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Research Article

Antibacterial and Hemolytic Activities of Brominated 2-Phenitidine Derivatives

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Abstract: The present study describes the antibacterial and hemolytic activities of some brominated 2-phenitidine derivatives. These synthetic molecules were screened against selected bacterial strains, i.e., gram positive and negative using streptomycin as control. The cytotoxicity of these molecules was also tested using triton as reference. The study revealed that the analyzed molecules displayed moderate potential as antibacterial agents; however, these might serve as valued hemolytic agents.

Keywords: 2-phenitidine derivatives, antibacterial agents, cytotoxicity, hemolytic agents

1. INTRODUCTION

Currently, pathogenic microbes are posing life threatening infections to human health in all over the world. The microorganisms are contributing morbidity and mortality in immune-compromised patients [1]. Especially, the increasing resistance of bacterial strains is a challenge as public health crises and has to overcome [2]. One of most noteworthy Gram positive bacterial starins, *Staphylococcus aureus* is commensal and part of human microbiota. This strain has potential to establish broad spectrum interactions with human host. It is capable of colonizing at multiple body sites. It is known to ground various hospital allied diseases including wide range of skin related and tissue infections to acute issues such as endocarditic and bacteraemia. It is also recognized for food poisoning leads to gastroenteritis as well as invader to defense mechanism. It has characteristic to acquire resistance to antimicrobial agents [3-8]. An enterohemorrhagic strain of *Escherichia coli* (O157:H7) is found ubiquitously on farm lands. Such pathogens breed in gastrointestinal tract of healthy animals like cattle. In this way, *E. coli* approaches the human food chains *via* their manure. This strain produces shiga-like toxin and become causative agent of several diseases including bloody stools and hemolytic uremic syndrome [9]. This strain is showing resistance to antibiotics which is a pressing global problem nowadays [10].

Sulfonamides constitute a variety of artificial antibiotics being applied in veterinary medicine as growth promoters and for the treatment of bacterial infections such as digestive and of respiratory tract [11-12]. A variety of sulfonamides are available for therapy and prevention of specific bacterial diseases related to poultry. Sulfonamide derivatives are known for competitive antagonists of *p*-aminobenzoic acid, which is precursor of folic acid both in protozoan and bacterial cells. Folic acid a coenzyme is destined to produce nucleic acids in these cells. Therefore, sulfonamides are characterized as hindering agents of bacterial activity [13, 14]. Sulfonamides possess a bacteriostatic effect. These are useful in therapy of bacterial infections for instance eye infection and

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urinary tract infection [15]. This class of compounds possesses diverse pharmacological properties like antibacterial. In this way it found a key position in medicinal chemistry [16-17]. Hence, the main focus of our study was to explore some potential inhibitors of pathogenic bacteria and possible hemolytic agents to save the humans from peril of microbes.

2. MATERIALS AND METHODS

2.1. Chemistry

We have previously reported the synthesis and structural characterization of studied brominated 2-phenetidine derivatives, **1-13** (Fig. 1) [18]. Those earlier synthesized samples were subjected to current study.

2.2. Microbial Strains

Samples were tested against microbial strains in accordance with the reported method [19].

2.3. Disc Diffusion Method

The antibacterial activity was employed on compounds by the reported disc diffusion method [20].

