Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 15, pp. 1781-1784, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00317-4

PREPARATION, ANTIMICROBIAL EVALUATION AND MUTAGENICITY OF DIFFERENTLY SUBSTITUTED [2-HYDROXYARYL]-[1-METHYL-5-NITRO-1*H*-2-IMIDAZOLYL]METHANOLS.

Y. Arredondo,[a] M. Moreno-Mañas,*[a] R. Pleixats,[a] C. Palacín,[b] M.M. Raga,[b] J.M. Castelló,[b] J.A. Ortiz*[b]

[a] *Department of Chemistry, Universitat Autònoma de Barcelona. Bellaterra, 08193-Barcelona. Spain. Fax: 343- 581 12 65. E-mail: iqorb@cc.uab.es.*

[b] *Centro de Investigación Grupo Ferrer. Juan de Sada, 32. 08028-Barcelona. Spain. Fax: 343- 411 27 64.*

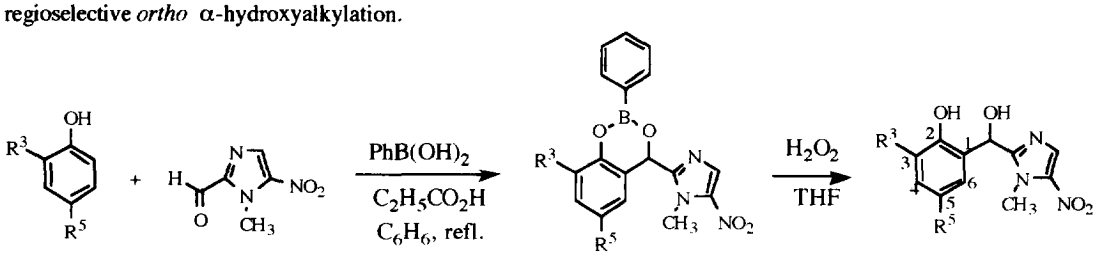
Abstract: An efficient preparation of the titled compounds is described, their antimicrobial activity and mutagenic properties being evaluated. Some of the studied compounds are non mutagenic and present a MIC as low as some of the usual standards in the field. Copyright © 1996 Elsevier Science Ltd

Introduction

The classical nitroimidazolic antimicrobial agents,[1] such as metronidazole and others, are very active against anaerobic bacteria and have a high and widespread use all over the world. However, they present serious problems of mutagenicity. The *in vivo* reduction of the nitro group is a common required step for both the antibiotic and the mutagenic activities. Thus, the separation of both effects is a goal of prime importance.[2] The mechanism of action of nitroimidazoles[3] begins with a monoelectronic reduction of the nitro group to give a radical-anion, which can be deactivated by oxygen, thus hampering the subsequent steps of the process. This suggests that new nitroimidazolic antimicrobial agents bearing phenolic antioxidant moieties would have a protecting effect on this radical-anion. Two interesting compounds, abunidazole,[4] **1a**, and EU-11100,[5] **1b**, which present substituted phenolic rings containing a *tert*-butyl lipophilic group, have been reported in the patent literature. With these precedents in mind we undertook the preparation of a family of compounds **1**, differently substituted in the *ortho* and/or *para* positions with respect to phenolic hydroxyl group. Bulky and strong lipophilic groups were chosen as substituents in the carbocyclic ring.

Synthesis

The reaction scheme is detailed below and our results are given in the Table. The described preparations of abunidazole,[4] **1a**, and EU-11100,[5] **1b**, required the condensation of 1-methyl-5-nitroimidazole-2-carboxaldehyde, **2**, with the corresponding aryloxymagnesium halides formed in a reaction between phenols and a Grignard reagent. In order to avoid the use of these type of reagents we turned to other methodologies for the regioselective *ortho* α-hydroxyalkylation.



