Nitroimidazoles, XIV*):

Synthesis of 4-Nitroimidazoles with 1-Substituents Containing Acid, Ester or Phenol Functions, and Radiosensitizing Efficiency of Some of These Compounds

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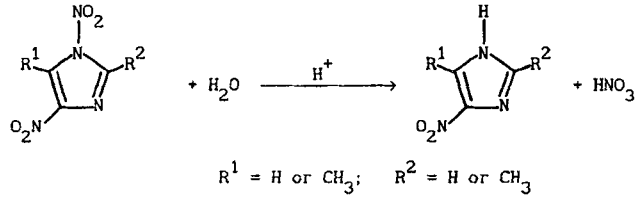
1,4-Dinitroimidazole and 1,4-dinitro-2-methylimidazole were reacted with aminocarboxylic acids and their esters, aminosulfonic acids, and aminophenol to obtain the corresponding 1-substituted-4-nitroimidazoles. The radiosensitizing efficiency of some esters of 2-(4-nitro-1-imidazolyl)alkanecarboxylic acids was tested.

Nitroimidazole, 14. Mitt.: Synthese von N^1-substituierten 4-Nitroimidazolen mit Säure-, Ester- und Phenol-Funktionen und ihre strahlensensibilisierenden Eigenschaften

1,4-Dinitroimidazole und 1,4-Dinitro-2-methylimidazole werden mit Aminocarbonsäuren und ihren Estern, Aminosulfonsäuren und Aminophenolen zu den entspr. N^1-substituierten 4-Nitroimidazolen umgesetzt. Die strahlensensibilisierenden Eigenschaften einiger Ester der 2-(4-Nitro-1-imidazolyl)-alkancarbonsäuren wurden untersucht.

Since mid-seventies synthesis and characteristics of nitroimidazoles have been the subject of persisting interest. This is related to the useful biological activity of a number of nitroimidazoles. In particular, numerous papers have been devoted to the synthesis and investigations of 2-nitro- and 5-nitro-derivatives which are generally considered more active than 4-nitro-derivatives[1]. There is a growing interest in 4-nitroimidazoles (which exhibit lower mutagenity than 2- or 5-nitroimidazoles[2]) as immunosuppressants[3], inhibitors of aldehyde dehydrogenase[4], radiosensitizers of tumor cells[5,6], as well as radiotherapeutic synergetics[7]. 4-Nitroimidazoles are also used in the synthesis of some nucleosides[8].

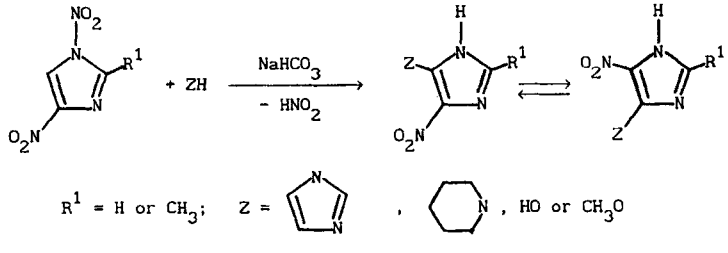


Scheme 1

In the course of our investigations we have found that convenient starting materials for the synthesis of many otherwise not easily available, tautomeric 4(5)-nitroimidazoles or 1-substituted 4-nitroimidazoles are 1,4-dinitroimidazoles. In the present paper the possibility of obtaining 1-substituted-4-nitroimidazoles by the reaction of 1,4-dinitroimidazoles with aliphatic or aromatic amino acids or their esters, and also with 3-amino-phenol has been investigated. We expected to obtain so far not described nitroimidazoles of low cytotoxicity and perhaps significant radiosensitizing efficiency towards hypoxic tumor cells.

Chemistry

1,4-Dinitroimidazoles are susceptible to an attack of nucleophiles. The reaction depends on the kind of nucleophile and the conditions. Examples are given in Schemes 1-3. In an acidic medium denitration in position 1[9] is preferred (Scheme 1).



Scheme 2

*) Part XIII: H. Llempen and J. Suwinski, Polish J. Chem. *66* (1992), in press.

