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Review

Nuclear Quadrupole Resonance spectroscopy in studies of biologically active molecular systems—a review

J.N. Latosińska *

Institute of Physics, Adam Mickiewicz University, Umultowska 85, 61-614 Poznań, Poland

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Abstract

The main idea of this work was to give an overview of the NQR capabilities in the search for a correlation between electronic structure and biological activity of certain compounds, mainly drugs. A correlation between the parameters characterising biological activity and the NQR spectral parameters describing chemical properties of a given compound, permits drawing conclusions on biological effectiveness of compounds from a certain group. The quadrupole coupling constants, which are very well correlated with atomic charges, can be treated as descriptors in QSAR. The information inferred from NQR study on local electron density distribution together with analysis of charge distribution, provides excellent means for determination of reactive sites and hence, can indicate possible promising directions to be followed in drugs design.

Keywords: NQR spectroscopy; Electronic structure; Biologically active molecular systems

Contents

1. Introduction

It is well known that the activity of a compound is a thermodynamical parameter enabling a description of real systems by the same equations as used for ideal ones, understood as the ability of a substance to enter into chemical reactions, whereas the biological activity is expressed as the ratio of the dose of a given compound to the effect it causes. Biological activity or activity of a compound is usually associated with the intensity of its action, although these notions are not equivalent. If one of the two drugs characterised by the same maximum effect produces it after a smaller dose it is more active, but when it produces a stronger effect—it is not only more active but its biological activity is greater. The mode of action of a given chemical substance depends on its dose and concentration in a given system. It can produce beneficial effects, poisoning, reversible or irreversible (death) disturbances of the functioning of an organism. In compara-

* Tel.: +48 61 8295277; fax: +48 61 8257758.
E-mail address: jolanta.latosinska@amu.edu.pl.

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tive studies, toxicity of a substance LD_{50} is measured as the amount of the substance expressed in mg/kg of body weight that would cause death of 50% of the experimental animals, while for assessment of the therapeutic effect the single dose SD effect is measured.

Although the concept of a relationship between the chemical structure of a chemical compound and its biological activity appeared over a 100 years ago, prediction of the compound activity on the basis of its chemical structure still comes across serious difficulties.

A breakthrough was the discovery by Crum-Brown and Fraser (1869) [1], that many alkaloids upon changing the structure into quaternary, lost the pharmacologically desired activity and showed antagonistic behaviour. Later studies have brought about new concepts and hypotheses, but till the present the predictions made only on the basis of chemical structure are ambiguous. However, it does not mean that attempts at finding an approximate correlation between the biological activity, the chemical structure and characteristics of a compound have been abandoned.

Attempts at the theoretical solution of the problem were made in [2–5]. It was established that the chemical structure of a compound and its physical and chemical properties had substantial influence on its activity both therapeutic and toxic. A relatively insignificant change in the chemical structure (introduction or exchange of substituents) affected the electron density distribution within the whole molecule (polar effects – induction effects, direct electrostatic interaction and coupling effects – mesomeric effects), and therefore could influence the potency and mode of action of the drug.

Although it is known that the chemical structure as well as physical and chemical properties of a given chemical compound determine its biological and chemical activity, it is very difficult to predict the particular effect of an electron donating or accepting substituent. In some cases, the effect can be inferred, e.g. substitution with $-NO_2$ or $-CN$ groups leads to increasing toxicity, but, as follows from analysis of imidasole or steroid derivatives, this is not always true. The question what makes a given compound therapeutic or toxic remains still open. Despite a large body of evidence relating activity of a given drug with its structure, there are no simple rules, which would allow drawing conclusions on biological activity of a compound on the basis of its chemical structure. It is known that formulation of a general theory explaining the activity of drugs is at present impossible because of the complex character of the problem, but from the point of view of assessment of pharmaceutical activity and toxicity, the search for methods that would help design drugs is of outmost interest.

In the aspect of the assessment of pharmacological activity and toxicity it is of profound interest to look for the research techniques which would allow prediction of the physical and chemical properties and activity of drugs on the basis of their chemical structure. Recently, much attention has been paid to look for a correlation between the dipolar moment of compounds, in particular drugs, and their biological activity [2–4], which has stimulated the search for such a correlation between electron density distribution in a molecule of a given compound and its activity [5].

The application of the NMR, EPR, UV and IR spectroscopies in investigation of biologically active systems has been widely reported in literature [6–8], while the papers devoted to the NQR spectroscopy are scarce although this method provides the most accurate description of the substitution-related changes in the electron density in molecules [9].

Over recent times, there has been a considerable resurgence of interest in nuclear quadrupole resonance (NQR) spectroscopy, largely because the use of nuclei as a probe of local atomic arrangement has several unique advantages over more conventional NMR methods. First of all NQR can be used for a very accurate determination of local distribution of electron density in a molecules, giving more accurate results than NMR chemical shifts [9]. Moreover, NQR is particularly suitable for this purpose because it does not require the use of magnetic fields or irradiation so it does not produce changes in the molecule studied. The parameters obtained from ^{14}N NQR spectra (quadrupole coupling constant and asymmetry parameter) bring the information on the electron density distribution in the neighbourhood of the nuclei studied, on the bonds in which a given isotope is involved so on the population of the bonds [10,11], while ^{35}Cl NQR spectra allow only a determination of the z component of the electric field gradient (EFG) and in some particular cases using the nutation technique, an asymmetry parameter.

The main idea of this work was to give a literature overview on the hitherto attempts at a comprehensive interpretation of a relationship between chemical structure and physical properties of drugs described by NQR parameters and their biological activity.

2. NQR principles

NQR spectroscopy can be approximately described as solid-state NMR without magnetic field [10,11]. Moreover, experimentally NQR measurements can be performed on the same instruments as used for solid-state NMR, of course with no external magnetic field applied. Unfortunately, not all atomic nuclei can be NQR probes—only those whose spin is >1. Another inconvenience is that the frequency range is extremely large, stretching from less than 100 kHzs (for ^{2}H) to 2500 MHz (for ^{127}I), which requires sophisticated apparatus.

