Asian Journal of Chemistry Vol. 22, No. 5 (2010), 3404-3412

Synthesis of Some Pramoxine-Based Compounds as Possible Local Anesthetic and Anticholinergic Agents

KAAN KUCUKOGLU* and MEHMET TANOL†
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ataturk University, 25240 Erzurum, Turkey
Fax: (90)(442)2360962; Tel: (90)(442)2315221; E-mail: kucukogluk@hotmail.com

A pair of pramoxine-based compounds, K-94 and K-95, which are potential local anesthetic and anticholinergic agents, were synthesized using a previously synthesized Schiff base of 2-diethylamine chloride and potassium carbonate. Of the compounds synthesized, compound K-94 was new. Moreover, R_M, a physicochemical parameter, has been calculated for both compounds in order to investigate the effect of these structural changes on the lipophilic and steric characteristics of these molecules. It also provides relevant data for the correlations with structure-activity relationship studies. Both of these compounds, phenylimino derivative, K-94, having an R_M of 0.91 and phenethylimino derivative, K-95, having an R_M of 0.87, showed similar values with procaine having R_M value of 0.66.

Key Words: Pramoxine, Anticholinergic effect, Schiff base.

INTRODUCTION

Today, the pharmaceutical industry and chemical industry are nested. This occurs because a large part of active compounds are firstly synthesized in laboratories by chemical reactions that are composed of various steps. Then, after proving their pharmacological effect, they are produced in drug factories and marketed. Drugs identified as local anesthetics are a drug group that are produced in the same ways and useful for various purposes in medicine. The drugs included in this drug group, in general, consist of an ester and amide structure. The pramoxine molecule that is the starting point of present study (Fig. 1) has been used as a local anesthetic drug.

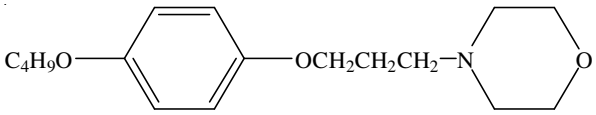


Fig. 1. Chemical structure of pramoxine

†Department of Pharmaceutical Chemistry, School of Pharmacy, The University of Kansas, Kansas, USA.

Pramoxine, also known as pramocaine, is different from other local anesthetic compounds in chemical structure. Active groups which are carboxylic acid derivatives like ester and amide are not present in this molecule. The drug molecule consists of ether functional groups that are much less active on a chemical level. The presence of the free radical morpholine in this compound is the cause of the reduction of the factor of toxicity and pharmacological activity. In pramoxine, toxicity is reduced but the local anesthetic affect is maintained[1].

Pramoxine is a local anesthetic drug used for topical anesthesia. Hydrochloride salt is preferred because of its easy dissolution in water, thus the drug is readily absorbed through the skin. Topical anesthetics are drugs used for removing pain and itching induced by sun burn or other minor burns, insect bites, poisonous plants and minor cut and scratch conditions[2]. Pramoxine, like other local anesthetics, reduces permeability of sodium ions in neuronal membranes, blocks to start and to improve neuronal impulses and thus inhibits depolarization of neurons[9]. Pramoxine relieves itching by affecting on the neuronal membrane of the end of neurons without myelin layer. The characteristic chemical structure of pramoxine is responsible for minimum cross-sensitization. Pramoxine rarely causes skin sensitivity so it is preferred as a topical anesthetic drug[3]. It is used for an antipruritic drug as well[2]. It can reduce the sense of itching by blocking transfer of impulses along fibers of sensor neurons[4]. However, the anesthetic effect of pramoxine is not the direct cause of the antipruritic effect. This is because this effect only appears on the cold pain threshold and is a hypoalgesic affect[5-7]. It has been shown that pramoxine lotion could treat histamine-induced pruritus. However, the usefulness of treating clinical pruritus needs more investigations[8]. According to the literature research, it is established that there are fewer local anesthetic compounds that carry the ether functional group than others. However, by using this structure, new pharmacological agents can be introduced. To this end, synthesis of some pramoxine-based derivatives has been achieved and their local anesthetic effect studied to clarify the relationship between biological activity and the structures of these derivatives[9]. On the synthesized compounds for these purposes, some modifications have been done on the *p*-position according to the aromatic group of local anesthetic molecules which had a lipophilic character on local anesthetic molecules that carry the ether functional group. Instead of the anilide functional group on this position, Schiff bases were formed by using bulkier amines sterically[10].

