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Preparation, Antimicrobial Evaluation, and Mutagenicity of [2-Hydroxyaryl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanols, [5-*tert*-Butyl-2-methylaminophenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol, and [2-Hydroxyaryl]-[1-methyl-5-nitro-1*H*-2-imidazolyl] ketones

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Abstract—Efficient preparations of the titled compounds are described, their antimicrobial activity and mutagenic properties being evaluated. Some of the studied compounds are nonmutagenic and present a MIC as low as some of the usual standards in the field.
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Introduction

The classical nitroimidazole antimicrobial agents,[1] such as metronidazole and others, are very active against anaerobic bacteria and have a world-wide use. However, they present serious problems of mutagenicity. The in vivo reduction of the nitro group is a step commonly required 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 nitroimidazole antimicrobial agents bearing phenolic antioxidant moieties would have a protecting effect on this radical anion. Two interesting compounds, abunidazole[4] (**4a**) and EU-11100[5] (**4b**), with 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 **4**, differently substituted in the *ortho* and/or *para* positions with respect to phenolic hydroxyl group, as well as related ketones **5** and aniline **14**. Bulky and strong lipophilic groups were chosen as substituents in the carbocyclic ring.

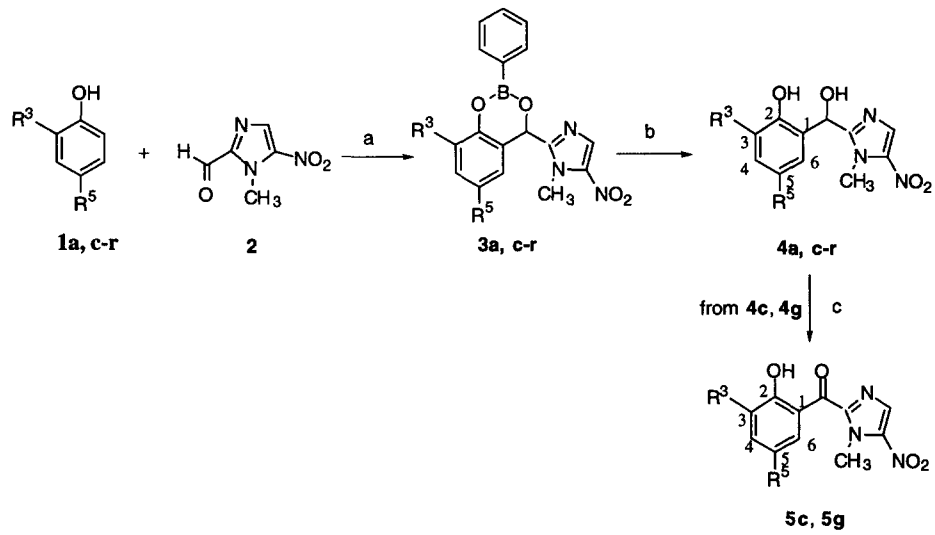
Results and Discussion

Synthesis

The described preparations of abunidazole[4] (**4a**) and EU-11100[5] (**4b**) 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 find an alternative to the use of these type of reagents we turned to other methodologies for the regioselective *ortho* α-hydroxyalkylation.

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 **4**. Thus, the aldehyde **2** was reacted with differently substituted phenols **1** in the presence of a little excess of phenylboronic acid and a catalytic amount of propionic acid in refluxing benzene (Scheme 1). The 4*H*-1,3,2-benzodioxaborins **3** thus obtained were oxidized with hydrogen peroxide in tetrahydrofuran at room temperature to afford the desired products **4**[8,9] (Scheme 1).

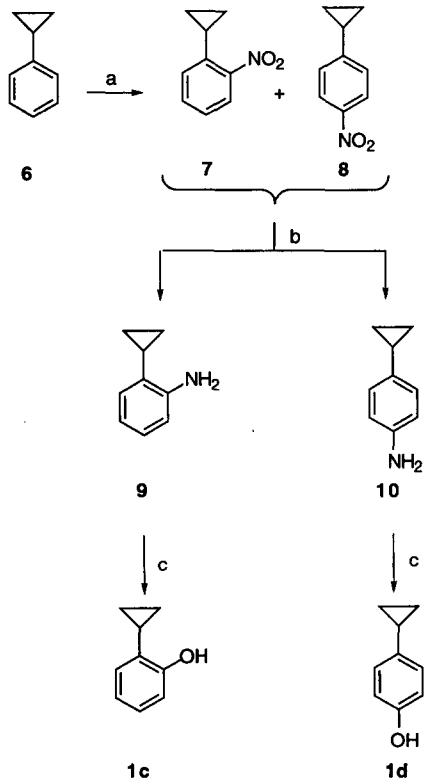
Aldehyde **2** was prepared in 72% yield from commercially available 2-hydroxymethyl-1-methyl-5-nitroimidazole by oxidation with manganese(IV) oxide in chloroform.[10] The non-commercial phenols **1g–r** bearing 1- and 2-adamantyl, cyclohexyl and norbornyl groups in *ortho* or *para* positions were synthesized by thermal reaction of non-alkylated phenols with the corresponding alkyl bromides as described elsewhere.[11] The *ortho*- and *para*-cyclopropylphenols, **1c** and **1d** (Scheme 2) were obtained from cyclopropylbenzene, **6**, by a modified version of a described reaction sequence[12] including nitration,[13] hydrogenation of the resulting mixture of nitro compounds **7** and **8**, chromatographic



Scheme 1. Preparation of alcohols **4** and ketones **5**. (a) $PhB(OH)_2$ (1.1 equiv), $C_2H_5CO_2H$ (0.4 equiv), C_6H_6, reflux; (b) H_2O_2, THF, rt; (c) MnO_2, $CHCl_3$, rt.

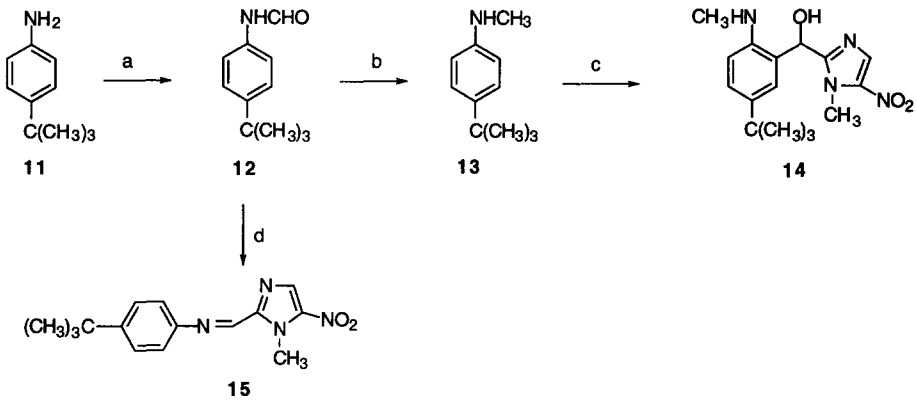
separation of the isomeric cyclopropylanilines **9** and **10**, and conversion to the corresponding phenols via diazotization and hydrolysis[14] of the arenediazonium salt.

