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New 2-alkylidenemethyl-5-nitrothiophenes: preparation *via* $S_{RN}1$ reactions and *in vitro* antiprotozoan activity

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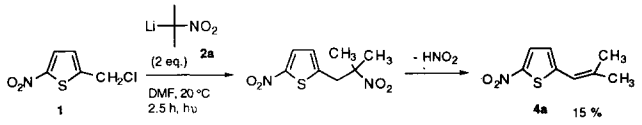
Summary — The reaction of 2-chloromethyl-5-nitrothiophene with the 2-nitropropane anion has been reinvestigated and extended to various nitronate anions to afford good yields of new 5-nitrothiophenes bearing a trisubstituted ethylenic double bond at the 2-position. These compounds were evaluated as potential antiprotozoan agents and some derivatives were found to have the same activity as that of reference compounds.

nitrothiophene derivative / antiprotozoan activity / $S_{RN}1$

Introduction

The antiprotozoan activity of various 5-nitrothiophene derivatives is well established. In 1981 Gayral [1] demonstrated that the 5-nitrothiophene skeleton was more active against protozoa than 5-nitrofuran. Recently, we reported the significant antiparasitic activity of hydrazones [2], aldimines [3] and oxime ethers [4] in the nitrothiophene series.

In 1979 Newcombe and Norris [5] described the reaction of 2-chloromethyl-5-nitrothiophene **1** with 2-nitropropane anion **2a** to give 2-isopropylidenemethyl-5-nitrothiophene **4a** by an $S_{RN}1$ C-alkylation followed by a base-promoted nitrous acid elimination. Nevertheless, this ethylenic derivative appeared to decompose to polar, tarry materials under the experimental conditions (in dimethylformamide at 20°C over 2.5 h with substrate concentration 0.25 M and salt concentration 0.5 M), which presumably accounts for the poor material balance in this reaction.



As a continuation of our program directed toward the preparation of new pharmacological compounds by $S_{RN}1$ reactions [6–7], we have reinvestigated the $S_{RN}1$ reaction of this reductive alkylating agent in order to increase yields and study extension to other nitronate anions.

Chemistry and pharmacology

The $S_{RN}1$ reaction [8] is a chain reaction involving radical and radical-anion intermediates as illustrated in eqns [1]–[4] in which R represents a range of sp^3–C groups, X a suitable nucleofuge and Nu^- a nucleophile.

$$RX + Nu^- \longrightarrow RX^{\bullet -} + Nu^{\bullet} \quad [1]$$

$$RX^{\bullet -} \longrightarrow R^{\bullet} + X^- \quad [2]$$

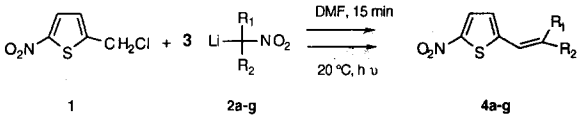
$$R^{\bullet} + Nu^- \longrightarrow RNu^{\bullet -} \quad [3]$$

$$RNu^{\bullet -} + RX \longrightarrow RNu + RX^{\bullet -} \quad [4]$$

The initiation step (eqn [1]) involves electron capture by the substrate RX to form a radical-anion. Dissociation of the intermediate radical-anion (eqn [2]) gives an anion and a reactive radical which rapidly reacts with the nucleophile to form a new radical-anion (eqn [3]). The chain is completed by single electron transfer from the product radical-anion to RX

(eqn [4]). The radical Nu$^{\bullet}$ generated in the initiation step is formed in very small quantities in a chain reaction and products from this species are generally not observed.

In the one 5-nitroimidazole series [9] we have described the influence of the donor/acceptor ratio (Nu^-/RX) on the reaction of 1-methyl-2-chloromethyl-5-nitroimidazole with 2-nitropropane anion. The use of an excess of nitronate anion was found to increase the C-alkylation yield. By using 3 equivalents of lithium salt of 2-nitropropane **2a** for 1 equivalent of 2-chloromethyl-5-nitrothiophene under the same conditions described by Newcombe and Norris (in dimethylformamide at 20°C under inert atmosphere and catalysis with light) but over 15 min, we have isolated 2-isopropylidenemethyl-5-nitrothiophene **4a** in 71% yield.

