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Some 2-Amino-benzo[de]isoquinolin-1,3-diones as Antimicrobial Agents

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A new series of 2-amino-benzo[de]isoquinolin-1,3-diones was evaluated *in vitro* for their antimicrobial activity. The tested compounds were investigated against a variety of species of Gram negative bacteria (*E. coli* RCMB 010052 and *Pseudomonas aeroginosa* RCMB 010043) and Gram positive bacteria (*Bacillis subtilis* RCMB 010067 and *Streptococcus pneumoniae* RCMB 010010). Furthermore, they were screened against several types of fungi as *Syncephalastrum racemosum* (RCMB 05922), *Aspergillus fumigatus* (RCMB 02568), *Candida albicans* (RCMB 05036) and *Geotricum candidum* (RCMB 05097). The minimum inhibitory concentration (MIC) of the tested molecules was determined using broth double dilution method (Serially diluted technique) in proper nutrient. The findings revealed that compounds **1**, **3**, **7**, **10**, **12**, **13**, **14** and **16** have shown as potential antimicrobial agents and could be useful as corner stones for further development to design more potent antimicrobial agents.

Keywords: 2-Amino-benzo[de]isoquinolin-1,3-diones, Amphotericin B, Ampicillin, Gentamicin, Antimicrobial.

INTRODUCTION

Looking back on the history of human diseases, communicable diseases have considered for a huge proportion of diseases as a whole. It was not until the latter half of the 19th century that microorganisms were found to be responsible for a variety of communicable diseases that had been plaguing humanity from ancient days. Discovering and development of new drugs resulted in the overly optimistic view that communicable diseases would be conquered in the near future. However, in response to the development of antimicrobial agents, microorganisms that have acquired resistance to drugs through a variety of mechanisms have emerged and continue to plague human beings[1]. The 1*H*-benzo[de]isoquinolin-1,3-diones are versatile reagents and play an important role for construction of many bioactive compounds that are known to possess a wide range of biological effects such as antitumor and histone deacetylase inhibitors (HDAC) agents[2-7]. Despite promising results have been obtained in this trend, most of the compounds were deserted because of their convoluted preparation[5]. Based on these points and as a part of our interest in the search for novel antimicrobial agents[8,9], we herein report the biological evaluation of sixteen 2-amino-benzo[de]isoquinolin-1,3-dione derivatives at different bacterial strains and fungi media.

EXPERIMENTAL

Antimicrobial activity: The target molecules were individually examined against different fungal and a panel of Gram-positive and negative bacterial pathogens. Antimicrobial tests were carried out by the agar well diffusion method[10] using 100 µL of suspension containing 1×10^8 CFU/mL of pathological tested bacteria and 1×10^4 spore/mL of fungi spread on nutrient agar (NA), sabourand dextrose agar (SDA) and potato dextrose agar (PDA) respectively[11]. After the media had cooled and solidified, wells (6 mm in diameter) were made in the solidified agar and loaded with 100 µL of tested compound solution prepared by dissolving 5 mg of the chemical compound in 1 mL of dimethyl sulfoxide. The inculcated plates were then incubated for 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. Ampicilin (50 µg/mL), gentamycin (50 µg/mL) and amphotericin (50 µg/mL) were used as standard drugs for Gram-positive bacteria, Gram-negative bacteria and fungi respectively. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated (Table-1).

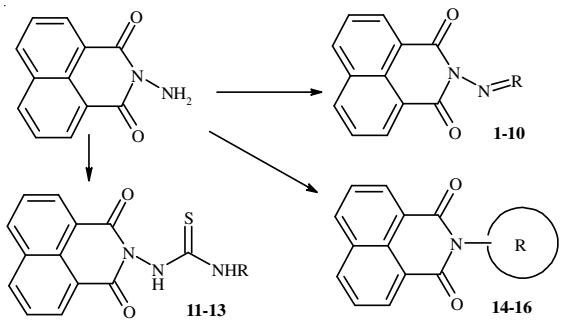
Measurement of minimum inhibitory concentration: The bacteriostatic activity of the active molecules (having inhibition zones) was evaluated using the two fold serial dilution technique[11]. The twofold serial dilutions of the tested compounds solutions were prepared using proper media broth. The final concentrations of the solutions were 500-0.007 µg/mL.

