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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and physiological evaluation of new *N*-nitroso-2,6-dicarbethoxy-3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxides

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Abstract A series of new *N*-nitroso-2,6-dicarbethoxy-3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxides **4a–r** were synthesized and evaluated for antibacterial and antifungal activity. Most of them registered significant antibacterial and antifungal activity. The newly synthesized compounds have been characterized on the basis of elemental analysis, infrared, and nuclear magnetic resonance spectroscopic data.

Keywords Thiazine · Antibacterial activity · *N*-nitroso compounds

Introduction

Heterocycles play an important role in biochemical processes because the side groups of the most typical and essential constituents of living cells, DNA and RNA are based on aromatic heterocycles (Balaban *et al*., 2004). Many organosulphur compounds exhibit diverse pharmacological activities of various types. Clinically useful organosulphur compounds fall under aryl sulphonic acid derivatives, phenothiazines, benzothiazipines and 1,2,4-benzothiadiazines. Another medicinally important sulphur- and nitrogen-containing heterocyclic system is tetrahydro-1,4-thiazine. Derivatives of tetrahydro-1,4-thiazine are associated with diverse pharmacological activities (Katritzky, 1985; Barbachyn *et al*., 1996; Kumazawa *et al*., 1997; Sundari *et al*., 2007; Muhammad *et al*., 2009). Among the nitrogen heterocyclic compounds, *N*-nitroso compounds are important because, they are the most versatile chemical carcinogens known, serving as models for almost every kind of human cancers (Lijinsky, 1992; Pablo, 2009; Dennie *et al*., 2010; Yet Hua *et al*., 2011). Therefore, we anticipated that the molecules containing the hybrid structure resulting from the incorporation of *N*-nitroso group into tetrahydrothiazine-1,1-dioxide core might exhibit useful biological activity.

Tetrahydro-1,4-thiazine-1,1-dioxides exhibit a wide range of biological activities (Srivastava *et al*., 2004; Moriyama *et al*., 2004). Baliah and Rangarajan (1954) first reported an elegant method of preparation of 3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxides by condensing sulphonyl diacetic acid with arylaldehydes in the presence of ammonium acetate in acetic acid/ethanol as solvent. Pandiarajan and Christopher Newton (1994) studied the conformational behaviour of some substituted 1,4-thiazine-1,1-dioxides by ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectroscopic techniques.

In our present study, the required 2,6-dicarbethoxy-3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxides were prepared in high yield by a modification of the procedure described by Baliah and Rangarajan (1954) using water as reaction medium in place of acetic acid for the first time. Direct nitrosation with HNO_2 afforded the *N*-nitroso compounds **4a–r** (Scheme 1).

Results and discussions

All the synthesized compounds **4a–r** answered positive tests for nitrogen and sulphur and all of them have been characterized by their spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR). The IR spectrum of the **4a** (a representative

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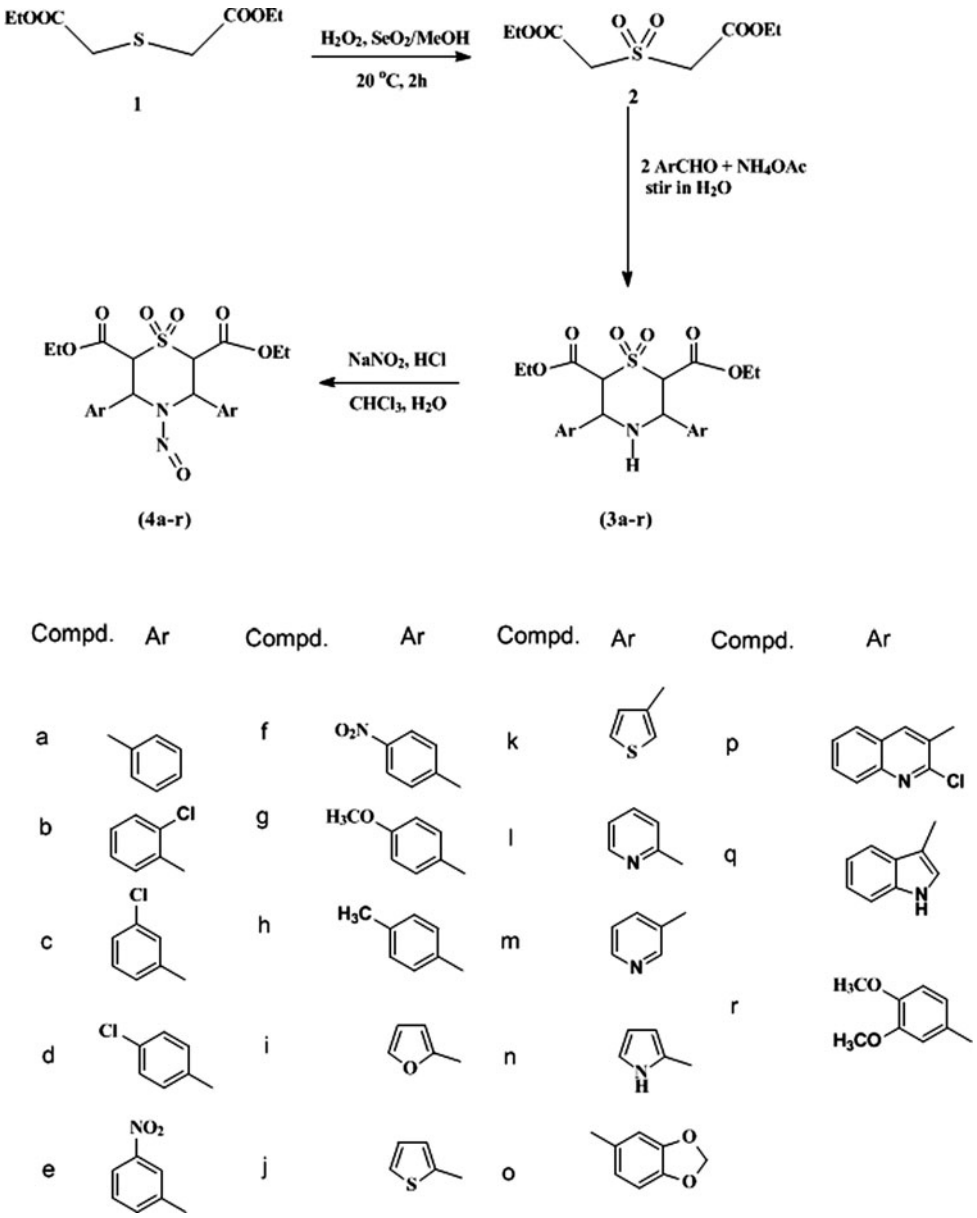
Scheme 1 Protocol for synthesis of the title compounds (**4a**–**r**)



