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α-Brominated 4-Hydroxy-3,5-dinitroacetophenones: Potent Inhibitors of the Erythrocyte Anion Transport Protein

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4-Hydroxy-3,5-dinitroacetophenone anion inhibits erythrocyte anion transport with a K_{50} of 220 μM in 165 mM KCl, pH 7.3, at 0°C. Substitution of bromine atoms on the α-carbon greatly enhances the inhibitory potency of this class of compounds. Thus, α-bromo-4-hydroxy-3,5-dinitroacetophenone **(III)** anion inhibits chloride transport in erythrocyte ghosts with K_{50} values of 13 and 5.3 μM when $[Cl]_o$ = 165 and 16.5 mM KCl, respectively, and $[Cl]_i$ = 165 mM. α,α-Dibromo-4-hydroxy-3,5-dinitroacetophenone **(IV)** anion has K_{50} values of 0.73 and 0.36 μM under identical conditions. **IV**, which can cause >99.9% inhibition without allowing nonspecific leakage of Cl^- across the membrane, is one of the most potent monoanion inhibitors of erythrocyte anion transport yet reported. The crystal structure of **III** has been determined [$P2_12_12_1$, a = 9.805(1), b = 20.691(2), c = 5.013(1) Å, R = 0.042, R_w = 0.045]. The substituted aromatic ring and the α-bromoacetophenone group form a planar molecule. The bromine atom is within 0.0844(1) Å of the plane of the aromatic ring, and the C–Br bond is quite short [1.914(6) Å]. Both of these observations suggest considerable delocalization of the bromine lone pair electrons into the π system of the molecule.

INTRODUCTION

The physiologically important exchange of chloride and bicarbonate across the red cell membrane is mediated by band 3, also known as capnophorin (*1*), an integral membrane protein with a molecular mass of ~100,000 D (*2–4*). Recent interest in structure/function studies of capnophorin has been stimulated by the determination (*5*) of the amino acid sequence of the murine protein by sequencing of the cloned cDNA.

A large number of structurally different compounds inhibit anion transport in erythrocytes (*2, 6, 7*). Phloretin **(I)**, a neutral hydrophobic structure with a large dipole moment, appears to represent one general class of inhibitors. Not only does it act as a high affinity (K_{50} = 2 μM) inhibitor of anion transport (*7*), but it also effectively inhibits other mediated transport processes such as hexose and urea

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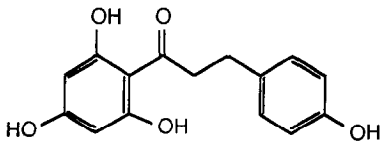

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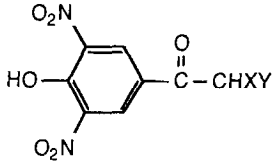
transport (*8, 9*). A second general class of inhibitors includes hydrophobic anions, such as 2,4,6-trinitrocresolate (*7*) and 4,4′-dinitro-2,2′-stilbenedisulfonate (*10*). These inhibitors appear to bind to the anion transport protein and prevent anion binding and/or translocation (*11*).

We have synthesized 4-hydroxy-3,5-dinitroacetophenone **(II)**, as well as its α-bromo **(III)** and α,α-dibromo **(IV)** derivatives, as possible inhibitors of anion transport. These compounds contain the acetophenone moiety that allows for effective inhibition of anion transport by phloretin and its analogs (*12*). In addi-



II: X = Y = H

III: X = Br, Y = H

IV: X = Y = Br

tion, because the conjugated nitro and carbonyl moieties lower the pK_a of the phenolic proton to approximately 2, each compound is a hydrophobic anion at neutral pH. This paper describes the synthesis of and inhibition of erythrocyte anion transport by **II–IV**, as well as the crystal structure of one of these inhibitors, **III**.

