Pergamon

Tetrahedron Letters 42 (2001) 751–754

TETRAHEDRON LETTERS

Novel *N*-ferrocenylmethyl, *N*′-methyl-2-substituted benzimidazolium iodide salts with in vitro activity against the *P. falciparum* malarial parasite strain NF54

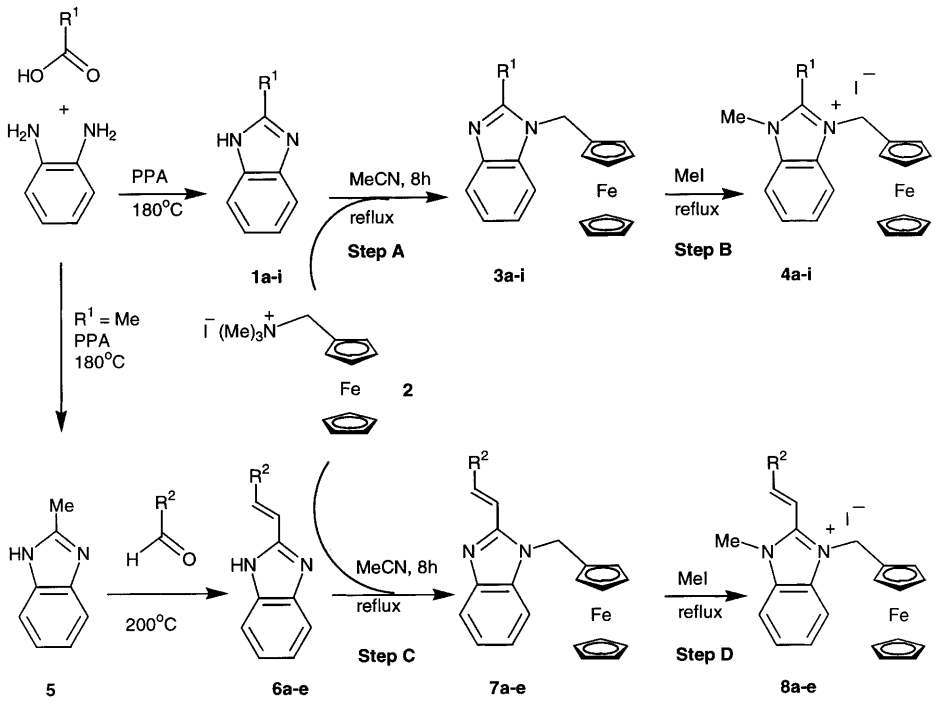
Joshua Howarth* and Keith Hanlon

School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland

Received 10 October 2000; revised 9 November 2000; accepted 15 November 2000

Abstract—Herein we disclose results of our research into a novel class of benzimidazolium compounds active against malarial parasites. We have discovered that *N*-ferrocenylmethyl, *N*′-methyl-2-aryl (or styryl) benzimidazolium iodide salts show excellent in vitro activity against the *P. falciparum* malarial parasite strain NF54.

Malaria, amongst all known diseases, has the most profound and devastating consequence for mankind wherever it is found. At present the disease is found in 102 countries and is responsible for over 500 million clinical cases each year, resulting in over two million deaths in the same annual period.[1] Given the parasite's resistance to most classical methods of chemotherapeutic treatment[2] the search is on for novel classes of compound that the parasite shows no resistance to, such as the naturally occurring compound artemisinin, and its derivatives.[3]



Scheme 1.

Keywords: *Plasmodium falciparum*; antimalaria; benzimidazolium salts.

* Corresponding author. Tel.: 353 1 7005312; fax: 353 1 7005503; e-mail: joshua.howarth@dcu.ie

0040-4039/01/$ - see front matter
PII: S0040-4039(00)02106-7

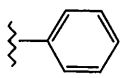
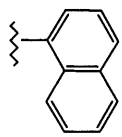
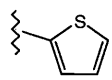
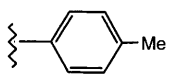
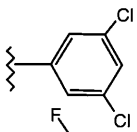
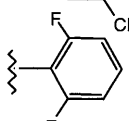
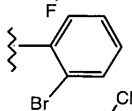
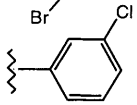
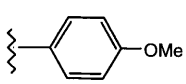
At the turn of the 19th century, Ehrlich focussed on the malarial parasite staining infected cells selectively over non-infected cells with methylene blue and acridine orange. The question we asked was why do these dyes selectively stain infected cells, and could the mechanism be put to use against the parasite?

Methylene blue is a cationic redox dye and when we examine the physiological consequences of malarial infection in red blood cells the answer becomes apparent. Infected red blood cells are under a high degree of endogenous oxidative stress stemming from the parasitic presence, and various physiological processes come into play to relieve this stress, primarily in the provision of substrates for reduction in a process known as the hexose monophosphate shunt (HMS). In infected cells HMS activity increases 24-fold and, as a consequence, the introduction of an alternative reductive target into the system, namely a redox dye, will prompt the uptake of the compound by the infected red blood cell. We can therefore conclude that infected cells have a high affinity for methylene blue.[4]

Ehrlich also noted the therapeutic effects of methylene blue,[5] and it has recently come to light that methylene blue is an antimalarial enzyme inhibitor, which is active against the glutathione reductase enzyme of the malarial parasite.[6] Other research has been undertaken examining the role of methylene blue as an antimalarial, given that it was up until 1985 prescribed as chemotherapy for the disease,[7] presently disused through toxicity concerns.

