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Quantitative Structure–Cytotoxicity Relationship Analysis of 5-Trifluoromethyloxazole Derivatives by a Semiempirical Molecular-orbital Method with the Concept of Absolute Hardness

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Abstract. *The most stable conformation of twelve 5-trifluoromethyloxazole derivatives was calculated by CONFLEX 5. The optimized structure was determined by CAChe Worksystem 4.9 PM3 method, in the presence (COSMO) or absence (non-COSMO) of water. Higher correlation coefficients for all descriptors were found under COSMO, as compared with non-COSMO conditions. Good correlation was found between the cytotoxicity of these compounds and the electron affinity, ionization potential, highest occupied molecular orbital energy (E_{HOMO}), lowest unoccupied molecular orbital energy (E_{LUMO}), absolute hardness (η) and reactivity index (ω). On the other hand, there was generally no clear-cut correlation between CC_{50} and the heat of formation, stability of hydration, dipole moment, absolute electron negativity (χ), molecular weight, maximum length of molecule, with some exceptions. The cytotoxic activity of 5-trifluoromethyloxazole derivatives became maximum at log p=4.6. The concept of absolute hardness is applicable in estimating the cytotoxicity of 5-trifluoromethyloxazoles, using an η-χ activity diagram.*

1,3-Oxazole, also known simply as oxazole, is one of the most prominent heterocyclic compounds and the oxazole motif occurs within the framework of many important pharmacophores and natural products (1). The benefit of introducing the trifluoromethyl group into organic molecules, especially in the area of drugs or pesticides, is now well documented (2). Therefore, trifluoromethylated nitrogen heterocyclic compounds are an attractive class of compounds because of their potential biological activities, and many 5-membered aromatic nitrogen heterocyclics have been prepared (3). Outstanding applications of such molecules have been found in the pharmaceutical field, as illustrated by celecoxib (antiarthritic) bearing a trifluoromethyl substituent on the pyrazole-ring, a recent drug used in the treatment of human diseases (4). As an extension of the search for highly selective antitumor agents against human promyleocytic leukemia (HL-60), human squamous cell carcinoma cell lines (HSC-2, HSC-3, HSC-4) and human glioblastoma cells (T98G), here the quantitative structure-activity relationship (QSAR) of newly synthesized 5-trifluoromethyloxazole derivatives (5, 6) (Figure 1) was investigated, using conventional and recent techniques of computational chemistry such as the concept of chemical hardness (7-9). The chemical hardness is a parameter that determines the softness and hardness of the test compound. In generally, the softness means the higher reactivity whereas the hardness means the poor reactivity. "Soft" molecule tends to affect profoundly on the biological system, whereas "hard" molecule is expected to have little or no biological activity.

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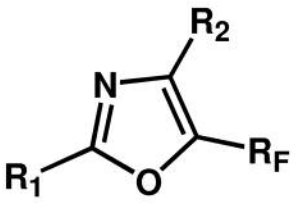
Key Words: 5-Trifluoromethyloxazole derivatives, QSAR, cytotoxicity, semiempirical molecular-orbital method, absolute hardness.

Materials and Methods

Calculation. The most stable conformation of twelve 5-trifluoromethyloxazole derivatives was calculated by CONFLEX 5 (Confluex Co. Ltd., Tokyo). The optimization of the structure was achieved using a semiempirical molecular-orbital method (PM3), using a CAChe Worksystem 4.9 (Fujitsu Co. Ltd., Tokyo) PM3 method, in the presence (COSMO) or in the absence (non-COSMO) of water (Figure 2). The following chemical descriptors were used: heat of formation (COSMO, non-COSMO) (Kcal/mole), stability of



Compd.	R_1	R_2	R_F
[1]	C_6H_5	C_6H_5	CF_3
[2]	2-Thienyl	C_6H_5	CF_3
[3]	C_6H_5	$CH_2C_6H_5$	CF_3
[4]	tert-Butyl	$CH_2C_6H_5$	CF_3
[5]	$C_6H_5CH=C(CH_3)$	$CH_2C_6H_5$	CF_3
[6]	C_6H_5	$(CH_2)_3OH$	CF_3
[7]	4-ClC_6H_4	$(CH_2)_3OH$	CF_3
[8]	4-$CH_3OC_6H_4$	$(CH_2)_3OH$	CF_3
[9]	2-$CH_3OC_6H_4$	$(CH_2)_3OH$	CF_3
[10]	$C_6H_5CH=C(CH_3)$	$(CH_2)_3OH$	CF_3
[11]	$C_6H_5CH=C(CH_3)$	$(CH_2)_2CHO$	CF_3
[12]	H	$(CH_2)_3NHCOC_6H_5$	CF_3

