Research Article

Synthesis and Analgesic Activity of 1-(4-P-Toluidino)-2-Phenylamino-1, 3, 5-Triazine-6-yl-2-Methyl Imidazole

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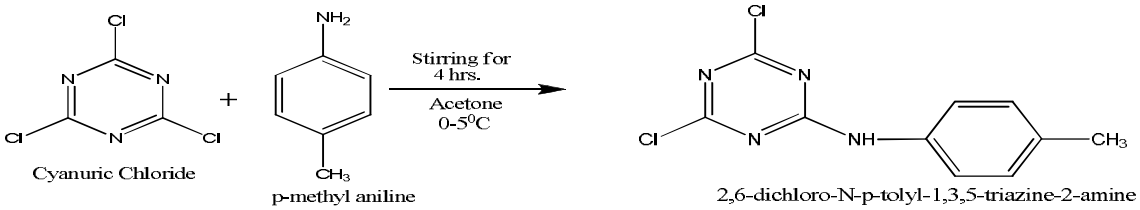
ABSTRACT
Triazine is the chemical species of six-membered heterocyclic ring compound with three nitrogens replacing carbon-hydrogen units in the benzene ring structure. The names of the three isomers indicate which of the carbon- hydrogen units in the benzene ring position of the molecules have been replaced by nitrogens called 1,2,3-triazines. The triazine derivative of 1-(4-p-toludino)-2-(phenylamino)-1,3,5-triazine-6-yl-2-methyl imidazole was synthesized by condensation method by using various amines. The final synthesized compound structure elucidated by spectral analysis and screened for analgesic activity.

Keywords: Triazine, Phenylamine, spectral analysis, analgesic.

INTRODUCTION
Triazine is the chemical species of six-membered heterocyclic ring compound with three nitrogens replacing carbon-hydrogen units in the benzene ring structure. The names of the three isomers indicate which of the carbon- hydrogen units in the benzene ring position of the molecules have been replaced by nitrogens called 1,2,3-triazines.1,2,4-triazines and 1,3,5-triazine respectively[4].

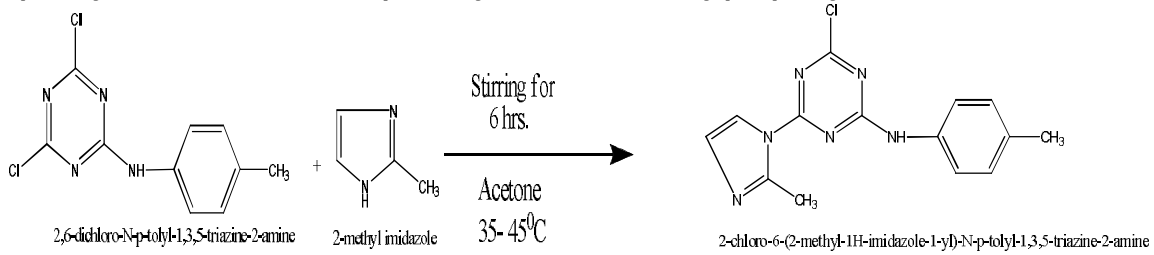
MATERIALS AND METHODS
Scheme of the work
Step 1: Synthesis of 2, 6-dichloro-N-p-tolyl-1, 3, 5-triazine-2-amine



Cyanuric Chloride — p-methyl aniline — 2,6-dichloro-N-p-tolyl-1,3,5-triazine-2-amine

The Chlorine atom of 2, 4, 6-trichloro-1, 3, 5-triazine was replaced by nucleophillic reagent eg. p-methyl aniline. 2,6-dichloro-N-p-tolyl-1,3,5-triazine-2-amine has been prepared by treating 2,4,6-trichloro-1,3,5-triazine in acetone with p-methyl aniline at 0-5°c and stirring for 4 hrs.

