

# Synthesis and Biological Activities of 2-Carboxyphenyloxamoylamino Acids, their Salts with 2-ethoxy-6,9-Diaminoacridine and D-glucosamine

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## Abstract

As a result of this work, 17 new compounds were obtained. This group of compounds (2-carboxyphenyloxamoylamino acids) alone or with biologically active base showed diverse biological activity-Anti-inflammatory, antimicrobial and hepatoprotective activity- which can be used in medicine after further investigations.

**Keywords:** Synthesis; 2-carboxyphenyloxamoylamino acids; Salts of rivanol and D-glucosamine; Antimicrobial; Anti-inflammatory; Hepatoprotective activity

## Introduction

N-acylamino acid derivatives are of significant interest due to their biological activities [1-4]. Our research in the chemistry and biological activity of oxamoylamino acids and their salts with biologically active base resulted in establishment of two methods for their synthesis one of which gave optically active compounds [5]. It was also found that, a representative of this chemical group possesses diverse biological activities, including antioxidants, hepatoprotective, immunosuppressant, anti-inflammatory, antimicrobial, diuretic, hypoglycemic and antimalarial activities [5-9]. In continuation of research in this field we arrived to synthesize new derivatives of phenyloxamoylamino acid (2-carboxyphenyloxamoylamino acid), and to obtaining their salts with biologically active base and to study the biological activities of the obtained compounds.

## Experimental

### Chemistry

The UV-spectra were measured on a UV-160 IPC (SHIMADZU) spectrophotometer using samples ( $10^{-4}$  mol) dissolved in ethanol. The IR spectra were obtained on a IR-FTIR-8300 (SHIMADZU) spectrophotometer in tablets of potassium bromide, at the range of 4000-400  $\text{cm}^{-1}$ . The HNMR- spectra were recorded on spectrophotometer «Varian Mercury-VX-200 (200 MHz)», using DMSO- $d_6$  as solvent and TMS as the internal standard [1]. The ionization constants for acids 1-5 were determined by potentiometric titration in 50% aqueous dioxane. The measurements were performed on FTI-6 UNIVERSAL DIGITAL pH-meter (ENGLAND). The melting temperatures were determined on melting point apparatus SMP3 (England). The purity of the targeted products was checked by TLC on Silufon 0,25 mm silica gel 60 F<sub>254</sub> (Merk, Germany).

#### I. 1 2-Carboxyphenyloxamoylglycine (1)

11.53 g (0.05 mol) of ethyl ester of 2-Carboxyphenyloxamic acid in 50 ml absolute methanol was added to 3.53 g (0.05 mol) glycine in 15 mL methanolic solution of sodium methoxide, sodium methoxide solution, was obtained from 0.1 g atom sodium metal and 15 ml absolute methanol. The reaction mixture keeps standing to neutral pH, as detected by universal indicator. The formed precipitate dissolved in minimum volume of water and acidified with H:Cl to pH 2 with 1:1

HCl. The resulting precipitate was filtered, dried and crystallized from ethanol. Similarly compounds 2-5 were obtained. UV  $\lambda_{\text{max}}$  nm(log  $\epsilon$ ): 208 (4.17), 231 (4.19), 268 (3.88), 307 (3.83); IR (KBr,  $\text{cm}^{-1}$ ): 3415, 3377  $\gamma$ NH; 2949-2650  $\gamma$ OH(COOH); 1730  $\gamma$ CO(COOH); 1680  $\gamma$ CO; 1517 CONH; 1600  $\gamma$ C=C; <sup>1</sup>HNMR (DMSO- $d_6$ ,  $\delta$ ): 3.88-4.20d (2H,  $\text{CH}_2$ ); 7.10-7.34t (1H,  $\text{arom}$ ); 7.54-7.69t (1H,  $\text{arom}$ ); 7.9-8.2d (1H,  $\text{arom}$ ); 8.51-8.75d (1H,  $\text{arom}$ ); 9.11-9.35t (1H, NHCH<sub>2</sub>); 12.42-12.65s (1H, NHCO).

#### II. 2-Carboxyphenyloxamoyl- $\beta$ -alanine (2)

UV  $\lambda_{\text{max}}$  nm(log  $\epsilon$ ): 208 (4.12), 231 (4.15), 268 (3.82), 307 (3.78); IR (KBr,  $\text{cm}^{-1}$ ): 3300, 3215  $\gamma$ NH; 2959-2528  $\gamma$ OH (COOH); 1720  $\gamma$ CO(COOH); 1678  $\gamma$ CO; 1514 CONH; 1587  $\gamma$ C=C; <sup>1</sup>HNMR (DMSO- $d_6$ ,  $\delta$ ): 1.24-1.49t (2H,  $\text{CH}_2$ , COOH); 4.22-4.40m (2H, NHCH<sub>2</sub>CH<sub>2</sub>); 7.10-7.35t (1H,  $\text{arom}$ ); 7.40-7.65t (1H,  $\text{arom}$ ); 7.93-8.09d (1H,  $\text{arom}$ ); 8.50-8.75d (1H,  $\text{arom}$ ); 8.85-9.10-t (1H, NHCH<sub>2</sub>); 12.33-12.57s (1H, NHCO).

