UDC 615.272.349:547.491.2

K.M. Al-rahawi[1], M. Al-haj[2], V.N. Petyunina[3], G. Al-daidamoni[1], W. Al-madhagi[1], R. Al-robaidi[1]

Synthesis, antimalarial and antimicrobial activities of arensulphohydrazidooxalyl- and arensulphonyloxamoylamino acids salts with 2-ethoxy-6,9-diaminoacridine and chloroquine

[1]Sana'a University (Yemen),
[2]Aden University, (Yemen),
[3]Kharkov Medical University (Ukraine)

Ключові слова: *синтез, аренсульфогідразидооксаліламінокислоти, аренсульфонілоксамоїламінокислоти, хлорохін, етакридин, антималярійна й антимікробна активності.*

Ключевые слова: *синтез, аренсульфогидразидооксалиламинокислоты, аренсульфонилоксамоиламинокислоты, хлорохин, этакридин, антималярийная и антимикробная активности.*

Key words: *synthesis, arensulphohydrazidooxalylamino acids, arensulphonyloxamoylamino acids, chloroquine, rivanol, antimalarial and antimicrobial activities.*

Здійснено синтез і вивчено антималярійну й антимікробну активності аренсульфогідразидооксаліл- й аренсульфонілоксамоїламінокислот і їх солей з хлорохіном й етакридином. Установлено, що активність солей, як правило, перевищує активність вихідних лікарських засобів.

Осуществлен синтез и изучены антималярийная и антимикробная активности аренсульфогидразидооксалил- и аренсульфонилоксамоиламинокислот и их солей с основаниями хлорохина и этакридина. Установлено, что активность солей, как правило, превосходит активность исходных лекарственных средств.

Arensulphohydrazidooxalyl- and arensulphonyloxamoylamino acids and their salts with chloroquine and aethacridine were synthesized . Representative compounds were screened for antimalarial, antimicrobial activity. It was established that the obtained salts are more active than the standards drugs: chloroquine and aethacridine.

Interest in search for new biologically active salts is inspired by the fact that among them many biologically active agents were discovered, including antimicrobial [1,2,3], antioxidant [4,5], antiviral [6]. In continuation of the previous work on the search for antimicrobial activity among arensulphohydrazidooxalyl-and arensulphonyloxamoyl amino acids [2], chloroquine salts of these two chemical groups were synthesized and their antimalarial activity was studied. Moreover the antimicrobial activity of previously obtained compounds [2] (arensulphohydrazidooxalyl-, arensulphonyloxamoyl amino acids, benzensulphohydrazido- aresulphomidoxalic acid and their salts with 2-ethoxy-6,9-diaminoacridine, compounds 1–11 and 12–22. *Table 1, 2*) was studied.

Experimental chemical part

The UV-spectra were carried out on a UV-160 IPC (Shimadzu), the IR-spectra were recorded on IR-FTIR-8300 (Shimadzu) ranged 4000-500 cm^{-1} using samples prepared as KBr-disks. The melting temperatures were determined on melting point apparatus SMP3 (ENGLAND).

7-chloro-4-[{4-(diethylamino)-1-methylbutyl}amino]-quinolinium benzene-sulphomidooxalylglycinate (23)

Ethanolic solution of 0.32 g (0.001 M) of chloroquine was added to an ethanolic solution of 0.30 g (0.001 M) of benzensulphomidooxalylglycin. The reaction mixture was allowed to stand until pH became neutral. The excess of ethanol was evaporated on a water-bath, then the formed residue was collected. Analogous procedure compounds 24-33 were synthesized.

Result and discussion

Salts of arensulphohydrazidooxalyl- and arensulphonyloxamoyl amino acids (23–30 with chloroquine were obtained by mixing of alcoholic solution of equivalent amounts of acids 1-11 and the chloroquine base according to the following scheme:

