"Design and Synthesis of Pyrimidine derivatives as Potential Anti- Inflammatory Agents".

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Abstract:
Pyrimidines are aromatic heterocyclic compounds that contain two nitrogen atoms at positions 1 and 3 of the six-membered ring. Numerous natural and synthetic pyrimidines are known to exist. They display a range of pharmacological effects including antioxidants, antibacterial, antiviral, antifungal, antituberculosis, and anti-inflammatory. This review sums up recent developments in the synthesis, anti-inflammatory effects, and structure–activity relationships (SARs) of pyrimidine derivatives. Numerous methods for the synthesis of pyrimidines are described. Anti-inflammatory effects of pyrimidines are attributed to their inhibitory response versus the expression and activities of certain vital inflammatory mediators namely prostaglandin E2, inducible nitric oxide synthase, tumor necrosis factor-a, nuclear factor kB, leukotrienes, and some interleukins. Literature studies reveal that a large number of pyrimidines exhibit potent anti-inflammatory effects. SARs of numerous pyrimidines have been discussed in detail. Several possible research guidelines and suggestions for the development of new pyrimidines as anti-inflammatory agents are also given. Detailed SAR analysis and prospects together provide clues for the synthesis of novel pyrimidine analogs possessing enhanced anti-inflammatory activities with minimum toxicity

INTRODUCTION

Medicinal or pharmaceutical chemistry is a discipline at the intersection of chemistry that involves identification, synthesis and development of new chemical entities suitable for therapeutic use. It also includes the study of existing drugs, their biological properties, and their quantitative structure-activity relationships (QSAR).

Heterocyclic compounds are cyclic organic substances which contain at least one atom other than carbon in the ring system. It is evident that more than a third of the known organic compounds are heterocyclic. Many antiANTI-INFLAMATORY, anti-inflammatory, antidiabetics, antimalarial, alkaloids, vitamins, antibiotics and dye stuffs are heterocyclic, including building blocks of life like nucleic acid also. Five and six-membered heterocyclic ring containing compounds form an important part of medicinal chemistry as large number of drugs which exist as of today bear these nuclei.

Anti-inflamatory, a major health impediment for both developed and underdeveloped countries, involves

abnormal cell growth with the potential to invade or spread to other parts of the body. According to the World Health Organization (WHO) report, 8.8 million people died of Anti-inflamatory globally in 2015 [1]. Most Anti-inflamatory s are recognized by uninhibited growth of cells without demarcation due to the deregulation of crucial enzymes and proteins controlling cell division and proliferation. obesity, poor nutrition, physical inactivity and /or excess alcohol consumption are responsible for 20% of all Anti-inflamatory diagnosed . Infection due to Human papilloma virus (HPV), hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and Helicobacter pylori (H. pylori) are also a cause of Anti-inflamatory. Although much progress has been aspired from the identification to the treatment of Anti-inflamatory, factors like poor patient compliance, drug resistance and drug induced toxicities has provided a strong impetus for the discovery and development of novel Anti-inflamatory chemotherapeutic hybrids of clinical significance [2]. Consequently, in the development of effectual and discerning antiinflamatorydrugs having low incidence of side effects, toxicity and emergence of drug resistance is of high priority [2]. To address this issue, combination therapy was considered, where numerous cytotoxic hybrids were pooled in antiinflamatorybehaviour regimes that endorse improved results with fewer side effects [2].

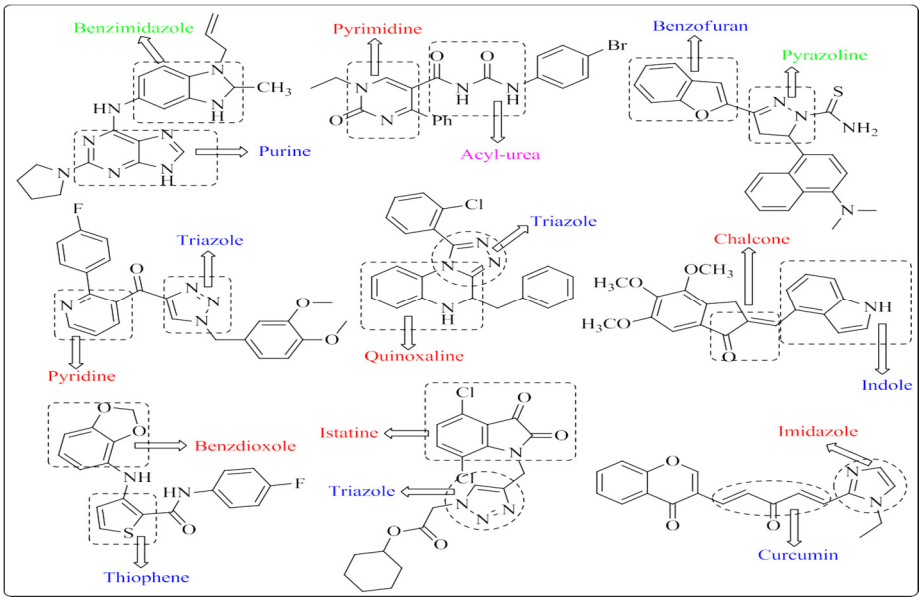
Hybrid Concept

The combination of two or more pharmacophores into a single molecule is an effective and commonly used direction in modern medicinal chemistry for the exploration of novel and highly active compounds. **Bleomycin** isolated as an antibiotic from *Streptomyces verticilus* is an efficient antiinflamatoryagent and an outstanding prototype of hybrid molecule obtained from nature. It consists of three important core units (i) Deoxyribonucleic acid (DNA) binding unit, (ii) metal binding unit, and, (iii) carbohydrate core [3,4].