example) displayed a band at 1039 cm^{-1} for N–N stretching and the presence of N=O group is evident from a band at 1446 cm^{-1} (Looney *et al*., 1957). The stretching bands at 1332 cm^{-1} (S=O unsymmetrical stretch) and 1121 cm^{-1} (S=O symmetrical stretch) revealed the presence of sulphone moiety (Silverstein *et al*., 1983).

The NMR (^{1}H and ^{13}C NMR) spectra of the synthesized compounds **4a**–**r** bear close resemblance in their spectral characteristics. The ^{1}H NMR spectrum of **4a** (a representative example) displayed a triplet for six protons at δ 1.03 and a four-proton quartet at δ 4.08 for methyl and methylene groups of two ethoxycarbonyl groups, respectively. Of the pair of doublets each integrating for two protons, the one at δ 4.21(J = 10.2 Hz) is assigned to the enantiotopic protons, H-2 & H-6 while the other one at δ 4.70 (J = 10.5 Hz) is due to the enantiotopic protons, H-3 & H-5. The magnitude of the coupling constant is consistent with *trans* diaxial orientation of H-2 & H-6 and H-3 & H-5 and suggests that the heterocyclic ring adopts a rigid chair conformation. The complex multiplet due to ten protons in the region δ 7.44–7.93 is associated with the aromatic protons of two phenyl groups at C-3 and C-5. The ^{13}C NMR spectrum of **4a** registered only two signals for heterocyclic ring carbons at δ 66.92 (C-3/C-5) and 73.78 (C-2/C-6) revealing high degree of symmetry in the molecule. A comparison of the spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR) of **4a** with that of **3a** disclosed that the ^{1}H NMR spectrum of the former (**4a**) was identical with that of the latter (**3a**) in all respects but for the absence of one-proton broad signal (δ 2.10) of the latter due to NH proton. Thus, based on the above spectroscopic

data, compound **4a** has been characterized as *N*-nitroso-2,6-dicarbethoxy-3,5-diphenyltetrahydro-1,4-thiazine-1,1-dioxide.

In vitro antibacterial and antifungal activity

Biochemical studies help to test the efficacy and existing toxicity of the compounds that possess antimicrobial properties. In search of new antimicrobial compounds, we have synthesized, a series of *N*-nitroso-2,6-dicarbehoxy-3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxide, **4a–r** and screened them for their in vitro antibacterial and antifungal activity using disc diffusion method (Barry, 1976; Chandrasekaran *et al*., 2005). Antibacterial activity of the compounds **4a–r** has been evaluated against microbial strains of two Gram-positive (*Staphylococcus aureus* MTCC 96 & *Streptococcus pyogenes* MTCC 389), two Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 2853 & *Escherichia coli* MTCC 722), and one fungi (*Candida albicans* MTCC 227). Amikacin (the antibacterial drug) and nystatin (the antifungal drug) were used as standards for comparison. The activity was measured as a function of zone of inhibition in mm and the results were compared with those of the reference drug by measuring their zone of inhibition and activity index (Table 1).

