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DESIGN AND SYNTHESIS OF PYRIMIDINE DERIVATIVES AS A POTENT UREASE INHIBITOR

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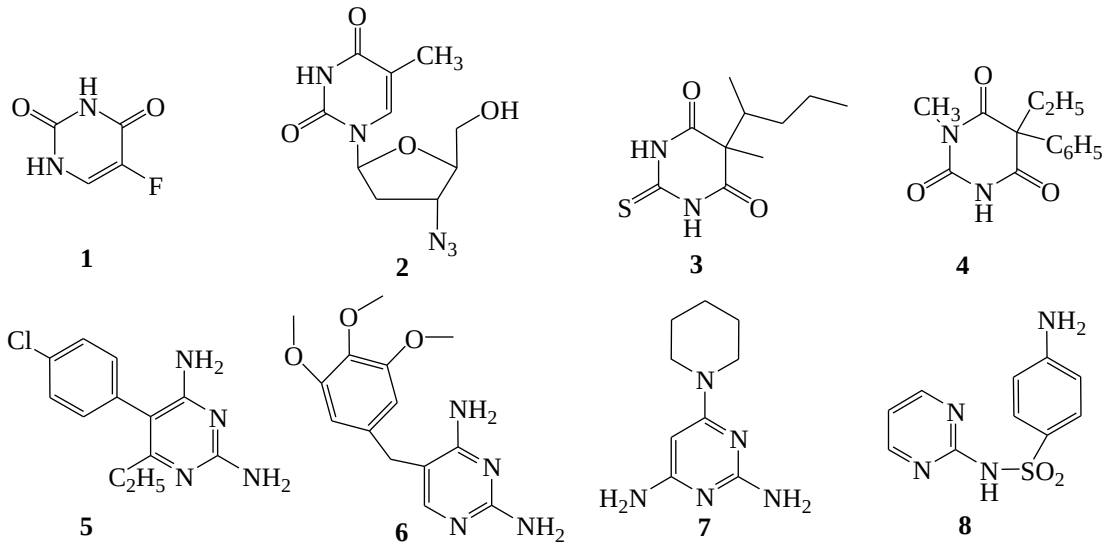
ABSTRACT

*Synthesis and importance of pyrimidine containing are having wide range of applications in pharmaceuticals. Salicylaldehyde **(I)** and ethyl cynoacetate **(II)** in presence of excess ammonium acetate in ethanol on reaction yielded the corresponding 2-oxo-2H-chromene-3-carboxamidine **(III).** Compound **(III)** was cyclized with ethyl acetoacetate and various aldehydes in presence of sodium ethoxide gave Ethyl-4-(4-aryl)-6-methyl-2- (2-oxo-2H-chromen-3-yl) pyrimidine-5-carboxylate **(IVa-l).** All newly synthesized compounds were characterized by IR, ^{1}HNMR, mass spectroscopy All synthesized compounds were evaluated for their in vitro urease inhibition activity.*