The samples must be highly pure and must be available in much greater amount than that required for the NMR study. The NQR spectroscopy gives weak signals and the measurement often is a real challenge for the experimenter. However, on the other hand, the results permit interpretation of subtle electronic effects and the accuracy of analysis is much better than that ensured by solid-state NMR [9].

On the basis of the resonance frequencies obtained in the NQR experiment the quadrupole coupling constant e^2Qqh^{-1},

MHz, and the asymmetry parameter are determined [10]. The interaction of the nuclear quadrupole moment with the electric field gradient is described by the constant e^2Qqh^{-1} (i.e. the z component of the tensor of interaction of the nuclear quadrupole moment with the electric field gradient), whereas the asymmetry of the tensor of the electric field gradient at the quadrupole nucleus is described by $\eta = [(e^2Qqh^{-1})_{xx} - (e^2Qqh^{-1})_{yy}]/(e^2Qqh^{-1})_{zz}$.

The quadrupole coupling constant calculated from NQR frequency is proportional to charge on quadrupole nuclei and gives information on electron distribution in a molecule, whereas the asymmetry parameter, similarly as in CP/MAS, provides information on the direction and order of chemical bonds. When the nuclear spin is a whole number, NQR provides indirect information on the population of chemical bonds, while otherwise on the degree of the double bond character of chemical bonds in which the quadrupole nuclei is involved [11].

3. NQR studies of biologically active compounds

First of all a correlation was searched for between the NQR parameters and the Hammett (or Taft) constants that is the parameters important for QSAR [11]. NQR literature gives many reports on attempts at correlating the NQR parameters and the Hammett or Taft constants [11], but only a few reports on attempts at finding a correlation between NQR parameters and biological activity of compounds. In the 70s, for some groups of drugs (derivatives of sulphonamides, anilines, nitrogen mustards) a good correlation was found between the physical and chemical properties (Hammett and Taft constants), biological activity expressed as LD_{50}, ED_{90}, minimum inhibiting concentration (MIC) and the parameters obtained directly (frequency, quadrupole coupling constant) or indirectly (population of chemical bonds) from NQR spectra. Intense studies were carried out only by Subbarao and Bray (1979) for a group of sulfonamides and aniline derivatives [12]. A correlation between NQR data and biological activity of sulphonamides in vitro found by Bray and co-workers supported the earlier Seydel and co-workers' conclusion [13–16] about a relation between the electronic properties of amine groups in anilines and the biological activity of sulphonamide prompted by the correlation diagrams between the chemical shifts from the ^{1}H NMR spectra and biological activity [17]. Similar attempts were undertaken for nitrogen mustard derivatives, however, the number of compounds studied in this group proved insufficient. So far a trend was found—toxic and anticancerous activities of nitrogen mustards and their toxicity was shown to increase with decreasing density of their free electron pair. No correlation was established between biological activity and population of isatine bonds calculated from ^{14}N NQR data, which suggested that the electron surrounding of nitrogen was not responsible for antimyotic activity of this class of compounds.

The results reported by Bray suggested that NQR spectroscopy could be useful for determination of physical and chemical properties and biological activity of compounds. Unfortunately, the scope of the studies was very limited and the methods limited only to NQR (no correlations with the results obtained from any other spectroscopies). Bray studied only three classes of compounds [18–20], whereas interesting conclusions were also obtained from the study of methane halogen derivatives, bromine salts, barbiturates, derivatives of *p*-aminobenzoesic acid and adenosine [21].

NQR studies of simple biologically active (highly toxic) compounds—halogen derivatives of methane were initiated in 1951 by Livingstone [22,23]. Selected compounds from this group were also studied by Allen (1953) [24], Gutowsky and McCall (1960) [25], Smirnov and Volkov (1970) [26], Kume and Nakamura (1976) [27], Bray and co-workers (1977, 1984) [28,29], Lucken and co-workers (1981) [30] and Semin et al. (1985) [31]. However, none of these authors was concerned with a correlation between the chemical structure and biological activity of the compounds. On the basis of the results published in [22–31], the parameters characterising the electronic structure of halogen derivatives of methane were determined and correlated with the data on biological activity taken from [32–36]. The quadrupole coupling constants (QCC, e^2Qqh^{-1}) obtained from NQR spectra and the maximum admissible concentrations (AC) in mg/l for halogen derivatives of methane were found well correlated [21]. Biological activity (toxicity) data for CCl_4, chloroform, di and monochloromethane suggested a decreasing toxicity of these substances with decreasing quadrupole coupling constant, although this was not the case for chloromethane. The quadrupole coupling constants were well correlated with the toxicity of halons (correlation coefficient 0.99—chloromethane excluded), so it seemed that they could be used for assessment of halons toxicity, [21], Table 1 and Fig. 1.

A similar correlation was found between the NQR data on ^{81}Br isotope [26,27,37–40] and single doses (SD) [25–29] expressed in grams, for bromide salts. The effectiveness of the compound increased with growing quadrupole coupling constant [21], Table 2 and Fig. 2 (correlation coeffcient 0.91).