We also wanted to evaluate the activity of ketones resulting from the oxidation of alcohols **4**. Thus, ketones **5c** (R^3 = cyclopropyl) and **5g** (R^3 = 2-adamantyl) were obtained by treatment of **4c** and **4g** with manganese(IV) oxide in chloroform at room temperature (Scheme 1). The antimicrobial activity (see Table 1) of **5c** and **5g** was lower than that of the corresponding alcohols **4c** and **4g**, and for this reason no more ketones were prepared.



Scheme 2. Preparation of phenols **1c–d**. (a) $Cu(NO_3)_2 \cdot 3H_2O$, AcOH/Ac_2O, rt; (b) (i) H_2 (2 atm), PtO_2, EtOH, rt; (ii) chromatographic separation; (c) (i) H_2SO_4, H_2O, $NaNO_2$; (ii) H_2O, $Cu(NO_3)_2 \cdot 3H_2O$, Cu_2O.

Next, we turned to anilines as nucleophilic aromatic reagents to react with aldehyde **2**. To our knowledge there were no precedents about the regioselective *ortho* hydroxyalkylation of primary anilines by reaction with carbonyl compounds. Only the *ortho* condensation of primary anilines with nitriles has been recently published.[15] However, secondary anilines have been reported[16] to condense at the *ortho* position with aldehydes in the presence of boron trichloride. When the experimental conditions of Nagata et al. [7] (phenylboronic acid, catalytic amount of propionic acid in refluxing benzene) were adopted in the reaction of **2** with *p*-chloroaniline, only the corresponding Schiff base was isolated. Thus, we prepared *N*-methyl-4-*tert*-butylaniline, **13**, (Scheme 3) by formylation of 4-*tert*-butylaniline, **11**, and reduction of the formamide **12** with lithium aluminium hydride. Treatment of **13** with boron trichloride in refluxing benzene followed by addition of aldehyde **2** and triethylamine afforded the condensation product **14** in 65 % yield (Scheme 3). We should mention that *N*-methyl-2-*tert*-butylaniline did not react with **2** under analogous conditions, possibly due to steric reasons. On the other hand, the condensation of formamide **12** with **2** in the presence of one equivalent of phenylboronic acid and catalytic propionic acid under refluxing xylene yielded the imine **15**. As the antimicrobial activity (see Table 1) of compound **14** was lower than that of the analogous phenol **4a** no more reactions of **2** with substituted anilines were undertaken.



Scheme 3. Preparation of **14**. (a) HCO_2H, reflux; (b) $LiAlH_4$, Et_2O, reflux; (c) (i) BCl_3, C_6H_6, reflux; (ii) **2**, Et_3N, C_6H_6, rt; (d) **2**, $PhB(OH)_2$, $C_2H_5CO_2H$, xylene, reflux.

Antimicrobial activity and mutagenicity

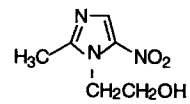
Minimum inhibitory concentrations (MIC) were determined by a standardized agar dilution technique.[17] The anaerobic bacteria studied were the following: one strain of *Bacteroides fragilis* ATCC 25285 (American Type Culture Collection), four strains of *Bacteroides fragilis* of clinical origin (366/H, 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. More details are given in the Experimental section.

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 **4c–r**, **5c**, **5g** and **14** are summarized in Table 1, together with the activity of other known drugs such as metronidazole, abunidazole, **4a**, and EU-11100, **4b**.

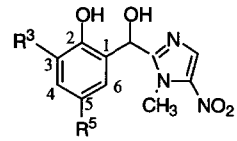
The mutagenic activities of compounds, **4**, **5** and **14** were evaluated by the *Salmonella typhimurium* reverse mutation assay (Ames test). The assay was carried out using the standard plate incorporation test described by Ames et al.[18] The *Salmonella* strain used, TA-100, was supplied by Professor B. N. Ames (Berkeley, CA, U.S.A.). More details are given in the Experimental section.

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 **4**, **5** and **14** are presented in Table 1. 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 **4g**, **4h** and **4m** which had been carefully treated with sodium hydrogenosulfite to eliminate residual traces of the intermediate aldehyde **2**, and then recrystallized. The purified samples (containing <0.01% of **2**) were not mutagenic.

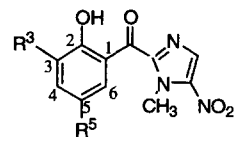
We also show in Table 1 the calculated[19] values of log *P* for all compounds. Alcohols **4g–h** and **4m**, which have good activity and which do not present mutagenic



metronidazole



4a-r



5c, g

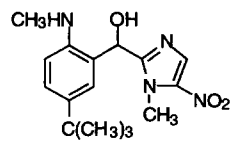


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Table 1. Antimicrobial activity, mutagenicity and calculated log *P* of compounds **4c—r**, **5c**, **5g**, and **14**. Comparison with metronidazole, abunidazole **4a**, and EU-11100, **4b**

			Antimicrobial activity (MIC, μmol/L)												
Compound	**R^3**	**R^5**	**1[b]**	**2[b]**	**3[b]**	**4[b]**	**5[b]**	**6[b]**	**7[b]**	**8[b]**	**9[b]**	**10[b]**	**(Geometric mean)**	**Mutagenicity[a]**	***C* log *P***
Metronidazole			1.46	1.46	1.46	1.46	0.70	0.70	1.46	2.92	2.92	1.46	1.45	+++	−0.70
4a	H	*tert*-Butyl	13.11	13.11	—	13.11	—	—	—	—	—	—	13.11	++/+++	1.33
4b	*tert*-Butyl	MeO	26.23	26.23	26.23	26.23	13.11	—	52.46	52.46	26.23	26.23	28.3	–	1.43
4c	Cyclopropyl	H	13.84	13.84	13.84	13.84	—	—	—	—	—	—	13.84	+++	0.44
4d	H	Cyclopropyl	3.46	3.46	3.46	3.46	—	—	—	—	—	—	3.46	+++	0.44
4e	H	Cl	3.53	3.53	3.53	3.53	—	—	—	—	—	—	3.53	+++	0.51
4f	Cl	H	3.53	3.53	3.53	3.53	—	—	—	—	—	—	3.53	+++	0.18
4g	2-Adamantyl	H	2.61	5.22	2.61	2.61	1.30	—	5.22	10.44	5.22	5.22	3.83	–	2.75
4h	H	1-Adamantyl	2.61	2.61	2.61	2.61	0.65	1.30	2.61	5.22	5.22	—	2.41	–	2.75
4i	1-Adamantyl	H	5.22	5.22	5.22	10.44	0.31	1.30	5.22	20.88	10.44	—	4.45	+	2.75
4j	2-Adamantyl	F	4.99	4.99	4.99	4.99	0.62	2.49	4.99	9.97	4.99	—	3.96	(+)	3.19
4k	2-Adamantyl	Cl	9.58	9.58	9.58	9.58	1.20	2.39	4.79	38.33	9.58	4.79	6.77	(+)	3.76
4l	2-Adamantyl	MeO	4.84	4.84	4.84	4.84	1.21	2.42	2.42	9.68	4.84	9.68	4.21	(+)	2.85
4m	H	2-Adamantyl	2.61	2.61	2.61	2.61	0.52	0.65	0.65	5.22	2.61	2.61	1.80	–	2.75
4n	Cyclohexyl	H	6.04	6.04	6.04	6.04	0.91	1.51	12.08	1.51	12.08	12.08	4.66	+	2.12
4o	H	Cyclohexyl	12.08	12.08	12.08	12.08	0.60	3.02	24.17	12.08	12.08	24.17	8.95	(+)	2.12
4p	*exo*-2-Norbornyl	H	5.83	5.83	5.83	5.83	—	5.83	5.83	2.91	5.83	1.46	4.63	+/−	1.88
4q	H	*exo*-2-Norbornyl	2.91	2.91	2.91	2.91	1.46	—	2.91	1.46	2.91	0.36	1.98	+/−	1.88
4r	2-Adamantyl	MeCO	9.41	9.41	9.41	—	—	4.70	—	18.82	9.41	4.70	8.52	(—)	2.73
5c	Cyclopropyl	H	223.0	223.0	223.0	223.0	—	—	223.0	223.0	27.87	223.0	171.9	(+)	2.42
5g	2-Adamantyl	H	168.0	168.0	168.0	168.0	—	—	168.0	168.0	168.0	168.0	168.0	—	4.73
14	H	*tert*-Butyl	201.2	201.2	201.2	201.2	—	—	—	—	—	—	201.2	(+)	0.93

