Voltammetric behavior, biocidal effect and synthesis of some new nanomeric fused cyclic thiosemicarbazones and their mercuric(II) salts

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Received 14 May 2011; accepted 22 July 2011
Available online 31 July 2011

KEYWORDS
Macrocyclization; Nanomerics sulfur–metal salts/complexes; Molluscicidal agents; Cyclic voltammetry; Mechanism of electrode reaction

Abstract New nanomeric 3-thioxo-5-methoxy-4,5-dihydro-6-methyl-9-unsubstituted/substituted-1,2,4-triazino[5,6-b]indoles (2a–c) and 3-thioxo-5-methoxy-4,5-dihydro-6,7-dihydroxy-1,2,4-triazino[5,6-c]cyclobut-6-ene (3) were prepared via reaction of thiosemicarbazide with 5-unsubstituted-and/substituted-indol-2,3-diones and/or 3,4-dihydroxyxyclobutane-1,2-dione in methanol–concentrated HCl at room temperature. A series of mercury(II)–ligand salts e.g. compound 4b and Hg(II) complexes 5a,b and 6 of cyclic Schiff base were prepared. Structures of these compounds were established by elemental analysis and spectral measurements. The redox characteristics of selected compounds were studied for use as chelating agents for stripping voltammetric determination of mercuric(II) ions in aqueous media. The compounds were also screened for their use as molluscicidal agents against Biomphalaria Alexandrina Snails responsible for Bilharziasis.

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1. Introduction

In recent years (Mukkerjee and Sarker, 1988; Pasini and Casella, 1979; Ofiong and Martelli, 1994; Zhong et al., 1998; Beer et al., 1997; Hambley et al., 2001; Hunzenkamp et al., 2000; Yildiz et al., 2005) great attention has been directed toward designing macrocyclic compounds and their salts for several purposes e.g. as chelating agents for selective separation, removal and subsequent determination of trace and ultra trace heavy metal ions in complicated matrices. Electron-deficient moieties inserted within the back-bone of nanomeric macrocyclic complex species have opened the door on host/guest chemistry and electron transfer as supra-molecular chemistry (Bradshaw, 1997).

Calixarenes (I) are phenols metacyclophanes having immense potential for developing molecular receptors for recognition of ions and small organic molecules. Chawla and Pathak (2000) have reported that addition of calix[n]arenes to methylene blue markedly speeded sensitized photooxygenation of 4,5-diphenylimidazoazoles. The remarkable effect of
added calix[n]arene is dependent upon the chain-length of appended alkyl groups (Foroughifar et al., 2009). The effect was more pronounced in calix[11]arenes compared to calix[12]arenes and calyx araines having the same function group R (Chandra, 2005). Condensation of squaric acid and its esters with aliphatic or aromatic amines yielded the corresponding bis amide (Hamaue and Minami, 1999), while cyclocondensation of squaric acid with amino guanidine in 1:1 molar ratio have afforded 2-alkylthio-4-cloro-5-methyl-N\(\text{-}\)(1,2-dihydroxy-2-cyclobuta[e][1,2,4-triazin-3-yl]) benzenesulfonamide (Pandey and Sengupta, 2006). These compounds have acted as potent inhibitors against HOP-62 non small cell lung cancer line (in vitro anti-tumor activity) (Pandey and Sengupta, 2006).

Recently, a series of nanomeric molecules and some of their metal salts have been reported (Xu et al., 2006; Ali et al., 2008). Thus, the present article is focused on: (i) The synthesis of new nanomers derived from cyclocondensation of squaric acid and cyclic bi-carbonyl compounds; (ii) evaluating the molluscicidal properties of the prepared compounds; and finally (iii) studying the redox characteristics of selected compounds for use as selective reagents in stripping voltammetry for mercury determination in wastewater samples.

2. Experimental

2.1. Apparatus

A Perkin Elmer (Lambda EZ-210) double beam spectrophotometer (190–1100 nm) with 1 cm (path width) quartz cell was used for recording the electronic spectra of the compounds. A Perkins Elmer model RXI-FT-IR system 55529 was used for recording the electronic spectra of the compounds. A Brucker advance pounds. A Perkins Elmer model RXI-FT-IR system 55529 was used for recording the electronic spectra of the com-

2.2. Reagents and materials

Analytical reagent grade chemicals were used as received. Low density polyethylene (LDPE) bottles, Nalgene were used for collection of various water samples. LDPE bottles were carefully cleaned first with hot detergent, soaked in 50% HCl (Analar), HNO\(_3\) (2.0 mol IPP-198), subsequently washed with dilute HCl (0.5 mol IPP-199) and finally rinsed with distilled water. In cyclic voltammetry, solutions were made in N,N-dimethylformamide (DMF) as supporting electrolyte.

