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Synthesis of New Uracil-5-Sulfonamide Derivatives and Immuno-Stimulatory Effect of a Chemically Modified Hemolymph of *Biomphalaria alexandrina on Schistosoma mansoni* Infected Mice

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Some *N-p*-substituted phenyl uracil-5-sulphonamide derivatives have been synthesized to be evaluated as molluscicides against *Biomphalaria alexandrina* snails, the intermediate host of *Schistosoma mansoni*. *Schistosoma mansoni* infected mice were treated with hemolymph obtained from pre-treated *Biomphalaria alexandrina* snails with the products **4a**, **10a**, **10b** and **4b** or obtained from non-treated snails. The selection of the concentration based on the predetermined dose which caused mortality of less than 50% of snails/24 h. LC_{50} of compounds **4a**, **10a**, **10b** and **4b** was 50, 100, 200 and 50 ppm respectively. The result showed that immunostimulatory effect of treated hemolymph with compounds **4a**, **10a** and **4b** was related to significant protective effects (44.4, 34.6 and 50.4% reduction in worm burden respectively). In addition, mean total worm burdens were significantly reduced in non treated hemolymph by 33.8%. The effect of hemolymph obtained from treated or non treated snails on *S. mansoni* adult worms antigens was studied by indirect immunofluorescence technique using chronic mouse sera (CMS). The results indicated that there was a strong reaction with epitopes in gut epithelium, tubercles, teigument and subtegumental musculature of untreated and treated *S.mansoni* adult worms antigens. Therefore, treatment of hemolymph obtained from pre-treated snails with compounds **4a**, **10a**, and **4b** can stimulate specific immune response and induce protective effects against *S. mansoni* infection.

Key words Uracil-5-sulphonamide derivatives, *Schistosoma mansoni, Biomphalaria alexandrina,* Haemolymph, Immunofluorescence technique

INTRODUCTION

As the chemotherapy of bilharziasis has been met with the toxicity problem, combating the disease through control of snails is still considered as main factor. Among the hundreds of chemicals tested as molluscicides, Bayluscide is still the molluscicide of choice. It inhibits different enzyme systems within the snail's body, mainly those of respiration and carbohydrate metabolism. On the other hand, it was found that a series of uracil-5-sulphonamide-*p*-phenyl derivatives possess moluscicidal activity. This activity may be ascribed to the uracil moiety which is enzymetically oxidized within the snail's bodies to give products related to nucleic acid that are known to possess potent molusscicdal activity.

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Several 5-substituted thiouracil possess chemotherapeutic importance especially against cancer, bacteria parasites (AbdelHamid and Fathall, O. A., 1993; Fathalla, O. A., 1992; Fathalla, O. A., 1999; Fathalla, O. A. *et al.,* 2000), also it was found that α,β-unsaturated ketones and chalcones have chemotherapeutic activity (Kamel *et al.*, 1985; Ebied *et al.,* 1991). Besides it has been reported that thiosemicarbazones (Hassaneen *et al.,* 1995) possess strong biological activity against microorganisms. Synthesis and biological evaluation of certain substituted thiazoles were also studied Ebied *et.al* 1996). The chemistry of pyridons and aminopyridines has been increasing intereste since many of these compounds found useful applications as chemotherapeutic agents (Abbady *et al.*, 1986).

The fact that the relationship between the parasite and its intermediate host which is highly specific may be of interest in studying the protective immunity of antigens separated from these intermediate hosts. (Grzych, *et al.*,

1987) indicated that *S. mansoni* and its intermediate host *B. glabrata* share a common glyceamic determinate previously demonstrated to be active in immunity to Schistosomasis (Dissous *et. al.*, 1986; Dissous *et al.*, 1990).

In the present work, uracil-5-sulphonamide-*p*-phenyl derivatives were prepared through the reaction of uracil-5-sulphonylchloride in order to overcome the escape mechanism adapted by the parasite to evade the immunological reaction of the host, a new strategy had been built up based upon the modification of the parasite antigen by chemical means. Also the present study was conducted to investigate the immuno-stimulatory effect of hemolymph obtained from a chemically treated *B. alexandrina* snails on the *S. mansoni* infected mice.

MATERIALS AND METHODS

All melting points are uncorrected and were taken on electro-thermal capillary melting point apparatus. Infrared spectra were performed on a Schimadzu IR spectrophotometer 435 and Unican Sp 1000 infracord spectrophotometer using KBr discs.

^{1}H-NMR spectra was obtained on a varian A-60 (200 MHZ) spectrometer using TMS as an internal standard. The mass spectra were recorded on GCMS-QP 1000Ex Schimadzu Gas chromatography MS appratus. All reactions were followed and checked by TLC (aluminium sheets) using chloroform-methanol. These (9:1 V/V) eluent and the plates were sprayed with iodine. Microanalysis were performed at the micro analytical Unit, Faculty of Science, Cairo University, Giza and National Research Centre.

5-(*p*-Acetylphenyl)uracil sulphonamide (1)

It was prepared according to the procedure described in literature (Fathalla, 1992).

5-*N*-[4-(4'-aryl-3'-cyano-2'-(amino or hydroxy)-6'-pyridyl) phenyl]uracilsulfonamide derivatives (2a, b and 3a, b) :

A mixture of **1** (0.003 mol), the appropriate aldehyde, namely 4-nitrobenzaldehyde and 4-*N*,*N*-dimethylaminobenzaldehyde (0.003 mol), malononitrile or ethyl cyanoacetate (0.021 mol) and ammonium acetate in *n*-butanol (50 mL) was refluxed for 8-10 h. The reaction mixture was concentrated till its half volume, then cooled and left overnight. The precipitate was filtered off, dried then recrystallized from the proper solvent to give the *N*-*p*-substituted phenyl uracil-5-sulphonamide derivatives of type **2a, b** and **3a, b** (Table I, II & Scheme 1).

