SYNTHESIS, CHARACTERIZATION, AND IN VITRO ANTIMALARY ACTIVITY OF DIHYDROXYLATION DERIVATIVES OF TRICLOSAN

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Introduction

INTRODUCTION

To date, malaria remains one of the most devastating diseases of tropical and subtropical areas and is caused by the protozoan parasites of the Plasmodium genus. Worldwide, malaria causes over 500 million new cases each year, with approximately 3 billion people living under the threat of malaria. The disease results in as many as 2.7 million deaths annually, with children mostly affected. In addition, malaria has a striking correlation with social and economic disruption on a grand scale [1-5].

Chemoresistance is of increasing concern, primarily for Plasmodium falciparum, the parasite responsible for cerebral malaria, which is the most serious type of malaria infection. Indeed, this chemoresistance is believed to be a major factor in the worldwide upsurge of malaria [6]; therefore, there is an urgent need for new and potent antimalarial treatments [7].

The chlorinated biphenyl ether triclosan [5-chloro-2-(2,4-dichlorophenoxyl)phenol ether] (Fig. 1) is an antimicrobial component sometimes added to consumer products such as toothpastes, mouthwashes, deodorant soaps, lotions, and children toys [8]. Nevertheless, FDA nowadays has a restriction on triclosan as the emerging resistance issues. Triclosan's effectiveness as an antimicrobial agent is believed to be due to its ability to inhibit the enzyme enoylacyl carrier protein reductase, which is involved in bacterial lipid biosynthesis [9-11]. In vitro studies confirm that triclosan is effective at killing Plasmodium falciparum and curing mice infected with the rodent malaria species Plasmodium berghei, as well as acute bacterial infection [12,13]. Appropriation as their potential efficacy against malaria parasites needs to be developed steadily to get the structure that is pharmacologically suitable for use in humans.

Continuing the work of earlier studies attempting to identify new and potent antimalarial treatments [14-20], in this study, we synthesized modified structures of triclosan and evaluated their antimalarial activities.

EXPERIMENTAL

Synthesis of compounds

General methods

All starting materials were obtained from Sigma-Aldrich, Wako Pure, or Tokyo Chemical Industries and used as supplied. Solvents for chemical synthesis were acquired from commercial sources and used without further purification unless otherwise stated. Flash column chromatography was performed using Merck silica gel 60, whereas reactions and chromatography fractions were performed using Merck thin-layer chromatography (TLC) plates 60 F254. Compounds were visualized by an ultraviolet lamp (254 and 360 nm). Melting points (°C) were determined with a Yanaco micro melting point apparatus and remained uncorrected. Specific rotation, [α]D, was measured on a Jasco Digital Polarimeter. Mass analysis was performed with an electrospray mass JEOL JMS-AX 700 spectrometer, and gas chromatography was coupled with a mass spectrometer of high-resolution. 1H and 13C NMR spectral analyses were performed on a JEOL JNM-ECP500 (500 MHz), with tetramethylsilane as the internal standard. Chemical shifts are reported in units of (δ) ppm. The following abbreviations were used...

ABSTRACT

Objective: The emergence of malaria as a global health problem over the past few decades, accompanied by the rise of chemoresistant strains of Plasmodium falciparum, has emphasized the need for the discovery of new therapeutic drugs against this disease. In this study, enantiomerically enriched (enantioenriched) analogs of triclosan were synthesized and evaluated for antimalarial activity against P. falciparum cultures.

Methods: Enantioselective dihydroxylation of the olefin in amide seven was performed efficiently using chiral quinine ligand (DHQ2)PHAL to yield enantioenriched dihydroxy propionamide derivative (+)-1 in moderate yields. In a similar way, the chiral quinidine ligand (DHQD)PHAL was used as a stereoselectivity agent yielded the desired enantioenriched (−)-1. The enantio-enriched products were used for further in vitro assay, and accordingly the percent enantiomeric excess (% ee) was not determined. The structures of compounds were proven by spectral data (1H NMR, 13C NMR, and mass spectra).

Results: The phenol moiety at the C1 position of triclosan was chemically substituted with a methoxy group, in conjunction with an introduced stereocenter in a 2,3-dihydroxy-propionamide group at C2' position. Unmodified triclosan inhibited the P. falciparum cultures with an IC50 value of 27.2 µM. By contrast, the triclosan analogs, compounds (+)-1 and (−)-1, inhibited the P. falciparum cultures with IC50 values of 0.034 and 0.028 µM, respectively.

Conclusion: Collectively, our preliminary in vitro results suggest that these triclosan analogs have potent antimalarial activity and represent a promising new treatment strategy on further development.