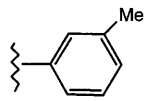
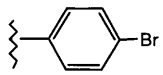
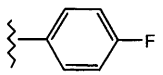
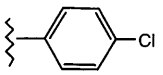
We decided to look for alternative cationic redox systems that might play a similar role as methylene blue and the other dyes as suggested above. We have an interest in azolium salts in the areas of ionic liquids[8] and anion receptors.[9] These azolium salts may be classified as cationic redox systems as there is obvious charge separation between the cationic azolium centre and the counterion. Based on our earlier studies on the development of antimalarial compounds involving bestatin analogues[10] we synthesised two series of benzimidazolium iodide salts, nine 2-aryl derivatives **4a**–**i**,[11] and five 2-styryl derivatives **8a**–**e**,[12] Scheme 1.

Table 1.

Compound	R^1	Yield% Step A	Yield% Step B	IC50 μM X	IC50 μM Y
1/3/4 a		67	94	1.2	0.44
b		73	99	16.6	0.28
c		63	96	18.4	0.04
d		71	91	17.5	0.28
e		73	93	21.6	0.31
f		76	92	25.0	0.38
g		75	80	18.1	0.47
h		64	84	25.0	0.84
i		54	87	14.5	0.32

X = benzimidazole derivative; **Y** = benzimidazolium derivative

Table 2.

Compound	R^2	Yield% Step C	Yield% Step D	IC50 μM X	IC50 μM Y
6/7/8 a	Me	67	90	18.0	0.08
b		73	85	32.0	0.12
c		63	82	32.0	0.08
d		71	64	2.0	0.09
e		73	73	22.0	0.13

X = benzimidazole derivative; **Y** = benzimidazolium derivative

When compounds **4a**–**i** and **8a**–**e** were tested for antimalarial activity against the *Plasmodium falciparum* NF54 strain the compounds were active with IC50s as given in Tables 1 and 2, respectively. Given that the IC50s for two of the current drugs used against this particular strain, chlorquine and artemeter, are 0.02 and 0.025 μM, respectively, these initial results would indicate that the *N*-ferrocenylmethyl, *N′*-methyl-2-aryl (or styryl) benzimidazolium iodide salts may have future potential as compounds in the fight against malaria. To the best of our knowledge these are the first examples of benzimidazolium salts shown to have activity against malaria, although clotrimazole has been shown to have powerful growth inhibiting effects in the *P. falciparum* parasite.[13] We have included the results for the same test carried out on the parent *N*-ferrocenylmethyl benzimidazole, compounds **3a**–**i** and **7a**–**e**. It is interesting to note that the activities for these non-quaternised compounds are several fold less, which in our opinion substantiates our hypothesis concerning the HMS above.

The mechanism of the antimalarial activity is unknown, however mechanisms shown for other imidazole-based drugs have been related to inhibition of catalase and peroxidase activities.[14] Alternatively a number of azino fused benzimidazolium salts have been utilised as DNA intercalating agents[15] and it has been shown that a positive charge is generally needed for activity.[16] To this end we are currently synthesising further derivatives and investigating the mechanism through which these molecules obtain their activity.

Acknowledgements

The authors would like to acknowledge the contribution to this research by the Tropical Disease Research section of the World Health Organisation, Enterprise Ireland, and Arch Chemicals BV.

References

1. Institute of Medicine, *Malaria, Obstacles and Opportunities*, a report compiled by the Committee for the Study on Malaria Prevention and Control; National Academy Press: Washington, DC 1991; pp. 1–2.
2. (a) Moran, J. S.; Bernard, K. W. *J. Am. Med. Assoc.* **1989**, *262*, 245; (b) Fullerton, D. S. *Med. Lett.* **1990**, *31*, 13.
3. Meshnick, S. R.; Thomas, A.; Ranz, A.; Xu, C. M.; Pan, H. Z. *Mol. Biochem. Parasitol.* **1991**, *49*, 181.
4. Atamna, H.; Pascarmona, G.; Ginsberg, H. *Mol. Biochem. Parasitol.* **1994**, *67*, 79.
5. Ehrlich, P. *The Collected Papers of Paul Ehrlich*; Pergamon Press: Oxford, 1956.
6. Faeber, P. M.; Arscott, L. D.; Wiliams, C. H.; Becker, K.; Schimer, R. H. *FEBS Lett.* **1998**, *422*, 311.
7. Atamna, H.; Kengliak, M.; Shalmiev, G.; Dehavo, E.; Pescarmona, G.; Ginsberg, H. *Biochem. Pharmacol.* **1996**, *51*, 693.
8. (a) Howarth, J. *Tetrahedron Lett.* **2000**, *41*, 6627; (b) Howarth, J.; Dallas, A. *Molecules* **2000**, *5*, 851.
9. (a) Thomas, J.-L.; Howarth, J.; Hanlon, K.; McGuirk, D. *Tetrahedron Lett.* **2000**, *41*, 413; (b) Howarth, J.; Thomas, J.-L.; Hanlon, K.; McGuirk, D. *Synth. Commun.* **2000**, *30*, 1865.
10. Lloyd, D. G. *The Design and Synthesis of Novel Antimalarial Agents*, Ph.D Thesis, Dublin City University, 1999.
11. General experimental for aryl series **4a**–**i** using the synthesis of **4a** as an example. To a mixture of 2-phenylbenzimidazole[17] **1a** (2.5 g, 13.0 mmol) and potassium carbonate (2.7 g, 19.5 mmol) in acetonitrile (150 ml) was added (trimethylammonium)ferrocenylmethyl iodide **2** (10.0 g, 13.0 mmol). The mixture was

