Chapter 12

Quantitative Structure–Activity Relationship Study of Aromatic Trifluoromethyl Ketones

In Vitro Inhibitors of Insect Juvenile Hormone Esterase

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The in vitro inhibitory activity of 41 aryl substituted 3-phenyl-1,1,1-trifluoro-2-propanones against insect juvenile hormone esterase has been related to various electronic, hydrophobic and steric parameters using linear stepwise regression analysis. The pI_{50} values were found to be significantly correlated to the total lipophilicity of the molecule and the corrected molar volume (partial molar refractivity) of the substituents at the different substituent positions.

Trifluoromethyl ketones (TFKs) have been found to inhibit various hydrolytic enzymes (1-6). Series of aliphatic and aromatic trifluoromethyl ketone sulfides (7-10) proved to be exceptionally powerful inhibitors of insect juvenile hormone esterase (JHE), an enzyme of key importance in insect metamorphosis. The trifluoroketone moiety is believed to behave as a transition state mimic (11,12) of juvenile hormones (JHs), substrates of the enzyme. The β sulfur atom is anticipated to mimic the α-β double bond present in all natural JH substrates. In earlier structure-activity relationship (SAR) studies (7,11) clear correlation was found among the molar I_{50} values of these compounds against JHE and the calculated molar refractivity of the inhibitors. Examining 18 substituted 3-phenylthio-1,1,1-trifluoro-2-propanones, regression equations were obtained between the inhibitory activities and the Hammett (σ), Taft (E_s) steric and Hansch (π) hydrophobicity constants (11). In the hope of increasing the significance of these equations and to better distinguish between the importance of various substituent positions, several new compounds of the related structure were synthesized, a much larger set of substituent parameters was applied, and instead of the arbitrary choice of these values, the variables were selected into the equations by a more sophisticated tool, linear stepwise regression analysis.

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Stepwise Regression Analysis

Considering the data set of the biological activity as dependent variable (Y_i) and different substituent parameters as independent variables ($X_{j,i}$), their correlation is approached by a linear equation (Equation 1):

$$Y_i = \beta_0 + \beta_1 X_{1,i} + \beta_2 X_{2,i} + \ldots + \epsilon_i \qquad (1)$$

where β_0 is the constant of the equation, β_is are the various coefficients of the independent variables and ϵ_i is the error factor, a random deviation. Several multiple regression analysis techniques are known to fit the equation to the actual data set ($X_{j,i}$,Y_i), differing from each other in the mode or sequence of how to consider the different independent variables one by one. The stepwise regression analysis (SRA) procedure is known to be one of the best of the variable selection techniques and is very useful when there is a great number of independent variables to consider. The process is discussed thoroughly elsewhere (13, for a computational method see 14,15), hereby we mention only its brief summary.

The variables (X_j) are included in the equation one at a time, entering always the one which, at that point, is the most correlated to Y, and the significance of the new linear equation, obtained by selecting the new parameter in, is checked at each step. To enter a new parameter the partial correlation coefficient (F) has to exceed a critical value, set at the beginning of the process. Also, any other X_j variable selected in the equation at a previous stage is discarded if its partial F value decreases below a partial F criterion due to entering the new independent variable. If no more variables meet the partial F criterion, the procedure stops resulting in Equation 1. The system also terminates if no significant variables can be selected at all, if all independent variables are selected, if the variable to be added to the model is the one that just was deleted from it, and - in some cases - if the regression steps exceed a certain number. In order to "protect" the variables already selected in the equation, the partial F criterion can be set to be higher for selecting a new X_j in (entry F) than for discarding one out (exit F), to avoid overflow, however, it is advisable to set these two criteria to the same value, i.e. to 95% confidence level (α=0.05).

