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STRUCTURE–DISTRIBUTION RELATIONSHIPS OF RADIOPHARMACEUTICALS

CORRELATION BETWEEN THE REVERSED-PHASE CAPACITY FACTORS FOR Tc-99m PHENYLCARBAMOYLMETHYLIMINODIACETIC ACIDS AND THEIR RENAL ELIMINATION

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SUMMARY

Capacity factors (k') have been obtained for twenty-eight different phenyl substituted phenylcarbamoylmethyliminodiacetic acids (HIDAs) using reversed-phase high-performance liquid chromatography (HPLC). They have been shown to be a useful measure of the lipophilicity of the ligands and their technetium-99m complexes. When k' for the ligands or log P_{oct} for the Tc-99m complexes was plotted against theoretical lipophilicity, three groups of HIDAs were observed. The membership of each group is determined by the degree of *ortho* substituion. The effect of lipophilicity on protein binding allows the use of ligand capacity factors to predict the routes of elimination of various Tc-HIDAs.

INTRODUCTION

The use of radiopharmaceuticals as *in vivo* diganostic agents is based on their pharmacokinetic properties and in almost all cases pharmacological activity is undesirable. The most common radionuclide used in nuclear medicine today is the short lived gamma emitter, technetium-99m ($t_{1/2}$ 6.0 h). This is produced from a generator as the daughter of a longer lived parent, molybdenum-99. Technetium is present in the 0.9% NaCl generator eluate at a concentration of about 10^{-6}–10^{-7} M^1 and is almost exclusively in the form of pertechnetate, TcO_4^-. The pertechnetate is reduced and bound to a variety of chelating ligands in order to change its *in vivo* distribution. These ligands are designed so that the injected dose distributes in the body in the manner necessary to achieve the high target to background ratios that are required for good images.

A goal of radiopharmaceutical development is to determine structure–distribution relationships (SDRs) which are analogous to structure–activity relationships. The correlations required for the development of quantitative SDRs can be divided into two groups; in one instance *in vivo* distribution is correlated with physiochemical data, in the other its correlated with chemical structure.

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Phenyl substituted technetium-99m complexes of phenylcarbamoylmethyliminodiacetic acids (Tc-HIDAs) are routinely used in nuclear medicine. After intravenous injection they are excreted by the functioning hepatobiliary system and are used for the differential diagnosis of acute cholecystitis[2]. High-performance liquid chromatography (HPLC) analysis of the radioactivity in the bile shows that the Tc-HIDAs are excreted essentially unchanged[3]. Re-injection of excreted radioactivity gives essentially the same distribution[4]. Although the complexes are somewhat susceptible to air oxidation *in vivo*, their *in vivo* stability is high as excretion of unchanged drug still occurs in patients with compromised hepatobiliary systems up to 24 h after injection[2]. The ideal hepatobiliary agent should possess rapid hepatobiliary excretion with high specificity for the liver and negligible renal clearance. This paper reports physiochemical data on a series of HIDAs and their technetium complexes. These data have been correlated with biological data obtained with the Tc-HIDA complexes.

EXPERIMENTAL

Materials

The ligands were synthesized using the general method reported by Burns *et al.*[5]. This involves the reaction of the appropriate aniline derivative with the anhydride of nitrilotriacetic acid prepared *in situ*. The ligands were analyzed by infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR), elemental analysis and other accepted analytical methods with satisfactory results. The technetium-99m complexes were made using standard radiopharmaceutical procedures.

Methods

The radiochemical purity was measured by HPLC. The HPLC system consisted of two Altex 110A pumps controlled by an Altex 420 microprocessor and was run in an isocratic mode with a Rheodyne 7125 or 7126 injector. Flow-rates of 1.0 ml min^{-1} were employed and gave maximum pressures of *ca.* 1500 p.s.i. with the columns used. Two in-line detectors were coupled in series using low-volume tubing. The first was a Schoeffel SF 770 absorbance detector set at 235 nm and 0.4 a.u.f.s. The second was a flow-through gamma detector having a 10-μl active volume inside a shielded 3 × 3 in. NaI(Tl) well type scintillation detector. The output from the crystal was fed into both a multichannel analyzer (MCA) in multiscaler mode and a chart recorder via a digital to analogue converter. This allowed the simultaneous acquisition of analogue and digital data (Fig. 1).

