Advanced Pharmaceutical Bulletin

Adv Pharm Bull, 2016, 6(3), 377-384
doi: 10.15171/apb.2016.049
http://apb.tbzmed.ac.ir

TUOMS Publishing Group

Research Article

Preliminary Safety Assessment of New Azinesulfonamide Analogs of Aripiprazole using Prokaryotic Models

Beata Powroźnik[1], Karolina Słoczyńska[1], Krzysztof Marciniec[2], Paweł Zajdel[3], Elżbieta Pękala[1*]

[1] *Department of Pharmaceutical Biochemistry, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland.*
[2] *Department of Organic Chemistry, Medical University of Silesia, Jagiellońska 4, 41-200 Sosnowiec, Poland.*
[3] *Department of Medicinal Chemistry, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland.*

Article info

Article History:
Received: 13 May 2016
Revised: 13 July 2016
Accepted: 4 August 2016
ePublished: 25 September 2016

Keywords:
· Ames test
· Antimutagenicity
· Aripiprazole
· Isoquinoline-sulfonamides
· Quinoline-sulfonamides
· *Vibrio harveyi* assay

Abstract

***Purpose*:** Determination of the mutagenic potential of new biologically active compounds is of great concern for preliminary toxicity testing and drug development.

Methods: The mutagenic and antimutagenic effects of some quinoline- and isoquinoline-sulfonamide analogs of aripiprazole (**1**-**8**), which display potent antidepressant, anxiolytic, and antipsychotic properties, were evaluated using the *Vibrio harveyi* assay and OSIRIS Property Explorer software. Additionally, the Ames test was used as the reference.

Results: *In silico* prediction showed that compounds **5** (*N*-(3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propyl)quinoline-7-sulfonamide) and **6** (*N*-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)quinoline-7-sulfonamide) trigger a mutagenic structural alert. However, this was not confirmed by *in vitro* assays, as none of the tested compounds displayed mutagenic activity against all tested strains of bacteria. Moreover, compounds **1**-**8** displayed a protective effect against the mutagenicity induced by a direct acting mutagen NQNO. The most beneficial antimutagenic properties showed compound **5** which exhibited strong antimutagenic properties in all tested *V. harveyi* strains. High antimutagenic potency of this compound was confirmed in the Ames TA100 assay system.

Conclusion: Newly synthesized azinesulfonamide analogs of aripiprazole may be considered as genotoxically safe as they do not display mutagenic activity on the tester strains. Moreover, the tested compounds demonstrated significant antimutagenic properties that can be valuable for prevention of the NQNO genotoxicity. Additionally, it appears that the *Vibrio harveyi* assay can be applied for primary mutagenicity and antimutagenicity assessment of chemical substances, thus, representing a useful alternative tool for compounds safety evaluation.

Introduction

Over the last decades the involvement of reactive oxygen species (ROS) in the molecular mechanisms related to psychiatric disorders such as depression or schizophrenia became evident.[1-3] In rat models of depression a decreased activity of antioxidant enzymes such as glutathione peroxidase (GSH-Px) followed by increased lipid peroxidation were observed.[4-6] Furthermore, in patients with depression the elevated plasma ROS levels were observed to effectively amplify oxidative stress.[7,8] All of these observations solidified the oxidative stress hypothesis of depression.

As the removal of ROS is a vital element of antimutagenesis strategy, it was further suggested that the improvement in antimutagenic defense system may be one of the mechanisms underlying the neuroprotective effects of antidepressant drugs during depressive disorder therapies. Therefore, the development of new compounds with antidepressant properties which additionally display antimutagenic and possibly chemopreventive properties is of great practical and therapeutic significance.[9]

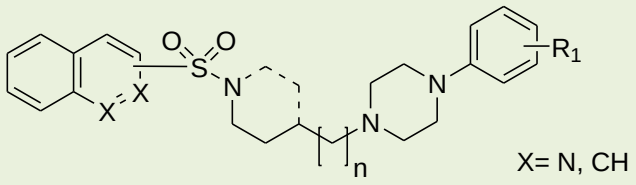
To evaluate mutagenic activity of new compounds many short-term and highly sensitive tests were introduced.[10,11] A general strategy behind mutagenicity testing is to apply a diversified set of tests to cover all of the main mutagenicity endpoints. Another big challenge for mutagenicity assessment is the prospect for using alternative assays to animal testing i.e. *in vitro* and *in silico* test methods.

