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Synthesis and evaluation of the antiplasmodial activity of tryptanthrin derivatives

Liliane Abodo Onambele [a], Herbert Riepl [b], Rainer Fischer [a], Gabriele Pradel [a], Aram Prokop [c,1,*], Makoah Nigel Aminake [a,d,1,**]

[a] Institute of Molecular Biotechnology, RWTH Aachen University, 52074 Aachen, Germany
[b] HochschuleWeihenstephan-Triesdorf, Organic-analytical Chemistry, 94315 Straubing, Germany
[c] Department of Paediatric Oncology, Children's Hospital Cologne, 50735 Köln, Germany
[d] Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa

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ABSTRACT

Malaria remains one of the most deadly diseases threatening humankind and is still affecting a significant proportion of the world population, especially in Africa. Chemotherapy is a vital component of the fight against the disease and new antimalarial agents are urgently needed to curb the spread of malaria parasites that are resistant to existing drugs. The natural product tryptanthrin is known for its wide range of activities, including antiplasmodial activity, but its poor solubility has undermined its development as potent antimicrobial and antiprotozoan agent. The aim of this work was to synthesize analogues of tryptanthrin and to evaluate their antiplasmodial activity against the asexual and sexual blood stages of *Plasmodium falciparum*. Our results suggest that most tryptanthrin analogues retained their antiplasmodial activity against chloroquine-sensitive and chloroquine-resistant malaria parasites in the nanomolar range (30–100 nM). The antiplasmodial activity of the most active compound NT1 (IC_{50}: 30 nM; SI: 155.9) was similar in both strains and close to that of chloroquine (IC_{50}: 20 nM) on the sensitive strain. The antiplasmodial activity was improved with derivatization, thus pointing out the necessity to explore tryptanthrin using medicinal chemistry approaches. Ten (10) of the tested derivatives met the criteria, allowing for advancement to animal testing, i.e., SI > 100 and IC_{50} < 100 nM. In addition to their activity on the asexual stages, tryptanthrin and two selected derivatives (NT1 and T8) prevented the maturation of gametocytes at their IC_{90} concentrations, indicating a transmission-blocking potential. Moreover, NT1 was able to impair gametogenesis by reducing the exflagellation of microgametes by 20% at IC_{90}, while tryptanthrin and T8 had no influence on exflagellation. The results of this study confirm that tryptanthrin and its derivatives are potential antimalarial candidates with abilities to kill the intraerythrocytic asexual stages and prevent the formation of sexual stages of the parasite.

1. Introduction

Malaria, a disease caused by protozoan parasites of the genus *Plasmodium*, remains one of the most devastating diseases in the world. Despite encouraging progress in the control of malaria, there was an estimated 627,000 malaria-related deaths worldwide in 2012. Most of the estimated cases occurred in sub-Saharan Africa (90%), with children under 5 years of age representing the most affected group (77%). Out of the five human pathogenic *Plasmodium* species, *Plasmodium falciparum* is the most virulent strain, responsible for almost all malaria-related death cases in Sub-Saharan Africa (World Health Organization, 2013). In the absence of an effective vaccine, the use of chemotherapy represents the most important pillar of malaria eradication besides vector control and prophylaxis. The World Health Organization now advises to apply artemisinin combination therapies (ACTs) as a new first line treatment, which shows very good clinical efficacy, especially towards *P. falciparum* malaria (World Health Organization, 2011). Despite the reduction of the parasite's sensitivity to artemisinins that has been reported in South East Asia, ACTs continue to cure patients as long as the partner drug is still effective. Nevertheless, resistance has been reported against both components of multiple ACTs in a province of Cambodia (World Health Organization, 2013). To maintain strict control of the disease, it is therefore crucial for humans to remain a step ahead of the

* Corresponding author. Department of Paediatric Oncology, Children's Hospital Cologne, 50735 Köln, Germany. Tel.: 0027-214066389; fax: 0027-216505195.
E-mail address: prokopa@kliniken-koeln.de (A. Prokop).
** Corresponding author. Institute of Molecular Biotechnology, RWTH Aachen University, 52074 Aachen, Germany. Tel.: 0027-214066389; fax: 0027-216505195.
E-mail address: nigel.makoah@uct.ac.za (M.N. Aminake).
[1] These authors contributed equally.

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malaria parasite by continually seeking for novel antimalarial compounds to overcome drug-resistant malaria parasites. In this regard, *in vitro* screens for compound activity constitute a key component of antimalarial drug discovery programs. To properly treat malaria, the new hit must be highly potent against the asexual blood stages of both drug-sensitive and drug-resistant parasites and be able to prevent parasite transmission to the mosquito.

