2-Nitroimidazole Dual-Function Bioreductive Drugs: Studies on the Effects of Regioisomerism and Side-Chain Structural Modifications on Differential Cytotoxicity and Radiosensitization by Aziridinyl and Oxiranyl Derivatives

Matthew A. Naylor,* Michael D. Threadgill,† Paul Webb, Ian J. Stratford, Miriam A. Stephens, E. Martin Fielden, and Gerald E. Adams

MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 ORD, United Kingdom, and School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom. *Received March 12, 1992*

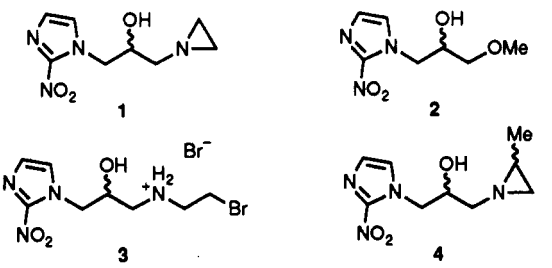
A series of 2-nitroimidazoles bearing side chains terminating in or containing aziridinyl and oxiranyl groups has been prepared, and the compounds were evaluated in vitro as hypoxia-selective bioreductively-activated cytotoxins and selected compounds tested for their radiosensitizing properties toward hypoxic mammalian cells. Compounds were either the regioisomers of analogues of the potent dual-functional 2-nitroimidiazole α-[(1-aziridinyl)-methyl]-2-nitro-1*H*-imidazole-1-ethanol (RSU-1069, 1) with additional methyl groups or related oxiranes of varying side-chain length and type. Oxiranyl derivatives showed little differential toxicity, and those tested were less effective as radiosensitizers, and although these properties were influenced by side-chain length, differences were not great. Aziridinyl compounds related to 1 but with increased side-chain lengths were unstable. Methylation of 1 in various regions had little effect on radiosensitization and no clear advantages over 1 as differential cytotoxic drugs. Progressive methylation at C-3 was found to increase toxicity but decrease hypoxia selectivity. Incorporation of a cyclohexane side chain in 1,2-*cis*-2,3-*trans*-3-aziridin-1-yl-2-hydroxy-1-(2-nitroimidazol-1-yl)cyclohexane (**26**) abolished hypoxia-selective toxicity and unexpectedly reduced radiosensitizing efficiency. Of the aziridines, 1-(2-nitro-1-imidazolyl)-2-methyl-3-(1-aziridinyl)-2-propanol (**20**) was comparable in efficacy to 1 as a bioreductively-activated cytotoxin with slightly lower aerobic toxicity; however, the prodrugs of 1 remain as preferred candidates for clinical evaluation.

Introduction

α-[(1-Aziridinyl)methyl]-2-nitro-1*H*-imidazole-1-ethanol (RSU-1069, 1) is the most effective of a large number of nitroheterocyclic compounds that have been synthesized and evaluated as radiosensitizers and bioreductively-activated cytotoxins.[1-3] Compounds of this type owe their selective cytotoxicity toward hypoxic cells within tumors to their bioreductive properties. Activation in vivo is by anaerobic enzymatic reduction to toxic metabolites.[4] The radiosensitizing activity of 2-nitroimidazoles is via fast free-radical mechanisms and is related to their one-electron reduction potentials.[5]

Compound (1) is an alkylating analogue of misonidazole (2)—a drug which failed clinically due to neurotoxicity.[6,7] Preliminary clinical investigation of 1 has, however, revealed gastrointestinal toxicity, which is severely dose-limiting.[8] A large number of other nitroheterocyclic moieties have been evaluated as carriers of aziridinyl and other alkylating functionalities; however, these have failed to produce comparable efficacy to the 2-nitroimidazoles as hypoxia-selective cytotoxins and any increase in radiosensitizing potency has generally been accompanied by higher toxicity.[9-12] Other approaches have been to reduce the observed toxicity of 1, and this has been achieved most effectively with the prodrug α-[[(2-bromoethyl)amino]-methyl]-2-nitro-1*H*-imidazole-1-ethanol (RB6145, **3**), which is the current candidate for clinical evaluation.[13,14] With the exception of substitution of the aziridine ring, which results in lower toxicity, but also a reduction in selective bioreductive differential toxicity, there have been no reports of 2-nitroimidazoles closely related to 1 or its substituted analogues such as α-[(2-methylaziridinyl)-methyl]-2-nitro-1*H*-imidazole-1-ethanol (RSU-1131, **4**).[2] We therefore present here the synthesis and evaluation of two classes of potentially alkylating 2-nitroimidazoles. (i) Aziridines: Regioisomers of **4** in which methyl substitution of the propyl side chain has been carried out are of interest to evaluate the steric influence of methyl substitution, which might be expected to influence reductase enzyme recognition and/or DNA binding. Such substituents may also be expected to influence the pK_a and consequent reactivity of the aziridine moiety. A close analogue of 1

Chart I



(1) Adams, G. E.; Ahmed, I.; Sheldon, P. W.; Stratford, I. J. Radiation sensitization and chemopotentiation: RSU-1069 a compound more efficient than misonidazole in vitro and in vivo. *Br. J. Cancer* **1984**, *49*, 571-577.
(2) O'Neill, P.; Jenkins, T. C.; Stratford, I. J.; Silver, A. R. J.; Ahmed, I.; McNeil, S. S.; Fielden, E. M.; Adams, G. E. Mechanism of action of some bioreducible 2-nitroimidazoles: comparison of in vitro cytotoxicity and ability to induce DNA strand breakage. *Anti-Cancer Drug Des.* **1987**, *1*, 271-280.
(3) Adams, G. E.; Ahmed, I.; Sheldon, P. W.; Stratford, I. J. RSU-1069, a 2-nitroimidazole containing an alkylating group: high efficiency as a radio- and chemosensitizer in vitro and in vivo. *Int. J. Radiat. Oncol. Biol. Phys.* **1984**, *10*, 1653-1656.
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(5) Adams, G. E.; Flockhart, I. R.; Smithen, C. E.; Stratford, I. J.; Wardman, P.; Watts, M. E. Electron-affinic sensitization. VII. A correlation between structures, one-electron reduction potentials and efficiencies of nitroimidazoles as hypoxic cell radiosensitizers. *Radiation Res.* **1976**, *67*, 9-20.
(6) Asquith, J. C.; Watts, M. E.; Patel, K.; Smithen, C. E.; Adams, G. E. Electron-affinic sensitization. V. Radiosensitization of hypoxic bacteria and mammalian cells in vitro by some nitroimidazoles and nitropyrazoles. *Radiat. Res.* **1974**, *60*, 108-118.
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† University of Bath.