The *in vitro* Ames/*Salmonella* test is a key tool for mutagenicity assessment and a substantial element of the official genotoxicity testing package[12] required for accomplishing the preclinical evaluation. The test is suitable for the detection of molecules that cause mutations such as frame-shifts or base-pair substitutions.[13] In the last several decades, several rapid bacterial mutagenicity tests have been developed and optimized, such as the *Vibrio harveyi* assay.[14,15] The test involves a series of genetically modified strains of a

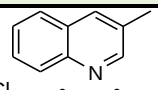
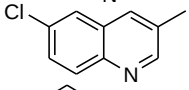
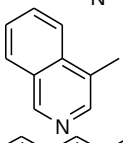
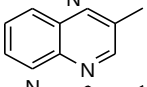
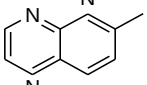
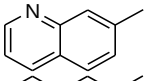
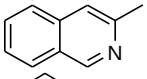
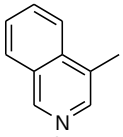
***Corresponding author:** Elżbieta Pękala, Tel: +48 12 620 55 98, fax: +48 12 657 02 62, Email: elzbieta.pekala@uj.edu.pl

marine bacterium *Vibrio harveyi*. The sensitivity of this assay was found to be similar to, or even somewhat higher than, that of the commonly used the Ames test.[16] To prevent genotoxic risk it is pertinent to identify potential mutagens in order to minimize human exposure to them, as well as to enhance the exposure to antimutagenic agents. Thus, the present study was designed to evaluate the mutagenic and antimutagenic properties of the newly synthesized quinoline- and isoquinoline-sulfonamide analogs of aripiprazole (**1**–**8**) (Table 1) which behave as multimodal dopamine/serotonin agents and display antidepressant, anxiolytic, and/or antipsychotic properties.[17-19] Focusing on the application of alternative *in vitro* and *in silico* test methods to predict compounds mutagenicity in the present study the *Vibrio harveyi* assay and OSIRIS Property Explorer software were employed. Additionally, the Ames test was used as the reference.

Table 1. Binding of the quinoline- and isoquinoline-sulfonamides (**1–xx**) for the 5-HT and D receptors, and their pharmacological behavior.[17,18]



1 - 8

Compd	Azinyl	Spacer	n	R	K_i [nM]						Forced swim test Potential antidepressant activity
					5-HT_{1A}	5-HT_{2A}	5-HT_6	5-HT_7	D_2	D_3[b]	MED [mg/kg][c]
1		Flexible	1	3-Cl	52	22	139	56	40	70/94	10
2		Flexible	1	3-Cl	208	56	155	88	NT[d]	NT	NT
3		Rigidified	2	4-Cl	3210	59	16650	47	60	67/93	30
4		Flexible	1	2,3-diCl	23	34	1331	30	9	94/99	10
5 PZ-549		Flexible	0	2,3-diCl	14	47	257	12	16	90/99	5
6		Flexible	1	2,3-diCl	17	22	301	31	11	99/101	10
7		Flexible	1	2,3-diCl	34	35	454	56	17	95/98	5
8		Flexible	1	2,3-diCl	59	90	220	21	8	93/95	20
	Aripiprazole	–	–	2,3-diCl	5.6	21	90	26	0.8	9.7	NA[e]

[a] K_i values (SEM ± 22) based on three independent binding experiments; [b] Screening procedure – displacement % at $10^{-7}/10^{-6}$; [c] MED – minimal effective dose; [d] NT – not tested; [e] NA – not active.

Materials and Methods

Tested compounds and chemicals

The tested compounds were synthesized in the Department of Medicinal Chemistry, Jagiellonian University Medical College by Zajdel et al.[17,18] The compounds structures were established previously on the basis of CHN elemental analysis and spectral data (^{1}HNMR and mass spectra).

Dimethylsulfoxide (DMSO) of high grade purity (99,9%) was obtained from Merck, (Darmstadt, Germany). 4-nitroquinoline-*N*-oxide (NQNO) chosen as positive control, was purchased from Sigma (Seelze, Germany).

The examined compounds were dissolved in DMSO to obtain the desired final test concentrations (*i.e.* 40 ng/ml for the *Vibrio harveyi* assay; 40 and 500 ng/ml for the Ames test) which were established in pilot experiments. It was demonstrated previously that in the *Vibrio harveyi* assay very low concentrations of mutagens could be detected.[20] As regards the Ames test, two compounds concentrations were employed, namely the one used in the *Vibrio harveyi* assay and the higher one commonly used in the assay.[10,21,22]

Bacterial strains

Four *Vibrio harveyi* strains were used in the experiments: wild-type BB7 and genetically modified strains: BB7M, BB7X and BB7XM.[14-16] These strains were kindly provided by Prof. G. Węgrzyn (University of Gdańsk, Poland). *Salmonella typhimurium* tester strain TA100, as described by Maron and Ames[13] and Mortelmans and Zeiger,[10] was kindly provided by Dr. T. Nohmi (National Institute of Hygienic Sciences, Tokyo, Japan).

