A Substituent Constant Analysis of the Interaction of Substituted Naphthalene Monoimides with DNA

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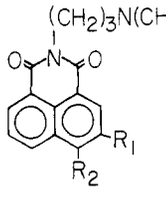
In a continuing analysis of substituent effects in intercalator–DNA interactions, an unsubstituted naphthalene monoimide, **1**, with a 3-(dimethylamino)propyl group on the imide nitrogen has been prepared along with 3- and 4-nitro- (**2** and **3**) and 3- and 4-amino- (**4** and **5**) substituted derivatives. These derivatives allow an evaluation of the importance of the Hammett substituent constant and of the substituent position on the binding of naphthalene monoimides to DNA. Viscosity and spectrophotometric analyses indicate that all five compounds bind to DNA by intercalation. The 4-nitro compound gives a smaller viscosity increase and binds only approximately one-third as strongly as the 3-nitro derivative. It is postulated that this difference is due to the significant angle that the 4-nitro group makes with the intercalated monoimide ring system. The 3-NO_2 group can assume a coplanar configuration with the monoimide ring system, allowing more favorable interactions with DNA base pairs, larger viscosity increases, and stronger binding to DNA. The binding constants of the 3-substituted monoimides are in the order **2** > **4** > **1** and, thus, do not follow a substituent constant pattern. The T_m values from thermal melting of DNA, on the other hand, are in the order **2** > **1** > **4**, suggesting that the enthalpy contributions are significantly different for the binding of the three compounds to DNA. van't Hoff plots support this finding and indicate that both enthalpy and entropy contribute significantly to the binding free energy of **1** and **2** while the binding of **4** is primarily an enthalpic process. Plots of T_m and 65 °C log K values as a function of substituent constant for **1**, **2**, and **4** are linear. CPK model building studies suggest that **4** can form a hydrogen bond with the 5′ diester oxygen of the sugar–phosphate backbone of DNA in an intercalation complex. This would lead to more favorable energetics of binding but a loss of mobility and/or available binding configurations with a resulting enthalpy–entropy compensation in the binding free energy of **4**. This series of compounds dramatically illustrates the steric and hydrogen bonding complexity that can arise in attempts to design drugs to favorably interact with a DNA intercalation site as a potential bioreceptor.

Braña et al.[1,2] have shown that 3-nitronaphthalene monoimides with cationic substituents on the imide nitrogen have significant antitumor activity and have proposed that the mode of action of these compounds involves interaction with DNA. Waring et al.[3] have shown that compounds of this type bind to DNA, increase the length of sonicated DNA, and cause unwinding of closed circular superhelical DNA, characteristics typical of intercalating compounds.[4] Yen et al.[5] have found that naphthalene monoimides and diimides with a variety of cationic substituents bind to DNA by intercalation and have a range of binding constants.

Although effects of changes in the cationic substituent on the interaction of naphthalene monoimides with DNA have been investigated,[1,2,5] there has been no study of substituent effects at the naphthalene imide ring on DNA binding. If DNA is indeed the biological target of antitumor naphthalene imides, then such a study should prove quite valuable in developing new compounds in this series. As a first step in this study we report here the synthesis and DNA interaction properties of 3- and 4-nitro (**2**, **3**) and 3- and 4-amino (**4**, **5**) derivatives of a cationic naphthalene monoimide (**1**). The nitro derivatives were used because of the antitumor activity of the 3-nitro compounds.[1,2] The amino substituents were chosen because the large difference in Hammett substituent constant between the amino and nitro groups[6] should allow a reasonable test of the importance of this parameter in the interaction of naphthalene monimides with DNA. A similar study with naphthothiopheneethanolamines indicated that ring substituent effects were quite important in DNA binding.[7]

Results

Chemistry. Three of the substituted naphthalene imides **1**, **2**, and **5** were prepared by coupling the corresponding commercially available substituted naphthalene-1,8-dicarboxylic anhydride with excess 3-(dimethylamino)propylamine (Scheme I).



Compound	R1	R2
1	H	H
2	NO_2	H
3	H	NO_2
4	NH_2	H
5	H	NH_2

The direct reduction of 3-nitronaphthalene-1,8-dicarboxylic anhydride (**7**) to the 3-amino derivative occurs in very low yield. This anhydride **7** was converted to disodium 3-nitro-1,8-naphthalenedicarboxylate and then reduced to the amino derivative **8** (Scheme II). The disodium carboxylate **8** was converted to the desired compound **4** by acidification followed directly by heating with 3-(dimethylamino)propylamine without isolation of the anhydride.