Hybrid molecules are defined as compounds having more than one pharmacologically active scaffolds, each acting on different or the same target through different mode of action. This concept can be considered as extension of the combination therapy, where-in two different drugs are administered simultaneously.

Sharma *et al*. reported the synthesis and antiinflamatoryevaluation of benzimidazole-purine hybrids [5]. Koca *et al*. reported pyrimidin-acylurea hybrids and evaluated antiinflamatoryactivity against human bone osteosarcoma (Saos-2) and human breast (MCF-7) cell lines [6]. Qin *et al*. synthesized pyrazoline-benzofuran hybrids with their anti-proliferation activity against various Anti-inflamatory [7]. Kamal *et al*. and developed pyridine-triazoles and tested for their cytotoxicity against Ht-29, DU-145 and A549 cell lines with IC_{50} value in the range of 0.1-4.1 µM) [8]. 1,2,4-Triazolo-quinoxalines analogues were designed, synthesized and tested for their antiinflamatorypotential against several Anti-inflamatory cell lines by Issa *et al* [9]. Chen *et al*. synthesized chalcone-based hybrids and tested their anti-proliferative activity against A549, HeLa, Bel-7402, PC-3 and K562 cell lines with IC_{50} values in the range of 0.026-0.035 µM [10]. Mudududdla *et al*. prepared a series of thiophen-based hybrids and examined their *in vitro* vascular endothelial growth factor receptors (VEGFR) inhibition activity [11]. The hybrids of isatin-triazole motifs synthesized by Yu *et al*. exhibited good Anti-inflamatory activity against various cell lines [12]. Chen *et al*. evaluated the anti-proliferative activity of curcumin-imidazol hybrids against several human Anti-inflamatory cell lines [13]. The reported hybrids molecules are given in (**Figure-1).**

Figure-1: Hybrids reported as Anti-inflamatory agents



Rationale:

Pyrimidine, a six membered heterocyclic ring is considered to play a vital role in drug discovery process and has diverse biological and pharmacological activities. Significant work has been done in the field of development of pyrimidine based antiinflamatorydrug candidates (**Figure-2**).

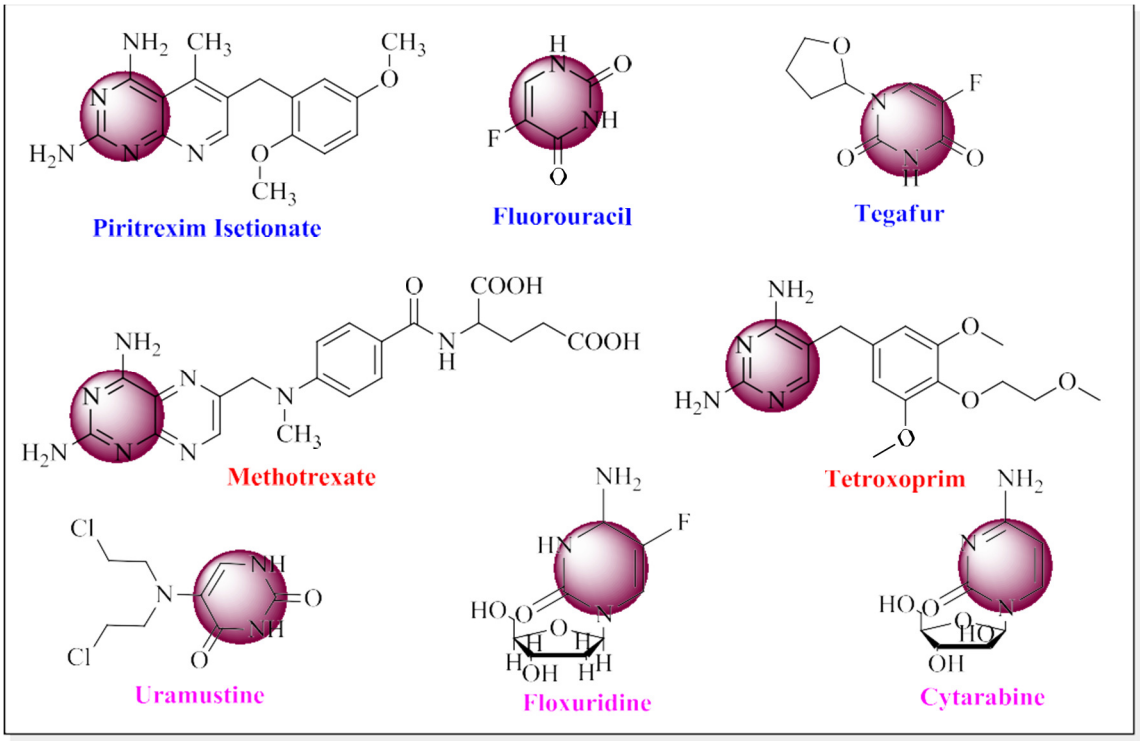


Figure-2: Pyrimidine containing anti-neoplastic drugs

Enough work has been reported enumerating the antiinflamatorypotential of pyrimidine (**Figure-3**). Wang *et al.* developed Pyrido[3,2-*d*]pyrimidines with nonclassical lipophilic antifolates targeting dihydrofolate reductase [14]. Qu *et al*. developed sulfonamide- substituted diphenylpyrimidine derivative with antitumor activity [15]. Pyrazolo[3,4- *d*]pyrimidines synthesised by Elshaier *et al.*, exhibited good degree of hepatocellular carcinoma and analgesic activity [16]. Sulfonamide-substituted diphenylpyrimidines synthesised by Liu *et al.*, showed good activity against B lymphoma cell lines with IC_{50} of 6.49μM [17].

