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Antitumor and Quantitative Structure Activity Relationship Study for Dihydropyridones Derived from Curcumin

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Abstract: Problem statement: Pyridones are known to have variety of biological activities like antitumor, antibacterial, antiinflamatory and antimalarial activities. This study presented antitumor evaluation of dihydropyridones derived from curcumin, as well as curcumin for comparison. **Approach:** The compounds evaluated for a preliminary estimation of the in vitro tumor inhibiting activity against 11 of tumor cell lines by using Microculture Tetrazolium assay (MTT) method. The method is based on the metabolic reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. The cell lines of tumor subpanels were incubated within five concentrations (0.01-100 $\mu g\ mL^{-1}$) of each tested compound for 48 h. **Results:** Antitumor biological activities represented as CC_{50} were within the range >100-17±1 against leukaemia (MT4). The CC_{50} values were found to increase with increasing chain length of the substituent on the nitrogen atom. **Conclusion:** Antitumor activities of the tested dihydropyridones can be enhanced by increasing chain length of the substituent on the nitrogen atom.

Key words: Dihydropyridones, curcumin, leukemia (MTT), QSAR, logP

INTRODUCTION

Six-membered nitrogen heterocycles are key units in medicinal chemistry and versatile intermediates in organic synthesis (Dong *et al*., 2005; Comins and Ollinger, 2001). Dihydropyridones are important intermediates for the synthesis of natural products, particularly alkaloids (Elias *et al*., 2008) and they have been extensively investigated as valuable building block for the construction of piperidines, perhydroquinolens, indolizidines, quinolizidines and other alkaloid systems, with a wide range of a biological and pharmacological activities. These compounds known for their antiproliferative and antitubolin activities (Magedov *et al*., 2008) and as potential selective inhibitors of receptor tyrosyn kinase (Hu *et al*., 2008; Goodman *et al*., 2007). Their ability to induce leukaemic cell differentiation have been demonstarated (Pierce *et al*., 1981). In addition they have potent antimalarial activity (Yeats *et al*., 2008) and good anticonvulsant activity against acutely elicited Seizures (Revas *et al*., 2009). On the other hand curcumin is a principal curcuminoid of Indian curry and has known for its antitumor (Ran *et al*., 2009; Wohlmuth *et al*., 2010; Ljngman, 2009), antioxidant, antiinflamatory (Takahashi *et al*., 2009; Kuhad *et al*., 2007; Michaelidou and H-Litina, 2005) and antiarthritic properties (Patil *et al*., 2009).

Very little was published about the antitumor activities of dihydropyridones and the aim of this study is to investigate the relationship between structure and antitumor activity of a series of dihydropyridones derived from curcumin.

MATERIALS AND METHODS

The screened pyridones were synthesized by the reaction of curcumin and amines elsewhere (Elias *et al*., 2008). These compounds as well as curcumin were evaluated for preliminary estimation of the in vitro tumor inhibiting activity against a panel of tumor cell lines consisting of $CD4^+$ human T-cells containing an integrated Human T-Leukaemia Virus type 1(HTLV-1), $CD4^+$ human acute lumphoblastic leukaemia, human splenic B-lymphoblastoid cells, human acute B-lymphoblastic leukaemia, human skin melanoma, human

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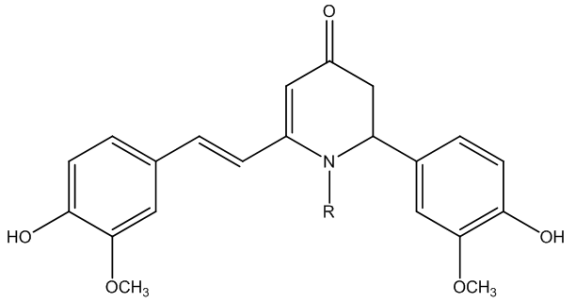

Fig. 1: General structure for the studied compounds

breast adenocarcinoma, human lung squamous carcinoma, human heptatocellular carcinoma, human prostate carcinoma, human foreskin fibroblasts and human lung fibroblasts, using microculture assay (MTT) method (Tang *et al*., 2010). This method is based on the metabolic reduction of 3-(4,5-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The cell lines of tumor subpanels were incubated within five concentrations (0.01-100 μg mL^{-1}) of each tested compound for 48 h. Molecular descriptors for the studied compounds, logP, Hydration energy (ΔH), Refractivity (Ref) and Polaraizability (POL) were calculated using HyperChem 8.5 program, after geometry optimization with the semi empirical RM1 Hamiltonian. The general molecular structure of the studied molecules is shown in Fig. 1.

