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Bioorganic & Medicinal Chemistry Letters 29 (2019) 1572–1575

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones

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ARTICLE INFO

Keywords:
2-Chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones
Anti-malarial
Anti-bacterial
Anti-tuberculosis
Cytotoxicity
HeLa cells

ABSTRACT

A series of novel, substituted 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones have been prepared and shown to exhibit promising concentration-dependent activity against human SH-SY5Y cells, *Plasmodium falciparum*, *Mycobacterium tuberculosis* and *P. aeruginosa*. Substituent effects on observed bioactivity have been explored; the *para*-fluorophenyl derivative **3d** exhibited activity across the range of the bioassays employed, indicating the potential of the 2-chloro-3-[(4-arylthiazol-2-yl)amino]-1,4-naphthoquinone scaffold in the development of novel, broad spectrum therapeutics.

Compounds containing the 1,4-naphthoquinone moiety are commonly found in antibiotics isolated from marine microorganisms, particularly bacteria and sponges.[1–7] These compounds have been reported to exhibit wide-ranging pharmacological properties including anti-microbial,[1,2,8–12] anti-cancer,[2,3,13–15] anti-viral,[16] trypanocidal,[17] anti-fungal,[18] anti-parasitic,[19] anti-malarial[20] and anti-mycobacterial[21] activity. The main mechanism for the pharmacological action of 1,4-naphthoquinone derivatives depends on their capacity to form radicals *in vivo*, especially *via* the metabolic effects of the cytochrome P450 enzyme complex in the liver.[22] This may explain the anti-oxidant, anti-cancer and anti-diabetic activities exhibited by various 1,4-naphthoquinone derivatives. Nicotinamide adenosine diphosphate (NADP), or its reductive analogue, NADPH-dependent quinone oxidoreductase (NQO1),[15,23,24] are key targets in the design and development of quinone-based anti-cancer agents. The 5,8-quinolinedione analogues, streptonigrin **1**[25] and lavendamycin,[26] have been shown to inhibit the latter enzyme.

Pettit et al.[2] reported the anti-cancer activity of cribrostatin **2** against various cancer cell lines, including pancreas-adenocarcinoma BXPC-3, breast-adenocarcinoma MCF-7, CNS glioblastoma SF-268, lung-NSC NCI-H460, colon-adenocarcinoma KM20L2, prostate DU-145 and mouse leukemia P388; the GI_{50} values were mostly in the 0.21–1 μg/mL range. Atamanyuk et al.[27] evaluated the anti-neoplastic and anti-mycobacterial potential of 1,4-naphthoquinone-derived 3,11-dihydro-2*H*-benzo[6,7]thiochromeno[2,3-d][1,3]-thiazole-2,5,10-triones, while Cai et al.[8] reported the total synthesis of hygrocin A and B, which contain the 1,4-naphthoquinone motif and exhibit moderate antibacterial activity against *Neisseria gonorrheae* (a Gram-negative bacterium) and *Aspergillus fumigates* (a fungus). Stasevych et al.[28] have used disc-diffusion susceptibility anti-microbial assays to demonstrate the capacity of certain 2-substituted-3-mercapto-1,4-naphthoquinones to selectively inhibit either *Escherichia coli* (Gram-negative) or *Staphylococcus aureus* (Gram-positive) bacteria.

In this communication, we report the preparation and biological

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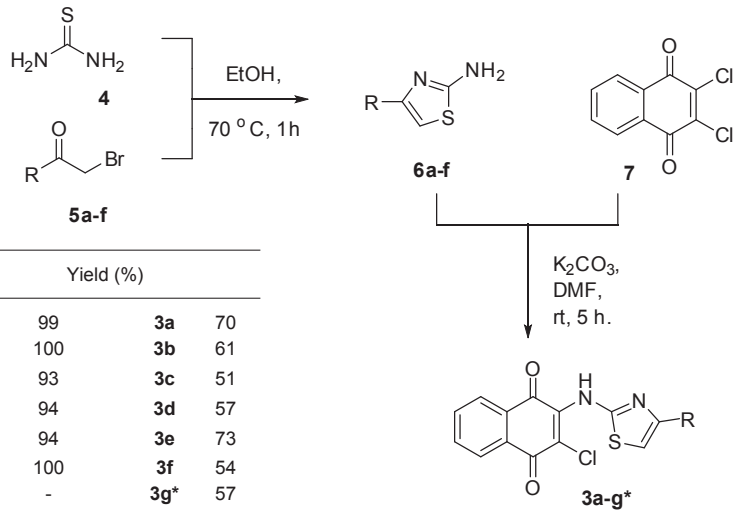
https://doi.org/10.1016/j.bmcl.2019.05.001
Received 1 March 2019; Received in revised form 30 April 2019; Accepted 2 May 2019
Available online 03 May 2019
0960-894X/



