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Original paper

Synthesis of thieno[3,2-*g*]quinoxalines as potential amoebicides

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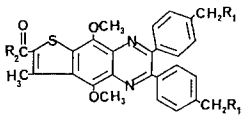
Summary – A route for the synthesis of thieno[3,2-*g*]quinoxalines and their derivatives is described. The antiamoebic activity of these compounds has been preliminaly assayed against *Entamoeba histolytica*. Compounds **1a, b, g, l, m, n, o** displayed activity against hepatic amoebiasis and compounds **1 a, b, l** showed activity against intestinal amoebiasis in animal models.

Résumé – **Synthèse de thiéno[3,2-*g*]quinoxalines, composés à activité antiamibienne potentielle.** *Une série de dérivés de thiéno[3,2-*g*]quinoxalines a été synthétisée et les produits ont été évalués pour leurs effets sur* Entamoeba Histolytica. *Sept composés* ***Ia, b, g, l, m, n, o*** *sont actifs contre l'amibiase hépatique et les dérivés* ***Ia, b, l*** *sont également actifs contre l'amibiase intestinale chez les animaux de laboratoire.*

thieno[3,2*g*]quinoxalines / hepatic amoebiasis / intestinal amoebiasis

Introduction

Substituted quinoxalines displayed antiamoebic, antitrichomonad and antiswine dysenteric properties [1, 2]. In continuation of our programme to find novel amoebicides [3, 4], we were interested in synthesizing thieno[3,2-*g*]quinoxalines **1** and studying their antiamoebic activities in the animal models. Herein we report a simple synthesis of the desired compounds and their derivatives.



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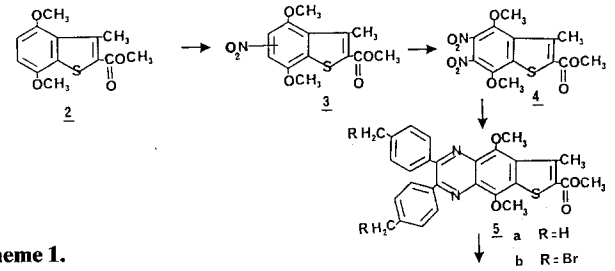
Chemistry

The synthesis of 2,3-diphenyl thienoquinoxalines **1** was accomplished starting with the known benzothiophene derivative **2** [5] as shown in Scheme 1.



Scheme 1.

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Direct one pot dinitration of **2** resulted in the formation of **4** in 20% yield. However, various trial experiments revealed that the yield of the dinitro compound could be improved significantly to 62% by carrying out the reaction in 2 steps. Treatment of **2** with concentrated nitric acid in acetic acid provided the mono nitro compound, methyl 5 or 6-nitro-4,7-dimethoxy-3-methylbenzo[*b*]thiophene-2-carboxalate **3**. Further nitration with concentrated nitric acid in trifluoroacetic acid yielded the required dinitro benzothiophene derivative **4** in 80% yield. Dinitro benzothiophene **4** was reduced to the corresponding *o*-phenylenediamine, followed by condensation with 4,4′-dimethylbenzil or 4,4′-dibromomethylbenzil in the presence of acetic acid to furnish the desired thienoquinoxaline **5a** or **5b**, respectively. Various aminomethyl derivatives were then prepared by reacting **5b** with appropriate amines. As a structural modification, the ester group present in thienoquinoxaline **5b** was converted to amide **1** [R_2 = N] through the corresponding carboxylic acid. The physical constants, yields and analytical data are given in Table I.

Results and Discussion

In vitro *study*

The compounds **1a–o** were initially tested for their *in vitro* activity against *Entamoeba histolytica* using a polyxenic culture.

The result of the test performed with the present compounds is documented in Table II. All the compounds displayed *in vitro* activity in the range of 10–100 μg/ml except **1j** and **1i** (Table II). Standard antiamoebic

compounds such as nitroimidazoles and diloxanide furoate showed *in vitro* activity in the range of 2–5 μg/ml in the above tests.

Animal study

Out of 15 compounds tested against hepatic amoebiasis in golden hamsters, 7 exhibited *in vivo* activity, of which **1l** cured 100% of the treated animals at 125 mg/kg × 4 *per os* (Table II). Compound **1l** also showed activity against intestinal amoebiasis in weanling Wistar rats. It cured 50% of the treated rats at 250 mg/kg × 4 *per os*. Two other compounds, namely **1a** and **1b**, displayed partial activity against hepatic amoebiasis at a dose of 150 mg/kg × 4 and showed activity against intestinal amoebiasis at a dose of 250 mg/kg × 4.

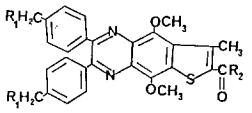
In summary, the activity of these derivatives is relatively low as compared with the standard antiamoebic drugs. Nevertheless, their *in vivo* activity indicated their possible use as leads for further optimisation.

