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SYNTHESIS AND SAR STUDIES OF 1-SUBSTITUTED-*n*-(4-ALKOXYCARBONYLPIPERIDIN-1-YL)ALKANES AS POTENT ANTIARRHYTHMIC AGENTS#

Ravish C. Tripathi[a], Suresh K. Pandey[a], K. Kar[b], M. Dikshit[b] and Anil K. Saxena[a,*]

[a]*Division of Medicinal Chemistry*
[b]*Division of Pharmacology*
Central Drug Research Institute, Lucknow-226 001 (India).

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Abstract: Synthesis and SAR studies of the title compounds have resulted in the identification of structural and physicochemical parameter (Vw) contributing for antiarrhythmic activity. Among the two most promising compounds **3a & 3b**, the **3a** has shown antiarrhythmic activity comparable to quinidine.

Cardiac arrhythmias result due to disturbances in generation, speed of conduction and termination of electrical impulse. Each heart beat is the result of highly integrated electro-physicological behaviour of multiple ion channels on multiple cardiac cells. Functions of ion channels are disturbed by factors such as acute ischemia, sympathetic stimulation or myocardial scarring to create abnormalities of cardiac rhythm i.e. arrhythmias[1,2]. Antiarrhythmic drugs generally supress these abnormalities of cardiac rhythm by blocking flow through specific ion channels or by altering autonomic functions. Major antiarrhythmic drugs which are available today either interact with the receptor[3,4] or nonspecifically act by their accumulation in the membrane[5-9]. Most of these drugs contain a basic nitrogen, a lipophilic aromatic ring capable of intercalating between alkyl chains of phospholipids and interconnecting alkyl chain with substituents capable of hydrogen bonding.

In order to explore new chemical entities (NCE's) which may be used as orally long acting and safe antiarrhythmic agents, the title compounds incorporating the essential structural requirements, have been synthesized and screened for antiarrhythmic activity. Using the common structural pattern related to antiarrhythmic activity for superimposition and physicochemical property like van der Waals volume (Vw), attempts have been made to establish the structural-activity relationships.

The key intermediate esters (**2a-d**) were synthesized by esterification of 4-piperidine carboxylic acid (**1**). The esters (**2a-d**) on condensation with 1-chloro-3-(4-fluorobenzoyl)propane yielded the corresponding 1-(4-fluorobenzoyl)propylpiperidin-4-carboxylic acid esters (**3a-d**)[10]. The condensation of the ester **2a** with 1-chloro-3-(4-substituted piperazin-1-yl)propanes yielded the corresponding condensation product **4a-d**[10]. Condensation of **2a** with 1-chloro-3-(3-acetamidophenoxy)propane yielded the compound **5**[10] (scheme 1).

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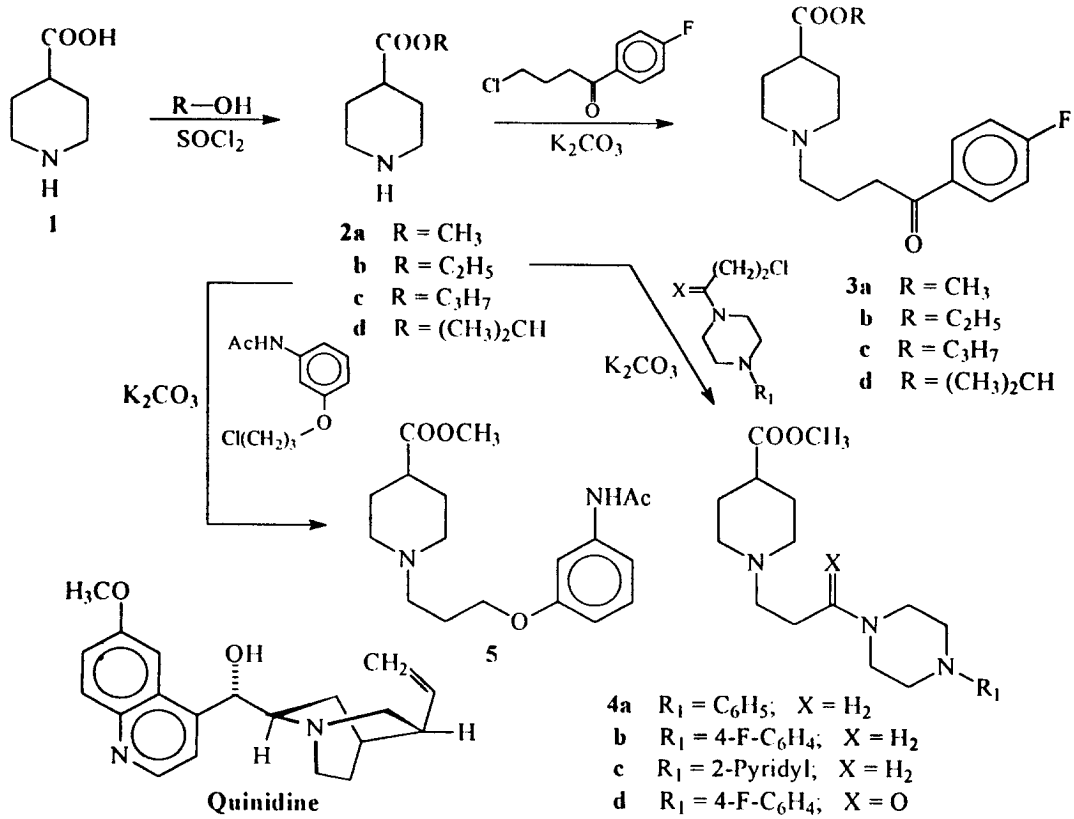
Scheme 1