It was supposed that enhanced tolerance to a given drug was related to increasing quadrupole coupling constant, however, the number of the compounds studied hitherto had been too low to draw such a general conclusion. Unfortunately, the lack of data on the toxicity of bromine salts prevented

Table 1
^{35}Cl NQR frequencies, quadrupole coupling constants, e^2Qqh^{-1}, and the maximum admissible concentration (AC) in mg/l of halogen derivatives of methane

No.	Compound	e^2Qqh^{-1} (MHz)	AC (mg/l)
1	CCl_4	81.85	100
2	$CHCl_3$	76.56	225
3	CH_2Cl_2	71.93	400
4	CH_3Cl	68.40	160

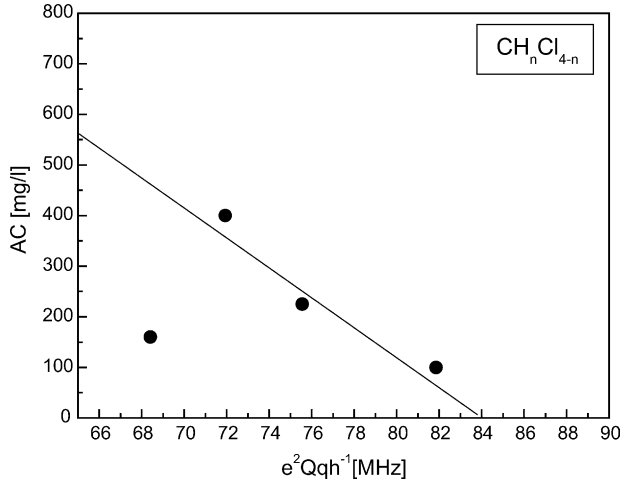

Fig. 1. Toxicity of halons (AC) vs. the quadrupole coupling constant.

Table 2
Quadrupole coupling constants (e^2Qqh^{-1}) and single doses (SD) for bromide salts

No.	Compound	e^2Qqh^{-1} (MHz)	SD (g)
1	Sodium bromide	2.4	0.5–3.0
2	Calcium bromide	78.7	0.5–2.0
3	Ammonium bromide	416.6	0.3–2.0

checking a correlation between QCC and lethal doses LD_{50}, which could be an interesting point in the discussion.

The above dependencies were observed for the compounds of simple chemical structure. The question arose whether it was a significant factor and whether with increasing number of atoms in a molecule the correlation between the electronic structure and biological activity would disappear. To answer this question, a similar study was undertaken for compounds of more complex chemical structure—derivatives of *p*-aminobenzoic acid. The literature data necessary for such a qualitative analysis were take from [29,41–43]. These data include populations of nitrogen atom bonds [41,42] and single doses pro-dosi (SD) [43] for selected derivatives of *p*-aminobenzoic acid, which are particularly important for drugs of prolonged activity: tetracaine, procaine, benzocaine and lidocaine. It is known that a single dose does not have to be related to the strength of a drug

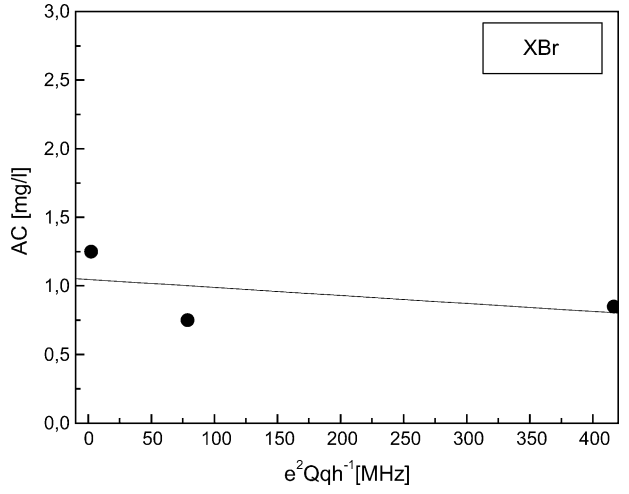

Fig. 2. Single dose of bromine salt (SD) vs. quadrupole coupling constant.

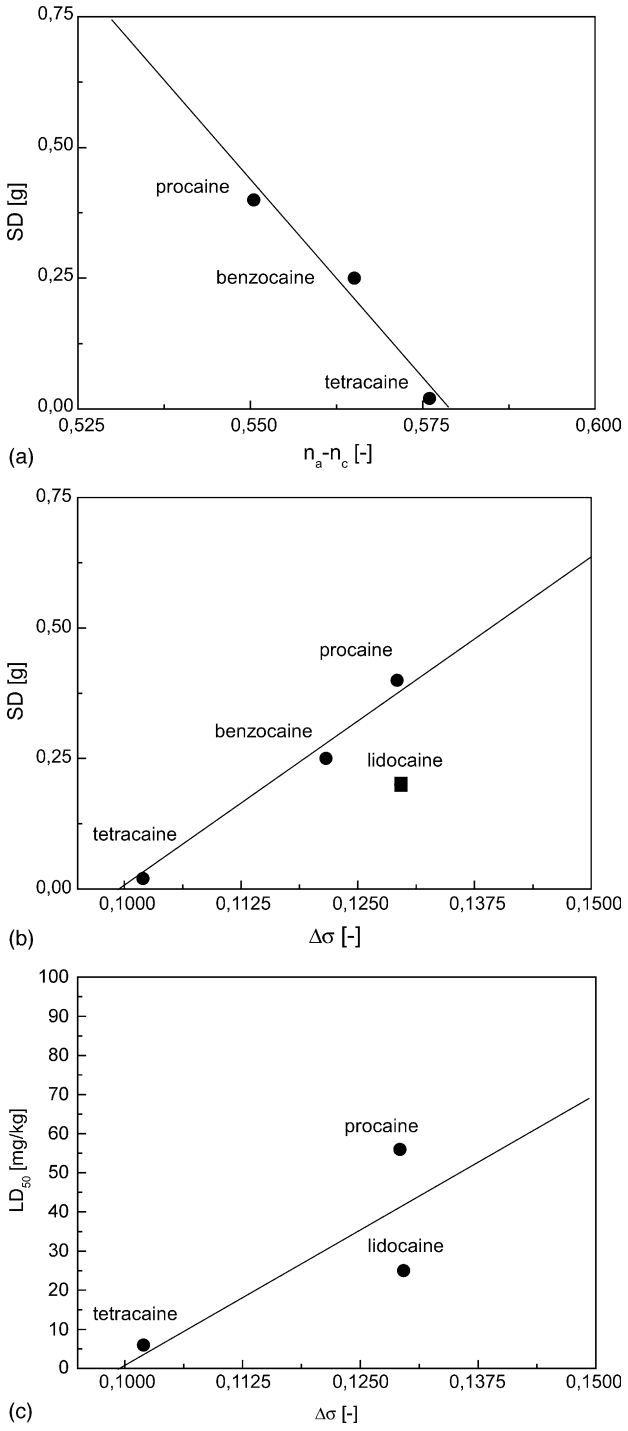

Fig. 3. (a) Single dose of *p*-aminobenzoic acid (SD) versus the difference in the bond population ($\Delta\sigma$); (b) single dose vs. the difference between the populations of the lone electron pair on nitrogen atom and average population of σ-bond $n_a - \sigma$, for selected derivatives of *p*-aminobenzoic acid; (c) lethal dose LD_{50} for mice vs. the difference in the bond population ($\Delta\sigma$).