In the light of this information, the purpose of this study is to discover new compounds which have potential as local anesthetic and anticholinergic activity, with some modifications on structure-activity rules by choosing pramoxine as a key compound.

EXPERIMENTAL

Chemicals used in this study were as follows: β-phenethylamine, 4-hydroxy-benzaldehyde, 2-diethylaminoethyl chloride hydrochloride (Merck, Darmstadt, Germany), aniline, potassium carbonate (Fluka, Steinheim, Switzerland), sodium

bicarbonate, sodium sulphate (Merck, Darmstadt, Germany), ethanol, dichloromethane, acetone and ethyl acetate (Riedel-de Haen, Seelze, Germany). The reactions were followed by TLC analysis (Merck Art No. 5715: Silica gel 60 F_{254}, 0.25 cm thickness). The spots belonging to the compounds were established by using UV light at 254 nm and 366 nm. Merck silica gel 60, 20-50 μm was used in flash chromatography as an adsorbant matter.

UV spectra of compounds were recorded on a Shimadzu 160-A spectrometer. IR spectra were obtained for a solution in $CHCl_3$ on Perkin-Elmer 457 IR Grating spectrophotometer. ^1H NMR spectra were recorded at 400 MHz on a Bruker GmbH DPX-400, high performance digital FT NMR spectrophotometer. Chemical shift values were given on δ scale and interaction constants were given as Hz. EI-MS spectra were recorded on a Fisons Instruments, VG Platform II LC-MS spectrometer.

Glass plates which were covered with Kieselgel HF_{254} and saturated with 1-octanol: ether (5:95) (v/v) solvent system as a stationary phase were used for establishing R_M values. Isopropanol:buffer solution system was used as a mobile phase and the best discrimination was observed in the system having (100:10) ratio.

For establishing R_M and R_f values, 20 cm × 20 cm-size glass plates were covered with 0.3 mm-thickness Kieselgel HF_{254} by using Camag thin layer spreader. After the plates were activated for 1 h in the etuv at 105 ºC, they were saturated in the chromatography tank within 1-octanol:ether (5:95) (v/v) for 14 h. At the end of this time, plates were taken out and held at room temperature to evaporate the ether.

For preparing the buffer solution, 2.04 g of potassium dihydrogen phosphate, 5.372 g of disodium hydrogen phosphate·$12H_2O$ were weighed and mixed with 100 mL of water. Thus, each solution was prepared at 0.15 M concentration. For preparing 100 mL buffer solution, 19.6 mL of 0.15 M potassium dihydrogen phosphate solution and 80.4 mL of 0.15 M disodium hydrogen phosphate solution were mixed and the prepared solution pH was found to be 7.8. This prepared solution was used as buffer solution in the study.

Solutions of each synthesized compounds were prepared in 10^{-2} M concentrations with methanol and three drops of the prepared solutions were put on the plate, respectively. The plate was than put into the chromatography tank within isopropanol: buffer solution (100:10) (v/v). When the solvent system reached the 10 cm height that had been drawn on the plate previously, it was taken out and dried. The spots of each studied compounds on the plate were established correctly by using UV lamp and R_f values were found. These values were put in the following formula and the R_M values of the studied compounds were calculated.

$$R_M = \log\left(\frac{1}{R_f} - 1\right)$$

Synthesized Schiff bases

4-(Phenethylimino-methyl)phenol: β-Phenethylamine (2.424 g, 0.02 mol) and 4-hydroxybenzaldehyde (2.442 g, 0.02 mol) were refluxed for 1 h in ethanol (10 mL).

At the end of the reaction, the flask was cooled and synthesized Schiff base was precipitated, filtered off, washed with cold ethanol and crystallized from ethanol (3.589 g, 80 %) (Table-1).

TABLE-1
DATA OF THE COMPOUNDS S-94 AND S-95
CARRIES SCHIFF BASE FUNCTIONAL GROUP

Code of compounds	Closed formula	Aril (Ar)	m.w.	Mol ratio amine derivative: 4-hydroxybenzaldehyde	Reflux time (min)	Crystallisation solvent	Yield (%)
S-94	$C_{15}H_{15}NO$	$C_6H_5CH_2CH_2$	225.29	1:1	60	Ethanol	80
S-95	$C_{13}H_{11}NO$	C_6H_5	197.23	1:1	15	Ethanol:Water (1:1)	66

4-Phenyliminomethyl phenol: 4-Hydroxybenzaldehyde (3.664 g, 0.03 mol) was dissolved in ethanol (10 mL). Aniline (2.794 g, 0.03 mol) was added to this solution and the mixture was stirred for 15 min. Synthesized Schiff base was filtered off and crystallized from ethanol-water mixture (1:1) (3.9 g, 66 %) (Table-1).