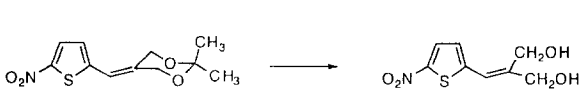


1 2a-g 4a-g

The use of these optimal experimental conditions for the reaction of other different cyclic and heterocyclic nitronate anions **2b–g** has afforded good yields of new 5-nitrothiophene derivatives **4b–g** bearing a trisubstituted ethylenic double bond at the 2-position.

The nitroalkanes were commercially available or prepared from secondary amines by oxidation with *m*-chloroperbenzoic acid [10] by refluxing in 1,2-dichloroethane for 3 h. These compounds were used as lithium salts and with 2-chloromethyl-5-nitrothiophene gave the ethylenic derivatives described in table I. The 5-nitro-1,3-dioxane salt was prepared from the previously described 2,2-dimethyl-5-hydroxymethyl-5-nitro-1,3-dioxane [11] after treatment with lithium methoxide, which induced a formaldehyde split-off.

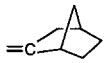
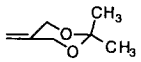
Derivative **4g** was readily subject to ring opening [12] by heating in methanol with ion-exchange resin (Dowex 50X 8–50) to give the corresponding 2-hydroxymethyl-3-(5-nitrothienyl)-2-propen-1-ol **5**.

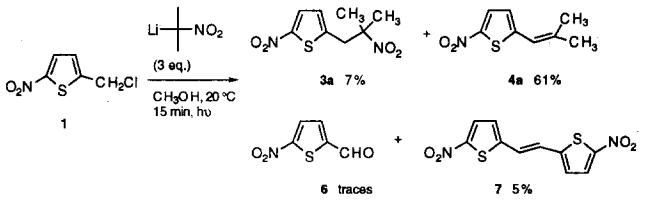


4g 5 93%

When the reaction of 2-chloromethyl-5-nitrothiophene and 2-nitropropane anion was performed in methanol, the C-alkylation yield decreases slightly because the ethylenic derivative **4a** was accompanied by other products: the C-alkylation product **3a**; the O-alkylation resulting product or 5-nitro-2-thiophenecarboxaldehyde **6**; and the alkene 1,2-bis-(5-nitro-2-thienyl)ethylene **7**.

Table I. New 2-alkylidenemethyl-5-nitrothiophenes.

No	*NT = CR_1R_2*	*Yield (%)*	*mp(°C)*	*Formula*
4b		97	87	$C_{10}H_{11}NO_2S$
4c		85	66	$C_{11}H_{13}NO_2S$
4d		64	54	$C_{12}H_{15}NO_2S$
4e		43	oil	$C_{13}H_{17}NO_2S$
4f		89	82	$C_{12}H_{13}NO_2S$
4g		84	oil	$C_{11}H_{13}NO_4S$



1 3a 7% 4a 61% 6 traces 7 5%

A possible route leading to the formation of the derivative **7** is given by the attack of the intermediate carbanion, formed from 2-chloromethyl-5-nitrothiophene **1** with the 2-nitropropane anion as a base, on the chloride **1** and dehydrohalogenation of this product.

The new compounds were identified by ^{1}H-NMR analysis and their purity established by controls on TLC and microanalysis. They were also tested *in vitro* against *Entamoeba histolytica*, *Trichomonas vaginalis*, promastigotes of 2 *Leishmania* strains.

Results and discussion

As shown in table II, all the compounds showed good antiprotozoan activity, except for the derivatives **3a** and **7**.

Table II. Protozoocid activity of thiophene derivatives.

