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SYNTHESIS OF POTENTIAL & BIOLOGICALLY ACTIVE PYRIMIDINE HYDRAZONES DERIVATIVES

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ABSTRACT

The development of innovative compounds, pyrimidine hydrazones have been shown to possess a diverse variety of biological activities viz. antimicrobial, anticonvulsant, anti-oxidant, anti-inflammatory, analgesic, antimalaral, anticancer, antiviral, cardio protective etc., Pyrimidine constitutes an important class of heterocycles in drug discovery. Hydrazones is a class of organic compounds with the structure $R_1R_2C=NNH_2$. They are formed usually by the action of hydrazine on ketones or aldehydes & have efficient CNS depressant, analgesics activity. In the same context, Schiff bases of pyrimidne hydrazones were prepared by the reaction of "4-(4-Chloro-phenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid hydrazide" with citral, camphor, furfuraldehyde & menthone individually by refluxing them in the presence of glacial acetic acid (as solvent) for at least 2hrs to yield their respective derivatives. The present studies revealed that Pyrimidine hydrazone derivatives could be used to synthesize the compounds having potent biological activities such as anticancer, antimicrobial, antioxidant, CNS depressant, analgesic & antiviral activities. All the prepared derivatives were under investigation for their antimicrobial activity and likely to posses the same & desired action.

KEYWORD: Pyrimidine Hydrazones, antimicrobial, antioxidant and Schiff Bases.

INTRODUCTION

Pyrimidine

Heterocyclic aromatic organic compound like benzene or pyridine, having two nitrogen atoms at positions 1 and 3 of the six-membered ring; they have isomers in the forms of diazine.[1]

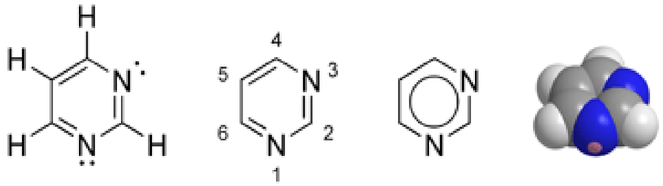
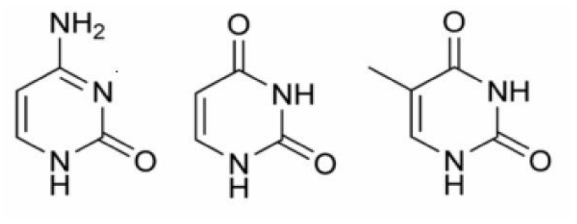


Figure 1.

IUPAC Name:	1, 3-Diazine, m-Diazine
Formula:	C4H4N2
Molecular Mass:	80.088 g mol-1
Density:	1.016 g cm-3
Solublity:	Alcohol, Water
Melting Point:	20-22 °C

Types of Pyrimidine

Three nucleobases found in nucleic acids, cytosine (C), thymine (T), and uracil (U), are pyrimidine derivatives.[1]



C **T** **U**

Figure 2.

Hydrazone

An organic compounds having structure of $R_1R_2C{=}NNH_2$ and are associated to ketones and aldehydes by substitution of the oxygen by means of NNH_2 functional group. They are designed basically by the feat of hydrazine on ketones or aldehydes.[2]

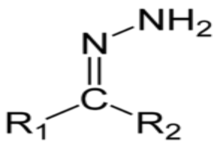


Figure 3.

Hydrazones have antioxidant, antimicrobial, antimalarial, antiviral actions and if they are allowed to fuse with Pyrimidine they produce CNS activity too. Therefore the above data clearly showed that pyrimidine hydrazones are potent biologically active compounds.

Schiff base

A **Schiff base**, invented by Hugo Schiff, is a compound with a functional group that consists of a C=N double bond by means of nitrogen atom connected to an aryl or alkyl group and having general formula of $R^1R^2C{=}NR$.[3] where R is an organic side chain. In this definition, *Schiff base* is identical to **azomethine**.[3]

General structure of a Schiff base General structure of an azomethine

Figure 4.

