Available online on 15.06.2022 at ijmspr.com

International Journal of Medical Sciences and Pharma Research

Open Access to Medical and Research

Open Access **Research Article**

Synthesis, Characterization and Antidiabetic Activity of New Pyrimidine Fused Derivatives

Pankaj Patidar[1]*, Prerna Chaturvedi [1], Neetesh Kumar Jain[2]

[1] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Oriental University Indore-India*

[2] *Department of Pharmacology, Faculty of Pharmacy, Oriental University Indore-India*

Article Info:

Article History:

Received 18 April 2022
Reviewed 02 June 2022
Accepted 08 June 2022
Published 15 June 2022

Cite this article as:

Patidar P, Chaturvedi P, Jain NK, Synthesis, Characterization and Antidiabetic Activity of New Pyrimidine Fused Derivatives, International Journal of Medical Sciences & Pharma Research, 2022; 8(2):45-49

DOI: http://dx.doi.org/10.22270/ijmspr.v8i2.37

*Address for Correspondence:

Pankaj Patidar, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Oriental University Indore-India

Email: pankajpatidar565@gmail.com

Abstract

Various evidences suggest that common health problem of modern society, *viz. diabetes mellitus*, obesity, cardiovascular diseases etc. The prevalence of *diabetes mellitus* in majority increases with age and obesity; over 20 % adults (≥ 60 years or older) have *diabetes*. Synthesis of starting materials by Noevenagel Condensation then A reaction mixture of different benzylidine propanednitriles **1a-d** (10 mmol), alkylisothiourea salts (11.00 mmol) and sodium acetate anhydrous (2.50 g, 24.40 mmol) in pyridine (50 ml) was refluxed for 3 hrs, and crystallized from ethanol give compound **2a-d.** then 2-Methylthiopyrimidines **2a-d** (5.0 mmol) was heated under reflux overnight with 5 mL of the appropriate amine (2-phenylethylamine or cyclohexylamine, and crystallized from appropriate solvent give compound **3a-d**. synthesized derivatives characterized by IR, NMR and Mass spectrophotometry and evaluated for anti-diabetic activity.

Keywords: *diabetes mellitus,* anti-diabetic activity, pyrimidine derivatives, Noevenagel Condensation.

INTRODUCTION:

A great deal of human health is affected by consumption of high-calorie diet and luxurious life style. Various evidences suggest that common health problem of modern society, *viz. diabetes mellitus*, obesity, cardiovascular diseases etc., are associated with intake of energy-rich food combined with decreased level of physical activity[1].

Recently, the World Health Organization (WHO) reported approximately 135 million people of the total world population to be affected by *diabetes mellitus*. The number has been predicted to rise up to 300 million by the year 2025[2].

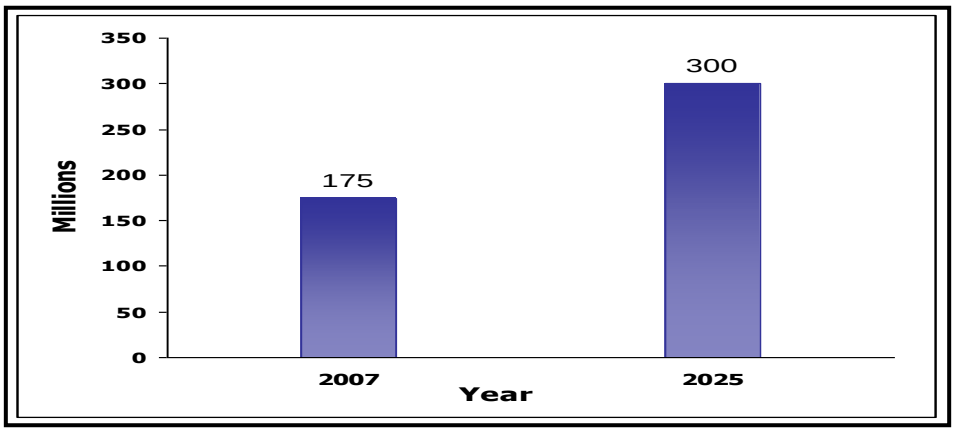


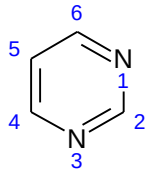
Fig. 1: Total world population to be affected by *diabetes mellitus*

The prevalence of *diabetes mellitus* in majority increases with age and obesity; over 20 % adults (≥ 60 years or older) have *diabetes*. At present more than 1 billion people are reportedly overweight; about 300 million people can be classified as obese[3, 4].

Thus, *diabetes mellitus* can be a debilitating metabolic disorder that cumulates in a range of progressive secondary complications (discussed in following chapters). Time has now arrived for the sections of society concerned with the health and welfare to take some gigantic steps for curbing the widespread of this disorder so as to have a society with more percentage of people leading a better life. *Diabetes mellitus* was recognized as early as 1500 BC by Egypt physicians, who described it as a disease associated with the passage of much urine[5].

