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Synthesis and antibacterial activity study of a novel class of cationic anthraquinone analogs

Jianjun Zhang [a], Nathan Redman [a], Anthony Phillip Litke [a], Jia Zeng [b], Jixun Zhan [b], Ka Yee Chan [a], Cheng-Wei Tom Chang [a,*]

[a] Department of Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, UT 84322-0300, USA
[b] Department of Biological Engineering, Utah State University, 4105 Old Main Hill, Logan, UT 84322-4105, USA

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ABSTRACT

Reported previously by our group, one-pot cycloaddition using naphthoquinone, sodium azide and alkyl halides can lead to the formation of both 1-alkyl-1*H*- and 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones. Herein, the effect of leaving group and additive in dictating the selectivity between the formation of 1-alkyl-1*H*- and 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones has been further investigated. In the process of investigating the factors that control the selectivity and the biological activity associated with these two compounds, a novel class of antibacterial cationic anthraquinone analogs has been developed. Although these compounds are structurally similar, different antibacterial profiles are noted. One lead compound, **4e** manifests high potency (MIC < 1 μg/mL) and selectivity against Gram positive (G+) pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA) while exerting only modest activity against Gram negative (G−) bacteria. Other lead compounds (**4f** and **4g**) exhibit broad antibacterial activity including MRSA and vancomycin-resistant *Enterococcus faecalis* (VRE) that is comparable to other commercially available cationic antiseptic chemicals. This unique difference in antibacterial profile may pave the way for the development of new therapeutic agents.

1. Introduction

Molecules containing naphthoquinone and anthraquinone scaffolds have long attracted great interest due to their important biological and pharmaceutical applications leading to the development and discovery of numerous therapeutics (Fig. 1).[1,2] For example, 1,4-naphthoquinone derivatives have been studied for their diverse biological activities including uses as antibacterial,[3,4] antifungal,[5] antimalarial,[6] and antitumor agents,[7,8] or being employed as inhibitors against vitamin K dependent carboxylase,[9] protein kinase,[10] coenzyme Q,[11] and even as growth stimulator for bifidobacteria.[12] Anthraquinone, which bears the structural core of anthracycline, has also attracted great interest due to its applications as antibiotics or anticancer agents.[13] Finally, naphthoquinone and anthraquinone are known to uncouple mitochondria oxidative phosphorylation leading to mechanistic investigations in their redox chemistry and related applications, such as the use as new material for photocell.[14]

The syntheses of naphthoquinone and anthraquinone derivatives, in general, require multi-step processes and begin from various starting materials.[15] Using naphthoquinone, **1**, sodium azide, and alkyl halides, we have recently reported a concise one-pot divergent synthesis of 1-alkyl-1*H*- and 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones (**2** and **3**), both of which can be viewed as analogs of anthraquinone or naphthoquinone fused with 1,2,3-triazole (Fig. 2).[16] Since a wide range of biological activities and

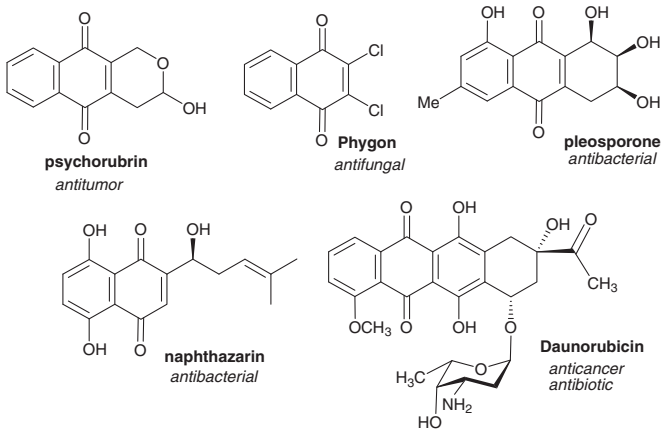

Figure 1. Examples of molecules containing naphthoquinone and anthraquinone scaffolds.