[a] +, ++ and +++indicate different levels of mutagenicity in the Ames test:[18] 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.

[b] The meanings of the different numbers are: 1, *Bacteroides fragilis* 388; 2, *B. fragilis* 781; 3, *B. fragilis* 11344; 4, *B. fragilis* 1009; 5, *P. anaerobicus* ATCC 27337; 6, *P. magnus* ATCC 29328; 7, *C. perfringens* ATCC 13124; 8, *B. vulgatus* ATCC 8482; 9, *B. fragilis* ATCC 25285; 10, *P. melaninogenica* ATCC 25845.

character, have higher values than those calculated for commercialized antimicrobial agents metronidazole, abunidazole and EU-11100. Compounds **4c–f** have lower values of log *P* but they are mutagenic. For a discussion on the influence of log *P* (which is a measure of the lipophilicity of the compound determined by using the octanol/buffer partition model system) on the pharmacokinetic properties of the drug (absorption, distribution and clearance) see Smith et al.[20]

Conclusion

In conclusion, compound **4m** was the best antimicrobial candidate due to its superior activity and its lack of mutagenicity. However, all in vivo data, that is, low oral bioavailability (1.9% for **4m**) and the lack of efficacy in the infection model used,[21] probably related to a high value of log *P* (see Table 1) preclude its systemic use, more research in the field being required before an optimal molecule will be found.

Experimental

^{1}H NMR (^{13}C NMR) were recorded at 250 MHz (62.5 MHz) using TMS as internal standard. The chemical shift values are given in δ (ppm).

2-Formyl-1-methyl-5-nitroimidazole (2). Manganese (IV) oxide (50.0 g, 0.575 mol) was added to a solution of 2-hydroxymethyl-1-methyl-5-nitroimidazole (15.0 g, 0.095 mol) in chloroform (350 mL). The mixture was left at room temperature under stirring for 18 h. Then, it was filtered through celite and the solvent from filtrate was evaporated to afford **2** as a yellow solid (10.8 g, 72% yield). Mp 93–95 °C (literature value:[22] mp 97–98 °C); IR (KBr) 1704, 1533, 1484, 1374, 1351 cm^{-1}; ^{1}H NMR ($CDCl_3$) 4.31 (s, 3H), 8.04 (s, 1H), 9.87 (s, 1H).

2- and 4-Nitrocyclopropylbenzene (7 and 8). A solution of cyclopropylbenzene, **6**, (1.01 g, 8.46 mmol) in acetic acid (1 mL) and acetic anhydride (2 mL) was added dropwise to a stirred mixture of powdered copper(II) nitrate trihydrate (2.25 g, 9.31 mmol), acetic acid (2 mL) and acetic anhydride (5 mL), an exothermic reaction taking place. The stirred mixture was left at room temperature for 3 h (TLC monitoring), then it was poured into ice water and it was extracted with dichloromethane. The organic layer was washed with diluted ammonium hydroxide and with water, it was dried with anhydrous sodium sulfate and the solvent evaporated, yielding a 2:1 mixture (^{1}H NMR) of **7** and **8** as a yellow oil (1.11 g), which was hydrogenated without further purification. IR (film): 1606, 1523, 1346 cm^{-1}. The reaction of **6** with fuming nitric acid in acetic acid/acetic anhydride[23] at 0 °C gave an analogous mixture but the yield was lower (48%).

2- and 4-Cyclopropylanilines (9 and 10). A stirred mixture of **7** and **8** (3.3 g, 19.6 mmol, 2:1 molar ratio), absolute ethanol (15 mL) and platinum(IV) oxide (0.12 g) was hydrogenated at room temperature under pressure (2 atm) for 18 h (TLC monitoring). The mixture was filtered through celite and the solvent from filtrate was evaporated. The residue was chromatographed through a silica gel column, eluting with mixtures of hexanes and ethyl acetate of increasing polarity. 2-Cyclopropylaniline, **9** (1.61 g, 62%) was eluted first; IR (film): 3466, 3376 cm^{-1}; ^{1}H NMR ($CDCl_3$) 0.52–0.58 (m, 2H), 0.81–0.88 (m, 2H), 1.57–1.68 (m, 1H), 3.56 (broad s, 2H), 6.65 (m, 2H), 6.97–7.02 (m, 2H). Then, 4-cyclopropylamine, **10**, was obtained (0.21 g, 8%); IR (film) 3438, 3361 cm^{-1}; ^{1}H NMR ($CDCl_3$) 0.49–0.55 (m, 2H), 0.76–0.83 (m, 2H), 1.69–1.80 (m, 1H), 3.02 (broad s, 2H), 6.55 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H). Also 5% Pd–C (K-0203) can be used as catalyst under analogous conditions (ethanol, 2 atm of hydrogen pressure). Higher pressures (2.5–3.0 atm) caused partial opening of the cyclopropyl ring. Sodium ditionite in water/tetrahydrofuran medium can also be used as reducing agent.

