Med Chem Res (2007) 16:402–407
DOI 10.1007/s00044-007-9059-y

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological activities of some N-hydroxymethylene and N-methoxymethylene thiazines

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Received: 21 February 2007 / Accepted: 12 November 2007 / Published online: 19 December 2007

Abstract A series of new 3,5-diaryl-N-hydroxymethylene-1,4-thiazine-1,1-dioxides and 3,5-diaryl-N-methoxymethylene-tetrahydro-1,4-thiazines-1,1-dioxides were synthesized and their antimicrobial activities were tested against Gram-positive (*Bacillus subtilis*, *Streptococci*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella penumoniae*) and against the fungi *Penicillium inclobium* and *Aspergillus flavus*. The synthesized compounds have been characterized on the basis of elemental analyses, infrared spectroscopy (IR), and nuclear magnetic resonance (NMR).

Keywords Thiazine · Antibacterial activity · N-methoxymethylene derivatives

Introduction

The importance of heterocyclic compounds has long been recognized in the field of synthetic organic chemistry. In recent years, the literature has been enriched with progressive findings about the synthesis and pharmacological action of heterocycles. The thiazine nucleus of benzothiazines, phenothiazines, and tetrahydro-1,4-thiazines constitute an important class of sulfur and nitrogen heterocycles. They are associated with diverse pharmacological properties, such as antibacterial, antifungal, and antihistaminic activities (Kartrizky, 1985;Ramana Reddy *et al.*, 1990; Bhaskar Reddy, 2000; Sundari, 2004). In this work, we have focused our interest on incorporating N-methoxymethylene into the thiazine (nucleus) moiety in

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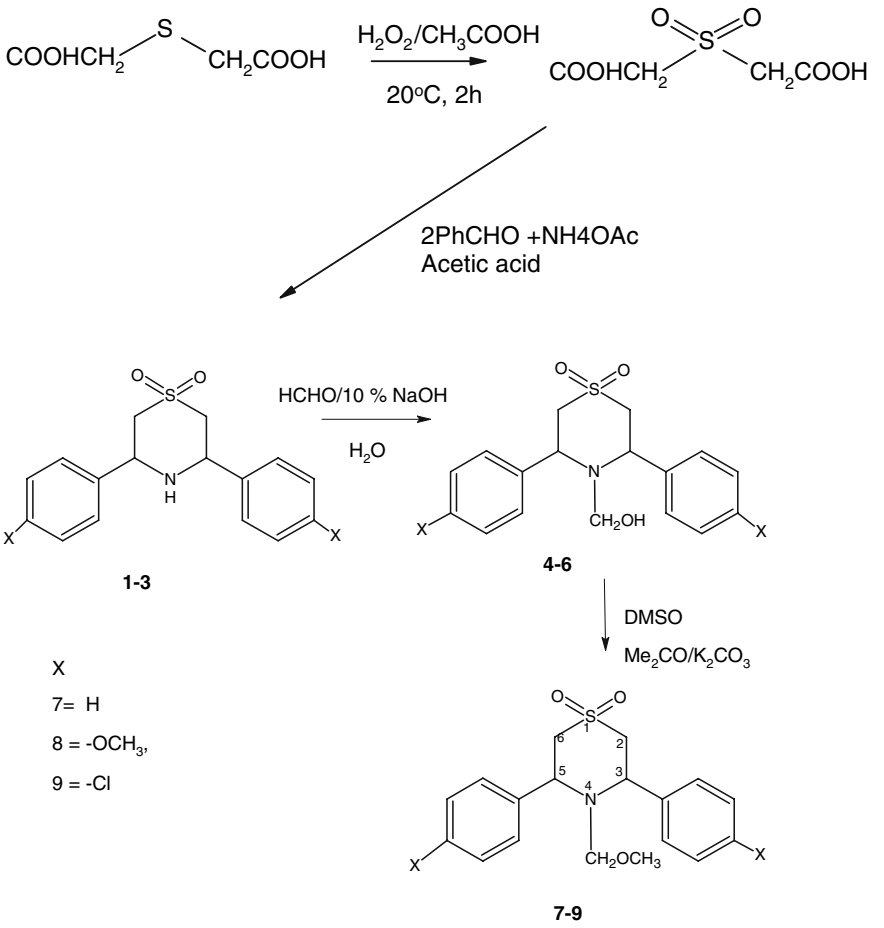
one framework, hoping to obtain few compounds that possess better antimicrobial activity. Ten new compounds have been synthesized and characterized.