Generally, some difference (ϵ_i) exists between the Y_i values observed and calculated, the error in prediction might be caused by errors in measuring Y or by not considering all responsible parameters in the initial model. As it has been stated by Pope (16), SRA does not necessarily result in an equation with direct physical meaning since imitative variables may correlate well with the response. Therefore high correlations obtained by this method do not necessarily indicate primary physical connection and *vice versa*, no correlation does not prove a lack of physical relationship. For predictive purposes, however, imitative models are suitable as well. Stowe and Mayer (17) also warns of the possible limitations of various statistical methods for the selection of significant variables. Using ANOVA, t-test and SRA

methods, the authors found significant correlation between the result of drawing tokens form a sack and various *ad hoc* variables. The error estimation, the validation test at each step and the consciousness at the conclusions are important to avoid misinterpretations (16,17) as well as to the correct evaluation of correlation coefficients (18). SRA has been successfully applied for QSAR purposes of pesticides (19-27) as well as in other areas of chemistry.

Synthesis and Enzyme Inhibition Assay of Trifluoromethyl Ketones

The title compounds, aryl substituted 3-phenyl-1,1,1-trifluoro-2-propanones of Structure A, were synthesized from the appropriate thiophenol using 3-bromo-1,1,1-trifluoro-2-propanone (BTFA) and triethylamine according to the previously published procedure (7,9). In general, the products were formed at good yields (80-95%), however, several compounds containing nitro and amino substituents on the aromatic ring were difficult to synthesize. Under the above reaction conditions, nitrothiophenols are oxidized to disulfides. Aminothiophenols, meanwhile, appear to react with BTFA on the carbonyl group, probably forming hemiaminals. Thus, the reaction of 4-nitro-thiophenol, 2-nitro-4-trifluoromethyl-thiophenol and 4-amino-thiophenol with BTFA was unsuccessful to give trifluoromethyl ketones of Structure A. Other amide, amine and imine derivatives (compounds 37-41, see Table I) were, although at a lower yield, successfully synthesized. Compounds 35 and 36 were gifts from Drs. Tim Schierling and Ken Musker (Chemistry Department, University of California, Davis).

The radiopartition assay (28,29) was applied to monitor the *in vitro* inhibitory activity of the title compounds against JHE. The enzyme activity is monitored by measuring the hydrolysis rate of the substrate, juvenile hormone. Diluted hemolymph from L_5D_2 larvae of *Trichoplusia ni* (cabbage looper) was the source of the enzyme, a mixture of ^{3}H-labeled and unlabeled JH-III was used as a substrate. Ethanol or acetone were used as solvents for the TFK inhibitors in the assay. The chemical structures and inhibitory potencies of the aromatic trifluoromethyl ketones involved in this study are summarized in Table I.

Stepwise Regression Analysis on Trifluoromethyl Ketones

In a previous study of 18 aromatic trifluoroketones, their inhibitory activity against JHE was correlated to the structure using four substituent parameters (11). The descriptors were chosen more or less arbitrarily and the effect of the different substituent positions could be determined semi-empirically, mainly by examining how the various data-points fit the equation. Important conclusions could, however, be drawn based on the empirical and intuitive method: the authors found that *meta*- and *para*-substituted compounds fit the regression line better and offered better selectivity against JHE.

A broad range of different electronic, hydrophobic and steric substituent parameters are available in the literature (30). In the present study 11 physicochemical parameters were

compiled from standard tables (31) and applied to stepwise regression analysis. The parameters involved in the study are Hansch-Fujita's lipophilicity parameter (π) (32), Hammett constants in the *meta* and *para* positions (σ_m, σ_p) (33), molar refractivity (MR) (31), Swain and Lupton's electronic parameters characterizing the field and resonance effects (F,R) (34), Taft's steric parameter (E_s) (35). STERIMOL steric parameters representing the smallest and largest width of the substituent perpendicular to the bond connecting its α-atom to the rest of the compound (B1,B4) (36) and two indicator variables describing proton acceptor-donor properties (H-AC, H-DO: the parameters are 1 if the appropriate property applies to the substituent, otherwise their value is 0).

