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Novel triazolo[4,3-*a*]quinazolinone and bis-triazolo[4,3-*a*:4,3′-*c*]quinazolines: synthesis and antitoxoplasmosis effect

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Abstract

Several quinazoline derivatives containing substituted thiosemicarbazido and *S*-methylisothiosemicarbazido groups at the 2-position and at both the 2- and 4-positions have been synthesized. Treatment of the *S*-methylthiosemicarbazides with morpholine or diethylamine did not give the corresponding guanidines. Instead, they underwent cyclodesulfurization into the condensed ring systems, [1,2,4]triazolo[4,3-*a*]quinazolinones and bis-[1,2,4]triazolo[4,3-*a*:4′,3′-*c*]quinazolines. Evaluation of the products for antitoxoplasmosis effect by studying the ultrastructure morphology of the organisms using scanning electron microscopy (SEM) indicated their efficacy in causing structural deformity of *Toxoplasma gondii*. Such a deformity plays an important role in obstructing the entry of the organisms into host cells.

Keywords: Quinazoline; Thiosemicarbazide; *S*-Methylisothiosemicarbazide; Cyclodesulfurization; Triazolo and bis-triazoloquinazoline; Antitoxoplasmosis effect

1. Introduction

Structure–activity relationship (SAR) studies on antiprotozoan compounds revealed that the antimalarial activity is associated with the attachment of alkylamino and arylamino groups to various heterocyclic nuclei, as in the 2-arylamino-4-dialkyl-aminoalkylamino-6-methylpyrimidines [1] and 2-arylamino-4-dialkylaminoalkylamino-quinazolines [1,2]. Other studies demonstrated that the presence of free amino group at the 2- and 4-positions of pyrimidine and quinazoline was effective in inducing strong antimalarial properties against sensitive and drug-resistant lines of *Plasmodia* [3–9]. Among the more potent antimalarial agents of this class of compounds are 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline [6,10,11] and 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine (pyrimethamine), which is in current clinical use against murine toxoplasmosis [12]. Replacement of the 6-substituted arylamino function in the dichlorobenzylaminoquinazoline derivative by arylthio, arylsulfinyl or arylsulfonyl groups was also effective in producing highly active antimalarial agents [13]. On the other hand, attachment of an urea or thiourea residue to the 2-position of pyrimidine led to inactive compounds [14]. In contrast, substitution by an arylguanidine group gave products with appreciably enhanced activity against experimental malarial infection [15]. Introduction of a thiosemicarbazide or semicarbazide function in the 2-position of quinoline also led to potent antimalarials [16]. These compounds represent analogues of the clinically active quinoline antimalarials, e.g. Chloroquine and Primaquine.

These observations aroused interest in the investigation of this structural class and it seemed desirable to prepare and test some analogues in which the typical antimalarial basic diamino groups, which were thought to play a key role in conferring optimal antiplasmodial effects among folate antagonists [17], have been re-

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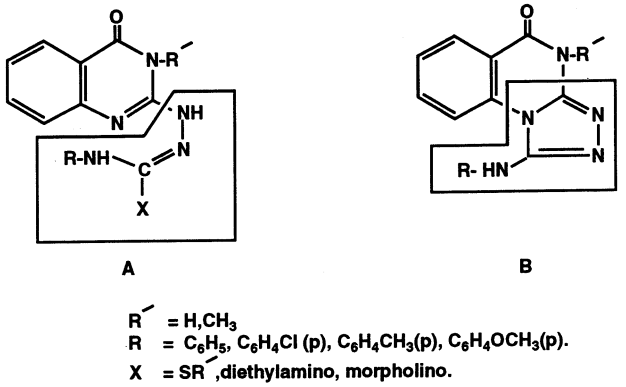


Scheme 1. Aminoguanidine structure in a flexible chain (**A**) or rigid heterocyclic ring (**B**).

placed by the more basic hydrazino moiety in the 2-position or in the 2- and 4-positions of quinazoline. In addition, it was also desirable to check the effect of conversion of such hydrazino group into a thiosemicarbazido moiety and an aminoguanidine function (Scheme 1, **A**). The synthesis of these compounds by reacting the intermediate *S*-methylisothiosemicarbazide derivative of the appropriate quinazoline with morpholine or diethylamine, as reported [18], followed a different pathway. The *S*-methylisothiosemicarbazide underwent cyclodesulfurization to the corresponding triazoloquinazoline derivatives (Scheme 1, **B**). These condensed heterocyclic products retained the aminoguanidine structure [19] but in a rigid form (**B**) if compared with the designed compounds (**A**). The products were evaluated in vitro for their antitoxoplasmosis activity and the results are reported in this paper.

2. Chemistry

The synthesis of the triazoloquinazolinones **4** and the ditriazoloquinazolines **9** was carried out as depicted in Schemes 2 and 4. The 2-hydrazinoquinazolin-4-one (**1**), prepared according to the method of Claesen and Vanderhaeghe [20], was condensed with the appropriate arylisothiocyanate in boiling ethanol to generate the 1-(4-oxoquinazolin-2-yl)-4-aryl-3-thiosemicarbazides (**2a–d**).

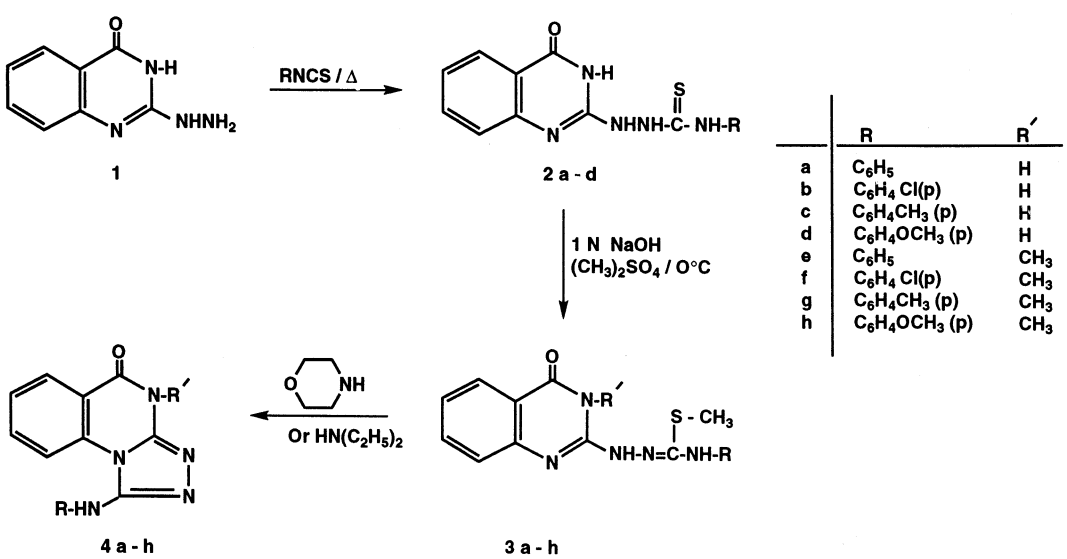
The use of iodomethane in absolute methanol to affect *S*-methylation [18,21] of these thiosemicarbazides was unsuccessful. However, reacting **2a–d** with dimethyl sulfate in aqueous sodium hydroxide [22,23] gave a mixture of the 4-oxoquinazolin-2-yl-*S*-methylisothiosemicarbazides (**3a–d**) and the corresponding *N*,*S*-dimethyl derivatives **3e–h** which were separated by preparative TLC. The ^{1}H NMR spectra of the *S*-methyl derivatives **3a** and **3b** showed a singlet assigned to quinazoline N–H at δ 11.04 and 11.10, respectively. This proton signal was absent in the spec-tra of *N*,*S*-dimethyl derivatives **3e** and **3h**, which showed a singlet resonating at δ 3.48 and 3.47, respectively, assigned to the three protons of the N–CH_3. The spectra also confirmed that 1-(4-oxoquinazolin-2-yl)-4-aryl-3-*S*-methylisothiosemicarbazides **3a–h** existed as

