Arch Pharm Res Vol 23, No 2, 128-138, 2000 Medicinal Chemistry & Natural Products

Synthesis of New Uracil-5-Sulphonamide-*p*-Phenyl Derivatives and Their Effect on *Biomphalaria alexandrina* Snail's Nucleoproteins

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(Received June 1, 1999)

In continuation of the previous work (Fathalla, 1992) on the synthesis of some heterocycles containing uracil moiety, we report herein the incorporation of uracil moiety into cyanopyridine thione, thiosemecarbazone, semicarbazone, cyanopyridine, aminocyano pyridine, isoxazoline, pyrazoline, pyrimidine, triazolo pyrimidine, pyran, selena and thiazole derivatives which might modify their biological activities.

The biological studies revealed that the chemical compound **III f** showed high molluscicdal activity than other compounds. The profile of the nucleoprotein extracted from chemically (compound **IIIc, e, f** and **g**) treated or UV-irradiated *B.alexandrina* snails did not show appreciable differences when compared to non-treated (native) snails by using SDS-PAGE, where no obvious qualitative or quantitative differences were observed. Immunization of experimental animals with the nucleoprotein extracted from native, chemically (compound **III f** & **g**) treated or physically treated *B.alexandrina* snails induced significant protection against challenge with normal *S.mansoni* cercariae, as compared to the non-immunized challenged control. As well as , a decrease in the number of granuloma formation and the size range of granuloma was also observed in immunized animals. It is concluded that, compounds **III f** and **g** have a potent molluscicidal activity. They also induced chemical modification comparable to that induced by physical treatment in the snail's nucleoprotein, which could possibly be used in immunization against *S.mansoni* infection.

Key words: Uracil-5-sulphonamide-*p*-phenyl derivatives, *Biomphalaria alexandrina* Snails, Nucleoproteins

INTRODUCTION

This study was undertaken in view of the fact that several 5-substituted uracils and 1,3-dimethyluracils possess chemotherapeutic importance especially against cancer and bacteria (Abdel-Hamid, Fathalla 1993, and Fathalla, 1992). Also it was found that α,β-unsaturated ketones and chalcones have hemotherapeutic activity (Kamell *et al.*,1985 and Ebeid *et al.*,1991). Besides, it has been reported that hyrazones (Hassaneen *et al.*, 1995) possess strong biological activity against microorganisms.

MATERIALS AND METHODS

All melting points are uncorrected and were determined in capillary tube. The IR spectra were recorded in potassium bromide on a Backman Infrared Spectrometer Model PU 9712 using KBr discs. The ^{1}H NMR spectra were obtained on Joel EX 270 MHz Spec-trometer with tetramethylsilane as an internal standard. The Mass spectra (MS) were recorded on SSQ 7000 Mass Spectrometer.

5-(*p*-Acetylphenyl)uracilsulphonamide (II)

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

5-(*p*-Phenyl-4`-aryl-3`-cyano-2`-mercapto-6`-pyridyl)uracilsulphonamide derivatives III a-g

A mixture of (0.003 mole) of **II** and (0.003 mole) of the appropriate aldehyde and (0.003 mole) of thiocyanoacetamide and (0.021 mole) of ammonium acetate in (50 ml) *n*-butanol was refluxed for 8-12 h. The reaction mixture was concentrated till its half volume, then cooled and left over night. The precipitate was filtered off, dried then recrystallized from the proper solvent to give the

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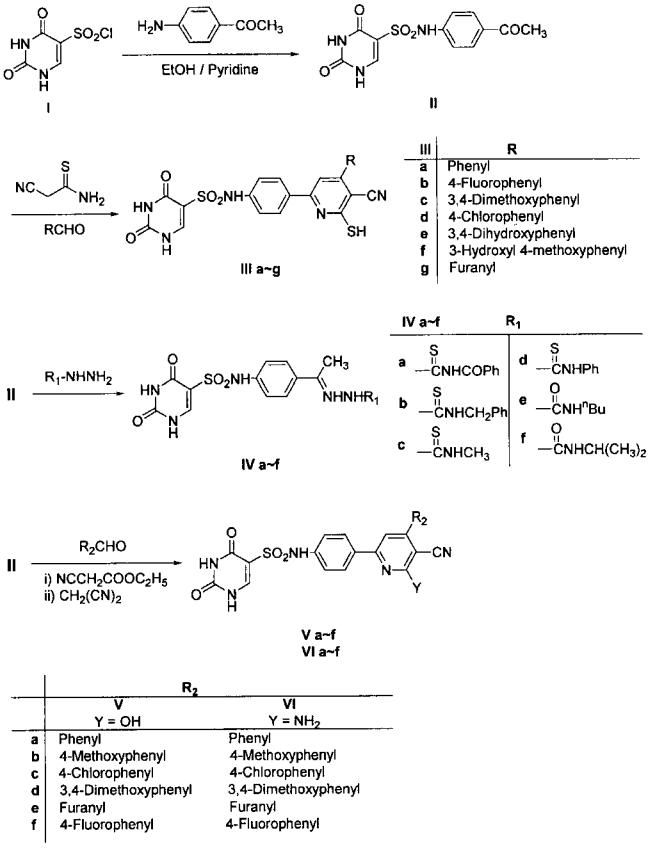


Scheme 1. Preparation of Compounds **II**~**VI**

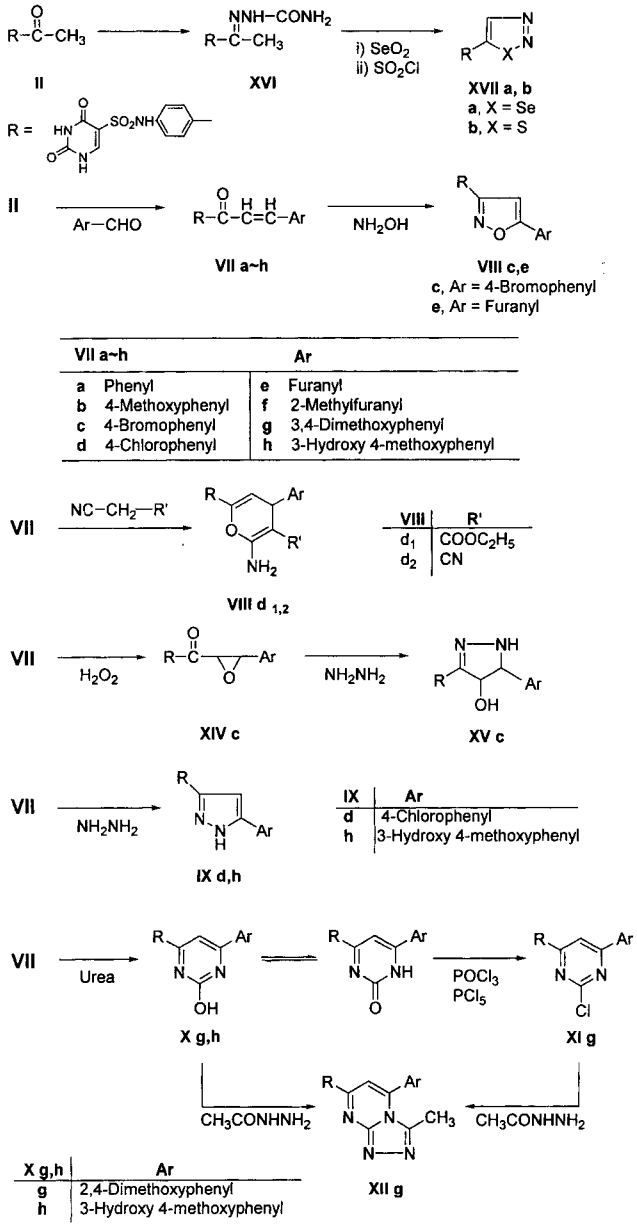
corresponding pyridine-2(1*H*)-thions of type **III a-j** (Scheme 1 and Table I & II).

