(1–39) and -(1–10) in steroidogenesis.[10] ACTH-(11–24), however, retains most of the effect of native ACTH on magnesium accumulation. It appears, therefore, that in ACTH different sequences of residues are responsible for adenylyl cyclase activation and the major portion of magnesium accumulation. Residues 21–24 are probably the ones which play the most important role in magnesium accumulation, since ACTH-(1–20) stimulates this activity only minimally, while ACTH-(1–24) is as potent as the parent hormone.

The mechanism by which ACTH stimulates magnesium accumulation appears to be analogous to the action of α-adrenergic agents. The bulk of the effect of both ACTH and adrenergic agents on magnesium accumulation is blocked by α-adrenergic antagonists. The effect of ACTH-(11–24), which lacks a stimulatory effect on adenylyl cyclase, is similar to that of the α-adrenergic agonist, methoxamine, which also does not stimulate this enzymatic activity. Both substances fail to cause the rapid initial accumulation seen with norepinephrine, native ACTH, and isoproterenol. This accumulation is small in net amount and inhibited by β blockade. While the bulk of magnesium accumulation seen after hormone stimulation of adipocyte plasma membranes appears to operate through an α-adrenergic receptor, cAMP may mediate a much smaller accumulation of magnesium seen immediately after hormone stimulation.

Experimental Section

Materials. ACTH-(1–30) was obtained from Calbiochem, La Jolla, Calif. ACTH-(1–24) (Synacthen) and phentolamine (Regitine) was obtained from Ciba-Geigy Corp., Summit, N.J. ACTH-(1–20) was synthesized in our laboratories by the solid-phase method as previously described.[6] ACTH-(11–24) was generously supplied by Dr. W. Rittel, Ciba Geigy Corp., Basel, Switzerland. Norepinephrine was obtained from Sigma Chemical Co. L-Propranolol was obtained from Ayerst Laboratories, New York, N.Y., and methoxamine hydrochloride from the Wellcome Research Laboratories, Research Triangle Park, N.C.

Bioassays. Rat adipocytes were isolated as described by Cushman[7] using chromatographically purified collagenase obtained from Worthington Biochemical Corp., Freehold, N.J. Plasma membrane vesicles containing the fluorescent ligand 8-hydroxyquinoline-5-sulfonic acid were prepared from rat adipocytes and Mg^{2+} accumulation determined from the increase in fluorescence of the ligand as previously described.[1] ^{63}Ni was used to measure the release of fatty acid from isolated adipocytes by the method of Ho[8] as modified by Draper et al.[9]

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Quantitative Structure–Activity Relationships of Chymotrypsin. On the Predictive Value of Correlation Equations

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It is shown that inhibition constants against chymotrypsin for new congeners of the type $R_1C(=O)R_2$ are well predicted by a correlation equation published earlier.

Now that the field of quantitative structure–activity relationships (QSAR) has reached a modest level of maturity, it becomes of increasing interest to begin to more rigorously assess the predictive value of correlation equations. Toward this end we have recently surveyed the literature and summarized examples where previously formulated equations were later found to give good correlations with new data.[2] Rouot et al.[3] have recently illustrated the forecasting ability of QSAR with clonidine analogues. In addition, we have now shown that a QSAR for 102 antimalarials[4] gives good results for over 100 new analogues.[5] In this report we wish to show that a QSAR formulated[6] for the inhibition of chymotrypsin by inhibitors of the type $R_1C(=O)R_2$ applies to new data.

$$\log 1/C = 0.35\Sigma MR - 0.0099(\Sigma MR)^2 + 0.74(I\text{-}1) + 0.83(I\text{-}2) - 0.36(I\text{-}3) - 0.77(I\text{-}4) + 0.66 \quad (1)$$
$$n = 103;\ r = 0.944;\ s = 0.290$$

Equation 1 was formulated from data obtained by Baker and his students. In this equation, C is the molar concentration of inhibitor producing 50% inhibition and ΣMR refers to the sum of MR for R_1 and R_2 (scaled by 0.1). The compounds are ketones, amides, or esters. R_2 represents Me of methyl ketones or NHR or OR of amides or esters. In the large majority of cases, $R_1 = -CH_2OC_6H_4\text{-}X$. The

