Application of Regression Analysis to Hypoglycemic Activities of 12 Piperidinesulfamylsemicarbazides and Activity Predictions for 12 Analogs

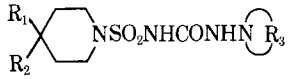
By W. RONALD SMITHFIELD and WILLIAM P. PURCELL

A regression analysis is applied to the hypoglycemic activity data for a series of 12 piperidinesulfamylsemicarbazides. The results are used to estimate the hypoglycemic activity for 12 untested analogs. The following seven untested compounds are predicted to have greater hypoglycemic activity than chlorpropamide: 1,1-pentamethylene-4-(4-ethyl-4-methyl-1-piperidinesulfamyl)semicarbazide; 1,1-pentamethylene-4-(4-methoxy-4-methyl-1-piperidinesulfamyl)semicarbazide; 1,1-pentamethylene-4-(4,4-diethyl-1-piperidinesulfamyl)semicarbazide; 1,1-pentamethylene-4-(4-ethyl-4-methoxy-1-piperidinesulfamyl)semicarbazide; 1,1-pentamethylene-4-(4,4-dimethoxy-1-piperidinesulfamyl)semicarbazide; 1,1-hexamethylene-4-(4-methoxy-4-methyl-1-piperidinesulfamyl)semicarbazide; 1,1-hexamethylene-4-(4-ethyl-4-methoxy-1-piperidinesulfamyl)semicarbazide.

THE POSSIBILITY of designing molecules for specific therapeutic uses by the application of regression analyses to structure-activity data (1–3) holds considerable interest for the authors. Recently, Free and Wilson's method (3) has been applied to an analogous series of cholinesterase inhibitors (4) synthesized in these laboratories.

Several prerequisites are necessary for the satisfactory application of regression analysis to structure-activity data: (*a*) the series to be studied should be composed of closely related analogs (thereby increasing the probability of having a similar mechanism of action), (*b*) accurate biological data measured under uniform conditions must be available, and (*c*) the activity parameter chosen must be intrinsically additive. Naturally, it is also desirable to have a maximum number of degrees of freedom and, therefore, to have a high ratio of the number of observations to the number of unknown terms in the linear equations.

McManus and Gerber (5) have recently reported activity data which appear suitable for the application of the regression analysis technique. They evaluated a series of 12 substituted piperidinesulfamylsemicarbazides (I) as possible hypoglycemic agents, and the activity data (Table I), including standard deviations, are presented as the maximum per cent fall in blood glucose at a dose level of 100 mg./Kg. in groups of 8–10 rats of the Sprague-Dawley strain. The authors analyzed these results using a method similar to that reported by Free and Wilson (3) with the purpose of (*a*) calculating and ranking group contributions to activity for the series of piperidinesulfamylsemicarbazides and (*b*) estimating the hypoglycemic activity of analogous molecules which have not been screened.



I

REGRESSION ANALYSIS

If one assumes that the substituents on the molecules evaluated by McManus and Gerber (5) make a constant and additive contribution to the over-all activity, a simultaneous equation may be written for each of the 12 observations (Eqs. 1–12).

$$a[\mathrm{H}] + b[\mathrm{H}] + c[\mathrm{C_5}] + \mu = 14.8 \quad \text{(Eq. 1)}$$

$$a[\mathrm{H}] + b[\mathrm{H}] + c[\mathrm{C_6}] + \mu = 11.1 \quad \text{(Eq. 2)}$$

$$a[\mathrm{H}] + b[\mathrm{Me}] + c[\mathrm{C_5}] + \mu = 26.1 \quad \text{(Eq. 3)}$$

$$a[\mathrm{H}] + b[\mathrm{Me}] + c[\mathrm{C_6}] + \mu = 33.9 \quad \text{(Eq. 4)}$$

$$a[\mathrm{Me}] + b[\mathrm{Me}] + c[\mathrm{C_5}] + \mu = 39.1 \quad \text{(Eq. 5)}$$

$$a[\mathrm{Me}] + b[\mathrm{Me}] + c[\mathrm{C_6}] + \mu = 34.9 \quad \text{(Eq. 6)}$$

$$a[\mathrm{Me}] + b[\mathrm{Et}] + c[\mathrm{C_6}] + \mu = 42.0 \quad \text{(Eq. 7)}$$

$$a[\mathrm{Et}] + b[\mathrm{Et}] + c[\mathrm{C_6}] + \mu = 34.4 \quad \text{(Eq. 8)}$$

$$a[\mathrm{C_2}] + b[\mathrm{C_2}] + c[\mathrm{C_5}] + \mu = 35.6 \quad \text{(Eq. 9)}$$

$$a[\mathrm{C_2}] + b[\mathrm{C_2}] + c[\mathrm{C_6}] + \mu = 30.8 \quad \text{(Eq. 10)}$$

$$a[\mathrm{C_{2.5}}] + b[\mathrm{C_{2.5}}] + c[C_6] + \mu = 25.0 \quad \text{(Eq. 11)}$$

$$a[\mathrm{H}] + b[\mathrm{OCH_3}] + c[\mathrm{C_6}] + \mu = 24.8 \quad \text{(Eq. 12)}$$