* Corresponding author. Tel.: +1 435 797 3545.
E-mail addresses: tom.chang@usu.edu, chang@cc.usu.edu (Cheng-Wei Tom Chang).

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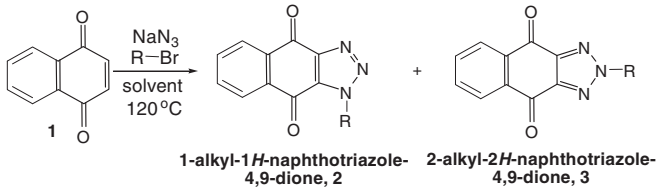


Figure 2. One-pot divergent synthesis of 1-alkyl-1*H*- and 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones.

practical applications can be associated with these compounds, we began to investigate the factors that can govern the selectivity between these two classes of compound.

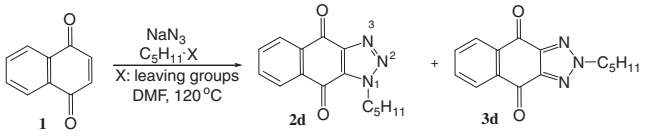
2. Results and discussion

2.1. Investigation of the effect in controlling selectivity

In our one-pot divergent synthesis of 1-alkyl-1*H*- and 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones, we have investigated the effect of solvents on the product ratio by using DMF, and toluene as the solvents. We have noted that less polar solvents, such as toluene, favor slightly the formation of 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones.[16] However, the cycloaddition reactions run in toluene offer less satisfactory yields, probably, due to the degradation of the products or intermediates. Therefore, we started to look for other factors, such as leaving group and additive that may offer the selectivity without scarifying the overall yields of both 1-alkyl-1*H*- and 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones.

We employed pentyl group as the alkyl group to be incorporated. Various leaving groups including bromide, chloride, tosylate (TsO), mesylate (MsO), and trifluoroacetate (CF_3CO_2), were investigated in the optimal condition established previously (Table 1). The pK_a of the conjugated acids of leaving groups has been used as the measurement of leaving group capability.[17] Based on the documented pK_a's of HI (−10), HBr (−9), HCl (−8), TsOH (−2.8), MsOH (−2.6), and trifluoroacetic acid (0.23),[18] we estimate that iodide is the best leaving group and trifluoroacetate is the poorest. From the ratio of **2d**/**3d** determined by ^{1}H NMR, we observed an approximate trend that better leaving groups (Br) favor the formation of 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones while poor leaving groups (MsO and CF_3CO_2) prefer the formation of 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones. Bromide is a good leaving group for the formation of 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones offering a higher ratio of **2d**/**3d**. Iodide is even a better leaving group as compared to chloride or bromide. The results from addition of TBAI, which can generate iodide as the best leaving group in situ, further corroborate this observation (entries 1 vs 2 and 3 vs 4). The poorest leaving group, CF_3CO_2, gave the lowest ratio of **2d**/**3d**, or better relative yield for the formation of 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-dione (entry 7). Nevertheless, the overall yields when poor leaving groups were employed decreased dramatically probably due to the degradation of products or intermediates.

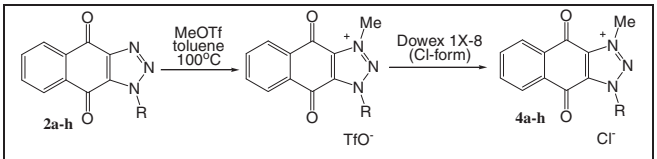
Table 1
The ratio of **2d**/**3d**



Entry	Leaving group (X)/additive	1*H*:2*H* ratio (**2d**/**3d**)	Overall yield (%)
1	Bromide/TBAI	7.12:1.00	98
2	Bromide	5.00:1.00	75
3	Chloride/TBAI	1.27:1.00	76
4	Chloride	1.02:1.00	99
5	Tosylate (TsO)	1.58:1.00	75
6	Mesylate (MsO)	1.17:1.00	29
7	Trifluoroacetate (CF_3CO_2)	1.00:1.06	38

