Notes

The Influence of Physicochemical Parameters on the Biliary Excretion of a Series of Nitroimidazoles

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The relationship between physicochemical parameters and biliary excretion of nitroimidazoles was investigated. The unmetabolized form of each drug was detected in the bile by means of a UV procedure. A highly significant reversed parabolic relationship was shown between the R_m values and the biliary excretion of the test compounds. In other words, the compounds closer to the optimal R_m value are excreted less than those characterized by higher or lower R_m values. Since the R_m values seem to account for both the lipophilic and polar character of nitroimidazoles, the reversed parabola could be due to plasma protein binding and/or some protein binding within the hepatocyte. In fact, both the lipophilic and polar character seem to play an important role in protein binding of chemicals.

The nitroimidazoles, which play an important role in chemotherapy for their antibacterial, antitrichomonal, and antiamoebic activity,[1,2] have been shown to have mutagenic or carcinogenic effects.[3] Structure–activity relationships have demonstrated the importance of physicochemical parameters in biological activity of nitroimidazoles.[4-7] In previous papers we had studied the relationship between R_m values, retention times, and log P values of a series of 5-nitroimidazoles as an expression of their lipophilic and/or polar character.[8,9] More recently we have shown the influence of the R_m values on the mutagenic activity in vitro of nitroimidazole compounds[10] and a parabolic relationship between log P values and urinary excretion of the same compounds.[11]

The purpose of the present study was to investigate the relationship between physicochemical parameters and biliary excretion of a series of nitroimidazoles. In fact, very little is known about the biliary excretion of drugs.[12] Moreover, the bile may be considered as a source of mutagenic metabolites produced in the liver.[13,14]

Results

Compounds were given by iv infusion to rats. The amounts of the unchanged form of each drug were detected in the bile samples collected at 0–1, 1–2, 2–3, 3–4, 4–5, and 5–6 h after the treatment. The total 1, 2, 3, 4, 5, and 6 h biliary excretion of the unchanged form of each drug was then calculated, and the data are reported in Table II as log BR values, where BR is the percent (×10) of the administered dose recovered from the bile. The log BR values showed that the unchanged forms of all the 12 compounds were present in the bile collected at 0–1 h. While for compounds **11**, **12**, and **5** the unmetabolized forms excreted in that period accounted for 16.7%, 14.3%, and 13.0%, respectively, of the administered dose, for compounds **8**, **6**, and **7** the unmetabolized forms excreted in the same time accounted only for 1.7%, 1.9%, and 2.0%, respectively, of the administered dose. Both eq 1 and 2 describing the relationship between log P values and the biliary excretion in the first hour after application showed very low correlation coefficients. As a further step in the analysis we turned our attention to the R_m values and obtained eq 3 and 4. The introduction of the R_m squared 1 h

$$\log \mathrm{BR} = 1.722\ (\pm 0.108) - 0.205\ (\pm 0.186) \log P \quad (1)$$

$$n = 12 \quad r = 0.330 \quad s = 0.354 \quad F = 1.22 \text{ ns}$$

$$\log \mathrm{BR} = 1.698\ (\pm 0.156) - 0.250\ (\pm 0.278) \log P + 0.096\ (\pm 0.428) \log P^2 \quad (2)$$

$$n = 12 \quad r = 0.337 \quad s = 0.372 \quad F = 0.580 \text{ ns}$$

1 h

$$\log \mathrm{BR} = 1.750\ (\pm 0.131) - 0.186\ (\pm 0.220) R_m \quad (3)$$

$$n = 12 \quad r = 0.258 \quad s = 0.362 \quad F = 0.72 \text{ ns}$$

$$\log \mathrm{BR} = 1.745\ (\pm 0.061) - 1.622\ (\pm 0.260) R_m + 1.467\ (\pm 0.244) R_m^2 \quad (4)$$

$$n = 12 \quad r = 0.902 \quad s = 0.170 \quad F = 19.73 \quad P < 0.005 \quad R_{m0} = 0.553$$