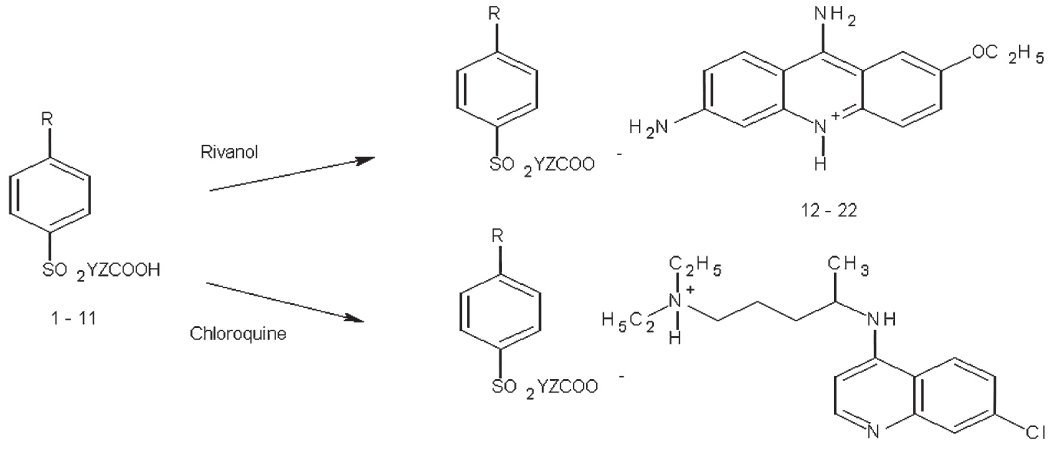

Table 1

The structures of the previously synthesized compounds 1-11

Compd	R	Y	Z
1	H	NH	$CONHCH_2$-
2	H	NH	$CONHCH(CH_3)$
3	H	NH	$CONHCH_2CH_2$-
4	H	NH	$CONHCH_2CH_2CH_2$-
5	NH_2	NH	$CONHCH_2$-
6	NH_2	NH	$CONHCH_2CH_2CH_2$-
7	H	NHNH	$CONHCH_2$-
8	H	NHNH	$CONHCH_2CH_2CH_2$-
9	H	NH	CO
10	NH_2	NH	CO
11	H	NHNH	CO

*The physical constants of the compounds 1-11 were published in [2].

The obtained salts 23–30 (*table 2*), are white, crystalline substances, readily soluble in water with formation of a neutral solution.

UV spectra of salts 23–30 are attributed to the absorption of cation and exactly identical to the chloroquine phosphate spectrum this is the evidence that the salt is formed at aliphatic nitrogen atom.

The IR-spectra of the compounds 23–30 display stretching absorption bands of the all functional groups corresponding to the proposed structures. They contain absorption bands due to stretching vibrations of NH group (3300–3100 cm^{-1}). They also show strong absorption bands arising from asymmetric and symmetric stretching of SO_2 at (1350,1348 $см^{-1}$) and (1173,1150 $см^{-1}$) respectively. On the other hand, the IR spectra of compounds 27,28 show stretching vibration of NH_2-group at (3484,3486 $см^{-1}$) respectively. Because of ionization, giving the COO^- group, resonance is possible between the C-O bonds. In consequence the characteristic carbonyl absorption vanishes and is replaced by two bands between 1610 cm^{-1} and 1550 cm^{-1} [7].

In order to explain the influence of amino acid fragment upon the antimalarial activity, salts without amino acid fragments (31,32,33)were obtained. The synthesis was performed by interaction of the arensulphohydrazidooxalic- and arensulphomidooxalic acid (9,10,11) with chloroquine base, as described in the above scheme. The salts 31–33 (*table 2*) are white hygroscopic substances, soluble in water and insoluble in organic solvents.

Table 2

Physical Constants of the new salts 23–33 and the previously synthesized salts 12–22