Culture media and growth conditions

In the Ames test the minimal medium described previously by Maron and Ames[13] was used. In case of the *Vibrio harveyi* assay BOSS medium was employed.[23] All strains were stored at ⁻80°C in 10% glycerol (final concentration).

Mutagenicity assays

Mutagenicity assays were performed as described previously by Maron and Ames[13] and Czyż et al.[16] All experiments were carried out in triplicate and the results were expressed as mutagenic index (M.I. = the number of revertant colonies induced in the tested sample/ the number of spontaneous revertants in the negative control).[14,15,24] A compound was considered mutagenic when the M.I. was equal or greater than 2.

Antimutagenicity assays

Antymutagenicity assays were performed according to previously described procedures.[10,13-15,25] Triplicate plates were set up with each test compound concentration and the entire experiment was repeated twice. The inhibition of mutagenicity was expressed as percentage decrease of mutant colonies and calculated using the following equation: Percent Inhibition = $100 - [(R_1/R_2) \times 100]$, where R_1 is the number of mutants per plate induced by test compound plus mutagen and R_2 is the number of mutants per plate induced by mutagen alone.[26,27] A 25–40% inhibition was defined as moderate antigenotoxicity, 40% or higher inhibition as strong antigenotoxicity, and 25% or less inhibition as no antigenotoxicity.[24]

In silico toxicity prediction

The OSIRIS Property Explorer was used to predict mutagenicity of the compounds in the present study.[28-30]

Results

Mutagenic activity

Table 2 presents a number of mutants per plate and mutagenic index (M.I.) for selected quinoline- and isoquinoline-sulfonamide analogs of aripiprazole (**1**–**8**) evaluated in *V. harveyi* strains. Additionally, Table 2 was supplemented with the results of *in silico* mutagenicity prediction of the tested compounds. On the basis of the OSIRIS prediction results it was noted that two of the tested compounds, namely **5** and **6** trigger a mutagenic structural alert.

Table 2. Mutagenic activity of azinesulfonamides in the *Vibrio harveyi* test and by using OSIRIS Property Explorer.

Compd	*Vibrio harveyi* test								*In silico*
	BB7[a]		BB7X[a]		BB7M[a]		BB7XM[a]		OSIRIS
	Mean ± S.D.	M.I.[c]	Mean ± S.D.	M.I.[c]	Mean ± S.D.	M.I.[c]	Mean ± S.D.	M.I.[c]	M[d]
DMSO[b]	21 ± 4		17 ± 3		28 ± 2		16 ± 4		
NQNO[b]	**42 ± 9**	**2**	**34 ± 8**	**2**	**58 ± 4**	**2.1**	**36 ± 7**	**2.3**	
1	31 ± 6	1.5	27 ± 5	1.6	43 ± 5	1.5	27 ± 6	1.7	-
2	22 ± 8	1.0	17 ± 5	1.0	46 ± 8	1.6	16 ± 3	1.0	-
3	25 ± 7	1.2	15 ± 4	0.9	37 ± 9	1.3	23 ± 6	1.4	-
4	27 ± 5	1.3	19 ± 5	1.1	33 ± 6	1.2	22 ± 7	1.4	-
5	17 ± 3	0.8	6 ± 3	0.4	24 ± 2	0.9	15 ± 5	0.9	+/-
6	15 ± 6	0.7	18 ± 6	1.1	32 ± 6	1.1	24 ± 7	1.5	+/-
7	31 ± 5	1.5	14 ± 6	0.8	29 ± 1	1.0	17 ± 6	1.1	-
8	25 ± 6	1.2	22 ± 4	1.3	34 ± 5	1.2	27 ± 4	1.7	-
Aripiprazole	12 ± 3	0.6	3 ± 2	0.2	10 ± 3	0.4	5 ± 2	0.3	-

[a]Number of revertants; [b]NQNO (nitroquinoline-*N*-oxide, 40 ng/ml) - positive control; DMSO - negative control;[c]M.I. (mutagenic index): number of induced revertants / number of spontaneous revertants; M[d]: mutagenicity.

The current *in vitro* study demonstrated that in a concentration of 40 ng/ml all of the tested compounds exhibited no mutagenic activity in *V. harveyi* BB7, BB7M, BB7X and BB7XM strains. Similarly, as shown

in Table 3 the tested compounds were non mutagenic in the Ames TA100 mutagenicity assay when tested in two concentrations *i.e.* 40 and 500 ng/ml.

Table 3. Mutagenic activity of azinesulfonamides in the Ames test.

