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ORIGINAL RESEARCH

Antioxidant, cytotoxicity, and QSAR study of 1-adamantylthio derivatives of 3-picoline and phenylpyridines

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Abstract A series of isomeric α- and β-(1-adamantylthio)pyridines were previously documented to possess interesting antimicrobial and antimalarial activities. In this study, the antioxidant and cytotoxic potentials of 1-adamantylthio-3-methyl and 2-,3-,4-phenylpyridines (**1–10**) were investigated. The tested compounds were shown to exhibit interesting superoxide (SOD)- and free radical (DPPH)-scavenging activities as well as cytotoxic activities. Particularly, β-(1-adamantylthio)-4-phenylpyridine (**8**) was shown to be the most potent antioxidant and cytotoxic compound. QSAR studies revealed that dipole moment (μ) and electrophilic index (ω_i) were the most important descriptors accounting for the observed SOD activities. Compounds with high μ and ω_i values were observed to display high SOD activity. Inversely, compounds with the lowest atomic polarizability (MATS4p) exhibited the highest DPPH activity. Other quantum chemical descriptors such as atomic masses (GATS4m), ω_i, and LUMO energy were also well correlated with cytotoxicity. The findings demonstrated that thiopyridine **8** is a potential lead compound that should be further investigated in drug discovery efforts. The QSAR results offer good prospect for the rational design of novel compounds with robust bioactivities.

Keywords 1-adamantylthiopyridines · Antioxidant · Cytotoxicity · QSAR · Multiple linear regression

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Introduction

Recently, a series of isomeric α- and β-(1-adamantylthio)-3-picoline and phenylpyridines with antimicrobial and antimalarial activities have been reported (Prachayasittikul *et al.*, 2009; Lawung *et al.*, 2010). In particular, 4-phenylpyridine and 3-picoline containing 1-adamantylthio group at β (*3*- or *5*-)-position were shown to be effective antimicrobials against both methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* isolates with low cell (L929 cells) cytotoxicity (Lawung *et al.*, 2010). It was also found that some thiopyridines with antimicrobial action also displayed antioxidant property. As it is known that free radicals (e.g., superoxide) produced under oxidative stress in the body is the causative agent of many diseases, such as cancer, aging, heart disease, and inflammation (Sakurai *et al.*, 2008; Valko *et al.*, 2007; Banerjee *et al.*, 2005). Thiopyridines that possess antioxidative activities are 3-substituted pyridines (R = OEt, OAc, OH,

NAc_2, Br, CN, COOH, and $CONH_2$) containing 1-adamantylthio group at either α- (2- and 6-) or β-position (Prachayasittikul *et al.*, 2008). To search for novel antioxidants having thiopyridine core structure, it is therefore crucial to investigate the therapeutic potential of other substituted pyridine compounds bearing non-polar substituents (R) such as alkyl and aryl groups. The present study reports the antioxidant effects as well as cytotoxicities of 1-adamantylthio-3-methyl and 2-,3-,4-phenylpyridines (**1–10**, Fig. 1). To understand the substituent effects of the pyridine ring toward their bioactivities, quantitative structure–activity relationship (QSAR) of the compounds was investigated.

Materials and methods

General

^{1}H-NMR spectra were recorded on a Bruker AM 400 instrument with a 400/100 MHz operating frequency using deuterochloroform solution with tetramethylsilane as an internal standard. Column chromatography was carried out using silica gel 60 (0.063–0.200 mm). Thin layer chromatography (TLC) was performed on silica gel 60 PF_{254} (cat. No. 7747 E., Merck). Melting points were determined on an Electrothermal melting point apparatus (Electrothermal 9100) and are uncorrected. Reagents for cell culture were as follows: RPMI-1640 (Rosewell Park Memorial Institute medium, Gibco and Hyclone laboratories, USA); HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), L-glutamine, penicillin, streptomycin, sodium pyruvate, and glucose (Sigma, USA); Ham's/F12 (Nutrient mixture F-12); DMEM (Dulbecco's Modified Eagle's Medium); FBS (fetal bovine serum, Hyclone laboratories, USA); gentamicin sulfate (Government Pharmaceutical Organization, Thailand); and MTT (3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Aldrich, USA). Vitamin E, DPPH and bovine erythrocyte SOD were obtained from Sigma Chemical Co. (USA). DMSO was purchased from Fluka.