No	*MIC (μg/ml) Entamoeba histolytica*	*MIC (μg/ml) Trichomonas vaginalis*	*MIC (μg/ml) Leishmania*	
			tropica	*infantum*
3a	50	50	25	25
4a	1	5	5	5
4b	1	5	5	5
4c	5	5	10	10
4d	5	10	10	25
4e	10	10	25	25
4f	10	10	10	10
4g	5	5	10	10
5	5	5	10	10
6	50	10	5	5
7	50	25	25	25
Metronidazole	5	5		
Pentamidine			5	5

Compounds **4a** and **4b** displayed good *in vitro* activity against *E histolytica*, *T vaginalis* and against promastigotes of 2 *Leishmania* strains. Both compounds were superior to metronidazole in their activity against *E histolytica* and comparable with metronidazole against *T vaginalis*. Against the 2 *Leishmania* strains, both compounds were comparable with the reference compound pentamidine. The other derivatives were slightly less active, except for compound **6** with a better leishmanicidal activity than amoebicidal and trichomonacidal.

The structure–activity relationships show the importance of the alkylidene group at the 2-position. Substitution on the thiophene ring by an alkylnitromethyl group decreased the antiprotozoan activity compared with 2-alkylidenemethyl group; compound **4a** with an isopropylidenemethyl group was more efficient than compound **3a** with a 2-methyl-2-nitropropyl group. For the 2-alkylidenemethyl-5-nitrothiophenes, the size of the substituent was also important: the activity decreased for larger than 5-membered rings. Compound **4b** with cyclopentyl group was more active than compounds **4c–g** with medium (6- to 8-membered) rings. Replacement of methyl groups of compound **4a** by hydroxymethyl groups or the incorporation of a dioxane group decreased the activity.

These results coincide with the increase in resonance conjugation in the molecular structure of the most potent antiparasitic compounds as described in 5-nitroimidazole series [13, 14]. In conclusion, we have described the preparation and the interesting *in vitro* antiprotozoan activity of a new series of 2-alkylidenemethyl-5-nitrothiophenes.

Experimental protocols

Chemistry

Melting points were recorded on a Büchi apparatus using glass capillary tubes and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker 200 MHz instrument and chemical shifts are reported in δ units (ppm) relative to internal TMS. Microanalyses for C, H, N were performed by the Microanalytical Section of the Saint-Jérôme Faculty and were within ± 0.4% of theoretical values.

2-Hydroxymethyl-5-nitrothiophene [5] was prepared and converted into 2-chloromethyl-5-nitrothiophene by literature procedures.

The lithium salt of 2-nitropropane **2a** [15], nitroalkanes **2b–f** [16] and 2,2-dimethyl-5-nitro-1,3-dioxane **2g** [16] were prepared as previously described.

General procedure for $S_{RN}1$ reactions

Reaction in dimethylformamide. The lithium salt of 2-nitropropane (0.86 g, 9 mmol) was added under nitrogen and under anhydrous conditions to a solution of 2-chloromethyl-5-nitrothiophene **1** (0.53 g, 3 mmol) in 20 ml dry DMF. The reaction mixture was then irradiated with 2 x 60 W fluorescent lamps from a distance of 10 cm. After stirring at rt for 24 h, the reaction mixture was poured into water (200 ml). The aqueous solution was extracted with benzene (3 x 40 ml) and ether (1 x 40 ml). The organic extracts were washed with water (3 x 100 ml), dried over $MgSO_4$ and evaporated under reduced pressure. Purification by chomatography on a silica-gel column eluting with chloroform gave 0.40 g (71%) 2-isopropylidenemethyl-5-nitrothiophene **4a**.

Yellow solid, mp 67–69°C (lit [5] mp 68–71°C), ^{1}H-NMR ($CDCl_3$) δ: 2.00 (s, 3H), 2.05 (s, 3H), 6.39 (s, 1H), 6.81 (d, J = 4.3 Hz, 1H), 7.84 (d, J = 4.3 Hz, 1H).

