Synthesis and Biological Evaluation of 2-Chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones

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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 *Mycobacterium tuberculosis,* human SH-SY5Y cells, *Plasmodium falciparum* 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.

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Graphical abstract

IC$_{50}$ values for **3e** (R=NO$_2$):
4 nM (SH-SY5Y); 20.3 μM (*Pf*LDH)
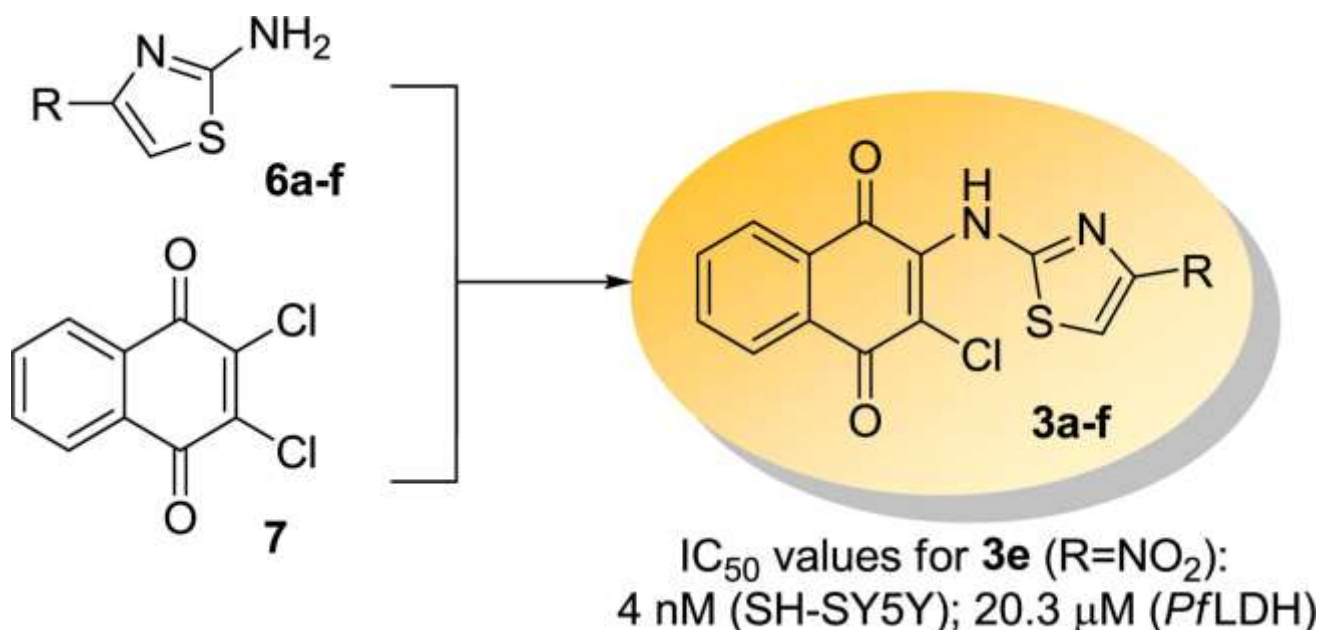
1. Introduction

