Structure–Activity Relationship of Glycine Betaine Analogs on Osmotolerance of Enteric Bacteria

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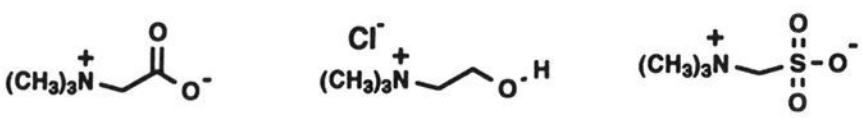
Received August 24, 1992

Bacterial cells have the ability to accumulate compatible solutes within the cytoplasm to maintain their osmolarity above that of the extracellular milieu. Glycine betaine (GB) and its biosynthetic precursor choline (Chol) are the major compatible solutes that bacteria accumulate when osmotically challenged. Different osmotically triggered active transport mechanisms have been identified for GB and Chol. In the present study we examined the bioisosteric replacement of the carboxylic group of GB with sulfonic, phosphonic or benzenesulfonamido groups. The sulfonic acid analog (sulfobetaine, compound **3**) showed osmoprotectant activity equivalent to that of GB. In addition, we tested the possibility of utilizing GB/Chol transport systems to deliver cytotoxic analogs of GB into three strains of *E. coli* that differed in their salt resistance. We found that N^1-betainyl-N^4-(haloacetyl)sulfanilamides (compounds **17c–e**) that are GB analogs containing alkylating side chain within their structures inhibited the bacterial growth of the tested standard and salt sensitive strains of *E. coli*. We also showed that the (*N*-methyl-cyclic ammonio)methanesulfonates (compounds **21a–c**) are able to block Chol transport system in both the standard and the salt-sensitive *E. coli* strains used. At the concentration used (0.1 mM), none of the tested compounds showed any significant effect on the salt-resistant strain used.

Bacterial cells need to adapt to the marked fluctuation in the osmotic conditions of their surroundings in order to survive. This is accomplished by a series of osmoadaptive mechanisms that tend to maintain the internal osmotic pressure slightly higher than that of the extracellular environment.[1,2]

The osmoadaptive mechanisms in enteric bacteria are controlled by osmotolerance genes that upon osmotic challenge trigger regulatory responses.[2–6] These include accumulation of osmolytes (compatible solutes), which support the intracellular turgor in bacteria. Compatible solutes include potassium ions, proline, glutamate, and trehalose.[1,3,7–9] The osmotic regulatory responses also include the synthesis and uptake of osmoprotectant molecules. The major osmoprotectant molecule in enteric bacteria is glycine betaine (GB, **1**).[1–3,10–13] Because of its dipolar character, GB is thought to form a protective "shell" around the protein excluding ions that may otherwise induce adverse conformational changes.[1,2] Under high osmotic conditions, osmoprotectants help maintain protein metabolic functions, in addition to providing positive turgor to the cytoplasm.[14] Upon osmotic stress, bacteria take up GB from the environment or synthesize it from choline (Chol, **2**) via a series of oxidative steps (Figure 1).[1,3,11] Different, osmotic-triggered, active transport mechanisms for GB and its precursor Chol have been identified in several types of bacteria.[1–3,15–17]



1 **2** **3**

Compounds that can interfere with GB uptake or its synthesis from Chol would be expected to have antibacterial activity in fluids of high osmolarity such as urine (which we found to contain GB[18,19]). The transport systems for GB and Chol could also be used to deliver toxic analogs into bacterial cells under conditions of osmotic stress. The therapeutic potential of such compounds prompted us to study the structural requirements for the osmoprotectant activity and the uptake of GB. We have previously shown that the quaternary ammonium end of GB could be replaced, with complete retention of the osmoprotectant activity, by other permanently charged cationic moieties such as dimethylsulfonium group.[20] The goal of the present study is to pursue this investigation by the bioisosteric replacement of the carboxylic end of the molecule with sulfonic, phosphonic, of *N*-arylsulfonyl amide groups and also to further examine the replacement of the quaternary ammonium portion of the molecule with quaternary cyclic ammonium moieties.

The aromatic ring of the *N*-arylsulfonyl series provides us with a vehicle to which different electrophilic moieties (e.g. potentially toxic alkylating groups) could be linked. This allows for the investigation of the possibility of

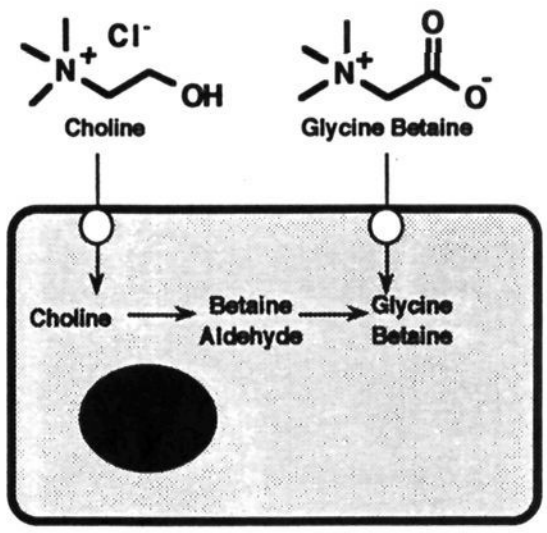


Figure 1. The metabolically linked glycine betaine–choline transport system in enteric bacteria.