Compounds **3a-d**, **4a-d** & **5** were tested at 8 μmolar concentration in isolated guinea pig auricle by Dawas method[11]. The auricles were dissected free from the heart immediately after killing the guinea pig (300-600 gm) and suspended in an organ bath of 40 ml capacity containing oxygenated locke's solution at 37°C. The solution contained NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.5 and dextrose 1.0 gm/lit. The auricles were fixed in a pair of stimulating platinum electrodes kept vertically immersed inside the bath, the thread tide at the other end of the auricle was fixed with the starling's heart liver writing on smoked paper. The auricle was allowed to equilibriate for a period of 30 min., and subsequently stimulated by rectangular wave pulses (0.5 msec. duration) at a frequency increasing 0.2 pulses per second every two second at a voltage five times higher than the threshold (1.5 to 3.0 v) using a gross stimulator (S-4). The maximum rate of stimulation at which the auricle just failed to follow each stimulus was determined before and after addition of test compound in bath. Doses of the compound were given as concentration of the salt per ml of bath solution and allowed to remain in contact with the auricle for 10 min. The antiarrhythmic effect of each dose was calculated as the percentage reduction of the maximal rate of stimulation in minimum of two experiments at each dose and the mean values are reported in Table 1.

Table 1 : Physicochemical data and antiarrhythmic activity of the compounds and quinidine sulphate (QS).

Compds.	M.P.(°C)	Yield (%)	Molecular Formula	% Inhibition[a] (P)	Log{P/ (100-P)}	RMS[b]	Vw[c]
3a	162	30	$C_{17}H_{22}O_3NF.HCl$	24.33 ± 2.85	-0.493	0.902	2.522
3b	190	25	$C_{18}H_{24}O_3NF.HCl$	25.66 ± 1.67	-0.462	0.902	2.676
3c	178	34	$C_{19}H_{26}O_3NF.HCl$	10	-0.954	0.902	2.830
3d	203	28	$C_{19}H_{26}O_3NF.HCl$	12	-0.865	0.902	2.780
4a	oil	35	$C_{20}H_{31}O_2N_3$	6	-1.195	2.251	2.993
4b	240	38	$C_{20}H_{30}O_2N_3F.3HCl$	Not significant	-	2.435	3.039
4c	160	36	$C_{19}H_{30}O_2N_4.3HCl$	Not significant	-	2.232	2.950
4d	oil	35	$C_{20}H_{28}O_3N_3F$	10	-0.954	2.278	2.960
5	85	40	$C_{18}H_{26}O_4N_2$	7	-1.123	1.898	2.899
QS	-	-	$C_{40}H_{48}O_4N_4.H_2SO_4.2H_2O$	29.68 ± 1.00	-0.375	-	2.691

[a]-The number of experiments for compounds **3a**, **3b** & QS are >3; [b] - Root mean square deviation: source compound is quinidine, target compounds are **3a-d**, **4a-d** & **5**; [c] - Vw values ($Å^3$) are scaled by a factor of 0.01.

The compound **3a** was compared with quinidine sulphate (QS) for its *in vivo* anti-arrhythmic activity in aconitine induced arrhythmia in rats and Ouabain induced arrhythmia in guinea pigs (Table 2 & 3). The experiments were carried out in male rats (100-200g) or guinea pigs (200-250g) anaesthetized with urethane (1.5 g/kg *i.p.*). The jugular vein was cannulated for infusion of aconitine nitrate or ouabain in saline at the rate of 3.9 μg/min. in rats or guinea pigs respectively. ECG changes (Lead II) were monitored and recorded on a polygraph before and after the administration of the test compound and during the infusion. Control groups received saline only. The results were expressed in terms of aconitine or ouabain required for the onset of early arrhythmia (appearence of ecotopic beat, EA), Ventricular tachycardia (VT), Ventricular fibrillation (VF) and cardiac arrest (CA).