2-Cyclopropylphenol (1c). Crushed ice was added to a solution of **9** (2.421 g, 0.018 mol) in 26% sulfuric acid (24 mL) (a solid is formed). The stirred mixture was maintained at 0–5 °C and a solution of sodium nitrite (1.380 g, 0.020 mol) in water (17 mL) was slowly added. After the addition, the mixture was left at the same temperature for 15 min (potassium iodide/starch assay for nitrite ion). Urea was added to remove excess of sodium nitrite. A solution of copper(II) nitrate trihydrate (50.011 g, 0.207 mol) in water (400 mL) was added to the freshly prepared solution of diazonium salt maintained at 0 °C; then copper(I) oxide (2.341 g, 0.016 mol) was added to the vigorously stirred mixture, gas evolution being observed. The reaction mixture was left at room temperature for 20 min (GLC monitoring), then extracted with dichloromethane. The organic layer was extracted with 10% aqueous sodium hydroxide, the alkaline aqueous phase acidified with concentrated hydrochloric acid and extracted with dichloromethane. The organic layer was washed with water, dried with anhydrous sodium sulfate and the solvent was evaporated to afford **1c** (1.003 g, 41%) as a brown oil; IR (film) 3546 (sharp), 3455 (broad) cm^{-1}; ^{1}H NMR ($CDCl_3$) 0.59–0.65 (m, 2H), 0.90–0.97 (m, 2H), 1.73–1.84 (m, 1H), 5.43 (s, 1H), 6.79–6.85 (m, 2H), 7.03–7.13 (m, 2H).

4-Cyclopropylphenol (1d). This was prepared from **10** in 51% yield as for **1c**: mp 75–76 °C (hexanes) (literature value:[24] mp 68–72 °C); IR (film) 3384–3255 (broad) cm^{-1}; ^{1}H NMR ($CDCl_3$) 0.51–0.57 (m, 2H), 0.79–0.87 (m, 2H), 1.73–1.83 (m, 1H), 4.63 (broad s, 1H), 6.67 (d, J = 8.4 Hz, 2H), 6.90 (d, J = 8.4 Hz, 2H).

6-*tert*-Butyl-4-(1-methyl-5-nitro-2-imidazolyl)-2-phenyl-4*H*-1,3,2-benzodioxaborin (3a). A solution of 4-*tert*-butylphenol **1a** (3.06 g, 20.mmol), aldehyde **2** (3.09 g, 20 mmol), phenylboronic acid (2.73 g, 22 mmol), and propanoic acid (7.39 g, 100 mmol) in anydrous benzene (60 mL) was heated under reflux for 20 h (TLC monitoring), with azeotropic removal of water with a Dean–Stark separator. The solvent was evaporated and the residue crystallized on addition of diethyl ether. The white solid was filtered and washed with diethyl ether to yield **3a** (5.47 g, 70%); mp 203–205 °C (ethyl acetate); IR (KBr) 1601, 1531, 1507, 1475, 1439, 1373, 1327, 1306, 1277, 1251, 1183, 1135, 1087, 826, 696 cm^{-1}; ^1H NMR ($CDCl_3$) 1.22 (s, 9H), 3.96 (s, 3H), 6.53 (s, 1H), 6.84 (d, J = 2.6 Hz, 1H), 7.11 (d, J = 8.8 Hz, 1H), 7.37–7.41 (m, 3H), 7.45–7.52 (m, 1H), 7.91 (d, J = 1.5 Hz, 1H), 7.94 (d, J = 1.5 Hz, 1H), 7.94 (s, 1H); ^{13}C NMR ($CDCl_3$) 31.20, 33.39, 34.25, 68.98, 117.92, 119.08, 122.39, 127.33, 127.74, 131.55, 131.89, 134.33, 139.62, 146.18, 146.73, 150.33. Anal. calcd for $C_{21}H_{22}N_3O_4B$: C, 64.47; H, 5.67; N, 10.74; found: C, 64.12; H, 5.62; N, 10.42.

[5-*tert*-Butyl-2-hydroxyphenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4a). To an ice-cooled and stirred solution of **3a** (2.01 g, 5.12 mmol) in tetrahydrofuran (40 mL) was slowly added 36% aqueous solution of hydrogen peroxide (5 mL) and the reaction mixture was left under stirring at room temperature for 2 h (TLC monitoring). Then ethyl acetate was added and the solution was successively washed with water, 40% aqueous sodium hydrogen sulfite and water. The organic layer was dried with anhydrous sodium sulfate, the solvent was evaporated and the solid residue washed with dichloromethane-diethyl ether to afford **4a** (0.80 g, 52%); mp 157–159 °C (methanol–diethyl ether); IR (KBr) 3538 (sharp), 3136 (broad), 2959 (broad), 1541, 1507, 1474, 1392, 1378, 1273 cm^{-1}; ^1H NMR ($CDCl_3$) 1.24 (s, 9H), 3.43 (broad s, 2H), 3.96 (s, 3H), 6.14 (s, 1H), 6.79 (d, J = 8.4 Hz, 1H), 7.14 (d, J = 2.2 Hz, 1H), 7.21 (dd, J = 8.4 Hz, J = 2.2 Hz, 1H), 7.93 (s, 1H); ^{13}C NMR (DMSO-d_6) 31.74, 33.39, 34.08, 62.89, 114.53, 124.23, 125.22, 126.28, 132.08. 138.92, 140.98, 151.76, 154.45.

[3-Cyclopropyl-2-hydroxyphenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4c). A solution of **1c** (2.14 g, 0.016 mol), aldehyde **2** (2.97 g, 0.019 mol), phenylboronic acid (2.37 g, 0.019 mol), and propanoic acid (5.93 g, 0.080 mol) in anhydrous benzene (55 mL) was heated under reflux for 12 h (TLC monitoring), with azeotropic removal of water using a Dean–Stark separator. The solvent was evaporated and ethyl acetate was added to the residue. The organic solution was washed with saturated aqueous sodium hydrogen carbonate solution and with water. The organic phase was dried with anhydrous sodium sulfate and the solvent was evaporated to afford **3c** (5.02 g, 84%, orange foam), which was used for the subsequent reaction without further purification.

To an ice-cooled and stirred solution of **3c** (1.5 g, 4.0 mmol) in tetrahydrofuran (20 mL) was slowly added 36% aqueous solution of hydrogen peroxide (3 mL) and the reaction mixture was left under stirring at room temperature for 3.5 h (TLC monitoring). Ethyl acetate was added and the organic solution was successively washed with water, 40% aqueous sodium hydrogen sulfite and water. The organic layer was dried with anhydrous sodium sulfate, the solvent was evaporated and the solid residue washed with dichloromethane–diethyl ether to give **4c** (0.82 g, 71%); mp 168–170 °C (ethanol–diethyl ether); IR (KBr) 3501 (sharp), 3191 (broad), 1530, 1381 cm^{-1}; ^1H NMR (CD_3OD): 0.62–0.68 (m, 2H), 0.95–1.02 (m, 2H), 1.96–2.07 (m, 1H), 4.06 (s, 3H), 6.32 (s, 1H), 6.87 (apparent t, J = 7.4 Hz, 1H), 6.96 (dd, J = 7.4 Hz, J = 1.5 Hz, 1H), 7.16 (dd, J = 7.4 Hz, J = 1.5 Hz, 1H), 7.98 (s, 1H); ^{13}C NMR (DMSO-d_6) 7.39, 7.56, 9.20, 33.07, 63.71, 119.15, 124.20, 124.60, 126.91, 129.64, 131.51, 138.64, 152.44, 153.74. Anal. calcd for $C_{14}H_{15}N_3O_4$: C, 58.13; H, 5.23; N, 14.53; found: C, 57.99; H, 5.22; N, 14.17.