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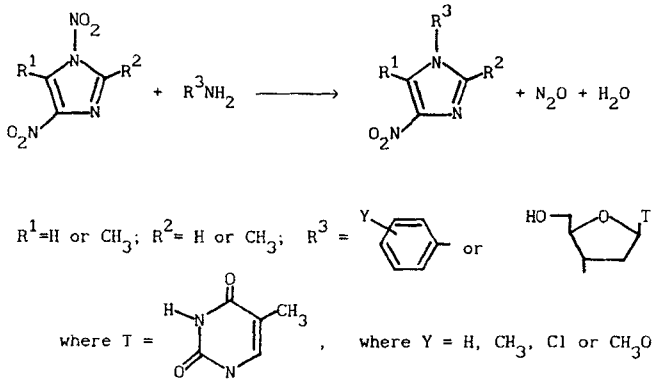


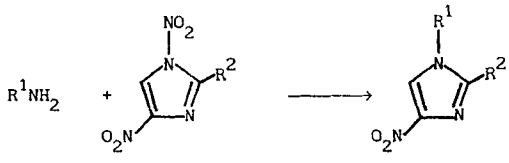
Scheme 3

In a basic medium, nucleophiles such as water, alcohols, secondary amines, and dissociating azoles react with 1,4-dinitroimidazoles towards corresponding 5(4)-substituted-4(5)-nitroimidazoles. Thus, a base-catalyzed, so-called *cine* nucleophilic substitution is then observed with nitrite ions being released[10,11] (Scheme 2).

Some compounds containing primary amino group react with 1,4-dinitroimidazoles in neutral or very weak alkaline aqueous-methanol solutions forming corresponding 1-substituted-4-nitroimidazoles[12,13]. Their formation is not catalyzed by bases and is accompanied by the release of nitrous oxide (Scheme 3).

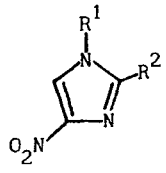
This novel reaction has been applied in the present work. The amino compounds used here (Scheme 4) may be divided into four groups differing in their acid or base characteristics which influenced the method of performing the reaction of these compounds with 1,4-dinitroimidazoles. Therefore, four procedures have been applied as it is shown in Table 1. Details are given in the Experimental Part.

In unbuffered aqueous solutions, the aminosulfonic and α-aminoalkanecarboxylic acids catalyze denitration of 1,4-dinitroimidazoles, themselves not undergoing any change. That is why the reaction of 1,4-dinitroimidazoles with amino acids was carried out at pH ≈ 9, achieved through successive addition of KOH-solution with the progress of the reaction. In these conditions the concentration of the particles with the free amino group was high enough to make their reaction with 1,4-dinitroimidazole faster than its alkaline hydrolysis. The yields of the 2-(4-nitro-1-imidazo-



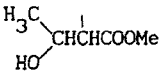
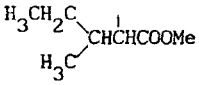
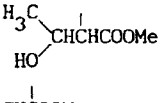
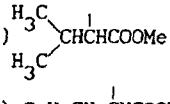
R^1 = Subst. alkyl or aryl rests shown in the Table 1; R^2 = H or CH_3.
Scheme 4

Table 1. Yields and physico-chemical properties of the obtained nitroimidazoles



Compd. number	R^1	R^2	Procedure	Yield [%]	M.p. [°C] (Solvent)	Formula	Found / Calcd.	C	H	N
1	$-CH_2COOH$	Me	A	83	246 dec. (H_2O)	$C_6H_7N_3O_4$	Found	38.93	3.81	22.70
							Calcd.	38.77	4.01	22.57
2	(RS) $CH_3CHCOOH$	Me	A	81	108-110 (H_2O)	$C_7H_9N_3O_4 \cdot 0.5H_2O$	Found	40.38	5.33	20.18
							Calcd.	40.96	5.87	19.84
3	(S) $CH_3CHCOOH$	Me	A	86	98-100 (H_2O)	$C_7H_9N_3O_4 \cdot 0.5H_2O$	Found	40.38	5.33	20.18
							Calcd.	40.81	5.60	19.95
4	$KO_3SCH_2CH_2-$	H	A	82	254-256 ($MeOH+H_2O$)	$C_5H_6KN_3O_5S$	Found	23.16	2.33	16.21
							Calcd.	23.23	2.52	16.00
5	$-CH_2COOMe$	H	B	64	141-142 (MeOH)	$C_6H_7N_3O_4$	Found	38.93	3.81	22.70
							Calcd.	39.00	3.92	22.40
6	(RS) $CH_3CHCOOMe$	H	B	72	116-118 (MeOH)	$C_7H_9N_3O_4$	Found	42.22	4.56	21.10
							Calcd.	42.31	4.76	21.22
7	(S) $CH_3CHCOOMe$	H	B	70	85-86 (MeOH)	$C_7H_9N_3O_4$	Found	42.22	4.56	21.10
							Calcd.	42.13	4.80	21.18
8	(R) $CH_3CHCOOMe$	H	B	76	84-86 (MeOH)	$C_7H_9N_3O_4$	Found	42.22	4.56	21.10
							Calcd.	42.32	4.75	21.31

Tab. 1 Cont.