The synthesis of the 4-nitronaphthalene imide **3** is shown in Scheme III. The starting material acenaphthene

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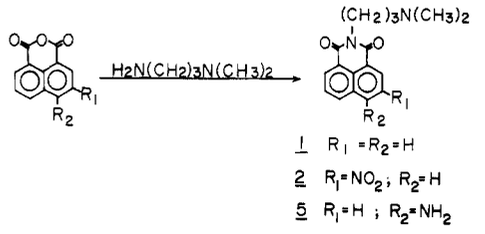
Scheme I

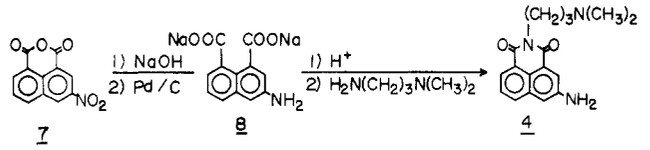


1 $R_1 = R_2 = H$

2 $R_1 = NO_2$; $R_2 = H$

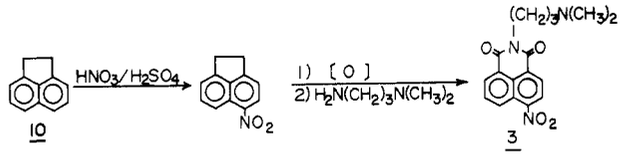
5 $R_1 = H$; $R_2 = NH_2$

Scheme II



7 8 4

Scheme III



10 3

(10) was nitrated as previously reported.[8] It should be noted that the reaction temperature should be held below 30 °C to avoid undesired multinitration. The 4-nitroacenaphthene was oxidized and dehydrated to form 4-nitro-1,8-naphthalic anhydride. The anhydride was reacted with the appropriate amine to give the 4-nitro analogue **3**.

Viscosity Studies. Compounds **1–5** all increase the viscosity of DNA in a manner quite similar to other well-characterized intercalators.[3-5] Viscometric titrations with the two nitro derivatives **2** and **3**, which gave the most different results, are shown for comparison in Figure 1. Viscometric titrations of DNA with all of the other compounds were more similar to the titration of **2** and gave a plateau at an η/η_0 value of 2.0 ± 0.05 for all of the compounds. The total viscosity increase for **3** was, thus, lower than for the other derivatives.

Spectrophotometric Studies. Compounds **1–5** all have absorption bands above 300 nm. The wavelengths and extinction coefficients in this region are collected for all of the compounds in Table I. Addition of DNA to these compounds was monitored by observing spectral changes in the region above 320 nm where overlap with DNA absorption is negligible. On addition of DNA all compounds showed significant hypochromicity and spectral shifts to longer wavelengths (Table I). A titration of **1** with DNA, which is typical for **1–5**, is shown in Figure 2. All compounds gave isosbestic behavior over the DNA concentration range used in this work, and the isosbestic points are also included in Table I.

Spectrophotometric Binding Studies. With use of a large excess of DNA, extinction coefficients for compounds bound to DNA can be determined (Experimental Section). These bound values were determined for **1–5** at the maximum wavelength of the compound free in solution and these values are listed in Table I. Using bound and free extinction coefficients and spectral results for a titration at a range of concentrations of **1–5** and DNA, Scatchard binding plots can be constructed.[3,5,9] Scatchard plots for **2** and **3**, for comparison, are shown in Figure 3.

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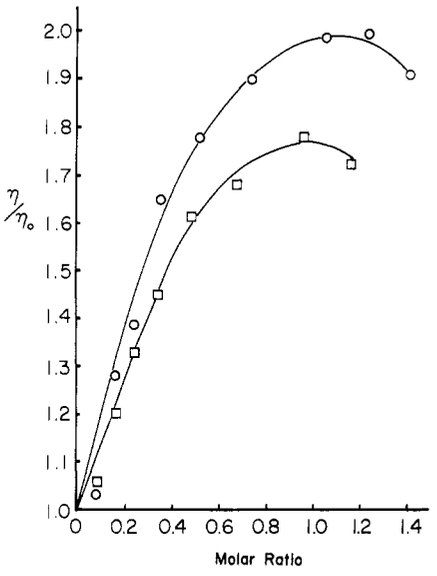

Figure 1. Viscometric titrations of sonicated calf thymus DNA with **2** (○) and **3** (□) are shown. The ratio of specific viscosity of the complexed DNA to that of DNA alone is plotted as a function of the molar ratio of compound to DNA base pairs. The titrations were done at 30 °C in MES 00 buffer. The initial DNA-P concentration was 2.16×10^{-4} M for **2** and 2.41×10^{-4} M for **3**. The lines were drawn to aid visualization of the data sets and have no theoretical significance.

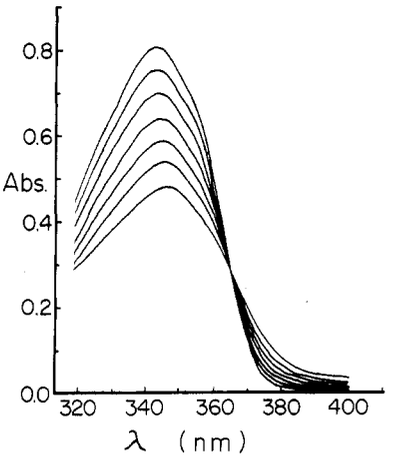

Figure 2. The effect of DNA on the spectrum of **1** is shown. From top, spectrum of free compound (5.92×10^{-6} M), spectra with compound/DNA-P ratios 0.15, 0.066, 0.036, 0.022, 0.014, 0.0067. Scans were done in MES 10 buffer at 25 °C from 410 to 320 nm in cells with a 10-cm lightpath.