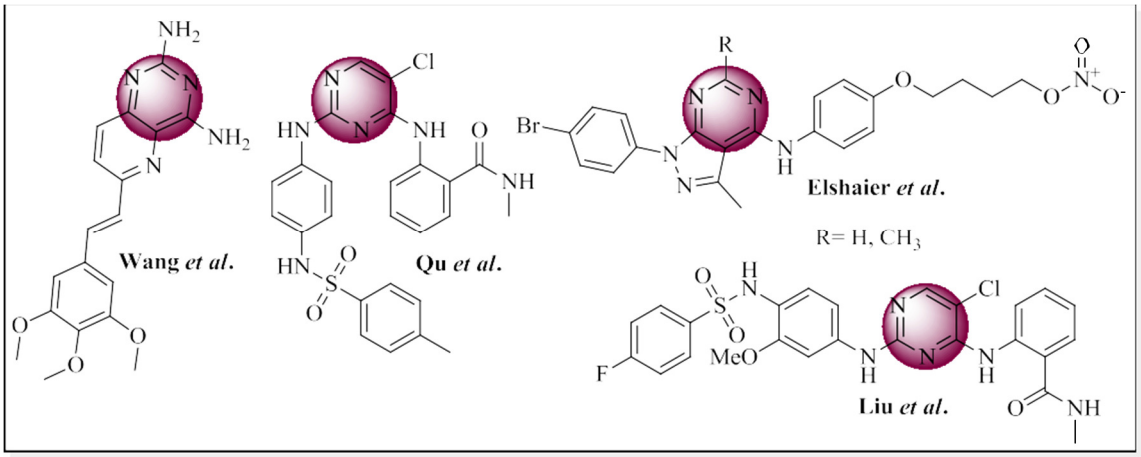


Figure-3: Pyrimidine containing anti-neoplastic derivatives

Pyrazoline, an unsaturated five-membered ring, contains two adjacent nitrogen atoms. It is present as an active moiety in a number of compounds, which has been known to possess wide spread biological properties [18]. Significant work has been done in the field of development of pyrazoline based drug candidates (**Figure-4**).