The antibacterial screening data revealed that all the tested compounds showed moderate to good antibacterial activity as compared to that of the standard. Compounds **4j**, **4k**, **4m** and **4p** showed significant inhibition in the order **4j** > **4k** > **4m** = **4p** against *S. aureus*, whereas compounds **4f**, **4h**, **4a** and **4j** showed significant inhibition in the order **4f** = **4h** > **4a** = **4j** against *S. pyogenes*, compounds **4g**, **4e** and **4j** registered significant activity against *P. aeruginosa* in the order **4g** > **4e** > **4j**; of these, **4g** showed greater activity than that of the standard; **4f**, **4h**, **4i** **4c**, **4e**, **4k** showed significant inhibition in the order **4f** = **4h** > **4i** > **4c** = **4e** = **4k** against *E. coli*; of these **4f** and **4h** registered comparable activity to that of the standard. Of all compounds of this series, **4j** showed most significant activity against Gram-positive strains and **4e** against Gram-negative strains. Compound **4g** registered excellent inhibitory activity against *P. aeruginosa*. Overall, antibacterial activity of the synthesized compounds **4a–r** is significant as compared to that of the standard (amikacin).

Screening of above compounds at a concentration of 10 μg/ml for antifungal activity by the same methodology was carried out against the fungal strain, *Candida albicans* using nystatin as a control. Substitution by thiophen-2-yl (**4j**) group results in an increase in activity and the activity index is greater than one. Compounds **4j**, **4p**, **4d**, **4b**, **4c**, **4m** and **4q** have emerged as moderately effective antifungal agents against *C. albicans*. Overall, the antifungal activity of synthesized compounds is significant as compared to that of the nystatin. The results of antimicrobial activity of the tested compounds are listed in Table 1.

Table 1 Antimicrobial activity of compounds **4a–r**

Compound no.	Zone of inhibition in mm at 10 μg/ml				
	S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*C. albicans*
4a	11 (0.61)	15 (0.75)	12 (0.71)	13 (0.76)	13 (0.69)
4b	9 (0.50)	10 (0.50)	13 (0.76)	12 (0.71)	16 (0.84)
4c	11 (0.61)	8 (0.40)	14 (0.82)	10 (0.59)	15 (0.79)
4d	10 (0.56)	10 (0.50)	13 (0.76)	12 (0.71)	16 (0.84)
4e	12 (0.67)	9 (0.45)	14 (0.82)	15 (0.88)	13 (0.69)
4f	13 (0.72)	17 (0.85)	17 (1.0)	11 (0.65)	12 (0.63)
4g	11 (0.61)	13 (0.65)	12 (0.71)	18 (1.11)	13 (0.69)
4h	11 (0.61)	17 (0.85)	17 (1.0)	9 (0.53)	11 (0.58)
4i	12 (0.67)	11 (0.55)	15 (0.88)	10 (0.59)	14 (0.74)
4j	17 (0.94)	15 (0.75)	13 (0.76)	14 (0.82)	20 (1.11)
4k	15 (0.83)	9 (0.45)	14 (0.82)	13 (0.76)	14 (0.74)
4l	13 (0.72)	11 (0.55)	10 (0.59)	9 (0.53)	12 (0.63)
4m	14 (0.78)	8 (0.40)	12 (0.71)	11 (0.65)	15 (0.79)
4n	13 (0.72)	14 (0.70)	11 (0.65)	12 (0.71)	11 (0.58)
4o	9 (0.50	11 (0.55)	8 (0.47)	12 (0.71)	10 (0.53)
4p	14 (0.78)	12 (0.60)	9 (0.53)	10 (0.59)	18 (0.95)
4q	8 (0.44)	9 (0.45)	12 (0.71)	11 (0.65)	15 (0.79)
4r	12 (0.67)	8 (0.40)	13 (0.76)	11 (0.65)	14 (0.74)
Amikacin	18	20	17	17	–
Nystatin	–	–	–	–	19

Zone of growth inhibition (mm) (activity index)

(Activity index) = Inhibition zone of the compound/Inhibition zone of the standard drug

Acute toxicity studies (LD_{50} determination)

Acute toxicity studies were undertaken to assess the lethal dose 50 (LD_{50}) of the compound. LD_{50} of a synthetic drug is determined as per OECD (Organization for Economic Co-operation and Development) guidelines. The observed values of LD_{50} revealed that the active compounds were non-toxic to mice even at a dose of 300 mg/kg.

Experimental section

General procedures

Melting points were recorded in open capillary tube and are uncorrected. IR spectra were recorded in KBr on a 8400S Shimadzu FT-IR Spectrophotometer and NMR spectra were scanned on a Bruker 300 MHz Spectrometer in $CDCl_3$/DMSO-d_6 using TMS as internal standard. The chemical

shifts are cited in δ-scale. Elemental analyses were carried out on Perkin-Elmer 2400 Series-II instrument. Purity of synthesized compounds was checked by TLC using silica gel-G and spots were detected in an iodine chamber.

