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Original article

10*H*-Phenothiazines: A new class of enzyme inhibitors for inflammatory diseases

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Abstract

The phenothiazine nucleus is known for their inhibitory activity towards the regulatory enzymes that are contributing to diseases such as asthma, autoimmune diseases including allergic rheumatoid and encephalomyelitis. In this study a number of substituted phenothiazines were synthesized and screened for their biological activity against the regulatory enzymes involved in inflammatory diseases. Our results show that the newly synthesized compounds **4a–c** and **5a–c** exhibited promising target specific enzyme inhibition against phosphodiesterase, prostaglandin dehydrogenase and superoxide dismutase activity depending on steric factors of the molecules.

Keywords: Enzyme inhibition; Phenothiazine; Prostaglandin synthetase; Target specific

1. Introduction

The pathophysiological functions of acute and chronic inflammatory diseases such as asthma, arthritis, autoimmuno disorders are due to production of a variety of chemical mediators such as leukotrienes, cytokines and prostaglandins released from biosynthetic cascade of arachidonic acid catalyzed via phospholipase A2 (PLA2), lipoxygenase and cyclooxygenase enzymes [1–3]. These regulatory enzymes are believed to play an important role in initiating and amplifying the inflammatory disorders in the body, contributing to diseases such as asthma (bronco contraction), autoimmune disorders, etc. [4–6]. Phenothiazines form an important class of heterocyclic compounds possessing wide spectrum diverse biological activities like antitumor, antimalarial, antipsychotic, anti-inflammatory, etc. [7–9]. The phenothiazines contain an interesting heterocyclic ring skeleton with two carbocyclic/aromatic rings connected to each other via a sulfide and an imino bridge which facilitates several types of reactions, substitution at the nitrogen, electrophilic substitution on the aromatic rings, *N*-oxidation and photochemical reactions, etc. [10–12]. Due to the increased importance of these heterocyclic compounds, attempts were made during the past few years in the synthesis of new generation of 10*H*-phenothiazines that exert their biological activity through modulation [13–15]. The wide range of biological activities exhibited by these phenothiazine analogues inspired us to prepare several 10*H*- and 2,10-dihydro-1*H*-phenothiazine derivatives and to evaluate them for selective enzyme inhibition studies.

2. Results and discussion

2.1. Chemistry

For the synthesis of title compounds, the key intermediates 4-hydroxy-*N*,*N*′,4-trimethyl-6-oxo-2-phenylcyclohexane-1,3-dicarboxamide (**2a**), 4-hydroxy-2-(2-methylphenyl)-*N*,*N*′,4-trimethyl-6-oxocyclohexane-1,3-dicarboxamide (**2b**), 2-(2-chlorophenyl)-4-hydroxy-*N*,*N*′,4-trimethyl-6-oxocyclohexane-1,3-dicarboxamide (**2c**), 2-(4-chlorophenyl)-4-hydroxy-*N*,*N*′,4-trimethyl-6-oxocyclohexane-1,3-dicarboxamide (**2d**), 4-hydroxy-2-(2-methoxyphenyl)-*N*,*N*′,4-trimethyl-6-oxocyclohexane-1,3-dicarboxamide (**2e**), 4-hydroxy-2-(4-methoxyphenyl)-*N*,*N*′,4-trimethyl-6-oxocyclohexane-1,3-dicarboxamide (**2f**), 2-(1,3-benzodioxol-5-yl)-4-hydroxy-*N*,*N*′,4-trimethyl-6-oxocyclohexane-1,