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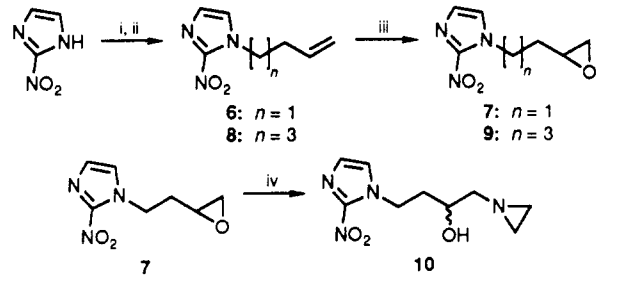
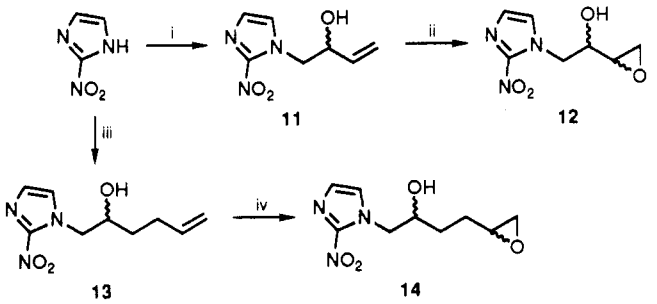
Scheme I[a]



[a] Reagents: (i) Bu^{t}OK/DMF, (ii) $Br(CH_2)_nCH_2CH{=}CH_2$, (iii) MCPBA/CH_2Cl_2, (iv) aziridine/EtOH.

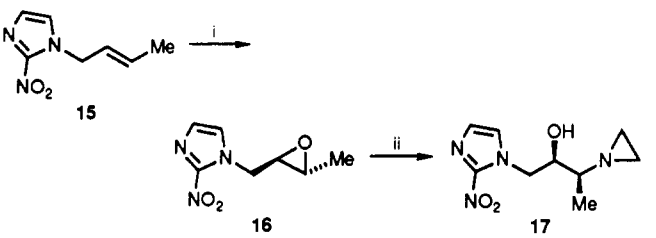
Scheme II[a]



[a] Reagents: (i) ethenyloxirane/DMF, (ii) MCPBA/CH_2Cl_2, (iii) 4-butenyloxirane, (iv) MCPBA/$CHCl_3$.

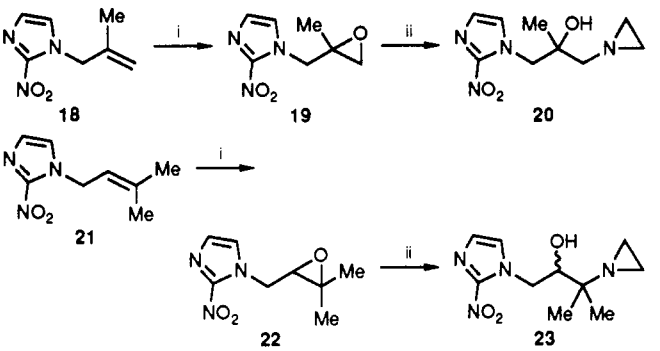
in which the 1-aziridinyl and 2-hydroxy groups are fixed in an antiperiplanar (trans-diaxial) conformation has also been synthesized by utilizing a cyclohexyl side chain in order to gain further information on structure–activity within this series, in particular steric and conformational effects. (ii) Oxiranes: One further way of moderating the toxicity of such alkylating 2-nitroimidazoles is to introduce

Scheme III[a]



[a] Relative stereochemistry only is shown. Reagents: (i) MCPBA/CH_2Cl_2, (ii) aziridine/EtOH/Et_3N.

Scheme IV[a]



[a] Reagents: (i) MCPBA/CH_2Cl_2, (ii) aziridine/EtOH/Et_3N.

a somewhat less reactive alkylating function. There have been no data published to date on differential toxicities of corresponding oxiranes although some radiosensitization data is available,[15] and we have therefore synthesized and evaluated a number of closely related and previously unreported oxiranyl analogues of varying side-chain lengths, and including the desoxy derivatives, which have been studied in view of recent reports that a hydroxyl group β to the imidazole is not required for activity in this class of bioreductive agent.[16,17]

Synthetic Chemistry

All compounds were synthesized from the common precursor 2-nitroimidazole (azomycin), which was available commercially in bulk quantity. This material was best utilized as its potassium salt in DMF, often in the presence of a crown ether. The alkenylnitroimidazoles **6** and **8** were thus synthesized from appropriate bromoalkenes in DMF with 18-crown-6 (Scheme I), whereas the 2-hydroxyalkenyl derivatives **11** and **13** were synthesized from 2-nitroimidazole and the appropriate epoxyalkene in refluxing DMF (Scheme II).

Reaction of oxirane **7** with refluxing aziridine in ethanol proceeded to give the chain-extended version of **1**, but this compound (**10**) was too unstable to isolate in a satisfactorily pure form for biological evaluation. Oxiranes **9**, **12**, and **14** gave only complex darkened mixtures under these

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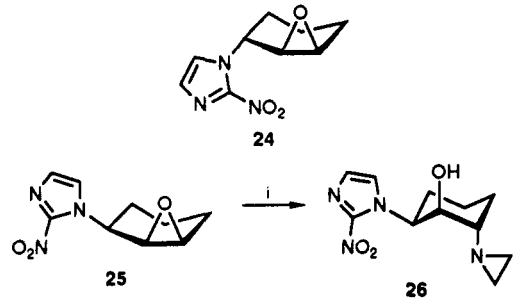
Scheme V[a]



[a] Reagents: (i) aziridine/EtOH/Et_3N.

and a variety of other conditions in the presence of aziridine.

