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STUDY OF LOCAL ANESTHETIC ACTIVITY OF SOME DERIVATIVES OF 3-AMINO-BENZO-[*d*]-ISOTHIAZOLE*

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On the basis of computer prediction of biological activity by PASS and toxicity by DEREK, the most prospective 18 alkylaminoacyl derivatives of 3-amino-benzo-[*d*]-isothiazole were selected. Their local anesthetic action was assessed using an *in vitro* preparation of the isolated peroneal nerve of the frog. The local anesthetics action of the compounds was assessed according to the time required for each compound to reduce the amplitude of the evoked compound action potential (CAP). Lidocaine was used as the control compound. The results show that the tested compounds can be divided into three groups: (a) compounds with action similar to lidocaine, (b) compounds with action lower than lidocaine and (c) compounds which block completely the evoked CAP, but after the compound was removed and replaced with normal saline showed no recovery of the potential at all. QSAR studies showed that polarizability, polarity and presence of five-membered rings in molecules have a positive influence on local anesthetic activity, while contributions of aromatic CH and singly bonded nitrogen are negative. Since estimations from PASS probabilities to find local anesthetic activity in the most active compounds were less than 50%, these compounds may be considered as new chemical entities (NCEs).

Keywords: Local anesthetics; 3-Amino-benzo-[*d*]-isothiazole; Compound action potential; Sciatic nerve; *In vitro*; Computer prediction

INTRODUCTION

Local anesthetics, compounds inactivating the voltage gated sodium channels of myelinated or unmielinated axons, are widely used pharmaceutical agents, due to their effects in many acute and chronic pain-generating situations. Following the classical scheme of Lofgren [1], structural requirements for a local anesthetic are: a lipophilic (aromatic) head, a hydrophilic end bearing a tertiary amine, and an intermediate substituted alkyl chain. Compounds having

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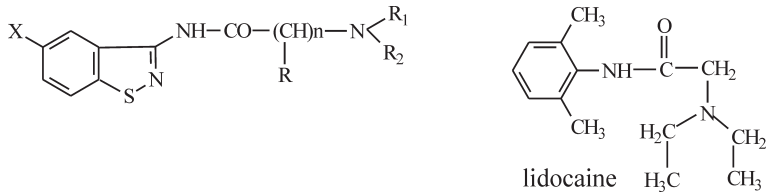


FIGURE 1 General formula of the tested and reference compounds.

an amino group linked to a heterocyclic nucleus as the lipophilic moiety display a greater activity and less toxicity than benzene analogues [2,3]. Some derivatives of 2-aminobenzothiazole [4–7] and 2-aminothiazoles [8–12] as well as 3-amino-benzo-[*d*]-isothiazole [13–14] are reported to posses local anesthetic activity. Keeping these facts in mind we have synthesized different thiazole and isothiazole derivatives. Several compounds among these heterocyclic derivatives were found to possess local anesthetic properties [13–19]. Among these compounds a number of 3-(alkylaminoacyl)a-mino-benzo[*d*]isothiazoles carrying different anesthesioforic basic moieties, lidocaine-like proved to be active in infiltration and trunkular anaesthesia [17].

The purpose of this work is to assess and compare the possible local anesthetic action of 18 3-(alkylaminoacyl)amino-benzo[*d*]isothiazoles previously synthesized [17] (Fig. 1), using an *in vitro* preparation based on the isolated in saline peroneal nerve of the frog. A similar preparation has previously been used to assess and classify successfully the action of other local anesthetics [20–22] and the neurotoxicity of other compounds [23–27]. To increase the chance for selecting the most prospective designed structures (probable anesthetic activity, high structural novelty, small likelihood of toxic action) predictions of biological activity and toxicity have been carried out with computer programs PASS [28] and DEREK[‡], respectively.

MATERIALS AND METHODS

Chemistry

The starting amine (R = H, CH_3) was prepared according to the method previously described [18,19]. The key intermediate B, 3-(haloacyl)amino-benzo-[*d*]-isothiazoles, were synthesized by reacting amine A with haloacylhalogenides. The final products were obtained by reaction between the various amines and the 3-(haloacyl)amino-benzo-[*d*]-isothiazoles B (Fig. 2). The designed and selected for testing hydrocloride compounds **1**–**18** (Table I) were obtained by dissolving the free bases, showed in Fig. 1, in anhydrous benzene saturated with gaseous hydrogen chloride. All compounds were obtained in good yields and crystallized from EtOH/Et_2O [17].