#### III. 2-Carboxyphenyloxamoyl- $\alpha$ -alanine (3)

UV  $\lambda_{\text{max}}$  nm(log  $\epsilon$ ): 208(4.19), 231(4.22), 268(3.89), 307(3.85); IR (KBr,  $\text{cm}^{-1}$ ): 3342, 3400  $\gamma$ NH; 2971-2771  $\gamma$ OH (COOH); 1700  $\gamma$ CO(COOH); 1670  $\gamma$ CO; 1514 CONH; 1585  $\gamma$ C=C; <sup>1</sup>HNMR (DMSO- $d_6$ ,  $\delta$ ): 1.25-1.51d (3H,  $\text{CH}_3$ ); 4.22-4.45m (1H,  $\text{CH}$ ); 7.10-7.33t (1H,  $\text{arom}$ ); 7.42-7.65t (1H,  $\text{arom}$ ); 7.90-8.15d (1H,  $\text{arom}$ ); 8.50-8.75d (1H,  $\text{arom}$ ); 9.00-9.25d (1H, NHCH<sub>2</sub>); 12.40-12.63s (1H, NHCO).

#### IV. 2-Carboxyphenyloxamoyl- $\gamma$ -aminobutyric acid (4)

UV  $\lambda_{\text{max}}$  nm(log  $\epsilon$ ): 210(4.26), 231(4.33), 267(4.01), 307(3.95); IR (KBr,  $\text{cm}^{-1}$ ): 3346, 3323  $\gamma$ NH; 2961-2532  $\gamma$ OH (COOH); 1720  $\gamma$ CO(COOH); 1674  $\gamma$ CO; 1516 CONH; 1570  $\gamma$ C=C; <sup>1</sup>HNMR (DMSO- $d_6$ ,  $\delta$ ): 1.55-1.80p (2H,  $\beta$ CH<sub>2</sub>); 2.05-2.29t (2H,  $\alpha$ CH<sub>2</sub>); 3.10-3.25t (2H  $\gamma$ CH<sub>2</sub>);

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7.10-7.34t (1H<sub>arom</sub>); 7.54-7.78t (1H<sub>arom</sub>); 7.95-8.19d (1H<sub>arom</sub>); 8.55-8.79d (1H<sub>arom</sub>); 9.0-9.24t (1H, NHCH<sub>2</sub>); 12.42-12.65s (1H, NHCO).

### V. 2-Carboxyphenyloxamoylserine (5)

UV  $\lambda_{max}$  nm(log  $\epsilon$ ): 210(4.21); 228(4.25), 268(4.01), 307(3.82); IR (KBr, cm<sup>-1</sup>): 3446 $\gamma$ OHass. 3360,3280  $\gamma$ NH; 2900-2638 $\gamma$ OH(COOH); 1700  $\gamma$ CO(COOH); 1685 $\gamma$ CO; 1517 CONH; 1590 $\gamma$ C=C; <sup>1</sup>HNMR (DMSO-d<sub>6</sub>,  $\delta$ ): 3.72-3.94d (2H,CH<sub>2</sub>OH); 4.19-4.40q (1H,CH); 5.00-5.21s (1H,OH); 7.10-7.32t (1H<sub>arom</sub>); 7.55-7.78t (1H<sub>arom</sub>); 7.93-8.15d (1H<sub>arom</sub>); 8.50-8.71d (1H<sub>arom</sub>); 8.80-9.05t (1H, NHCH); 12.40-12.55 (1H, NHCO).

### VI. 2-Ethoxy-6,9-diaminoacridinium 2-carboxyphenyloxamoylglycinate (6)

To an ethanolic solution of 0.266 g (0.001 mol) of 2-Carboxyphenyloxamoylglycine, an ethanolic solution of 0.252 g (0.001 mol) 2-ethoxy-6,9-diaminoacridinium base was added. The mixture allowed to stand to neutral pH, as detected by universal indicator. The formed precipitate (salt) was filtered, washed with diethyl ether and dried. Salt 7-10 were obtained similarly.