R	Yield (%)			
Ph	**6a**	99	**3a**	70
p-ClPh	**6b**	100	**3b**	61
p-BrPh	**6c**	93	**3c**	51
p-FPh	**6d**	94	**3d**	57
p-NO_2Ph	**6e**	94	**3e**	73
COOEt	**6f**	100	**3f**	54
COOH	-	-	**3g***	57

Scheme 1. Synthesis of 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones. *Compound **3g** obtained by subsequent hydrolysis of **3f**.

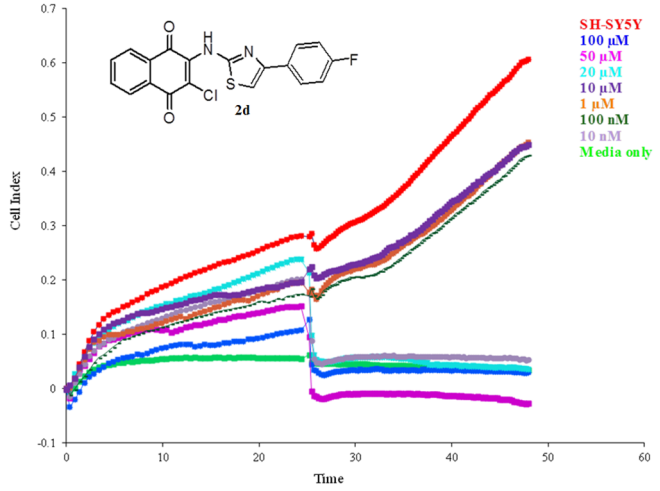
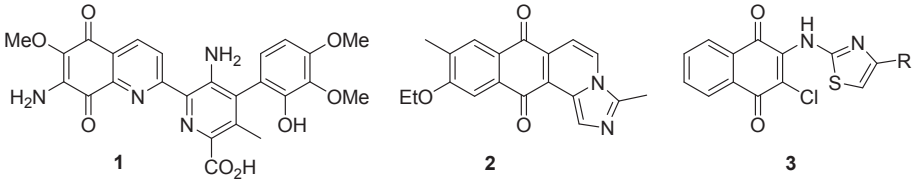


Fig. 1. Scans of Cell Index (CI) of SH-SY5Y *vs* time (h) for different concentrations of 2-chloro-3-[4-(4-fluorophenyl)thiazol-2-ylamino]-1,4-naphthoquinone **3d** over 48 h using an xCELLigence RTCA-SP instrument.

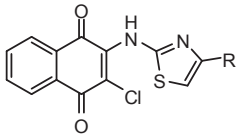
evaluation of a series of 1,4-naphthoquinone-derived 3-(thiazolylamino)naphthoquinones **3a–g** as potential multifunctional anti-cancer, anti-mycobacterial and anti-malarial agents.



The preparation of the known 2-aminothiazoles **6a–f**,[9–11] required as precursors for the targeted 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones **3a–f**, was accomplished by conventional Hantzsch condensation of thiourea **4** with the α-haloketones **5a–f** (Scheme 1). After completion of each reaction, the reaction mixture was poured into ice-cold water to precipitate the thiazole derivatives **6a–f** in excellent yields (93–100%). With the precursors in hand, studies were undertaken to optimise reaction conditions for the synthesis of the targeted compounds (**3**). These included the use of: i) different solvents [*viz.*, polar protic solvents (EtOH and MeOH), non-polar aprotic solvents (THF, toluene and methylene chloride) and polar aprotic solvents (DMSO and DMF)]; ii) different bases (triethylamine, pyridine or K_2CO_3); iii) different temperatures (ambient to 110 °C); iv) different reaction times (1–48 h); and v) microwave-assisted conditions with or without solvent at 150 °C for 10 min. Successful nucleophilic displacement of one of the chlorine atoms in 2,3-dichloro-1,4-naphthoquinone **7** by each of the 2-aminothiazoles **6a–f** was finally achieved using DMF in the presence of K_2CO_3 to obtain, without heating, the desired 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones **3a–f**. All of the compounds were fully characterised using 1- and 2-D NMR, IR and HRMS methods; the 'parent' system **3a**[12,17] is known but the analogues **3b–g** are new compounds. Although overall substitution of chloride by the nucleophilic 2-aminothiazole is achieved in these reactions, the proposed mechanism involves conjugate-addition followed by elimination of HCl.[12,29] The 3-[(4-carbethoxythiazol-2-yl)amino]-2-chloro-1,4-naphthoquinone **3f** was hydrolysed in methanolic KOH to give the corresponding acid **3g** in moderate yield (53%). Experimental details and characterisation data for all of the compounds synthesised in this study are provided in the Supplementary Data file.

