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Naphtho[2,3-*b*][1,4]-thiazine-5,10-diones and 3-substituted-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thioalkanoate derivatives: Synthesis and biological evaluation as potential antibacterial and antifungal agents

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Abstract—A series of 3-substituted-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thio-alkanoate derivatives **3**–**21** and naphtho[2,3-*b*][1,4]-thiazine-5,10-diones **24** were synthesized and evaluated for their antibacterial and antifungal activities. The structure–activity relationships of these compounds were studied and the results show that the compound **24a** exhibited better antibacterial activity than Gentamycin in vitro against *Staphylococcus aureus*. In addition **24a** also imparted marked antifungal activity in vitro against *Cryptococcus neoformans*, *Sporothrix schenckii*, and *Trichophyton mentagraphytes* when compared with Fluconazole. Compounds **15**, **18**, **19**, and **21** also exhibited significant antibacterial activity in vitro against *S. aureus*.

The quinone group forms the basis of biological activity of a number of clinical and experimental drugs that are associated with antitumor, antimalarial, antifungal, and antibacterial studies.[1–11] The clinical significance of this class of compounds has stimulated the synthesis and biological evaluation of new agents retaining the 'core' quinone moiety.[12–19]

The diverse biological effects caused by incorporation of nitrogen or sulfur atoms in five- or six-membered heterocyclic ring retaining the 'core' chromophore have been one of the mainstay of structural and chemical modifications of this class of compounds.[1–21] This has already led to development of lead molecules **I**–**III** (Fig. 1) having pronounced biological effects.[1,3,10,19]

In the course of a medicinal chemistry program aimed at the synthesis of new quinone derivatives having both N and S atoms in a six-membered ring as part of heterocyclic quinone, we have synthesized a series of 3-substituted-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thioalkanoate derivatives **2**–**21** and naphtho[2,3-*b*][1,4]-thiazine-5,10-diones **24**.

The evaluation of antibacterial activities of compounds **2**–**24** against various strains of the bacteria, for example, *Staphylococcus aureus*, *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* was carried out according to the broth microdilution technique described by NCCLS.[22,23] The minimum inhibitory concentration (MIC) and IC_{50} of each compound were determined against test isolates using this technique. The MICs and IC_{50}s of standard antibacterial drugs Gentamycin and Ampicillin were determined in 96-well tissue culture plates using Mueller–Hinton broth.

Table 1 shows antibacterial activity of compounds **2**–**24**. The antibacterial activity was compared with those of Gentamycin and Ampicillin which were used as positive control in all tests with MIC and IC_{50} values expressed in μg/mL. Compound **24a** showed better activity than Gentamycin against *S. aureus*. It also showed

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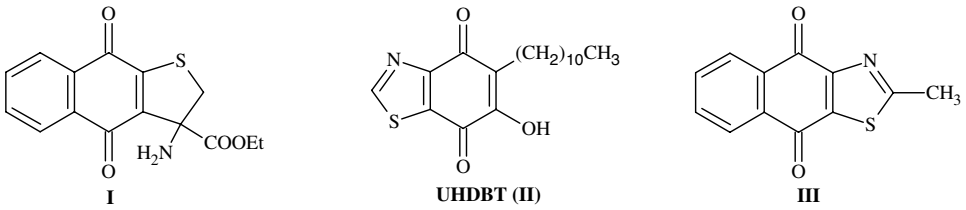


Figure 1.