Synthesis of diethyl 2,2′-sulphonyldiacetate, **2** (Kwan *et al*., 1989)

To a stirred solution of diethyl 2,2′-thiodiacetate **1** (1.0 mol) in methanol (20 ml) containing a pinch of selenium dioxide, 30% H_2O_2 (10 ml) was added dropwise and stirring continued while maintaining the temperature of the reaction mixture at 20°C for about 2 h. The contents were extracted with dichloromethane (3 × 25 ml) and removal of the solvent afforded diethyl 2,2′-sulphonyldiacetate **2** as thick oil.

Yield: 88%; ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.36 (t, 6H, –OCH_2CH_3), 4.32 (q, 4H, –OCH_2CH_3), 4.39 (s, 4H, CH_2).

General procedure for the synthesis of 2,6-dicarbethoxy-3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxides (**3a**–**r**)

A mixture of diethyl 2,2′-sulphonyldiacetate **2** (0.01 mol), aromatic aldehydes **a**–**r** (0.02 mol), ammonium acetate (0.02 mol) and a few drops of alcohol were made as a homogeneous paste. Then it was stirred with 10 ml of water at room temperature for about 3 h and left overnight. The reaction mixture was diluted with excess of water. The solid that separated was filtered, washed with water and dried; crystallization from aqueous alcohol gave pure 2,6-dicarbethoxy-3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxides, **3a**–**r**.

*2,6-dicarbethoxy-3,5-diphenyltetrahydro-1,4-thiazine-1,1-dioxide (**3a**)*

Colourless solid; m.p. 183–184°C (aq. alcohol); yield: 92%.

IR (KBr) ν_{max} (cm^{-1}): 3328 (NH str.), 1730 (ester C=O), 1323 (S=O unsym. str.), 1129 (S=O sym. str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.02 (t, 6H, –OCH_2CH_3), 2.10 (s, 1H, NH) 4.08 (q, 4H, –OCH_2CH_3), 4.22 (d, 2H, J = 10.5 Hz, H-2/H-6), 4.72 (d, 2H, J = 10.5 Hz, H-3/H-5), 7.24–7.44 (m, 10H, Ar-H). ^{13}C NMR (75 MHz): δ 13.72 (OCH_2CH_3), 61.94 (C-3, C-5), 62.49 (OCH_2CH_3), 72.69 (C-2, C-6), 127.87, 128.78, 129.12, 130.33, 137.70 (*ipso* carbon), 161.09 (C=O of ester).

General procedure for the synthesis of *N*-nitroso-2,6-dicarbethoxy-3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxide (**4a**–**r**)

To a stirred solution of 2,6-dicarbethoxy-3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxides **3a**–**r** (0.03 mol) in chloroform (7 ml) containing conc. hydrochloric acid (1 ml) and water (1 ml), solid sodium nitrite (0.03 mol) was added in portions during 0.5 h and the stirring was continued for another 0.5 h. After the completion of the reaction, the organic layer was washed with water and dried over anhydrous calcium chloride. The products **4a**–**r** that obtained on removal of the solvent were crystallized from ethanol–ethyl acetate mixture (1:2 v/v).

*N-nitroso-2,6-dicarbethoxy-3,5-diphenyltetrahydro-1,4-thiazine-1,1-dioxide (**4a**)*

Colourless solid; m.p. 163°C (aq. alcohol); yield: 81%;

IR (KBr) ν_{max} (cm^{-1}): 1734 (ester C=O), 1332 (S=O unsym. str.), 1121 (S=O sym. str.), 1446 (N=O), 1039 (N–N str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.03 (t, 6H, –OCH_2CH_3), 4.08 (q, 4H, –OCH_2CH_3), 4.21 (d, 2H, J = 10.2 Hz, H-2/H-6), 4.70 (d, 2H, J = 10.5 Hz, H-3/H-5), 7.44–7.93 (m, 10H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 18.56 (OCH_2CH_3), 66.92 (C-3, C-5), 67.49 (OCH_2CH_3), 73.78 (C-2, C-6), 127.26, 128.56, 133.47, 148.44 (*ipso* carbon), 153.12, 165.79 (C=O of ester). Anal. calcd.for $C_{22}H_{24}N_2O_7S$: C, 57.38; H, 5.25; N, 6.08%. Found C, 57.18; H, 5.18; N, 5.88%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(2-chlorophenyl)tetrahydro-1,4-thiazine-1,1- dioxide (**4b**)*

Creamy white solid; m.p.182°C (aq. alcohol); yield:78%.

IR (KBr) ν_{max} (cm^{-1}): 1736 (ester C=O), 1321 (S=O unsym. str.), 1124 (S=O sym. str.), 1453 (N=O), 1044 (N–N str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.04 (t, 6H, –OCH_2CH_3), 4.11 (q, 4H, –OCH_2CH_3), 4.28 (d, 2H, J = 10.2 Hz, H-2/H-6), 4.72 (d, 2H, J = 10.5 Hz, H-3/H-5), 7.77–8.11 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 19.08 (OCH_2CH_3), 66.25 (C-3, C-5), 67.42 (OCH_2CH_3), 74.52 (C-2, C-6), 128.90, 128.63, 135.12, 150.06 (*ipso* carbon), 165.06 (C=O of ester). Anal. calcd.for $C_{22}H_{22}Cl_2N_2O_7S$: C, 49.91; H, 4.19; N, 5.29%. Found C, 49.72; H, 4.08; N, 5.18%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(3-chlorophenyl)tetrahydro-1,4-thiazine-1,1-dioxide (**4c**)*

Colourless solid; m.p. 127°C (aq. alcohol); yield: 79%.