Thiopyridine derivatives **1–10**

Compounds **1–10** were prepared according to the literature (Prachayasittikul *et al.*, 1985, 1991) and confirmed by ^{1}H-NMR spectra. These compounds comprise 2-(1-adamantylthio)-3-picoline (**1**), 6-(1-adamantylthio)-3-picoline (**2**), 5-(1-adamantylthio)-3-picoline (**3**), 6-(1-adamantylthio)-3-phenylpyridine (**4**), 6-(1-adamantylthio)-2-phenylpyridine (**5**), 4-(1-adamantylthio)-2-phenylpyridine (**6**), 2-(1-adamantylthio)-4-phenylpyridine (**7**), 3-(1-adamantylthio)-4-phenylpyridine (**8**), 2-(1-adamantylthio)-3-phenylpyridine (**9**), and 5-(1-adamantylthio)-2-phenylpyridine (**10**).

Cytotoxicity assay

Cytotoxicity was tested using the modified method of Tengchaisri *et al.* (1998). Cancer cells were grown in Ham's/F12 medium containing 2 mM L-glutamine supplemented with 100U/mL penicillin, streptomycin, and 10% FBS, except for HepG2 cell which was grown in DMEM. In brief, cell lines suspended in RPMI-1640 containing 10% FBS were seeded with 1×10^4 cells (100 μL)

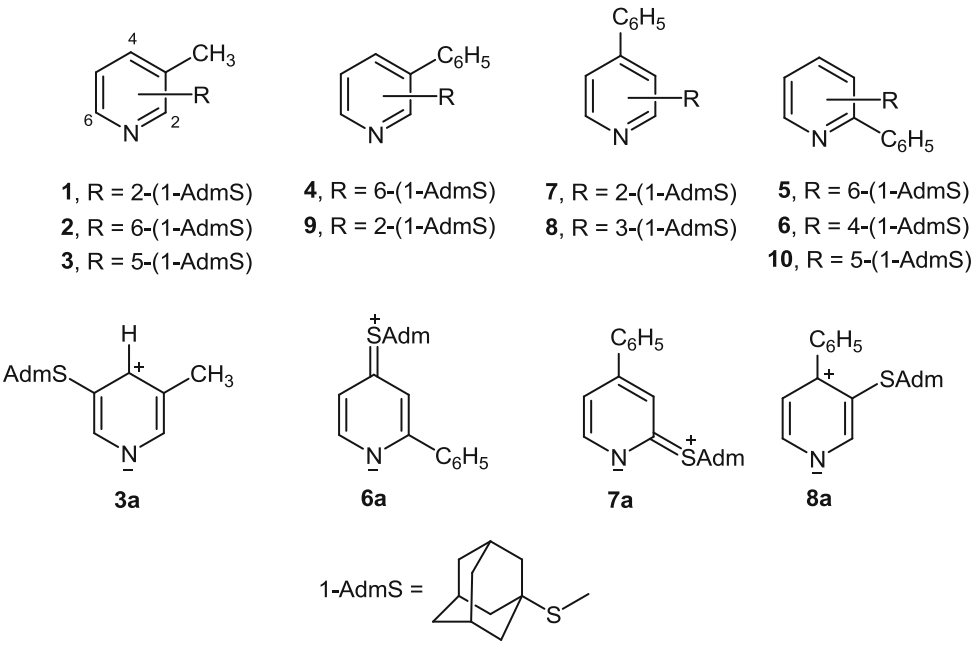

Fig. 1 Molecular structures of 1-adamantylthiopyridine derivatives **1–10**

per well in a 96-well plate, and incubated under humidified atmosphere (95% air and 5% CO_2) at 37°C. After 24 h, additional medium (100 μL) containing the test compound, and vehicle was added to a final concentration of 50 μg/mL, 0.2% DMSO, and then further incubated for 3 days. Cells were subsequently fixed with 95% EtOH, stained with crystal violet solution, and lysed with a solution of 0.1 N HCl in MeOH, after which its absorbance was measured at 550 nm. On the other hand, A549 and HepG2 cells were stained with MTT. IC_{50} values were determined as the drug and sample concentrations at 50% inhibition of the cell growth. Four tested cancer cells were T-lymphoblast (MOLT-3, acute lymphoblastic leukemia), Human cholangiocarcinoma cancer cells (HuCCA-1), Human lung carcinoma cell line (A549), and Human hepatocellular carcinoma cell line (HepG2).