Formation of chalcones 4a, b :

A mixture of **1** 0.003 mol and 0.003 mol of the appropriate aromatic aldehyde namely 4-nitrobenzaldeyde and 4-*N*,*N*-dimethylaminobenzaldehyde in 50 mL 10% ethanolic sodium hydroxide solution was shaken at room temperature for 24 h, then refluxed for 1 h, cooled and poured onto ice-cold water. The precipitate that appeared after neutralization with dilute HCl was filtered off and recrystallized from the proper solvent to give **4a, b** (Table I, II & Scheme 1).

Table I. analysis and data of compounds **2a, b, 3a, b, 4a, b, 5a, b, 6a, b, 7a, b, 8a, 9a,** and **10a, b**

Comp. No.	M.p. °C		Molecular Formula (M.W.)	Analysis		
	Solvent	Yield %		Calcd./found %		
				C	H	N
2a	>300 DMF	67	$C_{22}H_{15}N_7O_6S$ (505.47)	52.27 52.08	2.99 2.71	19.40 19.28
2b	>300 DMF	70	$C_{24}H_{21}N_7O_4S$ (503.55)	57.24 57.09	4.20 4.12	19.48 19.37
3a	<300 M	65	$C_{22}H_{13}N_6O_7S$ (505.44)	52.28 52.19	2.59 2.43	16.63 16.54
3b	>300 M	72	$C_{24}H_{19}N_6O_5S$ (503.51)	57.25 57.19	3.80 3.62	16.69 16.52
4a	>300 DMF	73	$C_{19}H_{14}N_4O_7S$ (442.4)	51.58 51.35	3.19 3.12	12.76 12.54
4b	>300 DMF	70	$C_{21}H_{20}N_4O_5S$ (440.47	57.26 57.15	4.58 4.43	12.72 12.61
5a	>300 A	50	$C_{25}H_{18}N_4O_9S$ (550.44)	54.55 54.43	3.30 3.21	10.18 10.12
5b	>300 A	52	$C_{27}H_{23}N_4O_7S$ (547.58)	59.22 59.18	4.23 4.18	10.23 10.17
6a	>300 DMF	50	$C_{22}H_{18}N_4O_7S$ (482.46)	54.77 54.59	3.76 3.61	11.61 11.49
6b	>300 DMF	52	$C_{24}H_{24}N_4O_5S$ (480.53)	59.98 59.79	5.03 4.94	11.66 11.54
7a	>300 DMF	55	$C_{22}H_{18}N_5O_7S$ (496.47)	53.22 53.14	3.65 3.54	14.11 14.05
7b	>300 DMF	57	$C_{24}H_{24}N_5O_5S$ (494.54)	58.28 58.21	4.89 4.75	14.10 14.07
8	>300 A	40	$C_{28}H_{19}N_5O_6S$ (537.54)	62.56 62.45	3.56 3.49	13.03 13.01
9	>300 A	30	$C_{22}H_{19}N_5O_7S$ (492.44)	53.66 53.54	2.87 2.78	14.22 14.16
10a	>300 DMF	70	$C_{34}H_{25}N_{11}O_7S$ (731.17)	55.85 55.78	3.45 3.34	21.07 21.01
10b	>300 DMF	68	$C_{35}H_{28}N_{10}O_9S$ (764.72)	54.97 54.84	3.69 3.84	18.32 18.25

A=Acetic acid, M=Methanol and DMF=Dimethylformamide.

Ethyl-6-substituted-4-[4'-(tetrahydro-2,4-dioxo-5-pyrimidinylsulfonylamino) phenyl]-2-oxo-3-cyclohexan-1-carboxylate (5a, b)

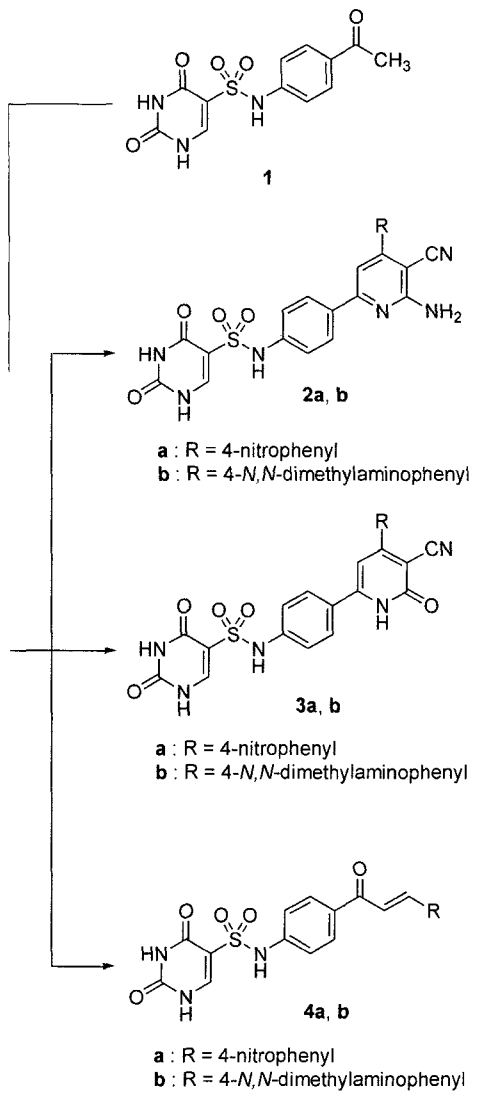
Table II. IR & Mass and ^{1}H-NMR spectral data for some of the newly synthesized compounds