9	(RS) $CH_3CHCOOEt$	H	B	78	87-88 (MeOH)	$C_8H_{11}N_3O_4$	45.07 45.42	5.20 5.12	19.71 20.02
10	(2RS,3RS) 	H	B	37	132-133 (MeOH)	$C_8H_{11}N_3O_5$	41.93 41.71	4.84 5.03	18.33 18.14
11	(S) $HOCH_2CHCOOMe$	H	B	56	108-109 (MeOH)	$C_7H_9N_3O_5$	39.08 38.85	4.22 4.62	19.53 19.81
12	(S) 	H	B	48	58-59 (MeOH)	$C_{10}H_{15}N_3O_4$	49.79 49.80	6.27 4.61	17.42 17.54
13	$-CH_2COOMe$	Me	B	71	151-152 ($MeOH+H_2O$)	$C_7H_9N_3O_4$	42.22 42.32	4.75 4.56	21.10 21.31
14	(RS) $CH_3CHCOOMe$	Me	B	66	107-108 ($MeOH+H_2O$)	$C_8H_{11}N_3O_4$	45.07 45.05	5.20 5.41	19.71 19.71
15	(S) $CH_3CHCOOMe$	Me	B	68	104-104 ($MeOH+H_2O$)	$C_8H_{11}N_3O_4$	45.07 45.20	5.20 5.39	19.71 19.90
16	(R) $CH_3CHCOOMe$	Me	B	71	103-105 ($MeOH+H_2O$)	$C_8H_{11}N_3O_4$	45.07 45.42	5.20 5.12	19.71 20.02
17	(RS) $CH_3CHCOOEt$	Me	B	39	52-53 (MeOH)	$C_9H_{13}N_3O_4$	47.58 47.86	5.77 6.05	18.49 18.70
18	(2RS,3RS) 	Me	B	84	132-133 (MeOH)	$C_9H_{13}N_3O_5$	44.45 44.76	5.39 5.68	17.28 17.21
19	(S) $HOCH_2CHCOOMe$	Me	B	68	161-162 (MeOH)	$C_8H_{11}N_3O_5$	41.93 42.20	4.84 5.05	18.33 18.65
20	(S) $p\text{-}HOC_6H_4CH_2CHCOOMe$	Me	B	86	191-192 (MeOH+MeCN)	$C_{14}H_{15}N_3O_5$	55.08 55.41	4.95 5.23	13.76 13.39
21	(S) 	Me	B	69	104-105 (MeOH)	$C_{10}H_{15}N_3O_4$	49.79 50.12	6.27 6.42	17.42 17.71
22	(S) $C_6H_5CH_2CHCOOMe$	Me	B	92	129-130 (MeOH)	$C_{14}H_{15}N_3O_4$	58.13 57.87	5.23 5.48	14.53 14.65
23	$o\text{-}HOOC\text{-}C_6H_4\text{-}$	H	C	63	248 dec. ($DMF+H_2O$)	$C_{10}H_7N_3O_4$	51.51 51.61	3.03 3.22	18.02 17.17
24	$m\text{-}HOOC\text{-}C_6H_4\text{-}$	H	C	75	282 dec. ($DMF+H_2O$)	$C_{10}H_7N_3O_4$	51.51 51.73	3.03 3.17	18.02 18.21
25	$p\text{-}HOOC\text{-}C_6H_4\text{-}$	H	C	50	292 dec. ($DMF+H_2O$)	$C_{10}H_7N_3O_4$	51.51 51.58	3.03 3.20	18.02 18.24
26	$m\text{-}HOOC\text{-}C_6H_4\text{-}$	Me	C	83	205 dec. ($DMF+H_2O$)	$C_{11}H_9N_3O_4$	53.47 53.47	3.67 3.88	17.01 16.96
27	$p\text{-}HOOC\text{-}C_6H_4\text{-}$	Me	C	70	281 dec. ($DMF+H_2O$)	$C_{11}H_9N_3O_4$	53.47 53.86	3.67 3.65	17.01 17.19
28	$p\text{-}EtOOC\text{-}C_6H_4\text{-}$	H	D	68	162-163 ($MeOH+H_2O$)	$C_{12}H_{11}N_3O_4$	55.17 55.55	4.24 4.43	16.02 15.90
29	$p\text{-}EtOOC\text{-}C_6H_4\text{-}$	Me	D	67	226-228 ($MeOH+H_2O$)	$C_{13}H_{13}N_3O_4$	56.73 57.04	4.76 5.00	15.27 15.46
30	$m\text{-}HO\text{-}C_6H_4\text{-}$	H	D	98	214-215 ($DMF+H_2O$)	$C_9H_7N_3O_3$	52.69 53.05	3.44 3.80	20.48 20.72
31	$m\text{-}HO\text{-}C_6H_4\text{-}$	Me	D	97	226-228 ($DMF+H_2O$)	$C_{10}H_9N_3O_3$	54.80 55.15	4.14 4.48	19.17 19.41
32	$p\text{-}KO_3S\text{-}C_6H_4\text{-}$	H	A	97	>350 ($MeOH+H_2O$)	$C_9H_6KN_3O_5S$	35.17 35.27	1.97 2.06	13.67 13.60

lyl)alkanecarboxylic acids were good. The products of the reaction of 1,4-dinitroimidazoles with amino sulfonic acids could be isolated only in the form of potassium salts.