Semicarbazone and thiosemicarbazone derivatives IV a-f

A mixture of compound **II** (0.003 mole) and (0.003 mole) the appropriate alkyl semicarbazides and / or aryl or alkyl thiosemicarbazides in (40 ml) absolute ethanol containing a few drops of piperidine was refluxed for 8-14 h. By cooling, the formed product was filtered off, dried then recrystallized from the proper solvent to give the title compounds of type **IV a-f** (Scheme 1 and Table I & II).

5-(*p*-Phenyl-4`-aryl-3`-cyano-2`-hydroxy-6`-pyridinyl)uracilsulphonamide and 5-(*p*-phenyl-2`-amino-4`-arylpyridyl-3`-cyano-2`-hydroxy-6`-pyridinyl)uracilsulphonamide derivatives V a-f , VI a-f

A mixture of **II**(0.003 mole), the appropriate aldehyde (0.003 mole), ethyl cyanoacetate or malononitrile (0.021 mole) and ammonium acetate in *n*-butanol (50 ml) was refluxed for 8-12 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 (Scheme 1 and Table I & II).



Scheme 2. Preparation of Compounds **VII**~**XVII**

5-(*p*-Phenyl-4-cinnamyl)uracilsulphonamie derivatives VII a-h

They were prepared according to the known method (Essawy and Wasfy, 1994).

5-(p-Phenyl-5`-arylisoxazol-3`-yl)uracilsulphonamide VIII

A solution of **VII c,e** (0.005 mole) and hydroxylamine hydrochloride (0.005 mole) in ethanol (20 mL) containing sodium hydroxide (0.4 g) was refluxed for 8-12 h. The product was isolated by concentration of the alcoholic solution, to give **VIII c,e** on recrystallisation from the proper solvent (Scheme 2 and Table I & II).

Pyrazoline derivatives IX d, h

The compounds **IX d, h** were prepared by known method (Essawy and Wasfy 1994).

Pyrimidine derivatives Xg,h

An alcoholic solution of urea (0.01 mole)in (10 ml) of ethanol was added to a solution of VII g, h (0.01 mole) and potassium hydroxide (0.2 mole) in absolute ethyl alcohol and the mixture was refluxed for 6 h. The solvent was removed under reduced pressure and the residue dissolved in water (50 ml). After neutralization with HCl solution, the precipitate was recrystallized (Table I & II).

2-Chloropyrimidine (XI g)

A mixture of compound **Xg** (0.01 mole) and PCl_5 (0.01 mole) in $POCl_3$ (20 ml) was heated on a steam bath for 3 h and the reaction mixture poured gradually onto crushed ice. The solid obtained was recrystalized to give the 2-chloropyrimidine derivative as pale yellow powder (Table I & II).

Triazolo[3,4-b]pyrimidine (XII g)

A mixture of compound **Xg** (0.01 mole) and acetylhydrazide (0.01 mole) in *n*-butanol (50 ml) was heated under reflux for 24 h. The solid that separated after cooling was filtered off, to give pall yellow needles. An authentic sample of **XIIg** was prepared by the known metheds (Essawy and Wasfy, 1994) mp and mixed mp. determination showed no depression.

Pyran derivatives XIII d 1, 2

A mixture of chalcone **VIId** (0.01 mole), ethyl cyanoacetate and/or malononitrile (0.01 mole) and pyridine (20 ml) was refluxed for 5 h, cooled and poured onto ice-HCl solution. The solid product formed was collected by filteration and crystallized from the proper solvent (Table I & II).

The epoxidation of chalcone (XIVc)

A solution of chalcone **VIIIc** (0.01 mole) in acetone (50 mL) and methyl alcohol (15 ml) was mixed with 8% aqueous sodium hydroxide (12 ml) followed by the addition of hydrogen peroxide (30%, 5 ml). The solution was shaken and heated to the boiling point during 1 h, then allowed to stand overnight at room temperature, Water was then added and the solution extracted with ether. The ether layer was evaporated and the residue was recrystallized from petroleum ether (b.p. 60-80°) to give white needles (m.p. 105-107°) in 67% yield.

4-Hydroxypyrazoline derivative XVc

The epoxide **VIIIc** (0.01 mole) and hydrazine hydrate (0.01 mole) were refluxed in ethanol for 10h. The solid that separated on cooling, was filtered and recrystallized from the proper solvent to give **XVc**. (Table I & II).

Semicarbazone XVI

To a solution of **II** (0.01 mole) in ethanol (50 ml) was added a solution of semicarbazide hydrochloride (0.01 mole) and sodium acetate (0.02 mole) in water (20 ml). The reaction mixture was refluxed for 1h, evaporated to half of its volume and then poured onto ice-water. The separated solid was filtered off, washed with water, dried and recrystallized from acetic acid to give **XVI** as pale yellow crystals (m.p >300).

1,2,3-Selenadiazole (XVIIa)

The semicarbazide **XVI** (0.56 mole) was dissolved in boiling glacial acetic acid (40 ml) and powdered selenium dioxide (0.9 gm) added portionwisely with stirring. After complete addition, the reaction mixture was refluxed during stirring for 1h, cooled and poured onto ice-water and extracted with ether, the extract was washed with 10% $NaHCO_3$ solution and water, and then dried over anhydrous ($MgSO_4$). The ether was removed and the residue was recrystallized from dimethylformamide/water (mp>300).

1,2,3-Thiazole (XVIIb)

Thionyl chloride (10 ml) was gradually added to the semicarbazone **XVI** (0.005 mole) and the mixture was gently warmed and then left 24 h at room temperature. An $NaHCO_3$ ice-cooled saturated solution was then added and the product was extracted with ether, and the extract was warked up as usual. The residue was crystallized from dimethylformamid/water as yellow crastals.

