ACS Combinatorial Science

Research Article

pubs.acs.org/acscombsci

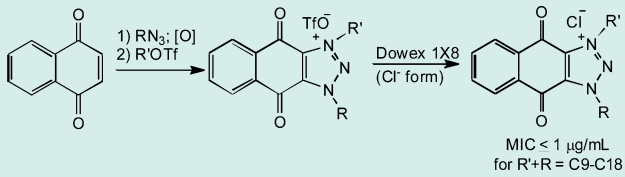
Library Synthesis and Antibacterial Investigation of Cationic Anthraquinone Analogs

Marina Y. Fosso, Ka Yee Chan, Rylee Gregory, and Cheng-Wei Tom Chang*

Department of Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, Utah 84322-0300, United States

Supporting Information

ABSTRACT: We report the parallel synthesis of a series of novel 4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride salts, which are analogs to cationic anthraquinones. Three synthetic protocols were examined leading to a convenient and facile library synthesis of the cationic anthraquinone analogs that contain double alkyl chains of various lengths (C_2−C_{12}) at N-1 and N-3 positions. The antibacterial activities of these compounds were evaluated against Gram-positive bacterium *Staphylococcus aureus* and Gram-negative bacterium *Escherichia coli*. The antibacterial activities of these compounds were expected to be associated with the structural features of naphthoquinone, cation and lypophilic alkyl chain and, interestingly, they showed much higher levels of antibacterial activities against G+ than G− bacteria. In addition, when the total number of carbon atoms of the alkyl groups at both N-1 and N-3 positions lies between 9 and 18, the bactericidal activity against *S. aureus* increased with increasing alkyl chain length at both N-atoms with MIC ≤ 1 μg/mL.



KEYWORDS: *anthraquinone analogs, Staphylococcus aureus, Escherichia coli, antibacterial activity*

INTRODUCTION

The alarming prevalence of drug-resistant bacteria, combined with the continuous emergence of infectious diseases, has triggered a considerable increase in the scope of antibacterial research.[1−3] Numerous efforts have been devoted to the development of new antibacterial agents and nature often provides leads that help to guide the discovery of new antibiotics. Naphthoquinones and anthraquinones are commonly found compounds that display a vast range of biological activities.[4−6] As part of our research program on the synthesis of these biologically active molecules, our group has developed the naphthoquinone derivatives 1-alkyl-1*H*-naphtho[2,3-*d*]-[1,2,3]triazole-4,9-diones.[7] The insolubility of these naphthoquinone derivatives in aqueous media and thus their non-availability for biological testing have led us to the discovery of a class of cationic anthraquinone analogs.[8] The profound antibacterial activity exhibited by some members of this class of compounds, notably against G+ bacteria, has therefore prompted us to improve on the preparation of its precursor 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione and develop a parallel synthesis for the library of 1,3-dialkyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride salts. Through the study of the antibacterial activity resulting from the incorporation of various alkyl groups at the N-1 and N-3 positions, detailed structure−activity relationship can be elucidated.

RESULTS AND DISCUSSION

1,4-Naphthoquinones are unique reagents in organic chemistry, but the syntheses of their derivatives usually impose the need for multiple steps and various starting materials.[9] We have recently discovered that a thermodynamically controlled cycloaddition of 1,4-naphthoquinone (or simply naphthoquinone), **1**, with azido compounds, followed with an oxidation using excess naphthoquinone, affords 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones (Scheme 1).[7] This simple but versatile reaction can provide structurally diverse molecules depending on the order of the addition of the different reagents or the reaction conditions.