Table 3
Bond populations and single doses (SD) for derivatives of *p*-aminobenzoic acid

No.	Compound	$n_a - \sigma$	$\Delta\sigma$	$n_a - n_c$	$n_a - n_b$	SD (g)
1	Tetracaine·HCl	0.5420	0.1020	0.5760	0.4740	0.02
2	Procaine·HCl	0.4644	0.1292	0.5505	0.4213	0.40
3	Benzocaine·HCl	0.4840	0.1216	0.5651	0.4435	0.20–0.30
4	Lidocaine·HCl	0.2893	0.1296	0.3350	0.2054	0.20

activity, but this is the case for anaesthetics for which the smaller the SD the stronger the anaesthetic. It was difficult to carry out the analysis on the basis of limited data available, however, it could be concluded that there was a good correlation between single dose and the difference in the bond populations [21], Table 3 and Fig. 3a and b (correlation coeffcients 0.84 for both depeneces SD($n_a - \sigma$) and SD($\Delta\sigma$), where $n_a - \sigma$ is a difference in population of the free electron pair of nitrogen and inequivalent nitrogen bond, $\Delta\sigma$ a difference in the nitrogen bonds population).

Table 4
Lethal doses of *p*-aminobenzoic acid derivatives LD_{50} determined in animal tests (mouse)

	LD_{50} (mg/kg)		
	Lidocaine, lidocaine·HCl	Tetracaine, tetracaine·HCl	Procaine, procaine·HCl
Intravenous	25	6	56
	22	7.5	–
Intraperitoneal	102	20	124
	119	70	–
Oral	520	–	500
	220	–	–
Subcutaneous	265	25	530
	285–390	35	800
Intramuscular	–	–	–
	260	–	–

Table 5
Bond populations for N1 and N3 nitrogen atoms in the barbiturates studied

No.	Compound	N1			N3		
		$n_b - n_c$	$n_a - n_c$	$n_a - n_b$	$n_b - n_c$	$n_a - n_c$	$n_a - n_b$
1	Amobarbitale	0.118	0.336	0.218	0.118	0.336	0.218
2	Aprobarbitale	0.121	0.340	0.219	0.121	0.340	0.219
3	Barbitale	0.133	0.330	0.197	0.133	0.330	0.197
4	Butabarbitale	0.129	0.333	0.204	0.129	0.333	0.204
5	Phenobarbitale	0.151	0.347	0.196	0.151	0.247	0.196
6	Secobarbitale	0.129	0.337	0.208	0.129	0.337	0.208
7	Hexobarbitale	0.030	0.361	0.331	0.114	0.361	0.247
8	Mephobarbitale	0.029	0.363	0.334	0.133	0.352	0.219
9	Metharbitale	0.027	0.352	0.325	0.122	0.342	0.220

Table 6
Lethal doses for man (LD_{50}), toxic concentrations in human blood (CT), biological half lifetimes (BHL) and single doses (SD) for selected barbiturants

No.	Compound	LD_{50} (mol/g)	CT (mg/100 cm^3)	BHL (h)	SD (0.1 g)
1	Amobarbitale	>1.5	0.9	14–42	1.0–2.0
2	Aprobarbitale	–	–	14–42	0.4–1.0
3	Barbitale	2–4	5.0–8.0	24–96	3.0–4.0
4	Butabarbitale	–	–	14–42	0.15–0.3
5	Phenobarbitale	1.5–5.0	1.7–9.0	24–96	1.0–2.0
6	Secobarbitale	–	–	–	0.5–2.0
7	Hexobarbitale	2.0	0.8	3–8	3.0–5.0
8	Mephobarbitale	2.0	–	14–42	2.5
9	Metharbitale	–	–	–	2.0

Table 7
Lethal dose of the barbiturates studied (LD_{50}) obtained from laboratory tests on mouse

Test type	LD_{50} (mg/kg)						
	Amobarbitale	Aprobarbitale	Barbitale	Phenobarbitale	Secobarbitale	Hexobarbitale	Metharbitale
Intravenous	–	–	–	–	–	75	–
Intraperitoneal	175	85	178	88	116	270	500
Oral	345	–	–	168	–	468	–
Subcutaneous	–	–	630	300	160	350	–
Intrapleural	–	–	–	–	–	340	–

