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Design, synthesis and biological evaluation of new oxazines with potential antiparasitic activity

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Abstract—A series of new oxazines with potential antiparasitic activity was prepared using Diels–Alder reactions, based on terpenes derived from eucarvone as dienes and nitrosoarenes with different electronic characteristics as dienophiles. The biological activity was evaluated with in vitro assays against *Plasmodium falciparum*, *Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense*. Some of these oxazines have activities in the 20–50 μM range, and may be leaders for the development of novel antiparasitic drugs with improved pharmacological properties.

Parasitic infectious diseases due to pathogenic protozoa affect more than 3 billion people in the world and occur mainly in underdeveloped countries. The most important protozoan infections are malaria, trypanosomiasis and leishmaniasis.[1] Malaria is principally caused by *Plasmodium falciparum*, and is transmitted by the bite of *Anopheles* mosquitoes. This infection, which affects predominantly tropical countries, can be considered the most important due to its high morbidity and mortality rates.

Both the extensive and intensive use of drugs to treat malaria have induced a 'selection' of resistant parasites. This has frustrated attempts to eradicate the disease by means of chemotherapy, and increased the risk of turning obsolete the drugs currently used.[2]

During the 1980s, the survey of plants used in Chinese traditional medicine against 'fevers' was resumed. The active metabolite artemisinine was isolated from *Artemisia annua* L. (Asteraceae) (Qinghaosu) (Fig. 1) and found to be one of the few natural *endo*-peroxides known.

This novel active product with a sesquiterpene-lactone has a structure completely different from the quinine derivatives that had been the main group used.[3,4] Pharmacological studies established that the *endo*-peroxide group was responsible for this activity. The interaction of the peroxide bond with Fe^{II} species within the protozoan food vacuole catalyzes the generation of cytotoxic free radicals, which are supposed to be the active species.[5,6] While this has not been conclusively demonstrated, the hypothesis is soundly based on the results of biomimetic studies.[7–9]

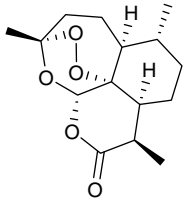

Figure 1. Chemical structure of artemisinine.

The activity derived from the peroxide bond of these natural compounds encourages the study of the antimalarial properties of other structurally related compounds.

Standard dissociation energies of peroxide bonds (–**O**–**O**–) and of nitroso bonds (–**N**–**O**–) are 33.2 and 35.8 kcal/mol, respectively.[10] Although the bond dissociation energy may vary somewhat when these bonds constitute part of more complex moieties, they are labile and might be prone to homolytic cleavages. Oxazines might follow the same reaction pattern of *endo*-peroxides (isosteric –**N**–**O**– bond cleavage and free radical

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generation), resulting in a similar biological activity. Our group has already reported the design, synthesis and biological evaluation of 12 oxazines obtained by Diels–Alder cycloaddition using dienes with the benzotropolone structure (tri-*O*-methylpurpurogallin) and nitrosobenzene as dienophile.[11]

In this Letter, the design, synthesis and biological evaluation of another 12 oxazines using the same hetero Diels–Alder reaction is reported. As dienes, eucarvone and its reduced and acetylated derivatives were used. As dienophiles, nitrosobenzene and *p*-substituted derivatives (aldehyde, chloro and methyl groups) were used (Fig. 2).

Eucarvone was synthesized from carvone using hydrogen bromide in glacial acetic acid according to Corey and Burke's technique.[12] The product obtained was reduced with $NaBH_4$ in methanol, resulting in a racemic mixture of the corresponding alcohols. The stereoselective acetylation of eucarvol was catalyzed by *Candida antarctica* lipase (Chirazyme L5 Lyo) and carried out using vinyl acetate as acyl donor agent. Kazlauskas's rule predicts that the *R* isomer will be esterified preferably.[13] This way, we have (*S*)-eucarvol and (*R*)-acetyleucarvol as dienes for the Diels–Alder cycloaddition.