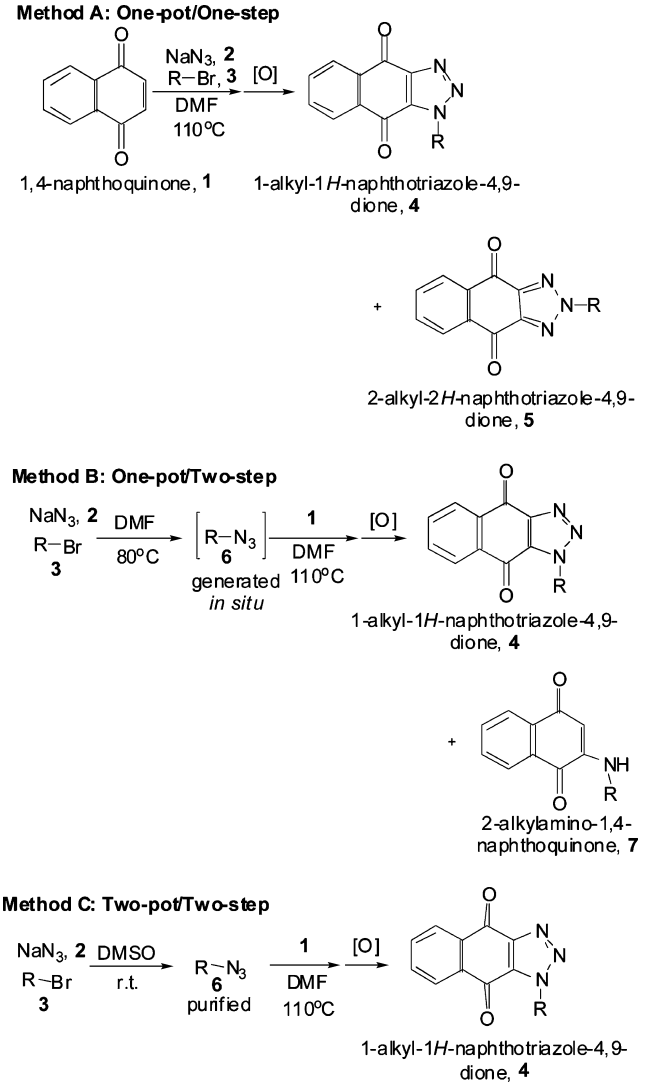
For example, when naphthoquinone **1**, sodium azide **2**, and alkyl bromides **3**{*1*}/**3**{*3*−*5*} were allowed to react in DMF in a one-pot/one-step [3 + 2] cycloaddition, compounds **4**{*1*}/**4**{*3*−*5*} were obtained. This method also afforded the byproducts 2-alkyl-2*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones **5**{*1*}/**5**{*3*−*5*}(Scheme 1, Method A).[7] Although this protocol provided a one-pot divergent synthesis of both compounds in a unique and simple fashion, difficulty in separating them arose as they displayed almost identical R_f values on TLC plate and was therefore a major drawback in scaling up this protocol.

To circumvent this problem, we decided to approach the synthesis of 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones **4**{*1*−*5*} in a one-pot/two-step fashion, whereby alkyl azides **6**{*1*−*5*} were first prepared in situ by reaction of sodium azide **2** and alkyl bromides **3**{*1*−*5*} before being allowed to react with naphthoquinone **1** (Scheme 1, Method B). To our surprise, this also gave rise to the byproducts 2-(alkylamino)-naphthalene-1,4-diones **7**{*1*−*5*}. It should be noted that several compounds of chemset **7** have been extensively studied for

Received: December 12, 2011
Revised: January 24, 2012
Published: February 10, 2012

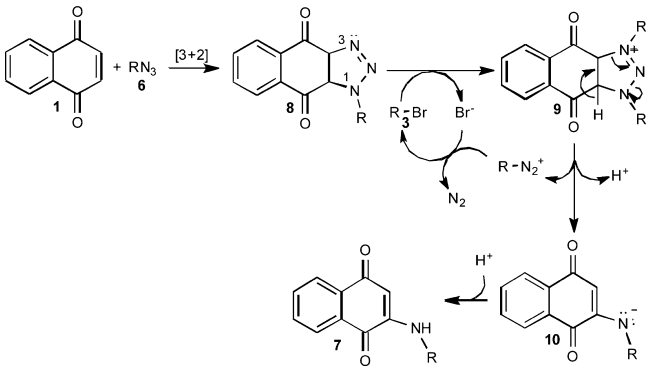
   dx.doi.org/10.1021/co2002075 | *ACS Comb. Sci.* 2012, 14, 231−235

Scheme 1. Synthesis of 1-Alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones 4{*1*−*5*}



their pharmacological activities as antimycobaterial agents[10] and inhibitors of coenzyme Q.[11] We suggest that the formation of chemset **7** results from the presence of an excess amount of alkyl bromides **3**{*1*−*5*} in the reacting vessel (Scheme 2).