–HN–N=C(SCH$_3$)-NHR

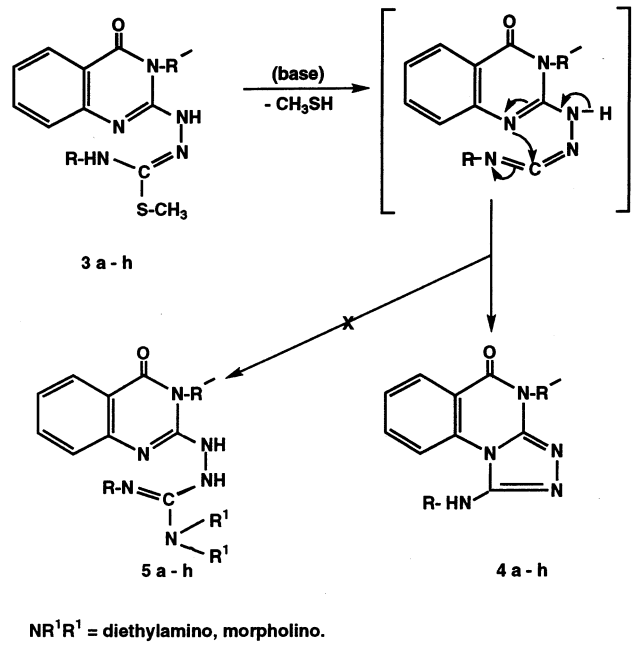
rather than the isomeric

–HNNH–C(SCH$_3$)=NR

as previously reported [18]. This was evidenced by the presence of the N^4-proton at δ 8.38–9.45 and the disappearance of the hydrazino N^2-proton at δ 7.82.



Scheme 2. Synthesis of triazoloquinazolinones **4a–h**.



Scheme 3. The proposed cyclodesulfurization mechanism of *S*-methylisothiosemicarbazides **3a–h** into the triazoloquinazolines **4a–h**.

Heating the *S*-methylisothiosemicarbazides **3a–h** under reflux with morpholine or diethylamine, as reported [18], did not produce the required aminoguanidines **5a–h**. Instead, compounds **3a–h** underwent cyclodesulfurization into the triazoloquinazolines **4a–h** according to the proposed mechanism in Scheme 3.

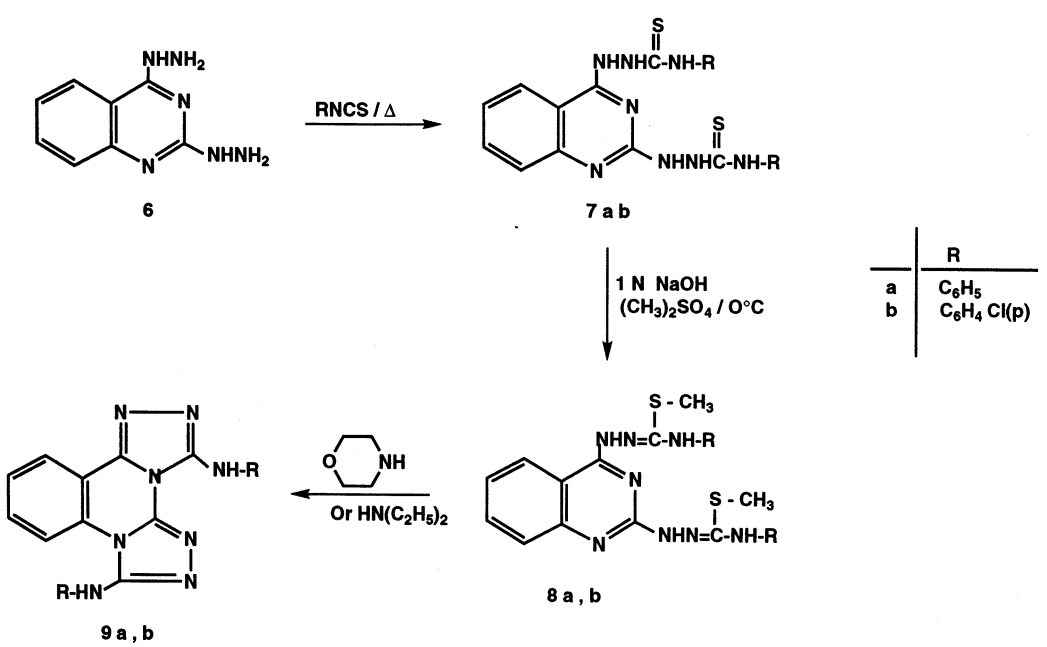
The ^{1}H NMR spectra of triazoloquinazolines **4a–h** lacked the singlet for S–CH_3 present in the *S*-methylisothiosemicarbazides **3a–h** and the signals for the morpholine or diethylamine part of the aminoguanidine moiety **5a–h**. In fact, the spectra of **4e–h** showed only one NH proton resonating at δ 8.02–9.04. The 3,7-bis-(substituted amino)[1,2,4]ditriazolo[4,3-*a*:4′,3′-*c*]quinazolines (**9a**,**b**) were likewise synthesized starting from the 2,4-dihydrazinoquinazoline **6** [20] (Scheme 4).

Compound **6** was reacted with the appropriate isothiocyanate and *S*-methylated by dimethyl sulfate in alkaline medium to produce the di-*S*-methylisothiosemicarbazides **8a**,**b**. The products were treated with morpholine or diethylamine to undergo cyclodesulfurization to compounds **9a**,**b**. The structure of the products was assessed by elemental analyses, IR and for some representative examples, by ^{1}H NMR and mass spectra.