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 antimicrobial,[1,2,8-12] anti-cancer,[2,3,13-15] antiviral,[16] trypanocidal,[17] antifungal,[18] antiparasitic,[19] antimalarial[20] and antimycobacterial[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. Quinone-based compounds have also been reported to act as potent inhibitors of nicotinamide adenosine diphospate (NADP) or its reductive analogue, NADPH-dependent quinone oxidoreductase (NQO1).[15,23,24] This enzyme is a key target in the design and development of quinone-based anti-cancer agents, and acts mainly by catalysing NAD(P)H-dependent reduction in biological systems.[15] The 5,8-quinolinedione analogues, streptonigrin **1** [25] and lavendamycin,[26] have been shown to inhibit this 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. 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). While Stasevych *et al.*[28] have used disc-diffusion susceptibility antimicrobial 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 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.
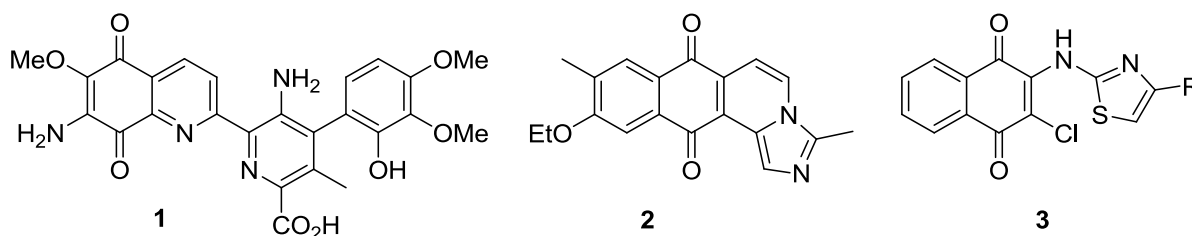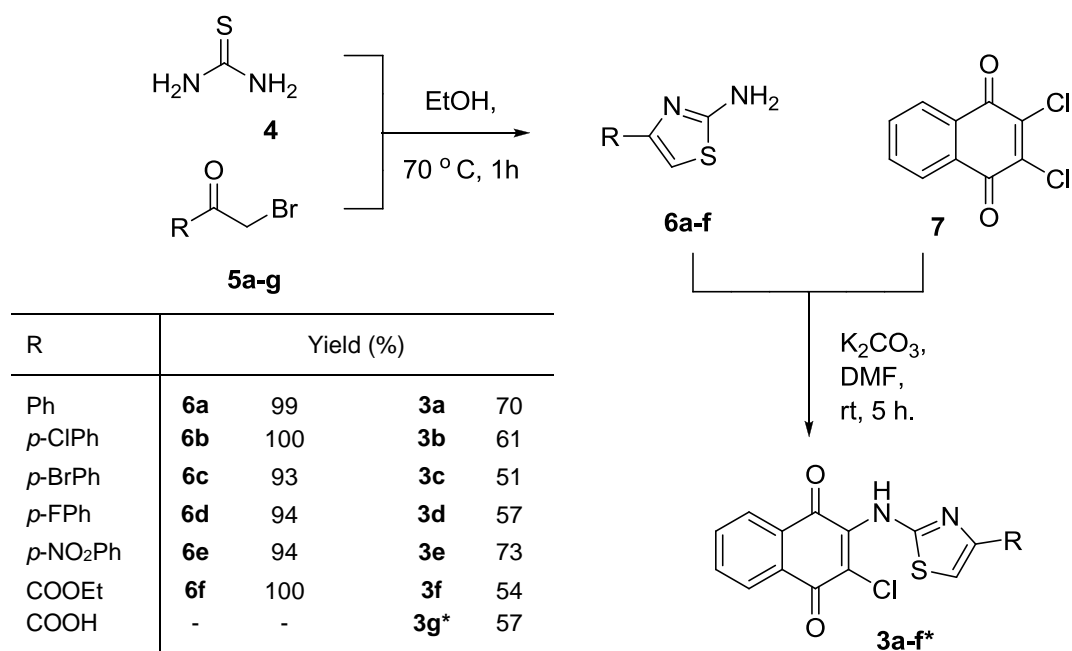

2. Results and discussion.

2.1. Chemistry

The preparation of the known 2-aminothiazoles **5a-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 **4a-f** with thiourea **3** (Scheme 1). After completion of each reaction, the reaction mixture was poured into ice-cold water to precipitate the thiazole derivatives **5a-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 (**2**). 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 protic apolar 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 hours); and v) microwave-assisted conditions with or without solvent at 150 °C for 10 minutes. Successful nucleophilic displacement of one of the chlorine atoms in 2,3-dichloro-1,4-naphthoquinone by each of the 2-aminothiazoles **5a-f** was finally achieved using DMF in the presence of potassium carbonate (K_2CO_3) to obtain, without heating, the desired 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones **2a-f**. All of the compounds were fully characterised using 1- and 2-D NMR, IR and HRMS methods; the 'parent' system **2a**[12,17] is known but the analogues **2b-f** 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 **2f** was hydrolysed in methanolic KOH to give the corresponding acid **2g** in moderate yield (53%).

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-NO2Ph	**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**.