Synthesized ether derivatives

Diethyl-{2-[4-(phenethylimino methyl)phenoxy]ethyl}amine: 4-(Phenethylimino methyl)phenol (0.901 g, 0.004 mol) and K_2CO_3 (0.691 g, 0.005 mol) were added to 2-diethylaminoethyl chloride (0.814 g, 0.006 mol) and the mixture was refluxed for 1 h in acetone (8 mL). At the end of the reaction, the solvent was removed *in vacuo*. The mixture was extracted with distilled water and dichloromethane. The aqueous phase was extracted by using dichloromethane twice. Organic phases were joined and washed with distilled water twice and dried with Na_2SO_4. The mixture was purified using flash chromatography with a solvent of ethyl acetate (1.02 g, 79 %) (Table-2).

TABLE-2
DATA OF THE COMPOUNDS K-94 AND K-95

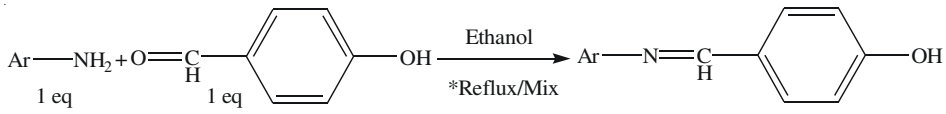
Code of compounds	Closed formula	Aril (Ar)	m.w.	Mol ratio2-diethylamino chloride: Schiff base: potassium carbonate	Reflux time (min)	Purification method (Solvent:Ethyl acetate)	Yield (%)
K-94	$C_{21}H_{28}N_2O$	$C_6H_5CH_2CH_2$	324.46	1.6:1:1.33	60	Flash chromatography	79
K-95	$C_{19}H_{24}N_2O$	C_6H_5	296.41	1.6:1:1.33	1	Flash chromatography	65

Diethyl-{2-[4-(phenylimino methyl)phenoxy]ethyl}amine: 4-(Phenylimino methyl)phenol (0.592 g, 0.003 mol) and K_2CO_3 (0.415 g, 0.003 mol) were added to 2-diethylaminoethyl chloride (0.678 g, 0.005 mol) and the mixture was refluxed for 1 h in acetone (7 mL). At the end of the reaction, the solvent was removed *in vacuo*. The mixture was extracted with distilled water and dichloromethane. The aqueous phase was extracted by using dichloromethane twice. Organic phases were

joined and washed with distilled water twice and dried with Na_2SO_4. The mixture was purified using flash chromatography with a solvent of ethyl acetate (0.579 g, 65 %) (Table-2).

RESULTS AND DISCUSSION

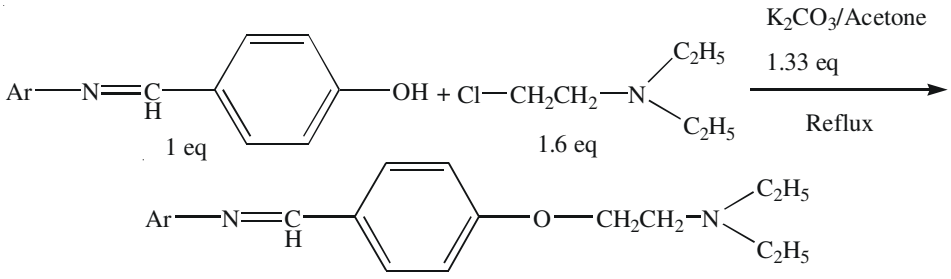
Synthesized Schiff bases: The synthetic route used for the preparation of the Schiff base-structured compounds S-94 and S-95 were used for the synthesis of the resultant compounds designed for this study is shown in **Scheme-I**.



Scheme-I: Synthetic pathway for the synthesis of the compounds S-94 and S-95. *: It was refluxed for 1 h for synthesis of S-94 compound, it was enough to mix for 15 min without heating for synthesis of S-95 compound

Synthesis: For the general synthesis of compounds consisting of the Schiff base functional group, 4-hydroxybenzaldehyde was mixed in 10 mL ethanol and then amine derivative was added. The compound was mixed at room temperature and/or refluxed until the desired synthesis was completed. At the end of the reaction, the precipitated material was filtered and then crystallized by using suitable solvent and/or mixture of solvents. If necessary, crystallization process was done by being held at a temperature of -4 ºC for 1 day. Crystals were filtered and dried at room temperature (**Scheme-I**).