Comp. No.	IR (KBr) Cm^{-1}	Mass. (R.I)	^{1}H-NMR (DMSO-d_6), p.p.m.
2a	3445, 3370, 3225 (NH_2, NH), 3118(C-H aromatic), 2210(CN), 1710, 1695(2CO of uracil), 1341 (NO_2) 1327, 1262 (-N-SO_2-) and 1200 for (-SO_2-), 837(C-N).	505.5	5.6(2H, b, NH_2 exchangeable with D_2O)6.1, 7.2 (4H, dd, aromatic), 6.3-7.2 (4H, dd, aromatic), 7.4 (1H, s, C=CH), 8.3 (1H, s, of uracil), 9.2 (1H, NH exchangeable with D_2O).
2b	3395, 3363, 3198 (NH_2, NH), 3118(C-H aromatic), 2210(CN), 1710, 1695(2CO of uracil), 1327, 1262 (-N-SO_2-) and 1200 for (-SO_2-).	503.5	2, 1, 2.3(6H, s, 2 N-CH_3)5.6(2H, b, NH_2 exchangeable with D_2O), 6.1, 7.2 (4H, dd, aromatic), 6.3-7.2 (4H, dd, aromatic), 7.4 (1H, s, C=CH), 8.3 (1H, s, of uracil), 9.2 (1H, NH exchangeable with D_2O
3a	3280 (NH), 3270, 3050(C-H aromatic), 2212 (CN), 1715(CO), 1700, 1690(2CO of uracil), 1350 (NO_2), 1345, 1327(-N-SO_2-) and 1200 for (-SO_2-), 835(C-N).	505.4	6.1, 7.2 (4H, dd, aromatic), 6.3-7.2 (4H, dd, aromatic), 7.6 (1H, s, C=CH), 8.3 (1H, s, of uracil), 9.2 (1H, NH exchangeable with D_2O).
3b	3325 (NH), 3289, 3134(C-H aromatic), 2217 (CN), 1714(CO), 1700, 1690(2CO of uracil), 1345, 1327(-N-SO_2-) and 1200 for (-SO_2-).	503.5	2, 3, 2.4(6H, s, 2 N-CH_3), 6.1, 7.2 (4H, dd, aromatic), 6.3-7.2 (4H, dd, aromatic), 7.5 (1H, s, C=CH), 8.3 (1H, s, of uracil), 9.2 (1H, NH exchangeable with D_2O
4a	3420 (NH), 3112, 2980 (C-H aromatic), 1720(CO CH), 1700, 1690 (2 CO of uracil), 1342 (NO_2), 1320, 1235 (-N-SO_2-), and 1200 for (-SO_2-)835(C-N).	442.4	5.6(2H, b, NH_2 exchangeable with D_2O)6.1, 7.2 (4H, dd, aromatic), 6.3-7.2 (4H, dd, aromatic), 6.9 (2H, s, CH=CH), 7.4 (1H, s, C=CH), 8.3 (1H, s, of uracil), 9.5 (1H, NH exchangeable with D_2O).
4b	3340 (NH), 3230, 3076 (C-H aromatic), 1715 (CO CH), 1707, 1695 (2 CO of uracil), 1323, 1220 (-N-SO_2-) and 1200 for (-SO_2-).	440.5	2.1, 2, 3(6H, s, 2 N-CH_3), 6.1, 7.1 (4H, dd, Ar-H), 6.2, 7.4 (4H, dd, Ar-H), 6.7 (2H, s, CH=CH), 7.4 (1H, s, -C=CH), 8.3 (1H, s, of uracil), 9.7(1H, NH exchangeable with D_2O).
5a	3330 (NH), 3227, 3136 (C-H aromatic), 1732(CO of ester), 1720 (CO), 1707, 1695 (2 CO of uracil), 1345 (NO_2), 1323 1220 (-N-SO_2-), 1200 (-SO_2-) and 838 for (C-N).	550.4	1, 2(2H, s, of cyclohexane), 1.7, (3H, s, CH_3), 2.6, 3.4(4H, s, of cyclohexane), 3.5 (2H, d, CH_2), 6.1, 7.2 (4H, dd, Ar-H), 6.3-7.2 (4H, dd, Ar-H), 8.3 (1H, s, of uracil), 9.2 (1H, NH exchangeable with D_2O).
5b	3343 (NH), 3223, 3078 (C-H aromatic), 1734(CO of ester), 1723 (CO), 1710, 1705 (2 CO of uracil), 1323, 1220 (-N-SO_2-), and 1200 for (-SO_2-).	547.6	1, 2(2H, s, of cyclohexane), 1.7, (3H, s, CH_3), 2.6, 3.4(4H, s, of cyclohexane), 3.5 (2H, d, CH_2), 6.3, 7.5 (8H, m, Ar-H), 8.2 (1H, s, of uracil), 9.3 (1H, NH exchangeable with D_2O).