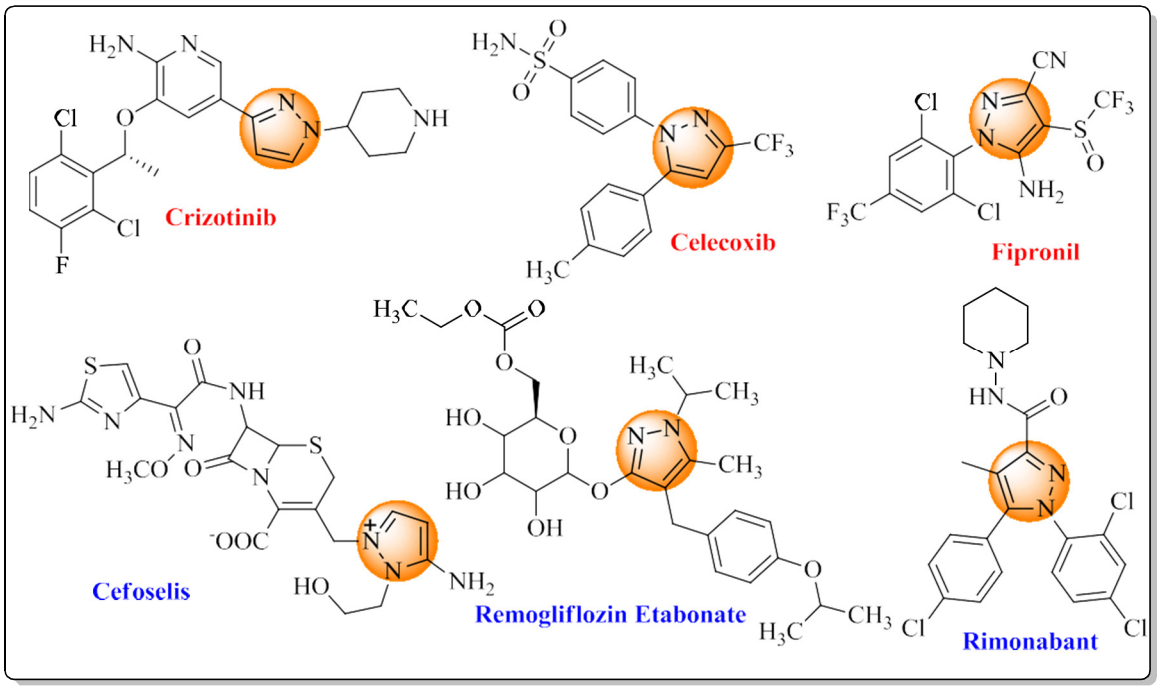


Figure-4: Pyrazoline containing drugs

Various work has been reported enumerating the antiinflamatorypotential of pyrazolines (**Figure-5**). Prada *et al*. synthesized quinazoline based dihydropyrazoles compounds and determined their antiinflamatoryactivity against various Anti-inflamatory cell lines [19]. Yang *et al*. reporte antiinflamatoryactivity of pyrazoline with inhibitory concentration IC_{50} of 0.04 µM against human melanoma cell line [20].George *et al*. reported Pyrazoline derivatives and tested them for their antiproliferative activity against with IC_{50} value of 8.33, 1.67 and 10 µM against HepG-2, MCF-7 and CaCo-2 Anti-inflamatory cell lines, respectively [21]. Altintop *et al*. synthesized a series of thiazolyl-pyrazoline derivatives and evaluated their antiinflamatory potential against A549 cell line [22].

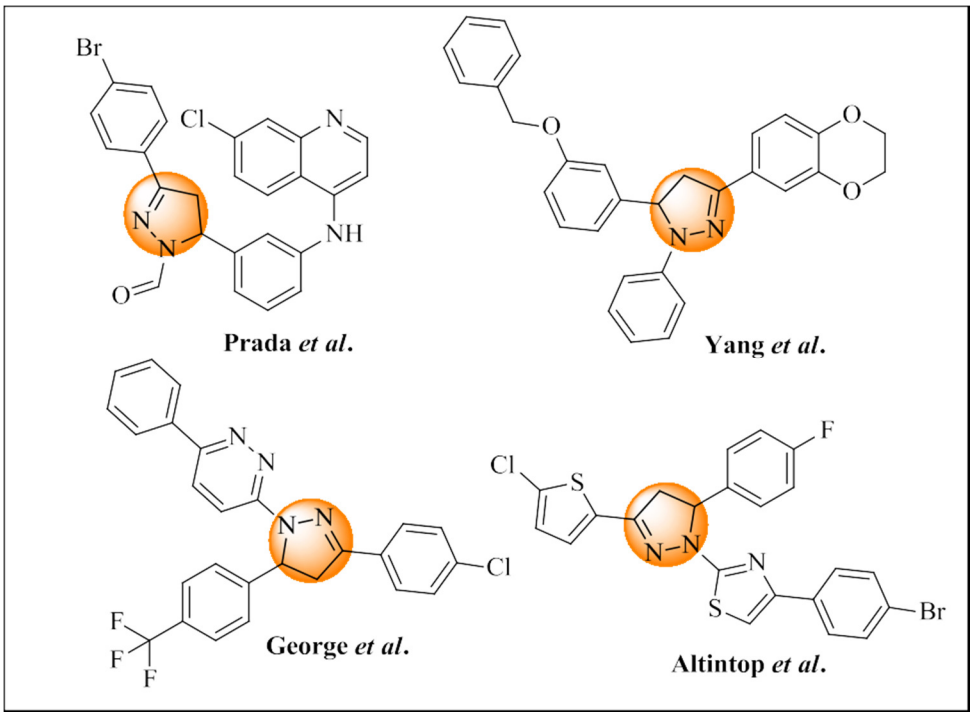


Figure-5: Pyrazoline containing anti-neoplastic derivatives

Taking the pharmacological relevance of pyrimidine and pyrazoline, it was incorporated with other bioactive features, on the premise that their presence in tandem in a single molecular framework can significantly contribute to the antiinflamatoryactivity of the resulting molecules.

Keeping this hybrid concept in mind and considering the importance of above moieties, pyridazinone and isobutyl/pentyl was clubbed with pyrimidine whereas pyrazole was clubbed with pyrazoline nucleus (**Figure-6**):

[A] PYRIMIDINE DERIVATIVES:

(i) **SCHEME-1**: Pyrimidine-Pyridazinone hybrids (**WPP Series**)
(ii) **SCHEME-2**: Pyrimidine-Isobutyl/Pentyl hybrids (**WPBA & WPPA Series**)

[B] PYRAZOLINE DERIVATIVES

(iii) **SCHEME-3**: Pyrazole-Pyrazoline hybrids (**WSPP Series**)