Table 1. Structures and in vitro antibacterial activity of compounds **2**–**24** (MIC, μg/mL) and (IC_{50}, μg/mL)

Compound	R	R^1	R^2/Ar	R^3	*S. aureus* MIC (IC_{50})	*S. faecalis* MIC (IC_{50})	*K. pneumoniae* MIC (IC_{50})	*P. aueruginosa* MIC (IC_{50})	*E. coli* MIC (IC_{50})
2c	H	C_2H_5	[a]	[a]	12.5 (6.98)	12.5 (9.24)	50.0 (33.24)	50.0 (44.42)	12.5 (8.56)
2d	CH_3	C_2H_5	[a]	[a]	12.5 (7.95)	12.5 (9.52)	50.0 (28.34)	50.0 (48.31)	12.5 (6.34)
4	CH_3	C_2H_5	$(CH_2)_2$- C_6H_3-3,4-$(OMe)_2$	[a]	12.5 (9.52)	>50 (>50)	50.0 (42.54)	50.0 (46.24)	50.0 (44.28)
5	H	C_2H_5	C_6H_5	[a]	12.5 (11.82)	25.0 (18.64)	12.5 (9.24)	25.0 (22.82)	6.25 (4.32)
7	H	C_2H_5	$CH_2C_6H_5$	[a]	12.5 (11.59)	>50 (>50)	25.0 (22.34)	>50 (>50)	12.5 (9.20)
12	H	C_2H_5	$CH_2CH(CH_3)_2$	[a]	25.0 (21.56)	25.0 (19.54)	50.0 (38.52)	50.0 (42.52)	50.0 (39.26)
13	H	C_2H_5	$CH(CH_3)_2$	[a]	25.0 (18.46)	25.0 (20.64)	50.0 (46.88)	50.0 (35.92)	25.0 (24.98)
15	CH_3	C_2H_5	CH_2CH_2OH	[a]	6.25 (4.82)	6.25 (5.42)	12.5 (11.58)	25.0 (24.54)	3.12 (2.24)
18	CH_3	C_2H_5	H	[a]	6.25 (6.16)	12.5 (8.54)	12.5 (11.68)	50.0 (34.52)	1.56 (1.18)
19	CH_3	C_2H_5	$C(CH_3)_3$	[a]	6.25 (**3.98**)[b]	6.25 (5.62)	6.25 (4.82)	**6.25 (5.32)**[b]	6.25 (4.38)
21	CH_3	C_2H_5	NH_2	[a]	6.25 (5.65)	25.0 (22.28)	6.25 (5.28)	**6.25 (4.62)**[b]	6.25 (5.20)
24a	CH_3	[a]	[a]	CH_3	**3.12 (1.92)**[b]	3.12 (2.58)	3.12 (1.98)	50.0 (49.32)	1.56 (0.96)
Gen					6.25 (4.46)	0.78 (0.62)	0.78 (0.34)	25.0 (24.64)	0.18 (0.11)
Amp					0.90 (0.74)	0.02 (0.01)	0.09 (0.08)	50.0 (48.74)	0.02 (0.01)

Gen, Gentamycin; Amp, Ampicillin.
[a] Activity not reported.
[b] Entries in bold font indicate more activity of the compound than Gentamycin.

Table 2. Structures and in vitro antifungal activity of compounds **2**–**24** (MIC, μg/mL) and (IC_{50}, μg/mL)