3a, c-k **2** **4a, c-k** **1a, c-k**

Scheme

The so called metal-template catalysis[6] for *o*-functionalization of phenols has been investigated by several authors. Nagata *et al* [7] reported the related regioselective *ortho*-hydroxyalkylation of phenols based on the trapping of the dihydroxy compound in the form of a phenylboronic acid cyclic ester. This method has been very convenient in our preparation of **1**. Thus, the aldehyde **2** was reacted with differently substituted phenols **3** in the presence of a little excess of phenylboronic acid and a catalytic amount of propionic acid in refluxing benzene. The 4*H*-1,3,2-benzodioxaborins **4** thus obtained were oxidized with hydrogen peroxide in tetrahydrofuran at room temperature to afford the desired products **1**.[8, 9]

Aldehyde **2** was prepared in 72 % yield from commercially available 2-hydroxymethyl-1-methyl-5-nitroimidazole by oxidation with manganese(IV) oxide in chloroform.[10] The non commercial phenols **3** bearing alkyl groups in *ortho* or *para* positions were synthesized by thermal reaction of non alkylated phenols with the corresponding alkyl bromides.[11]

Assay methods

1.- *Salmonella typhimurium* reverse mutation assay (Ames test)

The assay was carried out using the standard plate incorporation test described by Ames *et al.*[12]. The *Salmonella* strain used, TA-100, was supplied by Professor B.N. Ames (Berkeley). Briefly, 0.1 ml of the appropriate bacterial culture containing approximately 2.10^8 cells, together with 0.5 ml of S-9 (IFFA-CREDO) mix, was combined with 0.1 ml of test solution and 2 ml of histidine deficient agar. This mixture was layered onto 25 ml of pre-poured Vogel-Bonner minimal agar. Triplicate plates were used at each dose level. The plates were incubated for 72 h at 37°C and the number of revertant colonies counted on a IUL Countermat automatic colony counter. The products, dissolved in dimethylsulfoxide, were tested at the following concentrations: 5000, 2000, 800, 320 and 128 µg/plate, and the positive control assessed was 2-aminofluorene at 20 µg/plate.

A compound is considered to be mutagenic if a statistically significant dose-related increase in the number of revertant colonies, of at least twice the concurrent solvent control, is obtained in two separate experiments. The results of this mutagenicity test for the new compounds **1c-k** are presented in the Table. The first assays gave weak mutagenic character or not clear results, as mutagenicity was only observed at high doses and accompanied with doubtful bacterial background lawn. Thus, two of the requirements to define the mutagenic character of a compound were not fulfilled (dose-related increase in the number of revertants in the presence of normal bacterial background lawn). However, the number of revertants was superior to the control and the mutagenic character could not be excluded. In order to clarify the results the Ames test was repeated with compounds **1c**, **1d**, and **1i** which had been carefully treated with sodium hydrogeno sulfite to eliminate residual traces of the intermediate aldehyde **2**, and then recrystallized. The purified samples (containing < 0.01% of **2**) were not mutagenic.

2. Antimicrobial activity

Minimum inhibitory concentrations (MIC) were determined by a standardized agar dilution technique.[13] The anaerobic bacteria studied were the following: one strain of *Bacteroides fragilis* ATCC 25285 (American Type Culture Collection), four strains of *Bacteroides fragilis* of clinical origin (366/H, 781/L, 1134 and 1009), one *B. vulgatus* ATCC 8482, one *Clostridium perfrigens* ATCC 13124, one *Prevotella melaninogenica* ATCC 25845, one *Peptostreptococcus anaerobius* ATCC 27337 and one *Peptostreptococcus magnus* ATCC 29328, identified by standard criteria and kept frozen in glycerol-Caso broth until use. The susceptibilities of the isolates to the tested drugs were determined in Wilkins-Chalgren agar. The products were initially dissolved in dimethylsulfoxide and then dilutions were made in water. Metronidazole was used as standard. Plates containing serial doubling dilutions of antimicrobial agents ranging from 0.03 to 64 µg/ml were inoculated with a Steer's replicator to give a final inoculum of 10^5 CFU. They were incubated in an anaerobic chamber (Gaspak with Anaerocult A Merck) for 48 h at 37°C. The control plates contained 2 mL of a mixture DMSO-H_2O 1:2, the same mixture that was used in the samples having the higher concentration of DMSO.

MIC was defined as the lowest concentration of drug that inhibited growth. The antimicrobial activity (geometric mean of MIC values in μmol/l) of new compounds **1c-k** are summarized in the Table, together with the activity of other known drugs such as metronidazole, abunidazole, **1a**, and EU-11100, **1b**.