Compd	R	Y	Z	Yield, %	M.p., ^0C	Found, %N	Empirical Formula	Calculated % N
12	H	NH	$CONHCH_2$-	-	-	-	-	-
13	H	NH	$CONHCH(CH_3)$	-	-	-	-	-
14	H	NH	$CONHCH_2CH_2$-	-	-	-	-	-
15	H	NH	$CONHCH_2CH_2CH_2$-	-	-	-	-	-
16	NH_2	NH	$CONHCH_2$-	-	-	-	-	-
17	NH_2	NH	$CONHCH_2CH_2CH_2$-	-	-	-	-	-
18	H	NHNH	$CONHCH_2$-	-	-	-	-	-
19	H	NHNH	$CONHCH_2CH_2CH_2$-	-	-	-	-	-
20	H	NH	CO	-	-	-	-	-
21	NH_2	NH	CO	-	-	-	-	-
22	H	NHNH	CO	-	-	-	-	-
23	H	NH	$CONHCH_2$-	79	91-93	13.89	$C_{28}H_{36}N_5O_6SCl$	13.88
24	H	NH	$CONHCH(CH_3)$	68	94-96	13.52	$C_{29}H_{39}N_5O_6SCl$	13.57
25	H	NH	$CONHCH_2CH_2$-	92	89-91	13.52	$C_{29}H_{39}N_5O_6SCl$	13.57
26	H	NH	$CONHCH_2CH_2CH_2$-	72	91-93	13.29	$C_{30}H_{40}N_5O_6SCl$	13.27
27	NH_2	NH	$CONHCH_2$-	69	93-95	13.44	$C_{28}H_{36}N_6O_6SCl$	13.48
28	NH_2	NH	$CONHCH_2CH_2CH_2$-	69	83-85	13.61	$C_{30}H_{40}N_6O_6SCl$	13.66
29	H	NHNH	$CONHCH_2$-	90	62-64	13.42	$C_{28}H_{37}N_6O_6SCl$	13.53
30	H	NHNH	$CONHCH_2CH_2CH_2$-	95	99-100	12.89	$C_{30}H_{41}N_6O_6SCl$	12.95
31	H	NH	CO	70	92-94	10.00	$C_{26}H_{33}N_4O_5SCl$	10.21
32	NH_2	NH	CO	62	91-93	12.15	$C_{26}H_{35}N_5O_5SCl$	12.40
33	H	NHNH	CO	85	101-102	11.95	$C_{26}H_{34}N_5O_5SCl$	12.00

*Physical constants of the compounds 12-22 were published in [2].

UV spectra of salts 31–33 are attributed to the absorption of cation and exactly identical to the chloroquine's spectrum. This is the evidence that the salts were formed at aliphatic nitrogen atom. The IR spectra of salts 31–33 exhibit the same stretching absorption bands which are present in compounds 23–30.

Antimicrobial activity

A-Agar well diffusion bioassay:

Thirteen of the previously obtained compounds (5,6,10,12–21) in DMF (*table 1*) were tested for their *in vitro* antimicrobial action against *S.aureus* ATCC 25923, B.subtilis ATCC 6633, *E.coli* ATCC 25922 , *P.aeruginosa* ATCC-9027 and *Candida albicans* ATCC -885-653 following the modified *agar well-diffusion* method of Perez et al [8] using rivanol as standard drug. Microbial growth was determined by measuring the diameter of inhibition zone in mm.

Data presented in *table 3*, generally, revealed that compounds 12–19, 21 gave a strong activity (30–35 mm) against gram positive organisms when compared to rivanol, whereas compounds 5, 6 were less active (19–27 mm). The most active compound relative to *S.aureus* was compound 13 (34.66 mm**)**, while compounds 12,17 and 18 showed the highest activity against *B.subtilis* (33.50 mm; 33.20 mm; 35.30 mm, respectively). Compounds 17,18 showed strong activity against C. albicans (35.3 mm; 38.3 mm, respectively) in comparison with rivanol. Against gram negative organisms, only compounds 14 and 19 showed strong activity compared with rivanol. Thus compound 14 showed strong activity against *P. aureg* (31.3 mm), while compound 19 showed strong activity against *E. coli* (33.3 mm).

From the presented results it was found that combination of the acids with rivanol have crucial effect on increasing the antimicrobial activity of the rivanol.

B-Dilution method

The minimum inhibitory concentration (MIC) was determined by tube dilution method for each of the test organisms [9]. Compounds 5,6,10,12–21 were tested against *C. albicans* ATCC -885-653 and compounds 10, 21 were only tested against clinically isolated *P. aeruginosa*, which were resistant to 7 antibiotics (Cephotaxime, Cefoperazone, Ceftazidine, Ceftriaxone, Ceftizoxime, Cefepime, Aztreonam) and sensitive to four antibiotics (Ciprofloxacin, Enoxacin, Amikacin, Azlocillin). The results of antibacterial activity against *S.aureus* ATCC 25923, B.subtilis ATCC 6633, *E.coli* ATCC 25922 were previously obtained [2].