Table I. Spectrophotometric Results

no.	λ_F,[a] nm	λ_B,[b] nm	ϵ_F,[c] M^{-1} cm^{-1}	ϵ_B,[d] M^{-1} cm^{-1}	% H[e]	IP,[f] nm
1	345	347	13600	5975	56.1	366
2	336	340	9175	4675	49.1	372[g]
3	355	361	11350	5450	51.9	387
4	346	353	9750	4100	57.9	439, 366, 360
5	432	445	12075	6475	46.4	478

[a] λ_F is the wavelength of maximum absorbance of the free compound. [b] λ_B is the wavelength of maximum absorbance of the compound bound to DNA. [c] ϵ_F is the extinction coefficient of the free compound in MES 10 buffer. Error is ±100. [d] ϵ_B is the extinction coefficient of the compound bound to DNA in MES 00 buffer at the wavelength of maximum absorbance of the free compound. Error is ±100. [e] % H is the percent hypochromicity of the compound on addition of DNA calculated according to the equation % H = $(\epsilon_F - \epsilon_B)/\epsilon_F$. [f] IP is the isosbestic point for titration of the compound with DNA. [g] The spectrum of the free compound does not pass through the isosbestic point.

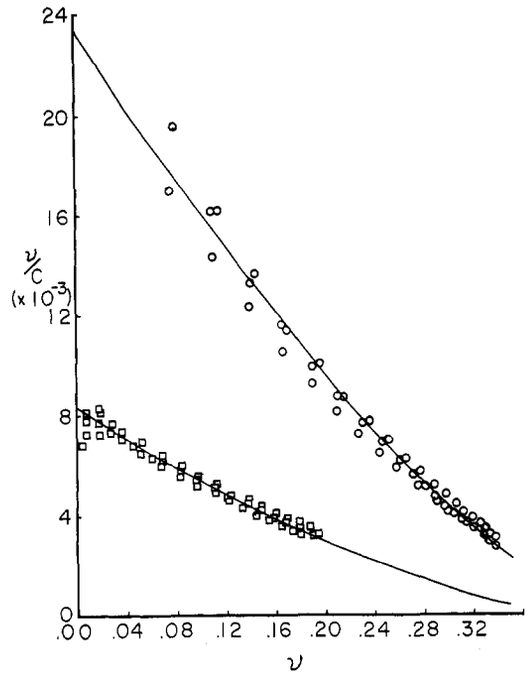

Figure 3. Scatchard plots of **2** (○) and **3** (□) are shown. The ratio of compound to DNA base pairs (ν) divided by the free compound concentration (ν/C) is plotted as a function of ν. The solid lines indicate computer fits of the data with use of the McGhee–von Hipple model[10] with the n and K values from Table II. Titrations were done at 25 °C in MES 10 buffer with use of initial DNA-P concentrations of 4.15×10^{-4} M and 4.00×10^{-4} M for **2** and an initial DNA-P concentration of 8.26×10^{-4} M for **3**.

Table II. Spectrophotometric Binding Data and Thermal Melting Points of Complexed DNA

no.	substit	$10^{-3}K^a$	n^b	T_m,[c] °C
1	H	8.9	2.6	71.9
2	3-NO_2	23.4	2.1	77.6
3	4-NO_2	8.3	2.3	71.7
4	3-NH_2	11.2	2.5	70.6
5	4-NH_2	16.2	2.4	70.6

[a] K is the relative binding affinity of the compound for calf thymus DNA in MES 10 at 25° C. It was determined from a computer fit of the data using the site exclusion binding model[10] and is reported in terms of base-pair molarity. [b] n is the binding site size determined from the same computer fit used for determination of K. [c] T_m is the thermal melting point of calf thymus DNA complexed with monoimide in MES 00 buffer. The T_m values of uncomplexed DNA was 57.7 °C.

Plots such as these for **1–5** were analyzed by using the site exclusion model[10] for intercalator binding[9] to determine the equilibrium constants and binding site sizes. These results are presented in Table II. The solid lines in Figure 3 are for the site exclusion model[10] using the best fit values from Table II, which were determined by using a nonlinear least-squares computer program.

Binding measurements for **1**, **2**, and **4** were determined at several temperatures as described in the Experimental Section. Results were calculated as described above and were used to construct the van't Hoff plot shown in Figure 4. Binding enthalpies, determined from the van't Hoff slopes, along with calculated entropies are presented in Table III.

Thermal Melting. The effects of **1–5** on the thermal denaturation curve of DNA have also been determined. The T_m value, the point where the absorption change of DNA in the thermal transition has reached the 50% point, is included for free DNA and DNA with **1–5** in Table II.