Scheme 2. Mechanistic Explanation for the Formation of Chemset 7



Following the initial cycloaddition of **1** with the alkyl azide chemset **6**, it is possible to have an S_N2 nucleophilic substitution via N-3 of the triazoline adduct **8** toward the alkyl bromides **3**{*1*−*5*}. The unstable molecule **9** can undergo decomposition to give the intermediate species **10**. Reprotonation of **10** affords the byproduct chemset **7**. In this proposed mechanism, the remaining alkyl bromide from previous step can actually function as a *catalyst* that facilitates the formation of chemset **7**.

In light of these results, we expected that a third alternative, a two-pot/two-step synthesis, whereby the alkyl azides **6**{*1*−*5*} were prepared separately and allowed to react with naphthoquinone **1** in another reacting vessel, would provide chemset **4**, selectively (Scheme 1, Method C). As expected, this method generated only the desired chemset **4** with yields comparable to the other two methods. More importantly, chemset **4** compounds produced in Method C could be isolated by precipitation, avoiding the use of a column chromatography (Table 1).

Table 1. Comparison of Methods A, B, and C in the Preparation of Chemset 4

	alkyl bromides 3	1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones, 4	yield (%) method A[a]	method B[b]	method C
1	*n*-pentyl bromide 3{*1*}	4{*1*}[a]	41	26	40
2	*n*-hexyl bromide 3{*2*}	4{*2*}	na	49	49
3	*n*-octyl bromide 3{*3*}	4{*3*}[a]	52	30	62
4	*n*-decyl bromide 3{*4*}	4{*4*}[a]	64	28	54
5	*n*-dodecyl bromide 3{*5*}	4{*5*}[a]	68	57	68

[a]reference 7. [b]obtained as inseparable mixtures of **4** and **7**. The yield of **4** is estimated from the integral ratio of the ^{1}H NMR.

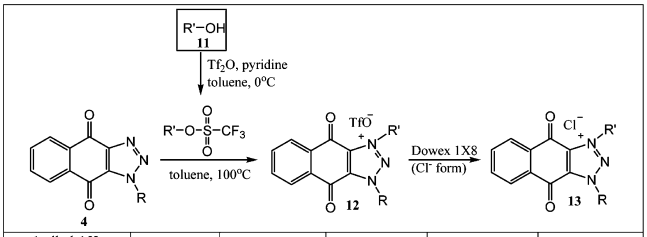
Our initial class of cationic anthraquinone analogs was obtained by methylation at the N-3 position of the triazole motif of compounds **4**{1−5}.[8] In order to investigate the effect of the chain length at N-3 position, we synthesized analogs with various chain lengths at both nitrogen atoms (N-1 and N-3) using alkyl triflates (ROTf) prepared *in situ* from the corresponding alcohol **11**{1−6} (Scheme 3). After alkylation at N3, the TfO^- anion of chemset **12** was exchanged with Cl^- anion using ion-exchange resin to yield chemset **13**. This protocol enabled the parallel synthesis of the 24 novel 1,3-dialkyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride salts using alcohols **11**{1−6} and the naphthoquinone derivatives 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]-triazole-4,9-diones **4**{1−5} (Figure 1).

Antibacterial Study. The 1,3-dialkyl-4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride salts represented in chemset **13** each bears the characteristics of naphthoquinone, cation, and lypophilic alkyl chain, and were therefore expected to show similar biological activity to a series of previously synthesized cationic anthraquinone analogs.[8]

The compounds were tested against *Escherichia coli* (ATCC 25922, G-) and *Staphylococcus aureus* (ATCC 25923, G+) using neomycin, kanamycin, vancomycin, amikacin and hexadecyltrimethylammonium bromide (HTB) as the controls. The