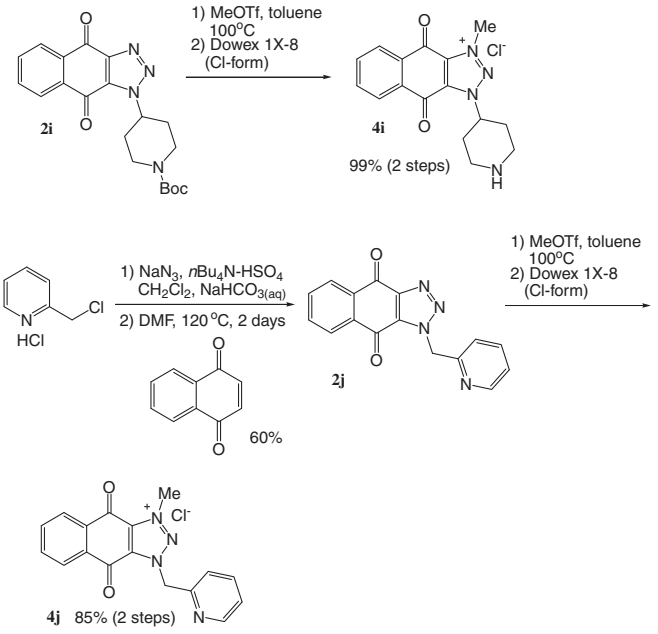
2.2. Synthesis of cationic anthraquinone analogues

In our initial studies on the synthesis of 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones, carbohydrates were incorporated as the alkyl group (R) group.[19] Unfortunately, the carbohydrate moieties were unsuitable for the synthesis of 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones following our one-pot protocol.[16] Only alkyl groups are applicable in the synthesis of both 1-alkyl-1*H*- and 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones. However, both 1-alkyl-1*H*- and 2-alkyl-2*H*-alkylated naphtho[2,3-*d*]triazole-4,9-diones were completely insoluble in aqueous media making these two classes of compounds not suitable for biological evaluation. Thus, our effort was directed to improve the solubility for these anthraquinone analogs. One of the feasible approaches is to convert these molecules into cationic compounds via methylation at the N-3 of the triazole motif. After several attempts, methylation using MeOTf proves to be effective in methylating the 1-alkyl-1*H*-alkylated naphtho[2,3-*d*]triazole-4,9-diones but not the 2-alkyl-2*H*-alkylated naphtho[2,3-*d*]triazole-4,9-diones (Scheme 1). The latter appears to yield intermediate or product that is too unstable to be isolated upon methylation. Similar results were noted with carbohydrates or adamantane attached at N-1 of 1-alkyl-1*H*-alkylated naphtho[2,3-*d*]triazole-4,9-diones. After methylation, the TfO^- anion was exchanged with Cl^- using ion-exchange resin. This protocol enables the synthesis of compounds 4a–**h**. The synthesis of compound **4i** began with a Boc-protected 1-(4-(*N*-*tert*-butoxycarbonylpiperidinyl))-1*H*-naphtho[2,3-*d*]triazole-4,9-dione (**2i**)[19] (Scheme 2). During the methylation step, the by-product TfOH also triggered the deprotection of Boc group and provided the desired product after ion-exchange. The synthesis of **4j** started with the synthesis of 2-picolyl azide followed by the cycloaddition protocol as described previously (Scheme 2).[19] The resulting cycloaddition product **2j** can be converted to the



Compound	R	Product	Yield (%)
2a[16]	CH_3	**4a**	81%
2b[16]	CH_2CH_3	**4b**	82%
2c[16]	$CH_2(CH_2)_2CH_3$	**4c**	84%
2d[16]	$CH_2(CH_2)_3CH_3$	**4d**	84%
2e[16]	$CH_2(CH_2)_6CH_3$	**4e**	99%
2f[16]	$CH_2(CH_2)_{10}CH_3$	**4f**	88%
2g[16]	$CH_2(CH_2)_{14}CH_3$	**4g**	43%
2h[16]	Bn	**4h**	85%

Scheme 1.