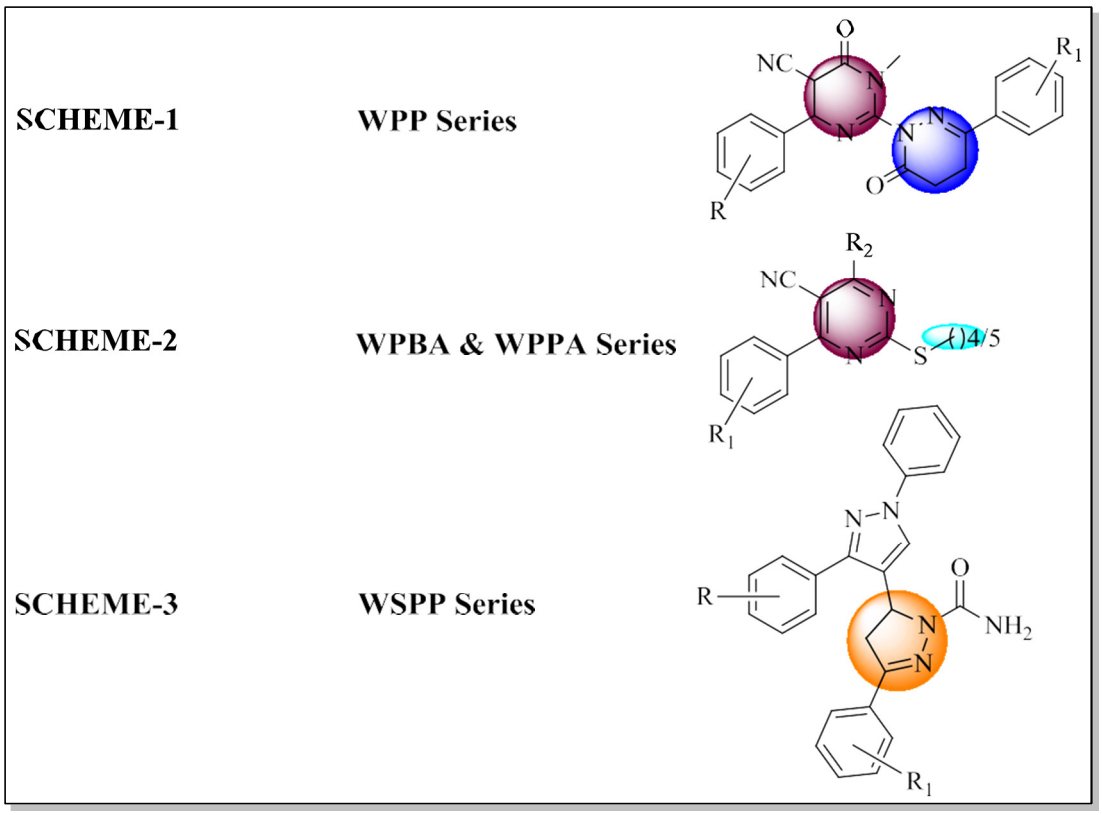


Figure-6: Representation of title compounds

All the synthesized compounds were structurally confirmed on the basis of IR, ^{1}H-NMR, ^{13}CNMR and mass spectral data and evaluated for their antiinflamatoryactivity. They were also tested for their toxicity studies.

ANTIANTI-INFLAMATORY

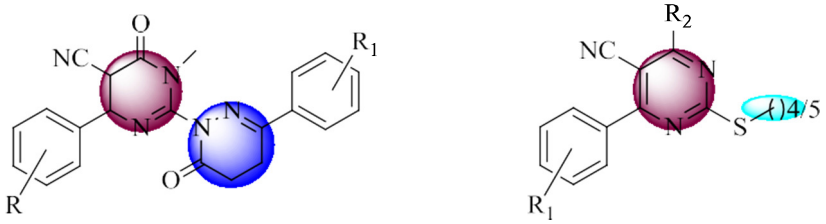
Anti-inflamatory refers to uncontrolled cell division/growth of cells which have the potential to spread /invade to adjoining cells or various organs of the body by means of blood stream or lymphatic system. Since few decades, Anti-inflamatory has been considered as the most dreadful disorder and is the major cause of death round the globe with an ever increasing rate. It is not restricted to a specific culture or population of a particular age [1-3]. Two-third of the Anti-inflamatory cases mainly occurs in middle and low income countries with 16.4 million cases in 2012, which is expected to rise to 21.4 million by 2030 [4,5]. Approximately, 14.1 million new Anti-inflamatory cases come into light every year causing 15.7% deaths which nearly account to 8.8 million people. Males were found to be affected with commonly prostate, lung, stomach and colorectal Anti-inflamatory whereas females were found to be affected with breast, lung, cervical and colorectal Anti-inflamatory [6,7]. Acute lymphoblastic leukemia and brain tumors are found affecting children round the globe whereas in the African continent, children were affected with non-Hodgkin lymphoma. The threat of Anti-inflamatory upsurges considerably with age and several Anti-inflamatory occur more frequently in developed countries [8-10].

MATERIAL & METHOD

PYRIMIDINE DERIVATIVES:

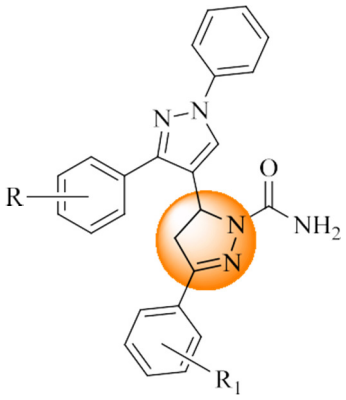
Pyrimidine hybrid with Pyridazinone (SCHEME-1)