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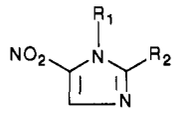
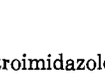
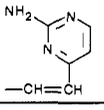
Table I. Structural Formulas and Physicochemical Parameters of the Nitroheterocyclic Compounds



no.	name	R_1	R_2	R_m	log P	$\sum MR_{1,2}$	wavelength, nm
1	5-nitroimidazole	H	H	−0.19	−0.16	0.21	295
2	2-methyl-5-nitroimidazole	H	CH_3	0.26	0.49	0.67	310
3	metronidazole	CH_2CH_2OH	CH_3	0.08	−0.10	1.75	320
4	ipronidazole	CH_3	$CH(CH_3)CH_3$	0.72	1.06	2.06	320
5	1-methyl-2-formyl-5-nitroimidazole	CH_3	CHO	−0.16	−0.69	1.25	310
6	carnidazole	$CH_2CH_2NHC(S)OCH_3$	CH_3	0.81	0.90	3.80	320
7	tinidazole	$CH_2CH_2SO_2CH_2CH_3$	CH_3	0.35	−0.36	3.31	315
8	ornidazole	$CH(CH_2Cl)CH_2OH$	CH_3	0.33	0.60	2.69	320
9	ronidazole	CH_3	CH_2COONH_2	−0.07	−0.38	2.36	310
10	nimorazole		H	0.97	0.07	3.34	305
11	1-methyl-2-(hydroxymethyl)-5-nitroimidazole	CH_3	CH_2OH	−0.14	−0.03	1.28	310
12	azanidazole	CH_3		1.31	0.85	5.07	380

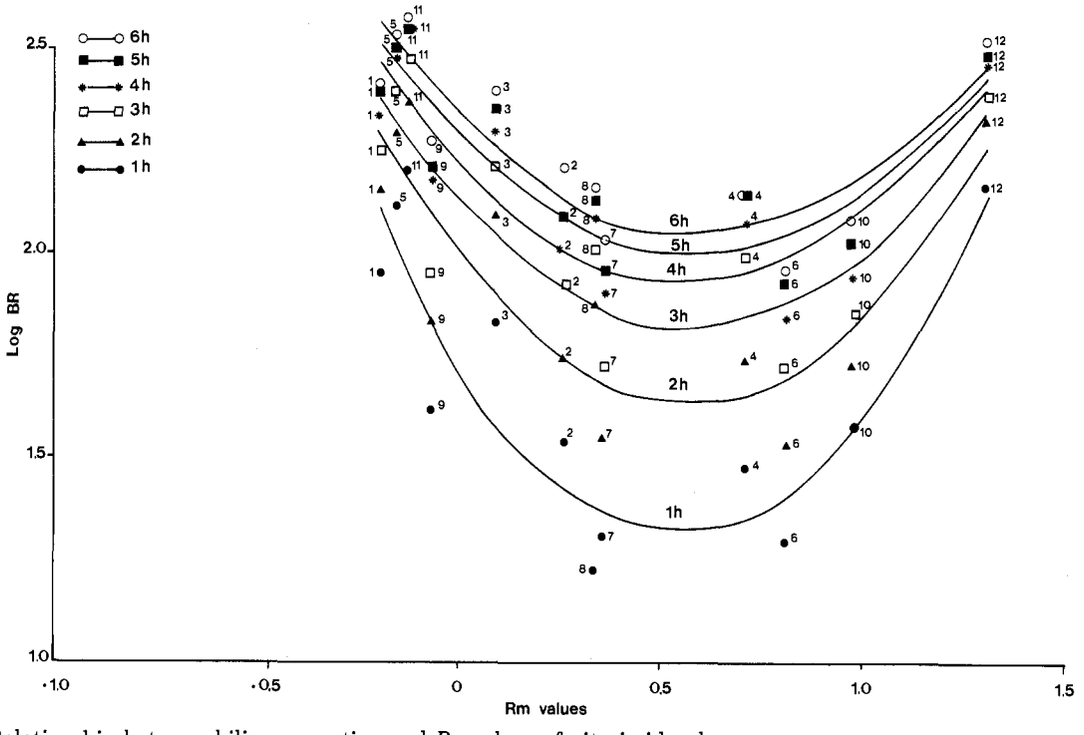


Figure 1. Relationship between biliary excretion and R_m values of nitroimidazoles.

term giving eq 4 provides a highly significant improvement over eq 3. In fact, if we consider that we are dealing with an in vivo experiment, the correlation coefficient of eq 4 is very good. In particular, eq 4 shows a reversed parabolic dependence of the biliary excretion on the R_m values (Figure 1).

