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Unraveling the anti-virulence potential and antifungal efficacy of 5-aminotetrazoles using the zebrafish model of disseminated candidiasis

Natasa Radakovic¹, Andrea Nikolić², Nataša Terzić Jovanović³, Pavle Stojković², Nada Stankovic¹, Bogdan Šolaja⁴, Igor Opsenica^{2*}, Aleksandar Pavic^{1*}

¹Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, 11042 Belgrade 152, the Republic of Serbia

²University of Belgrade – Faculty of Chemistry, PO Box 51, Studentski trg 16, 11158 Belgrade, the Republic of Serbia

³University of Belgrade - Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, Njegoševa 12, 11000 Belgrade, the Republic of Serbia

⁴Serbian Academy of Sciences and Arts, Knez Mihailova 35, 11000 Belgrade, the Republic of Serbia

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Table S1

Supplementary Table S1. Lethal and teratogenic effects observed in zebrafish (*Danio rerio*) embryos at different hours post fertilization (hpf).

Category	Developmental endpoints	Exposure time (hpf)				
		24	48	72	96	120
Lethal effect	Coagulated eggs ^a	•	•	•	•	•
	Lack of the heart beating	•	•	•	•	•
Teratogenic effect	Malformation of head	•	•	•	•	•
	Malformation of eyes ^b	•	•	•	•	•
	Malformation of sacculi/otoliths ^c	•	•	•	•	•
	Malformation of chorda	•	•	•	•	•
	Malformation of tail ^d	•	•	•	•	•
	Scoliosis	•	•	•	•	•
	Yolk edema	•	•	•	•	•
	Yolk deformation	•	•	•	•	•
	Growth retardation ^e		•	•	•	•
	Hatching			•	•	•
Hepatotoxicity	Yolk absorption			•	•	•
	Liver darkening			•	•	•
Cardiotoxicity	Pericardial edema		•	•	•	•
	Heart morphology			•	•	•
	Heart beating rate (beat/min)				•	•

^aNo clear organs structure is recognized.

^bMalformation of eyes was recorded for the retardation in eye development and abnormality in shape and size.

^cPresence of none, one or more than two otoliths per sacculus, as well as reduction and enlargement of otoliths and/or sacculi (otic vesicles).

^dTail malformation was recorded when the tail was bent, twisted or shorter than to control embryos as assessed by optical comparison.

^eGrowth retardation was recorded by comparing with the control embryos in a body length (after hatching, at and onwards 72 hpf) using by optical comparison using an inverted microscope (CKX41; Olympus, Tokyo, Japan).

Figure S1.

Supplementary Figure S1. The graphic representation of the numeric proportion of the 5-aminotetrazoles according to their (A) capability and potency (B) to suppress (partially and totally) filamentation of *C. albicans* SC5314 at the doses from 1.3 to 10 μM on the given number out of six employed media (Lee, YPS, YPS+uridine, Spider, SLAD and GlcNAc).

Figure S2.

Supplementary Figure S2. The dose-dependent potential of adamantane (**AD01**) in inhibiting *Candida albicans* SC5314 filamentation *in vitro* in 7 different media. The anti-virulence activity of **AD01** in relation to parental 5-aminotetrazole (**9**) and the most potent derivatives from the tested series containing ethyladamantane moiety (**5d**) is shown.

^1H NMR (400 MHz, $\text{DMSO}-d_6$)

5d

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$)

¹H NMR (400 MHz, DMSO-*d*₆)

6c

¹³C NMR (100 MHz, DMSO-*d*₆)

¹H NMR (500 Hz, CDCl₃)

7o

0.00

¹³C NMR (125 Hz, CDCl₃)

¹H NMR (400 Hz, CDCl₃)

9.41
8.48
8.48
8.47
7.79
7.79
7.78
7.27
4.40
4.38
4.37
2.09
2.07
2.05
2.04
2.02
2.00
1.04
1.02
1.00
0.00

¹³C NMR (100 Hz, CDCl₃)

160.32
160.16
150.05
106.71
77.48
77.16
76.84
49.39
22.44
11.11

¹H NMR (400 Hz, CDCl₃)

7q

¹³C NMR (100 Hz, CDCl₃)

¹H NMR (400 Hz, CDCl₃)

¹³C NMR (100 Hz, CDCl₃)

¹H NMR (400 Hz, CDCl₃)

¹³C NMR (100 Hz, CDCl₃)

¹H NMR (400 Hz, CDCl₃)