2.3. Biological studies

2.3.1. Anti-cancer activity

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 (Figure 3) 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 (media only). The carboxylic acid **3g** and the precursor ester **2f** exhibited the lowest inhibitions with IC_{50} values > 100 µM and 31.1 µM, respectively (Table 1). The phenyl-substituted analogues, however, exhibited significant cytotoxicity, with the unsubstituted phenyl (**2a**), *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 NADH 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**. The expected mechanism of action may involve the formation of free radicals by the 1,4-naphthoquinones, which then act as NADP or NADPH dependent quinone oxidoreductase 1 (NQO1) inhibitors, which are key targets for quinone-based anti-cancer agents.[15,30,31] Further studies are required to determine the morphological effects of these compounds on both cell lines.
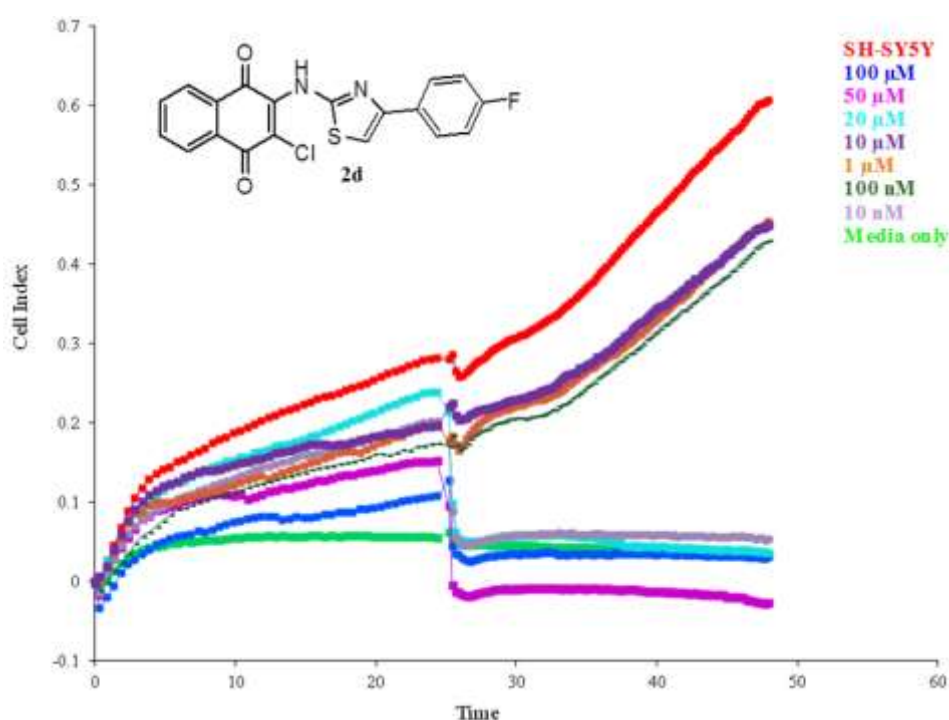

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Figure 1. Scans of Cell Index (CI) of SH-SY5Y *vs* time (h) for different concentrations of 2-chloro-3-[4-(4-fluorophenyl)thiazol-2-ylamino]-1,4-naphthoquinone **3d** over 48 h at using an xCELLigence RTCA-SP instrument.

2.3.2. *Anti-tuberculosis activity*

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 (**2a**: MIC_{90} = 9.19 µM and MIC_{99} = 10.2 µM); **2d**: 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 contribute to their superior absorption across the mycolic-rich lipophilic cell wall of *M. tuberculosis*.

2.3.3. *Antimalarial activity*

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 Supporting Information 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%

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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.

2.3.4. Antibacterial activity

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.

3. Conclusions

A series of 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones derivatives **3a-g** have 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*-substitutuents, 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.

Table 1. Biological evaluation of the 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones **3a-g**.
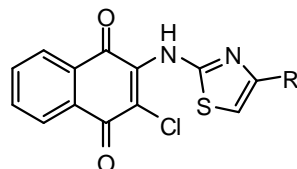
	R	*Cytotoxicity*		*Anti-TB*		*Antimalarial*	*Antimicrobial*				
		SH-SY5Y IC$_{50}$ (µM)	HeLa % Viability[a]	MIC$_{90}$ (µM)	MIC$_{99}$ (µM)	*Pf*LDH IC$_{50}$ (µM)	Zone of inhibition (mm)				
							2000 µM	1000 µM	100 µM	10 µM	1 µM
3a	Ph	1.8	80	9.19[b]	10.2[b]	-	13.2	11.4	9.1	5.3	-
3b	*p*-ClPh	2.7	80	> 20.0	> 20.0	-	12	9.7	7.6	-	-
3c	*p*-BrPh	-	55	> 20.0	> 20.0	50.6	-	-	-	-	-
3d	*p*-PhF	1.5	75	9.37[b]	10.4[b]	44.7	19.5	15	11	8	5
3e	*p*-NO$_2$Ph	0.004	80	> 20.0	> 20.0	20.3	14.1	11	7.2	-	-
3f	COOEt	31.1	82	19.5	> 20.0	-	-	-	-	-	-
3g	COOH	> 100	75	> 20.0	> 20.0	-	-	-	-	-	-
Controls											
Chloroquine		-	-	-	-	0.0143	-	-	-	-	-
Rifampicin		-	-	0.0015	0.00167	-					
Ampicillin		-	-	-	-	-			24.7 mm at 25 µg		
Streptomycin		-	-	-	-	-			20.0 mm at 10 µg		

