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Original paper

Genotoxicity in oxazolidine derivatives: influence of the nitro group

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Summary — Tris-(hydroxymethyl)-aminomethane reacts with various aromatic or heterocyclic aldehydes, leading to new mono- or bicyclic oxazolidine derivatives. The importance of molecular surroundings of the nitro group has been evaluated by the genotoxic study of synthetised compounds using metronidazole and dimetridazole as reference compounds.

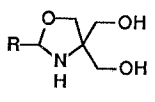
Résumé — Étude génotoxique de dérivés de l'oxazolidine: influence du groupe nitro. *Le tris-(hydroxyméthyl)aminométhane réagit avec divers aldéhydes aromatiques ou hétérocycliques pour conduire à de nouveaux composés mono- ou bicycliques dérivés de l'oxazolidine. L'étude génotoxique des produits synthétisés comparativement au métronidazole et dimétridazole, a permis d'apprécier l'importance de l'environnement moléculaire du groupement nitro.*

oxazolidine / genotoxicity / TRIS / aldehyde / nitro groups

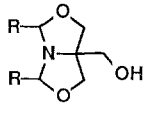
Introduction

There is now persuasive evidence that DNA damage caused by genotoxic agents may lead to mutagenesis and growing evidence that it may lead to carcinogenesis [1]. We used the oxazolidine skeleton [2] to show how molecular mutagenic potency can be increased by exchanging substituting groups. The oxazolidine skeleton is present in many anticonvulsant drugs [3] and the presence of a hydroxymethyl group increases the water–solubility of compounds which bear this combined skeleton. The bioisostery between nitroheterocyclic compounds (furan or imidazole) and benzenic ring was the basic principle behind this toxicomodulation.

The novel genotoxic agents obtained, mono- or bicyclic oxazolidine derivatives, have one of the following structures:



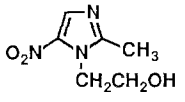
1



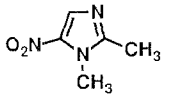
2

We confirmed that a pharmacotoxicophore group (as defined by Royer [4]), the nitro group, is a structural requirement of this genotoxicity.

Reference compounds were metronidazole and dimetridazole, used medicinally in surgical and gynaecological sepsis, notably in the treatment of trichomonal vaginitis and intestinal or hepatic amebiasis [5]. While several drugs in this group of 5-nitroimidazoles are currently available and in wide use, as a group they have the disadvantage of being mutagenic [6].



metronidazole



dimetridazole

Chemistry

Tris-(hydroxymethyl)-aminomethane (TRIS) reacts with substituted aromatic aldehydes, providing Schiff bases and then, by cyclization, oxazolidinic com-

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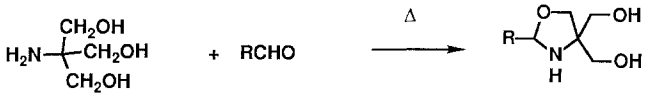
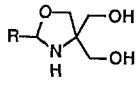
pounds **1** (table I). In these reactions, the proposed method is azeotropic distillation in which benzene or toluene is used as the water-entraining agent.

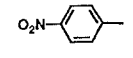
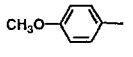
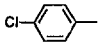
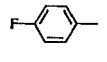
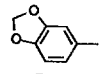
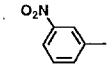
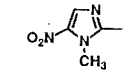


1

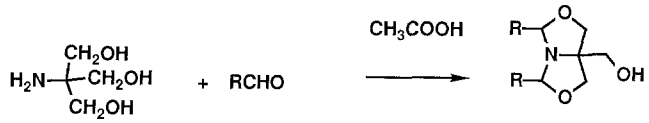
If the aromatic aldehyde is substituted with the electron-withdrawing group (NO_2, Cl) the reaction occurs; nevertheless acid catalyst (BF_3•Et_2O) is necessary when the aromatic ring is substituted by electron-donating group (CH_3O, methylenedioxy). With the substituting group described above, the isolated compound results from the reaction of one mol of amino alcohol with one mol of aldehyde. However, the condensation of TRIS with fluoro-aromatic aldehydes leads to the formation of a mixture of two derivatives: the mono and bicyclic compounds **1** and **2**. In this case, the formation of **2** (R:pF–C_6H_4) can be explained by the reaction of **1** with a second mol of aldehyde. The difference in the two reaction rates precludes the selective formation of pure **1**, but the different solubilities of bicyclic compounds **2** and **1** (R:pF–C_6H_4) allow the formation of the latter with a medium yield.

Table I. 2-Substituted -4,4-*bis*-(hydroxymethyl)-oxazolidines.