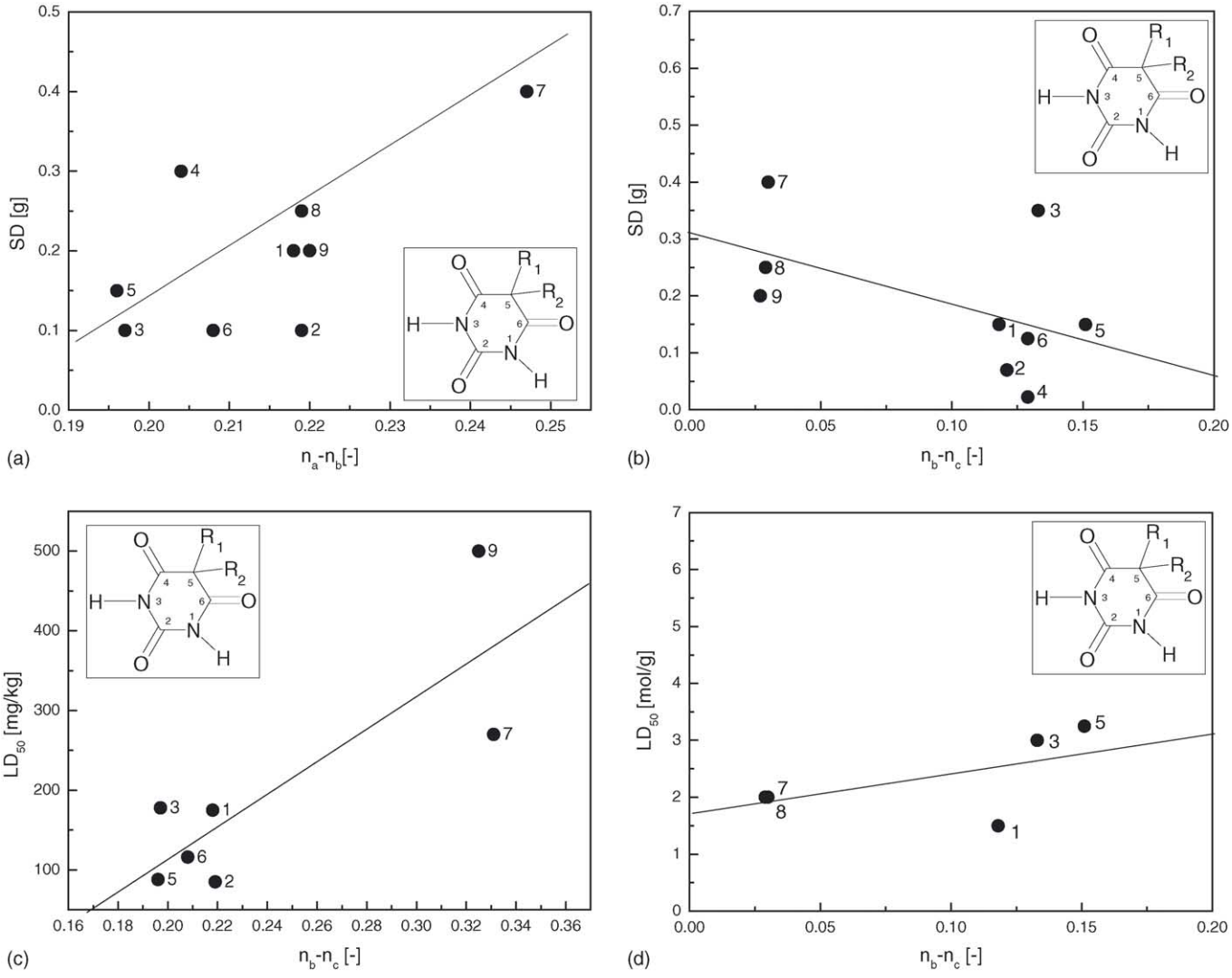

Fig. 4. (a) Single dose of barbiturates (SD) vs. the difference between the populations of free electron pair on nitrogen atom and population of NH-bond ($n_a - n_b$); (b) single doses of barbiturates (SD) vs. the difference between the populations of NH and NC bonds ($n_b - n_c$) (c) lethal dose of barbiturates (LD_{50} for human) vs. the difference between the populations of the lone electron pair on nitrogen atom and population of NH-bond ($n_a - n_b$); (d) lethal dose of barbiturates (LD_{50} for mouse) vs. the difference between the populations of NH and NC bonds.

It seemed interesting to compare the parameters describing the electronic structure, those referring to biological activity SD and the lethal dose LD_{50}. The therapeutic index is defined as the ratio of the lethal dose to the dose producing pharmacological effect in 50% of animals tested. The toxicity LD_{50} in mg/kg [44–47] and the lethal dose LD_{50} were found to be correlated with the difference in the nitrogen bonds population, Table 4. The correlation coefficient was 0.931 for $LD_{50} = 758.5\Delta\sigma - 68$ (Fig. 3c) and smaller 0.670 for $LD_{50} = 9317.2(n_a - n_c) - 813.4$. The results suggested that the parameter significant from the point of view of biological activity was the difference in the population of inequivalent nitrogen atom bonds.

Particularly interesting results were obtained for another group of anaesthetics—derivatives of barbituric acid. The acid was not anaesthetic itself, but its derivatives containing higher aliphatic or aromatic radicals showed this kind of activity. Medical therapy uses mainly 5,5-bisubstituted derivatives of barbituric acid containing substituents in position 1 of the ring or derivatives of 2-thiobarbituric acid [28,48]. For this group of compounds, Bray did not find a correlation between the NQR data and the biological activity of barbiturates [48], however, it was achieved in [21] on the basis of the biological activity data presented in Tables 5–7. It was shown that relatively small structural changes significantly affect the properties of barbiturates, Fig. 4a. The alkyl or aryl substituents at the C3 atom were found responsible for the anaesthetic activity. The strength and time of barbiturates activity was found to depend on the length of the aliphatic chain and the kinds of substituents. These changes were correlated with those in the barbiturate ring. A comparison of the differences in the bond population determined from ^{14}N NQR frequencies with the single doses expressed in grams showed that the activity of barbiturates was correlated with changes

Table 8
Parameters characterising the electronic structure of narcotics

No.	Compound	^{14}N NQR		Biological data
		e^2Qqh^{-1} (MHz)	η	LD_{50} (mg)
1	Cocaine	5.081	0.043	1150 (200–300)
2	Cocaine·HCl	1.187	0.263	–
3	Heroine	5.031	0.028	50–70 (10)
4	Codeine	5.039	0.020	800
5	Codeine·phosphate	1.200	0.350	–

in electronic density of the lone electron pair on the nitrogen atom and with changes in the population of the inequivalent bond [21]. The dependence of the single dose on the difference in bond populations of N3 nitrogen bond ($n_a - n_b$) or ($n_b - n_c$) was described by the least square method and the line was approximated by the following equation:

$$SD = 4.41(n_a - n_b) + 0.74 \quad \text{or}$$
$$SD = 12.05(n_b - n_c) - 3.10 \qquad (1)$$

with the correlation coefficient 0.842 or 0.720 and standard deviation 0.060 or 1.10, respectively, Table 5 and Fig. 4a.

