Correlation between lipophilicity and antimicrobial activity of some 2-(4-substituted phenyl)-3(*2H*)-isothiazolones

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ABSTRACT

Background and purpose of the study. 3(*2H*)-Isothiazolones have shown antimicrobial activity and have been used as preservative in different products. However, reports on their structure-antimicrobial activity relationship are scanty. The aim of this study was to determine the relationship between lipophilicity and antimicrobial activity of several 2- (4-substituted phenyl) -3(*2H*)-isothiazolones of which some have shown antibacterial activities similar or higher than gentamycin, ceftazidime, ceftriaxone, ciprofloxacin , and antifungal activites similar or higher than itraconazole and ketoconazole as reference drugs.

Methods. Partition coefficient (log $P_{o/w}$) of the tested compounds was determined experimentally by a reversed- phase high performance liquid chromatography method using octadecyl-poly(vinyl alcohol) (ODP) column and methanol-water gradient as mobile phase and theoretically by Clog *P* and ALOGPS computer programs.

Results. The HPLC and theoretical log $P_{o/w}$ values showed potential correlations which indicate that both experimental and theoretical methods are equally suitable to predict lipophilicity of 3(*2H*)-isothiazolones. There were also significant correlations between MICs (minimum inhibitory concentrations) of 5-chloro substituted 3(*2H*)- isothiazolnes against *Salmonella typhimurium* and *Escherichia coli* and experimentally determined log $P_{o/w}$ values, as well as MICs of 5-unsubstituted 3(*2H*)- isothiazolnes against *E. coli* and ALOGPS values. The antifungal activity of the tested compounds against *Tricophyton. mentagrophytes* and *M.canis* increased with increase in the experimental and theoretical log P values , but this increase was only significant for the activity against *Microsporum. canis.*

Keyword: 3*(2H)*-isothiazolones, Log $P_{o/w}$, Clog P, ALOGP, Reversed-phase column chromatography

INTRODUCTION

3(*2H*)-Isothiazolones have shown antimicrobial effects and have been used as preservatives for the control of living organisms in different products such as cosmetics, paints, soaps, fabrics, leather, swimming pools , etc (1). Some of these compounds have also demonstrated inhibition of telomerase activity, a very selective target for design of anti-tumour agents, (2) and inhibition of cartilage breakdown in arthritis by blockade of a metalloproteinase enzyme (3).

While there are many reports on the use of 3(*2H*)-isothiazolones as biocidal agents (4-7), reports on their structure-antimicrobial activity relationship are scanty. Based on the results of theoretical studies on a few number of 3(*2H*)-isothiazolone, it has been concluded that structural characteristics do not likely play an important role in their mechanism of actions but determine the reactivity of these compounds toward interaction with intracellular sulphur containing proteins, enzymes, or simple molecules such as glutathione. This interaction leads to ring opening and disulfide bond formation and as a result impairment of the cell functions (8). Previously, synthesis, antibacterial (9), and antifungal (10) activities of several known, and novel 2-(4-substituted phenyl)-3(*2H*)-isothiazolones substituted at the 4-position of the phenyl moiety with groups different in hydrophobicity, size, steric and electronic parameters with or without chlorine-substituent at the C_5 position of the isothiazolone ring was described. Most of these compounds exhibited moderate to high activities against tested microorganisms, and in comparison with the reference drugs some of compounds exhibited

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comparable or higher activities. Xia *et al* studied both density functional theory (DFT) and stepwise multiple linear regression of a number of these compounds against *Escherichia coli* and concluded that S (1) and N (2) atoms are the active sites (11). In continuation of our studies on development of novel 3(*2H*) – isothiazolones, investigation of the relationship between their antimicrobial activities and other physicochemical properties appeared of interest.

Since lipophilicity as a physicochemical parameter plays an important role on biological activity and drug design (12, 13) and there are many reports on the relationship between antimicrobial activity and lipophilicity of different classes of compounds (14-18), the aim of this study was to assess contribution of lipophilicity parameters of 3 (*2H*)-isothiazolones to their antibacterial and antifungal activities.