KEYWORDS: *Indian Education System , improved education, challenges.*

INTRODUCTION

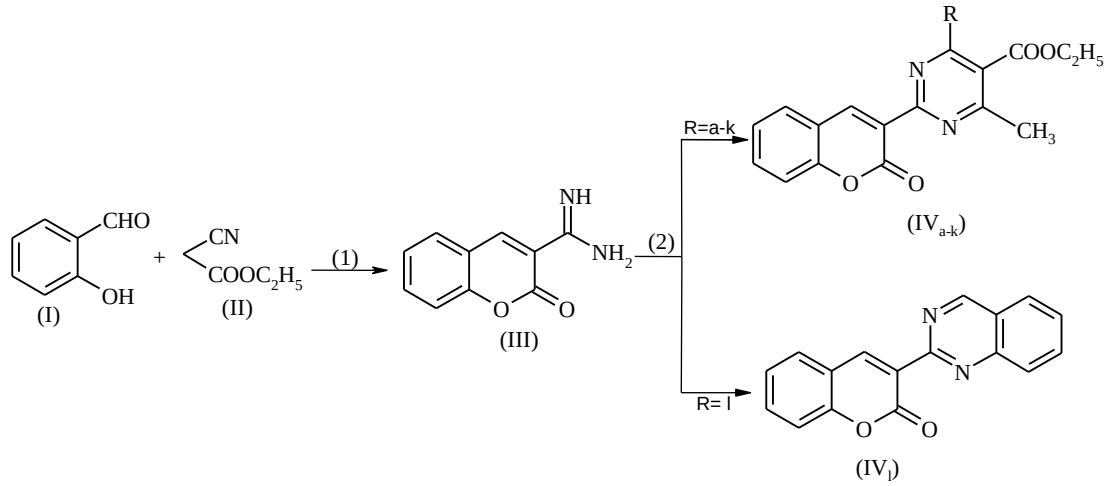
Over the years, the pyrimidine ring system represents in nature as an integral structure of Thymine, Cytosine and Uracil, which turned out to be an important pharmacophore, interacting with the synthesis and function of nucleic acids [15] (e.g., the cytostatic drug 5-fluorouracil (**1**) [16] and the HIV drug zidovudine (**2**) [17]. Ultrashort-acting barbiturates (**3**) are commonly used for anesthesia[18], whereas methylphenobarbital(**4**) [19] still is in use as antiepilepticum (anticonvulsants). Some of the diaminopyrimidines, such as pyrimethamine (**5**) [20], trimethoprim (**6**) [21] are powerful antimalaria drugs. Also, Trimethoprim (**6**) is a potent bacteriostatic antibiotic mainly used in the prophylaxis and treatment of urinary tract infections whereas minoxidil(**7**) [22] is used as antihypertensive. Sulfadiazine (**8**) [23] is one of the chemotherapeutics containing a pyrimidine moiety.



Some of the Bioactive Pyrimidines

Experimental

In present study, we herein report the synthesis and importance of substituted Pyrimidines containing coumarin moiety. Salicylaldehyde **(I)** and ethyl cynoacetate **(II)** in presence of excess ammonium acetate in ethanol on reaction yielded the corresponding 2-oxo-2H-chromene-3-carboxamidine **(III).** Compound **(III)** was cyclised with ethyl acetoacetate and various aldehydes in presence of sodium ethoxide gave Ethyl-4-(4-aryl)-6-methyl-2-(2-oxo-*2H*-chromen-3-yl) pyrimidine-5-carboxylate **(IVa-l).**



Reagents and Conditions:

(1) CH_3COONH_4, EtOH, Reflux. (2) Ethyl acetoacetate, RCHO, Sodium ethoxide

Where R is:

a = C_4H_3S

b = C_4H_3O

c = C_6H_5

d = 4-MeO-C_6H_4

e = 3,4 $(MeO)_2$-C_6H_3

f = 4-OH-3MeO-C_6H_3

g = 4-EtO-3MeO-C_6H_3

h = 4-OH-3,5$(OMe)_2$-C_6H_2

i = 2,4,6 $(MeO)_3$-C_6H_2

j = 4-N,N$(Me)_2$-C_6H_4

k = 3-NO_2-C_6H_4

l = 2-Cl-C_6H_4

Synthesis of 2-oxo-2H-chromene-3-carboxamidine (III):

A mixture of ethyl cyanoacetate (3 mmol), salicylaldehyde (3 mmol) and ammonium acetate (5 mmol) in ethanol (20 ml) was refluxed for 2 hr. The pale orange crystals were precipitated during the reaction which collected and washed with hot ethanol. It gives crude amidine containing coumarin. The crude compound was treated with 50% HCl and reprecipitated by ammonia to afford compound **IV**.

General procedure for synthesis of Ethyl-4-(4-aryl)-6-methyl-2-(2-oxo-2H-chromen-3-yl) pyrimidine-5-carboxylate (IVa-k):

In 50 ml of absolute ethanol (0.45 gm; 20 mmol) freshly cut sodium metal was added in small pieces. After the complete dissolution of sodium metal in ethanol, 2-oxo-2H-chromene-3-carboxamidine **(III)** (1.88 gm; 10 mmol), 10 mmol various aldehydes and (1.56 gm; 10mmol) ethylacetoacetate was added. The mixture was reflux for 3hr. The progress of reaction was monitored by TLC. After completion of reaction, reaction mixture was cooled to room temperature and poured to ice cold water containing dil. HCl. The separated solid was filtered and dried and recrystalized from ethanol.

