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Synthesis and biological evaluation of novel 6-nitro-5-substituted aminoquinolines as local anesthetic and anti-arrhythmic agents: molecular modeling study

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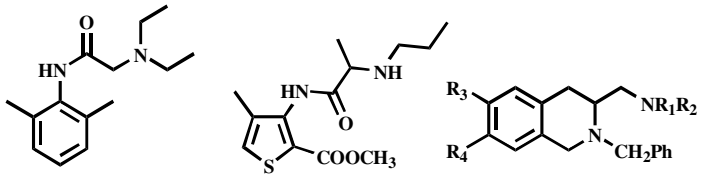
Abstract—A series of 6-nitro-5-[1-oxo-2-(substituted amino)ethylamino and 2-(substituted amino)propylamino] quinoline (**4a-i** and **5a-i**) was synthesized and evaluated for their local anesthetic and anti-arrhythmic activity. The detailed synthesis, spectroscopic, and biological data are reported. Molecular modeling methods are used to study the local anesthetic activity of lidocaine and the active compounds by means of the AM1 method. The superposition of the stable conformations of these compounds was studied using the HyperChem 5.11 program.

1. Introduction

Local anesthetics are of a wide use in minor surgical procedures in dentistry and ophthalmology.[1,2] Lidocaine **I** and carticaine **II** (Fig. 1) are anilide containing local anesthetics. Agents of this class are stable toward hydrolysis, more potent, having a lower frequency of side effect and induce less local irritation than the ester-type local anesthetics such as procaine.[3] They are characterized by the following structural features: (a) a lipophilic moiety usually of aromatic nature; (b) an intermediate amide chain, and (c) a hydrophilic part often a substituted amino group. These structural requirements are essential for the local anesthetic activity.[3]

In the course of research on drugs with anti-arrhythmic activity, it was found that 1,2,3,4-tetrahydro-*N*-alkyl-2-(phenylmethyl)-3-isoquinoline methanamine[4] **III** (Fig. 1) exhibited good potency. It was of interest to synthesize a series of compounds having general structures **4a**–**i** and **5a**–**i** in which the thiophene ring of carticaine **II** and 1,2,3,4-tetrahydro isoquinoline moiety of **III** was replaced by 6-nitroquinoline ring. Substitution with 2-acetyl, 2-propionyl derivatives have been utilized to represent non-branched and branched chains and also the type of substituents in hydrophilic basic center were chosen based on several reported find-ings.[5–12] This investigation is presented to evaluate the effect of these structural alteration on the local anesthetic and



I II III

Figure 1. The major reported local anesthetic.

Keywords: Local anesthetics; Antiarrhythmic agents; Xylocaine; 6-Nitro-quinoline; Molecular modeling.
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anti-arrhythmic activity of the parent compound lidocaine (xylocaine) as a trial to find new lead compounds with short onset and long duration of action.

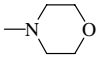
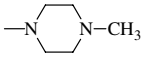
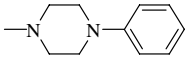
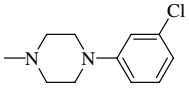
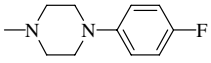
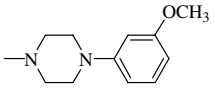
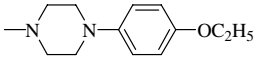
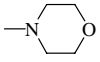
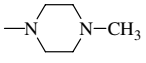
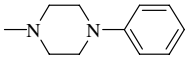
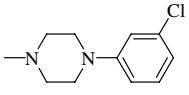
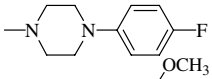
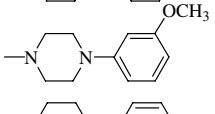
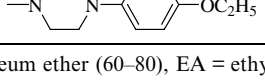
2. Results and discussion

2.1. Chemistry

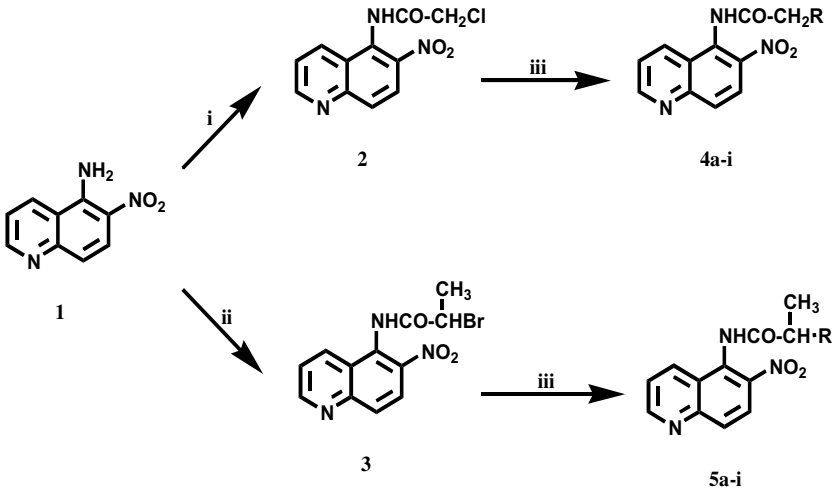
The starting 5-amino-6-nitroquinoline **1** was alkylated using chloroacetyl chloride or 2-bromopropionyl chloride in dry toluene to produce the amide derivative 6-nitro-5–(1-oxo-2-chloro-ethylamino)quinoline **2** and 6-nitro-5([1-oxo-2-bromopropylamino)quinoline **3**, respectively. The chlorine was displaced with a variety of appropriate amines to produce the target compounds 6-nitro-5-[1-oxo-2-(substituted amino)ethylamino]quinolines **4a**–**i** and 6-nitro-5-[1-oxo-2-(substituted amino)propylamino]quinolines **5a**–**i**, respectively (Table 1, Scheme 1).