To determine radiochemical purity a reversed-phase system was used consisting of a 250 × 4.0 mm μBondapak C_{18} column (Altech) eluted with 0.025 *M* phosphate buffer (pH 6.8) with methanol as modifier in approximately 40:60 proportions. Sample volumes were 2–20 μl.

The lipophilicity of the ligands was measured using a similar reversed-phase system except that 1 % aqueous acetic acid–methanol (60:40) was the eluent used. The individual ligands were dissolved in acetone–methanol (2:1) at approximately 10 mg ml^{-1} and 2–20 μl were injected.

Hepatobiliary specificity was measured in rats 30 min after injection by sacrificing the rats and determining the radioactivity in selected organs[6].

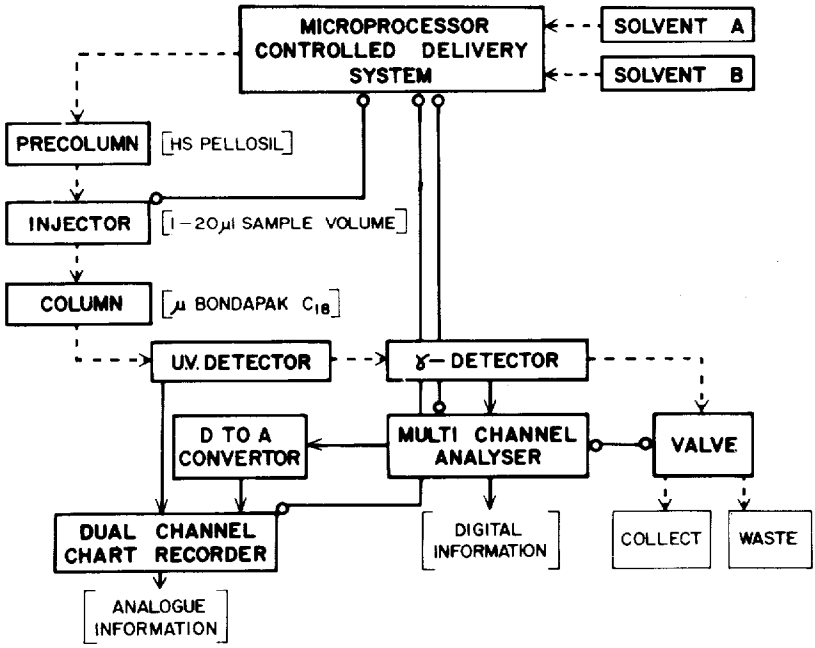


Fig. 1. Schematic diagram of the HPLC system used in these studies.

The measured lipophilicity of the ligands was taken to be the capacity factor (k') for each of the ligands under identical chromatographic conditions[7]. Acetone was used to determine the system's dead volume between the injector and detector.

The relative theoretical lipophilicity ($\Sigma\pi$) was obtained using the tabulations of Hansch and Leo[8] and is the sum of the fragmentary π values of the substituents on the phenyl ring of the ligand. It does not include the lipophilicity contributed by the remainder of the ligand which is assumed to be constant. Capacity factors and theoretical lipophilicities of the ligands together with previously reported log P_{oct} values for selected Tc-complexes[9] are shown in Table I.

RESULTS

The ligands studied are listed in Table I which is a summary of the results obtained with both ligands and their technetium complexes. All technetium complexes had greater than 90% of the radioactivity in a single peak as measured by HPLC and are assumed to be bischelate complexes with Tc(III)[10,11]. Radiochemical purities were obtained using HPLC by integrating the counts in each peak seen on the MCA. Such a procedure will give false results if a portion of the radioactivity remains on the column. Of the two common radiochemical contaminants of technetium radiopharmaceuticals, TcO_4^- and $TcO_2(OH)_n$[12,13], the latter species can form colloids and be filtered out by HPLC columns. To check for such species the total counts collected after injection of a pure sample of essentially unretained $^{99m}TcO_4^-$ stock solution was compared with comparable samples of the Tc-99m complex. Greater

