Original Article

Synthesis and Antihistaminic Potential of Some Novel Substituted Dinitrophenothiazine Derivatives

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Abstract

Background: Phenothiazine consists of a three-ring structure compound in which two benzene rings are connected with nitrogen and sulfur atoms at nonadjacent sides. Phenothiazine and its substituted derivatives are abundantly able to produce a variety of important pharmacological and valuable therapeutic effects, and till now, these are under profound investigational processes. **Objective:** To synthesize and evaluate the antihistaminic potential of some newly synthesized dinitrophenothiazine derivatives. **Materials and Methods:** Different derivatives have been synthesized by the appropriate chemical scheme using dinitrophenothiazine as a basic nucleus. The completion of the chemical reactions has been monitored by thin-layer chromatography. The chemical structures of the newly synthesized products (P1–P25) were affirmed by elemental analysis and by spectral (infra-red, ^{1}H nuclear magnetic resonance, and mass spectroscopy) findings and further examined for antihistaminic potential in guinea pigs. The synthesized products were also evaluated for their acute toxicity study and were found nontoxic. **Results:** The majority of the synthesized products of the dinitrophenothiazine series, namely, P07, P11, P12, P13, P15, P16, P17, P18, P19, and P20, have shown antihistaminic activity and compared with mepyramine (standard drug) at 0.8 µg/mL. Among the synthesized products, P18 was found to exhibit maximum antihistaminic activity. However, all the synthesized compounds were found to elicit a significant antihistaminic effect when compared with the standard drug. **Conclusion:** Therefore, dinitrophenothiazine compounds could be a good starting point to develop efficacious and potent analogues, as an antihistaminic agent in the treatment of allergic disorders.

Keywords: *Antihistaminic activity, dimethylformamide, dinitrophenothiazine, diphenyl ether, mepyramine*

Introduction

Histamine modulates the physiological activities in the gut and also functions as the neurotransmitter.[1,2] It is reported to elevate the capillaries' permeability to some proteins and leukocytes and also permits them to combat the pathogens in the site of infected tissues.[2] Histamine is a key mediator in allergic disorders where it generates most of its actions through H_1 receptors. H_1 antihistamine manifests rapid relief from various allergic symptoms and is well authenticated as the main therapeutic agent in the treatment of several allergic problems.[3] The role of histamine in inflammation and gastric acid suppression is terrifically expressed in the human body.[4]

Phenothiazine and its numerous derivatives possess important medicinal properties, and alkyl substituent on heterocyclic nitrogen atom accounts for their diversified biological activities,[5-7] such as neuroleptic, antihistaminic, antimicrobial, anticancer, antimalarial, antitubercular, analgesic, and anti-inflammatory.[8-11] It has been well evidenced that the therapeutic effect of phenothiazine-derived drugs is mainly due to inhibition of the dopamine receptors in the central nervous system. However, several kinds of shortcomings arise while using phenothiazine-originated drugs. The major adverse effect was seen, i.e., extrapyramidal manifestation accompanying tardive dyskinesia, dystonia, akathisia, parkinsonism, and weight gain. Moreover, the minor adverse effect is also seen such as sedation, constipation, pruritus, dry eyes, dry mouth, photosensitivity, urinary retention, etc.[12-16] Phenothiazine derivatives are also availed as an antipsychotic drug; a hydrogen atom attached with the carbon-2 (C-2) and nitrogen-10 (N-10) atoms have been substituted with numerous chemical groups and the side chain attached at the N-10 position of the phenothiazine ring, i.e., aliphatic side chain, piperazine, or piperidine moiety.[17-20] It was speculated that a 200 mg/kg dose was considered an effective dose with

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the least toxic effect.[21-26] Keeping in view the biological importance of phenothiazine and continuing our present research work envisage the synthesis of a few newer dinitro-derivatives of phenothiazine with the given scheme.[27-31] The newly generated novel compounds were characterized and evaluated for *in vivo* antihistaminic activity.

Materials and Methods

General

Products have been synthesized according to the illustrated chemical scheme. Melting points of all newly synthesized products were ascertained by using the open capillary method. These were found uncorrected. The purity of the products has been determined by thin-layer chromatography (TLC) techniques using silica gel G of 0.5 mm of thickness as stationary phase and n-hexane and ethyl acetate in a ratio of 8.5:1.5 and acetone and benzene (8:2) have been as mobile phase and visualized in iodine vapors and ultra-violet light.[27,31,32]

Elemental analysis along with spectral data was obtained from the Department, School of Physical Sciences Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi, and Jamia Hamdard University, New Delhi.

Synthesis of compounds P1–P25

Chemical scheme I

Using the chemical scheme, I, a series of products from product number 1 to product number 22 (P1–P22) have been designed and synthesized. The reaction completion has been checked by the TLC. The obtained products from the chemical scheme I have been characterized by spectral and elemental analysis. After characterization of the synthesized products, they have been evaluated for antihistaminic activity.[27,28] The reaction scheme is given below [Table 1 and Figure 1].

Chemical scheme II

Using chemical scheme II, the second series of products have been designed and synthesized. The compounds P22–P25 were synthesized by the chemical scheme II.

Synthesis of dinitrophenothiazine derivative

The synthetic procedure of the dinitrophenothiazine derivatives has been carried out mainly in two steps. The products from P01 to P21 have been prepared by chemical scheme I.