Synthesized ether derivatives: The synthetic route used for the preparation of the resultant compounds K-94 and K-95 which composed of the ether functional group is shown in **Scheme-II**.



(Ether derivatives)

Scheme-II: Synthetic pathway for the synthesis of the compounds K-94 and K-95

Synthesis: 2-Diethylaminoethyl chloride hidrochloride which was used for the synthesis of the resultant compounds was mixed for 1 h in a solution of 10 % aqueous $NaHCO_3$ to reduce the presence of salt. At the end of the reaction, aqueous

solution was extracted with dichloromethane, the solvent was removed *in vacuo*. The residue was dried and used in the synthesis of the resultant compounds.

In the general synthesis of the resultant compounds, 2-diethylaminoethyl chloride, a suitable Schiff base and K_2CO_3 were refluxed for 1 h in a suitable volume of acetone. At the end of the set time, the solvent was removed *in vacuo* and the mixture was extracted with distilled water and dichloromethane. An aqueous phase was extracted by using dichloromethane two times. The organic phases were joined and washed with distilled water twice and dried with Na_2SO_4. The resultant mixture was purified using flash chromatography with a solvent of ethyl acetate (**Scheme-II**).

Of the synthesized compounds, compound K-95 has been previously synthesized, however, the synthesis method in present study was different from that in the literature[11]. K-94 is a new compound. The structures of the synthesized compounds were confirmed by ^{1}H NMR (Table-3), UV, IR and MS data (Table-4).

TABLE-3
^{1}NMR SPECTRA OF THE SYNTHESIZED COMPOUNDS K-94 AND K-95

Compound	^{1}H-NMR ($CDCl_3$)
K-94	δ: 1.10 (t, 3H, 2x N-CH_3, *J* = 6.9 Hz), 2.68 (q, 2H, 2x N-CH_2, *J* = 6.9 Hz), 2.92 (t, 2H, C1-H_2, *J* = 5.9 Hz), 3.00 (t, 2H, C2''-H_2, *J* = 7.3 Hz), 3.82 (t, 2H, C1''-H_2, *J* = 7.3 Hz), 4.12 (t, 2H, C2-H_2, *J* = 5.9 Hz), 6.92 (d, 2H, C3'-H, C5'-H, *J* = 8.2 Hz), 7.18-7.30 (m, 5H, C2'''-H, C3'''-H, C4'''-H, C5'''-H, C6'''-H), 7.63 (d, 2H, C2'-H, C6'-H, *J* = 8.3 Hz), 8.10 (s, 1H, N=CH).
K-95	δ: 1.03 (q, 3H, 2x N-CH_3, *J* = 7.5 Hz), 2.60-2.68 (m, 2H, 2x N-CH_2), 2.88 (t, 2H, C1-H_2, *J* = 6.1 Hz), 4.08 (t, 2H, C2''-H_2, *J* = 6.4 Hz), 6.98 (d, 2H, C3'-H, C5'-H, *J* = 8.0 Hz), 7.18 (d, 2H, C2'-H, C6'-H, *J* =7.9 Hz), 7.33-7.41 (m, 2H, C3'''-H, C5'''-H), 7.78-7.86 (m, 3H, C2'''-H, C4'''-H, C6'''-H), 8.35 (s, 1H, N=CH).

TABLE-4
UV, IR AND MS RESULTS FOR THE SYNTHESIZED COMPOUNDS K-94 AND K-95

Compound	MW	UV(MeOH)		MS m/z M^+	IR($CHCl_3$)
		λ_{max}	log ε		C=O stretching
K-94	324.46	222.8 268.6	2.85 2.91	324	1646
K-95	296.41	221.6 282.6	2.58 2.70	296	1625

Product characterization: The absorption maximum observed in UV spectrum of the K-94 compound was observed at 222.8 nm. This peak was the E band that had suffered a bathochromic shift due to benzene chromophore and that was derived from $\pi \rightarrow \pi^*$ transition. The peak which was seen at 268.6 nm was the K band that related to the imine functional groups. In the IR spectrum of K-94 an absorption peak at 1646 cm^{-1} was observed, suggesting a stretching band of C=N (azomethine band). An absorption peak was also observed at 1249 cm^{-1}, suggesting a C-O asymmetric stretching band which related to the aromatic ether functional group. These results confirmed the presence of the functional groups that were expected in our