IR (KBr) ν_{max} (cm^{-1}): 1738 (ester C=O), 1326 (S=O unsym. str.), 1118 (S=O sym. str.), 1448 (N=O), 1039 (N–N str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.03 (t, 6H, –OCH_2CH_3), 4.10 (q, 4H, –OCH_2CH_3), 4.25 (d, 2H, J = 10.2 Hz, H-2/H-6), 4.78 (d, 2H, J = 10.5 Hz, H-3/H-5), 7.72–8.11 (m, 8H, Ar-H). ^{13}C NMR (75 MHz,

$CDCl_3$ + DMSO): δ 19.68 (OCH_2CH_3), 66.12 (C-3, C-5), 67.33 (OCH_2CH_3), 74.49 (C-2, C-6), 129.07, 129.75, 135.23, 149.51 (*ipso* carbon), 164.48 (C=O of ester). Anal. calcd.for $C_{22}H_{22}Cl_2N_2O_7S$: C, 49.91; H, 4.19; N, 5.29%. Found C, 49.69; H, 4.12; N, 5.11%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(4-chlorophenyl)tetrahydro-1,4-thiazine-1,1-dioxide (**4d**)*

Colourless solid; m.p. 196°C (aq. alcohol); yield: 82%.

IR (KBr) ν_{max} (cm^{-1}): 1726 (ester C=O), 1328 (S=O unsym. str.), 1122 (S=O sym. str.), 1436 (N=O), 1044 (N–N str.). ^1H NMR (300 MH_Z, $CDCl_3$): δ 1.04 (t, 6H, $-OCH_2CH_3$), 4.12 (q, 4H, $-OCH_2CH_3$), 4.27 (d, 2H, $J = 10.2$ Hz, H-2/H-6), 4.71 (d, 2H, $J = 10.5$ Hz, H-3/H-5), 7.69-8.18 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 19.12 (OCH_2CH_3), 67.02 (C-3, C-5), 67.48 (OCH_2CH_3), 73.88 (C-2, C-6), 129.10, 129.72, 134.12, 149.12 (*ipso* carbon), 164.80 (C=O of ester). Anal. calcd.for $C_{22}H_{22}Cl_2N_2O_7S$: C, 49.91; H, 4.19; N, 5.29%. Found C, 49.74; H, 4.07; N, 5.19%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(3-nitrophenyl)tetrahydro-1,4-thiazine-1,1-dioxide (**4e**)*

Pale yellow solid; m.p. 166°C (aq. alcohol); yield: 80%.

IR (KBr) ν_{max} (cm^{-1}): 1738 (ester C=O), 1323 (S=O unsym. str.), 1132 (S=O sym. str.), 1453 (N=O), 1046 (N–N str.). ^1H NMR (300 MH_Z, $CDCl_3$): δ 1.08 (t, 6H, $-OCH_2CH_3$), 4.14 (q, 4H, $-OCH_2CH_3$), 4.31 (d, 2H, $J = 10.2$ Hz, H-2/H-6), 4.81 (d, 2H, $J = 10.5$ Hz, H-3/H-5), 7.81–8.22 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 19.68 (OCH_2CH_3), 66.94 (C-3, C-5), 67.12 (OCH_2CH_3), 74.52 (C-2, C-6), 128.88, 129.10, 134.78, 148.18 (*ipso* carbon), 165.19 (C=O of ester). Anal. calcd.for $C_{22}H_{22}N_4O_{11}S$: C, 48.00; H, 4.03; N, 10.18%. Found C, 47.91; H, 3.99; N, 10.10%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(4-nitrophenyl)tetrahydro-1,4-thiazine-1,1-dioxide (**4f**)*

Pale yellow solid; m.p. 203°C (aq. alcohol); yield: 83%.

IR (KBr) ν_{max} (cm^{-1}): 1734 (ester C=O), 1328 (S=O unsym. str.), 1122 (S=O sym. str.), 1450 (N=O), 1038 (N–N str.). ^1H NMR (300 MH_Z, $CDCl_3$): δ 1.07 (t, 6H, $-OCH_2CH_3$), 4.13 (q, 4H, $-OCH_2CH_3$), 4.30 (d, 2H, $J = 10.5$ Hz, H-2/H-6), 4.81 (d, 2H, $J = 10.5$ Hz, H-3/H-5), 7.78–8.16 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 20.09 (OCH_2CH_3), 67.01 (C-3, C-5), 67.86 (OCH_2CH_3), 74.19 (C-2, C-6), 128.90, 129.75, 136.06, 150.51 (*ipso* carbon), 166.06 (C=O of ester). Anal. calcd.for $C_{22}H_{22}N_4O_{11}S$: C, 48.00; H, 4.03; N, 10.18%. Found C, 47.95; H, 3.95; N, 10.12%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(4-methoxyphenyl)tetrahydro-1,4-thiazine-1,1-dioxide (**4g**)*

Colourless solid; m.p. 139°C (aq. alcohol); yield: 72%.