Snails

Biomphlaria alexandrina snails (6-8 mm diameter) were used thoughout the present study. Snails were collected from irrigation canals near Abou-Rawash, Giza Governorate, Egypt. Snails were tested for infection and infected ones were discarded. The non-infected snails were maintained in ambient temperature in white plastic containers full to their 2/3 with dechlorinated water which was well aerated. Snails were divided into six major groups. Group one kept as non-treated control. Group two, subjected to UV irradiation. Group thee, four, five and sex were treated with chemical compounds **III c, e, f** and **g**, respectively.

Molluscicidal activity of the testing compounds

Compounds **III c, e, f** and **g** were dissolved in tween-80 and used in toxicity assay as aqueous suspension. Concentrations of 10 to 500/ml(ppm), weight/volume were prepared. A glass beaker containing 100 ml dechlorinated tap water and 10 snails were used. Beaker were covered with a perforated plastic cover. The results were recorded after 24 h. Dead snails were counted and the relation between the concentration and the mortality

Table I. Analysis and physical data for compounds

Comp. No.	Yield %	M.p°C of Cryst.	Sol.	Mol. Formula/ Mol. Wt	Analysis Calcd./Found% C	H	N
II	72	260-262	A	$C_{12}H_{11}N_3O_5S$ (309.30)	46.60 46.34	3.59 3.31	13.59 13.42
IIIa	71	>300	A	$C_{22}H_{15}N_5O_4S_2$ (477.51)	55.33 55.26	3.17 3.01	14.67 14.47
IIIb	75	>300	A	$C_{22}H_{14}FN_5O_4S_2$ (495.50)	53.32 53.09	3.85 3.54	14.14 14.01
IIIc	67	>300	A	$C_{24}H_{19}N_5O_6S_2$ (537.56)	53.62 53.43	3.56 3.24	13.03 13.12
IIId	65	>300	A	$C_{22}H_{14}ClN_5O_4S_2$ (511.96)	51.61 51.34	2.76 2.43	13.68 13.52
IIIe	71	>300	A	$C_{22}H_{15}N_5O_6S_2$ (509.51)	51.86 51.54	2.97 2.65	13.75 13.63
IIIf	67	>300	A	C23H17N5O6S2 (523.54)	52.76 52.46	3.27 3.15	13.45 13.32
IIIg	65	>300	A	$C_{20}H_{13}N_5O_5S_2$ (467.47)	51.38 51.27	2.80 2.61	14.98 14.65
IVa	64	>300	DMF	$C_{20}H_{18}N_6O_5S_2$ (486.52)	49.37 49.24	3.37 3.23	17.28 17.12
IVb	63	>300	DMF	$C_{20}H_{20}N_6O_4S_2$ (472.54)	50.83 50.53	4.27 4.12	17.79 17.54
IVc	64	>300	DMF	$C_{14}H_{16}N_6O_4S_2$ (396.44)	42.41 42.19	4.07 4.04	21.20 21.07
IVd	70	>300	DMF	$C_{19}H_{18}N_6O_4S_2$ (458.51)	49.77 49.52	3.96 3.65	18.33 18.21
IVe	62	>300	DMF	$C_{17}H_{22}N_6O_5S$ (438.47)	46.56 46.32	5.06 5.01	19.17 19.04
IVf	64	>300	DMF	$C_{16}H_{20}N_6O_5S$ (408.44)	47.05 47.01	4.94 4.71	20.58 20.41
Va	67	>300	M	$C_{22}H_{15}N_5O_5S$ (461.46)	57.26 57.19	3.28 3.13	15.18 15.09
b	65	>300	M	$C_{23}H_{17}N_5O_6S$ (491.48)	56.20 56.14	3.49 3.25	14.25 14.15
c	64	>300	M	$C_{22}H_{14}ClN_5O_5S$ (495.90)	53.28 53.19	2.85 2.54	14.12 14.07
d	65	>300	M	$C_{24}H_{19}N_5O_7S$ (521.50)	55.27 55.16	3.67 3.45	13.43 13.32
e	63	>300	M	$C_{20}H_{13}N_5O_6S$ (451.41)	53.21 53.04	2.90 2.75	15.52 15.35
f	65	>300	M	$C_{22}H_{14}FN_5O_5S$ (479.44)	55.11 55.04	2.94 2.76	14.61 14.34
VI a	68	>300	A	$C_{22}H_{16}N_6O_4S$ (460.47)	57.29 57.38	3.47 3.50	18.14 18.25
b	64	>300	A	$C_{23}H_{18}N_6O_5S$ (490.49)	56.32 56.32	3.49 3.70	17.03 17.14
c	69	>300	A	$C_{22}H_{15}ClN_6O_4S$ (494.92)	53.27 53.39	3.02 3.06	17.00 16.98
d	65	>300	A	$C_{24}H_{20}N_6O_6S$ (520.52)	55.38 55.13	3.87 3.54	16.15 16.36
e	62	>300	A	$C_{20}H_{14}N_6O_5S$ (450.43)	53.54 53.33	3.09 3.13	18.46 18.66
f	76	>300	A	$C_{22}H_{15}FN_6O_4S$ (478.46)	55.17 55.22	3.06 3.16	17.36 17.57
VII a	71	>300	DMF	$C_{19}H_{15}N_3O_5S$ (397.40)	60.14 60.02	3.80 3.49	10.57 10.35
b	70	>300	DMF	$C_{20}H_{17}N_3O_6S$ (427.43)	56.20 56.02	4.01 4.31	9.83 9.58