Compound N°	R	Yield %	m.p.°C	formula	MW	solvent of reaction
1a		90	102	$C_{11}H_{14}N_2O_5$	254.24	C_6H_6
1b		80	118	$C_{12}H_{17}NO_4$	239.27	toluene
1c		89	97	$C_{11}H_{14}NO_3Cl$	243.69	toluene
1d		65	81	$C_{11}H_{14}NO_3F$	227.24	toluene
1e		80	109	$C_{12}H_{15}NO_5$	253.26	toluene
1f		62	75	$C_{11}H_{14}NO_3F$	227.24	toluene
1g		90	84	$C_{11}H_{14}N_2O_5$	254.24	toluene
1h		92	144	$C_9H_{14}N_4O_5$	258.24	C_6H_6

The compound 5-nitrofurfural does not condense with TRIS under the stringent conditions because it decomposes. But two equivalents of 5-nitrofurfural with 1 equivalent of TRIS in the presence of 1 equivalent of glacial acetic acid lead to the bicyclic compound 2 with an excellent yield at room temperature. A similar product is obtained from nitroaromatic aldehydes (table II).



2

In an attempt to synthetise compound **1** by using the same experimental protocol but with only one mole of aldehyde, the acidic salt of TRIS is the only isolated compound. The equilibrium between TRIS and its protonated form is displaced to the latter form, prohibiting any reaction. With an excess of aldehyde, the equilibrium is displaced and the bicyclic oxazolidines **2** are obtained.

These mild experimental conditions allow formation of new derivatives from thermically unstable aldehydes. The conditions described in the literature [7–9] lead to the rapid degradation of these nitroaldehydes.

The ^1H NMR spectra of the isolated compounds **2** have showed a *trans* configuration. The two protons H_A and H_B are not magnetically equivalent, the more deshielded H_B is the *cis* with respect to the C^1C^2 band and H_A is the *trans*. Moreover, H_A and H_B in this *trans* isomer, form a diedral angle of 90° and are not coupled through the nitrogen atom.

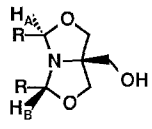
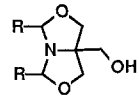
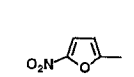


Table II. 1-Aza-2,8-bisubstituted-5-hydroxymethyl-3,7-dioxabicyclo(3.3.0)-octane.



Compound N°	R	Yield %	m.p. °C	Formula	MW	solvent of reaction
2a		90	237	$C_{18}H_{17}N_3O_7$	387.35	CH_2Cl_2
2b		90	141	$C_{14}H_{13}N_3O_9$	367.27	CH_2Cl_2

Genotoxic activity

The mutagenic potencies of the molecules were determined by two short *in vitro* tests: the *Salmonella* microsomal assay (Ames test) and the SOS chromotest. These potencies were compared to those of metronidazole or 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole and dimetridazole or 1,2-dimethyl-5-nitroimidazole.

Qualitative assays: spot tests

The *Salmonella* microsomal assay and the SOS chromotest were performed as spot tests on the 12 compounds. The results are shown in table III. Four compounds elicited mutagenic and genotoxic activities: metronidazole, dimetridazole and derivatives **1h** and **2b**. The other derivatives showed neither mutagenic nor toxic activities.

Table III. The Ames test and the SOS chromotest. Spot tests.

Compound/strain		*Ames test*								*SOS chromotest*	
(dose in μg)		*TA 97*		*TA 98*		*TA 100*		*TA 102*		*PQ 37*	
		–S9	*+S9*	*–S9*	*+S9*	*–S9*	*+S9*	*–S9*	*+S9*	*–S9*	*+S9*
1a	640	–	–	–	–	–	–	–	–	–/T++	–/T++
	64	–	–	–	–	–	–	–	–	–	–
1b	700	–	–	–	–	–	–	–	–	–	–
	70	–	–	–	–	–	–	–	–	–	–
1c	440	–	–	–	–	–	–	–	–	–	–/T+
	44	–	–	–	–	–	–	–	–	–	–
1d	640	–	–	–	–	–	–	–	–	–	–
	64	–	–	–	–	–	–	–	–	–	–
1e	300	–	–	–	–	–	–	–	–	–/T+	–
	30	–	–	–	–	–	–	–	–	–	–
1f	500	–	–	–	–	–	–	–	–	–	–
	50	–	–	–	–	–	–	–	–	–	–
1g	100	–	–	–	–	–	–	–	–	–	–
	10	–	–	–	–	–	–	–	–	–	–
ICR 191	1	+									
2,4,7TNFone	1			+/T++							
BaP	5				+						+
NaN_3	3					+					
MitC	1							+/T++		+/T++	
1h	540	+/T+++	+/T+++	+/T+	–	+/T+++	+/T+++	+	+	+/T+	+/T+
	54	+	+	–	–	+	+	–	–	+	+
2a	100	–	–	–	–	–	–	–	–	–	–
	10	–	–	–	–	–	–	–	–	–	–
2b	500	+/T+++	+/T+++	+/T+++	+/T+++	+/T+++	+/T+++	+/T++	+/T++	+/T+++	+/T+++
	50	+	+	+	+	+	+/T++	+	+	+/T++	+/T++
Metro[1]	500	+	+	–	–	+	+	+	+	+	+
	50	–	–	–	–	–	–	–	–	–	–
Dimet[2]	500	+	+	+	+	+	+	+	+	+	+
	50	+	+	–	–	+	+	+	+	–	–
ICR 191	1	+									
2,4,7TNFone	1			+/T++							
BaP	5				+						+
NaN_3	3					+					
MitC	1							+/T++		+/T++	

+ = Mutagenic activity; – = no mutagenic activity; T = toxic activity; T+ = diameter of the inhibition zone: $\phi < 10$ mm; T++ = diameter of the inhibition zone: 10 mm$< \phi < 15$ mm; T+++ = diameter of the inhibition zone: $\phi > 15$ mm. Spot tests of the *Salmonella* microsomal assay and the SOS chromotest are described in *Experimental protocols*. [1]Metronidazole; [2]dimetridazole.

