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Synthesis of some Thiochromone Derivatives and Activity Against *Plasmodium falciparum* In-vitro

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Abstract

A series of thiochromone derivatives were synthesized from 3-cyano-2-methylthio-1-thiocromone with hydrazine and guanidine derivatives, and tested for antimalarial activity. Pyrimethamine was used as a reference drug.

3-Amino-[1]-benzothiopyran-[2,3-c]-pyrazol-4-one showed significant antimalarial activity against *Plasmodium falciparum* in-vitro. The remaining compounds had only modest activity.

Malaria is a mosquito-borne protozoal disease and is endemic in many tropical and subtropical regions. *Plasmodium falciparum* and *Plasmodium vivax* are the most common causes of human malaria. *P. vivax* causes the classic recurrent/relapsing febrile illness, widely recognized as typical of malaria. Although it causes severe morbidity, it is rarely fatal. *P. falciparum* presents a much more variable picture. Symptoms may be severe or deceptively mild but the disease is fatal in approximately 1% of cases. Death may follow an acute infection (endotoxic shock or cerebral malaria) or may be the result of severe anaemia following chronic infection. In 1997 the World Health Organization estimated that world-wide up to 300 million cases and 3 million deaths are caused by malaria each year. The emergence and spread of parasite resistance to present antimalarial drugs urges for novel compounds to be discovered and developed. Recently we have reported a series of new quinolone, pyridopyrimidone and phenothiazine derivatives with antimalarial activity (Domínguez et al 1996a, b, 1997). We have now synthesized some thiochromone derivatives and tested for in-vitro antimalarial activity against a chloroquine-resistant strain of *Plasmodium falciparum* from Venezuela.

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Material and Methods

Chemistry

Melting points were recorded using a Thomas Hoover Capillary Melting Point Apparatus and are uncorrected. NMR was recorded using a Jeol JNM EX 270 FT NMR spectrometer at 67·8 MHz (^{13}C) and 270·05 MHz (^{1}H) and reported in ppm (δ) downfield from tetramethylsilane as internal standard. Infrared spectra were determined as KBr pellets on a Shimadzu model 470 spectrophotometer. Mass spectra were recorded on a Hewlett Packard 5995 mass spectrometer. The purity of all compounds was determined by thin-layer chromatography using several solvents of different polarity. All solvents were distilled and dried in the usual manner. Reactions were generally performed under a nitrogen atmosphere.

O-*Chlorobenzoylacetonitrile (**1**)*

This was prepared according to a method reported by Rudorf (1977).

*3-Cyan-2-methylthio-1-thiocromone (**2**)*

A mixture of **1** (27·8 mmol) and KOH (55·6 mmol) in dioxane (30 mL) was stirred at room temperature for 30 min, and then treated with carbondisulphide (27·8 mmol). After 1 h, methyl iodide (27·8 mmol) in dioxane (1 mL) was added to the reaction mixture and stirred at room temperature for a further 2 h. After the reaction mixture was refluxed for 1 h, solvent was removed under vacuum. The resulting

residue was added to water to give a solid which was filtered and recrystallized from ethanol to yield **2** (83%). Mp 229–230°C (Rudorf 1977: 233–234°C). IR (KBr, cm^{-1}): 2205 (CN); 1645 (CO); ^{1}H NMR (d_6-DMSO): 2·84 (s, 3H, SCH_3), 7·61–7·76 (m, 3H, Ar), 8·45 (d, 1H, J: 8·16 Hz, C–H_5); ^{13}C NMR: 14·61 (SCH_3), 178·1 (CO), 125·91 (C_8), 128·83 (C_5), 129·33 (C_6), 132·8 (C_7); MS m/e 233 (M^+ 96%).

*General procedure for the synthesis of 3-amino-[1]-benzothiopyrano-[2,3-c]-pyrazol-4-one derivatives **3** and **4***

A mixture of **2** (5·4 mmol) was treated separately with hydrazine hydrate or phenyl hydrazine (6 mmol) in refluxing pyridine (8 mL) for 4 h. The solvent was removed under vacuum. The resulting residues were added to water and the solid obtained was collected and crystallized from ethanol.

*3-Amino-[1]-benzothiopyrano-[2,3-c]-pyrazol-4(1*H*)-one (**3**).* Yield 91%. Mp 257–259°C (Rudorf & Augustin 1981: 260–261°C). IR (KBr, cm^{-1}): 1632 (CO), 3416, 3282, 3113 (NH); ^{1}H NMR (d_6-DMSO): 6·62 (br, 2H, NH_2), 7·47–7·51 (m, 3H, Ar), 8·4 (d, 1H, J:7·91 Hz, C–H_5), 12·18 (br, 1H, NH); ^{13}C NMR: 177·35 (CO), 151·40, 145·04, 135·80, 131·42 (C_7), 130·07, 127·85 (C_5), 126·75 (C_6), 125·46 (C_8), 99·56; MS m/e 217 (M^+).