Table I. Continued

Comp. No.	Yield %	M.p°C of Cryst.	Sol.	Mol. Formula/ Mol. Wt	Analysis Calcd./Found% C	H	N
c	65	>300	DMF	$C_{19}H_{14}BrN_3O_5S$ (476.31)	47.91 47.74	2.96 2.75	8.82 8.76
d	72	>300	DMF	$C_{19}H_{14}ClN_3O_5S$ (431.85)	52.84 52.65	3.27 3.15	9.73 9.56
e	75	>300	DMF	$C_{17}H_{13}N_3O_6S$ (387.36)	52.71 52.51	3.38 3.24	10.85 10.56
f	65	>300	DMF	$C_{18}H_{15}N_3O_6S$ (401.39)	53.85 53.59	3.77 3.54	10.47 10.32
g	70	>300	DMF	$C_{21}H_{19}N_3O_7S$ (457.45)	55.13 55.01	4.19 4.02	9.19 9.32
h	69	>300	DMF	$C_{20}H_{17}N_3O_7S$ (443.43)	54.17 54.03	3.86 3.51	9.48 9.36
VIII c	63	>300	A	$C_{19}H_{13}BrN_4O_5S$ (489.31)	46.64 46.38	2.68 2.43	11.45 11.24
e	62	>300	A	$C_{17}H_{12}N_4O_6S$ (400.37)	50.99 50.71	3.02 3.12	13.99 13.67
IX d	65	>300	A	$C_{19}H_{14}ClN_5O_4S$ (443.87)	64.92 64.71	3.18 3.01	15.78 15.45
IX h	67	>300	A	$C_{20}H_{17}N_5O_6S$ (455.45)	52,74 52.53	3.76 3.43	15.38 15.23
X g	76	>300	A	$C_{22}H_{19}N_5O_7S$ (497.48)	53.11 53.03	3.85 3.48	14.08 14.01
X h	70	>300	A	$C_{21}H_{17}N_5O_7S$ (483.46)	52.17 52.03	3.55 3.34	14.49 14.23
XI g	68	>300	A	$C_{22}H_{18}ClN_5O_6S$ (515.93)	51.21 51.01	3.52 3.32	13.58 13.42
XII g	65	>300	A	$C_{24}H_{21}N_7O_6S$ (535.54)	53.82 53.54	3.95 3.61	18.31 18.20
$XIII_1$	64	>300	A	$C_{24}H_{20}ClN_4O_7S$ (543.96)	52.99 52.74	3.71 3.51	10.30 10.24
$XIII_2$	56	>300	A	$C_{22}H_{15}ClN_5O_5S$ (496.91)	53.17 53.03	3.04 3.12	14.10 14.03
XV c	68	>300	A	$C_{19}H_{14}BrN_5O_5S$ (504.33)	45.25 45.31	2.80 2.52	13.89 13.61
XVI	65	>300	A	$C_{13}H_{14}N_6O_5S$ (366.36)	42.62 42.32	3.85 3.45	22.94 22.53
XVII a	64	>300	DMF/Wt	$C_{12}H_9SeN_5O_4S$ (398.26)	36.19 36.02	2.28 2.15	17.59 17.43
XVII b	67	280-282	DMF/Wt	$C_{12}H_9N_5O_4S_2$ (351.36)	41.02 41.13	2.02 2.23	19.93 19.54

A=acetic acid, M=methanol & DMF=dimethyl formamide

rates was calculated. The selection of the concentration used was based on the predetermined dose which caused mortality of less than 50% of snails/24 h.

Extraction of nucleoproteins

Extraction of nucleoproteins from native β. *alexandrina* snails either subjected to UV-irradiation or treated with chemical compounds **IIIc, e, f** and **g** was carried out according to the known method (Nabih, 1981). The protein content in the prepared nucleoprotein from the six-experimental groups was determined by Bradford's

