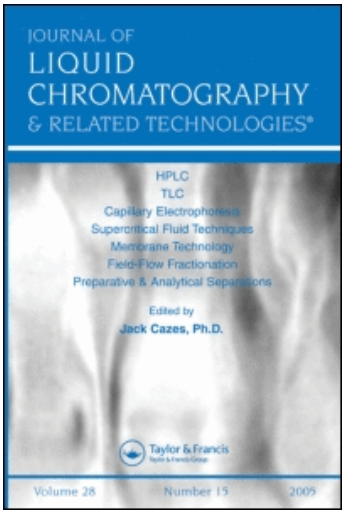

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K. Valkól[a]; T. Friedmann[b]; J. Báti[a]; A. Nagykáldi[c]
[a] Biological Research Center, Hungarian Academy of Sciences, Institute of Enzymology, Budapest [b] Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary [c] Department of Pharmacy, Semmelweis University of Medicine, Budapest, Hungary

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REVERSED-PHASE CHROMATOGRAPHIC SYSTEM AS A MODEL FOR CHARACTERIZING THE OFFSET RATE OF ACTION OF AZIDOMORPHINES IN GUINEA-PIG ILEUM

K. Valkó[1], T. Friedmann[2], J. Báti[1], A. Nagykáldi[3]

[1]Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, H-1502 Budapest, POB 7.

[2]Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary

[3]Department of Pharmacy, Semmelweis University of Medicine, Budapest, Hungary

ABSTRACT

The relationship between the offset rate of action and physicochemical properties of azidomorphines was investigated. The offset rate of action of 12 compounds was measured in isolated preparations of guinea-pig ileum. Physicochemical properties of azidomorphines were characterized by their hydrophobicity parameters, determined by reversed-

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phase high-performance liquid chromatography and by their activity in lowering surface tension.

The offset rate of action of azidomorphine derivatives in guinea-pig ileum proved to be inversely related to their hydrophobicity, as shown by correlation analysis.

A reversed-phase chromatographic system was developed which appears to be suitable for the estimation of the offset rate of action of azidomorphines.

INTRODUCTION

The investigation of quantitative relationships between biological activity and physicochemical properties of drugs /1/, /2/ can help in the understanding of the mechanism of pharmacological action.

The azidomorphine derivatives synthetized by Bognár and Makleit /3/ have advantageous pharmacological effects in certain tests /4/, /5/. The relationship between the chemical structure and pharmacological activity of the derivatives has already been studied /6/. It has been shown by Friedmann and knoll /7/ that 3-ethoxy-N-cyclopropylmethyl-azidomorphine exhibits an extremely slow offset of inhibitory action in the isolated myenteric

plexus-longitudinal muscle strip of the guinea-pig ileum.

Kosterlitz et al. /8/ demonstrated the role of hydrophobicity in the rate of offset of the action of different narcotic analgesics. Herz and Teschemacher /9/ and Wüster and Herz /10/, /11/ demonstrated the importance of surface tension-lowering activity of loperamid, an opiate-like antidiarrheal agent, in its extremely long lasting action on the guinea-pig ileum.

The question arose whether the offset rate of action of the investigated azidomorphines could be explained solely by their hydrophobic character and surface activity or other specific interactions at the receptor should also be invoked.

In the present study the rate of offset of the inhibitory action was measured in the isolated preparation of guinea-pig ileum. Hydrophobicity of the selected azidomorphine derivatives was caracterized by reversed-phase high-performance liquid chromatographic (RP-HPLC) retention data as they are often used in the literature (/12/, /13/, /14/) according to eq. 1

$$\log K = a \log k' + b \qquad (1)$$

where K is the partition coefficient of a compound, k' is the capacity ratio measured in a given RP-HPLC system, and a and b are constants.

The surface tension-lowering effects, $\Delta\sigma$, of the compounds were characterized by the difference of the surface tension of the pure buffer and the drug solution.

The relationship between the biological and physicochemical data was tested by correlation analysis.

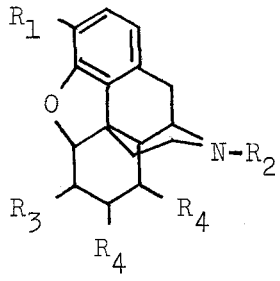
MATERIALS AND METHODS

The chemical structures of the compounds studied are shown in Table 1.