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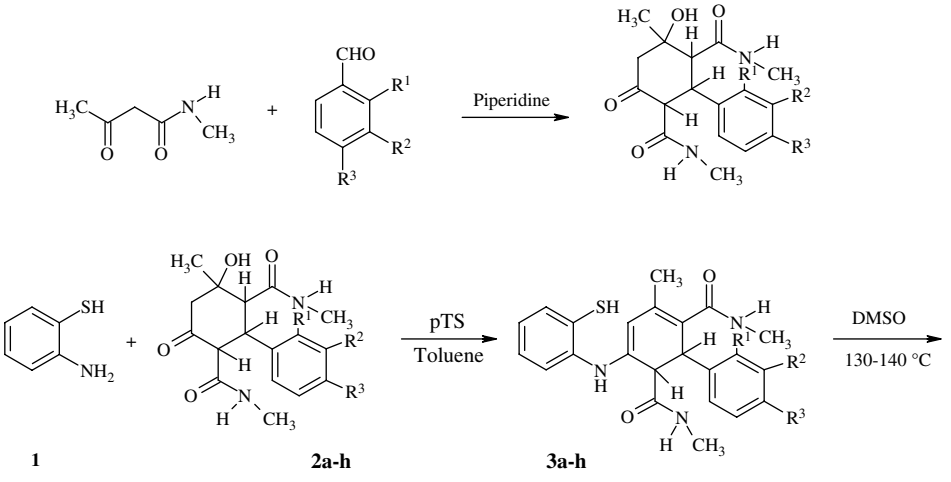
3-dicarboxamide (**2g**) and 4-hydroxy-2-(4-nitrophenyl)-*N*,*N*′,4-trimethyl-6-oxocyclohexane-1,3-dicarboxamide (**2h**) were prepared by stirring an ethanol solution of *N*-methyl-3-oxobutanamide with the corresponding aromatic aldehyde in the presence of catalytic amount of piperidine at room temperature in 52–66% yield. The reaction of 2-aminothiophenol (**1**) with 4-hydroxy-*N*,*N*′,*4*-trimethyl-6-oxo-2-arylcyclohexane-1,3-dicarboxamides (**2a–h**) in the presence of *p*-toluenesulfonic acid in dry toluene gave the corresponding enamines, 2-phenyl-*N*,*N*′,4-trimethyl-6-[(2-sulfanyl)amino]cyclohexa-3,5-diene-1,3-dicarboxamide (**3a**), 2-(2-methylphenyl)-*N*,*N*′,4-trimethyl-6-[(2-sulfanyl)amino]cyclohexa-3,5-diene-1,3-dicarboxamide (**3b**), 2-(2-chlorophenyl)-*N*,*N*′,4-trimethyl-6-[(2-sulfanyl)amino]cyclohexa-3,5-diene-1,3-dicarboxamide (**3c**), 2-(4-chlorophenyl)-*N*,*N*′,4-trimethyl-6-[(2-sulfanyl)amino]cyclohexa-3,5-diene-1,3-dicarboxamide (**3d**), 2-(2-methoxyphenyl)-*N*,*N*′,4-trimethyl-6-[(2-sulfanyl)amino]cyclohexa-3,5-diene-1,3-dicarboxamide (**3e**), 2-(4-methoxyphenyl)-*N*,*N*′,4-trimethyl-6-[(2-sulfanyl)amino]-cyclohexa-3,5-diene-1,3-dicarboxamide (**3f**), 2-(1,3-benzodioxol-5-yl)-*N*,*N*′,4-trimethyl-6-[(2-sulfanyl)amino]cyclohexa-3,5-diene-1,3-dicarboxamide (**3g**) and 2-(4-nitrophenyl)-*N*,*N*′,4-trimethyl-6-[(2-sulfanyl)amino]cyclohexa-3,5-diene-1,3-dicarboxamide (**3h**) (Scheme 1). The physical data of these enamines **3a–h** are given in Table 1. 2-Aryl-*N*,*N*′,4-trimethyl-6-[(2-sulfanyl)amino]cyclohexa-3,5-diene-1,3-dicarboxamides **3a–h** on heating in dimethyl sulfoxide gave a mixture of the corresponding 2-aryl-*N*,*N*′,4-trimethyl-2,10-dihydro-1*H*-phenothiazine-1,3-dicarboxamides (**4a–h**) and 2-aryl-*N*,*N*′,4-trimethyl-



(a) $R^1 = R^2 = R^3 = H$ (d) $R^1 = H$, $R^2 = R^3 = Cl$ (g) $R^1 = H$, $R^2 = R^3 = -O\text{-}CH_2\text{-}O-$

(b) $R^1 = CH_3 R^2 = R^3 = H$ (e) $R^1 = OCH_3$, $R^2 = R^3 = H$ (h) $R^1 = R^2 = H$, $R^3 = NO_2$

(c) $R^1 = Cl$, $R^2 = R^3 = H$ (f) $R^1 = R^2 = H$, $R^3 = OCH_3$

Scheme 1. Synthesis of substituted 1*H*-2,10-dihydro- and 10*H*-phenothiazines.

