SYNTHESIS AND EVALUATION OF THE TRYPANOCYDAL ACTIVITY OF 4-ALKYLAMINO-6-NITROQUINOLINES

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Abstract: A straightforward synthesis of a group of 4-alkylamino-6-nitroquinolines starting from a common intermediate, 5,8-dimethoxy-6-nitro-4(1H)quinolone **3**, is described. These compounds were tested *in vitro* as potential anti-trypanosomal agents. Some derivatives were found to have a significant activity, but less efficient than the control drug.

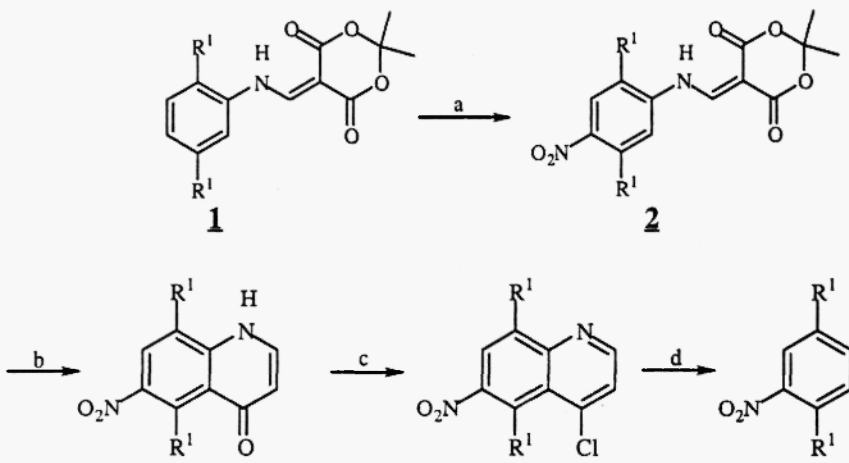
Introduction

The interesting pharmacological properties of the 4-aminoquinoline system have stimulated the synthesis of numerous derivatives.[1,2] Most efforts have been dedicated to the development of new antimalarial agents,[3] and there are no report on the anti-trypanosomal activity of 4-aminoquinoline derivatives. Recently it has been described some primaquine analogues, having the 8-aminoquinoline system, with potent anti-*Trypanosoma cruzi* activity *in vitro*.[4] Chagas´desease, a zoonosis caused by the flagellate protozoan *Trypanosoma cruzi* is of major importance in Central and South America where almost 20% of the population live at risk of infection.[5] Two nitro heterocyclic compounds, nifurtimox and benznidazol, are used for the therapy of acute infections with *Trypanosoma cruzi*, both presenting toxic effects and are mutagenic.[6] In the search for novel compounds with antiparasitic activity, we were interested in the synthesis of 4-alkylamino-6-nitroquinolines to evaluate its anti-trypanosomal activity.

Results and Discussion

A convenient method to synthesize 4-aminoquinolines[7,3d] is the nucleophilic displacement of the chlorine atom of 4-chloroquinolines, which are readily obtained from 4(1H)-quinolones.[8] To

prepare the required nitroquinolone **3**, the regioselective nitration of the arylamino methylene Meldrum's acid derivative **1**[9] was used. Reaction of compound **1** with nitric acid supported in silica gel,[10] in dichloromethane at room temperature gave **2** in 96% yield. Conversion of compound **2** to the corresponding 4(1H)-quinolone **3** was accomplished in boiling diphenyl ether in 80% yield. Heating quinolone **3** with an excess of phosphorus oxychloride yielded 4-chloroquinoline **4** (68%). Reaction of **4** with amines gave the corresponding 4-alkylamino-6-nitroquinolines **5**-**9** in 37-68% yields. (Scheme 1)



5 R^1= OMe, R^2= *n*-propylamino
6 R^1= OMe, R^2= 2-hydroxyethylamino
7 R^1= OMe, R^2= piperidino
8 R^1= OMe, R^2= morpholino
9 R^1= OMe, R^2= 2-(hydroxyethylamino)-ethylamino

a) HNO_3/SiO_2, CH_2Cl_2; b) Ph_2O, 240-250 °C; c) $POCl_3$; d) Alkylamine

Scheme 1

All compounds were screened against *T. cruzi* epimastigotes Tulahuén strains. *T. cruzi* epimastigotes were grown at 28 °C in Diamond's monophasic medium with blood replaced by 4μM hemin.[11,12] Fetal calf serum was added to 4% final concentration. The compounds were dissolved in dimethylsulfoxide and were added in concentrations of 100 and 50 μM to the culture, using a 20 μM solution of 1-[(5-nitrofurfurylidene)amino]-2-methyltetrahydro-1,4-thiazine 1,1-dioxide (nifurtimox) as control. *T. Cruzi* epimastigote growth was followed by nephelometry using culture

flasks with side-arms tube.[12,13] The inhibition growth percentage data were calculated on the seventh day of culture (exponential phase).