2.3. Organic syntheses

2.3.1. N-Methyl-indol-2,3-dione derivatives (1a–c)

Equimolar amounts of methyl iodide and isatin, 5-nitro/5-fluoroisatin in ethanolic KOH (5%) w/v) was stirred at room temperature for 4 h. The solution was left to cool overnight. The solid precipitates were filtered off, washed and dried to give 1a–c, respectively.

Compound 1a was crystallized from ethanol as pale yellow crystals. Yield = 75%, m.p. 180 °C. Analytical data: Found: C, 66.69; H, 4.21, N, 8.39; Calculated for C\(_9\)H\(_8\)N\(_2\)O\(_2\): C, 67.7; H, 4.34; N, 8.69.

Compound 1b was crystallized from ethanol as yellow crystals. Yield (71%), m.p. 226 °C. Analytical data: Found: C, 51.82; H, 2.72; N, 13.44; Calculated for C\(_9\)H\(_7\)N\(_2\)O\(_2\): C, 52.42; H, 2.91; N, 13.59.

Compound 1c was crystallized from ethanol as faint yellow crystals. Yield (69%), m.p. 212 °C. Analytical data: Found: C, 59.67; H, 3.31; N, 7.73; F, 10.49. Calculated for C\(_9\)H\(_7\)N\(_2\)O\(_2\): C, 60.33; H, 3.35; N, 7.82; F, 10.61.

2.3.1.1. 3-Thioxo-5-methoxy-4,5-dihydro-6-ethyl-9-unsubstituted/substituted-1,2,4-triazino[5,6]indoles (2a–c). A mixture of compound 1a–c (1.0 mmol) in dry methanol (50 mL) and thiosemicarbazide (1.0 mmol) in concentrated HCl (10 mL) was stirred for 6 h at room temperature. The produced solids were filtered off, washed with methanol and dried to give 2a–c, respectively.

Compound 2a was crystallized from ethanol as yellow crystals. Yield = 55%, m.p. 220 °C. Analytical data: Found: C, 52.64; H, 4.78; N, 22.33; S, 12.76. Calculated for [C\(_9\)H\(_7\)N\(_3\)SO]: C, 53.22; H, 4.83; N, 22.58; S, 12.90.

Compound 2b was crystallized from ethanol as yellow crystals. Yield = 58%, m.p. 305 °C. Analytical data: Found: C, 44.55; H, 3.71; N, 23.62; S, 10.80; Calculated for [C\(_9\)H\(_7\)N\(_3\)SO]: C, 45.05; H, 3.75; N, 23.89; S, 10.92.

Compound 2c was crystallized from ethanol as yellow crystals. Yield = 65%, m.p. 250 °C. Analytical data: Found: C, 49.07; H, 4.13; N, 21.05; S, 12.03; F, 7.14. Calculated for [C\(_9\)H\(_7\)N\(_3\)SO]: C, 49.62; H, 4.13; N, 21.05; S, 12.03; F, 7.14. IR: \(\text{cm}^{-1}\) (Int.%) 270 (M+4, 18.9, 149 (100), 95 (35)

2.3.1.2. 3-Thioxo-5-methoxy-4,5-dihydro-6-ethyl-9-unsubstituted/substituted-1,2,4-triazino[5,6]-cylohex-ene (3 ). Equimolar amounts of squaric acid and thiosemicarbazide in dry methanol (100 mL)–concentrated HCl (10 mL) was stirred at room temperature for 6 h. The solution was left to cool overnight and the resultant solid precipitate was filtered off, washed with methanol and dried to give compound 3. Compound 3 was crystallized from ethanol as yellow crystals. Yield = 82%, m.p. 178–180 °C. Found: C, 35.39; H, 3.42; N, 20.87; S, 15.74; Calculated for C\(_7\)H\(_7\)N\(_3\)SO\(_3\), (201). C, 35.82; H, 3.48; N, 20.89, S, 15.93. M(\(\text{c} \text{m}\)) 129 (23.15), 157 (5.18), 129 (23.15), 58 (100). IR: \(\text{cm}^{-1}\) 3429, 3308 (2 OH), 2947, 1614 (Me, C–N), 1317, 1167 (NCS & C–S), 1046 (–O–Me); \(\text{H}\)
3.2.2. Synthesis of compound 4a and mercury(II) compounds (4b, 5a.b)
A mixture of compound 2b or 2c (2 mmol) in dry methanol (100 mL) and mercuric(II) chloride (1.0 mmol) was stirred at room temperature for 12 h. The solution mixtures were left to cool overnight and the resultant solid precipitates were filtered off, washed with methanol to give compounds of type 5a.b. On diluting the filtrates, yellow precipitates were washed and dried in vacuum to give type of compounds 4a.b, respectively.