3. Experimental

3.1. Chemistry

Melting points, in open capillary tubes, were taken using a Griffin melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu 408 spectrophotometer as KBr discs or Nujol mulls (ν cm^{-1}). The ^{1}H NMR spectra (DMSO-d_6) were recorded on a Varian Gemini 200 (200 MHz) spectrophotometer. The chemical shift values, reported in δ (ppm), are relative to TMS as the internal standard. Abbreviations used are: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; dist., distorted. The mass spectra (MS) were run on a Shimadzu GCMS QP 1000 EX



Scheme 4. Synthesis of bis-triazoloquinazolinones **9a**,**b**.

gas chromatograph–mass spectrometer (GC–MS). Homogeneity of the products was determined by ascending TLC on silica gel coated glass plates visualized by iodine vapors. Preparative TLC was performed on 20×20 cm plates coated with 30 g silica gel 60 GF 254 for TLC, Adwic laboratory chemicals. A duo-UV lamp, Desega Heidelberg, Germany, was used for location of the spots. Elemental analyses were performed at the Microanalytical Unit, Faculty of Science, Cairo University, Egypt. Analyses (C, H, N) were within $\pm 0.4\%$ of the theoretical values.

*3.1.1. 2-Hydrazinoquinazolin-4-one (**1**)*

A mixture of 2-chloroquinazolin-4-one [1] (1.2 g, 6.6 mmol), potassium carbonate (2 g), 85% hydrazine hydrate (2 ml) and H_2O (6 ml) was heated under reflux for 2 h. The mixture was cooled to room temperature (r.t.), neutralized with acetic acid to deposit 2-hydrazinoquinazolin-4-one (**1**) which was crystallized from ethanol (0.7 g, yield 60%); m.p. > 300°C; reported m.p. dec. 360°C [20]. IR: *v* 3000 (br, NH and NH_2 mixed with OH of the tautomeric form), 1680 (C=O), 1610 (C=N), 1600 and 1505 cm^{-1} (C=C Ar).

*3.1.2. 1-(4-Oxoquinazolin-2-yl)-4-aryl-3-thiosemicarbazides (**2a–d**)*

A solution of 2-hydrazinoquinazolin-4-one (**1**) (0.5 g, 2.8 mmol) in ethanol (200 ml) was treated with 1.5 equiv. of the appropriate isothiocyanate. The mixture was heated under reflux for 2 h, concentrated and cooled to r.t. The separated product was filtered and crystallized from ethanol.

2a: m.p. > 300°C; yield 92%. *Anal.* ($C_{15}H_{13}N_5OS$) C, H, N. IR: *v* 3400, 3268 (NH), 1700 (C=O), 1660 (C=N), 1595 (C=C Ar), 1560, 1330, 1050 and 875 cm^{-1} (NCS amide I, II, III and IV mixed vibrational bands, respectively). ^1H NMR: δ 6.9–7.5 (m, 6H, Ar–H, N^1H), 7.82 (s, 1H, N^2–H), 8.01–8.31 (m, 3H, quinazolinone–C_6–C_8–H), 8.48 (dd, 1H, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, quinazolinone C_5–H), 9.66 (s, 1H, N^4–H), 13.00 (s, br, 1H, quinazolinone NH).

2b: m.p. > 300°C; yield 94%. *Anal.* ($C_{15}H_{12}ClN_5OS$) C, H, N. IR: *v* 3300 (NH), 1690 (C=O), 1660 (C=N), 1610 (C=C Ar), 1550, 1340, 1075 and 830 cm^{-1} (NCS amide I, II, III and IV mixed vibrational bands, respectively).

2c: m.p. > 300°C; yield 90%. *Anal.* ($C_{16}H_{15}N_5OS$) C, H, N. IR: *v* 3255 (NH), 1700 (C=O), 1650 (C=N), 1590 (C=C Ar), 1545, 1340, 1070 and 870 cm^{-1} (NCS amide I, II, III and IV mixed vibrational bands, respectively).

2d: m.p. 267–269°C; yield 93%. *Anal.* ($C_{16}H_{15}N_5O_2S$) C, H, N. IR: *v* 3250 (NH), 1690 (C=O), 1660 (C=N), 1590 (C=C Ar), 1535, 1335, 1060 and 835 cm^{-1} (NCS amide I, II, III and IV mixed vibrational bands, respectively).

*3.1.3. 1-(4-Oxoquinazolin-2-yl)-4-aryl-3-S-methylisothiosemicarbazides (**3a–d**) and 1-(3-methyl-4-oxoquinazolin-2-yl)-4-aryl-3-S-methylisothiosemicarbazides (**3e–h**)*

3.1.3.1. Using iodomethane in dry methanol. A solution of the thiosemicarbazide derivative **2a–d** (0.5 g) in dry methanol (20 ml) was treated with an equimolar amount of iodomethane. The solution was kept overnight at r.t. and evaporated to dryness under reduced pressure. The residue was treated with ethanol (10 ml) and scratched to deposit into solid. This was filtered and, after purification by preparative TLC and developing with benzene:chloroform:methanol 5:2.5:0.5, gave a negligible amount of the desired product.

3.1.3.2. Using dimethyl sulfate in 1 N NaOH. A solution of the thiosemicarbazide derivative **2a–d** (0.5 g) in 1 N NaOH (10 ml) was treated with dimethyl sulfate (0.2 g, 1.6 mmol) while stirring in an ice-bath. The reaction mixture was stirred for a further 1 h at 4–5°C. The obtained yellow sticky mass was scratched and stirred at r.t. for another 30 min, filtered and washed with water. Separation of the mixture into its components by preparative TLC with benzene:chloroform:methanol 5:2.5:0.5, as developing solvent, gave the 4-oxoquinazolin-2-yl-*S*-methylisothiosemicarbazides (**3a–d**) and the corresponding *N*,*S*-dimethyl derivatives **3e–h**. The physicochemical properties of compounds **3a–h** are listed in Table 1.

IR of **3a**: *v* 3350 (NH), 1675 (C=O), 1645 (C=N), 1590 (C=C Ar) and 1380 cm^{-1} (S–CH_3). ^1H NMR of **3a**: δ 2.62 (s, 3H, S–CH_3) 6.84–7.36 (m, 5H, Ar–H), 7.41–7.58 (m, 3H, quinazolinone C_6–C_8–H), 8.03 (dd, 1H, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, quinazolinone C_5–H), 8.38 (s, 1H, N^1–H). 9.83 (s, dist., 1H, N^4–H), and 11.04 (s, 1H, quinazolinone NH). IR of **3b**: *v* 3320 (NH), 1685 (C=O), 1630 (C=N), 1590 (C=C Ar) and 1375 cm^{-1} (S–CH_3). ^1H NMR of **3b**: δ 2.33 (s, 3H, S–CH_3) 6.80–7.53 (m, 4H, *p*-ClC_6H_4–H), 7.63–7.83 (m, 3H, quinazolinone C_6–C_8–H), 8.19 (dd, 1H, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, quinazolinone C_5–H), 9.54 (s, 1H, N^1–H).