Table 1
Physical data of 2-aryl-*N*,*N*′,4-trimethyl-6-[(2-sulfanylphenyl)amino]cyclohexa-3,5-diene-1,3-dicarboxamides (**3a–h**)

Compound	M.p. (°C)	Yield (%)	IR (KBr) (cm^{-1})
3a	244–246	64	3280 (NH), 2920 (CH_3), 1640, 1610 (CONH), 1550 (C=C)
3b	245–247	60	3280 (NH), 3060 (CH_3), 1640, 1610 (CONH), 1550 (C=C)
3c	225–227	50	3295 (NH), 3080 (CH_3), 1650, 1615 (CONH), 1550 (C=C), 1080 (C–Cl)
3d	247–249	64	3290 (NH), 2940 (CH_3), 1640, 1620 (CONH), 1580 (C=C), 1085 (C–Cl)
3e	246–248	56	3290 (NH), 2960 (CH_3), 1650, 1610 (CONH), 1550 (C=C)
3f	240–242	65	3290 (NH), 2960 (CH_3), 1650, 1620 (CONH), 1590 (C=C), 1250 (C–O–C)
3g	248–250	61	3280 (NH), 2905 (CH_3), 1640, 1610 (CONH), 1550 (C=C), 1038 (O–C–O)
3h	257–259	59	3285 (NH), 2960 (CH_3), 1650, 1620 (CONH), 1525, 1345 (NO_2)

10*H*-phenothiazine-1,3-dicarboxamides (**5a–h**) (Scheme 1) [16]. The reaction is believed to proceed via the disulfide intermediate in which the disulfur bond (S–S) is cleaved by the intramolecular nucleophilic attack of the enamine system present in **3a–h** leading to the formation of 2-aryl-*N*,*N*′,4-trimethyl-2,10-dihydro-1*H*-phenothiazine-1,3-dicarboxamides (**4a–h**). The ^{1}H NMR spectra of the representative compound **4f** exhibited a singlet at δ 1.87 ppm assignable to methyl protons on C4 and two doublets integrating for 1H each at δ 3.15 and 4.12 ppm ($J = 4.8$ Hz) attributable to protons on C1 and C2, respectively. The CONH protons exchangeable with deuterium were observed at δ 5.60 and 6.02 ppm. The IR (KBr) spectra of the compounds **4a–h** showed NH stretching around 3330 cm^{-1} and the amide carbonyl stretch at 1650 and 1620 cm^{-1}. The ^{1}H NMR spectra of 2-aryl-*N*,*N*′,4-trimethyl-10*H*-phenothiazine-1,3-dicarboxamides **5a–h** revealed the absence of methine proton signals for C1 and C2 and downfield shift of C4–CH_3 signal to δ 2.3 ppm, conforming the ring aromatization.

2.2. Biological activities

It is well known that the prostaglandins and leukotrienes are important mediators/regulators of chemomodulatory diseases such as asthma and chronic inflammatory disease. In principal the pharmacological activity of anti-inflammatory compounds is by inhibition of the enzymes that participate in arachidonic acid metabolism. Phenothiazines are important heterocyclic systems that have wide range of biological activities.