The rate of offset of inhibitory action was measured on myenteric plexus-longitudinal muscle prepared according to Paton and Vizi /15/. The muscle strip was suspended in a 5 ml organ bath (Krebs solution) and was field stimulated by 0.1 Hz supramaximal square wave pulses of 1 msec duration voltage. The Krebs solution had the following composition (mM): NaCl 113, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.5, $MgSO_4$ 1.2.

TABLE 1.

CHEMICAL STRUCTURE OF SOME AZIDOMORPHINE DERIVATIVES



Serial number	Name	R_1	R_2	R_3	R_4
1.	Azidomorphine	OH	CH_3	N_3	H
2.	Azidocodeine	OCH_3	CH_3	N_3	H
3.	N-cyclopropylmethyl-azidomorphine	OH	CH_2-cyclopropyl	N_3	H
4.	Azidoethylmorphine	OC_2H_5	CH_3	N_3	H
5.	N-phenylethyl-azido-ethylmorphine	OC_2H_5	CH_2CH_2-Ph	N_3	H
6.	N-phenylethyl-azido-morphine	OH	CH_2CH_2-Ph	N_3	H
7.	Acetylazidomorphine	$OCOCH_3$	CH_3	N_3	H
8.	Norazidoethylmorphine	OC_2H_5	H	N_3	H
9.	N-cyclopropylmethyl-azidoethylmorphine	OC_2H_5	CH_2-cyclopropyl	N_3	H
10.	Norazidomorphine	OH	H	N_3	H
11.	Normorphine	OH	H	OH	double bound
12.	Morphine	OH	CH_3	OH	"

All the investigated compounds exhibited opiate agonist activity. The offset rate of action was characterized by the $t_{1/2}$ value, which indicates the time needed for the 65-75 percent inhibition caused by the compound to decrease to one-half after washout with 100 ml Krebs solution. Pairs of compounds (one of them always azidomorphine) were tested on the preparation. The logarithm of the $t_{1/2}$ values relative to azidomorphine were used in correlation analysis ($\log t_{1/2}^{*}$).

The chromatographic retention data were measured on a LiChrosorb RP-18 (250 mm ID 4.6 mm dp 10 µm, Chromatronix) column. The parts of the HPLC equipment are listed in Table 2.
Detection was carried out at 280 nm. The temperature was 22 $^{o}C \pm 2\ ^{o}C$. The flow rate of the eluent was 1.51 ml/min. Pressure was $3 - 5 \times 10^{5}\ Nm^{-2}$.

The retention time values of compounds and the dead times were measured at least at three different concentrations of acetonitrile in the eluent. Sodium nitrate was used to determine the dead time. The eluent consisted of 5 to 80% acetonitrile and 0.05M phosphate buffer, pH 2. At this pH value compounds were in cationic form. Sodium-butylsulphonate (Aldrich Milwauke Wisc. USA) was applied as counter

TABLE 2.

HPLC EQUIPMENT

Injector: Rheodyne Model 7010 Sample Injection Valve
Detector: ISCO Model 226 Absorbance Monitor
Pump: Labormim Liquopump Model 312
Integrator: Chinoin Digint Model 24
Recorder: Endim Model 621.01
Calculations: Apple II+ microcomputer

ion in 0.005 M concentration in the eluent. Under such circumstances symmetric peaks were obtained after 20 µl injection of about 0.01% solutions of the drugs in the eluent. The logarithm of the capacity ratios (log k') was calculated at different concentrations of acetonitrile in the eluent (OP%). The slope and intercept ($\log k'_o$) of the log k' vs. OP% straight lines were also considered as characteristic of hydrophobic properties of drugs on the basis of an earlier study /16/, which showed good correlation between the 1-octanol/water partition coefficient and RP-HPLC retention data for different types of compounds.

The regression analysis yielded the slope and the intercept ($\log k'_o$) of the following equation:

$$\log k' = \text{'slope'}\ OP\% + \log k'_o \qquad (2)$$

To measure surface tension, compounds were dissolved at 10^{-3} M concentration in 0.067 M phosphate buffer, pH 7.4, prepared from KH_2PO_4 and NaOH. The surface tension of the buffer and the drug solutions was measured in a Traube stalagmometer. The mass of 60 drops of drug solution (m_x) was measured and compared to the mass of 60 drops of distilled water (m_w). The surface tension (σ_x) of the given solution was calculated according to eq. 3:

$$\sigma_x = \frac{m_x}{m_w}\,\sigma_w \qquad (3)$$

where the σ_w is the surface tension of distilled water (72.57 mN/m). Measurements were carried out at 24.6 °C.