Methyl and ethyl esters of 2-aminoalkanecarboxylic acids were used as hydrochlorides. Immediately before the reaction with 1,4-dinitroimidazoles, the hydrochlorides were treated with an equimolar quantity of $NaHCO_3$. The yields of the final esters obtained varied from poor to high ones. This was caused rather by the differences in physical properties of the products than by other reasons. The pH in the above reactions was not controlled.

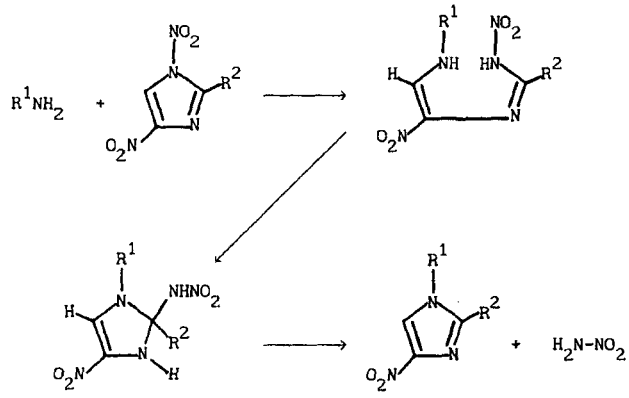
The reaction of aminobenzoic acids with 1,4-dinitroimidazoles was carried out in *Britton-Robinson* buffer pH = 7. The purpose of buffering was to prevent acidification of the solution with progress of the reaction.

3-Aminophenol and esters of amino benzoic acids reacted with 1,4-dinitroimidazoles in unbuffered aqueous-methanol solutions to give high yields of the corresponding 1-aryl-4-nitroimidazoles.

The structure of all new 1-substituted-4-nitroimidazoles has been confirmed by elemental analysis, ^{1}H-NMR, and UV spectra (Tables 1 and 3). In none of the reactions, apart from respective 1-substituted-4-nitroimidazoles, the formation of 5(4)-substituted-4(5)-nitroimidazoles has been noticed, and thus the *cine* substitution reaction has not been observed.

Some of the starting amino alkanecarboxylic acids and esters were optically active*). The chirality centre in these compounds was directly linked to the amino group. From these compounds and 1,4-dinitroimidazoles optically active products were obtained. The physico-chemical properties (with the exception of the sign of optical rotation) of the products obtained from enantiomeric α-amino esters were identical within the experimental error, proving that also the products are enantiomers. This result shows that in the reaction course there was no break of the bond between the asymmetric carbon atom and the amino nitrogen atom. The nitrogen atom in position 1 of the imidazole ring in the product thus comes from the amino group of the nucleophile. This has been proved by the use of ^{15}N-labelled aminoacetic acid[14].

Therefore, the formation of 1-substituted-4-nitroimidazoles from 1,4-dinitroimidazoles and primary amino compounds occurs most probably by nucleophilic addition of the amino compound to dinitroimidazole, ring opening, ring closure, rearomatization with nitroamide elimination and decomposition of the latter to nitrous oxide and water[15] (Scheme 5).



Scheme 5

Biology

Some of the esters of 2-(4-nitro-1-imidazolyl)alkanecarboxylic acids have been subjected to radiological tests. In experiments *in vitro* on cells of the line V79-379A of a Chinese hamster, chronic cytotoxicity of the esters tested in aerobic conditions has been estimated, as well as their radiosensitizing efficiency in the conditions of hypoxia.

In order to determine the chronic aerobic cytotoxicity (C_c) cells suspended in *Eagle's* medium (+13% calf serum +2% fetal calf serum) were placed on dishes and incubated for 2 h at 37°C to attach to glass. The medium was then replaced with a fresh one, supplemented with an appropriate concentration of the nitroimidazole tested, and dishes were incubated 7 days at 37°C for colony formation

Table 2. Chronic aerobic cytotoxicity (C_c) and radiosensitizing effectiveness (ER) of methyl 2-(4-nitro-1-imidazolyl)propanates.

	Surviving fraction/ER				
	Concentration (mM)				
Compound	0.2	0.5	1.0	2.0	C_c
6	0.87	0.90/1.07	0.83/1.16	0.37	1.75
7	0.90	0.91/1.07	0.83/1.19	0.41	1.75
8	0.84	0.90/1.08	0.80/1.16	0.41	1.75
Metronidazole		/1.21	/1.34		
Misonidazole		/1.66	/1.90		

*) A preliminary report on the synthesis of chiral 2-(4-nitro-1-imidazolyl)alkanecarboxylic acids and esters has been sent for rapid publication to "Tetrahedron: Asymmetry" (Ref. 15).