Lipophilicity is usually expressed quantitatively as log *P* where *P* is non-aqueous/ aqueous phases partition coefficient (12) and can be characterized by different techniques including solvent/water partitioning (19, 20), chromatographic methods (21), immobilized artificial membrane (22), electrokinetic and calculation methods (23). In this study the lipophilicity of the tested compounds was determined experimentally by chromatography and theoretically by Clog *P* and ALOGPS methods (23) and correlation of each method with others as well as with antimicrobial activity of the tested compounds was investigated. Experimentally, a reported reversed-phase high-performance liquid chromatography method (24) was employed which is simple, quick, versatile, and has been demonstrated to directly estimate log $P_{o/w}$ values with fair accuracy and good precision.

MATERIALS AND METHODS

Chemicals

3(*2H*)-isothiazolone (Table 1) were synthesized as reported previously (9) by addition of dichloromethane solution of sulfuryl chloride as an oxidizing agent to the solution of the corresponding dithiodipropionamides in the same solvent at 0-10 °C. HPLC-grade methanol and toluene were supplied by Merck (Germany).Triphenylene was purchased from Sigma-Aldrich (USA). All other reagents were of analytical grade.

Chromatography

A Breeze® high-performance liquid chromatography system (Waters Corporation, USA) consisting of a model 1525 binary HPLC pump and dual wavelength UV-visible detector model 2487 was employed to measure the log $P_{o/w}$. Samples were injected with a 10 µL Rheodyne® 7725i fixed-loop injector system (Rheodyne Inc, USA). The chromatographic method employed a methanol- buffer (different pHs) gradient elution from 10 up to 100 percent of methanol within 9.7 minutes at a flow rate of 1.5 mL/minute. A six minutes time was considered for reconditioning of the stationary phase. A short octadecyl–poly(vinyl alcohol) (ODP) column (20×4 mm I.D , 5 µm, 25 nm pore size ODP-50 cartridge column, Supelco, USA) was used as stationary phase. Chromatography experiments were carried out at ambient temperature and the peaks of tested compounds as well as triphenylene and toluene as internal standards were detected simultaneously at 260 and 285 nm. A solution of triphenylene in methanol was prepared by adding 20 mg of triphenylene to 2 mL of toluene, followed by the addition of 200 ml methanol. About 1 mg of each tested compound was added to 1 mL of the above solution separately, and then 2 µL was injected. The log $P_{o/w}$ values were determined from the following formula:

$$\log p_{test} = \frac{(\log p_{toluene} - \log p_{triphenylene}).t_{test} + t_{toluene}.\log p_{triphenylene} - t_{triphenylene}.\log p_{toluene}}{t_{toluene} - t_{triphenylene}}$$

where log $P_{toluene}$, log $P_{triphenylene}$, log P_{test}, $t_{toluene}$, $t_{tripheylene}$ and t_{test} are logarithm of octanol-water partition coefficients of toluene , triphenylene , and tested compounds and their corresponding retention times under chromatography condition, respectively. The chromatography and hence measurement of the log $P_{o/w}$ was performed under the pH conditions that suppress ionization. However, because pKa values of the tested compounds were not known exactly, three different measurements were performed at pH 2, 7 and 10 for each compound. Buffers of pH 7 and 10 were made from 0.01 *M* sodium phosphate adjusted to the required pH and trifluoroacetic acid 0.026 *M* was used as pH 2 buffer. No significant difference was found between the measured retention times of the tested compounds under different pH conditions due to negligible ionization of all compounds except for compound **1a** which showed considerable ionization in neutral to basic conditions. Therefore log $P_{o/w}$ for this compound was calculated by using the values of retention times at pH 2.

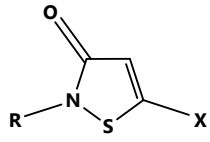
Theoretical calculation of log $P_{o/w}$

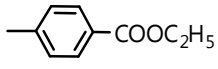
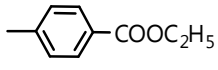
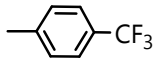
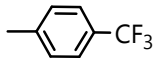
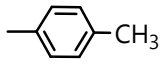
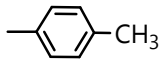
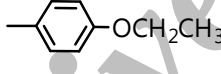
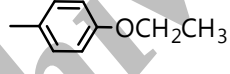
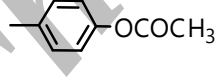
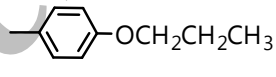
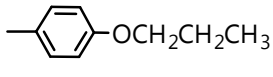
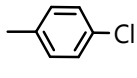
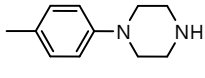
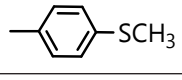
Two theoretical methods for determination of log $P_{o/w}$ (Clog *P* and ALOGPS) were utilized for calculation of log *P* values of the tested compounds using ALOGPS software (VCCLAB, Virtual Computational Chemistry Laboratory, http://www.vcclab.org, 2005) and Chem Bio Draw Ultra 11.0 (Cambridge Soft, USA).