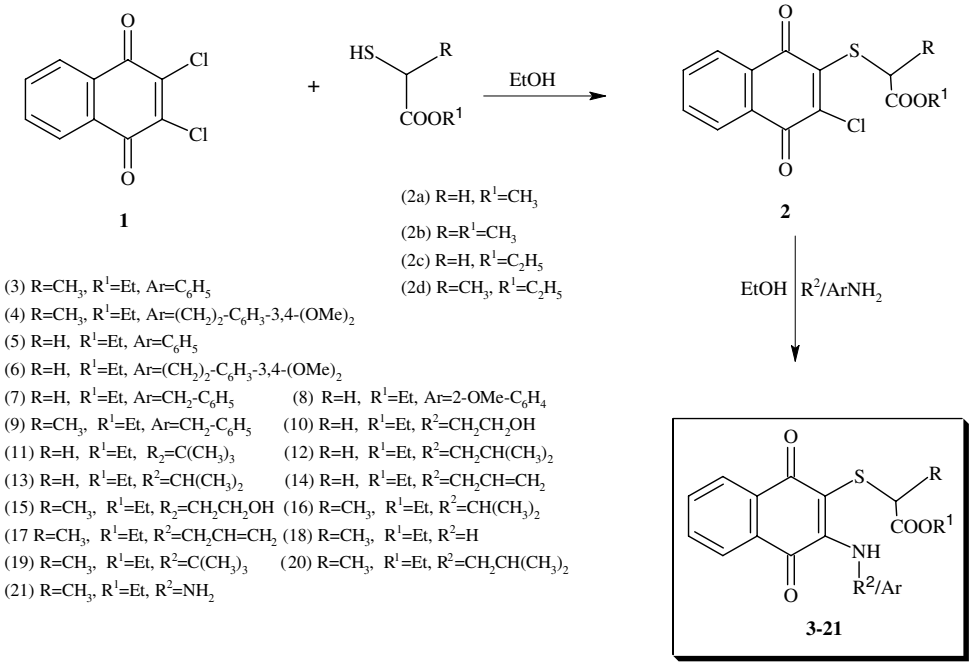
Compound	R	R^1	R^2/Ar	R^3	*C. albicans* MIC (IC_{50})	*C. parapsilosis* MIC (IC_{50})	*C. neoformans* MIC (IC_{50})	*A. fumigatus* MIC (IC_{50})	*S. schenckii* MIC (IC_{50})	*T. mentagraphytes* MIC (IC_{50})
2c	H	C_2H_5	[a]	[a]	>50 (>50)	>50 (>50)	12.5 (10.94)	>50 (>50)	25.0 (18.74)	>50 (>50)
2d	CH_3	C_2H_5	[a]	[a]	>50 (>50)	>50 (>50)	12.5 (11.46)	>50 (>50)	25.0 (22.68)	>50 (>50)
4	CH_3	C_2H_5	$(CH_2)_2$-C_6H_3-3, 4-$(OMe)_2$	[a]	>50 (>50)	>50 (>50)	>50 (>50)	>50 (>50)	>50 (>50)	25.0 (14.00)
5	H	C_2H_5	C_6H_5	[a]	>50 (>50)	>50 (>50)	>50 (>50)	>50 (>50)	50.0 (46.72)	25.0 (23.00)
7	H	C_2H_5	$CH_2C_6H_5$	[a]	>50 (>50)	>50 (>50)	>50 (>50)	>50 (>50)	>50 (>50)	6.25 (4.50)
12	H	C_2H_5	$CH_2CH(CH_3)_2$	[a]	>50 (>50)	>50 (>50)	>50 (>50)	>50 (>50)	50.0 (22.82)	12.5 (9.80)
13	H	C_2H_5	$CH(CH_3)_2$	[a]	50.0 (44.28)	25.0 (23.24)	12.5 (11.28)	50.0 (44.94)	50.0 (38.58)	12.5 (9.80)
15	CH_3	C_2H_5	CH_2CH_2OH	[a]	25.0 (23.28)	25.0 (22.54)	12.5 (6.92)	25.0 (23.92)	25.0 (21.94)	>50 (>50)
18	CH_3	C_2H_5	H	[a]	25.0 (24.20)	25.0 (21.24)	12.5 (12.00)	25.0 (16.58)	25.0 (17.92)	>50 (>50)
19	CH_3	C_2H_5	$C(CH_3)_3$	[a]	50.0 (38.27)	25.0 (22.20)	12.5 (10.29)	25.0 (21.82)	12.5 (10.92)	3.12 (2.80)
21	CH_3	C_2H_5	NH_2	[a]	50.0 (24.24)	25.0 (17.34)	25.0 (13.32)	25.0 (22.36)	25.0 (22.00)	6.25 (4.50)
24a	CH_3	[a]	[a]	CH_3	6.25(5.30)	12.5 (10.38)	3.12 (1.54)	6.25(3.98)	3.12 (2.84)	1.56 (0.90)
Flu					0.5 (0.13)	1.0 (0.21)	1.0 (0.46)	2.0 (1.06)	2.0 (1.45)	1.0 (0.63)
Am					0.12(0.09)	0.12 (0.11)	0.06 (0.04)	0.50(0.38)	0.12 (0.08)	0.12 (0.09)

Flu, Fluconazole; Am, Amphotericin-B.
[a] Activity not reported.

