and 4b exhibited significant biological activities (Table 1). The inhibition zones declined as the compounds concentration decreased. Very low or no antimicrobial activities observed at the lowest concentration used (500 μg/ml). Amongst all compounds tested, compound number 3 has the highest inhbition toward gram + bacteria *S. aureus* at a concentration 30000 and 15000 μg/ml (Table 1). Compounds 3 and 4a possessed

Table 1. Diameter (mm) of growth inhibition zones* of tested microorganisms

Compd. No.	Conc. µg/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*A. spinosa*
3	30,000	3.4	5.1	11.6	3.5	16.2
	15,000	2.1	3.5	6.5	2.1	11.1
	5,000	1.6	1.8	3.7	1.8	0
	1,000	0	0.9	2.6	0	0
	500	0	0.6	0	0	0
4a	30,000	5.1	4.0	7.2	12.3	32.0
	15,000	3.0	2.2	4.0	8.5	21.1
	5,000	1.7	1.8	3.1	1.5	11.3
	1,000	1.0	0.7	1.1	0	4.8
	500	0	0	0.8	0	0
4b	30,000	2.8	4.5	5.5	3.5	6.0
	15,000	1.8	2.5	2.5	2.1	3.0
	5,000	1.1	0	0	1.8	0
	1,000	0.8	0	0	1.1	0
	500	0	0	0	0	0
4c	30,000	0	3.7	0	2.8	0
	15,000	0	2.1	0	2.0	0
	5,000	0	0	0	0.8	0
	1,000	0	0	0	0.5	0
	500	0	0	0	0	0
4d	30,000	0	3.7	0	0	0
	15,000	0	2.2	0	0	0
	5,000	0	1.5	0	0	0
	1,000	0	0.8	0	0	0
	500	0	0	0	0	0

* Average of three measurements for each concentration.

considerable inhibition against gram - bacteria, *E. coli* and *P. aeruginosa* more than other tested compounds, and their activites were higher than that of Penicillin, which is used as standard antibiotic (Table 1 and 2).

Compound 4a exhibited a higher antifunigal activity comparing with the other tested compounds, moreover, it shows an inhibition zone greater than that of mycostatin, the commercial antifungus product (Table 1 and 2). Surprisingly compounds 4c and 4d possessed low activities toward all microorganism tested, although thiadiazole compound showed significant antimicrobial activity (Abdul-

Hafidh *et al.* 1987).

From the above results we can derive the conclusion that compounds number 3, 4a, and 4b have considerable inhibition toward tested microorganism similar or greater than that of commercial antibiotics used, but it need further work for commercial recommendation.

Table 2. Effects of antibiotic on tested organisms

Antibiotic	*E. coli*	*P. aeruginosa*	*S. aureus*	*C. albican*	*A. spinosa*
Amoxicillin	13.0*	3.2**	30.0		
Chloramphenicon	26.0	2.0	17.8		
Penicillin	2.2	3.0	34.4		
Mycostatin				24.6	2.6
Phenol				4.3	1.2

* Diameter of inhbition zone in (mm).

** Average of three measurements for each compound.

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تحضير ودراسة فعالية المركبات
" 2 ـ سبستيتوتد ـ 7 ـ كلورو ـ 5,4 ـ ثنائي أكسو بايرانو [3,4-e] [1,3] ـ أكسازين ـ 5,4 ـ ثنائي الكيتون " ضـد بعـض الأحيـاء الدقيقـة

علي هاشم الموسوي[1] و جاسم علي الراوي[2] و محمد سلمان العجيلي[3]

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[2]قسم الكيمياء ـ كلية العلوم و [3]قسم الكيمياء ـ كلية التربية ـ جامعة الموصل ـ الموصل ـ العراق

حُضّرت المركبات 2-N- خلات الأنيل، (4a)، 2-N ـ خلات الايثيل تيورسيل (4b)، 2-N ـ ايثيل كارهزيت (3)، 2-N ـ باي برازينو (4c) و 2-N ـ " 2 ـ أمينو ثيازول،(4d)، 7 ـ كلورو -5,4- ثنائي أكسو بيزانو [3,4-e] [1,3] ـ أكسازين ـ 5,4 ثنائي الكيتون "، مخبرياً وذلك باتباع طرق تحضير معروفة مسبقاً لكل مركب من هذه المركبات، وتم التحقق من صحة تحضير هذه المركبات.

الغاية من البحث هي دراسة التأثير المضاد لهذه المركبات الكيميائية على ثلاثة أنواع من البكتيريا: *E.coli* و *P.aeruginos* و *S.aureus* وعلى نوعين من الفطريات *C.albican* و *A.aspinosa* وبنتيجة البحث تبين ما يلي :

ان المركبات التي أرقامها (3) و (4a) و (4b) تبدي نشاطاً حيوياً مؤثراً وينقص التأثير كلما نقص التركيز، وينعدم التأثير ضد البكتيريا عندما يقل التركيز عن (500 µg/ml) ومن بين جميع المركبات فان المركب الذي رقمه (3) له التأثير الاقوي على البكتيريا *S.aureus* وذلك عندما يكون تركيز هذا المركب (15000 - 30000 µg/ml) كما أن للمركبين (3) و (4a) تأثير فعال ضد نوعي البكتيريا *E.coli* و *P.aeruginos* أقوى من

تأثير بقية المركبات على هذين النوعين من البكتيريا. بل وأن تـأثير هـذين المركبـين أقوى من تأثير البنسلين (المأخوذ كعينة قياسية) على نوعي البكتيريا المذكورين.

يبدي المركب (4a) تأثيراً فعالاً ضد الفطريات (مقارنة بتأثير المركبات الأخرى المجـرية) وأمـا المركبـين (4c) و (4d) فتأثـيرهما شبـه معدوم عـلى كل من البكتـيريا والفطريات.

وبالنتيجة يستخلص ان المركبات التي أرقامها (3) و (4a) و (4b) أعطت نتائج مشجعة كمؤثر ضد البكتيريا والفطريات المذكـورة تأثيـراً موازيـاً أو أكبر من تـأثير المضـاد الحيوي التجـاري المستعمل في الاسـواق. ولكن لابد من متـابعة العمـل لإمكانية انتاج مثل هذه المركبات تجارياً.