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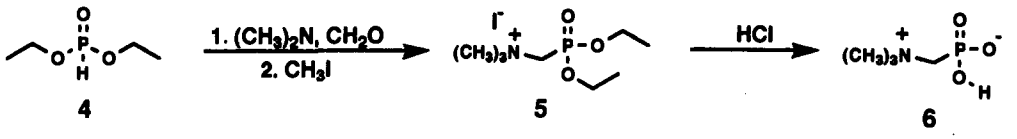
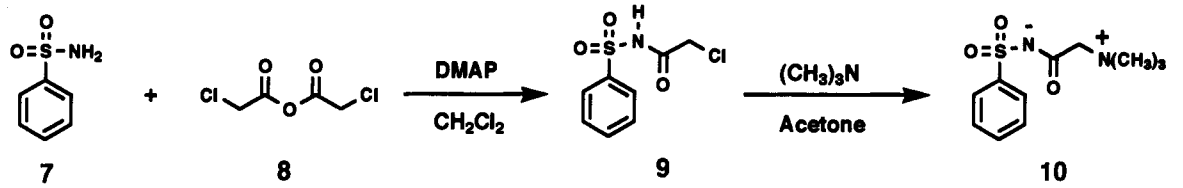
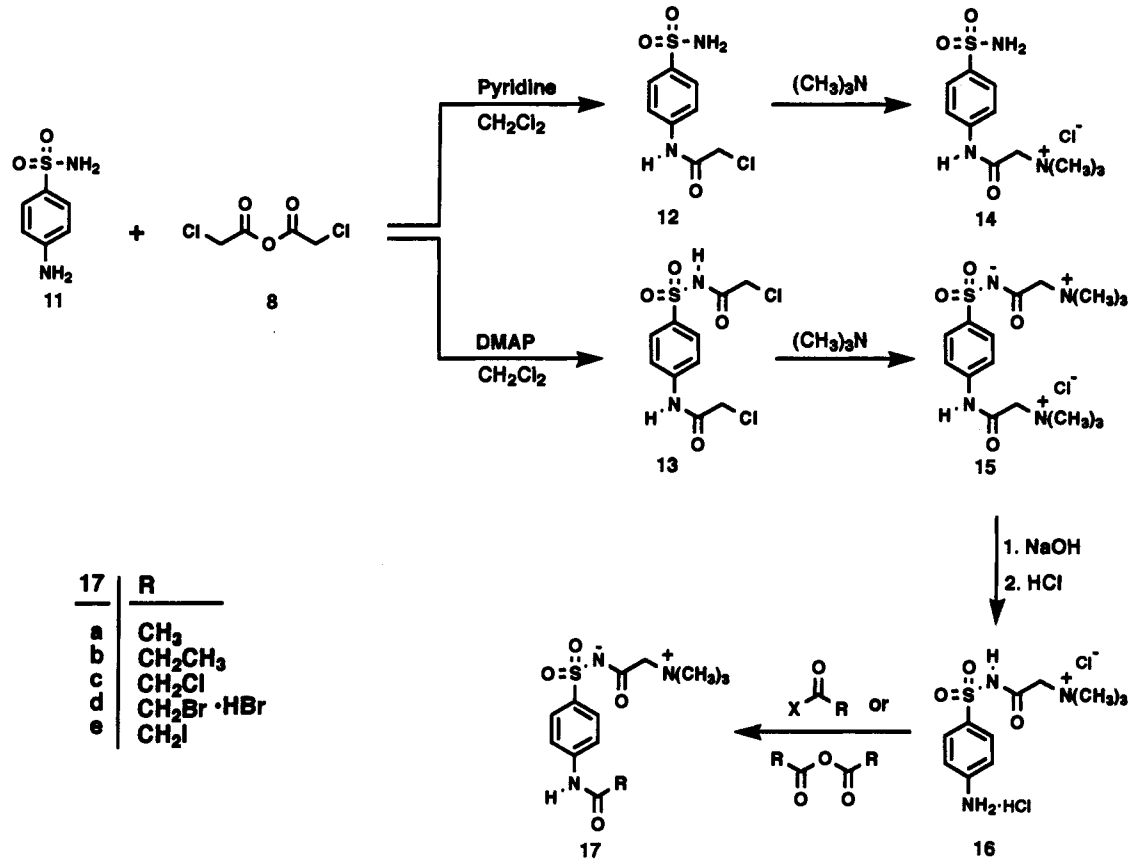
Scheme I



Scheme II



Scheme III



17	R
a	CH_3
b	CH_2CH_3
c	CH_2Cl
d	$CH_2Br \cdot HBr$
e	CH_2I

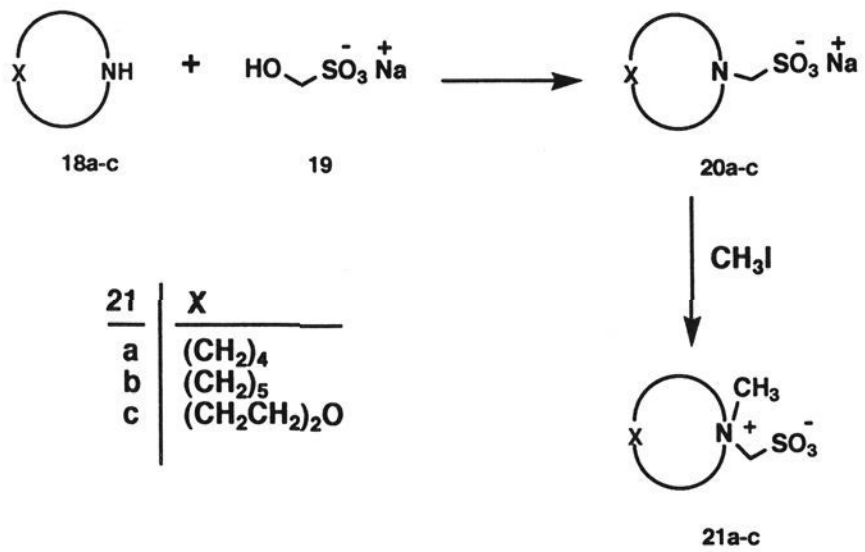
utilizing GB/Chol transport system to deliver such cytotoxic moieties into bacterial cells. This will be a reasonable way to interfere with the osmoadaptive mechanisms in bacteria and may lead to a development of a new class of antibacterial agents.

Chemistry

(Trimethylamino)methanesulfonate (sulfobetaine, 3) was synthesized by the method of King and Skonieczny.[21] (Trimethylamino)methanephosphonate (phosphobetaine, 6) was obtained by acid hydrolysis of the diethyl ester precursor 5. That was obtained by reacting diethyl phosphite with formaldehyde and dimethylamine followed by quaternarization with methyl iodide (Scheme I).