IR (KBr) ν_{max} (cm^{-1}): 1745 (ester C=O), 1328 (S=O unsym. str.), 1114 (S=O sym. str.), 1451 (N=O), 1042 (N–N str.). ^1H NMR (300 MH_Z, $CDCl_3$): δ 1.04 (t, 6H, $-OCH_2CH_3$), 3.74 (s, 6H, $-OCH_3$) 4.08 (q, 4H, $-OCH_2CH_3$), 4.21 (d, 2H, $J = 10.2$ Hz, H-2/H-6), 4.73 (d, 2H, $J = 10.5$ Hz, H-3/H-5), 7.63-8.39 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 18.79 (OCH_2CH_3), 66.00 (OCH_3), 67.25 (C-3, C-5), 67.43 (OCH_2CH_3), 74.81 (C-2, C-6), 128.42, 128.63, 134.09, 149.83 (*ipso* carbon), 152.49, 166.34 (C=O of ester). Anal. calcd.for $C_{24}H_{28}N_2O_9S$: C, 55.38; H, 5.42; N, 5.38%. Found C, 55.26; H, 5.25; N, 5.32%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(4-methylphenyl)tetrahydro-1,4-thiazine-1,1-dioxide (**4h**)*

Colourless solid; m.p. 145°C (aq. alcohol); yield: 76%.

IR (KBr) ν_{max} (cm^{-1}): 1732 (ester C=O), 1324 (S=O unsym. str.), 1122 (S=O sym. str.), 1448 (N=O), 1046 (N–N str.). ^1H NMR (300 MH_Z, $CDCl_3$): δ 1.05 (t, 6H, $-OCH_2CH_3$), 2.30 (s, 6H, CH_3), 4.10 (q, 4H, $-OCH_2CH_3$), 4.27 (d, 2H, $J = 10.5$ Hz, H-2/H-6), 4.88 (d, 2H, $J = 10.5$ Hz, H-3/H-5), 7.66–8.18 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 18.54 (OCH_2CH_3), 23.32 (CH_3), 67.94 (C-3, C-5), 68.33 (OCH_2CH_3), 74.82 (C-2, C-6), 127.75, 128.17, 133.12, 148.51 (*ipso* carbon), 164.89 (C=O of ester). Anal. calcd.for $C_{24}H_{28}N_2O_7S$: C, 59.00; H, 5.78; N, 5.73%. Found C, 58.91; H, 5.65; N, 5.62%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(phur-2-yl)tetrahydro-1,4-thiazine-1,1-dioxide (**4i**)*

Pale brown solid; m.p. 167°C (aq. alcohol); yield: 69%.

IR (KBr) ν_{max} (cm^{-1}): 1736 (ester C=O), 1330 (S=O unsym. str.), 1118 (S=O sym. str.), 1454 (N=O), 1042 (N–N str.). ^1H NMR (300 MH_Z, $CDCl_3$): δ 1.11 (t, 6H, $-OCH_2CH_3$), 4.12 (q, 4H, $-OCH_2CH_3$), 4.32 (d, 2H, $J = 10.5$ Hz, H-2/H-6), 4.81 (d, 2H, $J = 10.5$ Hz, H-3/H-5), 6.56–7.42 (m, 6H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 18.11 (OCH_2CH_3), 66.23 (C-3, C-5), 67.11 (OCH_2CH_3), 73.12 (C-2, C-6), 112.14, 114.23, 141.58, 150.12 (*ipso* carbon), 164.77 (C=O of ester). Anal.

calcd.for $C_{18}H_{20}N_2O_9S$: C, 49.09; H, 4.58; N, 6.36%. Found C, 49.01; H, 4.47; N, 6.29%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(thiophen-2-yl)tetrahydro-1,4-thiazine-1,1-dioxide (**4j**)*

Colourless solid; m.p. 156–157°C (aq. alcohol); yield: 74%.

IR (KBr) ν_{max} (cm^{-1}): 1730 (ester C=O), 1326 (S=O unsym. str.), 1124 (S=O sym. str.), 1450 (N=O), 1034 (N–N str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.13 (t, 6H, –OCH_2CH_3), 4.19 (q, 4H, –OCH_2CH_3), 4.30 (d, 2H, J = 10.5 Hz, H-2/H-6), 4.83 (d, 2H, J = 10.5 Hz, H-3/H-5), 6.66–7.39 (m, 6H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 18.07 (OCH_2CH_3), 66.12 (C-3, C-5), 67.26 (OCH_2CH_3), 74.08 (C-2, C-6), 125.52, 126.12, 141.26 (*ipso* carbon), 164.18 (C=O of ester). Anal. calcd.for $C_{18}H_{20}N_2O_7S_3$: C, 45.75; H, 4.27; N, 5.93%. Found C, 45.59; H, 4.22; N, 5.79%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(thiophen-3-yl)tetrahydro-1,4-thiazine-1,1-dioxide (**4k**)*

Colourless solid; m.p. 168°C (aq. alcohol); yield: 80%.