dx.doi.org/10.1021/co2002075 | *ACS Comb. Sci.* 2012, 14, 231−235

Scheme 3. Synthesis of Chemset 13



1-alkyl-1*H*-naphtho-triazole-4,9-diones, **4**	R	Alcohol **11**	R'	Product **13**	Yield (%)
4{1}	C_5H_{11}	**11**{1}	C_2H_5	**13**{1,1}	80
		11{2}	C_4H_9	**13**{1,2}	53
		11{3}	C_5H_{11}	**13**{1,3}	64
		11{5}	C_8H_{17}	**13**{1,5}	74
		11{6}	$C_{10}H_{21}$	**13**{1,6}	58
4{2}	C_6H_{13}	**11**{1}	C_2H_5	**13**{2,1}	82
		11{2}	C_4H_9	**13**{2,2}	95
		11{3}	C_5H_{11}	**13**{2,3}	62
		11{4}	C_6H_{13}	**13**{2,4}	90
		11{5}	C_8H_{17}	**13**{2,5}	88
		11{6}	$C_{10}H_{21}$	**13**{2,6}	39
4{3}	C_8H_{17}	**11**{1}	C_2H_5	**13**{3,1}	51
		11{2}	C_4H_9	**13**{3,2}	99
		11{5}	C_8H_{17}	**13**{3,5}	29
		11{6}	$C_{10}H_{21}$	**13**{3,6}	81
4{4}	$C_{10}H_{21}$	**11**{1}	C_2H_5	**13**{4,1}	99
		11{2}	C_4H_9	**13**{4,2}	87
		11{6}	$C_{10}H_{21}$	**13**{4,6}	93
4{5}	$C_{12}H_{25}$	**11**{1}	C_2H_5	**13**{5,1}	99
		11{2}	C_4H_9	**13**{5,2}	93
		11{3}	C_5H_{11}	**13**{5,3}	99
		11{4}	C_6H_{13}	**13**{5,4}	88
		11{5}	C_8H_{17}	**13**{5,5}	52
		11{6}	$C_{10}H_{21}$	**13**{5,6}	99

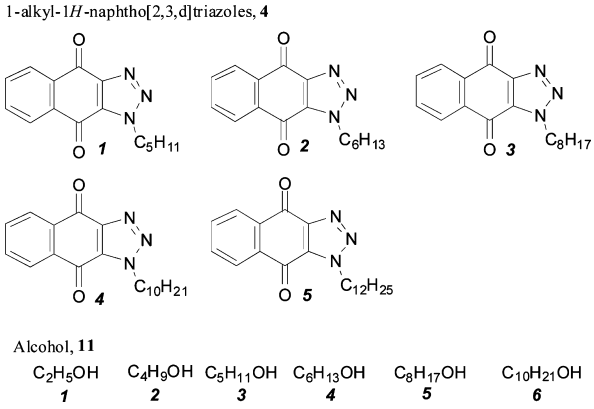


Figure 1. 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazoles **4**{1−5} and alcohols **11**{1−6}.

minimum inhibitory concentrations (MIC's) determined in standard fashion using serial 2-fold dilutions are listed in Table 2. The results show that these cationic compounds are more active against Gram positive (G+) bacteria than Gram negative (G−) bacteria, which is consistent with the antibacterial profile of naphthoquinone[12] and cationic antiseptic agents such as HTB and cetrimonium bromide.[13]

For cationic anthraquinone analogs with a methyl group at N-3 position, we had previously observed that the antibacterial activity against *S. aureus* slightly increased with the number of carbon atoms in the alkyl group at N-1, reaching a maximum at octyl and then decreasing as the chain length was extended to 16 carbons.[8] However, the installation of a different alkyl group at N-3 had a profound influence on antibacterial activity (Figure 2a). In general, highly potent compounds (MIC values below or equal to 1 μg/mL against *S. aureus*) were obtained when the total number of carbon atoms of the alkyl groups on both nitrogen atoms was between 9 and 18. This synergistic effect of alkyl groups suggests that overall lipophilicity is an