The general routes for the synthesis of the *N*-arylsulfonyl amide derivatives are summarized in Schemes II and III. Benzenesulfonamide (7) was *N*-chloroacetylated with chloroacetic anhydride (8) and 4-(dimethylamino)pyridine (DMAP) to give (*N*-chloroacetamido)benzenesulfonamide (9). Treatment of 9 with trimethylamine in refluxing acetone yielded the target compound *N*-betainylbenzenesulfonamide (10). The synthesis of analogs of compound 10 (compounds 14, 17a–e) started with sulfanilamide (11). Treatment of 11 with 8 and pyridine following the standard sulfonamide acylation procedures yielded only the N^4-chloroacetylated compound 12. Using DMAP instead of pyridine in this reaction we were able to get the N^1,N^4-dichloroacetylated analog compound 13. Reaction of 12 or 13 with trimethylamine in refluxing acetone gave the corresponding betainyl derivatives 14 and 15. Treatment of 15 with 1 equiv of NaOH followed by neutralization with HCl cleaved the N^4-betainyl group to give N^1-betainylsulfanilamide dihydrochloride (16). Treatment of 16 with the appropriate acid anhydride and pyridine gave the corresponding analogs 17a–c in good yields. Compound 17d was obtained by reacting 16 with bromoacetyl bromide and pyridine. Compound 17e was obtained by reacting 16 with iodoacetic anhydride in

Scheme IV



absence of the base. The target compounds **21a–c** were synthesized by the treatment of the commercially available sodium hydroxymethanesulfonate (**19**) with the appropriate cyclic amine to give the intermediates **20a-c** that were then quaternarized to the target compounds by the treatment with methyl iodide.

Biological Evaluation

The effect of the new analogs on the osmotolerance of enteric bacteria was tested against three strains of *E. coli*, a standard strain *E. coli* K10, a salt-resistant strain *E. coli* 31-, and a salt-sensitive strain *E. coli* ATCC 25922. The cells were grown in minimal media (MM) containing increasing concentrations of NaCl (0.1–1.0 M in 0.1 M increments) and the ability of the compounds to enhance or inhibit the bacterial salt tolerance was tested. Also the effect of the tested compounds on the osmoprotectant ability of GB, and its biosynthetic precursor Chol, was tested to determine if we can interfere with the uptake of either.

Results and Discussion

Target compounds were tested against three different strains of *E. coli* grown in different osmotic environments. The compounds were tested at a concentration of 0.1 mM for their ability to interfere with the bacterial maximum salt tolerance.

Maximum salt tolerance of the different strains of *E. coli* grown in MM, or in MM containing GB or Chol (0.1 mM) are recorded in Table I. Maximum salt tolerance of bacteria grown in MM containing the test compounds alone or in combination with GB or Chol are also recorded in Table I. The effect of representative members of the novel compounds on the maximum salt tolerance of *E. coli* 31, K10, or ATCC25922 is graphically presented in Figures 2–4 respectively.

The results indicated that bioisoteric replacement of the carboxylic group of GB with a sulfonic group (sulfobetaine; compound **3**) produces an osmoprotectant with equipotent activity to GB in both the standard and the salt resistant strains used (Figures 2 and 3). It showed some toxicity toward the salt-sensitive strain, which was completely reversed by GB and to a lesser extent by Chol (Figure 4). The phosphonic acid analog (compound **6**) did not show any osmoprotectant activity or toxicity toward any of the tested strains (Table I). This indicates that the sulfonic, and not the phosphonic, group can replace the carboxylic group part of GB with complete retention of its osmoprotectant activity.

Table I. Effect of the Tested Compounds on Bacterial Maximum Salt Tolerance

		maximum salt tolerance (M NaCl)[a]		
compd	media	*E. coli* 31	*E. coli* K10	*E. coli* ATCC25922
	MM	0.65 ± 0.05	0.49 ± 0.03	0.42 ± 0.04
	MM + GB	0.92 ± 0.04	0.90 ± 0.04	0.83 ± 0.05
	MM + Chol	0.88 ± 0.04	0.81 ± 0.04	0.82 ± 0.02
3	MM	0.83 ± 0.06	0.83 ± 0.06	0.27 ± 0.06
	MM + GB	0.90 ± 0.00	0.93 ± 0.06	0.86 ± 0.06
	MM + Chol	0.90 ± 0.00	0.90 ± 0.10	0.33 ± 0.10
6	MM	0.60 ± 0.00	0.40 ± 0.00	0.33 ± 0.06
	MM + GB	0.90 ± 0.00	0.87 ± 0.06	0.80 ± 0.00
	MM + Chol	0.83 ± 0.00	0.77 ± 0.06	0.77 ± 0.06
10[c]	MM	0.73 ± 0.06	0.56 ± 0.06	0.50 ± 0.00
	MM + GB	0.90 ± 0.00	0.90 ± 0.00	0.83 ± 0.06
	MM + Chol	0.90 ± 0.00	0.86 ± 0.06	0.80 ± 0.00
17a[d]	MM	0.70 ± 0.00	0.57 ± 0.07	0.40 ± 0.00
	MM + GB	0.90 ± 0.00	0.90 ± 0.00	0.86 ± 0.07
	MM + Chol	0.80 ± 0.00	0.83 ± 0.07	0.80 ± 0.00
17c	*MM*	*0.63 ± 0.06*	*0.30 ± 0.00*	*0.10 ± 0.00*
	MM + GB	0.90 ± 0.00	0.86 ± 0.06	0.86 ± 0.06
	MM + Chol	0.80 ± 0.00	0.80 ± 0.00	0.80 ± 0.00
21a	MM	0.70 ± 0.00	0.50 ± 0.00	0.13 ± 0.06
	MM + GB	0.93 ± 0.06	0.97 ± 0.06	0.87 ± 0.06
	MM + Chol	0.86 ± 0.06	0.60 ± 0.00	0.20 ± 0.00
21b	MM	0.43 ± 0.05	0.35 ± 0.06	0.13 ± 0.06
	MM + GB	0.93 ± 0.05	0.90 ± 0.00	0.86 ± 0.06
	MM + Chol	0.88 ± 0.05	0.50 ± 0.00	0.20 ± 0.00
21c	MM	0.77 ± 0.06	0.63 ± 0.06	0.20 ± 0.10
	MM + GB	0.97 ± 0.06	0.97 ± 0.06	0.83 ± 0.06
	MM + Chol	0.90 ± 0.00	0.87 ± 0.06	0.23 ± 0.06

[a] Maximum salt tolerance was measured as the maximum NaCl molar concentration that showed 50% bacterial growth (average of three observations ±SD, see Experimental Section). [b] Media consists of minimum media (MM), MM + 0.1 mmol GB or MM + 0.1 mmol Chol with and without 0.1 mmol of the tested compound. [c] Compounds **14**, **15**, and **16** showed similar activity to that of **10**. [d] Compound **17b** showed similar activity to that of **17a**. [e] Compounds **17d–e** showed similar activity to that of **17c**.