From the results summarized in Tables 2 and 3, it is apparent that all the derivatives of 2,10-dihydro-1*H*-phenothiazine (**4a–h**) and 10*H*-phenothiazine compounds (**5a–h**) exhibit broad inhibitory activity towards regulating enzymes amplifying the inflammatory disorders. The compounds, 2-phenyl-*N*,*N*′,4-trimethyl-2,10-dihydro-1*H*-phenothiazine-1,3-dicarboxamide (**4a**), 2-(2-methylphenyl)-*N*,*N*′,4-trimethyl-2,10-dihydro-1*H*-phenothiazine-1,3-dicarboxamide (**4b**) and 2-(2-chlorophenyl)-*N*,*N*′,4-trimethyl-2,10-dihydro-1*H*-phenothiazine-1,3-dicarboxamide (**4c**), exhibited promising phosphodiesterase, prostaglandin synthetase and superoxide dismutase inhibition activity, when compared to standard drug aspirin. Within the 10*H*-phenothiazine derivatives **5a–h** studied the compounds 2-phenyl (**5a**), 2-(2-methyl-phenyl) (**5b**) and 2-(2-chlorophenyl) (**5c**) have shown better enzyme inhibitory activity due to hydrophilic nature of the compounds. Whereas compounds **4d–h** and **5d–h** have shown reduced enzyme inhibitory activity which may be due to increased lipophilicity of the molecule. It appears that substitution at C1- and C2-positions of the 10*H*-phenothiazines and presence of a 2-phenyl ring without substituents in *meta*- and *para*-positions were important structural requirements for exhibiting better chemomodulatory action in inflammatory disorders. Enzyme specificity and inhibitory potency of the phenothiazine derivatives studied can be related to molecular volumes, steric and electronic factors.

3. Conclusion

In vitro studies on enzyme inhibitory activities of newly synthesized analogues 2,10-dihydro-1*H*-phenothiazine (**4a–c**) and 2-aryl-10*H*-phenothiazine derivatives (**5a–c**) exhibited promising in vitro enzyme inhibitory activity and further structural variation of these compounds could result in designing a potent lead molecules in the therapy of many inflammatory diseases.

Table 2
Physical and biological activity data of 2-aryl-*N*,*N*′,4-trimethyl-2,10-dihydro-1*H*-phenothiazine-1,3-dicarboxamides (**4a–h**)

Compound	M.p.	Yield	Mol. formula	Found (calculated) (%)			IC_{50} (μM)			
				C	H	N	PE	PS	GT	SOD
4a	244–246	55	$C_{24}H_{23}N_3O_2S$	68.1 (68.1)	5.8 (5.7)	10.4 (10.4)	16	18	20	15
4b	246–248	56	$C_{25}H_{25}N_3O_2S$	68.8 (68.7)	6.1 (6.0)	10.2 (10.0)	18	21	22	17
4c	224–226	46	$C_{23}H_{22}ClN_3O_2S$	63.1 (62.9)	5.1 (5.0)	9.5 (9.6)	13	16	19	18
4d	258–260	58	$C_{23}H_{22}ClN_3O_2S$	61.3 (61.3)	5.1 (4.9)	9.5 (12.4)	26	28	>35	31
4c	226–227	31	$C_{24}H_{25}N_3O_3S$	66.2 (66.2)	5.8 (5.8)	9.7 (9.7)	>30	20	>30	>30
4f	236–238	32	$C_{24}H_{25}N_3O_3S$	64.5 (64.5)	5.8 (5.9)	9.0 (9.0)	21	20	25	18
4g	158–160	36	$C_{24}H_{23}N_3O_4S$	64.1 (64.1)	5.2 (5.2)	9.3 (9.4)	>30	28	>30	>30
4h	224–226	48	$C_{23}H_{22}N_4O_4S$	63.5 (63.4)	5.6 (5.4)	10.6 (10.5)	>30	28	36	32

Enzyme inhibition activity: (a) phosphodiesterase activity (PE), (b) prostaglandin synthetase activity (PS), (c) γ-glutamyltranspeptidase activity (GT), (d) superoxide dismutase (SOD). IC_{50} – concentration of the substrate where fifty percent (50%) of the enzyme activity was inhibited. Values represent average of three independent experiments and IC_{50} values more than 20 μM of substrate concentration were significantly different with respect to that of the control $P < 0.01$; $n = 3$.