6a	3335 (NH), 3219, 3059 (C-H aromatic), 1723 (CO), 1712, 1697 (2 CO of uracil), 1345(NO_2), 1322, 1215 (-N-SO_2-), 1200 for (-SO_2-) and 837 for (C-N).	482.4	1.3(2H, s, of cyclohaxane), , 2.5, 3.2 (4H, s, of cyclohexane), 6.3, 7.5 (8H, m, Ar-H), 8.4 (1H, s, of uracil), 9.6 (1H, NH exchangeable with D_2O).
6b	3335 (NH), 3219, 3059 (C-H aromatic), 1723 (CO), 1712, 1697 (2 CO of uracil), 1322, 1215 (-N-SO_2-) and 1200 for (-SO_2-).	480.5	1, 2(2H, s, of cyclohexane), 2.6, 3.3 (4H, s, of cyclo-hexane), 3.4, 3, 6 (6H, s, 2N-CH_3), 6.1, 7.4 (8H, m, Ar-H), 8.3 (1H, s, of uracil), 9.4 (1H, NH exchangeable with D_2O).
7a	3335 (NH), 3219, 3059 (C-H aromatic), 1723 (CO), 1712, 1697 (2 CO of uracil), 1345(NO_2), 1322, 1215 (-N-SO_2-), 1200 (-SO_2-) and 837 for (C-N).	496.5	1.2(2H, s, of cyclohexane), 2, 3, 3.4 (4H, s, of cyclohaxane), 6.3, 7.5(4H, dd, Ar-H), 6.1, 7(4H, dd, Ar-H), 8.3 (1H, s, of uracil), 9.1, 11.3(2H, s, NH, OH exchangeable with D_2O).
7b	3435(OH), 3335(NH), 3219, 3059 (C-H aromatic), 1723 (CO), 1712, 1697 (2 CO of uracil), 1600(CN), 1345, 1322, 1215 (-N-SO_2-), 1200 (-SO_2-) and 837 for (C-N).	494.5	1.2(2H, s, of cyclohexane), 2, 3, 3.4 (4H, s, of cyclohaxane), 3.6 (6H, s, OCH_3), 6.3, 7.5(4H, dd, Ar-H), 6.1, 7 (4H, dd, Ar-H), 8.3 (1H, s, of uracil), 9.1, 11.3(2H, s, NH, OH exchange -able with D_2O).
8	3335 (NH), 3219, 3059 (C-H aromatic), 1723 (CO), 1712, 1697 (2 CO of uracil), 1345(NO_2), 1322, 1215(-N-SO_2-)1200(-SO_2-) and 837 for (C-N).	537.5	6.1, 7.2 (4H, dd, Ar-H), 6.3-7.2 (4H, dd, Ar-H), 6.9 (2H, s, CH=CH), 7.4 (1H, s, C=CH), 8.3 (1H, s, of uracil), 9.5 (1H, NH exchangeable with D_2O).
9	3335 (NH), 3219, 3059 (C-H aromatic), 1723 (CO), 1712, 1697 (2 CO of uracil), 1345(NO_2), 1322, 1215 (-N-SO_2-), 1200 (-SO_2-) and 837 for (C-N).	492.4	2.9 (4H, s, 2 CH_2), 6.1, 7.2 (4H, dd, Ar-H), 6.3-7.2 (4H, dd, Ar-H), 6.9 (1H, s, C=CH), 8.3 (1H, s, of uracil) 5.6, 9.5 (2H, 2NH exchangeable with D_2O).
10a	3335 (NH), 3219, 3059 (C-H aromatic), 2218(CN), 1723 (CO), 1712, 1697 (2 CO of uracil), 1345(NO_2), 1322, 12'5(-N-SO_2-), 1200 (-SO_2-) and 837 for (C-N).	731.2	2.3(3H, s, C-CH_3), 3.1(3H, s, N-CH_3), 4.8, 9.7(2H, s, exchangeable with D_2O), 7.1(1H, s, -C=CH), 7.-7.5 (13H, m, Ar-H), 8.3(1H, s, of uracil).
10b	3335 (NH), 3219, 3059 (C-H aromatic), 1734(CO), 1723 (CO), 1712, 1697 (2 CO of uracil), 1600(C=N), 1322, 1215(-N-SO_2-), 1200 (-SO_2-) and 837 for (C-N).	764.7	2.2(3H, s, C-CH_3), 3.3(3H, s, N-CH_3), 3.8(3H, s, OCH_3), 4.7, 9.5(2H, s, exchangeable with D_2O), 7.1(1H, s, -C=CH), 7.-7.5 (13H, m, Ar-H), 8.3(1H, s, of uracil).