Quantitative technique of the Salmonella *microsomal assay*

Dose–response relationships of the 4 mutagenic compounds selected from the preliminary screening were determined by the plate incorporation technique in the Ames test. *Salmonella* tester strains TA 97, TA 98, TA 100 and TA 102 were used with or without S9 MIX. Complete data are showed in table IV. Calculated mutagenic potencies are presented in table VI. The 4 molecules displayed mutagenic activities on all strains with and without metabolic activation. Tester strain TA 100 showed the highest sensitivity to the products. Derivative **2b** was found to be the most mutagenic compound and its mutagenic potency increased following metabolic activation.

Quantitative technique of the SOS chromotest

The 4 mutagenic compounds were also tested by the quantitative technique of the SOS chromotester using strain *E coli* PQ 37. Complete data are presented in table V and calculated SOS inducing power are included in table VI. In the Ames test, the 4 molecules

Table IV. Salmonella microsomal assay: mutagenic activity of the 4 selected molecules.

Compound dose / plate		*Revertants / plate*							
		TA 97		*TA 98*		*TA 100*		*TA 102*	
nmol	*μg*	*–S9*	*+S9*	*–S9*	*+S9*	*–S9*	*+S9*	*–S9*	*+S9*
Metronidazole:									
0.00	0	248±29	255±21	38±8	38± 8	167± 15	159± 6	307±18	369±22
292.23	50	323±10	308± 2	35±7	38±10	693± 16	653± 57	481±14	671±24
584.28	100	422± 8	393±27	32±3	65± 3	1079± 58	1168± 42	678±25	809±31
876.42	150	538±13	524±16	47±6	76±12	1743± 62	1614±116	844±17	1010±31
1168.56	200	617±38	622±19	56±7	87± 4	2301±155	2346±102	971±61	1194±26
ICR 191:									
1.11	0.5	1468							
2,4,7 TNFone:									
1.59	0.5			4547					
BaP:									
19.82	0.5				498				
NaN3:									
30.76	2.0					421			
MitC:									
1.50	0.5							2150	
Dimetridazole:									
0.00	0	160±20	173±26	22±4	33± 3	133± 14	155± 16	272±17	336±10
354.28	50	328±33	354±31	31±2	37± 3	807± 66	1006± 64	422±19	549± 7
708.50	100	542±47	513±41	27±6	49± 5	1489± 33	1548± 42	623±10	669±12
1062.85	150	670±56	634±20	54±8	60± 5	2411± 68	2319±109	713±37	897±29
1417.13	200	876±55	715±32	63±9	79± 3	2854±105	3082±117	909±35	966±43
ICR 191:									
1.11	0.5	1239							
2,4,7 TNFone:									
1.59	0.5			4346					
BaP:									
19.82	0.5				459				
NaN3:									
30.76	2.0					540			
MitC:									
1.50	0.5							2437	

Data points are the means of triplicate plates ± standard deviation.
The plate incorporation assay is described in *Experimental protocols*.
– = Not done.

Table IV. *(continued)*

Compound dose / plate		*Revertants / plate*							
		TA 97		*TA 98*		*TA 100*		*TA 102*	
nmol	*μg*	*–S9*	*+S9*	*–S9*	*+S9*	*–S9*	*+S9*	*–S9*	*+S9*
1h:									
0	0	176±10	195± 2	22± 4	36± 3	174± 16	164± 8	305± 29	305±29
19.36	5	248±14	242±13	–	–	293± 12	252± 22	–	–
38.72	10	308± 8	279± 5	–	–	410± 23	340± 4	–	–
58.09	15	341± 4	333±17	–	–	512± 7	416± 12	–	–
77.45	20	407± 9	391± 6	–	–	620± 22	506± 16	–	–
193.62	50	–	–	88± 8	88± 8	–	–	600± 12	653±43
387.24	100	–	–	187±17	157±14	–	–	866± 51	810±30
580.86	150	–	–	253±16	211± 6	–	–	989±114	947±90
774.47	200	–	–	351± 7	265±13	–	–	1352± 88	1271±69
ICR 191:									
1.11	0.5	1840							
2,4,7 TNFone:									
1.59	0.5			3921					
BaP:									
19.82	0.5				671				
NaN_3:									
30.76	2.0					674			
MitC:									
1.50	0.5							1816	
2b:									
0	0	139± 5	164±30	16± 3	32± 5	108± 10	98± 4	239± 20	334±23
13.61	5	534±34	457±40	48± 6	68±12	395± 34	493± 43	450± 26	776±44
27.23	10	671±50	677±57	55± 9	85± 8	583± 18	680± 59	520± 20	844±36
40.84	15	726±41	813±39	73±12	112±17	689± 22	860± 60	701± 24	1105±52
54.46	20	787±31	926±60	88±16	143±26	toxic	1245± 72	738± 20	1260±67
ICR 191:									
1.11	0.5	770							
2,4,7 TNFone:									
1.59	0.5			6037					
BaP:									
19.82	0.5				802				
NaN_3:									
30.76	2.0					635			
MitC:									
1.50	0.5							2580	

