Binding of Certain Nonsteroid Antiinflammatory Agents and Uricosuric Agents to Human Serum Albumin

William J. Dunn, III

Department of Medicinal Chemistry, University of Illinois at the Medical Center, Chicago, Illinois 60680. *Received October 24, 1972*

The I_{50} for displacement from human serum albumin (HSA) of 5-dimethylaminonaphthalene-1-sulfonamide (DNSA) by a set of nonsteroid antiinflammatory agents and uricosuric agents is analyzed using the Hansch model and the 1-octanol–water system as a reference for lipophilic character. The set of displacing agents was divided into three subsets and each subjected to regression analysis. A structural feature common to one series was the carboxy group and to the other the benzenesulfonamido pharmacophore. The results indicate that the displacing ability of each series is highly dependent upon lipophilic character and that the binding site is highly specific for the benzenesulfonamido group.

The binding of drugs to extracellular protein, in particular, the albumin fraction of blood protein, has long been recognized as an important factor for consideration in drug design. Binding to serum albumin is known to effect the bioavailability and level of response of certain pharmaceuticals. For example, phenylbutazone will displace warfarin bound to serum albumin *in vivo* and drastically affect its blood levels and its anticoagulant effects.[1] Other drugs are known to bind to specific sites on human serum albumin.[2,3]

The binding of drugs to specific sites is the result of a number of possible types of interactions, *e.g.*, electrostatic, hydrogen bonding and hydrophobic bonding. The objectives of protein binding studies approached from a physicochemical point of view are to isolate and quantitate the various contributions to binding that result from these aforementioned interactions. An appropriate operational model for quantitating protein binding of drugs in terms of their physicochemical properties is the extrathermodynamic model of Hansch.[4]

The compound 5-dimethylaminonaphthalene-1-sulfonamide (DNSA) has been shown to bind *in vitro* to a specific site on human serum albumin (HSA).[2] Various drugs with an affinity for this site can compete with DNSA and displace it from this site. Whitehouse, *et al.*,[3] have reported the I_{50} for displacement of DNSA from HSA by approximately 60 compounds of various structure types, some of which are important nonsteroid antiinflammatory agents and uricosuric drugs. This report deals with the analysis of recently reported[3] data for the binding of certain carboxylic acids, sulfonamides, and sulfonylureas to HSA using this approach.

Method. The binding data analyzed in this study were used as reported[3] and are given in Tables I and II. 1-Octanol–water partition coefficients were taken from the literature,[5] obtained experimentally in this laboratory according to published procedures,[6] or estimated taking advantage of the additive-constitutive nature of log P.[6] The log P values obtained in this laboratory are reported as the average of at least three determinations and are reported as that for the neutral form of the drug. C is the concentration of the drug in moles per liter required to displace 50% of the 5-dimethylaminonaphthalene-1-sulfonamide bound to human serum albumin at pH 7.4 and 37°. The regression equations were obtained using a nonweighted multiple linear regression program and the IBM 370/155 computer of the Research Resources Laboratory of the University of Illinois at the Medical Center.

Results and Discussion

The structure types represented in the study are carboxylic acids, sulfonyl compounds, phenylbutazone and compounds related to it, and a group of miscellaneous neutral compounds. Attempts to treat all compounds in one equation resulted in a poor correlation indicating that the structurally different agents should be treated separately. The set was divided into three subsets: (1) compounds containing the carboxy group, (2) compounds containing the benzenesulfonamido group, and (3) compounds containing neither group. The carboxy subset contained 29 compounds, the benzenesulfonamido subset contained