Ethyl-4-methyl-2-(2-oxo-2H-chromen-3-yl)-6-(thiophen-2yl)pyrimidines-5-carboxylate (IVa) ^{1}H –NMR (δ, ppm)**(**DMSO-d_6) 1.02-1.06 (3H, m, -CH_3), 2.38 (3H, s, Pyrimidyl-CH_3), 4.34 - 4.36 (2H, m, CH_2), 6.89- 6.94(2H, m, CH), 7.04 -7.09 (2H, m, Ar-H), 7.15 (1H,d, CH), 7.28-7.34 (2H, m, Ar-H), 7.57 (1H, s, Ar-CH). MS (m/z) 393.13, 434.08

Ethyl-4-(furan-2-yl)-6-methyl-2-(2-oxo-2H-chromen-3-yl) pyrimidines-5- carboxylate (IVb) ^{1}H – NMR 1.09-1.15(3H, m, -CH_3), 2.38 (3H,s,Pyrimidyl-CH_3), , 4.32-4.37(2H, m, CH_2), 6.45(2H, d, Furanyl-CH) 7.00 – 7.12 (2H, m, Ar-H of coumarin ring), 7.19(1H,d, CH), 7.24-7.29 (2H, m, Ar-H), 7.61 (1H, s, Ar-CH) MS (m/z) 377

Ethyl-6-methyl-2-(2-oxo-2H-chromen-3-yl)-phenyl-pyrimidines-5- carboxylate (IVc) ^{1}H –NMR 1.00 -1.12 (3H, m, -CH_3), 2.40 (3H,s,Pyrimidyl-CH_3), 4.21-4.27 (2H, m, OCH_2), 6.78 (2H,d, Ar-H), 7.04 – 7.20 (3H, m, Ar-H), 7.22-7.32 (2H, m,Ar-H), 7.54 (2H, d, Ar-H), 7.73 (1H, s, Ar-CH) MS (m/z) 387, 409.

IVd:Ethyl-4-(4-methoxyphenyl)-6-methyl-2-(2-oxo-2H-chromen-3-yl)pyrimidine-5-carboxylate- Yield: 75%; **M.P. :**240-244; **IR (cm^{-1})** 1752,1678, 1564, 1441, 1245,1158, 1030; **^{1}H – NMR (**δ, ppm) **(**DMSO-d_6) 0.98-1.07(3H, m, -CH_3), 2.42 (3H, s, Pyrimidyl-CH_3), 3.96 (3H, s, OCH_3), 4.30-4.38(2H, m, CH_2), 6.72(2H,d, Ar-H), 6.94 – 7.12 (2H, m, Ar-H of coumarin ring), 7.28-7.36 (2H, m,Ar-H), 7.61 (2H, Ar-H), 7.66 (1H, s, Ar-CH); **MS (m/z)** 417.

IVe:Ethyl-4-(3,4-dimethoxyphenyl)-6-methyl-2-(2-oxo-2H-chromen-3-yl)pyrimidine-5-carboxylate- Yield: 68%; **M.P. :**231-234; **IR (cm^{-1})** 1749,1667, 1547, 1435, 1145, 1025, 960; **^{1}H –NMR (**δ, ppm) **(**DMSO-d_6) 1.06-1.10 (3H, m, -CH_3), 2.44 (3H,s,Pyrimidyl-CH_3), 3.89 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 4.26-4.33(2H, m, CH_2), 6.81 (1H,d, Ar-H), 6.94 – 7.12 (4H, m, Ar-H), 7.29 (2H, d, Ar-H), 7.64 (1H, s, Ar-CH) ; **MS (m/z)** 447, 488.

IVf: Ethyl-4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2-(2-oxo-2H-chromen-3-yl) pyrimidine-5-carboxylate- Yield: 69%; **M.P. :2**62-263; **IR (cm^{-1})** 3200, 1747, 1648, 1525, 1435, 1159, 1017; **^{1}H –NMR (**δ, ppm) **(**DMSO-d_6) 1.03-1.06 (3H, m, -CH_3), 2.41 (3H,s,Pyrimidyl-CH_3), 3.86 (3H, s, OCH_3), 4.31-4.36 (2H, m, CH_2), 6.77 (1H, d, Ar-H), 6.83 – 6.89 (2H, m, Ar-H), 7.09 (2H, d, Ar-H), 7.22 (2H, d, Ar-H), 7.69 (1H, s, Ar-CH), 8.10 (1H, bs, OH); **MS (m/z)** 433