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

Comp. No.	IR (KBr) cm^{-1}	Mass (R.I)	^{1}HNMR (DMSO-d_6), p.p.m.
II	3450(NH), 3250, 2980(C-H aromatic), 1725 (-$COCH_3$ aromatic), 1710,1690 (2 CO of uracil),1350,1325 (-N-SO_2) and 1200 for (-SO_2-).	309.5	3.4(3H,s,Ar-$COCH_3$), 7.4-7.9(4H,dd,aromatic), 8.1(1H,s, of uracil), 10.6(1H,s, NH exchangeable with D_2O).
III a	3410(NH), 3310, 3150 (C-H aromatic), 2210(CN), 1700, 1690 (2CO of uracil), 1340, 1320(-N- SO_2-) and 1200 for (-SO_2-).	477.8	2.1(1H,s,S-H),6.8-7.2(4H,dd, aromatic),7.4 (1H,s, aromatic), 7.4-7.9(5H,m,phenyl), 8.3(1H, s, of uracil), 10.6(1H.s, NH exchangeable with D_2O).
III c	3350(NH), 3260, 3100 (C-H aromatic), 2208(CN), 1705, 1695 (2CO of uracil), 1345, 1325(-N-SO_2-) and 1200 for (-SO_2-).	537.9	2.1(1H,s,S-H), 3.3(3H,s, OCH_3), 3.5(3H,s, OCH_3), 6.9-7.3 (4H,dd, aromatic), 7.6-8(4H,t, aromatic), 8.2(1H,s, of uracil), 9.5(1H,s, NH exchangeable with D_2O).
III e	3610, 3500, 3420,(2OH), (NH), 3250, 3210 (C-H aromatic), 2210 (CN), 1710, 1695 (2CO of uracil), 1335, 1325 (-N-SO_2-)and 1200 for (-SO_2-).	509.4	2.2(1H,s, S-H), 6.5-7.2(4H,dd, aromatic), 7.5-8.2(4H, t, aromatic), 8.3(1H,s, of uracil), 9.1, 10.4, 11.2(3H,s,NH, 2 OH exchangeable with D_2O).
IV a	3390(NH), 3200, 3150 (C-H aromatic), 2215(CN), 1705 (CO), 1690,1685(2CO of uracil), 1335, 1320 (-N- SO_2-) and 1200 for (-SO_2-).	486.7	2.4(3H,s,C-CH_3), 6.5-7.2(4H,dd, aromatic), 8.1(1H,s,of uracil), 7.3-8 (5H,m, aromatic) 9.3,10.2,11.3(3H,s, 3NH exchangeable with D_2O).
IV d	3410 (NH), 3210, 3131(C-H aromatic), 1685,1602 (2 CO of uracil),1360, 1332(-N-SO_2-)and 1200 for (-SO_2-).	458.6	2.4(3H,s,C-CH3), 6.3-7.1(4H,dd, aromatic), 8.2(1H,s, of uracil), 7.3-8.1(5H,m, aromatic), 9.2, 10.3,11.5(3H,s, 3NH exchangeable with D_2O).
V b	3272(NH), 3200, 3110 (C-H aromatic), 2216 (CN), 1700 (CO), 1695, 1685 (2CO of uracil), 1363, 1332 (-N-SO_2-) and 1200 for (-SO_2-).	491.3	3.3(3H,s, OCH_3), 6.1-7(4H,dd,aromatic), 6,3-7.2(4H, dd, aromatic), 7.4 (1H,s, -C=CH-), 8.3 (1H,s,of uracil), 9.4, 11.1 (2H, s, NH, OH exchangeable with D_2O).
V c	3280 (NH), 3210, 3150 (C-H aromatic), 2210(CN), 1710 (CO), 1695,1685 (2CO of uracil), 1350, 1320 (-N-SO_2-) and 1200 for (-SO_2-).	496.2	6,3-7.2 (4H, dd, aromatic), 6.1-7 (4H, dd, aromatic) 7.5 (1H,s, -C=CH-), 8.2(1H,s, of uracil), 8.6,10.2(2H,s, NH, OH exchangeable with D_2O).
V d	3240(NH), 3290, 3040(C-H aromatic), 2213(CN), 1710 (CO), 1694, 1680 (2CO of uracil), 1350, 1320 (-N-SO_2-) and 1200 for (-SO_2-).	521.5	3.2, 3.3(6H,s, OCH_3), 6.3-7.2(7H,t, aromatic) 7.4(1H,s,-C=CH-), 8.2(1H,s,of uracil), 9.3(1H, s, NH exchangeable with D_2O), 10.5(1H, s, OH exchangeable with D_2O).
VI b	3450, 3340, 3220(NH_2, NH), 3205, 3115(C-H aromatic), 2215(CN), 1710, 1695(2CO of uracil), 1325, 1310(-N-SO_2-) and 1200 for (-SO_2-).	490.7	3.4(3H,s, OCH_3), 5.9(2H,b, NH_2 exchangeable with D_2O) 6.2-7(4H, dd, aromatic), 6.4-7.1(4H,dd, aromatic), 7.5 (1H, s,-C=CH-), 8.1(1H,s, of uracil), 9.1(1H,s, NH exchangeable with D_2O).
VI c	3430, 3350, 3210(NH_2,NH), 3200, 3150(C-H aromatic), 2210(CN), 1710, 1690 (2CO of uracil), 1325,1318(-N-SO_2-) and 1200 for(-SO_2-).	494.9	6.1(2H,b, NH_2 exchangeable with D_2O), 6.3-7.2(4H, dd, aromatic), 6.4-7.1(4H, dd, aromatic), 7.3(1H,s,-C= CH-), 8.2(1H,s,of uracil), 9.3(1H, s, NH exchangeable with D_2O).
VI f	3440, 3320, 3220(NH_2,NH), 3200, 3160(C-H aromatic), 2218(CN), 1710,1690 (2CO of uracil),1325, 1320(-N-SO_2-) and 1200 for (-SO_2-).	478.4	5.9(2H,b, NH_2 exchangeable with D_2O) 6.3-7(4H,dd, aromatic), 6.4-7.1(4H,dd, aromatic) 7.4(1H,s,-C=CH-), 8.2(1 H, s, of uracil), 9.3 (1H, s, NH exchangeable with D_2O).
VII b	3423(NH), 2942, 2838(C-H aromatic), 1710(-COCH-) 1690, 1656 (2CO of uracil), 1328, 1282 (-N-SO_2-),1200 for (-SO_2-).	427.8	3.3(3H,s, OCH_3), 6.1-7(4H,dd, aromatic),,6.3-7.2(4H, dd, aromatic), 6.3,7.4(2H,s, -CH=CH-), 8.2(1H,s, of uracil), 9.3 (1H, s, NH exchange-able with D_2O).
VII f	3340(NH), 3010, 2890(C-H aromatic), 1720(-CO CH-), 1700, 1690(2CO of uracil), 1320, 1230(-NSO_2-), 1200 for (-SO_2-).	401.5	2.3(3H,s,C-CH_3), 5.8, 6.9(2H,s,-CH=CH-), 6.1, 6.5 (2H, s, 2-C=CH-), 6.3-7.2(4H,dd, aromatic), 8.2(1H,s,of uracil), 9.3(1H ,s, NH exchangeable with D_2O).
VII h	3405(NH) 3150, 2910(C-H aromatic), 1715(-CO CH-), 1700, 1695(2CO of uracil), 1315, 1240(-N-SO_2-) and 1200 for (-SO_2-).	443.7	3.2(3H,s, OCH_3), 6.3-7.2(7H,m, aromatic) 8.2(1H,s,of uracil), 9.3 (1H, s, NH exchangeable with D_2O), 10.5 (1H, s, OH exchangeable with D_2O).
VIII e	3425(NH), 3160, 2920(C-H aromatic), ,1705, 1690 (2CO of uracil), 1620 (C=N), 1315, 1240 (-N-SO_2-) and 1200 for (-SO_2-).	400.9	6.1-7.5(4H, dd, aromatic), 6.5-7.6(4H, m, aromatic), 8.2 (1H,s, of uracil), 9.3(1H, s, NH exchangeable with D_2O), 10.3 (1H,s, OH exchangeable with D_2O).
IX h	3510(OH), 3395(NH), 3240, 2910(C-H aromatic), 1700, 1895 (2CO of uracil) 1620 (C=N),1315, 1240 (-N-SO_2-) and 1200 for (-SO_2-).	455.8	3.4(3H,s, OCH_3), 6.2,7(7H, m, aromatic), 6.5(1H,s, -CH =C-), 8.1(1H,s, of uracil), 9.1 (1H ,s, NH exchangeable with D_2O), 10.5(1H,s, OH exchangeable with D_2O)..
X g	3420(NH), 3240, 2930 (C-H aromatic), 1720(-CONH-), 1710, 1695 (2CO of uracil), 1315, 1240 (-N-SO_2-) and 1200 for (-SO_2-).	497.8	3.1, 3.4(6H,s, OCH_3), 6.1-7.3(8H,m, aromatic), 8.1(1H,s, of uracil), 8.5, 9.3(2H,s, 2NH exchangeable with D_2O).
XI g	3405(NH), 3150, 2923(C-H aromatic), ,1700, 1685 (2CO of uracil), 1315, 1240(-N-SO_2-) and 1200 for (-SO_2-).	515.2	3.1, 3.4(6H, s, 2 OCH_3), 6.2,7(4H,dd, aromatic), 7.1-7.5(4H,m, aromatic), 8.1(1H,s, of uracil), 9.1(1H,s, NH exchangeable with D_2O).