Table I. Parameters Used in the Formulation of Eq 2[a]

No.	Log $1/K_i$ Obsd[b]	Log $1/K_i$ Calcd[c]	$\lvert\Delta \log 1/K_i\rvert$	ΣMR	*I*-1	*I*-2	*I*-3	*I*-4	*I*-5	R_1	R_2	Ref
1	1.72	2.20	0.48	4.12	0	0	0	0	1	H	NHC_6H_4-4-$COCH_3$	7c
2	1.90	2.07	0.17	3.63	0	0	0	0	1	H	NHC_6H_4-4-CN	7c
3	2.06	2.12	0.06	3.79	0	0	0	0	1	H	NHC_6H_4-4-OCH_3	7c
4	2.17	2.12	0.05	3.79	0	0	0	0	1	H	NHC_6H_4-3-OCH_3	7c
5	2.40	1.93	0.47	3.10	0	0	0	0	1	H	NHC_6H_5	7c
6	2.42	2.10	0.32	3.74	0	0	0	0	1	H	NHC_6H_4-4-NO_2	7c
7	2.52	2.05	0.47	3.56	0	0	0	0	1	H	NHC_6H_4-4-CH_3	7c
8	3.10	2.89	0.21	3.60	0	1	0	0	1	H	NHC_6H_4-3-Cl	7c
9	3.40	2.89	0.51	3.60	0	1	0	0	1	H	NHC_6H_4-4-Cl	7a
10	1.90	2.28	0.38	4.42	0	0	0	0	1	$CH_2NHCOC_6H_5$	NH_2	7a
11	2.64	2.45	0.19	5.12	0	0	0	0	1	CH_2CH_2-3-(C_8H_6N)	NH_2	7a
12	2.74	2.57	0.17	5.59	0	0	0	0	1	CH_3	$NHCH_2CH_2$-3-(C_8H_6N)	7a
13	2.92	2.55	0.37	5.54	0	0	0	0	1	CF_3	$NHCH_2CH_2$-3-(C_8H_6N)	7a
14	2.10	2.05	0.05	4.91	0	0	1	0	1	CF_3	$NHCH(COO^-)CH_2C_6H_5$	7d
15	2.35	2.16	0.18	5.38	0	0	1	0	1	CF_3	$NHCH(COO^-)CH_2C_6H_4$-3-CH_3	7d
16	2.35	2.89	0.54	4.99	0	1	1	0	1	CF_3	$NHCH(COO^-)CH_2C_6H_4$-2,4-F_2	7d
17	2.57	2.87	0.30	4.90	0	1	1	0	1	CF_3	$NHCH(COO^-)CH_2C_6H_4$-3-F	7d
18	2.82	2.16	0.65	5.38	0	0	1	0	1	CF_3	$NHCH(COO^-)CH_2C_6H_4$-4-CH_3	7d
19	2.89	2.87	0.02	4.90	0	1	1	0	1	CF_3	$NHCH(COO^-)CH_2C_6H_4$-4-F	7d
20	3.00	3.06	0.06	5.70	0	1	1	0	1	CF_3	$NHCH(COO^-)CH_2C_6H_4$-4-Br	7d
21	1.82	2.05	0.23	3.54	0	0	0	0	1	$CH_2C_6H_5$	NH_2	7b
22	1.89	2.05	0.16	3.55	0	0	0	0	1	CH_3	NHC_6H_5	7b
23	1.92	2.29	0.37	4.47	0	0	0	0	1	$CH_2CH_2CH_2C_6H_5$	NH_2	7b
24	1.94	2.30	0.36	4.49	0	0	0	0	1	CH_3	$NHCH_2CH_2C_6H_5$	7b
25	2.00	2.42	0.42	4.97	0	0	0	0	1	CH_2Cl	$NHCH_2CH_2C_6H_5$	7b
26	2.00	1.92	0.08	3.08	0	0	0	0	1	C_6H_5	NH_2	7b
27	2.08	2.13	0.05	3.85	0	0	0	0	1	4-C_9H_6N	NH_2	7b
28	2.12	2.18	0.06	4.02	0	0	0	0	1	CH_3	$NHCH_2C_6H_5$	7b
29	2.15	2.17	0.02	4.01	0	0	0	0	1	$CH_2CH_2C_6H_5$	NH_2	7b
30	2.18	2.18	0.00	4.02	0	0	0	0	1	CH_2CH_3	NHC_6H_5	7b
31	2.20	2.30	0.10	4.49	0	0	0	0	1	CH_2CH_3	$NHCH_2C_6H_5$	7b
32	2.25	2.24	0.01	4.26	0	0	0	0	1	2-$C_7H_5N_2$	OCH_3	7b
33	2.31	2.30	0.01	4.50	0	0	0	0	1	CH_2Cl	$NHCH_2C_6H_5$	7b