2.4. Hemolytic Activity

The reported method [19, 21] was used to study the

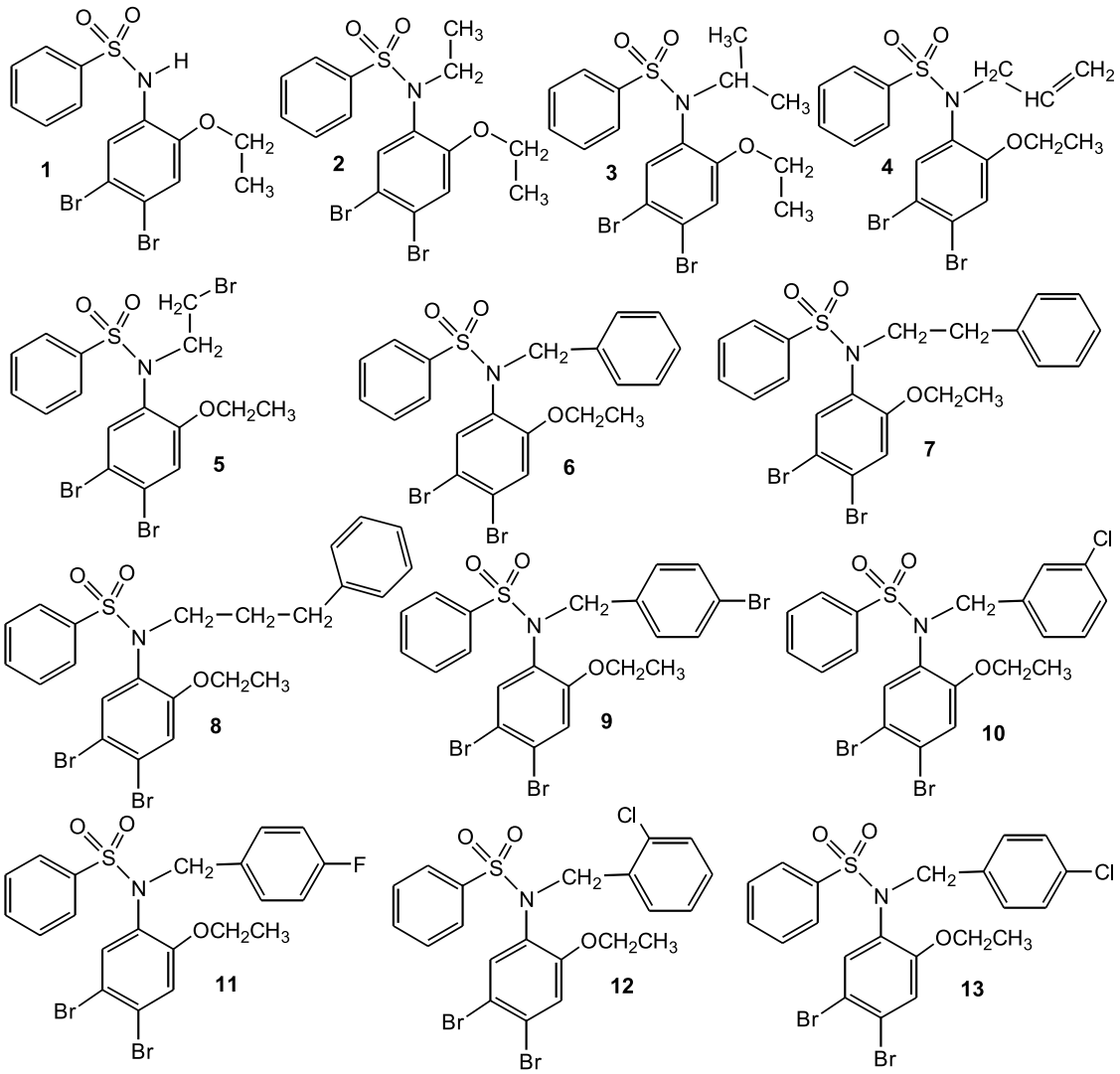

Fig. 1. Structures of brominated 2-phenetidine derivatives, **6a-m.**

hemolytic activity of the compounds. Samples were incubated at 37°C. Triton X-100 (0.1% v/v) was taken as positive control and phosphate buffer saline (PBS) was taken as negative control. Absorbance of compounds was observed at 576 nm using μ Quant (Bioteck, USA). The % RBCs lysis for each sample was calculated [21].

3. RESULTS AND DISCUSSION

3.1. Anti-bacterial Activity

Amongst the studied molecules, *N*-(4,5-dibromo-2-ethoxyphenyl)benzenesulfonamide (**1**), *N*-ethyl-*N*-(4,5-dibromo-2-ethoxyphenyl) benzenesulfonamide (**2**) and *N*-benzyl-*N*-(4,5-dibromo-2-ethoxyphenyl) benzenesulfonamide (**6**) exhibited antibacterial activity against the selected bacterial strains. The descending order of compounds activity was observed as **2** > **1** > **6** against the chosen strains of bacteria relative to standard streptomycin sulfate used throughout the assay. The greater activity of **2** as compared to **1** could be attributed to the incorporated ethyl moiety at *N*-atom in former which was not present in latter un-substituted molecule. Similarly, promising activity was shown by **6** which might be ascribed to the substitution of benzyl group on nitrogen atom in this molecule. The substantial data is evident of better activity demonstrated by compounds **1** and **2** against gram positive strain but compound **6** was comparatively more active against gram negative species *Pasturella multocida* and *Escherichia coli*. Remaining ten compounds possess very low/ no activity against bacterial species under study (Table 1).