The cytotoxic activity of compounds **3a–g** were examined in two cancer cell lines, *viz.*, HeLa cervical adenocarcinoma and SH-SY5Y neuroblastoma cells. The SH-SY5Y cells showed a dose-dependent response to the compounds, with higher concentrations (particularly at 100 µM) inhibiting the growth of the cells, while lower concentrations showed similar effects to the untreated SH-SY5Y control. The xCELLigence RTCA-SP scans of the effects of different concentrations of compound **3d** on SH-SY5Y cells (Fig. 1) are typical of the series of compounds examined. At lower concentrations (100 nM–1 µM), this compound (**3d**) resulted in similar patterns of growth to that observed with the untreated control, but at higher concentrations (10–100 µM) inhibited the growth of the cells at levels comparable to the negative control (*i.e.*, media without the test sample). The carboxylic acid **3g** and the precursor ester **3f** exhibited the lowest inhibitions with IC_{50}

Table 1
Biological evaluation of the 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones **3a-g**.



	R	Cytotoxicity		Anti-TB		Anti-malarial	Anti-microbial				
		SH-SY5Y IC_{50} (μM)	HeLa % Viability[a]	MIC_{90} (μM)	MIC_{99} (μM)	*Pf*LDH IC_{50} (μM)	Zone of inhibition (mm)				
							2000 μM	1000 μM	100 μM	10 μM	1 μM
3a	Ph	1.8	80 ± 5.9	9.19[b]	10.2[b]	–	13.2 ± 0.6	11.4 ± 0.9	9.1 ± 0.4	5.3 ± 0.5	–
3b	*p*-ClPh	2.7	80 ± 2.1	> 20.0	> 20.0	–	12 ± 0.7	9.7 ± 0.5	7.6 ± 1	–	–
3c	*p*-BrPh	–	55 ± 1.8	> 20.0	> 20.0	50.6	–	–	–	–	–
3d	*p*-PhF	1.5	75 ± 2.6	9.37[b]	10.4[b]	44.7	19.5 ± 0.5	15 ± 0.4	11 ± 0.6	8 ± 0.7	5 ± 0.3
3e	*p*-NO_2Ph	0.004	80 ± 3.6	> 20.0	> 20.0	20.3	14.10 ± 0.8	11 ± 013	7.2 ± 0.7	–	–
3f	COOEt	31.1	82 ± 2.8	19.5	> 20.0	–	–	–	–	–	–
3g	COOH	> 100	75 ± 1.8	> 20.0	> 20.0	–	–	–	–	–	–
Controls											
Chloroquine		–	3.6 ± 0.1	–	–	0.0143	–	–	–	–	–
Rifampicin		–	–	0.0015	0.00167	–					
Ampicillin (25 μg/disc)		–	–	–	–	–	24.7 ± 1.2				
Streptomycin (10 μg/disc)		–	–	–	–	–	20.0 ± 0.8				

[a] At 20 μM.
[b] MIC values approximately 10 μM or lower.

values > 100 μM and 31.1 μM, respectively (Table 1). The phenyl-substituted analogues, however, exhibited significant cytotoxicity, with the unsubstituted phenyl (**3a**), *para*-chlorophenyl (**3b**), *para*-fluorophenyl (**3d**) and *para*-nitrophenyl (**3e**) derivatives exhibiting IC_{50} values of 1.8, 2.7, 1.5 and 0.004 μM, respectively. These results suggest that the presence of the phenyl substituent and its electronegative *para*-substituents, particularly *p*-NO_2, may increase the binding affinity of these compounds to the binding pocket of the SH-SY5Y cells.