Table I. Binding of Benzenesulfonamido Compounds to HSA

		Log 1/C		
	Log P	Obsd	Calcd	$\lvert\Delta$ log 1/$C\rvert$
1. *p*-(Dipropylsulfamyl)benzoic acid (probenicid)	3.21[a]	2.60	2.56	0.04
2. N^1-Acetylsulfanilamide (sulfacetamide)	0.53[b]	3.12	3.15	0.03
3. Sulfapyrimidine (sulfadiazine)	0.13[b]	3.52	3.53	0.01
4. 1-[*p*-(1-Hydroxyethyl)phenylsulfonyl]-3-cyclohexylurea (1-hydroxyhexamide)	1.82[c]	3.52	3.88	0.36
5. *dl*-Hydroxyhexamide	1.82[c]	3.60	3.88	0.28
6. 1-(*p*-Acetylphenylsulfonyl)-3-cyclohexylurea (acetohexamide)	1.98[d]	3.70	3.78	0.08
7. 1-Butyl-3-*p*-tosylurea (tolbutamide)	2.34[a]	3.70	3.53	0.17
8. Sulfamethoxypyradazine (sulfapyradazine)	0.40[b]	3.82	3.87	0.05
9. Sulfamethoxypyradazine (sulfamethizole)	0.54[b]	3.98	3.93	0.05
10. Sulfamethylisoxazole (sulfaisoxazole)	1.15[b]	4.00	4.05	0.05
11. Sulfaphenylpyrazole (sulfaphenazole)	1.57[b]	4.12	3.98	0.14
12. Sulfadimethoxypyrimidine (sulfamethoxine)	1.56[b]	4.22	3.97	0.25
13. Sulfaethylthiadiazole (sulfaethidole)	1.04[e]	4.30	4.05	0.25

[a] Experimental value, this laboratory. [b] Reference 5. [c] Log P = log $P_{\text{acetohexamide}}$ + log $P_{\text{1-phenylethanol}}$ − log $P_{\text{acetophenone}}$ = 1.98 + 1.42[a] − 1.58 = 1.82. [d] Log P = log $P_{\text{tolbutamide}}$ − $\pi_{C_4H_9}$ − π_{CH_3} + $\pi_{c\text{-}C_6H_{11}}$ + π_{COCH_3} = 2.34 − 2.00 − 0.50 + 2.51 − 0.37 = 1.98. [e] Log P = log $P_{\text{sulfamethizole}}$ + π_{CH_2} = 0.54 + 0.50 = 1.04.

Table II. Binding of Carboxy Compounds to HSA

	Log P	Log $1/C$ Obsd	Log $1/C$ Calcd	$\lvert\Delta \log 1/C\rvert$
1. Hexahydrosalicylic acid	0.77[a]	1.92	1.97	0.05
2. *p*-Hydroxybenzoic acid	1.58[b]	2.20	2.77	0.57
3. 4-Aminosalicylic acid	1.07[c]	2.26	2.28	0.02
4. *O*-Acetylsalicylic acid	1.23[b]	2.70	2.44	0.26
5. 2,4-Dihydroxybenzoic acid	1.44[e]	2.75	2.64	0.11
6. α-(4-Chlorophenoxy)isobutyric acid	2.59[d]	3.10	3.56	0.46
7. 2,3-Dihydroxybenzoic acid	2.24[b]	3.30	3.31	0.01
8. Salicylic acid	2.26[b]	3.35	3.33	0.02
9. 4-Allyloxy-3-chlorophenylacetic acid (mervan)	2.48[e]	3.50	3.48	0.02
10. 2-(4-Chlorophenyl)thiazol-4-ylacetic acid (fenclozic acid)	3.29[e]	3.52	3.97	0.45
11. D-2-(6-Methoxy-2-naphthyl)propionic acid (naproxen)	3.18[e]	3.63	3.91	0.28
12. α-(4-Isobutylphenyl)propionic acid (ibuprofen)	3.51[e]	3.70	4.07	0.37
13. 2-Phenoxybenzoic acid	3.89[f]	3.70	4.23	0.53
14. 2,6-Dihydroxybenzoic acid	2.20[b]	3.80	3.28	0.52
15. β-(4,5-Diphenyloxazol-2-yl)propionic acid	4.19[e]	3.92	4.33	0.41
16. 2,3-Dichloro-4-(2-methylenebutyryl)phenoxyacetic acid	3.24[g]	4.00	3.94	0.06
17. 3,5-Di-*tert*-butyl-6-hydroxysalicylic acid	5.56[h]	4.00	4.52	0.52
18. 1-(4-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid (indomethacin)	3.08[e]	4.30	3.88	0.42
19. 3,4,5,6-Tetrafluorosalicylic acid	3.44[i]	4.30	4.04	0.26
20. 3-Hydroxycinchophen	4.43[j]	4.40	4.39	0.01
21. Cinchophen	4.04[k]	4.40	4.28	0.12
22. *N*-(2,3-Xylyl)anthranilic acid	4.70[l]	4.40	4.45	0.05
23. *p*-Isobutylphenylacetic acid	3.21[m]	4.40	3.93	0.47
24. *N*-(2,6-Dichloro-*m*-tolyl)anthranilic acid	6.31[n]	4.45	4.44	0.01
25. *N*-(α',α',α'-Trifluoro-*m*-tolyl)anthranilic acid	4.88[o]	4.45	4.47	0.03
26. 2-(3,4-Dichlorobenzamido)phenoxyacetic acid	3.36[p]	4.46	4.00	0.46
27. 4-*p*-Chlorophenyl-3-trifluoromethylphenoxyacetic acid	4.99[q]	4.45	4.49	0.04
28. 3,5-Di-*tert*-butylsalicylic acid	5.62[r]	5.00	4.52	0.48
29. 3,5-Diiodosalicylic acid	4.56[e]	5.00	4.42	0.58

