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"Synthesis and Antihistaminic Activity of Some Novel Dinitrophenothiazine Derivatives"

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Abstract: Phenothiazine are one of the heterocyclic compounds with very important pharmacological activities. Phenothiazine is a benzo derivatives of thiazine now most commonly used as an intermediate chemical in the manufacturing of various psychiatric drugs.In this view there have been synthesized some novel dinitrophenothiazine derivatives from chloronitrobenzene derivatives and with nitroaromatic amine derivatives in presence of dimethylformamide with anhydrous potassium carbonate and copper powder. The product formed here than reacted with sulphur and iodine in presence of diphenylether. The newly formed dinitrophenothiazine derivatives showed better and marked antihistaminic activity.
Keywords: Dinitrophenothiazine, Dimethylformamide, Diphenylether, Antihistaminic activity.

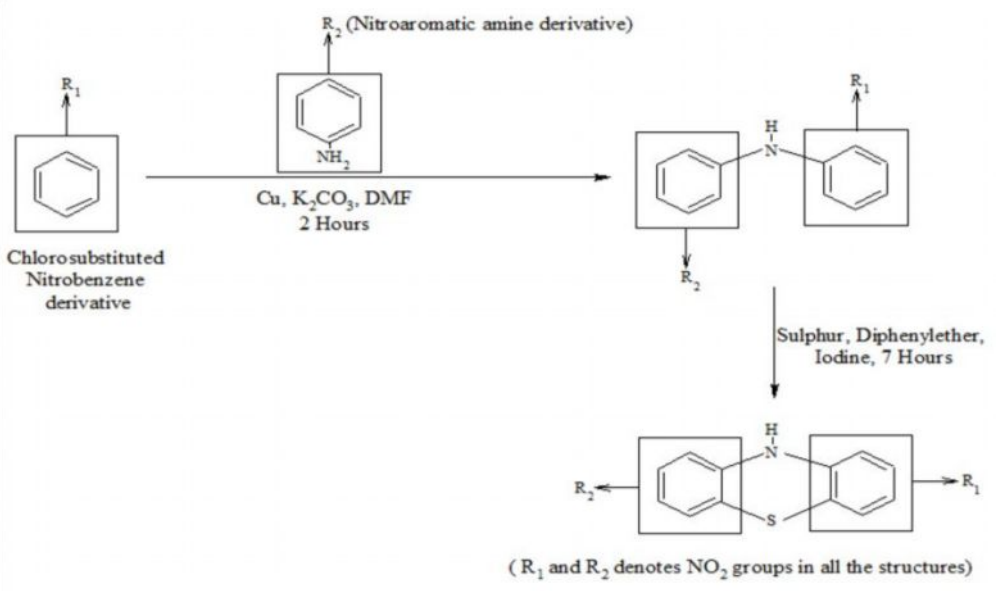
Introduction

In the heterocyclic system containing tricyclic ring which contain sulphur and nitrogen atom at five and ten position respectively are very important class in medicinal chemistry. Phenothiazines are pharmaceutically very active and potent compounds and possess numerous pharmacological activities.[5, 6, 20] Their antibacterial, antifungal, antipsychotic and antihistaminic etc. properties have been widely reported and also accepted successfully. The area of interest in phenothiazine relevant compounds has given an enormous amount of research on new structural modification to improve the wide spectrum of antibacterial and antihistaminic activity as well as to improve their bioavailability and therapeutic index potential parameters (margin of safety).[7,8,21] Chlorpromazine was one of the first compound used as a neuroleptic to treat symptoms of psychosis. The earlier phenothiazine derivative, methylene blue, in addition to many other applications has been used as a treatment for parasitic, bacterial and viral infections, for cancer treatment and most recently for the treatment of dementia such as Alzheimer's disease (AD).[9]

In this study the synthesis of several substituted dinitrophenothiazine derivatives based on the Ullman coupling of chloronitrobenzene derivatives with different nitroaromatic amine derivatives takes place. It provided better yield.[10, 11, 22] In my research work the use of diphenylether used as specifically as a solvent decreases the reaction time period, increase in the % yield and improves the purity and safety parameter up to a valuable extent. All the newly synthesized products have been reported to give rise to remarkable and valuable antihistaminic activity, their antibacterial activity have been also reported successfully.[12, 23, 24]

Experimental:

Chemical Scheme: All the compound of dinitrophenothiazine series will be prepared by the following chemical scheme successfully.



(Preparation of the compounds and their Antihistaminic Activity)

Formation of the products have been checked by thin layer chromatography (TLC) on silica gel-G plates of 0.5mm thickness and spots were properly located by iodine. All the melting points were determined by capillary melting point apparatus. All the newly synthesized compounds were characterized by IR (using the KBr pellets) and ^{1}H NMR spectra data dissolved in DMSO using TMS as an internal standard.