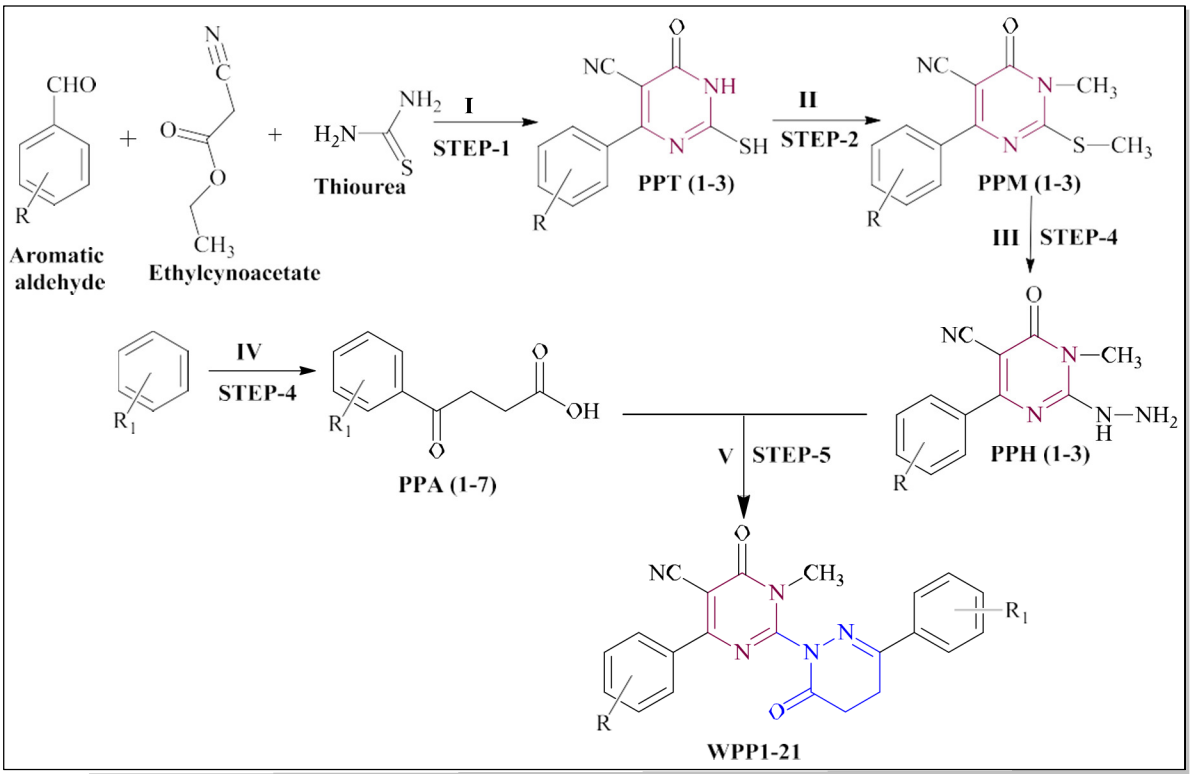
Pyrimidine hybrid with Iso-butyl/Iso-pentyl moiety (SCHEME-2)



PYRAZOLINE DERIVATIVES:

Pyrazoline hybrid with Pyrazole (SCHEME-3)




CHO
Aromatic aldehyde
Ethylcynoacetate
Thiourea
I
STEP-1
PPT (1-3)
II
STEP-2
PPM (1-3)
III
STEP-4
IV
STEP-4
PPA (1-7)
V
STEP-5
PPH (1-3)
WPP1-21

PYRIMIDINE HYBRID WITH PYRIDAZINONE
Chemistry

Reactions and Conditions: (i)K_2CO_3,Absolute ethanol, reflux; (ii) K_2CO_3,Dry DMF, Methyl iodide, stirring; (iii) Hydrazine hydrate,Absolute ethanol, reflux; (iv) Succinic anhydride, $AlCl_3$, DCM, R.T; (v) Absolute methanol, sodium acetate, reflux

SCHEME-I: WPP SERIES

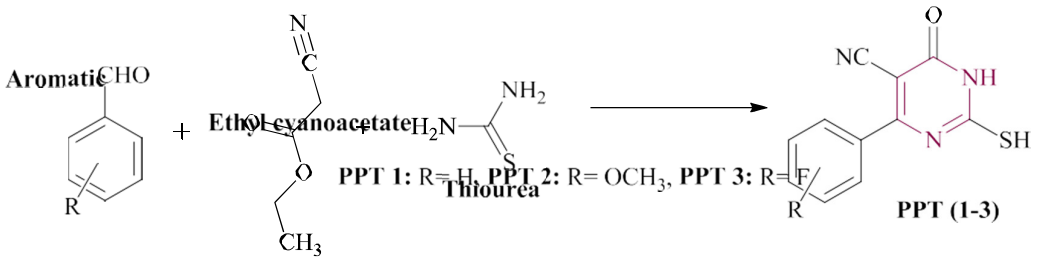
Table-1: Substitution of (**WPP 1-21**) Series

Comp. Code	R	R1	Comp. Code	R	R1
WPP 1	H	H	**WPP 12**	4-OCH_3	4-F
WPP 2	H	4-C_2H_5	**WPP 13**	4-OCH_3	4-Cl
WPP 3	H	4-CH_3	**WPP 14**	4-OCH_3	4-OCH_3
WPP 4	H	4-Br	**WPP 15**	4-F	H
WPP 5	H	4-F	**WPP 16**	4-F	4-C_2H_5
WPP 6	H	4-Cl	**WPP 17**	4-F	4-CH_3
WPP 7	H	4-OCH_3	**WPP 18**	4-F	4-Br
WPP 8	4-OCH_3	H	**WPP 19**	4-F	4-F
WPP 9	4-OCH_3	4-C_2H_5	**WPP 20**	4-F	4-Cl
WPP 10	4-OCH_3	4-CH_3	**WPP 21**	4-F	4-OCH_3
WPP 11	4-OCH_3	4-Br			

STEP I: Synthesis of 2-mercapto-6-oxo-4-aryl-1,6-dihydropyrimidine-5-carbonitrile (PPT 1-3)

Aromatic aldehyde (0.01 mole), ethylcyanoacetate (0.01 mole; 1.06 mL) and thiourea (0.01 mole; 0.76 gm) were dissolved in absolute ethanol and the reaction mixture was refluxed for 2 h in presence of potassium carbonate (0.03 mol; 4.14 gm). After the completion of reaction, the solvent was concentrated and the separated solid was filtered, washed with cold water and dried. The filtrate was poured into ice-cold water with stirring and neutralized with glacial acetic acid, which resulted in further separation of solid. This was filtered, washed with water. The solid obtained was re-crystallised from ethanol: water mixture (50:50).