Figure 1. *The structure of 5-trifluoromethyloxazol derivatives used.*

hydration (=COSMO – non-COSMO) (ΔH) (Kcal/mole), dipole moment (D), electron affinity (eV), ionization potential (eV), log P as an index of hydrophobicity, highest occupied molecular orbital energy (E_{HOMO}; eV), lowest unoccupied molecular orbital energy (E_{LUMO}; eV), maximum length of molecule (Å), absolute hardness (η; eV), absolute electron negativity (χ; eV), reactivity index (ω; eV)(7) (Table I). The following equations were used to determine η, χ and ω:

$\eta = (E_{LUMO} - E_{HOMO})/2$

$\chi = -(E_{LUMO} + E_{HOMO})/2$

$\omega = \chi^2/2\eta$

Assay for cytotoxic activity. Near confluent human promyleocytic leukemia (HL-60), human squamous cell carcinoma cell lines (HSC-2, HSC-3, HSC-4) and human glioblastoma cells (T98G) were cultured for 24 hours in RPMI 1640 (only for HL-60 cells) or Dulbecco's modified Eagles's medium (DMEM) (for all other cells) supplemented with 10% fetal bovine serum containing different concentrations of each 5-trifluoromethyloxazole derivative, as described previously (10). The 50% cytotoxic concentration (CC_{50}) of each compound was determined from the dose-response curve. The QSAR between CC_{50} and each descriptor delineated from the molecular structure was investigated by a CAChe Worksystem 4.9 project reader.

Results and Discussion

The CC_{50} values of twelve 5-trifluoromethyloxazole derivatives against HL-60, HSC-2, HSC-3, HSC-4 and T98G cells (experimental values), and chemical descriptors such as heat of formation, stability of hydration (=COSMO – non-COSMO) (ΔH) (Kcal/mole), dipole moment (D), electron affinity (eV), ionization potential (eV), log P, E_{HOMO} (eV), E_{LUMO} (eV), maximum length of molecule (Å), molecular weight, η (eV), χ (eV), ω (eV) (determined by calculation) are listed in Table I (a) (COSMO) and Table I (b) (non-COSMO). The correlation between the CC_{50} and each descriptor was then investigated (Figure 3). The correlation coefficient between the CC_{50} and each chemical descriptor (non-COSMO or COSMO) is listed in Table II. There was no correlation between the CC_{50} and heat of formation, χ or molecular weight, regardless of non-COSMO or COSMO conditions in any of the five cell lines. There was only minor correlation between the CC_{50} and ΔH, dipole moment and the maximum length of the molecule except for T98G cells (ΔH, dipole moment) (Figure 3C-1), Table II(a) COSMO), HL-60 (maximum length, COSMO/non-COSMO, Table II).

On the other hand, correlation was found between the CC_{50} and electron affinity under COSMO in all cell lines, especially in HL-60 cells (r^2=0.857) (Figure 3A-1), except for HSC-3 cells. In non-COSMO, the correlation between these parameters was found only in HL-60 and HSC-2 cells in non-COSMO (Table II). Similarly, correlation was found between the CC_{50} and ionization potential in HL-60 (r^2=0.750) (Figure 3A-2), HSC-4 (r^2=0.544) in COSMO, and in HSC-4 (r^2=0.422) in non-COSMO. E_{HOMO} (Figure 3A-3, B-1) and ionization potential gave the identical absolute values, whereas E_{LUMO} (A-4, B-2, C-2) and electron affinity gave the same absolute values. Good correlation was found between the CC_{50} and η (Figure 3B-3, C-3), both in non-COSMO (except for HSC-3 cells) and COSMO [especially in HL-60 cells (r^2=0.903) (Figure 3A-5), but not in HSC-2 and HSC-3 cells]. Good correlation was found between CC_{50} and ω (Figure 3C-4) especially in HL-60 cells (r^2=0.761) (Figure 3A-6), but not in HSC-3 (COSMO) and HSC-3, HSC-4 and T98G cells (non-COSMO).