### VII. 2-Ethoxy-6, 9-diaminoacridinium 2-carboxyphenyloxamoyloxalate (16)

The equivalent mole of an ethanolic solution of 2-ethoxy-6,9-diaminoacridine and 2-carboxyphenyloxamic acid were mixed together and the reaction mixture allowed to stand to pH neural. The formed precipitate, was filtered, washed with diethyl ether and dried. C<sub>24</sub>H<sub>22</sub>N<sub>4</sub>O<sub>6</sub>, Yield, 3.88 g (85%); mp., 250°C.

### VIII. 2-D-(+)-glucosamonium 2-carboxyphenyloxamoylglycinate (11)

0.862 g (0.004 mole) of D(+)-glucosaminium chloride was dissolved by heating in potassium hydroxide solution, obtained from 0.224 g (0.004 mol) of potassium hydroxide and 10 mL 50% aqueous ethanol. The precipitate of potassium chloride was filtered, then the filtrate was added to a solution of 1.064 g (0.004 mol) of 2-carboxyphenylglycine in 15 mL ethanol. The reaction mixture was allowed to stand for one night. The formed precipitate (salt) was filtered, washed with diethyl ether and dried. Other salts (12-15) were similarly obtained.

### IX. 2-D-(+)-glucosamonium 2-carboxyphenyloxamine (17)

0.862 g (0.004 mol) of D(+)-glucosaminium chloride was dissolved by heating in potassium hydroxide solution, obtained from 0.224 g (0.004 mol) of potassium hydroxide and 10 mL 50% aqueous ethanol. The precipitate of potassium chloride was filtered, then the filtrate was added to a solution of 1.5 g (0.004 mol) of 2-carboxyphenylxamic acid in 15 mL ethanol. The reaction mixture was allowed to stand for one night. The formed precipitate (salt) was filtered, washed with diethyl ether and dried. C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>O<sub>10</sub>, Yield, 1.3 g (84%); mp., 160°C.

## Biological activity assays

### Antimicrobial activity

**i. Agar well diffusion bioassay:** Six of new compounds 6-10,16 in DMF (Table 3) were tested for their in vitro antimicrobial action against five microorganisms (i.e. S.aureus ATCC 25923, B.subtilis ATCC 6633, E.coli ATCC 2592, P. aeruginosa ATCC-9027, Candida albicans ATCC -885-653), following agar well-diffusion method, [10] and using the antiseptic rivanol as standard drug. Microbial growth was determined by measuring the diameter of inhibition zone in mm. DMF alone

showed no inhibition zone.

**ii. Dilution method:** The minimal inhibitory concentrations of synthesized compounds 6-10, 16 were determined by the tube dilution method [11].

**Anti-inflammatory activity:** Animals were obtained from Animal house, Faculty of Science, Sana'a University, Yemen. Male Swiss albino mice weighing 25-30 g were used. All animal experiments were performed according to the ethical guidelines suggested by the Institutional animal ethics committee (IAEC).

Carrageenan-induced paw edema in mice was used as an acute inflammatory model as described by Winter et al. [12]. 50  $\mu$ L of a 1% carrageenan solution was injected subcutaneously into the sub plantar surface of the right hind paws of the mice using a 25-gauge needle. Seventy two mice were used and were divided into 8 groups (n=9 in each group) as follows: Group I served as control (vehicle treated) and received normal saline (0.3 ml/kg i.p intraperitoneal) only, Group II served as a positive control and received diclofenac sodium (10 mg/kg i.p), Groups (III-VIII) served as test groups and received 10 mg/kg i.p., of compounds (ii,1-5) one hour before carrageenan injection. The differences in weight between right and left hind paws represent the amount of edema developed in the right hind paw [13].

Edema was calculated using the following formula;

$$EWD=EW_R-EW_L$$

Where; EWD=Edema weight difference between right and left hind paw.

$EW_R$ =Edema weight of right hind paw (test).  $EW_L$ =Edema weight of left hind paw (control). Percentage Inhibition of Edema (PI) was calculated from equation:

$$PI=\frac{EW_C-EW_T}{EW_C}\times 100$$

Where;  $EW_C$ =Edema Weight difference of Control animal's paw, and  $EW_T$ =Edema Weight difference of test animal's paw.

### i. Statistical analysis

All results were expressed as the arithmetic mean  $\pm$  SE. Differences between groups were evaluated by analysis of variance (ANOVA) complemented by Student's *t*-test. A difference was considered significant at P value less than 0.05.

**Hepatoprotective activity:** This study was performed to assess the hepatoprotective activity of the synthesized salts 2-D-(+)-glucosamonium-2-carboxyphenyloxamoylamino acids (compounds 11-15,17) in rats against carbon tetrachloride (CCl<sub>4</sub>) as hepatotoxin using silymarin as standard hepatoprotective.