The surface tension-lowering activity ($\Delta\sigma_{pH=7.4}$) of compounds was expressed as the difference in σ_x values of the drug and the buffer solutions.

Several compounds were insoluble in the buffer at 10^{-3} M concentration, therefore surface tension-lowering activity of the compounds was also measured in 0.05 M KH_2PO_4 (pH=4.6) solutions, in which all investigated compounds dissolved. The differ-

ences of the surface tension ($\Delta\sigma_{pH=4.6}$) for the drug and the KH_2PO_4 solutions were also used in the correlation analysis.

All other chemicals were reagent grade preparations obtained from REANAL (Hungary). Acetonitrile was purified by us for chromatographic purposes.

RESULTS AND DISCUSSION

The biological and physicochemical data obtained for the 12 compounds are listed in Table 3.

Table 4 shows the correlation coefficient for each pair of variables.

Since washing of the ileum preparation failed to abolish the inhibitory action of compounds 5 and 9 ($t_{1/2} > 120$ min), these two compounds were omitted from the correlation analysis.

Our finding that the action of drugs 5 and 9 was completely antagonized by naloxone excluded the possibility that the extremely long-lasting effect of the two compounds was due to covalent binding to opiate receptor sites. The fact that subsequent washing of the preparation (removal of naloxone) restored inhibition showed that the drugs were still present in the preparation near the receptor.

TABLE 3.

THE BIOLOGICAL AND PHYSICOCHEMICAL DATA OF AZIDOMORPHINE DERIVATIVES

Ser. num.	log $t^{*}_{1/2}$	'slope'	log k'_o	log k'_{40}	$\Delta\sigma^{*}$ pH=4.6	$\Delta\sigma^{*}$ pH=7.4
1.	0.000	-0.0311	0.975	-0.259	0.38	-0.19
2.	0.294	-0.0064	0.499	0.188	0.95	1.75
3.	0.468	-0.0056	0.358	0.078	1.17	-§
4.	0.642	-0.0088	0.862	0.501	1.65	3.02
5.	>1	-0.0250	2.164	>0.6	0.79	-§
6.	0.734	-0.0150	1.124	0.527	1.97	-§
7.	0.060	-0.0080	0.594	0.272	2.19	0.93
8.	0.641	-0.0106	0.880	0.454	0.86	1.12
9.	>1	-0.0194	1.644	>0.6	0.32	5.32
10.	0.009	-0.0271	0.851	-0.202	1.46	-§
11.	-0.523	-0.0277	0.344	-0.703	-0.30	-§
12.	-0.229	-0.0298	0.467	-0.653	0.91	0.38

*$\Delta\sigma$ values are given in mN/m.

§$\Delta\sigma_{pH=7.4}$ values could not be measured for these compounds because they were insoluble in the buffer (pH=7.4) at 10^{-3} M concentration.

TABLE 4.

CORRELATION COEFFICIENTS OF PAIRS OF VARIABLES

	'slope'	log k'_o	log k'_{40}	$\Delta\sigma_{pH=4.6}$
log $t^{*}_{1/2}$	0.711	0.537	0.925	0.582
'slope'		-0.086	0.802	0.530
log k'_o			0.524	0.382
log k'_{40}				0.693

It can be seen from Table 4 that the values of log $t^{*}_{1/2}$ show good correlation with the logarithm of the capacity ratios measured at 40% acetonitrile in the eluent (log k'_{40}) as described by eq. 4:

$$\log t^{*}_{1/2} = 0.843 \log k'_{40} + 0.192 \qquad (4)$$

n=10 R=0.925 s=0.168 F=47.3 $F_{(1,8\ p=0.95)}=5.3$

where n is the number of compounds considered in the calculations, R is the correlation coefficient, s is the standard error of the estimate, and F is the F-test value.