The word *diabetes* (Greek word for siphon) was coined by Greek physician Aretaeus, the Cappadocian, around 2 AD. Aretaeus noticed that patient with diabetes suffer from siphoning of structural components form body into urine - although it was known from centuries that urine of patient with diabetes was sweet, it was not until 1674 that a physician named Wills coined the term diabetes mellitus (latter from the Greek word Honey)[5]. *Diabetes mellitus* is a chronic disorder characterized by hyperglycaemia resulting due to irregularity in metabolism of carbohydrates, proteins and fats, -an ultimate consequence of complete/relative insufficiency of insulin secretion or insulin action[5].

The nomenclature of pyrimidines is straightforward. However, like other heterocyclics, tautomeric hydroxyl groups yield complications since they exist primarily in the cyclic amide form. For example, 2-hydroxypyrimidine is more properly named 2-pyrimidone [structures]. A partial list of trivial names of various pyrimidines exists.[6]



pyrimidine

Physical properties are shown in the data box. A more extensive discussion, including spectra, can be found in Brown *et al.*[7, 9]

According to literature review I Concluded that following requirement must be in structure for their anti-diabetic activity. Pyrimidine moiety, electrophilic amino group at 4thposition of pyrimidine nucleus, substituted ethylamino group with phenyl ring at position 2nd of pyrimidine ring, The presence of nitrile group at position 5th responsible for desired activity Substituted halogen or alkyl aromatic ring at position 6th responsible for their desired activity.

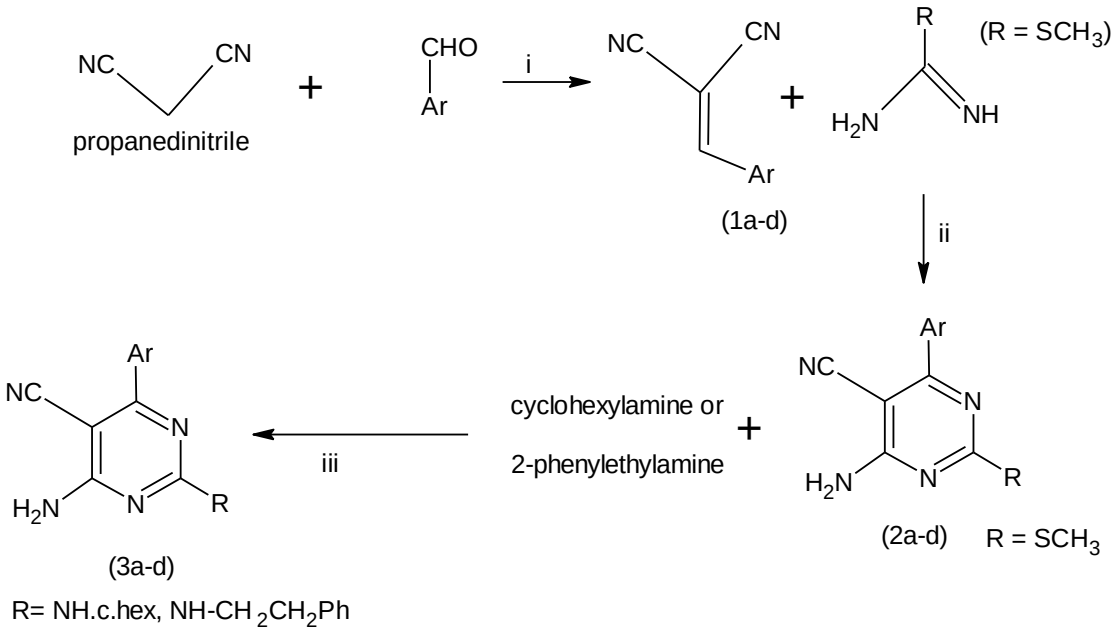
MATERIALS AND METHODS:

Chemical and reagents:

- **Chemical and reagents**

All chemicals were provided from our college. All solvents were redistilled before use. Reactions were routinely monitored by thin layer chromatography and spots were visualized by exposure to iodine vapour or UV light. All the synthesized compounds were purified by recrystallization. Melting points were determined by using open capillary method.