Compd	Concentration (ng/ml)	Ames test TA100[a]	
		Mean ± S.D.	M.I.[c]
DMSO[b]		10 ± 5	
NQNO[b]	40	**31 ± 4**	**3.1**
	500	**72 ± 10**	**7.2**
1	40	5 ± 2	0.5
	500	8 ± 3	0.8
2	40	3 ± 1	0.3
	500	12 ± 4	1.2
3	40	4 ± 2	0.4
	500	6 ± 2	0.6
4	40	8 ± 2	0.8
	500	18 ± 4	1.8
5	40	5 ± 2	0.5
	500	9 ± 3	0.9
6	40	7 ± 2	0.7
	500	9 ± 3	0.9
7	40	5 ± 2	0.5
	500	8 ± 2	0.8
8	40	4 ± 2	0.4
	500	6 ± 3	0.6
Aripiprazole	40	14 ± 4	1.4
	500	19 ± 4	1.9

[a]Number of revertants; [b]NQNO (nitroquinoline-*N*-oxide, 40 ng/ml, 500ng/ml) - positive control; DMSO - negative control; [c]M.I. (mutagenic index): number of induced revertants / number of spontaneous revertants (positive assay when M.I.≥2); [d]M: mutagenicity

Antimutagenic activity

The antimutagenic effects of the selected quinoline- and isoquinoline-sulfonamide derivatives (**1**–**8**) were examined against NQNO in *V. harveyi* BB7, BB7M, BB7X, BB7XM and in *S. typhimurium* TA100 strains. All of the tested compounds effectively reduced a number of mutations induced by a direct acting agent NQNO in all bacterial strains used in the experiment (Tables 4 and 5).

In the *Vibrio harveyi* antimutagenicity assay compounds **4** and **5** demonstrated the highest antimutagenic activity against NQNO induced mutagenicity. The inhibition rates for these compounds were between 40% and 84%. It is worth noting, that only compound **5** exhibited strong antimutagenic properties in all tested *V. harveyi* strains. Aripiprazole also strongly inhibited the mutagenicity induced by NQNO in *V. harveyi* BB7XM assay system. In the remaining three strains the compound demonstrated moderate antimutagenic potential. Three of the tested compounds *i.e.* **2**, **3** and **8** strongly reduced NQNO mutagenicity in *V. harveyi* strains BB7 and BB7XM. The inhibition percentages of these substances ranged from 41 to 65. For the remaining two strains these compounds demonstrated moderate antimutagenic activity (inhibition percentages between 28 and 38), except for compound **3** which showed weak antimutagenic potency in BB7M strain. Finally, compounds **6** and **7** strongly suppressed mutagen activity in one tested strain and moderately inhibited mutagenicity in the other three *V. harveyi* strains (Table 4).

Table 4. Antimutagenic activity of azinesulfonamides in the *Vibrio harveyi* test.

Compd	*Vibrio harveyi* test							
	BB7[a]		BB7X[a]		BB7M[a]		BB7XM[a]	
	Mean ± S.D.	Inhib. (%)[c]	Mean ± S.D.	Inhib. (%)[c]	Mean ± S.D.	Inhib. (%)[c]	Mean ± S.D.	Inhib. (%)[c]
DMSO	16 ± 4		13 ± 5		18 ± 4		11 ± 2	
NQNO	**38 ± 6**		**32 ± 3**		**47 ± 5**		**34 ± 5**	
1	17 ± 7	(55)	19 ± 5	(41)	34 ± 4	(28)	18 ± 4	(47)
2	15 ± 5	(61)	20 ± 6	(38)	31 ± 3	(34)	20 ± 3	(41)
3	17 ± 7	(55)	23 ± 3	(28)	36 ± 2	(23)	12 ± 3	(65)
4	19 ± 6	(50)	17 ± 7	(47)	28 ± 5	(40)	16 ± 5	(53)
5	6 ± 5	(84)	8 ± 2	(75)	9 ± 4	(81)	14 ± 5	(59)
6	18 ± 2	(53)	25 ± 3	(22)	31 ± 7	(34)	23 ± 3	(32)
7	24 ± 4	(37)	23 ± 5	(28)	29 ± 5	(38)	18 ± 2	(47)
8	14 ± 6	(63)	20 ± 6	(38)	34 ± 4	(28)	12 ± 2	(65)
Aripiprazole	21 ± 2	(45)	17 ± 5	(47)	22 ± 4	(53)	13 ± 2	(62)

[a]Number of revertants; [b]NQNO (nitroquinoline-*N*-oxide, 40 ng/ml) - positive control; DMSO - negative control; [c]The values in parenthesis are the inhibition rates (%) of mutagenicity.

The results presented in Table 5 show that in *S. typhimurium* TA100 strain the strongest antimutagenic effect was observed for compound **5** with inhibition percentages of 48% (40 ng/ml) and 43% (500 ng/ml).