Biological Action

Frogs (*Rana ridibunda*), of either sex, weighing between 100 and 120 g were used. The animals were sacrificed with deep anesthesia. The nerve (sciatic and peroneal) was dissected and mounted across a three-chambered bath made of Plexiglas. Then chambers

[‡]http://www.chem.leeds.ac.uk/luk/

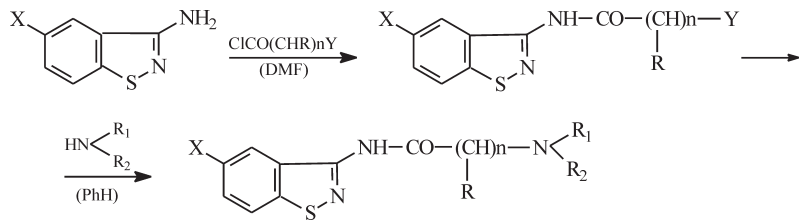


FIGURE 2 Synthetic pathway for preparation of the tested compounds.

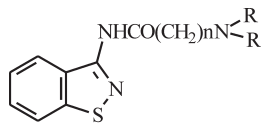
were filled with oxygenated physiological solution (100% O_2) of the following concentration (in mM l-1): 111 NaCl, 2.41 KCl, 10 HEPES, 2 $CaCl_2$, and 10 glucose, pH = 7.2. All the experiments were performed at 20.0° ± 0.5°C. A similar recording bath was used to assess the local anesthetic action of other compounds [20–22]. Using standard electrophysiological methods the evoked nerve compound action potential (CAP) of the isolated nerve was recorded (Fig. 3). Samples of the evoked CAPs were stored to a computer every 1 min throughout the experiment. The value of the integral of the CAP was calculated and measured automatically by the computer. This parameter contains information about the amplitude and the duration of the CAP. For the assessment of the local anesthetic action, the records were obtained until the CAP reached normal values (equilibration period), then it was incubated in saline where the compound under investigation was diluted at the desirable concentration (exposure period), and finally the nerve was washed and incubated in normal saline (recovery period). After the end of each experiment the percentage values of the CAP vs. time diagrams were plotted for the equilibration period, the exposure period and the recovery period (see Fig. 4). The outcome of many trials using of the same concentration of the compound ($n = 6$) were averaged and plotted as a single curve. The data were expressed as a mean ± S.E.M. Statistical analysis was performed using the one-way Anova test with post test (Dunnett) between the values of the averaged curves. The parameters used to evaluate the local anesthetic activity of each compound were: (a) the time required to decrease the CAP to 50% of the control values; this value is called as Inhibitory Time 50 (IT_{50}), and measured in min: (b) the time required to reduce the CAP to 0%, called IT_{100} and also measured in min: (c) the time required for the CAP to recover to the 50% of the control value after the replacement with control saline, called as Recovery Time 50 (RT_{50}), measured also in min.

Methods for Prediction of Biological Activity and Toxicity

Prediction of anesthetic activity was obtained with PASS [26,29,30][¶]. PASS version 1.603 predicts 783 types of biological activity on the basis of structural formulae of the compounds. Prediction is based on structure–activity relationships knowledgebase developed by the analysis of the training set included more than 45,000 known biologically active compounds. The number of local anesthetics in PASS training set is 408. The accuracy of prediction of local anesthetic activity is 84.9% (leave-one-out cross-validation). The result of prediction is presented as a list of activities with appropriate *P*a and *P*i, which are the estimates of probability to be active and inactive, respectively.