2.1.1. Evaluation of local anesthetic activity. All the synthesized compounds were subjected to three different

Table 1. Recrystallization solvents, melting points, yield % and molecular formulae of compounds (**2**–**5**)

Compd. no.	R	Yield (%)	MP (°C)	Solvent[a]	Molecular formulae
2	—	95	215–217	E	$C_{11}H_8ClN_3O_3$
3	—	85	248–250	E	$C_{12}H_{10}BrN_3O_3$
4a	–NHEt	52	211–213	P	$C_{13}H_{14}N_4O_3$
4b		60	255–257	EA	$C_{16}H_{18}N_4O_3$
4c		63	264–266	EA	$C_{15}H_{16}N_4O_4$
4d		58	250–252	P	$C_{16}H_{19}N_5O_3$
4e		45	225–227	EA	$C_{21}H_{21}N_5O_3$
4f		55	228–230	C	$C_{21}H_{20}ClN_5O_3$
4g		58	270–272	C	$C_{21}H_{20}FN_5O_3$
4h		50	210–212	C/E	$C_{22}H_{22}N_5O_4$
4i		49	240–242	E	$C_{23}H_{25}N_5O_4$
5a	–NHEt	57	212–214	P	$C_{14}H_{16}N_4O_3$
5b		46	220–222	C	$C_{17}H_{20}N_4O_3$
5c		59	240–242	E	$C_{16}H_{18}N_4O_4$
5d		52	218–220	EA	$C_{17}H_{21}N_{15}O_3$
5e		48	225–227	E/C	$C_{22}H_{23}N_5O_3$
5f		50	228–230	C	$C_{22}H_{22}ClN_5O_3$
5g		55	242–244	E	$C_{22}H_{22}FN_5O_3$
5h		48	198–200	E/C	$C_{23}H_{25}N_5O_4$
5i		42	238–240	E/C	$C_{24}H_{27}N_5O_4$

[a] E = ethanol, P = petroleum ether (60–80), EA = ethyl acetate, C = chloroform.



Scheme 1. Reagents: (i) chloroacetyl chloride; (ii) 2-bromopropionyl chloride; (iii) ethylamine, piperidine, morpholine, 4-methylpiprazine, 4-phenylpiprazine, 4-(2-chlorophenyl)piprazine, 4-(4-fluorophenyl)piprazine, 4-(2-methylphenyl)piprazine or 4-(4-ethoxyphenyl)piprazine.

models for evaluation of their local anesthetic activity, namely, frog limb withdrawal, rabbit corneal reflex and guinea-pig wheal derm tests. Xylocaine (Positive control), normal saline (Negative control) and dimethylsulfoxide; DMSO; (Solvent) were also subjected to the three models parallel to the compounds. Among the tested compounds, only compounds **4g** and **5d** have shown local anesthetic activity in the three models, with onsets of action close to that of xylocaine. The solvent (DMSO) did not show activity that may interfere in the interpretation of the results.

2.1.2. Evaluation of anti-arrhythmic activity. Compounds **4g** and **5d** that have shown local anesthetic activity were tested for their anti-arrhythmic activity against calcium chloride induced arrhythmia in mice. Xylocaine and DMSO were also tested parallel to the compounds. However, compounds **4g**, **5d**, and xylocaine have shown a significant decrease in the magnitude of the initial bradycardia than control group (Normal saline). In addition, they protected animals from the incidence of fibrillation and death. DMSO has shown no activity. The results are shown in Figure 2.

2.1.3. Acute toxicity determination. Compounds **4g** and **5d** that have shown local anesthetic and anti-arrhythmic activities were subjected further for determination of their LD_{50}. However, no deaths were found in the groups injected by the two compounds at doses of 1, 10, 100, 500 and 1000 mg/kg, respectively.