Step I

Nitro-substituted chlorobenzene derivative (3.14 g) and substituted nitroaniline derivative (2.76 g) were taken in dimethylformamide (DMF) (20 mL) in a round bottom flask of 250 mL capacity. Anhydrous potassium carbonate (3 g) and copper powder (0.3 g) were incorporated into the flask, and the reaction was refluxed for 2 h on an oil bath. The product so obtained was filtered and washed with hot DMF (10 mL). The filtrate obtained was poured into ice-cold water approximately 200 mL in a beaker. The solid products were collected, air-dried, and recrystallized from toluene.

Table 1: Substituted R and R′ groups in chemical scheme I

Product number	R	R′
P_1	7-NO_2	3-NO_2
P_2	7-NO_2	2-NO_2
P_3	7-NO_2	1-NO_2
P_4	8-NO_2	2-NO_2
P_5	6-NO_2	2-NO_2
P_6	6-NO_2	1-NO_2
P_7	9-NO_2	3-NO_2
P_8	9-NO_2	2-NO_2
P_9	9-NO_2	1-NO_2
P_{10}	7,9-dinitro	H
P_{11}	7,9-dinitro	1-Cl
P_{12}	7,9-dinitro	2-Cl
P_{13}	7,9-dinitro	3-Cl
P_{14}	6,8-dinitro	H
P_{15}	6,8-dinitro	1-Cl
P_{16}	6,8-dinitro	2-Cl
P_{17}	6,8-dinitro	3-Cl
P_{18}	6,8-dinitro	2,3-dichloro
P_{19}	7,9-dinitro	1,3-dichloro
P_{20}	7,9-dinitro	1,2-dichloro
P_{21}	7,9-dinitro	3-Cl

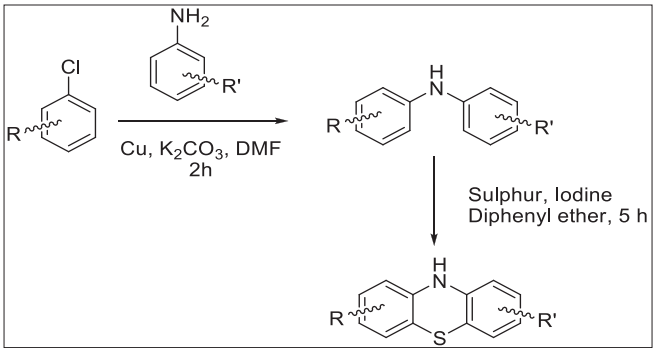

Figure 1: Synthesis of P1–P22 by chemical scheme I

Step II

To the product obtained from the first step was added 20 mL diphenyl-ether, sulfur powder (0.64 g), and 0.3 g of iodine and subjected to reflux for 5 h and then distilled under vacuum to remove the excess solvent. The reaction mixture cooled and the product separated as an orange crystalline mass. It was recrystallized from a toluene–acetone mixture to get the pure product. Similarly, all other dinitrophenothiazine derivatives were successfully prepared by using the above two steps, respectively.

Chemical scheme II

The formation of the dinitrophenothiazine compounds has been done mainly in two steps. By using chemical scheme II, the products (P22–P25) were prepared in two steps [Table 2 and Figure 2].

Step I

Nitro-substituted aniline derivative (3.14 g) and chlorosubstituted chlorobenzene (2.76 g) were taken in DMF

(20 mL) in a round bottom flask of 250 mL capacity. Anhydrous potassium carbonate (3 g) and copper powder (0.3 g) were incorporated into the flask, and the reaction was refluxed for 2 h on an oil bath. The mixture was filtered and the residue obtained was given washings with hot DMF (10 mL). The filtrate obtained was poured into ice-cold water approximately 200 mL in a beaker. The solid products were collected, air-dried, and recrystallized from toluene.

Table 2: Substituted R″ and R‴ groups in chemical scheme II

Product number	R″	R‴
P_{22}	6,8-dinitro	H
P_{23}	6,8-dinitro	1-Cl
P_{24}	6,8-dinitro	2-Cl
P_{25}	6,8-dinitro	3-Cl

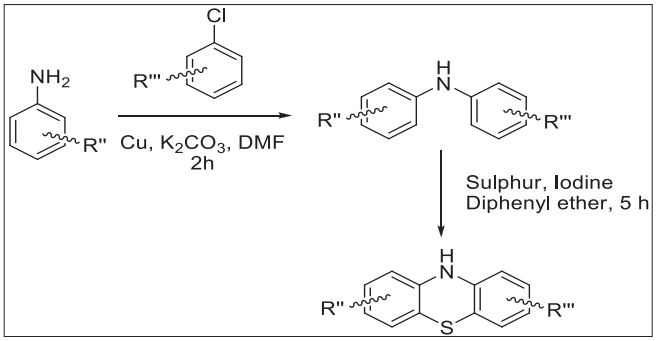

Figure 2: Synthesis of P22–P25 by chemical scheme II

Step II

To the product obtained from the first step was added 20 mL diphenyl-ether, sulfur powder (0.64 g), and 0.3 g of iodine and subjected to reflux for 5 h and then distilled under vacuum to remove the excess solvent. The reaction mixture cooled and the product separated as an orange crystalline mass. It was recrystallized from a toluene-acetone mixture to get the pure product. Similarly, all other dinitrophenothiazine derivatives were successfully prepared by using the above two steps, respectively.

The yield, % yield, retention (Rf) values, and melting points of the products (1–21) synthesized from chemical scheme I are summarized in Table 3.

The yield, % yield, Rf values, and meting points of the products (22–25) estimated from chemical scheme II are illustrated in Table 3.