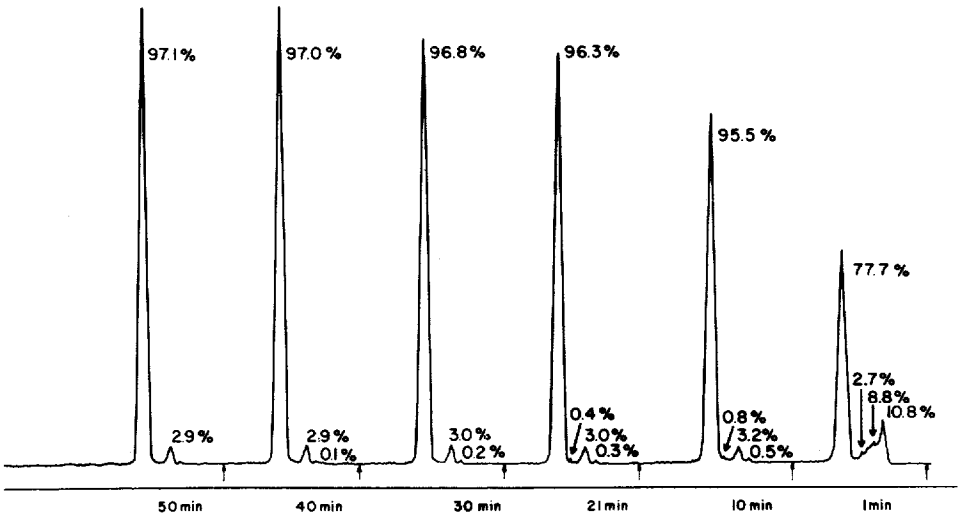


Fig. 2. Radiochromatogram of a Tc-99m-mebrofenin kit at various after reconstitution.

than 97% of the injected kit radioactivity was routinely recovered in the eluate. No interaction between the stainless steel in the system and the injected radioactivity has been observed.

The retention of the Tc-complexes was greater than that of the ligands alone because of the increase in lipophilicity that occurs when two ligand molecules bind to one technetium atom via their carboxyl groups. The "no carrier added" nature of the preparations, and technetium radiopharmaceuticals in general, means that no mass trace was obtained by HPLC corresponding to the technetium complexes.

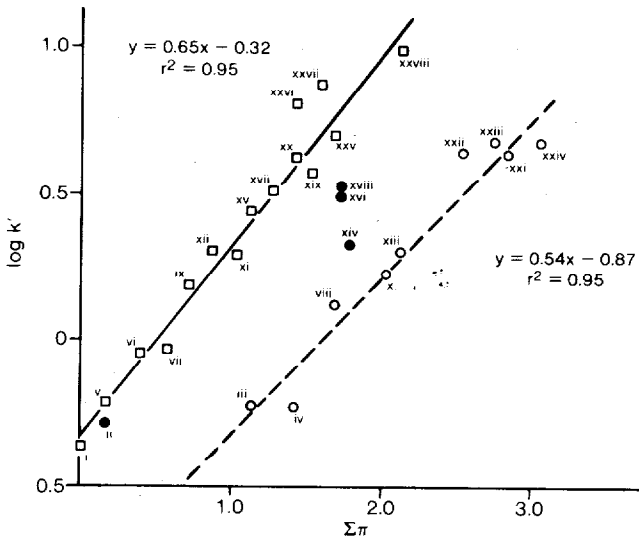
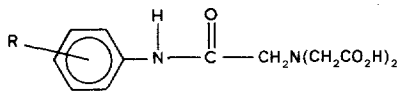


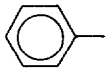
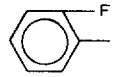
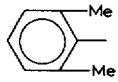
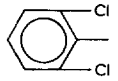
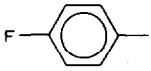
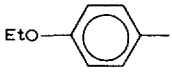
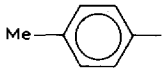
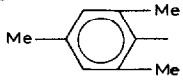
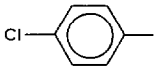
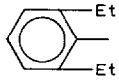
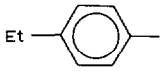
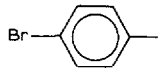
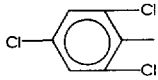
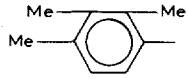
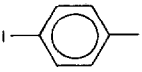
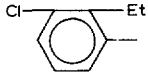
Fig. 3. Correlation between log k' for the ligands and their theoretical lipophilicity. □ = No *ortho* substituents; ● = mono-*ortho* substitution; ○ = di-*ortho* substitution.