The cytotoxic activity of 5-trifluoromethyloxazole derivatives became maximum at log P=4.6 (Figure 3A-8), slightly higher than the optimal log P values reported for prenylalcohol, vitamin K_2 (11), gallic acid (12) and coumarin (13) derivatives (log P of 2-3).

The correlation between the electron structure and the cytotoxicity of 5-trifluoromethyloxazole derivatives was next investigated, using the η-χ activity diagram (Figure 4). In HL-60 cells, compounds with higher cytotoxicity (lower CC_{50}) are within the area surrounded by the box. Their cytotoxicity strongly depended on the η value, but not on the χ value. Compounds with higher cytotoxicity (lower CC_{50}) had a lower η value (η<4.29). Compounds with lower cytotoxicity (higher CC_{50}) had a higher η value (η>4.62). The value of η determined by this method may be useful in estimating the cytotoxic activity of newly synthesized 5-trifluoromethyloxazole derivatives.

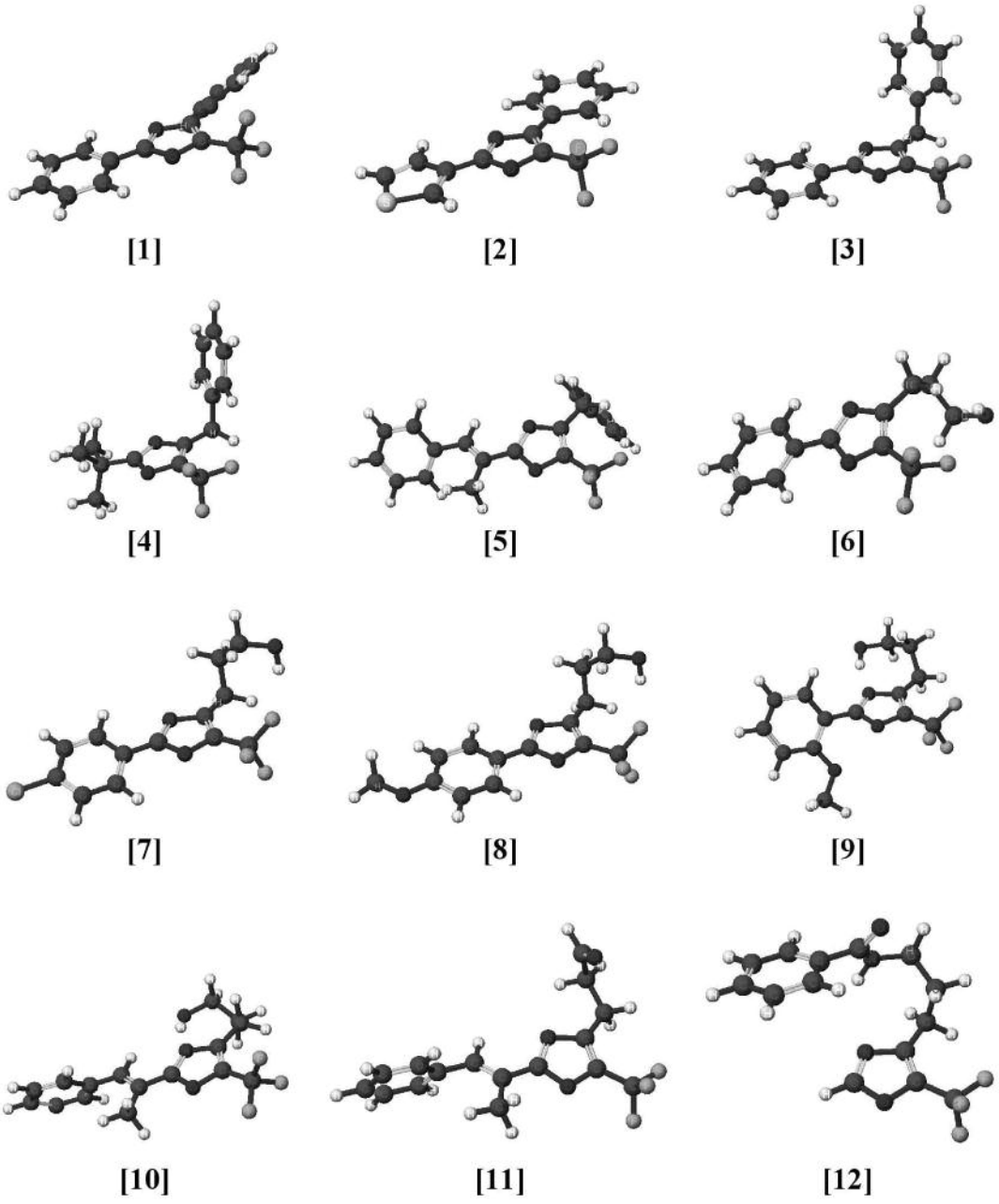


Figure 2. *The most stable conformation of 5-trifluoromethyloxazol derivatives.*

We performed the QSAR analysis of the CC_{50} values of 5-trifluoromethyloxazole derivatives. Higher correlation coefficients were generally found under COSMO, as compared with non-COSMO conditions. This suggests that calculations should be performed under biomimetic conditions, although a longer calculation time is required. The concept of absolute hardness is applicable in estimating the cytotoxicity of 5-trifluoromethyloxazoles, using chemical descriptors η, ω or the η-χ activity diagram.

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Table I. *CC_{50} and chemical descriptors for 5-trifluoromethyloxazole derivatives.*