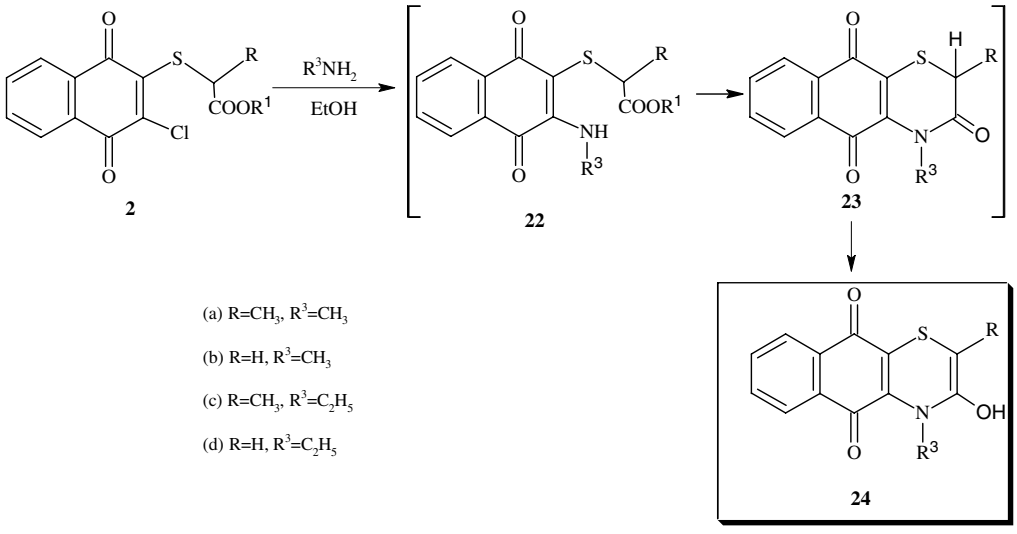
significant activity against *S. faecalis*, *K. pneumoniae*, and *P. aueruginosa*. Compounds **15**, **18**, **19**, and **21** had similar activity when compared with Gentamycin against *S. aureus*. Compounds **19** and **21** exhibited better activity than Gentamycin against *P. aueruginosa*. Both compounds **18** and **24a** showed marked activity against *E. coli* when compared with Gentamycin. On comparison of antibacterial activity of the compounds referred to in Table 1, it was observed that none of the compounds showed better activity than Ampicillin.

The study of structure–activity relationship in **2**–**24** revealed that cyclic analog **24a** showed pronounced antibacterial activity against most of the bacteria referred to in Table 1 than the acyclic chloro and amino esters **2**–**21**. Substitution of R^2 by $C(CH_3)_3$ and NH_2 in acyclic esters **19** and **21** leads to marked increase in antibacterial activity.

The antifungal activity of compounds **2**–**24** against various strains of pathogenic fungi, for example,



Scheme 1.



Scheme 2.

Candida albicans, *Candida parapsilosis* (ATCC 22019), *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Sporothrix schenckii*, and *Trichophyton mentagraphytes* was carried out according to the broth microdilution technique described by NCCLS.[22,23] The minimum inhibitory concentration (MIC) and IC_{50} of each compound were determined against test isolates using this technique.

The antifungal activity was compared with those of standard drugs Fluconazole and Amphotericin-B. MIC and IC_{50} of standard drugs referred to in Table 2 and the compounds were determined in 96-well tissue culture plates using RPMI 1640 media buffered with MOPS (3-[*N*-morpholino]-propane sulfonic acid) (Sigma Chemical Co.).