TABLE I

EXPERIMENTAL AND PREDICTED LIPOPHILICITIES AND BIOLOGICAL DATA ON THE COMPOUNDS TESTED

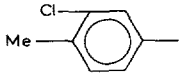
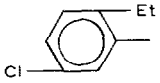
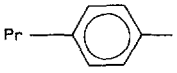
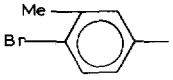
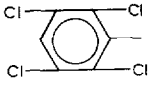
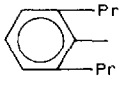
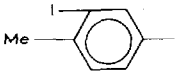
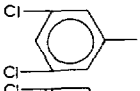
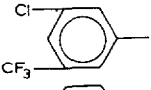
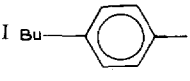
Me = Methyl, Et = ethyl, Pr = propyl, Bu = butyl.



	R	*Log k'* *(ligand)*	*Log P_{oct}** *(complex)*	*Σπ*	*Renal clearance*** *(%)*	*Hepatic clearance**** *(%)*
I		−0.37		0.00		
II		−0.30		0.14		
III		−0.24	−1.42	1.12	15.1	63.4
IV		−0.24		1.42		
V		−0.22	−1.02	0.14		
VI		−0.05		0.38		
VII		−0.04	−0.42	0.56		
VIII		0.10	−1.12	1.68	6.8	88.1
IX		0.18		0.71		
X		0.21	−0.85	2.04	8.2	83.8
XI		0.28	−0.03	1.02		
XII		0.29		0.86		
XIII		0.29		2.13		
XIV		0.32	−1.43	1.68	17.6	72.6
XV		0.43		1.12		
VI		0.49		1.73	17.9	69.9

(Continued on p. 96)

TABLE I *(continued)*

	R	*Log k′ (ligand)*	*Log P_{oct}* (complex)*	*Σπ*	*Renal clearance** (%)*	*Hepatic clearance*** (%)*
XVII		0.50		1.27	7.0	77.3
XVIII		0.53		1.73	11.3	83.2
XIX		0.56	0.24	1.53		
XX		0.61		1.42	4.8	91.9
XXI		0.62		2.84		
XXII		0.63		2.54	1.2	94.8
XXIII		0.66		2.75	2.7	90.1
XXIV		0.66	−0.38	3.06	7.4	89.6
XXV		0.69		1.68	4.7	86.8
XXVI		0.80		1.42	28.6	50.4
XXVII		0.86		1.58	3.6	89.1
XXVIII		0.98	0.64	2.13	3.6	86.0

* From ref. 9.
** Kidneys + bladder and contents.
*** Liver + gastrointestinal tract.

HPLC demonstrated that a radioactive reaction intermediate was present during the formation of the final product[14]. The reaction was normally complete in 15–30 min at room temperature (Fig. 2). All animal studies were performed using samples having greater than 90% of the radioactivity in the final product.

Three groups of ligands are observed when log k' is plotted against the theoretical lipophilicity (Fig. 3). The membership of a group is determined by whether the