The offset rate of acetylazidomorphine (compound 7) was nearly the same as that of azidomor-

phine (number 1), although their hydrophobic characters differed markedly. Consequently, there were great differences between the measured and calculated (by eq. 4) log $t^{*}_{1/2}$ values for acetylazidomorphine. An explanation of this phenomenon may be that acetylazidomorphine was hydrolyzed to azidomorphine by the tissue's cholinesterases.

Omission of its data from the calculations in eq. 4 gave significantly better correlations (Fig. 1.).

$$\log t^{*}_{1/2} = 0.900 \log k'_{40} + 0.230 \qquad (5)$$

$n=9 \quad s=0.103 \quad R=0.976 \quad F=137.7 \quad F_{(1,7\ p=0.95)}=5.6$

The log k' values of compounds measured on reversed-phase chromatographic columns are usually taken to be proportional to the logarith of partition coefficients of the compound in two immiscible solvents /12/, /13/, /14/. Riley et al. /13/ underlined the relevance of liquid-liquid distribution phenomena of solute retention in ion-pair liquid chromatographic systems as well. Thus, the significant relationship between log $t^{*}_{1/2}$ and log k'_{40} suggests that the half-time of the offset of the investigated compounds in guinea-pig ileum depends on their hydrophobic character.

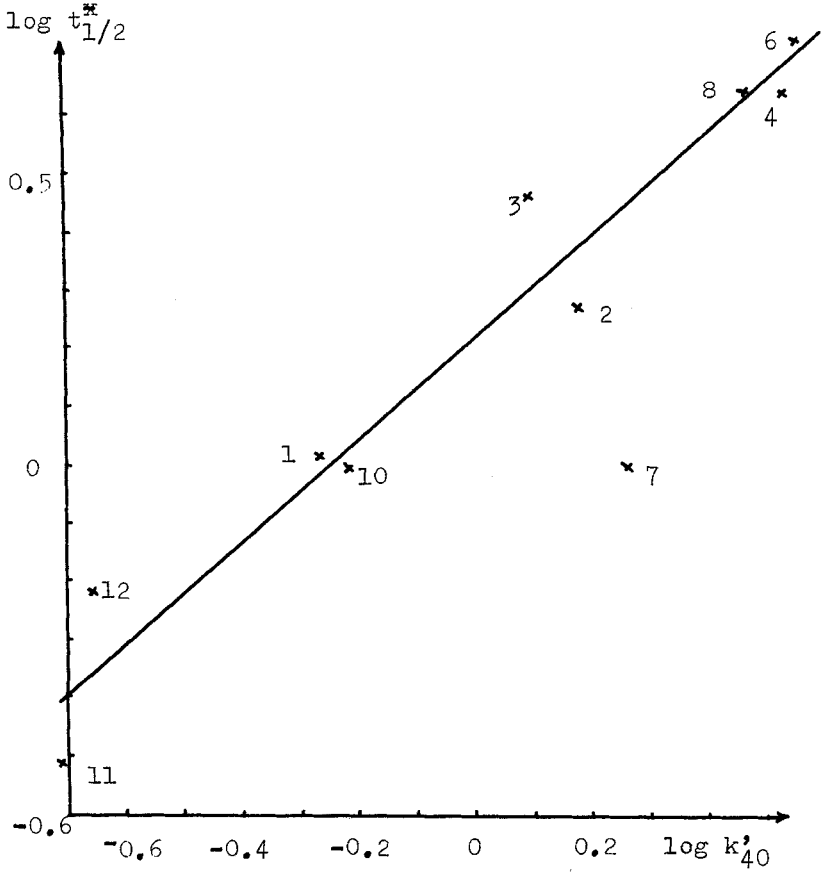


FIGURE 1.

THE RELATION OF log k' VALUES TO THE log $t^{*}_{1/2}$ VALUES ACCORDING TO EQ. 5.