(a) COSMO

Compound	CC_{50}					Heat of formation (Kcal/mol)	ΔH	Dipole moment (D)
	HL-60	HSC-2	HSC-3	HSC-4	T98G			
[1]	43.0	177.0	138.0	140.0	150.0	−112.707	−8.392	3.120
[2]	45.0	195.0	196.0	137.0	188.0	−104.602	−8.218	2.443
[3]	52.0	152.0	90.0	130.0	180.0	−119.426	−8.705	3.315
[4]	120.0	363.0	257.0	231.0	296.0	−167.015	−7.751	2.543
[5]	36.0	162.0	88.0	82.0	151.0	−112.777	−9.301	3.258
[6]	52.0	250.0	246.0	255.0	279.0	−200.871	−11.896	5.161
[7]	35.0	141.0	140.0	123.0	190.0	−207.606	−12.261	3.943
[8]	35.0	195.0	261.0	150.0	225.0	−241.844	−14.202	6.082
[9]	44.0	280.0	221.0	200.0	267.0	−237.769	−12.784	3.959
[10]	29.0	128.0	120.0	111.0	168.0	−193.299	−11.256	2.078
[11]	74.0	330.0	249.0	211.0	344.0	−181.013	−15.037	5.731
[12]	142.0	275.0	260.0	320.0	330.0	−196.773	−18.597	6.378

Compound	Electron affinity (eV)	Ionization potential (eV)	E_{HOMO}	E_{LUMO}	η	χ	ω	M.W.	Length (Å)	log P
[1]	1.228	9.622	9.622	−1.228	4.197	5.425	3.506	289.0	12.075	5.184
[2]	1.228	9.483	9.483	−1.228	4.098	5.386	3.540	295.0	11.230	3.782
[3]	1.167	9.739	−9.739	−1.167	4.286	5.453	3.469	303.0	11.341	5.436
[4]	0.689	9.943	−9.943	−0.689	4.627	5.316	3.054	283.0	10.681	5.514
[5]	1.130	9.491	−9.491	−1.130	4.181	5.310	3.373	343.0	12.788	6.256
[6]	1.170	9.748	−9.748	−1.170	4.289	5.459	3.474	271.0	12.314	3.468
[7]	1.262	9.553	−9.553	−1.262	4.146	5.407	3.527	306.0	11.371	3.986
[8]	1.154	9.337	−9.377	−1.154	4.111	5.266	3.372	301.0	12.878	3.215
[9]	1.023	9.550	−9.550	−1.023	4.263	5.287	3.278	301.0	10.227	3.215
[10]	1.122	9.532	−9.532	−1.122	4.205	5.327	3.374	311.0	12.078	4.288
[11]	1.051	9.547	−9.547	−1.051	4.248	5.299	3.305	401.0	12.194	3.791
[12]	0.539	10.016	−10.016	−0.539	4.738	5.278	2.939	298.0	9.466	2.664