2.2. Discussion

The newly synthesized compounds, which are rationally synthesized to have local anesthetic activity, were subjected to three different models for their local anesthetic activity. In the first method, frog limb withdrawal method, only compounds **4g** and **5d** have shown local anesthetic activity, with onset of action close to that of xylocaine. In this method, the preparation may lose its viability after 20 min. No recovery occurred within 20 min for the tested compounds and

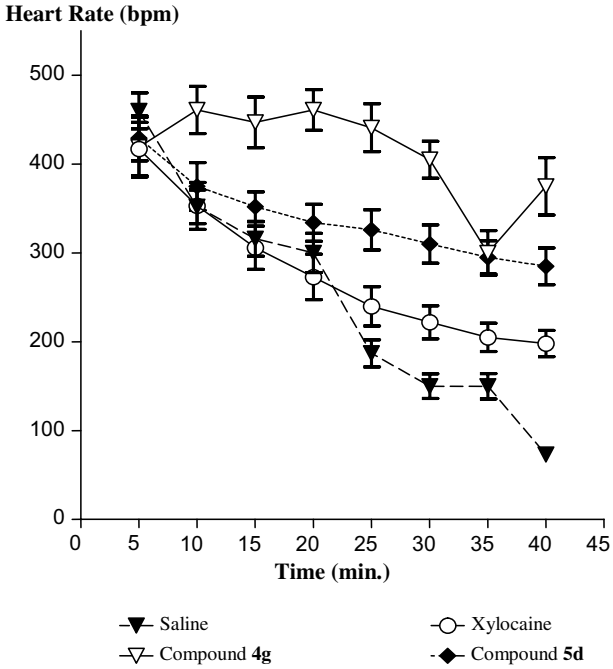


Figure 2. Effect of pretreatment with normal saline, xylocaine **I**, compounds **4g** and **5d** on the change in heart rate induced by i.p. injection of $CaCl_2$ in mice at different time intervals. Drugs were injected i.p. 25 min before $CaCl_2$ injection. Values are expressed as mean ± SEM (six animals in each group).

xylocaine, so it was not possible to determine the duration of action. However, some other tested compounds have shown local anesthetic activity, in some and not all preparations, with onsets close to 20 min, so it cannot be reported a local anesthetic activity for these compounds.

In rabbit corneal reflex, also compounds **4g** and **5d** exhibited a local anesthetic activity with onset and duration of action very close to those of xylocaine. This method tests the activity of the local anesthetics, which is useful in topical anesthesia. Then, another

criterion in these compounds can be added by this method.

The guinea pig derm wheal test gave us another proof for the close similarity in pharmacodynamics between these compounds and xylocaine as local anesthetics. The two compounds also showed a local anesthetic activity with onset of action close to xylocaine. The duration of action of the xylocaine and the two compounds exceeded 60 min. However, the difference in onset and duration of action in our assays from those reported by other workers may be due to the difference in experimental approaches.

In the screening of their anti-arrhythmic activity, also compounds **4g** and **5d** have only shown activity. Unlike the local anesthetic activity, the activity of the two compounds seems to be superior on that of xylocaine. However, this can be explained on the difference in pharmacokinetic profiles of these compounds than those of xylocaine or it may be due to difference in Na-channels subtypes blocked by these agents.

In the models used for screening of the local anesthetic activity and anti-arrhythmic activity, dimethyl sulfoxide, the solvent in which the test compounds were dissolved had shown no activity, indicating that the local anesthetic and anti-arrhythmic activities of the tested compounds were due to the compounds themselves rather than DMSO.

3. Molecular modeling analysis

An attempt to gain a better insight on the molecular structures of the active compounds **4g** and **5d** compared with standard drug lidocaine **I**, conformational analysis of the target compounds has been performed by use of the MM+[13] force field (calculations in vacuo, bond dipole option for electrostatics, Polak-Ribiere algorithm, RMS gradient of 0.1 kcal/Å mol). as implemented in HyperChem 5.1.[14] The starting atomic coordinate of the target compounds were obtained from the X-ray data of lidocaine **I**.[15] Calculation of the isopotential molecular surface was performed with hyperchem 5.1. In order for these ligands to bind to the same receptor site they would all have to allow for conformation that show (i) equal distance of the carbonyl oxygen to the amine nitrogen, (ii) equal (strain angle) torsion angles between the CO- and the NH-group, and (iii) equal distance between the hydrophobic part; aromatic ring and hydrophilic part; tertiary nitrogen.[3,16] Accordingly, the molecular parameters of the most stable conformers of compounds **4g**, **5d** and standard drug lidocaine **I**, such as, non-bonded distance ($\mathbf{d_1}$) between the centroid of aromatic ring and terminal nitrogen, non-bonded distance ($\mathbf{d_2}$) between the carbonyl group and terminal nitrogen (O═C–C–N), the plane angle (τ_1) represented by the torsional angle between the aromatic core and amide moiety ∠C═C–N–CO, the strain angle (τ_2) represented by the torsional angle between the carbonyl group and terminal nitrogen ∠O═C–C–N, were obtained by full geometry optimization with semi-empirical AM1[17] molecular orbital method, as shown in Figure 3 and Table 3. Compounds **4g** and **5d** exhibit strong structural similarity to lidocaine **I** and therefore should yield good geometrical and electrostatic correspondence as indicated by their molecular parameters (Table 3). The results show that the occurrence of two major conformations for **4g**, **5d** and lidocaine **I**, anti-conformers and *syn*-conformers (Fig. 3). The anti-conformers for compounds **4g** and **5d** such as **4g-Anti**, **5d-Anti**, respectively, have the same molecular parameters and are identical with anti-conformer of lidocaine **I-Anti**, in which the non-bonded distance ($\mathbf{d_1}$) was 5.6 Å, the non-bonded distance ($\mathbf{d_2}$) was 3.6 Å and the strain angle was 143°–144° (Table 3). The variation in the plane angles between **4g-Anti**, **5d-Anti** (120.6°–121.6°) and **I-Anti** (113.6°) may be due to the occurrence of strong hydrogen bond between NH and O═N═O group (2.2 Å). Similarly, the *syn*-conformers for compounds **4g** and **5d** such as **4g-Syn**, **5d-Syn**, respectively, have approximate molecular parameters and are identical with *syn*-conformer of lidocaine **I-Syn**, in which the non-bonded distance ($\mathbf{d_1}$) was 6.2–6.4 Å, the non-bonded distance ($\mathbf{d_2}$) was 3.0–3.1 Å. The differences in the strain angles and plane angle of **4g-Syn**, **I-Syn** compared with **5d-Syn** may be due to the steric effect of α-methyl group in compound **5d** which prohibit the free rotation around amide moiety. As clear from the calculation, due to a low barrier of rotations which were lower than 1 kcal/mol, all possible isomers of compounds **4g**, **5d**, and lidocaine **I** can be existing. It is noteworthy to say that the structural similarity among the compounds **4g**, **5d**, and lidocaine **I** was responsible for similar biological activity as evident from the experimental data (Table 2, Fig. 2). It is worth mentioning that even though the calculations were done in vacuo the data closely resembles a conformation of lidocaine that has been found to be one possible preferred conformation in solution.[18] This finding justifies the assumption made throughout this work that the calculated conformations are also representatives of solution structures.