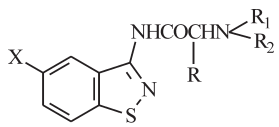
[¶]http://www.ibmh.msk.su/PASS

TABLE I Structures and lipophilicity of the compounds selected for testing



(A)

Compound number	*N(R)₂*	*n*	*C log P*	*IA log P*	*SRC log P*	*mean log P*
N1	$N(CH_3)_2$	1	1.28	1.85	0.88	1.34
N3	$N(C_2H_5)2$	1	2.258	2.44	1.86	2.19
N5	Morpholine	1	1.444	1.64	0.5	1.19
N6	Morpholine	2	1.641	1.86	0.59	1.36
N8	Piperidine	1	2.713	2.83	2.24	2.59
N10	Pyrrolidine	1	2.154	2.34	1.75	2.08
N15	$NHCH_3$	2	1.094	1.21	0.76	1.02
N18	NHC_2H_5	2	1.623	1.65	1.25	1.51



(B)

Compound number	*N(R₁R₂)*	*R*	*X*	*C log P*	*IA log P*	*SRC log P*	*mean log P*
N2	$N(CH_3)_2$	CH_3	H	1.509	1.92	1.3	1.58
N4	$N(C_2H_5)_2$	CH_3	H	2.567	2.62	2.28	2.49
N7	Morpholine	CH_3	H	1.753	1.89	0.91	1.52
N9	Piperidine	CH_3	H	3.022	2.97	2.66	2.88
N12	Pyrrolidine	CH_3	H	2.463	2.48	2.17	2.37
N14	NH-C_3H_7	CH_3	H	2.151	2.12	2.07	2.11
N16	$NHCH_3$	CH_3	H	1.093	1.21	1.09	1.13
N19	Piperidine	H	H	3.521	3.31	3.21	3.35
N20	NH-C_3H_7	CH_3	CH_3	2.65	2.5	2.62	2.59
N21	$N(CH_3)_2$	CH_3	CH_3	2.008	2.29	1.85	2.05

An important criterion for selecting the more prospective compounds is their novelty. If the *P*a value is high, e.g. *P*a > 0.7, one may often find close analogs of known pharmaceutical agents. For instance, in the case of standard anesthetic agent Lidocaine *P*a $= 0.95$. If *P*a < 0.7 the chance to find the activity in experiment is less, but the compound is not so similar to known pharmaceutical agents. The less the *P*a value, the more the chance to find that active compound is a New Chemical Entity (NCE). Based on this criterion, the majority of selected compounds have *P*a < 0.7.

Toxicities of compounds were predicted using DEREK ver. 6.0.0[‡]. DEREK predicts a number of toxicities, including carcinogenicity, mutagenicity, irritation, respiratory sensitization, skin sensitization and thyroid toxicity. It indicates the likelihood of a compound possessing such toxicities by classification into one of six categories, namely certain, probable, plausible, implausible, improbable or impossible. It is considered that only certain and probable categories may be an obstacle for further investigations.

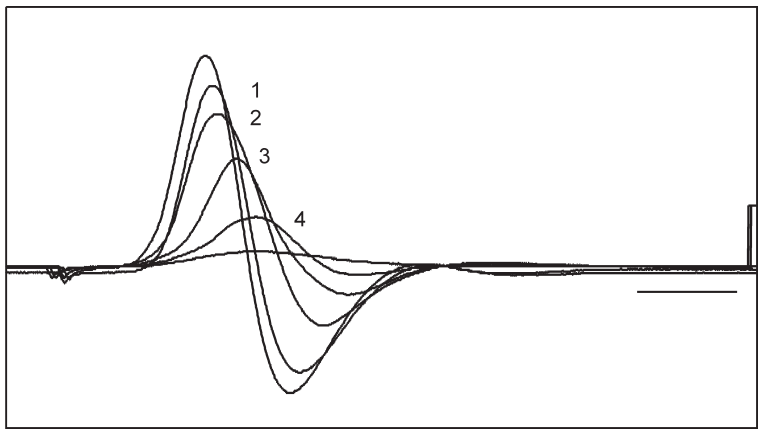


FIGURE 3 Action of 1% lidocaine on the isolated peroneal nerve of the frog (*Rana rindibunda*). The nerve was incubated in physiological solution (saline) where 1% of lidocaine was diluted. The overlapping recordings obtained 1–4 min after exposure to lidocaine, show that there was a gradual decrease of the nerve CAP and finally the action potential was completely eliminated. In this case the physiological saline in the perfusion chamber was used as a vehicle for the tested compounds and as soon as this saline was replaced with normal there was a complete recovery of the CAP. Data not shown. Horizontal scale bar: 1 msec. Vertical scale bar: 4 mV.