target compound[12]. In the ^{1}H NMR spectrum of the K-94 compound, taken in $CDCl_3$ (Table-3), the protons of terminal non-substituted benzene ring were observed as a multiplet at δ 7.18-7.30 ppm (5*H*). Each dublet observed in aromatic area related to 4-disubstituted benzene ring (J = 8.3 Hz). Of these protons, the protons on the *o*-position to phenolic oxygen were observed at δ 7.63 ppm (2*H*) and the protons on the *o*-position to sp^2 carbon that belonged to the Schiff base were observed at δ 6.92 ppm (2*H*). Presence of a sharp singlet at δ 8.10 ppm (1*H*) suggested the proton on the sp^2 carbon belonged to the Schiff base. The signals of four methylene groups that had different chemical environments in the compound were observed at δ 2.92, 3.00, 3.82 and 4.12 ppm. The protons of the methylene group on the nitrogen atom (6*H*) were observed as a triplet at δ 1.10 ppm (J = 6.9 Hz). In the mass spectrum of the K-94 compound, the molecular ion peak observed at m/z 324 was in accordance with the proposed structure. The M^+-15 peak that originated to break off a methyl group from the molecule was observed at m/z 309. The peak observed at m/z 86 belonged to N,N-dimethyl-N-methylene cation. Tropilium cation which originated to break off the terminal non-substitued benzyl group was observed[13] at m/z 91.

In the UV spectrum of the K-95 compound, two peaks were observed at 221.6 and 282.6 nm, respectively. Of the peaks, the absorption maximum observed at 221.6 nm was an E band that had suffered bathochromic shift due to benzene chromophore and that was derived from $\pi \rightarrow \pi^*$ transition. In the UV spectrum, a K band that related to the imine functional group was observed at 282.6 nm and this peak was in the characteristic band for the target molecule. Because the imine functional group present in this compound conjugated two benzene chromophores, the K band of the K-95 compound suffered a larger bathochromic shift in comparison with the K band observed in the UV spectrum of K-94 but the intensity of the band decreased. In the IR spectrum of K-95, the absorption peak was observed at 1625 cm^{-1} suggesting a stretching band of C=N of azomethine. The absorption peak observed at 1255 cm^{-1} suggested a C-O asymmetric stretching band related to the aromatic ether functional group. The presence of these two peaks in the spectrum confirmed the formation of the functional groups that were expected in present target compound[14]. When the ^{1}H NMR spectrum of compound K-95, taken in $CDCl_3$, was examined (Table-3), it was observed that none of the protons of the terminal non-substituted benzene ring gave a multiplet, as was seen in the spectrum of K-94. Of these protons, the protons on the *o*- and *p*-position to the nitrogen atom connected to the benzene ring gave a multiplet (3*H*) at δ 7.78-7.86 ppm and the protons on the *m*-position to the nitrogen atom connected to the benzene ring gave a multiplet (2*H*) at δ 7.33-7.41 ppm. The protons of the *p*-disubstituted benzene ring that existed in the molecule gave two dublets and had different chemical shifts due to having a different chemical environ-ment. Of the dublets, the dublet belonging to the protons on the *o*-position to sp^2 carbon originated from the Schiff base was first observed at δ 6.98 ppm (2*H*). The other dublet observed at δ 7.18 ppm belonged to the protons on the *o*-position to phenolic oxygen. The signal suggested that the proton on the sp^2 carbon that originated from the Schiff base in the molecule was observed at

δ 8.35 ppm (1*H*). This singlet shifted by 0.25 ppm more to the lower area according to the singlet suggested in the spectrum of K-94. This may be due to the ethyl chain between imine nitrogen and terminal benzene ring did not exist in K-95 molecule. The signals of two methylene groups existing in the molecule were observed at δ 2.88 and 4.08 ppm as a triplet, respectively. A multiplet observed at δ 2.60-2.68 ppm (4*H*) belonged to the methylene protons of two ethyl substituent on the terminal nitrogen atom. The protons belonging to the terminal methyl group were observed at δ 1.03 ppm (6*H*) as a quartet. The mass spectrum of the K-95 compound molecular ion peak observed at m/z 296 was in accordance with the proposed structure. The M^+-15 peak that originated to break off a methyl group from the molecule was observed at m/z 281. The peak belonging to N,N-dimethyl-N-methylene cation was observed[13] at m/z 86.