Further evidence for identical binding of lidocaine and compounds **4g**, **5d** comes from QSAR data that were derived from the QSAR-Properties module of HyperChem using the anti-conformations depicted in Figure 3, where the hydrophobic ($\log P$), dipole moment, and steric properties (refractivity) were calculated for the conformations depicted. QSAR studies have been carried out on a variety of general anesthetics resulting in an optimum hydrophobicity close to $\log P = 2.3$, regardless of the class of anesthetics.[19] As clear from the calculation, $\log P$ values of lidocaine and **5d** are well in this range while **4g** is of somewhat lower value. In addition, measurements of global molecular parameters (surface area, volume, refractivity) also reflect their similarity.

Moreover, the anti-conformers **4g-Anti**, **5d-Anti**, and lidocaine **I-Anti** resulting from computational chemistry analysis as a representative example and the X-ray structure of lidocaine were superimposed in order to reveal the similarities and differences in structure (Fig. 4).

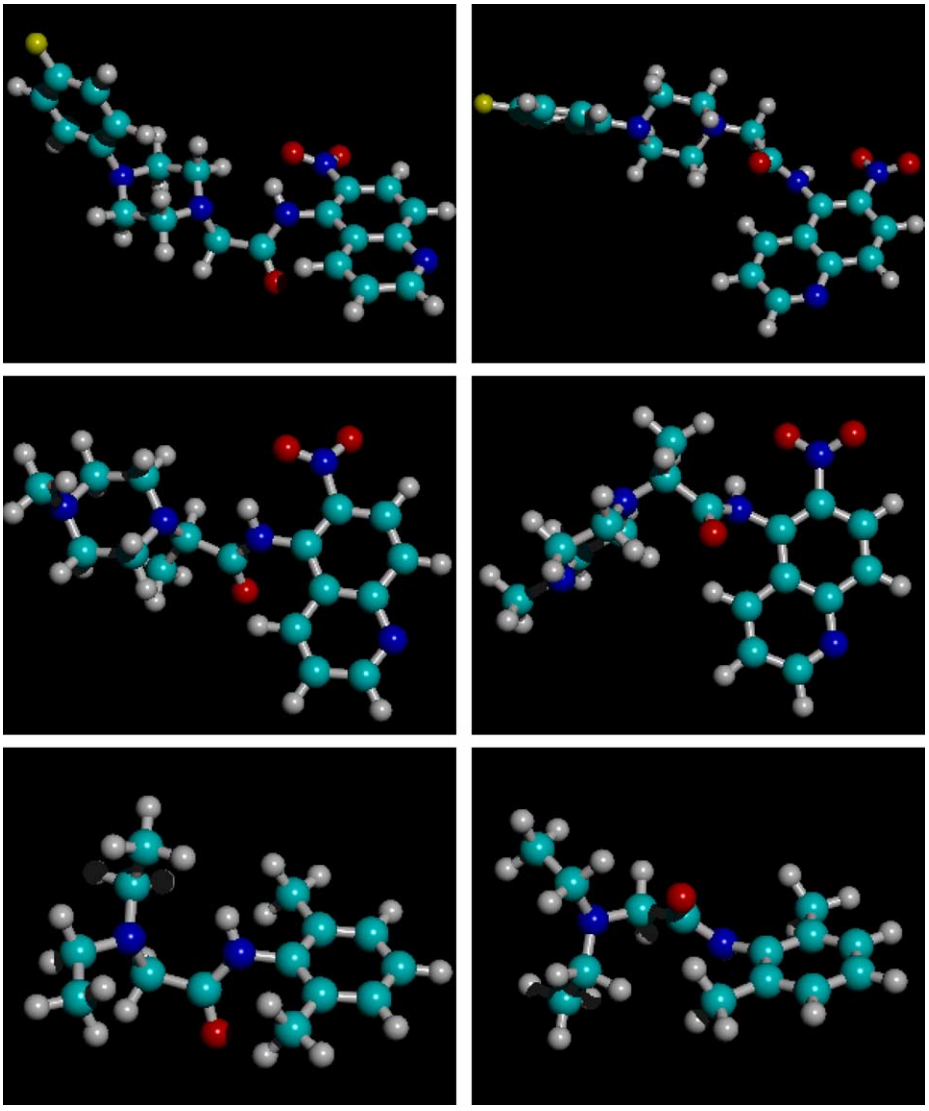


Figure 3. Ball and stick rendering for the most stable conformers of compound **4g**, **5d**, and **I** (from above to below, respectively) showing anti-conformers on the left and *syn*-conformers on the right.

The fitting was carried out with respect to polar groups including the carbonyl group and tertiary nitrogen group. The results show that some deviation of the superimposed structures was observed in the region of the hydrophilic amine group only. Furthermore semi-empirical calculations of the conformers displayed in Figure 5 also demonstrate their closely related charge distribution and electrostatic potential suggesting a similar interaction of the molecules with a potential protein binding site. As a result we find that lidocaine, **4g** and **5d** all can adopt conformations that have identical distances and orientations of the assumed major protein binding groups (N–H, C═O) and that show sufficient agreement of the overall structure and of the electrostatic potential pattern. Consequently these conformations permit approach to a protein surface in much the same way connecting to a binding site that fits all three ligands. They can thus be regarded as the active binding conformations. Further reorientation at the site will lead to an induced fit different for the specific ligands and responsible for variations in their binding affinity.