The extension of the chain led to a decrease in the electronic density of the lone electron pair (n_a), and introduction of alkyl substituents caused a decrease in the population of the lone electron pair and an increase in the population of the inequivalent bond of the nitrogen atom (n_b). The effect of the changes in the population of the lone pair was dominant. The differences in the activity of barbital and phenobarbital containing aliphatic and aromatic groups as substituents, were mainly a result of differences in the population of the inequivalent nitrogen atom. Similarly as for the derivatives of *p*-aminobenzoic acid, a relationship between the NQR parameters and the lethal dose was checked for barbiturates, Fig. 4c and d. The lethal doses estimated for human and obtained from the tests on animals are given in Tables 6 and 7 [35,49]. The results suggested a possible correlation between the electronic structure and biological activity of anaesthetics.

This supposition could be generalised over narcotics. The results of the measurements of quadrupole coupling constants on ^{14}N nuclei by NQR in pure narcotics: cocaine, codeine and heroine [50–52] as well as the lethal doses taken from Ref. [49]. A comparison of the differences in the quadrupole coupling constants determined from ^{14}N NQR frequencies with the lethal doses expressed in grams revealed that the activity of narcotics was correlated with changes in the electronic density on the nitrogen atom. The correlation between the lethal dose and the quadrupole coupling constant on nitrogen was found (the correlation coefficient 0.838 and standard deviation 430) Table 8 and Fig. 5 [21].

The question was whether this supposition could be generalised over the groups of compounds other than anaesthetics or narcotics.

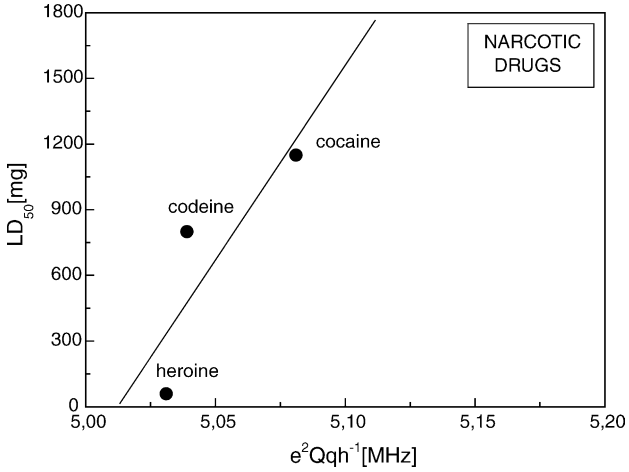

Fig. 5. The lethal dose of selected narcotics (LD_{50}) vs. the quadrupole coupling constants.

The most important finding implied by the studies was that in the search for anticancerous drugs from the group of 4N-derivatives of cytosine, the more effective would be those in which the aromatic substituents were strongly separated by the $-CH_2-CH_2$ chain [53]. It was found that the substitution at position 1H of imidazole led to a redistribution of the electron density from the nitrogen atom –N= onto –NR– [54]. On the basis of the results obtained for two groups of compounds: 4N-derivatives of cytosine (anticancerous drug) and imidazole derivatives (anticancerous through bacteriostatic activity depending on the substituents), it was found that the substitution with substituents causing redistribution of π-electron density led to a change in the biological activity of the compounds [53–54,21].

A question arose whether the results of these studies could be generalised not only over other drugs but over biologically active systems as well.

In view of the results obtained for a small group of herbicides, it seemed that the answer was positive. Encouraging results were obtained for a group of phenoxyl herbicides—derivatives of phenoxyacetic acid substituted at positions 2, 4 and 5 [2,4-dichlorophenoxyacetic acid (2,4-D); 4-chloro-2-methyl phenoxyacetic acid (MCPA); 2,4,5-trichloro phenoxyacetic acid (2,4,5-T)] and their metabolites (2,4-dichlorophenol, 4-chloro-2-methylphenol and 2,4,5-trichlorophenol). The ^{35}Cl NQR quadrupole coupling constants [55–58] and lethal doses LD_{50} [35], percentage of erythrocyte hemolysis and the number of free SH groups responsible for damage to the membrane proteins [58] for the hitherto studied herbicides were taken into account. These data indicated that the toxicity of phenoxyl herbicides was correlated with ^{35}Cl NQR frequencies. The data for 2,4-dichlorophenol and 2,4-D significantly deviated from the line of this correlation obtained by the least square method, which was approximated as:

$$LD_{50} = 927e^2Qqh^{-1} - 32052 \qquad (2)$$

Table 9
The ^{35}Cl NQR frequencies and lethal doses LD_{50} for the phenoxyl herbicides studied

No.	Compound	e^2Qqh^{-1} (MHz)	Erythrocyte haemolysis in saline solution (%)	LD_{50} (mg/kg)
1	2,4-Dichlorophenol	70.160	2.6	580
2	2,4-Dichlorophenoxyacetic acid (2,4-D)	70.292	2.08	370
3	2,4-Dichlorophenoxy propanoic acid (2,4-DP)	70.680	–	800
4	4-Chloro-2-methyl phenol	69.787	5.45	–
5	4-Chloro-2-methyl-phenoxyacetic acid (MCPA)	70.734	1.80	700
6	1,2,4-Trichlorobenzene	70.806	–	756
7	2,4,5-Trichlorophenol	72.490	4.21	–
8	2,4,5-Trichloro-phenoxyacetic acid (2,4,5-T)	73.347	2.60	–

Compounds 1, 4, 7 are metabolites of herbicides 2, 5, 8.