Tryptanthrin (indolo-[2, 1-*b*]-quinazoline-6, 12-Dione) is an alkaloid originally isolated from *Isatis tinctoria*, a medicinal plant in temperate climate zones which has been used since antiquity as a source for indigo dye production (Honda et al., 1980; Seifert and Unger, 1994; Lee et al., 2007). Tryptanthrin was also identified as the active principle of a traditional Japanese herbal remedy used to treat fungal infections (Honda and Tabata, 1979). Following these discoveries, a number of biological activities have been reported for tryptanthrin and its derivatives, including antibacterial activity (Kataoka et al., 2001), particularly against *Mycobacterium tuberculosis* (Mitscher and Baker, 1998; Lee et al., 2007; Hwang et al., 2013), and activities against the protozoan parasites *Plasmodium falciparum* (Bhattacharjee et al., 2004), *Leishmania donovani* (Bhattacharjee et al., 2002), *Trypanosoma brucei* (Scovill et al., 2002), and *Toxoplasma gondii* (Krivogorsky et al., 2008). In addition, the inhibitory activities of tryptanthrin against cyclooxygenase (COX)-2 (IC_{50} = 64 nM), 5-lipoxygenase (LOX) (IC_{50} = 0.15 μM), nitric oxide synthase (NOS), and prostaglandin E(2) expression at the cellular level opened a vista for a possible lead for anti-inflammatory agents (Jahng, 2013). Activity against tumor and cancer cells has also been reported (Yu et al., 2007, 2010; Yang et al., 2013) and tryptanthrin derivatives were recently reported to inhibit indoleamine 2,3-dioxygenase with therapeutic activity in Lewis Lung cancer (LLC) tumor-bearing mice (Yang et al., 2013). However, tryptanthrin has not been extensively taken into consideration for pharmaceutical development because of its poor solubility. The aim of this study was to generate and synthesize tryptanthrin analogues with improved solubility and to evaluate their antiplasmodial activity against the asexual and sexual blood stages of *P. falciparum in vitro*.

2. Experimental section

2.1. Procedures for the synthesis of tryptanthrin derivatives

2.1.1. General synthesis of 8-substituted tryptanthrins from 5-R-isatins (R = Me, F, Cl, Br, NO_2)

Method A: 8-R-isatin (0.011 mol) was suspended in 10–15 ml dry pyridine. After addition of 5 drops of triethylamine and 2.2 g isatoic acid anhydride (MW = 163.13, 0.013 mol), the solution was heated to approximately 60 °C for 15 min. Then, 1.5 ml diisopropylcarbodiimide was added, and the solution was heated to reflux for an additional 15–20 min. During refluxing, the dark solution gave a crop of yellow crystals. These were isolated by suction and washed with cold methanol.

8-Bromotryptanthrin, 8-Bromo-indolo [2,1-b] quinazoline-6,12-dione (T2)

Synthesis according to method A: From 2.9 g of 5-bromoisatin (MW = 226, 0.013 mol).

Yield: 1.1 g, 51%, yellow crystals recrystallized from chloroform.

Mp.: 276 °C.

MS (Cl): 328.8 (15%), 327.8 (96.8), 326.8 (14.5), 325.8 (100), 299.8 (24.35), 297.8 (23.75), 247.8 (10.7), 218. 8 (18.9), 190.9 (44.1), 163.8 (15.6).

IR (KBr, cm^{-1}): 3067.9 (w), 1731 (m), 1674 (vs), 1592 (m), 1458 (m), 1429 (w), 1338 (m), 1300 (m), 1262 (w), 1181 (m), 1127 (w), 1041 (w), 872 (w), 772 (m), 687 (w).

^{1}H-NMR ($CDCl_3$, ppm): 8.28 (d, J = 8.23 Hz, 1H), 8. 18 (dd, J = 8.23 Hz, 1.5 Hz, 1H), 7.7 ("m", 2H), 7.6 ("q", 2H), 7.4 ("t", 1H).

^{13}C-NMR (d_6-DMSO, ppm): 181.56, 157.98, 146.45, 145.15, 140.04, 135.7, 130.33, 130.3, 127.45, 127.43, 124.6, 123.47, 119.47, 119.28.

$C_{15}H_7O_2N_2Br$: calc.: C: 55.0, H: 2.1, O: 10, N: 8.8, Br: 24; found: C: 54.73, H: 2.2, N: 8.52, Br: 23.44.

8-Chlorotryptanthrin, 8-Chloro-indolo [2,1-b] quinazoline-6,12-dione (T7)

Synthesis according to method A: From 2.2 g of 5-chloroisatin (MW = 181, 0.012 mol).

Yield: 1.3 g, 38%, yellow crystals recrystallized from acetone.

Mp.: 287 °C.

MS (Cl): 282 (100), 283 (35.85), 284 (34.12), 285 (11.84), 254 (11.24), 226 (4.95), 191 (24.52), 164 (2.81).