IR (KBr) ν_{max} (cm^{-1}): 1739 (ester C=O), 1334 (S=O unsym. str.), 1120 (S=O sym. str.), 1448 (N=O), 1040 (N–N str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.03 (t, 6H, –OCH_2CH_3), 4.15 (q, 4H, –OCH_2CH_3), 4.28 (d, 2H, J = 10.5 Hz, H-2/H-6), 4.78 (d, 2H, J = 10.5 Hz, H-3/H-5), 6.72–7.28 (m, 6H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 18.67 (OCH_2CH_3), 65.77 (C-3, C-5), 67.22 (OCH_2CH_3), 73.86 (C-2, C-6), 126.21, 126.76, 140.21 (*ipso* carbon), 165.11 (C=O of ester). Anal. calcd.for $C_{18}H_{20}N_2O_7S_3$: C, 45.75; H, 4.27; N, 5.93%. Found C, 45.66; H, 4.17; N, 5.84%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(pyridin-2-yl)tetrahydro-1,4-thiazine-1,1-dioxide (**4l**)*

Pale brown solid; m.p. 108–110°C (aq. alcohol); yield: 85%.

IR (KBr) ν_{max} (cm^{-1}): 1732 (ester C=O), 1328 (S=O unsym. str.), 1128 (S=O sym. str.), 1454 (N=O), 1044 (N–N str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.11 (t, 6H, –OCH_2CH_3), 4.22 (q, 4H, –OCH_2CH_3), 4.31 (d, 2H, J = 10.5 Hz, H-2/H-6), 4.84 (d, 2H, J = 10.5 Hz, H-3/H-5), 7.31–8.28 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 17.68 (OCH_2CH_3), 66.14 (C-3, C-5), 67.12 (OCH_2CH_3), 74.12 (C-2, C-6), 127.90, 128.75, 129.12, 144.51 (*ipso* carbon), 166.33 (C=O of ester). Anal. calcd.for $C_{20}H_{22}N_4O_7S$: C, 51.94; H, 4.79; N, 12.11%. Found C, 51.81; H, 4.67; N, 12.04%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(pyridin-3-yl)tetrahydro-1,4-thiazine-1,1-dioxide (**4m**)*

Pale brown solid; m.p. 198–200°C (aq. alcohol); yield: 83%.

IR (KBr) ν_{max} (cm^{-1}): 1732 (ester C=O), 1326 (S=O unsym. str.), 1130 (S=O sym. str.), 1450 (N=O), 1038 (N–N str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.03 (t, 3H, –OCH_2CH_3), 4.09 (q, 2H, –OCH_2CH_3), 4.27 (d, 2H, J = 10.5 Hz, H-2/H-6), 4.79 (d, 2H, J = 10.5 Hz, H-3/H-5), 7.29–8.12 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 18.84 (OCH_2CH_3), 65.67 (C-3, C-5), 66.98 (OCH_2CH_3), 73.55 (C-2, C-6), 128.33, 129.34, 144.78 (*ipso* carbon), 166.78 (C=O of ester). Anal. calcd.for $C_{20}H_{22}N_4O_7S$: C, 51.94; H, 4.79; N, 12.11%. Found C, 51.79; H, 4.77; N, 12.01%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(1H pyrrole-2-yl)tetrahydro-1,4-thiazine-1,1-dioxide (**4n**)*

Pale grey solid; m.p. 129°C (aq. alcohol); yield: 77%.

IR (KBr) ν_{max} (cm^{-1}):1737 (ester C=O), 1328 (S=O unsym. str.), 1120 (S=O sym. str.), 1444 (N=O), 1046 (N–N str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.03 (t, 6H, –OCH_2CH_3), 2.1 (broad s, 1H, NH) 4.12 (q, 4H, –OCH_2CH_3), 4.27 (d, 2H, J = 10.5 Hz, H-2/H6), 4.80 (d, 2H, J = 10.5 Hz, H-3/H-5), 6.25–6.59 (m, 6H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 19.12 (OCH_2CH_3), 66.12 (C-3, C-5), 67.11 (OCH_2CH_3), 73.54 (C-2, C-6), 111.21, 115.22, 126.2, 141.81 (*ipso* carbon), 164.06 (C=O of ester). Anal. calcd.for $C_{18}H_{22}N_4O_7S$: C, 49.31; H, 5.06; N, 12.78%. Found C, 49.21; H, 4.97; N, 12.63%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(1,3-benzodioxol-6-yl)tetrahydro-1,4-thiazine-1,1-dioxide (**4o**)*

Pale yellow solid; m.p. 216–218°C (aq. alcohol); yield: 81%.