Table 1
Physical data of compounds **3a–h**

Comp.	M.p. (°C)	Yield (%)	Formula (MW)
3a	291–293	24	$C_{16}H_{15}N_5OS$ (325)
3b	279–280	20	$C_{16}H_{14}ClN_5OS$ (359.5)
3c	265–267	25	$C_{17}H_{17}N_5OS$ (339)
3d	252–255	21	$C_{17}H_{17}N_5O_2S$ (355)
3e	223–225	27	$C_{17}H_{17}N_5OS$ (339)
3f	275–277	28	$C_{17}H_{16}ClN_5OS$ (373.5)
3g	260–262	26	$C_{18}H_{19}N_5OS$ (353)
3h	237–239	30	$C_{18}H_{19}N_5O_2S$ (369)

9.80 (s, dist., 1H, N^4–H), and 11.10 (s, dist., 1H, quinazolinone NH). IR of **3c**: *v* 3225 (NH), 1680 (C=O), 1635 (C=N), 1580 (C=C Ar) and 1375 cm^{-1} (S–CH_3). IR of **3d**: *v* 3295 (NH), 1685 (C=O), 1670 (C=N), 1585 (C=C Ar) and 1360 cm^{-1} (S–CH_3). IR of **3e**: *v* 3410 (NH), 1685 (C=O), 1655 (C=N), 1605 (C=C Ar) and 1360 cm^{-1} (S–CH_3). ^{1}H NMR of **3e**: δ 2.47 (s, 3H, S–CH_3) 3.48 (s, 3H, N–CH_3), 6.90–7.39 (m, 6H, 5Ar–H + N^1–H)), 7.46–7.56 (m, 3H, quinazolinone C_6–C_8–H), 8.09 (dd, 1H, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, quinazolinone C_5–H) and 8.98 (s, 1H, N^4–H). IR of **3f**: *v* 3350 (NH), 1688 (C=O), 1635 (C=N), 1570 (C=C Ar) and 1375 cm^{-1} (S–CH_3). IR of **3g**: *v* 3250 (NH), 1695 (C=O), 1645 (C=N), 1595 (C=C Ar) and 1360 cm^{-1} (S–CH_3). IR of **3h**: *v* 3320 (NH), 1680 (C=O), 1655 (C=N), 1580 (C=C Ar) and 1365 cm^{-1} (S–CH_3). ^{1}H NMR of **3h**: δ 2.43 (s, 3H, S–CH_3), 3.47 (s, 3H, S–CH_3), 3.80 (s, 3H, OCH_3), 6.77–7.26 (m, 4H, 4Ar–H), 7.46–7.61 (m, 3H, quinazolinone C_6–C_8–H), 8.03 (dd, 1H, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, quinazolinone C_5–H), 8.39 (s, dist., 1H, N^1–H) and 8.98 (s, 1H, N^4–H).

*3.1.4. 1-Substituted amino[1,2,4]triazolo[4,3-a]quinazolin-5-ones (**4a–d**) and 1-substituted amino-4-methyl[1,2,4]triazolo[4,3-a]quinazolin-5-ones (**4e–h**)*

*3.1.4.1. Reaction of the S-methylisothiosemicarbazides **3a–h** with diethylamine or morpholine.* A solution of the appropriate *S*-methylisothiosemicarbazide **3a–d** or the N^3,*S*-dimethyl derivatives **3e–h** (250 mg) in morpholine or diethylamine (5 ml) was stirred at 100°C for 6–8 h. The mixture was then evaporated to dryness under reduced pressure and the residue was purified by preparative TLC using benzene:ethyl acetate 9:1 as developing system, to give the triazoloquinazolines **4a–d** and **4e–h**.

The physicochemical properties of these compounds are reported in Table 2.

IR of **4a**: *v* 3410 (NH), 1675 (C=O), 1625 (C=N), 1570 (C=C Ar). ^{1}H NMR of **4a**: δ 6.73–7.57 (m, 9H, Ar–H + quinazolinone NH + quinazolinone C_6–C_8–H), 8.14 (dd, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, 1H, quinazolinone C_5–H), 9.52 (s, 1H, NH phenyl). MS of **4a**: $M^{\bullet+}$ 277 absent, $M^+ - O$ ($M - 16$) 261 (23%), 55 (100%). IR of **4b**: *v* 3320 (NH), 1675 (C=O), 1620 (C=N), 1570 (C=C Ar). ^{1}H NMR of **4b**: δ 6.59 (d, $J = 10.9$ Hz, 2H, Ar–H *meta* to Cl), 6.84 (d, $J = 10.9$ Hz, 2H, Ar–H *ortho* to Cl), 7.06–7.44 (m, 4H, quinazolinone NH + C_6–C_8–H), 7.91 (dd, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, 1H, quinazolinone C_5–H), 8.53 (s, 1H, NH–Ar). IR of **4c**: *v* 3300 (NH), 1690 (C=O), 1630 (C=N), 1570 (C=C Ar). ^{1}H NMR of **4c**: δ 6.69 (d, $J = 10.9$ Hz, 2H, Ar–H *ortho* to CH_3), 7.06 (d, $J = 10.9$ Hz, 2H, Ar–H *meta* to CH_3), 7.24–7.69 (m, 4H, quinazolinone NH + C_6–C_8–H), 8.40 (dd, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, 1H, quinazolinone C_5–H), 9.22 (s, 1H, NH–Ar). IR of **4d**: *v* 3390 (NH), 1680 (C=O), 1620 (C=N), 1580 (C=C Ar). ^{1}H NMR of **4d**: δ 3.75 (s, 3H, OCH_3), 6.82 (d, $J = 10.9$ Hz, 2H, Ar–H *meta* to CH_3), 7.06 (m, 3H, Ar–H *ortho* to CH_3 + quinazolinone NH), 7.21–7.60 (m, 4H, quinazolinone C_5–C_8–H), 8.60 (s, 1H, NH–Ar). IR of **4e**: *v* 3410 (NH), 1680 (C=O), 1625 (C=N), 1570 (C=C Ar). ^{1}H NMR of compound **4e**: δ 3.60 (s, 3H, N–CH_3), 6.83–6.93 (m, 3H, Ar–H), 7.18–7.26 (m, 2H, Ar–H), 7.53 and 7.78 (2t, $J = 16.6$ Hz, 2H, quinazolinone C_6 and C_7–H), 8.09 (d, 1H, $J = 8.6$ Hz, quinazolinone C_8–H), 8.22 (dd, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, 1H, quinazolinone C_5–H), 9.04 (s, 1H, NH). MS of compound **4e**: $M^{\bullet+}$ 291 (89%), $M^{\bullet+} - H$ 290 (100%). IR of **4f**: *v* 3320 (NH), 1675 (C=O), 1620 (C=N), 1570 (C=C Ar). ^{1}H NMR of **4f**: δ 3.61 (s, 3H, N–CH_3), 7.27 (d, $J = 10.9$ Hz, 2H, 2 Ar–H *meta* to Cl), 7.37 (d, $J = 10.9$ Hz, 2H, 2Ar–H *ortho* to Cl), 7.53–7.81 (m, 4H, quinazolinone C_6–C_8–H + NH), 8.25 (dd, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, 1H, quinazolinone C_5–H). IR of **4g**: *v* 3345 (NH), 1680 (C=O), 1620 (C=N), 1655 (C=C Ar). ^{1}H NMR of compound **4g**: δ 2.27 (s, 3H, CH_3), 3.81 (s, 3H, N–CH_3), 6.69 (d, $J = 10.9$ Hz, 2H, Ar–H *ortho* to CH_3), 7.01 (d, $J = 10.9$ Hz, 2H, Ar–H *meta* to CH_3), 7.60–8.04 (m, 4H, quinazolinone C_6–C_8–H + NH), 8.40 (dd, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, 1H, quinazolinone C_5–H). IR of **4h**: *v* 3300 (NH), 1690 (C=O), 1630 (C=N), 1570 (C=C Ar). ^{1}H NMR of **4h**: δ 3.71 (s, 3H, OCH_3), 3.78 (s, 3H, N–CH_3), 6.71–6.90 (m, 4H, Ar–H), 7.46–7.61 (m, 3H, quinazolinone C_6–C_8–H), 8.02 (s, dist., 1H, NH), 8.36 (dd, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, 1H, quinazolinone C_5–H).