Table. Antimicrobial activity and mutagenicity of compounds **1**. Comparison with metronidazole, abunidazole and EU-11100.

Compound	R^3	R^5	mp (°C)	MIC (geometric mean) (μmol/l)	Mutagenicity[a]
Metronidazole				1.46	+++
Abunidazole, **1a**	H	^{t}Bu	157-159	13.11	++/+++
EU-11100, **1b**	^{t}Bu	MeO		28.20	--
1c	2-adamantyl	H	154-157	3.84	--
1d	H	1-adamantyl	192-195	2.43	--
1e	1-adamantyl	H	182-184	4.46	+
1f	2-adamantyl	F	176-178	3.97	(+)
1g	2-adamantyl	Cl	165-168	6.78	(+)
1h	2-adamantyl	MeO	164-167	4.21	(+)
1i	H	2-adamantyl	173-175	1.80	--
1j	cyclohexyl	H	foam	4.68	+
1k	H	cyclohexyl	168-170	8.82	(+)

[a] +, ++, +++ indicate different levels of mutagenicity in the Ames test[12]: the number of revertant colonies obtained increases 1.5-2, 5 and 10 times respectively; (+) low levels of mutagenicity could possibly be related to the presence of > 0.01% of **2** in the tested sample; -- indicates no evidence of mutagenicity.

In conclusion, compound **1i** was the best antimicrobial candidate due to its superior activity and its lack of mutagenicity. However, all *in vivo* data, i.e. low oral bioavailability (1.9 % for **1i**) and the lack of efficacy in the infection model used,[14] probably related to a high value of log P[15], preclude the possibility of its systemic use.

Acknowledgements. The group at UAB gratefully acknowledges financial support from CICYT through Project PTR93-0048, and Barisintex S.A. for a generous gift of 2-hydroxymethyl-1-methyl-5-nitroimidazole.

References and Notes.

1. For the synthesis and evaluation of nitroimidazoles see, for example: a) Cosar, C.; Crisan, C.; Horclois, R.; Jacob, R.M.; Robert, J.; Tchelitcheff, S.; Vaupré, R. *Arzneim.-Forsch.* **1966**, *16*, 23. b) Butler, K.; Howes, H.L.; Lynch, J.E.; Pirie, D.K. *J. Med. Chem.* **1967**, *10*, 891. c) Giraldi, P.N.; Mariotti, V.; Nannini, G.; Tolosini, G.P.; Dradi, E.; Logemann, W.; De Carneri, I.; Monti, G. *Arzneim.-Forsch.* **1970**, *20*, 52. d) Hoffer, M.; Grunberg, E. *J. Med. Chem.* **1974**, 17, 1019. e) Winkelmann, E.; Raether, W.; Gebert, U.; Sinharay, A. *Arzneim.-Forsch.* **1977**, *27*, 2251. f) Neville, M.C.; Verge, J.P. *J. Med. Chem.* **1977**, *20*, 946.
2. Woogd, C.E. *Mutation Research* **1981**, *86*, 243.
3. Edwards, D.I. "Nitroimidazole drugs-action and resistance mechanisms/Mechanisms of action" *J. Antimicrob. Chemoth.* **1993**, *31*, 9.
4. Tessitore, P.T. *Eur. Pat.* 111, 657 (1983).