Three other compounds **1**, **4** and **8** were strong inhibitors of the mutagenicity induced by a direct-acting mutagen NQNO only in one tested concentration. Compound **1** in a lower concentration inhibited NQNO mutagenicity by 48%, whereas compound **4** when tested in higher concentration reduced NQNO - induced mutagenic effect by 51%. Aripiprazole used as a reference psychotropic drug inhibited the mutations induced by NQNO ranging from 27% to 30%, which indicates a moderate antimutagenic effect. Additionally, compounds **2**, **3** and **7** displayed moderate antimutagenic activity in both tested concentrations. The inhibition percentages for these compounds were between 25 and 37. Only compound **6** when tested in lower concentration exhibited weak antimutagenic effect with inhibition rate of 18%.

Table 5. Antimutagenic activity of azinesulfonamides in the Ames test.

Compd	Concentration (ng/ml)	Ames test	
		TA100[a]	
		Mean ± S.D.	Inhib. (%)[c]
DMSO		12 ± 5	
NQNO	40	**33 ± 4**	
	500	**75 ± 11**	
1	40	17 ± 6	(48)
	500	48 ± 9	(36)
2	40	23 ± 10	(30)
	500	55 ± 8	(27)
3	40	24 ± 2	(27)
	500	56 ± 8	(25)
4	40	25 ± 4	(24)
	500	37 ± 6	(51)
5	40	17 ± 6	(48)
	500	43 ± 3	(43)
6	40	27 ± 2	(18)
	500	56 ± 13	(25)
7	40	24 ± 3	(27)
	500	47 ± 7	(37)
8	40	25 ± 3	(24)
	500	43 ± 9	(43)
Aripiprazole	40	23 ± 2	(30)
	500	55 ± 2	(27)

[a]Number of revertants; [b]NQNO (nitroquinoline-*N*-oxide, 40 ng/ml, 500 ng/ml) - positive control; DMSO - negative control; [c]The values in parenthesis are the inhibition rates (%) of mutagenicity.

Discussion

Determination of a mutagenic potential of new compounds is an essential component of regulatory toxicology.[12,31,32] Its highly recommended to perform genetic toxicology screening studies in the early stage of product development to minimize potential mutagenic activity of new molecules and to prioritize structural modifications.

In the present study we evaluated mutagenic and antimutagenic properties of the new bioactive quinoline- and isoquinoline-sulfonamide derivatives of long-chain arylpiperazines using the combination of both *in silico* and *in vitro* methods. With a view to develop new alternative approaches to safety testing we employed the *Vibrio harveyi* assay to assess compounds muta- and antimutagenicity. This assay, based on the detection of colonies of neomycin-resistant mutants appearing frequently after a contact with mutagens, was shown to be of sensitivity equal to or higher than that of the Ames test, depending on the nature of a tested mutagen.[20] Using the *V. harveyi* assay it is possible to detect significantly lower concentrations (such as 40 ng/ml) of typical chemical mutagens than when employing the Ames test. Additionally, in the present study the standard Ames test was used to check the reliability of OSIRIS Property Explorer and *Vibrio harveyi* prediction.

Structural modifications within evaluated compounds (**1**–**8**) comprised diversification of the kind of azinyl moiety, introduction of a flexible (three- and four methylene groups spacer) and semi-rigid alkylene linker, and variation of the position of the halogen atoms in the phenylpiperazine moiety. Quinoline- and isoquinoline-sulfonamides of LCAPs containing monochloro-substituted phenylpiperazine (**1**, **2**) were classified as multireceptor 5-HT_{1A}/5-HT_{2A}/5-HT_7/D_2/D_3 ligands, which behaved as 5-HT_{2A}/5-HT_7/D_2 antagonists. Compound **3** was classified as 5-HT_{2A}/5-HT_7/D_2/D_3 ligand. However, azinesulfonamides containing 2,3-diClPhP (**4**–**8**) were classified as potent, multireceptor 5-HT_{1A}/5-HT_{2A}/5-HT_7/D_2/D_3 ligands and behaved as 5-HT_{1A} receptor partial agonist/5-HT_{2A} and 5-HT_7 receptor antagonist/D_2 receptor partial agonists. Such receptor profile and the functional properties of the investigated agents were similar to those reported for aripiprazole. It's worth noting, that aripiprazole, a reference drug approved for the treatment of schizophrenia and depression, was classified as a neuroprotective agent based on non-clinical studies using transformed cell lines and *in vivo* stress and lesion paradigms.[33,34] Additionally, aripiprazole inhibited chronic mild stress induced accumulation of ROS.[4]

Firstly, the tested compounds were submitted to *in silico* toxicity screening using the OSIRIS program. It was found that compounds containing 7-quinolinyl fragment, namely **5** (*N*-(3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propyl)quinoline-7-sulfonamide) and **6** (*N*-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)quinoline-7-sulfonamide) trigger a mutagenic structural alert and should be consider potentially hazardous. Subsequently, all the tested compounds were evaluated *in vitro* using the *Vibrio harveyi* assay and the Ames test. Contrary to the preliminary *in silico* data, none of the compounds showed mutagenic activity on *V. harveyi* BB7, BB7M, BB7X and BB7XM and *S. typhimurium* TA100 strains.