The tubes were then inoculated with the test organisms, grown in their suitable broth for tested pathogenic bacteria (1×10^8 CFU/mL for bacteria and 1×10^4 spore/mL for fungi); each 5 mL received 0.1 mL of the above inoculum and was incubated at 37 °C for 24 h for bacteria and fungi at 28 °C for 48 h. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC).

Statistical analysis: The experiment was carried out in triplicate and the data was expressed as mean ± SD. Difference in zone of inhibition between tested compounds and reference drugs were compared using Anova one way test with a difference being significant where $p < 0.001$ or $p < 0.05$.

RESULTS AND DISCUSSION

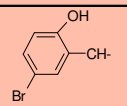
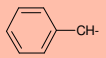
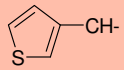
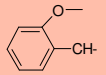
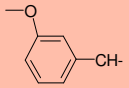
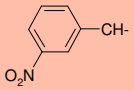
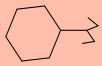
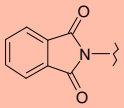
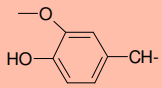
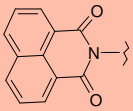
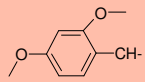
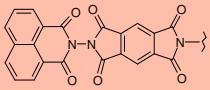
Previously[12-15], we have mentioned the synthetic strategy for preparation of compounds **1-16** as shown in **Scheme-I** and Table-1. *In vitro*, compounds **1-16** were assayed for their antimicrobial activity against *Syncephalastrum racemosum* (RCMB0-05922), *Geotricum candidum* (RCMB-05097), *Aspergillus fumigatus* (RCMB 02568), *Candida albicans* (RCMB-05036), *Bacillis subtilis* (RCMB-010067), *Streptococcus pneumoniae* (RCMB 010010), *Pseudomonas aeruginosa* (RCMB -010043) and *Escherichia coli* (RCMB-010052).



Scheme-I

In Tables 2 and 3, the obtained results revealed that, the structural modifications carried out on 2-amino-benzo[de]-isoquinolin-1,3-dione may have a considerable impact on the antibacterial and antifungal activities on all elaborated derivatives (**1-16**). Many of investigated molecules demonstrated comparable antimicrobial activities with respect to the parent compound and the control drugs (amphotericin B, ampicillin and gentamycin). Based on their preliminary antimicrobial screening results (Table-2), compounds **1**, **3**, **5**, **7**, **9-14** and **16** were selected for further studies in terms of MIC-evaluation (Table-3). Consequently, compound **3** exhibited the highest potency, higher than or equal to that of reference drugs, against RCMB-02568, RCMB-05922, RCMB-05097, RCMB-010010, RCMB-010067 and RCMB-010052. However, it was inactive against RCMB-05036 and showed slight activity against RCMB-010043 relative to gentamycin. Depending on MIC-values, compounds **1**, **7**, **12** and **13** were found to possess comparable potency to that of Amphotericin B (0.98 g/mL) against RCMB-05922 with MIC-values of 1.95, 3.90, 0.98 and 1.95, respectively. In terms of selectivity, compoud **10** showed a potent activity against Gram-negative bacteria of RCMB-010043 type in regards to gentamycin (MIC = 31.25).