QSAR Methods

QSAR analysis was performed with the use of many structural, quantum chemical, electronic, steric, topological, electrotopological and hydrogen bonding descriptors. Lipophilicities of the compounds were calculated as log *P* values (*P* is the octanol-water partition coefficient) using three different computer programs, namely C log *P* ver. 1.0.0[§], Interactive Analysis[‖] and KOWWIN ver. 1.75[#]. These three values and their average are given in Table I. Mean values of log *P* were used in QSAR analysis. Log *D* values (*D* is a distribution coefficient) at pH = 7.2 were calculated using ACD/log *D* software, ver. 6.0[**]. Numerous other physicochemical and structural descriptors were calculated using MDL QSAR[††], TSAR[‡‡], ABSOLV[¶¶] and HYBOT[§§]. QSAR analysis was performed with the stepwise regression routine from MDL QSAR and Minitab ver. 13.1.

RESULTS AND DISCUSSION

Biological Evaluation

The isolated nerve preparation is suitable for the assessment of local anesthetics. The evoked CAP is graded in amplitude reflecting the summation of the external currents generated by the action potential of each activated nerve fiber in the nerve. In this case the electrical stimulation of the nerve, by the stimulating electrodes, activates the voltage-gated sodium channels (VGSCs) of each individual nerve fiber to generate the recorded CAP. The VGSCs

[§]http://www.biobyte.com
[‖]http://www.logp.com
[#]http://www.epa.gov/oppt/exposure/docs/episuitedl.htm
[**]http//www.acdlabs.com
[††]http://www.mdli.com
[‡‡]http://www.accelrys.com
[¶¶]http://www.sirius-analytical.com
[§§]http://www.ibmh.msk.su/hybot