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

4. Experimental

All reagents were purchased from Sigma-Aldrich (South Africa) and used without further purification. Melting points were recorded, uncorrected, using Reichert slide warmer hot plate microscopy. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance II 600 MHz, Bruker Avance III HD 400 MHz and Bruker Fourier 300 MHz spectrometers. Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum 400 Frontier / FT-IR spectrometer; compounds were analysed neat. High resolution mass spectra (HRMS) were recorded on a Waters API Q-TOF Ultima spectrometer (University of Stellenbosch, Stellenbosch, South Africa).

4.1. Synthesis

4.1.1. Preparation of the 2-aminothiazoles 6a-f.

Following reported methodology,[9,10] mixtures of thiourea **4** (1.2 mmol) and the haloketones **5a-f** (1 mmol) in EtOH (2 mL) were stirred at 70 ^{o}C for 1h. The reaction mixtures were cooled to room temperature, poured into ice-cold water and the resulting precipitates formed were filtered off and dried to give the known compounds:

 amino-4-phenylthiazole **6a** (0.208 g, 94%) as a bright yellow solid, mp 287-288 ^{o}C (Lit.[12,17] 285-286 ^{o}C); 2-amino-4-(4-chlorophenyl)thiazole **6b** (0.210 g, 100%) as a white solid, mp 162-165 ^{o}C (Lit.[12] 163-164 ^{o}C); 2-amino-4-(4-bromophenyl)thiazole **6c** (0.236 g, 93%) as a white solid, mp 179-181 ^{o}C (Lit.[12] 180-181 ^{o}C); 2-amino-4-(4-fluorophenyl)thiazole **6d** (0.191 g, 98%) as a white solid, mp 122-124 ^{o}C (Lit.[13] 120 ^{o}C); 2-amino-4-(4-nitrophenyl)-thiazole **6e** (0.208 g, 94%) as a bright yellow solid, mp 287-288 ^{o}C (Lit.[12,17] 285-286 ^{o}C); and ethyl 2-aminothiazole-4-carboxylate **6f** (0.172 g, 100%) as a white solid, mp 172-174 ^{o}C (Lit.[18-20] 172 ^{o}C).

4.1.2. The general procedure for the preparation of 2-chloro-3-[(thiazol-2-yl)amino]-1,4-naphthoquinones 2-(2-chloroacetamido)thiazoles 3a-f involved a modification of the method reported by Tandon and Maurya[29] and is illustrated by the following example.

A mixture of 2-amino-4-phenylthiazole **5a** (0.5 mmol, 0.088 g) and K$_2$CO$_3$ (0.745 mmol, 0.103 g) in DMF (5 mL) was stirred at room temperature for 30 min. Thereafter, 2,3-dichloro-1,4-naphthoquinone (0.45 mmol, 0.103 g) was added and the mixture stirred for 5 h

at room temperature. The reaction progress was monitored by TLC using a hexane-ethyl acetate (4:1) solvent system. Ethyl acetate (10 mL) was added, and the mixture was washed with 2% HCl (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The crude product was purified using column chromatography on silica gel G, eluting with hexane-ethyl acetate (7:3) to give 2-Chloro-3-[(4-phenylthiazol-2-yl)amino]-1,4-naphthoquinone **3a** (0.116 g, 70%) as an amber solid, mp 152-154 °C;[12,17] [HRMS: *m/z* calculated for $C_{19}H_{12}N_2O_2SCl$ (MH$^+$) 367.0308. Found 367.0294]; ν_{max} / cm^{-1} 1650 (C=O), 1666 (C=O), 3256 (NH); δ_H (400 MHz; CDCl$_3$) 8.22 (1H, dd, *J* = 7.7 Hz, 1.0 Hz, ArH), 8.15 (1H, dd, *J* = 7.6 Hz, 1.0 Hz, ArH), 8.00 (1H, br s, NH), 7.83 (2H, dd, *J* = 7.6 Hz, 1.4 Hz, ArH), 7.80 (1H, dd, *J* = 7.5 Hz, 1.3 Hz, ArH), 7.74 (1H, td, *J* = 7.6, 1.3 Hz, ArH), 7.41 (2H, t, *J* = 7.6 Hz, ArH), 7.33 (1H, t, *J* = 7.4 Hz, ArH) and 7.26 (1H, s, thiazolyl-H); δ_C (100 MHz; CDCl$_3$) 179.7 (C=O), 177.6 (C=O), 159.1, 152.1, 141.3, 135.2, 134.1, 133.8, 132.2, 130.1, 128.9, 128.5, 127.5, 127.3, 126.4, 121.7 and 110.1 (ArC and thiazolyl-C).