The title compounds **(PPT 1-3)** were synthesized in accordance with the reported method [1] shown in **SCHEME-1**.

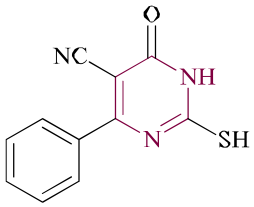


Reactions and Conditions: K_2CO_3,Absolute ethanol, reflux

aldehydes

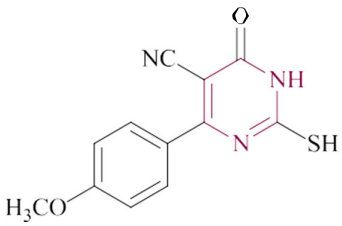
EXPERIMENTAL DETAILS

Synthesis of 2-mercapto-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile



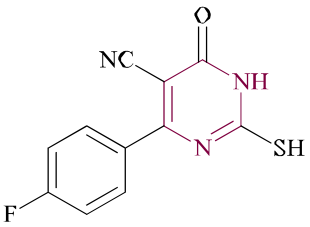
Benzaldehyde	0.01mole; 1.01 mL
Ethyl cyanoacetate	0.01mole; 1.06 mL
Thiourea	0.01 mole; 0.76 gm
Potassium Carbonate	0.03 mole; 4.14 gm
Ethyl alcohol	20 mL
Nature of Compound	Yellow powder
% Yield	92
Solvent System	I
R_f	0.72
Observed (Reported) Melting Point	292-95°C (299-300°C) Re-crystallisation
Solvent	Methanol

Synthesis of 2-mercapto-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5- carbonitrile



Anisaldehyde	0.01mole; 1.21 mL
Ethyl cyanoacetate	0.01mole; 1.06 mL
Thiourea	0.01 mole; 0.76 gm
Potassium Carbonate	0.03 mole; 4.14 gm
Ethyl alcohol	20 mL
Nature of Compound	Yellow powder
% Yield	89
Solvent System	I
R_f	0.76
Observed (Reported) Melting Point	182-86°C (293-94°C) Re-crystallisation
Solvent	Methanol

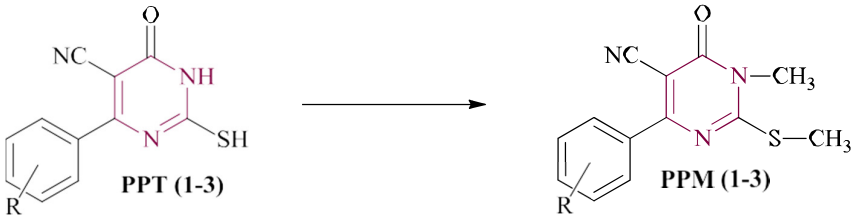
Synthesis of 4-(4-fluorophenyl)-2-mercapto-6-oxo-1,6-dihydropyrimidine-5-carbonitrile



4-Fluorobenzaldehyde	0.01mole; 1.05 mL
Ethyl cyanoacetate	0.01mole; 1.06 mL
Thiourea	0.01 mole; 0.76 gm
Potassium Carbonate	0.03 mole; 4.14 gm
Ethyl alcohol	20 mL
Nature of Compound	Yellow powder
% Yield	81
Solvent System	I
R_f	0.68
Observed (Reported) Melting Point	280-83°C (285-87°C) Re-crystallisation
Solvent	Methanol

STEP II: Synthesis of 1-methyl-2-(methylthio)-6-oxo-4-substituted Phenyl-1,6-dihydropyrimidine-5-carbonitrile (PPM 1-3)

The title compounds **(PPM 1-3)** were synthesized according to the reported method [2] by using **SCHEME-1**.

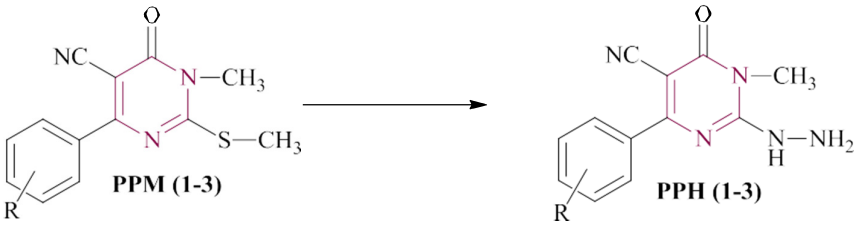


PPM 1: R=H, **PPM 2:** R= OCH_3, **PPM 3:** R= F

Reactions and Conditions: K_2CO_3,Dry DMF, Methyl iodide, stirring

STEP III: Synthesis of 2-hydrazinyl-1-methyl-6-oxo-4-substituted phenyl-1,6- dihydropyrimidine-5-carbonitrile (PPH 1-3)

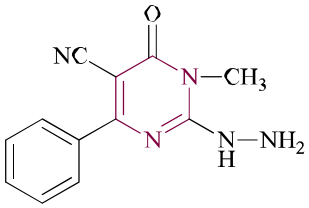
The title compounds **(PPH 1-3)** were also synthesized according to the reported method [2] by using **SCHEME-1**.