5-substituted-3-[4'-(tetrahydro-2,4-dioxo-5-pyrimidinylsulfonylamino)phenyl]-2-cyclohexan-1-ones (6a, b) :

General method

Ethylacetoacetate (0.01 mol) was added to an alcoholic solution of sodium ethoxide (prepared by dissolving 0.03



Scheme 1. Preparation of **2**, **3**, and **4**

g, 0.0013 g atom of sodium in 15 mL of absolute ethanol) and the reaction mixture was stirred for 1 h. The appropriate chalcone (0.01 mole) **4a, b** was add to the reaction mixture and the resulting solution was refluxed for 3 h. The hot solution was poured onto cold dilute hydrochloric acid and the solid products of ethyl-4-[uracil-5-sulphonamide)]-6-substituted aryl-2-oxo-3-cyclohexene-1-carboxylates **5a, b**) were separated respectively.

To 0.006 mol of this solid product was added to ethanolic sodium hydroxide solution (0.1 mol) in 50 mL of EtOH : 5 mL of H_2O was added, and the reaction mixture was refluxed for 3 h. The reaction mixture was cooled, acidified with dilute hydrochloric acid and extracted with benzene. The benzene layer was washed with water, dried over $MgSO_4$ and benzene was evaporated under vacuum to give solid product. Recrystallization from appropriate solvent afforded **6a**, **b** in quantitative yields (Tables I, II & Scheme 2).

5-Substituted-3-[4-(tetrahydro-2,4-dioxo-5-pyrimidinyl-sulfonylamino)phenyl]-2-cyclohexene-1-ketoximes (7a, b)

General method

Two grams (0.028 mol) of hydroxylamine hydrochloride were dissolved in ethanol. A solution of 2.5 g (0.03 mol) sodium acetate and 10.7 g (0.03 mol) of **6a** or **6b** in 50 mL of ethanol was added and the reaction mixture was refluxed for 7 h, then cooled and filtered. The solid obtained recrystallized from the proper solvent to give **7a** or **7b** (Tables I, II & Scheme 2).

1,2-Dihydro-1-(4-nitrophenyl)-3-[4'-tetrahydro-2,4-dioxo-5-pyrimidinylsulfonylamino)phenyl] carbazole (8)

To a solution of **6a** (0.01 mol) in (20 mL) of glacial acetic acid was added (1.08 g, 0.01 mol) of phenylhydrazine. The reaction mixture was heated under reflux for 6 h, cooled poured into ice water to give a pale yellow precipitate. Recrystallization from acetic acid gave 40% yield of compound **8** (Table I, II & Scheme 2).

1,5,6,7-tetrahydro-6-(4-nitrophenyl)-4-[4'(tetrahydro-2, 4-dioxo-pyrimidinyl sulfonylamino)phenyl]-2*H*-azepin-2-one (9)

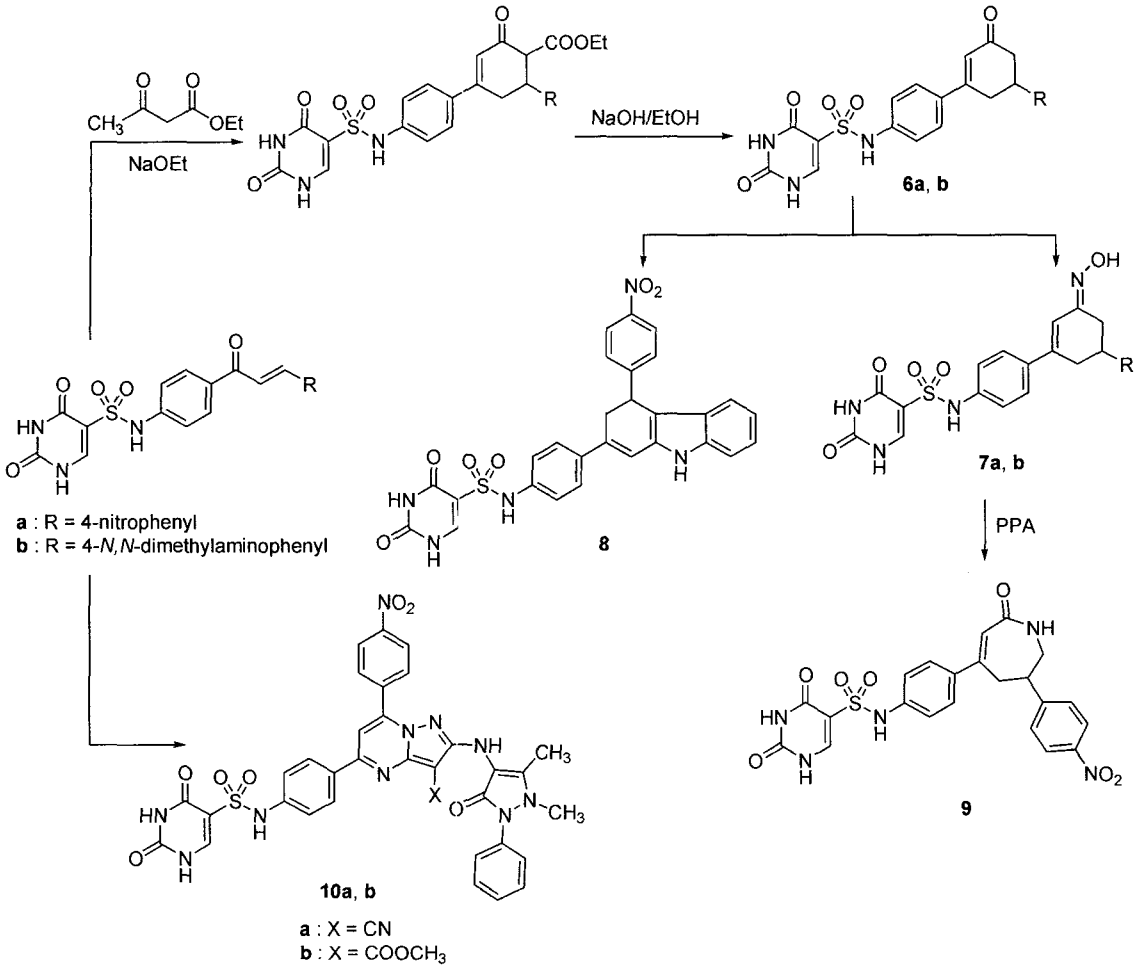
The oxime **7a** (0.007 mole) was heated with 60 g of polyphosphoric acid (1:1) for 10 min at 140 °C. The mixture was cooled and treated with water. The product was separated by extraction with chloroform which was dried over anhydrous sodium sulphate. After complete evaporation of chloroform, the residue was treated with hot benzene and charcoal. Recrystallization from acetic acid gave 30% of compound **9** (Tables I, II & Scheme 2).