Analytical data of synthesized products

P1 (3,7-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_7N_3O_4S$; molecular weight: 289.27; infra-red (IR): KBr, cm^{-1}: 3481 (NH), 1300 (C-N), 1631 (C=C

Table 3: Physical data of the synthesized compounds

Product number	Compound name	Yield (g)	% yield	Rf_1 (n-hexane: ethyl acetate)	Rf_2 (acetone: benzene)	Melting point (°C)
P1	3,7-dinitrophenothiazine	3.57	61.76	0.41	0.52	109–111
P_2	2,7-dinitrophenothiazine	3.37	58.30	0.47	0.57	107–109
P_3	1,7-dinitrophenothiazine	3.25	56.22	0.49	0.61	111–113
P_4	2,8-dinitrophenothiazine	3.40	58.82	0.44	0.64	106–108
P_5	2,6-dinitrophenothiazine	3.62	62.62	0.43	0.59	107–109
P_6	1,6-dinitrophenothiazine	3.27	56.57	0.42	0.63	112–114
P_7	1,7-dinitrophenothiazine	3.30	57.09	0.45	0.65	113–115
P_8	1,8-dinitrophenothiazine	3.21	55.53	0.39	0.48	105–107
P_9	1,9-dinitrophenothiazine	3.33	57.61	0.40	0.51	106–108
P_{10}	1,3-dinitrophenothiazine	3.29	56.92	0.38	0.47	110–112
P_{11}	9-chloro-1,3-dinitrophenothiazine	3.36	66.93	0.65	0.72	114–116
P_{12}	8-chloro-1,3-dinitrophenothiazine	3.34	66.53	0.63	0.69	113–115
P_{13}	7-chloro-1,3-dinitrophenothiazine	3.32	66.13	0.61	0.67	112–114
P_{14}	2,4-dinitrophenothiazine	3.54	61.24	0.59	0.64	107–109
P_{15}	1-chloro-6,8-dinitrophenothiazine	3.57	71.11	0.70	0.74	116–118
P_{16}	8-chloro-2,4-dinitrophenothiazine	3.59	71.51	0.72	0.76	118–120
P_{17}	7-chloro-2,4-dinitrophenothiazine	3.51	69.22	0.61	0.67	117–119
P_{18}	2,3-dichloro-6,8-dinitrophenothiazine	4.19	75.35	0.76	0.79	120–122
P_{19}	1,3-dichloro-7,9-dinitrophenothiazine	4.15	74.64	0.72	0.74	122–124
P_{20}	1,2-dichloro-7,9-dinitrophenothiazine	4.12	74.10	0.68	0.72	119–121
P_{21}	3-chloro-7,9-dinitrophenothiazine	3.49	69.52	0.65	0.69	114–116
P_{22}	2,4-dinitrophenothiazine	3.24	65.45	0.52	0.59	109–111
P_{23}	1-chloro-6,8-dinitrophenothiazine	4.43	80.98	0.72	0.76	116–118
P_{24}	2-chloro-6,8-dinitrophenothiazine	4.41	80.62	0.70	0.74	115–117
P_{25}	3-chloro-6,8-dinitrophenothiazine	4.45	81.35	0.74	0.76	118–120

Note: All the synthesized products from the above two mentioned schemes have appeared in the form of pale yellowish color

of aromatic ring), (1587, 1300) NO_2; ^{1}H nuclear magnetic resonance (NMR) (dimethyl sulfoxide [DMSO]-d_6, 400 MHz) (ppm): 8.81 (s, 1H, Ar-6, 8), 7.96-7.90 (m, 2H, Ar-1 and 9), 3.40 (broad hump of N-H); electro spray ionization (ESI)-mass spectroscopy (MS) *(m/z)*: 290 $[M+H]^+$, 274 [M+H-O], 139 [nitroaniline+1], 122 [nitrobenzene M-167]; elemental analysis: estimated (%): C, 49.83; H, 2.44; N, 14.53 and observed (%): C, 49.63; H, 2.24; N, 14.33.

P2 (2,7-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_7N_3O_4S$; molecular weight: 289.27; IR: KBr, cm^{-1}: 3332 (NH), 1344 (C-N), 1602 (C=C of aromatic ring), (1519, 1309) NO_2; ^{1}H NMR (DMSO-d_6, 400 MHz) (ppm): 7.34-7.30 (brd, 2H, Ar-1, 3), 7.10-7.06 (m, 2H, Ar-6, 8), 6.93-6,76 (2H, Ar-1 and 9); ESI-*MS (m/z)*: 290 $[M+H]^+$, 274 [M+H-O], 139 [nitroaniline+1], 122 [nitrobenzene M-167]; elemental analysis: estimated (%): C, 49.83; H, 2.44; N, 14.53 and observed (%): C, 49.63; H, 2.24; N, 14.33.

P3 (1,7-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_7N_3O_4S$; molecular weight: 289.27; IR: KBr, cm^{-1}: 3332 (NH), 1346 (C-N), 1604 (C=C of aromatic ring), (1519, 1313) NO_2; ^{1}H NMR (DMSO-d_6, 400 MHz) (ppm): 8.24-8.22 (brd, 1H, Ar-2), 7.74-7.21 (brd, 2H, Ar-3, 8), 5.80 (s, 1H, Ar-4), 3.32 (s, 1H, N-H); ESI-MS *(m/z)*: 290 $[M+H]^+$, 165, 166 [M-nitrobenzene (123, 124)]; elemental analysis: estimated (%): C, 49.83; H, 2.44; N, 14.53 and observed (%): C, 49.63; H, 2.24; N, 14.33.