Assessment of in vitro antimicrobial activity

In vitro antibacterial activities of the synthesized compounds as their minimum inhibitory concentration (MIC) against Gram-positive (*Staphylococcus aureus* ATCC 29737, *Staphylococcus epidermidis*

Table 1. Log *P* values of 3 (*2H*)-isothiazolones **1a-9a** and **2b-7b, 10b** and **11b** by different methods.



Compound	X	R	HPLC Log *P*	ALOGPS	C log*P*
1a	Cl	H	2.3	0.3	-0.27
2a	Cl		3.31	2.85	2.97
2b	H		2.28	2.15	2.26
3a	Cl		3.43	3.23	3.36
3b	H		2.48	2.57	2.64
4a	Cl		2.75	2.71	2.97
4b	H		1.44	2.39	2.26
5a	Cl		2.85	3.06	2.95
5b	H		1.63	2.56	2.21
6a	Cl		2.55	2.58	1.85
7a	Cl		3.31	3.36	3.48
7b	H		3.26	2.53	2.74
8a	Cl		3.26	3.03	3.18
9a	Cl		2.24	1.77	1.95
10b	H		1.60	1.50	1.76
11b	H		2.31	2.30	2.32

ATCC 12229, *Bacillus subtilis* ATCC 12711) and Gram-negative (*Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 19430 and *Pseudomonas aeruginosa* ATCC 9027) bacteria were determined by the conventional agar dilution method using Mueller Hinton agar medium (9).

Table 2. Antimicrobial activity (presented as MIC in μM) of compounds **1a-9a** and **2b-7b,10b** and **11b** against various bacterial andfungal species.

Compound	*Pseudomonas aeruginosa ATCC 9027*	*Salmonella typhimurium ATCC 19430*	*Escherichia coli ATCC 8739*	*Bacillus subtilis ATCC 12711*	*Staphylococcus Epidermidis ATCC 12229*	*Staphylococcus aureus ATCC 29737*	*Tricophyton mentagrophytes*	*Microsporum canis*	*Candidia albicans*	*A .niger*
1a	0.7	7.4	7.4	9.2	18.4	9.2	110.6	184.4	4.4	184.4
2a	37.1	185.4	185.4	18.5	9.3	46.3	74.2	18.5	370.8	nd
2b	80.2	200.6	200.6	2.0	5.0	80.2	100.3	80.2	401.1	nd
3a	0.4	44.7	89.4	35.8	17.9	17.9	71.5	71.5	nd	nd
3b	81.6	81.6	81.6	0.8	0.8	81.6	101.9	101.9	nd	407.8
4a	0.04	22.2	22.2	4.4	2.2	22.2	22.2	88.6	22.2	221.5
4b	104.6	104.6	104.6	1.0	10.5	104.6	78.4	104.6	5.2	261.4
5a	7.8	39.1	19.6	39.1	2.0	39.1	19.6	78.2	391.1	195.5
5b	11.3	90.4	22.6	2.3	4.5	45.2	67.8	113.0	451.9	226.0
6a	0.04	37.1	4.6	37.1	9.3	18.5	55.6	92.7	370.8	370.8
7a	37.1	37.1	37.1	0.9	3.7	9.3	9.3	74.1	185.4	nd
7b	85.0	85.0	85.0	4.2	8.5	85.0	21.2	85.0	212.5	212.5
8a	2.4	1.2	1.2	1.2	0.6	1.2	81.3	5.1	5.1	2.4
9a	1.0	1.0	1.0	42.3	1.0	42.3	0.2	16.9	4.2	2.0
10b	nd*	nd	nd	nd	nd	nd	22.4	112.0	53.7	56.0
11b	89.6	89.6	89.6	0.6	9.0	89.6	110.6	184.4	4.4	184.4

*Not determined

In vitro antifungal activity of the studied compounds against clinical isolates of *Aspergillus niger*, *Candidia albicans*, *Tricophyton mentagrophytes* and *Microsporum canis* were investigated by broth macro dilution method as reported elsewhere (10).