Keywords: Triclosan, Synthetic analogs, Plasmodium falciparum, Antimalarial.
4-Chloro-1-fluoro-2-nitro-benzene (3)  
A three-neck round-bottomed flask was charged with m-CPBA (3.16 g, 14.5 mmol) and continued stirring at 0°C for 4 h. The reaction was quenched by the addition of water (15 mL) and NaOH, and the mixture was filtered through a plug of silica gel to obtain product 7 (45 mg, 73%). TLC Rf 0.37 (hexane/EtOAc 4:1); [M+Na] m/z 371.0327 [M+Na] m/z 371.0327; [M+H] m/z 369.0266 [M+H] m/z 369.0266; [M+H]+ m/z 369.0266; [M+Na]+ m/z 369.0266.

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N-[5-Chloro-2-(4-chloro-methoxy-phenoxo)-phenyl]-acrylamide (7)  
To a solution of triethylamine (0.03 mL, 0.42 mmol), dry THF (1.5 mL) under N₂ was added to give the amine compound 6 (50 mg, 0.14 mmol) at 0°C. After stirring for 15 min, acryloyl chloride (0.02 mL, 0.28 mmol) was introduced, and the mixture was allowed to warm to RT for 3 h. The mixture was then diluted with EtOAc, 1 M HCl solution, saturated NaHCO₃ solution, and a brine solution. The solution was then dried over anhydrous MgSO₄ and the crude extract was purified by column chromatography on a silica gel using 10:1 hexane/EtOAc to obtain product 7 (45 mg, 73%) as an off-white liquid. TLC Rf 0.37 (hexane/EtOAc 4:1); [M+Na] m/z 371.0327 [M+Na] m/z 371.0327; [M+H] m/z 369.0266 [M+H] m/z 369.0266; [M+H]+ m/z 369.0266; [M+Na]+ m/z 369.0266.
quenched by the addition of water (1.5 mL) and Na₂SO₄ (0.5 g, 4.0 mmol). The resulting mixture was extracted using CH₂Cl₂. The combined CH₂Cl₂ layers were dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The crude residue was then purified by column chromatography on silica (gradient elution 100:0–98:2, CHCl₃/CH₂OH) to give an enantiomer mixture of products (–)-1 (1.54 mg, 70%) as a white solid, m.p.: 158°C. The resulting mixture of products (–)-1 was then crystallized from a mixture of CHCl₃ and CH₂OH (3:1, 150 mg) and then recrystallized from a mixture of CHCl₃ and CH₂OH (1:1, 150 mg) to give an enantiomer mixture of products (–)-1 (1.54 mg, 70%) as a white solid, m.p.: 158°C. The resulting mixture of products (–)-1 was then crystallized from a mixture of CHCl₃ and CH₂OH (3:1, 150 mg) and then recrystallized from a mixture of CHCl₃ and CH₂OH (1:1, 150 mg) to give an enantiomer mixture of products (–)-1 (1.54 mg, 70%) as a white solid, m.p.: 158°C.

**Determination of the antimalarial activity**

The antimalarial activity of the synthesized molecules was evaluated against *P. falciparum* strain 3D7 (Eijkman Institute for Molecular Biology, Indonesia) using sensitive chloroquine and the procedure described by Budimulya [21]. *P. falciparum* 3D7 in human red blood cells (RBCs) (3% initial parasite density, and 4% hematocrit) was cultured with the test compound (added as 150 µL dimethyl sulfoxide solution) in 1350 µL of the medium (RPMI-1640, 25 µg/mL gentamycin, 50 µg/mL hygromycin, 25 mM Hepes buffer, 25 mM NaHCO₃, and 10% human serum) using a 96-well microtiter plate at 37°C. Tests were carried out simultaneously for three molecules in duplicate. A sealed incubation chamber continuously gassed with a mixture of 2% O₂, 8% CO₂, and 90% N₂ was used. Increases in the proportion of infected RBCs were assessed at the end of the 48 h incubation period in control samples and at various concentrations of each drug using Giemsa stained slides. Control samples contained *P. falciparum* 3D7 without any test compounds. The growth of the parasite was monitored by performing a blood smear fixed with MeOH and stained with a Giemsa stain. The antimalarial activity of each compound was expressed as an IC₅₀ value, defined as the concentration of the compound causing 50% inhibition of parasite growth relative to the untreated control.