In contrast, compounds **3a–g** exhibited relatively low cytotoxicity against the HeLa cell line at a concentration of 20 μM (Table 1), indicating selective inhibition of SH-SY5Y cells by the phenyl-substituted compounds **3a–e**. Further studies are required to determine the mechanism of action of these compounds and their morphological effects on both cell lines.

The results of the anti-TB bioassay of the 2-chloro-3-[(thiazol-2-yl) amino]-1,4-naphthoquinones **3a–g** against the virulent *M. tuberculosis* $H_{37}Rv$ strain are presented in Table 1 (dose-response curve are provided in the Supporting Information file). While the carboxylic acid **(3g)** and ester (**3f**) derivatives exhibited low levels of anti-TB activity, with MIC_{90} and MIC_{99} values > 20 μM, certain phenyl derivatives showed promising anti-TB activities with MIC_{90} and MIC_{99} values < 10 μM (**3a**: MIC_{90} = 9.19 μM and MIC_{99} = 10.2 μM); **3d**: MIC_{90} = 9.39 μM and MIC_{99} = 10.4 μM). All of the naphthoquinone derivatives (**3**) were found to have favourable (< 5) Log P values, but the predicted aqueous solubilities (0.15 mg/mL and 0.08 mg/mL) of compounds **3a** and **3d**, respectively, were significantly higher than those of their aryl-substituted analogues **3b**, **3c** and **3f** which may enhance their absorption across the mycolic-rich lipophilic cell wall of *M. tuberculosis*.

Whole-cell *Pf*LDH bioassays were conducted to determine the antimalarial activities of the 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones **3a–g**, using 20 μM as the baseline concentration before determining IC_{50} values for compounds with significant levels of inhibition. Chloroquine was used as the positive control. The results showed that the 'parent' compound **3a**, and the *p*-chlorophenyl (**3b**) and ethyl ester (**3f**) analogues exhibited low levels of inhibition against *P. falciparum* at the tested concentration, with values of 10%, 30% and 40% respectively, while analogues **3c–e** exhibited 80–90% inhibition of parasite viability at the tested concentration; IC_{50} values of 50.6, 44.7 and 20.3 μM were determined for the latter compounds, **3c**, **3d** and **3e** respectively (Table 1). The acid derivative **3g**, on the other hand, enhanced the growth *P. falciparum*, recording 115% viability of the cells. Graphs showing the IC_{50} plots and the % parasite viabilities with related standard deviations are provided in the Supplementary Data file. The results clearly reveal the importance of the thiazole substituent.

i) The acyl derivatives are clearly the least promising, with the ethyl ester **3f** exhibiting the lowest inhibition (10%) and its carboxylic acid derivative **3g** actually stimulating proliferation of *P. falciparum* by 15%
ii) Compared to the 'parent' system **3a** (R = Ph), the introduction of the electronegative *para*-substituents Br, F and NO_2 in the phenyl group in compounds **3c**, **3d** and **3e**, respectively, appears to decrease the viability of the parasite significantly.

The results of the disc diffusion susceptibility studies of the 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones **3a–g** against an environmental strain of *P. aeruginosa* are summarised in Table 1. As was the case with the antimalarial bioassays, neither the carboxylic acid **3g** nor the ester precursor **3f** showed any inhibition potential, even at the highest concentration (2000 μM). The phenyl derivatives **3a**,**b**,**d**,**e**, on the other hand, exhibited dose-dependent antibacterial activity, with the *para*-fluorophenyl derivative **3d** exhibiting the highest inhibition across the different concentrations tested by clearing zones of 5 mm, 8 mm, 11 mm, 15 mm and 19.5 mm at 1 μM, 10 μM, 100 μM, 1000 μM and 2000 μM, respectively.

A series of 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones derivatives **3a–g** have thus been successfully synthesised in moderate to good yield (51–73%). Various compounds exhibited concentration-dependent activities in each of the biological studies. The presence of the phenyl ring, particularly when bearing electronegative *para*-substituents, clearly increases the levels of antimalarial and antibacterial activity, with the *para*-fluorophenyl derivative **3d** proving to be

consistently active across all of the bioassays. These results indicate the potential of the title compounds **3a–e** to serve as lead compounds in the development of novel, multifunctional therapeutics.

Acknowledgments

The authors wish to thank Rhodes University for bursary support (to E.O.O.) and the South African Medical Research Council (SAMRC) and Rhodes University for research funding.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.05.001.

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