*3-Amino-2-phenyl-[1]-benzothiopyrano-[2,3-c]-pyrazol-4(2*H*)-one (**4**).* Yield 75%. Mp 217–219°C (Rudorf & Augustin 1981: 220–222°C). IR (KBr, cm^{-1}): 1635 (CO), 3393, 3325, 3086 (NH); ^{1}H NMR: ($CDCl_3$) 5·99 (s, 2H, NH_2), 7·39–7·60 (m, 8H, Ar), 8·47 (d, 1H, J: 7·67 Hz, C–H_5). ^{13}C NMR: 178·86 (CO), 148·77, 137·31, 136·59, 132·09 (C_7), 130·22 ($C_{3',5'}$), 123·93 ($C_{2',6'}$), 128·80, 128·58 (C_5), 127·33 (C_6), 126·07 (C_8), 101·30; MS m/e 293(M^+).

*General procedure for the synthesis of 4-amino-[1]-benzothiopyrano-[2,3-d]-pyrimidin-5-one derivatives **5** and **6***

Compound **2** (2·3 mmol), was treated separately with guanidine hydrochloride (2·8 mmol) or cyanoguanidine (3 mmol), potassium carbonate (4·6 mmol) and DMF (10 mL) under reflux for 5 h. The mixture was cooled and poured into water and the solid obtained was collected and crystallized from ethanol.

*2,4-Diamino-[1]-benzothiopyrano-[2,3-d]-pyrimidin-5-one (**5**).* Yield 81%. Mp 330–332°C (Rudorf & Augustin 1981: 334–336°C). IR (KBr, cm^{-1}): 1616 (CO), 1670, 3440, 3385, 3170 (NH); ^{1}H NMR (d_6-DMSO): 7·13 (br, 2H, NH_2), 7·39 (m, 1H, C–H_9), 7·53 (dd, 1H, J:8·16 Hz, C–H_7), 7·58 (m, 1H, C–H_8), 7·82 (d, 1H, J: 4·72 Hz, NH), 8·31 (d, 1H, J: 7·91 Hz, C–H_6), 9·45 (d, 1H, J: 4·82 Hz, NH); ^{13}C NMR: 179·72 (CO), 164·91, 162·31, 134·67, 133·15 (C_8), 130·53, 129·25 (C_6), 127·52 (C_9), 126·65 (C_7), 101·14; MS m/e 244 (M^+).

*2,4-Diamino-2-cyanamino-[1]-benzothiopyrano-[2,3-d]-pyrimidin-5-one (**6**).* Yield 52%. Mp > 360°C. IR (KBr, cm^{-1}): 1190 (CN), 1642 (CO), 3437, 3253, 3215 (NH); ^{1}H NMR (d_6-DMSO): 7·19 (br, 1H, NHCN), 7·56 (dd, 1H, J: 7·67 Hz, C–H_9), 7·74 (m, 2H, C–H_7), 8·17 (d, 1H, NH, J: 5·01 Hz), 8·34 (dd, 1H, J: 7·67 Hz, C–H_8), 8·71 (d, 1H, J: 7·91 Hz, C–H_6), 9·58 (d, 1H, NH, J: 5·16 Hz); ^{13}C NMR: 179·83 (CO), 166·55, 163·89, 134·5, 133·5 (C_8), 130·76, 129·31 (C_6), 128·04 (C_9), 127·1 (C_7), 102·76; MS m/e 269 (M^+). $C_{12}H_7N_5OS$. 1/2 H_2O; C (51·93), H (2·67), N (25·47).

Biological evaluation

Field isolates. Isolates of *Plasmodium falciparum* were collected between June and December 1996 in Bolívar and Amazonas States, Venezuela, from six patients with acute *falciparum* malaria who presented voluntarily at the Dirección General Sectorial de Malariología y Saneamiento Ambiental. Parasitaemias (parasites/1000 erythrocytes) ranged from 0·1 to 1·7%. Venous blood samples were collected in Vacutainer tubes (Becton Dickinson, Rutherford, NJ) before treatment. Blood samples were transported at room temperature and were used within 12 h of collection. Giemsa-stained thin blood films were examined to confirm infection with *P. falciparum* and to determine parasite density. The erythrocytes were washed three times in RPMI 1640 medium (Gibco, Grand Island, NY) by centrifugation. The washed erythrocytes were resuspended (haematocrit 1·5%) in RPMI 1640 medium supplemented with 10% human serum and buffered with 25 mM HEPES and 0·25% $NaHCO_3$.