Table 3: Spectroscopic properties of the obtained compounds **1 - 32**

1. UV: 314.6(7250); H_2O. NMR: 13.0(s,1H,COOH); 8.20(s,1H,Imid.), 4.91(s,2H,CH_2); 2.23(s,3H,CH_3); DMSO-d_6.
2. UV: 314.7(8450); H_2O. NMR: 13.0(bs.,1H,COOH); 8.37(s,1H,Imid.), 5.12(q,1H,CH,J=7.23Hz); 2.31(s,3H,CH_3-Imid.); 1.70(d,3H,CH_3, J=7.23Hz); DMSO-d_6.
3. UV: 314.6(8450); H_2O. NMR: 13.0(bs.,1H,COOH); 8.37(s,1H,Imid.); 5.13(q,1H,CH,J=7.23Hz); 2.32(s,3H,CH_3-Imid.); 1.71(d,3H,CH_3, J=7.23Hz); DMSO-d_6. $[\alpha]_{546}^{20}$=39.0°, c=1, DMF.
4. UV: 298.9(6250); H_2O. NMR: 8.37(d,1H,Imid.,J=1.37Hz); 7.81(d, 1H,Imid.,J=1.37Hz); 4.31(t,2H,CH_2-N,J=7.23Hz); 2.96(t,2H,CH_2--S,J=7.23Hz); DMSO-d_6.
5. UV: 287.0(7200); MeOH. NMR: 8.51(d,1H,Imid.,J=2.3Hz); 8.20(d, 1H,Imid.,J=2.3Hz); 5.32(s,2H,CH_2);3.62(s,3H,OCH_3); Pyridine-d_5.
6. UV: 286.7(7200); MeOH. NMR: 8.05(d,1H,Imid.,J=1.47Hz); 7.62(d, 1H,Imid.,J=1.47Hz);5.12(q,1H,CH-N,J=7.30Hz); 3.74(s,3H,OCH_3); 1.75(d,3H,CH_3,J=7.30Hz); MeCN-d_3.
7. UV: 287.0(7200); MeOH. NMR: 8.01(d,1H,Imid.,J=1.50Hz); 7.58(d, 1H,Imid.,J=1.50Hz); 5.10(q,1H,CH-N,J=7.23Hz); 3.70(s,3H,OCH_3); 1.75(d,3H,CH_3,J=7.23Hz); MeCN-d_3. $[\alpha]_{546}^{25}$ = + 34.00°, c=1, MeCN.
8. UV: 286.6(7600); MeOH. NMR: 7.99(d,1H,Imid.,J=1.56Hz); 7.58(d, 1H,Imid.,J=1.56Hz); 5.05(q,1H,CH-N,J=7.23Hz); 3.71(s,3H,OCH_3); 1.74(d,3H,CH_3,J=1.23Hz); MeCN-d_3. $[\alpha]_{546}^{25}$ = - 34.00°, c=1, MeCN.
9. UV: 287.1(7500), MeOH. NMR: 8.05(d,1H,Imid.,J=1.56Hz); 7.61(d, 1H,Imid.,J=1.56Hz); 5.09(q,1H,CH-N,J=7.29Hz); 4.20(q,2H,CH_2,J= 7.02Hz); 1.75(d,3H,CH_3,J=7.29Hz); 1,24(t,3H,CH_3,J=7.02Hz); MeCN-d_3.
10. UV: 287.5(8250); MeOH. NMR: 8.07(d,1H,Imid.,J=1.46Hz); 7.63(d, 1H,Imid.,J=1.46Hz); 4.95(d,1H,CH-N,J=3.41Hz); 4.12-4.75(m,1H, CH-O); 3.78(s,3H,OCH_3); 3.65(d,1H,OH,4.74Hz); 1.01(d,3H,CH_3,J= 6.60Hz); MeCN-d_3.
11. UV: 287.6(7800); MeOH. NMR: 8.10(d,1H,Imid.,J=1.47Hz); 7.66(d, 1H,Imid.,J=1.47Hz); 5.13(q,1H,CH-N,J_1=5.13Hz, J_2=3.13Hz); 3.93--4.25(m,2H,CH_2); 3.77(s,3H,OCH_3); 3.25(bs.,1H,OH); MeCN-d_3. $[\alpha]_{546}^{25}$ = + 37.12°, c=1.25, MeOH.
12. UV: 286.2(8200); MeOH. NMR: 8.09(d,1H,Imid.,J=1.47Hz); 7.64(d, 1H,Imid.,J=1.47Hz); 4.76(d,1H,CH-N,J=8.55Hz); 3.76(s,3H,OCH_3); 0.76-1.33(m,9H,$CH_3CH_2CHCH_3$); MeCN-d_3. $[\alpha]_{546}^{25}$=+72.40°,c=2, MeOH.