**RESULTS AND DISCUSSION**

Compound 6 was prepared by three chemical reactions in accordance with a previously reported procedure [18] (Fig. 2). Primary amine 2 in a commercially available form was oxidized with m-CPBA to form the corresponding nitrobenzene 3. Diphényl ether derivative 5 was obtained by the coupling of the methoxy phenol 4 and nitrobenzene 3 in the presence of K₂CO₃ and 18-crown-6 in DMF. The nitro group in 5 was easily reduced with Sn/HCl by reflux in ethanol to afford the known compound 6.

In dry THF, the amidation of 6 with acrylic chloride afforded the desired amide 7. The amidation of 6 with acrylic and carboximide reagents was also attempted but was unsuccessful to give the desired amide 7. Diamagnetic anisotropy, which is commonly associated with asymmetric monosubstituted alkenes, appeared on the terminal olefin group of compound 7. Each of the three hydrogen atoms contributed equivalently to form an exceptional splitting pattern (Fig. 3). The resulting peak of each hydrogen atom was expressed in terms of a chemical shift 6.44 ppm (δ, J = 0.37), 6.30 ppm (δ, J = 10.1 Hz), and 5.78 ppm (δ, J = 10.4 Hz and 1.2 Hz).

Enantioselective dihydroxylation of the olefin in amide 7 was performed efficiently using the Sharpless et al. asymmetric dihydroxylation reaction [22] and the chiral quinine ligand [DHQD]PHAL, to yield enantioenriched dihydroxy propanamide derivative (+)-1 in moderate yields, attributed to a specific rotation, [α] (+) 4.0 ([α] = 0.40, in CH₂OH). Similarly, when the catalyst was changed to the quinidine derivative [DHQD]PHAL, the opposite selectivity was observed, and it yielded the desired enantioenriched (–)-1 with a specific rotation, [α] (–) 3.2 ([α] = 0.37, in CH₂OH). As reported by Sharpless et al. and associates in 2001, these chiral ligands have proven to be superior to others for the dihydroxylation of olefins with aliphatic substrates [22]. The enantioenriched products were used for further *in vitro* assays, with the percent of enantiomeric excess (% ee) not determined. The structures of compounds were proven by spectral data (1H NMR, 13C NMR, and mass spectra).

All inhibitors were tested for their inhibition of growth of the blood stages of the parasite *P. falciparum* 3D7 culture for 48 h in human blood. The activities of the test compounds compared with those of reference drugs are presented in Table 1. From the biological test results generated, analog (–)-1 appears to possess activity nearly as active as analog (+)-1 at lower micromolar concentrations. Qualitatively, this is understood by the fact that the specific configurations (*R*, *rectus* and *S*, *sinister*) of the enantiomers of these analogs are both active. This is likely because each of these cinchona ligands produced high enantiomer excess of a certain configuration [23].

Our results indicate that both of the enantioenriched analogs (+)-1 and (–)-1 of the 2'-chloro- and phenol-modified series exhibited better antimalarial activity profiles than the unsubstituted triclosan. Hence, triclosan inhibited *P. falciparum* cultures with an IC₅₀ value of 27.2 µM, whereas the analogs (+)-1 and (–)-1 inhibited the cultures with IC₅₀ values of 0.034 and 0.020 µM, respectively. In comparison, the IC₅₀ value for chloroquine, a well-established drug for the treatment of malaria, is 0.0003 µM. Therefore, given our activity results, it will be necessary to

![Fig. 3: The splitting pattern of hydrogen atoms on the vinyl group of amide 7](image-url)
Table 1: Experimental IC₅₀ values of the inhibitors against Plasmodium falciparum strain 3D7

| Compound | Experimental IC₅₀ (µM)* |
|----------|-------------------------|
| (+)-1, \( \left[a\right]^{27}_{D} = +4.0^{*} \) (c=0.40) | 0.034 |
| (-)-1, \( \left[a\right]^{27}_{D} = -3.2^{*} \) (c=0.37) | 0.028 |
| Triclosan | 27.2 |
| Chloroquine | 0.0003 |

*The specific rotations determined in CH₃OH, IC₅₀ taken from the Giemsa stained slide method (MIC method)

add more chemical attributes to these molecules to make them active at the nanomolar level.

CONCLUSION

We have synthesized novel triclosan analogs that appear to have potent antimalarial activity. The design of these new analogs was initiated with the introduction of chirality, and the analogs were synthesized in five steps to obtain a moderate yield. These favorable preliminary in vitro results demonstrating the potency of the synthesized compounds, (+)-1 and (-)-1, indicate that these compounds, particularly with additional modification, may prove to be promising antimalarial leads based on the concept of drug enantioselectivity. Future efforts will seek to define the mode of action of these potent antimalarials while further optimizing their activity with a wider range of substituent groups that may offer improved antimalarial activity.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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