Table I. Inhibition of insect juvenile hormone esterase by arylthio-trifluoropropanones of structure A

compound number	substituent	Molar I_{50} [M][a]	compound number	substituent	Molar I_{50} [M][a]
1[b]	-	8.2×10^{-6}	21	3,4-Me_2	1.1×10^{-8}
2	2-Br	6.8×10^{-7}	22	3,5-Me_2	1.8×10^{-6}
3	3-Br	8.8×10^{-7}	23	2-Et	2.9×10^{-6}
4[b]	4-Br	4.1×10^{-7}	24[b]	2-iPr	3.4×10^{-7}
5[b]	2-Cl	2.1×10^{-6}	25[b]	4-tBu	7.5×10^{-9}
6[b]	3-Cl	4.0×10^{-7}	26	2-Me-4-tBu	3.0×10^{-9}
7[b]	4-Cl	1.3×10^{-6}	27	3-Br-4-Me	1.3×10^{-8}
8[b]	4-F	8.4×10^{-6}	28	2-MeO	2.0×10^{-6}
9[b]	2,5-Cl_2	2.3×10^{-8}	29[b]	3-MeO	1.3×10^{-6}
10	2,6-Cl_2	7.8×10^{-8}	30[b]	4-MeO	1.3×10^{-6}
11[b]	3,4-Cl_2	2.0×10^{-8}	31[b]	3-CF_3	2.5×10^{-6}
12[b]	2,4,5-Cl_3	3.2×10^{-8}	32[b]	4-OH	6.1×10^{-6}
13	2,3,5,6-F_4	1.2×10^{-6}	33	2-COOH	$> 10^{-4}$
14	F_5	1.1×10^{-5}	34	2-CH2OH	7.0×10^{-6}
15[b]	2-Me	4.3×10^{-6}	35	4-Me_2N	9.0×10^{-8}
16[b]	3-Me	1.3×10^{-7}	36	4-Me_3N^+	$>> 10^{-4}$
17[b]	4-Me	1.1×10^{-7}	37	4-MeCONH	$> 10^{-4}$
18	2,4-Me_2	9.1×10^{-7}	38	2-PhNH	3.0×10^{-8}
19	2,5-Me_2	1.7×10^{-7}	39	4-PrNHCOO	$> 10^{-4}$
20	2,6-Me_2	1.2×10^{-7}	40	2(2-HOPh)CH=N	$> 10^{-4}$
			41	2(2-thienyl)CH=N	$> 10^{-4}$

[a] JH III was used as a substrate for JHE from *Trichoplusia ni* (cabbage looper).
[b] Data from Hammock *et al.* (7).

As it has been pointed out by Topliss and Edwards (37), the higher the number of the possible independent variables to consider in a QSAR study, the more probable the occurrence of chance correlations. Therefore, in order to enter a large number

of parameters into the SRA, the number of compounds needed to be increased. Thus, 23 more compounds of Structure A were synthesized.

Thirty-five compounds provided exact numerical I_{50} values which could be used in the computations. There are 15 various substituents appearing in five possible substituent positions (numbered I-V in Structure A) in these compounds. In a program, written in BASIC, a descriptor matrix was created by copying the appropriate substituent parameters into the corresponding substitution position-compartments of the descriptor matrix if the actual substituent is present in the molecule in the actual position. From the Hammett constants, the equation $\sigma_o = \sigma_p$ was assumed (31,38,39), thus σ_p was applied for positions I, III and V, and σ_m for positions II and IV. Therefore, 10 parameters were used to enter the descriptor matrix for each substituent position. Two additional parameters, the total lipophilicity [$\sum\pi$] and its square value [$(\sum\pi)^2$] were also calculated for each compound and were added to the data-set.

Five parameters in the data-set were found to be unchanged for all 35 compounds and removed from the matrix. These parameters are H-DO for positions II, IV and V and H-AC for positions IV and V. After the redundant elements had been removed, the resulting [35x47] matrix was correlated to the vector of the biological activity. To perform the linear stepwise regression analysis, the STEPWISE procedure of the SAS statistical package (40) and BASIC programs were used.