Reaction in methanol. 2-Nitropropane (1.78 g, 20 mmol) was mixed with a methanolic solution of sodium (0.46 g, 0.02 g-atom) for 30 min. A solution of 2-chloromethyl-5-nitrothiophene **1** (1.17 g, 6.6 mmol) in 20 ml methanol was added. The reaction was allowed to proceed for 15 min at rt under an inert atmosphere and in the presence of light (2 x 60 W fluorescent lamps). Methanol was distilled off on a rotatory evaporator under reduced pressure and 40 ml water was added to the residue which was then extracted with chloroform. The extracts were dried and evaporated to give a residual oil. Purification by column chromatography (SiO_2) using $CHCl_3$ as eluent gave: 0.74 g (61%) of 2-isopropylidenemethyl-5-nitrothiophene **4a**; 110 mg (7%) of 2-(2-methyl 2-nitropropyl)-5-nitrothiophene **3a**; yellow solid, mp 61–62°C [5], ^{1}H-NMR ($CDCl_3$) δ: 1.67 (s, 6H), 3.46 (s, 2H), 6.82 (d, J = 4.2 Hz, 1H), 7.78 (d, J = 4.2 Hz, 1H); 46 mg (5%) of 1,2-bis-(5-nitro-2-thienyl)ethylene **7**, red solid, mp > 300°C (ether), ^{1}H-NMR (DMSO-d_6) δ: 7.45 (d, J = 4.4 Hz, 2H), 7.66 (s, 2H), 8.12 (d, J = 4.4 Hz, 2H); and traces of 5-nitro-2 thiophenecarboxaldehyde **6**.

Extension to other nitronate anions using experimental conditions in dimethylformamide. This protocol led to the following compounds: 2-cyclopentylidenemethyl-5-nitrothiophene **4b**, 97% yield, yellow solid, mp 87°C (methanol), ^{1}H-NMR ($CDCl_3$) δ: 1.72 (m, 4H), 2.49 (t, J = 7.3 Hz, 2H), 2.52 (t, J = 7.3 Hz, 2H), 6.57 (s, 1H), 6.77 (d, J = 4.3 Hz, 1H), 7.83 (d, J = 4.3 Hz, 1H); 2-cyclohexylidenemethyl-5-nitrothiophene **4c**, 85% yield, yellow solid, mp 66°C (methanol), ^{1}H-NMR ($CDCl_3$) δ: 1.65 (m, 6H), 2.29 (t, J = 5.5 Hz, 2H), 2.62 (t, J = 5.5 Hz, 2H), 6.26 (s, 1H), 6.79 (d, J = 4.3 Hz, 1H), 7.81 (d, J = 4.3 Hz, 1H); 2-cycloheptylidenemethyl-5-nitrothio-

phene **4d**, 64% yield, yellow solid, mp 54°C (methanol), ^{1}H-NMR ($CDCl_3$) δ: 1.59 (m, 4H), 1.79 (m, 4H), 2.46 (t, J = 5.2 Hz, 2H), 2.60 (t, J = 5.2 Hz, 2H), 6.44 (s, 1H), 6.81 (d, J = 4.3 Hz, 1H), 7.84 (d, J = 4.3 Hz, 1H); 2-cyclooctylidenemethyl-5-nitrothiophene **4e**, 43% yield, yellow oil, ^{1}H-NMR ($CDCl_3$) δ: 1.45–1.64 (m, 6H), 1.80–1.93 (m, 4H), 2.40 (t, J = 6.0 Hz, 2H), 2.58 (t, J = 6.0 Hz, 2H), 6.45 (s, 1H), 6.80 (d, J = 4.3 Hz, 1H), 7.84 (d, J = 4.3 Hz, 1H); 2-(2-norbornylidenemethyl-5-nitrothiophene **4f**, 89% yield, yellow solid, mp 82°C (methanol), ^{1}H-NMR ($CDCl_3$) δ: 1.25–1.84 (m, 6H), 2.44 (broad s, 1H), 2.62 (m, 2H), 2.90 (broad s, 1H), 6.54 (s, 1H), 6.77 (d, J = 4.3 Hz, 1H), 7.83 (d, J = 4.3 Hz, 1H); 2-(2,2-dimethyl-1,3-dioxane-5-ylidenemethyl)-5-nitrothiophene **4g**, 84% yield, yellow oil, ^{1}H-NMR ($CDCl_3$) δ: 1.45 (s, 6H), 4.37 (s, 2H), 4.66 (s, 2H), 6.35 (s, 1H), 6.82 (d, J = 4.3 Hz, 1H), 7.85 (d, J = 4.3 Hz, 1H).