Baliah and Rangarajan (1954) synthesized 3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxides (**1**) by the condensation of sulfonyl diacetic acid with aryl aldehydes and ammonia. The N-methyl derivatives of substituted tetrahydro-1,4-thiazine-1,1-dioxides were synthesized by Pandiarajan and Newton Benny (1994).

In the present study we synthesized some 3,5-diaryl-N-hydroxymethylene-tetrahydro-1,4-thiazine-1,1-dioxides **4–6**. This transformation is carried out by treating thiazine with hot ethanol, formalin, and 10% sodium hydroxide solution.

The N-methoxymethylene derivatives **7–9** were prepared from their respective N-hydroxymethylene thiazine with dry acetone, dimethyl sulfate and anhydrous potassium carbonate by refluxing for four hours (Scheme 1). The structure of these compounds was confirmed by elemental, IR, and ^{1}H and ^{13}C NMR spectral analysis. The chemical data for compounds are given in Table 1.



Scheme. 1

Table 1 Chemical data of the synthesized compounds 4–9

Compounds	Melting point	Yield (%)	Molecular formula	Elemental analysis		
				C (%)	H (%)	N (%)
4	199	42	$C_{17}H_{17}NO_3S$	64.12	6.01	4.36
5	217	40	$C_{19}H_{23}NO_5S$	60.40	6.10	3.68
6	219	52	$C_{17}H_{17}Cl_2NO_3S$	56.86	4.40	3.60
7	199	48	$C_{18}H_{21}NO_3S$	65.20	6.32	4.12
8	201	52	$C_{19}H_{22}NO_4S$	63.21	6.05	3.69
9	210	56	$C_{18}H_{19}NO_3SCl$	53.81	4.68	3.30

Biological activity

The screening of newly synthesized antimicrobial chemotherapeutic compounds provides potentially effective treatments for many dreadful diseases. Biochemical studies help to test the efficacy and existing toxicity of the compounds that possess antimicrobial properties. Compounds **7–9** were evaluated for their antibacterial activity in vitro against the Gram-positive cocci, such as *Bacillus subtitles* and *Streptococci*, and Gram-negative bacilli, including *Klebseilla pneumoniae, Escherchiae coli*, using the disc method with concentrations of 25, 50, and 100 μg/ml. Acetone was used as a control and norfloxacin was used as the standard.

To test for antibacterial activity, plates containing nutrient agar were seeded with different organisms at a concentration of $2–3 \times 10^{-7}$ colony forming units (CFU) using a sterile swab. The filter paper discs containing the synthesized compounds were placed at different positions with the help of fine-pointed forceps. The plates were incubated at 37°C for 24 hours and the zone of inhibition was measured.

The antifungal activity of the synthesized compounds **7–9** was also tested. The subculture and the viable count were carried out by the same procedure as for the antibacterial studies. The temperature maintained at 28 ± 1°C and the results were noted after 72–96 hours. The concentration of the test compound was as described previously, and solvent and griesoflavin (standard drug) were used for the antifungal studies.

The compounds with chloro- (25 μg/ml) or methoxy- (50 μg/ml) groups on the phenyl moiety were found to have appreciable activity when compared to unsubstituted compounds (at concentrations greater than 100 μg/ml) (Table 2).

Table 2 Antibacterial activity of compounds 7–9

Compound	K. pneumoniae	E. coli	B. subtilis	S. aureus	P. inclobium	A. flavus
7	7	9	6	7	7	6
8	11	11	12	12	11	10
9	16	16	15	15	14	12
Norfloxacin	22	22	22	22	–	–
Griesoflavin	–	–	–	–	22	22

Zone of inhibition: inactive <8 mm; moderate 9–12 mm; active >12 mm

Acute toxicity studies (LD_{50} determination)

Acute toxicity studies were undertaken to identify lethal dose 50 (LD_{50}) of the compounds **3** and **6**, respectively. According to the method of Miller and Tainter (1944), with little modification except that different doses were used, colony-bred male albino mice weighing 20–30 g were taken and divided into five groups of ten animals each.

The observed percentage mortality results were converted into probit values, which were then plotted against dose. The LD_{50} values of the active compounds were found to be nontoxic in mice at oral doses of up to 1000 mg/kg.