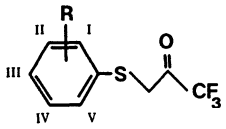
Running SRA on the full sample gave the following equation:

$$\begin{aligned} pI_{50} = {} & 0.910(\pm0.390)\ \text{H-DO}_I + 0.153(\pm0.046)\ MR_{II} \\ & - 1.653(\pm0.720)\ \sigma_{mII} + 0.088(\pm0.020)\ MR_{III} \\ & + 1.561(\pm0.900)\ \sigma_{mIV} + 0.169(\pm0.084)\ MR_V \\ & + 0.712(\pm0.143)\ \textstyle\sum\pi + 4.885 \end{aligned} \qquad (2)$$

$n = 35$ $\quad r = 0.888$ $\quad s = 0.51$ $\quad F = 14.41$

(In the equations, n represents the number of datum points used to derive the equation, r is the multiple correlation coefficient, s is the standard deviation from regression, F is the F statistic for variance of each additional variable, the values in parentheses after the equation coefficients are for construction of confidence intervals and the Roman numbers in subscript refer to the substituent position.)

The partial r value is the highest for $\sum\pi$ and MR_{III} (0.739 and 0.325, respectively) and does not exceed 0.183 for any of the other variables in the equation. This means that the total lipophilicity alone explains over 54% of the total variance (Figure 1). The predicted pI_{50} values and the standard error of the prediction are listed in Table II. and shown in Figure 2. The fact that σ_m has opposite signs in positions II and IV is seemingly contradictory, but the weight of σ_{mIV} and MR_V is very low in the equation, therefore they can be omitted resulting in Equation 3.



Structure A

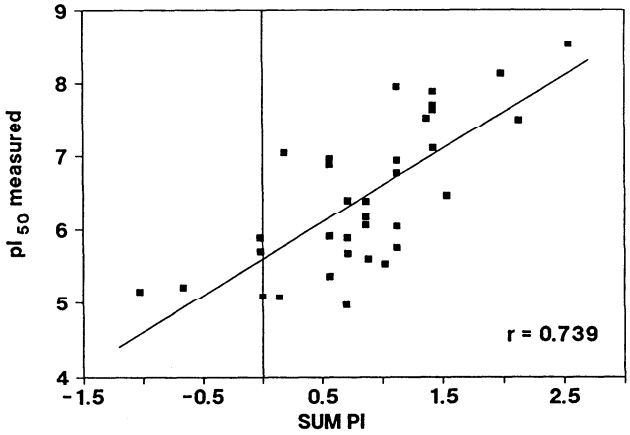


Figure 1. Correlation between the total lipophilicity and the inhibitory potency.

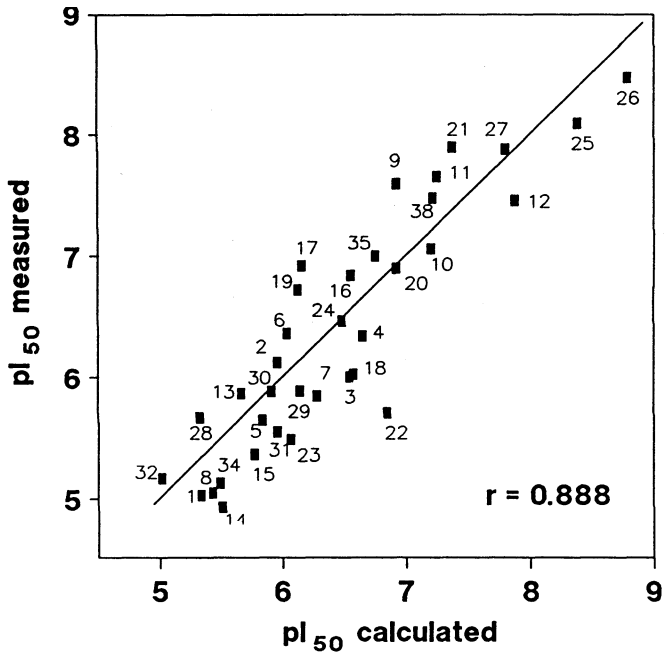


Figure 2. Correlation between the calculated and measured pI_{50} values.

$$-pI_{50} = 0.796(\pm0.412)\ \text{H-DO}_{I} + 0.108(\pm0.046)\ MR_{II} - 1.292(\pm0.702)\ \sigma_{mII} + 0.071(\pm0.020)\ MR_{III} + 0.842(\pm0.140)\ \sum\pi + 5.220 \quad (3)$$

$n = 35$ $r = 0.862$ $s = 0.54$ $F = 16.79$

Requirements for good activity (ranked according the absolute value of the corresponding path coefficients given in brackets):

Large overall hydrophobicity ($\sum\pi$) [0.626];
in position III: large volume (MR) [0.368];
in position II: large volume (MR) [0.263];
in position II: electron-donating substituent [-0.194];
in position I: proton-donor substituent [0.189].