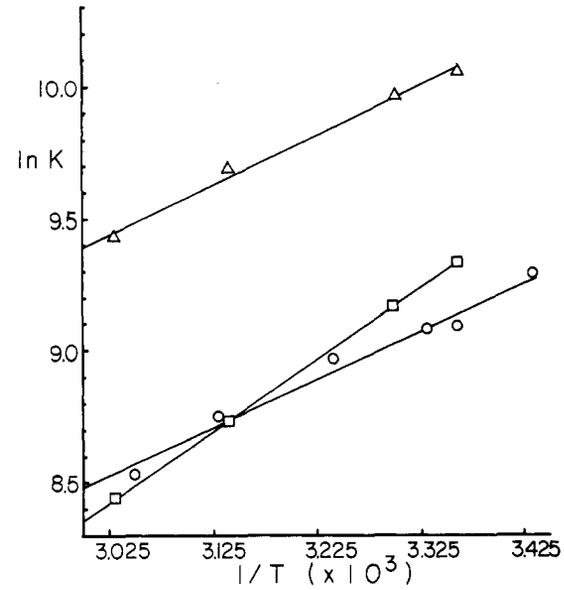

Figure 4. A van't Hoff plot for **1** (○), **2** (△), and **4** (□) is shown. Ln K is plotted as a function of $1/T$, and ΔH is obtained from the slope. Titrations were done in MES 10 with use of initial DNA-P concentrations ranging from 4×10^{-4} to 1.1×10^{-3} M.

Table III. Binding Enthalpies and Entropies for the Interaction of **1**, **2**, and **4** with DNA

no.	ΔH,[a] kcal/mol	ΔS, eu
1	−3.6	5.4
2	−3.8	7.7
4	−5.4	0.68

[a] Values were determined from the slopes of the van't Hoff plot in Figure 4. The correlation coefficient for each line is greater than 0.975.

Discussion

The spectral shifts in the presence of DNA and the viscometric titrations of DNA with **1–5** indicate that all of these compounds interact strongly with DNA base pairs in an intercalation complex. This agrees with the other results on unsubstituted[5] and 3-NO_2[1] naphthalene imides and is not unexpected for the planar monoimide ring system and cationic side chain.

Our initial binding results with **1–5** at 25 °C are collected in Table II. The n values, basically the base pairs covered per bound monoimide,[9] are in the range (2.35 ± 0.2) generally found for intercalators. The equilibrium constants at this temperature vary by over a factor of 3. The unsubstituted compound **1** is near the low end of the binding scale with the 3-nitro derivative **2** having the highest equilibrium constant. The 4-nitro compound **3** has a K value slightly less than the K for **1**. This result was, at first, somewhat surprising since **3** has a slightly greater Hammett substituent constant than **2**.[6] An examination of CPK space filling models of **2** and **3** along with X-ray structures for 1- and 2-nitronaphthalene derivatives, however, suggests that the 4-nitro substituent is rotated significantly out of the monoimide plane while the 3-nitro group can be coplanar with the monoimide ring system. It has been reported, for example, that the oxygen atoms of the nitro groups in 1,5-dinitronaphthalene make a 48.7° angle with the naphthalene plane.[11] In contrast, the nitro groups of 2,6-dinitronaphthalene are coplanar with the

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(11) Wyckoff, R. W. G., Ed. "Crystal Structures"; Interscience: New York, 1971; Vol. 6, p 403.

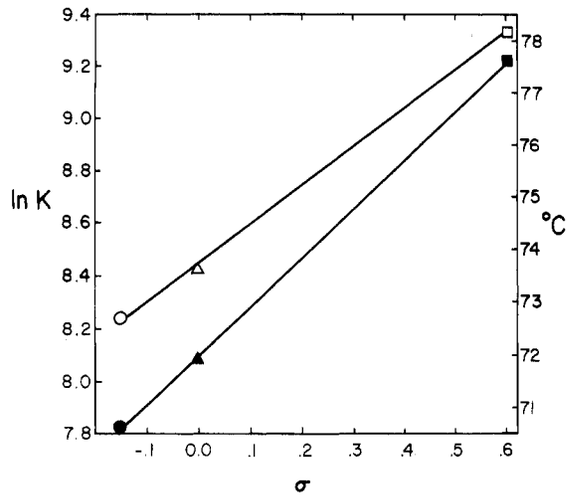

Figure 5. Plots of T_m (closed symbols, right ordinate) and ln K at 65 °C (open symbols, left ordinate) vs. Hammett substituent constant[6] for 1 (△, ▲), 2 (□, ■), and 4 (○, ●) are shown.

naphthalene aromatic ring system.[12] A nonplanar nitro group in **3** should greatly perturb stacking in an intercalated complex and would, therefore, significantly lower the equilibrium constant. We feel that it is this steric effect that accounts for the lower K for **3** relative to **2**. We have previously found steric effects to be quite important in the intercalation of quinoline derivatives.[13] A nonplanar structure for **3** along with a perturbation of its intercalation complex would also explain the significantly lower viscosity increase for **3** relative to **2** as shown in Figure 1. CPK model building studies of aminonaphthalenes suggest that any rotation of the amino group in **5** should be significantly less than the angle for the nitro group in **3**. This lack of steric repulsion in **5** would explain its increased binding constant and larger increase in viscosity, relative to **3**, when titrated into DNA.