Regioisomers of 4 and derivatives with further methyl substitutions in the hydroxypropyl side chain were synthesized via appropriately substituted oxiranes, which were themselves target compounds. Thus, as for the above compounds, appropriately substituted bromoalkenes were treated with potassium 2-nitroimidazole in DMF, and the resulting alkenes epoxidized with MCPBA to give the oxiranes **16**, **19**, and **22** (Schemes III and IV). Oxirane **16** was shown by NMR to be a mixture of isomers (approximately 10:1 trans:cis) corresponding to the isomeric content of the starting material. Oxiranyl synthons **16**, **19**, and **22** reacted cleanly with aziridine in refluxing ethanol containing 1% triethylamine to afford aziridines **17**, **20**, and **23**. Some selectivity was evident in the reaction of aziridine with the 2,3-disubstituted oxirane **16** in that **17** comprised an inseparable mixture of *erythro* and *threo* diastereoisomers in the ratio 4.5:1, as shown by ^1H NMR. Assignments and connectivity were established by a COSY experiment; the spectroscopic dispersion was sufficient at 250 MHz to permit measurement of most coupling constants for both stereoisomers. Karplus analysis of these data suggested that, in the predominant conformation for both *erythro*-**17** and *threo*-**17**, the aziridinyl group was gauche to the OH, in contrast to the conformation of compound **26** below.

The correct regioisomeric identity of compound **23** was established by the observation of a 6.2-Hz doublet for the OH proton in the 270-MHz NMR spectrum. This shows that the alcohol is secondary rather than tertiary and that the structure is as shown in Scheme IV rather than the regioisomer. Thus, it is demonstrated that the aziridine nucleophile has attacked the oxirane **22** at the more substituted carbon atom under basic conditions, in contrast to the regioselectivity usually observed for nucleophilic attack on substituted oxiranes. This regioselectivity may be the result of an electronic effect of the neighboring 2-nitroimidazole moiety, favoring ring opening at the distal epoxide carbon. The (2,3-epoxycyclohexyl)-2-nitroimidazoles **24** and **25** were synthesized as previously described.[18] The diastereoisomer **24** could be isolated in pure form for biological evaluation by virtue of its unreactivity toward nucleophilic attack by aziridine. Epoxide **25** reacts to give the corresponding aziridinyl derivative **26** (Scheme V).

Biological Evaluation in Vitro

Selective toxicity to hypoxic V79-379A cells was determined for all compounds using the MTT assay as has been described elsewhere.[12,19] These results are presented in Tables I and II, where C_{50}(air), the concentration required to kill 50% of the aerobic cells under the conditions of the assay, are divided by $C_{50}(N_2)$ values, concentrations required to kill hypoxic cells, to give differential toxicity ratios, which enable quantitative comparisons of bioreductive activities of drugs.

Table I. Biological Data for Oxiranyl 2-Nitroimidazoles

compd no.	$C_{1.6}$ (mM)[a]	C_{50}(air) (mM)[b]	$C_{50}(N_2)$ (mM)[c]	ratio[d]
5	0.25[e]	0.55	0.27	2.0
7	nd[f]	0.5	0.25	2.0
9	nd	1.2	0.7	1.7
12	0.2	1.2	0.36	3.3
14	0.4	0.6	0.4	1.5
16	nd	10.0	3.3	3.0
19	nd	0.2	0.2	1.0
24	nd	0.28	0.18	1.5

[a] Concentration required to give enhancement ratio = 1.6. [b] Concentration required to kill 50% of aerobic cells. [c] Concentration required to kill 50% of cells under N_2. [d] C_{50}(air)/$C_{50}(N_2)$. [e] Data taken from ref 15. [f] Not determined.

Table II. Biological Data for Aziridinyl 2-Nitroimidazoles

compd no.	$C_{1.6}$ (mM)[a]	C_{50}(air) (mM)[b]	$C_{50}(N_2)$ (mM)[c]	ratio[d]
1	0.1	0.3	0.015	20.0
4	0.08	0.79	0.065	12.2
17	0.12	0.2	0.01	20.0
20	nd[e]	0.55	0.02	27.5
23	0.07	0.1	0.008	12.5
26	0.45	0.35	0.35	1.0

[a] Concentration required to give enhancement ratio = 1.6. [b] Concentration required to kill 50% of aerobic cells. [c] Concentration required to kill 50% of cells under N_2. [d] C_{50}(air)/$C_{50}(N_2)$. [e] Not determined.

The oxiranyl derivatives **5**, **7**, **9**, **12**, **14**, **16**, **19**, and **24** showed only small additional toxicities as a result of bioreduction, and were indeed less effective than **2**. The high aerobic toxicity of oxiranes has also limited the bioreductive potential of other nitroheterocyclic compounds such as pyrroles and furans.[10,12] Those compounds that do not show high aerobic toxicity, such as **12** and **16**, are therefore likely to be limited in hypoxia selectivity by the reduced alkylating potential of the oxirane ring compared to the aziridine ring of their close analogues **1** and **17**, under similar conditions. Of the oxiranes most closely related to **1**, the four-carbon side chain of **12** resulted in greater differential cytotoxicity than a six-carbon side chain (**14**), although both differential values were low. A similar comparison can be made between compounds **7** and **9**. A side-chain length study of aziridinyl derivatives was not possible due to the instability of chain-extended versions of **1**, but these results indicate that elongation of the side chain does not improve the bioreductive potential. In contrast, studies on 2-nitroimidazoles with varying N-1 side-chain lengths terminating in morpholine, piperidine, and pyrrolidine showed that changes in sensitizing efficiency and, in some cases, cytotoxicity can occur.[20]

Aziridinylnitroimidazoles **17**, **20**, and **23** displayed differential cytotoxicities in the range 15–25; these figures are considered insignificantly different from values obtained

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for 1. Methyl substitution of the hydroxypropyl side chain therefore has little influence on bioreductive activity. Only 23 shows a more significant drop in activity, but more notable is the increase in aerobic toxicity in this example. Indeed the results for 1, 17, and 23 indicate progressive methylation at C-3 of the side chain increases toxicity in air and decreases hypoxia selectivity as a result. This could be due to progressive increases in the aziridine pK_a and hence increases in its reactivity to impractical levels. Consequently compound 23 has similar differential toxicity to the derivative containing a substituted aziridine (4), although this compound is known to contain a less potent alkylating group. All bioreductive activity was abolished when the side-chain stereochemistry was fixed in the trans-diaxial conformation of a cyclohexane substituent in compound 26. Aerobic toxicity was at a level attributable to the aziridine ring, with no additional toxicity under hypoxic conditions. Stereochemical and steric constraints on reductase enzyme recognition and binding are therefore in evidence and a cyclohexane skeleton will not be tolerated.