P4 (2,8-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_7N_3O_4S$; molecular weight: 289.27; IR: KBr, cm^{-1}: 3361 (NH), 1298 (C-N), 1631 (C=C of aromatic ring), (1598, 1444) NO_2; ^{1}H NMR (DMSO-d_6, 400 MHz) (ppm): 8.24-8.22 (brd, 2H, Ar-1,3), 7.74-7.72 (m, 2H, Ar-7, 9), 7.35-7.25 (m, 1H, Ar-4), 6.93 (s, 1H, Ar-6), 3.32 (s, 1H, N-H); ESI-MS *(m/z)*: 290 $[M+H]^{+,+}$, 274 $[M+H-O]^+$; elemental analysis: estimated (%): C, 49.83; H, 2.44; N, 14.53 and observed (%): C, 49.63; H, 2.24; N, 14.33.

P5 (dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_7N_3O_4S$; molecular weight: 289.27; IR: KBr, cm^{-1}: 3354 (NH), 1344 (C-N), 1602 (C=C of aromatic ring), (1519, 1309) NO_2; ^{1}H NMR (DMSO-d_6, 400 MHz) (ppm): 8.24-8.22 (brd, 2H, Ar-1, 3), 7.74-7.72 (brd, 2H, Ar-7, 8), 7.35-7.27 (brd, 1H, Ar-9), 6.93 (s, Ar-4); ESI-MS *(m/z)*: 290 $[M+H]^+$, 182 $[M-2NO_2\text{-}NH]^{+,+}$; elemental analysis: estimated (%): C, 49.83; H, 2.44; N, 14.53 and observed (%): C, 49.63; H, 2.24; N, 14.33.

P6 (1,6-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_7N_3O_4S$; molecular weight: 289.27, IR: KBr, cm^{-1}: 3481 (NH), 1300 (C-N), 1631 (C=C of aromatic ring), (1587, 1300) NO_2; ^{1}H NMR (DMSO-d_6, 400 MHz) (ppm): 8.23-8.21 (brs, 1H, Ar-2), 7.73-7.71 (brs, 1H, Ar-7), 7.35-7.25 (m, 2H, Ar-3,4), 7.23-7.18 (s, 1H, Ar-8), 6.93 (s, 2H, Ar-4, 9), 3.34 (s, 1H, NH); ESI-*MS (m/z)*: 290 $[M+H]^+$, 228 $[M-2NO_2\text{-}O]^+$, 182 $[M-2NO_2\text{-}NH]^+$; elemental analysis: estimated (%): C, 49.83; H, 2.44; N, 14.53 and observed (%): C, 49.63; H, 2.24; N, 14.33.

P7 (3,9-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_7N_3O_4S$; molecular weight: 289.27; IR: KBr, cm^{-1}: 3481 (NH), 1300 (C-N), 1631 (C=C of aromatic ring), (1587, 1300) NO_2; ^{1}H NMR (DMSO-d_6, 400 MHz) (ppm): 8.24-8.22 (brd, 2H, Ar-2,4), 7.74-7.21 (brd, 1H, Ar-8), 7.35-7.25 (m, 2H, Ar-6,7), 6.93 (s, 1H, Ar-4), 3.32 (s, 1H, N-H); ESI-MS *(m/z)*: 290 $[M+H]^+$; elemental analysis: estimated (%): C, 49.83; H, 2.44; N, 14.53 and observed (%): C, 49.63; H, 2.24; N, 14.33.

P8 (2,9-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_7N_3O_4S$; molecular weight: 289.27; IR: KBr, cm^{-1}: 3334 (NH), 1266 (C-N), 1625 (C=C of aromatic ring), (1521, 1349) NO_2; ^{1}H NMR (DMSO-d_6, 400 MHz) (ppm): 8.23-8.21 (m, 2H, Ar-1,3), 7.73-7.70 (brs, 1H, Ar-4), 3.34 (s, 1H, NH); ESI-MS *(m/z)*: 290 $[M+H]^+$, 182 $[M-2NO_2\text{-}NH]^+$; elemental analysis: estimated (%): C, 49.83; H, 2.44; N, 14.53 and observed (%): C, 49.63; H, 2.24, N; 14.33.

P9 (1,9-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_7N_3O_4S$; molecular weight: 289.27; IR: KBr, cm^{-1}: 3328 (NH), 1265 (C-N), 1625 (C=C of aromatic ring), (1522, 1348) NO_2; ^{1}H NMR (DMSO-d_6, 400 MHz) (ppm): 8.24-8.18 (d, 1H, Ar-7), 7.89-7.69 (m, 2H, Ar-3,6), 6.68 (s, 1H, Ar-5), 6.55 (s, 1H, Ar-4), 3.30 (s, 1H, NH); ESI-MS *(m/z)*: 290 $[M+H]^{+,+}$, 274 $[M+H-O]$, 182 $[M-2NO_2\text{-}NH]^+$, 139 [nitroaniline+1], 122 [nitrobenzene M-167]; elemental analysis: estimated (%): C, 49.83; H, 2.44; N, 14.53 and observed (%): C, 49.63; H, 2.24; N, 14.33.