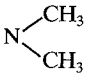
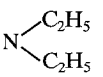
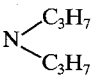
Experimental protocols

Chemistry

Melting points are uncorrected. IR spectra were recorded on Perkin–Elmer 157 Spectrophotometer. Chemical shifts (δ) are in parts per

Table I. Thieno[3,2-*g*]quinoxalines 1.



1	R_1	R_2	mp (°C)	Yield (%)	Molecular formula[a]
a		OCH_3	158–59[b]	59	$C_{37}H_{40}N_4O_4S$
b		OCH_3	165[b]	68	$C_{39}H_{44}N_4O_4S$
c		OCH_3	148[b]	43	$C_{41}H_{48}N_4O_4S$
d		OCH_3	176–77[b]	29	$C_{39}H_{46}N_6O_4S$
e		OCH_3	82[b]	45	$C_{43}H_{52}N_4O_4S$
f		OCH_3	173–74[b]	47	$C_{33}H_{36}N_4O_4S$
g		OCH_3	141[b]	41	$C_{37}H_{44}N_4O_4S$
h		OCH_3	95–96[b]	31	$C_{41}H_{52}N_4O_2S$
i		OCH_3	92[b]	29	$C_{41}H_{44}N_4O_4S$
j		OH	>220[c,d]	64	$C_{38}H_{42}N_4O_4S$
k		NH_2	213–14[d]	61	$C_{38}H_{43}N_5O_3S$
l			192[d]	60	$C_{41}H_{51}N_4O_3S$
m		$N(C_2H_5)_2$	80–81[d]	55	$C_{42}H_{51}N_5O_3S$
n			94[d]	58	$C_{40}H_{45}N_5O_3S$
o			162–63[d]	19	$C_{41}H_{41}N_5O_3SH_2O$

[a]Satisfactory elemental analyses were obtained C, ±0.17 – ±0.3; H, ±0.08 – ±0.3; N, ±0.03 – ±0.4.
Solvent for crystallisation: [b]hexane; [c]aq. alcohol; [d]methanol.

million relative to tetramethylsilane. Coupling constants (J values) are in Hertz (Hz). ^{1}H NMR spectra were run on a Varian T-60 Spectrometer.

5- or 6-Nitro-4,7-dimethoxy-3-methylbenzo[b]thiophene-2-carboxylate ***3***
To the well stirred suspension of 5 g (18.8 mmol) of **2** [5] in 50 ml of glacial acetic acid, 1 ml of conc. nitric acide (70%) was added dropwise at room temperature. At the end of the addition a yellow product was formed. The stirring was continued for a further 30 min. The reaction was then filtered, washed with 10 ml of cold acetic acid and then thoroughly washed with water affording **3** (4.5 g, 77%) as a yellow solid: mp 182–183°C, IR: ν 1730 cm^{-1} ($-COOCH_3$); ^{1}H NMR δ 2.95 (s, 3H, $Ar-CH_3$); 3.90, 3.95 and 4.00 (3 × s, 9H, 2 × OCH_3 and $-COOCH_3$); 7.25 (s, 1H, Ar–H).

Methyl-5,6-dinitro-4,7-dimethoxy-3-methylbenzo[b]thiophene-2-carboxylate ***4***
A solution of 4.5 g (14.5 mmol) of **3** in 30 ml of trifluoroacetic acid was cooled in an ice-bath. 2 ml of conc. nitric acid (70%) was then added dropwise and the resulting dark red solution was stirred for a further 2 h. The reaction mixture was then diluted with ice-water. Filtration of the obtained solid, followed by washing with 5 ml of cold acetic acid and then with water, yielded **4** (4.1 g, 80%), mp: 196–197°C. IR: ν 1730 cm^{-1} ($-COOCH_3$), ^{1}H NMR (TFA-d) δ 2.8 (s, 3H, $Ar-CH_3$); 3.90 and 3.95 (2 × s, 9H, 2 × $Ar-OCH_3$ and $-COOCH_3$). Anal. calc. for $C_{13}H_{12}N_2O_8S$: C: 43.82; H: 3.39; N: 7.86; found: C: 43.84; H: 3.03; N: 7.41