(b) Non-COSMO

Compound	CC_{50}					Heat of formation (Kcal/mol)	Dipole moment (D)	Electron affinity (eV)
	HL-60	HSC-2	HSC-3	HSC-4	T98G			
[1]	43.0	177.0	138.0	140.0	150.0	−104.315	2.533	1.118
[2]	45.0	195.0	196.0	137.0	188.0	−96.384	2.184	1.253
[3]	52.0	152.0	90.0	130.0	180.0	−110.721	2.599	1.062
[4]	120.0	363.0	257.0	231.0	296.0	−159.264	2.198	0.549
[5]	36.0	162.0	88.0	82.0	151.0	−103.476	2.73	0.979
[6]	52.0	250.0	246.0	255.0	279.0	−188.975	3.817	1.173
[7]	35.0	141.0	140.0	123.0	190.0	−195.345	2.972	1.327
[8]	35.0	195.0	261.0	150.0	225.0	−227.642	4.715	1.100
[9]	44.0	280.0	221.0	200.0	267.0	−224.985	3.478	1.014
[10]	29.0	128.0	120.0	111.0	168.0	−182.043	2.163	1.189
[11]	74.0	330.0	249.0	211.0	344.0	−165.976	4.441	0.944
[12]	142.0	275.0	260.0	320.0	330.0	−178.176	4.035	0.858

Table I. *continued*

Table I. *continued*

Compound	Ionization potential (eV)	log P	E_{HOMO}	E_{LUMO}	η	χ	ω	M.W.	Length (Å)
[1]	9.466	5.184	–9.466	–1.118	4.174	5.292	3.355	289.0	12.075
[2]	9.365	3.782	–9.365	–1.253	4.056	5.309	3.475	295.0	11.230
[3]	9.631	5.436	–9.631	–1.062	4.285	5.346	3.336	303.0	11.341
[4]	9.696	5.514	–9.696	–0.549	4.574	5.122	2.868	283.0	10.681
[5]	9.023	6.256	–9.450	0.979	4.235	5.215	3.210	343.0	12.788
[6]	9.742	3.468	–9.742	–1.173	4.285	5.457	3.476	271.0	12.314
[7]	9.561	3.986	–9.561	–1.327	4.117	5.444	3.599	306.0	11.371
[8]	9.318	3.215	–9.318	–1.100	4.109	5.209	3.301	301.0	12.878
[9]	9.534	3.215	–9.534	–1.014	4.260	5.274	3.265	301.0	10.227
[10]	9.577	4.288	–9.577	–1.189	4.194	5.383	3.455	311.0	12.078
[11]	9.522	3.791	–9.522	–0.944	4.304	5.248	3.200	401.0	12.194
[12]	9.739	2.664	–9.739	–0.858	4.441	5.298	3.161	298.0	9.466

Table II. *Correlation coefficients between CC_{50} against the indicated cells and each chemical descriptor.*

(a) COSMO

Cell line	Heat of formation (Kcal/mole)	ΔH	Dipole moment (D)	Electron affinity (eV)	Ionization potential (eV)	log P
HL-60	0.002	0.136	0.109	0.857	0.750	C
HSC-2	0.071	0.073	0.123	0.448	0.249	C
HSC-3	0.321	0.283	0.383	0.267	0.086	C
HSC-4	0.161	0.345	0.363	0.556	0.544	C
T98G	0.247	0.401	0.413	0.470	0.261	C

Cell line	E_{HOMO}	E_{LUMO}	η (eV)	χ (eV)	ω (eV)	M.W.	Length (Å)
HL-60	0.750	0.857	0.903	0.100	0.761	0.003	0.462
HSC-2	0.249	0.448	0.391	0.161	0.459	0.020	0.182
HSC-3	0.086	0.267	0.192	0.189	0.290	0.006	0.092
HSC-4	0.544	0.556	0.662	0.045	0.496	0.029	0.357
T98G	0.261	0.470	0.410	0.169	0.476	0.042	0.210

(b) Non-COSMO

Cell line	Heat of formation (Kcal/mole)	ΔH	Dipole moment (D)	Electron affinity (eV)	Ionization potential (eV)	log P
HL-60	0.001	0.136	0.026	0.598	0.387	C
HSC-2	0.067	0.073	0.125	0.577	0.164	C
HSC-3	0.309	0.283	0.394	0.168	0.157	C
HSC-4	0.143	0.345	0.235	0.255	0.442	C
T98G	0.226	0.401	0.375	0.315	0.273	C

Cell line	E_{HOMO}	E_{LUMO}	η (eV)	χ (eV)	ω (eV)	M.W.	Length (Å)
HL-60	0.387	0.598	0.711	0.142	0.499	0.003	0.453
HSC-2	0.164	0.577	0.534	0.265	0.534	0.020	0.170
HSC-3	0.157	0.168	0.145	0.088	0.157	0.006	0.087
HSC-4	0.442	0.255	0.453	0.004	0.179	0.029	0.348
T98G	0.273	0.315	0.417	0.050	0.258	0.042	0.195

C: r^2 could not be determined due to the parabolic curve.