Comparison of the activity of compounds **2**–**24** referred to in Table 2 with that of antifungal drug Fluconazole showed that the cyclic compound **24a** had marked activity against fungi *C. neoformans, S. schenckii*, and *T. mentagraphytes*. Amongst the acyclic compounds **2**–**21**, only compound **19** showed significant antifungal activity against *T. mentagraphytes*. Other compounds whose MIC was >75 mg/mL are not reported in Table 2 as these were considered to be inactive compounds.

To the best of our knowledge naphtho[2,3-*b*][1,4]-thiazine-5,10-diones **24** have not been synthesized earlier. The precursors, 3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thioalkanoic acid esters **2** required for the synthesis of **24**, have been synthesized according to

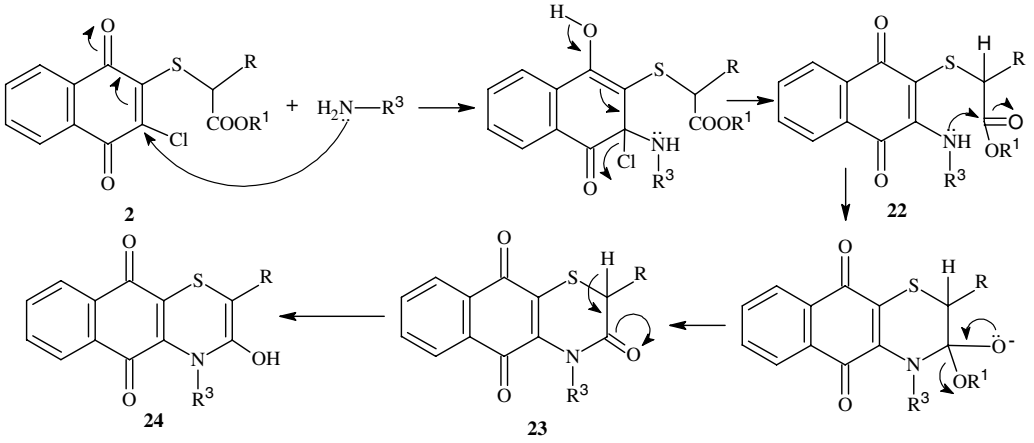


Figure 2.

Scheme 1. The reaction of 2,3-dichloro-1,4-naphthoquinone **1** with thioalkanoic acid methyl and ethyl esters afforded 3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thio methyl and ethyl alkanoic acid esters **2**.[24] This reaction involves nucleophilic displacement of Cl atom in 2,3-dichloro-1,4-naphthoquinone **1** with sulfur nucleophile. Further nucleophilic displacement reaction of **2** with primary aliphatic and aromatic amines having long chain or bulky substituents resulted in the formation of compounds **3**–**21**[25] as exhibited in Scheme 1.

However, the nucleophilic displacement reaction of **2** with methyl and ethyl amine afforded naphtho[2,3-*b*][1,4]-thiazine-5,10-diones **24(a**–**d)**[25] as the only isolated products as exhibited in Scheme 2. However, the reaction of **2** with other bulky aliphatic primary and aromatic amines did not result in formation of **24** due to steric hindrance. The bulky chain R^3 thus could not be introduced in **24**. New routes to synthesis of **24** having bulky substituents are underway and will be published in full paper. The mechanism of formation of **24** from **2** is shown in Figure 2 and involves nucleophilic displacement of Cl by NHR^3 followed by intramolecular nucleophilic addition-elimination and cyclization leading to intermediates **22** and **23**. All the new compounds **2**–**24** were characterized by IR, ^{1}H NMR, and ^{13}C NMR spectroscopic data. Elemental analytical data for each of these compounds were found to be in agreement with calculated value.

In conclusion, we have synthesized a series of 3-substituted-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thioalkanoate derivatives **3**–**21** and naphtho[2,3-*b*][1,4]-thiazine-5,10-diones **24**. Amongst the promising compounds, compound **24a** showed better antibacterial activity than Gentamycin in vitro against *S. aureus*. Compounds **15**, **18**, **19**, and **21** have also shown significant antibacterial activity in vitro against *S. aureus* as well as against other bacteria. Compound **24a** exhibited marked antifungal activity compared to the other members of the library against *C. neoformans*, *S. schenckii*, and *T. mentagraphytes*.