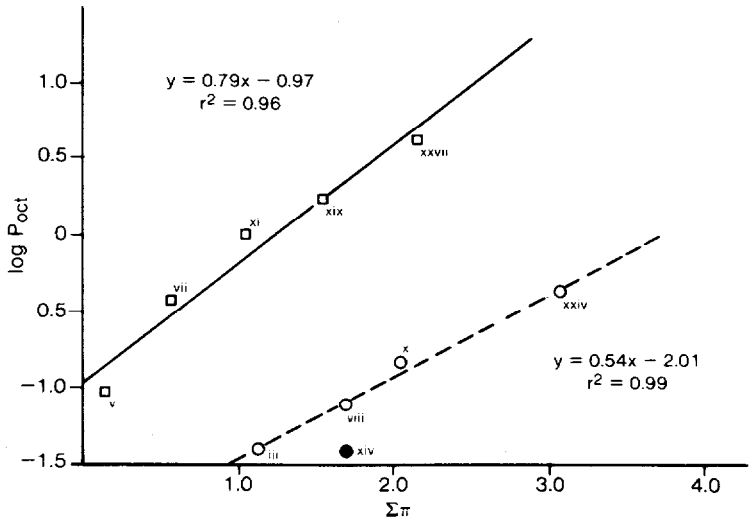


Fig. 4. Correlation between log P_{oct} for the Tc-complexes and their theoretical lipophilicity. □ = No *ortho* substituents; ● = mono-*ortho* substitution; ○ = di-*ortho* substituents. Practical data from ref. 9.

ligand has 0, 1 or 2 *ortho* substituents. The slopes of the lines joining the members of the two main groups are comparable. A similar pattern emerges when log P_{oct} of the Tc complex is plotted against the theoretical lipophilicity of the corresponding ligand (Fig. 4).

A plot of the renal clearance of a complex *vs.* the measured lipophilicity for the corresponding ligand is shown in Fig. 5. A straight line can be drawn through the data encompassing the two main groups of ligands. Mono-*ortho* ligands fall off this line.

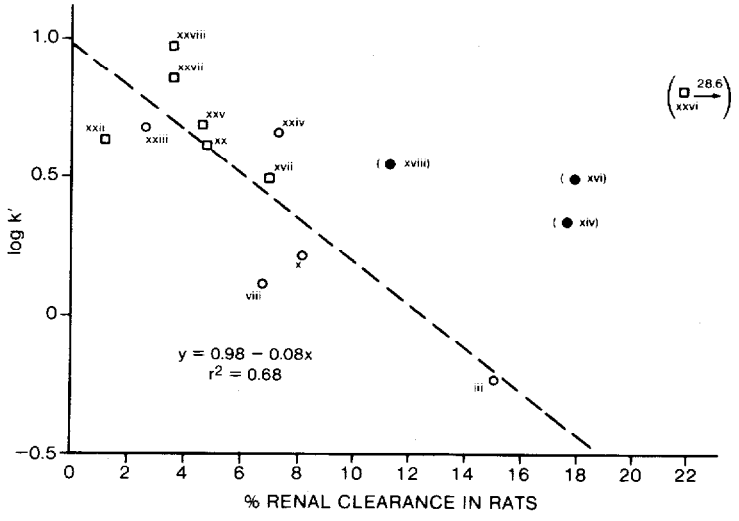


Fig. 5. Correlation between log k' for the ligands and renal clearance for the Tc-complexes. □ = No *ortho* substituents; ● = mono-*ortho* substitution; ○ = di-*ortho* substituents.

DISCUSSION

When developing hepatobiliary radiopharmaceuticals a fine line must be drawn between the lipophilicity required for low renal clearance and excessive lipophilicity which contributes to slow hepatocellular transit times[9]. SDRs are needed that prompt the synthesis of an agent that is specifically and rapidly excreted by the liver. The problem is exacerbated by the fact that the structure of most technetium radiopharmaceuticals is unknown because of the difficulties inherent in working with technetium compounds[15].

In the case of the Tc-HIDAs more is known of the structure than most other radiopharmaceuticals (*e.g.* stoichiometry oxidation state, overall charge[10]). However the arrangement of the central core of atoms is still uncertain because of an inability to isolate and characterize the pure complex in macroscopic amounts. As can be seen in Fig. 2 the radiochromatogram shows the reaction leading to the final bischelate species proceeds by at least one intermediate which at equilibrium in this preparation constitutes *ca.* 3% of the total radioactivity. This phenomenon is general for the HIDAs and occurs with syntheses using chromium as well as technetium. The rate of formation of the intermediate is rapid but the rate of conversion to the final product varies depending upon reaction conditions and the substitution pattern of the HIDA[14]. The two species have also been observed using gel permeation chromatography[16] and it has been suggested that they are mono- and bischelate complexes. Using mg amounts of Tc-99 we have found it difficult to drive the reaction through to completion. We have observed a Tc = 0 stretch in the IR spectrum of the intermediate which is not present in the final product but we cannot confirm the assignment of a monochelate structure to the intermediate.