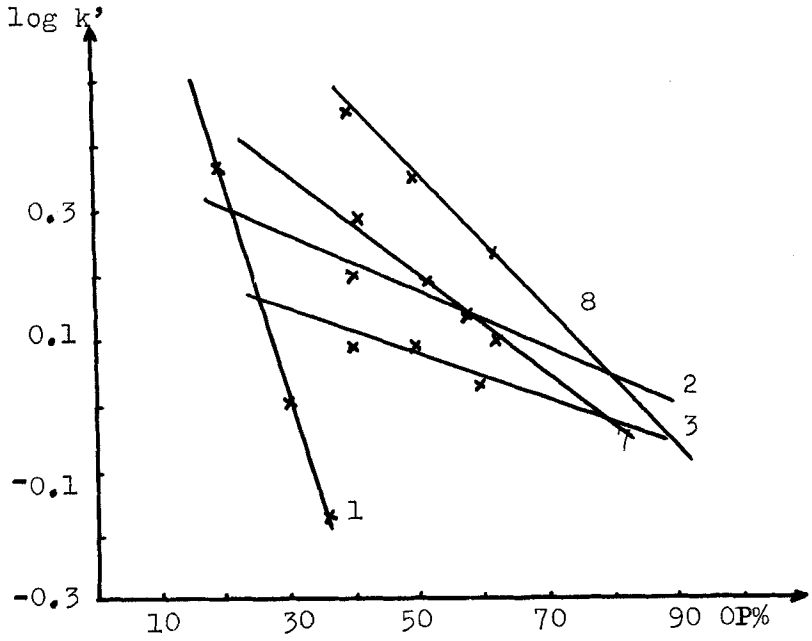
The whole set of compounds could be analyzed only when 40 - 50% acetonitrile was used in the eluent. At lower concentrations of acetonitrile hydrophobic compounds (No. 5, 6, 4, 8 and 9) could not be eluted, whereas at higher acetonitrile con-

centration more hydrophilic compounds (No. 12, 11, 1) had no retention on the column. At 40% acetonitrile concentration only two compounds could not be eluted, No. 5 and 9, the ones that could not be washed out from the ileum preparation.

The relationships between log $t^{*}_{1/2}$ and log k'_{40} values (eq. 4 and 5) are significant according to the F-test. These results indicate that a well-defined reversed-phase chromatographic system can provide a useful means to estimate the rate of offset of azidomorphine derivatives in the isolated guinea-pig ileum.

However, one may question the use of log k' values as the measure of hydrophobicity when log k' vs. OP% straight lines are not parallel for all compounds, rather they intersect each other. In such cases different ranks of log k' values can be obtained at different percentages of acetonitrile in the eluent. It was pointed out earlier /16/ that in such instances the hydrophobic properties of compounds can be characterized by the slope and the intercept (log k'_0) of the log k' vs. OP% straight line.

In case of the compounds investigated the OP% vs. log k' straight lines intersected each other as can be seen in Fig. 2.



Numbers refer to the compounds in Table 1.

FIGURE 2.

THE OP% vs. log k' STRAIGHT LINES FOR FIVE AZIDOMORPHINE DERIVATIVES

Therefore, the statistical parameters of the relation of hydrophobicity to the offset rate were also calculated from the 'slope' and log k'_o values of compounds:

$$\log t^{*}_{1/2} = 29.906\,\text{'slope'} + 0.908 \log k'_o + 0.087 \quad (6)$$

$n = 10 \quad R = 0.931 \quad s = 0.172 \quad F = 22.8 \quad F_{(2,7\ p=0.95)} = 4.7$

The calculated and measured log $t^{*}_{1/2}$ values of acetylazidomorphine were again different. Omitting

this compound from eq. 6 we obtained eq. 7:

$$\log t^{*}_{1/2} = 33.390\text{'slope'} + 0.862 \log k'_{o} + 0.219 \quad (7)$$

$n=9 \quad s=0.112 \quad R=0.975 \quad F=57.9 \quad F_{(2,6\ p=0.95)}=5.1$

Considering eq. 1 and 2, it can be derived /16/ that the quotient of the regression coefficient referring to the 'slope' and log k'_{o} values in eq. 7 give the concentration of acetonitrile in the eluent (OP%) at which the biological partition system can be best modelled. As it equals 38.7%, it is conceivable why log k' values referring to 40% acetonitrile concentration showed good correlation with log $t^{*}_{1/2}$ values.

Wüster and Herz /10/, /12/ have pointed out that surface activity of opiates can be an important factor in their accumulation in isolated organs. Therefore, we have determined the surface tension-lowering activity of azidomorphines at two different pH values. At near-physiological hydrogen-ion concentration none of the compounds showed significant surface tension-lowering activity. The highest value was only 5.32 (see Table 3) for compond 9. As this compound exhibited also a very slow offset rate of action, some tendency for the ΔG- vs. log $t^{*}_{1/2}$ relationship cannot be completely excluded.