Interestingly, in general mutagenic indexes was lower in *S. typhimurium* TA100 strain than in *V. harveyi* strains. This phenomenon could be explained in terms of a higher sensitivity of the *Vibrio harveyi* mutagenicity assay in comparison to the Ames test.

All of the tested azinesulfonamide derivatives of long-chain arylpiperazines displayed a protective effect against the mutagenicity induced by a direct acting mutagen NQNO in the *Vibrio harveyi* and the Ames assays with inhibition percentages ranging from 22 to 84 in *V. harveyi* assay and from 18 to 51 in the Ames test. The most beneficial antimutagenic properties showed compound **5** which exhibited strong antimutagenic properties in all tested *V. harveyi* strains. High antimutagenic potency of this compound was confirmed in the Ames TA100 assay system. Moreover, antimutagenic effects of compounds **7** and **8** obtained in *Vibrio harveyi* were closely related to the Ames test. In can be concluded that antimutagenicity data are comparable between *Vibrio harveyi* and *Salmonella* assays. Only in case of compounds **3**, **6** and aripiprazol small discrepancy exists between the data obtained in these two antimutagenicity tests.

The inhibitory effects of tested azinesulfonamides **1**–**8** on the mutagenicity of NQNO indicated that these compounds may protect the bacterial genome against genotoxicity induced by directly acting mutagens. Although the tested compounds may exert their antimutagenic actions by more than one mechanism, it seems probable that tested compounds facilitate or stimulate the bacterial transmembrane export system to eliminate the mutagen. Alternative mechanism may involve uptake of mutagen into bacteria.[35] In addition, as NQNO is an oxidative mutagen that undergoes redox recycling to generate ROS[36] the antimutagenic action of tested compounds may be attributed to the inhibition of free radicals formation. However, further studies are required in order to establish the exact mechanism of these compounds action.

Conclusion

Newly synthesized azinesulfonamide analogs of aripiprazole may be considered as genotoxically safe as they do not display mutagenic activity on the tester strains. Moreover, the tested compounds demonstrated significant antimutagenic properties that can be valuable for prevention of the NQNO genotoxicity. The present study showed that although the results of *in silico* analysis are informative and accurate for some structural classes, they have often limited application for prediction of mutagenic properties of the novel classes of compounds. Thus, experimental verification of structural alerts for such compounds should be always considered. Additionally, it appears that the *Vibrio harveyi* assay can be applied for primary mutagenicity and antimutagenicity assessment of chemical substances, thus, representing a useful alternative tool for compounds safety evaluation. Finally, the obtained preliminary mutagenicity and antimutagenicity results encourage further search in the group of quinoline- and isoquinoline-sulfonamide derivatives of long-chain arylpiperazines as potential psychotropic agents that additionally display antimutagenic properties.

Acknowledgments

The project was supported from the Jagiellonian University, Medical College (Grant no. K/ZDS/005488) and partially by Grant no. K/DSC/001410. We thank Prof. G. Węgrzyn and Dr. T. Nohmi for providing bacterial strains for the experiment.

Ethical Issues

Not applicable.

Conflict of Interest

The authors declare no conflict of interests.

References

1. Ng F, Berk M, Dean O, Bush AI. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. *Int J Neuropsychopharmacol* 2008;11(6):851-76. doi: 10.1017/S1461145707008401
2. Wood SJ, Yücel M, Pantelis C, Berk M. Neurobiology of schizophrenia spectrum disorders: the role of oxidative stress. *Ann Acad Med Singapore* 2009;38(5):396-401.
3. Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. *Prog Neuropsychopharmacol Biol Psychiatry* 2011;35(3):676-92. doi: 10.1016/j.pnpbp.2010.05.004
4. Eren I, Naziroğlu M, Demirdaş A. Protective effects of lamotrigine, aripiprazole and escitalopram on depression-induced oxidative stress in rat brain. *Neurochem Res* 2007;32(7):1188-95. doi: doi: 10.1007/s11064-007-9289-x
5. Lucca G, Comim CM, Valvassori SS, Réus GZ, Vuolo F, Petronilho F, et al. Increased oxidative stress in submitochondrial particles into the brain of rats submitted to the chronic mild stress paradigm. *J Psychiatr Res* 2009;43(9):864-9. doi: 10.1016/j.jpsychires.2008.11.002
6. Lucca G, Comim CM, Valvassori SS, Réus GZ, Vuolo F, Petronilho F, et al. Effects of chronic mild stress on the oxidative parameters in the rat brain. *Neurochem Int* 2009;54(5-6):358-62. doi: 10.1016/j.neuint.2009.01.001
7. Kodydková J, Vávrová L, Zeman M, Jirák R, Macášek J, Staňková B, et al. Antioxidative enzymes and increased oxidative stress in depressive women. *Clin Biochem* 2009;42(13-14):1368-74. doi: 10.1016/j.clinbiochem.2009.06.006
8. Michel TM, Frangou S, Thiemeyer D, Camara S, Jecel J, Nara K, et al. Evidence for oxidative stress in the frontal cortex in patients with recurrent depressive