General Procedure for the Preparation of Dinitrophenothiazine derivatives (Product 1-7)

A mixture of chloronitrobenzene derivative and nitroaromatic amine derivative were added in dimethylformamide (DMF) taking anhydrous potassium carbonate and copper powder as catalyst, then reflux for 2hours. The intermediate product has been formed. This product is then treated with sulphur taking diphenylether as solvent and iodine has been added in small amount. Then it was refluxed continuously for 7 hours.[13, 25, 26.] Then the final product has been formed that is dinitrophenothiazine derivatives.

Similarly all the seven dinitrophenothiazine derivatives have been formed in this manner.

Physical Data of the compounds(Product1-Product7)

Compound	R^1	R^2	Molecular formulae	M.Range(^{0}C)	Yield (%)
Product1	$2NO_2$	$6NO_2$	$C_{12}H_7N_3O_4S$	210-212	55.1
Product2	$2NO_2$	$8NO_2$	$C_{12}H_7N_3O_4S$	215-217	57.4
Product3	$2NO_2$	$9NO_2$	$C_{12}H_7N_3O_4S$	217-219	60.8
Product4	$4NO_2$	$6NO_2$	$C_{12}H_7N_3O_4S$	207-209	64.2
Product5	$4NO_2$	$7NO_2$	$C_{12}H_7N_3O_4S$	214-216	56.2
Product6	$4NO_2$	$8NO_2$	$C_{12}H_7N_3O_4S$	208-210	62.3
Product7	$4NO_2$	$9NO_2$	$C_{12}H_7N_3O_4S$	218-220	57.2

IR and ^{1}HNMR Spectral Data of the Compounds (Products 1-7)

Compounds	IR (KBr pellets) cm^{-1}	^{1}HNMR
Product 1	3432-------N-H stretch 3334----------N-H Asym. stretch 3095 (C-H stretch aromatic) 1625 (C= C stretch) 1521(C-S-stretch), 1349 (O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz) 9.63-8.14 (brs, 2H-H1&3), 8.11-7.95-(s,1H-H-7), 7.84-7.82 (s,1H-H-4),7.68-7.66 (s,1H-H8)7.64-7.61(s,1H-H-9) & 2.53 (brd hump1H-NH)
Product 2	3432---NH stretch 3321-------- N-H Asym. stretch 3029 (C-H stretch aromatic) 1625 (C= C stretch) 1484 (C-S-stretch),1348 (O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz)7.33-7.27(brs,2H H1 & 3), 7.25-7.24(brs,2H-H 7 & 9), 7.22-7.20 (s,1H-H-4),6.92-6.90 (s,1H-H6), & 3.31 (brd hump1H-NH)
Product 3	3482---NH stretch	^{1}HNMR(DMSO-D_6-400MHz)7.34-7.32(brs, 2H-H1 &

	3362-------- N-H Asym. stretch 3107 (C-H stretch aromatic) 1631 (C= C stretch) 1471 (C-S-stretch), 1299 (O-N-O stretch)	3), 7.30-7.10 (s,1H-H-4), 7.08-7.06 (s,1H-H-6), 6.98-6.96 (s,1H-H-7) & 3.51 (brd hump1H-NH)
Product 4	3482---NH stretch 3361-------- N-H Asym. stretch 3220 (C-H stretch aromatic) 1637 (C= C stretch) 1481 (C-S-stretch),1182 (O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz) 8.94 (s, 1H-H3), 8.56 (s,1H-H-1), 7.64 (s,1H-H-8),7.35-7.11(s,1H-H-9, & 3.40 (brd hump1H-NH)
Product 5	3457---NH stretch 3354-------- N-H Asym. stretch 3224 (C-H stretch aromatic) 1606 (C= C stretch) 1465 (C-S-stretch), 1309(O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz) 8.24-8.18 (brs,2H-H-6 &8),8.16-7.91(s,1H-H-3), 7.89-7.69 (s,1H-H-1), 7.67-6.68 (s,1H-H-2), 6.57-6.55(s,1H-H-9) & 3.36 (brd hump1H-NH)
Product 6	3487---NH stretch 3326-------- N-H Asym. stretch 3012 (C-H stretch aromatic) 1614 (C= C stretch) 1337 (C-S-stretch), 1157(O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz) 7.37-7.35 (brs, 2H-H 6 &7),7.33-7.12 (s,1H-H 3)7.10-7.08 (s,1H-H-1), 6.98-(s,1H-H-2), 6.96 (s,1H-H-6) & 3.32(brd hump1H-NH)
Product 7	3412---NH stretch 3319-------- N-H Asym. stretch 3031 (C-H stretch aromatic) 1589 (C= C stretch) 1489 (C-S-stretch), 1234 (O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz) 7.37-7.35 (s,1H-H-3), 7.33-7.30(s,1H-H-8), 7.24-7.12 (s,1H-H-1),7.10-7.09 (s,1H-H-2), 6.98 (s,1H-H-6), 6.96 (s,1H-H-7) & 2.47(brd hump1H-NH)