**a) Animals:** Fifty four male albino rats weighing 175- 225 g. maintained under good husbandry conditions (Temp. 23  $\pm$  2°C. relative humidity 55  $\pm$  10% and 12 h light dark cycle) were used for all studies. Animals were allowed to take standard laboratory feed and tap water. Ethical committee in accordance with animal experimentation and care has approved all animal procedures.

**b) Evaluation of hepatoprotective activity:** Fifty four male albino rats were divided into nine equal groups; control, silymarin, carbon tetrachloride (CCl<sub>4</sub>) and test groups.

Group (1) The rats of control group received three doses of 5% acacia mucilage each (1 ml/Kg, per oral) at 12 h intervals ( 0 h, 12 h

and 24 h).

Group (2) The rats of silymarine group received three doses of silymarine (25 mg /kg) at 0 h, 12 h and 24 h. CCl<sub>4</sub> (1.25 ml /kg i.p) was administered 30 min. after the first dose of silymarine.

Group (3) The rats of CCl<sub>4</sub> group received three doses of vehicle at 12 h intervals and a single dose of CCl<sub>4</sub> (1.25 ml/kg i.p) diluted in liquid paraffin (1:1) 30 min after the administration of the first dose of vehicle .

Group (4, 5, 6, 7, 8 and 9) were given the first dose of the tested compounds at a dose of (25 mg/kg p.o) in acacia mucilage at 0 h. which was followed by a dose of CCl<sub>4</sub> (1.25 ml/kg p.o) after 30 min., while at 12 h and 24 h the second and third dose of the test compounds (25 mg/kg p.o) were administered.

After 36 h of administration of CCl<sub>4</sub> blood was collected and serum was separated and used for determination of biochemical tests.

**c) Measurement of biochemical parameters:** Liver function was investigated by estimation of alanine aminotransferase ALT [14], aspartate aminotransferase AST [14], and alkaline phosphatase ALP [15], Serum total protein T.P. was determined according to Henry 1964 [16], serum albumin was measured according to Doumas and Biggs, 1976 [17], Serum total bilirubin T.B. and serum direct bilirubin D.B. were determined according to Walters and Gerarde [18].

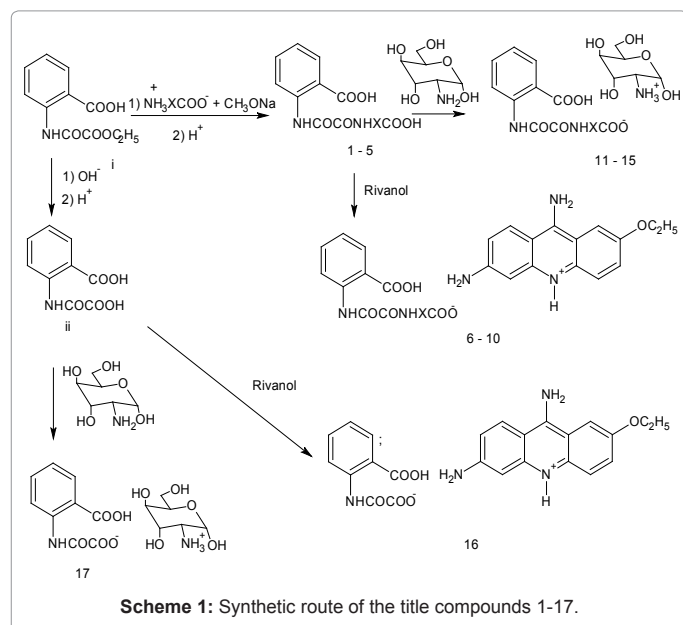
**d) Statistical analysis:** All results were expressed as the arithmetic mean ± SE. Differences between groups were evaluated by analysis of variance (ANOVA) complemented by Student's t-test. A difference was considered significant at P value less than 0.05.

## Results and Discussion

### Chemistry

The synthesis of 2-carboxyphenyloxamoylamino acids 1-5 was performed by acylation of ethyl ester of 2-carboxyphenyloxamic acid, compound i [19], with amino acids in the presence of sodium methoxide and absolute methanol as shown in the scheme 1.