All the tested compounds inhibit the growth of the parasites, but none of them was more active than nifurtimox. The presence of a chlorine atom in the molecule seems to be important, so we are currently studying the synthesis of 4-alkylamino-6-chloroquinolines in the search for more active compounds. (Table 1)

Table 1. Inhibition effect of compounds **3**-**9** on *T. Cruzi* (Tulahuén strains) culture growth.

Compound	[Compound] μM	I (%)
3	100	60
4	100	90
	50	78
5	100	78
	50	56
6	100	72
	50	54
7	100	84
	50	61
8	100	43
9	100	68
Nifurtimox	20	88

Experimental

Melting points were determined on a Kofler apparatus and are not corrected. IR spectra were obtained on a Bruker Model Vector 22 spectrophotometer. ^{1}H and ^{13}C spectra were recorded on a Bruker AM-200 spectrometer, using tetramethylsilane as internal reference. Column chromatography were performed on Merck silica gel 60 (70-230 mesh). Elemental analyses were carried out on a FISONS EA 1108 CHNS-O analyzer. Accurate MS measurements were determined at the SERC Mass Spectrometry Centre, Leicester University.

5-{[(2,5-Dimethoxy-4-nitrophenylamino)]methylidene}-2,2-dimethyl-4,6-dioxo-1,3-dioxane **2**.

To a solution of 5-{[(2,5-dimethoxyphenylamino)]methylidene}-2,2-dimethyl-4,6-dioxo-1,3-dioxane **1** (5.0 g, 16.3 mmol) in dichloromethane (25 ml) was added nitric acid adsorbed on silica

gel (8.3 g)[10] and the mixture was stirred at room temperature for 1 h. After the solid was filtered off, the solvent was removed and the residue was recrystallized from ethanol to give compound **2** (5,5 g, 96%) , mp 193-194 °C , lit.[9] 192-194 °C.

5,8-Dimethoxy-6-nitro-4(1H)quinolone **3**.

A mixture of **2** (0.75 g, 2.13 mmol) and phenyl ether (40 g) was heated at 240-250 °C for 15 min. After cooling at room temperature the reaction mixture was diluted with petroleum ether (150 ml) and filtered to give compound **3** (0.44 g, 80%). mp 68-69 °C. ^{1}H-NMR [$(CD_3)_2SO$] δ: 3.96 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 6.17 (dd, 1H, *J*=1.2, 7.5 Hz, H-2), 7.72 (s, 1H, H-7), 7.82 (dd, 1H, *J*=6.0, 7.5 Hz, H-3), 11.64 (m, 1H, NH); ^{13}C-NMR [$(CD_3)_2SO$] δ: 56.8, 63.2, 105.7, 113.0, 119.6, 135.6, 137.8, 138.1, 144.1, 146.1, 176.0. IR (KBr) cm^{-1}: 3180, 1675, 1520,1350. *Anal.* Calcd for $C_{11}H_{10}N_2O_5$: C, 52.80, H, 4.03, N, 11.20. Found: C, 53.05, H, 4.20, N, 11.10.

4-Chloro-5,8-dimethoxy-6-nitroquinoline **4**

Freshly distilled $POCl_3$ (30 ml) was slowly added to compound **3** (3.0 g, 10.1 mmol) and the resulting solution was heated under reflux for 2h. After cooling the mixture was poured into ice-water, treated with charcoal and filtered. The filtrate was neutralized with sodium bicarbonate and extracted with dichloromethane (3x75 ml). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (dichloromethane-ethyl acetate 19:1) to give compound **4** (2.20 g 68%); mp 144-144.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.92 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 7.32 (s, 1H, H-7), 7.40 (d, 1H, *J*=4.7 Hz, H-3), 8.80 (d, 1H, *J*=4.7 Hz, H-2); ^{13}C-NMR ($CDCl_3$) δ: 56.8, 64.8, 103.1, 122.4, 126.1, 139.6, 142.5, 143.7, 143.9, 150.9, 152.5. IR (KBr) cm^{-1}: 1520, 1350, 1060, 800. FAB-MS *m/z*: 269.03287 (Calcd for $C_{11}H_9ClN_2O_4$ $[M + H]^+$ 269.03290).