displayed genotoxic activity with and without the S9 MIX. Although the compounds showed a toxic activity against the bacterial strain at the tested doses (the alkaline phosphatase activity decreased with increasing doses); the SOS inducing power could be calculated. Derivative **2b** showed the highest SOS inducing power; similar results were found with the Ames test. However, no increasing activity could be detected with derivative **2b** following metabolic activation.

Discussion and Conclusion

Four mutagenic molecules selected from a preliminary screening were assayed quantitatively to determine their mutagenic and genotoxic potencies using two short-term tests: the Ames test and the SOS chromotest. The compounds were: metronidazole, dimetridazole, derivatives **1h** and **2b**. The four compounds displayed mutagenic and genotoxic activities, suggesting that they may induce frame-

shift and base-pair mutations in the *Salmonella* strains and the SOS error-prone system of reparation in the *Escherichia* strain. The mutagenic and genotoxic properties of these 4 compounds are probably associated with the monoalkylations of DNA [10]. In both assays, derivative **2b** was found to be the most active molecule. Increasing mutagenic activity of derivative **2b** was shown with metabolic activation in the Ames test; however, this phenomenon was not reported with the SOS chromotest. This discrepancy in the results may be due to the lack of sensitivity of the SOS chromotest with the metabolic fraction [11–13].

Substitution of a hydroxyethyl group by a methyl group on the imidazole ring slightly increases the mutagenic and the genotoxic potencies (1.5 and 2.0 revertants/nmol for TA 100, 0.027 and 0.037 IF/nmol for PQ 37) in position 1. Methyl group substitution in

Table V. SOS chromotest: genotoxic activity of the 4 selected molecules.

Compound		*–S9 Mix*			*+S9 Mix*		
Dose / tube							
nmol	*µg*	U_β	U_p	*IF*	U_β	U_p	*IF*
Metronidazole:							
0.00	0.0	2.61±0.11	13.79±0.49	1.00	1.09±0.02	3.89±0.02	1.00
116.86	20.0	12.50±0.23	13.37±0.06	4.93	4.15±0.15	3.69±0.10	3.99
175.28	30.0	14.00±0.14	12.43±0.10	5.94	5.20±0.23	3.30±0.22	5.61
233.71	40.0	16.69±0.29	11.75±0.10	7.49	5.39±0.40	2.95±0.05	6.48
292.14	50.0	17.17±0.05	10.01±0.15	9.04	5.47±0.05	2.68±0.25	7.31
Dimetridazole:							
0.00	0.0	2.61±0.11	13.79±0.49	1.00	1.09±0.02	3.89±0.02	1.00
141.71	20.0	17.06±0.69	13.87±1.61	6.54	4.38±0.25	3.06±0.26	5.09
212.57	30.0	18.21±0.20	9.73±0.08	9.86	4.82±0.08	2.58±0.04	6.63
283.43	40.0	18.85±0.56	8.42±0.37	11.81	5.16±0.17	2.32±0.06	7.91
354.28	50.0	19.19±0.45	7.15±0.21	14.16	5.31±0.10	2.07±0.05	9.13
MitC:							
1.50	0.5	21.74	9.74	11.76	ND	ND	ND
BaP:							
3.96	1.0	ND	ND	ND	8.73	3.18	9.76
1h:							
0.00	0.0	1.62±0.02	10.74±0.12	1.00	1.22±0.04	5.10±0.04	1.00
3.87	1.0	2.38±0.06	10.96±0.34	1.44	1.70±0.05	5.24±0.31	1.35
5.81	1.5	2.78±0.09	11.08±0.36	1.67	1.87±0.04	5.23±0.08	1.50
7.74	2.0	2.93±0.04	10.64±0.27	1.83	2.02±0.12	5.19±0.06	1.62
9.68	2.5	2.93±0.03	9.70±0.20	2.01	2.23±0.15	4.90±0.09	1.90
2b:							
0.00	0.0	1.62±0.02	10.74±0.12	1.00	1.22±0.04	5.10±0.04	1.00
2.72	1.0	4.26±0.02	10.21±0.29	2.77	2.42±0.10	4.27±0.21	2.37
4.08	1.5	4.66±0.07	7.53±0.11	4.11	2.25±0.07	3.40±0.09	2.77
5.45	2.0	4.45±0.03	7.12±0.29	4.16	2.35±0.09	3.27±0.04	2.99
6.81	2.5	4.37±0.04	5.96±0.13	4.87	2.49±0.11	2.98±0.05	3.50
MitC:							
1.50	0.5	15.34	8.10	12.57	ND	ND	
BaP:							
3.96	1.0	ND	ND	ND	11.23	4.87	9.64