Scheme 2.

corresponding cationic adduct **4j** via the same procedure. Overall, above investigations on the factors for selective synthesis of 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-dione is helpful in optimizing the synthesis of the novel cationic analogs (**4a–j**).

2.3. Antibacterial study and discussion

With the structural character of both cation and naphthoquinone, the resulting molecules may have duel properties found in cationic antibiotics and naphthoquinone-based therapeutics. A series of these cationic 1-alkyl-3-methyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones were tested against various Gram positive (G+) and Gram negative (G−) bacteria including *Escherichia coli* (G−, ATCC 25922), *Staphylococcus aureus* (G+, ATCC 25923), *Klebsiella pneumoniae* (G−, ATCC 13883), *Pseudomonas aeruginosa* (G−, ATCC 27853), *Mycobacterium smegmatis* (G+, ATCC 14468), methicillin-resistant *S. aureus* (G+, ATCC 33591) (MRSA), vancomycin-resistant *Enterococcus faecalis* (G+, ATCC 51299) (VRE), and *E. faecalis* (G+, ATCC 29212). Similar cationic compounds, such as benzyldimethylhexadecylammonium chloride (BDC), hexadecylpyridinium bromide (HPB), hexadecyltrimethylammonium bromide (HTB), and clinical used antibiotics were employed as the controls. Due to the relatively modest solubility of BDC and HPB in aqueous media, most of the assay employed only HTB as the control.

In general, these compounds are much more active against G+ bacteria than G− bacteria. From the minimum inhibition concentration (MIC) generated from compounds with linear alkyl group, we were impressed with the high potency of some of the compounds against, in particular, the Gram positive bacteria (Table 2). For example, against *S. aureus* and MRSA, the MIC's for **4e**, **4f**, and **4g** are lower than all the controls employed in this study. The MIC's of **4e** and **4f** are even in mid-nanomolar range. Compounds with non-linear alkyl groups, **4h** (Bn), **4i** (4-piperidinyl), and **4j** (2-picolyl) are less active than those with linear alkyl groups. Although compound **4h** seems to be the most active one among these three, which could imply the positive role of lipophilicity of the Bn group, the pool of this type of compounds is too small to make affirmative conclusion. Rather clearer structure–activity relationship (SAR) can be deduced from the compounds with linear alkyl groups. The antibacterial activity slightly increases as the number of carbon of the alkyl group increases. However, significant antibacterial activity emerges as the chain length reaches eight carbons (octyl group, **4e**), and such high activity remains even with sixteen carbons (hexadodecyl group, **4g**). Nevertheless, compound **4e** (C8) has different antibacterial profile as compared to compounds **4f** (C12) and **4g** (C16) particularly against G− bacteria and *E. faecalis*. The former compound, **4e** shows high antibacterial activity selectively toward G+ bacteria except *E. faecalis*. Compounds with shorter linear alkyl chain than **4e** also display similar antibacterial profile as **4e**. The latter two compounds, **4f** and **4g** manifest rather broad antibacterial activity against G− and G+ strains comparable to the commonly used cationic control, HTB.

Enterococci are facultative anaerobic organisms that can thrive in both oxygen-rich and oxygen-deficient environments.[20] The lack of activity of **4e** against *E. faecalis* is specially interesting since it implies that **4e** and compounds with shorter alkyl chains (**4a–d**) have a different antibacterial mode of action from **4f** and **4g**. Commercially available cationic antibacterial agents, such as HTB, carry a lipophilic alkyl chain with length around twelve to eighteen carbons, which often lowers the solubility of these agents in aqueous media. The shorter chain length of **4e** is potentially advantageous since it can be quite soluble in aqueous media unlike HTB. Finally, all the cationic compounds including HTB are not very activity against *P. aeruginosa*, which is known to exert drug resistance via lowering its membrane permeability.[21]

Two structural motifs are expected to be responsible for the observed antibacterial activity: the cationic anthraquinone analog and the alky groups at the N-1 position. As mentioned previously, the neutral 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones, compound **2a–j** are insoluble in aqueous media making it difficult to evaluate the antibacterial activity of these compounds. We have previously prepared 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones with carbohydrates attached to the N-1 position which enables these compounds to have moderate to excellent solubility in aqueous media (Fig. 3).[19] However, these compounds were found to be inactive against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) in the diffusion assay with no observable zone of inhibition. Therefore, the cationic form of the anthraquinone analog is crucial for the antibacterial activity.