4. Experimental

4.1. Synthesis

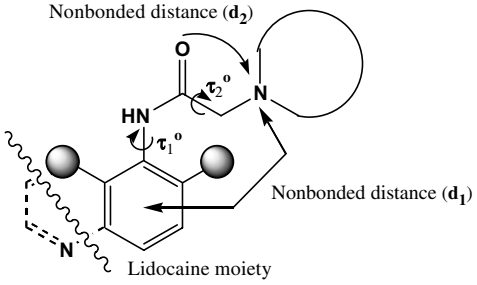
Melting points are recorded on a Fischer–Johns melting point apparatus (°C, uncorrected) IR Spectra were recorded on Mattson 5000 FT-IR Spectrophotometer (in cm^{-1}). ^1H NMR spectra were recorded on a Varian EM −390 (90 MHz) instrument using TMS as an internal standard (chemical shift in δ ppm). Microanalytical data (C, H, N) agreed with proposed structures within ±0.4% of the theoretical values.

4.1.1. 6-Nitro-5-(2-chloro1-oxo-ethylamino)quinoline 2. A solution of 5-amino-6-nitroquinoline (1.89 g, 1.19 mL, 0.01 mol) in dry toluene (50 mL) was stirred at room temperature, while chloroacetyl chloride (1.69 g, 0.015 mol) was added dropwise. The reaction was then heated under reflux for 6 h, solvent was removed under vacuum and the obtained solid crystallized

Table 2. Local anesthetic activity of the synthetized compounds and xylocaine

Compd.	Concentration		Local anesthetic activity	Frog limb withdrawal onset (min)	Rabbit corneal reflex onset (min)	Guinea-pig wheal derm onset (min)
	% w/v	Mol × 10^{-2}				
Xylocaine	0.542	2	+	4.3 ± 0.58	5.03 ± 0.06	3.06 ± 0.27
4a	0.875	2	–	—	—	—
4b	0.625	2	–	—	—	—
4c	0.625	2	–	—	—	—
4d	0.650	2	–	—	—	—
4e	0.800	2	–	—	—	—
4f	0.800	2	–	—	—	—
4g	0.625	2	+	5.1 ± 0.4	5.67 ± 0.57	4.13 ± 0.31
4h	0.800	2	–	—	—	—
4i	0.875	2	–	—	—	—
5a	0.650	2	–	—	—	—
5b	0.800	2	–	—	—	—
5c	0.650	2	–	—	—	—
5d	0.700	2	+	4.2 ± 0.2	6.0 ± 0.59	3.28 ± 0.074
5e	0.850	2	–	—	—	—
5f	0.875	2	–	—	—	—
5g	0.625	2	–	—	—	—
5h	0.575	2	–	—	—	—

Table 3. Selected molecular parameters of compounds **4g**, **5d**, and lidocaine as calculated by semi-empirical AM1



Conformer	Plane angle τ_1^0 (C=C–N–CO)[a]	Strain angle τ_2^0 (N–CO–C–N)[b]	Non-bonded distance ($\mathbf{d_1}$)[c]	Non-bonded distance ($\mathbf{d_2}$)[d]	H_f (kcal/mol)[e]
4g-Anti	120.6	144.0	5.7	3.6	28.73
4g-Syn	−121.6	−35.38	6.4	3.0	29.57
5d-Anti	121.5	143.2	5.7	3.6	37.86
5d-Syn	−114.2	−66.6	6.2	3.1	38.78
I-Anti	113.6	144.9	5.7	3.6	−25.83
I-syn	−119.2	−48.5	6.3	3.0	−25.52

[a] Torsional angel between the plane of aromatic nucleus and amide moiety.
[b] Torsional angel between the amide carbonyl and *tert*-nitrogen.
[c] Non-bonded distance between aromatic centroid and *tert*-nitrogen.
[d] Non-bonded distance between CO and *tert*-nitrogen.
[e] Heat of formation.