References

1 Pippel DJ, Mapes CM and Mani NS: Reactions between Weinreb amides and 2-magnesiated oxazoles: a simple and efficient preparation of 2-acyl oxazoles. J Org Chem *72*: 5828-5831, 2007.

2 Schlosser M: CF_3-bearing aromatic and heterocyclic building blocks. Angew Chem Int Ed Engl *45*: 5432-5446, 2006.

3 Kirk KL: Fluorine in medicinal chemistry: recent therapeutic applications of fluorinated small molecules. J Fluorine Chem *127*: 1013-1029, 2006.

4 Singh SK, Vobbalareddy S, Kalleda SR, Casturi SR, Mullangi R, Ramanujam R, Yeleswarapu KR and Iqbal J: *N*-Acylated sulfonamide sodium salt: a prodrug of choice for the bifunctional 2-hydroxymethyl-4-(5-phenyl-3-trifluoromethyl- pyrazol-1-yl)benzene-sulfonamide class of COX-2 inhibitors. Bioorg Med Chem Lett *16*: 3921-3926, 2006.

5 Kawase M, Miyamae H and Kurihara T: A general method for the preparation of 5-trifluoromethylated oxazoles from α-amino acids. Chem Pharm Bull *46*: 749-756, 1998.

6 Kawase M, Saito S and Kurihara T: Convenient synthesis of α-trifluoromethylated cycloins from α-hydroxy or α-amino acids. Chem Pharm Bull *48*: 1338-1343, 2000.

7 Kobayashi S, Tanaka A and Sameshima K: Relationship between electron structure toxicity and biological activity of environmental hormones. Chemi Chem Industry *52*: 41-44, 1999 (in Japanese).

8 Kobayashi S, Sameshima K, Ishii Y and Tanaka A: Toxicity of dioxins: role of absolute hardness-absolute electronegativity diagram (η-χ diagram) as a new measure in risk assessment. Chem Pharm Bull *43*: 1780-1790, 1995.

9 Kobayashi S, Hamashima H, Kurihara M, Miyata N and Tanaka A: Hardness controlled enzymes and electronegativity controlled enzymes: role of an absolute hardness-electronegativity (η-χ) activity diagram as a coordinate for biological activities. Pharm Bull *46*: 1108-1115, 1998.

10 Das U, Kawase M, Sakagami H, Ideo A, Shimada J, Molnar J and Dimmock JR: Reversal of multidrug resistance and selective cytotoxic properties displayed by some 3-(3,4,5-trimethoxyphenyl)-2-propenyl derivatives. Bioorg Med Chem *15*: 3373-3380, 2007.

