CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

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7.1 Introduction

New and re-emerging infectious diseases for which no effective therapy is available and the development of resistance of many pathogens to currently used drugs are of utmost concern. In the developing countries, malaria, tuberculosis (TB) and human immunodeficiency virus (HIV) are the three major infectious diseases. They account for approximately half of the mortality caused by infectious diseases, which is almost half of the mortality in the developing countries (Mahidol *et al.,* 2002). Malaria has been responsible for much of human suffering and misery. Every year there are more than 300 million cases of malaria in the world and malaria kills more than one million people. Over the last ten years, the malaria situation has been worsening in many areas of the world. The need to find new antimalarials is pressing, due to the resistance of the human malarial parasite, *Plasmodium falciparum* to the presently available common antimalarial drugs. Treatment has thus become both less effective and much more expensive. The problem is further aggravated by the resistance of the vector anopheline mosquitoes to the most effective and least toxic insecticides, which had been used to kill them.

The potential of natural products as therapeutic agents in the treatment of malaria is enormous and the research work in this area has been the subject of some recent reviews.

7.2 Bioassay guided fractionation of the ethanol crude extract and isolated compounds

The crude extract of the leaves of *C. steenkampianus* was prepared in ethanol. The isolation of the compounds was guided with antibacterial and antimalarial bioassays. The correlation between the active antibacterial and active antimalarial fractions reported by Prozesky, 2004, Boonphong, 2007 and Zdzislawa, 2007 was verified. The compounds were isolated with silica and Sephadex chromatography. Six compounds were isolated: an indane, steenkrotin A, steenkrotin B, quercetin, tamarixetin and eriodictyol. These compounds were identified with NMR, LCMS, IR and X-ray crystallography.

7.3 Biological evaluation of the compounds

Quercetin showed the highest antiplasmodial activity among the compounds isolated. Its activity was the best against D10 and Dd2 strains of the parasite. It also displayed moderate antibacterial activity and best antioxidant activity. It showed no anti-HIV activity. Quercetin is an established medicine of value with increasing application in health care. Its biological activities (antibacterial and antioxidant) reported in this work were as documented in the literature (Kumarasamy *et al.,* 2002; Brahmachari and Gorai, 2006). Of the compounds isolated, quercetin showed the highest toxicity value but less toxicity than that of chloroquine.

The antiplasmodial activity shown by steenkrotin A is very interesting and promising. Even though its activity is less than that of quercetin against the parasite, it is more active against resistant strains than sensitive ones. The potential of steenkrotin A as an antiplasmodial agent needs further studies and development. Several derivatives of the compound needs to be synthesised and tested for improved activity. Steenkrotin A showed moderate antibacterial activity and weak antioxidant activity. No activity was observed against reverse transcriptase. Its toxicity value is similar to that of quercetin.

Steenkrotin B did not show antiplasmodial activity on the strains of *P. falciparum* used. However, it showed moderate antibacterial activity and moderate antioxidant activity, and no anti-HIV activity at the concentration in which it was tested. The activity of steenkrotin A and steenkrotin B on bacteria is promising and indicated antibiotic activity against Gram negative and positive bacteria. The antiplasmodial activity of steenkrotin B needs to be researched further because of the presence of an endoperoxide in its molecule. The high activity of artemisinin against malarial parasites has been linked to the endoperoxide in its structure. In order to optimise the possible antimalarial activity of steenkrotin B, many derivatives need to be made and tested for activity. The fact that artemisinin is active against *P. falciparum* while steenkrotin B is not, may mean that even though both contain endoperoxides they are in different chemical environments. Steenkrotin B showed the best overall toxicity value, being the lowest of all the compounds isolated. However, more studies are required to identify other biological properties of this compound.

The indane did not show antiplasmodial activity at the concentrations in which it was tested. However, it showed moderate antibacterial activity, slight anti-HIV activity and weak antioxidant activity. The indane showed the second best toxicity value in this study. It had low cytotoxicity but moderate antibacterial and weak anti-HIV activity.

The biological study of the activities of tamarixetin and eriodictyol were not done because their quantities isolated were very small. However, their antioxidant, antimalarial and antibacterial activities are well documented (Prozesky, 2004; Mahidol *et al.,* 2002; Newman *et al.,* 2003). The toxicity (ID_{50}) of tamarixetin was 53.8, which is relatively low.

Only three compounds (indane, steenkrotin A and steenkrotin B) were tested against *Mycobacterium tuberculosis*. None of them show antimycobacterial activity at the concentration at which they were tested.

The crude ethanol extract showed moderate antimalarial, antioxidant and antibacterial activity. These bioactivities can be linked to the compounds (flavonoids, diterpenes, triterpenes etc) of the extract. Without doubt *C. steenkampinus* like other species of *Croton* (Pooley, 1993) posse*ss*es medicinal properties. Its low cytotoxicity unlike others species (Mahidol *et al.,* 2002) makes it worthy of further studies *in vivo*.

7.4 References

Boonphong, S., Puangsombat, P., Baramee, A., Mahidol, C., Ruchirawat, S. and Kittakoop, P. 2007. Bioactive compounds from *Bauhinia purpurea* possessing antimalaral, antimycobacterial, antifungal, anti-inflammatory, and cytotoxic activities. J. Nat. Prod. 70:795-801.

Brahmachari, G. and Gorai, D. 2006. Progress in the research on naturally occurring flavones and flavonols: An overview. Curr. Org. Chem. 10:873-898.

Kumarasamy, Y., Fergusson, M.E., Nahar, L. and Satyajit, S.D. 2002. Bioactivity of Moschamindole from *Centaurea moschata*. Pharm. Biol. 40(4):307-310.

Mahidol, C., Prawat, H., Prachyawarakon, V. and Ruchirawat, S. 2002. Investigation of some bioactive Thai medicinal plants. Phytochem. Rev. 1:287-297.

Newman, D.J., Cragg, G.M. and Snader, K.M. 2003. Natural products as sources of new drugs over the period 1981-2002. J. Nat. Prod. 66 (7):1022-1037.

Pooley, E. 1993. The complete field guide to trees of Natal, Zululand & Transkei. P.222. Natal Foral Publications Trust, Durban.

Prozesky, E.A. 2004. Antiplasmodial and chloroquine resistance reversal properties of a new diterpene from *Croton steenkampianus.* PhD thesis. University of Pretoria, South Africa.

van der Westhuyzena, C.W and Parkinson, C.J. 2005. Synthesis of a Structurally Constrained Endoperoxide having Antimalarial Activity from Santonin. S. Afr. J. Chem. 58:41–45. Viewed 20 January 2008, <http://journals.sabinet.co.za/sajchem/>.

Zdzislawa, N. 2007. A review of anti-infective and anti-inflammatory chalcones. Eur. J. Med. Chem. 42(2):125-137.