In Eqs. 1–12 the brackets indicate activity contributions, while the letters *a*, *b*, and *c* refer to positions R_1, R_2, and R_3, respectively. Therefore, $a[\mathrm{H}]$ is the activity contribution of a hydrogen atom at position R_1; $a[\mathrm{C_2}]$ is the activity contribution of half of a tetramethylene group at position

Received December 21, 1966, from the Department of Medicinal Chemistry, College of Pharmacy, University of Tennessee, Memphis, TN 38103

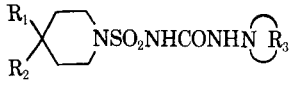
Accepted for publication February 13, 1967.

The authors acknowledge support by grant GB-4453 from the National Science Foundation and research contract DA-49-193-MD-2779 from the U. S. Army Medical Research and Development Command. Computer facilities were provided by grant HE-09495 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

The authors thank Dr. C. W. Sheppard and Mrs. A. B. McEachran, University of Tennessee Medical Units Computer Center, for help in processing the data.

Contribution No. 143 from the Army Research Program on malaria.

TABLE I—OBSERVED HYPOGLYCEMIC ACTIVITY FOR 12 SUBSTITUTED PIPERIDINESULFAMYLSEMICARBAZIDES AND PREDICTED ACTIVITIES FOR 12 SIMILARLY SUBSTITUTED DERIVATIVES



No.	Substitution at R_1[a] H	CH_3	C_2H_5	OCH_3	$(CH_2)_2$	$(CH_2)_{2.5}$	Substitution at R_2[a] H	CH_3	C_2H_5	OCH_3	$(CH_2)_2$	$(CH_2)_{2.5}$	Substitution at R_3 $(CH_2)_5$	$(CH_2)_6$	Activity[b] Obs.[c]	Calcd.
1	+	...	...	...	...	...	+	...	...	...	...	...	+	...	14.8 ± 7.0	14.8
2	+	...	...	...	...	...	+	...	...	...	...	...	...	+	11.1 ± 1.9	14.1
3	+	...	...	...	...	...	...	+	...	...	...	...	+	...	26.1 ± 2.6	27.4
4	+	...	...	...	...	...	...	+	...	...	...	...	...	+	33.9 ± 1.5	26.7
5	+	...	...	...	...	...	...	...	+	...	...	...	+	...	...	26.0
6	+	...	...	...	...	...	...	...	+	...	...	...	...	+	...	25.3
7	+	...	...	...	...	...	...	...	...	+	...	...	+	...	...	25.0
8	+	...	...	...	...	...	...	...	...	+	...	...	...	+	24.8 ± 3.1	24.3
9	...	+	...	...	...	...	...	+	...	...	...	...	+	...	39.1 ± 2.8	39.9
10	...	+	...	...	...	...	...	+	...	...	...	...	...	+	34.9 ± 3.3	39.2
11	...	+	...	...	...	...	...	...	+	...	...	...	+	...	...	38.5
12	...	+	...	...	...	...	...	...	+	...	...	...	...	+	42.0 ± 3.3	37.8
13	...	+	...	...	...	...	...	...	...	+	...	...	+	...	...	37.5
14	...	+	...	...	...	...	...	...	...	+	...	...	...	+	...	36.8
15	...	...	+	...	...	...	...	...	+	...	...	...	+	...	...	37.2
16	...	...	+	...	...	...	...	...	+	...	...	...	...	+	34.4 ± 2.8	36.5
17	...	...	+	...	...	...	...	...	...	+	...	...	+	...	...	36.2
18	...	...	+	...	...	...	...	...	...	+	...	...	...	+	...	35.4
19	...	...	...	+	...	...	...	...	...	+	...	...	+	...	...	35.1
20	...	...	...	+	...	...	...	...	...	+	...	...	...	+	...	34.4
21	...	...	...	...	+	...	...	...	...	...	+	...	+	...	35.6 ± 2.4	33.5
22	...	...	...	...	+	...	...	...	...	...	+	...	...	+	30.8 ± 3.7	32.8
23	...	...	...	...	...	+	...	...	...	...	...	+	+	...	...	25.7
24	...	...	...	...	...	+	...	...	...	...	...	+	...	+	25.0 ± 3.7	25.0