The ΔG values of every compound could be measured at pH 4.6, but these values did not show correlation either with log $t^{*}_{1/2}$ or with log k' values. The ΔG values measured in the two buffers did not correlate either, which suggests that the degree of protonation of the compounds markedly influences their surface activity.

In conclusion, it can be stated that the hydrophobic properties of azidomorphines play an important role in the half-time of offset of action on longitudinal muscle of guinea-pig ileum. On this basis a well-defined chromatographic system was established by which the offset of action in guinea-pig ileum can be estimated.

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REFERENCES

1. Hansch, C. and Fujita, T. ρ-σ-π Analysis. A Method for the Correlation of Biological Activity and Chemical Structure, J. Amer. Chem. Soc. 86 1616, 1964.

2. Seydel, J. K. and Schaper, K. J., Chemische Struktur und Biologische Aktivität von Wirkstoffen, Verlag Chemie, Weinheim, New York, 1979.

3. Bognár, R. and Makleit, S., Conversion of Tozyl and Mezyl Derivatives of the Morphine Group, Acta Chim. Acad. Sci. Hung. 58 203, 1968.

4. Knoll, J., Fürst, S. and Kelemen, K., The Pharmacology of azidomorphine and azidocodeine, J. Pharm. Pharmacol. 25 929, 1973.

5. Knoll, J., Fürst, S. and Makleit, S., The Pharmacology of N-substituted Azidomorphines, Arch. Int. Pharmacodyn. 228 268, 1977.

6. Dinya, Z. and Makleit, S., Morfinszármazékok elektron és térszerkezetének vizsgálata és összefüggésük a farmakológiai hatással, /Hung./, Kémiai Közlemények 39 141, 1973.

7. Friedmann, T. and Knoll, J., Opiate Receptors and the Neurochemical Correlates of Pain, Ed. Fürst, S., Akadémia, Pergamon Press, Budapest, 1979. p. 45.

8. Kosterlitz, H. W., Leslies, F. M. and Waterfield, A. A., Rates of Onset and Offset of Action of Narcotic Analgesics in Isolated Preparations, Eur. J. Pharmacol., 32 10, 1975.

9. Herz, A. and Teschemacher, T., Activities and Sites of Antinociceptive Action of Morphine-

Like Analgesics and Kinetics of Distribution Following Intravenous, Intracerebral and Intraventricular Application, Advan. Drug Res. 6 79, 1971.

10. Wüster, M. and Herz, A., Proceedings of the International Narcotics Research Club Meeting, Ed. Kosterlitz, H. W., Aberdeen, North-Holland Publishing Comp., 1976, p. 447.

11. Wüster, M. and Herz, A., Opiate Agonist Action of Antidiarrheal Agents in Vitro and in Vivo - Finding in Support for Selective Action, Naunyn-Schmiedeberg's Arch. Pharmacol., 301 187, 1978.

12. Unger, S. H., Cook, J. R. and Hollenberg, J. S., Simple Procedure for Determining Octanol-Aqueous Partition, Distribution and Ionization Coefficients by Reversed-Phase High-Pressure Liquid Chromatography, J. Pharm. Sci. 67 1364, 1978.

13. Riley, C. M., Tomlinson, E. and Jefferies, T. M. Functional Group Behaviour in Ion-Pair Reversed-Phase High-Performance Liquid Chromatography Using Surface - Active Pairing Ions, J. Chromatogr., 185 197, 1979.

14. Brent, D. A., Sabatka, J. J., Minick, D. J. and Henry, D. W., A Simplified High-Pressure Liquid Chromatography Method for Determining Lipophilicity for Structure-Activity Relationships, J. Med. Chem. 26 1014, 1983.

15. Paton, W. D. M. and Vizi, E. S., The Inhibitory Action of Noradrenaline and Adrenaline on Acetylcholine Output by Guinea-Pig Longitudinal Muscle Strip, Br. J. Pharmacol. 35 10, 1969.

16. Valkó, K., General Approach for the Estimation of Octanol/Water Partition Coefficient by Reversed- Phase High-Performance Liquid Chromatography, J. Liquid Chromatog. (in press)