Table 3

Antimicrobial activity of compounds 5,6,10,12–21 (inhibition zone (in mm) / minimum inhibitory concentration (μg/ml)

C.albicans	P.aeruginosa	E.coli	B.subtilis	S.aureus	Compd
33.00 ± 0.13	29.70 ± 0.09	32.00 ± 0.12	30.70 ±0.09	29.20 ± 0.17	Rivanol
50.00±0.06	< 50	*	*	*	
16.70± 0.03	19.30± 0.03	19.80 ±0.11	26.70 ±0.46	19.30 ± 0.01	5
< 50	-	*	*	*	
21.80 ± 0.12	20.70 ± 0.06	16.50 ±0.08	21.50±0.13	23.10±0.04	6
< 50	-	*	*	*	
20.03 ± 0.23	24.10 ± 0.02	27.10 ± 0.02	29.00 ±0.06	25.70 ± 0.03	10
< 50	50.0±0.08	*	*	*	
33.00 ± 0.07	28.10 ± 0.06	29.10 ± 0.29	33.50 ± 0.07	31.8 ±0.1	12
< 50	-	*	*	*	
33.50 ± 0.08	23.50 ± 0.19	31.60 ± 0.29	32.00 ± 0.12	34.66 ± 0.09	13
< 50	-	*	*	*	
32.30 ± 0.03	31.30 ± 0.03	31.60 ± 0.14	31.30 ± 0.07	33.70±0.07	14
< 50	-	*	*	*	
33.30 ± 0.67	28.20 ±0.04	2.87 ± 0.41	32.2 ± 0.1	32.80 ± 0.08	15
25.0±0.12	-	*	*	*	
33.00 ± 0.14	26.70 ± 0.04	31.80 ± 0.13	32.50 ± 0.03	29.80 ± 0.13	16
< 50	-	*	*	*	
35.30 ± 0.12	27.80 ± 0.02	32.80 ± 0.19	33.20 ± 0.07	32.50 ± 0.10	17
< 50	-	*	*	*	
38.30 ± 0.09	30.50 ± 0.15	28.70 ± 0.33	35.30 ± 0.03	30.00 ± 0.03	18
50.00±0.07	-	*	*	*	
33.50 ±0.04	26.80 ± 0.04	33.30 ± 0.12	31.70 ± 0.03	30.00 ± 0.77	19
< 50	-	*	*	*	
26.80 ± 0.12	23.8 ± 0-.06	31.00 ± 0.21	29.60 ± 0.03	27.30 ± 0.03	20
< 50	-	*	*	*	
31.80 ± 0.11	24.80 ± 0.04	31.0 ± 0.1	32.80 ± 0.02	33.70 ± 0.17	21
25.00±0.09	50.00±0.12	*	*	*	

*The results of antibacterial activity against *S.aureus*, *B.subtilis* and *E.coli* were published in [2].

From the data presented in table 3 it is clear that compounds 15 and 21 possess strong activity (MIC 25 μg/ml) against *C.albicans* as compared to standard (Rivanol). Compound 18 showed similar activity to the standard (MIC 50 μg/ml). Other compounds were found to be inactive at prepared concentration.

Against clinically isolated *P.aeruginosa*, compounds 21, 10 showed strong activity with MIC of 50μg/ml whereas rivanol was inactive at this concentration.

Antimalarial activity

The chloroquine sensitive *P.falciparum* isolated from Yemeni patients was continuously cultured following the protocol of Trager and Jensen [10]. Quantitative assessment of anti-plasmodial activity *in vitro* was undertaken by means of the Desjadins et al. [11].

IC_{50}, IC_{90}, IC_{99} values were determined graphically by plotting the concentration versus percentage of inhibition. The values extrapolated from graphs are given in *table 4*.

As observed in *table 4*, compounds 24,28,30,32,33 showed the highest potency among the compounds screened, with IC_{50} values of 0.3, 0.1, 0.19, 0.2, 0.22 nM, respectively, whereas IC_{50} value of chloroquine was 0.38 nM. The normal human RBC's used for the culture when exposed at all the concentrations showed no lysis which suggests that the test compounds have no lytic activity on the normal human cells.

Table 4

Antimalarial activity of compounds 1–9, 11, 23–33 by schizont maturation assay against chloroquine sensitive strain of *P. falciparum* isolated from Yemeni patient

Compound	IC(nM)		
	IC_{50}	IC_{90}	IC_{99}
1	3.00	5.00	5.2
2	0.80	3.80	4.3
3	0.80	4.10	9.2
4	0.80	3.80	4.3
7	4.05	8.20	16.3
8	2.19	15.20	30.15
9	3.20	6.20	6.8
11	2.38	19.64	20.03
23	0.40	4.00	4.7
24	0.30	1.20	1.4
25	0.40	2.40	7.0
26	0.40	1.20	2.4
27	0.40	3.40	4.7
28	0.10	1.90	5.4
29	0.84	2.01	4.0
30	0.19	3.86	7.53
31	0.60	4.50	5.2
32	0.20	2.20	3.8
33	0.22	3.86	8.66
Chloroquine	0.38	1.00	1.15

Conclusion

As a conclusion, eleven new compounds were synthesized (compounds 23-33 **)** and their structures were characterized.