[a] The 4,4-tetramethylene group is denoted by two units of $(CH_2)_2$, and the 4,4-pentamethylene group is denoted by two units of $(CH_2)_{2.5}$. [b] Activities are reported (5) as maximum per cent blood glucose lowering at dose of 100 mg./Kg. McManus and Gerber (5) found chlorpropamide to have an activity of 35 ± 3.3%. [c] Observed values are reported with standard deviations.

R_1 and is necessarily paired with $b[C_2]$; $a[C_{2.5}]$ is the activity contribution of half of a pentamethylene group at position R_1 and is paired with $b[C_{2.5}]$; $c[C_5]$ is the activity contribution of a pentamethylene group at position R_3; $c[C_6]$ is the activity contribution of a hexamethylene group at position R_3, *etc.*; μ represents the activity contribution of the parent structure. In each equation the sum of the appropriate group contributions and μ are set equal to the maximum per cent fall in blood glucose at a dose level of 100 mg./Kg. as reported by McManus and Gerber (5).

If one assumes that positions R_1 and R_2 are equivalent in the substituted piperidinesulfamylsemicarbazides, the following six simplifying equations may be written:

$$a[H] = b[H] \quad \text{(Eq. 13)}$$

$$a[Me] = b[Me] \quad \text{(Eq. 14)}$$

$$a[Et] = b[Et] \quad \text{(Eq. 15)}$$

$$a[C_2] = b[C_2] \quad \text{(Eq. 16)}$$

$$a[C_{2.5}] = b[C_{2.5}] \quad \text{(Eq. 17)}$$

$$a[OCH_3] = b[OCH_3] \quad \text{(Eq. 18)}$$

Substitution of these relationships into Eqs. 1–12 results in 12 equations with nine unknowns. In addition, two restrictions (Eqs. 20, 21) result from the summation to zero (3) of the group contributions at positions R_1 and R_2 and position R_3:

$$5a[H] + 2b[H] + 3a[Me] + 4b[Me] + a[Et] + 2b[Et] + 2a[C_2] + 2b[C_2] + a[C_{2.5}] + b[C_{2.5}] + b[OCH_3] = 0 \quad \text{(Eq. 19)}$$

or, from Eqs. 13–18,

$$7a[H] + 7a[Me] + 3a[Et] + 4a[C_2] + 2a[C_{2.5}] + a[OCH_3] = 0 \quad \text{(Eq. 20)}$$

and

$$4c[C_5] + 8c[C_6] = 0 \quad \text{(Eq. 21)}$$

The 14 simultaneous equations with nine unknowns were solved by the method of least squares by employing the program Scrap and the IBM 1620 computer.

RESULTS AND DISCUSSION

Simultaneous solution of the 12 linear equations gives the calculated activity contribution for each group (Table II) and the value for the constant, μ (35.25%).

The appropriate group contributions and μ may be used in Eq. 22 to calculate hypoglycemic activities (Table I) and

$$\text{hypoglycemic activity} = \mu + \Sigma \text{ group contributions} \quad \text{(Eq. 22)}$$

the agreement between calculated and observed values may be used as a test for the analysis.

From Table II one can rank[1] the groups at positions R_1 and R_2 in order of decreasing contribution to hypoglycemic activity as follows: $CH_3 > C_2H_5 > OCH_3 > (CH_2)_2 > (CH_2)_{2.5} > H$. The hydrogen substituent has a deleterious effect on activity (−8.99%), while the methyl group contributes most to activity (3.56%). It is interesting to note that the addition of one methylene group produces a substantial reduction in contribution between the tetramethylene group, $(CH_2)_4$ (2 ×

[1] Free and Wilson have emphasized that "The rank order of related substituents within a position would be expected to have meaning" (3).

TABLE II—SUBSTITUTENT GROUP CONTRIBUTION TO HYPOGLYCEMIC ACTIVITY

Position(s)	Group[a]	Contribution[b]
R_1 and R_2	H	−8.99
R_1 and R_2	CH_3	3.56
R_1 and R_2	C_2H_5	2.20
R_1 and R_2	OCH_3	1.17
R_1 and R_2	$(CH_2)_2$	0.38
R_1 and R_2	$(CH_2)_{2.5}$	−3.54
R_3	$(CH_2)_5$	−2.47
R_3	$(CH_2)_6$	−3.17

[a] See *Footnote a*, Table I. [b] See *Footnote b*, Table I.