Table 2
Physical data of compounds **4a–h**

Comp.	M.p. (°C)	Yield (%)	Formula (MW)
4a	230–231	20	$C_{15}H_{11}N_5O$ (277)
4b	250–252	29	$C_{15}H_{10}ClN_5O$ (311.5)
4c	252–254	35	$C_{16}H_{13}N_5O$ (291)
4d	245–247	25	$C_{16}H_{13}N_5O_2$ (307)
4e	246–247	35	$C_{16}H_{13}N_5O$ (291)
4f	270–272	40	$C_{16}H_{12}ClN_5O$ (325.5)
4g	268–270	45	$C_{17}H_{15}N_5O$ (305)
4h	258–259	45	$C_{17}H_{15}N_5O_2$ (321)

*3.1.5. 2,4-Dihydrazinoquinazoline (**6**)*

A mixture of 2,4-dichloroquinazoline [1] (2.5 g, 12.6 mmol) and 50% hydrazine hydrate (20 ml) was heated under reflux for 2 h. The reaction mixture was cooled to r.t., filtered and crystallized from methanol, m.p. 224–225°C (reported 226–227°C) [19]; yield 40%. IR: *v* 3250 and 3050 (NH and NH_2), 1620 (C=N), 1580 and 1500 cm^{-1} (C=C Ar).

*3.1.6. 2,4-Di-(4-substituted thiosemicarbazido)-quinazolines (**7a,b**)*

A solution of 2,4-dihydrazinoquinazoline (**6**) (0.5 g, 2.6 mmol) in ethanol:chloroform (4:1) was treated dropwise while stirring with phenyl or *p*-chlorophenyl isothiocyanate (10 mmol), the reactants were stirred overnight at r.t. The obtained yellowish-white precipitate was filtered, washed with light petroleum 60–80°C and crystallized from ethanol. **7a**: m.p. 296–298°C; yield 90%. *Anal.* ($C_{22}H_{20}N_8S_2$) C, H, N. IR of **7a**: *v* 3450 (NH), 1620 (C=N), 1590, 1495 (C=C Ar), 1545, 1380, 1080 and 810 cm^{-1} (NCS amide I, II, III and IV mixed vibrational bands, respectively). **7b**: m.p. > 300°C; yield 85%. *Anal.* ($C_{22}H_{18}Cl_2N_8S_2$) C, H, N. IR of **7b**: *v* 3250 (NH), 1640 (C=N), 1615, 1490 (C=C Ar), 1530, 1370, 1090 and 828 cm^{-1} (NCS amide I, II, III and IV mixed vibrational bands, respectively). ^{1}H NMR of **7b**: δ 7.39–7.49 (m, 8H, $2C_6H_4Cl$(p)), 7.58–7.80 (m, 3H, quinazolinone C_6–C_8–H), 8.33 (dd, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, 1H, quinazolinone C_5–H), 9.84–9.99 (m, 4H, $2N^1$–H and $2N^2$–H), 10.24 (s, 2H, $2N^4$–H).

*3.1.7. 2,4-Di-(4-substituted-3-S-methylisothiosemicarbazido)quinazolines (**8a,b**)*

A solution of the dithiosemicarbazide derivative **7a**,**b** (0.5 g) in 1 N NaOH (10 ml) containing a few drops of ethanol was treated with an equimolar amount of dimethyl sulfate while stirring in an ice-bath. Stirring was continued for 1 h and the obtained yellow sticky mass was scratched, and stirred for another 30 min. The product was filtered, washed with water and purified by preparative TLC using benzene:ethyl acetate 8:2 as developing solvent.

8a: m.p. 195–197°C; yield 30%. *Anal.* ($C_{24}H_{24}N_8S_2$) C, H, N. IR of **8a**: *v* 3350 (NH), 1635 (C=N), 1590, 1490 (C=C Ar) and 1310 cm^{-1} (S–CH_3).

8b: m.p. 205–207°C; yield 33%. *Anal.* ($C_{24}H_{22}Cl_2N_8S_2$) C, H, N. IR of **8b**: *v* 3300 (NH), 1620 (C=N), 1575, 1500 (C=C Ar) and 1315 cm^{-1} (S–CH_3). ^{1}H NMR: δ 2.44 (s, 6H, 2S–CH_3), 7.15–7.42 (m, 8H, $2C_6H_4Cl$(p)), 7.86–8.19 (m, 3H, quinazolinone–C_6–C_8–H), 8.35 (dd, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, 1H, quinazolinone C_5–H), 9.73–9.93 (m, 2H, $2N^1$–H) and 10.38 (s, dist., 2H, $2N^4$–H).

*3.1.8. 3,7-bis-(Substituted amino)[1,2,4]ditriazolo-[4,3-a:4′,3′-c]quinazolines (**9a,b**)*

A solution of di-*S*-methylisothiosemicarbazide derivative **8a**,**b** (250 mg) in excess morpholine or diethylamine (3 ml) was heated on a water bath for 6 h. The mixture was then evaporated to dryness under reduced pressure and the residue purified by preparative TLC using benzene:ethyl acetate 9:1 as developing solvent.