MATERIALS AND METHODOLOGY

Synthesis of pyrimidine hydrazone derivatives

Procedure

Synthesis of Pyrimidine hydrazone derivatives can be divided into following 3 steps:

Step (1)

Synthesis of ethyl 6-methyl-2-oxo-4-aryl-1, 2, 3, 4-tetrahydro pyrimidin-5-carboxylates.

Urea, ethylacetoacetate and aromatic aldehyde (0.1 mol) were mixed (equimolar amount) in ethanol (25 mL app.). A catalytic amount of conc. HCl (1ml) was added to the mixture, which was then refluxed for three hours. The contents were kept in refrigerator overnight. The solid separated out was filtered off. The filtrate was further refluxed on a water bath for 1.5 hour. A solid separated out on cooling was filtered and recrystallized from ethanol.

Where aromatic aldehyde used are as:

1). Benzaldehyde (C_6H_4CHO)

2). ó-Chloro-benzaldehyde (2-Cl-C_6H_4CHO)

3). þ-Chloro-benzaldehyde (4-Cl-C_6H_4CHO)

4). þ-Br-benzaldehyde (4-Br-C_6H_4CHO)

Step (2)

Synthesis of 6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidin-5-carbohydrazides.

To 0.1 mol of (i) in ethanol (25 mL), hydrazine hydrate was added, followed by a catalytic amount of conc. H_2SO_4 (3 drops). The mixture was refluxed for two hours. Excess solvent was removed and, on cooling, a solid was formed. The solid was crystallized from ethanol.

Step (3)

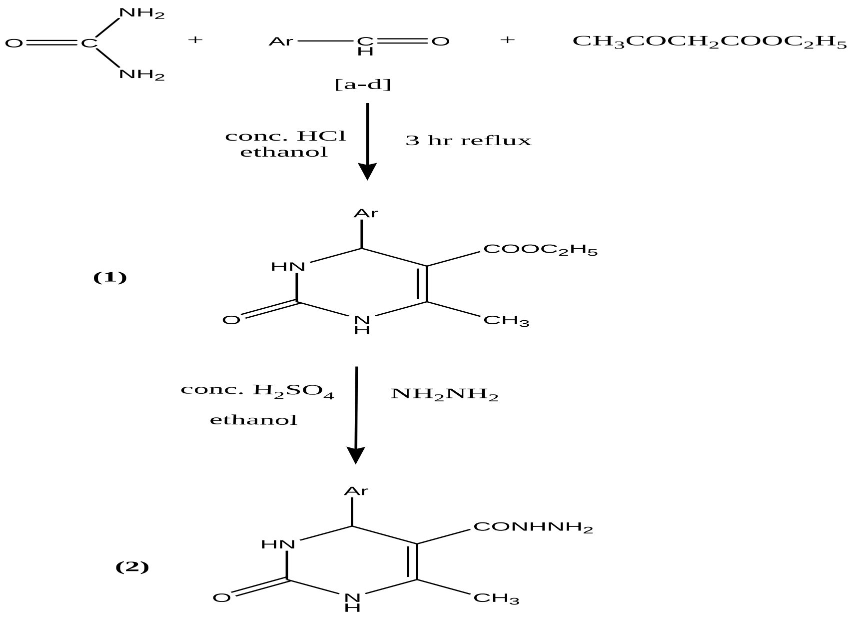
Synthesis of various Schiff bases of the above obtained pyrimidine hydrazones

On adding the final product of above ***step 2*** with ***Terpenes*** like camphor, citral, menthone and furfuraldehyde, we obtain following desired Schiff bases for the derivatives of Pyrimidine Hydrazone *(i.e.* ***A, B, C & D****)* respectively as per proposed scheme which are described & elaborated below on the synthetic scheme part:-

Scheme 1

Series of desired pyrimidine hydrazone derivatives will be synthesized as follows:-

Where: Ar = a: C_6H_5; **b:** 4-Br-C_6H_5; **c:** 2-Cl-C_6H_4; **d:** 4-Cl-C_6H_3