Compounds 9, 17, 22 and 23 are outliers because the residual of their measured and calculated pI_{50} value deviate most. Omission of the four outliers introduced more parameters and significantly improved the statistics of the new equation, Equation 4.

$$-pI_{50} = 0.960(\pm0.296)\ \text{H-DO}_{I} + 0.165(\pm0.034)\ MR_{II} - 1.633(\pm0.529)\ \sigma_{mII} - 0.608(\pm0.300)\ \pi_{III} + 0.117(\pm0.026)\ MR_{III} + 0.115(\pm0.064) MR_{V} + 0.984(\pm0.141)\ \sum\pi + 4.803 \quad (4)$$

$n = 31$ $r = 0.943$ $s = 0.38$ $F = 26.42$

Requirements for good activity (ranked according the absolute value of the corresponding path coefficients given in brackets):

Large overall hydrophobicity ($\sum\pi$) [0.754];
in position III: large volume (MR) [0.627];
in position II: large volume (MR) [0.403];
in position III: hydrophilic substituent [-0.341];
in position II: electron-donating substituent [-0.252];
in position I: proton-donor substituent [0.189];
in position V: large volume (MR) [0.138].

The partial r value is still the highest for $\sum\pi$ and MR_{III}, the total lipophilicity alone explains over 60% of the total variance and it is also visible that it has a particular importance in the *para* position. Since σ_m has a negative coefficient, electron donating substituents are preferred in the *meta* position. MR has almost the same coefficients for positions II, III and V, the latest, however, has the lowest absolute path coefficient [0.138]. Therefore, compounds 3,4-disubstituted with lipophilic substituents are expected to be highly active.

Test of Significance

A way to avoid chance correlations is to increase the size of the sample. The partial F value, however, is also informative, in

Table II. Observed and predicted pI_{50} values and statistics of prediction

compd. number	pI_{50} obs.	pI_{50}[a] predicted	pI_{50}[b] pred.	compd. number	pI_{50} obs.	pI_{50}[a] predicted	pI_{50}[b] pred.
1	5.09	5.31 ± 0.16	5.33	**19**	6.77	6.10 ± 0.21	4.40[c]
2	6.17	5.92 ± 0.27	5.90	**20**	6.94	6.89 ± 0.35	6.06[c]
3	6.06	6.48 ± 0.27	8.39[c]	**21**	7.95	7.34 ± 0.21	6.70
4	6.39	6.61 ± 0.13	6.63	**22**[d]	5.75	6.82 ± 0.22	8.20[c]
5	5.68	5.81 ± 0.15	6.21[c]	**23**[d]	5.54	6.03 ± 0.16	6.31
6	6.40	6.00 ± 0.22	5.87	**24**	6.47	6.40 ± 0.21	6.38
7	5.89	6.25 ± 0.12	6.28	**25**	8.13	8.36 ± 0.29	8.47
8	5.08	5.40 ± 0.15	5.43	**26**	8.52	8.76 ± 0.31	8.89
9[d]	7.64	6.90 ± 0.30	7.09	**27**	7.89	7.83 ± 0.23	7.82
10	7.11	7.17 ± 0.38	6.54	**28**	5.70	5.29 ± 0.16	5.20
11	7.70	7.21 ± 0.24	6.77[c]	**29**	5.89	6.14 ± 0.26	6.23
12	7.50	7.84 ± 0.31	8.58[c]	**30**	5.89	5.90 ± 0.18	5.90
13	5.91	5.64 ± 0.28	5.39[c]	**31**	5.60	5.83 ± 0.26	6.41
14	4.97	5.53 ± 0.28	4.79	**32**	5.22	4.99 ± 0.22	5.90[c]
15	5.37	5.71 ± 0.14	5.74	**34**	5.16	5.48 ± 0.40	3.46[c]
16	6.89	6.53 ± 0.22	6.09	**35**	7.05	6.72 ± 0.28	5.42
17[d]	6.96	6.11 ± 0.12	5.91	**38**	7.52	7.19 ± 0.40	6.19
18	6.04	6.51 ± 0.13	6.89[c]				