[a] Log P = log $P_{\text{cyclohexanol}}$ + (log $P_{\text{lactic acid}}$ − log P_{ethanol}) = log $P_{\text{cyclohexanol}}$ + $\pi_{\text{–COOH α to –CHOH}}$ = 1.23 − 0.62 + 0.16 = 0.77. [b] Reference 5. [c] Log P = log $P_{\text{salicylic acid}}$ + $\pi_{4\text{-NH}_2}$ = 2.26 − 1.19 = 1.07. [d] Log P = log $P_{\text{4-chlorophenoxyacetic acid}}$ + $2\pi_{\text{branched CH}_3}$ = 1.99 + 0.60 = 2.59. [e] Experimental value, this laboratory. [f] Log P = log $P_{\text{diphenyl ether}}$ + π_{COOH} = 4.21 − 0.32 = 3.89. [g] Log P = log $P_{\text{phenoxyacetic acid}}$ + $\pi_{2\text{-Cl}}$ + $\pi_{3\text{-Cl}}$ + $\pi_{\text{–COC(=CH}_2)\text{C}_2\text{H}_5}$ = 1.26 + 0.59 + 0.76 + 0.63 = 3.24. [h] Log P = log $P_{\text{2,6-dihydroxysalicylic acid}}$ + $2\pi_{\text{–C(CH}_3)_3}$ = 2.20 + 2(1.68) = 5.56. [i] Log P = log $P_{\text{salicylic acid}}$ + $\pi_{\text{tetrafluoro}}$ = 2.26 + 1.18 = 3.44. [$\pi_{\text{tetrafluoro}}$ = log $P_{\text{2,3,4,5-tetrafluorophenol}}$ − log P_{phenol} = 2.64 − 1.46 = 1.18]. [j] Log P = log $P_{\text{naphthalene}}$ + π_{phenyl} + π_{COOH} + π_{OH} = 2.03 + 1.89 + 0.12 + 0.39 = 4.43. [k] Log P = log $P_{\text{3-hydroxycincophen}}$ − π_{OH} = 4.43 − 0.39 = 4.04. [l] Log P = log $P_{\text{anthranilic acid}}$ + $\pi_{\text{phenyl from diphenylamine}}$ + $\pi_{2\text{-CH}_3}$ + $\pi_{3\text{-CH}_3}$ = 1.21 + 2.60 + 0.39 + 0.50 = 4.70. [m] Log P = log $P_{\text{phenylacetic acid}}$ + $\pi_{i\text{-C}_4\text{H}_9}$ = 1.41 + 1.80 = 3.21. [n] Log P = log $P_{\text{anthranilic acid}}$ + π_{phenyl} + $\pi_{2\text{-CH}_3}$ + $2\pi_{\text{Cl from 2-chloroaniline}}$ = 1.21 + 2.60 + 0.50 + 2.00 = 6.31. [o] Log P = log $P_{\text{anthranilic acid}}$ + π_{phenyl} + $\pi_{3\text{-CF}_3}$ = 1.21 + 2.60 + 1.07 = 4.88. [p] Log P = log $P_{\text{phenoxyacetic acid}}$ + log $P_{\text{benzamide}}$ + $\pi_{3\text{-Cl}}$ + $\pi_{4\text{-Cl}}$ = 1.26 + 0.64 + 0.70 + 0.76 = 3.36. [q] Log P = log $P_{\text{phenoxyacetic acid}}$ + $\pi_{\text{phenyl from biphenyl}}$ + $\pi_{4\text{-Cl}}$ + $\pi_{3\text{-CF}_3}$ = 1.26 + 1.96 + 0.70 + 1.07 = 4.99. [r] Log P = log $P_{\text{salicylic acid}}$ + $2\pi_{\text{–C(CH}_3)_3}$ = 2.26 + 2(1.68) = 5.62.