Generally, the 2-carboxyphenyloxamoylaminoacids 1-5 (Table 1)



Com.	X	Yield, %	M.p.,C <sup>0</sup>	Empirical formula	*R <sub>f</sub>	pK <sub>a</sub>	
						I	II
1	CH <sub>2</sub>	67	228-230	C <sub>11</sub> H <sub>10</sub> N <sub>2</sub> O <sub>6</sub>	0.89	5.2	6.1
2	CH <sub>2</sub> CH <sub>2</sub>	54	238-240	C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> O <sub>6</sub>	0.80	5.3	6.0
3	CHCH <sub>3</sub>	56	233-235	C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> O <sub>6</sub>	0.81	5.3	6.3
4	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>	81	221-223	C <sub>13</sub> H <sub>14</sub> N <sub>2</sub> O <sub>6</sub>	0.89	5.5	6.7
5	CHCH <sub>2</sub> OH	58	195-197	C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> O <sub>7</sub>	0.75	4.5	6.0

\*The TLC systems: n-butanol-acetic acid-water (4:1:1)

**Table 1:** Physical Constants of 2-Carboxyphenyloxamoylamino acid (1-5).

Compd.	X	Yields,%	mp., C <sup>0</sup>	Empirical formula
6	CH <sub>2</sub>	80	251-253	C <sub>26</sub> H <sub>25</sub> N <sub>5</sub> O <sub>7</sub>
7	CH <sub>2</sub> CH <sub>2</sub>	81	268-270	C <sub>27</sub> H <sub>27</sub> N <sub>5</sub> O <sub>7</sub>
8	CH <sub>2</sub> CH <sub>3</sub>	81	193-195	C <sub>27</sub> H <sub>27</sub> N <sub>5</sub> O <sub>7</sub>
9	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>	81	258-260	C <sub>28</sub> H <sub>29</sub> N <sub>5</sub> O <sub>7</sub>
10	CHCH <sub>2</sub> OH	81	196-198	C <sub>27</sub> H <sub>27</sub> N <sub>5</sub> O <sub>8</sub>
11	CH <sub>2</sub>	52	155 dec.	C <sub>18</sub> H <sub>26</sub> O <sub>11</sub> N <sub>3</sub>
12	CH <sub>2</sub> CH <sub>2</sub>	98	180 dec.	C <sub>19</sub> H <sub>27</sub> O <sub>11</sub> N <sub>3</sub>
13	CH <sub>2</sub> CH <sub>3</sub>	87	115 dec.	C <sub>19</sub> H <sub>27</sub> O <sub>11</sub> N <sub>3</sub>
14	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>	65	185 dec.	C <sub>20</sub> H <sub>29</sub> O <sub>11</sub> N <sub>3</sub>
15	CHCH <sub>2</sub> OH	20	117-119	C <sub>19</sub> H <sub>27</sub> O <sub>12</sub> N <sub>3</sub>

**Table 2:** Physical Constants of salts (6-10) and salts (11-15).

are pale yellow crystalline substances, soluble in hot water, ethanol, alkaline solution and insoluble in most organic solvents. The purity and identity of the synthesized compounds were checked by using thin layer chromatography eluted with two systems of solvents (Table 1). Since the obtained substances (1-5) are acids the ionization constants (pKa) were measured (Table 1), the obtained results confirm that the synthesized compounds are dibasic acids. The values corresponded to high acidity (pKa I) belong to the aliphatic carboxylic group's ionization.

The proposed structures of compounds 1-5 were confirmed by the UV, IR and <sup>1</sup>H NMR spectroscopic data.

The UV spectra of compounds (1-5) contain four absorption bands at 208-210, 228-231, 267-268 and 307 nm. The first three bands originates from π-π\* -electronic transition of benzene ring. The 307 nm band originate from n-π\* -electronic transition characteristic for N-acylanthranilic acid derivatives [20].

In accordance with the number and position of proton's signal, the result of <sup>1</sup>H NMR spectra of 2-carboxyphenyloxamoylamino acids 1-5 corresponds with the proposed structures. It should be noted that proton's signal of Ar-NHCO of compounds 1-5 is shifted from high field 10.24-10.90 ppm (signals characterized for other substituted phenyloxamoylamino acids) to low field 12.33-12.65 due to the effect of hydrogen bonding between the amide's hydrogen and ortho-carboxylic group [6,8,21].

Based on the synthesized acids 1-5, salts with the base of rivanol 6-10 and D-glucosamine 11-15 were obtained.

The obtained salts 6-10 (Table 2) are yellow crystalline substances, soluble in hot water and insoluble in most organic solvents.

UV spectra of salts 6-10 are attributed to the absorption of cation and are definitely identical to the ethacridine lactate's spectrum. This is the evidence that the salts were formed at ring's nitrogen atom. The IR spectra of salts 6-10 and 16 exhibit stretching absorption bands of the initial acids without the stretching absorption band of CO (COOH), the absence of this band in the salts may be due to the resonance effect between C-O bonds [22]. Furthermore, they exhibit absorption bands

belonging to the acridine's rings (1491-1500  $\text{cm}^{-1}$ ; 1446-1455  $\text{cm}^{-1}$ ; 1370-1395  $\text{cm}^{-1}$ ).