P10 (7,9-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_7N_3O_4S$; molecular weight: 289.27, IR: KBr, cm^{-1}: 3362 (NH), 1299 (C-N), 1631 (C=C of aromatic ring), (1588, 1471) NO_2, ^{1}H NMR (DMSO-d_6, 400 MHz) (ppm): 8.23-8.21 (m, 2H, Ar-6, 8), 7.27-7.25 (s, 1H, Ar-2,3,4), 6.93-6.91 (brs, 3H, Ar-2,3,4), 3.34 (s, 1H, NH); ESI-MS *(m/z)*: 290 $[M+H]^+$, 274 [M+H-O], 182 $[M-2NO_2\text{-}NH]^+$, 139 [nitroaniline+1]; elemental analysis: estimated (%): C, 49.83; H, 2.44; N, 14.53 and observed (%): C, 49.63; H, 2.24; N, 14.33.

P11 (1-chloro-7,9-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_6ClN_3O_4S$; molecular weight: 322.97; IR: KBr, cm^{-1}: 3326 (NH), 1268 (C-N), 1614 (C=C of aromatic ring), (1579, 1337) NO_2, 733 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz) (ppm): 7.37-7.33 (m, 2H, Ar-6,8), 7.12-7.08 (m, Ar-3, 4), 3.34 (s, 1H, NH); ESI-MS *(m/z)*: 323 $[M]^+$, 290 $[M+2\text{-}Cl]^+$, 274 [M+2-Cl-O+H], 182 $[M-2NO_2\text{-}NH\text{-}Cl]^+$, 139 [nitroaniline+1]; elemental analysis: estimated (%): C, 44.52; H, 1.87; N, 12.98 and observed (%): C, 44.32; H, 1.67; N, 12.78.

P12 (2-chloro-7,9-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_6ClN_3O_4S$; molecular weight: 322.97; IR: KBr, cm^{-1}: 3481 (NH), 1300 (C-N), 1631 (C=C of aromatic), 1300, 1587 (NO_2), 750 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz)

(ppm): 7.37-7.33 (m, 2H, Ar-6,8), 7.12-7.08 (m, 1H, Ar-1), 6.98-6.96 (m, Ar-3, 4), 3.34 (s, 1H, NH); ESI-MS *(m/z)*: 325 $[M+2]^+$, 323 $[M]^+$, 139 [nitroaniline+1], 121 [nitrobenzene]; elemental analysis: estimated (%): C, 44.52; H, 1.87; N, 12.98 and observed (%): C, 44.32; H, 1.67; N, 12.78.

P13 (3-chloro-7,9-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_6ClN_3O_4S$; molecular weight: 322.97; IR: KBr, cm^{-1}: 3326 (NH), 1268 (C-N), 1614 (C=C of aromatic), (1579, 1337) (NO_2), 733 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 7.12-7.08 (m, 1H, Ar-2), 6.98-6.96 (m, Ar-1, 4); ESI-MS *(m/z)*: 323 $[M]^+$, 247 [M-Cl-NO_2], 139 [nitroaniline+1], 121 [nitrobenzene]; elemental analysis: estimated (%): C, 44.52; H, 1.87; N, 12.98 and observed (%): C, 44.32; H, 1.67; N, 12.78.

P14 (6,8-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_7N_3O_4S$; molecular weight: 289.27; IR: KBr, cm^{-1}: 3328 (NH), 1265 (C-N), 1625 (C=C of aromatic), (1522, 1348) (NO_2); ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 8.23-8.21 (s, 2H, Ar-7, 9), 7.73-7.23 (m, 4H, Ar-1,2,3,4), 3.34 (s, 1H, NH); ESI-MS *(m/z):* 290 $[M+H]^+$, 139 [nitroaniline+1], 123 [nitrobenzene M-167]; elemental analysis: estimated (%): C, 49.83; H, 2.44; N, 14.53 and observed (%): C, 49.63; H, 2.24; N, 14.33.

P15 (1-chloro-6,8-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_6ClN_3O_4S$; molecular weight: 322.97; IR: KBr, cm^{-1}: 3332 (NH), 1344 (C-N), 1602 (C=C of aromatic), (1519, 1309) NO_2, 736 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 7.37-7.33 (m, 2H, Ar-7, 9), 7.12-7.10 (s, 1H, Ar-2), 6.98-6.96 (m, 2 H, Ar-3,4); ESI-MS *(m/z)*: 324 $[M+2]^+$, 323 $[M+H]^+$; elemental analysis: estimated (%): C, 44.52; H, 1.87; N, 12.98 and observed (%): C, 44.32; H, 1.67; N, 12.78.

P16 (2-chloro-2,4-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_6ClN_3O_4S$; molecular weight: 322.97; IR: KBr, cm^{-1}: 3354 (NH), 1300 (C-N), 1606 (C=C of aromatic), (1465, 1309) NO_2, 758 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 7.37-7.33 (m, 2H, Ar-9,7), 7.12-7.10 (m, 1H, Ar-1), 6.98-6.96 (m, 2H, Ar-3,4), 3.34 (s, 1H, NH); ESI-MS *(m/z)*: 324 $[M+2]^+$, 323 $[M+H]^+$; elemental analysis: estimated (%): C, 44.52; H, 1.87; N, 12.98 and observed (%): C, 44.32; H, 1.67; N, 12.78.

P17 (3-chloro-6,8-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_6ClN_3O_4S$; molecular weight: 322.97; IR: KBr, cm^{-1}: 3332 (NH), 1344 (C-N), 1602 (C=C of aromatic), (1519, 1309) NO_2, 736 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 7.34-7.30 (m, 2H, Ar-7,9), 7.10-7.08 (s, 1H, Ar-2), 6.98-6.97 (m, 2H, Ar-1,4); ESI-MS *(m/z)*: 324 $[M+2]^+$, 323 $[M+H]^+$; elemental analysis: estimated (%): C, 44.52; H, 1.87; N, 12.98 and observed (%): C, 44.32; H, 1.67; N, 12.78.