13 compounds, and the miscellaneous subset contained 19 compounds. Attempts to correlate binding with log P resulted in eq 1 and 2 for the 29 compounds containing the carboxy group and eq 3 and 4 for the 13 compounds containing the benzenesulfonamido group.

For 50% displacement of DNSA from HSA by carboxy compounds

$$\log 1/C = 2.17\ (\pm 0.43) + 0.48\ (\pm 0.12) \log P \qquad (1)$$

where $n = 29$, $r = 0.84$, and $s = 0.44$ and

$$\log 1/C = 1.06\ (\pm 0.67) + 1.26\ (\pm 0.42) \log P - 0.12\ (\pm 0.06)(\log P)^2 \qquad (2)$$

where $n = 29$, $r = 0.91$, $s = 0.36$, and $\log P_0 = 5.48$ (4.69–7.80).

For 50% displacement of DNSA from HSA by sulfonamido compounds

$$\log 1/C = 3.83\ (\pm 0.48) - 0.09\ (\pm 0.29) \log P \qquad (3)$$

where $n = 13$, $r = 0.21$, and $s = 0.48$ and

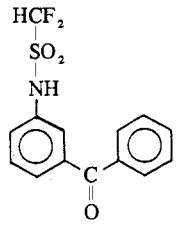
$$\log 1/C = 3.64\ (\pm 0.21) + 0.74\ (\pm 0.28) \log P - 0.34\ (\pm 0.10)(\log P)^2 \qquad (4)$$

where $n = 13$, $r = 0.92$, $s = 0.20$, and $\log P_0 = 1.10$ (0.89–1.29).

In these equations the values in parentheses are those of the 95% confidence interval for the appropriate regression coefficient and $\log P_0$ is the log P which gives optimum affinity for the binding site. Including the squared term in eq 2 is significant at the 99% level of confidence, $F_{1,26}\ \alpha_{0.01} = 7.72$. Including this term in eq 4 is also significant at this level of confidence, $F_{1,10} = 45.40$ and $F_{1,10}\ \alpha_{0.01} = 10.04$.

The compounds in the third subset (phenylbutazone and derivatives of it, amides, and other neutral compounds) could not be treated with the carboxy compounds, with the benzenesulfonamido compounds, or alone as one group with significant results. In this third subset were two binding agents that contained the sulfonyl group, 3-benzoyl-1,1-difluoromethane sulfonanilide (I) and 2-phenyl-5-trifluoromethylbenzothiophen-3(2*H*)-one 1,1-dioxide (II). Neither of these could be included in eq 4 with the benzenesulfonamido subset.

Equation 2 explains 82% of the variance in log $1/C$ ($r^2 = 0.82$) leaving 18% to other factors such as experimental error and the approximate nature of the 1-octanol–water model. Equation 4 accounts for 82% of the variance in log $1/C$ for the benzenesulfonamides. Including the variable pK_a in the appropriate equation for those compounds for which pK_a's



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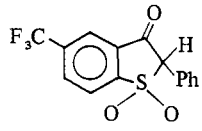

II

could be found in the literature or could be estimated from Hammett σ constants[7] did not significantly reduce the variance. This accounted for all of the benzenesulfonamides and all but three of the carboxylic acids.

