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Experimental and Theoretical Study on Lipophilicity and Antibacterial Activity of Biphenylamine Derivatives

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Abstract: The lipophilicity of the biphenylamine derivatives were determined by normal phase TLC. The lipophilicity determined by TLC were correlated with theoretically calculated various log P values and the best theoretically calculated log P value was chosen based on the correlation coefficient value and also antibacterial activity of compounds were correlated with lipophilicity of the compound determined by normal phase TLC.
Key words : Lipophilicity (RM0), Antibacterial activity and Theoretically calculated log P.

Introduction

The most popular scale to measure the lipophilicity of organic compounds is the logarithm of the partition coefficient of compound (called the log *P* parameter) between1 n-octanol and water, introduced by Hansch and Leo[1]. The lipophilicity of a substance is one of the parameters which influences its biological activity and is well-known prime physiochemical descriptor to QSAR study[2-4]. The hydrophobic interactions of drugs with their receptors, pharmacokinetic behavior of drug molecules and toxicological properties as well as pharmaceutical aspects like solubility are examples of a steadily increasing number of topics in which lipophilicity plays an important role. The determination of the partition coefficient by direct measurement using the shake-flask method faces problems such as poor reproducibility, length of time for experiment, it needs a reasonable quantity of compound and it needs very pure compound because impurity influence the partition coefficient value. The lipophilicity of the compounds also were determined in the reverse phase and normal phase TLC and this is the alternative to shake-flask partition coefficient method. The advantage of TLC method are purity of the compound is immaterial, requires very less quantity and short time. The nonpolar stationary phase and polar mobile phase is called as reverse phase TLC and vice versa is called as normal phase TLC. The water , very high polar , and water soluble organic solvents are used as mobile phase in reverse phase TLC and in the case of normal phase TLC polar solvent like methanol, and nonpolar like hexane are used as mobile phase[14]. The both method , reverse phase and normal phase TLC, are used in the lipophilicity determination and the solvent composition for mobile phase is chosen by trial and error method. The good linear correlations are obtained between lipophilicity determined in the TLC and log P (partition coefficient) determined in other method, theoretically calculated log P.

Experimental

The compounds were prepared by using standard procedure and characterized with help of IR,Mass, ^{1}H NMR. The merck F_{624} TLC plate were employed for R_f value determination. The plate developed for 60 mm height and product spots were indentified under UV light at short UV wave length. The theoretical log P values are determined via on-line at VCCLAB.org[37] website and linear regression analysis were carried out using labfit software. The best theoretical log P value was chosen base on correlation coefficient value of linear regression analysis. The nutrient broth was used as media to test anti bacterial activity. The media (2.8gm in 100ml water) , isotonic solution (0.9% NaCl), petri dishes, loop and test tube were autoclaved in pressure cooker and the loading

of media and culture were done under sterile air (laminar flow) after that 8 mm diameter well were made in petri plates and 25 µl of drug dissolved in DMSO in the concentration of 20µg / 1µl was loaded then incubated at 40° C for 24 hour and zone of inhibition was measured.

Result and Discussion

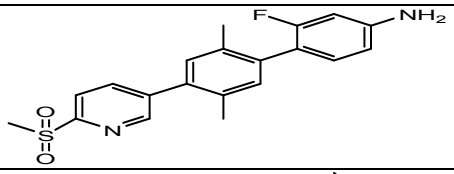
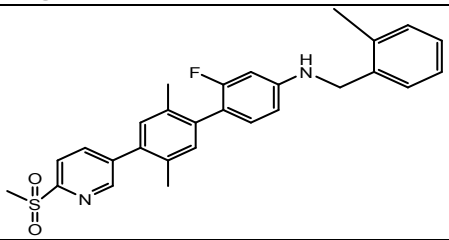
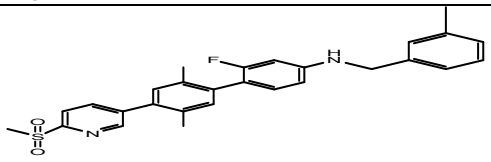
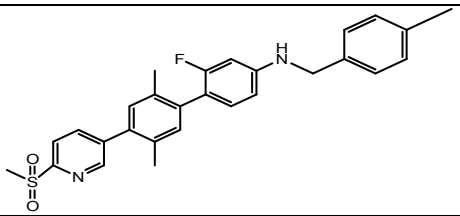
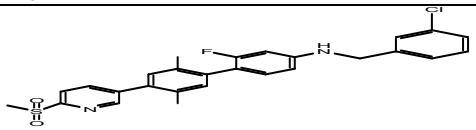
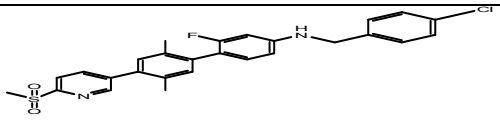
The lipophilicity (R_{M0}) is obtained from R_f values by the following equations 1 and 2 and the R_M value is calculated from the R_f value by the equation 1[5-9]. The lipophilicity value is obtained by the extrapolation to zero concentration of polar component in the graph drawn between R_M and concentration of polar component in mobile phase. The R_{M0} and b in the equation 2 are represents intercept and slope of the graph drawn between C and R_M. The C in the equation 2 is the concentration of polar component in the mobile phase. The b in the equation 2 is called as specific hydrophobic surface area of compound and the lipophilicity determined in TLC are being correlated with theoretically calculated log P , log P determined in the other and biological activity of the compound.