Experimental section

General

Melting points were recorded in open capillary tube and are uncorrected. Melting points and other data are recorded in Table 1. IR spectra were recorded in KBr on a Perkin–Elmer spectrophotometer and ^{1}H NMR spectra on a Bruker AMXC-500 FT NMR spectrometer in $CDCl_3$ using TMS as an internal standard. The chemical shifts have been expressed in δ ppm. The purity of the compounds was checked on silica gel-coated aluminum plates (Merck).

General method for the preparation of 3,5-diaryl-tetrahydro-1,4-thiazine-1,1,-dioxide (**1–3**)

All of the parent 3,5-diaryl-tetrahydro-1,4-thiazine-1,1-dioxides were prepared according to the procedure of Baliah and Rangarajan (1954) by the condensation of sulfonyl diacetic acid with araldehydes and ammonium acetate.

General method for the preparation of 3,5-diaryl-N-hydroxymethylene-tetrahydro-1,4-thiazine-1,1-dioxides (**4–6**)

To the hot solution of the respective thiazines (**1–3**) (0.01 M) dissolved in 20 ml of ethanol, 30 ml of formalin solution was added. A white precipitate was obtained. Ten milliliters of 10% sodium hydroxide solution was added and shaken vigorously to obtain a clear solution. This was kept overnight, and then sufficient water was added to obtain a white precipitate. This was filtered, washed with enough cold water to make it free from alkali, and then dried. The crude product was recrystallized from aqueous ethanol. Homogeneity of the product was checked by thin-layer chromatography (TLC).

3,5-Diphenyl-N-hydroxymethylene-tetrahydro-1,4-thiazine-1,1-dioxide (**4**)

IR: cm^{-1} (KBr) 1127.24, 1301.27, 1361.18 (SO_2), 3346.48 (-OH). Mass: *m/z* 313 (M^+) (M.F.: $C_{17}H_{17}NO_3S$). ^{1}H NMR: 3.16–3.38 H_2 and H_6 (m), 4.40 H_3 and H_5 (t),

7.25–7.45 aryl (m), 2.10 N-CH_2 (s), 4.60 CH_2OH (s). ^{13}C NMR: 59.81 (C_2 and C_6), 59.47 (C_3 and C_5), 70.10 (CH_2OH), 127.17–140.73 (aromatic).

3,5-Bis(p-methoxyphenyl)-N-hydroxymethylene-tetrahydro-1,4-thiazine-1,1-dioxide (**5**)

IR: cm^{-1} (KBr) 1115.09, 1301.30, 1353.68 (SO_2), 3438.34 (-OH). Mass: *m/z* (M^+) 371.45 (M.F.: $C_{19}H_{23}NO_5S$) ^{1}H NMR: 3.30 H_2 and H_6 (d), 4.40 H_3 and H_5 (t), 6.8, 7.11 aryl (d), 3.8 -OCH_3 (s), 2.10 N-CH_2 (s), 4.60 CH_2OH (s). ^{13}C NMR: 55.84(C_2 and C_6), 55.74(C_3 and C_5), 58.82 (O-CH_3), 70.73 (CH_2OH), 114.84–162.40 (Aromatic).

3,5-Bis(p-chlorophenyl)-N-hydroxymethylene-tetrahydro-1,4-thiazine-1,1-dioxide (**6**)

IR: cm^{-1} (KBr) 1121.19, 1302.62, 1355.99 (SO_2), 3440.28 (-OH). Mass: *m/z* 386.29 (M.F.: $C_{17}H_{17}C_{l2}NO_3S$) ^{1}H NMR: 3.16–3.35 H_2 and H_6 (m), 4.41 H_3 and H_5 (t), 7.25–7.45, Aryl (m), 2.10 N-CH_2(s), 4.61 CH_2OH (s). ^{13}C NMR: 58.58 (C_2 and C_6), 58.74 (C_3 and C_5), 58.82 (O-CH_3), 70.73 (CH_2OH), 114.84–162.40 (aromatic).

General method of preparation of 3,5-diaryl-N-methoxymethylene-tetrahydro-1,4-thiazine-1,1-dioxides (**7–9**)

About 0.005 M of N-hydroxymethylene thiazine (**4–6**) was dissolved in 25 ml of dry acetone and 10 ml of dimethyl sulfate was added, followed by 3 g of anhydrous potassium carbonate. This mixture was refluxed for four hours. After cooling to room temperature the inorganic salt was filtered off and the filtrate was concentrated and poured onto crushed ice. The solid obtained was filtered, washed with water and recrystallized from methanol to yield 62% colorless crystalline compound. The homogeneity of the product was determined by TLC.