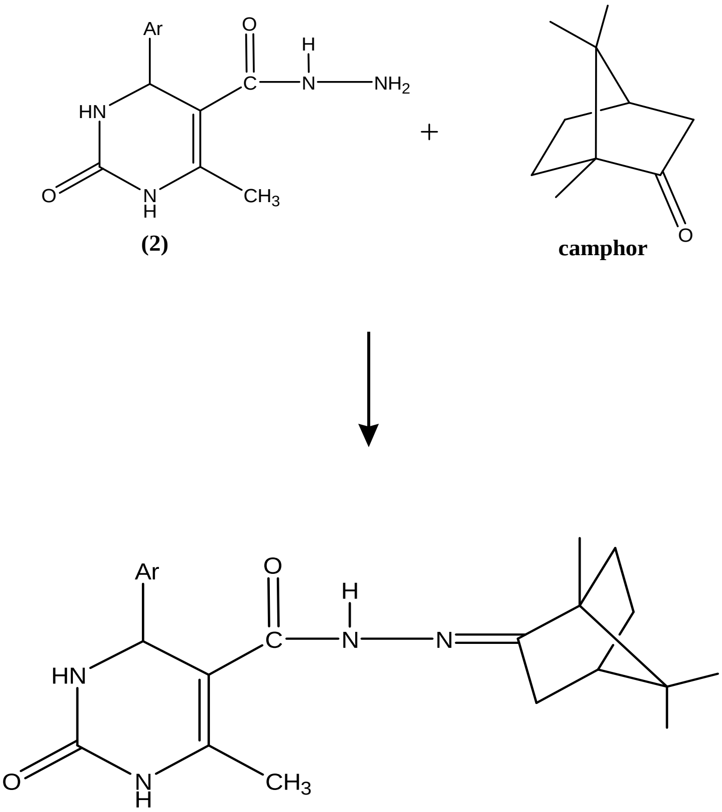


Step (3)

On adding the final product of above ***step ii*** with camphor & citral to yield the desired Schiff bases for the derivatives of Pyrimidine Hydrazone respectively as per scheme which are described & elaborated below on the synthetic scheme part:

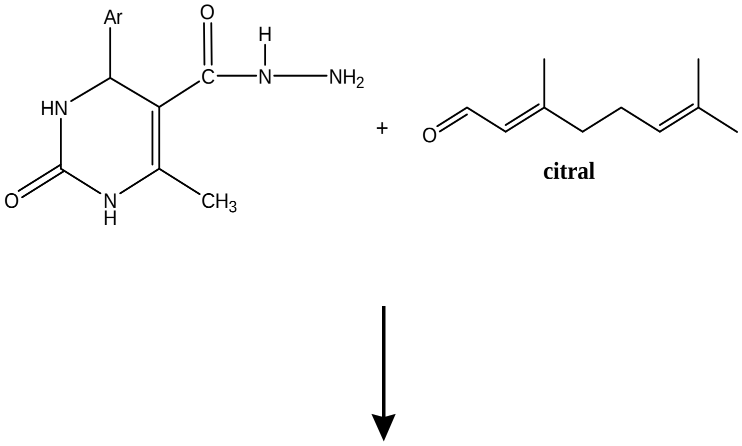
Where: Ar = a: C_6H_5; **b:** 4-Br-C_6H_4; **c**: 2-Cl-C_6H_4; **d:** 4-Cl-C_6H_4;

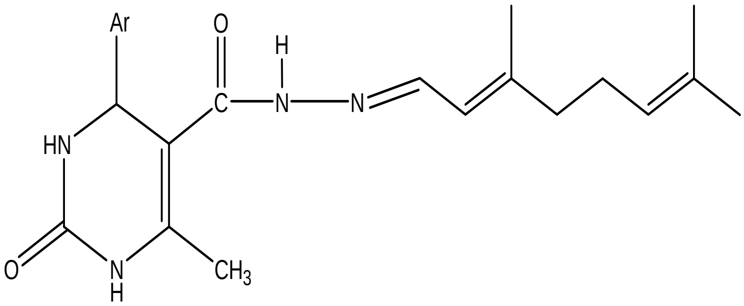
A).