P18 (2,3-dichloro-6,8-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_5Cl_2N_3O_4S$; molecular weight: 358.16; IR: KBr, cm^{-1}: 3329 (NH), 1265 (C-N), 1625 (C=C of aromatic), (1587, 1348) NO_2, 740 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 7.35 (s, 2H, Ar-7,9), 7.18-7.10 (m, 1H, Ar-1), 6.97 (s, 1H, Ar-4); ESI-MS *(m/z)*: 360 $[M+2]^+$, 359 $[M+H]^+$, 290 $[M+2-2Cl]^+$; elemental analysis: estimated (%): C, 40.24; H, 1.41; N, 11.73 and observed (%): C, 40.04; H, 1.21; N, 11.53.

P19 (1,3-dichloro-7,9-dinitrophenothiazine)

Molecular formula: $C_{12}H_5Cl_2N_3O_4S$; molecular weight: 358.16; IR: KBr, cm^{-1}: 3361 (NH), 1298 (C-N), 1631 (C=C of aromatic), 1598, 1444 (NO_2), 698 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 8.81-8.63 (m, 2H, Ar-2,4), 7.95-7.91 (m, 2H, Ar-6,8); ESI-MS *(m/z)*: 360 $[M+2]^+$, 359 $[M+H]^+$; elemental analysis: estimated (%): C, 44.52; H, 1.87; N, 12.98 and observed (%): C, 44.32; H, 1.67; N, 12.78.

P20 (1,2-dichloro-7,9-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_5Cl_2N_3O_4S$; molecular weight: 358.16; yield: 48%; IR: KBr, cm^{-1}: 3332 (NH), 1346 (C-N), 1604 (C=C of aromatic), 1313, 1519 (NO_2), 736 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 7.46-7.42 (m, 2H, Ar-6,8), 7.20 (s, 1H, Ar-3) ,6.92 (s, 1H, Ar-4), 2.53 (s, 1H, NH); ESI-MS *(m/z)*: 360 $[M+2]^+$, 359 $[M+H]^+$; elemental analysis: estimated (%): C, 44.52; H, 1.87; N, 12.98 and observed (%): C, 44.32; H, 1.67; N, 12.78.

P21 (3-chloro-7,9-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_6ClN_3O_4S$; molecular weight: 322.97; IR: KBr, cm^{-1}: 3326 (NH), 1268 (C-N), 1614 (C=C of aromatic), 1337, 1579 (NO_2), 733 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 7.12-7.08 (m, 1H, Ar-2), 6.98-6.96 (m, Ar-1,4); ESI-MS *(m/z)*: 325 $[M+2]^+$, 324 $[M+H^+]$, 323 $[M]^+$; elemental analysis: estimated (%): C, 44.52; H, 1.87; N, 12.98 and observed (%): C, 44.32; H, 1.67; N, 12.78.

P22 (6,8-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_7N_3O_4S$; molecular weight: 289.27; IR: KBr, cm^{-1}: 3328 (NH), 1265 (C-N), 1625 (C=C of aromatic), (1348, 1522) NO_2; ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 8.23-8.21 (s, 2H, Ar-7, 9), 7.73-7.23 (m, 4H, Ar-1,2,3,4), 3.34 (s, 1H, NH); ESI-MS *(m/z)*: 290 $[M+H]^+$, 139 [nitroaniline+1], 121 [nitrobenzene-1 M-168]; elemental analysis: estimated (%): C, 49.83; H, 2.44; N, 14.53 and observed (%): C, 49.63; H, 2.24; N, 15.35.

P23 (1-chloro-6,8-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_6ClN_3O_4S$; molecular weight: 322.97; IR: KBr, cm^{-1}: 3481 (NH), 1300 (C-N), 1631 (C=C of aromatic), 1300, 1587 (NO_2), 750 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 7.37-7.33 (m, 2H, Ar-7,9), 7.12-7.10 (s, 1H, Ar-2), 6.98-6.96 (m, 2H, Ar-3,4); ESI-MS *(m/z)*: 325 $[M+2]^+$, 324 $[M+H^+]$, 323 $[M]^+$; elemental analysis: estimated (%): C, 44.52; H, 1.87; N, 12.98 and observed (%): C, 44.32; H, 1.67; N, 12.78.

P24 (2-chloro-6,8-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_6ClN_3O_4S$; molecular weight: 322.97; IR: KBr, cm^{-1}: 3361 (NH), 1298 (C-N), 1631 (C=C of aromatic), 1598, 1444 (NO_2), 689 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 7.37-7.33 (m, 2H, Ar-9,7), 7.12-7.10 (m, 1H, Ar-1), 6.98-6.96 (m,

2H, Ar-3,4), 3.34 (s, 1H, NH); ESI-MS *(m/z)*: 325 $[M+2]^+$, 324 $[M+H^+]$, 323 $[M]^+$; elemental analysis: estimated (%): C, 44.52; H, 1.87; N, 12.98 and observed (%): C, 44.32; H, 1.67; N, 12.78.

P25 (3-chloro-6,8-dinitro-10H-phenothiazine)

Molecular formula: $C_{12}H_6ClN_3O_4S$; molecular weight: 323.71; IR: KBr, cm^{-1}: 3329 (NH), 1265 (C-N), 1625 (C=C of aromatic), 1587, 1348 (NO_2), 740 (C-Cl); ^1H NMR (DMSO-d_6, 400 MHz) (ppm): 7.37-7.34 (m, 2H), 7.10 (s, 1H), 6.98-6.96 (m, 2H); ESI-MS *(m/z)*: 325 $[M+2]^+$, 324 $[M+H^+]$, 323 $[M]^+$, 290 $[M+2-Cl]^+$; elemental analysis: estimated (%): C, 44.52; H, 1.87; N, 12.98 and observed (%): C, 44.32; H, 1.67; N, 12.78.