Statistical calculations

Spearman's rank correlation test was used to evaluate the correlation between log $P_{o/w}$ derived by the HPLC method and those obtained by theoretical calculations as well as correlation between MIC values and the lipophilicity of the compounds. All statistical calculations were performed by using Statistica 7.0 (Statsoft Inc., USA) statistical package.

RESULTS AND DISCUSSION

Log $P_{o/w}$ values have been found to correlate very highly with a number of pharmacological phenomena including antibacterial (14-16) and antifungal activities (17, 18) . In the previous paper correlation between lipophilicity measures and antimycobacterial activity of 2-hydroxyacetamides was described (16). In this study contribution of lipophilicity parameters of 3 (*2H*)-isothiazolones to their antibacterial and antifungal activities was investigated .The log $P_{o/w}$ values obtained experimentally by the HPLC method and theoretically by Clog *P* and ALOGPS programs are give in table 1 and the results of antimicrobial activity are presented in table 2. In general lipophilicity measures of the most active compounds were below 3 and with exception of the activity against *B. subtilis* ,compounds substituted at the C_5 of the isothiazolone ring with chlorine compared with un-substituted analogues showed higher antibacterial activities and as expected had higher log $P_{o/w}$ values. Similar findings of lower antibacterial activity of 5-chloro derivatives in comparison with corresponding unsubstituted analogues against *B. subtilis* for other 2-(4-substituted phenyl)-3(*2H*)-isothiazolones substituted at the 4-position of the phenyl ring with nitro,methoxy, and N,N-dimethyl has been reported previously (9).

A rather good and statistically significant correlation was observed between the values calculated by programs and experimental data (Figure 1) as proved by Spearman correlation coefficients (ρ) which were greater than 0.80 (P-value less than 0.0002). In agreement with results of similar investigations (14, 15), findings of this study indicate that both experimental and theoretical methods are equally suitable to predict lipophilicity of 3(*2H*)-isothiazolones.

Correlations between various lipophilicity measures and MICs against different bacterial species were not statistically significant when all isothizolones were included into the analysis (Figure 2).The best correlations were found between MIC against *S. aureus* and ALOGPS (ρ=-0.44, P-value=0.1086) and also HPLC log *P* values (ρ=-0.43, P-value=0.1045). However a significant correlation between MIC values