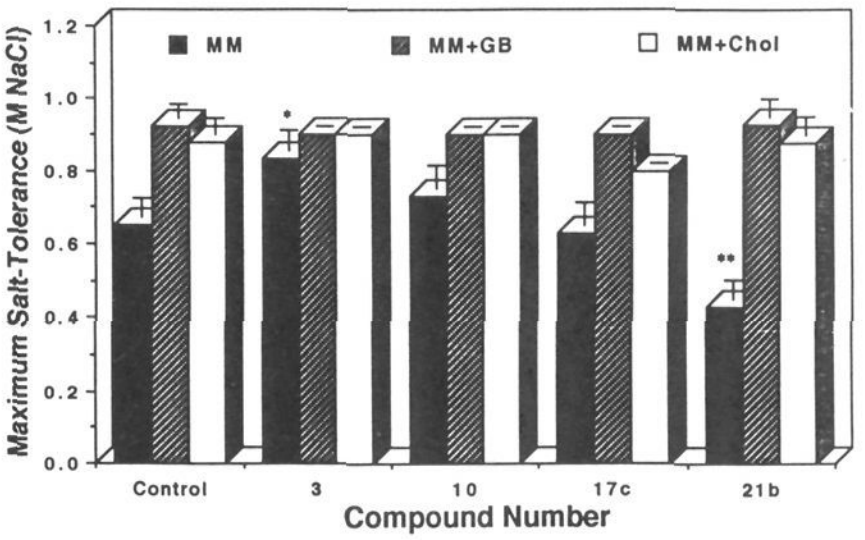


Figure 2. The effect of the tested compounds on the maximum salt tolerance of the salt resistant strain *E. coli 31*; bars represent the SDM ($n \geq 3$). (*) Significant from its control at $p < 0.01$ (paired student T-test). (**) Significant from its control at $p < 0.05$ (paired student T-test).

The *N*-benzenesulfonyl amide analog of GB (compound **10**) showed slight osmoprotectant activity at the concentration used (Table I, Figure 4). That indicates that the GB/Chol transport system can recognize the *N*-arylsulfonyl amide group as bioisostere of the carboxylic group of GB. This type of bioisosteric replacement of the carboxylic group has been utilized before in different systems.[22,23] Addition of 4-amino or 4-betainyl amide groups on the aromatic ring of **10** (compounds **16** and **15**, respectively) did not enhance its osmoprotectant activity (Table I). The N^4 regioisomer of compound **16** (compound **14**) showed similar activity to that of **10** (Table I). When the alkylating

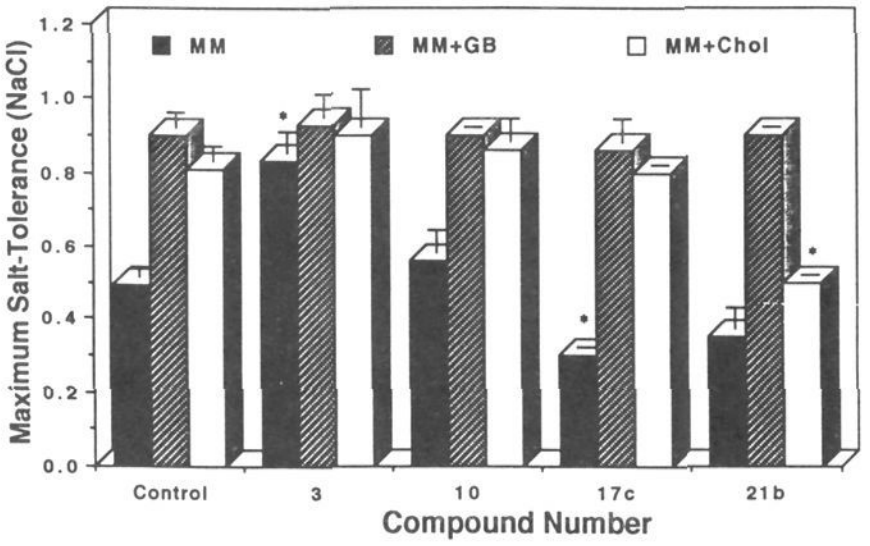


Figure 3. The effect of the tested compounds on the maximum salt tolerance of the standard strain *E. coli K10*; bars represent SDM ($n \geq 3$). (*) Significant from its control at $p < 0.01$ (paired student *T*-test).

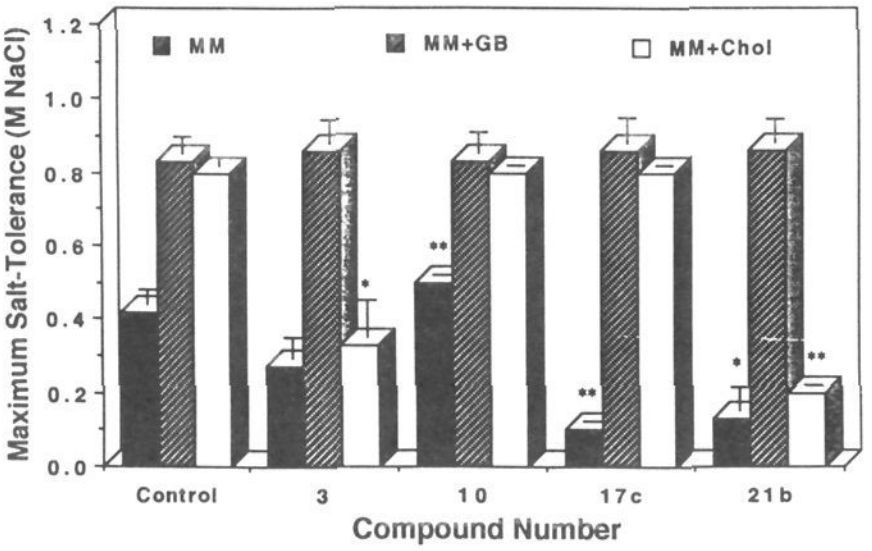


Figure 4. The effect of the tested compounds on the maximum salt tolerance of the salt sensitive strain *E. coli* ATCC 25922; bars represent SDM ($n \geq 3$). (*) Significant from its control at $p < 0.05$ (paired student T-test). (**) Significant from its control at $p < 0.01$ (paired student T-test).