Reaction of 4a, b with 1-phenyl-2,3-dimethyl-4-[5-amino-4-(cyano)-1*H*-pyrazolyl amino]-pyrazolin-5-one or 1-phenyl-2,3-dimethyl-4-[5-amino-4-(carbomethoxy)-1*H*-pyrazolylamino]- pyrazolin-5-one (10a, b)

A solution of **4a**, **b** (0.01 mol) and (0.01 mole the antipyrene derivatives) and 1 mL of pipridine in DMF (10 mL) were refluxed for 8 h. The mixture was cooled, most of DMF evaporated under vacuum, 20 mL of ethanol were added, filter, and recrystallized from DMF to give **10a**, **b** (Tables I, II & Scheme 2).

Testing for the molluscidal activity

Biomphalaria alexandrina snails (6-8 mm diameter) were collected from irrigation canals near Abou-Rauash, Giza Governorate, Egypt. Snails were tested for infection and infected ones were discarded. Snails were maintained in the laboratory under suitable conditions of aeriation feeding and temperature.



Scheme 2. Synthesis of new uracil-5-sulfonamide

Snails were divided into five groups. Group one kept as non-treated control. Group two, three, four and five were treated with chemical compounds **4a**, **10a, 10b** and **4b** respectively. The chemical compounds **4a**, **10a**, **10b** and **4b** were dissolved in tween 80 (T-80) and used in toxicity assay as aqueous suspension. Concentrations of 10 to 200 mL (part per million), weight per volume were prepared. A glass beaker containing 100 mL de-chlorinated tap water and 10 snails were used. The beaker was covered with a perforated plastic cover, The results were recorded after 3 to 24 h. following application of chemical compounds. Dead snails were counted and the relation between the concentration and the mortality rates were calculated. The selection of the concentration used was based on the predetermination dose which caused mortality of less than 50% of snails/24 h.

Hemolymph preparation

To obtain haemolymph, we removed the treated snails with chemical compounds (**4a**, **10a**, and **4b**) as well as non-treated snails from the beaker and put each group in petri-dishes without water. The snails surface was carefully cleaned with an ether-soaked swab and dried with a sterile cotton swab. After cleaning the area, the haemolymph obtained by cardiac puncture of each experimental group. It was kept on ice until used.

Detection of total protein content

The protein content of haemolymph obtained from treated and non-treated snails was determined colourimetrically according to Bradford (1976).

Infection and treatment

Fourty mice were exposed to 100 cercariae of *Schistosoma mansoni* strain by tail immersion method (Oliver and Stire Walt., 1952).

Mice were divided into five groups. Four weeks after exposure, three groups were injected intraperitioneally (i.p.) with treated haemolymph. Treated haemolymph was obtained from three groups of treated snails with chemical compounds **4a**, **10a**, and **4b** respectively. Fourth group of mice was injected by haemolymph obtained from non-

treated snails. Fifth group of mice was injected by phosphate buffer snails (PBS pH 7.3).

Anti schistosomal effect of treated haemolymph on *S. mansoni* infected mice

Two weeks post-treatment the experimental groups were sacrified by intraperitoneal injection of sodium pentobarbital and adult worms were recovered by portal perfusion (Duvall and Dewitt., 1967)). The rate of worm court reduction ® was calculated by comparing the mean worm burdens in control (C) and treated (T) mice using the formula :

$$R = (C - T)/C \times 100$$

The relative sex ratio of worm burdens perfused from both liver and intestine was calculated according to the formula of the known method (Fallon *et al.*, 1994).

The worms were collected and used for preparation of paraffin section according to (Afifi., 1986).

Indirect immuno-fluorescence (IF) staining of adult schistosome worms

The assay was done as described by (Nash., *et al.*, 1974) with some modification. The worm sections from the five groups were adhered on glass slides, covered with chronic mouse sera (1:100 in PBS-0.3% T-20) and incubated for 1 h in a humid chamber at room temperature The sections were washed three times for 5 min each with PBS-0.3% T-20 (washing buffer) and then covered with FITC-goat antimouse Ig G conjugate (Sigma) (1:50 in PBS-0.3% T-20-4% BSA). Worms were incubated for 1 h in a dark chamber and then washed three times with washing buffer. The slides were examined using a fluorescence microscope.

RESULTS AND DISCUSSION

No major changes have occurred during the past 20 years regarding the therapeutic tools available to the clinician for the treatment of Schistosomiasis (Cioli., *et al.*, 1995).

The current WHO model list of essential drugs for Schistosomiasis is metrifonate (tablet, 100 mg), oxamniquin (Capsule, 250 mg, Syrup, 250 mg in 5 mL) and praziquantel (Tablet, 600 mg). A syrup formulation of PZQ is now available from (EIPICO, Egypt) (Cioli., *et al.*, 1998).

Chemotherapy remains the cornerstone of intervention but rapid reinfection demands frequent re-treatment and emphasizes the need for a more long-term approach. The existence of at least partially protective immunity in exposed humans would make a vaccine a logical complement to during therapy (Bergquist and Colle., 1998).