Table 2. Minimum Inhibitory Concentrations (μg/mL)

compound	R	R′	*E. coli*	*S. aureus*
neomycin B			8	1
kanamycin			4	1−2
vancomycin			64−125	0.5
amikacin			0.125	0.5
HTB			1	0.5−1
14[a]	C_5H_{11}	CH_3	8−16	2
13{*1,1*}	C_5H_{11}	C_2H_5	≥250	2−4
13{*1,2*}	C_5H_{11}	C_4H_9	≥250	0.5
13{*1,3*}	C_5H_{11}	C_5H_{11}	≥250	1
13{*1,5*}	C_5H_{11}	C_8H_{17}	32−64	0.125
13{*1,6*}	C_5H_{11}	$C_{10}H_{21}$	8−16	1−2
15[b]	C_6H_{13}	CH_3	125−250	1−2
13{*2,1*}	C_6H_{13}	C_2H_5	125−250	1
13{*2,2*}	C_6H_{13}	C_4H_9	125−250	1
13{*2,3*}	C_6H_{13}	C_5H_{11}	125	1−2
13{*2,4*}	C_6H_{13}	C_6H_{13}	32−64	0.5−1
13{*2,5*}	C_6H_{13}	C_8H_{17}	4−8	0.5−1
13{*2,6*}	C_6H_{13}	$C_{10}H_{21}$	2	0.25−0.5
16[a]	C_8H_{17}	CH_3	16−32	0.032−0.064
13{*3,1*}	C_8H_{17}	C_2H_5	≥250	0.25−0.5
13{*3,2*}	C_8H_{17}	C_4H_9	64	1−2
13{*3,5*}	C_8H_{17}	C_8H_{17}	≥250	2−4
13{*3,6*}	C_8H_{17}	$C_{10}H_{21}$	32−64	1−2
17[a]	$C_{10}H_{21}$	CH_3	32	0.032
13{*4,1*}	$C_{10}H_{21}$	C_2H_5	≥250	0.125−0.25
13{*4,2*}	$C_{10}H_{21}$	C_4H_9	64−125	0.25
13{*4,6*}	$C_{10}H_{21}$	$C_{10}H_{21}$	125−250	16−32
18[a]	$C_{12}H_{25}$	CH_3	16−32	0.064−0.125
13{*5,1*}	$C_{12}H_{25}$	C_2H_5	32	0.125
13{*5,2*}	$C_{12}H_{25}$	C_4H_9	≥250	0.5−1
13{*5,3*}	$C_{12}H_{25}$	C_5H_{11}	125−250	0.25−0.5
13{*5,4*}	$C_{12}H_{25}$	C_6H_{13}	125	0.5−1
13{*5,5*}	$C_{12}H_{25}$	C_8H_{17}	125−250	2−4
13{*5,6*}	$C_{12}H_{25}$	$C_{10}H_{21}$	>250	16−32

[a]Ref 8. [b]Compound **15** was synthesized according to the protocol described in ref 8.

important factor in the antibacterial activity. In fact, antiseptic agents with lipophilic alkyl chains have been noted for their ability to disrupt the bacterial membrane of *S. aureus*.[14] It should also be noted that those cationic antiseptic agents generally have a C_{12} or longer hydrophobic tail length. This new library therefore combines shorter-chain and longer-chain compounds. On the other hand, no general trend could be deduced from the MIC values against *E. coli* based on the chain length, suggesting that lipophilicity might not be so important for the antibacterial activity of this class of compounds against G- bacteria (Figure 2b).

■ CONCLUSION

In conclusion, we have developed a new and improved protocol for the synthesis of 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones. To further investigate the effect of alkyl substitution at N-3 position, we constructed a library of 4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-3-ium chloride salts and tested them against a representative Gram positive and Gram negative bacterium. Members of this library exhibited nanomolar-level antibacterial activity against *S. aureus* when the total number of carbon atoms from both alkyl chains ranged