EXPERIMENTAL

Synthesis. **II** was synthesized from 4-hydroxy-3-nitroacetophenone **(V)** in 70% isolated yield by the method of Bartlett (*13*); mp 119–120.5°C (95% ethanol); lit. mp 123–123.5°C (*13*). **V** was synthesized from 4-hydroxyacetophenone **(VI)** (Aldrich Chemical Co.) as previously described (*13*). Several attempts to prepare **II** by direct dinitration of **VI** ended in failure, with a recurring problem being the production of considerable amounts of picric acid.

III was synthesized from **II** by bromination with $CuBr_2$ by the method of King and Ostrum (*14*). **II** (3.39 g, 15.0 mmol) was dissolved in 40 ml hot ethyl acetate in

a 300-ml round-bottom flask equipped with a magnetic stir bar. $CuBr_2$ (6.70 g, 30.0 mmol) was then added, and the stirred mixture was heated under reflux until no more HBr gas was evolved. The off-white CuBr was removed by suction filtration, and the filtrate was rotary evaporated to remove the solvent. Crude **III** was dissolved in 15 ml hot benzene, treated with activated charcoal, and suction filtered, and the hot benzene solution was treated with an equal volume of hexane. The pale yellow crystalline product was isolated by suction filtration and air dried. Yield, 60%; mp 92–94°C. *Elemental anal.* Calcd for $C_8H_5BrN_2O_6$: C, 31.50; H, 1.65; Br, 26.19. Found (Galbraith Laboratories): C, 31.46; H, 1.62; Br, 26.33.

By an adaption of the procedure of King and Ostrum (*14*), **IV** was synthesized from **II** by bromination with excess $CuBr_2$ (5 : 1 mole ratio of $CuBr_2$: **II**). After recrystallization from benzene : hexane, **IV** was isolated in 66% yield, mp 93–95°C. *Elemental anal.* Calcd for $C_8H_4Br_2N_2O_6$: C, 25.03; H, 1.05; Br, 41.62; N, 7.30. Found (Galbraith): C, 24.95; H, 0.97; Br, 41.63; N, 7.15.

We note in passing that this method of synthesizing α,α-dibromoacetophenones directly from acetophenones seems to be of general utility. Thus, by the procedure outlined above, acetophenone, 4-bromoacetophenone, 4-hydroxyacetophenone, 4-methoxyacetophenone, 4-nitroacetophenone, 4-hydroxy-3-nitroacetophenone, and 4-methoxy-3-nitroacetophenone were converted to their corresponding α,α-dibromoacetophenones in 56–82% yields, with no attempt to optimize yields.

Chloride exchange flux. Chloride efflux of washed human red blood cells, prepared from freshly drawn heparinized blood, or of resealed ghosts, prepared by the method of Bjerrum *et al.* (*3*), was determined by the Millipore filtering technique of Dalmark and Wieth (*15*). Cells or ghosts were first loaded with $^{36}Cl^-$ (as $Na^{36}Cl$, Radiochemical Centre (Amersham, England), sp act 500 μCi/mmol) by suspension in 0.3–0.6 μCi/ml $^{36}Cl^-$ in 165 mM KCl, 2 mM KPO_4, pH 7.3, for sufficient time to allow isotopic equilibration. The packed cells, with $[Cl]_i$ = 165 mM, were then injected into the efflux medium (165 mM KCl, or 16.5 mM KCl, 22.5 mM citrate, and 180 mM sucrose (to maintain isotonicity), 2 mM KPO_4, pH 7.3), with the indicated concentrations of **II, III,** or **IV** at 0°C, and samples were withdrawn at appropriate time intervals and the extracellular medium counted for $^{36}Cl^-$ to give the pseudo first-order rate coefficients of efflux, k (s^{-1}), under each set of conditions. In the absence of inhibitors, the half-times for $^{36}Cl^-$ efflux were approximately 17 s for whole cells and approximately 29 s for ghosts.