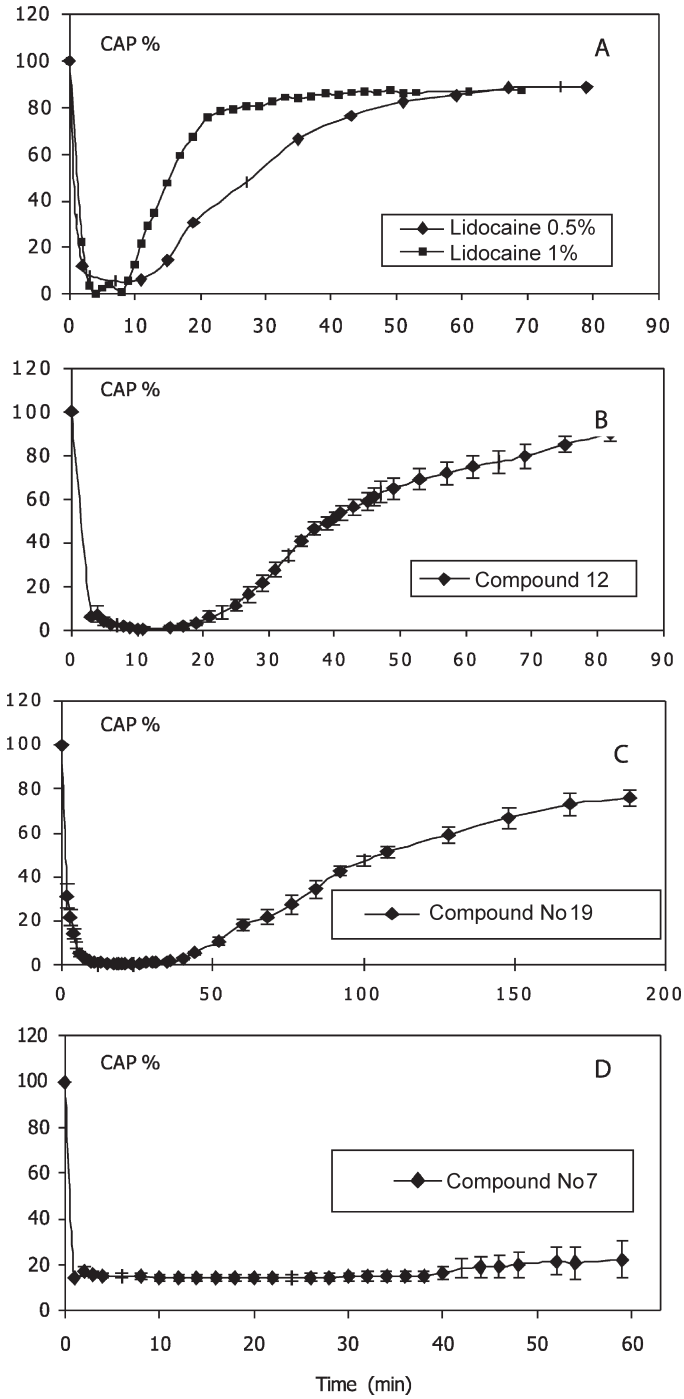


FIGURE 4 The assessment of the local anesthetic action of lidocaine and the other compounds under investigation using the isolated peroneal nerve of the frog *Rana rindibunda*. (**A**) The percentage value of the energy (*E*) of the digitised CAP vs. time diagrams were plotted for the incubation period in 0.5% (squares) and 1% of lidocaine. As soon as the CAP was eliminated, the preparation was washed with normal saline. There was a quick recovery of the CAP to normal values, those before incubation. (**B**) As in **A** but the nerve was incubated in compound No 12. (**C**) As in **A** but the nerve was incubated in compound No 19. (**D**) as in **A** but the nerve was incubated in the compound No 7. The outcome of many trials using the same concentration of the compound ($n = 6$) were averaged and plotted as a single curve. The data were expressed as a mean ± S.E.M.

TABLE II Local anesthetic activity, experimental and predicted data

Compound number	*IT50 (min)*	*IT100 (min)*	*RT(min)*	*RA (min)*	*Prediction, Pa %*
N1 1%	18	27		8.88	64
N2 1%	7	*20%	57	22.6	59
N3 1%	4	14		40	81
N3 0.5%	1.5	8.3	28	67	59
N4 1%	2.5	*10%	29.3	64	78
N5 0.5%	3	7.6	34	33	41
N6 1%	7.4	24		21.6	36
N7 1%	0.6	*10%		266.7	40
N8 1%	19	32.5		8.4	45
N8 0.1%	4.3	25	51.7	37	
N9 1%	0.2	4.8		800	44
N10 0.1%	1.25	9.4	12		45
N10 0.5%	5	*15%	36		
N10 1%	1.3	15	28.4	123	
N12 1%	1.8	9	38.2	88.9	44
N14 1%	8.2	18.9		19.5	52
N14 0.1%	13	*15%	76.7		
N15 1%	10.3	*35%	47.7	15.5	36
N16 1%	2.4	27		66.7	45
N18 1%	9	23	160	17.8	44
N19 1%	4.5	10	108	35.6	59
N20 1%	0.2	6	79	800	41
N21 1%	2	11	61	80	45
Lidocaine 1%	1	4.4	5.8	100	95
Lidocaine 0.5%	1.6		7.6		

IT_{50}: the time (min) required to decrease the CAP to 50% of the control values; IT_{100}: the time (min) required to reduce the CAP to 0%; RT_{50}: the time (min) required for the CAP to recover to the 50% of the control value after the replacement with control saline; RA—$(100*IT50_{\text{lidocaine 0.5\%}}/IT50_{\text{compound}})$.
* Displays a minimal value of CAP %, which was shown during the experiment.

are the main target of the local anesthetics [31]. Thus, the isolated sciatic nerve of the frog in the tree-chamber recording bath is a reliable preparation allowing quantitative and qualitative assessment of the action of local anesthetics (see, for example, [20–22]).

The Action of Lidocaine

The local anesthetic action of the synthesized compounds, shown in Table II, was compared with the action of a standard local anesthetic, lidocaine. Lidocaine, as all local anesthetics, act on the voltage-gated sodium channels of the axons (myelinated or unmyelinated) and causes the elimination of the action potential [31]. The local anesthetic action of lidocaine, final concentration 1%, on the isolated nerve is shown in Fig. 3. In this case the physiological saline in the perfusion chamber was used as a vehicle for the tested compounds. The detail course of the amplitude of the CAP is given in Fig. 4A. From this curve it is clear that the time required for lidocaine to decrease the CAP to 50% of the control values, the IT_{50}, is 1.6 ± 0.7 min. The time required to reduce the CAP to 0%, the IT_{100}, is 4.4 ± 0.5 min and the time required to recover to the 50% of the control value, the RT_{50}, is 5.8 ± 0.6 min. These values and those obtained from the same experiment using 0.5% lidocaine are summarized in Table II.