Thus compound **24a** is the lead compound for both antibacterial and antifungal activities. Further work on compound **24a** is in progress.

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24. *General procedure for the synthesis of 3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thioalkanoic acid esters* **2(a–d)**. A mixture of 2,3-dichloro-1,4-naphthoquinone **1** (10 mmol) and thioalkanoic acid methyl and ethyl esters **2** (12 mmol) in abs. EtOH (50 mL) was refluxed with vigorous stirring for 8–10 h at 80–90 °C. The resulting solution was allowed to crystallize at room temperature to give solid which was further crystallized with ethanol to give **2** in 95–97% yield. Compound **2c**; orange crystals, 97% yield; mp 96 °C; IR (KBr): 1590 and 1669 (>C=O of quinone), 1737 (>C=O of COOEt) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.19 (t, 3H, CH_3), 3.64 (s, 2H, SCH_2), 4.13 (q, 2H, OCH_2), 7.62 (m, 2H, C_6-H and C_7-H), 8.16 (m, 2H, C_5-H and C_8-H); Anal. Calcd for $C_{14}H_{11}ClO_4S$ (310.75) C, 54.11; H, 3.57; S, 10.32. Found: C, 54.28; H, 3.64; S, 10.48.
25. *General procedure for the synthesis of 3-alkyl and arylamino-1,4-dioxo-1,4-dihydronaphthal-ene-2-yl-thioalkanoic acid esters* **3–21** and *naphtho[2,3-b][1,4]-thiazine-5,10-diones* **24(a–d)**. Primary amines (aliphatic/aromatic) (12 mmol) were added to a stirred solution of 3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thioalkanoic acid esters **2** (10 mmol) in abs. EtOH (100 mL). The reaction mixture was refluxed with stirring for 5–26 h at 20–90 °C. The resulting solution was concentrated in vacuo and the residue was subjected to column chromatography on silica gel using EtOAc/hexane (1:20) and the product was crystallized with suitable solvent to give **3–21** in 55–67% yield. Compound **13**; orange crystals after crystallization with EtOH/hexane; 62% yield; mp 46–48 °C; IR (KBr): 1593 and 1675 (>C=O of quinone), 3310 (NH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.16 (t, 3H, CH_3), 1.32 (d, 6H, 2× CH_3), 3.56 (s, 2H, SCH_2), 4.08 (q, 2H, OCH_2), 4.75 (m, 1H, CH), 6.58 (br s, 1H, NH), 7.66 (m, 2H, C_6-H and C_7-H), 8.08 (m, 2H, C_5-H and C_8-H). Anal. Calcd for $C_{17}H_{19}NO_4S$ (333.40): C, 61.24; H, 5.74; N, 4.20; S, 9.62. Found: C, 61.40; H, 5.66; N, 4.38; S, 9.78. *3-Hydroxy-2,4-dimethyl-4H-naphtho[2,3-b][1,4]-thiazine-5,10-dione* **24a**; orange crystals after crystallization with EtOH; 58% yield; mp 186 °C; ^1H NMR ($CDCl_3$): δ 1.57 (s, 3H, CH_3), 1.59 (s, 1H, OH), 3.47 (s, 3H, NCH_3), 7.74 (m, 2H, C_6-H and C_7-H), 8.09(m, 2H, C_5-H and C_8-H); ^{13}CNMR ($CDCl_3$): δ 14.50, 35.00, 36.99, 99.98, 126.49, 127.16, 131.20, 131.96, 132.52, 134.03, 134.19, 141.54, 166.07, 180.63; Anal. Calcd for $C_{14}H_{11}NO_3S$ (273.31): C, 61.52; H, 4.06; N, 5.12; S, 11.73. Found: C, 61.68; H, 4.14; N, 5.30; S, 11.84.