In-vitro assay of field isolates. Drug effects on *P. falciparum* proliferation were measured as inhibition of ^{3}H-hypoxanthine (New England Nuclear, Boston, MA) incorporation into nucleic acid, using the method described by Desjardin et al (1979). All compounds were dissolved in 10% dimethylsulph-

oxide (DMSO), subsequently diluted with RPMI 1640 medium and tested at 1×10^{-6} M in two parallel columns of a flat-bottomed 96-well microtitre plate (Falcon plastics, Oxnard, CA). The final concentration of isotope in the medium was 2 μCi mL^{-1}. Two hundred microlitres of the culture were added to each well of the microculture plates containing the drugs and the isotope. Plates were incubated at 37°C in candle jars for 24 and 48 h. Just before reinvasion erythrocytes were frozen at 20°C. Cells were lysed in an automatic cell harvester (LKB, Wallac oy, Finland). DNA was retained in a glass filter, pore size 1 μm (LKB Wallac oy, Finland), and the radioactivity incorporated in the DNA was measured in a beta counter (Beta Plate, LKB, Wallac oy, Finland). Normal non-infected erythrocytes were used as an additional control to measure radioactivity incorporation independent of parasite. The final concentration of DMSO never exceeded 1% (v/v). DMSO alone had no effect on the proliferation of parasites. Control wells containing infected erythrocytes without drugs and DMSO at the same concentrations used by dissolving drugs were included. The 50% inhibitory concentration (IC50) was defined as the drug concentration corresponding to 50% inhibition of schizont development in the control well.

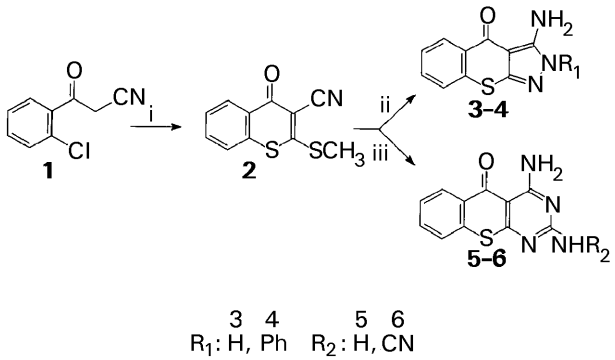

Figure 1. Structures and synthetic pathways of 3-amino-[1*H*]-benzothiopyrano[2,3-a]-pyrazol-4-one and 2,4-diamino-[1]-benzothiopyrano[2,3-d]-pyrimidin-5-one analogues. i. KOH, dioxane, CS_2, CH_3I; ii. $RNHNH_2$, pyridine; iii. $R'CH_3N_2$, DMF, K_2CO_3.

Table 1. Inhibition of biological activity by thiochromone derivatives.

Compound	IC50 (μM)
Pyrimethamine	26·4
3	25·2
4	>100
5	>100
6	>100

IC50 was extrapolated from curves of percentage activity vs concentration for each compound.

Results and Discussion

3-Cyano-2-methylthio-1-thiochromone **2** (Figure 1), was prepared according with the procedure used by Rudorf (1977), with some modifications. Reaction of **2** with hydrazine hydrate or phenyl hydrazine in pyridine, gave the expected 3-amino-[1]-benzothiopyrane-[2,3-c]-pyrazol-4-one derivatives **3** and **4** in good yields. The ^{1}H NMR spectrum of compounds **3** and **4** showed a singlet at 6·62–5·99 ppm due to the NH_2 protons. Two doublets in the region of 7·81 and 8·47 (J = 7·91 and 7·67 Hz) were assigned to proton 5, and compound **3** showed a broad singlet at 12·18 ppm due to the NH proton. Similarly, 4-amino-[1]-benzothiopyrano-[2,3-d]-pyrimidin-5-one derivatives **5** and **6** were obtained by treating **2** with guanidine hydrochloride or dicyandiamide, and potassium carbonate in DMF. The ^{1}H NMR spectra of compounds **5** and **6** showed a singlet in the region 7·13 and 7·14 ppm due to NH_2 and NH proton, respectively; a doublet in the region of 8·31 and 8·71 ppm (J = 7·91 Hz) was assigned to proton 6. The ^{1}H NMR for compounds **5** and **6** showed two doublets around 9·45, 7·82 and 9·58, 8·17 ppm, respectively, which were assigned to the amino group linked to C-4 (J = 4·86 Hz). This provides clear evidence of the presence of hydrogen bonding between one of the NH_2 protons and the carbonyl group attached to the carbon at position 5. We have previously found the same phenomenon in some quinolones (Domínguez et al 1998). This might be related to antimalarial activity.

Biological results

Isolates of *P. falciparum* from South Venezuela were characterized with respect to their susceptibility to chloroquine. All were resistant to 100 μM chloroquine (unpublished result). Table 1 shows the effect of thiocromone derivatives on the erythrocytic cycle of six different *P. falciparum* isolates. Compound **3** (IC50 25·2 μM) had higher activity, while compounds **4**–**6** showed no significant antimalarial activity compared with the parent compound, pyrimethamine. This is consistent with our previous observations using the quinolone analogues, indicating that the presence of hydrogen bonding between one of the NH_2 protons and the carbonyl group attached to the carbon at C-5 reduces the antimalarial activity of 2, 4-diamino- [1] -benzothiopyrano- [2,3-d]-pyrimidin-

5-one derivatives. Clearly compound **3** merits further investigation.

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