Compound 4a was crystallized from ethanol as yellow crystals. Yield = 18%, m.p. 240 °C. Found: C, 30.39; H, 1.19; N, 17.66; S, 8.05; Cl, 8.93; Calculated for [C20H12N10S2O4Cl2Hg]; C, 36.34; H, 1.51; N, 17.69; S, 8.09; Cl, 8.97. IR: v cm⁻¹ 3215, 3170 (2 NH), 2880, 1485 (CH₃), 1165 (C–S).

Compound 4b was crystallized from ethanol as yellow crystals. Yield = 48%, m.p. 238 °C. Analytical data: Found: C, 31.94; H, 2.39; N, 14.90; S, 8.51; F, 5.05; Cl, 9.45. Calculated for [C20H12N4S2F2Cl2Hg]; C, 32.56; H, 1.62; N, 15.19; S, 8.61; F, 5.11; Cl, 9.65. M/S: 740 (M+3, 15.21), 150 (100). IR: v cm⁻¹ 3210, 3180 (N²H, N⁴H²), 2920, 1440 (CH₃), 1165 (C–S).

Compound 5a was crystallized from ethanol as pale yellow crystals. Yield = 35%, m.p. 236 °C. Analytical data: Found: C, 32.87; H, 2.19; N, 19.00; S, 8.44; Calculated for C₂₀H₁₆N₁₀S₂O₄Hg; C, 35.58; H, 1.78; N, 16.06; S, 9.50; F, 5.59. Calculated for C₂₀H₁₆N₁₀S₂O₄HgCl; C, 35.82; H, 2.38; N, 16.70; S, 9.59; F, 5.69. IR: v cm⁻¹ 161.83, 130-128, 110, 38.53 and 17.76 ppm.

Compound 5b was crystallized from ethanol as pale yellow crystals. Yield = 48%, m.p. 270 °C. Analytical data: Found: C, 35.58; H, 1.78; N, 16.06; S, 9.50; F, 5.59; Calculated for C₂₀H₁₆N₁₀S₂O₄HgCl₂; C, 35.82; H, 2.38; N, 16.70; S, 9.59; F, 5.69. IR: v cm⁻¹ 161.83, 130-128, 110, 38.53 and 17.76 ppm.

3.3. Di[4,5-dihydro-6,7-di(hydroxyl-1,2,4-triazino-[5,6-c])cyclobut-6-ene]-3-thioato-f-mercuric chloride 6
A mixture of 3 (2 mmol) and mercuric(II) chloride in dry methanol (100 mL) (1.0 mmol) in 2:1 and 1:1 molar ratios was stirred for 12 h at room temperature. The mixtures were left to cool overnight and the resultant solid were filtered off, washed with methanol to give compounds of type 6.

Compound 6 was crystallized from ethanol as yellow crystals. Yield = 65%, m.p.225 °C. Analytical data: Found: C, 19.42; H, 1.28; N, 13.59; S, 10.26; Cl, 11.38. Calculated for [C₂₀H₁₄N₆S₂O₄HgCl₂]; C, 61.4, M+3; C, 19.63; H, 1.3; N, 13.74; S, 10.47; Cl, 11.62. IR: v cm⁻¹ 3429, 3380 (2 OH), 3180, 3166 (2 NH), 1360 (CNS), 1155 (C=S); ¹H NMR (δ ppm): δ: 8.8 (1H s, NH), 5.7, 5.2 (each s, 2 OH) and 7.8 (s, 1H of C₂NHNH-1,2,4-triazine).