The oxazines were synthesized through Diels–Alder reaction using these dienes and nitrosobenzene, 4-nitrosobenzaldehyde, 4-chloronitrosobenzene and 4-nitrosotoluene as dienophiles (Fig. 2). Yields observed are reported in Figure 2. Compound **6** was previously synthesized and characterized by Hart et al.[14] Compounds **7**–**17** were characterized by IR, MS, ^1H and ^{13}C NMR.

The dienes were treated with a slight excess of the substituted nitrosoarenes at room temperature, using tetrahydrofuran as solvent. The Diels–Alder reaction was clean and resulted in good yields (70–98%). In most cases the reactions were completed in less than 24 h. In the cases of adducts **10**–**17**, **15** and **17**, only the *anti* stereoisomer was obtained. Oxazines **14** and **16** were obtained as mixtures of the *anti* and *syn* stereoisomers in a *anti*/*syn* adduct ratio = 9/1. The regiochemistry of these hetero Diels–Alder reactions is the one shown in Figure 2, which was established from the ^1H and ^{13}C

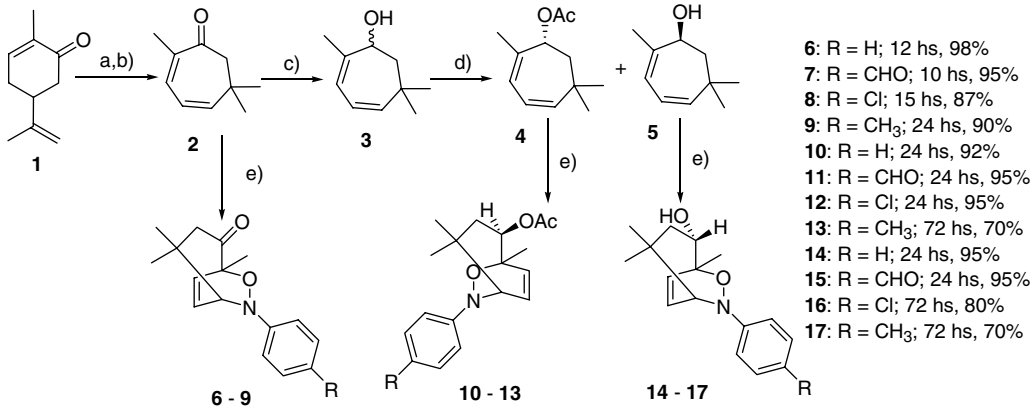

Figure 2. Route to oxazines **6**–**17**. Reagents and conditions: (a) HBr, HAc, −30 °C; (b) KOH, MeOH, reflux; (c) $NaBH_4$, MeOH, rt; (d) $CH_3COOCHCH_2$, Chirazime L5 Lyo, 30 °C, 48 h; (e) NOPhR, THF, rt yields.

Table 1. In vitro antiprotozoal activity of oxazines **6**–**17** against *P. falciparum*, *T. cruzi* and *T. brucei rhodesiense*

Compound	*P. falciparum* (IC_{50}, μM)	*T. cruzi* (IC_{50}, μM)	*T. brucei rhodesiense* (IC_{50}, μM)	Cytotoxicity (μM)
6	n.a.	112.7	13.9	110
7	n.a.	n.a.	103	310
8	n.a.	n.a.	73	120
9	43.9	31.7	53.8	60
10	n.a.	n.a.	n.a.	740
11	n.a.	62.3	n.a.	250
12	99.5	28.3	59.2	180
13	n.a.	39.5	94.7	450
14	n.a.	77.3	23.9	450
15	n.a.	n.a.	52.2	220
16	n.a.	37.8	19.7	110
17	72.9	42.5	32	42.2

Chloroquine (IC_{50} 3.0 nM), benznidazol, (IC_{50} 4.7 μM), pentamidine (IC_{50} 1.7 μM) were used as positives for *P. falciparum*, *T. cruzi* and *T. brucei rhodesiense* respectively. Cytotoxicity was assayed against KB cells, using Podophyllotoxin (LD_{50} 0.2 nM) as a standard. All determinations were performed in triplicate.