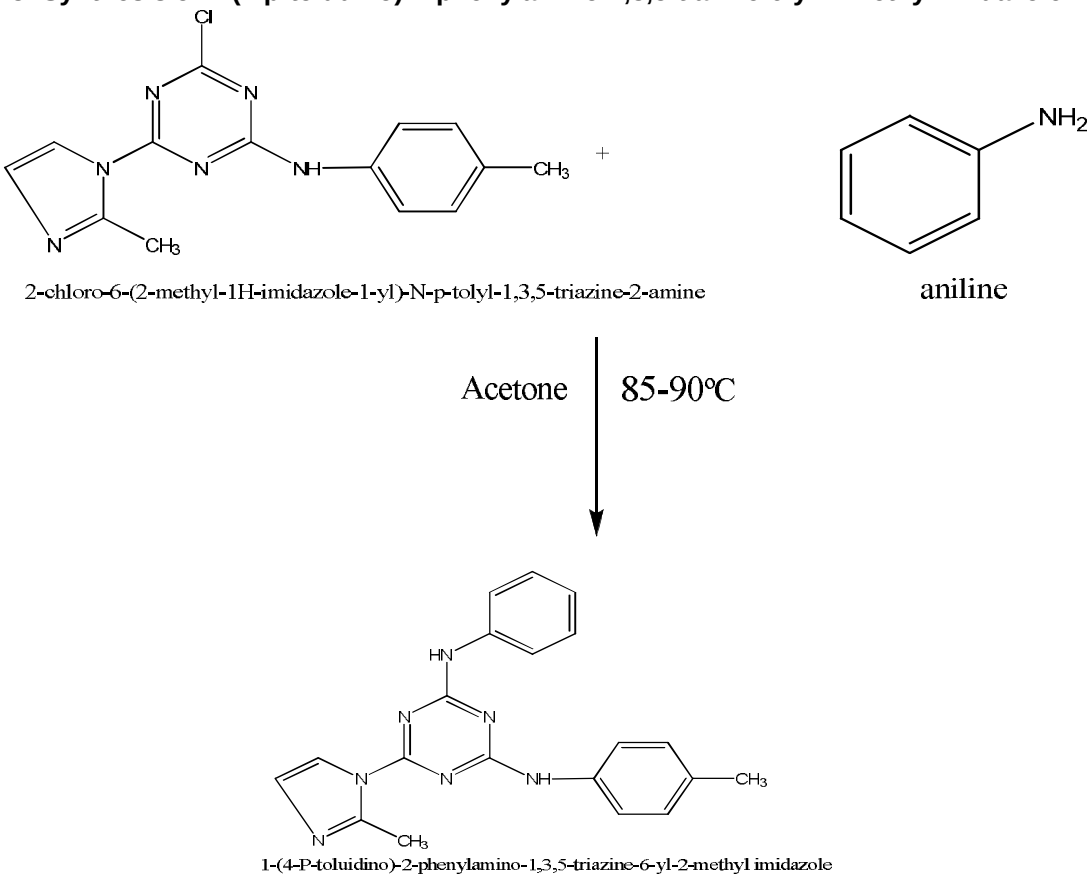
Step 2: Synthesis of 2-chloro-6-(2-methyl-1H-imidazole-1-yl)-N-p-tolyl-1, 3, 5-triazine-2-amine



2,6-dichloro-N-p-tolyl-1,3,5-triazine-2-amine — 2-methyl imidazole — 2-chloro-6-(2-methyl-1H-imidazole-1-yl)-N-p-tolyl-1,3,5-triazine-2-amine

2-chloro-6-(2-methyl-1H-imidazole-1-yl)-N-p-tolyl-1,3,5-triazine-2-amine has been prepared by treating 2,6-dichloro-N-p-tolyl-1,3,5-triazine-2-amine in acetone with 2-methyl imidazole at 35-45°c and stirring for 6 hrs.

Step 3: Synthesis of 1-(4-p-toludino)-2-phenylamino-1,3,5-tiazine-6-yl-2-methyl imidazole



Compound -2 (2-chloro-6-(2-methyl-1H-imidazole-1-yl)-N-p-tolyl-1,3,5-triazine-2-amine) (0.01 mole) was dissolved in acetone (50 ml) then it was added to Aniline (0.01 mole) in acetone (50 ml) and contents are to be stirred for 3 hours at 85-90°C poured in to ice water and neutralized with sodium carbonate solution to get the product. Then it was filtered, washed, dried, and recrystallized from ethanol.