*Structural studies of **III**.* **III** was crystallized from ethanol/water (2/1) solvent by vapor diffusion with water. Approximate lattice constants were measured from Weissenberg photographs, and the space group was determined to be either $P2_12_12_1$ or $P2_12_12$ from systematic absences $h00$, h odd, and $k00$, k odd.

Final cell parameters (Table 1) and an orientation matrix for data collection were obtained by least-squares refinement on an Enraf-Nonius CAD4 computer-controlled, κ-axis diffractometer equipped with a graphite crystal, incident-beam monochromator. Angles of 25 reflections in the range $10° < \theta < 12°$ were measured by the computer-controlled diagonal slit method of centering.

Data were collected using the ω-2θ scan technique to a maximum 2θ of 55°. The scan rate varied from 6 to 29° min^{-1} (in ω). The variable scan rate allows rapid data

TABLE 1

Crystal Data for
α-Bromo-4-hydroxy-3,5-dinitroacetophenone

Empirical formula	$C_8H_5BrN_2O_6$
Formula weight	305.05
$F(000)$	600
Space group	$P2_12_12_1$
Z	4
a	9.805 (1) Å
b	20.691 (2) Å
c	5.013 (1) Å
V	1017.0 (2) Å^3
D_X	1.99 g ml^{-1}
λ(Mo K_α)	0.71073 Å
μ(Mo K_α)	40.2 cm^{-1}
Temperature	23 ± 1°C

collection for intense reflections where a fast scan rate is used and assures good counting statistics for weak reflections where a slow scan rate is used. The scan width (in degrees) was determined as $0.7 + 0.35 \tan \theta$ to correct for the separation of the K_α doublet; moving-crystal moving-counter background counts were made by scanning an additional 25% above and below this range. Thus the ratio of peak counting time to background time was 2 : 1. The counter aperture was also adjusted as a function of θ. The horizontal aperture width ranged from 2.0 to 2.4 mm; the vertical aperture was set at 6.0 mm. The diameter of the incident beam collimator was 0.7 mm and the crystal-to-detector distance was 21 cm. For intense reflections an attenuator was automatically inserted in front of the detector; the attenuator factor was 20.0.

The intensities of representative reflections measured every 25 min remained constant within experimental error throughout data collection, and no decay correction was applied. The standard deviation on intensities is given by

$$\sigma^2(F_o^2) = [S^2(C + R \cdot B) + (pF_o^2)^2]/\mathrm{Lp}^2,$$

where S is the scan rate, C is the total integrated peak count, R is the ratio of scan time to background counting time, B is the total background count, Lp is the Lorentz-polarization factor, and the parameter p is a factor introduced to downweight intense reflections. Here p was set to 0.040 (*16*).[3]

A total of 2025 reflections were collected, of which 1748 were unique and not systematically absent. Intensities of equivalent reflections, but not Friedel pairs, were averaged. After averaging, 14 reflections were rejected because their intensities differed significantly from the average. The agreement factors for the averaging of the 376 observed and accepted reflections was 2.2% based on intensity and 1.5% based on F_o. Only the 1120 reflections having intensities greater than 3.0 times their standard deviations were used in subsequent calculations.

[3] All calculations were performed on a PDP-11/44 computer using SDP-PLUS software.

The structure was solved by direct methods (*17*). Scattering factors were taken from Cromer and Waber (*18*); anomalous dispersion corrections were included in F_c (*19*). Hydrogen atoms bonded to carbon were added to the structure factor calculations at idealized positions (C–H distances 0.95 Å); these positions were not refined. The hydroxyl hydrogen was located in a difference map and also included in structure factor calculations but not refined. Absorption corrections were made using the program DIFABS (*20*). In the refinement the quantity minimized was $\Sigma w(|F_o| - |F_c|)^2$ where the weights, w, were $4F_o^2/[\sigma^2(F_o^2)]$. The final cycle of refinement included 154 variable parameters and converged (largest parameter shift 0.01 times its estimated standard deviation) with the final value of $R = \Sigma(||F_o| - |F_c||)/\Sigma|F_o|$ being 0.042 and $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$ being 0.045. The highest peak in the final difference Fourier map (0.68 eÅ^{-3}) was near the bromine atom. The standard deviation of an observation of unit weight was 1.45. Plots of $\Sigma w(|F_o| - |F_c|)^2$ versus $|F_o|$, order of reflection observation, $(\sin\theta)/\lambda$, and various classes of indices showed no unusual trends.