The Action of the Synthesized Compounds

In an attempt to study the possible local anesthetic activity of the synthesized compounds (see Table II) a standard concentration of 1% of each individual compound was compared with the action of 1% of lidocaine on the isolated peroneal nerve (Fig. 4A).

The results show that one of the most active compounds, having a similar action to lidocaine, was compound N12. Application of 1% of compound N12 on the isolated nerve caused a rapid inhibition of the CAP (Fig. 3B), with an $IT_{50} = 1.8$ min, $IT_{100} = 9$ min, and $RT_{50} = 38$ min, which are almost identical to those described for lidocaine (see Table II). Using the same procedure it was demonstrated that compounds N4, N5, N10, N20 and N21 show a similar action to that of lidocaine (see Table II). For these compounds the IT_{50} is between 1 and 3 min, the IT_{100} is between 7.6 and 15 min and the RT_{50} is between 28 and 79 min, as shown in Table II. These compounds were classified as group A.

Another group of compounds, group B, consisting of compounds N2, N3, N15, N18 and N19, showed lower activity than those in group A (Table II). For example, application of compound N2 to the isolated peroneal nerve causes a slow inhibition of CAP (Fig. 4C curve a), with an IT_{50} of 7 min, and RT_{50} of 57 min. The compounds classified in group B have an IT_{50} of 4.5 to 10.3 min, IT_{100} is 10–23 min and RT_{50} between 48 and 160 min. Finally there are compounds, like compound N7, shown in Fig. 3D, which cause a very fast inhibition of the CAP, with IT_{50} 0.6 min, but after the nerve was washed with normal saline there was no recovery at all, even after 24 h. These compounds (N1, N6, N7, N9, N14, N16, shown in Table II), were classified as group C.

It is interesting to note that in most cases, there is a concentration-dependent reduction of CAP (Table II). For example compound N10 in concentration of 1, 0.5 and 0.1% has an IT_{50} value of 1.3, 5 and 1.25 min, respectively. Regarding the RT_{50}, it was shown that low concentrations give a faster recovery time. Another example is compound N8: at a concentration of 1% there is no recovery of the CAP at all, but at a concentration of 0.1%, recovery was observed with an RT_{50} of 51.7 min.

Using the facility of the method, to allow long period of recording, over 50 h, the possible toxicity of the tested compounds was investigated. None of the examined compounds, except those of group C, had a neurotoxic action. For almost all the compounds there was a 100% recovery after 24 h. It should be mentioned that in most cases there was a concentration-dependent decrease of the CAP.

From the structural point of view, the most effective compounds (group A) are acetamides derivatives, where between lipophilic and hydrophilic moieties there is a chain with one methylene group. Increasing the chain by one more methylene group leads to less potent compounds. Activity and lipophilicity for this group of compounds are in agreement. For example, the most active compound is compound N20 (800%) with the highest mean $\log P$ value (2.59). Regarding compounds from group B there is the same observation concerning the length of intermediate chain. The most active compound of this group is compound N3. Compound N18 has almost the same structure as compound N3, with the only difference in the intermediate chain, being a propionamide derivative. Even though the lipophilicity of this compound is higher than of compound N3, it is less potent than the latter. Clearly, therefore, not only lipophilicity, but the structure of compounds also, plays a role in their activity.

For group C compounds, no correlation is observed between structure and activity, nor between lipophilicity and activity. This is probably due to the fact that these compounds completely block the CAP, but they do show any recovery at all, an indication of possible neurotoxic activity caused by the irreversible binding of the compound with the VGSC.

Prediction of Biological Activity and Toxicity

Table II displays the experimental activities of the compounds, and their activities predicted by PASS. Local anesthetic activity was predicted by PASS for the all studied compounds.

That is, PASS has proved 100% accurate in its predictions of local anesthetic activity of these compounds. There is no correlation between predicted values of probability and experimental values. The explanation of this fact is that the calculated *P*a value is not proportional to the potency of the compound. It is rather the probability of belonging to the class of "actives". It gives us a similarity assessment of tested compounds with classic local anesthetics from the PASS training set. Since lidocaine is a classic local anesthetic, *P*a = 95%. The most active compounds under study (N7, N9 and N20) have *P*a values from 40 to 45%. This suggests that these compounds differ significantly from classic local anesthetics and that they may be NCEs.