Pharmacological activity

Antihistaminic activity

The products have been assessed for their antihistaminic activity on the ileum of guinea pig, weighing around 400–500 g. The chosen guinea pigs have been forfeited by exsanguination and stunning. The abdomen was clipped using the cutter and then raised the cecum to find the ileocecal junction. The required length was detached quickly and kept on the watch glass accompanying the Tyrode solution. Taking appropriate precautions, further mesentery was carried out and the ileum was dissected into small portions of 2–3 cm in length. To the selected portion of the ileum, a thread was tied to the top and bottom ends, and further, the tissue was placed in the organ bath accompanying Tyrode solution, fixed at 37°C, which was fizzed with oxygen. Before incorporating drugs into the organ bath, a tightness of 0.5 g was executed, and the tissue was allowed to equilibrate for 30 min.[27,33-39]

Preparation of histamine

Histamine was dissolved in physiological saline. This saline was recommended as it is known to be compatible with human tissue and tonicity with body fluid.[27,29]

Preparation of test solution

DMSO was taken as a solvent, and test samples (10 mg) have been dissociated in it. To attain a concentration of 0.2, 0.4, and 0.8 µg/mL, separate solutions were prepared along with dextrose normal saline (DNS) solution.[27,29]

Preparation of standard solution[27,40-43]

Ten mg of mepyramine (standard drug)[43] was dissolved in the DNS solution. Different solution of the standard has been synthesized to attain a concentration of 0.2 µg/mL, 0.4 µg/mL, and 0.8 µg/mL successively. Kymograph was used to monitor responses. The graph was plotted by taking the concentration of the standard or test on the X-axis and % inhibition on the Y-axis. The % inhibition of histamine effect was estimated and values have been illustrated in the respective table. % histamine inhibition was calculated by the following formula:

% inhibition of histamine = (a−b/a) × 100,

where a = height of histamine response (in cm)

b = height of standard or test response (in cm).

Results and Discussion

Physical characterization data of all products

A novel series of dinitrophenothiazine derivatives have been synthesized with appreciable yields according to the given chemical schemes. These compounds were synthesized by condensing either nitro-substituted chlorobenzene with substituted nitroaniline or with nitro-substituted anilines with chlorosubstituted chlorobenzene. The physical characterization of obtained products including meting point, Rf value, yield, and % yield has been reported in Table 3. All the newly synthesized products, i.e., dinitrophenothiazine derivatives were characterized based on elemental analysis, and spectral results of IR, NMR, as well as mass spectra have been discussed in above-given section "Analytical data of synthesized products."

The IR spectrum showed the characteristic peak at 3481 (NH), 1300 (C-N), 1631 (C=C of aromatic ring), 1587, 1300 cm^{-1} (C-NO_2) stretches; ^1H NMR spectra confirmed the presence of aromatic protons showing signals at (δ) 8.24-8.22 and 7.74-7.21. A broad singlet at δ 3.32 corresponding to the NH signal was also observed supporting the presence of the phenothiazine skeleton in the molecule. The molecular weight of the compounds has been ascertained by MS. Elemental analysis has provided pertinent findings, and the values were in the range of ± 0.4%.

In general, it was seen that the characterized products exhibited IR spectrum at 3481, 1300, 1631, 1587, and 1300 cm^{-1} corresponding to the presence of NH, C-N, C=C, C-NO_2 stretches in the compound. The obtained compounds exhibited the molecular ion peak at $[M+1]^+$ and [M+2] corresponding to their molecular weight P1–P25 and molecular formula. Also compounds that are chloro derivatives (P11–12, 13, 15, 16, 17, 21, 23, 24, and 25) exhibited very small [M+2] along with molecular ion peak. All synthesized compounds showed major fragments at 139 corresponding to the loss of phenyl and substituted phenyl moiety, and also loses of 15, 46, 81 and 122, 123 corresponding to the loss of various functional moieties present in the nucleus were also observed in regular pattern. All the characteristic signals have been summarized in section "Analytical data of synthesized products."

Pharmacological study

Statistical evaluation

All the values of % inhibition of histamine were manifested as mean ± standard error of the mean (SEM) and were examined for significance by two-way analysis of variance (ANOVA) (m observations per cell), and groups were compared by Tukey's test for individual comparison of groups with standard. *P* value was calculated and found moderately significant at the $P < 0.05$ level.[27,41,42] The newly synthesized dinitrophenothiazine derivative compounds have been tested for *in vivo* antihistaminic potential. The results are summarized in Table 4 and Figure 3.