In the present work, uracil-5-sulphonamide-*p*-phenyl derivatives were prepared through the reaction of uracil-5-sulphonylchloride (Fathalla, O.A, 1992) with *p*-amino-acetophenone to give 5-(*p*-acetylpheyl)uracilsulphonamide (**1**) followed by condensation with active methylenes namely, ethyl cyanoacetate or malononitrile and some aldehydes namely, 4-nitrobenzaldehyde or 4-*N*,*N*-dimethylamino-benzaldehyde in the presence of ammonium acetate to afford new cynopyridine derivatives **2a**,**b**, **3a**,**b** respectively (Tables I, II & Scheme 1).

Claisen-Schmidt condensation of **1** with aromatic aldehydes, namely, *p*-nitrobenzaldehyde, *p*-*N*, *N*-dimethyl amino-benzaldehyde, in presence of 10% sodium hydroxide in ethanol afforded the corresponding chalcones (Fathalla, OA 1992) **4a**,**b** (Table I, II & Scheme 1).

Compounds **4a**,**b** were allowed to react with ethylacetoacetate in presence of alcoholic sodium ethoxide solution to give oily products of ethyl-4-(uracil-5-sulphonamide)-2-oxo-3-cyclohexane-1-carboxylate (**5a,b**) which upon decarboxylation by heating with alcoholic sodium hydroxide afforded the corresponding substituted cyclohexanones namely, 5'-substituted-3-[uracil-5-sulphonamide]-2-oxo-cyclohexanes (**6a, b**) respectively in quantitative yields.

The reaction mixture of **6a, b** with ethanolic solution of hydroxylamine hydrochlodide in presence of sodium acetate affected the formation of the corresponding oximes **7a, b** respectively (Tables I, II & scheme 2).

Reaction of 4-nitrophenyl-3-[6-(uracil-5-sulphonamide)]-1-oxo-2-cyclohexane (**6a**) with phenylhydrazine in boiling acetic acid afforded the corresponding 1, 2-dihydro-1-(4-nitrophenyl)-3-[6-(uracil-5-sulphonamide)]carbazole (**8**) according to the reported method (Kamel *et al.*, 1996) (Tables I, II & Scheme 2).

Heating of the oxime **7a** with polyphosphoric acid underwent Beckmann rearrangement with the formation of 1,5,6,7-tetrahydro-6-(*p*-*N*,*N*-dimethylaminophenyl)-4-[(uracil-5-sulphonamide)]-2*H*-azepin-2-one (**9**) (Table I, II & Scheme 2).

Reaction of **4a,b** with 1-phenyl-2,3-diethyl-4-[5-amino-4-(cyano)-1*H*-pyrazolyl amino]-pyrazolin-5-one (Fathalla, O.A. and Zaki, M.E.A 1998)or 1-phenyl-2,3-dimethyl-4-[5-amino-4-(carbmethoxy)-1*H*-pyrazolylamino]-pyrazolin-5-one (Shalaby, A.M. *et. al.*, 1998) afforded products in type **10a, b** respectively. (Tables I, II & Scheme 2).

Effect of some new uracil derivatives on *Biomphalaria alexandrina* snails

The molluscicidal properties of four prepared chemical compounds **4a**, **10a**, **10b** and **4b** were investigated against *Biomphalaria alexandrina* snails.

The molluscicidal activity of prepared compounds effect on the snails was increased by increasing the concentration of the molluscicide solutions.

The observed results showed that the maximum snail death (100%) was recorded after exposuse to compounds **4a**, **10a**, **10b** and **4b** for 24 h with the concentrations of 60, 120, 500 and 60 ppm respectively.

The presented here reported that the prepared compounds **4a** and **4b** more active molluscides (LC_{50} at 50 ppm at 24 h) in comparison to the other prepared compounds **10a** and **10b** which are less active respectively. These observation suggested that this behaviour may be attributed to slower rate of release of active constituents of these prepared compounds or to their higher stability in the water. These data are agreement with (Abd el-Hamid and Rizk, 1997; Salama *et al.*, 1996).

Levels ol protective immunity in control and treated infected mice

S. mansoni infected mice were treated on the day 28th post infection with haemolymph obtained from treated *B. alexandrina* with new uracil derivatives (compounds **4a**, **10a**, and **4b**). Hemalymph obtained from non treated snails (H_o) treated infected mice had a 33.8 % reduction in worm burden compared to un-treated infected animals ($P < 0.05$) was observed. There was a greater mortality of female than male worms as shown with the elevated relative sex ratio (RSR). Hemolymph obtained from compound **4a** treated snails (**H4a**) reduced the worm burden by 44.36% with a significantly decreased in the mean number of worm burden as compared to control infected mice ($P<0.05$). There was no difference between the mortality of female and male.

The dose of haemolymph obtained from compound **10a** treated snails (**H10a**) reduced the worm burden by 34.6%. The mean worm burden was decreased significantly as compared to un-treated infected control ($P<0.05$) Hemolymph obtained from compound **10b**-treated snails (**H4b**) reduced the worm burden by (50.4%). In contrast to the death of predominatly female worms in the **H4a** and **H10a** groups, there was more male death in **H4b**-treated group, with a lowering in the RSR (Table IV).

There was a significant decrease in the mean worm recovery in **H4a** and **H4b** experimental groups as compared to **H0** groups but not significantly decreased ($p< 0.05$) in the mean worm burden in **H10a** group as compared to **H0** group.