(Table 1). ^{1}HNMR (DMSO-d_6) δ 4.49 (s, 2H, CH_2), 6.8 (s, 1H, NH), 7.0–7.5 (m, 5H, ArH).

4.1.2. 6-Nitro-5-(2-bromo1-oxo-propylamino)quinoline 3. 2-Bromopropionyl chloride (2.57 g, 1.51 mL, 0.015 mol) was added dropwise to a stirred solution of compound **1** (1.89 g, 0.01 mol) in dry toluene (50 mL). The reaction mixture was then heated under reflux for 8 h. The solvent was evaporated in vacuum. The obtained residue was triturated with toluene, filtered, dried, and crystallized (Table 1). ^{1}HNMR (DMSO-d_6) 1.90–1.93 (d, 3H, J = 7.0 Hz, $CHCH_3$), 4.50–4.71 (q, 1H, J = 7.0 Hz, –$CHCH_3$), 7.0 (s, 1H, NH), 7.42–7.80 (m, 5H, ArH).

4.1.3. 6-Nitro-5-[1-oxo-2-(substituted amino)ethylamino]-quinolines 4a-i. A solution of **2** (2.65 g, 0.01 mol) in dry toluene (50 mL) was stirred at room temperature while (0.04 mol) of the appropriate amine was added dropwise, the reaction mixture was heated under reflux for 8–10 h. The solvent was then evaporated in vacuo, the obtained residue was washed with cold toluene and then crystallized (Table 1). ^{1}HNMR (DMSO-d_6) δ, **4a**: 1.18–1.22 (t, 3H, J = 7.0 Hz, –CH_2CH_3), 2.80–3.02 (q, 2H, J = 7.0 Hz, CH_2CH_3), 3.50 (s, 2H, CH_2), 7.02 (s, 1H, NH), 7.42–7.90 (m, 5H, ArH). **4b**: 1.32–1.67 (m, 6H, piperidine–H), 3.36–3.70 (m, 4H, piperidine–H), 3.24 (s, 2H, CH_2), 6.88 (s, 1H, NH), 7.42–7.94 (m, 5H, ArH). **4c**: 2.40–2.70 (m, 4H, morpholine–H), 3.26 (s, 2H, CH_2), 3.80–3.92 (m, 4H, morpholine–H), 7.0 (s, 1H, NH), 7.52–8.0 (m, 5H, ArH). **4d**: 2.26 (s, 3H, CH_3), 2.28–2.34 (m, 8H, piperazine–H), 3.25 (s, 2H, CH_2) 6.90 (s, 1H, NH), 7.44–7.95 (m, 5H, ArH). **4e**; δ

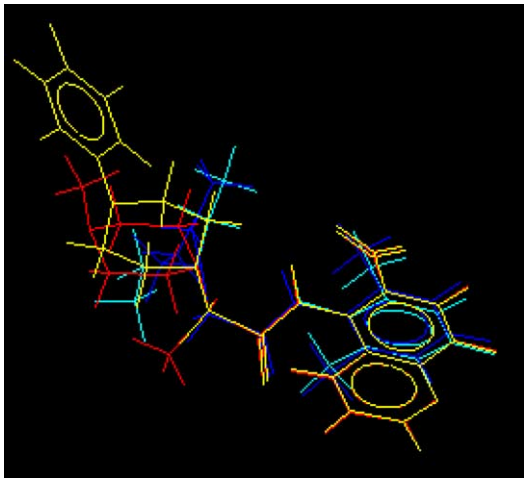


Figure 4. Superimposition of lowest energy anti-conformers **4g-Anti** (yellow), **5d-Anti** (red), **I-Anti** (cyan), respectively, and X-ray structure of lidocaine (blue).

2.70–3.0 (m, 4H, piperazine–H), 3.24 (s, 2H, CH_2), 3.40–3.62 (m, 4H, piperazine–H), 7.0 (s, 1H, NH), 7.40–8.08 (m, 10H, ArH). **4f**: 2.28–2.32 (m, 4H, piperazine–H), 3.30 (s, 2H, CH_2), 3.44–3.62 (m, 4Hpiperazine–H), 6.90 (s, 1H, NH), 7.24–7.88 (m, 9H, ArH). **4g**: 2.31–2.41 (m, 4H, piperazine–H), 2.60–2.69 (m, 4H, piperazine–H), 3.40 (s, 2H, CH_2), 6.6 (s, 1H, NH), 7.0–7.72 (m, 9H, ArH). **4h**: 2.33–2.38 (m, 8H, piperazine–H), 3.32 (s, 2H, CH_2), 4.02 (s, 3H, OCH_3), 6.80 (s, 1H, NH), 7.20–7.92 (m, 9H, ArH). **4i**: 1.19–1.22 (t, 3H, J = 7.0 Hz, CH_2CH_3), 2.62–3.0 (m, 8H, piperazine–H), 3.20 (s, 2H, CH_2) 3.30 (s, 2H, CH_2), 3.98–4.10 (q, 2H, J = 7.0 Hz, CH_2CH_3), 7.0 (s, 1H, NH), 7.41–7.98 (m, 9H, ArH).