Aziridinyl-2-nitroimidazoles 1, 4, 17, 23, and 26, together with oxiranyl derivatives 12 and 14, were assayed for their ability to sensitize hypoxic V79 cells in vitro to γ-radiation, carried out as described previously.[12,13] The results are expressed in Tables I and II as $C_{1.6}$ values, the concentration of nitroimidazole required to give an enhancement ratio of 1.6.

The two oxiranyl derivatives evaluated were the compounds most closely related to 1 and containing a β-hydroxy substituent on the side chain. There was a 2-fold difference in these compounds sensitizing efficiencies, with 12, containing a 4-carbon side chain, being the more effective. This indicates that the chain length for maximum potency as a dual-function sensitizer may be quite small (four or five carbon atoms).

There was little effect on sensitizing efficiency within the series of aziridinyl derivatives upon methyl substitution of either the side chain or aziridine ring, although a similar small enhancement in sensitization was observed with compounds 4 and 23. Increases in aziridine pK_a as a result of these substitutions therefore may not be significant enough to improve tumor uptake, as has been observed with the more basic 2-nitroimidazoles including those bearing more substituted aziridines.[21] In contrast, the cyclohexyl derivative 26 was markedly less potent than its acyclic analogues. The contribution of the aziridine function toward radiosensitization has therefore been much reduced in 26 and its potency is even lower than that of non-alkylating nitroimidazoles such as 2. This is unexpected in view of the normal contribution the aziridine moiety exhibits toward aerobic toxicity in the bioreductive assay. The activity of 23 indicates that steric crowding at C-3 of the side chain is tolerated; hence the inactivity of 26 must be conformational in origin, not steric.

Conclusions

Although reduction of the alkylating potency of 1 by methyl substitution of the aziridine retains significant bioreductively-activated cytotoxicity, this is not the case for corresponding oxiranes. These derivatives do not have the alkylating potential required for bifunctional electrophilicity under hypoxic conditions and show little promise for further development.

Single methyl substitutions of the hydroxypropyl side chain of 1 has little effect on its activity, although aerobic toxicity was influenced by progressive C-3 methylations. No obvious advantages over 1 were apparent with these derivatives although 20 was slightly less toxic to aerobic cells and may be worthy of further study in vivo. Major structural modification of the side chain, for example with the cyclohexane ring of 26, abolishes activity, presumably by inhibiting bioreducibility. The prodrug 3 remains the candidate of this series of 2-nitroimidazoles for Phase I clinical evaluation.

Experimental Section

NMR spectra were obtained at 60 MHz with JEOL PMX60SI and at 270 MHz with JEOL GX270 spectrometers, using $SiMe_4$ as internal standard. Mass spectra were obtained with a VG 7070 spectrometer in either the electron impact (EI) mode at 70 eV or in the chemical ionizatioin mode (CI) with 2-methylpropane. Melting points are uncorrected. Elemental analyses were determined by Butterworth Laboratories Ltd., Teddington, Middlesex, U.K. Solutions in organic solvents were dried by treatment with Na_2SO_4 and filtration. Solvents were removed by evaporation under reduced pressure. Dichloromethane (CH_2Cl_2) was dried over calcium chloride and passed through neutral alumina prior to use. Dimethylformamide (DMF) and acetonitrile (CH_3CN) were anhydrous commercial grades. Silica gel radial chromatography was carried out on a Chromatotron (TC Research, Norwich, U.K.). 3-Chloroperbenzoic acid (MCPBA) either was the commercial grade (55%) or was purified by treatment with phosphate buffer (pH 7.0) and dried over P_2O_5 to obtain material of approximate 85% purity. 1-Potassio-2-nitroimidazole was obtained by treatment of azomycin with 1.1 equiv of potassium *tert*-butoxide (*t*-BuOK) in refluxing DMF, the mixture cooled, and the solid salt washed with CH_2Cl_2. Oxirane 5 was synthesized as described previously.[22] Compounds were racemic unless otherwise stated. Where elemental analyses and/or accurate mass spectra are not given due to compound form, instability, or lack of molecular ion, other mass spectral and NMR data were satisfactory and compounds were chromatographically homogeneous.

1-(3,4-Epoxybutyl)-2-nitroimidazole (7). 1-Potassio-2-nitroimidazole (3.03 g, 20 mmol) in dry CH_3CN (50 mL) was stirred with 4-bromo-1-butene (10 mL, 98.5 mmol) in the presence of 18-crown-6 (0.12 g, 0.45 mmol) for 16 days. The suspension was evaporated to dryness, redissolved in $CHCl_3$ (200 mL), and washed with H_2O (3 × 200 mL). The solution was then evaporated to dryness to afford 3.32 g (99%) of 6 as a pale yellow oil: NMR ($CDCl_3$) δ 2.55 (q, 2 H, J = 7.5 Hz, Im-CH_2CH_2CH=CH_2), 4.5 (t, 2 H, J = 7.5 Hz, Im-CH_2CH_2CH=CH_2), 5.3 (m, 3 H, 3 × olefinic-H), 7.1 (s, 1 H, Im-H), 7.25 (s, 1 H, Im-H). This material (3.32 g, 19.9 mmol) was dissolved in CH_2Cl_2 (100 mL) and MCPBA (55%, 7.3 g, 23.3 mol) added. The solution was heated under gentle reflux for 6 h, cooled, diluted with CH_2Cl_2 (100 mL), and washed with 10% Na_2SO_3(aq) (3 × 150 mL), H_2O (2 × 150 mL) and saturated $NaHCO_3$(aq) (2 × 150 mL). The solution was dried and evaporated, and the residue was purified on silica gel eluting with $CHCl_3$, to give 1.63 g (44.8%) of 1-(3,4-epoxybutyl)-2-nitroimidazole (7): mp 37–39 °C; NMR ($CDCl_3$) δ 2.0 (q, 2 H, J = 7.5 Hz, Im-CH_2CH_2), 2.4 (dd, 1 H, J = 3 and 4.5 Hz) and 2.9 (t, 1 H, J = 4.5 Hz) oxiranyl CH_2, 3.1 (m, 1 H, oxiranyl-H), 4.5 (t, 2 H, J = 7.5 Hz, Im-CH_2), 7.1 (s, 1 H, Im-H), 7.2 (s, 1 H, Im-H). Anal. ($C_7H_9N_3O_3$) C, H, N.