Our results are in agreement with (Chiriboga *et al.*, 1971) who demonstrated that haemolymph obtained from *B. glaberta*, the snail vector of *S. mansoni*, exerts a curative effect when injected intraperitoneally into mice infected with *S. mansoni*. The curative effect is manifested by reduction in the number of worms. Some protection was obtained against *S. mansoni* infection in mice immunized with *B. pfeifferi* hepatopancreas treated with ascorbic acid copper sulphate (Dodin., 1966; Schwick *et al.*, 1980) Study the effect of homogenate of hepatopancreas of *B. alexandrina* reduced worm burden (57.6%) after exposure to *S. mansoni*. The extract of *B. alexandrina* previously treated with thioxanthens derivatives was induced significant reduction in the mean number of worm burden (El-

Table III. Percentage of mortality at 24 h of the molluscicidal activity of the prepared compounds on *Biomphalaria alexandrina* Snails

Conc.of moll. Sol. (ppm)	4a	10a	10b	4b
10	30	–	–	30
20	30	–	–	30
30	40	–	–	40
40	40	–	–	40
50	50	–	–	50
60	100	30	–	100
70	–	30	–	–
80	–	40	–	–
90	–	40	–	–
100	–	50	–	–
120	–	100	–	–
140	–	–	20	–
160	–	–	20	–
180	–	–	20	–
200	–	–	50	–
300	–	–	60	–
400	–	–	70	–
500	–	–	100	–

Table IV. Mean worm recovery of *Schistosoma mansoni* infected mice treated with haemolymph obtained from chemically treated *B. alexandrina* snails.

Experimental groups	Mean worm+ Recovery (± S.D)	RSR++	Protection %	P+++ value
Cont. Untreated	66 ± 11.5	1	–	–
H^0	44 ± 4	1.3	33.8	0.05
H^{4a}	37 ± 1	1	44.36	0.05
H^{10a}	43 ± 1.5	1.2	34.6	0.05
H^{4b}	33 ± 3	0.9	50.4	0.5

*Experimental group was treated by un-chemically modified haemolymph.

Experimental group was treated by chemically modified haemolymph using compound **4a.

***Experimental group was treated by chemically modified haemolymph using compound **10a**.

****Experimental group was treated by chemically modified haemolymph using compound **4b**.

+SD = Standard deviation of the difference of the means.

++RSR = Relative sex ratio.

+++Student's test (P<0.05).

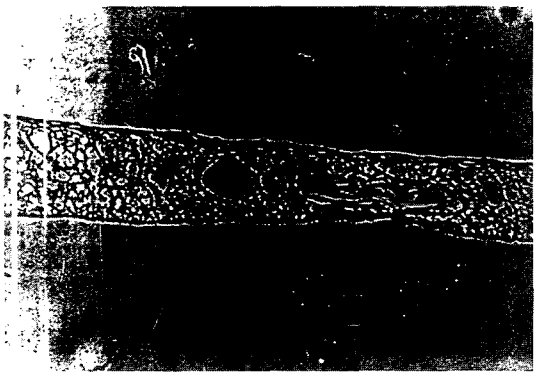


Fig. 1. T.S. in adult *Schistosoma mansoni* worms obtained from mice treated with hemolymph of *Biomphalaria alexandrina* snails treated with chemical compounds **4a**, **10a** and **4b**.

Fig. 2. Indirect Immunofluorescent reaction on the tegumental surface and gut of *S. mansoni* adult worms obtained from mice non treated with hemolymph or treated with hemolymph treated with chemical compounds **4a**, **10a**, and **4b**.

Hamsnary *et al.*, 1996).

Discoous showed that snails haemolymph was reduced the number of worms in vaccinated mice.

Recently, *S. mansoni* glyceraldehyde-3-phosphate dehydrogenase (Sm37-GAPDH) is reported as one of the main Schitosoma-vaccine candidates and as a target for chemotherapeutic treatments (Argiro *et al.*, 2000).

Mice were vaccinated with recombinant *S. japonicum* cathepsin D aspartic protease. Mean total worm burden were significantly reduced in vaccinated mice by 21-38%, and significant reductions in female worm burdens were also reduced 22-40% (Verity *et al.*, 2001).

In our study, the preferential mortality of female worms as a result of **H4a** and **H10b** treatment is agreement with the study that the effect of praziquantel on female worms (Fallon *et al.*, 1994).

It is concluded that the haemolymph obtained from treated snails with compound **H4a** and **H4b** induced protection against *S. mansoni* infection (44.36% and 50.4% respectively).

Light microscopy observations

Sections of 6 mm thickness were cut and stained with hematoxylin and eosin. There was no changes have been observed in the structure of the tegumental and subtegumental tissues of male and female worms, Post-treatment with haemolymph obtained from a chemically modified *B. alexandrina* snails with compounds **4a**, **10a**, and **4b** as compared with non-treated group Fig. 2.

Indirect immunoflorescence detection

The fluorescent reaction was used to detect immunogenicity on the surface tegument of adult treated worms as a result of treatment.

The fluorescent reaction observed with adult *S. mansoni* worms of infected untreated group as well as **4a**, **10a**, and **4b** groups. There was a binding of CMS with different parts of the worms was intense in the gut epithelium tubercles, tegument and subtegumental musculature of worms normal mouse sera (NMS) showed a negative fluorescent reaction with all treatments (Fig. 2).

These observation are in agreement with many investigations, where praziquantel (PZQ) has been shown to increase parasite antigens exposure at the surface of both juvenile and adult scistosomes both *in vivo* and *in vitro* (Fallon *et al.*, 1996).

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