RESULTS

Inhibition of anion transport. To determine whether **II, III,** and **IV** are effective inhibitors of chloride transport in whole red cells, efflux measurements were carried in 165 mM KCl in the presence of 500 μM inhibitor. Compared to controls in the absence of inhibitor, **II** reduced chloride flux by 70.0%, while **III** and **IV** caused 98.1 and 99.93% inhibition, respectively, of chloride exchange (data not shown). Assuming linear Dixon plots of these data, which seems to be a valid treatment as shown below, we estimated the K_{50} of **II, III,** and **IV** to be approximately 220, 12, and 0.5 μM, respectively. Thus **II** is a moderately effective inhibitor of anion transport, but addition of bromine atoms to the side chain greatly increases the affinity of the inhibitor for the anion transport system. We investigated the inhibitory potency of **III** and **IV** more thoroughly.

Figure 1 shows the effects of various concentrations of **IV** on $^{36}Cl^-$ efflux in whole red cells. Fluxes were determined in 165 mM KCl in the presence of 1–500 μM inhibitor, and a Dixon plot, k_o/k_i vs **[IV],** shows that **IV** is a model inhibitor of anion transport. Extrapolation gives a K_{50} of 0.43 μM, in general agreement with the previous estimate given above. Perhaps more importantly, even at the highest **[IV],** where $k_o/k_i \cong 1400$, the data still fit a linear plot. Not only does **IV** greatly inhibit anion transport, but even at >99.9% inhibition there is no nonspecific leakage of chloride ions across the membrane.

We determined the effects of the brominated compounds on chloride exchange in red cell ghosts. Figure 2 shows a Dixon plot of data obtained using **IV** for inhibition. The data suggest that in 165 mM KCl, **IV** inhibits with a K_{50} of 0.73 μM. If $[KCl]_o$ is decreased from 165 to 16.5 mM, K_{50} not unexpectedly decreases—to a value of 0.39 μM (Fig. 2). A Dixon plot of efflux data on ghosts in 165 mM KCl in the presence of 0–100 μM **III** indicates a K_{50} of 13 μM, while a similar experiment using 16.5 mM extracellular KCl and 0–10 μM **III** gives a K_{50} of 5.3 μM under these

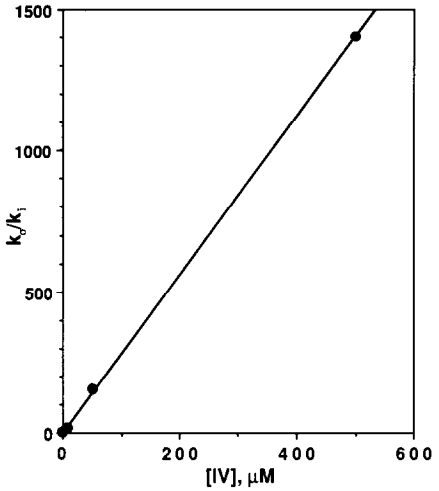


FIG. 1. Dixon plot of the inhibition of chloride flux in whole red blood cells by α,α-dibromo-4-hydroxy-3,5-dinitroacetophenone **(IV)** anion. Rate coefficients were determined in the absence of inhibitor (k_o) and in the presence (k_i) of the indicated concentrations of inhibitor. The data are plotted as k_o/k_i vs **[IV].** The data fit a straight line: $y = 1.203 + 2.810x$, $r = 1.000$.