Table 3
Physical and biological activity data of 2-aryl-*N*,*N*′,4-trimethyl-10*H*-phenothiazine-1,3-dicarboxamides (**5a–h**)

Compound	M.p.	Yield	Mol. formula	Found (calculated) (%)			IC_{50} (μM)			
				C	H	N	PE	PS	GT	SOD
5a	253–255	16	$C_{23}H_{21}N_3O_2S$	63.2 (63.1)	4.8 (4.8)	10.0 (10.1)	20	18	20	14
5b	258–260	15	$C_{24}H_{23}N_2O_2S$	69.1 (69.1)	5.5 (5.6)	10.1 (10.1)	18	21	25	18
5c	321–323	10	$C_{23}H_{20}ClN_3O_2S$	63.2 (63.2)	4.6 (4.6)	9.6 (9.6)	15	15	18	20
5d	345–347	15	$C_{23}H_{20}ClN_3O_2S$	61.1 (61.6)	4.5 (4.5)	12.4 (12.5)	18	23	20	18
5e	318–320	15	$C_{24}H_{23}N_3O_3S$	66.5 (66.5)	5.3 (5.4)	9.8 (9.7)	25	22	28	>30
5f	267–269	15	$C_{24}H_{23}N_3O_3S$	64.8 (64.8)	5.5 (5.4)	9.1 (9.1)	22	18	21	19
5g	265–267	10	$C_{21}H_{21}N_3O_4S$	64.4 (64.4)	4.8 (4.7)	9.4 (9.4)	20	24	18	20
5h	260–262	12	$C_{23}H_{20}N_4O_4S$	62.3 (61.9)	4.5 (4.3)	10.3 (10.2)	22	26	22	>30
Acetyl salicylic acid (aspirin)							22	24	36	28

Enzyme inhibition activity: (a) phosphodiesterase activity (PE) (b) prostaglandin synthetase activity (PS) (c) γ-glutamyltranspeptidase activity (GT) (d) superoxide dismutase (SOD). IC_{50} – concentration of the substrate where fifty percent (50%) of the enzyme activity was inhibited. Values represent average of the three independent experiments and IC_{50} values more than 20 μM of substrate concentration were significantly different with respect to that of the control $P < 0.01$; $n = 3$.

4. Experimental details

Melting points were determined on a Buchi capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 283B spectrophotometer in a potassium bromide pellet. The NMR spectra were recorded on a Jeol FX 200 FT NMR spectrometer using tetramethylsilane as internal standard. Mass spectra were recorded on a VG-70 70H micromass spectrometer. Product purification was carried out by column chromatography using Acme silica gel (100–200 mesh). Elemental analyses were carried out on a Perkin–Elmer 240 B analyzer.

*4.1. Synthesis of 2-aryl-N,N′,4-trimethyl-6-[(2-sulfanylphenyl)amino]cyclohexa-3,5-diene-1,3-dicarboxamide (**3a–h**)*

A mixture of 2-aminothiophenol (**1**) (0.03 mol), 2-aryl-4-hydroxy-*N*,*N*′,4-trimethyl-6-oxocyclohexane-1,3-dicarboxamide (**2a–h**) (0.03 mol), dry toluene (150 ml) and catalytic amount of *p*-toluenesulfonic acid (0.5 g) was refluxed until 2 mol equiv. of water were collected in a Dean–Stark apparatus. The product that separated was filtered, washed with methanol and taken for further reaction. The compounds thus synthesized and their physical properties are given in Table 1.

*4.2. Synthesis of 2-aryl-N,N′,4-trimethyl-2,10-dihydro-1H-phenothiazine-1,3-dicarboxamide (**4a–h**) and 2-aryl-N,N′,4-trimethyl-10H-phenothiazine-1,3-dicarboxamide (**5a–h**)*

A mixture of 2-aryl-*N*,*N*′,4-trimethyl-6-[(2-sulfanylphenyl)amino]cyclohexa-3,5-diene-1,3-dicarboxamide (**3a–h**) (0.01 mol) and dry dimethyl sulfoxide (10 ml) was heated at 110–140 °C for 2–5 h, cooled and poured into water. The product mixture separated out was filtered, purified through hexane:ethyl acetate gradient column chromatography. The products **4a–h** and **5a–h** were further recrystallized from methanol and characterized by IR, ^{1}H NMR and mass spectral studies.