1-(5,6-Epoxyhexyl)-2-nitroimidazole (9). 1-Potassio-2-nitroimidazole (1.51 g, 10 mmol) in dry CH_3CN (50 mL) was stirred with 6-bromo-1-hexene (1.9 mL, 14 mmol) for 8 h in the presence of 18-crown-6 (0.1 g, 0.38 mmol). The solution was then heated under reflux for 12 h, cooled, and evaporated to dryness. The residue was redissolved in EtOAc (250 mL) and washed with H_2O (3 × 150 mL) and saturated NaCl(aq) (150 mL), dried, and evaporated to give 1.76 g (90%) of 1-(5-hexenyl)-2-nitroimidazole (8) as a yellow oil: NMR ($CDCl_3$) δ 1.8 (m, 6 H, Im-$CH_2(CH_3)_3$),

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4.5 (t, 2 H, J = 3.6 Hz, Im-CH_2), 5.25 (m, 3 H, CH=CH_2), 7.2 (br, 2 H, 2 × Im-H). This material (1.76 g, 9.0 mmol) was dissolved in CH_2Cl_2 (100 mL) and stirred with MCPBA (55%, 4.14 g, 13.2 mmol) at 25 °C for 6 h. A further 1.7 g (5.4 mmol) of MCPBA was then added and stirring continued for 16 h. The solution was diluted with CH_2Cl_2 (150 mL) and washed with 10% Na_2SO_3(aq) (3 × 150 mL), H_2O (2 × 150 mL), and saturated $NaHCO_3$(aq) (2 × 150 mL), dried, and evaporated. The residue was purified on silica, eluting with EtOAc, to afford 1.5 g (79%) of **9** as a pale yellow solid, recrystallized from aqueous ethanol: mp 29–31 °C; NMR ($CDCl_3$) δ 1.8 (m, 6 H, Im-$CH_2(CH_2)_3$), 2.5 (dd, 1 H, J = 3.6 and 4.8 Hz) and 2.9 (t, 1 H, J = 4.8 Hz), oxiranyl 6-CH_2, 3.1 (m, 1 H, oxiranyl 5-CH), 4.6 (t, 2 H, J = 7.5 Hz, Im-CH_2), 7.2 (s, 1 H, Im-H), 7.3 (s, 1 H, Im-H). Anal. ($C_9H_{13}N_3O_3$·0.25H_2O) C, H, N.

1-(2-Hydroxy-3,4-epoxybutyl)-2-nitroimidazole (12). 2-Nitroimidazole (3.0 g, 26.5 mmol) was dissolved in 10 mL of DMF and refluxed with 10 g (142 mmol) of butadiene monoxide and 0.2 g (1.4 mmol) of K_2CO_3. After 20 min the DMF was removed and the residue redissolved in H_2O (100 mL) and extracted with Et_2O (3 × 100 mL). The combined ether extracts were evaporated, and the residue was recrystallized from EtOAc/$CHCl_3$ to afford 2.5 g (52%) of 1-(2-hydroxy-3-butenyl)-2-nitroimidazole (**11**): mp 92–93 °C (lit.[23] mp 90–92 °C). This material (0.5 g, 2.7 mmol) was dissolved in 10 mL of dry CH_2Cl_2 together with 1.0 g (85%, 4.9 mmol) of MCPBA and the solution heated under reflux for 1.5 h. The solution was then cooled and evaporated, and the residue was purified on silica (eluting with EtOAc) to give 0.35 g (65%) of **12** as a pale yellow solid, recrystallized from EtOH: mp 131–132 °C (lit.[23] mp 134–136 °C); NMR ($(CH_3)_2SO$) δ 2.1 (t, 1 H, J = 2.4 Hz) and 2.7 (dd, 1 H, J = 2.4 and 4.8 Hz), oxiranyl 4-CH_2, 3.0 (m, 1 H, oxiranyl 3-CH), 3.9 (m, 2 H, C*H*O*H*), 4.8 (dd, 2 H, J = 3.6 and 7.5 Hz, Im-CH_2), 7.0 (s, 1 H, Im-H), 7.3 (s, 1 H, Im-H).

1-(2-Hydroxy-5,6-epoxyhexyl)-2-nitroimidazole (14). 2-Nitroimidazaole (3.46 g, 30.6 mmol) and Na_2CO_3 (0.72 g, 6.8 mmol) were heated under reflux in DMF (35 mL) with 1,2-epoxy-5-hexene (3.75 mL, 33.2 mmol) for 15 min with stirring. The solution was then poured into EtOH (100 mL) and filtered, and the solid material was washed with EtOH (150 mL). The filtrate was evaporated to dryness and the residue redissolved in EtOAc (250 mL) and washed with H_2O (4 × 200 mL) and saturated NaCl(aq) (200 mL), dried, and evaporated. The residue was purified on silica, eluting with EtOAc, to afford 2.42 g (37.5%) of 1-(2-hydroxy-5-hexenyl)-3-nitroimidazole as a yellow solid (**13**): mp 81–83 °C; NMR ($CDCl_3$) δ 1.8 (dt, 2 H, J = 1.5 and 7.5 Hz, CH_2=$CHCH_2CH_2$), 2.4 (dt, 2 H, J = 2.4 and 7.5 Hz, CH_2=$CHCH_2CH_2$), 4.2 (br, 2 H, J = ca. 10 Hz, Im-CH_2), 4.35 (m, 2 H, C*H*O*H*), 5.7 (m, 3 H, CH_2=C*H*), 7.0 (s, 1 H, Im-H), 7.2 (s, 1 H, Im-H).