Table 4: SEM (±) of % inhibition of histamine action

Product	% inhibition		
	Doses		
	0.2 µg/mL	0.4 µg/mL	0.8 µg/mL
P07	10.09 ± 1.25	22.84 ± 1.42	42.82 ± 2.19
P11	12.63 ± 1.58	24.45 ± 1.57	44.19 ± 1.34
P12	13.76 ± 1.20	24.55 ± 1.68	44.06 ± 1.49
P13	16.77 ± 1.46	24.40 ± 1.49	42.13 ± 1.34
P15	16.17 ± 1.73	26.06 ± 1.35	43.73 ± 1.12
P16	14.95 ± 1.19	25.08 ± 1.32	41.64 ± 1.21
P17	13.90 ± 1.27	23.70 ± 1.34	43.53 ± 0.74
P18	15.27 ± 1.17	24.95 ± 1.95	47.11 ± 1.20
P19	15.80 ± 1.30	23.13 ± 1.25	46.05 ± 1.17
P20	17.89 ± 1.40	25.35 ± 1.25	45.49 ± 0.58
Control	8.83 ± 1.20	19.11 ± 1.14	47.66 ± 0.91
Standard	22.20 ± 1.10	37.08 ± 1.24	71.95 ± 1.28

Note: % inhibition is speculated as mean inhibition ± SEM, n = four guinea pigs, $P < 0.050$ compared with standard. Data were analyzed by using two-way ANOVA (m observations per cell) followed by Tukey's test

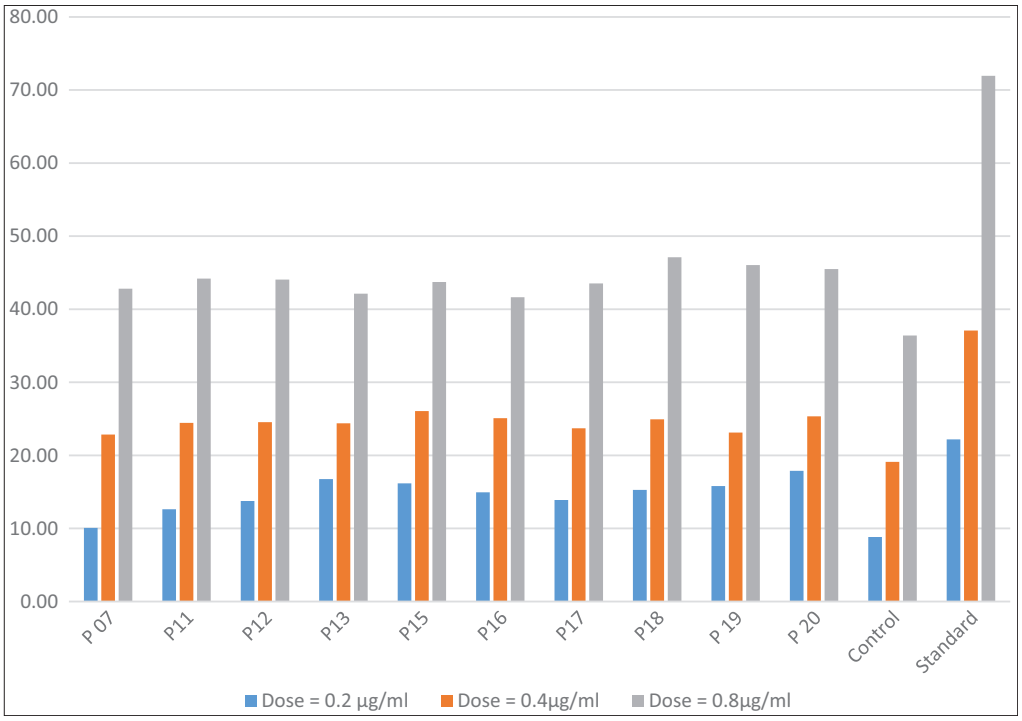

Figure 3: Comparison of the antihistaminic activity of the synthesized products

% inhibition of histamine action

Figure 3 shows the % inhibition of histamine action. All screened products have shown the strong, moderate, and weak % of histamine inhibition effectively at a dose of 0.8 µg/mL, 0.4 µg/mL, and 0.2 µg/mL, respectively. However, product 7 showed a minimum effect, which indicated that the introduction of the chloro group in the basic skeleton has a pronounced effect on the activity. Hence, the introduction of another chloro group in the basic Skelton P18, P19, and P20 and effect was found to be much higher, with P18 being a highly effective compound in the series as compared to other compounds. These newly synthesized products have been compared with the standard drug mepyramine at all dose levels. Thus, phenothiazine derivatives can be used as one of the choices for the antihistaminic drug.

Conclusion

Phenothiazine moiety belongs to the crucial class of therapeutic compounds and is widely exploited for new investigations and developments. A major modification in the substitution of the phenothiazine ring often produces a considerable difference in therapeutic activities because of the versatile properties of the substituent.

In our research study, it could be concluded that the synthetic methods are appropriate with better yields. It is found that

dinitrophenothiazine derivatives showed a wide spectrum of antihistaminic activity, exhibiting an equal inhibition of the effect of histamine. The majority of the synthesized products of the dinitrophenothiazine series have shown efficacious and auspicious antihistaminic activity, as exhibited by mepyramine (standard drug). Some newer synthesized products, i.e., P07, P11, P12, P13, P15, P16, P17, P18, P19, along with P 20, showed a great extent of antihistaminic activity at 0.8 µg/mL, which is equal to the known standard drug. Among the series, compound P18 was found to be showing maximum antihistaminic effect. It can be stated from research findings that these newly synthesized dinitrophenothiazine derivatives are promising new antihistaminic agents that can be exploited for the treatment of allergic disorders after detailed toxicity studies.

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Nil.

Conflict of interest

There are no conflicts of interest.

Ethical statement

Consent of all the authors was taken before submitting the article for publication. Further this material is the authors' own original work, which has not been previously published elsewhere and is not currently being considered for publication elsewhere. The protocol was approved by the institutional review board at each center. The study includes animal models and the study was conducted in accordance with CPSCEA norms and approved by IAEC.

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