The chain length of the linear alkyl groups also plays important role with the optimal number of carbon ranging from C8 to C16. Commonly used antiseptic quaternary ammonium compounds often contain linear lipophilic alkyl chains (C12–C18). The amphiphilic property of these cationic agents allows the molecules to exert their antibacterial activity by disrupting the bacterial membrane.[22] Since compound **4e** (C8) also exert excellent antibacterial activity as compared to **4f** and **4g**, membrane disruption that is related to the lipophilicity of the commercially used cationic antiseptic agents may not offer sole explanation for the observed activity of **4e**. We speculate that it is a combination of the cationic nature and the octyl (C8) group that contributes to the exceptionally high antibacterial activity of **4e** via an unknown mode of action, which seems to be specific toward G+ bacteria. Noticeable antibacterial activities, which are also specific toward G+ bacteria for compounds with shorter alkyl chains (**4a–d**, C2–C5) implies that the cationic anthraquinone moiety plays an important role in the antibacterial activity of these compounds. As the chain length extended to C12 and C16, the increased lipophilicity of **4f** and **4g** begins to exert broad antibacterial activity like other cationic antiseptic agents. The antibacterial profile of **4f** and **4g** falls in-line with other cationic compounds suggests that the dominant antibacterial mode of action is still on the perturbation of bacterial membrane for these two compounds. Thus, the lead compound with octyl group, **4e** insinuates an optimal combination of cationic anthraquinone moiety and the lipophilicity of octyl group to

Table 2
Minimum inhibitory concentration (MIC)[a]

Entry	Compounds	*E. coli*[b]	*S. aureus*[c]	*K. pneumoniae*[d]	*S. aureus*[e]	*P. aeruginosa*[f]	*M. smegmatis*[g]	*E. faecalis*[h]	*E. faecalis*[i]
1	Neomycin	4	1	2	125	64	ND [j]	16–32	125–250
2	Kanamycin	2–4	1–2	0.5–1	125–250	125–250	1–2	ND	ND
3	Vancomycin	⩾250	1	⩾250	0.5–1	⩾250	ND	1–2	125
4	Amikacin	1	0.5–1	1	16	0.25	0.25	32–64	⩾250
5	BDC	ND	2–4	ND	1	ND	ND	ND	ND
6	HPB	ND	2–4	ND	4	ND	ND	ND	ND
7	HTB	1	0.5–1	1	2	16–32	1	2–4	4–8
8	**4a**	32–64	4–8	64	4–8	125	32–64	⩾250	125–250
9	**4b**	16–32	1–2	32–64	1–2	⩾250	16–32	⩾250	⩾250
10	**4c**	16–32	2–4	32–64	1–2	⩾250	16	⩾250	⩾250
11	**4d**	8–16	2	32	1–2	⩾250	8–16	⩾250	125–250
12	**4e**	8	0.032–0.064	8–16	0.5–1	⩾250	1	125–250	125–250
13	**4f**	2–4	0.032	2–4	0.25–0.5	16	1–2	4–8	4–8
14	**4g**	0.5–1	0.125–0.25	2–4	0.25–0.5	⩾250	2–4	2–4	4–8
15	**4h**	8–16	2	8–16	0.5–1	125–250	8–16	⩾250	⩾250
16	**4i**	32–64	2–4	64	2–4	64	16	⩾250	⩾250
17	**4j**	16–32	2–4	16–32	2–4	⩾250	16	⩾250	⩾250

[a] Unit: μg/mL.
[b] *Escherichia coli* (ATCC 25922).
[c] *Staphylococcus aureus* (ATCC 25923).
[d] *Klebsiella pneumoniae* (ATCC 13883).
[e] *Staphylococcus aureus* (ATCC 33591) (MRSA).
[f] *Pseudomonas aeruginosa* (ATCC 27853).
[g] *Mycobacterium smegmatis* (ATCC 14468).
[h] *Enterococcus faecalis* (ATCC 29212).
[i] *E. faecalis* (ATCC51299) (VRE).
[j] ND: not determined.