The above material (1.06 g, 5.3 mmol) was dissolved in $CHCl_3$ (100 mL) and 55% MCPBA (3.6 g, 11.5 mmol) added. The solution was stirred for 1 h at 25 °C and then heated under reflux for 1.5 h, cooled, and evaporated. The residue was purified on silica, eluting with EtOAc, to afford 0.83 g (73%) of 1-(2-hydroxy-5,6-epoxyhexyl)-2-nitroimidazole (**14**): mp 76–78 °C; NMR ($CDCl_3$) δ 1.8 (m, 4 H, CH_2CH_2-oxirane), 2.5 (dd, 1 H, J = 2.4 and 4.8 Hz) and 2.8 (t, 1 H, J = 4.8 Hz), oxirane 6-CH_2, 3.0 (m, 1 H, oxirane 5-CH), 4.35 (m, 4 H, Im-CH_2CH(O*H*)), 7.0 (s, 1 H, Im-H), 7.2 (s, 1 H, Im-H); CIMS, m/z = 228 (M + H).

***erythro*-1-(3-Aziridin-1-yl-2-hydroxybutyl)-2-nitroimidazole (17).** 1-Potassio-2-nitroimidazole (2.26 g, 15 mmol) and 1-bromo-2-butene (2 mL, 19.4 mmol) were refluxed in 20 mL of DMF for 5 min, cooled, poured into EtOH (100 mL), and filtered. The solid material was washed with hot EtOH (100 mL) and the filtrate evaporated, dried, and redissolved in CH_2Cl_2 (200 mL) and washed with H_2O (4 × 250 mL) and saturated NaCl(aq) (250 mL). The solution was dried and evaporated, and the residue was treated with activated charcoal (MeOH, reflux, 5 min), filtered, and evaporated to dryness to give 2.05 g (61.4%) of 1-but-2-enyl-2-nitroimidazole (**15**) as a yellow oil, which was used without further purification: NMR ($CDCl_3$) δ 1.8 (d, 3 H, J = 3.6 Hz, CH=CHC*H*$_3$), 5.1 (br d, 2 H, J = 4.8 Hz, Im-CH_2), 5.8 (m, 2 H, CH=CH), 7.15 (s, 1 H, Im-H), 7.2 (s, 1 H, Im-H).

The alkene **15** (0.84 g, 5.0 mmol) was dissolved in CH_2Cl_2 (50 mL) and MCPBA (55%, 1.7 g, 5.5 mmol) added. The solution was heated under reflux for 6 h, cooled, and diluted with CH_2Cl_2 (100 mL). The organic material was washed with 10% Na_2SO_3(aq) (2 × 100 mL), saturated $NaHCO_3$(aq) (2 × 150 mL), H_2O (2 × 150 mL), and saturated NaCl(aq) (200 mL), dried, and evaporated. The residue was purified by silica gel radial chromatography, eluting with CH_2Cl_2, to give 0.47 g (51.1%) of 1-(2,3-epoxybutyl)-2-nitroimidazole (**16**) as a pale yellow gum: NMR ($CDCl_3$) δ 1.3 (d, 3 H, J = 4.8 Hz, oxirane 3-CH_3), 2.8 (dd, 1 H, J = 2.4 and 4.8 Hz, oxirane 3-CH), 3.1 (m, 1 H, oxirane, 2-CH), 4.2 (dd, 1 H, J = 7.5 and 14.4 Hz), and 4.9 (dd, 1 H, J = 3.6 and 14.4 Hz), Im-CH_2, 7.1 (s, 1 H, Im-H), 7.15 (s, 1 H, Im-H); EIMS 183.0635 (calcd for $C_7H_9N_3O_3$ 183.0644).

The oxiranyl synthon **16** (0.46 g, 2.5 mmol) was dissolved in EtOH/1% Et_3N (20 mL) and heated under reflux with 1*H*-aziridine (**CAUTION!**) (2.0 g, 46.5 mmol) for 8 h. The solution was evaporated and the residue purified on silica (eluting with $CHCl_3$/MeOH/Et_3N, 98:1:1) to give, after recrystallization from EtOH (1% Et_3N), 0.46 g (81%) of **17** as an off-white solid: mp 113–114 °C; NMR ($CDCl_3$/$(CD_3)SO$) δ 1.3 (s, 3 H, CH(C*H*$_3$)), 1.35 (dd, 2 H, J = 2 and 4 Hz, aziridine-CH_2), 1.8 (dd, 2 H, J = 2 and 4 Hz, aziridine-CH_2), 2.6 (m, 1 H, C*H*(CH$_3$)), 4.0 (m, 1 H, C*H*(OH)), 4.4 (dd, 1 H, J = 9.6 and 19.2 Hz) and 4.8 (dd, 1 H, J = 2.4 and 12 Hz), Im-CH_2, 7.1 (s, 1 H, Im-H), 7.3 (s, 1 H, Im-H). *erythro*-**17**: NMR ($CDCl_3$, 250 MHz) δ 1.17 (d, 3 H, J = 7 Hz, CH_3), 1.31 (ca. q, 1 H, J = ca. 4 Hz) and 1.37 (ca, q, 1 H, J = ca. 4 Hz), 1.67 (ca. q, 1 H, J = ca. 4 Hz) and 1.69 (ca, q, 1 H, J = ca. 4 Hz), aziridine-H_4, 1.54 (dq, 1 H, J = 3 and 7 Hz, C*H*CH$_3$), 3.7 (br 1 H, OH), 4.03 (dd, 1 H, J = 3 and 10 Hz, CHOH), 4.08 (dd, 1 H, J = 10 and 13 Hz) and 4.72 (d, 1 H, J = 13 Hz), imidazole-CH_2, 7.14 (s, 1 H, Im-4-H), 7.29 (s, 1 H, Im-5-H). *threo*-**17**: NMR δ 1.22 (d, 3 H, J = 7 Hz, CH_3), 1.37 (m, 1 H) and 1.40 (m, 1 H) and 1.90 (ca. q, 1 H, J = ca. 4 Hz) and 1.92 (ca. q, 1 H, J = ca. 4 Hz), aziridine-H_4, 1.66 (dq, 1 H, J = 6 and 7 Hz, C*H*CH$_3$), 3.6 (br, OH), 3.91 (ddd, 1 H, J = 2.5 and 6 and 10 Hz, C*H*OH), 4.26 (dd, 1 H, J = 10 and 14 Hz) and 4.87 (dd, 1 H, J = 2.5 and 14 Hz), imidazole-CH_2, 7.13 (br s, 1 H, Im-4-H), 7.27 (br s, 1 H, Im-5-H); CIMS m/z = 227 (M + H), 197, 180. Anal. ($C_9H_{14}N_4O_3$·0.25H_2O) C, H, N.