2.4. Cyclic voltammetric experiments
An accurate concentration (2.0 mmol) of the compound 3 or its mercury(II) salt or complex and the supporting electrolyte tetramethyl ammonium chloride (TEAC) (200 mmol) in DMF were transferred into the electrochemical cell composed of Pt working (small surface area), Pt (large surface area) and Ag/AgCl as working, counter and reference electrodes, respectively. The test solution was then stirred with nitrogen gas for 5 min to release oxygen and the voltammograms were then recorded at various scan rates (50–1000.0 mV/s) in the potential range −1.5 to +1.0 V vs. Ag/AgCl electrode.

3. Results and discussion

3.1. Chemical characterization
Preliminary investigations of coordination chemistry of the prepared organic ligands containing nitrogen and/or nitrogen–sulfur atoms, have shown that, heterocyclic ligands react with mercuric(II) chloride in a mono dentate or bi dentate fashion via SH or NH. Condensed 1,2,4-triazin-3-thiones are known heterocyclic thione exists in two thione and thiol tautomeric forms (Maya et al., 2000; Bellon et al., 2000; Singh and Patel, 2003; Rourk, 1999; Abdel-Monem, 2010; Nicholson and Shain, 1964). These compounds coordinate as neutral ligand via sulfur atom (Singh and Patel, 2003) or as bi dentate ligand (S, N), S, N bridging by loss of a proton (N–S).

Interaction of cyclic dicarbonyl reagents as 1-methyl-indole-2,3-diones (1a–c) and thiosemicarbazide in dry methanol–HCl (2.0 mol L⁻¹), yielded 3-thioxy-5-methoxy-4,5-dihydro-6-methyl-9-unsubstituted/substituted-1,2,4-triazino[5,6-b]indoles (2a–c) (Scheme 1).

Structures of 2a–c were established from their elemental analysis and spectral measurements. IR spectrum of 2c showed the stretching bands characteristics to NH, NH and C=O functional groups. ¹H NMR spectra recorded signals at δ 11.3, 9.2, 3.48, and 2.5 ppm due to NH²S, NH²H, OH3, and Me–N protons with aromatic protons at 7.5, 7.18, and 6.9 ppm. ¹³C NMR spectrum of 2c recorded resonated signals at 178.19, 111.55, 55.48, 38.51, and 18.02 ppm confirming the proposed structure. M/S spectrum of 2c showed m/z at 270 (M+4) as molecular ion with a base peak at 149 assigned to C₆H₅NF (1-methyl-5-fluoroindole) radical.
Treatment of 2b and/or 2c with mercuric(II) chloride in 2:1 molar ratios in absolute methanol, yielded complexes 4a, b (Scheme 2) and 5a, b (Scheme 3). Structure of 5b was also deduced from its mass spectrum. In mass spectrum of 5b two peaks at m/z 670 and 235 (100%) were observed and were attributed to the molecular ion C_{20}H_{16}N_{8}S_{2}F_{2}Hg and a base peak as C_{10}H_{6}N_{4}SF species, respectively. IR spectrum of 5b recorded lack of peaks assigned to NH, and SH functional groups which confirm the formation of a type complex. 1H NMR showed only protons of aromatic and N-methyl moieties. 13C NMR showed signals at δ: 170.48, 164.37, 162.79, 162.51, 144, 123.65, 117.61, 113.63, and 36.04 ppm. Structure of 4 was also deduced from their elemental analysis and spectral measurements. Mass spectrum of 4b showed two peaks at m/z 740 (M+3), corresponding to (C_{20}H_{12}N_{8}S_{2}F_{2}Cl_{2}Hg+3) as molecular ion and at m/z 150 (M+1) represented C_{9}HNF as base peak. The IR spectrum recorded vibration bands at 3210 and 3180 cm\(^{-1}\) due to NH, N\(_4\)H with lack of thiol band, which confirms that complexation of mercury with ligand forming S–Hg. 13C NMR spectrum exhibited mainly a resonated signals at δ 17.76, 38.53, 110, 128–130 and 161.83 ppm confirming structure of 4b.