[5-Cyclopropyl-2-hydroxyphenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4d). This was prepared from **1d** and **2** via **3d** as for **4c**. Compound **3d** (71% yield); mp 107–109 °C (washed with diethyl ether); IR (KBr) 1532, 1372, 1324 cm^{-1}; ^1H NMR ($CDCl_3$) 0.52–0.59 (m, 2H), 0.84–0.92 (m, 2H), 1.72–1.83 (m, 1H), 3.94 (s, 3H), 6.51 (s, 1H), 6.58 (d, J = 2.2 Hz, 1H), 6.98 (dd, J = 8.4 Hz, J = 2.2 Hz, 1H), 7.07 (d, J = 8.4 Hz, 1H), 7.35–7.52 (m, 3H), 7.90 (d, J = 1.5 Hz, 1H), 7.94 (d, J = 1.5 Hz, 1H), 7.95 (s, 1H). Compound **4d** (62% yield); mp 134–135 °C (methanol–dichloromethane); IR (KBr) 3543 (sharp), 3226 (broad), 1538, 1507, 1388 cm^{-1}; ^1H NMR (CD_3OD) 0.59–0.65 (m, 2H), 0.87–0.94 (m, 2H), 1.82–1.93 (m, 1H), 4.03 (s, 3H), 6.25 (s, 1H), 6.72 (d, J = 8.4 Hz, 1H), 6.92 (dd, J = 8.4 Hz, J = 2.2 Hz, 1H), 7.26 (d, J = 2.2 Hz, 1H), 7.95 (s, 1H); ^{13}C NMR (DMSO-d_6) 8.73, 8.78, 14.79, 33.36, 62.55, 114.93, 125.14, 125.37, 126.88, 132.04, 133.58, 138.94, 151.82, 154.33. Anal. calcd for $C_{14}H_{15}N_3O_4$: C, 58.13; H, 5.23; N, 14.53; found: C, 58.06; H, 5.11; N, 14.36.

[5-Chloro-2-hydroxyphenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4e). This was prepared from **1e** and **2** via **3e** as for **4c**. Compound **3e** was obtained in 63% yield and alcohol **4e** in 31% yield from **3e**. Data for **4e**: mp 157–159 °C (methanol–dichloromethane); IR (KBr) 3245 (broad), 1538, 1388 cm^{-1}; ^1H NMR (CD_3OD) 4.09 (s, 3H), 6.21 (s, 1H), 6.78 (d J = 8.4 Hz, 1H), 7.18 (dd, J = 8.4 Hz, J = 2.6 Hz, 1H), 7.58 (d, J = 2.6 Hz, 1H), 7.93 (s, 1H); ^{13}C NMR (DMSO-d_6) 32.99, 61.55, 116.12, 122.32, 126.98, 127.75, 129.20, 131.47, 138.61, 152.49, 153.07. Anal. calcd for $C_{11}H_{10}ClN_3O_4$: C, 46.57; H, 3.55; N, 14.81; found: C, 46.35; H, 3.45; N, 14.41.

[3-Chloro-2-hydroxyphenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4f). This was prepared from **1f** and **2** via **3f** as for **4c**. Compound **3f** was obtained in 85% yield and alcohol **4f** in 51% yield from **3f**. Data

for **4f**: mp 163–165 °C (chloroform); IR (KBr) 3425 (broad), 3124 (broad), 1536, 1382 cm^{-1}; ^1H NMR (CD_3OD) 4.09 (s, 3H), 6.28 (s, 1H), 6.97 (t, J = 8.1 Hz, 1H), 7.34 (dd, J = 8.1 Hz, J = 1.5 Hz, 1H), 7.52 (dd, J = 8.1 Hz, J = 1.5 Hz, 1H), 7.94 (s, 1H); ^{13}C NMR (DMSO-d_6) 33.08, 62.93, 120.04, 120.23, 126.25, 128.44, 130.09, 131.39, 138.72, 149.27, 153.08. Anal. calcd for $C_{11}H_{10}ClN_3O_4$: C, 46.57; H, 3.55; N, 14.81; found: 46.48; H, 3.50; N, 14.71.

[3-(2-Adamantyl)-2-hydroxyphenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4g). See ref 9.

[5-(1-Adamantyl)-2-hydroxyphenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4h). This was prepared from **1h** and **2** via **3h** as for **4c** (0.4 equiv of propanoic acid were used). Compound **3h** was obtained in 84% yield and alcohol **4h** in 88% yield from **3h**. Data for **4h**: mp 192–195 °C; IR (KBr) 3394–3331 (broad), 1538, 1504, 1374 cm^{-1}; ^1H NMR (CD_3OD) 1.76 (apparent s, 6H), 1.86 (apparent s, 6H), 2.02 (apparent s, 3H), 3.93 (s, 3H), 6.20 (s, 1H), 6.68 (d, J = 8.4 Hz, 1H), 7.11 (dd, J = 8.4 Hz, J = 2.6 Hz, 1H), 7.45 (d, J = 2.6 Hz, 1H), 7.87 (s, 1H); ^{13}C NMR (DMSO-d_6) 28.57, 33.27, 35.30, 36.45, 43.20, 62.79, 114.50, 123.78, 124.62, 126.22, 132.03, 138.81, 141.37, 151.71, 154.40. Anal. calcd for $C_{21}H_{25}N_3O_4$: C, 65.78; H, 6.57; N, 10.96; found: C, 65.39; H, 6.28; N, 10.75.

[3-(1-Adamantyl-2-hydroxyphenyl]-[1-methyl-5-nitro-1-*H*-2-imidazolyl]methanol (4i). This was prepared from **1i** and **2** via **3i** as for **4c** (0.4 equiv of propanoic acid were used). Compound **3i** was obtained in 72% yield and alcohol **4i** in 86% yield from **3i**. Data for **4i**: mp 182–184 °C; IR (KBr) 3376 (broad), 1540, 1378 cm^{-1}; ^1H NMR (CD_3OD) 1.68 (apparent s, 6H), 1.98 (apparent s, 3H), 2.03 (apparent s, 6H), 3.92 (s, 3H), 6.12 (s, 1H), 6.76 (t, J = 7.7 Hz, 1H), 7.05 (apparent dd, J = 7.7 Hz, J = 6.2 Hz, 2H), 7.99 (s, 1H); ^{13}C NMR (DMSO-d_6) 28.60, 33.60, 36.63, 36.77, 39.36, 40.27, 66.09, 119.50, 125.86, 126.09, 127.07, 131.54, 137.78, 139.33, 153.21, 154.05. Anal. calcd for $C_{21}H_{25}N_3O_4$: C, 65.78; H, 6.57; N, 10.96; found: C, 65.27; H, 6.37; N, 11.13.