Table II. Continued

Comp. No.	IR (KBr)cm^{-1}	Mass (R.I)	^{1}HNMR (DMSO-d_6), p.p.m.
XII g	3405(NH), 3150, 2910(C-H aromatic), ,1700, 1895 (2CO of uracil), 1620(C=N), 1315, 1240 (-N-SO_2-) and 1200 for (-SO_2-).	535.8	2.3(3H, s, C-CH_3), 3.2,3.6(6H,s, 2OCH_3), 6.1-7.5(8H,m, aromatic), 8.2(1H, s, of uracil), 9.1(1H,s, NH exchgangeable with D_2O).
XIII d2	3405, 3320(NH,NH_2), 3150, 2910(C-H aromatic), 2215(CN), 1700, 1895 (2CO of uracil), 1315, 1240(-N-SO_2-) and 1200 for (-SO_2-).	496.9	6.2-7.6(9H, m, aromatic),), 6.4(2H,b, NH_2) 8.3(1H,s, of uracil), 9.5(1H,s, NH exchgangeable with D_2O).
XV c	3410, 3320(2NH), 3210, 2950(C-H aromatic), 1690, 1675(2CO of uracil), 1340, 1240(-N-SO_2-), 1200 for (-SO_2-).	504.5	6.1-7.3(4H, dd, aromatic), 6.3-7.5(4H, dd, aromatic), 8.1(1H,s, of uracil), 8.6, 9.1(1H,s, 2NH exchangeable with D2O), 10.2(1H, s, OH exchgangeable with D_2O).
XVI	3420-3150(NH_2), (NH), 1790, 1650(CO of uracil), 1640(NH_2), 1620(C=N), 1315, 1250(-N-SO_2-) and 1200 for (-SO_2-).	366.3	2.4(3H, s, C-CH_3), 5.8(2H, b, NH_2), 6.4-7.1(4H, dd, aromatic), 8.1(1H,s, of uracil), 9.1, 10.2(2H,s, 2NH exchangeable with D_2O).
XVII b	3410(NH), 2925(C-H aromatic), 1705, 1690 (2CO of uracil), 1620(C=N), 1340, 1250(-N-SO_2-), 1200(-SO_2-) and 765-700 for (C-S).	351.5	6.5(1H,s, -CH=C-), 6.7-7.4(4H, dd, aromatic), 8.2(1H, s, of uracil), 9.5(1H, s, NH exchangeable with D_2O).

method (Bradford, 1967).

Sodium dodecyl sulphate-poly acrylamide gel electrophesis assay (SDS- PAGE)

The electrophesis assay was carried out by the known method (Lammli, 1970). The prepared nucleoprotein (100 mg) from different experimental treatments were separated on polyacrylamide gel of 10% concentration.

Immunization of experimental animals

Fifty out-bred swiss albino mice were used thoughout the experimental time. Animals were divided into five groups. The first group kept as non-immunized control. The 2nd, 3rd, 4th and 5th groups were immunized subcutaneously (S.C), at zero day with antigen (Ag.) extracted-from native, chemically pretreated with III f & g compounds and UV-irradiated *B.alexandrina* snails, respectively. Different nucleoprotein preparations inoculated in association with complete frund's adjuvant (V/V), 150 µg protein/mouse. At the 3rd week post 1st immunization animals were boosted (S.C.) with nucleoprotein (100 µg protein/mouse). Control non-immunized group injected (S.C) with frunds adjuvant in association with streile phosphate buffer saline (PBS) and PBS alone at zero and 21st d, respectively. Two week post the 2nd immunization, both the immunized and control groups were challenged (S.C), with 150 normal *Schistosoma mansoni* cercariae/ouse. Seven weeks later all animals were perfused according to the known method (Duvall and Dewitt ,1967) and the number of worm burdens per mouse were counted and the percentage of protection was calculated.

Relative sex ratio (RSR)

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

Histopathological examination

Control-challenged and vaccinated challenged experimental groups at the 7th week post challenge were undertaken in histological technique.The histopathological sections of liver were investigated for the number and size range of granuloma formation according to Afifi's method (Afifi, 1986).

Statistical analysis

The statistical analysis was carried out by using two tail Students t-test according to the known method (Dean *et al.*, 1983).

RESULTS AND DISCUSSION

In view of the above findings it was of interest to undertake the synthesis of new 5-substituted uracil incorporated with all other moieties. Synthesis of the desired compounds was achieved by allowing uracil-5-sulphonyl chloride (Fathalla, 1992) to react with *p*-aminoacetophenone to give 5-(*p*-acetylphenyl) uracilsulphonamide (**II**). Compound **II** was reacted with thioacetamide and some various aldehydes namely benzaldehyde, *p*-flourobenzaldehyde, 3,4-dimethoxybenzaldehyde, *p*-chlrobenzaldehyde, 3,4-dihydroxybenzaldehyde, 3-hydroxy-4-methoxybenzaldehyde, and furfural to give the corresponding3-cyanopyridine-2-thione derivatives of type (**IIIa-g**) (Scheme 1). On the other hand, compound **II** was allowed to react with aryl or alkyl thiosemicarbazides, namely, benzoyl-, benzyl-, methyl-, phenyl-, thiosemicarbazides in absolute ethanol to give the corresponding thiosemicarbazone derivatives **IVa-d**.

In a similar manner, compound **II** reacted with alkylsemi-

carbazides namely *n*-butyl and isopropylsemicarbazides to give the corresponding semicarbazone derivatives **IVe, f** (Scheme 1). The obtained compound **II** reacted also with active methylenes, namely, ethyl cyanoacetate and malononitrile and some aldehydes namely, benzaldehyde, anisaldehyde, *p*-chlorobenzaldehyde, 3,4-dimethoxy benzaldehyde, furfural and *p*-flourobenzaldehyde in the presence of ammonium acetate to afford new cyanopyridine deivatives **Va-f,VIa-f** respectively (Scheme 1).

The Claisen-Schmidt condensation of **II** with various aromatic or heterocyclic aldehydes, namely, benzaldehyde, anisaldehyde, *p*-bromobenzaldehyde, *p*-chloro-benzaldehyde, furfural, 5-methylfufural, 3,4-dimethoxybenzaldehyde, 3-hydroxy, 4-methoxybenzaldehyde, in ethanolic sodium hydroxide furnished the corresponding derivatives **VIIa-h** in fairly good yield (Scheme 2).

A solution of **VIIc, e** in ethanolic KOH on treatment with hydroxylamine hydrochloride gave **VIIIc, e**. This reaction is analogous with what has been reported by Hassaneen (Hassaneen *et al.*, 1995). Cyclocondensation reaction of hydrazines and ureas with *a,b* unsaturated ketones was investigated (Abbady *et al.*,1986 and Hassaneen *et al.*, 1995). When allowed to react with hydrazine hydrate in ethanol in presence of a few drops of pipridine as a basic catalyst, the bezalacetophenone derivatives **VIId, h** afforded the corresponding pyrazoline derivatives **IXd, h**. On the other hand, compound **VIIg, h** when allowed to react with urea in the presence of KOH yielded pyrimidine derivatives **Xg, h** respectively.

It was reported that 3-aryl-S-triazolo[3,4-a]pyridazines have anxiolytic activity, and exhibit antidepressant activity. In this study, we planned to synthesize triazolopyrimidine for the same object. When compound **Xg** was refluxed with acetylhydrazine in *n*-butanol, it gave the triazolo[3, 4-b]pyrimidine derivative **XIIg**. The structure of compound **XIIg** was conclusively established by its identity with an authantic sample prepared by treatment of pyrimidine-2-one derivative **Xg** with $POCl_3/PCl_5$ mixture to yield 2-chloropyrimidine derivative **XIg** which was subsequently converted into **XIIg** by treatment with acetylhydrazine in refluxing *n*-butanol.