The 3D structures of the compounds **3a-d**, **4a-d**, **5** & quinidine were constructed, optimized for their geometries and finally minimized for their energy by employing Steepest Descent, Conjugate Gradients and Neuton-Raphson's algorathims in sequence followed by Quasi-Neuton-Raphson optimisation procedure using energy tollerance value of 0.001 $kcal/mol^{-1}$, using different modules of InsightII from the Biosym Technologies of San Diego[12] on INDY R-4000 work station. The substructure containing 4-methoxyphenyl ring and *tert.* N of quinidine was used as structure template for superimposition with the corresponding substructure present in different compounds **3a-d**, **4a-d** & **5**. The degree of superimposition described as RMS value for each molecule in terms of this structural pattern was measured. The van der Waals volumes (Vw) were calculated according to Moriguchi *et al.*[13] (Table 1). The QSAR studies were made on PC-486 using SYSTAT[14] (ver. 7.0) software.

Table 2^: Antiarrhythmic activity of 3a and QS against aconitine induced arrhythmias in rats.

Drug	Dose	Change in HR (1-5')	Amount of Aconitine used in			
			EA	VT	VF	CA
3a	10 mg/kg *i.v.*	16-34%	132.79 ±19.30	231.27 ±25.72	373.36 ±42.55	544.28 ±43.99
QS	10 mg/kg *i.v.*	18-30%	156.24 ±18.41	252.72 ±8.95	370.00 ±36.06	523.64 ±53.47
Control	-	-	115.74 ±11.97	173.33 ±26.98	250.71 ±55.71	340.52 ±40.99
Drug	Dose	Change in HR(10-30')	Amount of Aconitine used in			
			EA	VT	VF	CA
3a	25 mg/kg *p.o*	16-17%	159.29 ±25.30	226.74 ±21.37	356.09 ±38.80	457.72 ±41.83
QS	25 mg/kg *p.o*	11-17%	173.66 ±22.32	237.02 ±33.55	416.36 ±13.36	581.06 ±63.12
Control	-	-	101.19 ±14.52	144.21 ±15.59	231.00 ±14.98	326.89 ±13.93

Table 3^ : Antiarrhythmic activity of 3a and QS against ouabain induced cardiac arrhythmias in guinea pig.

Drug	Dose	Change in HR (1-5')	Amount of Ouabain used in		
			EA	VF	CA
3a	10 mg/kg *i.v.*	17-30%	101.35 ±5.01	138.04 ±8.18	175.76 ±8.50
QS	10 mg/kg *i.v.*	17-18%	128.33 ±4.41	167.78 ±9.10	187.59 ±4.33
Control	-	-	76.89 ±4.93	114.48 ±2.51	157.46 ±15.58
Drug	Dose	Change in HR (10-30')	Amount of Ouabain used in		
			EA	VF	CA
3a	20 mg/kg *p.o.*	10-35%	124.56 ±15.35	153.65 ±17.66	187.05 ±20.28
QS	20 mg/kg *p.o.*	18-27%	138.99 ±31.96	201.34 ±52.98	225.31 ±54.98
Control	-	-	111.04 ±35.21	146.25 ±48.75	169.26 ±50.11

^- Values are mean ±SE (n = 5); HR = Heart Rate; EA = Early arrhythmias; VT = Ventricular Tachycardia; VF = Ventricular Fibrillation; CA = Cardiac Arrest.

At 8 μmolar concentration, most of these molecules except compounds **4b** and **4c** showed good to moderate antiarrhythmic activity in the order **3a**~**3b** > **3d** > **3c** ~ **4d** > **5** > **4a** > **4c** ~ **4b**. The most active compounds **3a** and **3b** showed IC_{20} 4.75 nM and 5.41 nM, respectively as compared to quinidine IC_{20} 4.16 nM. The two compounds **4b** and **4c** which did not show significant activity at 8 μmolar concentration were tested at 25 μmolar concentration, at which compound **4c** showed 5% inhibition while **4b** still did not show significant activity.

A comparative study of energy optimized 3D-structures of these molecules in terms of the superimposition of the substructure containing 4-substituted phenyl ring and *tert.* N of piperidine ring of these molecules with the corresponding substructure of quinidine described as RMS value in Table 1 suggests that compounds **3a-3d** have better superimposition compared to other with the least RMS value 0.902 followed by the compounds **6** (1.898), **4c** (2.232), **4a**(2.251), **4d** (2.278) and **4b**(2.435). The variation in the activity of the four compounds **3a-3d** with the same RMS value may be explained in terms of the tollerence of the steric bulk Vw around the N atom which has similar order of activity variation being **3a** (126.9), **3b** (142.3), **3d** (152.7) and **3c** (157.7) comparable to quinidine (124.2). In addition to the identified substructure containing electron rich hydrophobic aromatic ring and *tert.* N in a proper orientation, the other contributing factor for activity seems to be the presence of electron rich atom capable for H-bonding near to the aromatic ring corresponding to the N atom in quinidine and oxygen atom of the C=O adjacent to aromatic ring in molecules **3a-d**, **4d** and of ether in **5**. The poor or no activity of the molecules **4a-4c** may be due to the absence of these factors in these molecules.