In a previous paper we had shown that the R_m values of 5-nitroimidazoles were very poorly correlated with the log P values.[8,9] In fact, the striking difference between eq 2 and 4 can be explained with eq 5, which shows a low

$$R_m = 0.107\ (\pm 0.152) + 0.827\ (\pm 0.256)\ \log P \quad (5)$$
$$n = 12 \quad r = 0.714 \quad s = 0.421 \quad F = 10.41$$
$$P < 0.025$$

correlation coefficient between the R_m and log P values of the present series of 12 compounds. As already reported for a larger series of 22 5-nitroimidazole derivatives, the introduction of the $\sum MR_{1,2}$ term giving eq 6 improved very much the correlation coefficient over eq 5.[8,9] The correlation coefficient between log P and $\sum MR_{1,2}$ is quite low ($r = 0.415$), showing the poor relatedness of the predictor variables in eq 6. In other words, the R_m values of 5-nitroimidazoles seem to account for both the lipophilic and polar character of the molecules.[8,9] The data of Table II on the cumulative biliary excretion over 2, 3, 4, 5, and 6

$$R_m = -0.187\ (\pm 0.120) + 0.383\ (\pm 0.118)\ \log P + 0.213\ (\pm 0.048) \sum MR_{1,2} \quad (6)$$
$$n = 12 \quad r = 0.922 \quad s = 0.204 \quad F = 25.41$$
$$P < 0.005$$

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Table II. Biliary Excretion of Nitroheterocyclic Compounds over 1-, 2-, 3-, 4-, 5-, and 6-h Periods (UV Analysis)

no.	0–1 h		log BR calcd	0–2 h		0–3 h		0–4 h		0–5 h		0–6 h	
	%	log BR		%	log BR	%	log BR	%	log BR	%	log BR	%	log BR
1	8.71	1.940 ± 0.01	1.741	14.12	2.150 ± 0.01	17.58	2.245 ± 0.01	20.89	2.320 ± 0.02	24.27	2.385 ± 0.02	26.18	2.418 ± 0.03
2	3.33	1.522 ± 0.02	1.599	5.54	1.743 ± 0.01	7.98	1.902 ± 0.01	10.20	2.009 ± 0.03	12.64	2.102 ± 0.02	16.19	2.209 ± 0.02
3	6.77	1.831 ± 0.03	1.724	12.46	2.095 ± 0.04	16.77	2.224 ± 0.03	19.69	2.294 ± 0.02	22.31	2.348 ± 0.02	24.55	2.390 ± 0.02
4	2.99	1.476 ± 0.08	1.542	5.37	1.730 ± 0.08	9.60	1.982 ± 0.09	11.69	2.068 ± 0.08	13.97	2.145 ± 0.08	13.97	2.145 ± 0.08
5	12.99	2.114 ± 0.02	1.916	19.18	2.283 ± 0.01	25.03	2.398 ± 0.06	29.16	2.465 ± 0.07	31.14	2.493 ± 0.06	32.00	2.505 ± 0.05
6	1.91	1.281 ± 0.09	1.551	3.43	1.535 ± 0.08	5.11	1.708 ± 0.09	7.00	1.845 ± 0.09	8.42	1.925 ± 0.08	8.94	1.951 ± 0.08
7	1.99	1.299 ± 0.04	1.801	3.47	1.540 ± 0.03	5.25	1.720 ± 0.04	7.58	1.880 ± 0.02	8.81	1.945 ± 0.02	10.47	2.020 ± 0.03
8	1.68	1.225 ± 0.02	1.583	7.33	1.865 ± 0.03	10.58	2.024 ± 0.02	12.32	2.091 ± 0.02	13.52	2.131 ± 0.03	14.72	2.168 ± 0.02
9	4.00	1.602 ± 0.09	1.807	6.96	1.842 ± 0.08	8.88	1.948 ± 0.05	15.49	2.190 ± 0.07	16.22	2.210 ± 0.07	19.72	2.295 ± 0.08
10	3.42	1.535 ± 0.03	1.681	5.25	1.720 ± 0.01	6.92	1.840 ± 0.02	8.71	1.940 ± 0.03	10.71	2.030 ± 0.04	11.75	2.070 ± 0.03
11	16.74	2.224 ± 0.04	1.706	23.27	2.367 ± 0.03	29.63	2.472 ± 0.04	34.59	2.539 ± 0.02	34.59	2.539 ± 0.02	37.77	2.577 ± 0.03
12	14.36	2.157 ± 0.02	1.555	21.08	2.324 ± 0.01	24.99	2.396 ± 0.01	28.45	2.454 ± 0.01	30.62	2.486 ± 0.02	33.12	2.520 ± 0.02