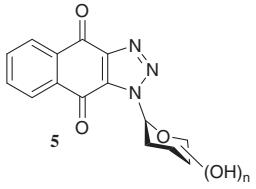


Figure 3. 1*H*-Naphtho[2,3-*d*]triazole-4,9-diones with N-1 carbohydrates.

achieve the observed significant and selective antibacterial activity. The interesting selectivity of these compounds against G+ bacteria may find additional applications. For example, it may be possible to employ compound **4e** as antibacterial agent and avoid *Clostridium difficile* infection (CDI).[23] *C. difficile* is a G+ anaerobic bacterium and is the most significant cause of pseudomembranous colitis, a severe infection of the colon, often appears after normal gut flora is eradicated by the use of antibiotics following surgery. Many associated deaths have been reported, especially among the elderly. The presence of beneficial bacteria within our intestines is necessary to help the human body develop properly and to remain healthy. Since compound **4e** is less active against G− bacteria, it may be possible to selectively 'kill' pathogenic G+ bacteria and avoid CDI. Finally, quaternary ammonium compounds, such as HTB, BDC or HPB are often considered too toxic, and are limited to topical uses as antiseptics, disinfectants, or preservatives.[24] The reduced lipophilicity of compound **4e** with shorter alkyl chains could have potential of being employed as effective antibiotics. Further investigations on the possible modes of antibacterial action are currently undertaken.

3. Conclusion

In conclusion, we have further investigated the effect of leaving groups in the chemoselectivity for the synthesis of 1-alkyl-1*H*- and 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones. In an effort of improving the bioavailability of the triazole constructs, the methylation approach proved be an effective method for 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones, which results the novel cationic anthraquinone analogs with impressive antibacterial activity. With the growing interest in using triazoles and 'Click' chemistry as the tools for constructing bioactive molecules, this methylation protocol may broaden the potential applications. The prominent activity and selectivity of **4e** may lead to the development of antibacterial agent and avoid CDI. The broad spectrum activity of **4f** and **4g** could have significance of being used as antiseptic agents. These cationic compounds that contain a combination of alkyl groups and the cationic anthraquinone or naphthoquinone moieties could pave the way for the development of novel antibiotics. Ongoing effort has been directed to the investigation of the possible antibacterial modes of action.

4. Experimental

4.1. General procedure of methylation

To a solution of starting material (ca. 0.05 g) in toluene (10 mL), MeOTf (4 equiv) was added. The reaction mixture was stirred at 100 °C for 24 h. After completion of the reaction, the solvent was removed and the crude product was loaded with a short column packed with Dowex 1X-8 resin (Cl^- form). The column was eluted with MeOH (ca. 20 mL). After removal of the solvent, the product was obtained as brownish solid.

4.2. 1-(2-Picolyl)-1*H*-naphtho[2,3-*d*]triazole-4,9-dione (2j)

This compound is synthesized using procedure described in Ref. 19. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.51 (d, J = 4.8 Hz, 1H), 8.34 (dd, J = 7.6, 1.4 Hz, 1H), 8.20 (dd, J = 7.5, 1.7 Hz, 1H), 7.85 (td, J = 7.2, 1.4 Hz, 1H), 7.79 (td, J = 7.6, 1.7 Hz, 1H), 7.70 (td, J = 7.6, 1.7 Hz, 1H), 7.32 (d, J = 7.9 Hz, 1H), 7.2 (m, 1H), 6.18 (s, 2H); ^{13}C ($CDCl_3$, 100 MHz) δ 177.0, 175.6, 153.4, 150.2, 145.7, 137.4, 135.4, 134.5, 134.1, 133.7, 133.1, 128.1, 127.6, 123.7, 122.3, 55.0; ESI/APCI calcd for $C_{16}H_{11}N_{14}O_2^+$ ([M+H]$^+$) m/z 291.0877; measure m/z 291.0879.