4.3. Evaluation of inhibition activity against enzymes

The different concentrations of **4a–h** and **5a–h** compounds were used to study IC_{50} values against enzymes known for their role in many inflammatory diseases i.e., phosphodiesterase, prostaglandin dehydrogenase, γ-glutamyltranspeptidase and superoxide dismutase. The phosphodiesterase inhibition activity was determined by measuring the production of inorganic phosphate in the presence of an excess of 5′-nucleotidase [17]. Arachidonic acid metabolites (prostaglandin E and leukotrienes) were analyzed by HPLC using reverse phase ODS-18 column with acetonitrile:water (70:30) as mobile phase and absorbance monitored at 280 nm using isolated soya bean lipooxygenase as a source of enzyme [18]. γ-Glutamyltranspeptidase inhibition was measured by standard procedure colorimetrically at 405 nm [19]. Superoxide dismutase (SOD) activity was measured by inhibition of superoxide generation (O_2^-) in cell membrane free system spectrophotometrically at 340 nm [20].

5. Spectral data

*5.1. 2-Phenyl-N,N′,4-trimethyl-2,10-dihydro-1H-phenothiazine-1,3-dicarboxamide (**4a**)*

^{1}H NMR ($CDCl_3$): δ 1.87 (s, 3H, C4–CH_3), 2.66 (d, $J = 4.3$ Hz, 3H, C_3–CONHCH_3), 2.73 (d, $J = 4.2$ Hz, 3H, C1–CONHCH_3), 3.12 (d, $J = 4.9$ Hz, 1H, C2–*H*), 4.12 (d, $J = 4.9$ Hz, 1H, C1–*H*), 5.33 (br, 1H, CON*H*), 5.78 (br, 1H, CON*H*) and 6.77–7.52 ppm (unresolved multiples, 9H, aromatic-*H*).

*5.2. 2-(2-Methylphenyl)-N,N′,4-trimethyl-2,10-dihydro-1H-phenothiazine-1,3-dicarboxamide (**4b**)*

^{1}H NMR ($CDCl_3$): δ 1.87 (s, 3H, C4–CH_3), 2.32 (s, 3H, CH_3 on C2–phenyl), 2.65 (d, $J = 4.2$ Hz, 3H, NH–CH_3), 2.71 (d, $J = 4.2$ Hz, 3H, NH–CH_3), 3.12 (d, $J = 4.9$ Hz, 1H, C2–*H*), 4.12 (d, $J = 4.9$ Hz, 1H, C1–*H*), 5.32 (br, 1H, CON*H*), 5.80 (br, 1H, CONH) and 6.78–7.54 ppm (unresolved multiplets, 8H, aromatic-*H*).

*5.3. 2-(2-Chlorophenyl)-N,N′,4-trimethyl-2, 10-dihydro-1H-phenothiazine-1,3-dicarboxamide (**4c**)*

^{1}H NMR ($CDCl_3$): δ 1.88 (s, 3H, C4−CH_3), 2.67 (d, $J = 4.2$ Hz, 3H, NHCH_3), 2.70 (d, $J = 4.3$ Hz, 3H, NHCH_3), 3.31 (d, $J = 4.8$ Hz, 1H, C2−*H*), 4.12 (d, $J = 4.8$ Hz, 1H, C1−*H*), 5.42 (br, 1H, CON*H*), 5.90 (br, 1H, CON*H*) and 6.77−7.56 ppm (m, 8H, aromatic-*H*).

*5.4. 2-(4-Chlorophenyl)-N,N′,4-trimethyl-2, 10-dihydro-1H-phenothiazine-1,3-dicarboxamide (**4d**)*

^{1}H NMR ($CDCl_3$): δ 1.88 (s, 3H, C4−CH_3), 2.66 (d, $J = 4.2$ Hz, 3H, NH−CH_3), 2.71 (d, $J = 4.3$ Hz, 3H, NH−CH_3), 3.29 (d, $J = 4.8$ Hz, 1H, C2−*H*), 4.11 (d, $J = 4.8$ Hz, 1H, C1−*H*), 5.51 (br, 1H, CON*H*), 6.04 (br, 1H, CON*H*) and 6.80−7.78 ppm (unresolved multiplets, 7H, aromatic-*H*).