disorder--a postmortem study. *Psychiatry Res* 2007;151(1-2):145-50. doi: doi: 10.1016/j.psychres.2006.04.013
9. Miadokova E, Masterova I, Vlckova V, Duhova V, Toth J. Antimutagenic potential of homoisoflavonoids from Muscari racemosum. *J Ethnopharmacol* 2002;81(3):381-6. doi: 10.1016/s0378-8741(02)00135-6
10. Mortelmans K, Zeiger E. The Ames *Salmonella*/microsome mutagenicity assay. *Mutat Res* 2000;455(1-2):29-60. doi: 10.1016/s0027-5107(00)00064-6
11. Podgórska B, Wegrzyn G. A modified *Vibrio harveyi* mutagenicity assay based on bioluminescence induction. *Lett Appl Microbiol* 2006;42(6):578-82. doi: doi: 10.1111/j.1472-765X.2006.01891.x
12. Gollapudi BB, Krishna G. Practical aspects of mutagenicity testing strategy: an industrial perspective. *Mutat Res* 2000;455(1-2):21-8. doi: 10.1016/s0027-5107(00)00114-7
13. Maron DM, Ames BN. Revised methods for the *Salmonella* mutagenicity test. *Mutat Res* 1983;113(3-4):173-215. doi: 10.1016/0165-1161(83)90010-9
14. Powroźnik B, Słoczyńska K, Canale V, Grychowska K, Zajdel P, Pękala E. Preliminary mutagenicity and genotoxicity evaluation of selected arylsulfonamide derivatives of (aryloxy)alkylamines with potential psychotropic properties. *J Appl Genet* 2016;57(2):263-70. doi: 10.1007/s13353-015-0322-5
15. Obniska J, Rapacz A, Rybka S, Powroźnik B, Pękala E, Filipek B, et al. Design, synthesis and biological activity of new amides derived from 3-methyl-3-phenyl-2,5-dioxo-pyrrolidin-1-yl-acetic acid. *Eur J Med Chem* 2015;102:14-25. doi: 10.1016/j.ejmech.2015.07.017
16. Czyż A, Jasiecki J, Bogdan A, Szpilewska H, Węgrzyn G. Genetically modified *Vibrio harveyi* strains as potential bioindicators of mutagenic pollution of marine environments. *Appl Environ Microbiol* 2000;66(2):599-605. doi: 10.1128/aem.66.2.599-605.2000
17. Zajdel P, Marciniec K, Maślankiewicz A, Satała G, Duszyńska B, Bojarski AJ, et al. Quinoline- and isoquinoline-sulfonamide derivatives of LCAP as potent CNS multi-receptor-5-HT_1A/5-HT_2A/5-HT_7 and D_2/D_3/D_4-agents: the synthesis and pharmacological evaluation. *Bioorg Med Chem* 2012;20(4):1545-56. doi: 10.1016/j.bmc.2011.12.039
18. Zajdel P, Marciniec K, Maślankiewicz A, Grychowska K, Satała G, Duszyńska B, et al. Antidepressant and antipsychotic activity of new quinoline- and isoquinoline-sulfonamide analogs of aripiprazole targeting serotonin 5-HT_1A/5-HT_2A/5-HT_7 and dopamine D_2/D_3 receptors. *Eur J Med Chem* 2013;60:42-50. doi: 10.1016/j.ejmech.2012.11.042
19. Zajdel P, Partyka A, Marciniec K, Bojarski AJ, Pawłowski M, Wesołowska A. Quinoline- and isoquinoline-sulfonamide analogs of aripiprazole: novel antipsychotic agents? *Future Med Chem* 2014;6(1):57-75. doi: 10.4155/fmc.13.158
20. Czyż A, Szpilewska H, Dutkiewicz R, Kowalska W, Biniewska-Godlewska A, Wegrzyn G. Comparison of the Ames test and a newly developed assay for detection of mutagenic pollution of marine environments. *Mutat Res* 2002;519(1-2):67-74. doi: 10.1016/s1383-5718(02)00112-2
21. Lepailleur A, Bureau R, Halm-Lemeille MP, Bouquet M, Pecquet R, Paris-Soubayrol C, et al. Assessment of the genotoxic and carcinogenic potentials of 3-aminothiophene derivatives using in vitro and in silico methodologies. *J Appl Toxicol* 2014;34(7):775-86. doi: 10.1002/jat.2938
22. Ozkan H, Gultekin DD, Sezen S, Ozgeris B, Gulluce M, Tümer F. Mutagenic assessment of three synthetic pyridine-diaryl ketone derivatives. *Toxicol Ind Health* 2015;31(12):1252-7. doi: 10.1177/0748233713491810
23. Klein G, Walczak R, Krasnowska E, Błaszczak A, Lipińska B. Characterization of heat-shock response of the marine bacterium *Vibrio harveyi*. *Mol Microbiol* 1995;16(4):801-11. doi: 10.1111/j.1365-2958.1995.tb02441.x
24. Gulluce M, Agar G, Baris O, Karadayi M, Orhan F, Sahin F. Mutagenic and antimutagenic effects of hexane extract of some Astragalus species grown in the eastern Anatolia region of Turkey. *Phytother Res* 2010;24(7):1014-8. doi: 10.1002/ptr.3059
25. Słoczyńska K, Pękala E, Wajda A, Węgrzyn G, Marona H. Evaluation of mutagenic and antimutagenic properties of some bioactive xanthone derivatives using *Vibrio harveyi* test. *Lett Appl Microbiol* 2010;50(3):252-7. doi: 10.1111/j.1472-765X.2009.02781.x
26. Birosova L, Mikulasova M, Vaverkova S. Antimutagenic effect of phenolic acids. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2005;149(2):489-91. doi: 10.5507/bp.2005.087
27. Pękala E, Liana P, Kubowicz P, Powroźnik B, Obniska J, Chlebek I, et al. Evaluation of mutagenic and antimutagenic properties of new derivatives of pyrrolidine-2,5-dione with anti-epileptic activity, by use of the *Vibrio harveyi* mutagenicity test. *Mutat Res* 2013;758(1-2):18-22. doi: 10.1016/j.mrgentox.2013.07.011
28. Sangamwar AT, Deshpande UD, Pekamwar SS, Vadvalkar SM. Improving decision making for drug candidates: A computational approach for benzthiazoles as antifungal. *Indian J Biotechnol* 2007;6(3):389-96.
29. RajaSekhar KK, RajendraPrasad Y, Shankarananth V, Harika KS, Rajani K, Padmavathamma M. *In silico* prediction of selected pharmacokinetic, biological and toxic properties of some 1, 3, 5-trisubstituted-2-pyrazolines derived from isonicotinic acid. *J Glob Trends Pharm Sci* 2011;2(4):489-512.
30. OSIRIS Property Explorer. [2 March 2016]; Available from: http://www.organic-chemistry.org.