[a] Prediction by Equation 2.
[b] Prediction by the "leave one out" method.
[c] Parameters, not involved in Equation 2, were introduced into the "leave one out" equation at r=0.900 total confidence level.
[d] Compounds have been omitted from Equation 4.

this respect it is the most important statistical parameter. It takes automatically into account the number of observations and variables (degrees of freedom). According to this parameter, the significances of Equation 3 and Equation 4 are very high ($p<0.001$), at the level of 0.1%. The high F values provided by the F-statistics for significance show that the equations are descriptive, also informative is the fact that the F value of each equation is gradually increasing when a new parameter is considered, which also suggests non-accidental correlation. Additional indication of significance is the fact if the residues of the regression follow normal distribution, it demonstrates that there is no further correlation in the sample.

On the other hand, theoretically there is no way to tell the difference between chance correlation and true correlation. Only

the development and exploration of a plausible hypothesis that corresponds to the meaning of the equation can test its relevance. In our case, the use of well established chemical descriptors which were found to be relevant with biological activities by many authors is certain guarantee for a real QSAR. Further guarantee is the test of the predictive power of the equation.

For this purpose the "leave one out" approach (also known as the jack-knife method (13,20)) was used: the activity of each compound was predicted by the equation obtained from linear regression analysis on the sample leaving out the compound in question. The calculated standard deviation of the difference between the calculated and measured values is, of course, larger than that obtained the usual way. Its increase, however, brings plausible information on the predictive power of the regression equation, as well as which compound's removal would increase the significance of the equation most. Also informative, which parameters and how frequently are considered in the equations obtained in the "leave one out" approach: their continuous and systematic appearance also suggests a "real" correlation.

Leaving the compounds out one by one 35 regression equations were obtained, each on different 34 membered (n=34) samples. $\sum\pi$ and MR_{III} appeared in all 35 equations, meanwhile MR_{II}, $H\text{-}DO_I$, σ_{mII}, MR_V and σ_{mIV} appeared only in 85.7%, 82.9%, 82.9%, 74.3% and 62.9% of the cases, respectively. In addition, occasionally additional parameters were introduced, i.e. $\sum\pi^2$, $H\text{-}AC_I$, π_{III} and R_{III}. The r values of the regression equations were between 0.865 and 0.950. The pI_{50} values predicted by Equation 2 and the "leave one out" method are listed in Table II.

Interpretation of the Regression Equations

It is interesting that in the individual substituent positions molar volume (MR) was found to be the relevant parameter rather than π, E_s or the STERIMOL descriptors. This fact and the positive coefficients for MR suggest that the enzyme-inhibitor interaction proceeds *via* London dispersion forces (31,41) and the binding to the enzyme is favored if the bulky substituents are in *meta* or *para* positions, which is in accordance with the earlier results (11). The parameter H-DO in position I seems to be important in the regression, it systematically appears in each equation. Its path coefficient and partial r value, however, is relatively low compared to those of $\sum\pi$ and MR_{III}. In addition, the $H\text{-}DO_I$ variable is not very useful because these indicator parameters are the most poorly defined and the number of proton donor substituents (H-DO=1) is rather small.

The simultaneous presence of $\sum\pi$ and MR could be somewhat contradictious, since π commonly relates to a desolvation step in binding to the enzyme, meanwhile correlation with MR does not appear to be associated with desolvation (31). However, it is quite commonly observed that π and MR parameters appear together in descriptive equations (42-45) and it indicates that the rate limiting step in the interaction with the enzyme is the binding step to it, rather than transport or the inhibition reaction itself (46,47).

The function of π is obvious: the higher the lipophilicity of the compound, the better binding is possible to the enzyme. The good correlation of the pI_{50} values with $\sum\pi$ shows the presence of a hydrophobic pocket in JHE close to the active site. Such a hydrophobic pocket has been recently evidenced by non-QSAR methods as well (10). In addition, since trifluoromethyl ketones, in general, predominantly exist in their hydrated form (geminal diol), a dehydration step and/or condensation with the enzyme active site is involved, as it has been proposed previously (4,9).