Among different physicochemical properties like lipophilicity (Log P), molar refractivity (MR), steric bulk (Vw), only Vw exhibited linear correlation of > 99% statistical significance ($F_{1,6}$=19.939; $F_{1,6,0.01}$= 18.60) (Eq. 1) suggesting that it has (-)ve contribution for activity and as the optimum has not been reached in the present set of molecules, there is a scope for improving the activity by reducing the steric bulk (Vw).

$$\text{Log } \{P/(100\text{-}P)\} = -1.739(\pm 0.389)Vw + 4.056(\pm 1.090) \quad \text{Eq. 1}$$
$$n = 8 \qquad r = 0.877 \qquad F = 19.939 \qquad S = 0.164$$

Among the most promising compounds **3a** and **3b**, the compound **3a** was evaluated in detail in comparison to quinidine sulphate (QS). It provided protection equivalent to QS against aconitine induced arrhythmia in rats at 10 mg/kg dose (Table 2). However, when administered orally, it was less active than QS, although, the difference was not statistically significant. The amount of ouabain required to induce early arrhythmia, ventricular fibrillation and cardiac arrest was found to be significantly increased in the guinea pigs treated with compound **3a** or QS at the dose of 10 mg/kg *i.v.* (Table 3). However, it did not provide significant protection against ouabain induced arrhythmia on oral administration at 20 mg/kg which may be due to its poor oral bioavailability.

In conclusion, the above studies not only provide new lead molecule for further exploration but also suggest model with structural and physicochemical requirements for modulation to get effective antiarrhythmic agent for development in future.

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10. Experimental procedure; A mixture of 4-carbomethoxypiperidine (0.421 g, 0.003 mol), appropriate halo compound (0.003 mol) and dry potassium carbonate (0.4 g, 0.003 mol) in dry acetone (50 ml) was refluxed for 36 - 48 hours. The reaction mixture was filtered, concentrated and chromatographed on silica gel column using 2% MeOH in $CHCl_3$ as eluant. Select data for one compound of each type is given below; **(3a)** was characterised as monohydrochloride. MS : m/z 307 (m^+). ^1H NMR (400 MHz, $CDCl_3$)δ: 1.64-1.75(m, 2H, CH_2), 1.84-2.06(m, 8H, piperidinyl-$N(CH_2CH_2)_2$), 2.24-2.33(m, 1H, COCH), 2.40(t, J=5.71 Hz, 2H, $COCH_2$), 3.00(t, J=5.71 Hz, 2H, NCH_2), 3.67(s, 3H, OCH_3), 7.10-7.16(m, 2H, ArH), 7.98-8.04(m, 2H, ArH); **(4a)** Oil. MS : m/z 345 (m^+). ^1H NMR (90 MHz, $CDCl_3$)δ: 1.80-2.00(m, 6H, $3xCH_2$), 2.05-2.15(m, 1H, CH), 2.30-2.45(m, 4H, piperidinyl-$N(CH_2)_2$), 2.50-2.70(m, 4H, piperazinyl-$N(CH_2)_2$), 2.80-3.00(m, 4H, piperazinyl-$N(CH_2)_2$), 3.05-3.20(m, 4H, $2xCH_2$), 3.65(s, 3H, OCH_3), 6.70-7.00(m, 4H, ArH); **(4d)** Oil. MS : m/z 377 (m^+). ^1H NMR (90 MHz, $CDCl_3$)δ: 2.20-2.30(m, 2H, $COCH_2$), 2.35-2.45(m, 2H, NCH_2), 2.50-2.60(m, 1H, COCH), 3.00-3.25(m, 8H, piperazinyl-$N(CH_2CH_2)_2$), 3.35-3.50(bs, 4H, $N(CH_2)_2$), 3.60-3.80(m, 7H, OCH_3 & $N(CH_2)_2$), 6.90-7.10(m, 4H, ArH); **(5)** Oil, MS : m/z 334 (m^+). ^1H NMR (90 MHz, $CDCl_3$)δ: 1.60-1.95(m, 8H, piperidinyl-$N(CH_2CH_2)_2$, 2.00-2.05(m, 2H, CH_2), 2.15(s, 3H, $COCH_3$), 2.40-2.50(m, 1H, COCH), 2.75-2.95(m, 2H, NCH_2), 3.65(s, 3H, OCH_3), 3.90-4.05(m, 2H, OCH_2), 6.60-7.30(m, 4H, ArH).
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