The obtained salts 11-15 (Table 2) appeared as white crystalline substances, readily soluble in water with the formation of neutral solution. Adding mineral acid into the solutions of the obtained salts leads to formation of the precipitate of initial compounds 2-carboxyphenyloxamoylaminoacids 1-5.

The UV spectra of the salts 11-15 and 17 were determined by anions and were found to be almost similar to the spectra of acids 1-5. The IR spectra of compounds 11-15 and 17 contain broad absorption bands due to the stretching vibration of associated NH and OH groups in the of region of 3430-3251  $\text{cm}^{-1}$ , CO amid I in the region of 1680-1660  $\text{cm}^{-1}$  and groups of stretching absorption bands in the region of 3000-2810  $\text{cm}^{-1}$ , arising from symmetrical and asymmetrical stretching in the ammonium groups. Moreover, the hydrocarbon ring of D-(+)-glucosamine exists in pyranose form and shows two bands in IR spectrum; asymmetric at 925-910  $\text{cm}^{-1}$  and symmetric at 775-765  $\text{cm}^{-1}$  due to the pyranose vibration.

To study the effect of the amino acids residues upon the biological activity, the salts 16,17, which don't contain amino acids fragments, were obtained.

The obtained salt 16 is a yellow crystalline substances, soluble in hot water, insoluble in most organic solvents. The obtained salt 17 is a white crystalline substance, soluble in water and insoluble in most organic solvents. The UV and IR spectra of compound 16 and 17 were almost identical to the UV and IR spectra of compounds 6-10 and compounds 11-15, respectively.

### Antimicrobial activity

**Well diffusion method:** From the data presented in table 3, in general it may be seen that compound 10 possesses strong activity (33 mm) against *S.aureus* when compared to rivanol, whereas compounds 8,10 possess strong activity (29.5 mm, 32 mm, respectively) against.

*B.subtilis*. Compound 10 possesses strong activity (32 mm) against *E. coli* in comparison with rivanol. Compounds 10, 16 possess strong activity (25.5 mm, 24.5 mm respectively) against *P.aeruginosa*. Compounds 8, 10 possess strong activity (33 mm, 32 mm) against *C. albicans* in comparison with rivanol.

From the presented results it was found that combination of acids with rivanol have clear effect on the antimicrobial activity of rivanol especially in compound 10 (2-ethoxy-6, 9-diaminoacridinum-2-

Compd	S.aureus	B.subtilis	E.coli	P.aeruginosa	C.albicans
Rivanol	22.5 ± 0.17 <50	24.5 ± 0.12 <50	26 ± 0.12 <50	19.5 ± 0.12 <50	30 ± 0.07 50 ± 0.06
6	12.5 ± 0.09 <50	18.5 ± 0.15 <50	14 ± 0.13 <50	16.5 ± 0.08 <50	19.5 ± 0.08 <50
7	12 ± 0.07 <50	19.5 ± 0.33 <50	14 ± 0.29 <50	12.5 ± 0.17 <50	18.5 ± 0.13 <50
8	21 ± 0.02 <50	29.5 ± 0.29 6.25 ± 0.17	26 ± 0.18 50 ± 0.04	19.5 ± 0.07 <50	33 ± 0.07 50 ± 0.12
9	17.5 ± 0.12 <50	23 ± 0.07 <50	14 ± 0.16 <50	16 ± 0.18 <50	24.5 ± 0.05 50 ± 0.08
10	33 ± 0.03 <50	32 ± 0.03 3.25 ± 0.06	32 ± 0.13 1.55 ± 0.05	25.5 ± 0.17 <50	32 ± 0.06 12.5 ± 0.13
16	14.5 ± 0.17	19.5 ± 0.77	<50	24.5 ± 0.08	17 ± 0.11

**Table 3:** Antimicrobial activity of compounds 6-10,16 [inhibition zone (in mm)/ minimum inhibitory concentration ( $\mu\text{g/ml}$ )].

carboxyphenyloxamoylserinate), which showed stronger antimicrobial activity than rivanol against all test microorganisms.

**Dilution method:** From the data presented in table 3 it is clear that compound 8 possesses strong activity against *B.subtilis*, (MIC 6.25  $\mu\text{g/ml}$ ) and against *E.coli* (MIC 50  $\mu\text{g/ml}$ ) when compared with rivanol. Compound 10 possesses strong activity against *B.subtilis* (MIC 3.25  $\mu\text{g/ml}$ ), *E.coli* (MIC 1.55  $\mu\text{g/ml}$ ), and against *C.albicans* (MIC 12.5  $\mu\text{g/ml}$ ) as compared to standard rivanol.