[3-(2-Adamantyl-5-fluoro-2-hydroxyphenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4j). This was prepared from **1j** and **2** via **3j** as for **4c** (0.4 equiv of propanoic acid were used). Compound **3j** was obtained in 73% yield and alcohol **4j** in 88% yield from **3j**. Data for **4j**: mp 176–178 °C; IR (KBr): 3371 (broad), 3208, 1540, 1382 cm^{-1}; ^1H NMR (CD_3OD) 1.64 (apparent d, J = 12.4 Hz, 2H), 1.80–2.03 (m, 10H), 2.29 (apparent d, J = 13.5 Hz, 2H), 3.25 (apparent s, 1H), 4.02 (s, 3H), 6.20 (s, 1H), 6.95 (dd, J_{H-F} = 8.8 Hz, J_{H-H} = 3.3 Hz, 1H), 7.11 (dd, J_{H-F} = 10.6 Hz, J_{H-H} = 3.3 Hz, 1H), 7.90 (s, 1H); ^{13}C NMR (DMSO-d_6) 26.86, 27.24, 29.89, 30.05, 31.94, 32.19, 33.06 (d, J_{C-F} = 2.8 Hz), 37.24, 38.83, 39.21, 42.76, 63.14, 110.81 (d, J_{C-F} = 24.0 Hz), 113.18 (d, J_{C-F} = 23.1 Hz), 129.83 (d, J_{C-F} = 7.4 Hz), 131.44, 134.89 (d, J_{C-F} = 6.5 Hz), 138.70, 147.85 (d, J_{C-F} = 2.8 Hz), 153.09, 155.77 (d, J_{C-F} = 234.0 Hz). Anal. calcd for $C_{21}H_{24}FN_3O_4$: C, 62.83; H, 6.02; N, 10.47; found: C, 62.49; H, 5.76; N, 10.53.

[3-(2-Adamantyl)-5-chloro-2-hydroxyphenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4k). This was prepared from **1k** and **2** via **3k** as for **4c** (0.4 equiv of propanoic acid were used). Compound **3k** was obtained in 66% yield and alcohol **4k** in 57% yield from **3k**. Data for **4k**: mp 165–168 °C; IR (KBr) 3305 (broad), 3139–3051 (broad), 1545, 1379 cm^{-1}; ^1H NMR (CD_3OD) 1.62 (apparent d, J = 12.4 Hz, 2H), 1.77–1.93 (m, 10H), 2.25 (apparent d, J = 10.2 Hz, 2H), 3.21 (apparent s, 1H), 4.00 (s, 3H), 6.15 (s, 1H), 7.16 (d, J = 2.6 Hz, 1H), 7.29 (d, J = 2.6 Hz, 1H), 7.86 (s, 1H); ^{13}C NMR (DMSO-d_6) 26.39, 26.76, 29.39, 29.54, 31.51, 31.74, 32.63, 36.76, 38.35, 38.71, 42.23, 62.61, 122.62, 124.08, 125.80, 129.58, 130.91, 134.44, 138.28, 150.35, 152.52. Anal. calcd for $C_{21}H_{24}ClN_3O_4$: C, 60.36; H, 5.79; N, 10.05; found: C, 59.98; H, 5.66; N, 9.77.

[3-(2-Adamantyl)-2-hydroxy-5-methoxyphenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4l). This was prepared from **1l** and **2** via **3l** as for **4c** (0.4 equiv of propanoic acid were used). Compound **3l** was obtained in 73% yield and alcohol **4l** in 76% yield from **3l**. Data for **4l**: mp 164–167 °C; IR (KBr) 3349, 3134–3099 (broad), 1542, 1380 cm^{-1}; ^1H NMR (CD_3OD) 1.63 (apparent d, J = 12.5 Hz, 2H), 1.80 (apparent s, 2H), 1.95–2.04 (m, 8H), 2.29 (apparent d, J = 10.3 Hz, 2H), 3.24 (apparent s, 1H), 3.72 (s, 3H), 4.00 (s, 3H), 6.21 (s, 1H), 6.75 (d, J = 2.9 Hz, 1H), 6.96 (d, J = 2.9 Hz, 1H), 7.91 (s, 1H); ^{13}C NMR (DMSO-d_6) 27.29, 27.69, 30.42, 30.57, 32.39, 32.66, 33.39, 37.70, 39.33, 39.67, 43.21, 55.33, 64.24, 109.33, 113.64, 129.05, 131.88, 134.65, 139.00, 145.91, 152.55, 153.94. Anal. calcd for $C_{22}H_{27}N_3O_5$: C, 63.91; H, 6.58; N, 10.16; found: C, 64.18; H, 6.36; N, 10.20.

[5-(2-Adamantyl)-2-hydroxyphenyl][1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4m). This was prepared from **1m** and **2** via **3m** as for **4c** (0.4 equiv of propanoic acid were used). Compound **3m** was obtained in 80% yield and **4m** in 81% yield from **3m**. Data for **4m**: mp 173–175 °C; IR (KBr) 3365 (broad), 1538, 1379 cm^{-1}; ^1H NMR (CD_3OD) 1.51 (apparent d, J = 12.1 Hz, 2H), 1.70–2.01 (m, 10H), 2.36 (apparent s, 2H), 2.90 (apparent s, 1H), 3.92 (s, 3H), 6.21 (s, 1H), 6.71 (d, J = 8.4 Hz, 1H), 7.10 (dd, J = 8.4 Hz, J = 2.2 Hz, 1H), 7.40 (d, J = 2.2 Hz, 1H), 7.87 (s, 1H); ^{13}C NMR (DMSO-d_6) 27.33, 27.63, 30.61, 30.64, 31.59, 33.27, 37.61, 38.66, 45.77, 62.74, 114.77, 125.78, 126.47, 126.52, 131.97, 133.84, 138.85, 151.49, 154.37. Anal. calcd for $C_{21}H_{25}N_3O_4$: C, 65.78; H, 6.57; N, 10.96; found: C, 65.38; H, 6.51; N, 10.61.

[3-Cyclohexyl-2-hydroxyphenyl]-[1-methyl-5-nitro-1-*H*-2-imidazolyl]methanol (4n). This was prepared from **1n** and **2** via **3n** as for **4c** (0.4 equiv of propanoic acid were used). Compound **3n** was obtained in 80% yield and alcohol **4n** in 74% yield from **3n**. Data for

4n: foam; IR (KBr) 3297 (broad), 1540, 1376 cm^{-1}; ^1H NMR ($CDCl_3$) 1.24–1.50 (m, 5H), 1.71–1.84 (m, 5H), 2.91–3.01 (m, 1H), 3.92 (s, 3H), 4.85 (s, 1H), 5.95 (s, 1H), 6.68 (dd, J = 7.7 Hz, J = 1.8 Hz, 1H), 6.81 (t, J = 7.7 Hz, 1H), 7.16 (dd, J = 7.7 Hz, J = 1.8 Hz, 1H), 7.81 (s, 1H), 8.56 (s, 1H); ^{13}C NMR ($CDCl_3$) 26.21, 26.92, 33.03, 33.59, 36.80, 68.88, 120.29, 123.08, 124.23, 127.67, 130.44, 136.96, 139.17, 152.20, 152.37. Anal. calcd for $C_{17}H_{21}N_3O_4$: C, 61.62; H, 6.39; N, 12.68; found: C, 61.10; H, 5.95; N, 12.42.