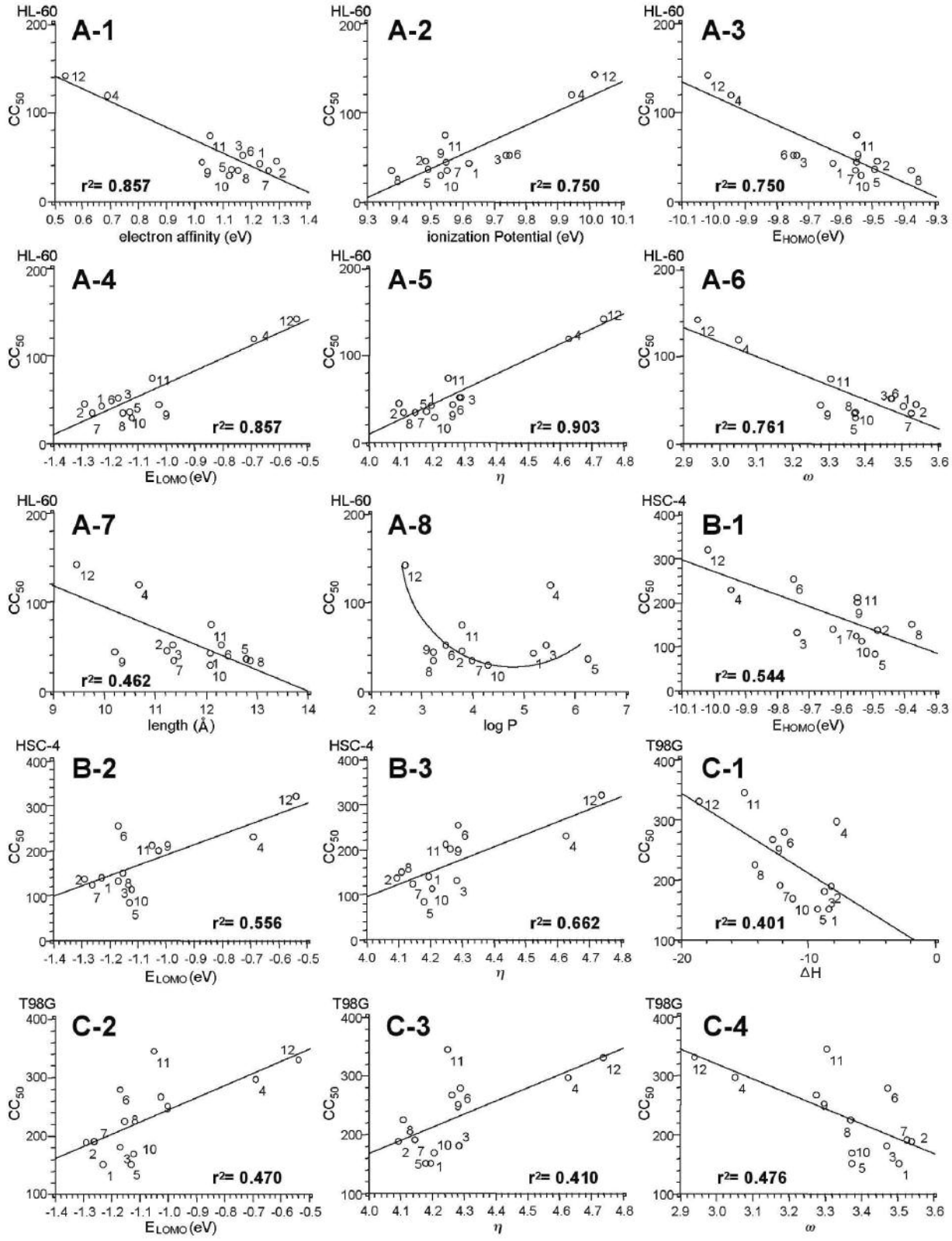

Figure 3. *Correlation between CC_{50} values and each chemical descriptor of 5-trifluoromethyloxazol derivatives against HL-60 (A-1 to A-8), HSC-4 (B-1 to B-3) and T98G (C-1 to C-4) cells.*

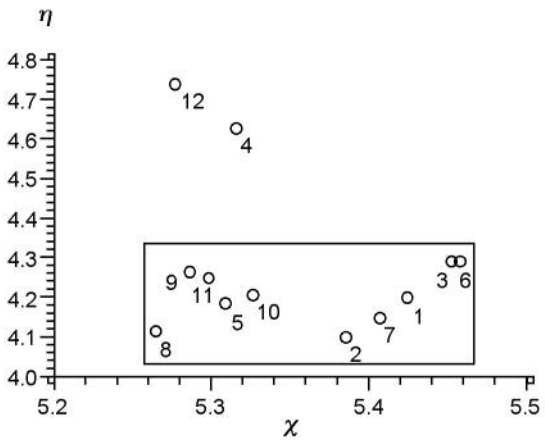

Figure 4. *The η-χ diagram of 5-trifluoromethyloxazol derivatives.*

11 Ishihara M, Takayama F, Toguchi M, Nakano K, Yasumoto E, Nakayachi T, Satoh K and Sakagami H: Cytotoxic activity of polyprenylalcohols and vitamin K_2 derivatives. Anticancer Res *20*: 4307-4314, 2000.
12 Ishihara M and Sakagami H: Application of semiempirical method to estimate the cytotoxic activity of gallic acid and its related compounds. Anticancer Res *23*: 2549-2552, 2003.
13 Ishihara M, Yokote Y and Sakagami H: Quantitative structure-cytotoxicity relationship analysis of coumarin and its derivatives by a semiempirical molecular-orbital method. Anticancer Res *26*: 2883-2886, 2006.

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