Because they are not subject to these severe steric limitations, the 3-substituted monoimides should allow a better evaluation of substituent effects in DNA interactions. An analysis of the binding of **1**, **2**, and **4** with DNA in terms of Hammett substituent constants[6] did not, however, at first appear promising. The nitro derivative **2** binds most strongly, followed by the amino, **4**, and unsubstituted, **1**, compounds (Table II). T_m experiments were performed next (results in Table II) and provided an interesting contrast to the 25 °C binding results. In the 3-substituted series, the T_m values decreased in the order nitro > unsubstituted > amino, suggesting a substituent constant correlation with stronger binding to DNA for derivatives with electron-withdrawing substituents. Similar results were found with a series of naphthothiopheneethanolamines.[7] For reference the T_m values for **1**, **2**, and **4** are plotted vs. the appropriate Hammett substituent constant[6] in Figure 5. The T_m values for the 4-substituted monoimides clustered in less than a two degree range around 71 °C (Table II).

The variation in the apparent binding order for the 3-substituted monoimides when using K values at 25 °C or T_m values in the 70–80 °C range suggests that enthalpy contributions are not the same for intercalation of each of these compounds. To investigate this point in more detail, the van't Hoff plots in Figure 4 were used to determine more detailed thermodynamic values for the binding of **1**, **2**, and **4** to DNA (Table III). Both the enthalpy and entropy contribute favorably to the binding of these compounds to DNA, but the relative contributions are quite different. The binding of the amino compound **4** to DNA, for example, is largely an enthalpic process. With **1** and **2**, both enthalpy and entropy are almost equally important in binding. The logarithms of equilibrium constants determined at 65 °C are also plotted in Figure 5 for reference. It can be seen that in this high-temperature region an excellent substituent constant correlation with the binding results is obtained.

Relatively few studies have been conducted on the importance of enthalpy and entropy in simple organic model systems. It is interesting to note, however, that in thermodynamic studies on the ionization of meta- and para-substituted benzoic acids that ΔS values vary linearly with σ while ΔH values do not vary in any discernible pattern.[14] Fernandez et al. have reported that ΔS is the most important thermodynamic parameter in substituent effects in the ionization of a series of phenol derivatives.[15] It would seem then that **4** has a higher enthalpy, lower entropy, and at low temperature a higher K value than would be expected from the results with **1** and **2**. These results along with the characteristics of the amino group relative to the proton and nitro groups of **1** and **2** suggest that **4** could form a hydrogen bond with the sugar–phosphate backbone of DNA in an intercalated complex. CPK model building experiments with **4** and a B form CPK model of DNA are consistent with this idea. In an intercalated complex **4** can form a hydrogen bond with the 5′ diester oxygen of the phosphate groups in the sugar–phosphate chains of DNA groups. These oxygens point into the intercalation cavity formed for intercalation into DNA. Obviously a similar hydrogen bond can not be formed in the intercalation complexes of **1** and **2**. A hydrogen bond of this type would increase the energetic contribution to binding (ΔH) and decrease the number of available configurations in the intercalated complex (or equivalently decrease the motion of the intercalated monoimide ring system between DNA base pairs) and, therefore, decrease the entropic contribution to ΔG. This enthalpy–entropy compensation is well documented in simple systems[16] and probably accounts for the reasonable agreement of T_m and 65 °C log K values with substituent constants as shown in Figure 5. For these reasons we feel that the high enthalpy, low entropy, and relatively high 25 °C equilibrium constant for the interaction of **4** with DNA is due to hydrogen-bonding interactions which can not occur with **1** and **2**.

These results with naphthalene monoimides binding to DNA dramatically illustrate the influence of substituent electronic effects, hydrogen bonding, and steric effects in intercalator–DNA interactions. This series of compounds also shows, once again, the severe steric constraints on the two base pair intercalation site in DNA, which we have previously seen with a series of quinoline derivatives.[13] Results with these compounds have also shown that evaluation of enthalpy–entropy compensation in the interaction of a series of intercalators with DNA may provide a clue to specific hydrogen bonds that can form with only certain compounds in the series. The basic result of this work, that, other things being equal, the strongest elec-

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(13) Davidson, M. W.; Griggs, B. G.; Boykin, D. W.; Wilson, W. D. *J. Med. Chem.* **1977**, *20*, 1117.
(14) Hammett, L. P. *J. Chem. Phys.* **1936**, *4*, 613.
(15) Fernandez, L. P.; Hepler, L. G. *J. Am. Chem. Soc.* **1959**, *81*, 1783.
(16) Leffer, J. E.; Grunwald, E. "Rates and Equilibria of Organic Reactions"; Wiley: New York, 1963; pp 315–402.

tron-withdrawing substituents give the largest equilibrium constants for binding to DNA, is in agreement with our previous finding with a series of naphthothiophene-ethanolamines.[6] It will be of interest to begin to explore the molecular basis for this increased interaction strength and to determine its generality.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra on all new compounds were recorded with a Perkin-Elmer 710B spectrometer. ^1H NMR and ^{13}C spectra were recorded on a JEOL-FX60Q or a JEOL-GX270 instrument. All spectra were in accord with the structures assigned. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA, and were within 0.4% of theoretical values.