[a] The parameters in this table plus those in Table IV of ref 2 were used to formulate eq 2. [b] From ref 7. [c] Calculated using eq 2.

exponential term contributes little (less than 1%) to the reduction in variance although it is statistically significant. The indicator variable *I*-1 takes the value of 1 for cases where $R_1 = NHC_6H_4SO_2F$. The SO_2F function increases inhibition by about fivefold, other things being equal. *I*-2 is an unusual parameter in that it assumes the value of 1 for cases where there are one or two halogens on the aromatic rings of either R_1 or R_2. For some strange reason, the effect of halogen (mostly Cl) is not additive. The variables *I*-3 and *I*-4 parameterize the presence of a COO^- function on R_1 and R_2, respectively. Equation 1 is a robust expression based on about 20 data points/variable covering a range of 2000 in $1/C$.

Since the publication of eq 1, we have come across new data[7] from other laboratories on similar inhibitors of chymotrypsin. However, in this new work inhibition is expressed in terms of K_i rather than I_{50}. Since I_{50} and K_i are similar parameters, we have combined the 33 new data points of Table I with the 103 of eq 1 by means of an indicator variable *I*-5.

$$\log 1/C = 0.33\ (\pm 0.15)\ \Sigma MR - 0.0083\ (\pm 0.0087)\ (\Sigma MR)^2 + 0.75\ (\pm 0.31)\ (I\text{-}1) + 0.82\ (\pm 0.13)\ (I\text{-}2) - 0.35\ (\pm 0.18)\ (I\text{-}3) - 0.78\ (\pm 0.18)\ (I\text{-}4) + 0.23\ (\pm 0.16)\ (I\text{-}5) + 0.77\ (\pm 0.55) \quad (2)$$

$$n = 136;\ r = 0.940;\ s = 0.292$$

In eq 2, *I*-5 is the correction factor placing log K_i on the same basis as log $1/C$. Note that all of the coefficients in the terms of eq 2 as well as the intercept are quite close to those of eq 1. Also, the quality of fit in terms of *r* and *s* is the same for the two equations. No data points have been omitted in the formulation of either eq 1 or 2.

Again we find that the exponential term is of very marginal importance although it is significant ($F_{1,128} = 3.54$); in fact, it reduces the variance in the data by only 0.3%. In the case of eq 1, it accounted for only 0.6% of the variance in log $1/C$.

In the development of eq 2, the 33 new data points were run as a set. This produced an equation having similar coefficients as eq 2 except that $(\Sigma MR)^2$ was not significant because only relatively low MR values were involved. Also, no structural features for *I*-1 or *I*-4 were present so that these variables could not be tested. The standard deviation for this three-variable equation was 0.310. Dropping two poor data points gave an equation with *s* of 0.272. The goodness of fit for the small set was about the same as the large, although the correlation was lower ($r = 0.79$) because of much less variance in the data in the small set.

Even though the K_i values of Table I have been determined in somewhat different ways in three laboratories, they are well fit in eq 2. On a closer inspection of the log K_i values in Table I it is found that they range from 1.72 to 3.4. Even adding the figure of 0.22 from *I*-5 to these values, they are still well within the range (1.47–4.85) used in the formulation of eq 1; therefore, it is not surprising that their activity is well predicted by eq 1 when account is taken of the difference between $1/C$ and $1/K_i$.