4.1.4. 6-Nitro-5-[1-oxo-2-(substituted amino)propylamino]-quinolines 5a–i. A solution of **3** (3.2 4 g, 0.01 mol) in dry toluene (50 mL) was stirred at room temperature, while (0.014 mol) of the appropriate amine was added while stirring. The reaction mixture was heated under reflux for 6–10 h. The solvent was then evaporated in vacuo, the obtained residue was washed with cold toluene and then crystallized (Table 1). ^{1}HNMR (DMSO-d_6) δ, **5a**: 1.0 6–1.20 (t, 3H, J = 7.2Hz, CH_2–CH_3), 1.33–1.36 (d, 3H, J = 7.2 Hz, CH_2CH_3), 3.18–3.30 (q, 2H, J = 7.0 Hz, CH_2CH_3), 4.20–4.32 (q, 1H, J = 7.0 Hz, $CHCH_3$), 7.2 (br s, 1H, NH), 7.4–7.8 (m, 5H, ArH). **5b**: 1.20–1.66 (m, 6H, piperidine–H), 1.44–1.46 (d, 3H, J = 7.0 Hz, $CHCH_3$), 2.60–3.20 (m, 4H, piperidine–H), 3.60–3.80 (q, 1H, J = 7.0 Hz, –$CHCH_3$), 7.0 (br s, 1H, NH), 7.40–7.90 (m, 5H, ArH). **5c**: 1.48–1.60 (d, 3H, J = 7.0 Hz, $CHCH_3$), 3.60–3.80 (m, 4H, morpholine–H), 4.22–4.26 (q, 1H, J = 7.0 Hz, $CHCH_3$), 6.40 (br s, 1H, NH), 7.22–7.82 (m, 5H, ArH). **5d**: 1.58–1.62 (d, 3H, J = 7.0 Hz, –$CHCH_3$), 2.28 (s, 3H, CH_3), 3.0–3.62 (m, 8H, piperazine–H), 4.12–4.14 (q, 1H, J = 7.0 Hz, –$CHCH_3$), 6.80 (br s, 1H, NH), 7.20–7.66 (m, 5H, ArH). **5e**: 1.48–1.52 (d, 3H, J = 7.0 Hz –$CHCH_3$), 2.80–3.0 (m, 4H, piperazine–H), 3.40–3.44 (m, 4H, piperazine–H), 4.22–4.24 (q, 1H, J = 7.0 Hz, $CHCH_3$), 6.80 (br, 1H, NH), 7.32–7.98 (m, 10 H, ArH). **5f**: 1.48–1.52 (d, 3H, J = 7.2 Hz, –$CHCH_3$), 2.82–3.12 (m, 4H, piperazine–H), 3.40–3.62 (m, 4H, piperazine–H), 4.20–4.24 (q, 1H, J = 7.2 Hz, –$CHCH_3$), 6.80 (br s, 1H, NH), 7.0–7-80 (m, 9H, ArH). **5g**: 1.47–149 (d, 3H, J = 7.2 Hz, –$CHCH_3$), 2.80–3.14 (m, 4H, piperazine–H), 3.42–3.48 (m, 4H, piperazine–H) 4.10–4.13 (q, 1H, J = 7.2 Hz, –$CHCH_3$), 7.10 (br s, 1H, NH), 7.42–8.0 (m, 9H, ArH). **5h**: 1.44–1.49 (d, 3H, J = 7.0 Hz, –$CHCH_3$), 2.30–2.36 (m, 8H, piperazine–H), 3.80 (s, 3H, OCH_3), 4.21–4.24 (q, 1H, J = 7.0 Hz, –$CHCH_3$), 6.88 (br s, 1H, NH), 7.22–7.88 (m, 9H, ArH) **5i**: 1.50–1.52 (d, 3H, J = 7.0 Hz, $CHCH_3$), 1.34–1.38 (m, 6H, –CH_2CH_3 and $CHCH_3$) 2.67–2.76 (m, 4H, piperazine–H), 3.22–3.26 (m, 4H, piperazine–H), 3.39–3.42 (q, 1H, J = 7.0 Hz, $CHCH_3$), 4.26–4.40 (q, 2H, J = 7.2 Hz, –CH_2CH_3), 6.88 (br s, 1H, NH), 7.2–7.92 (m, 9H, ArH).

4.2. Evaluation of local anesthetic activity

For each test compound six animals were used to test its activity in each of the following models of local anesthesia. Drugs used were Lidocaine hydrochloride (Xylocaine 2% solution, Astro zeneca Ph. Co., Sweden, purchased from the market), normal saline (Nile Pharmaceutical Co., Egypt, purchased from the market), Thiopental sodium vial (Eipico Ph. Co., Egypt, purchased from the market).