Synthetic Scheme:



Ar = 4-fluroC$_6$H$_4$, 4-NH$_2$C$_6$H$_4$, 4-BrC$_6$H$_4$, 4-OHC$_6$H$_4$,
i = ethanol, piperidine,heat
ii = pyridine, sodium acetate, reflux
iii = reflux

Synthetic Procedure:

Synthesis of starting material:

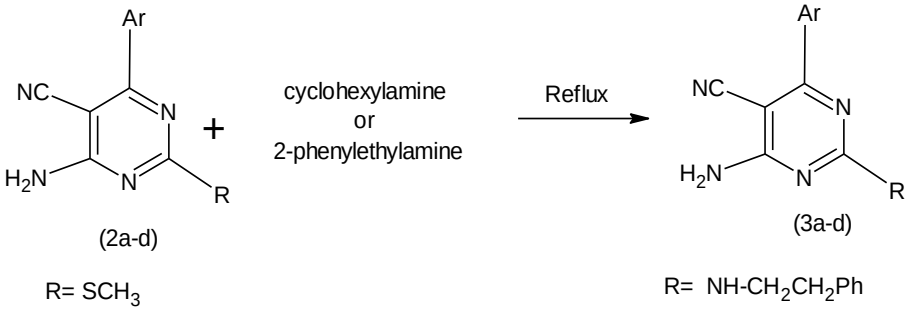
The (1 equiv.) substituted aldehyde, (2 equiv.) propandinitrile were dissolved into Pipridine (4 equiv.) was added into the mixture also at 0°C. The resulting mixture was stirred for 12 hours at room temperature. The resulting mixture was filtered; the filtrate was diluted with ethanol and washed with brine[10-13]. The organic layer was dried and concentrated. The resulting residue was purified by silica gel column

General procedure for 4-amino-6-aryl (4-substituted)-2-(methylsulfanyl) pyrimidine-5-carbonitrile (2a-d)

A reaction mixture of different benzylidine propanednitriles **1a-d** (10 mmol), salkylisothiourea salts **(**11.00 mmol) and sodium acetate anhydrous (2.50 g, 24.40 mmol) in pyridine (50 ml) was refluxed for 3 hrs. The reaction mixture was poured into water, acidified with dilute hydrochloric acid[14]. The formed precipitate was filtered and crystallized from ethanol give compound **2a-d.**

General procedure for 4-amino-2- (2-phenylethyl)amino)-6-(4-substitute dphenyl)pyrimidine-5-carbonitrile (3a-d)

2-Methylthiopyrimidines **2a-d** (5.0 mmol) was heated under reflux overnight with 5 mL of the appropriate amine (2-phenylethylamine or cyclohexylamine). The reaction mixture was cooled, diluted with water and acidified with dilute hydrochloric acid[15]. The formed precipitate was filtered and crystallized from appropriate solvent give compound **3a-d**. Sovent system: DMSO: Water (5:5)



Anti-diabetic activity:

Albino wistar male rats, weighing 180 – 250 g, were used for testing the anti-diabetic ability of synthesized compounds in comparison with standards -Rosiglitazone and Pioglitazone-using experimental animal models of diabetes mellitus. These were maintain at normal conditions of temperature (20-30 °C) and humidity (40–60 %), and were fed with standard diet twice a day. The blood glucose level was determined by using equipment Microplate Reader.

Induction of *Type 2 diabetes*:

Two groups of female rats were allowed breeding. One group was treated with alcohol (36 % body weight) during pregnancy for 18 days, and the other were similarly treated during nursing the pups for the same period. Both groups of pups were allowed growing separately till they attained age of 16 weeks. Subsequently, blood from each off spring was withdrawn and subject to OGTT[58]. The results are displayed in observation table given below.

Group I Control, Group II alcohol treatment during pregnancy, Group III alcohol treatment during nursing, the standards and test compounds were suspended in 1 % CMC to get suspensions of appropriate concentrations. The experimental animals were divided into following groups of 6 rats each. Group I (control): receiving 1 % CMC. Group II (std. 1): receiving Rosiglitazone (10 mg/Kg. Group III (std. 2) receiving Pioglitazone[16] (10mg/Kg) Group IV receiving 3a (10 mg/Kg) Group V receiving 3b (20 mg/Kg).Group VI receiving 3c (10 mg/Kg) Group VII: receiving 3d (10 mg/Kg).

Oral Glucose Tolerance Test:

Overnight fasted rats were anesthetized using anesthetic ether. Blood was withdrawn, from the *retro orbital plexus* situated in the eye-cavity of animals, twice at an interval of 30 min. After further 30 min, a glucose load (aqueous 50 % Dextrose) was orally fed to the animals corresponding to 2 g/Kg body weight. Blood was further withdrawn for subsequent 2 h, at an interval of 30 min[17-18]. The blood was subjected to centrifugation (2400 rpm, 10 min), serum separate and transfer to 96-well micro-titer plate, containing appropriate reagents, (as given below), and incubated for 10 min at 37 °C. The absorbance of the solutions was read at particular nm[17-18]

Table 1: Oral Glucose Tolerance Test

Solution	Blank	Standard	Test
Glucose Reagent	1.0 mL	1.0 mL	1.0 mL
Standard	- -	10 µL	- -
Sample	- -	- -	10 µl
Distilled Water	10 µl	- -	- -

The blood glucose levels present in the serum samples were calculate using the formula

Glucose (mg/dL) = $(A_T - A_B)/ (A_S - A_B)$ X 100 Where, A_T = Absorbance of test A_S= Absorbance of standard, A_B = Absorbance of blank . The observe blood-glucose levels show in following table were express as mean ± S.E.M. The obtain data was analyzed statistically by applying one-way ANOVA and *post hoc* Dunnet test, with $p< 0.05$ consider as significant.