***N*-[3-(Dimethylamino)propyl]-1,8-naphthalenedicarboximide (1).** 3-(Dimethylamino)propylamine (16 mL, 125 mmol) was added to a suspension of 10.0 g (50 mmol) of 1,8-naphthalic anhydride in 150 mL of ethanol. The reaction mixture was refluxed for 0.5 h and the solvent was removed under reduced pressure. A dark brown liquid was obtained which crystallized upon standing overnight. The product was recrystallized with ethanol to yield 10.1 g (72%) of a pale yellow crystal: mp 114–115 °C; ^{13}C NMR (Me_2SO-d_6) δ 163.3, 134.1, 131.2, 130.5, 127.3, 127.0, 122.0, 56.7, 44.9, 38.2, 25.5. Anal. ($C_{17}H_{18}N_2O_2$) C, H, N.

***N*-[3-(Dimethylamino)propyl]-3-amino-1,8-naphthalenedicarboximide (4).** 3-Nitro-1,8-naphthalic anhydride (5.1 g, 21 mmol) was dissolved in 300 mL of methanol solution containing 2 g of sodium hydroxide. The solution was added to 750 mg of 5% Pd/C and hydrogenation was carried out at 30 psi. After the requisite amount of hydrogen was adsorbed, the catalyst was removed by filtration and the solvent was removed under reduced pressure, leaving orange crystals. A mixture of 0.43 g (1.6 mmol) of the uncharacterized disodium 3-amino-1,8-naphthalenedicarboxylate, 0.5 mL (4 mmol) of 3-(dimethylamino)propylamine, and 16 mg of *p*-toluenesulfonic acid in 200 mL of benzene was allowed to reflux for 27 h. The solution was filtered hot, the solvent was removed under reduced pressure, and the resulting brown solid was collected (0.18 g, 36%). Recrystallization from methanol gave a solid which melted at 57–60 °C. The nonhydrated compound is reported to melt at 81–83 °C.[1] **4**: ^{13}C NMR (Me_2SO-d_6) δ 163.6, 163.4, 147.7, 140.5, 133.4, 131.2, 126.7, 125.2, 122.5, 121.7, 121.6, 120.6, 56.7, 44.9, 38.1, 25.6. Anal. ($C_{17}H_{19}N_3O_2 \cdot H_2O$) C, H, N.

***N*-[3-(Dimethylamino)propyl]-3-nitro-1,8-naphthalenedicarboximide (2).** 3-Nitro-1,8-naphthalic anhydride (5 g, 21 mmol), 3-(dimethylamino)propylamine (6.0 mL, 48 mmol), and 20 mL of tetrahydrofuran were placed in a thick-walled tube, and it was sealed. The reaction mixture was heated at 90 °C for 20 h. The reaction mixture was evaporated to dryness. The resulting oil was redissolved in 200 mL of CH_2Cl_2 and washed twice with 100 mL of saturated sodium bicarbonate solution and then extensively washed with water. The methylene chloride layer was dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting solid was dissolved in 50 mL of anhydrous methanol and the container was placed in an ice bath. A stream of HBr gas was bubbled into the solution until no further precipitation was observed. The resulting solid was recrystallized from methanol; 2.23 g (26.5%) of yellow solid was obtained: mp 269–270 °C; ^{13}C NMR (Me_2SO-d_6) δ 162.9, 162.4, 145.7, 136.3, 133.8, 130.7, 129.5, 129.4, 129.2, 122.6, 122.5, 54.5, 42.2, 37.3, 22.8. Anal. ($C_{17}H_{17}N_3O_4 \cdot HBr$) C, H, N.

***N*-[3-(Dimethylamino)propyl]-4-nitro-1,8-naphthalenedicarboximide (3).** Acenaphthene was nitrated, as previously reported,[7] to yield 4-nitroacenaphthene (75%); however, it should be noted that the reaction temperature should be held below 30 °C to obtain good yields. Oxidation of 4-nitroacenaphthene was carried out as reported[17] to yield 4-nitro-1,8-naphthalic anhydride (38%). 3-(Dimethylamino)propylamine (1.26 mL, 10 mmol) was added to a suspension of 1.0 g (4 mmol) of the anhydride in 15 mL of ethanol. The mixture was heated on a steam bath for 15 min; on cooling to room temperature yellow crystals were obtained, which on recrystallization from ethanol gave 0.7 g (56%): mp 106–109 °C; ^{13}C NMR (Me_2SO-d_6) δ 162.6, 161.8, 148.8, 131.4, 129.8, 129.3, 128.3, 128.0, 126.3, 123.9, 122.5, 56.6, 44.8, 38.5, 25.2. Anal. ($C_{17}H_{17}N_3O_4$) C, H, N.

***N*-[3-(Dimethylamino)propyl]-4-amino-1,8-naphthalenedicarboximide (5).** 3-(Dimethylamino)propylamine (1.5 mL, 12 mmol) was added to a suspension of 1 g (5 mmol) of 4-amino-1,8-naphthalic anhydride in 20 mL of absolute ethanol. After the solution was refluxed for 0.5 h and concentrated to 10 mL, an orange yellow solid was collected (0.92 g, 66%), which was purified by recrystallization from ethanol and then by bulb-to-bulb short-path sublimation to give a light yellow-orange crystal: mp 184–185 °C; ^{13}C NMR (Me_2SO-d_6) δ 163.6, 162.8, 152.4, 133.7, 130.8, 129.5, 129.1, 123.8, 121.7, 119.3, 108.1, 107.6, 56.9, 45.1, 37.9, 25.9. Anal. ($C_{17}H_{19}N_3O_2$) C, H, N.