arm (haloacetamido moiety) was attached to N^4 of compound **16** (compounds **17c-e**), they showed strong toxicity to both the standard (K10) and the salt sensitive (ATCC25922) *E. coli* strains (Table I). This toxicity was completely reversed by both GB and Chol (Figures 3 and 4). The fact that compounds **17c–e** did not show any toxicity at zero molar salt concentration indicates that these compounds are probably utilizing the osmotically triggered GB/Chol transport systems to get into the bacterial cells and perform their cytotoxicity. The presence of the natural substrates for these transport systems can prevent such toxicity. To prove that the toxicity is due to the electrophilic character and not due to the size of compounds **17c–e**, we tested compounds **17a** and **17b** in which the halogen atom is replaced by a hydrogen atom or a methyl group, respectively. The results indicated that neither **17a** nor **17b** possess any toxicity, in fact they showed slight osmoprotectant activity (Table I).

When we replace the trimethylammonium portion of the sulfobetaine (compound **3**) with other cyclic ammonium moieties, such as methylpyrrolidinium (compound **21a**), methylpiperidinium (compound **21b**), or methylmorpholinium (compound **21c**), we observed marked inhibition of the bacterial maximum salt tolerance of the standard and the salt-sensitive strains (Figures 3 and 4) and to a lesser extent in the salt-resistant strain (Figure 2). This effect was reversed by GB but not by Chol. This observation suggests that compounds **21a–c** may possess better affinity for the Chol than for the GB transport system. Recently we have shown, through the use of radiolabeled Chol, that (*N*-methylpiperidino)methanesulfonate (**21b**) can block Chol transport into the bacterial cells under high osmotic pressure.[24]

Conclusions

The carboxylic group of GB is bioisosterically replaceable with sulfonic but not phosphonic groups (compounds **3** and **6**). We were able to utilize the GB/Chol transport systems to deliver cytotoxic analogs of GB (compounds **17c–e**) into bacterial cells. The Chol transport system is inhibited by the (methyl-cyclic ammonio)methanesulfonate (compounds **21a–c**).

Experimental Section

Bacterial Strains. *E. coli* K10 was provided by L. T. Smith (University of California, Davis). *E. coli* ATCC 25922 was obtained from the American Type Culture Collection. *E. coli* 31 was obtained from clinical isolates from young women with urinary tract infections. Stock cultures, from a single colony of each isolate, were grown in Schaedler's broth containing 15% glycerin (Difco, Detroit, MI) and stored at 40 °C.

Media. Minimal medium consisted of glucose (2.0 g), K_2PO_4 (10.5 g), $KHPO_4$ (4.5 g), trisodium citrate (0.5 g), $MgSO_4$ (0.264 g), and $(NH_4)_2SO_4$ (1.0 g) per liter; the pH was adjusted to 7.2 prior to use. The osmolarity was 238 mOsmol/kg as determined by freezing point depression. The osmolarity of the minimal medium was elevated by the additional NaCl as indicated. GB and Chol were obtained from Sigma Chemical Co. (St. Louis, MO).

Growth Conditions. A single colony, isolated from MacConkey agar plates (BBL Laboratories, Cockeysville, MD), was subcultured in minimal media and incubated overnight at 37 °C. A 1:200 dilution of a 0.5 McFarland standard (final concentration $\sim 5 \times 10^5$ cfu/mL) was added to tubes containing minimum medium alone or with graded concentration of NaCl (0.1–1 M in 0.1 M steps). Growth was assessed by OD_{600nm} at 48 h using a Milton Roy spectrophotometer (Spectronic 601, Rochester, NY). The end point for salt tolerance was the maximum concentration of NaCl at which there was 50% or more growth (absorbance of ~0.5) compared with tubes in there was full growth.

Melting points were determined using Thomas-Hoover melting point apparatus and are uncorrected. Structures of all the new compounds were confirmed by ^{1}H-NMR, elemental analysis, and/or MS spectra. ^{1}H-NMR spectra were obtained with IBM AF/250 FTNMR (250 MHz) with D_2O as solvent and HOD as internal reference. Mass spectra were obtained with Kratos MS 25 RFA mass spectrometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. All analytical results for the indicated elements were ±0.4% of the theoretical values.

((*N,N,N*-Trimethylammonio)methyl)phosphonate (6). Diethyl((trimethylammonio)methyl)phosphonate iodide (**5**) was prepared according to the reported procedure (62%), mp 120–122 °C (lit.[25] mp 126 °C). The diethyl ester **5** (2.1 g, 6.4 mmol) was refluxed with concentrated HCl (10 mL) for 4 h, and the solvent was removed under vacuum. The resulting solid was dissolved in H_2O (150 mL), brought to ~pH 8 with 10% aqueous NaOH, and passed through a column of amberlite MB-1 desalting resin. Solvent was removed under vacuum to give **6** (0.74 g, 72%): mp 285 °C (lit.[25] mp 270 °C); ^{1}H NMR δ 3.23 (d, 2 H) and 3.1 (s, 9 H); MS *m/e* FAB 153 M^+. Anal. ($C_4H_{12}NO_3P$) C, H, N.