heated at reflux for 12 h, cooled to room temperature and water was added. The resulting suspension was extracted into chloroform, the organic layer was washed with water, dried over magnesium sulphate and evaporated under vacuum to leave an orange gum. The crude product was purified by column chromatography on silica gel using DCM:methanol (97:3) as eluent. Compound **3a** was obtained as a light orange solid. *N*-Ferrocenylmethyl-2-(phenyl)benzimidazole **3a** (1.0 g, 2.6 mmol) was stirred at room temperature with an excess of methyl iodide (5 ml). After approx. 20 min a fine yellow ppt. fell out of solution. The mixture was heated at reflux for 2 h, filtered and the precipitate washed with ether. Compound **4a** was obtained as a light yellow powder. In some cases it was necessary to convert the iodide salt to the corresponding hexafluorophosphate salt to obtain microanalysis: General example: The hexafluorophosphate salt was obtained by stirring the iodide **4b** (1.8 g, 3.1 mmol) and ammonium hexafluorophosphate (0.5 g, 3.1 mmol) in acetone (50 ml) for 24 h. Subsequent work up revealed the hexafluorophosphate salt as a light brown crystalline solid. All the aryl series **4a–i** gave correct spectroscopic and microanalytical data.

12. General experimental for styryl series **8a–e** using the synthesis of **8a** as an example. To a mixture of 2-propenylbenzimidazole[18] **6a** (3.0 g, 19.0 mmol) and potassium carbonate (3.4 g, 28.5 mmol) in acetonitrile (100 ml) was added (trimethylammonium)ferrocenylmethyl iodide (7.3 g, 19.0 mmol). The mixture was heated at reflux for 12 h, cooled to room temperature and water was added. The resulting suspension was extracted into chloroform, the organic layer was washed with water, dried over magnesium sulphate and evaporated under vacuum to leave a dark brown solid. The crude product was purified by column chromatography on silica gel using DCM:methanol (97:3) as eluent. The compound **7a** was obtained as a light orange solid. To a solution of *N*-ferrocenylmethyl-2-(propenyl)benzimidazole **7a** (1.5 g, 4.2 mmol) in acetonitrile (100 ml) was added (trimethylammonium)ferrocenylmethyl iodide (1.6 g, 4.2 mmol) and the solution was heated at reflux for 24 h. The reaction was cooled to room temperature, water was added and the suspension was extracted into chloroform. The organic layer was washed with water, dried ($MgSO_4$) and evaporated to leave a brown residue that was treated with ether to leave the iodide salt **8a** as a light brown solid. Where necessary, for the purposes of analysis, the iodide salts were converted to the hexafluorophosphate salt. For example the hexafluorophosphate salt of **8b** was obtained by stirring the iodide **8b** (0.4 g, 0.7 mmol) and ammonium hexafluorophosphate (0.12 g, 0.7 mmol) in methanol (50 ml) at room temperature for 24 h. The resultant orange/red crystalline precipitate was filtered and washed several times with methanol. All of the styryl series **8a–e** gave correct spectroscopic and microanalytical data.
13. Tiffert, T.; Ginsberg, H.; Krugliak, M.; Elford, B. C.; Lew, V. L. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 331.
14. De Nollin, S.; Van Belle, H.; Goossens, F.; Thone, F.; Borgers, M. *Antimicrob. Agents Chemother.* **1977**, *11*, 500.
15. Pastor, J.; Siro, J. G.; Garcia-Navio, J. L.; Vaquero, J. J.; Alvarez-Builla, J.; Gago, F.; de Pascual-Teresa, B.; Pastor, M.; Rodrigo, M. M. *J. Org. Chem.* **1997**, *62*, 5476.
16. Waring, M. J. *J. Mol. Biol.* **1970**, *54*, 247.
17. (a) Hein, D. W.; Alheim, R. J.; Leavitt, J. J. *J. Am. Chem. Soc.* **1957**, *79*, 427; (b) Phillips, M. A. *J. Chem. Soc.* **1930**, 1409.
18. Sullivan, W. *J. Med. Chem.* **1970**, *2*, 784.