Antioxidant assays

The compounds were tested for antioxidant property using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide dismutase (SOD) assays. The DPPH assay (Prachayasittikul *et al.*, 2010) was initiated by adding 1 mL of 0.1 mM solution of DPPH in methanol to a sample solution (0.45 mL, 1 mg/mL dissolved in DMSO). The reaction mixture was incubated for 30 min in a dark room. The absorbance at 517 nm was measured, and the percentage of radical scavenging activity was calculated from the following equation:

$$\%\,\text{Radical scavenging activity} = \left[1 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right] \times 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of the control reaction, and $\text{Abs}_{\text{sample}}$ is the absorbance of the tested compound.

The inhibition of the photoreduction of nitro blue tetrazolium (NBT) was performed to measure the SOD activity (Suksrichavalit *et al.*, 2009). The photochemically excited riboflavin was first reduced by methionine into a semiquinone, which donated an electron to oxygen to form a superoxide source. The superoxide readily converted NBT into a purple formazan product. As a result, the SOD activity was inversely related to the amount of formazan formation.

Data preparation

A dataset comprising of ten 1-adamantylthio derivatives of 3-picoline and phenylpyridines **1–10** (Fig. 1) were used in QSAR studies. The cytotoxic activity was subjected to negative logarithm transformation to the base of 10 for the numerical indicator of the IC_{50} activity ($-\log IC_{50}$ or pIC_{50}, where IC_{50} indicates the 50% inhibitory concentration that inhibits the cancer cell growth).

Descriptors calculation

The molecular structures of the compounds were constructed using GaussView (Dennington *et al.*, 2003). They were initially subjected to geometrical optimization in Gaussian 03W (Frisch *et al.*, 2004) by the semi-empirical method Austin Model 1 (AM1) followed by quantum chemical calculations at the DFT level using Becke's three-parameter hybrid method and the Lee–Yang–Parr correlation functional (B3LYP) together with the 6–31g(d) basis set. The quantum chemical descriptors used in this study were comprised of the total energy (E_{total}) of the molecule, the highest occupied molecular orbital energy (E_{HOMO}), the lowest unoccupied molecular orbital energy (E_{LUMO}), the total dipole moment (μ) of the molecule, the electron affinity (EA), the ionization potential (IP), the energy difference of LUMO and HOMO (HOMO-LUMO_{gap}), Mulliken electronegativity (χ), Hardness (η), Softness (S), Electrophilicity (ω), Electrophilic index (ω_i) and the mean absolute atomic charge (Q_m) (Parr *et al.*, 1978, 1999; Parr and Pearson, 1983; Karelson *et al.*, 1996; Thanikaivelan *et al.*, 2000). In addition, the optimized geometry of the compounds was subjected to further calculations using the Dragon software (Dragon 5.5, 2007) for generation of an additional 3,224 descriptors spanning 22 categories: 48 constitutional descriptors, 119 topological descriptors, 47 walk and path counts, 33 connectivity indices, 47 information indices, 96 2D autocorrelation, 107 edge adjacency indices, 64 burden eigenvalues, 21 topological charge indices, 44 eigenvalue-based indices, 41 randic molecular profiles, 74 geometrical descriptors, 150 RDF descriptors, 160 3D-MoRSE descriptors, 99 WHIM descriptors, 197 GETAWAY descriptors, 154 functional group counts, 120 atom-centered fragments, 14 charge descriptors, 29 molecular properties, 780 2D binary fingerprints and 780 2D frequency fingerprints.

Descriptor reduction

Descriptors with constant value and pairs of variables with correlation coefficient greater than 0.9 were discarded using the Unsupervised Forward Selection algorithm of UFS, version 1.8 (Whitley *et al.*, 2000) as described previously (Nantasenamat *et al.*, 2005). This is followed by using stepwise multiple linear regression (SPSS Statistics 18.0, SPSS Inc. USA) to obtain important descriptors for correlating with antioxidant and cytotoxic activities of 1-adamantylthio derivatives (**1–10**).

Multiple linear regression (MLR) and model evaluation

MLR was used for the construction of QSAR models and can be summarized by the following equation:

$$Y = B_0 + \sum B_n X_n$$

where Y is the pIC_{50} of the 1-adamantylthio derivatives, B_0 is the intercept, and B_n are the regression coefficients of the descriptors X_n. MLR was calculated using the Waikato Environment for Knowledge Analysis (Weka), version 3.4.5. (Witten *et al.*, 2011).