4.1.3.1. 2-Chloro-3-{[4-(4-chlorophenyl)thiazol-2-yl]amino}-1,4-naphthoquinone 3b

(0.110 g, 61%) as an amber solid, mp 186-187 °C; [HRMS: *m/z* calculated for $C_{19}H_{11}N_2O_2S^{35}Cl_2$ (MH$^+$) 400.9918. Found 400.9912]; ν_{max} / cm^{-1} 1656 (C=O), 1666 (C=O) and 3246 (NH); δ_H (400 MHz; CDCl$_3$) 8.22 (1H, dd, *J* = 7.7 Hz, 1.0 Hz, ArH), 8.15 (1H, dd, *J* = 7.7 Hz, 1.0 Hz, ArH), 8.00 (1H, br s, NH), 7.81 (1H, td, *J* = 7.6 Hz, 1.3Hz, ArH), 7.78-7.73 (3H, m, ArH), 7.37 (2H, d, *J* = 8.6 Hz, ArH) and 7.24 (1H, s, thiazolyl-H); δ_C (100 MHz; CDCl$_3$) 179.6 (C=O), 177.6 (C=O), 159.2, 150.8, 141.1, 135.1, 134.1, 133.7, 132.5, 132.1, 129.9, 129.0, 127.5, 127.4, 127.2, 121.9 and 110.2 (ArC and thiazolyl-C).

4.1.3.2. 2-Chloro-3-{[4-(4-bromophenyl)thiazol-2-yl]amino}-1,4-naphthoquinone 3c

(0.102 g, 51%) as an amber solid, mp 210-212 °C; [HRMS: *m/z* calculated for $C_{19}H_{10}N_2O_2SBr$ (MH$^+$-HCl) 412.0152. Found 412.0152 (^{79}Br)]; ν_{max} / cm^{-1} 1671 (C=O) and 1680 (C=O); δ_H (400 MHz; CDCl$_3$) 8.27 (2H, d, *J* = 8.8 Hz, ArH), 8.24 (1H, d, *J* = 7.9 Hz, ArH), 8.16 (1H, d, *J* = 7.3 Hz, ArH), 8.00 (2H, d, *J* = 8.8 Hz, ArH), 7.82 (1H, t, *J* = 7.1 Hz, ArH), 7.77 (1H, t, *J* = 7.1 Hz, ArH) and 7.46 (1H, s, thiazolyl-H); δ_C (100 MHz; CDCl$_3$) 179.7 (C=O), 177.6 (C=O), 159.2, 150.8, 141.0, 135.1, 133.7, 132.9, 132.0, 131.9, 129.9, 127.8, 127.4, 127.2, 122.3, 122.1 and 110.3 (ArC and thiazolyl-C).

4.1.3.3. 2-Chloro-3-{[4-(4-fluorophenyl)thiazol-2-y])amino}-1,4-naphthoquinone 3d

(0.098 g, 57%) as an amber solid, mp 171-173 °C; [HRMS: m/z calculated for $C_{19}H_{11}N_2O_2S^{35}ClF$ (MH$^+$) 385.0214. Found 385.0210]; v_{max} / cm^{-1} 1648 (C=O), 1671 (C=O) and 3252 (NH); δ_H (400 MHz; CDCl$_3$) 8.22 (1H, dd, J = 7.6 Hz, 1.1 Hz, ArH), 8.15 (1H, dd, J = 7.6 Hz, 1.2 Hz, ArH), 8.00 (1H, br s, NH), 7.82-7.78 (3H, overlapping m, ArH), 7.74 (1H, td, J = 7.5 Hz, 1.4 Hz, ArH), 7.18 (1H, s, thiazolyl-H) and 7.12-7.06 (2H, overlapping m, ArH); δ_C (100 MHz; CDCl$_3$) 179.7 (C=O), 177.7 (C=O), 163.0 ($^1J_{F,C}$ = 248 Hz), 159.2, 151.1, 141.2, 135.3, 133.8, 132.2, 130.4 ($^4J_{F,C}$ = 3.4 Hz), 130.0, 128.2 ($^3J_{F,C}$ = 8.2 Hz), 127.5, 127.3, 121.9.