The lipophilicity of a compound has traditionally been described by its organic/aqueous partition coefficient measured using "shake-flask" methods. More recently a variety of reversed-phase HPLC methods have been published[7,17–19]. If there are no modifying effects, a plot of the measured lipophilicity *vs.* the theoretical lipophilicity for a series of ligands should produce a straight line. When the log k' values for the HIDA ligands were plotted against their theoretical lipophilicities, the ligands separated into three groups whose membership was determined by the degree of *ortho* substitution (Fig. 3). The two main groups are obviously linearly related with similar slopes. These data suggest that occupation of the *ortho* positions causes a drop in the measured lipophilicity from that predicted by theory. (The alternative, an increase in the lipophilicity of *para*-substituted derivatives is unlikely.) In practical terms, 2,6-substituted derivatives possess slightly lower measured lipophilicities than the corresponding 4 substituted compounds. This is illustrated by the relative positions of the *o*-dimethyl or *o*-dichloro HIDAs (III, IV) *vs.* their *p*-methyl or *p*-chloro congeners (VII, IX). (Figure 3). This shortfall in measured lipophilicity is barely made up by the addition of a third substituent in the *para* position (*e.g.*, VIII, XIII *vs.* VII, IX). Similar results are obtained when the published log P_{oct} values of the complexes are plotted against $\Sigma\pi$ (Fig. 4).

The problem of a measured lipophilicity that is lower than that predicted by theory has been addressed before and is thought to be due to shelf shielding[20]. The groups are not artefacts of the HPLC system, nor are they confined to either the ligands or Tc complexes alone. The data in Figs. 3 and 4 confirm that the ring

substituents are the main determinants of the differences in the relative lipophilicity of the ligands and the Tc-complexes and allow the prediction of lipophilicity from tabulated π values.

Protein binding of a compound is known to influence its renal clearance. It is accepted that the main determinant of a compound's non-specific protein binding is its lipophilicity. Two groups of Tc-HIDAs have been previously described using an albumin affinity column assay to measure protein binding[21]. They are complexes with *para*-substituted ligands which show appreciable binding (which increases with the chain length of the substituent), and those with *ortho*-substituted ligands which show negligible binding. The low protein binding of complexes with di-*ortho*-substituted ligands is most likely caused by the lower than expected lipophilicity of these ligands that is described above. If this is so, there should be a correlation between measured lipophilicity and renal clearance. *In vivo* testing was performed to see if such a correlation could be found.

The results presented graphically in Fig. 4 demonstrate that there is indeed a simple linear relationship between the measured lipophilicity and renal clearance for the two main groups of ligands. As the lipophilicity increases the renal clearance decreases and the hepatic clearance increases (Table I). Thus, contrary to the opinion of Molter and Kloss[9] lipophilicity and protein binding can be used to predict the *in vivo* distribution of Tc-HIDAs. Additional factors may play a role in the elimination of complexes with mono-*ortho*-substituted ligands since their elimination is not predicted by the relationship shown in Fig. 5.

The hepatocellular transit time could not be predicted from either the ligand's lipophilicity or the protein binding of the complex. However, it has been determined that faster hepatocellular transit times can be achieved by designing derivatives with small alkyl substituents in the *ortho* position and with additional substituents in the *meta* and *para* positions to produce the required lipophilicity[22,23]. Ultimately this work has lead to the synthesis of a new hepatobiliary agent Tc-mebrofenin (XXII) that has excellent *in vivo* properties in rats and rabbits and which is exhibiting the predicted distribution in man[22,24].

CONCLUSION

The difficulties of handling "no carrier added" technetium-99m compounds have in the past hindered the collection of adequate data for such studies. Some of these problems have been circumvented in this work by assuming that the central core of the complexes is the same in all cases and that it exerts a constant effect on the remainder of the molecule. Thus much of the work of ranking the Tc-HIDAs has been done with the ligands alone. The validity of this procedure is confirmed by the correlations in Figs. 3 and 4. It is not possible, nor useful to use the ligands rather than the complexes to obtain *in vivo* data because it has been demonstrated that the ligands and the metal complexes are handled differently in the body[25].

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