9a: m.p. 260–262°C; yield 25%. *Anal.* ($C_{22}H_{16}N_8$) C, H, N. IR of **9a**: *v* 3400 (NH), 1620 (C=N), 1590, 1495 (C=C Ar). ^{1}H NMR: δ 6.86–7.25 (m, 10H, $2 \times C_6H_5$), 7.28–7.65 (m, 3H, quinazolinone C_6–C_8–H), 8.59 (dd, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, 1H, quinazolinone C_5–H), 10.21 (s, 2H, $2 \times NH$–C_6H_5).

9b: m.p. 285–287°C; yield 30%. *Anal.* ($C_{22}H_{14}Cl_2N_8$) C, H, N. IR of **9b**: *v* 3450 (NH), 1615 (C=N), 1595, 1495 (C=C Ar). ^{1}H NMR: δ 7.27–7.70 (m, 8H, $2 \times C_6H_4Cl$), 7.90–8.21 (m, 3H, quinazolinone C_6–C_8–H), 8.40 (dd, $J_o = 9.9$ Hz, $J_m = 1.8$ Hz, 1H, quinazolinone C_5–H), 9.11 (s, 2H, $2 \times NH$–C_6H_4Cl). MS: $M^{\bullet+}$ 461 absent, $M^{\bullet+} - 2 \times p$-chlorophenyl, -2H, 236 (37%), 55 (100%).

3.2. Antitoxoplasmosis properties

The antitoxoplasmosis property of the synthesized quinazoline derivatives was studied by examining their effect on the surface morphology of intact fixed organisms, as viewed by scanning electron microscopy (SEM).

3.2.1. Materials and methods

An RH virulent strain of *Toxoplasma gondii* was maintained in the Parasitology Laboratory, Faculty of Medicine, University of Alexandria, by several passages (3–4 day intervals) of tachyzoites intraperitoneally in Swiss albino mice (6–8 weeks old and about 20 g each). The tachyzoites were harvested from the peritoneal exudate on the 4th day of infection, washed three times and diluted to 10^3 with phosphate buffer solution (PBS) pH 7.4 [24]. They were then inoculated intraperitoneally at a dose of 0.1 ml/mouse in 50 Swiss albino mice, and were divided into ten groups (five mice each) [24].

3.2.2. Infected, non-treated control group

48 h post-inoculation, DMSO was injected subcutaneously in a dose of 0.1 ml/mouse. The parasite-rich peritoneal fluid was taken 5 days post-inoculation.

3.2.3. Standard group

Pyrimethamine, obtained by extraction of five pulverized Daraprim® tablets (pyrimethamine tablets BP 25 mg, Wellcome, England) with ethanol (50 ml) followed by evaporation of the solvent, was given orally as a single dose of 0.25 mg in 0.1 ml DMSO/mouse [25,26] 48 h after inoculation. The peritoneal fluid containing tachyzoites was collected 3 days after treatment.

3.2.4. Test groups

An amount of each of the test compounds (**1**, **2b**, **3f**, **4f**, **6**, **7b**, **8b** and **9b**) equivalent to the amount of pyrimethamine was given as a single dose in 0.1 ml DMSO/mouse. Peritoneal fluid containing tachyzoites was collected on the 5th day post inoculation.

3.2.5. Preparation of specimens for SEM

The collected suspension of *Toxoplasma* was washed twice with PBS (600 ml) then fixed in glutaraldehyde. Specimens were then washed three times by flooding with large volumes of sterile distilled water. The specimens were processed according to Klainer and Betsch method [27] and examined using a Jeol-JSM-25 SII scanning microscope.

4. Results

The tachyzoites of *T. gondii* in the control group appeared in SEM as elongate, often crescent-shaped with a round pole at one end and a more or less pointed pole at the other (Fig. 1). The site of the conoid resembled a compressed spring (Fig. 2). The surface of the tachyzoites had slender ridges radiating from the anterior to the posterior end. The cytostome appeared as indentation in the pellicle (Fig. 1). Each parasite was enclosed in a membrane with blebs at the periphery (Fig. 3). This membrane isolated the organism from the host cell cytoplasm. Figs. 4 and 5 show the entry of the *Toxoplasma* into the host cells by penetration through the conoid.

The tachyzoites of *T. gondii* in the standard group appeared smooth, homogeneous, regular with more or less tapered ends and elongated. They lost their crescent shape (Fig. 6). Some organisms appeared to have pores or dimples on their surface (Fig. 7).

In the test groups, treatment with 2-hydrazinoquinazolin-4-one (**1**), the *p*-chlorophenylthiosemicarbazide **2b**, *S*-methylisothiosemicarbazide **3f** and the monotriazoloquinazolinone **4f** caused some organisms to become smooth, homogeneous and lose their ridges. Their membrane became redundant with no blebs (Fig. 8)

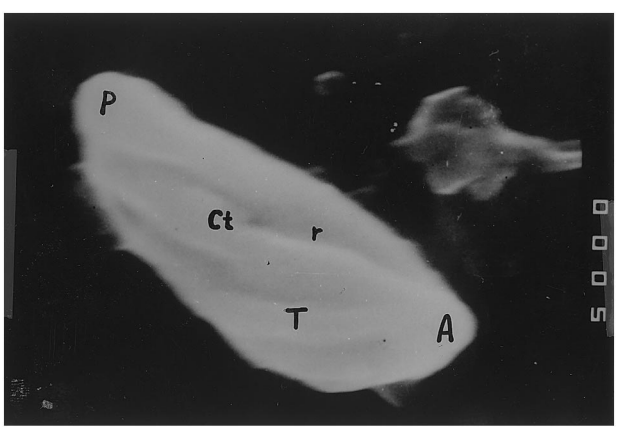


Fig. 1. Effect of the synthesized quinazoline derivatives on the surface morphology of intact fixed *T. gondii* as viewed by scanning electron microscopy (SEM). Abbreviations: T, tachyzoites; C, conoid; Ct, cytostome; b, blebs; H, host cell; A, anterior end; P, posterior end; r, ridges.

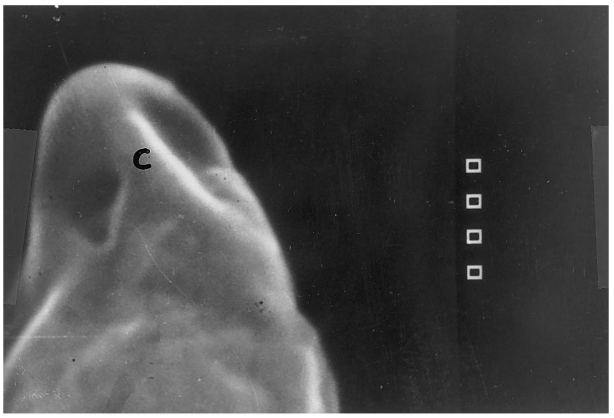


Fig. 2. Effect of the synthesized quinazoline derivatives on the surface morphology of intact fixed *T. gondii* as viewed by SEM. See Fig. 1 for abbreviations.