SBP-1a; SBP-2b; SBP-3c; SBP-4d

B).





SBP-5a; SBP-6b; SBP-7c; SBP-8d

PHYSICOCHEMICAL DATA

TABLE 1.1.

COMPOUND CODE	Ar	*Ketone Type*	*M.P. (^{0}C)*	*% YIELD*	*Molecular Formula*	*Molecular Weight*	*Rf*	*Color*
SBP-1a	C_6H_5	Camphor	195	65%	$C_{22}H_{28}N_4O_2$	380.48	0.75	White
SBP-2b	4-Br-C_6H_4	Camphor	210	73%	$C_{22}H_{27}BrN_4O_2$	459.38	0.72	Yellow
SBP-3c	2-Cl-C_6H_4	Camphor	195	61%	$C_{22}H_{27}ClN_4O_2$	414.93	0.63	Yellow
SBP-4d	4-Cl-C_6H_4	Camphor	185	65%	$C_{22}H_{27}ClN_4O_2$	414.93	0.68	White
SBP-5a	C_6H_5	Citral	65	92%	$C_{22}H_{28}N_4O_2$	380.48	0.63	Black
SBP-6b	4-Br-C_6H_4	Citral	205	88%	$C_{22}H_{27}BrN_4O_2$	458.13	0.81	Yellow
SBP-7c	2-Cl-C_6H_4	Citral	63	76%	$C_{22}H_{27}ClN_4O_2$	414.93	0.82	Black
SBP-8d	4-Cl-C_6H_4	Citral	205	80%	$C_{22}H_{27}ClN_4O_2$	414.93	0.84	Brown

PHARMACOLOGICAL EVALUATION

Analgesic activity

Animals

Swiss mice (20-25 g) were obtained from Central Animal Facility, Saroj Institute of management and Technology and kept at 25 ± 1 °C, 55 ± 5 % humidity along with 12 hr light/dark cycle. The animals were given standard pellet diet (Lipton rat feed, Ltd., Pune) and water *ad libitum* throughout the experimental period. The experiment was approved by the 'Institutional Animal Ethics Committee'. The animals were kept in large spacious hygienic cages during the course of experimental period.

Analgesic activity using hot plate

The experiment was carried out using Glassman's method.[4] using hot plate apparatus, maintained at 55±0.5 0C. The mice were divided into 14 groups of 6 animals each. The reaction time of mice to the thermal stimulus was the time interval between placing the animal above the hot plate and it licked its hind paw or jumped. The reaction time was measured prior to administration of synthesized compounds and drug administration (0 min). Group 1 was kept as normal control. The synthesized compounds were injected subcutaneously to mice of groups 2-14 at a dose of 5mg/kg. Mice of group 14 served as standard and were treated with morphine sulphate 5mg/kg. The reaction time was again measured at 0, 30, 60 min after the treatment. To avoid tissue damage to the mice paws, cut-off time for the response to the thermal stimulus was set at 60s. The increase in the reaction time against control was calculated.

TABLE 1.2. Evaluation of analgesic activity using Hot-plate method

TREATMENT	DOSE mg/kg body wt.	TIME (min.)	MEAN ±SEM
Control	5 mg/kg	0	3± 0.24
		30	4 ± 0.24
		60	3 ± 0.24
SBP-1a	5 mg/kg	0	3 ± 0.24
		30	7.6 ±0.31
		60	9.8 ± 0.44
SBP-2b	5 mg/kg	0	3 ± 0.24
		30	9.5 ± 0.21
		60	7.5 ± 0.4
SBP-3c	5 mg/kg	0	3 ± 0.24
		30	5.5 ± 0.21
		60	10.33 ± 0.2
SBP-4d	5 mg/kg	0	3 ± 0.24
		30	11.66 ± 0.39
		60	14.33 ± 0.62
SBP-5a	5 mg/kg	0	3 ± 0.24
		30	10.1 ± 0.56
		60	11.33 ± 0.46
SBP-6b	5 mg/kg	0	3 ± 0.24
		30	14.33 ± 0.95
		60	10.66 ± 0.31
SBP-7c	5 mg/kg	0	3 ± 0.24
		30	8.6 ± 0.46
		60	10.3 ± 0.46
SBP-8d	5 mg/kg	0	3 ± 0.24
		30	9.16 ± 0.29
		60	11 ± 0.54
Morphine	*5 mg/kg*	*0*	*3 ± 0.24*
		30	*14 ± 0.32*
		60	*10 ± 0.30*