Buffers. Two buffers were used: 1×10^{-2} MES and 1×10^{-3} M EDTA, adjusted to pH 6.0 (MES 00), and 1×10^{-2} M MES, 1×10^{-3} M EDTA, and 1×10^{-1} M NaCl, adjusted to pH 6.2 (MES 10).

DNA. Calf thymus DNA was purchased from Worthington Biochemical Corp. and was prepared as previously described.[5] DNA phosphate concentrations were determined from absorbance measurements using an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm.

Stock Solutions. Stock solutions of compounds of $\sim 1 \times 10^{-3}$ M concentration were prepared by weighing out the appropriate amount, adding an equivalent amount of ~1 M HCl, and then dissolving the compound in 1–3 mL of methanol. The methanol was evaporated and the solid residue dissolved in deionized water. **2**, a HBr salt, was dissolved directly in deionized water.

Viscosity. Viscometric titrations were conducted as previously described[5] with sonicated calf thymus DNA at 30 °C with a Cannon semimicro dilution viscometer using MES 00 buffer and an initial DNA-P concentration in the range 2.2×10^{-4} to 2.4×10^{-4} M. Known increments of compound were added to cover a compound/DNA-P molar ratio of ~0.04–0.7.

Spectrophotometric Studies. All absorbance measurements were performed with a Cary 219 UV–visible spectrophotometer. The wavelength of maximum absorbance (λ_f) was determined for each compound by scanning an $\sim 4.7 \times 10^{-5}$ M solution of compound in MES 10 buffer in a 1-cm cell from 400 to 300 nm (500–350 nm for **5**). The extinction coefficient (ϵ_f) of each compound was obtained by adding known increments of the $\sim 1 \times 10^{-3}$ M stock solution to a known volume of MES 10 buffer in a 10-cm cell and using a Beers law plot to determine ϵ_f. Only absorbance readings below 0.8 were used to determine ϵ_f. An isosbestic point (IP) determination was carried out for each compound ($\sim 7.69 \times 10^{-6}$ M) in MES 10 with use of a 10-cm cell. The appropriate wavelengths were scanned with no DNA added and with known increments of calf thymus DNA-P added.

Spectrophotometric Binding Studies. The spectrum of each compound bound to DNA was determined by scanning an $\sim 2 \times 10^{-4}$ M solution of compound in an excess of DNA in MES 00 with use of a 1-cm cell from 400 to 300 nm (500–350 nm for **5**). Th extinction coefficient (at λ_f) of each compound bound to DNA (ϵ_B) was determined by adding known increments of the $\sim 1 \times 10^{-3}$ M stock solution to a 1-cm cell containing a large excess of calf thymus DNA ($\sim 1 \times 10^{-2}$ M DNA-P) in MES 00 buffer. Absorbance readings below 0.8 were used to construct a Beers law plot to determine ϵ_B and all compounds gave linear plots under these conditions.

The relative binding affinity (K) of each compound for calf thymus DNA was determined by titrating $\sim 1 \times 10^{-3}$ M stock solution of compound into DNA in MES 10 buffer. For these titrations the Cary 219 spectrophotometer was interfaced to an Apple II microcomputer through a bidirectional digital communications port which calculated the average of 100 absorbance readings for each data point. Average absorbance values were converted by the computer to ν (moles compound bound/mole of DNA base pairs) and free ligand concentrations with use of ϵ_F and ϵ_B values determined as described. From these quantities a Scatchard plot was constructed by the computer and the best fit K and n values from the site exclusion model determined by using a nonlinear least-squares program. Titrations at various temperatures were carried out with the Cary 219 spectrophotometer equipped with a Neslab Exacal EX100 constant-temperature bath. The initial concentration of DNA used in each

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titration depended on the compound and the temperature (range = 1.8×10^{-3} to 4.0×10^{-4} M DNA-P).

Thermal Melting. Thermal melting studies were carried out in MES 00 buffer at 260 nm with a compound/DNA-P molar ratio of 0.2. A Cary 219 spectrophotometer equipped with a five-sample compartment with an automatic sample changer and a Neslab temperature bath and programmer was used to collect the data.

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Registry No. 1, 92078-84-7; 2, 69408-73-7; 3, 92078-85-8; 4, 69408-86-2; 5, 92078-86-9; 3-(dimethylamino)propylamine, 109-55-7; 1,8-naphthalic anhydride, 81-84-5; 3-nitro-1,8-naphthalic anhydride, 3027-38-1; disodium 3-amino-1,8-naphthalenedicarboxylate, 92078-87-0; acenaphthene, 83-32-9; 4-nitroacenaphthene, 1015-74-3; 4-nitro-1,8-naphthalic anhydride, 6642-29-1; 4-amino-1,8-naphthalic anhydride, 6492-86-0.