Table 3: Cont.

13. UV: 297.3(7450); MeOH. NMR: 7.80(s,1H,Imid.); 4.76(s,2H,CH_2); 3.73(s,3H,OCH_3); 2.29(s,3H,CH_3); MeCN-d_3.

14. UV: 297.5(7550); MeOH. NMR: 8.01(s,1H,Imid.); 5.05(q,1H,CH-N, J=7.32Hz); 3.74(s,3H,OCH_3); 2.35(s,3H,CH_3-Imid.); 1.74(d,3H,CH_3, J=7.32Hz); MeCN-d_3.

15. UV: 298.2(7550); MeOH. NMR: 7.93(s,1H,Imid.); 4.99(q,1H,CH-N, J=7.23Hz); 3.71(s,3H,OCH_3); 2.32(s,3H,CH_3-Imid.); 1.72(d,3H,CH_3, J=7.23Hz); MeCN-d_3. $[\alpha]_{546}^{25}$= +17.16°, c=1.25, MeOH.

16. UV: 297.8(7700); MeOH. NMR: 7.93(s,1H,Imid.); 4.99(q,1H,CH-N, J=7.23Hz); 3.71(s,3H,OCH_3); 2.32(s,3H,CH_3-Imid.); 1.03(d,3H, CH_3,J=7.23Hz); MeCN-d_3. $[\alpha]_{546}^{25}$= - 17.11°, c=1.25, MeOH.

17. UV: 298.2(7400); MeOH. NMR: 7.93(s,1H,Imid.); 4.96(q,1H,CH-N, J=7.03Hz); 4.16(q,2H,CH_2,J=7.23Hz); 2.32(s,3H,CH_3-Imid.); 1.71 (d,3H,CH_3,J=7.23Hz); 1.22(t,3H,CH_3,J=7.03Hz); MeCN-d_3.

18. UV: 297.9(7850); MeOH. NMR: 8.06(s,1H,Imid.); 4.85(d,1H,CH-N, J=3.71Hz); 4.37-4.72(m,1H,CH-O); 3.74(s,3H,OCH_3); 3.69(d,1H, OH,J=6.25Hz); 2.33(s,3H,CH_3-Imid.); 1.03(d,3H,CH_3,J=6.25Hz); MeCN-d_3.

19. UV: 298.5(7550); MeOH. NMR: 8.11(s,1H,Imid.); 5.05(q,1H,CH-N, J_1=5.86Hz,J_2=4.69Hz); 3.95-4.12(m,2H,CH_2); 3.77(3,3H,OCH_3); 3.51(t,1H,OH,J=6.02Hz); 2.34(s,3H,CH_3-Imid.); MeCN-d_3. $[\alpha]_{546}^{25}$= +48.46°, c=1.25, MeOH.

20. UV: 226.2(13100); 297.9(7750); MeOH. NMR: 8.03(s,1H,Imid.); 6.61-6.97(m,5H,HOC_6H_4); 5.05(q,1H,CH-N,J_1=10.43Hz,J_2=5.18Hz); 3.76(s,3H,OCH_3); 2.96-3.43(m,2H,CH_2); 2.01(s,3H,CH_3-Imid.); MeCN-d_3. $[\alpha]_{546}^{25}$= -157.00°, c=0.5, MeCN.

21. UV: 296.8(7800); MeOH. NMR: 8.06(s,1H,Imid.); 4.56(d,1H,CH,J= 9.52Hz); 3.75(s,3H,OCH_3); 2.38(s,3H,CH_3-Imid.); 2.31-2.51(m,1H, CH); 1.04(d,3H,CH_3,J=6.68Hz); 0.84(d,3H,CH_3,J=6.68Hz); MeCN-d_3. $[\alpha]_{546}^{25}$= +56.00°, c=1, MeOH.

22. UV: 298.3(7350); MeOH. NMR: 8.06(s,1H,Imid.); 7.02-7.31(m,5H, C_6H_5); 5.13(q,1H,CH-N,J_1=11.20Hz,J_2=4.44Hz); 3.77(s,3H,OCH_3); 3.06-3.54(m,2H,CH_2); 2.00(s,3H,CH_3-Imid.); MeCN-d_3. $[\alpha]_{546}^{25}$= -228.28°, c=1.5, MeOH.

23. UV: 290.2(9350); MeOH. NMR: 13.23(s,1H,COOH); 8.64(d,1H,Imid., J=1.37Hz); 8.00(d,1H,Imid.,J=1.37Hz); 7.47-8.09(m,4H,C_6H_4); DMSO-d_6.

Table 3: Cont.

24. UV: 290.4(11000); MeOH. NMR: 13.30(s,1H,COOH); 8.99(d,1H,Imid., J=1.20Hz); 8.47(d,1H,Imid.,J=1.20Hz); 7.55-8.20(m,4H,C_6H_4); DMSO-d_6.