4.3. Compound 4a

^{1}H NMR (CD_3OD, 300 MHz) δ 8.4 (m, 2H), 8.1 (m, 2H), 4.70 (s, 6H); ^{13}C NMR (CD_3OD, 75 MHz) δ 172.4 (2 carbons), 136.0 (2 carbons), 135.8 (2 carbons), 132.6 (2 carbons), 127.8 (2 carbons), 47.1 (2 carbons); ESI/APCI calcd for $C_{12}H_{10}N_3O_2^+$ ([M]$^+$) *m/z* 228.0768; measure *m/z* 228.0772.

4.4. Compound 4b

^{1}H NMR (CD_3OD, 300 MHz) δ 8.4 (m, 2H), 8.1 (m, 2H), 5.14 (q, J = 7.2 Hz, 2H), 4.71 (s, 3H), 1.74 (t, J = 7.2 Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 172.6, 172.4, 136.1, 135.9, 135.88, 135.5, 132.8, 132.6, 127.8, 127.7, 50.0, 39.8, 13.0; ESI/APCI calcd for $C_{13}H_{12}N_3O_2^+$ ([M]$^+$) *m/z* 242.0924; measure *m/z* 242.0929.

4.5. Compound 4c

^{1}H NMR (CD_3OD, 300 MHz) δ 8.4 (m, 2H), 8.0 (m, 2H), 5.09 (t, J = 7.6 Hz, 2H), 4.71 (s, 3H), 2.1 (m, 2H), 1.51 (m, 2H), 1.03 (t, J = 7.6 Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 172.5, 172.4, 136.1, 136.0, 135.9, 135.5, 132.8, 132.5, 127.8, 127.7, 54.0, 39.8, 30.7, 19.1, 12.3; ESI/APCI calcd for $C_{15}H_{16}N_3O_2^+$ ([M]$^+$) *m/z* 270.1237; measure *m/z* 270.1242.

4.6. Compound 4d

^{1}H NMR (CD_3OD, 300 MHz) δ 8.4 (m, 2H), 8.0 (m, 2H), 5.08 (t, J = 7.2 Hz, 2H), 4.72 (s, 3H), 2.1 (m, 2H), 1.5 (m, 4H), 0.96 (t, J = 6.9 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.7, 172.6, 136.3, 136.1, 136.0, 135.7, 132.9, 132.7, 127.9, 127.8, 54.4, 40.0, 28.6, 28.1, 21.9, 12.9; ESI/APCI calcd for $C_{16}H_{18}N_3O_2^+$ ([M]$^+$) *m/z* 284.1394; measure *m/z* 284.1399.

4.7. Compound 4e

^{1}H NMR (CD_3OD, 300 MHz) δ 8.4 (m, 2H), 8.1 (m, 2H), 5.08 (t, J = 7.6 Hz, 2H), 4.71 (s, 3H), 2.1 (m, 2H), 1.5 (m, 2H), 1.3 (m, 8H), 0.90 (t, J = 6.9 Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 172.6, 172.5, 136.2, 135.9, 135.87, 135.6, 132.9, 132.6, 127.8, 127.7, 54.2, 39.9, 31.6, 28.8, 28.7 (2 carbons), 25.9, 22.4, 13.2; ESI/APCI calcd for $C_{19}H_{24}N_3O_2^+$ ([M]$^+$) *m/z* 326.1863; measure *m/z* 326.1866.

4.8. Compound 4f

^{1}H NMR (CD_3OD, 300 MHz) δ 8.4 (m, 2H), 8.1 (m, 2H), 5.08 (t, J = 7.6 Hz, 2H), 4.71 (s, 3H), 2.1 (m, 2H), 1.4 (m, 2H), 1.3 (m, 16H), 0.88 (t, J = 6.5 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.5, 172.4, 136.1, 135.9 (2 carbons), 135.5, 132.8, 132.5, 127.8, 127.7, 54.2, 39.8, 31.7, 29.4 (2 carbons), 29.3, 29.1 (2 carbons), 28.8, 28.7, 25.8, 22.4, 13.1; ESI/APCI calcd for $C_{23}H_{32}N_3O_2^+$ ([M]$^+$) *m/z* 382.2489; measure *m/z* 382.2492.