Methyl-6,7-di-(4-methylphenyl)-4,9-dimethoxy-3-methylthieno[3,2-g]-quinoxalin-2-carboxylate ***5a***
A suspension of 0.6 g (1.7 mmol) of **4** in 10 ml of ethanol was reduced at 50 psi hydrogen pressure using Raney nickel as a catalyst, in a Parr hydrogenator, at room temperature. The catalyst was filtered off and washed with 5 ml of alcohol. Removal of solvent from the combined filtrate gave a solid. To the solution of this solid in 5 ml of acetic acid, 0.42 g (1.8 mmol) of 4,4-dimethylbenzyl was added and the reaction mixture was stirred at 70°C for 1 h. Dilution with water gave a solid which was filtered, washed with water, dried and purified by column chromatography on silica gel using chloroform as eluant to afford **5a** (0.64 g, 76%). mp: 256–257°C: IR : ν 1730 cm^{-1} ($-COOCH_3$); ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 6H, 2 $ArCH_3$); 3.10 (s, 3H, $ArCH_3$); 3.80 (s, 3H, $-COOCH_3$); 4.30 and 4.35 (2 × s, 6H, 2 × $ArOCH_3$); 6.95 (d, J = 8 Hz, 4H, Ar–H); 7.35 (d, J = 8Hz, 4H, Ar–H). Anal. calc. for $C_{29}H_{26}N_2O_4S$: C: 69.86; H: 5.26; N: 5.62; S: 6.43; found: C: 69.39; H: 5.14; N: 5.81; S: 6.72.

Methyl-6,7-di-[4-(bromomethyl)phenyl]-4,9-dimethoxy-3-methylthieno-[3,2-g]quinoxalin-2-carboxylate ***5b***
Similarly **5a** was prepared from 4,4′ di (bromomethyl) benzyl (1 g;

Table II. *In vitro* and *in vivo* antiamoebic activity of some thienoquinoxalines.

1	*In vitro*	*In vivo* (cured / treated)			
	MIC (μg / ml)	Hepatic[a]			Caecal[b]
		150 mg / kg × 4 *p.o.*	125 mg / kg × 4 *p.o.*	100 mg / kg × 4 *p.o.*	250 mg / kg × 4 *p.o.*
a	10	2 / 6	–	0 / 4	2 / 2
b	100	2 / 4	–	1 / 4	3 / 4
c	50	–	–	0 / 4	–
d	50	–	–	0 / 4	–
e	50	–	–	0 / 4	–
f	50	0 / 4	–	0 / 4	–
g	25	–	3 / 4	3 / 4	–
h	inactive	–	–	0 / 4	–
i	inactive	–	–	0 / 4	–
j	inactive	–	–	0 / 4	–
k	50	–	–	0 / 4	–
l	100	–	7 / 7	3 / 4	2 / 4
m	50	2 / 6	–	2 / 4	–
n	50	2 / 4	–	3 / 4	–
o	50	–	–	2 / 4	–
Metronidazole (Flagyl)	5	100% activity at 40 mg / kg × 4 against hepatic amoebiasis			
Diloxanide furoate	2	100% activity at 200 mg / kg × 4 against caecal amoebiasis			

[a]Activity against hepatic amoebiasis in hamster model; administration −2, +2, +24 and 48 h.
[b]Activity against intestinal amoebiasis in weanling rat model; administration −24, +48, 72 and +96 h.

2.5 mmol) in (0.75 g) 45% yield: mp: 230°C. IR: ν 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.16 (s, 3H, Ar−CH_3); 3.98 (s, 4H, 2 × CH_2−Br); 4.38, 4.40 and 4.42 (3 × s, 9H, 2 × OCH_3 and −$COOCH_3$); 7.30 (d, J = 8 Hz, 4H, Ar−H); 7.58 (d, J = 8 Hz, 4H, Ar−H).

Methyl-6,7-di-[4-(piperidinomethyl)phenyl]-4,9-dimethoxy-3-methyl-thieno-(3,2-g)-quinoxaline-2-carboxylate ***Ib***

To the well stirred suspension of 0.4 g (0.6 mmol) of **5b** in 2 ml of dimethylformamide, 0.4 ml of piperidine was added at room temperature. The resulting clear solution was stirred for further 30 min, water was added and the solid obtained was collected by filtration, washed with water and dried. Purification by chromatography over alumina using petroleum ether / ethyl acetate / benzene as eluant provided **1b** (0.28g, 68%), mp: 165°C. IR: ν 1730 cm^{-1} (−$COOCH_3$); ^1H NMR ($CDCl_3$) δ 1.20−1.80 (m, 12H, −CH_2−CH_2−CH_2−); 2.2−2.60 (m, 8H, NCH_2); 3.10 (s, 3H, $ArCH_3$); 3.45 (s, 4H, Ar−CH_2); 3.90 (s, 3H, $COOCH_3$); 4.35 and 4.40 (2 × S, 6H, $ArOCH_3$); 7.20 (d, J = 8 Hz, 4H, ArH); 7.50 (d, J = 8 Hz, 4H, ArH). Anal. calc. for $C_{39}H_{44}N_4O_4S$: C: 70.45; H: 6.67; N: 8.43; found: C: 70.63; H: 6.78; N: 8.32.

Compounds **1a, c−i** were prepared similarly (Table I).