25. UV: 229.2(13750); 292.0(12850); MeOH. NMR: 13.13(s,1H,COOH); 9.02(d,1H,Imid.,1.56Hz); 8.51(d,1H,Imid.,J=1.56Hz); 7.82-8.03 (m,4H,C_6H_4); DMSO-d_6.

26. UV: 294.3(10450); MeOH. NMR: 13.20(s,1H,COOH); 8.56(s,1H,Imid.); 7.65-8.09(m,4H,C_6H_4); 2.29(s,3H,CH_3); DMSO-d_6.

27. UV: 226.2(15650); 297.7(10350); MeOH. NMR: 13.15(s,1H,COOH); 8.57(s,1H,Imid.); 7.61-8.12(m,4H,C_6H_4); 2.34(s,3H,CH_3); DMSO-d_6.

28. UV: 233.4(12700); 285.3(14550); MeOH. NMR: 9.02(d,1H,Imid., J=1.56Hz); 8.53(d,1H,Imid.,J=1.56Hz); 7.85-8.13(m,4H,C_6H_4); 4.32(q,2H,CH_2,J=7.03Hz); 1.34(t,3H,CH_3,J=7.03Hz); DMSO-d_6

29. UV: 229.0(16800); 295.0(10800); MeOH. NMR: 8.56(s,1H,Imid.); 7.62-8.11(m,4H,C_6H_4); 4.31(q,2H,CH_2,J=7.00Hz); 2.31(s,3H,CH_3-~Imid.); 1.32(t,3H,CH_3,J=7.00); DMSO-d_6.

30. UV: 294.3(10450); MeOH. NMR: 9.96(s,1H,OH); 8.83(d,1H,Imid., J=1.55Hz); 8.34(d,1H,Imid.,J=1.55Hz); 6.76-7.42(m,4H,C_6H_4); DMSO-d_6.

31. UV: 298.3(9200); MeOH. NMR: 10.04(s,1H,OH); 8.51(s,1H,Imid.); 6.91-7.39(m,4H,C_6H_4); 2.30(s,3H,CH_3); DMSO-d_6.

32. UV: 225.2(14950); 300.2(11050); H_2O. NMR: 8.92(d,1H,Imid.,J= 1.47Hz); 8.41(d,1H,Imid.,J=1.47Hz); 7.70(m,4H,C_6H_4); DMSO-d_6.

In order to determine enhancement ratio (ER) cells in the medium with an appropriate concentration of the nitroimidazole were incubated for 1 h at 37°C in sealed ampoules under argon of high purity. Then cells were irradiated with ^{60}Co-γ rays. A single dose of 25 Gy was used for all the experiments. ERs were calculated on the basis of auxilliary surviving curves, obtained from the single experiment points and a constant value of so called extrapolation number.

The esters studied may be divided into two groups: 1-substituted derivatives of 4-nitroimidazole and 1-substituted derivatives of 2-methyl-4-nitroimidazole. The derivatives without a substituent C-2 of the imidazole exhibit moderate chronic cytotoxicity (C_c = 0.5 - 2 mM) and weak radiosensitizing abilities at nontoxic concentrations. These compounds are not only weaker radiosensitiziers of misonidazole but also of metronidazole. The derivatives containing a methyl group at C-2 are practically nontoxic (C_c > 5.0 mM) but neither do they exhibit radiosensitizing abilities.

In view of lit. reports about the importance of enantiomers on the biological activity of not only drugs[16] but also radiosensitizers[17] we may be surprised by the fact that (*R*), (*S*), and (*RS*) methyl 2-(4-nitro-1-imidazolyl)propanate do not differ as to their biological activity in the *in vitro* conditions (Table 2). Both aerobic cytotoxicities and the radiosensitizing effectiveness of these three compounds are very similar, they differ within the experimental error only. This does not exclude the importance of enantiomers on the activity of these compounds under *in vivo* conditions.

1-Aryl-4-nitroimidazoles obtained in the present research, namely (4-nitro-1-imidazolyl)benzoic acids, their esters and (4-nitro-1-imidazolyl)phenol have a too small solubility in water to make the investigation of their biological activity in standard conditions possible. However, 1-phenyl-2-methyl-4-nitroimidazole was tested for obtaining it C_c = 1.2 and ER = 1.5. On the basis of this it is assumed that 1-aryl-4-nitroimidazoles may be promising radiosensitizing compounds after suitable modification of their physical properties.

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Experimental Part

Chemistry

Starting compounds 1,4-dinitroimidazole and 1,4-dinitro-2-methylimidazole were obtained as described[9]. Amino-compounds were of commercial grade with the exception of hydrochlorides of α-amino esters. The latter were obtained from the corresponding α-amino acids and alcohols and $SOCl_2$.