5. Furlan, D. (Euroresearch S.r.L.). *Eur. Pat.* 535, 528 (1992).
6. Sartori, G.; Maggi, R.; Bigi, F.; Arienti, A.; Porta, C.; Predieri, G. *Tetrahedron* **1994**, *50*, 10587 and references cited therein.
7. Nagata, W.; Okada, K.; Aoki, T. *Synthesis* **1979**, 365.
8. Foguet R.; Moreno-Mañas, M.; Arredondo, Y.; Pleixats, R.; Raga, M.M.; Castelló, J.M.; Ortiz, J.A. *Spanish Pat.* 9500255 (1995).
9. Typical procedure: A solution of 2-(2-adamantyl)phenol (2.23 g, 9.8 mmol), 1-methyl-5-nitroimidazole-2-carboxaldehyde (1.68 g, 10.8 mmol), phenylboronic acid (1.43 g, 11.7 mmol) and propanoic acid (0.29 g, 3.9 mmol) in anhydrous benzene (60 mL) was heated under reflux for 45 min (TLC monitoring) with azeotropic removal of water using a Dean-Stark type separator. The solvent was evaporated and the crude oily residue crystallized by addition of diethyl ether. The solid was filtered and washed with diethyl ether to give the 4*H*-1,3,2-benzodioxaborin **4c** (white solid, 3.94 g, 86% yield). To an ice-cooled and stirred solution of **4c** (1.5 g, 3.2 mmol) in tetrahydrofuran (25 mL) was slowly added 36% aqueous solution of hydrogen peroxide (5 mL) and the reaction mixture was left under stirring at room temperature for 5 h (TLC monitoring). Ethyl acetate was added (100 mL) and the organic solution was washed with water (50 mL), vigorously stirred with 40% aqueous sodium hydrogeno sulfite (100 mL) for 48 h, washed again with water (4x100 mL), dried with anhydrous sodium sulfate and evaporated. The solid residue was washed with dichloromethane-diethyl ether to afford **1c** (0.84 g, 69% yield), mp 154-157°C; IR (KBr): 3359 (br), 3282, 1539, 1480, 1454, 1379, 1268, 1190, 833, 752 cm^{-1}; ^1H NMR (CD_3OD): 1.60 (apparent d, J = 12.4 Hz, 2H), 1.78 (br s, 2H), 1.89-1.96 (m, 8H), 2.28 (br s, 2H), 3.25 (br s, 1H), 3.94 (s, 3H), 6.20 (s, 1H), 6.82 (t, J = 7.7 Hz, 1H), 6.96 (dd, J = 7.7 and 1.8 Hz, 1H), 7.36 (d, J = 7.7 Hz, 1H), 7.90 (s, 1H); ^{13}C NMR ($(CD_3)_2SO$): 27.3, 27.7, 30.4, 30.5, 32.4, 32.6, 33.4, 37.7, 39.4, 39.6, 43.0, 64.9, 119.1, 125.2, 127.0, 127.2, 131.6, 132.9, 139.0, 152.6, 153.7. Anal. Calcd. for $C_{21}H_{25}N_3O_4$: C, 65.78; H, 6.57; N, 10.96. Found: C, 65.92; H, 6.29; N, 10.59. The other compounds were similarly prepared. They were characterized by spectroscopic means and all of them presented correct elemental analysis.
10. a) Shafiee, A.; Pirouzzadeh, B.; Ghasemian, F.; Parang, K. *J. Heterocyclic Chem.* **1992**, *29*, 1021. b) Rufer, C.; Kessler, H.-J.; Schröder, E. *J. Med. Chem.* **1971**, *14*, 94.
11. Y. Arredondo, M. Moreno-Mañas, R. Pleixats. Submitted.
12. a) Ames, B.N.; McCann, J.; Yamasaki, E. *Mut. Res.* **1975**, *31*, 347. b) Maron, D.M.; Ames, B.N. *Mut. Res.* **1983**, *113*, 173.
13. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A2. National Committee for Clinical Laboratory Standards, Vilanova, Pa. 1991.
14. A quantitative model for subcutaneous abscess formation in mice. Joiner, K.A.; Onderdonk, A.B.; Gelfand, J.A.; Bartlett, J.G.; Gorbach, S.J. *Br. J. Exp. Path* **1980**, *61*, 97. Metronidazole proved to be effective reducing the number of *B. fragilis* in the abscess from 10^8 to 10^3 while **1i** was not effective.
15. Estimated with the program ClogP for Windows. Version 1.0.0., 1995, BioByte Corp., Claremont, CA 91711. Calculating log P_{oct} from Structures: Leo, A.J., *Chem. Rev.* **1993**, *93*, 1281. ClogP for **1i** was 2.75 and for metronidazole was -0.70. The program also contains the experimental value (- 0.02) of logP for metronidazole.

(*Received in Belgium* 6 *May* 1996; *accepted* 28 *June* 1996)