Establishment of R_M and R_f values: R_M values were established by using the finally synthesized ether derivative compounds, K-94 and K-95 and procaine as a local anesthetic drug (Fig. 2a) and a MTD 46 K compound (Fig. 2b) (Table-5). It was found that the compound MTD 46 K had the most effective local anesthetic activity in its homologous series. The chemical name of MTD 46 K is N-[4-(4-morpholin-4-yl-butoxy)phenyl]acetamide and it had a similar structure to the synthesized compounds (Fig. 2b). Thus, it was found that one of the parameters that would help the correlation about the finally synthesized compounds gained lipophilic and steric characters in the biological tests. At the end of this study, K-94 and K-95 compounds gave values that were highly close to each other. The R_M values found for K-94 and K-95 were 0.91 and 0.87, respectively and the R_f values were established as 0.11 and 0.12, respectively. The observed values are presented in Table-5.

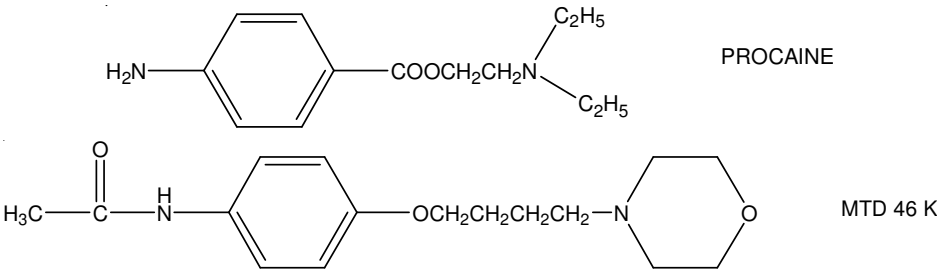


Fig. 2. Chemical structures of procaine and MTD 46 K

TABLE-5
THE ESTABLISHED R_M AND R_f VALUES OF STUDIED COMPOUNDS

Code of compounds	R_M	R_f
K-94	0.91	0.11
K-95	0.87	0.12
Procaine	0.66	0.18
MTD 46 K	0.29	0.34

Conclusion

In this study, some potential local anesthetic and anticholinergic compounds were synthesized by considering researches about local anesthetic compounds which consisted of the ether functional group and by protecting the groups required for base anticholinergic activity in the molecule.

Consequently, in present study, we first synthesized the compound K-94 which was not available in the literature, followed by K-95, which had been synthesized previously. These compounds were planned and synthesized as compounds having potential local anesthetic and anticholinergic effects. R_M and R_f values were found as a guide parameter for these potential pharmacological effects.

ACKNOWLEDGEMENT

The authors would like to thank Assoc. Prof. Dr. Zekai Halici (Faculty of Medicine, Ataturk University) for his valuable support.

REFERENCES

1. J.L. Schmidt, L.E. Blockus and R.K. Richards, *Curr. Res. Anesth. Analg.*, **32**, 418 (1953).
2. http://www.nlm.nih.gov/medlineplus/druginfo/uspdi/202042.html.
3. A.A. Fisher, *Curtis*, **62**, 122 (1998).
4. J.Z. Litt, in ed.: J. Bernhard, Itch Mechanisms and Management of Pruritus, McGraw-Hill, New York, pp. 383-397 (1994).
5. A. Ekblom, *Semin. Dermatol*., **14**, 272 (1995).
6. S.B. McMahon and M. Koltzenberg, *Trends Neurosci*., **15**, 497 (1992).
7. D.A. Simone, J. Ngeow, J. Whitehouse, L. Becerra-Cabal, G.J. Putterman and R.H. LaMotte, *Somatosens. Res*., **5**, 81 (1987).
8. G. Yosipovitch and H.I. Maibach, *J. Am. Acad. Dermatol*., **37**, 278 (1997).
9. M. Tanol, *Gazi Ecz. Fak. Der*., **3**, 81 (1986).
10. W.O. Foye, Principles of Medicinal Chemistry, Lea and Febiger/Philadelphia, edn. 3 (1989).
11. H.R. Meyer and M. Morf, US Patent, 4478598 (1984).
12. N. Ergenc, A. Gursoy and O. Ates, Ilaclarin Taninmasi and Kantitatif Tayini, Istanbul Universitesi Yayinlari No: 3566, Eczacilik Fakultesi No: 55, Genisletilmis IV, Baski, Istanbul, Turkey (1989).
13. R.M. Silverstein, G.C. Basler and T.C. Morrill, Spectrometric Identification of Organic Compounds, John and Sons Inc., New York, edn. 4 (1981).

(*Received*: 24 April 2009; *Accepted*: 13 January 2010) AJC-8286