RESULTS

The results of the antitumor activities, represented as CC_{50} (μM) are summarized in Table 1.

The activity values are within the ranges >100-17±1, >100-34±2 and >100-57±4 for leukaemia lymphoma, solid tumoer-derived cell lines and normal-cell lines respectively. The calculated molecular descriptors are gathered in Table 2.

The values of logP, Refractivity, Polarizibility increase with increasing molecular weight while hydration energy decreases with increasing molecular weight except for molecule 6.

DISCUSSION

All the tested compounds have antitumor activities less than those of curcumin against all tumor cell lines. This may be due to the lack to the β-diketone moiety in pyridones. It is obvious from Table 1 that the CC_{50} value is increased with increasing chain length of the substituent on the nitrogen atom. Comparing the activity of compound 1 with other pyridones showed

Table 1: Antitumor activities of the studied dihudropyridones in most sensitive tumor cell lines

Comp.	R	Tumor	Cell line	CC50 (μM)[a]
1	$-CH_3$	Leukaemia lymphoma	MT4[b]	>100
			CCRF-CEM[c]	>100
			WIL-2NS[d]	>100
			CCRF-SB[e]	>100
		Solid tumor-derived cell lines	SK-MEL-28[f]	>100
			MCF7[g]	>100
			SK-MES-1[h]	>100
			HepG2[i]	>100
			DU145[j]	>100
		Normal-cell lines	CRL-7065[k]	>100
			MRC-l[l]	>100
2	$-C_2H_5$	Leukaemia lymphoma	MT4[b]	54
			CCRF-CEM[c]	36±9
			WIL-2NS[d]	57±1.5
			CCRF-SB[e]	66±9
		Solid tumor-derived cell lines	SK-MEL-28[f]	>100
			MCF7[g]	>100
			SK-MES-1[h]	>00
			HepG2[i]	>100
			DU145[j]	>100
		Normal-cell lines	CRL-7065[k]	>100
			MRC-l[l]	>100
3	$-C_3H_7$	Leukaemia lymphoma	MT4[b]	51
			CCRF-CEM[c]	>100
			WIL-2NS[d]	>100
			CCRF-SB[e]	>100
		Solid tumor-derived cell lines	SK-MEL-28[f]	>100
			MCF7[g]	>100
			SK-MES-1[h]	>100
			HepG2[i]	>100
			DU145[j]	>100
		Normal-cell lines	CRL-7065[k]	>100
			MRC-l[l]	>100
4	$-C_4H_9$	Leukaemia lymphoma	MT4[b]	36
			CCRF-CEM[c]	20±2.5
			WIL-2NS[d]	26±6
			CCRF-SB[e]	36±11
		Solid tumor-derived cell lines	SK-MEL-28[f]	46±2
			MCF7[g]	>100
			SK-MES-1[h]	58±2
			HepG2[i]	53±0.5
			DU145[j]	53±0.3
		Normal-cell lines	CRL-7065[k]	>100
			MRC-l[l]	>100
5	$-C_6H_{13}$	Leukaemia lymphoma	MT4[b]	20
			CCRF-CEM[c]	17±1
			WIL-2NS[d]	24±1
			CCRF-SB[e]	25±1
		Solid tumor-derived cell lines	SK-MEL-28[f]	43±7
			MCF7[g]	47±8
			SK-MES-1[h]	45±10
			HepG2[i]	34±2
			DU145j	42±6
		Normal-cell lines	CRL-7065[k]	60±0.5
			MRC-l[l]	57±4
6	$-CH_2$-Ph	Leukaemia lymphoma	MT4[b]	53
			CCRF-CEM[c]	21±1
			WIL-2NS[d]	52±2
			CCRF-SB[e]	46±8
		Solid tumor-derived cell lines	SK-MEL-28[f]	76±8
			MCF7[g]	>100
			SK-MES-1[h]	>100
			HepG2[i]	>100

Table 1: Continued

		DU145[j]	56±13
	Normal-cell	CRL-7065[k]	>100
	lines	MRC-l[l]	>100
	Leukaemia	MT4[b]	18
	lymphoma	CCRF-CEM[c]	13±0.10
		WIL-2NS[d]	19±0.05
		CCRF-SB[e]	20±1.00
Curcumin	Solid tumor-	SK-MEL-28[f]	18±0.60
	derived cell	MCF7[g]	31±3.00
	lines	SK-MES-1[h]	22±2.00
		HepG2[i]	30±1.00
		DU145[j]	21±2.50
	Normal-cell	CRL-7065[k]	19±0.80
	lines	MRC-l[l]	17±2.00