h after the application allowed the calculation of the parabolic equations given in eq 7–11. The plots of Figure 1 and eq 7–11 compared with that of eq 4 show that the reversed parabolic relationship seems to characterize the biliary excretion of the unmetabolized forms of 5-nitroimidazole derivatives all over the period of 6 h. The increasing broadening of the parabolas is due to the higher excretion of the compounds with R_m values closer to the minimum of the parabola which takes place from 2 to 6 h. The lower correlation coefficients of eq 7–11 can be explained on the basis of the narrower range of the log BR values at 2, 3, 4, 5, and 6 h. In fact, $r^2 = 1 - s^2(n-3)/\text{Dev } y$, where Dev y is the sum of squares of the deviations of the y values from their mean.

2 h

$$\log BR = 1.996\ (\pm 0.059) - 1.314\ (\pm 0.251)R_m + 1.150\ (\pm 0.235)R_m^2 \quad (7)$$

$n = 12$ $r = 0.868$ $s = 0.164$ $F = 13.74$ $P < 0.005$ $R_{m0} = 0.571$

3 h

$$\log BR = 2.129\ (\pm 0.061) - 1.110\ (\pm 0.259)R_m + 0.960\ (\pm 0.243)R_m^2 \quad (8)$$

$n = 12$ $r = 0.819$ $s = 0.170$ $F = 9.16$ $P < 0.005$ $R_{m0} = 0.578$

4 h

$$\log BR = 2.236\ (\pm 0.046) - 1.058\ (\pm 0.194)R_m + 0.894\ (\pm 0.182)R_m^2 \quad (9)$$

$n = 12$ $r = 0.876$ $s = 0.127$ $F = 14.82$ $P < 0.005$ $R_{m0} = 0.592$

5 h

$$\log BR = 2.282\ (\pm 0.042) - 0.958\ (\pm 0.178)R_m + 0.817\ (\pm 0.167)R_m^2 \quad (10)$$

$n = 12$ $r = 0.874$ $s = 0.117$ $F = 14.50$ $P < 0.005$ $R_{m0} = 0.586$

6 h

$$\log BR = 2.330\ (\pm 0.039) - 0.937\ (\pm 0.165)R_m + 0.782\ (\pm 0.154)R_m^2 \quad (11)$$

$n = 12$ $r = 0.885$ $s = 0.108$ $F = 16.24$ $P < 0.005$ $R_{m0} = 0.599$

Since the 5- and 6-h parabolas are very close, we may assume that from any practical point of view the unchanged forms of all the compounds had been completely excreted in the bile in a 5-h period.

Discussion

In a previous paper we showed a parabolic relationship between the urinary excretion of a series of 5-nitroimidazoles and nitrothiazoles and their log P values as an expression of the lipophilic character of molecules.[11]

In particular, at 18 h after the treatment we obtained the following equation:

$$\log BR_{urinary} = 2.570\ (\pm 0.042) + 0.050\ (\pm 0.054)\log P - 0.964\ (\pm 0.089)\log P^2 \quad (12)$$

$n = 25$ $r = 0.928$ $s = 0.141$ $F = 68.38$ $P < 0.005$ $\log P_0 = 0.026$

which is very similar to eq 13 calculated for the present series of only 12 compounds:

$$\log BR_{urinary} = 2.568\ (\pm 0.041) + 0.093\ (\pm 0.073) \log P - 0.920\ (\pm 0.103) \log P^2 \quad (13)$$

$$n = 12 \quad r = 0.960 \quad s = 0.098 \quad F = 53.67 \quad P < 0.005 \quad \log P_0 = 0.050$$

As regards the biliary excretion, the situation seems to be quite different. In fact, while eq 1 and 2 show that the lipophilic character, as expressed by the log P values, does not play any role in the biliary excretion, eq 4 and 7–11 show a very significant influence of the R_m values. On the other hand, eq 6, relating R_m values with log P and $\sum MR_{1,2}$ values, seems to suggest that both the lipophilic and polar character are involved in the biliary excretion of this kind of compounds. However, we are dealing with a reversed parabolic relationship; i.e., the compounds closer to the optimal R_m value are less excreted than those characterized by higher or lower R_m values. In other words, the optimal lipophilic and polar character seems to prevent the biliary excretion. This could be due to the plasma protein binding and/or to some protein binding within the hepatocyte of the compounds closer to the minimum of the parabola. In fact, both the lipophilic and polar character seem to play an important role in protein binding of chemicals.