The obtained results confirmed that only the combination of 2-carboxyphenyloxamoyl serine with rivanol has strengthened the antimicrobial activity of the later. Herewith, the most active one is compound 10 (2-ethoxy-6, 9-diaminoacridinum-2-carboxyphenyloxamoyl serinate).

### Anti-inflammatory activity

Result of investigation (Table 4) showed that compounds ii, 3, 4 treated group possess higher % of inhibition of paw volume (61.1, 64.2, 77.8, respectively) than the standard drug, diclofenac (58.8). The best anti-inflammatory activity was exhibited by compound 4 (2-carboxyphenyloxamoyl- $\gamma$ -aminobutyric acid), which superior to the reference drug, Diclofenac sodium in 35%.

Previously, we synthesized 3,4-dimethylphenyloxamoylamino acids and investigated their anti-inflammatory activity. In present study, it was found that introduction of carboxylic group to ortho position instead of the 3,4-dimethyl-groups, increases the anti-inflammatory activity when compared to Diclofenac sodium. This result is predictable, because insertion of COOH group, produces the known anti-inflammatory chemical group, N-substituted anthranilic acid derivatives [23,24].

### Hepatoprotective activity

As is shown in table 5,  $\text{CCl}_4$  elicited a significant increase in the activities of serum ALT, AST, ALP which may be attributed to cellular leakage and loss of functional integrity of cell membrane of hepatocytes [25].

Rats in  $\text{CCl}_4$  group evoked a significant decrease in serum total proteins and albumin. This decline is due to disruption and disassociation of polyribosomes from endoplasmic reticulum following  $\text{CCl}_4$  administration [26]. A similar significant decrease in serum total proteins was noted by Achliya et al. [27] and Shanmugasundaram [28]. They attributed these results to depression of liver ability to synthesize the proteins due to  $\text{CCl}_4$ -induced hepatic damage.

Rats in  $\text{CCl}_4$  group showed significant increase in serum total and

NO.	Group Name (n=9)	EWD (M+Se)	PI (%)
I	Control (0.9% NaCl)	750.9 ± 0.47	- - -
II	Diclofenac	319.4 ± 0.32 ** *; N.S.	58.8
III	ii	292.0 ± 0.29 ** *; N.S.	61.1
IV	1	311.1 ± 0.75 ** *; N.S.	58.6
V	2	416.8 ± 0.56 ** *; N.S.	44.5
VI	3	268.8 ± 0.37 ** *; N.S.	64.2
VII	4	166.7 ± 0.49 ** *; N.S.	77.8
VIII	5	544.4 ± 0.47 *; N.S.	27.5

The statistical data were expressed as M ± SE. M, mean; SE, standard error; n, number of rats; \* or ★=P<0.05; \*\* or ★★=p<0.01; \*\*\* or ★★=p<0.001; \*compare between different groups and control group while ★ compare between different groups and diclofenac group.