$R_M = \log(1/R_f - 1)$ — 1

$R_M = R_{M0} + bC$ — 2

The name and structure of the compound taken for the study is shown below in the table 1.

Table 1: Name and structure of the compound.

Compound No	Structure of compound	Name of compound
1		2-fluoro-2',5'-dimethyl-4'-{6-(methylulfonyl) pyridin-3-yl}biphenyl-4-amine.
2		N-(2-methylbenzyl)-2-fluoro-2',5'-dimethyl-4'-{6-(methylsulfonyl) pyridin-3-yl}biphenyl-4-amine.
3		N-(3-methylbenzyl)-2-fluoro-2',5'-dimethyl-4'-{6-(methylsulfonyl) pyridin-3-yl}biphenyl-4-amine.
4		N-(4-methylbenzyl)-2-fluoro-2',5'-dimethyl-4'-{6-(methylsulfonyl)pyridin-3-yl}biphenyl-4-amine.
5		N-(3-chlorobenzyl)-2-fluoro-2',5'-dimethyl-4'-{6-(methylsulfonyl) pyridin-3-yl}biphenyl-4-amine.
6		N-(e-chlorobenzyl)-2-fluoro-2',5'-dimethyl-4'-{6-(methylsulfonyl) pyridin-3-yl}biphenyl-4-amine.

R_M value of all above compounds are determined in NP-TLC method by using ethylacetate and petroleum ether as mobile phase. The R_f values are taken in the triplicate and average value is taken for R_M value calculation. The R_f values for each compound is determined in five different composition of mobile phase and the R_f value of compounds 1 to 6 are shown in the table 2 and 3. The % in the tabular column indicates ethylacetate concentration and remaining portion indicates petroleum ether and mobile phase has been chosen trial and error method. The R_f values are converted to R_M by using equation 1.

Table 2 : R_f value of the compounds 1 to 6.

% of Ethylacetate in mobile phase	R_f					
	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5	Compound 6
20	0.18	0.23	0.24	0.24	0.20	0.21
30	0.22	0.28	0.27	0.28	0.26	0.28
40	0.26	0.33	0.34	0.34	0.31	0.30
50	0.31	0.38	0.38	0.39	0.38	0.37
60	0.35	0.44	0.44	0.44	0.41	0.41

Table 3: RM value of the compound 1 to 6.

% of Ethylacetate in mobile phase	R_M					
	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5	Compound 6
20	0.65	0.52	0.50	0.50	0.54	0.57
30	0.54	0.41	0.43	0.41	0.45	0.41
40	0.45	0.30	0.28	0.28	0.34	0.36
50	0.34	0.21	0.21	0.19	0.21	0.23
60	0.26	0.10	0.8	0.10	0.15	0.15

The lipophilicity (RM0) and is determined by equation 2 via graphical method and these values are shown in the table 4.

Table 4: lipophilicity of the compounds.

Compound No.	R_{M0}
1	0.84
2	0.724
3	0.724
4	0.724
5	0.746
6	0.752

The theoretically calculated six type of log P namely A log P, AC log P, mi logP, log P kowwin, X log P2 and X log P3 values for each compound are shown in the table 5.

Table 5: Various type of calculated log P values for each compounds.

Compound No.	A log P	AC log P	mi log P	log P knowin	X log P2	X log P3
1	4.01	3.38	4.19	3.28	4.48	3.82
2	6.23	5.62	7.06	6.13	7.36	6.61
3	6.23	5.62	7.08	6.13	7.36	6.61
4	6.23	5.62	7.11	6.13	7.36	6.61
5	6.6	5.92	7.31	6.23	7.55	6.88
6	6.61	5.92	7.34	6.23	7.55	6.88

The lipophilicity values determined in TLC were correlated with above theoretically calculated various log P by linear regression analysis one by one. The equations 3 to 8 are generated by linear regression analysis.