dx.doi.org/10.1021/co2002075 | *ACS Comb. Sci.* 2012, 14, 231−235

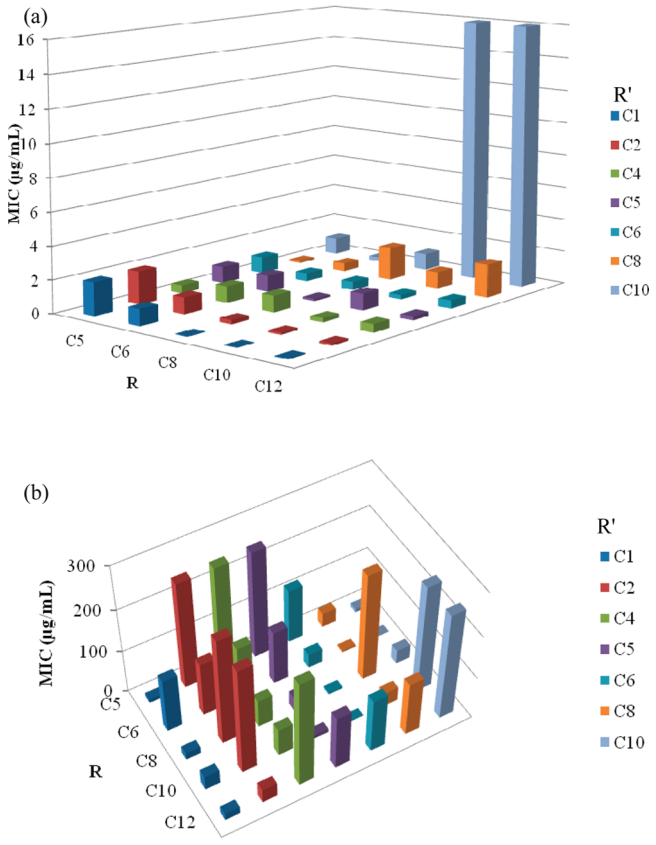


Figure 2. MIC values against (a) *S. aureus* and (b) *E. coli*.

between 9 and 18, suggesting a synergistic effect of the additional alkyl chain.

EXPERIMENTAL PROCEDURES

General Procedure for Cycloaddition of Naphthoquinone. *Method A.* is described in ref 7.

Method B. A solution of NaN_3 (~0.1 g) and alkyl bromide (2 equiv) in DMF (10 mL) was stirred at 80 °C for one day in a sealed vial. Then naphthoquinone (2 equiv) was added and the mixture was heated for another day at 110 °C. The solvent was evaporated and the crude product was purified by column chromatography (eluted from hexane:EtOAc = 100:0 to 50:50) to afford a mixture containing both 1-alkyl-1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-diones and 2-alkylamino-1,4-naphthoquinones. The 2-alkylamino-1,4-naphthoquinones were recovered after N-3 alkylation.

Method C. Alkyl azide (~0.3 g), which was obtained using the method described in ref 15, and naphthoquinone (2 equiv) were dissolved in DMF (10 mL) and the solution was stirred at 110 °C overnight in a sealed vial. The solvent was evaporated and cold ether (50 mL) was added. The solid that precipitated was collected by filtration through a Hirsh funnel and washed with more ether to afford the expected product as a pale brown solid.

General Procedure for N-3 Alkylation. The alcohol (2 equiv) and pyridine (4 equiv) were dissolved in anhydrous toluene (10 mL) and cooled in an ice−water bath before Tf_2O (4 equiv) was slowly added. The mixture was stirred at 0 °C for 2 h and the triazole (0.11 g, 1 equiv) was then added. This mixture was then refluxed at 110 °C for 6−8 h. After completion of the reaction, the solvent was removed and the crude product was purified by column chromatography (eluted with 300 mL Hexane/EtOAC (50/50), 200 mL pure EtOAc and finally 100 mL EtOAc/MeOH (80/20)) to afford the expected product which was then eluted through a small column packed with Dowex 1 × 8 (Cl^-) resin for ion exchange.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, and ^1H and ^{13}C NMR of the synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: 2-1-435-797-3545. Fax: 2-1-435-797-3390. E-mail: tom.chang@usu.edu.

Funding

This work is supported by USTAR TCG grant and URCO grant at USU.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

S. aureus, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; G+, Gram-positive; G−, Gram-negative; MIC, minimal inhibitory concentration

REFERENCES

(1) For a general review of antibacterial agents and bacterial resistance, see: (a) Wilson, D. N. The A−Z of Bacterial Translation Inhibitors. *Crit. Rev. Biochem. Mol. Biol.* **2009**, *44*, 393−433. (b) Chu, D. T. W.; Platner, J. J.; Katz, L. New Directions in Antibacterial Research. *J. Med. Chem.* **1996**, *39*, 3853−3874. (c) Neu, H. C. The Crisis in Antibiotic Resistance. *Science* **1992**, *257*, 1064−1073.