One interesting feature of the new data is a set of nine aldehydes. These points are somewhat less well fit than the others, their standard deviation being 0.375 compared to 0.292 for the set of 136.

Equation 2 accounts for 88.3% of the variance in the inhibition constants. This equation is based on the assumption that all of the members of this diverse set of

inhibitors are binding in the same way to the same site on the enzyme. The fact that less than 12% of the variance is unaccounted for by eq 1 indicates that a variety of binding modes seems unlikely. To further explore this possibility, we factored ΣMR into two terms (MR_{R_1}, MR_{R_2}) and fit the data to this more complex equation; no improvement in correlation occurred. The data were also factored according to the type of compound: ketone, ester, amide, or aldehyde. No significant differences were found among the different classes of inhibitors. These results tend to reinforce the view that, in general, only one mode of binding on a single site is occurring.

Equation 2 constitutes another example of the reliability of correlation equations in structure-activity studies.

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Quinazolines as Inhibitors of Dihydrofolate Reductase. 4. Classical Analogues of Folic and Isofolic Acids[1a,b]

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A series of classical quinazoline analogues of folic and isofolic acids was evaluated for inhibitory activity against the dihydrofolate reductases from rat liver and from *Streptococcus faecium*. Included in this group were the known active antitumor agents methasquin and chlorasquin as well as methotrexate. Two new compounds, N^{10}-formyl-5,8-deazaaminopterin and N^{10}-formyl-5,8-deazafolic acid, were synthesized specifically for this study. The latter displayed modest activity against L1210 leukemia in mice.

In the initial paper in this series quinazolines bearing relatively simple substituents were evaluated as inhibitors of rat liver dihydrofolate reductase.[2] It was observed that the 2,4-diamino configuration afforded optimum inhibitory activity and that small hydrophobic groups located in positions 5 and/or 6, but in particular the former, caused substantial increases in inhibitory potency. Next, a series of potential antimalarial agents and related compounds was evaluated against the dihydrofolate reductases from rat liver as well as from *Streptococcus faecium*.[3] As expected, compounds containing amino groups at both the 2 and 4 positions were the most effective inhibitors of either enzyme. The presence of a large hydrophobic substituent (i.e., arylthio) dramatically enhanced activity with attachment at the 6 position being more favorable than at position 5. For 6-substituted compounds replacement of the 4-NH_2 by 4-OH or 4-SH resulted in only modest decreases in activity toward the mammalian enzyme but more dramatic losses in potency were observed with the bacterial enzyme.

Recently, quinazoline analogues of pteroic and isopteroic acids were prepared and evaluated as inhibitors of the dihydrofolate reductases from the aforementioned sources.[1a] Several of the 2,4-diamino modifications were found to be exceptionally potent inhibitors of either enzyme. Interestingly, the compounds studied were not found to display the high degree of species specificity which was reported for drugs such as trimethoprim.[4] This observation suggested that compounds which closely resemble folic acid even though devoid of an amino acid residue bind to dihydrofolate reductase in a similar configuration to that assumed by classical inhibitors such as methotrexate (**1c**). A quantitative structure–activity relationship was recently formulated for quinazolines causing 50% inhibition of rat liver dihydrofolate reductase.[5] The equation derived for data generated in this laboratory was successful in correlating 101 out of 104 compounds. It was of interest, therefore, to determine whether structure–activity patterns could be elucidated for classical quinazoline analogues of folic acid (**1a**) and isofolic acid (**1b**).[6] Several of these, in particular chlorasquin and methasquin, have been evaluated as inhibitors of dihydrofolate reductase from *S. faecium*[7] as well as from human leukemia cells.[8] However, these studies did not employ a sufficient variety of analogues to allow the formulation of meaningful structure–activity patterns. In addition, the diethyl ester derivatives were not evaluated. Such information was deemed of particular importance since recent studies indicate that certain of these esters have potential utility as topical antipsoriatic agents.[9]

Chemistry. The chemical structures and physical properties of the compounds prepared for this study are summarized in Table I. The diethyl aspartates and glutamates **2**, **4**, **6**, **8**, **10**, and **12** were prepared by the reductive condensation of the required amino acid derivative with the appropriately substituted 6-cyano-