3.2. Hemolytic Activity

N-(4-chlorobenzyl-*N*-(4,5-dibromo-2-ethoxyphenyl)benzenesulfonamide (**13**) gave high hemolytic activity value (7.54±0.340 %) but much below the positive control. It is interpreted from data that the enhanced activity of compound **13** is imparted by 4-Chlorobenzyl group. Lowest hemolytic activity was recorded by *N*-isopropyl-*N*-(4,5-dibromo-2-ethoxyphenyl) benzenesulfonamide (**3**) (0.10±0.031 %) followed

Table 1 Antibacterial against the selected bacterial species and hemolytic activity by using the human erythrocytes of the compounds.

Sr. No	Sample	*S. aureus*	*B. Subtilis*	*P. multocida*	*E. coli*	Hemolytic activity (Mean % ± S.D)
1	**1**	16	18	18	18	0.37±0.031
2	**2**	20	22	20	20	0.39±0.000
3	**3**	-	-	-	-	0.10±0.031
4	**4**	-	-	-	-	0.26±0.062
5	**5**	-	-	-	-	0.50±0.278
6	**6**	14	14	16	16	0.91±0.062
7	**7**	-	-	-	-	0.13±0.062
8	**8**	-	-	-	-	0.85±0.093
9	**9**	-	-	-	-	0.96±0.000
10	**10**	-	-	-	-	0.74±0.062
11	**11**	-	-	-	-	3.43±0.093
12	**12**	-	-	-	-	3.41±0.371
13	**13**	-	-	-	-	7.54±0.340
14	Streptomycin	28	34	34	30	
15	PBS					0.00±0.000
16	Triton (toxicity)					100±0.000

by *N*-phenylethyl-*N*-(4,5-dibromo-2-ethoxyphenyl) benzenesulfonamide (**7**) (0.13±0.062 %) but higher than the negative control phosphate-buffered saline (PBS) (Table 1). The compounds which show highest hemolytic activity might be considered for the antitumor determination and the molecules which exhibit less hemolytic activity could be targeted as valued antibacterial compounds for further studies. On the whole, the studied brominated 2-phenitidine derivatives showed much little membrane destability within the range of the given minimum and maximum values and hence have pretty low cytotoxic impact. These molecules are thus valued for further studies.

4. CONCLUSIONS

Ever growing prevalence of resisting microbial strains towards sulfonamides inculcated the need for preparation of structurally modified products owing infection combating tendency. Among the studied brominated 2-phenitidine derivatives, a few demonstrated promising activity against both bacterial strains. The compounds **1**, **2** and **6** were found more active relative to other molecules in the series. The data also showed that all the molecules were active in hemolytic analysis. The molecule **13** exhibited relatively greater cytotoxic impact and molecules **3** and **7** have very low cytotoxicity. Overall, the studied molecules are less cytotoxic and hence are very appropriate entities for further studies.