7.96
7.52
7.52
7.50
7.49
7.36
7.34
7.34
7.32
7.25
7.00
6.98
6.96

4.56
4.55
4.54
4.54
4.52

1.99
1.86
1.85
1.84
1.83
1.82
1.75
1.72
1.70
1.67
1.64
1.58
1.57

-0.00

¹³C NMR (100 Hz, CDCl₃)

163.87

140.32

129.34

121.35

116.50

77.48
77.16
76.84

49.01

43.06
42.20

37.01
32.08

28.59

¹H NMR (500 Hz, CDCl₃)

¹³C NMR (125 Hz, CDCl₃)

HPLC analyses for purity

Compounds **5d**, **6c**, **7q**, **10c**, **11** were analyzed for purity (HPLC) using a Agilent 1200 HPLC system equipped with Quat Pump (G1311B), Injector (G1329B) 1260 ALS, TCC 1260 (G1316A) and Detector 1260 DAD VL+ (G1315C). HPLC analysis was performed in two diverse systems:

METHOD A

Poroshell 120 EC-C18 4.6 × 50 mm, 2.7µm, S.N. USCFU07797 was used as the stationary phase. Eluent was made from the following solvents: 0.2% formic acid in water (A) and methanol (B). The analysis were performed at the UV max of the compounds (at 254 or 330 nm) to maximize selectivity. Compounds were dissolved in mixture containing acetonitrile and 0.2% formic acid in water, final concentrations were ~ 1 mg/mL. Flow rate was 0.5 mL/min. Compounds were eluted using gradient protocol: 0 – 1.5 min 40% A, 1.5 – 10 min 40% A→ 15% A, 10 - 15 min 15% A→ 0% A, 15 - 20 min 0% A, 20 – 21 min 0% A→ 100% A, 21 - 22 min 100% 100% A→ 40% A.

METHOD B

Poroshell 120 EC-C18 4.6 × 50 mm, 2.7µm, S.N. USCFU07797 was used as the stationary phase. Eluent was made from the following solvents: 0.2% formic acid in water (A) and acetonitrile (B). The analysis were performed at the UV max of the compounds (at 254 or 330 nm) to maximize selectivity. Compounds were dissolved in mixture containing acetonitrile and 0.2% formic acid in water, final concentrations were ~ 1 mg/mL. Flow rate was 0.8 mL/min. Compounds were eluted using gradient protocol: 0 – 8 min 40% A→ 20% A, 8 - 15 min 20% A→ 100% A, 15 - 18 min 0% A, 18 – 19 min 0% A→ 100% A, 19 - 20 min 100% A.

Compounds **7o**, **7p**, **10a**, **10b** were analyzed for purity (HPLC) using a Agilent 1200 HPLC system equipped with Quat Pump (G1311B), Injector (G1329B) 1260 ALS, TCC 1260 (G1316A) and Detector 1260 DAD VL+ (G1315C). HPLC analysis was performed in two diverse systems:

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METHOD C

Poroshell 120 EC-C18 4.6 × 50 mm, 2.7µm, S.N. USCFU07797 was used as the stationary phase. Eluent was made from the following solvents: 0.2% formic acid in water (A) and acetonitrile (B). The analysis were performed at the UV max of the compounds (at 254 or 330 nm) to maximize selectivity. Compounds were dissolved in mixture containing acetonitrile and 0.2% formic acid in water, final concentrations were ~ 1 mg/mL. Flow rate was 0.8 mL/min. Compounds were eluted using gradient protocol: 0 – 10 min 100% A→ 20% A, 10 - 15 min 20% A→ 0% A, 15 - 18 min 0% A, 18 – 19 min 0% A→ 100% A, 19 - 20 min 100% A.

5d

Method A

Sample Name: NTAD1A

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.101	BB	0.2864	35.40524	1.56216	0.0815
2	15.734	BB	0.2116	19.28015	1.28078	0.0444
3	16.723	BB	0.2141	736.90088	52.39580	1.6968
4	18.044	BV	0.2138	49.92886	3.43118	0.1150
5	18.564	VV	0.2419	4.25522e4	2700.14111	97.9814
6	19.783	VB	0.1996	35.14485	2.53656	0.0809

Method B

Sample Name: NTAD1A

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.172	BB	0.0850	32.57227	4.55246	0.2065
2	1.824	BV	0.0701	24.25398	4.18655	0.1538
3	1.999	VV	0.0795	331.72202	61.46799	2.1032
4	2.948	VV	0.1230	48.42096	4.66856	0.3070
5	3.914	VV	0.1603	1.51170e4	1419.20862	95.8459
6	4.515	VV	0.0718	9.91224	1.66777	0.0628
7	9.344	BV	0.1172	20.89213	2.11607	0.1325
8	9.599	VV	0.1232	42.57890	4.14848	0.2700
9	10.625	VV	0.1343	105.56944	9.31268	0.6693
10	11.105	VV	0.1606	39.26785	2.87933	0.2490