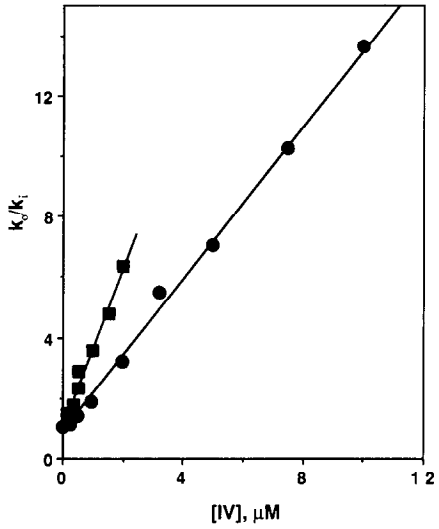


FIG. 2. Dixon plot of the inhibition of chloride flux in red cell ghosts by α,α,-dibromo-4-hydroxy-3,5-dinitroacetophenone **(IV)** anion. Rate coefficients were determined in the absence of inhibitor (k_o) and in the presence (k_i) of the indicated concentrations of inhibitor in a medium containing either 165 (circles) or 16.5 (squares) mM KCl. The data are plotted as k_o/k_i vs **[IV].** The data in 165 mM KCl fit a straight line: $y = 0.918 + 1.260x$, $r = 0.998$. The data in 16.5 mM KCl also fit a straight line: $y = 1.039 + 2.636x$, $r = 0.995$.

TABLE 2

K_{50} Values for the Inhibition of Anion Transport in Whole Red Cells or Ghosts by 4-Hydroxy-3,5-dinitroacetophenones

Inhibitor	Chloride flux in	[KCl] (mM)	K_{50} (μM)
II	Whole cells	165	220
III	Whole cells	165	10
	Ghosts	165	13
	Ghosts	16.5	5.3
IV	Whole cells	165	0.43
	Ghosts	165	0.73
	Ghosts	16.5	0.39

conditions (data not shown). A summary of K_{50} values for the inhibition of erythrocyte anion transport by **II, III,** and **IV** is given in Table 2.

To determine the specificity of the inhibition of anion transport by the hydroxynitroacetophenones, we tested for inhibition of glucose transport in whole red cells (60 or 100 mM glucose, 150 mM KCl, pH 7.2, 38°C) by **IV.** Ten micromolar **IV** causes a small (~15%) decrease in glucose transport, while no significant decrease (<5%) is seen with 1 μM inhibitor. At comparable concentrations of **IV,** chloride transport is inhibited by 95 and 65%, respectively. These data are best explained by a specific binding of the deprotonated form of **IV** to the anion erythrocyte transport protein.

*Crystal structure of **III.*** α-Bromo-4-hydroxy-3,5-dinitroacetophenone is a planar molecule consisting of an aromatic ring with conjugated nitro, hydroxy, and α-bromoacetyl substituents. A drawing of the structure, including the atom numbering system, is shown in Fig. 3. Positional and thermal parameters are given in Table 3. Tables of observed and calculated structure factors, anisotropic temperature factors, and hydrogen atom positions are available as supplementary material.[4] Interatomic distances are summarized in Table 4.

The carbon atoms of the aromatic ring deviate no more than 0.022(6) Å from the plane. Deviations of all nonhydrogen atoms in **III** from the least-squares plane of the aromatic ring are given in Table 5. Substituent N, C, and O atoms bonded directly to the ring deviate no more than 0.110(5) Å from the plane, the largest distance being that for the hydroxyl oxygen atom O(4). The two nitro groups differ markedly in their coplanarity with the aromatic ring: nitro group N(1), O(2), O(3) is twisted by only 10°, while nitro group N(2), O(5), O(6) is twisted by 42° from the molecular plane. Significant distortion from planarity is not unusual for aromatic nitro substituents when there is steric hindrance from a group in an adjacent position (*21*), in this case from the hydroxyl oxygen atom O(4). There is an

[4] See NAPS document No. 04638 for 17 pages of supplementary material. Order from ASIS/NAPS, Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, NY 10163-3513. Remit with the order, in U.S. funds only, $7.75 for photocopies or $4.00 for microfiche. Outside the U.S. and Canada, add $4.50 (photocopies) or $1.50 (microfiche) for postage.