[a]: Compound concentration required to reduce cell proliferation by 50% as determined by the MTT method. Data represent mean values (±SD); [b]: $CD4^+$ human T-cells containing an integrated HTLV-1; [c]: $CD4^+$ human acute T-lymphoblastic leukaemia; [d]: Human splenic lymphoplastoid cells; [e]: Human acute B-lymohoplastic leukaemia; [f]: Human skin melanoma; [g]: Human breast adenocarcinoma; [h]: Human lung squamous carcinoma; [i]: Human hepatocellular carcinoma; [j]: Human prostate carcinoma; [k]: Human foreskin fibroblasts; [l]: Human lung fibroblast

Table 2: Calculated molecular descriptors, observed activity against leukaemia (MT4) and the predicted activity for the studied dihydropyridones

No.	logP	Ref.	Pol.	ΔH	π	A_{obs}	A_{pred}	Resdual
2	3.29	114.03	43.11	-16.69	1.02	-0.238	-0.251	-0.013
3	3.67	118.56	44.64	-16.28	1.55	-0.232	-0.210	-0.013
4	4.16	123.16	46.78	-15.85	2.13	-0.192	-0.184	0.008
5	4.95	132.36	50.45	-15.01	3.10	-0.114	-0.121	-0.007
6	4.72	133.90	50.93	-17.76	2.01	-0.237	-0.237	0.000

Ref: Refractivity; Pol: Polarizability; ΔH: Hydration energy; π: Hydrophobicity constantof the substituent; A_{obs}: Observed biological activity expressed by Log ($1/CC_{50}$); Apred: Predicted biological activity

that the inclusion of a methylen or a phenyl group in the substituent moiety shifted the threshold of potency from inactive side towards activity in some of leukaemia lymphoma cell lines, particularly against the leukaemia cell lines MT4. For substituent longer than propyl group the compounds become active for most cell lines and in the case where R is hexyl group the antitumor activity becomes comparable to that of curcumin. Ignoring the data of compound 1 (CC_{50}>100 for all cell lines) we tried to correlate the activity of the compounds 2-6 represented by Log($1/CC_{50}$) against the leukaemia cell lines MT4 with the molecular descriptors, logP, refractivity, polarizability , hydration energy and carbon number of the substituent (C_n). Very good models with R^2 values 0.938, 0.957, 0.968, 0.957 and 0.955 respectively, were obtained when the data of compound 6 are not involved. The models are shown in Eq. 1-5:

$$\text{Log}(1/CC50) = 0.078\log P - 0.512 \quad (1)$$
$$R^2 = 0.938,\quad S^2 = 0.017,\quad F = 30.3$$

$$\text{Log}(1/CC_{50}) = 0.007\,\text{Ref} - 1.064 \quad (2)$$
$$R^2 = 0.957,\quad S^2 = 0.014,\quad F = 44.3$$

$$\text{Log}(1/CC_{50}) = 0.017\text{Pol} - 1.011 \quad (3)$$
$$R^2 = 0.968,\quad S^2 = 0.012,\quad F = 36.3$$

$$\text{Log}(1/CC_{50}) = 0.077\Delta H + 1.047 \quad (4)$$
$$R^2 = 0.957,\quad S^2 = 0.014,\quad F = 0.3$$

$$\text{Log}(1/CC_{50}) = 0.033C_n - 0.317 \quad (5)$$
$$R^2 = 0.955,\quad S^2 = 0.015,\quad F = 42.9$$

Equation 1-5 indicates a strong dependency of the activity on the alkyl chain length. However, when compound 6 involved in the regression equation poor models with low R^2 are predicted for all parameters except for ΔH. For example, in the case of the model including log P the correlation coefficient R^2 is 0.417, while for ΔH as a descriptor, a model with R^2 = 0.713 is obtained. This value became 0.957 when a double parameter regression equation including both ΔH and the hydrophobicity constant of the substituent (π) was used as shown in Eq. 6:

$$\text{Log}(1/CC_{50}) = 0.134\Delta H + 2.551\pi + 4.183 \quad (6)$$
$$R^2 = 0.957,\qquad S^2 = 0.015,\quad F = 22.3$$

The predicted biological activities for the dihydropyridones from Eq. 6 represented as Log ($1/CC_{50}$) are shown in Table 2.

CONCLUSION

This study has shown that the biological activity of the studied compounds increases with increasing chain length of the substituent on the nitrogen atom as well the activity could be predicted to good estimate on the basis of a model involving both hydration energy and the hydrophoibicity constant of the substituent.

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