***N*-(Chloroacetyl)benzenesulfonamide (9).** A suspension of benzenesulfonamide (**7**; 1.8 g, 11.4 mmol), chloroacetic anhydride (**8**; 2.1 g, 12.3 mmol), and DMAP (1.4 g, 11.4 mmol) in dry CH_2Cl_2 (20 mL) was heated at reflux for 5 h. The reaction mixture was cooled and washed with 1 N HCl (2 × 20 mL). The nonaqueous layer was concentrated at reduced pressure. Upon addition of hexane the product precipitated out of solution and was collected by filtration. Recrystallized from toluene gave **9** (2.4 g, 90%), mp 94–96 °C (lit.[26] mp 104–105 °C).

***N*-Betainylbenzenesulfonamide (10).** Trimethylamine (4 mL, 25% aqueous solution, 17 mmol) was added to **9** (1 g, 5.0

mmol) in acetone (30 mL), and the reaction mixture was heated at reflux for 5 h. The product that separated out was collected and recrystallized from H_2O to give colorless crystals of **10** (0.61 g, 56%): mp 280–283 °C; ^{1}H NMR δ 7.73 (d, J = 8 Hz, 2 H), 7.46 (m, 3 H), 3.79 (s, 2 H), and 3.04 (s, 9 H); MS m/e EI 256 M^+. Anal. ($C_{11}H_{16}NO_3S$) C, H, N.

N^3-(Chloroacetyl)sulfanilamide (12). A solution of 8 (5.6 g, 32.7 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a suspension of sulfanilamide (11; 5.0 g, 29.0 mmol) and pyridine (5.0 g, 63.0 mmol) in dry CH_2Cl_2 (100 mL), and the reaction mixture was heated at reflux for 4 h. The product that separated out was collected by filtration, washed with 1 N HCl, and recrystallized from MeOH to give colorless crystals of **12**, mp 218–220 °C (lit.[27,28] mp 217 °C).

N^1,N^3-Bis(chloroacetyl)sulfanilamide (13). A solution of 8 (30 g, 175.45 mmol) in dry CH_2Cl_2 (100 mL) was added over a period of 2 h to a cooled suspension of 11 (ice bath; 10 g, 58.0 mmol) and DMAP (15 g, 122.7 mol) in dry CH_2Cl_2 (200 mL). The reaction mixture was heated at reflux for 4 h, upon which a heavy precipitate separated out that was collected by filtration and washed with 1 N HCl. The solid was dissolved in 10% aqueous NaOH solution, treated with activated charcoal, and reprecipitated with 10% aqueous HCl. Recrystallization from MeOH gave yellowish crystals of **13** in almost quantitative yield; mp 246 °C (lit.[27] mp 215 °C). Anal. ($C_{10}H_{10}Cl_2N_2O_4S$) C, H, N.

N^3-Betainylsulfanilamide Hydrochloride (14). The same procedure as for **10** was applied using **12** (1 g, 4.02 mmol) as starting material. Recrystallization was done from H_2O/acetone to give 14 (0.66 g, 50%) as colorless crystals: mp 268 °C; ^{1}H NMR δ 7.78 (d, J = 7 Hz, 2 H), 7.58 (d, J = 7 Hz, 2 H), 4.2 (s, 3 H), and 3.25 (s, 9 H); MS m/e EI 271 M^+. Anal. ($C_{11}H_{17}N_3O_3S$·HCl) C, H, N.

N^1,N^3-Dibetainylsulfanilamide Hydrochloride (15). Trimethylamine (5.6 mL, 25% w/v, 23.2 mmol) was added to a suspension of **13** (1 g, 3.0 mmol), and the resulting solution was heated at reflux for 2 h. The product that separated out was collected and recrystallized from H_2O/acetone to give white clusters of **15** (0.9 g, 81%): mp 302 °C dec; ^{1}H NMR δ 7.73 (d, J = 8 Hz, 2 H), 7.52 (d, J = 8 Hz, 2 H), 4.15 (s, 2 H), 3.77 (s, 2 H), 3.21 (s, 9 H), and 3.03 (s, 9 H); MS m/e EI 270 M^+. Anal. ($C_{16}H_{26}N_4O_4S$·HCl) C, H, N.

N^1-Betainylsulfanilamide Dihydrochloride (16). A mixture of **15** (0.8 g, 2.0 mmol) and NaOH (0.1 g, 2.5 mmol) in H_2O (10 mL) was heated at reflux for 15 min and acidified to ~pH 4 with 10% aqueous HCl. The solvent was removed at reduced pressure, and the organic material was extracted from the residue with MeOH (2 × 25 mL). The product was precipitated with ether to give **16** (0.5 g, 74%), mp 248 °C (lit.[29] mp 254 °C).

General Procedure for the Synthesis of N^3-Acyl-N^1-betainylsulfanilamides (17a–e). N^3-Acetyl-N^1-betainylsulfanilamide (17a). A mixture of **16** (0.35 g, 1.0 mmol), acetic anhydride (0.122 g 1.2 mmol), and dry pyridine (0.2 g, 2.5 mmol) in dry CH_2Cl_2 (10 mL) was heated at reflux for 4 h during which the product separated out. It was collected by filtration and recrystallized from MeOH/ether to give **17a** (0.3 g, quantitative): mp 263–265 °C; ^{1}H-NMR δ 7.7 (d, J = 8 Hz, 2 H), 7.48 (d, J = 8 Hz, 2 H), 3.78 (s, 2 H), 3.04 (s, 9 H), and 2.03 (s, 3 H). Anal. ($C_{13}H_{19}N_3O_4S$·H_2O) C, H, N.

N^3-Propionyl-N^1-betainylsulfanilamide (17b). The same procedure as for **17a** was used except that propionic anhydride (0.15 g, 1.2 mmol) was used instead of acetic anhydride to give **17b** (0.35 g, 92%): mp 240–241 °C; ^{1}H NMR δ 7.7 (d, J = 8 Hz, 2 H), 7.48 (d, J = 8 Hz, 2 H), 3.78 (s, 2 H), 3.05 (s, 9 H), 2.3 (q, J = 7 Hz, 2 H), and 1.03 (t, J = 7 Hz, 3 H). Anal. ($C_{14}H_{21}N_3O_4S$·HCl) C, H, N.