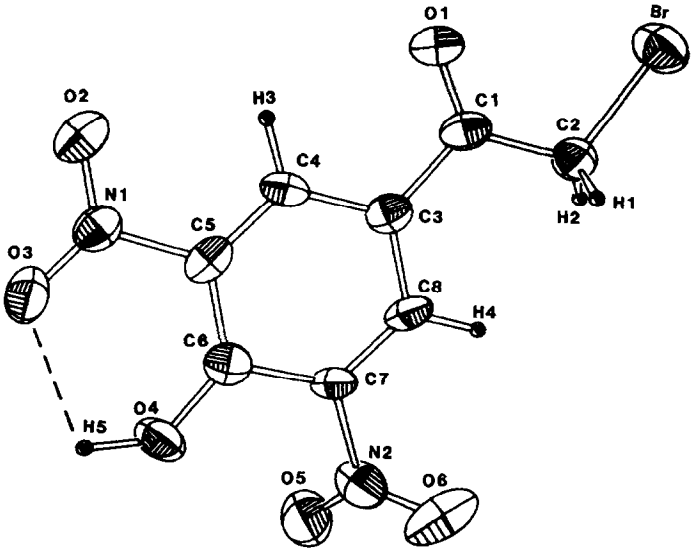


FIG. 3. Drawing of α-bromo-4-hydroxy-3,5-dinitroacetophenone **(III)** showing thermal ellipsoids at the 50% probability level.

TABLE 3

Fractional Coordinates and Estimated Standard Deviations for α-Bromo-4-hydroxy-3,5-dinitroacetophenone **(III)**

Atom	*X*	*Y*	*Z*	B^a (Å^2)
Br	−0.15559 (8)	0.83846 (4)	0.6914 (2)	4.37 (1)
O1	0.0944 (5)	0.9150 (2)	0.808 (1)	4.4 (1)
O2	0.5275 (5)	1.0148 (2)	0.771 (1)	3.7 (1)
O3	0.6701 (5)	0.9981 (2)	0.454 (1)	3.8 (1)
O4	0.6342 (5)	0.9009 (2)	0.131 (1)	3.5 (1)
O5	0.5747 (6)	0.7773 (3)	0.008 (1)	6.3 (2)
O6	0.3797 (6)	0.7859 (2)	−0.188 (1)	5.3 (1)
N1	0.5631 (6)	0.9877 (3)	0.568 (1)	2.9 (1)
N2	0.4640 (6)	0.8017 (3)	−0.024 (1)	3.6 (1)
C1	0.1144 (7)	0.8841 (3)	0.606 (1)	2.8 (2)
C2	0.0100 (7)	0.8403 (4)	0.489 (1)	3.0 (1)
C3	0.2481 (7)	0.8883 (3)	0.473 (1)	2.5 (2)
C4	0.3426 (7)	0.9336 (3)	0.566 (1)	2.4 (1)
C5	0.4685 (7)	0.9386 (3)	0.462 (1)	2.5 (1)
C6	0.5180 (7)	0.8977 (3)	0.255 (1)	2.6 (2)
C7	0.4215 (6)	0.8502 (3)	0.176 (1)	2.3 (1)
C8	0.2935 (7)	0.8466 (3)	0.275 (1)	2.5 (1)
H5[b]	0.7148	0.9277	0.1425	4.5

[a] Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter, defined as $(4/3) * [a^2 * B(1,1) + b^2 * B(2,2) + c^2 * B(3,3) + ab(\cos\gamma) * B(1,2) + ac(\cos\beta) * B(1,3) + bc(\cos\alpha) * B(2,3)]$.