Bulky Amine Analogues of Ketoprofen: Potent Antiinflammatory Agents

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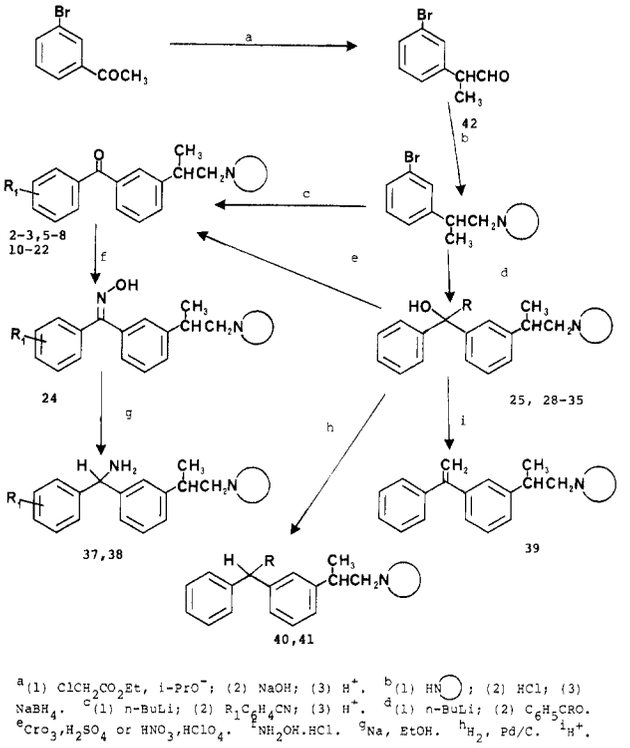
Replacement of the carboxyl group of 2-(3-benzoylphenyl)propionic acid (Ketoprofen) with various bulky amines has produced a series of highly active antiinflammatory agents that have reduced intestinal ulcerogenicity and have better therapeutic ratios in the 21-day adjuvant arthritis assay in rats than currently marketed nonsteroidal antiinflammatory drugs. Activity is maintained on reduction of these 2-(3-benzoylphenyl)propyl bulky amines to the corresponding alcohols or methylene analogues, on conversion of the ketone function to a primary amine or oxime, and on introduction of a 4-halo substitutent (Cl or F) on the terminal aromatic ring. Removal of the α-CH_3 group greatly reduces the antiiflammatory activity of the series. These compounds have been synthesized by the reductive amination of 2-(3-bromophenyl)propionaldehyde with the respective amine followed by lithiation of this product and condensation with the appropriate benzonitrile.

The literature in recent years is filled with reports of arylacetic acids that have shown significant antiinflammatory activity in animal models.[1] Unfortunately, many of these acids have exhibited considerable gastrointestinal intolerance in man. Consequently, the question was raised in our laboratories whether replacement of the acid moiety in the known clinically most active arylacetic acid structures by basic amine functions might not give compounds retaining the antiinflammatory activity of their acidic counterparts but with less incidence of gastrointestinal intolerance. This paper relates the synthesis and pharmacological properties of analogues of 2-(3-benzoylphenyl)propionic acid (Ketoprofen) where the carboxylic acid function has been replaced by a variety of amines. Many of these analogues exhibit potent antiinflammatory activity in both the carrageenan and adjuvant arthritis assays, limited gastrointestinal intolerance, low toxicity, and mild analgesic and antipyretic activities.

Chemistry. Preparation of the 2-(3-benzoylphenyl)-propylamines was most easily carried out by Scheme I. Conversion of 3-bromoacetophenone[2] to the corresponding propionaldehyde **42** proceeded via a Darzen's glycidic ester condensation. The appropriate amine and **42** were then reacted in benzene, water being azeotropically removed. The resulting enamines and imines were converted to their respective hydrochlorides and then reduced with sodium borohydride in DMF. Lithiation[3] of the distilled amines was followed either by reaction with an appropriate benzaldehyde or acetophenone (method B) to give alcohols **25** and **28–35** or by reaction with benzonitrile (method A) to give ketones **2**, **2a**, **3**, **5**, **6**, **8**, **10**, and **15–22** on acid hydrolytic workup. In some cases, the alcohols were oxidized to ketones **7** and **10** by using either chromium trioxide in sulfuric acid or a perchloric acid–nitric acid mixture in 1,2-dimethoxyethane[4] (methods C and D). Oximation of the ketones followed by reduction of the resulting oximes with sodium in ethanol (methods E and F) gave the corresponding amines **36–38**. Vinyl compound **39** was pre-

Scheme I



[a] (1) $ClCH_2CO_2Et$, i-PrO$^-$; (2) NaOH; (3) H^+. [b] (1) HN◯; (2) HCl; (3) $NaBH_4$. [c] (1) n-BuLi; (2) $R_1C_6H_4CN$; (3) H^+. [d] (1) n-BuLi; (2) C_6H_5CHO. [e] CrO_3,H_2SO_4 or HNO_3,$HClO_4$. [f] $NH_2OH.HCl$. [g] Na, EtOH. [h] H_2, Pd/C. [i] H^+.

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