Analogous to the recent report (Essawy and Wasfy, 1994), the base-catalysed condensation of **VIIIc** with ethyl cyanoacetate and/or malononitrile afforded the corresponding pyran derivatives **XIIId**$_{1,2}$. Similar to the behaviour of (El-Hashash,1984) treatment of the alcoholic solution of chalcone **VII** with hydrogen peroxide in alkaline medium yielded the corresponding *a,b*-epoxyketone **XIVc**. The formation of oxirane nucleus can be theoretically explained as the oxyaion (HO-O)derived from H_2O_2 attacks the *b*-carbon atom by the Michael type reaction, leading to afleeting intermediate of which the loss of a hydroxide ion producing the desired oxirane. Recently. it has been shown that the oxirane ring of *a,b*-epoxyketones is opened with hyrazines. It was found that the oxirane ring is readily opened when the *a,b*-epoxyketone **XIVc** was allowed toreact with hydrazine hydrate in boiling ethanol to furnish 4-hydroxypyrazoline derivative **XVc**. Similar to the recently reported results (Essawy and Wasfy, 1994), compound **II** was easily condensed with semicarbazide hydrochloride in an aqueous ethanolic solution to give the corresponding semicarbazone **XVI**. The purified semicarbazone was then subjected to oxidative cyclization (Hussein *et al.*, 1986) by either selenium dioxide in glacial acetic acid or thionyl chlodide to yield the selena-and thiadiazol derivatives XVIIa,b, respectively.

The biological effects of the new synthesized compounds

The prospects of immunological control of schistosomiasis by vaccination, represent a central approach for schistosome control. As well, the snail control represent an important approach in controlling the disease. Although chemotherapy is one of the most valuable methods for controling of schistosomiasis (WHO,1980), there is a pressing need for more selective and efficient molluscides for the control of the snail vector.

It has been declared that *S. mansoni* and their intermediate host *B. alexandrina* snail have some common epitopes of which are present on the surface of early stage of *S. mansoni* (Viera *et al.*, 1991). Dissous provided evidence that the important epitope of the 38 KDa. molecule is expressed by the infected intermediat host of *S.mansoni* (*B. galbrata*) (Dissous *et al.*, 1986) and was synthesized both in molluscc and by the parasite thoughout its life cycle. They added that parasitemolluscc protein determinants are shared and showen to induce antibodies (Abs.) to invertebrate determinants in mice and rabbits (Dissous *et al.*,1990).

The fact that the relationship between the parasite and its intermediate host is highly specific may be of interest in leading us to study the protective immunity of Ags. extracted from schistosome intermediate host *B. alexandrina* (nucleoprotein) in vaccination against schistosome infection. The present study was conducted to examin the molluscicidal activity of the new synthesized compounds **IIIc, e, f,** and **g** and UV-irradiation. The nucleoprotein profile extracted from pre-treated snails is also studied.

Molluscicidal activity of synthesized compounds and UV-irradiation

Molluscicidal activity of the four prepared chemical compounds with the code **IIIc, e, f** and **g** revealed that the chemical compound **IIIf** showed more potent molluscicidal activity (LC50 at 100 p.p.m for 24 h) in comparison to other compounds **III c, e, f** and **g** which are less potent (LC50 at 300, 200 and 200 p.p.m) respectively (Table III).

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

Conc of molluscicide solution (ppm)	IIIg	IIIf	IIIc	IIIe
10	-------	------	-------	------
20	-------	------	-------	------
30	-------	------	-------	------
40	------	------	-------	
50	------		------	-------
60	-------	3	-------	-------
70	-------	3	-------	-------
80	-------	4	-------	-------
90	-------	4	--------	-------
100	-------	5	-------	-------
120	-------	10	3	-------
140	2	-----	3	-------
150	2		3	2
180	2		2	2
200	5		2	5
300	6	---------	5	7
400	7		6	7
500	10	---------	10	10

The dose response curve of mortality over 15 days revealed that snails subjected to UV-irradiation for 1h showed that 25% of snails died within 15 days.

SDS-PAGE of the snail's nucleoprotein

The SDS-PAGE of nucleoprotein profile showed about 15 bands of molecular weights (m.wt.)in the range 250 KDa. to 25.500 KDa. The bands of m.wt.155,88 and 40 KDa. are most intensive bands expressed in control and physically (UV-irradiated) or chemically pre-treated snails (Fig. 1). The profile of nucleoprotein extracted from different experimental groups did not reveal appreciable differences, where no obvious qualitative or quantitative differences were observed.

Immunization experiments

The mean number of worm burdens and the percentage (%) of protection induced in immunized and control challenged experimental groups is tabulated in (Table IV). The precentage of protection against challenge infection is reflected in the reduction in the number of hepatic and intestinal perfused worms in immunized groups relative to the control challenged group. The recorded data revealed significant reduction ($P \leq 0.01$) in the mean no. of worm burdens in immunized animals (36.00±9.75; 34±10.90; 42.61±11.77 and 36.33±12.61) for native, UV-irradiated, **III f** and **II g** treated groups, respectively as compared to the control (74.33±12.66), and the corresponding percentage of protection are (51.56; 53.81, 42.67 and 51.12), respectively (Table IV). No considerable changes were observed in each immu-nized group as compared to each other.

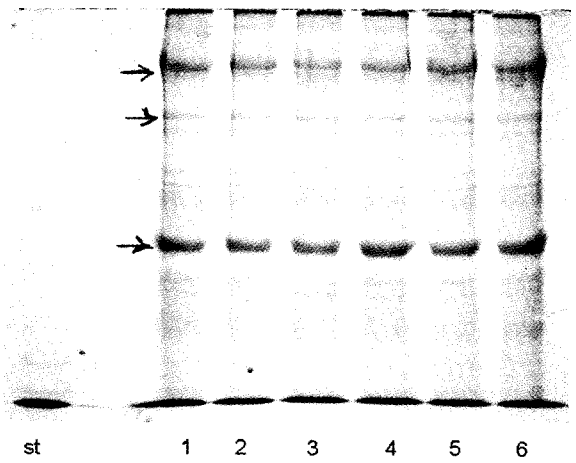


Fig. 1. Immunoelectrophoresis (SDS-PAGE) of nucleo-protein prepared from native snails pretreated with different chemical compounds and UV- irradiation. St:Standar molecular wight (Marker protein). 1:Control(non-treated nucleoprotein). 2:UV-irradiated nucleoprotein. 3:Compound c-treated nucleoprotein 4:compound e-treated nucleoprotein. 5:Compound f- treated nucleoprotein. 6: Compound g- treated nucleoprotein.