U_β: enzymatic units of β-galactosidase; U_p: enzymatic units of alkaline phosphatase; IF: induction factor expressed as: $(U_{\beta(d)} / U_{p(d)}) / (U_{\beta(0)} / U_{p(0)})$ where: $U_{\beta(d)}$ and $U_{p(d)}$ are the enzymatic units calculated for the dose d. $U_{\beta(0)}$ and $U_{p(0)}$ are the enzymatic units calculated for the control. Data points are the means of duplicate assays ± SD. The quantitative SOS chromotest is described in *Experimental protocols.*

dimetridazole by (4,4-*bis*-hydroxymethyl) oxazolidine leads to a significant increase in mutagenic and genotoxic potencies (5.73 revertants/nmol for TA 100 as compared with 13.75 revertants/nmol; 0.105 IF/nmol for PQ 37 as compared with 1.555 IF/nmol). Since derivatives **1a** and **1g** are not mutagenic, mutagenicity seems not to result from the nitro group but rather from the environment that bears this nitro group. Similarly, with **2** bicyclic derivatives, the most mutagenic and genotoxic compound is derivative **2b** (13.75 revertants/nmol for TA 100; 1.555 IF/nmol for PQ 37) whereas derivative **2a** is not mutagenic. These results confirm the observed mutagenic and carcinogenic risks involved in the use of nitro-heterocycles [14], especially metronidazole [15–17]. Aromatic ring substitution by other electron-withdrawing (F, Cl) or electron-donating (CH_3O, methylene dioxy) groups in compounds **1** does not modify the non-mutagenic character of these compounds.

Table VI. The Ames test and SOS chromotest: mutagenic potencies and SOS induction power of the 4 tested molecules.

Strain	*Mutagenic potency (revertants / nmol)*		*SOS induction power (IF / nmol)*	
	–S9	*+S9*	*–S9*	*+S9*
1h:				
TA 97	2.86	2.49		
TA 98	0.43	0.30		
TA 100	5.73	4.37		
TA 102	1.28	1.11		
PQ 37			0.105	0.089
Metronidazole:				
TA 97	0.27	0.27		
TA 98	< 0.03	0.03		
TA 100	1.50	1.50		
TA 102	0.48	0.56		
PQ 37			0.027	0.022
Dimetridazole:				
TA 97	0.50	0.39		
TA 98	0.03	0.03		
TA 100	2.00	2.02		
TA 102	0.44	0.45		
PQ 37			0.037	0.023
2b:				
TA 97	10.59	13.37		
TA 98	1.21	1.93		
TA 100	13.75	18.65		
TA 102	8.88	15.51		
PQ 37			1.555	0.418

The mutagenic potencies were defined as the slope of the dose-response curves calculated by regression analysis (expressed in revertants / nmol). The SOS induction powers were defined as the slope of the dose-response curves calculated by regression analysis (expressed in IF units / nmol).

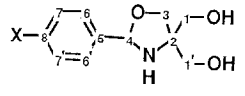
Experimental protocols

Chemical synthesis

Melting points were determined with a Büchi apparatus in capillary tubes and are uncorrected. The ^1H NMR spectra were recorded on a Varian EM 360 or XL 200 spectrometer and the ^{13}C NMR spectra on Brucker 200 MHz. Chemical shifts are reported in δ units (ppm) relative to internal TMS. Microanalysis were performed by Ecole Supérieure de Chimie de Marseille and were within 0.4% of theoretical values.

*2-Substituted-4,4-*bis*-(hydroxymethyl)-oxazolidines* ***1***

General procedure
A mixture of Tris-(hydroxymethyl)-aminomethane (6.06 g, 0.05 mol), the appropriate aldehyde (0.05 mol) and boron trifluoride etherate (0.71 g, 0.005 mol) if necessary, in 60 ml of benzene or toluene, was refluxed under a water separator for 6 h. After hot filtration of the unreacted aminoalcohol, methylene chloride was added to the organic solution. The solution which separated, was collected and crystallized from methylene chloride–petroleum ether (1:1). With compounds **1d** and **1f**, after hot filtration of the reaction mixture, the crystalline oxazolidine was yielded on standing.



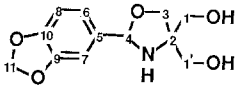
*2-*p*-Nitrophenyl-4,4-*bis*-(hydroxymethyl)-oxazolidine* ***1a***
^1H NMR, 60 MHz, DMSO–D_6 δ: 3.14 (d, J = 10 Hz, 1H, NH); 3.64 (t, J = 5 Hz, 4H, C^1H_2, $C^{1'}H_2$); 3.97 (s, 2H C^3H_2); 4.96 (t, J = 5 Hz, 2H, OH); 5.60 (d, J = 10 Hz, 1H, C^4H); 7.70–8.14 (AB, J_{AB} = 9 Hz, 4H, C^6H, $C^{6'}H$, C^7H, $C^{7'}H$).
^{13}C NMR, DMSO δ: 62.58 and 62.82 (CH_2, C^1, $C^{1'}$); 67.30 (C, C^2); 69.29 (CH_2, C^3); 90.40 (CH, C^4); 123.23 (CH, C^7, $C^{7'}$); 127.48 (CH, C^6, $C^{6'}$); 147.32 (C, C^5); 148.04 (C, C^8).