1-(2-Nitro-1-imidazolyl)-2-methyl-3-(1-aziridinyl)-2-propanol (20). To 1-potassio-2-nitroimidazole (5.72 g, 37.9 mmol) in 50 mL of DMF was added sodium iodide (0.1 g, 0.67 mmol) followed by β-methallyl chloride (10 mL, 101 mol) and the solution heated under reflux for 0.25 h. The solution was then cooled, poured into EtOH (100 mL), and filtered, and the solid material was washed with warm EtOH (150 mL). The combined filtrates were evaporated, and the residue was redissolved in $CHCl_3$ (250 mL), washed with H_2O (4 × 200 mL) and saturated NaCl(aq) (200 mL), dried, and evaporated to afford 6.95 g (82.2%) of 1-(2-methylprop-2-enyl)-2-nitroimidazole (**18**) as a pale yellow oil, which was used without further purification: NMR ($CDCl_3$) δ 1.8 (s, 3 H, $CH_2C(CH_3)$), 4.6 (bs, 1 H) and 5.0 (bs, 1 H), C(CH$_3$)=CH_2), 5.0 (s, 2 H, Im-CH_2), 7.2 (s, 2 H, 2 × Im-H). This material (3.42 g, 20.5 mmol) was dissolved in CH_2Cl_2 (100 mL) and heated under reflux with MCPBA (55%, 12.14 g, 38.8 mmol) for 3 h. The cooled solution was diluted with $CHCl_3$ (150 mL) and washed with 10% Na_2SO_3(aq) (3 × 200 mL), saturated $NaHCO_3$(aq) (2 × 200 mL), H_2O (2 × 200 mL), and saturated NaCl(aq) (200 mL). The solution was dried and evaporated and the residue purified on silica, eluting with EtOAc, to give 1.89 g (50.4%) of 1-(2-methyl-2,3-epoxypropyl)-2-nitroimidazole (**19**) as a yellow solid: mp 70–71 °C; NMR ($CDCl_3$) δ 1.4 (s, 3 H, oxirane 2-CH_3), 2.7 (dd, 2 H, J = 3.6 and 14.5 Hz, oxiranyl 3-CH_2), 4.7 (dd, J = 14.5 and 33 Hz, Im-CH_2), 7.2 (s, 1 H, Im-H), 7.25 (s, 1 H, Im-H). Anal. ($C_7H_9N_3O_3$) C, H, N. The oxirane **19** (1.26 g, 6.9 mmol) was heated under reflux in EtOH (1% Et_3N, 30 mL) with 1*H*-aziridine (2.0 g, 46.5 mmol) for 0.75 h. The solvent was then evaporated and the residue recrystallized from EtOH (1% Et_3N) to afford 0.93 g (59.8%) of **20** as a pale yellow solid: mp 108–110 °C; NMR ($CDCl_3$/$(CD)_3SO$) δ 1.2 (s, 3 H, C(OH)C*H*$_3$), 1.25 (dd, 2 H, J = 2 and 4 Hz, aziridine CH_2), 1.8 (dd, 2 H, J = 2 and 4 Hz, aziridine CH_2), 2.3 (dd, 2 H, J = 12 and 22 Hz, CH_2-aziridine), 4.7 (s, 2

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H, Im-CH_2), 7.05 (s, 1 H, Im-H), 7.3 (s, 1 H, Im-H). Anal. ($C_9H_{14}N_4O_3$) C, H, N.

1-(2-Nitro-1-imidazolyl)-3,3-dimethyl-3-(1-aziridinyl)-2-propanol (23). 1-Potassio-2-nitroimidazole (3.05 g, 20.2 mmol) in dry CH_3CN (50 mL) was stirred with 4-bromo-2-methylbut-2-ene (4.75 g, 31.8 mmol) and 18-crown-6 (0.26 g, 0.98 mmol) for 24 h. The suspension was evaporated and redissolved in $CHCl_3$ (200 mL) and washed with H_2O (4 × 75 mL) and saturated NaCl (75 mL) and then dried and evaporated to dryness. The residue was purified on silica gel eluting with $CHCl_3$ to afford 0.8 g (22%) of 1-(3-methylbutenyl)-2-nitroimidazole (**21**) as a pale yellow oil: NMR ($CDCl_3$) δ 1.8 (bs, 6 H, CH=C(CH_3)$_2$), 5.1 (bd, 2 H, Im-CH_2), 5.4 (m, 1 H, C*H*=C(CH_3)$_2$), 7.1 (s, 1 H, Im-H), 7.15 (s, 1 H, Im-H).