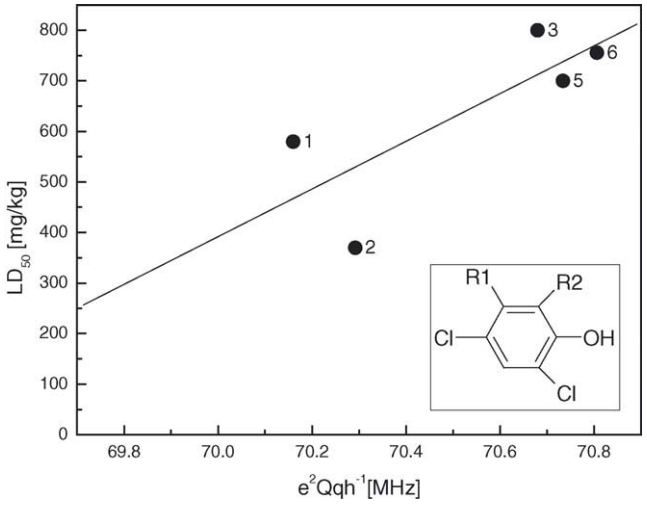

Fig. 6. The lethal dose of phenoxyl herbicides (LD_{50}) vs. the quadrupole coupling constant.

with the correlation coefficient of 0.776 and standard deviation of 125.8, Table 9 and Fig. 6 [59].

A too small number of the compounds studied could not justify the rejection of any of the points 1 or 2, which would much improve the fit. It is known that hydroxyl groups increased the toxicity of aromatic compounds, therefore, the presence of such a group could be supposed to be responsible for the experimentally measured toxicity of 2,4-dichlorophenol being higher than that estimated from Eq. (2). Definite solution of the problem would require investigation of a greater number of compounds from this group. Moreover percentage erythrocyte haemolysis in saline solution [58] was found well correlated with quadrupole coupling constants, Table 9 (correlation coefficient 0.82, standard deviation 0.34) [21].

4. Biological activity and resonance spectroscopies

The question was whether this supposition could be generalised not only over other groups of compounds but over data provided by other spectroscopic methods, and if yes, which of the methods provides the most accurate description of the electronic structure. The results obtained from the study of thiazides [9] provided a definite answer.

A good correlation between the degree of bonding of carbonic dehydratase after treatment with a certain sulfonamide and the ^{14}N NMR frequency measured on nitrogen nuclei of the amide group of the sulfonamide encouraged us to take up a study for a group of compounds of similar structure and different activity—sulfonamide derivatives of benzodithiazine known also as thiazides. The study was also prompted by the fact that literature gives many contrasting opinions on the electron density distribution and reactive sites in the thiazide molecules. Shinagawa [60,61] related the biological activity of thiazides with the π-electron density on the nitrogen atoms N(2), N(4) and the carbon atom C(7), Wohl [62,63] supposed that it was determined by the positive charge on the nitrogen atom N(4) and the rotational symmetry of the substituent, according to Cragoe [64] it was a consequence of the lipidophilous properties of the substituent, according to Beyer and Baer [65] of chlorouretic properties of thiazides, according to Orita it was related to the formal charge on the atom at C(7) [66] and according to Topliss—to the parameters of octanol–water division [67]. These were mostly theoretical works based on the results of calculations performed by simplified methods (Hückel method [62,63], semiempirical CNDO/2 [66]), and only a few of them were experimental reports on the physico-chemical properties of thiazides (parameters of octanol–water division [67]). In the recent review papers on thiazides [67–69], this problem was left unsolved and the authors emphasised that the mechanism of the thiazides activity was still unknown. In the papers published till 1997, the diuretic activity of thiazides was related to inhibition of carbonic anhydrase (CA). As follows from recent studies, the diuretic properties of thiazides were determined by the reflux absorption of sodium and chlorine based on the inhibition of the protein 115 kDa (built of 1021 amino acids) of the Na^+–Cl^- co-transporter sensitive to thiazides (TSC, NCC or SLC12A3) [64]. In 1999, Chang and Fujita [70] proposed a model of thiazides activity assuming they were competitive to chlorine at making bonds with the Na^+–Cl^- co-transporter. Also it was experimentally proved [69] that the Na^+–Cl^- co-transporter with a thiazide instead of chlorine could not permeate through cell membranes. The model was developed by Lloyd who used the CellML [71]. How-

Table 10
Parameters characterising the electronic structure of thiazides

No.	Compound	Spectroscopic parameters				Biological data
		^{13}C NMR	EPR		^{35}Cl NQR	
		$\delta_{C(2)}$ (ppm)	A_N (mT)	A_C (mT)		
1	HCTZ	55.7	1.18	2.10	72.958	25–150
2	TCTZ	69.3	1.11	3.30	73.800	5–20
3	ATZ	63.9	1.13	0.59	72.838	20–100
4	CTZ	150.3	1.18	0.59	74.220	1000–2000

ever, the Chang model did not explain the way of the bonding a thiazide with the Na^+–Cl^- co-transporter. The problem was important as, according to the recent results, thiazides were successful in therapy of osteoporosis and the interest in them was growing [72–75].

The correlated spectroscopic (magnetic resonance methods) and density functional theory (DFT) research reported in [5,9] permitted a comprehensive analysis of the electron density distribution and identification of the reactive sites in thiazide molecules.

Finally, it was established that the biological activity of thiazides was determined by the electron density distribution in the region N(2)–C(3)–N(4), as followed from the results of the experimental study on the chlorine atom at the position C(7) [76] and on all carbon atoms [77] in the ground state, and in direct neighbourhood of the radical (i.e. on the chlorine atoms N(2) and N(4), the chlorine atom from $-CHCl_2$ and C(3) from the radical [78]) and from the DFT calculations [9,79,80]. Moreover, the biological activity of the thiazides was correlated with the properties of substituents; the delocalisation effects decreased it, the induction effects modified it (decreased or increased depending on the substituent electron donor or acceptor properties), and the density changes of the lone electron pair at N(4) played the most important role N(4) [5,9]. As reported in [5] with increasing single dose of administered drug (corresponding to its decreasing biological activity), the spectroscopic parameter describing the polarisation effect also decreased. The appearance of the delocalisation effect on passing from HCTZ to CTZ was accompanied by a significant increase in the spectroscopic parameter, which induced a significant increase in the single dose and thus a drastic decrease in the biological activity. The same conclusions were drawn irrespective of the spectroscopic parameter analysed: quadrupole coupling constant, chemical shift, hyperfine coupling constants, Table 10 and Fig. 7 (correlation coefficients higher than 0.97 and standard deviations lower than 0.56).