31. Benfenati E, Benigni R, Demarini DM, Helma C, Kirkland D, Martin TM, et al. Predictive models for carcinogenicity and mutagenicity: frameworks, state-of-the-art, and perspectives. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 2009;27(2):57-90. doi: 10.1080/10590500902885593
32. Vanparys P, Corvi R, Aardema MJ, Gribaldo L, Hayashi M, Hoffmann S, et al. Application of *in vitro* cell transformation assays in regulatory toxicology for pharmaceuticals, chemicals, food products and cosmetics. *Mutat Res* 2012;744(1):111-6. doi: 10.1016/j.mrgentox.2012.02.001
33. Deutsch SI, Rosse RB, Schwartz BL, Mastropaolo J. A revised excitotoxic hypothesis of schizophrenia: therapeutic implications. *Clin Neuropharmacol* 2001;24(1):43-9. doi: 10.1097/00002826-200101000-00008
34. Lieberman JA, Bymaster FP, Meltzer HY, Deutch AY, Duncan GE, Marx CE, et al. Antipsychotic drugs: comparison in animal models of efficacy, neurotransmitter regulation, and neuroprotection. *Pharmacol Rev* 2008;60(3):358-403. doi: 10.1124/pr.107.00107
35. Ajith TA, Soja M. A Comparative study on the antimutagenicity of atorvastatin and lovastatin against directly acting mutagens. *Cell Biol Toxicol* 2006;22(4):269-74. doi: 10.1007/s10565-006-0064-6
36. Zhang J, Tian Q, Yung Chan S, Chuen Li S, Zhou S, Duan W, et al. Metabolism and transport of oxazaphosphorines and the clinical implications. *Drug Metab Rev* 2005;37(4):611-703. doi: 10.1080/03602530500364023