Cyclocondensation of squaric acid with thiosemicarbazide in dry methanol–HCl (2.0 mol L\(^{-1}\)) afforded 3-thioxo-5-methoxy-4,5-dihydro-6,7-dihydroxy-1,2,4-triazino (Beer et al., 1997; Hambley et al., 2001). Structure of 3 was established from their elemental analysis and spectral measurements. Mass spectrum of 3 showed mainly two peaks at m/z 202 (M+1) as molecular ion (C_{6}H_{7}N_{3}SO_{3}) and at m/z 58 as a base peak. IR spectrum recorded vibration bands at 3429 and 3388 cm\(^{-1}\) of two hydroxy groups at 2947, 1614 cm\(^{-1}\) of aliphatic CH\(_3\) and C’N functional groups. Another vibrations at 1371, 1167 and 1046 cm\(^{-1}\) attributed to CNS, C–S and O–Me functional groups. 1H NMR spectrum showed resonated signals at δ: 10.3, 9.7, and 6.085 and 5.9 ppm attributed to two NH and OH protons with peaks at 3.43 ppm of OMe proton. 13C NMR spectrum showed resonated signals at 18.01, 38.49, 38.76, 39.44, 55.46, 173.55, 181.42 ppm confirming the proposed structure. Complexation of 3 with mercuric(II) chloride in dry methanol in 1:1 and 2:1 molar ratios gave only one complex species 6 (Scheme 3). Elimination of MeOH and

Scheme 2

Scheme 3 Formation of 4b from 2c on reaction with HgCl\(_2\).

Scheme 4 Formation of 6 from 3 with HgCl\(_2\).
coordination of sulfur atom to mercury(II) ions as mono-dentate was noticed. IR spectrum showed the presence of two hydroxy and NH groups indicating complexation with mercury is in a pseudo-tetrahedral environment with organic ligands bonded to the metal ion. $^1$H NMR spectrum of 6 recorded signals $\delta$: 5.7, 5.2 ppm due to hydroxyl and C$_5$-H of 1,2,4-triazine with $\delta$ at 8.4, 8.8 ppm attributed to two NH and NH protons confirming the structure of complex 6 (Scheme 4).

3.2. Voltammetric behavior of selected compounds (3, 4c and 5)

Selection of the tested compounds 3, 4c and 5 was based on the influence of mercury(II) on the redox characteristic of the resulting species and also to see whether compound 3 or similar compounds can be used as effective chelating agents for trace metal determination employing stripping voltammetry. Cyclic voltammograms (CVs) of 3 and its nanomeric mercuric salts prepared in 1:1 (4c) and in 2:1 (5) molar ratios of 3 to mercury(II) in DMF–TEAC at Pt were carried out. The CVs of compound 3 versus Ag/AgCl reference electrode at various scan rates (50–500 mV s$^{-1}$) were carried out. Representative voltammograms are shown in Fig. 1. The CV at 50 mV s$^{-1}$ (Fig. 1) revealed one well-defined anodic peak at 0.75 V coupled with an ill defined cathodic peak at $\sim$1.2 V vs. Ag/AgCl electrode. At a scan rate $> 1000$ mV s$^{-1}$, the anodic and cathodic peaks became ill defined suggesting the instability of the electrogendated species at Pt working electrode i.e. slow kinetics and irreversible electrochemical process (Nicholson and Shain, 1964; Bowmaker et al., 1982; Bard and Faulkner, 1980).

The CVs of compound 4c at Pt working electrode versus Ag/AgCl reference electrode at various scan rates (100–3000 mV s$^{-1}$) were carried out and representative CVs at 100 and 300 mV s$^{-1}$ are shown in Fig. 2. The CV at 100 and 300 mV s$^{-1}$ revealed one well-defined anodic peak at $\sim$0.65 V and two ill defined anodic peaks at 0.3 and 0.80 V coupled with one well defined cathodic peak at $\sim$1.0 V. On increasing the scan rate from 400 to 3000 mV/s, one well defined electrode couple was noticed on the potential range from $\sim$1.3 to $\sim$0.3 V and the other anodic peaks disappeared suggesting slow kinetics electrochemical process (Bard and Faulkner, 1980). The observed cathodic and anodic shifts to more negative and positive potential, respectively, suggesting irreversible nature of the electrochemical process (Nicholson and Shain, 1964; Bowmaker et al., 1982; Bard and Faulkner, 1980). Plot of the square root of the scan rates versus anodic and cathodic peak currents increased linearly (Fig. 3).
indicating that the process is a diffusion controlled electrochemical process and favors electrode-coupled (EC) chemical reaction mechanism of the type EC (Bard and Faulkner, 1980). These data suggest the possible use of compound 4b in stripping voltammetric determination of mercury(II) in aqueous solution.