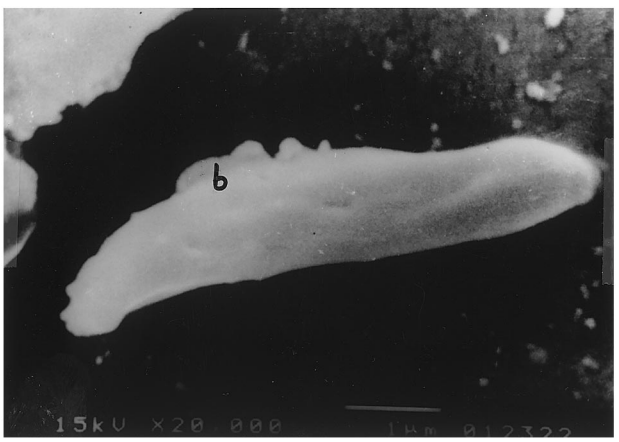


Fig. 3. Effect of the synthesized quinazoline derivatives on the surface morphology of intact fixed *T. gondii* as viewed by SEM. See Fig. 1 for abbreviations.

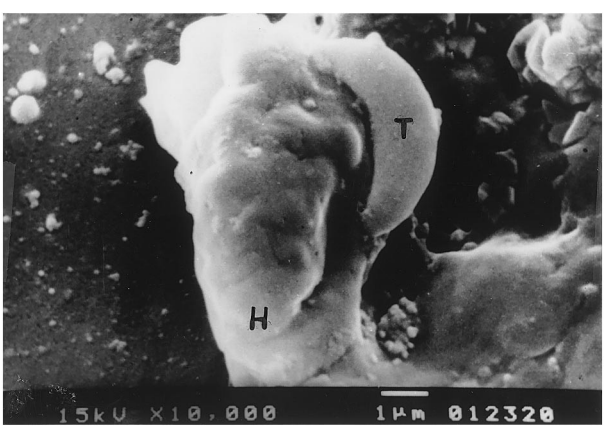


Fig. 4. Effect of the synthesized quinazoline derivatives on the surface morphology of intact fixed *T. gondii* as viewed by SEM. See Fig. 1 for abbreviations.

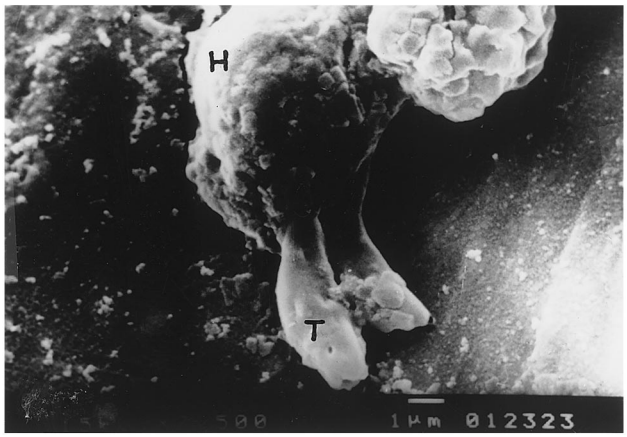


Fig. 5. Effect of the synthesized quinazoline derivatives on the surface morphology of intact fixed *T. gondii* as viewed by SEM. See Fig. 1 for abbreviations.

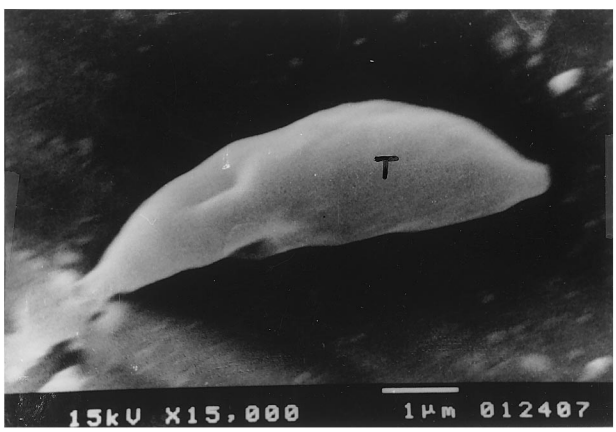


Fig. 6. Effect of the synthesized quinazoline derivatives on the surface morphology of intact fixed *T. gondii* as viewed by SEM. See Fig. 1 for abbreviations.

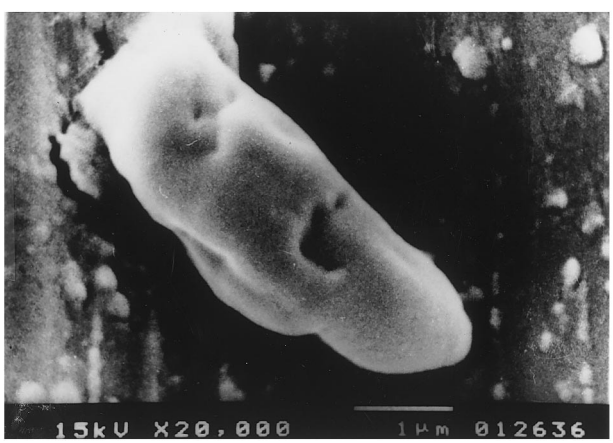


Fig. 7. Effect of the synthesized quinazoline derivatives on the surface morphology of intact fixed *T. gondii* as viewed by SEM. See Fig. 1 for abbreviations.

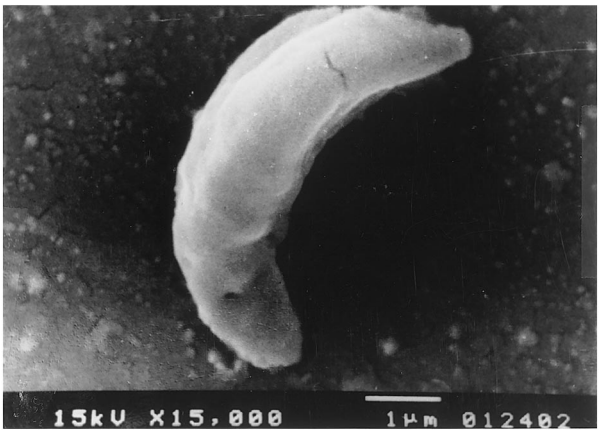


Fig. 8. Effect of the synthesized quinazoline derivatives on the surface morphology of intact fixed *T. gondii* as viewed by SEM. See Fig. 1 for abbreviations.