[b] Position found in difference map but not refined.

TABLE 4

Summary of Important Interatomic Distances for α-Bromo-4-hydroxy-3,5-dinitroacetophenone (III)[a]

Atom 1	Atom 2	Distance (Å)	Atom 1	Atom 2	Distance (Å)
Br	C2	1.914 (6)	N1	C5	1.474 (8)
O1	C1	1.210 (7)	N2	C7	1.480 (8)
O2	N1	1.212 (6)	C1	C2	1.489 (9)
O3	N1	1.215 (6)	C1	C3	1.474 (9)
O4	C6	1.299 (7)	C3	C4	1.398 (8)
O4	H5	0.97[b]	C3	C8	1.388 (9)
O3	H5	2.18[b]	C4	C5	1.344 (8)
O3[c]	H5	2.13[b]	C5	C6	1.427 (9)
O5	N2	1.208 (8)	C6	C7	1.420 (9)
O6	N2	1.208 (8)	C7	C8	1.352 (8)

[a] The number in parentheses is the estimated standard deviation in the least significant digit.

[b] The position of H5 was not refined.

[c] Symmetry-related atom at $1.5 - X$, $2 - Y$, $-0.5 + Z$.

TABLE 5

Deviations from the Least-Squares Plane of the Aromatic Ring for α-Bromo-4-hydroxy-3,5-dinitroacetophenone (III)[a]

Orthonormal equation

$$-0.3328\,X + 0.6390\,Y - 0.6935\,Z - 9.2736 = 0$$

(0.0025 0.0021 0.0019 0.0440)

Atom	X	Y	Z	Distance (Å)	Esd
C3	2.4328	18.3792	2.3732	0.0147	±0.0066
C4	3.3595	19.3172	2.8387	−0.0172	±0.0063
C5	4.5932	19.4216	2.3168	0.0008	±0.0066
C6	5.0785	18.5734	1.2775	0.0181	±0.0064
C7	4.1325	17.5907	0.8832	−0.0215	±0.0063
C8	2.8775	17.5175	1.3797	0.0050	±0.0061
N1	5.5209	20.4361	2.8484	−0.0283	±0.0056
O2	5.1723	20.9977	3.8638	−0.2576	±0.0048
O3	6.5707	20.6516	2.2761	0.1569	±0.0047
O4	6.2188	18.6403	0.6591	0.1103	±0.0046
N2	4.5491	16.5876	−0.1225	−0.1036	±0.0061
O5	5.6347	16.0825	0.0385	−0.8992	±0.0060
O6	3.7232	16.2613	−0.9407	0.5302	±0.0052
C1	1.1217	18.2936	3.0402	−0.0664	±0.0066
O1	0.9252	18.9317	4.0488	−0.2927	±0.0053
C2	0.0980	17.3863	2.4526	0.1021	±0.0071
Br	−1.5256	17.3486	3.4660	−0.0844	±0.0008

[a] $\chi^2 = 32.9$ for the plane of the first six atoms listed.

intramolecular hydrogen bond between the hydroxyl hydrogen H(5) and O(3) which is indicated by a dashed line in Fig. 3. There is also an intermolecular H-bond between H(5) and O(3) of a symmetry-related molecule. These H-bonding distances are included in Table 4.