4.2.1. Frog limb withdrawal method.[20] Frogs (30–40 g) were decapitated and the abdominal cavity was emptied through a small transverse incision. The cavity was filled with the test solution at a concentration of 20 mM dissolved in dimethyl sulfoxide (DMSO). The limb

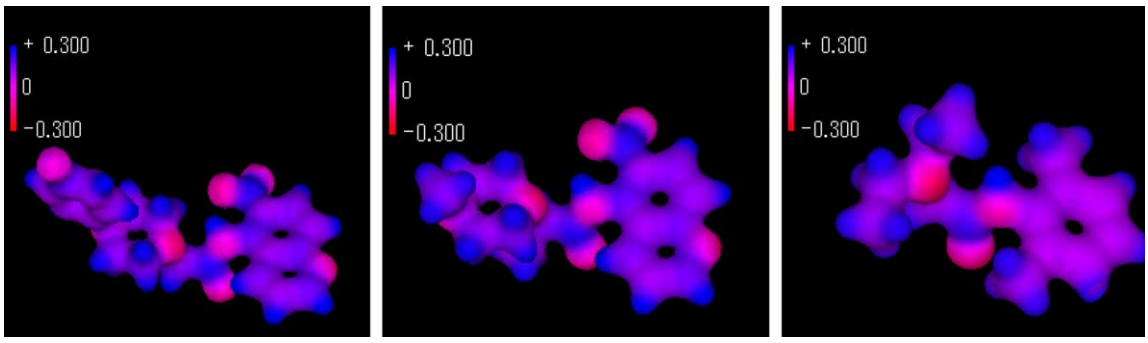


Figure 5. Electrostatic potential isosurface of the lowest energy Anti-conformers **4g-Anti** (left), **5d-Anti** (middle) and **I-Anti** (right), negative region colors red and positive region colors blue.

withdrawal reflex was tested prior to drug instillation and every 1 min thereafter for a maximum of 20 min using 0.1 N HCl solution. The onset time 'time of failure of withdrawal of the limb when immersed in HCl' was determined for each test compound, saline, DMSO, and the standard local anesthetic, lidocaine (20 mM, in saline).

4.2.2. Rabbit corneal reflex.[8,12] White New Zealand rabbits (3.4 kg) were placed individually in restraining cages. Test solutions (20 mM in DMSO) were instilled into the conjunctival sac in a volume of 0.5 mL during 1–2 min. The eyelid was closed manually for 2 min. The corneal reflex was tested using a pointer every 2 min. The onset time 'time of loss of corneal reflex' was determined for each test compound, saline, DMSO, and lidocaine (20 mM in saline).

4.2.3. Guinea-pig wheal derm test.[8,12] Guinea-pig (400–600 g) was used. Twenty four hours before the test, their hair was shaved on an area (5 × 5 cm) on the lower back. A solution of 0.1 mL of each test solution, lidocaine (0.5%w/v) and saline were injected intradermally to form a wheal. Each site was injected six times every 2 min for up to 60 min. The time elapsed following the injection of the test solution to the failure of the animal to respond by skin twitch was taken as the onset time for the local anesthesia. This time was recorded for each test drug.

4.3. Evaluation of the anti-arrhythmic activity[21]

Mice (20 g) were anesthetized with 50 mg/kg thiopental solution (0.5 g%) in distilled water, and prepared for recording of ECG. ECG lead **II** were recorded by the aid of subcutaneous needle electrode and the ECG records was displayed by Fukuda cardisuny, model B-501 III, Japan. The recording speed was 5 cm/s. After stabilization of the animals, cardiac arrhythmia were induced by a single bolos i.p. injection of 10% aqueous solution of $CaCl_2$ (50 mg/kg). The induced arrhythmia were then analyzed for magnitude of the initial bradycardia, incidence and duration of the induced fibrillation. After induction of the arrhythmia, each animal was allowed to recover completely (10–15 min) and each of the test compound, DMSO, and lidocaine was injected in a dose of 40 mg/kg intraperitoneally. The effect of the drug on the basal heart rate was then examined. Thirty minutes later, the arrhythmogenic dose of $CaCl_2$ was re-administered and the effect of the test solution on the induced arrhythmia parameters indicated above, was then evaluated as a percentage change in the measured parameters or as protection or non-protector against the induced fibrillation.

4.4. Acute toxicity determination[22]

Five groups of mice, each consists of six animals were used. The test compounds were injected intraperitoneally in a doses of 1, 10, 100, 500 and 1000 mg/kg, respectively. Twenty four hours later, the percentage mortality in each group was recorded and the lethal dose (LD_{50}).

5. Conclusion

We successfully conclude that 6-nitro-5-[1-oxo-2-(substituted amino)ethylamino and 2-(substituted amino)propylamino] quinolines, which carry cyclic amines (*N*-substituted piperazine) as hydrophilic basic center proved to have a good local anesthetic and anti-arrhythmic activity especially as they have shown to be very safe in the determination of their LD_{50}, They were considered to be lead compound deserving further investigation. Semi-empirical molecular orbital calculation shows lidocaine-like structural features for **4g** and **5d**, which can cause observed lidocaine-like activity in physiological experiments. The reported results should serve as an element to understand pharmacophore requirements for local anesthetics, the identification for which may lead to rational design of new ligands.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.02.050.

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