To evaluate the model, correlation coefficient (r) was used as a relative measure of predictive performance, which corresponded to the degree of correlation between the predicted and experimental values. Root mean square error (RMS) was used to measure the predictive error of the model. The RMS was calculated according to the following equation:

$$\text{RMS} = \sqrt{\frac{\sum_{i=1}^{n} (p_i - a_i)}{n}}$$

where p_i is the predicted output, a_i is the actual output, and n is the number of compounds in the data set.

Generation of dataset

The dataset was separated into two sets: the training and testing sets. The testing set was derived from the leave-one-out cross-validation (LOO-CV) where one sample was left out and used as the testing set, while the remaining $N - 1$ samples were used as the training set. This process was repeated iteratively until each sample of the dataset was used as the testing set.

Results and discussion

Antioxidant effect

1-Adamantylthiopyridines (**1–10**) were evaluated for their antioxidant property using DPPH and SOD assays. It was found that all the tested thiopyridines (**1–10**) displayed both DPPH and SOD activities (Table 1). Antioxidant activities of these compounds have not yet been reported in the literature. Significant results demonstrated that β-(1-adamantylthio) derivatives of 3-picoline (**3**) and 4-phenylpyridine (**8**) exhibited comparable SOD activity where compound **8** was the strongest antioxidant with 37.55% superoxide-scavenging activity. Such observation is in accordance to the antimicrobial activity of β-thiopyridine **8**, which has previously been shown to be the most potent compound among the tested compounds (Prachayasittikul *et al.*, 2009). In addition, thiopyridines **6**, **7,** and **10** were shown to be relatively strong antioxidants with 32% SOD activity. However, thiopyridine **6**, which bears the 1-adamantylthio group at γ-(or C-4) position, displayed the highest DPPH activity (30%).

Table 1 Antioxidant activity of thiopyridines **1–10**

Compound[a]	SOD activity (%)[b]	DPPH activity (%)[c]
1	10.83	19.60
2	28.61	7.69
3	36.92	7.96
4	18.00	10.62
5	25.86	7.37
6	32.15	30.25
7	32.03	6.95
8	37.55	12.25
9	26.66	11.10
10	31.64	8.77

[a] Tested concentration was 300 μg/mL, [b] superoxide dismutase (SOD) from bovine erythrocytes was used as a control in SOD assay (IC_{50} = 1.97 μg/mL), [c] vitamin E was used as a control in DPPH assay (IC_{50} = 4 μg/mL)

Cytotoxicity

The thiopyridines (**1–8**) were tested for their cytotoxicity against four cell lines (Table 2). Results showed that the tested compounds were active against MOLT-3 cell lines. The β-thiopyridine **8** exhibited the highest cytotoxicity followed by compound **3** with IC_{50} values of 8.02 and 15.84 μg/mL, respectively. It is notable that β-thio derivative of 4-phenylpyridine (**8**) exerted the strongest effect as antioxidant and cytotoxic compounds when compared to the β-thio derivative of 3-picoline (**3**). This is presumably due to the hydrophobic effect of the phenyl group that enhances its ability to absorb to cells. Moreover, both compounds **3** and **8** displayed cytotoxic effect toward all the tested cells.

QSAR modeling

The principle of QSAR has been described previously and is essentially the correlation of calculated physiochemical descriptors with their respective bioactivity (Nantasenamat *et al.*, 2009, 2010). Successful utilization of quantum chemical descriptors (Isarankura-Na-Ayudhya *et al.*, 2008; Piacham *et al.*, 2006, 2009; Prachayasittikul *et al.*, 2007; Suvannang *et al.*, 2011; Worachartcheewan *et al.*, 2011), and QSAR modeling (Nantasenamat *et al.*, 2005, 2007a, 2007b, 2008a, 2008b; Prachayasittikul *et al.*, 2011; Suksrichavalit *et al.*, 2008; Thippakorn *et al.*, 2009; Worachartcheewan *et al.*, 2009) for elucidation and modeling of biological and chemical properties has previously been demonstrated. The antioxidant and cytotoxic activities of 1-adamantylthio