DISCUSSION

When we set out to determine the crystal structures of members of this new class of anion transport inhibitor, we of course tried to obtain data on **IV** as well as **III.** As is often the case, the compound of major interest **(IV)** did not yield first to data analysis. However, **III** is a potent inhibitor of anion transport in its own right, with a K_{50} of 10–13 μM in 165 mM KCl at pH 7.3. The K_{50} is reduced to 5.3 μM when the extracellular chloride concentration is reduced 10-fold. The data suggest that **III** and **IV** inhibit by binding to the same site on the anion transport protein, although with slightly different affinities. **III** offers the additional possibility over **IV** that its α-bromoacetyl moiety might allow it to react covalently with a nucleophilic residue at its binding site on capnophorin, and thus **III** may serve as an effective affinity reagent for the erythrocyte anion transport protein. We have not explored this possibility.

On the basis of the crystal structure results given in Table 5, **III** is almost entirely planar, with only slight deviations from planarity due to the twisting of the nitro substituents on the aromatic ring. The planarity of the molecule extends also to the α-bromoacetyl group, and to the bromine atom, which lies within 0.0844(8) Å of the aromatic plane (Table 5). The Br atom and the carbonyl O(1) atom are completely eclipsed, e.g., the O(1)–C(1)–C(2)–Br torsion angle is 0°. The C(2)–Br bond [1.914(6) Å] is quite short. A search of the Cambridge Structural Database (*22*) revealed the following C–Br distances for various structural fragments: terminal aliphatic, 1.99 Å (23 observations); α-bromoacetyl or α-bromoacetoxy, 1.93 Å (115 observations); aromatic ring substituent, 1.90 Å (193 observations). In only 8 of the 115 α-bromocarbonyl fragments was the O–C–C–Br torsion angle less than 5° (or greater than 175°) and in those structures the mean C–Br bond length was shortened to ~1.90 Å, the bond length when Br is an aromatic ring substituent. Thus it appears that when the C–Br bond is approximately coplanar with the carbonyl group, the C–Br bond tends to be shorter. One plausible explanation is that the polarizable lone electron pairs of the Br atom are delocalized into the π electron system. The C(1)–C(2) distance (Table 4), which is between normal single bond and aromatic C–C bond distances, also indicates considerable π electron delocalization in this part of the molecule. These observations suggest that the π electron system of **III** extends from the substituents on the aromatic ring to the bromine atom at the other end of the molecule.

IV is a potent selective inhibitor of erythrocyte anion transport, for levels of reagent that cause almost complete loss of chloride exchange activity produce no significant decrease in glucose transport. Dixon plots of k_o/k_i vs **[IV]** are linear, suggesting that inhibition is due to binding to a single type of high affinity site on capnophorin. **IV** inhibits chloride transport in erythrocytes and ghosts with a K_{50}

of 0.4–0.8 μM (Table 2), thus its inhibitory effectiveness approaches or equals that of the most potent inhibitors reported in the literature. 4,4′-Diisocyano-2,2′-stilbenedisulfonate (DIDS),[5] the best reversible (as well as irreversible) inhibitor reported to date, has a K_{50} of 0.04 μM (*23*), while the dihydro derivative of DIDS has a K_{50} of ~0.1 μM at zero chloride concentration (*24*). Other stilbenedisulfonates have affinities that are comparable to, or less than, that of **IV** (see *2* and *4* for reviews). The stilbenedisulfonates appear to be competitive inhibitors of chloride transport (*24–26*); however, competitive inhibition is not a requirement for high-affinity inhibitors. Hydrophobic aromatic monoanions such as niflumate (*27*) and flufenamate (*28*), which have K_{50} values that approach those of **IV**, give noncompetitive inhibition (*29*).

We have not determined if **IV** is a transport substrate for capnophorin, nor if it blocks anion translocation by binding to an external or an internal site, or both. The less than twofold decrease in K_{50} as the $[Cl]_0$ is lowered from 165 to 16.5 mM suggests that the mutual binding of chloride and **IV** may be possible. However, our data on **IV** are only preliminary, and more work needs to be done to determine the nature of inhibition by this potent blocker of erythrocyte anion transport.

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[5] Abbreviation used: DIDS, 4,4′-diisocyano-2,2′-stilbenedisulfonates.

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