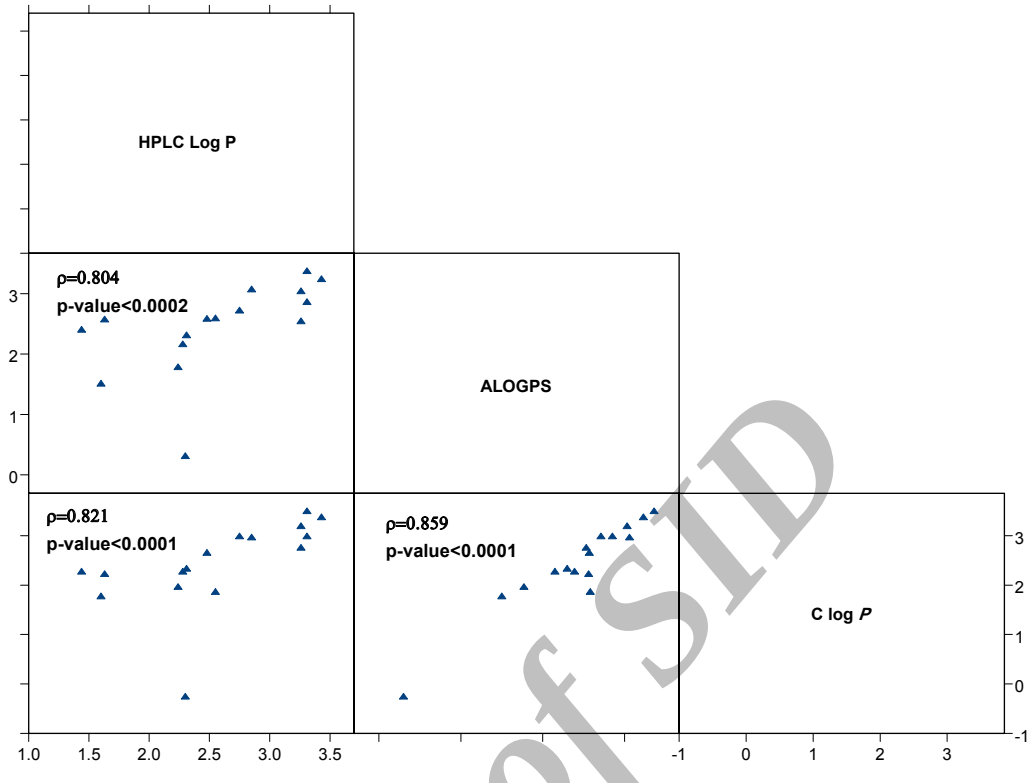


Figure 1. Correlation between log *P* values by calculation methods(estimated by ChemDraw and ALOGPS software) and HPLC method. Spearman correlation coefficient(ρ) and the level of significance are shown for each correlation.

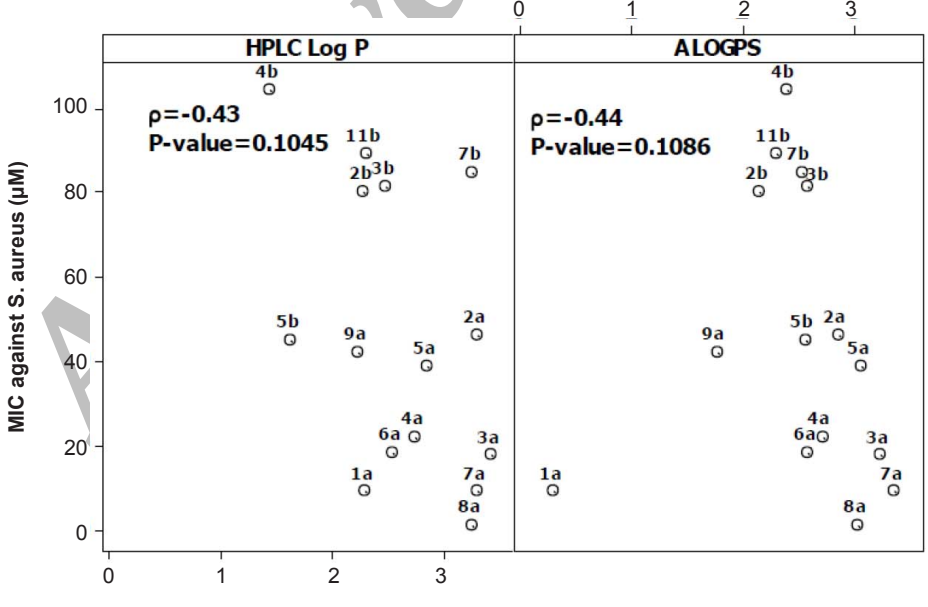


Figure 2. Correlation between MIC against *Staphylococcus aureus* and lipophilicity measures(HPLC Log *P* and ALOGPS)for all 3(*2H*)-isothiazolone derivatives(ρ is the Spearman correlation coefficient).

and lipophilicity parameters of some tested compounds were obtained when 5-chloro substituted and 5- unsubstituted compounds were analysed separately. For 5-chloro substituted 3*(2H)*-isothiazolnes, a significant correlation was obtained between MICs against *S. typhimurium* (ρ= 0.67, P-value = 0.049) and *E.coli* (ρ= 0.77, P-value= 0.015) and experimentally determined log $P_{o/w}$ values (Figure 3). In the case of 3(*2H*)-isothiazolnes without chlorine atom at the C_5 of

Figure 3. Correlation between MIC against *Salmonella typhimurium* and *Escherichia coli* with HPLC log *P* for 3(*2H*)-isothiazolone **1**a-**9**a.Spearman correlation coefficients(ρ) and levels of significance are also shown.