N^3-(Chloroacetyl)-N^1-betainylsulfanilamide (17c). The same general procedure as for **17a** was applied except that the anhydride used was 8 (0.2 g, 1.2 mmol) to give **17c** (0.3 g, 84%). Crystallization solvent was H_2O/acetone: mp 225–227 °C; ^{1}H NMR δ 7.75 (d, J = 8 Hz, 2 H), 7.55 (d, J = 8 Hz, 2 H), 4.18 (s, 2 H), 3.8 (s, 2 H), and 3.05 (s, 9 H); MS m/e EI 347 M^+. Anal. ($C_{13}H_{18}ClN_3O_4S$·H_2O) C, H, N.

N^3-(Bromoacetyl)-N^1-betainylsulfanilamide Hydrobromide (17d). The same general procedure as for **17a** was applied except that bromoacetyl bromide (0.24 g, 1.2 mmol) was substituted for acetic anhydride, and the reaction was carried out at room temperature to give **17d** (0.45 g, 93%). Crystallization solvent was MeOH/ether: mp 212–214 °C; ^{1}H NMR δ 7.74 (d, J = 8 Hz, 2 H), 7.53 (d, J = 8 Hz, 2 H), 3.95 (s, 2 H), 3.8 (s, 2 H), and 3.06 (s, 9 H). Anal. ($C_{13}H_{18}BrN_3O_4S$·HBr) C, H, N.

N^3-(Iodoacetyl)-N^1-betainylsulfanilamide (17e). The same general procedure as for **17a** was applied except that pyridine was omitted, iodoacetic anhydride (0.42 g, 1.2 mmol) was used instead of acetic anhydride, and the reaction was carried out at room temperature overnight to give **17e** (0.56 g, quantitative). Crystallization solvent was H_2O/acetone: mp 198–200 °C; ^{1}H NMR δ 7.72 (d, J = 7 Hz, 2 H), 7.50 (d, J = 7 Hz, 2 H), 3.79 (s, 4 H), and 3.05 (s, 9 H). Anal. ($C_{13}H_{18}IN_3O_4S$) C, H, N.

(*N*-Methylpyrrolidinio)methanesulfonate (21a). Pyrrolidine (0.79 g, 11.1 mmol) was added to a solution of sodium hydroxymethanesulfonate (**19**; 1.0 g, 7.4 mmol) in H_2O (20 mL), and the reaction mixture was stirred at room temperature for 3 h. The H_2O was then distilled off under reduced pressure to give *N*-pyrrolidinylmethanesulfonate (**20a**; 1.3 g, 90%). Quaternarization was carried out by stirring **20a** (0.5 g, 2.6 mmol) with iodomethane (0.4 g, 2.8 mmol) in aqueous methanol solution overnight. The zwitterion was obtained by passing an aqueous solution of the quaternary ammonium salt through a column of Amberlite MB-1 desalting resin. Distillation of H_2O under reduced pressure gave **21a** (0.3 g, 62%): mp 220–222 °C; ^{1}H-NMR δ 4.4 (s, 2 H), 3.7–3.5 (m, 4 H), 3.18 (s, 3 H), and 2.2–2.0 (m, 4 H). Anal. ($C_6H_{13}NO_3S$) C, H, N.

(*N*-Methylpiperidinio)methanesulfonate (21b). The same procedure as for **21a** was used except that piperidine (0.94 g, 11.0 mmol) was used instead of pyrrolidine to give **21b** (0.25 g, 53%): mp 224–226 °C; ^{1}H NMR δ 4.38 (s, 2 H), 3.62–3.55 (m, 2 H), 3.42–3.32 (m, 2 H), 3.22 (s, 3 H), 1.80 (m, 4 H), and 1.57–1.54 (m, 2 H); MS m/e FAB 194 M^+. Anal. ($C_7H_{15}NO_3S$) C, H, N.

(*N*-Methylmorpholinio)methanesulfonate (21c). The same procedure as for **21a** was used except that morpholine (0.97 g, 11.0 mmol) was used to give **21c** (0.22 g, 45%): mp 272–274 °C; ^{1}H NMR δ 4.55 (s, 2 H), 3.99 (t, J = 7 Hz, 4 H), 3.79–3.68 (m, 2 H), 3.6–3.5 (m, 2 H), and 3.39 (s, 3 H). Anal. ($C_6H_{13}NO_4S$) C, H, N.

References

(1) (a) Csonka, L. N. Physiological and Genetic Responses of Bacteria to Osmotic Stress. *Microbiol. Rev.* **1989**, *53*, 121–147. (b) Csonka, L. N.; Hanson, A. D. Prokaryotic Osmoregulation: genetic and physiology. *Annu. Rev. Microbiol.* **1991**, *45*, 569–606. (c) Brown, A. D. *Microbial Water Stress Physiology: Principles and Perspectives*; Wiley: Chichester, 1990.

(2) Higgins, C. F.; Cairney, J.; Stirling, D. A.; Sutherland, L.; Booth, I. R. Osmotic Regulation of Gene Expression. *Trends Biochem. Sci.* **1987**, *12*, 339–344.

(3) LeRudulier, D.; Strom, A. R.; Dandekar, A. M.; Smith, L. T.; Valentine, R. C. Molecular Biology of Osmoregulation. *Science* **1984**, *224*, 1064–1068.

(4) Andresen, P. A.; Kaasen, I.; Styrvold, O. B.; Boulnois, G.; Strom, A. R. Molecular Cloning, Physical Mapping and Expression of the *bet*-Genes Governing the Osmoregulatory Choline-Glycine Betaine Pathways of *Escherichia coli*. *J. Gen. Microbiol.* **1988**, *134*, 1737–1746.

(5) Lamark, T.; Kaasen, I.; Eshoo, M. W.; Falkenberg, P.; McDougall, J.; Strom, A. R. DNA Sequence and Analysis of the *bet*-Genes Encoding the Osmoregulatory Choline-Glycine Betaine Pathway of *Escherichia coli Mol. Microbiol.* **1991**, *5*, 1049–1064.