The changes in the QCC recorded by ^{35}Cl NQR on the chlorine atom very far from the site of substitution, revealed the same tendency as that obtained on the basis of the chemical shift values determined by ^{13}C CP/MAS NMR or hyperfine coupling constants by EPR; moreover, the NQR was found the most sensitive among the resonance methods applied [9]. Although the number of points presented in Fig. 7 illustrating the character of the dependence of spectroscopic

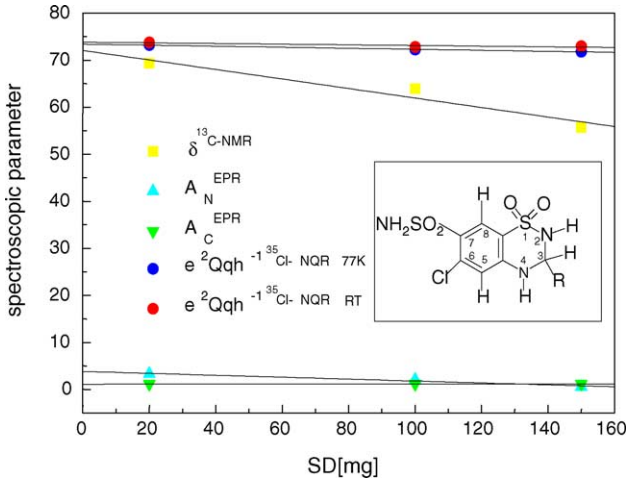

Fig. 7. The spectroscopic parameters (NQR, NMR, EPR) vs. the biological activity of the thiazides studied expressed by the single dose (SD).

parameters on the single dose is small (the small number of compounds studied) the tendency is clear.

As indicated by the results of biological study, the weakest from among the thiazides studied was CTZ, while the strongest diuretic was TCTZ with the electron acceptor group $-CHCl_2$ at C(3), whose replacement by hydrogen (HCTZ) or $-CH_2SCH_2CH{=}CH_2$ (ATZ) significantly reduced the biological activity. The Parr reactivities determined for thiazides by the DFT method in [79] suggested the highest chemical reactivity of TCTZ and the lowest of CTZ, while the electronegativity calculated for TCTZ was close to that of a chlorine atom, which in the light of the Chang model, explained the highest biological activity of TCTZ. The results given in [65] suggested that the source of the reactive electrons was the nitrogen atom N(4), and because of the type of radical, formed upon γ-irradiation [78], the most active site in the molecule was the carbon atom C(3) and these atoms played the main role in the process of the thiazide bonding with the Na^+–Cl co-transporter.

5. Summary

A good correlation of the parameters obtained from NQR spectra (resonance frequencies, quadrupole coupling constants for isotopes of chlorine and bromide, bond population

for nitrogen) with the data describing biological activity suggested that the distribution of electron density in the molecule could be one of the factors determining the compound activity. However, it would not always be possible to correlate the parameters obtained from NQR spectra with the data describing biological activity, because of the lack of the latter. Fortunately, in some cases it was possible to explain the increase or decrease in biological activity of some compounds, depending on the kind of substituent, by analysing the distribution of electron density. It is a very important conclusion because of the insufficient information currently available on the molecular background of the biological activity of many drugs for man and animals.

On the other hand, the information provided by NQR investigation was helpful in establishing directions of the search for new drugs or other biologically active compounds. NQR data indicated the possibility of formation of derivatives, that is provided the information necessary to identify the reactive sites, determine the influence of the positions of substituents on toxicity and effectiveness of a given group of compounds and moreover, helped establish the mechanism of their activity.

The results obtained in the hitherto studies confirmed the usefulness of NQR spectroscopy for determination of physical and chemical properties of compounds and prediction of their biological activity or toxicity. Moreover, the spectroscopic parameters (NMR, EPR, NQR) characterising the electronic effects were very well correlated with biological activity of the compounds studied. The information inferred from the NQR study on local electron density distribution together with analysis of charge distribution by the density functional DFT methods, could provide excellent means for determination of reactive sites and hence, could indicate possible promising directions to be followed in drugs design.

6. Conclusions

1. A good correlation between the parameters obtained from the NQR spectra (resonance frequencies, quadrupole coupling constants—for chlorine and bromide isotopes, and bond population for nitrogen atoms) and biological activity suggested that a distribution of electronic density in a molecule could be an important factor determining the activity of a drug.
2. A correlation between biological activity and parameters obtained from NQR spectra, characterising a given compound or its part, could permit a prediction of biological activity of new compounds from the same group. This fact may be of great importance for pharmacy and toxicology because the classical methods for determination of toxicity require a long time, therefore, in view of a rapidly growing number of newly synthesised compounds the use of the classical methods cannot ensure getting quick information about their toxicity.
3. NQR studies could be of importance in the search for new drugs as they provide the information on the possibility of formation of the derivative compounds (identification of the reactive sites), the influence of the substituent positions on the toxicity and efficiency of a given group of drugs and molecular mechanisms of their activity.
4. The studies performed so far indicated the usefulness of NQR spectroscopy for determination of physical and chemical properties of compounds and prediction of biological activity of drugs.
5. The information inferred from NQR study on local electron density distribution together with analysis of charge distribution by the density functional DFT methods, provided excellent means for determination of reactive sites and hence, could indicate possible promising directions to be followed in drugs design.

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