IR (KBr, cm^{-1}): 3069 (w), 1730 (s), 1674 (vs), 1594 (s), 1558 (m), 1459 (s), 1450 (w), 1341 (s), 1300 (s), 1263 (w), 1218 (w), 1182 (m), 1125 (m), 1042 (w), 915 (w), 877 (w), 843 (w), 772 (m), 742 (w), 787 (w). ^{1}H-NMR ($CDCl_3$, ppm): 8.55 (d, J = 8.98 Hz, 1H), 8.41 (dd, J = 8.23 Hz/1.5 Hz, 1H), 8.01 (d, J = 8.23 Hz, 1H), 7.84 (dt, J = 6.73 Hz/1.5 Hz, 2H), 7.72 (dd, J = 8.98 Hz/2.25 Hz, 1H), 7.67 (t, J = 6.5 Hz, 1H).

$C_{15}H_7O_2N_2Cl$: calc.: C: 63.8, H: 2.5, O: 11.3, N: 9.9, Cl: 12.4; found: C: 63.8, H: 2.5, N: 9.9, Cl: 12.5.

8-Fluorotryptanthrin, 8-Fluoro-indolo [2,1-b] quinazoline-6,12-dione (T6)

Synthesis according to method A: From 2.14 g of 5-fluoroisatin (MW = 165, 0.013 mol).

Yield: 1.9 g, 54%, yellow crystals recrystallized from 80 ml THF.

Mp.: 271.5 °C.

MS (CI, e/m): 267 (37.18), 266 (100), 238 (14.11), 210 (7.53), 148 (3.75), 130 (10), 120 (4. 82), 108 (21.93).

IR (KBr, cm^{-1}): 1722 (s), 1687 (s), 1592 (w), 1558 (w), 1484 (s), 1456 (w), 1351 (m), 1305 (w), 1269 (w), 1232 (w), 1185 (w), 1135 (w), 1116 (w), 1040 (w), 890 (w), 836 (m), 770 (s), 703 (w), 684 (w).

^{1}H-NMR ($CDC1_3$, ppm): 8.6 (dd, J = 8.98Hz/3.74 Hz, 1H), 8.4 (dd, J = 8.22Hz/1.5 Hz, 1H), 7.99 (d, J = 8.2 Hz, 1H), 7.82 (dt, J = 8.23 Hz/1.5 Hz, 1H), 7.65 (t, 7.48 Hz, 1H), 7.54 (dd, 6.73 Hz/2.99 Hz, 1H), 7.44 (dt, 8.97 Hz/2.99 Hz, 1H).

^{13}C-NMR ($CDCl_3$, ppm): 181.7, 162.35, 159.86, 157.87, 146.5, 144.28, 142.5, 135.22, 130.67 (d, 2JCF = 34.9 Hz), 127.54, 124.81 (d, $^2J_{CF}$ = 24.3 Hz), 124.15, 123.68, 123.33 (d, $^2J_{CF}$ = 7.7 Hz), 119.67 (d, $^2J_{CF}$ = 6.8 Hz), 112.05 (d, $^2J_{CF}$ = 25.26 Hz).

$C_{15}H_7O_2N_2F$: calc.: C: 67.66, H: 2.63, O: 12.03, N: 10.52, F: 7.14; found: C: 67.86, H: 2.77, N: 10.71, F: 6.44

8-Methyltryptanthrin, 8-Methyl-indolo [2,1-b] quinazoline-6,12-dione (T1)

Synthesis according to method A: From 2.1 g of 5-methylisatin (MW = 161.3, 0.013 mol).

Yield: 2.4 g, 70%, yellow crystals recrystallized from acetone.

Mp.: 270.5 °C.

MS (CI, m/e): 261.9 (100%), 233.8 (24.14), 204.8 (15.5), 130.8 (3.33), 129.8 (3.22), 101.8 (6.15)

IR (KBr, cm^{-1}): 3061 (w), 3029 (w), 2933 (w), 1724 (s), 1684 (vs), 1613 (w), 1593 (m), 1558 (w), 1482 (m), 1458 (m), 1340 (m), 1309 (m), 1297 (w), 1261 (w), 1227 (m), 1186 (w), 1154 (w), 1138 (m), 1041 (m), 947 (w), 886 (w), 830 (m), 796 (w), 774 (s), 705 (w), 688 (m), 659 (w).

^{1}H-NMR (d_6-DMSO, ppm): 8.33 (d, J = 8.23 Hz, 1H), 8.3 (d, J = 7.5 Hz, 1H), 7.93 (s, 1H), 7.92 (s, 1H), 7.7 ("m", 3H), 2.4 (s, 3H).

^{13}C-NMR (d_6-DMSO, ppm): 205.67, 182.6, 157.61, 146.55, 145.31, 144.05, 138.2, 136.75, 135.13, 129.87, 126.95, 124.85, 123.43, 122.4, 116.87, 20.55.

$C_{16}H_{10}O_2N_2$: calc.: C: 73.1, H: 4.0, O: 12.0, N: 11.1; found: C: 73.15, H: 3.78, N: 10.64.

8-Nitrotryptanthrin, 8-Nitro-indolo [2,1-b] quinazoline-6,12-dione (T4)

Synthesis according to method A: From 2.2 g of 5-nitroisatin (MW = 192, 0.011 mol).