Table 2 Cytotoxic activity of thiopyridines **1–8**

Compound	IC_{50} (μg/mL)[a]			
	MOLT-3	HuCCA-1	A549	HepG2
1	33.30 ± 0.900	Inactive	Inactive	30.00 ± 7.070
2	27.24 ± 4.880	Inactive	Inactive	Inactive
3	15.84 ± 0.330	34.00 ± 4.240	29.00 ± 1.414	26.50 ± 0.700
4	32.47 ± 0.710	Inactive	44.00 ± 5.656	Inactive
5	32.24 ± 3.370	Inactive	44.00 ± 5.656	48.50 ± 2.120
6	28.56 ± 0.440	49.00 ± 02.121	38.00 ± 8.485	Inactive
7	31.08 ± 1.180	Inactive	Inactive	46.50 ± 2.120
8	8.02 ± 0.610	29.00 ± 0.707	24.00 ± 0.707	21.50 ± 2.120
Doxorubicin	–	0.45 ± 0.070	0.39 ± 0.021	0.23 ± 0.001
Etoposide	0.019 ± 0.003	–	–	12.00 ± 0.005

[a] The assay was performed in triplicate, using doxorubicin and/or etoposide as reference drugs

MOLT-3 acute lymphoblastic leukemia cell line, HuCCA-1 human cholangiocarcinoma cancer cell, A549 human lung carcinoma cell line, HepG2 human hepatocellular carcinoma cell line

Table 3 Descriptors used for QSAR modeling of antioxidant and cytotoxic activities of 1-adamantylthio derivatives

Model	Activity	Symbol	Definition	Type
1	%SOD activity	μ	Dipole moment	Quantum chemistry
		χ	Mulliken electronegativity	Quantum chemistry
		ω_i	Electrophilic index	Quantum chemistry
2	%DPPH activity	MATS4p	Moran autocorrelation –lag 4/weighted by atomic polarizabilities	2D autocorrelation
		F03[C-S]	Frequency of C-S at topological distance 03	2D frequency fingerprints
3	MOLT-3 pIC_{50}	GATS4m	Geary autocorrelation -lag 4/weighted by atomic masses	2D autocorrelation
4	A549 pIC_{50}	ω_i	Electrophilic index	Quantum chemistry
		E_{HOMO}	Highest occupied molecular orbital energy	Quantum chemistry
5	HepG2 pIC_{50}	E_{LUMO}	Lowest unoccupied molecular orbital energy	Quantum chemistry
		PJI2	2D Petitjean shape index	Topological descriptor

SOD superoxide dismutase, MOLT-3 acute lymphoblastic leukemia cell line, A549 human lung carcinoma cell line, HepG2 human hepatocellular carcinoma cell line

derivatives **1–10** were used in QSAR studies as a function of the calculated molecular properties of the compounds. The multi-collinear and redundant descriptors obtained from the Dragon software package were removed from a volume of 3,224 descriptors to nine descriptors using UFS. The selected descriptors are composed of MW, F03[C-S], PJI2, GATS4m, Mor22m, MATS4p, G1v, R5m+, and RDF115m. An additional set of 13 quantum chemical descriptors was derived from Gaussian 03W which were combined with the nine descriptors obtained from the Dragon software. Selection of the most significant descriptors that are responsible for their corresponding antioxidant and cytotoxic activities was performed using stepwise multiple linear regression. Descriptors obtained from stepwise multiple linear regression were further used for construction of the QSAR model. MLR models were calculated using WEKA software.

QSAR studies of antioxidant activity

The important descriptors used for predicting the antioxidative (SOD) activity were found to be dipole moment, elcetrophilic index, and elctronegativity (Table 3), which were used in MLR analysis to derive the QSAR equation as shown in Table 4. We observed that compounds **3**, **6–8**, and **10** displayed higher SOD activity (Table 1) that correspondingly had greater dipole moment and elcetrophilic index, but lower elcetronegativity (Table 5). The *r* values for the training and testing sets were 0.8591 and 0.7059, respectively, while the RMS values were 4.0273 and 5.8706, respectively (Table 4). A plot of the experimental versus predicted values of the SOD activity is presented in Fig. 2a.