(2) For a general review of emerging infectious diseases, see: (a) Morens, D. M.; Folkers, G. K.; Fauci, A. S. The Challenge of Emerging and Re-emerging Infectious Diseases. *Nature* **2004**, *430*, 242−249. (b) Morse, S. S. Factors in the Emergence of Infectious Diseases. *Emerg. Infect. Dis.* **1995**, *1*, 7−15.

(3) Miller, A. A., Miller, P. F., Eds. *Emerging Trends in Antibacterial Discovery: Answering the call to Arms*; Caister Academic Press: Norfolk, VA, 2011.

(4) Verma, R. P. Anti-Cancer Activities of 1,4-Naphthoquinones: A QSAR Study. *Anticancer Agents Med. Chem.* **2006**, *6*, 489−499.

(5) Kumagai, Y.; Shinkai, Y.; Miura, T.; Cho, A. K. The Chemical Biology of Naphthoquinones and Its Environmental Implications. *Annu. Rev. Pharmacol. Toxicol.* **2012**, *52*, 221−247.

(6) Brase, S.; Encinas, A.; Keck, J.; Nising, C. F. Chemistry and Biology of Mycotoxins and Related Fungal Metabolites. *Chem. Rev.* **2009**, *109*, 3903−3990.

(7) Zhang, J.; Chang, C.-W. T. One-Pot Synthesis of 1- and 2-Substituted Naphtho[2,3-*d*][1,2,3]triazole-4,9-diones. *J. Org. Chem.* **2009**, *74*, 4414−4417.

(8) Zhang, J.; Redman, N.; Litke, P.; Zeng, J.; Zhan, J.; Chan, K. Y.; Chang, C.-W. T. Synthesis and Antibacterial Activity Study of a Novel Class of Cationic Anthraquinone Analogs. *Bioorg. Med. Chem.* **2011**, *19*, 498−503.

(9) Sartori, M. F. Heterocyclic Quinones from 2,3-Dichloro-1,4-naphthoquinones. *Chem. Rev.* **1963**, *63*, 279−296.

(10) Mital, A.; Sonawane, M.; Bindal, S.; Mahlavat, S.; Negi, V. Substituted 1,4-Naphthoquinones as a New Class of Antimycobacterial Agents. *Der Pharma Chemica* **2010**, *2*, 63−73.

(11) Dhaon, M. K.; Lehrman, S. R.; Rich, D. H.; Engelke, J. A.; Suttie, J. W. Derivatives of 2-Methyl-1,4-Naphthoquinones as Substrates and

Inhibitors of the Vitamin K-dependent Carboxylase. *J. Med. Chem.* **1984**, *27*, 1196−1201.
(12) Medentsev, A. G.; Akimenko, V. K. Naphthoquinone Metabolites of the Fungi. *Phytochem* **1998**, *47*, 935−959.
(13) For examples, see: (a) Thorsteinsson, T.; Másson, M.; Kristinsson, K. G.; Hjálmarsdottir, M. A.; Hilmarsson, H.; Loftsson, T. Soft Antimicrobial Agents: Synthesis and Activity of Labile Environmentally Friendly Long Chain Quaternary Ammonium Compounds. *J. Med. Chem.* **2003**, *46*, 4173−4181. (b) Maeda, S.; Kita, T.; Meguro, K. Synthesis of Novel 4,6-Di(substituted)amino-1,2-dihydro-1,3,5-triazine Derivatives as Topical Antiseptic Agents. *J. Med. Chem.* **2009**, *52*, 597−600. (c) Kanazawa, A.; Ikeda, T.; Endo, T. Synthesis and Antimicrobial Activity of Dimethyl- and Trimethyl-Substituted Phosphonium Salts with Alkyl Chains of Various Lengths. *Antimicrob. Agents Chemother.* **1994**, *38*, 945−952.
(14) Ioannou, C. J.; Hanlon, G. W.; Denyer, S. P. Action of Disinfectant Quaternary Ammonium Compounds against *S. aureus*. *Antimicrob. Agents Chemother.* **2007**, *51*, 296−306.
(15) Alvarez, S. G.; Alvarez, M. T. A Practical Procedure for the Synthesis of Alkyl Azides at Ambient Temperature in Dimethyl Sulfoxide in High Purity and Yield. *Synthesis* **1997**, *1997*, 413−414.