*2-*p*-Methoxyphenyl-4,4-*bis*-(hydroxymethyl)-oxazolidine* ***1b***
^1H NMR, 60 MHz, DMSO–D_6 δ: 2.90 (d broad, J = 8 Hz, 1H, NH); 3.60 (s, 4H, C^1H_2, $C^{1'}H_2$); 3.95 (s, 2H, C^3H_2); 5.00 (t, J = 4 Hz, 2H, OH); 5.50 (d broad, J = 5 Hz, 1H, C^4H); 6.90–7.55 (AB, J_{AB} = 9 Hz, 4H, C^6H, $C^{6'}H$, C^7H, $C^{7'}H$).
^{13}C NMR, DMSO δ: 55.03 (CH_3); 62.45 and 63.27 (CH_2, C^1, $C^{1'}$); 67.12 (C, C^2); 68.93 (CH_2, C^3); 91.37 (CH, C^4); 113.51 (CH, C^7, $C^{7'}$); 127.46 (CH, C^6, $C^{6'}$); 132.27 (C, C^5); 159.23 (C, C^8).

*2-*p*-Chlorophenyl-4,4-*bis*-(hydroxymethyl)-oxazolidine* ***1c***
^1H NMR, 60 MHz, DMSO–D_6 δ: 3.30–4.00 (m, 9H); 5.30 (s broad, 1H, C^4H); 7.30–7.50 (AB, J_{AB} = 6 Hz, 4H, C^6H, $C^{6'}H$, C^7H, $C^{7'}H$).
^{13}C NMR, DMSO δ: 62.48 and 63.05 (CH_2, C^1, $C^{1'}$); 67.22 (C, C^2); 69.09 (CH_2, C^3); 90.76 (CH, C^4); 128.08 (CH, C^7, $C^{7'}$, C^6, $C^{6'}$); 132.72 (C, C^5); 139.40 (C, C^8).

2-p-Fluorophenyl-4,4-bis-(hydroxymethyl)-oxazolidine ***1d***
^{1}H NMR, 60 MHz, DMSO–D_6 δ: 3.40–4.50 (m, 9H); 5.60 (s broad, 1H, C^4H); 7.00–7.80 (m, 4H, aromatics).
^{13}C NMR, DMSO δ: 62.47 and 63.12 (CH_2, C^1, $C^{1'}$); 67.20 (C, C^2); 69.05 (CH_2, C^3); 90.87 (CH, C^4); 114.64 and 115.07 (CH, C^7, $C^{7'}$); 128.21 and 128.37 (CH, C^6, $C^{6'}$); 136.58 (C, C^5); 164.36 and 187.64 (CF, C^8).

2-(3,4-Methylene-dioxyphenyl)-4,4-bis-(hydroxymethyl)-oxazolidine ***1e***



^{1}H NMR, 60 MHz, DMSO–D_6 δ: 2.90 (s broad, 1H, NH); 3.50–3.80 (m, 4H, C^1H_2, $C^{1'}H_2$); 3.90 (s, 2H, C^3H_2); 4.55–5.25 (m, 2H, OH); 5.50 (s broad, 1H, C^4H); 5.90 (s, 2H, $C^{11}H_2$); 6.70–7.35 (m, 3H, C^6H, C^7H, C^8H).
^{13}C NMR, DMSO δ: 62.45 and 63.19 (CH_2, C^1, $C^{1'}$); 67.05 (C, C^2); 68.97 (CH_2, C^3); 91.35 (CH, C^4); 100.89 (CH_2, C^{11}); 106.47 (CH, C^7); 107.77 (CH, C^8); 119.84 (C, C^9); 124.07 (C, C^{10}); 134.33 (CH, C^6); 147.10 (C, C^5).

2-(m-Fluorophenyl)-4,4-bis(hydroxymethyl)-oxazolidine ***1f***
^{1}H NMR, 60 MHz, C_6D_6 + ε DMSO–D_6 δ: 3.80 (s, 4H, C^1H_2, $C^{1'}H_2$); 4.00 (s, 2H, C^3H_2); 4.10 (s broad, 3H, OH, NH); 5.50 (s, 1H, C^4H); 6.70–7.70 (m, 4H, aromatics).

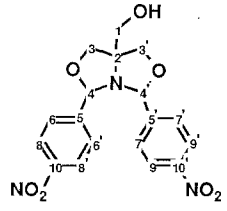
2-(m-Nitrophenyl)-4,4-bis-(hydroxymethyl)-oxazolidine ***1g***
^{1}H NMR, 60 MHz, DMSO–D_6 δ: 3.20 (d, J = 10 Hz, 1H, NH); 3.70 (t, J = 5 Hz, 4H, C^1H_2, $C^{1'}H_2$); 3.95 (s, 2H, C^3H_2); 5.00 (t, J = 5 Hz, 2H, OH); 5.65 (d, J = 10 Hz, 1H, C^4H); 7.30–8.50 (m, 4H, aromatics).