4.9. Compound 4g

^{1}H NMR (CD_3OD, 300 MHz) δ 8.4 (m, 2H), 8.1 (m, 2H), 5.08 (t, J = 7.6 Hz, 2H), 4.71 (s, 3H), 2.1 (m, 2H), 1.5 (m, 2H), 1.3 (m, 24H), 0.88 (t, J = 6.5 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.6, 172.5, 136.2, 136.1 (2 carbons), 135.6, 132.9, 132.6, 127.9, 127.8, 54.3, 39.9, 31.9, 29.6 (5 carbons), 29.4 (2 carbons), 29.2 (2 carbons), 28.9, 28.8, 26.0, 22.2, 13.2; ESI/APCI calcd for $C_{27}H_{40}N_3O_2^+$ ([M]$^+$) *m/z* 438.3115; measure *m/z* 438.3120.

4.10. Compound 4h

^{1}H NMR (CD_3OD, 300 MHz) δ 8.4 (m, 2H), 8.0 (m, 2H), 7.6 (m, 2H), 7.4 (m, 3H), 6.30 (s, 2H), 4.94 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.6, 172.5, 136.4, 136.2, 136.1, 135.3, 132.9, 132.7, 131.6, 129.8, 129.5 (2 carbons), 129.2 (2 carbons), 128.0, 127.9, 57.2, 40.1; ESI/APCI calcd for $C_{18}H_{14}N_3O_2^+$ ([M]$^+$) *m/z* 304.1081; measure *m/z* 304.1077.

4.11. Compound 4i

^{1}H NMR (CD_3OD, 300 MHz) δ 8.4 (m, 2H), 8.1 (m, 2H), 5.90 (tt, J = 8.2, 4.1 Hz, 1H), 4.75 (s, 3H), 3.7 (m, 2H), 3.55 (td, J = 14.4, 3.1 Hz, 2H), 2.6 (m, 4H); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.6, 172.5, 136.22, 136.16 (2 carbons), 136.0, 133.0, 132.5, 128.1, 128.0, 59.7, 42.2 (2 carbons), 40.4, 27.6 (2 carbons); ESI/APCI calcd for $C_{16}H_{17}N_4O_2^+$ ([M]$^+$) *m/z* 297.1346; measure *m/z* 297.1346.

4.12. Compound 4j

^{1}H NMR (CD_3OD, 300 MHz) δ 8.99 (d, J = 5.5 Hz, 1H), 8.68 (td, J = 1.7, 0.5 Hz, 1H), 8.4 (m, 3H), 8.2 (t, J = 6.0 Hz, 1H), 8.0 (m, 2H), 6.80 (s, 2H), 4.77 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.5, 172.2, 147.3, 145.2, 143.6, 136.5, 136.4, 136.3, 136.1, 132.8, 132.7, 128.6, 128.1, 128.0 (2 carbons), 53.4, 40.7; ESI/APCI calcd for $C_{17}H_{13}N_4O_2^+$ ([M]$^+$) *m/z* 305.1033; measure *m/z* 305.1034.

4.13. Procedure for MIC determination

A solution of selected bacteria was inoculated in the Trypticase Soy broth at 35 °C for 1–2 h. After which, the bacteria concentration was found, and diluted with broth, if necessary, to an absorption value of 0.08–0.1 at 625 nm. The adjusted inoculated medium (100 μL) was diluted with 10 mL broth, and then applied to a 96-well microtiter plate (50 μL). A series of solutions (50 μL each in twofold dilution) of the tested compounds was added to the testing wells. The 96-well plate was incubated at 35 °C for 12–18 h. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound needed to inhibit the growth of bacteria. The MIC results are repeated at least three times.

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