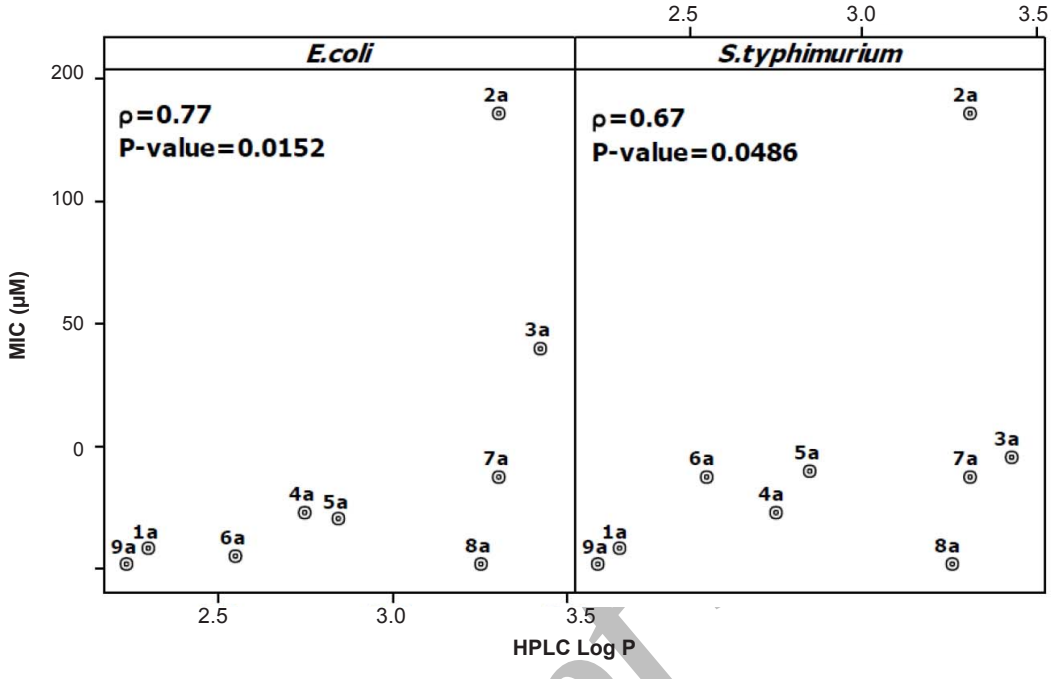
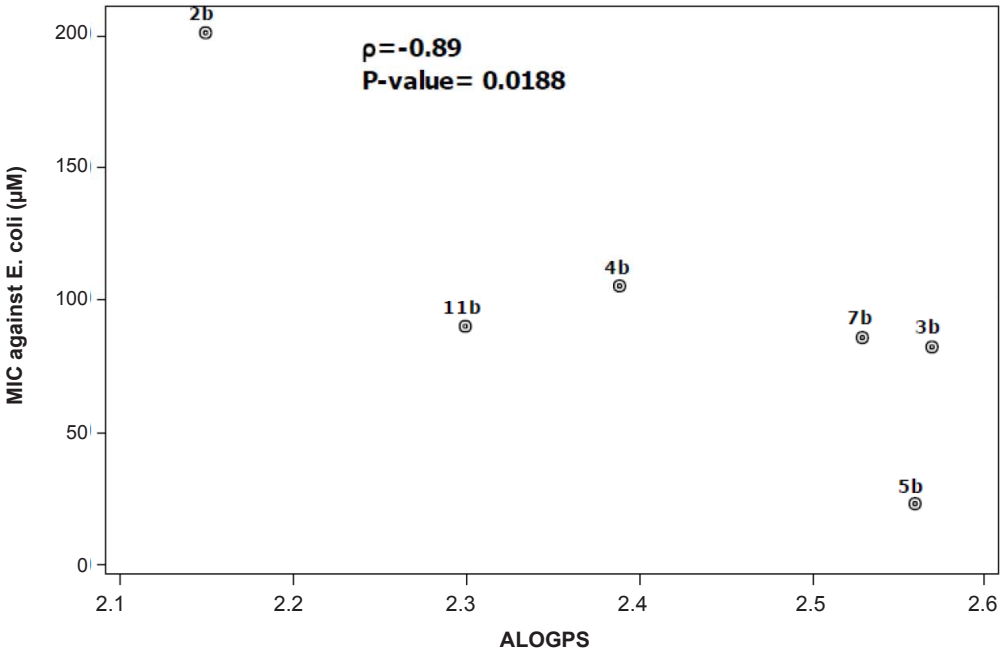


Figure 4. Correlation between MIC against *Escherichia coli* with log *P* calculated by ALOGPS software (ALOGPS) for 3(*2H*)-isothiazolone **2b-5b,7b** and **11b**(ρ is the Spearman correlation coefficient.).