Although the observed differences in the relative sex ratio between the experimental groups, it was not appreciablly different, where no potent effect on female rather than male was recorded. The effecany of UV-irradiation was relatively higher than the other treatments and native snails. Obvious differences in the number of granuloma formation was recorded, where the UV-irradiated and native groups showed relatively low number of granuloma formation and size range of granuloma as compared to the control, or **IIIf** and **g** treated Ags. where the size range of granuloma (0.2-1.1 mm and 0.2-0.8 mm) for both native and UV-irradiated groups respectively. Both **III f** and **g** compounds showed 0.5-1.8 and 0.5-1.7 mm granuloma size respectively (Table IV). A correlation is observed between the results of protection, male/female ratio and the histopathological observations.

The molluscicidal activity of the four prepared chemical compounds with the code **IIIc**, **e**, **f** and **g** as well as, UV-irradiation were tested against *B. alexandrina* snails. Compound **IIIf** showed more potent molluscicidal activity as compared to other compounds **IIIc**, **e** and **g** which are less potent ; which is in agreement with the findings that some amino uracil derivatives induced some alterations in the percentage of DNA bases and in the ratio of these bases, it was reported that tetrahydro-naphthalen-chalcone is more potent molluscicide in comparison to the other prepared compounds such as pyridine and cyclohexanone derivatives which are less potent (Abdel

Table IV. Levels of Protective Immunity and Hepatic Changes Induced in Control and Immunized Mice Challenged with Normal *S. mansoni* Cercariae

Treated Experimental groups	No.of challenge cercariae	Mean No.of woem burden X±S.D.	% of Protection	Statistical significance	RSR*	No.of Granuloma Formation	Size range of Granuloma (mm)
Control	150	74.33±12.06	–	–	–	++++	0.8–2 mm.
Native	150	36.00±9.76	51.56	P≤0.01	0.94	++	0.2–1.1 mm
UV-irradiated	150	34.33±10.09	53.81	P≤0.001	0.74	++	0.2–0.8 mm
III f- compound	150	42.61±11.77	42.67	P≤0.001	1.11	+++	0.5–1.8 mm
III g compound	150	36.33±12.61	51.12	P≤0.001	1.07	+++	0.5–1.7 mm

* Relative Sex Ratio. ++ Few, +++ Moderate, ++++ Numerous

Hamed and Rizk,1997, Salama *et al.,* 1996). Variation in the potency of these molluscicides may be attributed to the slower rate of release of the active constituents of these prepared compounds or to their diffstabilin water.

The SDS-PAGE of nuecleoprotein of snails either chemically or physically treated did not show obvious qualitative or quantitative differences in the separated bands when compared to the control, the separated bands were identical and have the same molecular weights. In contrast to this data, Tolba reported that physical (γ-irradiation) and chemical (Thioxanthone derivatives) treatments of *B. alexandrina* snails induced considerable modifications in their antigens (Tolba *et al.,*1995). Selected bands appeared, while others disappeared.

Our observations recorded in the present study revealed that the antigenic extract (nucleoprotein) from *S. mansoni* intermediate host *B. alexandrina* snails treated with either UV-irradiation, compounds **III f** and **g** or native non-treated snails induced significant levels of protection against challenge infection with normal cercariae. These observations are in accordance with different studies, that irradiation of snails may induce physically modified Ags. These Ags. could possibly stimulate immunologic reaction. As well, immunization of mice with protein isolated from chemically pre-treated snails, may confer considerable protection against *S. mansoni* infection. Whereas, protein extracted from native snails did not show immunological effect against schistosome infection as protein extracted from irradiated snails which induce marked decrease in the number of schistosomula of mice infected with schistosome. Our results are also in agreement with Tolba (Tolba *et al.,* 1995) where mice immunized with Ags. extracted from chemically treated, irradiated and native *B. alexandrina* snails developed resistance to challenge infection with *S. mansoni* cercariae. Similarly, vaccination with chemically modified Ags. extracted from *B. alexandrina* snails, significantly reduced the no. of worm burdens post challenge with normal cercariae and induced high level of IgG Abs. Response (El-Hamshary *et al.,*1995).

Despite the relatively low levels of protection induced by Ags. Extracted from the chemically (**IIIf** and **g**) treated snails than that extracted from UV-irradiated snails, it is proved that synthesized compounds **IIIf** and **g** induced comsiderable modification in the nucleoprotein Ags. comparable to that induced by UV-irradiation, and succeded to induce protection against *S. mansoni* infection.

The findings of other studies concerned with using Ag-extract from *S. mansoni* intermediat host in vaccination against schistosome infection could be put in line with our present finidings where Grzych indicated that *S. mansoni* and its intermediate host *B. glabrata* shared a common glycamic determinants which demonstrated to be active in immunization against schistosomiasis (Grzych *et al.,* 1987). The protein determinants shared by *B. glabrata* (Bg 39), crossreactivity was observed, identified and it was shown to induce Abs. specific for invertebrate determinants in mice and rabbits (Dissous *et al.,*1990). The complex biological materials which have been showed to reduce adult schistosome count in vaccinated mice are snails hemolymph, and hepatopancrease (Dissous *et al.,*1990 and Gammal-Elddin *et al.,*1996). Schwick stated that injection of mice with homogenate of hepatopancrease of *B. alexandrina* in conjugation with an adjuvant reduced worm burdens 57.6% after exposure to infection (Schwick, 1980).

In the same correspondance, most attempts to protected mice by immunization with non-living schistosome Ags. have been successful (Dean,1983 and James & Blois 1986). In some cases, purified Ags. have been used for protective immunization in mice, the levels of resistance achived is 40%. El-Ridi group reported that *S. mansoni* recombinant glyceraldhyde 3-phosphate dehydrogenase (3MG 3PDH) Ag. is a candidate vaccine for human schistosomiasis (El-Ridi *et al.,* 1998). This recombinant Ag. induces powerful cellular and humoral immune responses and this immune responses mediate significant protection against challenge infection and reduction in egg deposition. These above mentioned observations are in harmony with our present observations, but they are more or less agree with other studies using living attenuated parasite in vaccination against schistosome infection (Dean *et al.,*1983 and Smythies et al.,1992). The discrepancy of our observations with Dunne (Dunne *et al.,*1995) or other studies using attenuated living parasite

could be explained by the fact that the failuer of candidate immunogens is may be due to the correct presentation in the form or route which induce T-cell or cytokine response, because cytokine manipulation may offer a strategy for improving the action of non-living vaccine (Gazzinelli *et al.*,1991). Similarly, the panel of Ags. recoginzed by T-and /or B-lymphocytes of different mice strains differed considerably despite the fact that the condition of immunization and testing, were highly comparable (Al-Sherbiny *et al.*, 1995).

It is concluded that the prepared chemical compounds **III f** and **g** have a potent molluscicidal activity, and they induced chemical modification comparable to that induced by physical treatment in the snail's nucleo-protein, which could possibly used in immunization against *S. mansoni* infection.

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