6. REFERENCES

1. Rahman, M.M., M.M.I. Sheikh, S.A. Sharmin, M.S. Islam, M.A. Rahman, M.M. Rahman, & M.F. Alam. Antibacterial activity of leaf juice and extracts of *Moringa oleifera* Lam. against some human pathogenic bacteria. *Journal of Natural Sci*ence 8(2): 219-227 (2009).
2. Cushnie, T.P.T. & A.J. Lamb. Antimicrobial Activity of flavonoids. *International Journal of Antimicrobial Agents* 26: 343–356 (2005).
3. Rahman, M.M., A. Richardson & M. Sofian-Azirun**.** Antibacterial activity of propolis and honey against *Staphylococcus aureus* and *Escherichia coli*. *African Journal of Microbiology Research* 4(16): 1872-1878 (2010).
4. Barbagelata, M.S., L.P. Alvarez, C.M. Dotto, S.M. Lattar, D.O. Sordelli, & F.R. Buzzola. Aromatic Compound-dependent *Staphylococcus aureus* is safe in a nasal colonization leukopenic murine model. *International Journal of Microbiology* 2012: 1-7 (2012).
5. Pardo, L., V. Machado, M. Mollerach, M.I. Mota, L.P.N. Tuchscherr, P. Gadea, N. Gardella, D.O. Sordelli, M. Vola, F. Schelotto & G. Varela. Characteristics of community-associated methicillin resistance *Staphylococcus aureus* (CA-MRSA) strains isolated from skin and soft-tissue infections in uruguay. *International Journal of Microbiology* 2009: 1-5 (2009).
6. Morandi, S., M. Brasca, C. Andrighetto, A. Lombardi, and R. Lodi. Phenotypic and genotypic characterization of *Staphylococcus aureus* strains from Italian dairy products. *International Journal of Microbiology* 2009: 1-7 (2009).
7. Singh, K. & V.K. Singh. Expression of four methionine sulfoxide reductases in *Staphylococcus aureus*. *International Journal of Microbiology* 2012: 1-8 (2012).
8. Rajam, G., G.M. Hammons, G.M. Carlone, J.S. Sampson & E.W. Ades. A novel innate immune-enhancement strategy combined with IVIG rescues mice from fatal *Staphylococcus aureus* septicemia. *International Journal of Microbiology* 2011: 1-5 (2011).
9. Piper, C. P.G. Casey, C. Hill, P.D. Cotter & R.P. Ross. The Lantibiotic Lacticin 3147 prevents systemic spread of *Staphylococcus aureus* in Murine infection model. *International Journal of Microbiology* 2012: 1-6 (2012).
10. Duffitt, A.D. R.T. Reber, A. Whipple, & C. Chauret. Gene expression during survival of *Escherichia coli* O157:H7 in soil and water. *International Journal of Microbiology* 2011: 1-12 (2011).
11. Liu, R.P. He, Z. Li, & R. Li. Simultaneous determination of 16 sulfonamides in animal feeds by UHPLC-MS-MS. *Journal of Chromatographic Science* 49: 640-646 (2011).
12. Shao, B., D. Dong, Y. Wu, J. Hu, J. Meng, X. Tu, & S. Xu. Simultaneous determination of 17 sulfonamide residues in porcine meat, kidney and liver by solid-phase extraction and liquid chromatography-tandem mass spectrometry. *Analytica Chimica Acta* 546: 174-181 (2005).
13. Siddiki, A.Z., M.J. Karim, & E.H. Chawdhury. Sulfonamide resistance in chicken coccidiosis: A clinico-pathological study. *Bangladesh Journal of Microbiol*ogy 25(1): 60-64 (2008).
14. Ali, E.S.H., F.I. Nassar, A.M. Badawi & S.A. Afify. Physical properties and biological applications

of novel substituted biphenyl-sulfonamides. *International Journal of Genetics and Molecular Biology* 2(5): 78-91 (2010).

15. Nagaraja, P., S.D. Naik, A.K. Shrestha & A. Shivakumar. A sensitive spectrophotometric method for the determination of sulfonamides in pharmaceutical preparations. *Acta Pharm* 57: 333-342 (2007).
16. Owa, T., T. Okauchi, K. Yoshimatsu., N.H. Sugi, Y. Ozawa, T. Nagasu, N. Koyanagi, T. Okabe, K. Kitoh & H. Yoshino. A focused compound library of novel *N-(*7-Indolyl) benzenesulfonamides for the discovery of potent cell cycle inhibitors. *Bioorganic and Medicinal Chemistry Letters* 10: 1223-1226 (2000).
17. Dauban, P. & R.H. Dodd. Intramolecular Bromine-catalyzed Aziridination: a new direct access to cyclic sulfonamides. *Tetrahedron Letters* 42: 1037-1040 (2001).
18. Abbasi, M.A., A. Saeed, A. Rehman, K.M. Khan, M. Ashraf, & S.A. Ejaz. Synthesis of brominated 2-phenitidine derivatives as valuable inhibitors of cholinesterases for the treatment of alzheimer's disease. *Iranian Journal of Pharmaceutical Research* 13 (1): 87-94 (2014).
19. Powell, W.A., C.M. Catranis & C.A. Maynard. Design of self-processing antimicrobial peptide for plant protection. *Letters in Applied Microbiology* 31(2): 163-165 (2000).
20. CLSI (The clinical Laboratory Standard Institute). Agar dilution and disk diffusion susceptibility testing of campylobacter spp. *Journal of Clinical Microbiology* 45(8): 2758-2759 (2010).
21. Sharma, P. & J.D. Sharma. *In vitro* hemolysis of human erythrocytes by plant extracts with antiplasmodial activity. *Journal of Ethnophamacology* 74: 239-243 (2001).