isothiazolone ring, the only significant correlation as could be found from figure 4 was between MIC against *E. co*li and ALOGPS (ρ= -0.89, P-value=0.0189). Of 5-chloro substituted 3(*2H*)-isothiazolones compounds **2b** and **3b** with similar lipophilic parameter values and substituted at the 4-position of the phenyl rings with electron withdrawing groups and compounds **4b** and **5b** with similar lipophiolic parameter values but substituted with electron donating groups were highly active against *S. epidermidis* . From these results it may be concluded that electronic factor of these compounds either is not important for activity against this microorganism or its square

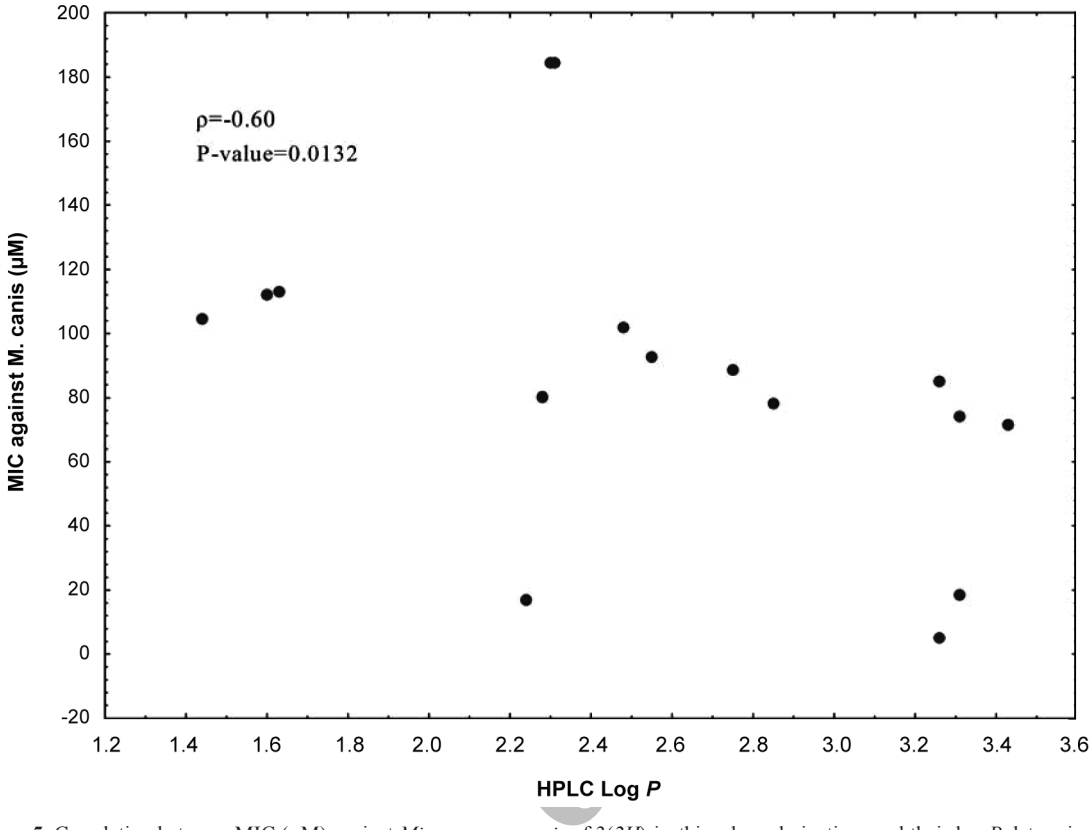


Figure 5. Correlation between MIC (μM) against *Microsporum canis* of 3(*2H*)-isothiazolone derivatives and their log *P* determined by HPLC(HPLC Log *P*).

term produces additive contribution to the activity.

The antifungal activity of the tested compounds against *T. mentagrophytes* and *M. canis* (Figure 5) increased by increase in log *P* values determined experimentally and theoretically but such an increase was only statistically significant for *M. canis* (ρ values better than -0.56 at P-levelst of less than 0.02). Similar direct relationship between antifungal activity against various fungal species and log *P* values has been reported previously (17).

As it was expected, in general 3(*2H*)-isothiazolones substituted at the 5- position with chlorine had log *P* values higher than the un-substituted analogues and consistent with our previous reports showed higher antifungal activities (10).

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