The parabolic dependence in displacing ability on log P results from the specificity in binding of these agents to HSA. The decrease in specificity for compounds with log P greater than the optimum could result from (1) an increase in the steric bulkiness of the compounds that parallels an increase in log P, or (2) an increased affinity for some other lipophilic site on the surface of HSA. An increase in steric bulkiness that parallels an increase in log P is usually observed in homologous series. Because the structures of the carboxylic acids subjected to analysis in this work are of such a diverse nature, it is improbable that an increase in lipophilicity has associated with it a parallel increase in size for the carboxy substituents. The same reasoning can be used to explain the parabolic dependence of binding on log P for the benzenesulfonamido binding agents. In this series, too, there is a great deal of structural diversity in the variable portion of the binding agents and it is doubtful that an increase in lipophilic character reflects an increase in steric bulkiness. The decrease in affinity for the DNSA binding site for the members of the two series with log P greater than the respective log P_0 probably results from competition for displacing agent by another lipophilic region on HSA.

The difference in log P_0 for the two series (1.10 and 5.48 for the benzenesulfonamides and carboxylic acids, respectively) can be explained by proposing different secondary binding sites for the two series. The affinity of the benzenesulfonamide agents with log $P > 1.10$ for its secondary site would be much greater than would be the affinity of the isolipophilic carboxylic acid for its secondary site.

A comparison of the intercepts of eq 2 and 4 reveals that there is a significant difference in the affinity of the carboxy compounds and the benzenesulfonamides for the DNSA binding site. The difference in intercepts indicates that the binding site is approximately 230 times more specific for the benzenesulfonamido than for the carboxy pharmacophore. Compounds I and II, which both contain the sulfonyl group, could not be correlated in eq 4. I contains an acidic sulfonamide function with $pK_a = 5.30$ (Dr. K. Harrington, Riker Laboratories, personal communication) but does not contain the benzenesulfonamido grouping, while II is a sulfone. The phenyl portion of the benzenesulfonamido pharmacophore and its position relative to the sulfonyl group in the binding agent must play a critical role in binding to the site. This is not unexpected since DNSA contains the benzenesulfonamido group and the high intercept of eq 4 could be interpreted to mean that a principal and constant component of binding for this series may be due to an interaction of this group with the site. This point is suggested by the fact that probenicid, the only compound in the set that contains both the carboxy and benzenesulfonamido pharmacophore, is accommodated by eq 4 and was a misfit in initial attempts to correlate its displacing ability by eq 2. This would also indicate that if binding by those compounds described by eq 4 occurs through the sulfonamido group, the specificity of this group is not dependent upon it being ionized since probenicid has no ionizable N–H group. Neither would hydrogen bond formation with the N–H group as donor play a role in the binding. The difference in displacing ability of the benzenesulfonamido compounds would be due to the difference in their hydrophobic character as measured by log P.

An incremental increase in hydrophobicity has a greater effect on the displacing ability of the carboxy compounds than on that of the benzenesulfonamido compounds as seen from a comparison of the regression coefficients of the log P terms in eq 2 and 4. This dependency is greater over a greater range of lipophilicity for the carboxy analogs than for the benzenesulfonamides. Optimum displacing ability for the carboxy compounds is associated with a log $P_0 = 5.48$ while that for the benzenesulfonamides is associated with log $P_0 = 1.10$. The carboxylic acids, although not as specific for the site, are at their optimum more potent displacing agents under these conditions.

Whitehouse, *et al.*,[3] in reporting the binding data showed that the ability of these agents to displace DNSA approximated their ability to displace uric acid from HSA. This would imply that the ability of benzenesulfonamido or carboxy compounds to displace uric acid from HSA could be adjusted to any desired level by using eq 2 and 4 as aids in their design.

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