PPH 1: R=H, **PPH 2:** R= OCH_3, **PPH 3:** R= F

Reactions and Conditions: Hydrazine hydrate, Absolute ethanol, reflux

Synthesis of 2-hydrazinyl-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile

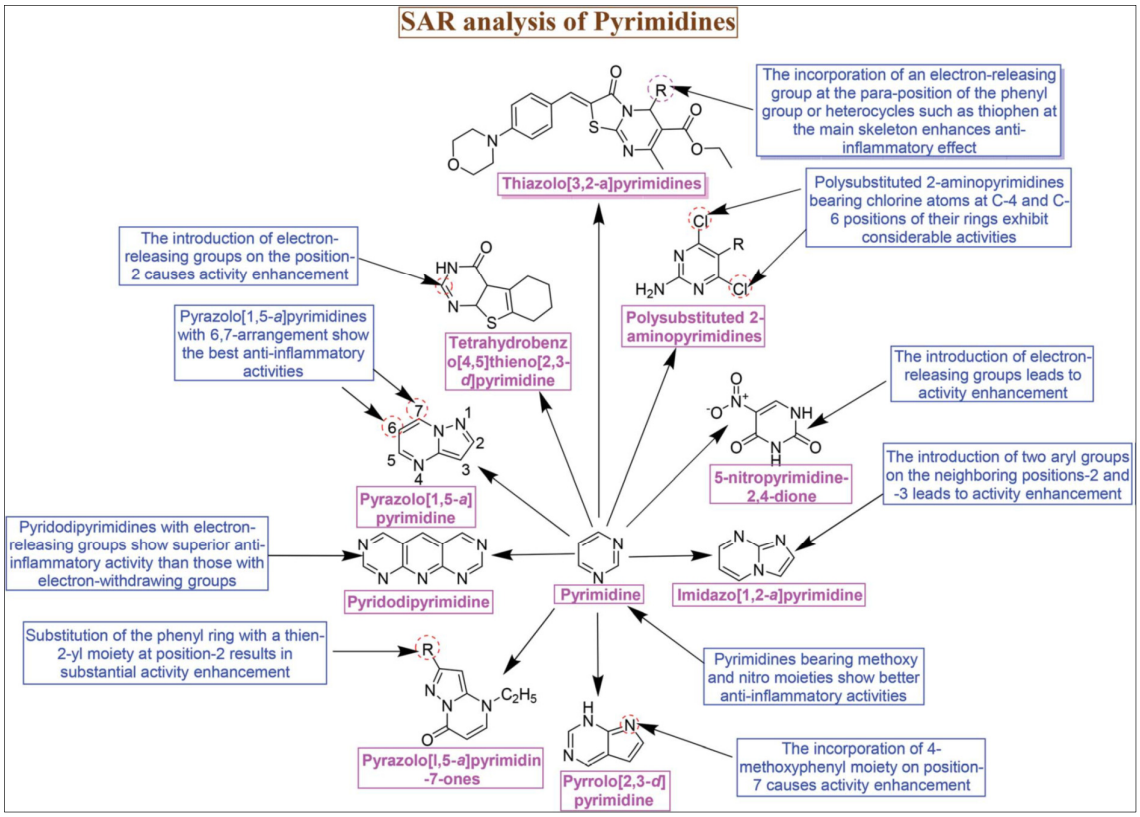


RESULT & DISCUSSION
PYRIMIDINE-PYRIDAZINONE DERIVATIVES

CHEMISTRY

Methodology

The Pyrimidine-pyridazinone hybrids (**WPP 1-21**) were synthesised using a five-step reactions. Initially 2-Mercapto-6-oxo-4-aryl-1,6-dihydropyrimidine-5-carbonitrile (**PPT 1-4**) were prepared by Biginelli condensation reaction of aromatic aldehydes, ethyl cyanoacetate and thiourea in presence of potassium carbonate (**STEP I**). Compounds obtained were treated with methyl iodide in presence of potassium carbonate in dry DMF (**STEP II**) to get 1-Methyl-2-(methylthio)-6-oxo-4-substituted phenyl-1,6- dihydropyrimidine-5-carbonitrile (**PPM 1-4**). These were further reacted with hydrazine hydrate (**STEP III**) to get 2-Hydrazinyl-1-methyl-6-oxo-4-substituted phenyl-1,6- dihydropyrimidine-5-carbonitrile (**PPH 1-4**). In a parallel pathway, the required 4-Oxo-4- phenylbutanoic acid (**PPA 1-7**) were synthesized from respective aromatic hydrocarbons using anhydrous aluminium chloride and succininc anhydride by following Friedal Craft's acylation reaction (**STEP IV**). The Pyridazinone derivatives (**WPP 1-21**), were obtained by the reaction (**STEP V**) of hydrazino derivatives (**PPH 1-4**) with acid derivatives (**PPA 1-7**). Removal of two moles of water (Dehydration) resulted in the formation of the desired title compounds. A sharp melting point and single spot in TLC established the purity of compounds.



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