IVg: Ethyl-4-(4-ethoxy-3-methoxyphenyl)-6-methyl-2-(2-oxo-2H-chromen-3-yl) pyrimidine-5-carboxylate - Yield: 64%; **M.P. :**270 – 272; **IR (cm^{-1})** 1756, 1635, 1510, 1435, 1145,860; **^{1}H –NMR (**δ, ppm) **(**DMSO-d_6) 1.12-1.18 (6H, m, -CH_3), 2.39 (3H,s,Pyrimidyl-CH_3), 3.86 (3H, s, OCH_3), 3.96-4.02 (2H, m, -CH_2), 4.21-4.25 (2H, m, CH_2), 6.73 (1H, d, Ar-H), 6.88 – 6.94 (2H, m, Ar-H), 7.073 (2H, d, Ar-H), 7.25 (2H, d, Ar-H), 7.68 (1H, s, Ar-CH); **MS (m/z)** 461, 502

IVh:Ethyl-4-(4-hydroxy-3,5-dimethoxyphenyl)-6-methyl-2-(2-oxo-2H-chromen-3-yl) pyrimidine-5-carboxylate - Yield: 73%; **M.P. :**267 – 268; **IR (cm^{-1})** 3243, 1756, 1423, 1145, 1069; **^{1}H –NMR (**δ, ppm) **(**DMSO-d_6) 0.98-1.03 (3H, m, -CH_3), 2.33 (3H, s, Pyrimidyl-CH_3), 3.94 (6H, s, OCH_3), 4.31 – 4.39 (2H, m, -CH_2), 6.56 (2H, s, Ar-H), 7.01 – 7.09 (2H, m, Ar-H), 7.29 (2H, d, Ar-H), 7.67 (1H, s, Ar-CH), 8.04 (1H, bs, OH).

IVi:Ethyl-4-(2,4,6-trimethoxyphenyl)-6-methyl-2-(2-oxo-2H-chromen-3-yl) pyrimidine-5-carboxylate Yield: 72%; **M.P. :**274 – 276; **IR (cm^{-1})** 1767, 1648, 1500,1435, 1245, 1015; **^{1}H –NMR (**δ, ppm) **(**DMSO-d_6) 0.98-1.03 (3H, m, -CH_3), 2.33 (3H, s, Pyrimidyl-CH_3), 3.69 (3H, s, OCH_3), 3.74 (6H, s, $(OCH_3)_2$), 4.37 – 4.41 (2H, m, -CH_2), 6.81 (2H, s, Ar-H), 7.04 – 7.08 (2H, m, Ar-H), 7.26 (2H, d, Ar-H), 7.71 (1H, s, Ar-CH).; **MS (m/z)** 477

IVj:Ethyl-4-(4-(dimethylamino)phenyl)-6-methyl-2-(2-oxo-2H-chromen-3-yl) pyrimidine-5-carboxylate- Yield: 78%; **M.P. :**249 – 251; **IR (cm^{-1})** 1739, 1715, 1610, 1510, 1445, 1234; **^{1}H –NMR (**δ, ppm) **(**DMSO-d_6) 0.94-1.01 (3H, m, -CH_3), 2.301 (3H, s, Pyrimidyl-CH_3), 2.84(6H, s, $(CH_3)_2$), 4.30 – 4.36 (2H, m, -CH_2), 6.91 (2H, d, Ar-H), 7.00 – 7.06 (2H, m, Ar-H), 7.30 (2H, d, Ar-H), 7.37(2H, d, Ar-H), 7.70 (1H, s, Ar-CH). **MS (m/z):** 430

IVk: Ethyl 4-methyl-6-(3-nitrophenyl)-2-(2-oxo-2H-chromen-3-yl)pyrimidine-5-Carboxylate- Yield: 71%; **M.P. :**257 – 258; **IR (cm^{-1})** Nujol 1758, 1730, 1620, 1524, 1358.**^{1}H –NMR (**δ, ppm) **(**DMSO-d_6) 0.98-1.07 (3H, m, -CH_3), 2.29 (3H, s, Pyrimidyl-CH_3), 4.34 – 4.39 (2H, m, -CH_2), 7.02 – 7.08 (2H, m, Ar-H), 7.22 (1H, d, Ar-H), 7.26(1H, d, Ar-H) 7.68 (1H, s, Ar-CH), 7.75 - 7.79 (2H, m, Ar-H), 8.09 (1H, d, Ar-H), 8.31 (1H, d, Ar-H).