Preparation of 2-hydroxymethyl-3-(5-nitrothienyl)-2-propen-1-ol **5**

A stirred mixture of 2-(2,2-dimethyl-1,3-dioxane-5-ylidenemethyl)-5-nitrothiophene **4g** (0.76 g, 3 mmol) and 0.3 g ion-exchange resin (Dowex 50X 8–50 Aldrich) in 60 ml methanol was boiled for 2 h. After filtration of resin and evaporation under reduced pressure, the residue was dissolved in ethyl acetate. The solvent was washed with water, dried over anhydrous $MgSO_4$. Removal of the solvent and recrystallization from methanol gave 0.6 g (93%) 2-hydroxymethyl-3-(5-nitrothienyl)-2-propen-1-ol **5** as a red solid, mp 78°C (methanol).

^{1}H-NMR ($CDCl_3$) δ: 1.99 (broad s, 2H), 4.45 (s, 2H), 4.60 (s, 2H), 6.65 (s, 1H), 6.96 (d, J = 4.3 Hz, 1H), 7.85 (d, J = 4.3 Hz, 1H).

Biology

In vitro amoebicidal activity was evaluated using the usual protocol [17] against *Entamoeba histolytica* (Rahman strain) cultivated on Jones' liquid medium [18]. The minimum inhibitory concentration (MIC) was determined after 48 h in culture, using metronidazole as the reference drug.

In vitro trichomonacidal activity was performed on a *Trichomonas vaginalis* wild strain grown in oxoid liquid medium (*Trichomonas medium* code CM 161). The MIC was determined after 48 h [19] using metronidazole as the reference drug.

Leishmanicidal activity was evaluated on *Leishmania infantum* strain (MCAN/FR/74 LPMA 57; WHO) and *L tropica* (MHOM/FR/65 LPMA 59; WHO). *Leishmania infantum* was originally isolated from the ganglia of dogs in Marseilles and *Leishmania tropica* from a human case of cutaneous leishmaniasis with numerous typical ulcerations. These isolates contained numerous *Leishmania* amastigotes and were cultivated in NNN (Novy, MacNeal, Nicolle) [20] and Tobie [21] media where they were transformed into promastigote forms. These strains were maintained in continuous culture in RPMI 1640 (Gibco) containing 10% heat-inactivated fetal calf serum. Streptomycin (50 mg/l) and penicillin G (50 units/ml) were also added (these concentrations did not affect *Leishmania* growth).

Promastigotes (10^6 *Leishmania*/ml) were inoculated in tubes containing 5 ml of the above-described medium and incubated at 24°C [22]. Subcultures were made once a week, and each subculture was checked for abundance and motility of promastigote forms. They were counted with a Malassez cell and the volume of inoculum was adjusted to distribute 10^6 *Leishmania*/ml. The test compounds were first dissolved in dimethylformamide (10 mg/ml) then distributed to the culture tubes to obtain final concentrations of 100, 50, 25, 10, 5, 1 and 0.5 mg/l. Dimethylformamide was completely inactive on the parasites at these concentrations. Each strain and each concentration was tested in triplicate. The minimal inhibitory concentrations (MIC) of the compounds were determined after the parasites had been in culture for 7 d, by checking for the presence or absence of promastigotes microscopically (x 400). The absence of promastigotes in the tubes was confirmed by retroculture. If the parasites did not recover, that concentration of a compound was considered leishmanicidal. The MIC for each compound was then compared with that of pentamidine determined under the same conditions.

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