4.1.3.4. 2-Chloro-3-{[4-(4-nitrophenyl)thiazol-2-yl]amino}-1,4-naphthoquinone 3e (0.136 g, 73%) as an amber solid, mp 212-214 °C; [HRMS: m/z calculated for $C_{19}H_{11}N_3O_4S^{35}Cl$ (MH$^+$) 412.0159. Found 412.0150]; v_{max} / cm^{-1} 1644 (C=O), 1674 (C=O) and 3248 (NH); δ_H (400 MHz; acetone-d_6) 8.28 (2H, d, J = 8.9 Hz, ArH), 8.19-8.14 (3H, overlapping m, ArH), 8.12 (1H, dd, J = 7.5 Hz, 1.3 Hz, ArH), 7.79 (1H, s, thiazolyl-H), 7.93 (1H, td, J = 7.5, 1.4 Hz, ArH) and 7.89 (1H, td, J = 7.5 Hz, 1.1 Hz, ArH); δ_C (100 MHz; acetone-d_6) 180.2 (C=O), 178.2 (C=O), 161.8, 149.6, 148.0, 143.6, 141.3, 135.7, 134.7, 132.7, 131.6, 127.7, 127.6, 127.5, 124.9, 124.2 and 114.6 (ArC and thiazolyl-C).

4.1.3.5. 3-[(4-Carbethoxythiazol-2-yl)amino]-2-chloro-1,4-naphthoquinone 3f (0.179 g, 54%) as an amber solid, mp 148-151 °C; [HRMS: m/z calculated for $C_{16}H_{12}N_2O_4S^{35}Cl$ (MH$^+$) 363.0206, Found 363.0197]; v_{max} / cm^{-1} 1654 (C=O), 1717 (C=O), 1731 (C=O) and 3263 (NH); δ_H (400 MHz; CDCl$_3$) 8.83 (br s, NH), 8.09 (1H, d, J = 7.6 Hz, ArH), 7.82 (1H, s, thiazolyl-H), 7.76 (1H, t, J = 7.4 Hz, ArH), 7.68 (1H, t, J = 7.4 Hz, ArH), 4.39 (2H, q, J = 7.1 Hz, OCH$_2$), 1.38 (3H, t, J = 7.1 Hz, CH$_3$); δ_C (100 MHz; CDCl$_3$) 184.3 (C=O), 180.7 (C=O), 161.1 (OC=O), 159.6, 144.2, 140.7, 135.3, 133.3, 132.7, 130.0, 127.0, 126.6, 121.0, 110.9 (ArC and thiazolyl-C), 61.8 (OCH$_2$) and 14.5 (CH$_3$).

4.1.4. 3-[(4-Carboxythiazol-2-yl)amino]-2-chloro-1,4-naphthoquinone 3g.

A mixture of 3-[(4-carbethoxythiazol-2-yl)amino]-2-chloro-1,4-naphthoquinone **2f** (0.091 g, 0.25 mmol) in MeOH (250 μL) and KOH (0.093 g, 0.5 mmol) in MeOH-H$_2$O was stirred at room temperature for 2 h.[38] Work-up afforded ***3-[(4-carboxythiazol-2-yl)amino]-2-chloro-1,4-naphthoquinone*** **2g** (0.048 g, 57%) as a light-grey solid, mp 189-191 °C; [HRMS: m/z

calculated for $C_{14}H_8N_2O_4S^{35}Cl$ (MH$^+$) 334.9893, Found 334.9881]; ν_{max} / cm^{-1} 1662 (C=O), 1713 (C=O), 2513-3507 (br, COOH) and 3161 (NH); δ_H (400 MHz; acetone-d_6) 8.12 (1H, dd, J = 7.3 Hz, 1.2 Hz, ArH), 8.08 (1H, dd, J = 7.1 Hz, 1.4 Hz, ArH), 8.03 (1H, s, thiazolyl-H), 7.93–7.82 (2H, m, ArH); δ_C (100 MHz; acetone-d_6) 216.2 (COOH), 169.3 (C=O), 167.9 (C=O), 152.3, 152.2, 151.9, 151.8, 125.4, 124.6, 121.7, 121.1, 117.2, 117.0 and 114.0 (ArC and thiazolyl-C).

Acknowledgments

Supplementary data

Supplementary data associated with this article can be found in the online version.

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