The QSAR equation of DPPH activity of the compounds was shown in Table 4. MATS4p and F03[C-S] were

Table 4 QSAR models of antioxidant and cytotoxic activities of 1-adamantylthio derivatives

Model	Equation	N	r_{Tr}	RMS_{Tr}	r_{CV}	RMS_{CV}
1	%SOD activity = 19.4005 (μ) − 19.0893 (χ) − 0.5242 (ω_i) + 1.3755	10	0.8591	4.0273	0.7059	5.8706
2	%DPPH activity = 3.8951 (F03[C-S]) − 57.5083 (MATS4p) − 10.8768	10	0.9235	2.6724	0.8361	3.8572
3	MOLT-3 pIC_{50} = 0.8597 (GATS4m) − 3.0758	8	0.7838	0.1270	0.5222	0.1914
4	A549 pIC_{50} = 7.9384 (E_{HOMO}) + 0.0089 (ω_i) − 0.073	5	0.9960	0.0093	0.9735	0.0277
5	HepG2 pIC_{50} = 1.9838 (PJI2) − 5.666 (E_{LUMO}) − 3.5625	5	0.9988	0.0069	0.9887	0.0239

SOD Superoxide dismutase, *MOLT-3* acute lymphoblastic leukemia cell line, *A549* human lung carcinoma cell line, *HepG2* human hepatocellular carcinoma cell line, pIC_{50} concentration of the compounds that inhibit 50% cell growth, N number of the data set, r_{Tr} correlation coefficient of training set, RMS_{Tr} root mean square error of training set, r_{CV} correlation coefficient of leave-one-out cross validation (LOO-CV) testing set, RMS_{CV} root mean square error of LOO-CV testing set

Table 5 Important set of descriptors accounting for antioxidant and cytotoxic activities of 1-adamantylthio derivatives (**1–10**)

Compound	μ	E_{HOMO}	E_{LUMO}	χ	ω_i	MATS4p	F03[C-S]	GATS4m	PJI2
1	0.8874	−0.2096	−0.0168	−0.1132	4.0860	−0.029	6	1.817	1
2	1.5048	−0.2065	−0.0210	−0.1137	12.2065	−0.03	5	1.912	0.8
3	2.8476	−0.2233	−0.0295	−0.1264	41.8498	−0.063	4	2.259	1
4	1.1578	−0.2054	−0.0333	−0.1194	7.7891	−0.007	5	1.662	1
5	1.1511	−0.2079	−0.0402	−0.1240	7.9021	−0.017	5	2.001	0.833
6	2.4716	−0.2265	−0.0460	−0.1362	33.8476	−0.376	5	1.914	0.833
7	3.8489	−0.2102	−0.0422	−0.1262	88.1945	−0.024	5	1.997	0.833
8	2.9576	−0.2192	−0.0425	−0.1309	49.4874	−0.044	5	2.231	1
9	0.9468	−0.2068	−0.0273	−0.1170	4.99292	−0.026	6	2.242	1
10	2.3882	−0.2233	−0.0488	−0.1361	32.6979	−0.020	4	1.654	1

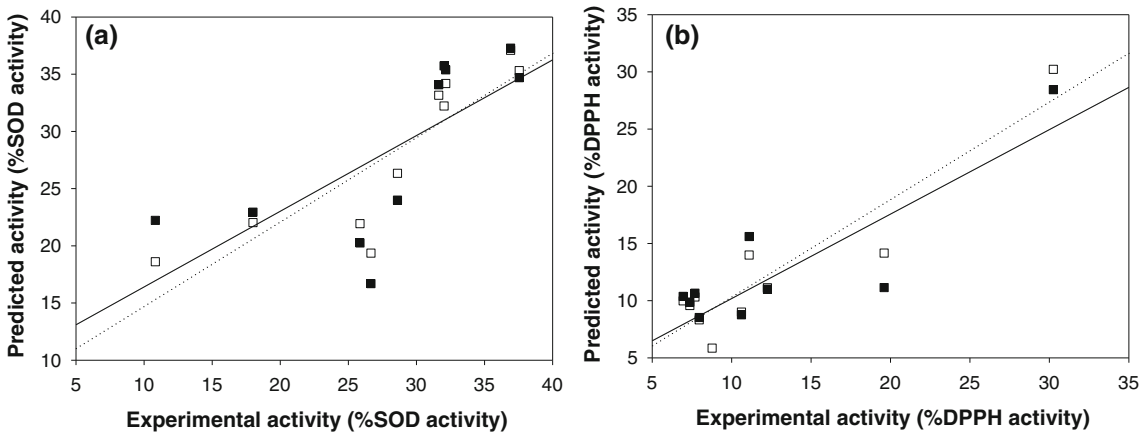

Fig. 2 Plot of the experimental and predicted SOD (**a**) and DPPH (**b**) activities for the training set (*white square* regression line is represented as *dotted line*) and the leave-one-out cross-validated testing set (*black square*; regression line is represented as *solid line*)

essential descriptors in predicting the compound's activity (Table 3). We found that compound **6** displayed the highest radical scavenging activity (Table 1) with correspondingly the lowest MATS4p (Table 5). The r values for the training and testing sets were 0.9235 and 0.8361, respectively, while the RMS values were 2.6724 and 3.8572, respectively. Figure 2b presents a plot of the experimental against the predicted DPPH activity of the compounds.