6,7-Di-[4-(piperidinomethyl)phenyl]-4,9-dimethoxy-3-methylthieno(3,2-g)-quinoxaline-2-carboxylic acid ***Ij***

To the solution of 0.22 g (0.3 mmol) of **1b** in 3 ml of tetrahydrofuran, an aqueous solution of sodium hydroxide (0.2 g / 4 ml) was added and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was then extracted with ether and the aqueous layer was carefully acidified to pH 6.5 with acetic acid. The precipitated **1j** was filtered, washed with water and dried (0.14 g, 64%); mp: >220°C (d). IR: ν 3570 cm^{-1} (OH); ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 1.30−2.00 (m, 12H, CH_2−CH_2−CH_2−CH_2); 2.55−2.80 (m, 8H, NCH_2); 3.10 (s, 3H, $ArCH_3$); 3.50 (s, 4H, Ar−CH_2); 4.20 and 4.25 (s, 6H, Ar−OCH_3); 7.20−7.60 (m, 8H, ArH). Anal. calc. for $C_{38}H_{42}N_4O_4S$. 1/2 H_2O: C: 69.16; H: 6.57; N: 8.49; found: C: 68.89; H: 6.30; N: 8.02.

6,7-Di-[4-(piperidinomethyl)phenyl]-4,9-dimethoxy-2-piperidoyl-3-methyl-thieno(3,2-g)quinoxaline ***Il***

To the stirred suspension of 1.0 g (1.5 mmol) of **1j** in 20 ml of dry tetrahydrofuran, 3 ml of freshly distilled thionyl chloride was added and the resulting solution was stirred at room temperature for 30 min. Solvent and excess of thionyl chloride was distilled under vaccum and the process repeated twice, each time adding 20 ml of dry tetrahydrofuran. The resulting solid was dissolved in 10 ml of dry *N,N*-dimethylformamide and 5 ml of piperidine was then added to the solution. After stirring for further 30 min, water was added to the reaction mixture and the solid obtained was filtered, washed with water and dried. Crystallisation from methanol gave **1l** (0.67 g, 61%); mp: 192°C. IR: ν 1650 cm^{-1} ($CON(CH_2)_5$); ^1H NMR ($CDCl_3$) δ 1.20−1.85 (m, 18H, −CH_2−CH_2−CH_2); 2.15−2.50 (m, 8H, N−CH_2); 2.60 (s, 3H, $ArCH_3$); 3.30−3.80 (m, 8H, Ar−CH_2 and $CONCH_2$); 4.30 and 4.40 (s, 6H, $ArOCH_3$); 7.20 (d, J = 8 Hz, 4H, ArH); 7.70 (d, J = 8 Hz, 4H, ArH). Anal. calc. for $C_{41}H_{51}N_4O_3S$: C: 72.42; H: 7.56; N: 8.24; found: C: 72.21; H: 7.48; N: 8.37.

Compounds **1k, m−o** were prepared similarly (Table I).

Biological evaluation

Materials and Methods

Minimum inhibitory concentration (MIC) was determined using polyxenic culture of *E. histolytica* BY 80 strain. Compounds were dissolved in DMSO and serial dilutions were made. An appropriate concentration of DMSO alone was taken during the experiments as DMSO control. MIC was determined by microscopic examination of 10 fields for motile *E. histolytica* from the sediments of the culture tube after thorough mixing with 0.5 ml of fresh medium. Two sets of controls were maintained, one without any test material and another with metronidazole or diloxanide furoate as the standard antiamoebic compound.

For *in vivo* drug administration compounds were prepared as a suspension with 0.5% aqueous carboxy methyl cellulose (tylose) and drenched orally by a stomach tube to respective animals. For hepatic infection study, the compounds were administered 2 h before the infection followed by a second dose 2 h after infection and then one dose each day for 2 consecutive days. Autopsy was performed on sixth day post infection and final results were obtained by microscopic and culture examination of the liver tissues from the treated hamsters. A compound was considered inactive when motile trophozites of *E. histolytica* were detected by such examination. The intestinal amoebiasis was studied in weanling Wistar rats. The rats were inoculated with 400,00 trophozites in the caecum through the ileocaecal junction after brief ether anaesthesia. The first dose of the test compounds was administered 24 h post infection followed by 3 more doses, one each day for 3 consecutive days. Autopsy was made on sixth day post infection and caecum was examined for amoebia first microscopically and if negative, then by culture of the affected tissues. In control groups, metronidazole-treated animals were considered as active drug control for hepatic study. Similarly, diloxanide furoate-treated animals were kept as active controls for intestinal amoebiasis. Every experiment had infected but untreated animals as controls for comparison.

Details of methodology for the *in vitro* and *in vivo* tests have been published elsewhere [6, 7].

Acknowledgments

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