**Table 4:** The anti-inflammatory effects of compounds ii, 1-5 comparing with Diclofenac.

N <sup>o</sup>	AST U/L	ALT U/L	A.P. U/L	Alb g/dl	T.P. g/dl	T.B mg/dl	D.B mg/dl
Gp <sub>1</sub> Control	86.00 <sup>d</sup> ± 1.14	66.83 ± 0.60	19.40 <sup>e</sup> ± 0.46	4.17 <sup>a</sup> ± 0.03	6.97 <sup>a</sup> ± 0.04	0.45 <sup>f</sup> ± 0.02	0.14 <sup>d</sup> ± 0.001
Gp <sub>2</sub> Silyl +CCl <sub>4</sub>	77.17 <sup>d</sup> ± 13.64	93.83 <sup>ab</sup> ± 15.72	18.58 <sup>e</sup> ± 0.39	3.95 <sup>b</sup> ± 0.04	6.87 <sup>a</sup> ± 0.03	0.53 <sup>ef</sup> ± 0.03	0.15 <sup>d</sup> ± 0.004
Gp <sub>3</sub> CCl <sub>4</sub>	119.83 <sup>a</sup> ± 16.14	105.00 <sup>a</sup> ± 2.22	38.42 <sup>a</sup> ± 0.74	2.97 <sup>e</sup> ± 0.042	5.98 <sup>b</sup> ± 0.05	1.14 <sup>a</sup> ± 0.06	0.42 <sup>a</sup> ± 0.006
Gp <sub>4</sub> 1+CCl <sub>4</sub>	95.80 <sup>bcd</sup> ± 1.66	74.80 <sup>cd</sup> ± 1.77	21.90 <sup>d</sup> ± 0.33	3.60 <sup>c</sup> ± 0.03	6.92 <sup>a</sup> ± 0.04	0.57 <sup>oe</sup> ± 0.01	0.15 <sup>d</sup> ± 0.009
Gp <sub>5</sub> 2+CCl <sub>4</sub>	89.60 <sup>cd</sup> ± 0.51	67.67 <sup>d</sup> ± 0.71	20.00 <sup>e</sup> ± 0.71	4.14 <sup>a</sup> ± 0.05	7.04 <sup>a</sup> ± 0.05	0.45 <sup>f</sup> ± 0.03	0.14 <sup>d</sup> ± 0.002
Gp <sub>6</sub> 3+CCl <sub>4</sub>	113.67 <sup>ab</sup> ± 1.76	90.50 <sup>abc</sup> ± 0.92	25.80 <sup>c</sup> ± 0.37	3.32 <sup>d</sup> ± 0.04	6.64 <sup>a</sup> ± 0.05	0.72 <sup>c</sup> ± 0.04	0.23 <sup>c</sup> ± 0.01
Gp <sub>7</sub> 4+CCl <sub>4</sub>	96.00 <sup>bcd</sup> ± 1.37	82.83 <sup>bcd</sup> ± 1.82	29.17 <sup>b</sup> ± 0.31	3.30 <sup>d</sup> ± 0.03	6.48 <sup>a</sup> ± 0.11	0.63 <sup>cd</sup> ± 0.01	0.16 <sup>d</sup> ± 0.01
Gp <sub>8</sub> 5+CCl <sub>4</sub>	112.50 <sup>abc</sup> ± 1.12	100.33 <sup>a</sup> ± 1.05	27.17 <sup>c</sup> ± 0.48	3.25 <sup>d</sup> ± 0.03	6.41 <sup>a</sup> ± 0.11	0.86 <sup>b</sup> ± 0.02	0.35 <sup>a</sup> ± 0.02
Gp <sub>9</sub> 6+CCl <sub>4</sub>	102.00 <sup>abc</sup> ± 1.41	104.17 <sup>a</sup> ± 1.54	29.00 <sup>b</sup> ± 0.37	3.34 <sup>d</sup> ± 0.02	6.38 <sup>a</sup> ± 0.05	0.61 <sup>de</sup> ± 0.01	0.15 <sup>d</sup> ± 0.009

Means within the same column having different alphabetical superscript letters are significantly different (P<0.01)

**Table 5:** Effects of the test compounds (11-15,17) on various biochemical parameters in rats with carbon tetrachloride induced hepatotoxicity.

direct bilirubin levels. Similar effects were bosomed by Nevin and Vijayammal [29]. They evaluated the toxic effect of CCl<sub>4</sub> on the liver and found that CCl<sub>4</sub> administered rats showed an elevation in serum bilirubin.

The test compounds 11-15 and 17 elicited a significant reduction in the levels of AST, ALT towards the normal value which is an indication of regeneration process.

Reduction of ALP levels with concurrent depletion of raised bilirubin level suggests the stability of the biliary function during injury with CCl<sub>4</sub>.

The protein and albumin levels were also raised, suggesting stability of endoplasmic reticulum leading to protein synthesis.

The data presented in table 5, showed that the rank order of potency was silymarin >compd 12 >compd 11, compd13 >compd14 >compd 17 and compd 15, this promising hepatoprotection activity of the tested compounds may be due to their antioxidant and free radical scavenging properties.

From the obtained result it was established that not only glucosamine salt of 3,4-dimethylphenyloxamoylamino acids [5], and 4-bromophenyloxamoylamino acid [30], possess hepatoprotective properties, but also glucosamine salt of 2-carboxyphenyloxamoylamino acids. This effect may be due to the glucosamine as hepatoprotective [31], and antioxidant [32], or to the combination of glucosamine and substituted phenyloxamoylamino acids in one molecule as salts [5,30].

Further study should be done to clarify the biological rule of each molecule and to study relationship between the D-glucosamine, amino acid moiety, the substituent on the aromatic ring and the hepatoprotective property.

## Conclusion

1. Seventeen new compounds were synthesized, their structures were established by using different physico-chemical methods.

2. TThe results of biological study clearly demonstrated that some compounds of 2-carboxyphenyloxamoylamino acids, their salts with rivanol and D-glucosamine possess strong anti-inflammatory, antimicrobial and hepatoprotective activities.

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