(6) Barron, A.; May, G.; Bremer, E.; Villarejo, M. Regulation of Envelope Protein Composition During Adaptation to Osmotic Stress in *E. coli*. *J. Bacteriol.* **1986**, *167*, 433–438.

(7) Booth, I. R.; Higgins, C. F. Enteric Bacteria and Osmotic Stress. *FEMS Microbiol. Rev.* **1990**, *75*, 239–246.

(8) Boos, W.; Ehmann, U.; Frokl, H.; Klein; W.; Rimmele, M.; Postma, P. Trehalose Transport and Metabolism in *Escherichia coli*. *J. Bacteriol.* **1990**, *172*, 3450–3461.

(9) Styrvoid, O. B.; Strom, A. R. Synthesis, Accumulation, and Excretion of Trehalose in Osmotically Stressed *Escherichia coli K-12 strains*. *J. Bacteriol.* **1991**, *173*, 1187–1192.

(10) Le Rudulier, D.; Bouilard, L. Glycine Betaine, an Osmotic Effector in *Klebsiella pneumoniae and Other Members of the Enterobacteriaceae*. *Appl. Environ. Microbiol.* **1983**, *46*, 152–159.

(11) Landfald, B.; Strom, A. R. Choline-Glycine Betaine Pathway Confers a High Level of Osmotic Tolerance in *Escherichia coli*. *J. Bacteriol.* **1986**, *165*, 849–855.

(12) Kunin, C. M.; Rudy, J. Effect of NaCl-Induced Osmotic Stress on Intracellular Concentrations of Glycine Betaine and Potassium in *Escherichia coli*, *Enterococcus faecalis* and *Staphylococci*. *J. Lab. Clin. Med.* **1991**, *188*, 217–224.

(13) Imhoff, J. F.; Rodriquez-Valera, F. Betaine is The Main Compatible Solute of Halophilic Eubacteria. *J. Bacteriol.* **1984**, *160*, 478–479.
(14) Paleg, L. G.; Douglas, T. J.; Van Daal, A.; Keech, D. B. Proline, Betaine and Other Organic Solutes Protect Enzymes Against Heat Inactivation. *Aust. J. Plant Physiol.* **1981**, *8*, 107–114.
(15) Perroud, B.; Le Rudulier, D. Glycine Betaine Transport in *Escherichia coli*: Osmotic Modulation. *J. Bacteriol.* **1985**, *161*, 393–401.
(16) May, G.; Faatz, E.; Villarejo, M.; Bremer, E. Binding Protein Dependent Transport of Glycine Betaine and its Osmotic Regulation in *Escherichia coli K-12*. *Mol. Gen. Genet.* **1986**, *205*, 225–233.
(17) Styrvold, O. B.; Falkenberg, P.; Landfald, B.; Eshoo, M. W.; Bjornsen, T.; Strom, A. R. Selection, Mapping and Characterization of Osmoregulatory Mutants of *Eschirechia coli* Blocked in the Choline-Glycine Betaine Pathway. *J. Bacteriol.* **1986**, *165*, 856–863.
(18) Chambers, S. T.; Kunin, C. M. The Osmoprotective Properties of Urine for Bacteria. *J. Infect. Dis.* **1985**, *152*, 1308–1315.
(19) Chambers, S. T.; Kunin, C. M. Isolation of Glycine Betaine and Proline Betaine from Human Urine. *J. Clin. Invest.* **1987**, *79*, 731–737.
(20) Chambers, S. T.; Kunin, C. M.; Miller, D. D.; Hamada, A. Dimethylthetin Can Substitute for Glycine Betaine as an Osmoprotectant Molecule for *Escherichia coli*. *J. Bacteriol.* **1987**, *169*, 4845–4847.
(21) King, J. F.; Skonieczny, S. Trimethylammoniomethanesulfinate and Trimethylammoniomethanesulfonate, the Simplest Sulfinic and Sulfonic Acid Betaines. *J. Phosphorous Sulfur* **1985**, *25*, 11–20.
(22) Schaaf, T. K.; Hess, H.-J. Synthesis and Biological Activity of Carboxyl-Terminus Modified Prostaglandin Analogues. *J. Med. Chem.* **1979**, *22*, 1340–1346.
(23) Matassa, V. G.; Maduskuie, T. P.; Shapiro, H. S.; Hesp, B.; Snyder, D. W.; Aharony, D.; Krell, R. D.; Kieth, R. A. Evolution of a Series of Peptidoleukotriene Antagonists: Synthesis and Structure/Activity Relationships of Substituted Indoles and Indazoles. *J. Med. Chem.* **1990**, *33*, 1781–1790.
(24) Kunin, C. M.; Tong, H. H.; Miller, D. D.; Abdel-Ghany, Y. S.; Poggi, M., Le Rudulier, D. Effect of Novel Compound, 1-Methyl-1-Piperidinio Methane Sulfonate (MPMS), on the Osmoprotectant Activity of Glycine Betaine, Choline and Proline in *Escherichia coli*. *Arch. Microbiol.*, submitted for publication.
(25) Myers, T. C.; Jibril, A. O. a series of w-Trimethylammoniumalkylphosphonic Acids and their Diethyl Ester Iodides. *J. Org. Chem.* **1957**, *22*, 180–182.
(26) Kretov, A. E.; Kremlev, M. M. *N*-Chloro and *N*-Trichloroacetyl Derivatives of Arenesulfonamides. *Ukrain. Khim. Zhur.* **1959**, *25*, 482–483.
(27) Whitney, P. L.; Folsch, G.; Nyman, P. O.; Malmastrom, B. G. Inhibition of Human Erythrocyte Carbonic Anhydrase B by Chloroacetyl Sulfonamides with Labeling of the Active Site. *J. Biol. Chem.* **1967**, *242*, 4206–4211.
(28) Jacobs, W.; Heidelberger, M. On Amides, Uramino Compounds and Urides Containing an Aromatic Nucleus. *J. Am. Chem. Soc.* **1917**, *39*, 2418–2443.
(29) Klotzer, W. New Reactions of Sulfanilamide and New N^1-Substituted Sulfanilamides. *Monatsh* **1956**, *87*, 313–318.