*5.5. 2-(2-Methoxyphenyl)-N,N′,4-trimethyl-2, 10-dihydro-1H-phenothiazine-1,3-dicarboxamide (**4e**)*

^{1}H NMR ($CDCl_3$): δ 1.88 (s, 3H, C4−CH_3), 2.65 (d, $J = 4.1$ Hz, 3H, NH−CH_3), 2.72 (d, $J = 4.1$ Hz, 3H, NH−CH_3), 3.31 (d, $J = 4.9$ Hz, 1H, C2−*H*), 3.79 (s, 3H, OCH_3), 4.14 (d, $J = 4.9$ Hz, 1H, C1−*H*), 5.53 (br, 1H, CON*H*), 6.04 (br, 1H, CON*H*) and 6.67−7.51 ppm (unresolved multiplets, 8H, aromatic-*H*).

*5.6. 2-(4-Methoxyphenyl)-N,N′,4-trimethyl-2, 10-dihydro-1H-phenothiazine-1,3-dicarboxamide (**4f**)*

^{1}H NMR ($CDCl_3$): δ 1.88 (s, 3H, C4−CH_3), 2.65 (d, $J = 4.1$ Hz, 3H, NH−CH_3), 2.71 (d, $J = 4.1$ Hz, 3H, NH−CH_3), 3.30 (d, $J = 4.8$ Hz, 1H, C2−*H*), 3.76 (s, 3H, OCH_3), 4.12 (d, $J = 4.8$ Hz, 1H, C1−*H*), 5.60 (br, 1H, CON*H*), 6.02 (br, 1H, CON*H*, exchangeable with deuterium) and 7.01−7.51 ppm (unresolved multiplets, 8H, aromatic-*H*). IR (KBr; ν cm^{-1}): 3330 (NH), 1650, 1620 (NHCO). MS: *m*/*z* 465 (M^+).

*5.7. 2-(1,3-Benzodioxol-5-yl)-N,N′,4-trimethyl-2, 10-dihydro-1H-phenothiazine-1,3-dicarboxamide (**4g**)*

^{1}H NMR ($CDCl_3$): δ 1.89 (s, 3H, C4−CH_3), 2.69 (d, $J = 4.2$ Hz, 3H, NH−CH_3), 2.74 (d, $J = 4.2$ Hz, 3H, NH−CH_3), 3.39 (d, $J = 4.8$ Hz, 1H, C2−*H*), 4.14 (d, $J = 4.8$ Hz, 1H, C1−*H*), 5.62 (br, 1H, CON*H*), 6.02 (s, 2H, −O−CH_2−O−), 6.10 (br, 1H, CON*H*) and 7.0−7.51 ppm (unresolved multiplets, 7H, aromatic-*H*).

*5.8. 2-(4-Nitrophenyl)-N,N′,4-trimethyl-2,10-dihydro-1H-phenothiazine-1,3-dicarboxamide (**4h**)*

^{1}H NMR ($CDCl_3$): δ 1.91 (s, 3H, C4−CH_3), 2.70 (d, $J = 4.3$ Hz, 3H, NH−CH_3), 2.76 (d, $J = 4.3$ Hz, 3H, NH−CH_3), 3.45 (d, $J = 4.9$ Hz, 1H, C2−*H*), 4.16 (d, $J = 4.9$ Hz, 1H, C1−*H*), 5.63 (br, 1H, CON*H*), 6.08 (br, 1H, CON*H*) and 7.0−7.94 ppm (unresolved multiplets, 8H, aromatic-*H*).

*5.9. 2-Phenyl-N,N′,4-trimethyl-10H-phenothiazine-1, 3-dicarboxamide (**5a**)*

^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H, C4−CH_3), 2.51 (d, $J = 4.3$ Hz, 3H, C3−CONH−CH_3), 2.57 (d, $J = 4.3$ Hz, C1−CONH−CH_3), 5.10 (br, 2H, CON*H* on C1 and C3), 6.77−7.72 (unresolved multiplets, 9H, aromatic-*H*) and 8.28 ppm (s, 1H, exchangeable with D_2O, N*H*).