Estimation of Antihistaminic Activity

The products (1-7) were evaluated for their anti-histaminic activity on guinea pig ileum.Guinea pig of either sex 400-500 g were used in this study. These animals were sacrificed by stunning and exsanguination. The abdomen were opened with scissors and lifted the caecum to trace the illeo-caecal junction. A required length of the long ileal portion was cut, removed and immediately placed on the watch glass containing tyrode solution.[14,15, 34] Then the mesentery was trimmed and with gentle care the contents of the ileum were cleaned by pumping the tyrode solution into the lumen of the ileum. The ileum was cut into segments of 2 to 3 cms,length. One piece of ileum was taken and a thread was tied to the top &bottom ends without closing the lumen and the tissue was mounted in the organ bath containing tyrode solution, maintained at 37^0C and bubbled with oxygen air. A tension of 0.5 g is applied and tissue is allowed to equilibrate for 30 minutes before adding drugs to the organ bath.[32,33]

The concentration dependent Response due to histamine were recorded using frontal writing lever. A contact time of 30 seconds and 5 minutes are kept for proper recording of the responses. Initially histamine dose was given with a concentration of 0.1 µg/ml, 0.4 µg/ml and 0.8 µg/ml.From that0.4 µg/ml concentration was selected as sub-maximal dose.[16, 17,27,28,31]

Test Solution Preparation: 10 mg of each test compound was dissolved in DMSO solvent. Different solutions were made with DNS (Dextrose Normal Saline), solution to get a concentration of 0.1 µg/ml, 0.2µg/ml, 0.4µg/ml and 0.8µg/ml.The responses were recorded on a kympograph.The graph was plotted taking a concentration of the test or standard on X-axis and % inhibition on Y-axis. The % inhibitions were calculated and values are shown in the table given below.[17,18, 19,29]

% histamine inhibition=a-b÷a×100, Where a=height of histamine response (in cm)

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

% Histamine inhibition of newly synthesized Dinitrohenothiazine derivatives

Sample code	% inhibition			
	0.1 µg/ml	**0.2 µg/ml**	**0.4 µg/ml**	**0.8 µg/ml**
BN_1	4.3	10.2	21.1	42.1
BN_2	11.2	25.4	49.1	65.25
BN_3	10.2	27.3	43.8	57.2
BN_4	7.1	20.8	32.35	48.56
BN_5	6.1	19.7	27.9	48.41
BN_6	9.1	22.21	31.8	46.81
BN_7	2.4	10.2	42.7	56.7

Results and Discussions

The seven novel dinitrophenothiazine were synthesized by coupling of chloro nitrobenzene derivative and nitroaromatic amine derivative in presence of dimethylformamide (DMF), copper powder and anhydrous potassium carbonate. In final step the intermediates have been treated with sulphur powder and very small amount of iodine taking Diphenylether as a solvent. The finally prepared dinitrophenothiazine compounds were purified by recrystallization or columnchromatography. The structure of the newly synthesized compounds were characterized on the basis of their spectral data.

The newly synthesized compounds showed moderate to considerable antihistaminic activity. Among the seven dinitrophenothiazine derivatives the main electron withdrawing substituents that is 2, 8 & 2, 9 and 4, 9 dinitrosubstitutedphenothiazine compounds (Product 2, product 3 and product7) showed better antagonistic activity. Although the other remaining compounds also possess marked antihistaminic activities.

The significant outcome of the study is the emergence of dinitro groups with phenothiazine nucleus as promising anti-histaminic agents. A further extension of this study in future may contribute to the development of other useful antihistaminic agents.These dinitrophenothiazine derivatives can also helpful to possess other types of biological activity.

Conclusion

It is concluded from the scheme that it is appropriate and efficient method for the synthesis of substituted dinitrophenothiazine with excellent yield that have been developed now. Other related libraries are under investigation. In conclusion the antihistaminic activity of dinitrophenothiazine derivative showed wide spectrum of activity exhibiting an equal inhibition of the histamine. The some compounds possessing activity almost equal to the reference drug like Diphenhydramine, promethazine etc.Some newer synthesized compound like compound C Compound D and Compound G showed up to great extent activity at 0.8µg/ml equal to the known reference drug like diphenhydramine and promethazine etc. The compound C and compound D as well as compound G showed nearby equal growth inhibition of histamine when compared with the standard drug Diphenhydramine and promethazine (65.25, 57.2 and 56.7).

It can be stated that these derivatives are promising new antihistaminic agents in treating allergic disorders. The present result is worth noticing because in recent years increasing rates of antihistaminic resistance among community for treating allergic disorders. It is observed that introduction of a chloro group at para position increases the activity exceedingly then ortho and meta position. So finally it is concluded that the synthesized compounds were evaluated for antihistaminic activity by standard procedures with slight modifications and the results are compared with a known standard drugs. Therefore finally it is concluded that the newly synthesized dinitrophenothiazine derivatives showed prominent antihistaminicpotential but some compounds also possesses potent antimicrobial activities besides the antihistaminic activity. By the conclusion we can also plan to do further studies on this series of compounds for diverse types of pharmacological activities.

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