[5-Cyclohexyl-2-hydroxyphenyl]-[1-methyl-5-nitro-1-*H*-2-imidazolyl]methanol (4o). This was prepared from **1o** and **2** via **3o** as for **4c**. Compound **3o** was obtained in 81% yield and alcohol **4o** in 72% yield from **3o.** Data for **4o**: mp 168–170 °C; IR (KBr) 3543 (sharp), 3129, 1538, 1506, 1393, 1377 cm^{-1}; ^1H NMR (CD_3OD) 1.28–1.45 (m, 5H), 1.67–1.77 (m, 5H), 2.31–2.44 (m, 1H), 3.92 (s, 3H), 6.18 (s, 1H), 6.65 (d, J = 8.0 Hz, 1H), 6.95 (dd, J = 8.0 Hz, J = 2.2 Hz, 1H), 7.26 (d, J = 2.2 Hz, 1H), 7.86 (s, 1H); ^{13}C NMR (DMSO-d_6) 25.52, 26.28, 32.94, 34.16, 34.35, 43.02, 62.39, 114.38, 125.42, 126.05, 126.26, 131.63, 137.61, 138.48, 151.65, 153.98. Anal. calcd for $C_{17}H_{21}N_3O_4$: C, 61.62; H, 6.39; N, 12.68; found: C, 61.31; H, 6.00; N, 12.43.

[2-Hydroxy-3-(2-*exo*-norbornyl)phenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4p). This was prepared from **1p** and **2** via **3p** in 49% overall yield as for **4c** (0.4 equiv of propanoic acid were used). Data for **4p**: foam; IR (KBr) 3355 (broad), 1540, 1376 cm^{-1}; ^1H NMR ($CDCl_3$) 1.19–1.63 (m, 7H), 1.82 (ddd, J = 11.3 Hz, J = 9.5 Hz, J = 1.8 Hz, 1H), 2.34 (broad s, 2H), 2.96 (dd, J = 5.8 Hz, J = 5.5 Hz, 1H), 3.85 (s, 3H), 6.09 (s, 1H), 6.56 (d, J = 7.7 Hz, 1H), 6.76 (t, J = 7.7 Hz, 1H), 7.17 (d, J = 7.7 Hz, 1H), 7.69 (s, 1H), 8.39 (broad s, 1H); ^{13}C NMR ($CDCl_3$) 28.96, 30.27, 33.58, 36.09, 36.18, 36.78, 38.28, 38.42, 40.43, 41.08, 41.17, 68.65, 68.69, 119.86, 122.91, 122.95, 124.05, 124.08, 126.82, 130.48, 136.33, 136.41, 139.21, 152.26, 152.79, 152.79, 152.83. Anal. calcd for $C_{18}H_{21}N_3O_4$: C, 62.96; H, 6.16; N, 12.24; found: C, 62.71; H, 6.00; N, 11.78.

[2-Hydroxy-5-(2-*exo*-norbornyl)phenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4q). This was prepared from **1q** and **2** via **3q** in 78% overall yield as for **4c** (0.4 equiv of propanoic acid were used). Data for **4q**: mp 175–178 °C; IR (KBr) 3454–2627 (broad), 3137, 1537, 1503, 1375 cm^{-1}; ^1H NMR (CD_3OD) 1.09–1.35 (m, 3H), 1.48–1.73 (m, 5H), 2.21 (apparent s, 1H), 2.27 (apparent s, 1H), 2.63 (dd, J = 6.6 Hz, J = 6.2 Hz, 1H), 3.93 (s, 3H), 6.18 (s, 1H), 6.65 (d, J = 8.4 Hz, 1H), 6.96 (dd, J = 8.4 Hz, J = 2.2 Hz, 1H), 7.29 (d, J = 2.2 Hz, 1H), 7.86 (s, 1H); ^{13}C NMR (DMSO-d_6) 28.66, 30.14, 33.23, 35.57, 35.64, 36.32, 38.60, 38.70, 43.23, 46.32, 62.59, 114.68, 126.07, 126.17, 126.54, 126.70, 126.76, 131.95, 137.22, 137.25, 138.80, 151.72, 154.30.

[5-Acetyl-3-(2-adamantyl)-2-hydroxyphenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (4r). This was prepared from **1r** and **2** via **3r** as for **4c** (0.4 equiv of propanoic acid were used). The crude mixture containing **4r** was chromatographed through silica gel, eluting with mixtures of dichloromethane-ethyl acetate of increasing polarity. The following fractions were obtained: 3-(2-adamantyl)-4-hydroxyacetophenone, **1r** (39% recovery); **4r** (75% yield based on consumed **1r**); mp 110–113 °C; IR (KBr) 3305–3054 (broad), 1668, 1596, 1543, 1376 cm^{-1}; ^1H NMR (CD_3OD) 1.65 (apparent d, J = 12.4 Hz, 2H), 1.79 (apparent s, 3H), 1.95–1.98 (m, 8H) 2.30 (apparent s, 2H), 2.51 (s, 3H), 4.02 (s, 3H), 5.45 (s, 1H), 6.22 (s, 1H), 7.84 (d, J = 2.2 Hz, 1H), 7.88 (s, 1H), 8.06 (d, J = 2.2 Hz, 1H); ^{13}C NMR (DMSO-d_6) 26.36, 27.26, 27.61, 30.32, 30.44, 32.33, 32.54, 33.47, 37.61, 39.17, 39.51, 42.79, 64.21, 126.79, 127.35, 127.41, 128.35, 131.58, 132.48, 139.11, 153.35, 157.36, 196.65.

(3-Cyclopropyl-2-hydroxylphenyl) (1-methyl-5-nitro-1*H*-2-imidazolyl) ketone (5c). Manganese(IV) oxide (0.41 g, 4.7 mmol) was added to a stirred mixture of **4c** (0.21 g, 7.2 mmol) in chloroform (40 mL). The reaction mixture was left under stirring at room temperature for 20 h (TLC monitoring); then, it was filtered and the solvent from filtrate was evaporated to afford **5c** (0.13 g, 65% yield) as an orange solid, which was washed with diethyl ether. Melting point 196–198 °C; IR (KBr) 3121, 1615, 1540, 1365, 1342 cm^{-1}; ^1H NMR ($CDCl_3$) 0.62–0.68 (m, 2H), 0.93–1.01 (m, 2H), 2.15–2.26 (m, 1H), 4.20 (s, 3H), 6.84 (t, J = 8.1 Hz, 1H), 7.14 (dd, J = 8.1 Hz, J = 1.5 Hz, 1H), 8.05 (dd, J = 8.1 Hz, J = 1.5 Hz, 1H), 8.06 (s, 1H), 12.10 (s, 1H); ^{13}C NMR ($CDCl_3$) 7.28, 9.06, 35.22, 118.19, 118.79, 131.09, 131.23, 132.86, 133.48, 144.66, 163.26, 187.92. Anal. calcd for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63; found: C, 58.63; H, 4.55; N, 14.51.