The above alkene (2.7 g, 14.9 mmol) was dissolved in CH_2Cl_2 (100 mL) and heated under reflux with MCPBA (6.29 g, 20.1 mmol) for 1 h, cooled, diluted with CH_2Cl_2 (100 mL), and washed with 10% Na_2SO_3(aq) (3 × 150 mL), H_2O (2 × 150 mL), and saturated $NaHCO_3$(aq) (2 × 150 mL). The solution was dried (Na_2SO_4) and evaporated, and the residue was purified on silica, eluting with EtOAc, to give 1.8 g (81.7%) of 1-(3,3-dimethyl-2,3-epoxybutyl)-2-nitroimidazole (**22**) as a pale yellow oil: NMR ($CDCl_3$) δ 1.4 (s, 3 H, oxirane 3-CH_3), 1.45 (s, 3 H, oxirane 3-CH_3), 3.2 (dd, 1 H, *J* = 3.6 and 8.4 Hz, oxirane 2-CH), 4.3 (dd, 1 H, *J* = 8.4 and 13.5 Hz), and 5.1 (dd, 1 H, *J* = 3.6 and 14.4 Hz), Im-CH_2, 7.15 (s, 1 H, Im-H), 7.3 (s, 1 H, Im-H).

The oxiranyl synthon **22** (1.09 g, 5.5 mmol) was dissolved in EtOH (1% Et_3N) (30 mL) and heated under reflux with 1*H*-aziridine (2.0 g, 46.5 mmol) for 10 h. Further aziridine (5 mL, 116 mmol) was then added, and heating was continued for another 4 h. The solution was then evaporated and the residue purified on silica (eluting with $CHCl_3$/MeOH/Et_3N (90:9:1)) to afford, after recrystallization from EtOH (1% Et_3N), 0.32 g (32.8%) of **23** as a pale yellow solid: mp 127–128 °C; NMR ($(CD_3)_2SO$) δ 0.78 (s, 3 H, C(CH_3)), 0.83 (s, 3 H, C(CH_3)), 1.4 (m, 4 H, 2 × aziridine CH_2), 4.25 (dd, 1 H, *J* = 10.7 and 13.5 Hz) and 4.92 (dd, 1 H, *J* = 1.5 and 13.5 Hz), Im-CH_2, 5.07 (d, 1 H, *J* = 6.2 Hz, OH), 7.14 (s, 1 H, Im-H), 7.59 (s, 1 H, Im-H); NMR ($CDCl_3$) δ 0.92 (s, 3 H, C(CH_3)), 0.98 (s, 3 H, C(CH_3)), 1.5 (m, 2 H) and 1.53 (m, 1 H) and 1.61 (m, 1 H), 2 × aziridine CH_2, 3.66 (dd, 1 H, *J* = 1.7 and 10.1 Hz, C*H*OH), 4.10 (dd, 1 H, *J* = 10.2 and 13.5 Hz) and 4.9 (dd, 1 H, *J* = 1.7 and 13.5 Hz), Im-CH_2, 4.2 (br, 1 H, OH), 7.08 (d, 1 H, *J* = 1.1 Hz, Im-H), 7.27 (d, 1 H, *J* = 1.1 Hz, Im-H). Anal. ($C_{10}H_{16}N_4O_3$) C, H, N.

1,2-*cis*-2,3-*trans*-3-Aziridin-1-yl-2-hydroxy-1-(2-nitroimidazol-1-yl)cyclohexane (26). 1-Cyclohex-2-enyl-2-nitroimidazole was prepared and epoxidized with MCPBA as described previously.[18] The anti-isomer **24** was isolated as unreacted starting material when a mixture of isomers was used for the following reaction. The syn-isomer **25** reacts with aziridine, but could be obtained in pure form when the above epoxidation was carried out with dried peroxy acid as described previously.[18]

The oxiranyl synthon **25** (0.5 g, 2.4 mmol) was refluxed in 40 mL of EtOH (1% Et_3N) with 1*H*-aziridine (2.0 g, 46.5 mmol) for 1.5 h. The solution was evaporated to dryness and the residue purified on silica, eluting with $CHCl_3$/MeOH/Et_3N (90:9:1), to give 0.15 g (26%) of **26** as a pale yellow solid, recrystallized from acetone: mp 204–206 °C; NMR ($(CD_3)_2SO$) δ 1.12 (dd, 1 H, *J* = 6.0 and 3.3 Hz, aziridine-H syn to N-cycohexane bond), 1.16 (dd, 1 H, *J* = 6.0 and 3.4 Hz, aziridine-H syn to *N*-cyclohexane bond), 1.45–1.90 (m, 7 H, 2 × aziridine-H anti to *N*-cyclohexane bond + 4ax,4eq,5ax,5eq,6eq-H), 2.17 (dq, 1 H, *J* = 11.4 and 3.4 Hz, 6ax-H), 3.34 (m, 1 H, 3-H), 3.91 (m, 1 H, 2-H), 5.11 (d, 1 H, *J* = 5.6 Hz, OH), 5.49 (d ca. t, 1 H, *J* = 11.9 and 3 Hz, 1-H), 7.15 (d, 1 H, *J* = 0.7 Hz, Im-4H), 7.67 (d, 1 H, *J* = 0.7 Hz, Im-5H); EIMS 252.1222 (calcd for $C_{11}H_{15}N_4O_3$ 252.1240).

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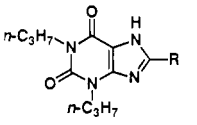
Communications to the Editor

7,8-Dihydro-8-ethyl-2-(3-noradamantyl)-4-propyl-1*H*-imidazo[2,1-*i*]purin-5(4*H*)-one: A Potent and Water-Soluble Adenosine A_1 Antagonist

Adenosine elicits a wide variety of physiological responses[1] via interactions with two major subtypes of extracellular receptors, designated as A_1 and A_2. The two receptor subtypes were originally defined in terms of different effects on adenylate cyclase. A_1 receptors exhibit relatively high affinity to adenosine in binding studies (nM) and some are coupled to and inhibit adenylate cyclase. In contrast, A_2 receptors exhibit low affinity to adenosine (μM) and some are coupled to, but stimulate adenylate cyclase.[2,3]

Considerable effort to search for selective antagonists has been invested in order to elucidate the physiological role of adenosine and develop therapeutic agents.[4,5] Although many selective adenosine A_1 antagonists such as 1 (DPCPX)[6] and 2 (KF15372)[7] are recently available



compound		R
1	(CPX)	cyclopentyl
2	(KF15372)	dicyclopropylmethyl
3	(KW-3902)	3-noradamantyl
10	(KFM19)	3-oxocyclopentyl

Figure 1. Chemical structure of xanthine derivatives.

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