6c

Method A

Sample Name: ntad2

DAD1 A, Sig=254,4 Ref=off (SANDRAIMELITIN.LOD 2019-12-23 16-14-24\TEST0000006.D)

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.044	BB	0.1489	5263.35205	543.79047	95.3204
2	16.757	BB	0.1116	248.54994	33.11275	4.5013
3	18.126	BB	0.1050	9.84383	1.38424	0.1783

Method B

Sample Name: NTAD2

DAD1 A, Sig=254,4 Ref=off (ANDREAIDEF_LC 2019-12-20 14-14-43\008-0201.D)

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.902	VV	0.0790	45.31862	7.00043	0.4103
2	0.952	VB	0.0587	37.74693	7.76697	0.3418
3	1.114	BV	0.0778	43.03737	8.54148	0.3897
4	1.502	VV	0.3004	37.46500	1.46233	0.3392
5	3.776	BB	0.1403	1.07363e4	1166.88354	97.2087
6	5.329	BV	0.1213	15.33884	1.50042	0.1389
7	5.702	VB	0.1481	28.74401	2.31057	0.2603
8	9.925	VV	0.1493	69.40450	5.51485	0.6284
9	13.722	VB	0.1399	19.68944	1.67141	0.1783
10	18.303	VB	0.1054	11.54834	1.29088	0.1046

7o

Method A

Sample Name: AMN25

DAD1 A, Sig=254,4 Ref=off (SANDRA\MELITIN.LOD 2019-12-23 16-14-24\TEST0000002.D)

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.360	BB	0.1213	73.26116	9.15863	0.5875
2	2.681	BB	0.1585	189.34917	17.19462	1.5186
3	5.562	BB	0.2310	1.20312e4	811.32727	96.4889
4	16.349	BV	0.1205	34.93004	4.40627	0.2801
5	16.628	VB	0.1667	22.41571	2.09747	0.1798
6	18.124	BB	0.0996	77.28111	11.62575	0.6198
7	18.852	BB	0.1147	27.08187	3.81919	0.2172
8	19.883	BBA	0.1043	13.47656	2.00738	0.1081

Method C

Sample Name: AMN25

DAD1 A, Sig=254,4 Ref=off (ANDREA\DEF_LC 2019-12-20 09-56-29\004-0401.D)

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.330	BV	0.0678	27.86647	5.05374	0.4775
2	1.397	VB	0.0346	11.92681	5.33171	0.2044
3	1.547	BV	0.1112	33.97068	3.62945	0.5820
4	8.813	BB	0.0818	5588.36133	1005.61603	95.7501
5	10.065	BB	0.0994	8.38761	1.01027	0.1437
6	10.888	VV	0.1023	14.84809	1.74416	0.2544
7	14.118	BV	0.0841	18.40096	2.66219	0.3153
8	16.064	BB	0.0820	12.53753	1.82889	0.2148
9	17.040	VB	0.0947	108.39198	16.07808	1.8572
10	17.861	BB	0.0932	11.71165	1.48994	0.2007

7p

Method A

Sample Name: AMN35

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.294	BB	0.1232	76.32647	9.35048	0.3748
2	2.609	BB	0.1261	127.84424	15.52577	0.6277
3	4.048	BB	0.1929	2.00457e4	1613.88257	98.4213
4	5.445	BB	0.2277	52.78683	3.62924	0.2592
5	16.378	BV	0.1132	19.17175	2.56573	0.0941
6	16.616	VB	0.1774	26.44459	2.31306	0.1298
7	19.878	BBA	0.1094	18.97297	2.65411	0.0932

Method C

Sample Name: AMN35

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.688	MM	0.0955	9003.33691	1571.09961	98.7723
2	8.585	MM	0.0950	18.72864	3.28733	0.2055
3	8.822	MM	0.1025	9.23486	1.50153	0.1013
4	9.210	MM	0.0882	5.96482	1.12664	0.0654
5	10.889	MM	0.1456	15.80468	1.80889	0.1734
6	12.532	MM	0.6791	44.32363	1.08787	0.4863
7	14.115	MM	0.1302	10.39877	1.33164	0.1141
8	14.516	MM	0.1510	7.45083	8.22332e-1	0.0817