(3-(2-Adamantyl)-2-hydroxyphenyl)-(1-methyl-5-nitro-1*H*-2-imidazolyl) ketone (5g). This was prepared from **4g** as for **5c**. The crude mixture was chromatographed through a silica gel column under pressure, with dichloromethane as eluent, to give **5g** (75% yield) as a yellow solid; mp 190–192 °C; IR (KBr) 3435 (broad), 3150, 1602, 1541, 1365 cm^{-1}; ^1H NMR ($CDCl_3$) 1.62 (apparent d, J = 11.8 Hz, 2H), 1.75 (apparent s, 2H), 1.85–2.02 (m, 8H), 2.35 (apparent s, 2H), 3.30 (apparent s, 1H), 4.18 (s, 3H), 6.89 (t, J = 8.1 Hz, 1H), 7.71 (d, J = 8.1 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 8.06 (s, 1H), 12.24 (s, 1H); ^{13}C NMR ($CDCl_3$) 27.71, 28.03, 30.68, 32.85, 35.24, 37.87, 39.81, 43.49, 118.31, 118.42, 131.13, 131.29, 134.81, 136.42, 144.82, 163.41, 188.17. Anal. calcd from $C_{21}H_{23}N_3O_4$: C, 66.13; H, 6.08; N, 11.02; found: C, 66.04; H, 5.94; N, 11.21.

***N*-(4-*tert*-Butylphenyl)formamide (12)**. A solution of 4-*tert*-butylaniline, **11**, (1.01 g, 6.77 mmol) in 99% formic acid (2 mL, 2.46 g, 0.053 mol) was heated under reflux for 48 h (TLC monitoring). The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with a saturated aqueous solution of sodium hydrogen

carbonate and with water, it was dried with anhydrous sodium sulfate and the solvent was evaporated to yield **12** (1.02 g, 87%) as a liquid which crystallized spontaneously. Mp 60–62 °C; IR (KBr) 3294, 3262, 1672, 1607 cm^{-1}; ^1H NMR ($CDCl_3$) (mixture of isomers) 1.30 (s, 9H), 1.31 (s, 9H), 7.03 (d, J = 8.8 Hz, 2H), 7.33–7.48 (m, 7H), 8.07 (apparent d, J = 10.2 Hz, 1H), 8.35 (d, J = 1.8 Hz, 1H), 8.65 (d, J = 11.3 Hz, 1H). The same compound was obtained in 90% yield by treating **11** with a mixture of 99% formic acid (3.8 equiv) and acetic anhydride (3.8 equiv) at room temperature for 2 h.

N-methyl-4-*tert*-butylaniline (13). A solution of **12** (1.06 g, 5.99 mmol) in anhydrous diethyl ether (15 mL) was added dropwise under argon atmosphere to a stirred mixture of lithium aluminium hydride (0.30 g, 7.79 mmol) and anhydrous diethyl ether (15 mL). The mixture was refluxed under argon for 4 h (TLC monitoring). Then the solvent was evaporated, ethyl acetate was added to the residue and the organic solution was washed with 15% aqueous solution of sodium hydroxide and with water. The organic layer was dried with anhydrous sodium sulfate and the solvent was evaporated to afford **13** (0.94 g, 97%) as a liquid; IR (film) 3411 cm^{-1}; ^1H NMR ($CDCl_3$) 1.28 (s, 9H), 2.82 (s, 3H), 6.58 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 8.8 Hz, 2H).

[5-*tert*-Butyl-2-methylaminophenyl]-[1-methyl-5-nitro-1*H*-2-imidazolyl]methanol (14). A solution of **13** (0.42 g, 2.58 mmol) in anhydrous benzene (10 mL) was slowly added under argon to an ice-cooled and stirred mixture of 1 M dichloromethane solution of boron trichloride (2.8 mL) and anhydrous benzene (10 mL). A change in the color of the solution was observed and acid vapours were released. The solution was heated under reflux for 3 h, then it was cooled and flushed with a strong flow of argon to eliminate the formed hydrogen chloride. To this stirred and ice-cooled solution was added dropwise a solution of **2** (0.43 g, 2.78 mmol) and triethylamine (0.52 g, 5.15 mmol) in anhydrous benzene (10 mL), and the reaction mixture was left at room temperature for 12 h. Then, it was poured into a mixture of ice and 2 M hydrochloric acid (25 mL) and extracted with ethyl acetate. The organic layer was washed with 2 M hydrochloric acid and with water, it was dried with anhydrous sodium sulfate and the solvent was evaporated to give **14** as a foam. It crystallized on addition of diethyl ether (0.54 g, 65% yield); mp 153–155 °C; IR (KBr) 3410, 3155 (broad), 3023, 1539, 1521, 1373 cm^{-1}; ^1H NMR ($CDCl_3$) 1.19 (s, 9H), 1.57 (broad s, 1H), 2.79 (s, 3H), 3.80 (s, 3H), 4.78 (broad s, 1H), 5.80 (s, 1H), 6.70 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 2.2 Hz, 1H), 7.28 (dd, J = 8.4 Hz, J = 2.2 Hz, 1H), 7.79 (s, 1H); ^{13}C NMR (DMSO-d_6) 29.60, 30.80, 32.83, 65.87, 109.16, 122.48, 122.85, 124.53, 130.63, 137.07, 138.70, 143.89, 152.14. Anal. calcd for $C_{16}H_{22}N_4O_3$: C, 60.36; H, 6.96; N, 17.60; found: 60.35; H, 7.03; N, 17.44.

2-(4-*tert*-Butylphenyliminomethyl)-1-methyl-5-nitroimidazole (15). This was formed in the reaction of **2** with formamide **12** and phenylboronic acid/propionic acid in refluxing xylene following an analogous procedure as described for the preparation of **3a**. Diethyl ether was added to the residue obtained after evaporation of the solvent, a yellow solid being formed; mp 122–124 °C; IR (KBr) 1534, 1362 cm^{-1}; ^1H NMR ($CDCl_3$) 1.31 (s, 9H), 4.51 (s, 3H), 7.21 (d, J = 8.8 Hz, 2H), 7.43 (d, J = 8.8 Hz, 2H), 8.08 (s, 1H), 8.57 (s, 1H).

Determination of antimicrobial activity

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.

Salmonella typhimurium reverse mutation assay (Ames test)

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.

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