Preparation of 4-alkylaminoquinolines. General Procedure

A mixture of chloroquinoline **4** (100 mg, 0.37 mmol), *n*-butanol (2.0 ml) and the amine (0.74 mmol) was heated to reflux for 4-5 h. The reaction mixture was concentrated under vacuum and the residue was purified by column chromatography on silica gel (dichloromethane-ethyl acetate 19:1)

4-*N*-Propylamino-5,8-dimethoxy-6-nitroquinoline **5** (52 mg, 48%), mp 197-198 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.0 (t, 3H, *J*=7.3, CH_3), 1.6-1.8 (m, 2H, H-2´), 3.1-3.3 (m, 2H, H-1´), 3.90 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3), 7.45 (d, 1H, *J*=4.7 Hz, H-3), 7.6 (s, 1H, H-7), 7.7 (s, 1H, NH), 8.80 (d, 1H, *J*=4.7 Hz, H-2); ^{13}C-NMR ($CDCl_3$) δ: 11.3, 24.8, 54.0, 56.5, 64.2, 108.6, 112.8, 120.7, 124.6, 142.0, 144.1, 146.7, 147.4, 151.4. IR (KBr) cm^{-1}: 3180, 1560, 1345. FAB-MS *m/z*: 291.12190 (Calcd for $C_{14}H_{17}N_3O_4$ 291.12189).

4-*N*-(2-Hydroxyethyl)amino-5,8-dimethoxy-6-nitroquinoline **6** (40 mg, 37%), mp 159-160 °C. ^{1}H-NMR [$(CD_3)_2SO$] δ: 2.7-2.9 (m, 2H, H-1´), 3.4-3.6 (m, 2H, H-2´), 3.84 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 4.9 (broad s, 1H, OH), 7.0 (d, 1H, *J*=5.3 Hz, H-3), 7.5 (s, 1H, H-7); 7.6 (broad s, 1H, NH), 7.8 (d, 1H, *J*=5.3 Hz, H-2), ^{13}C-NMR [$(CD_3)_2SO$] δ: 29.7, 31.9, 46.6, 56.3, 110.4, 112.9, 119.9, 125.9, 142.8, 144.5, 145.4, 146.8, 153.1. IR (KBr) cm^{-1}: 3260, 1540, 1340. FAB-MS *m/z*: 293.10115 (Calcd for $C_{13}H_{15}N_3O_5$ 293.10116).

4-Piperidin-5,8-dimethoxy-6-nitroquinoline **7** (80 mg, 68%) mp 180.5-181.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.7-2.0 (m, 6H, 3xCH_2), 2.6-2.9 (m, 2H, CH_2), 3.4-3.7 (m, 2H, CH_2), 3.84 (s, 3H, OCH_3), 4.07 (s, 3H, OCH_3), 6.94 (d, 1H, *J*= 5.3 Hz, H-3), 7.27 (s, 1H, H-7); 8.70 (d, 1H, *J*=5.3 Hz, H-2), ^{13}C-NMR ($CDCl_3$) δ: 24.1, 25.7, 54.2, 56.5, 64.1, 101.9, 110.3, 117.8, 140.1, 144.3, 145.0, 151.6, 152.3, 158.5. IR (KBr) cm^{-1}: 1530, 1380. FAB-MS *m/z*: 317.13754 (Calcd for $C_{16}H_{19}N_3O_4$ 317.13754).

4-Morfolin-5,8-dimethoxy-6-nitroquinoline **8** (80 mg, 67%) mp 142.5-143.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.3 (s, 4H, 2xCH_2) 3.94 (s, 4H, 2xCH_2), 3.84 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 7.4 (s, 1H, H-7); 7.7 (d, 1H, J 5.2 Hz, H-3), 8.8 (d, 1H, J 5.2 Hz, H-2), ^{13}C-NMR ($CDCl_3$) δ: 53.1, 56.6, 64.5, 66.6, 102.1, 110.2, 117.6, 140.6, 143.4, 144.7, 151.8, 152.6, 157.6. IR (KBr) cm^{-1}: 1510, 1375. FAB-MS *m/z*: 319.11683 (Calcd for $C_{15}H_{17}N_3O_5$ 319.11681).

4-(2-Hydroxyethylamino)ethylamino-5,8-dimethoxy-6-nitroquinoline **9** (60 mg, 48%) mp 201-202 °C. ^{1}H-NMR [$(CD_3)_2SO$] δ: 3.7 (m, 8H, 4xCH_2), 3.80 (s, 6H, 2xOCH_3), 3.94 (s, 3H, OCH_3), 4.90 (s, 2H, 2xNH), 6.9 (d, 1H, J 5.8 Hz, H-3), 7.5 (s, 1H, H-7); 8.3 (d, 1H, J 5.8 Hz, H-2), 11.2 (s, 1H, OH), ^{13}C-NMR [$(CD_3)_2SO$] δ: 46.7, 55.5, 55.6, 56.3, 57.5, 101.9, 107.2, 109.8, 122.8, 145.6, 147.4, 148.3, 149.6, 155.0, 156.0. IR (KBr) cm^{-1}: 3420, 3170, 1540, 1360. FAB-MS *m/z*: 336.14334 (Calcd for $C_{15}H_{20}N_4O_5$ 336.14336).

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