The literature is very sparse as regards quantitative structure–activity relationships describing the biliary excretion of drugs.[15,16] However, Seydel,[16] using log P values, calculated from the data of Ryrfeldt[17] a reversed parabola describing the biliary excretion of penicillins. In the present work compounds were administered iv and therefore the pharmacokinetic processes to be considered should be tubular reabsorption, plasma protein binding, and biotransformation. Since the endothelial cells of the hepatic sinusoids are not tightly connected and show relatively large gaps between them, there should be a free passage to the hepatocytes. Therefore, in order to be delivered from the hepatic sinusoids to the bile canaliculi, drugs should only pass through the hepatocytes by a process of passive diffusion.

The percent of the administered dose not recovered from the bile must have been biotransformed in the liver or excreted in the kidney. In our experiment, the enterohepatic cycle has been suppressed since the common bile duct had been cannulated. In normal animals, drugs endowed with physicochemical properties favored for passive diffusion across the intestinal barrier and excreted by the liver into bile would be capable of being almost completely reabsorbed in the small intestine. Such drugs would remain in the enterohepatic cycle until they are biotransformed or excreted in the urine.

Experimental Section

Chemicals. The nitroimidazole derivatives listed in Table I belong to a larger series of compounds that had been obtained from commercial sources and drug companies as previously reported.[11] All other chemicals and solvents were of reagent grade.

Physicochemical Parameters. The determination of the log P and R_m values as well as the calculation of the molar refractivity values summed over the R_1 and R_2 groups ($\sum MR_{1,2}$) have been described previously.[8,9]

Animal Experiments. The following procedure, which is similar to that of Levine,[18] was used for the collection of the bile. Male Sprague–Dawley rats weighing 350–450 g were anesthetized with urethane. The urethane was injected ip as a 15% solution in saline. The rats received between 1.25 and 1.5 g/kg, as required. After anesthesia was achieved, the animals were restrained on a dissecting board, and their temperature was maintained at 36–37 °C with an infrared lamp 60 cm above the animal. A small abdominal incision was made and the common bile duct was exposed and cannulated with use of polyethylene tubing (0.58 i.d. × 0.956 mm o.d. × 18 cm) with a beveled end. The cannula was then secured with surgical thread immediately below the ligation. As soon as the bile was flowing out, the incision was closed and covered with gauze sponges saturated with saline. The bile was collected in 2.0-mL polyethylene liquid nitrogen vials at room temperature. In the control animals, the bile was collected for six 1-h intervals. In the treated animals, the bile was collected for 0.5 h after cannulation prior to the injection of the drugs. After injection of the drug, the bile was collected for the same time intervals as with the untreated animals. All drugs were injected iv into either femoral vein in Me_2SO solution (100 μmol/mL^{-1} kg^{-1}). An incision was made to expose the vein, and the drugs were injected by means of a 25-gauge needle attached to polyethylene tubing. An infusion pump was used to ensure that the drugs were injected at a slow rate (ca. 1 mL/5 min). Each test compound was administered to groups of six animals. Preliminary experiments had been carried out in order to rule out the possibility of toxic effects of Me_2SO.

Detection of Test Compounds in Bile. The biliary concentration of the unchanged form of each drug was detected by means of UV analysis at the appropriate wavelength reported in Table I. A Perkin-Elmer 12 double-beam spectrophotometer was used. In a previous paper it was shown that the metabolites of each parent compound have different UV wavelengths.[19] Three or four different volumes of each bile sample were diluted to 2.5 mL with water in the UV cuvette and read against a blank. This was prepared with equivalent volumes of bile collected from the same animal during the 30 min after the cannulation and prior to the injection of the drug. Finally the concentration of the unmodified forms was determined by means of a standard curve for each compound.

Registry No. **1**, 3034-38-6; **2**, 696-23-1; **3**, 443-48-1; **4**, 14885-29-1; **5**, 4750-57-6; **6**, 42116-76-7; **7**, 19387-91-8; **8**, 16773-42-5; **9**, 7681-76-7; **10**, 6506-37-2; **11**, 936-05-0; **12**, 62973-76-6.

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