2-(1-Methyl-5-nitroimidazolyl)-4,4-bis-(hydroxymethyl)-oxazolidine ***1h***
^{1}H NMR, 60 MHz, DMSO–D_6 + C_6D_6 δ: 3.40–4.30 (m, 7H); 3.85 (s, 3H, N–CH_3); 5.15 (s broad, 2H, OH); 5.70 (d, J = 10 Hz, 1H, C^4H); 7.98 (s, 1H, imidazole).

1-Aza-2,8-bisubstituted-5-hydroxymethyl-3,7-dioxabicyclo(3,3,0)-octane ***2***

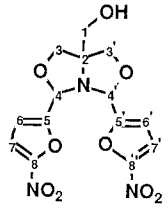
General procedure
The aldehyde (0.1 mol), the Tris-(hydroxymethyl)-aminomethane (6.06 g, 0.05 mol) and glacial acetic acid (3.05 g, 0.05 mol) were suspended in methylene chloride (50 ml) in the presence of molecular sieves (3 Å). The mixture was stirred at room temperature for 3 h. Methylene chloride was removed under reduced pressure and the residue was crystallized from petroleum ether, providing a product of satisfactory purity.

*1-Aza-2,8-*p*-nitrophenyl-5-hydroxymethyl-3,7-dioxabicyclo(3,3,0)-octane* ***2a***



^{1}H NMR, 200 MHz, DMSO–D_6 δ: 3.65 (d, J = 6 Hz, 2H, C^1H_2); 3.85–4.15 (AB, J_{AB} = 9 Hz, 4H, C^3H_2, $C^{3'}H_2$); 5.15 (s, 1H, C^4H); 5.20 (t, J = 6 Hz, 1H, OH); 5.65 (s, 1H, $C^{4'}H$); 7.30 (d, J = 10.5 Hz, 2H, C^6H, $C^{6'}H$); 7.55 (d, J = 10.5 Hz, 2H, C^7H, $C^{7'}H$); 8.05 (t, J = 10.5 Hz, 4H, C^8H, $C^{8'}H$, C^9H, $C^{9'}H$).
^{13}C NMR, DMSO–D_6 δ: 64.68 (CH_2, C^1); 72.56 (C, C^2); 74.36 (CH_2, C^3, $C^{3'}$); 91.15 and 92.16 (CH, C^4, $C^{4'}$); 122.78 and 123.07 (CH, C^6, $C^{6'}$, C^7, $C^{7'}$); 128.33 and 128.55 (CH, C^8, $C^{8'}$, C^9, $C^{9'}$); 141.60 (C, C^{10}, $C^{10'}$); 147.37 (C, C^5, $C^{5'}$).

1-Aza-2,8-(5-nitrofurfuryl)-5-hydroxymethyl-3,7-dioxabicyclo(3,3,0)-octane ***2b***



^{1}H NMR, 200 MHz, DMSO–D_6 δ: 3.85 (d, J = 6 Hz, 2H, C^1H_2); 4.05 (AB, J_{AB} = 10 Hz, 4H, C^3H_2, $C^{3'}H_2$); 5.15 (t, J = 6 Hz, 1H, OH); 5.45 (s, 1H, C^4H); 5.62 (s, 1H, $C^{4'}H$); 6.70 (d, J = 4 Hz, 1H, C^6H); 7.00 (d, J = 4 Hz, 1H, $C^{6'}H$); 7.60 (d, J = 4 Hz, 1H, C^7H); 7.70 (d, J = 4 Hz, 1H, $C^{7'}H$).
^{13}C NMR, DMSO–D_6 δ: 64.05 (CH_2, C^1); 74.03 (C, C^2); 74.18 (CH_2, C^3, $C^{3'}$); 86.06 and 87.49 (CH, C^4, $C^{4'}$); 111.96 and 113.65 (CH, C^6, $C^{6'}$); 112.98 (CH, C^7, $C^{7'}$); 151.12 and 151.70 (C, C^8, $C^{8'}$); 156.01 (C, C^5, $C^{5'}$).

Biological assays

The Ames test: principle of the method
The mutagenicity of the 12 molecules was determined by the *Salmonella* microsomal assay (Ames test) [18] using *Salmonella typhimurium* strains TA 97, TA 98, TA 100 and TA 102 with or without the metabolic activating mixture (S9 MIX). The tester strains were kindly provided by BN Ames.

These strains are auxotrophic for histidine and revert to prototrophy either by frameshift mutations (TA 97 and TA 98) or by base pair mutations (TA 100). In addition, strain TA 102 is able to detect various oxidative mutagens.

The spot test
The mutagenicity of the molecules was assayed qualitatively by the spot test. Briefly, 0.1 ml of tester strains from overnight cultures in Oxoid Nutrient broth No. 2 (NB2) and 0.5 ml of the activating mixtures: S9 MIX (if necessary), were mixed to 2.0 ml of molten soft agar. The top agar mixes were overlayed onto Vogel–Bonner salt minimal agar plates (VB). After hardening of the agar, various amounts of the compounds were spotted onto the plates. After two days of incubation at 37°C, the mutagenic activity of the compounds was evaluated by a ring of revertant colonies around the spot. The toxic activities could be evaluated by an area of growth inhibition.