Dipole moment is a descriptor that accounts for the asymmetric charge distribution in a molecule, which in the case of the investigated compounds is originated from an inductive effect of the N-atom of the pyridine ring. This causes the positive charge to be localized at the C-2, C-4, and C-6 positions while leaving negative charges at the N-1, C-3, and C-5 positions. The experimental results revealed that all tested thiopyridine compounds displayed

interesting SOD activity. The β-thiopyridine **8** is shown to be the strongest antioxidant (SOD), although having a lower μ than the α-thiopyridine **7**. This may be due to an isometric effect of 1-AdmS group on the pyridine ring where the 1-AdmS moiety of compound **7** deposits at the C-2 position contributing a lone pair electron of S atom to the positive charge at C-2 leading to thionium ion (**7a**). The ionic form **7a** can be written in many resonant forms, and thus, **7** gives higher degree of charge localization (at C-2, C-4, C-6, and S atom) than compound **8**. This can be seen that compound **8** contributes positive charge at C-2 or C-4 (**8a**) in such a way that the thiol group at C-3 could not donate its electron pair to positive charge in the ring. Similarly, thiopyridine **3** provides charge distribution (**3a**) as described for compound **8**. It was found that thiopyridines bearing electron-withdrawing substituent such as CN had higher μ than COOH and $CONH_2$ groups, which thereby contributes to higher SOD activity (Prachayasittikul *et al.*, 2010). On the other hand, compounds with lower μ exhibited better radical scavenging (DPPH) activity (Prachayasittikul *et al.*, 2010). Such notion has been observed for γ-(or 4-) thiopyridine **6**, which possessed the lowest MATS4p (atomic polarizability), and thus providing the highest DPPH activity (Table 5). It may be reasonable to assume that participation of the electron pair of S atoms to the positive charge at the C-4 position or at the position that is *para* to the N-ring atom of compound **6**, provided the molecule with a more symmetrical thionium (**6a**), which correspondingly yielded the highest DPPH activity.

Electrophilic index is a descriptor that accounts for the positively charge property in a molecule that also has high capability to scavenge electrons or superoxide anions. This high value of ω_i is observed for compounds **3**, **6–8**, and **10** that also display high SOD activity. Conversely, the higher SOD activity of compounds **3**, **6–8,** and **10** also possessed lower χ value (Table 5).

QSAR studies of cytotoxic activity

The important descriptors for predicting the cytotoxicity of MOLT-3, A549, and HepG2 cells were obtained from stepwise MLR as displayed in Table 3. The QSAR equation and model evaluations are presented in Table 4. We found that the r values obtained from LOO-CV for the testing set of MOLT-3, A549, and HepG2 cells were