The inhibitory potencies, suggested by the present equations, appear to be significant if found within the range of those represented in the sample ($I_{50} = 10^{-4} - 10^{-10}$ M). Extrapolations to lower ranges of I_{50}s are, however, not necessarily valid. I_{50} values for several 3,4-disubstituted 3-phenylthio-1,1,1-trifluoro-2-propanones, such as the 3,4-di-*tert*.butyl and 3,4-dicyclohexyl derivatives have been predicted even at the low range of $10^{-13} - 10^{-15}$ M. This prediction is against the observation that below $10^{-9} - 10^{-10}$ M range the inhibitory activity seems not to increase with the lipophilicity. In the present study, no square terms, such as $(\sum\pi)^2$, $(\sum\sigma)^2$, etc. showed significant correlation with the inhibitory potencies, therefore these correlations appear "truly" linear and not parabolic with these parameters. The seeming controversy can be explained by two reasons. First, the calculated molarity of the enzyme in diluted hemolymph is in the same range, $10^{-9} - 10^{-10}$ M, mentioned before. In other words, the most potent inhibitors show almost stoichiometric inhibition of the enzyme. I_{50} values below this level cannot be observed, even if the compounds are truly of higher inhibitory potency. I_{50}, which can be related to K_i under certain conditions, works well for moderately active compounds. For highly potent compounds one will observe smaller and smaller differences in I_{50} as the compounds approach stoichiometric inhibition. Second, the compounds in the present series might appear in a *quasi*-linear portion of a parabolic curve before reaching the $\sum\pi$ (or $\sum$MR) optimum.

Other predictions for the molar I_{50} values appear to be more realistic, i.e. that of 3-(4-phenoxy-phenyl)thio-1,1,1-trifluoro-2-propanone. (Some of the *para*-substituted derivatives of high predicted inhibitory potency are listed in Table III.) This also illustrates the importance of the professional interpretation: the selection of the plausible equation. The equations never guarantee strict cause-effect relationships, they only show the mathematical possibility of a model (while rejecting others) and give a useful guide for finding out the "real" explanation.

Although steric parameters in the *ortho* position have not been selected into the equation, the fact that *ortho* substituents often seem to decrease the inhibitory activity, relative to other compounds of the same lipophilicity, shows possible steric hindrance. It has been suggested (12) that the high specificity of JHE to the natural substrates (JHs) is due to a "recognition" of the α-β double bond in the substrate, and that the β sulfur in the title compounds is bioisosteric with the β sp^2 carbon of this double bond. The *ortho* substituents of the phenyl ring possibly

Table III. *Para*-substituted 3-phenylthio-1,1,1-trifluoro-2-propanones (Structure A) of high predicted inhibitory activity

substituent	I_{50} [M] predicted	substituent	I_{50} [M] predicted
4-Ph	1.43×10^{-9}	4-PhCH=N	9.12×10^{-10}
4-PhO	7.36×10^{-10}	4-PhN=CH	9.12×10^{-10}
4-$PhCH_2$	5.14×10^{-10}	4-PhN=N	6.68×10^{-10}
4-$PhOCH_2$	6.39×10^{-10}	4-PhCONH	2.43×10^{-9}
4-PhCO	2.33×10^{-9}	4-$PhSO_2NH$	1.35×10^{-9}
4-PhOCO	1.23×10^{-9}	4-PhCOO	7.92×10^{-10}
4-PhC≡C	9.42×10^{-11}	4-$PhSO_2O$	7.79×10^{-10}
4-PhCH=CH	7.38×10^{-11}	4-$PhSO_2$	4.67×10^{-9}
4-PhCOCH=CH	3.67×10^{-10}	4-PrCOCH=CH	2.41×10^{-9}
4-$(Ph)_2N$	2.38×10^{-13}	4-PrOCOCH=CH	9.61×10^{-10}

hinder the sulfur atom and therefore render its binding to the enzyme binding pocket, which is reflected in the relatively higher I_{50} values.

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