The quantitative assay
The mutagenic potency of the 2 compounds: **1h** and **2b**, was determined by the plate incorporation method using

metronidazole and dimetridazole as positive references. Briefly, 0.1 ml of the tester strains from an overnight culture in NB2, 0.5 ml of S9 MIX (if necessary) and various volumes of solution of the molecules in dimethylsulfoxide (DMSO) were mixed with 2.0 ml of molten soft agar. The mixtures were overlayed onto VB plates. Four doses were tested in triplicate for each compound. Controls for each experiment included: (i) 40 μl of DMSO as negative control for the determination of spontaneous revertants for each strain; (ii) positive controls: ICR 191 for tester strain TA 97; 2,4,7, TNFone for tester strain TA 98; NaN_3 for tester strain TA 100; Mit C for tester strain TA 102 and BaP with tester strain TA 98 for the S9 MIX. In addition, all the strains were checked for sodium deoxycholate and crystal violet sensitivities, UV light sensitivity, ampicilline resistance and tetracycline resistance (strain TA 102 only). Following a 48-h incubation period at 37°C, spontaneous and induced revertants were counted using an automatic colony counter (Interscience, Model 500A). The number of induced revertants was determined for each dose (observed revertants–spontaneous revertants). The linear portion of the dose–response curve was calculated by regression analysis using the software 'Statgraphics' (STSC, Uniware, version 2.6). The mutagenic potency was defined as the slope of the dose–response curve (expressed in revertants/nmol).

The SOS chromotest: principle of the assay
The SOS chromotest is a bacterial assay for genotoxins [19, 20]. Tester strain *Escherichia coli* PQ 37 was kindly provided by M. Hofnung. This strain carries a *sfiA::lacZ* gene fusion and contains a deletion for the constitutive *lacZ* gene. Hence, β-galactosidase activity reflects the induction level of the error-prone system of reparation (the SOS system). Furthermore, the inhibition of protein synthesis is estimated by measuring alkaline phosphatase activity (a constitutive enzyme).

The SOS spot test
The spot test was performed according to Quillardet and Hofnung [19]. Briefly, 0.1 ml of a logarithmic growing culture (2 x 10^8 cells/ml) and 0.4 ml of S9 mix if necessary were mixed to 2.5 ml of molten top agar. The mixtures were poured onto ST medium plates containing a specific substrate for β-galactosidase: 5 bromo-4 chloro-3 indolyl-β-D-galactoside (X-Gal). Various doses of the compounds dissolved in DMSO were spotted onto the plates and the plates were incubated overnight at 37°C. Induction of the *sfiA* gene was indicated by a blue ring around the spot. The positive controls were 0.5 μg of mit C and 2 μg of BaP with S9 MIX.

Quantitative technique
The quantitative technique was performed as described by Quillardet and Hofnung [19] and modified by De Méo *et al.* [12]. Briefly, an exponentially growing culture of the tester strain was diluted 1/10 with L medium or a modified S9 Mix solution (2% S9). The mixture was distributed in 0.4 ml fractions into disposable tubes containing 13 μl of the test substance. Four doses were tested in duplicate. After a contact of 2 h at 37°C with shaking, 2 separate aliquots of 0.2 ml from each tube were withdrawn for determination of β-galactosidase and alkaline phosphatase activities. The chromogenic substrates were O-nitrophenyl-β-D-galactopyranoside (ONPG) and *p*-nitrophenylphosphate (PNPP) respectively. Colour development was measured after 60 min at 420 nm and evaluated against a blank without bacterial cells. The positive controls were Mit C (0.5 μg) and BaP (1 μg) without and with metabolic activation respectively.

The number of enzymatic units was calculated using a simplification of Miller's formula [19]:

$$U = (OD_{420nm} \cdot 1000)/T$$

where OD_{420nm} was the optical density at 420 nm and T the time (min).

The induction factor (IF) at the concentration C of the tested product was given by the formula:

$$IF = (U_{\beta c} \cdot U_{p0})/(U_{\beta 0} \cdot U_{pc})$$

where $U_{\beta c}$ and U_{pc} were the number of enzymatic units of β-galactosidase and alkaline phosphatase respectively calculated at the concentration c of the tested product and $U_{\beta 0}$ and U_{p0} were the number of enzymatic units of β-galactosidase and alkaline phosphatase calculated in the control (without the tested product).

The SOS inducing power (SOSIP) was defined as the slope of the linear portion of the dose–response curve calculated by regression analysis by using the software 'Statgraphics' (expressed in IF/nmol).

Activation mixture
The liver homogenate (S9) was prepared from Sprague–Dawley rats treated with Aroclor 1254 (500 mg/kg body weight). For the Ames test, the protein concentration was 36.7 mg/ml as determined by the technique of Lowry *et al* [21]. The S9 MIX was a mixture of 10% S9 and a solution of cofactors (NADPH generating system) [18]. For the SOS chromotest, the protein concentration was 21.0 mg/ml and the S9 MIX was a mixture of 2% S9 and a solution of cofactors.

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