*5.10. 2-(2-Methylphenyl)-N,N′,4-trimethyl-10H-phenothiazine-1,3-dicarboxamide (**5b**)*

^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H, C4−CH_3), 2.31 (s, 3H, CH_3 on C2−phenyl), 2.51 (d, $J = 4.3$ Hz, 3H, NH−CH_3), 2.57 (d, $J = 4.2$ Hz, 3H, NH−CH_3), 5.30 (br, 2H, CON*H*), 6.72−7.20 (unresolved multiplets, 8H, aromatic-*H*) and 8.21 ppm (s, 1H, exchangeable with D_2O, N*H*).

*5.11. 2-(2-Chlorophenyl)-N,N′,4-trimethyl-10H-phenothiazine-1,3-dicarboxamide (**5c**)*

^{1}H NMR ($CDCl_3$): δ 2.30 (s, 3H, C4−CH_3), 2.52 (d, $J = 4.3$ Hz, 3H, NH−CH_3), 2.58 (d, $J = 4.2$ Hz, 3H, NH−CH_3), 5.32 (br, 2H, CON*H*), 6.71−7.42 (unresolved multiplets, 8H, aromatic-*H*) and 8.26 ppm (br, 1H, exchangeable with D_2O, N*H*).

*5.12. 2-(4-Chlorophenyl)-N,N′,4-trimethyl-10H-phenothiazine-1,3-dicarboxamide (**5d**)*

^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H, C4−CH_3), 2.50 (d, $J = 4.2$ Hz, 3H, NH−CH_3), 2.57 (d, $J = 4.2$ Hz, 3H, NH−CH_3), 5.36 (br, 2H, CON*H*), 6.71−7.62 (unresolved multiplets, 7H, aromatic-*H*) and 8.28 ppm (br, 1H, exchangeable with D_2O, N*H*).

*5.13. 2-(2-Methoxyphenyl)-N,N′,4-trimethyl-10H-phenothiazine-1,3-dicarboxamide (**5e**)*

^{1}H NMR ($CDCl_3$): δ 2.30 (s, 3H, C4−CH_3), 2.51 (d, $J = 4.1$ Hz, 3H, NH−CH_3), 2.58 (d, $J = 4.2$ Hz, 3H, NH−CH_3), 3.81 (s, 3H, OCH_3), 5.42 (br, 2H, CON*H*), 6.67−7.30 (m, 8H, aromatic-*H*) and 8.21 ppm (br, 1H, exchangeable with D_2O, N*H*).

*5.14. 2-(4-Methoxyphenyl)-N,N′,4-trimethyl-10H-phenothiazine-1,3-dicarboxamide (**5f**)*

^{1}H NMR ($CDCl_3$): δ 2.30 (s, 3H, C4−CH_3), 2.50 (d, $J = 4.1$ Hz, 3H, NH−CH_3), 2.58 (d, $J = 4.2$ Hz, 3H, NH−CH_3), 3.80 (s, 3H, OCH_3), 5.54 (br, 2H, CON*H*), 6.67−7.20 (unresolved multiplets, 8H, aromatic-*H*) and 8.94 ppm (br, 1H, exchangeable with D_2O, N*H*). IR (KBr; ν cm^{-1}): 3300 (NH), 1630 (NHCO). MS: *m*/*z* 463 (M^+).

*5.15. 2-(1,3-benzodioxol-5-yl)-N,N′,4-trimethyl-10H-phenothiazine-1,3-dicarboxamide (**5g**)*

^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H, C4–CH_3), 2.56 (d, $J = 4.2$ Hz, 3H, NH–CH_3), 2.60 (d, $J = 4.2$ Hz, 3H, NH–CH_3), 5.60 (br, 2H, CONH), 6.03 (s, 2H, O–CH_2–O), 6.60–6.91 (m, 7H, aromatic-H) and 8.90 ppm (br, 1H, exchangeable with D_2O, NH).

*5.16. 2-(4-Nitrophenyl)-N,N′,4-trimethyl-10H-phenothiazine-1,3-dicarboxamide (**5h**)*

^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H, C4–CH_3), 2.56 (d, $J = 4.2$ Hz, 3H, NH–CH_3), 2.68 (d, $J = 4.2$ Hz, 3H, NH–CH_3), 5.84 (br, 2H, CONH), 6.67–7.95 (unresolved multiplets, 8H, aromatic-H) and 8.56 ppm (br, 1H, exchangeable with D_2O, NH).

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