spectra of the products and later confirmed by X-ray diffraction studies performed on oxazine **6**.[15]

The in vitro biological activity of the new compounds was assayed against the protozoan parasites *P. falciparum*, *Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense*, and their toxicity evaluated against KB cells (Table 1).[16–18] It can be observed that few of these *anti* adducts have activity against *P. falciparum*, and those that show activity are less active than the standard drug by a factor of 10^3. The situation is more promising against *T. cruzi* and *T. brucei rhodesiense* as more of the adducts show some activity, and the activity in some cases is only ten times lower than the standards. Compound **16** is quite active on both parasites, as are compounds **12**, **13** and **14**, at a lower level. Compounds **9** and **12** show good results on *T. cruzi* and compound **6** is active on *T. brucei rhodesiense*. The compounds that show activity are those with less polar *p*-substituents. These results are being taken into consideration in experiments on the activity of the *syn* adducts which are being carried out. Now that some of the basic aspects of the synthetic reactions have been established, it is clear that the scheme is particularly interesting for a combinatorial approach to a vast group of potential drugs.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.02.032.

References and notes

1. Tracy, J. W.; Webster, L. T. Fármacos usados en la quimioterapia de infecciones causadas por protozoarios. In *Las Bases Farmacológicas de la Terapéutica*; Goodman & Gilman Ed.; Ed. Interamericana: México DF, 1996; Vol. II, pp 1025–1047.
2. Enserink, M. *Science* **2000**, *287*, 1956–1958.
3. Woerdenbag, H. J.; Pras, N.; Van Uden, W.; Wallaart, T. E.; Beekmann, A. C.; Lugt, C. B. *Pharm. World Sci.* **1994**, *16*, 169–180.
4. Phillipson, J. D.; O'Neill, M. J. *Biological Active Natural Products*; Oxford Science: Oxford, England, 1987.
5. Meshnick, S. R.; Taylor, T. E.; Kamchonwongpaisan, S. *Microbiol. Rev.* **1996**, *60*, 301–315.
6. Laurent, S. A.-L.; Robert, A.; Meunier, B. *Angew. Chem., Int. Ed.* **2005**, *44*, 2060–2063.
7. Posner, G. H.; Oh, C. H. *J. Am. Chem. Soc.* **1992**, *114*, 8328–8329.
8. Posner, G. H.; Oh, C. H.; Wang, D.; Gerena, L.; Milhous, W. K.; Meshnick, S. R.; Asawamahasakda, W. *J. Med. Chem.* **1994**, *37*.
9. Posner, G. H.; O'Neill, P. M. *Acc. Chem. Res.* **2004**, *37*, 397–404.
10. Pauling, L. *The Nature of the Chemical Bond*, 3rd ed.; Cornell, Univ. Press.: New York, 1960.
11. Ren, H.; Grady, S.; Gamenara, D.; Heinzen, H.; Moyna, P.; Croft, S. L.; Kendrick, H.; Yardley, V.; Moyna, G. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1851–1854.
12. Corey, E. J.; Burke, H. J. *J. Am. Chem. Soc.* **1956**, *78*, 174–180.
13. Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656–2665.
14. Hart, H.; Sambharamadom, K. R.; Willer, R. *J. Org. Chem.* **1979**, *44*, 1–7.
15. Russi, S.; Pardo, H.; Heinzen, H.; Días, E.; Moyna, P.; Mariezcurrena, R.; Suescun, L.; Mombrú, A. *Acta Crystallogr., Sect. C* **2000**, 672–673.
16. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
17. Budkner, F. S.; Verlinde, C. L.; La Flamme, A. C.; Van Voortis, W. C. *Antimicrob. Agents Chemother.* **1996**, *40*, 2592–2597.
18. Raz, B.; Item, M.; Grether-Buhler, Y.; Kaminski, R.; Brun, R. *Acta Trop.* **1997**, *68*, 139–147.