3,5-Diphenyl-N-methoxymethylene-tetrahydro-1,4-thiazine-1,1-dioxide (**7**)

IR: cm^{-1} (KBr) 1120.44, 1306.25, 1331.19 (SO_2), 1150–1085 (aliphatic ether linkage) Mass: *m/z* 331.42 (M.F.: $C_{18}H_{21}NO_3S$). ^{1}H NMR: 3.12–3.30 H_2 and H_6 (m), 4.41 H_3 and H_5 (t), 7.25–7.45, aryl (m), 2.10 N-CH_2(s), 3.92 CH_3 (s). ^{13}C NMR: 58.12(C_2 and C_6), 59.57(C_3 and C_5), 67.12 (CH_2O), 57.98 (OCH_3), 127.17–140.73 (aromatic).

3,5-Bis(p-methoxyphenyl)-N-methoxymethylene-tetrahydro-1,4-thiazine-1,1-dioxide (**8**)

IR: cm^{-1} (KBr) 1111.23, 1301.32, 1345.23 (SO_2), 1150–1085 (aliphatic ether linkage) Mass: m/z: 391.47 (M.F.: $C_{20}H_{25}NO_5S$) ^{1}H NMR: 3.30 H_2 and H_6 (d), 4.40

H_3 and H_5 (t), 6.8, 7.11 aryl (d), 3.8 -OCH_3 (s), 2.10 N-*CH_2* (s), 3.90 CH_3 (s). ^{13}C NMR: 55.40 (C_2 and C_6), 55.14 (C_3 and C_5), 58.42 (O-CH_3), 67.73 (CH_2O), 57.45 (OCH_3), 115.24–161.20 (aromatic) 132.49 (ipso).

3,5-Bis(p-chlorophenyl)-N-methoxymethylene-tetrahydro-1,4-thiazine-1,1-dioxide (**9**)

IR: cm^{-1} (KBr) 1113.13, 1302.33, 1342.38 (SO_2), 1150–1085 (aliphatic ether linkage) Mass: *m*/*z*: 400.31 (M.F.: $C_{18}H_{19}NO_3SCl_2$). ^1H NMR: 3.16–3.35 H_2 and H_6 (m), 4.41 H_3 and H_5 (t), 7.25–7.45 Aryl (m), 2.10 N-*CH_2* (s), 3.90 CH_3 (s). ^{13}C NMR: 55.78 (C_2 and C_6), 59.24 (C_3 and C_5), 67.03 (CH_2O), 114.84–162.40 57.12 (OCH_3), 128.20–129.41 (aromatic) 141. 80 (ipso).

Acknowledgements The authors are grateful to the university authorities for providing the necessary facilities to carry out this research work, and the sophisticated instrumentation facilities available at Madurai Kamaraj University, IISc., Bangalore for recording the spectra, as well as the Department of Microbiology and Central Animal House, Rajah Muthiah Medical College, for providing the facilities for biological evaluation.

References

Baliah V and Rangarajan T (1954) Synthesis and some heterocyclic compounds containing sulphur and nitrogen. J Chem Soc 3068

Bhaskar RD, Chandrasekhar BN, Padmaja A (2000) Part V: Synthesis of substituted 1,4-thiomorpholine-1,1-dioxides. Indian J Chem 39B:406

Kartrizky AR (1985) Advances in heterocyclic chemistry. Academic Press, London, 38:135

Miller LC, Tainter ML (1944) Experimental determination of $LD_{50.}$ Proc Soc Exp Biol Med 57:261

Pandiarajan K, Benny CN (1994) Conformational studies of some substituted tetrahydro-1,4-thiazine-1,1-dioxides using ^1H and ^{13}C NMR spectra. Indian J Chem 33B:1134

Ramana Reddy MV, Vijayalakshmi S, Ramana Reddy PV, Reddy S (1990) Synthesis of substituted 1,4-thiomorpholine-1,1-dioxides. Indian J Chem 29B:168

Sundari V, Valliappan R (2002) Synthesis and Biological screening of some 3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxide. Indian J Heterocyclic Chem 11:187; see also Indian J Heterocyclic Chem 2004;14:47,71