The CVs of compound 5 at various scan rates were recorded at various sweep rate 50–3000 mV/s. Representative CVs are shown in Fig. 4. The CV at 50 mV s\(^{-1}\) showed an ill defined (like a shoulder) anodic peak at \(-0.3\) in addition to another peak at \(-0.1\) V versus Ag/AgCl electrode. On the reverse scan, two well defined cathodic peaks were noticed at \(-0.3\) and \(-0.6\) V and are safely assigned to Hg\(^{II}\)/Hg\(^{I}\) and Hg\(^{I}\)/Hg\(^{0}\), respectively. Plots of the cathodic or anodic peak currents versus the square root were linear revealing that the reduction process is diffusion controlled process (Bard and Faulkner, 1980). The CV at 50 mV s\(^{-1}\) showed an ill defined anodic peak at \(-0.3\) in addition to another peak at \(-0.1\) V versus Ag/AgCl electrode. On the reverse scan, two well defined cathodic peaks were noticed at \(-0.3\) and \(-0.6\) V and are safely assigned to Hg\(^{II}\)/Hg\(^{I}\) and Hg\(^{I}\)/Hg\(^{0}\), respectively. Plots of the cathodic or anodic peak currents versus the square root were linear revealing that the reduction process is diffusion controlled process (Bard and Faulkner, 1980). The plot of the current function (cathodic peak current/square root of sweep rate) versus sweep rate decreased linearly indicating that the first reduction processes (Hg\(^{II}\)/Hg\(^{I}\)) proceeded according to the EC mechanism (Bard and Faulkner, 1980). Thus, compound 5 can be used in differential pulse cathodic stripping voltammetric determination of mercury(II) in wastewater. On raising the sweep rate from 500 to 1000 mV s\(^{-1}\), the cathodic and anodic peaks are shifted to more negative values confirming the irreversible nature of the electrochemical process. The cathodic peak current increased linearly on increasing the analyte concentration confirming the performance of compound 3 as efficient chelating agent for mercury(II) ions and other toxic hard toxic metal ions in stripping voltammetry.

### 3.3. Molluscicidal activity

Recently, 1,2,4-triazine derivatives have been used as molluscicidal agents against snails responsible for bilharzias (Abde-Rahman et al., 2010). In a similar manner, the prepared 1,2,4-triazine compounds and their mercury(II) complexes were screened. Solutions of the compounds (20–80 ppm) in dechlorinated tap water of pH 7–7.7 at 27°C were tested in the present study and their LC\(_{50}\) were calculated (WHO, 1953; WHO, 1965). Exactly 10 snails were exposed to each concentration for 24 h followed by another 24 h as a recovery period in dechlorinated water (WHO, 1965). Snails Biomphalaria alexandrina (shell in diameter 9–11 mm) was tested. The intermediate host of Schistosoma mansoni which helps in causing intestinal bilharzias the most common problem in Egypt was collected from the irrigation canals in Abu-Rawash, Giza Govern state. The snails were adapted to laboratory conditions for two weeks before use to be sure that the Snails are strong and healthy. Snails were kept in plastic aguaria containing de chlorinated tap water at 25–27°C. The efficiency of the compounds against snails followed the order: 6 > 4b > 5 > 2 and 2c > 2b > 2a. The electron barrier of the molecular structure of the compound participated in the enzymatic inhibition of the living processes for the Snails thereby causing break of a vital-cyclic of Snails. Hg and F atoms in the compounds enhanced the mortality of snails due to the high toxicity of mercury(II) and a deposition of protein in the vital-cell of Snails by the available fluorine atoms (Table 1).

### Acknowledgments

The authors would like to thank the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah for the financial support under grant number 33-97-430 H and the Chemistry Department for the facilities provided.

### References


### Table 1 Molluscicidal activity of the compounds against Biomphalaria Alexandria Snails

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<th>Compound. no.</th>
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<td>80</td>
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<td>Reference standard, Baylucide</td>
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Figure 4 CVs of compound 5 at 50 (I) and 100 mV s\(^{-1}\) versus Ag/AgCl reference electrode.
Voltammetric behavior, biocidal effect and synthesis


WHO, Expert Committee on Bilharziasis, 65 (1953) 33.

WHO, Snail Control Information of Bilharziasis Monograph Series 50 (1965) 124.