TABLE-1
SYNTHESIZED 2-AMINO-BENZO[DE]ISOQUINOLIN-1,3-DIONES

Comp.	R	Comp.	R
1	H_3C—CH-	**9**	
2		**10**	
3		**11**	
4		**12**	
5		**13**	
6		**14**	
7		**15**	
8		**16**	

Tables 2 and 3 provide evidence that characteristic chemical features of aldehyde, isothiocyanide and acid anhydride are key factors for their antimicrobial activity and may prove to be useful in elucidating the mechanism action of the target products in foreseeable research programms. Moreover, variation of the substituents on the benzene ring in aromatic aldehydes (**2-9**) led to different activity. Of particular compound **3** has shown the highest activity that could be attributed to the *ortho*-position of methoxyl group in regards to its *meta*-isomer **4**. In addition, insertion of *p*-hydroxyl group in compound **7** resulted in increase the activity with respect to compound **4**. On other hands, introduction of *p*-methoxyl group in compound **8** did not offer advantagous effect relative to compound **3**. Among all tested compounds, **10** was appeared only to have selective activity against RCMB-010043 that may be probably due to the presence of hetero moiety. Concerning isothiocyanide derivatives (**11-13**), compound **12** has shown the highest activity against RCMB-05922 strain. Furthermore, the acid anhydride derivative **14** was more active against RCMB-010052 in regards to **15** and **16**. Taken together, from the results given in Tables 2 and 3, it can be concluded that all investigated compounds displayed a moderate to high antimicrobial activities. Many of tested compounds **3**, **7**, **10**, **12**, **14** were found to exhibit the same behaviour as the reference antibiotics and compound **3** was more potent than amphotericin-B against RCMB-02568 and RCMB-05922. Accordingly, this would be reflected the importance for carrying out further antimicrobial studies on such compounds.

In conclusion, all examined compounds have shown moderate to high antimicrobial activity, particularly compound **3** that exhibited potency comparable to or higher than that of

TABLE-2
MEAN ZONE OF INHIBITION IN mm ± STANDARD DEVIATION

	Fungi				Gram positive Bacteria		Gram negitive Bacteria	
	(RCMB 02568)	(RCMB 05922)	(RCMB 05097)	(RCMB 05036)	(RCMB 010010)	(RCMB 010067)	(RCMB 010043)	(RCMB 010052)
Parent	12.6 ± 0.58	13.6 ± 0.25	15.2 ± 0.38	NA	14.6 ± 0.43	16.2 ± 0.53	NA	13.7 ± 0.25
1	20.3 ± 0.39#	21.6 ± 0.16*	24.6 ± 0.58	13.2 ± 0.58	13.9 ± 0.17	15.2 ± 0.22	NA	16.9 ± 0.25
2	10.6 ± 0.25	11.7 ± 0.34	16.5 ± 0.58	NA	16.0 ± 0.44	18.3 ± 0.67	NA	13.0 ± 0.46
3	25.9 ± 0.25*	26.1 ± 0.44**	29.2 ± 0.63*	NA	25.9 ± 0.25**	30.3 ± 0.63#	12.6 ± 0.44	20.3 ± 0.44*
4	15.7 ± 0.33	17.2 ± 0.25#	19.8 ± 0.34	19.3 ± 0.24	16.2 ± 0.15	19.8 ± 0.42	NA	17.4 ± 0.53
5	16.2 ± 0.36	17.0 ± 0.44#	17.6 ± 0.58	19.3 ± 0.58	18.1 ± 0.63	20.0 ± 0.32	NA	13.9 ± 0.46
6	13.6 ± 0.25	16.8 ± 0.34	16.5 ± 0.58	16.8 ± 0.44	16.0 ± 0.44	18.3 ± 0.67	NA	13.0 ± 0.46
7	22.3 ± 0.25#	20.3 ± 0.25*	25.8 ± 0.58#	20.3 ± 0.62	25.1 ± 0.44**	29.8 ± 0.58#	NA	19.6 ± 0.19#
8	17.6 ± 0.58	19.4 ± 0.25#	19.9 ± 0.38	NA	17.7 ± 0.43	19.3 ± 0.53	NA	13.7 ± 0.25
9	16.3 ± 0.44	18.4 ± 0.58#	19.1 ± 0.37	NA	18.9 ± 0.44	20.3 ± 0.58	NA	18.9 ± 0.63#
10	18.6 ± 0.36	19.3 ± 0.44#	21.4 ± 0.58	12.1 ± 0.20	23.8 ± 0.2#	32.4 ± 0.3#	17.3± 0.1#	19.9 ± 0.3#
11	17.8 ± 0.63	18.9 ± 0.44#	20.3 ± 0.25	13.6 ± 0.25	20.6 ± 0.63	22.4 ± 0.44	NA	16.9 ± 0.58
12	20.3 ± 0.25#	21.6 ± 0.34*	23.4 ± 0.58	13.8 ± 0.22	20.6 ± 0.44#	23.4 ± 0.67	NA	18.6 ± 0.46#
13	18.3 ± 0.34	21.1 ± 0.25*	21.3 ± 0.38	15.6 ± 0.44	18.6 ± 0.44	21.1± 0.58	NA	16.3 ± 0.33
14	19.0 ± 0.12	17.3 ± 0.26#	19.8 ± 0.26	18.9 ± 058	19.7 ± 0.17	20.9 ± 0.32	NA	19.9 ± 0.10#
15	18.7 ± 0.11	19.3 ± 0.23#	15.2 ± 0.27	15.8 ± 0.44	17.8 ± 0.17	19.8 ± 0.22	NA	NA
16	20.5 ± 0.22#	18.3 ± 0.26#	15.7 ± 0.15	20.3 ± 0.63	20.7 ± 0.22#	22.4 ± 0.36	NA	22.4 ± 0.36**
R-1	23.7 ± 0.1	19.7 ± 0.2	28.7 ± 0.2	25.4 ± 0.1	–	–	–	–
R-2	–	–	–	–	23.8 ± 0.2	32.4 ± 0.3	–	–
R-3	–	–	–	–	–	–	17.3 ± 0.1	19.9 ± 0.3