Physical characterization

- ✓ Molecular formula : $C_{20}H_{19}N_7$
- ✓ Molecular weight (gm) : 357.41
- ✓ Soluble in Methanol, Ethanol, DMSO and DMF.
- ✓ Melting point : 115ºC
- ✓ Melting points were determined using Veego Digital melting point apparatus.
- ✓ The purity of synthesis compound was monitored on TLC.
- ✓ Absorbent used : Precoated Silica gel- G plate
- ✓ Mobile Phase : Chloroform : Methnol (3:7)
- ✓ Rf value: 0.73

Biological screening

ANALGESIC ACTIVITY

MATERIALS AND METHODS

Acute toxicity

The acute toxicity study was carried out as per OECD-425 Guidelines. Mortality in each group within 24 hr was recorded. The animals were observed for a further 14 days for any signs for delayed toxicity. The compound has good margin of safety and did not show the lethal effects on the animals up to the doses of 500 mg/kg. Hence LD50 of triazine derivative considered as 500mg/kg, studies were carried out with 1/10 of the LD50 dose is 50mg/kg.

Evaluation of analgesic activity

Tail immersion method

Swiss albino mice were screened by exposure to the thermal stimulus. The mice showing positive response were divided in to four groups of six animals each. The animals of Group I, II, III and IV were received DMSO (1ml/kg/p.o.), indomethacin (10 mg/kg/p.o.) and triazine derivative i.e. (50 mg/kg) respectively. After half an hour of treatment, the tail of mice was dipped in warm water kept constant at 55±1^{o} C upto 2cm from the tip of the tail. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cut of time being 60 sec. The observations were made at 0 min, 30 min, 60 min, 120 min, and 180 min[9].

Acetic acid induced writhing test

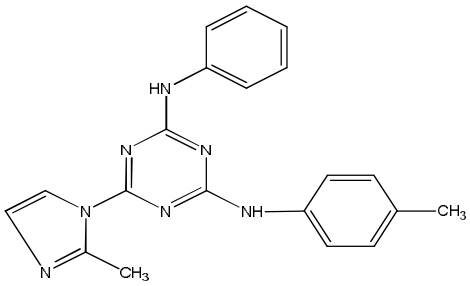
The triazine a derivative was evaluated for its analgesic activity by acetic acid induced writhing model. Swiss albino mice were divided in to four groups of six animals each. First group was served as a negative control received DMSO (1ml/kg). Second group served as positive control received indomethacin (10 mg/kg). While the third and fourth groups were administered orally with triazine derivative. Half an hour after the administration of above drugs 0.6% v/v acetic acid (10ml/kg) i.p was given to all animals and observed for 15minutes. The number of abdominal constriction (writhing) and stretching with a jerk of the hind limb was counted for 15 minutes after administering acetic acid[10].

% Protection = 1- (Experimental/control) x 100

Statistical analysis

One way analysis of variance (ANOVA) by Dunnett's method was employed using Graphpad instat 3.0 software for statistical analysis of the data. A probability value of < 0.01 was considered statistically significant. Values in the text and tables are represented as Mean ± SEM.

Spectral Analysis



IUPAC Name

1-(4-P-toluidino)-2-phenylamino-1, 3, 5-triazine-6-yl-2-methyl imidazole

IR Interpretation

I.R. Spectral data (KBr discs) (in Cm^{-1})	
N-H str.	3460.63
C=N str.	1508.06
=C-H str.	3523.31
C-N str.	1343.91

^{1}HNMR Interpretation

^{1}HNMR Spectral data Absorption position (in PPM)	
6.34 – 7.21	m, 19H, ArH
1.16	d, 3H, CH_3
2.35	s, 3H, CH_3
3.10, 2.85	d, 2H, CH_2
3.14	q, 1H, CH
4.0	s, 2H, NH
4.12	d, 1H, CH
4.13	t, 1H, CH

RESULTS AND DISCUSSION

Synthesis

The present study report the synthesis of triazine derivatives nucleophillic substitution of cyan uric chloride in p-methyl aniline was carried out stepwise at different temperature by various amines. The first step involve substitution of cyan uric chloride and the next by 2, 6-dichloro-N-p-tolyl-1, 3, 5-triazine-2-amine. The final triazine derivative in the synthesized compound 2 was replaced by aniline. Since the report regarding this compound suggest a triazine posses a good bioactive moiety[6].