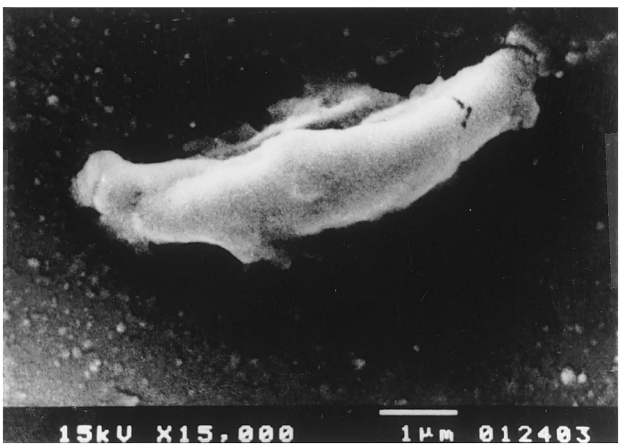


Fig. 9. Effect of the synthesized quinazoline derivatives on the surface morphology of intact fixed *T. gondii* as viewed by SEM. See Fig. 1 for abbreviations.

and appeared as if it has been peeled off leaving a disorganized tachyzoite with a disorganized conoid (Fig. 9).

In the infected group treated with 2,4-bis-(hydrazinoquinazoline) (**6**), 2,4-bis-(*p*-chlorophenylthiosemicarbazide) (**7b**), 2,4-bis-(*S*-methylisothiosemicarbazide) (**8b**) and the ditriazoloquinazoline (**9b**), the tachyzoites of *Toxoplasma* gradually lost their crescent shape and became triangular with a complete disorganized surface and absence of the conoid (Figs. 10–12).

5. Discussion

SEM allows the study of the surface of *T. gondii* at high magnification in three-dimensional perspectives. The present work examined the details of intact and treated organisms of *T. gondii* as well as the effect of

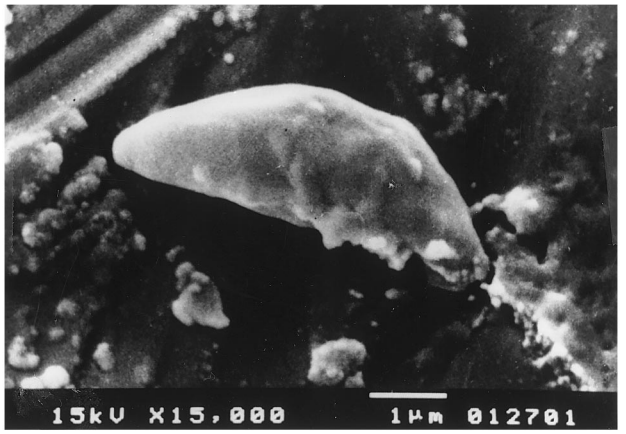


Fig. 10. Effect of the synthesized quinazoline derivatives on the surface morphology of intact fixed *T. gondii* as viewed by SEM. See Fig. 1 for abbreviations.

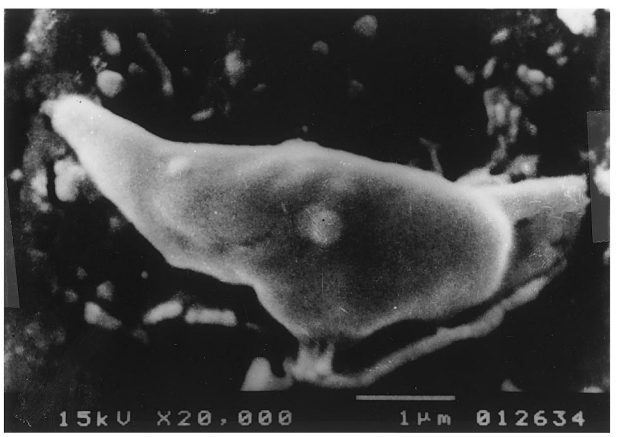


Fig. 11. Effect of the synthesized quinazoline derivatives on the surface morphology of intact fixed *T. gondii* as viewed by SEM. See Fig. 1 for abbreviations.

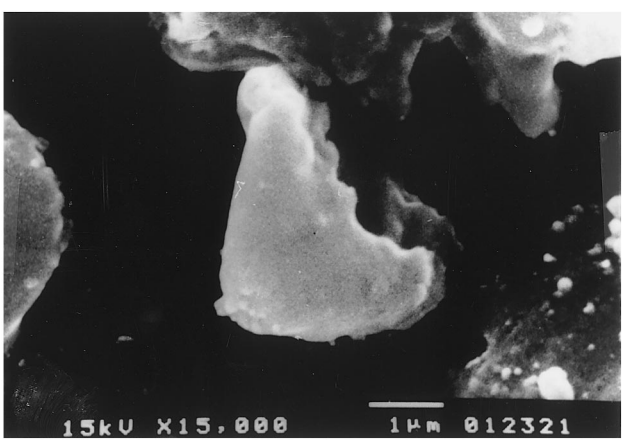


Fig. 12. Effect of the synthesized quinazoline derivatives on the surface morphology of intact fixed *T. gondii* as viewed by SEM. See Fig. 1 for abbreviations.

test compounds on the presence or absence of conoid. The latter is important for entry of the parasite in the host cell membrane [28].

In the groups treated with pyrimethamine and the monosubstituted quinazolin-4-ones **1**, **2b**, **3f** and **4f**, the shape of organisms became distorted. In the groups treated with the disubstituted quinazolines **6**, **7b**, **8b** and **9b**, the organisms became triangular in shape. The changes in conoid whether disorganized or completely absent, are an indication of the antitoxoplasmosis efficacy of the test compounds on the organisms, a property which is important for obstructing the entry of the organisms into the host cells and, in turn, causing their ultimate elimination. According to Hammouda et al. [26], the tachyzoites after treatment with pyrimethamine or spyramicin, change their shape and become rounded or triangular with loss of their conoid. These changes in structure may be secondary to the changes resulting from interference of the drugs in DNA synthesis of the parasite or interference with the folic acid cycle.

The results achieved in this study have also shown that the replacement of the amino group at the 2- or 2,4-positions of quinazolines by hydrazino (**1** and **6**), thiosemicarbazido (**2b** and **7b**) or *S*-methylisothiosemicarbazido (**3f** and **8b**) moieties retained the antiprotozoan activity. Conversion of these flexible chains into the rigid triazoloquinazoline structure, as in compounds **4f** and **9b**, also retained the antiprotozoan activity. These successful modifications have shed some light on new structural requirements for antitoxoplasmosis activity of quinazoline derivatives. Similar studies on pyrimidine ring systems are progressing.

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