**P < 0.001 highly significant; *P < 0.05 significant; #comparable or equal to the reference drug. The test was done using the diffusion agar technique, Well diameter: 6.0 mm, (100 µL was tested), RCMB: Regional center for mycology and biotechnology antimicrobial unit test organisms, NA: No activity, data are expressed in the form of mean ± SD. R-1: Amphotericin B, R-2: Ampicillin (Ve +) , R-3: gentamycin (Ve -)

TABLE-3
MIC-VALUES (µg/mL) OF TARGET COMPOUNDS (**1-16**) AGAINST TESTED MICROORGANISMS

	Fungi				Gram positive Bacteria		Gram negative Bacteria	
	(RCMB 02568)	(RCMB 05922)	(RCMB 05097)	(RCMB 05036)	(RCMB 010010)	(RCMB 010067)	(RCMB 010043)	(RCMB 010052)
1	3.9	1.95	0.12	125	125	62.5	NA	31.25
3	0.06	0.06	0.007	NA	0.06	0.007	125	3.9
5	62.5	31.25	15.63	7.81	15.63	3.9	NA	125
7	0.98	3.9	0.06	3.9	0.12	0.007	125	3.9
9	62.5	15.63	7.81	NA	62.5	15.63	NA	31.25
10	7.81	7.81	1.95	125	0.24	0.007	31.25	3.9
11	15.63	7.81	3.9	125	1.95	0.98	NA	31.25
12	3.9	0.98	0.49	125	1.95	0.49	NA	7.81
13	15.63	1.95	1.95	62.5	7.81	1.95	NA	62.5
14	7.81	31.25	3.9	7.81	3.9	1.95	NA	3.9
16	1.95	15.63	62.5	3.9	1.95	0.98	NA	15.63
R-1	0.24	0.98	0.007	0.12	–	–	–	–
R-2	–	–	–	–	0.24	0.007	–	–
R-3	–	–	–	–	–	–	31.25	3.9

*NA: No activity R-1: amphotericin B, R-2: ampicillin, R-3: gentamycin

reference antibiotics. Furthermore, as a heterocylic aldehyde-based compound **10**, displayed selective antibacterial activity against RCMB-010043 strain.

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