It was established that combination of benzensulphohydrazidoxalyl- arensulphonyloxamoylaminoacids and benzensulphahydrazido- arensulphamidoxalic acid with chloroquine and rivanol was important for improving the antimalarial and antimicrobial activity of chloroquine and rivanol, respectively. This advantage may be due to good penetration of the new salts into microorganisms, or to assistance of new mechanism of action.

Screening of the in vitro antimicrobial and antimalarial activity of the investigated compounds has evidenced that some of them appear to be novel and promising antimicrobial and antimalarial candidates.

References

1. *Alrahawi Kh.M.* Synthesis and antimicrobial activity of 3,4-dimethyloxamoylamino acid's salts with aminoacridines base / Kh.M. Alrahawi, G.P. Petyunin, I.L. Dikiy // Pharm. J. – 2008. – №2. – P. 73–76.
2. *Alrahaw Kh.M.* Synthesis and biological activity of arensulphonyloxamoyl- and arensulphohydrazidooxalyamino acids and their salts with 2-ethoxy-6,9-diaminoacridine / Kh.M. Alrahaw, G.P. Petyunin, I.L. Dikiy // Pharm. J. – 2008. – №5. – P. 62–66.
3. *Alrahawi Kh.* Synthesis and antibacterial activity of new N-R-oxamoylamino acid derivatives and their salts of 2-ethoxy-6,9-diaminoacridine / Kh. Alrahawi, A. Al-kaf, I.L. Dikiy // Yem. J. for Med. Sci. – 2007. – №3. – P. 35–40.
4. *Alrahawi Kh. M.* Synthesis and biological activity of 3,4-dimethyloxamoylamino acids and their salts with D(+)-Glucoseamine / Kh.M. Alrahawi, G.P. Petyunin, L.N. Maloshtan // J.Org. & Pharm. Chem. – 2008. – №3 (23). – P. 53–58.
5. *Halina K.* Antioxidative properties of pyrrolidinium and piperidinium salts / K. Halina, O. Małgorzata, B. Dorota // Z. Naturforsch. – 2002. – №57. – P. 344–347.
6. Synthesis and Antiviral Effect against Herpes Simplex Type 1 of 12-substituted Benzo[c]phenanthridinium Salts / [Enguerran V., Maryvonne A., Joel B., Michael A.]. // J.of Enzy.inhibition & M. Chem. – 2004. – Vol. 19, №6. – P. 481–487.
7. *Bellamy L.J.* The infra-red spectra of complex molecules / L.J. Bellamy. – London: Chapman and Hall, Vol.1. – P. 433–498
8. *Perez C.* An antibiotic assay by the agar well diffusion method / C. Perez, M. Pauli, P. Bazevque // An Acta biologia et medicine experimentalis. – 1990. – №15. – P. 113–115.
9. *Doughari J.H.* Antimicrobial Activity of Tamarindus indica Linn / J.H. Doughari // Trop. J. pharm. Res. – 2006. – №5 (2). – P. 597–603.
10. *Targer W.* Human malaria parasites in continuous culture / W. Targer, J.B. Jensen // Science. – 1976. – №193. – P. 673–675.
11. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique / [Desjardins R.E., Canfield C.J., Haynes J.D., Chulay J.D.] // Antimicrob. Agents Chemother. – 1979. – №16. – P. 710–718.

Informations about authors:

Prof. K.M. Al-rahawi; Medicinal Chemistry department, Sana'a University (Yemen).
E-mail : alrahawi_65@yahoo.com. Tel. mob.: 00967-777345947.
Assoc. Prof. V.N. Petyunina; Department of medical and bioorganic chemistry, Kharkov medical university.
E-mail: petyunin48@mail.ru, tel. mob. 0501838758.
Assoc. Prof. M. Al-haj; Medicinal chemistry department, Aden University (Yemen). Tel. mob.: 00967-777373515.
Assoc. Prof. G. Al-daidamoni; Pharmacognosy department, Sana'a University (Yemen).