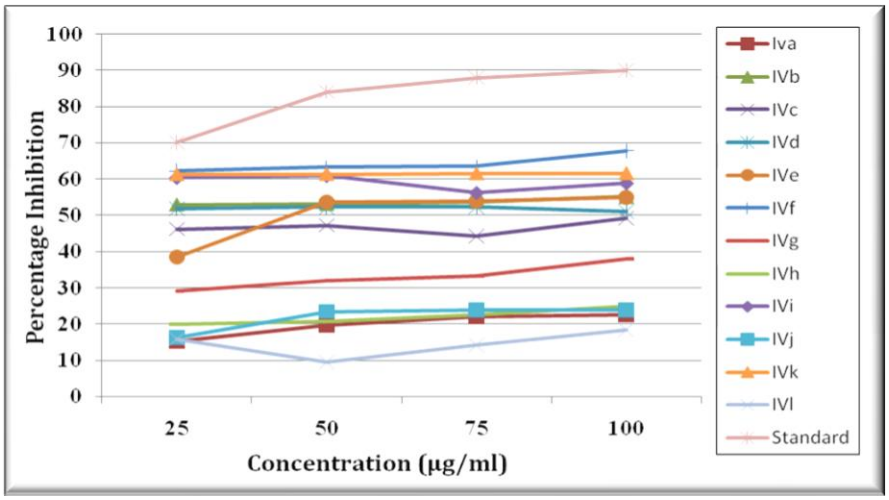
UREASE INHIBITION ASSAY :-

A solution comprising 25 µL of Jack bean Urease, 55 µL of buffer and 100 mM urea were incubated with 5 µL (0.5 mM conc.) of the test compounds at 30 °C for 15 min in well plates. The production of ammonia was measured by indophenol method and used to determine the urease inhibitory activity. The phenol reagent (45 µL, 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (70 µL, 0.5% w/v sodium hydroxide and 0.1% NaOCl) were added to each well and the increased absorbance at 630 nm was measured after 50 min, The percentage inhibition was calculated from the formula. Thiourea was used as the standard inhibitor.

$$\% \text{ Inhibition} = \frac{\text{Abs (standard-blank)} - \text{Abs (sample-blank)}}{\text{Abs (standard-blank)}} \times 100$$

Table 1: Quantitative Antiurease Activity of compounds IV a-l (% inhibition)

Sr. No.	Concentration(µg/ml)	% Inhibition ± 5			
		25	50	75	100
	Compounds				
1	IVa	15.23	19.66	22.08	22.56
2	IVb	52.92	53.09	53.60	55.08
3	IVc	46.10	47.15	44.12	49.17
4	IVd	51.82	52.16	52.22	51.05
5	IVe	38.48	53.56	53.93	54.94
6	IVf	62.13	63.15	63.34	67.76
7	IVg	29.13	31.87	33.40	37.96
8	IVh	19.78	20.54	22.40	24.91
9	IVi	60.32	60.83	56.08	58.77
10	IVj	16.21	23.43	23.89	23.91
11	IVk	61.25	61.32	61.49	61.56
12	IVl	15.80	9.37	14.20	18.42
13	Standard	70.00	77.18	80.04	82.15



Graphical representation of Antiurease Activity of compounds IVa-l

RESULTS AND DISCUSSION :-

Antiurease Activity :-

The synthesized compounds were screened for their in vitro antiurease activity. Thiourea was used as standard references and ethanol as a control. All tested compounds exhibited appreciable *in-vitro* antiurease activity.

In urease inhibition screening, compounds **IVf, IVi, IVk** exhibited excellent activity while compounds **IVb, IVd, IVj** showed moderate to poor activity with respect to standard. Compound **IVa, IVg, IVh, IVl** shows lowest activity among all the synthesized compounds. From above result, it conclude that the concentration effect of all the synthesized compounds was not so promising on urease inhibition activity.

The result of molecular docking study could explain that the enzyme binds to inhibitors with a binding mode identical to its substrate urea, through several hydrogen bonds.

CONCLUSION

We have synthesized Ethyl-4-(4-aryl)-6-methyl-2-(2-oxo-2H-chromen-3-yl) pyrimidine-5-carboxylate **(IVa-l)** and screened them for their urease inhibition activity. Among the synthesized compounds, compounds **IVf, IVi, IVk** exhibited excellent urease inhibition activity. Also we concluded that hydrogen bonding plays an important role between the inhibitor and enzyme in urease inhibition study.

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