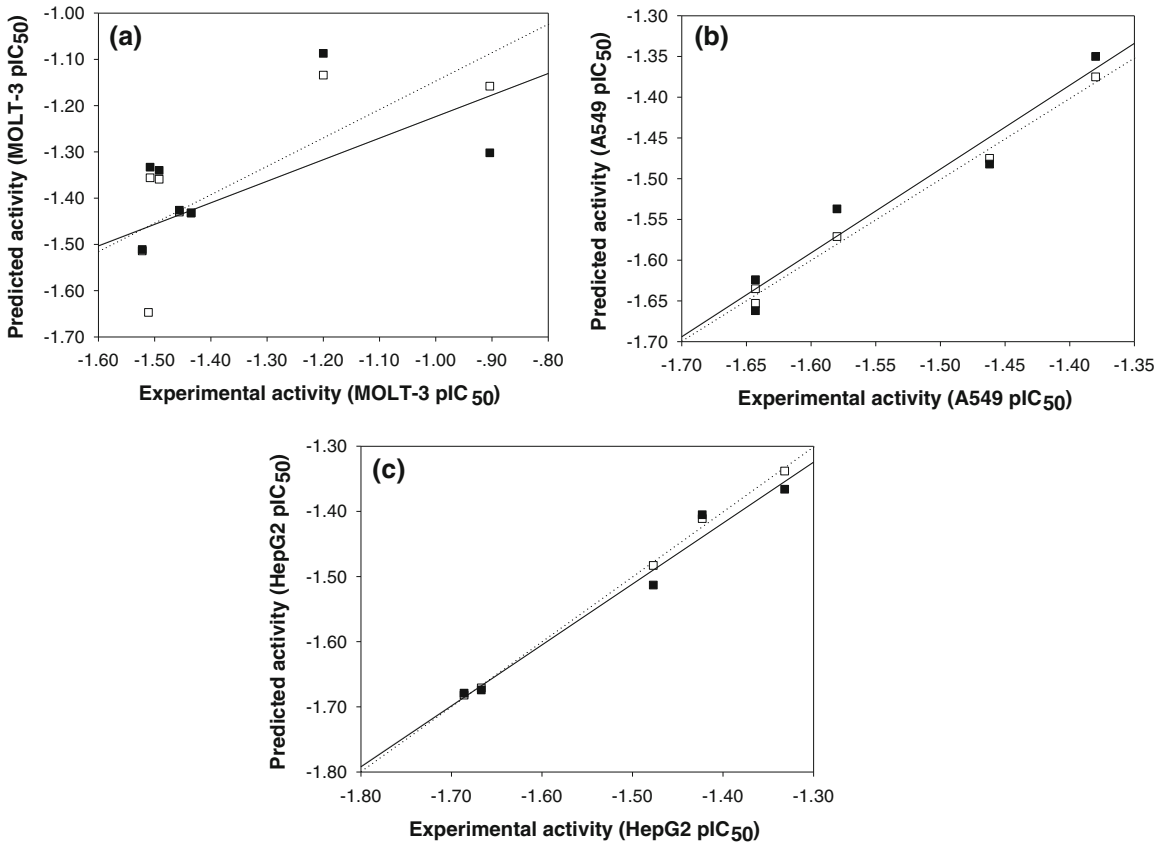

Fig. 3 Plot of the experimental and predicted anticancer activities (pIC_{50}) against MOLT-3 (**a**), A549 (**b**), and HepG2 (**c**) cell lines for the training set (*white square*; regression line is represented as *dotted line*); and the leave-one-out cross-validated testing set (*black square*; regression line is represented as *solid line*)

0.5222, 0.9735, and 0.9887, respectively, while the RMS values were 0.1914, 0.0277, and 0.0239, respectively. In Table 5, it is seen that the β-thiopyridines **3** and **8** showed high GATS4m where compound **8** had the highest cytotoxicity against MOLT-3. This could be due to the hydrophobic effect of phenyl group of thiopyridine **8** that enhances its absorption to cells as compared to compound **3** with CH_3 group. It was observed that compound **8** exhibited high ω_i value with the best inhibition of A549 cell growth. Molecules with high ω_i tends to be positively charged, for example, compound **8** (containing phenyl group that can delocalize positive charge at C-4 of pyridine ring to the *ortho*- and *para*- positions on the phenyl ring) as compared to compound **3**. Furthermore, compound **8** was also found to have low LUMO with correspondingly high cytotoxic activity against HepG2 cells. Although, compound **6** had lower LUMO than compound **8**, it was inactive against HepG2 cells, possibly because of the position of 1-adamantylthio and phenyl groups in the core structure. Plots of the experimental versus predicted activities of the compounds as anticancer against MOLT-3, A549, and HepG2 cell lines are shown in Fig. 3a–c, respectively.

Conclusions

Thiopyridines **1–10** were evaluated for their antioxidant and cytotoxic effects. The tested compounds exhibited both antioxidant (e.g., SOD and DPPH activities) and cytotoxic activities (e.g., MOLT-3, A549, and HepG2). Interestingly, β-(1-adamantylthio)-4-phenylpyridine (**8**) is the most potent antioxidant and cytotoxic compound. QSAR studies suggested that dipole moment and elcetrophilic index were the most significant descriptors for correlating the molecular structure of compounds with their respective SOD activities. Results indicated that molecules with high μ and ω_i values also had high SOD activity. Inversely, compounds with the lowest atomic polarizability (MATS4p) also had the highest DPPH activity. Other descriptors: atomic masses (GATS4m), ω_i, and LUMO energy were well correlated with cytotoxicity. The studies show that thiopyridine **8** is a potential lead compound that should be further developed and that QSAR models were useful in the design and modeling of related new molecules with interesting bioactivities.

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