0.38% = 0.76%), and the pentamethylene group, $(CH_2)_5$ (2 × −3.54% = −7.08%). At the R_3 position, both the pentamethylene and hexamethylene groups have a negative contribution (−2.47% and −3.17%, respectively).

It is apparent from Table I that reasonable agreement exists between the calculated and observed hypoglycemic activities. The *F*-ratio between the variance of the estimated activity and the variance of the observed activity is significant at the 99% level. The coefficient of the multiple correlation is 0.977. In four of the 12 compounds (No. *2*, *4*, *10*, and *12*), however, the differences between the calculated and observed values are greater than the standard deviations reported by McManus and Gerber (5).

The primary interest in this type of study is the selection of untested molecules which have a high probability of possessing useful therapeutic activity. The seven untested molecules which are predicted to be more active than chlorpropamide [activity as determined by McManus and Gerber (5), 35 ± 3.3%], are 1,1-pentamethylene-4-(4-ethyl-4-methyl-1-piperidinesulfamyl)semicarbazide; 1,1 - pentamethylene - 4 - (4 - methoxy - 4 - methyl-1 - piperidinesulfamyl)semicarbazide; 1,1 - hexamethylene - 4 - (4 - methoxy - 4 - methyl - 1 - piperidinesulfamyl)semicarbazide; 1,1-pentamethylene-4-(4,4-diethyl - 1 - piperidinesulfamyl)semicarbazide; 1,1-pentamethylene-4-(4-ethyl- 4 - methoxy-1-piperidinesulfamyl)semicarbazide; 1,1-hexamethylene-4-(4-ethyl-4-methoxy-1-piperidinesulfamyl)semicarbazide; 1,1-pentamethylene-4-(4,4-dimethoxy-1-piperidinesulfamyl)semicarbazide (No. *11*, *13*, *14*, *15*, *17*, *18*, and *19*, respectively). These compounds appear to be worthy of synthesis and testing as possible hypoglycemic agents.

REFERENCES

(1) Hansch, C., and Fujita, T., *J. Am. Chem. Soc.*, **86**, 1616(1964).
(2) Hansch, C., Deutsch, E. W., and Smith, R. N., *ibid.*, **87**, 2738(1965).
(3) Free, S. M., Jr., and Wilson, J. W., *J. Med. Chem.*, **7**, 395(1964).
(4) Purcell, W. P., *Biochim. Biophys. Acta*, **105**, 201 (1965).
(5) McManus, J. M., and Gerber, C. F., *J. Med. Chem.* **9**, 256(1966).

Kinetics of Reaction of Dehydroacetic Acid I

Reaction with Primary Amines I

By SHIGERU GOTO, SADAO IGUCHI, AKIRA KONO, and HIDEKO UTSUNOMIYA

Dehydroacetic acid (DHA), one of the officially recognized food preservatives in Japan, has been found in some instances to readily react with amino compounds under mild and physiological conditions to produce Schiff's base type compounds. The kinetic study of the reaction between DHA and β-phenethylamine (PE-NH), with the formation of 3-(1-phenethylamino)ethylidene-6-methyl-3H-pyran-2,4-dione, was investigated in various water–ethanol mixtures, and a linear relationship was observed between the reciprocal of the dielectric constant of the solvent and log *k*. The *k*–pH profiles for the reaction in 80 per cent ethanol solution at 40 and 60° were completed in the acetate buffer regions. No general acid-base catalysis was observed but salt effects were not negligible. The rate-determining step in 80 per cent ethanol solution was probably the attack of free PE-NH on the protonated carbonyl carbon atom in free DHA.

DEHYDROACETIC ACID (DHA) (I) has been accepted as one of the officially recognized food preservatives in Japan and is at present being used widely not only in some foodstuffs and refreshing drinks but also in pharmaceutical preparations as an antiseptic. It has been pointed out that one of the most remarkable chemical behaviors of DHA is its affinity toward compounds possessing an amino radical. When they coexist in solution, Schiff's base type compounds (II) are initially readily produced by the reaction between DHA and amino compounds, and then finally transformed, in some cases, to lutidone derivatives (V). This has been reported in the preceding papers (1, 2) and the mechanism described in Scheme I was proposed.

In some instances the conversion proceeds under mild and physiological conditions.

Other similar qualitative and synthetic investigations (3–5) have been published, but no

Received September 6, 1966, from the Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan.
Accepted for publication November 22, 1966.