R_{M0} = -0.0411 × A log P + 0.999 —(3)

R_{M0} = -0.0341 × mi log P + 0.9797 —(4)

R_{M0} = -0.0361× log P kowwin + 0.9574 —(5)

R_{M0} = -0.0369 × X log P2+ 0.1 —(6)

R_{M0} = -0.0349 × X log P3 + 0.9697 —(7)

R_{M0} = -0.0419 × AC log P + 0.9753 —(8)

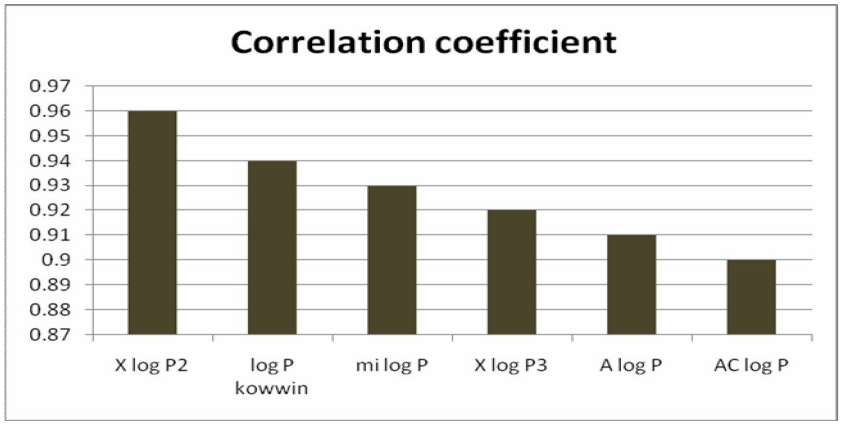
The correlation coefficient and standard deviation of the equations 3 to 8 are in the table 6.

Table 6 : Correlation and standard deviation.

Equation	Correlation coefficient	Standard deviation
Equation 3	0.91	0.02
Equation 4	0.93	0.01
Equation 5	0.94	0.01
Equation 6	0.96	0.01
Equation 7	0.92	0.01
Equation 8	0.9	0.02

The comparison of correlation coefficient of equation 3 to 8 is shown in the graph 7 and it reveals that x log P2 is having higher correlation coefficient than other log P hence theoretically calculated x log P2 can be used instead of lipophilicity wherever applicable in QSAR and QSPR study for above serious of the compound.

Graph 7 : Comparison of the correlation coefficient of the equation 3 to 8.



The compound 1 to 6 are shown the antibacterial activity against E.coli and psedomonas auregenoma. The activity of the compound are compared with gentamycin 10mg disc as standard by zone inhibition method. The DMSO solvent was used as drug control and the concentration of the drug used for anti bacterial activity is 25 µl (20 µg/µl). The inhibition zone are give in mm scale in table 7.

Table 7 : Inhibition diametre in mm scale.

Compound No	E.Coli	Psedumona auregenoma
1	16	15
2	14	13
3	14	13
4	14	13
5	15	14
6	15	14
Std,Gentamycin	23	20

The order of antibacterial avtivity of compounds for E.coli and Pseudomonas auregenoma are 1>5=5>2=3=4 and the order of activity of the compound is same order against E.Coli as well as pseudomonas auregenoma and order of antibacterial activity of compound shows that primary amine more active than secondary amine and chloro substituted is more active than methy substituted. The chloro and methyl sustitution in 2,3 and 4 does not makes any dofference in the biological activity of the compound. The lipophilicity of compound has been correlated with the inhibition zone of the compound for both of the bacteria E.Coli as well as pseudomonas auregenoma by the following equation.

Inhibition for E.Coli = $16.91 \times R_{M0} + 1.95$ —(9)

(r = 0.93, Std. deviation = 0.33)

Inhibition for Pseudomonas = $16.91 \times R_{M0} + 0.95$ —(10)

(r = 0.93, Std. deviation = 0.33)

Conclusions

1). The lipophilicity determined in TLC have good correlation with theoretically calculated .
2). xlog P2, so xlog P2 can be used as descriptor in QSAR and QSPR study in the place of lipophilicity.
3). The ethylacetate and petroleum ether can be used as mobile phase in normal phase TLC to determine the lipophilicity of the above serious of compound.
4). The liphibilicity of compound determined in the TLC have good correlation with its antibacterial activity.
5). It is understood that the compound having higher lipophilicity is exhibiting higher antibacterial activity.

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