Values represent the mean ± SEM of six animals for each group.
****significant at $p<0.05$, **significant at $p<0.01$ (Dunnett's test)***

ANALGESIC ACTIVITY (ACETIC ACID INDUCED WRITHING RESPONSE MODEL)

The compounds were selected for investigating their analgesic activity in acetic acid induced writhing response in Swiss albino mice, following the method of *Koster et al*,.[5] The 14 groups of mice having 6 each were used for the experiment. The 1st group served as control and received only vehicle & the groups between 2 to 12 received the tested compounds. The last two groups received reference drugs as Diclofenac at a dose of 20 mg/kg & indomethacin at a dose of 10mg/kg.[6] After 30 min, each mouse was administered 0.6% of an aqueous solution of acetic acid (10mL/kg) and the mice were then kept in transparent boxes for observation. The numbers of writhes were counted for 20min after acetic acid injection. The number of writhes in each treated groups was compared to that of control group. The number of writhes was recorded and the percentage protection was calculated using the following ratio***: % protection = (control mean- treated mean/control mean) ×100.***

TABLE 1.3. Evaluation of analgesic activity of acetic acid induced writhing response model

TREATMENT	**DOSE (Mg/kg body wt.)**	**NO. OF WRITHING RESPONSE (mean±SEM) N=6**	**% ANALGESIC ACTIVITY**
CONTROL	20 mg/kg	27.16 ± 0.29	--
SBP-1	20 mg/kg	5.1 ± 0.29	81%
SBP-2	20 mg/kg	5 ± 0.24	81.5%
SBP-3	20 mg/kg	11.16 ± 0.29	58.9%
SBP-4	20 mg/kg	6.33 ± 0.31	76.6%
SBP-5	20 mg/kg	7.5 ± 0.21	72.43%
SBP-6	20 mg/kg	18.16 ± 0.24	33.13%
SBP-7	20 mg/kg	16.66 ± 0.46	38.65%
SBP-8	20 mg/kg	12.33 ± 0.31	54.46%
DICLOFENAC	*20 mg/kg*	*6.67 ± 0.34*	*87.62%*

Each value represents the mean ±SEM (n=6)
Significant levels *p<0.01 as compared with respective control

RESULT AND DISCUSSION

All the Pyrimidine hydrazones derivatives were evaluated for their analgesic activity using various methodology and tools with the help of central analgesic and peripheral analgesic assays.

Acetic acid induced model

The compound ***SBP-1a, SBP-2b*** was found to be the most active among all tested compound with ***81%***, ***81.5%*** analgesic activity, when compared with reference standard drug i.e., Diclofenac & even more active than another reference drug using peripheral analgesic assay.

Hot plate model

The compounds ***SBP-5a, SBP-6b, SBP-8d*** was found to be pharmacologically suitable compound among all the tested compounds with analgesic activity, when compared with reference drug i.e., Morphine and even more active & efficacious.

From the Table 1.2 The results showed that the compound ***SBP-1a, SBP-2b, SBP-5a, SBP-6b, SBP-8d*** are active compound against both Peripheral and Central analgesic assays respectively & revealed the desired potency and reliable efficacy. Thus the present research paves the way for increasing potency and spectrum of activity in various infectious and non infectious diseases by combining two drugs. It will help in the research associated with rational combination with respect to pharmacological compatibility and therapeutic optimization.

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