IR (KBr) ν_{max} (cm^{-1}): 1738 (ester C=O), 1312 (S=O unsym. str.), 1130 (S=O sym. str.), 1442 (N=O), 1038 (N–N str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.05 (t, 6H, –OCH_2CH_3), 4.12 (q, 4H, –OCH_2CH_3), 4.31 (d, 2H, J = 10.5 Hz, H-2/H-6), 4.82 (d, 2H, J = 10.5 Hz, H-3/H-5), 6.04 (s, 4H, –OCH_2O–), 6.89–7.69 (m, 6H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 18.68 (OCH_2CH_3), 65.86 (C-3, C-5), 67.12 (OCH_2CH_3), 74.11 (C-2, C-6), 101.2 (–OCH_2O–), 125.32, 129.85, 148.12 (*ipso* carbon), 165.11 (C=O of ester). Anal. calcd.for $C_{24}H_{24}N_2O_{11}S$: C,

52.55; H, 4.41; N, 5.11%. Found C, 52.38; H, 4.36; N, 5.02%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(2-chloroquinolin-3-yl)tetrahydro-1,4-thiazine-1,1-dioxide (**4p**)*

Pale yellow solid; m.p. 228°C (aq. alcohol); yield: 93%.

IR (KBr) ν_{max} (cm^{-1}): 1741 (ester C=O), 1333 (S=O unsym. str.), 1122 (S=O sym. str.), 1446 (N=O), 1038 (N–N str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.05 (t, 6H, –OCH_2CH_3), 4.12 (q, 4H, –OCH_2CH_3), 4.24 (d, 2H, J = 10.5 Hz, H-2/H-6), 4.71(d, 2H, J = 10.5 Hz, H-3/H-5), 7.67–8.16 (m, 10H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 19.09 (OCH_2CH_3), 65.27 (C-3, C-5), 67.32 (OCH_2CH_3), 73.12 (C-2, C-6), 129.11, 129.87, 132.34, 139.11, 143.12, 152.33, 166.22 (C=O of ester). Anal. calcd.for $C_{28}H_{24}Cl_2N_4O_7S$: C, 53.26; H, 3.83; N, 8.87%. Found C, 53.18; H, 3.76; N, 8.79%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(1H-indol-2-yl)tetrahydro-1,4-thiazine-1,1-dioxide (**4q**)*

Creamy white solid; m.p. 164°C (aq. alcohol); yield: 77%.

IR (KBr) ν_{max} (cm^{-1}): 1738 (ester C=O), 1340 (S=O unsym. str.), 1132 (S=O sym. str.), 1456 (N=O), 1048 (N–N str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.07 (t, 6H, –OCH_2CH_3), 4.12 (q, 4H, –OCH_2CH_3), 4.29 (d, 2H, J = 10.5 Hz, H-2/H-6), 4.88 (d, 2H, J = 10.5 Hz, H-3/H-5), 7.12–7.78 (m, 10H, Ar-H), 11.02 (s, 2H, indole-NH). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 18.22 (OCH_2CH_3), 66.11 (C-3, C-5), 67.18 (OCH_2CH_3), 74.11 (C-2, C-6), 114.11, 115.12,116.78, 122.12, 129.90, 130.12, 131.12, 139.18 165.18 (C=O of ester). Anal. calcd.for $C_{26}H_{26}N_4O_7S$: C, 57.98; H, 4.87; N, 10.40%. Found C, 57.88; H, 4.69; N, 10.22%.

*N-nitroso-2,6-dicarbethoxy-3,5-bis(3,4-dimethoxyphenyl)tetrahydro-1,4-thiazine-1,1-dioxide (**4r**)*

Colourless solid; m.p. 158°C (aq. alcohol); yield: 84%.

IR (KBr) ν_{max} (cm^{-1}): 1732 (ester C=O), 1327 (S=O unsym. str.), 1125 (S=O sym. str.), 1449 (N=O), 1033 (N–N str.). ^{1}H NMR (300 MH_Z, $CDCl_3$): δ 1.09 (t, 6H, –OCH_2CH_3), 3.84 (s, 6H, –OCH_3), 3.88 (s, 6H, –OCH_3), 4.12 (q, 4H, –OCH_2CH_3), 4.31 (d, 2H, J = 10.5 Hz, H-2/H-6), 4.84 (d, 2H, J = 10.5 Hz, H-3/H-5), 7.69–8.09 (m, 6H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO): δ 18.11 (OCH_2CH_3), 60.12, 60.16, 65.12 (C-3, C-5), 67.12 (OCH_2CH_3), 72.11 (C-2, C-6), 114.12,116.12, 129.12, 131.34, 132.98, 140.34 (*ipso* carbon), 151.09, 152.66, 166.04 (C=O of ester). Anal. calcd.for $C_{26}H_{24}N_2O_{11}S$: C, 53.79; H, 5.56; N, 4.82%. Found C, 53.68; H, 5.39; N, 4.69%.

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