Synthesis of 1-substituted-4-nitroimidazoles

Procedure **A**

To 0.01 mole of α-amino acid in 60 ml of water at 20 - 25°C N KOH was added to pH = 9. To the stirred solution, 0.01 mole of 1,4-dinitroimidazole was added in one portion and next to the suspension N KOH was dropped in at such a rate as to maintain pH within 8.7 - 9.0. After the end of the reaction (stabilization of pH, after about 40 min) the solution obtained was acidified with conc. HCl to pH ~ 2, and the product precipitate was filtered. Additional quantities of the product were obtained after concentrating the filtrate to about 25 ml. The raw product was crystallized from water/charcoal.

Procedure **B**

To the suspension of 0.01 mole of α-aminoester hydrochloride in 15 ml of water-methanol (1:1) at -10°C, 0.01 mole of $NaHCO_3$ was added and the whole was mixed until the end of CO_2 emission (5 - 10 min). To the solutions obtained 0.01 mole 1,4-dinitroimidazole were added in one portion and the suspension obtained was stirred for 6 - 8 h. Next, 10 ml of water were added and the mixture was left at 4°C for several h. The precipitate was filtered off and recrystallized with addition of activated charcoal.

Procedure **C**

To 20 ml of Britton-Robinson buffer pH = 7 were added 0.01 mole of aminobenzoic acid and 10 ml of methanol, and next 0.01 mole of 1,4-dinitroimidazole. The suspension obtained was stirred for 4 h and then left until the next day. The precipitate was filtered off and recrystallized from DMF/water with an addition of activated charcoal.

Procedure **D**

To 40 ml of methanol-water (1:1) was added 0.01 mole of aminophenol or aminoester, and then while stirring, 0.01 mole of 1,4-dinitroimidazole. Stirring was continued for a few h and the mixture was left until the next day. The suspension was diluted with 30 ml of water and the precipitate filtered off. In the case of aminophenols the precipitate was recrystallized from DMF/water. In the case of amino esters the precipitate was heated in 30 ml of DMSO for 1 h in a boiling water bath, precipitated with water and recrystallized from diluted methanol.

Biology

Compounds tested: **6, 7, 8, 11, 14, 15, 18, 19.**

Testing methods:

Testing of the chronic aerobic cytotoxicity and radiosensitizing effectiveness in hipoxy was done in a standard way described for chloronitroimidazoles[6]. Some of the results are summarized in Table 2.

References

1 J.H. Boyer, Nitroazoles, VCH Publishers Inc., Deerfield Beach, Florida 1986.
2 C.E. Voogd, Mutation Research *86*, 243 (1981).
3 P. Galanaud, Pharmacologie Clinique, Paris 1978, p. 1781.
4 R. Klink, K.G.R. Pachler, and R. Gottschlich, Arzneim.-Forsch. *1985*, 1220.
5 J. Morgenstern, R. Otto, and S. Scheithauer, Ger. (East) DD, 260, 062 (1988); C.A. *110*, 231634r (1989).
6 M. Widel, J. Watras, J. Suwinski, and E. Salwinska, Neoplasma *34*, 241 (1987); C.A. *107*, 194206t (1987).
7 R. Chibber, I.J. Stratford, T. Ahmed, A.B. Robbins, D. Goodgame, and B. Lee, Int. J. Radiat. Oncol., Biol., Phys. *10*, 1213 (1984); C.A. *101*, 225872q (1984).
8 M. Grimmett, in Comprehensive Heterocyclic Chemistry, Vol. VI, (ed. A.R. Katritzky, C.W. Ress), Pergamon Press, Oxfort, 1984.
9 J. Suwinski and E. Salwinska, Polish J. Chem. *61*, 913 (1987); C.A. *109*, 128905k (1988).
10 J. Suwinski, E. Salwinska, and M. Bialecki, Stud. Org. Chem. (Amsterdam) (Chem. Heterocycl. Compd.), p. 543.
11 E. Salwinska, J. Suwinski, and M. Bialecki, Polish J. Chem. *65*, 323 (1991).
12 E. Salwinska and J. Suwinski, Polish J. Chem. *64*, 813 (1990); C.A. *115*, 71477q (1991).
13 M.S. Motavia, E.B. Pedersen, J. Suwinski, and C.M. Nielsen, Arch. Pharm. (Weinheim) *323*, 949 (1990).
14 J. Suwinski and W. Szczepankiewicz, J. Labelled Cmpds. (in press).
15 J. Suwinski and W. Szczepankiewicz, Tetrahedron: Asymmetry, *1991*, 941.
16 K.M. Williams and E. Lee, Drugs *30*, 333 (1985).
17 K.M. Williams, Clin. Pharmacol. Theor. *36*, 817 (1984); C.A. *102*, 109022j (1985).

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