7q

Method A

Sample Name: AMN39

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.368	BB	0.0990	70.86563	11.32424	0.8059
2	2.662	BB	0.1267	39.76822	4.89983	0.4522
3	12.914	BB	0.1455	28.28180	2.95876	0.3216
4	14.622	BV	0.1184	41.60281	5.48933	0.4731
5	15.021	VB	0.1186	8551.42871	1100.91699	97.2464
6	16.632	BB	0.1554	49.46365	4.60204	0.5625
7	19.898	BBA	0.0994	12.15687	1.88182	0.1382

Method B

Sample Name: amn39

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.669	VV	0.1179	63.13242	6.85867	0.2700
2	6.418	BB	0.1476	2.28175e4	1962.92371	97.5798
3	9.795	BV	0.1713	502.79858	36.71552	2.1502

10a

Method A

Sample Name: PAS02

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.400	BB	0.0987	72.79959	11.69057	0.2720
2	3.473	BB	0.2072	26.37526	1.86315	0.0986
3	6.437	BB	0.2286	18.02468	1.21830	0.0673
4	8.892	BB	0.2148	19.03928	1.27045	0.0711
5	10.689	BB	0.1800	2.64495e4	2268.87158	98.8285
6	13.253	BB	0.1771	29.07410	2.36959	0.1086
7	16.224	BV	0.1148	14.06170	1.80722	0.0525
8	16.462	VV	0.1409	18.12483	1.87221	0.0677
9	16.817	VB	0.1725	28.71340	2.64830	0.1073
10	18.102	BV	0.1036	72.12076	10.06022	0.2695
11	18.492	VB	0.1042	15.19089	2.15595	0.0568

Method C

Sample Name: PAS02

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.263	BB	0.0564	29.51749	7.27264	0.2217
2	1.559	BB	0.1338	29.60149	2.62995	0.2224
3	3.516	BB	0.1184	16.87066	1.69805	0.1267
4	7.224	BB	0.0762	8.37370	1.31692	0.0629
5	9.226	VB	0.0679	9.94040	1.77302	0.0747
6	10.107	BV	0.0775	8.25674	1.26746	0.0620
7	10.817	BB	0.0979	1.31070e4	2055.55835	98.4629
8	11.799	BB	0.0803	15.81879	2.37150	0.1188
9	12.345	BV	0.0935	7.89313	1.01808	0.0593
10	12.570	VB	0.1091	11.76956	1.27582	0.0884
11	14.053	BV	0.0800	11.29407	1.71161	0.0848
12	14.169	VB	0.0660	5.75810	1.05012	0.0433
13	16.200	BV	0.1014	49.52473	6.15569	0.3720

10b

Method A

Sample Name: PAS01

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.415	BB	0.0995	73.16942	11.61650	0.6612
2	2.721	BB	0.2116	26.82096	2.01048	0.2424
3	8.892	BB	0.2142	1.08410e4	779.60559	97.9661
4	10.933	BB	0.1450	10.96087	1.15157	0.0990
5	12.332	BB	0.2307	29.43125	2.03533	0.2660
6	16.222	VB	0.1185	46.46455	5.98893	0.4199
7	16.798	BB	0.1727	19.85674	1.80051	0.1794
8	18.103	BB	0.1065	18.37273	2.47801	0.1660

Method C

Sample Name: pas01

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.984	BV	0.0831	5398.60742	983.17981	96.7614
2	9.597	VV	0.1158	33.77315	3.44752	0.6053
3	9.926	VB	0.0656	6.91334	1.25786	0.1239
4	10.409	BB	0.0729	9.87399	1.63697	0.1770
5	11.102	BV	0.0924	45.91921	5.99354	0.8230
6	11.258	VB	0.0712	9.35344	1.58886	0.1676
7	12.444	VV	0.0927	10.23509	1.30871	0.1834
8	12.686	VB	0.1034	37.77139	4.36908	0.6770
9	14.755	VB	0.1042	13.33669	1.53820	0.2390
10	18.491	VB	0.0962	13.51706	1.66602	0.2423

10c

Method A

Sample Name: PAS03

Method B

Sample Name: PAS03

11

Method A

Sample Name: AMN32B

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.560	MM	0.0917	1.26707e4	2302.40698	97.6810
2	0.718	MM	0.0901	233.14105	36.26469	1.7973
3	10.907	MM	0.2955	57.90184	3.26630	0.4464
4	15.409	MM	0.1855	9.77067	8.77949e-1	0.0753

Method B

Sample Name: AMN32B

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.704	MM	0.1017	9275.26465	1520.10608	95.2814
2	11.945	MM	0.1257	459.34085	60.89954	4.7186