Physical Characterization

Melting points of the synthesized compound was taken in open capillary tubes and was uncorrected and were found to be in the range 95-115˚C.

TLC was performed using precoated silica gel plates of 0.25mm thickness. Eluents used were chloroform, methanol (3:7) spots were visualised in U.V. light.

At room temperature solubility of newly synthesized compounds were determined by various organic solvents and it was found that all compounds were freely soluble in Methanol, Ethanol, DMSO and DMF.

Structural Confirmation

The Infra-red spectroscopy was performed with KBr on perkin FT-IR instrument. Presence of stretching in the range 700 cm^{-1} to 3900 cm^{-1} indicating the presence of NH functional group. Stretching between 1500 cm^{-1} to 1600 cm^{-1} indicates the presence of C=N characteristics. C-N stretching between at 1300 cm^{-1} to 1400 cm^{-1}.

^{1}H NMR spectroscopy was recorded on Bruker 400 MZs Avance. ^{1}H NMR the chemical shifts were reported as parts per million downfield from tetra methyl silane and solvent used as DMSO. Presence of chemical shift in the range 6.34-7.21 (m, 19H, ArH), 2.85-3.10 (d, 2H, CH_2).

ANALGESIC ACTIVITY

Tail immersion method

The analgesic effect of Triazine derivative (50 mg/kg) were studied by using tail immersion method and it was compared with the Group I. The Triazine derivative shows significant and almost equal to that of the positive control at 60 minute of post treatment.

Table 1: The analgesic activity of Triazine derivative by tail immersion method

Groups	Treatment	Dose (mg/kg)	Post Treatment Reaction Times In Seconds				
			0 min	30 min	60 min	120 min	180 min
I	DMSO	1 ml	2.5 ± 0.094	2.5 ± 0.094	2.75± 0.094	2.5± 0.094	2.75± 0.094
II	Indomethacin	10	2.75 ± 1.08	7.12 ± 1.08*	7.75 ±1.08 *	8.20± 1.08*	8.75± 1.08*
III	Triazine derivative	50	2.50± 1.01	6.30± 1.01*	6.73± 1.01*	7.80± 1.01*	8.20± 1.01*

Values are expressed as mean ± SEM (N=6), P<0.01* considered significant with respect to the control group.

Acetic acid induced writhing

The analgesic effect of Triazine derivatives (50 mg/kg) were studied by acetic acid induced writhing method. The Triazine derivative shown significant (p<0.01) reduction in the number of writhes induced by acetic acid when compared to Group I, which served as negative control.

Table 2: The analgesic activity of Triazine derivative by acetic acid induced writhing response in mice

Groups	Treatment	Dose (mg/kg)	Mean number of writhing(15 mints)	Percentage of protection
I	DMSO	1 ml	47.00± 1.238	0
II	Indomethacin	10	9.16 ± 0.477 *	80.51
III	Triazine derivative	50	13.2 ± 0.87 *	71.91

Values are expressed as mean ± SEM (N=6), P<0.01* significant with respect to the control group.

DISCUSSION

Acetic acid induced writhing and Tail immersion methods are used to study the action on the peripheral nervous system. The analgesic effect of triazine derivative was studied using the above said methods and it was compared with the Group I (DMSO 1ml/kg). Our results showed that acetone extract possessed good analgesic activity than alcoholic extract. The activity of triazine derivative is significant and is equipotent to that of the positive control at 60 minute of post treatment. Increase in the immersion time of the tail in hot water suggests that the extracts probably inhibit the production of

substance p and bradykinin. Acetic acid which causes nociception by liberating endogenous substances including histamine, serotonin, bradykinin and prostaglandin, which may stimulates pain. Therefore the triazine derivative might inhibit the synthesis and release of these endogenous substances.

ACKNOWLEDGEMENT

We are grateful to thankful to Mr.Lakshmisundaram Sri Ramachandra Medical Centre, Chennai for providing IR, ^{1}HNMR Spectral data.

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