The only toxicity highlighted by DEREK for the present compounds was carcinogenicity, which was rated as plausible for all the compounds. The carcinogenicity of lidocaine was also rated as plausible, as was the likelihood of its producing methaemoglobinaemia. The rating of plausible is not regarded as an indication that development of a compound should be halted, but rather that experimental carcinogenicity testing should be carried out on each compound selected for development.

QSAR Analysis

There have been only a few published QSAR studies of local anesthetic activity. Recanatini *et al.* [32] correlated the local anesthetic activity of a series of 67 lidocaine derivatives with two indicator variables and $\log D$ (D = distribution coefficient at pH 7.4), and found $r^2 = 0.652$. Caliendo *et al.* [33] obtained a good correlation of the surface local anesthetic activity of a series of 12 *N*-[2-(alkylamino)ethyl]benzotriazol-*x*-yl acetamides with C$\log P$ ($r^2 = 0.882$), whilst Caliendo *et al.* [34] similarly found a good correlation of the surface local anesthetic activity of a series of 12 *N*-[2-(alkylamino)ethyl]benzotriazol-*x*-yl isobutyramides with C$\log P$ ($r^2 = 0.750$). In both studies by Caliendo *et al.*, the correlation of infiltration local anesthetic activity was considerably worse than that of the surface local anesthetic activity ($r^2 = 0.728$ and $r^2 = 0.546$, respectively).

The best QSAR that could be obtained for the compounds presented in Tables I and II was:

$$\begin{aligned} \log \mathrm{RA} = {} & 0.215\,\mathrm{Pol_{ZZ}} + 0.0368\,\mathrm{Oct_{ZZX}} - 1.77\,\mathrm{E_D(max)} + 0.425\,\mathrm{D_Y} \\ & - 0.794\,{}^1\chi^{\mathrm{v}} - 2.77 \\ n = 19 \quad & r^2 = 0.794 \quad Q^2 = 0.586 \quad s = 0.306 \quad \mathrm{F} = 10.0 \end{aligned} \tag{1}$$

where n = number of compounds used in the correlation, r = correlation coefficient, Q = cross-validated (leave-one-out procedure) correlation coefficient, s = standard error of the estimate, F = Fisher statistic, $\mathrm{Pol_{ZZ}}$ = ZZ component of polarizability, $\mathrm{Oct_{ZZX}}$ = ZZX component of octopole (a polarity term), $\mathrm{E_D(max)}$ = maximum hydrogen bond donor energy, $\mathrm{D_Y}$ = Y component of dipole moment, and ${}^1\chi^{\mathrm{v}} = 1^{\mathrm{st}}$ order valence molecular connectivity. All descriptors were significant at the 0.3% level or better, and there were no high pair-wise collinearities.

Despite the above published work [32–34] indicating that $\log P$ or $\log D$ appears to be important in local anaesthetic activity, our results do not support that, since neither term appears in equation 1. Neither does the local anaesthetic activity of our compounds correlate directly with either $\log P$ ($r^2 = 0.143$) or $\log D$ at pH 7.2 ($r^2 = 0.176$).
The QSAR that we obtained suggests the following:

1. The polarizability and polarity terms have positive coefficients, indicating that these factors increase local anaesthetic activity.
2. The 1st order valence molecular connectivity coefficient is negative. This descriptor correlates well with molecular size, and thus could indicate that larger molecules are less potent.
3. Hydrogen bond donor ability appears to increase local anaesthetic action, since the calculated bond energies are negative.

The above information should enable us to design more potent local anesthetic agents of this class.

CONCLUSIONS

Based on computer predictions, the most prospective 18 potential local anestetics were selected among the designed alkylaminoacyl derivatives of 3-amino-benzo-[*d*]-isothiazole. These compounds were synthesized and tested on *in vitro* preparation of the isolated peroneal nerve of the frog.

According to their local anesthetic action the tested compounds could be divided into three groups: (a) lidocaine-like action, (b) lower lidocaine-like action and (c) compounds with very fast inhibition time but without recovery at all.

From the QSAR analysis it was shown that polarity, polarizability and hydrogen bond donor ability increase local anaesthetic action while conversely, molecular size reduces it.

Since calculations from PASS probability *P*a to find the local anesthetic action in compounds N7, N9 and N20 is in range 40–45%, the most potent compounds could be New Chemical Entities (NCEs).

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