RESULT AND DISCUSSION:

Table 2: Data for Analysis of synthesize compound

S. No.	Compound	% yield	Rf value	Mol. formula
1	**1a**	73.64	0.65	$C_{10}H_5FN_2$
2	**1b**	61.27	0.71	$C_{10}H_7N_3$
3	**1c**	89.33	0.69	$C_{10}H_5BrN_2$
4	**1d**	71.01	0.81	$C_{10}H_6N_2o$
5	**2a**	63.03	0.78	$C_{12}H_9FN_4S$
6	**2b**	77.62	0.51	$C_{12}H_{11}N_5S$
7	**2c**	59.11	0.56	$C_{12}H_9BrN_4S$
8	**2d**	65.23	0.67	$C_{12}H_{10}N_4OS$
9	**3a**	82.53	0.91	$C_{19}H_{16}FN_5$
10	**3b**	87.02	0.84	$C_{19}H_{18}N_6$
11	**3c**	72.17	0.87	$C_{19}H_{16}BrN_5$
12	**3d**	57.11	0.70	$C_{19}H_{17}N_5O$

Table 3: Spectral Characteristics of synthesized compounds

Compound code	IR (cm^{-1})	Mass (m/e)	^{1}H-NMR δ value (ppm)
3a	Ar-C-H- 3001.34, 2949.26, 2918.40 NH_2- 3492.95 NH- 3575.89 CN- 1466.66 CH_2- 3051.49, 3026.41 C=N- 1211.34, 1384.94 C=C- 1624.12 C-F- 675.11	333.3 m+ peak 257.1 base peak	Ar-CH, 4H, m, δ 6.479-6.973 Ar-CH, 5H, m, δ 7.027-7.884 NH_2- 2H, s, δ 5.109 CH_2 - 2H, t, δ 3.600-3.657 CH_2 - 2H, d, δ 2.300- 2.704 NH- 1H, t, δ 2.049-2.250
3b	Ar-C-H- 2953.12 2922.25 stretch NH_2- 3481.63, 3355.18 stretch NH- 3365.90 stretch CN- 1456.30 stretch CH_2- 2866.32 stretch C=N- 1166.97 stretch C=C- 1674.39 stretch	330.1 M+ Peak 253.1 base peak	Ar-CH, 4H, m, δ 6.720-7.759 Ar-CH, 5H, m, δ 7.826-8.929 NH_2- 2H, s, δ 3.641 CH_2 - 2H, t, δ 2.851-3.110 CH_2 - 2H, d, δ 2.247-2.332 NH- 1H, t, δ 3.127-3.337 NH_2 – 2H, s, δ 9.742
3c	NH- 3422.25 NH_2- 3340.03, 3126.71 CN- 1512.24 C=N- 1435.09 CH_2- 2922.25, 2953.12, 3032.20 C-Br- 837.13 Ar- C-H- 2781.44, 2810.38 C=C- 1668.48	395.2 M+1 Peak 318.1 Base peak	Ar-CH, 4H, m, δ 7.026-7.511 Ar-CH, 5H, m, δ 7.535-7.8174 NH_2- 2H, s, δ 5.398 CH_2 - 2H, t, δ 3.865-3.739 CH_2 - 2H, d, δ 2.777-2.865 Br- 0H, s, δ 3.127
3d	NH- 3126.71 CN- 1512.24 C=N- 1365.65, 1313.57 CH_2- 3032.20, 3057.27 C-OH- 3538.29 Ar- C-H- 2920.32, 2953.12 C=C- 1579.75	331.0 M+ Peak 235.5 base peak	Ar-CH, 4H, m, δ 6.713-7.262 Ar-CH, 5H, m, δ 7.296-8.664 NH_2- 2H, s, δ 3.648 CH_2 - 2H, t, δ 3.645-3.693 CH_2 - 2H, d, δ 2.778-2.815 NH- 1H, t, δ 3.601 OH – 1H, s, δ 3.758

Screening for anti-diabetic activity of pyrimidine derivatives:

The popular experimental models -Alloxan-induced *diabetes* and Streptozotocin-induced *diabetes*- however, could not be used because in these models the induced *diabetes* is due to destruction of β-cells of *islet of Langerhans* in pancrease, therefore reducing the levels of circulating insulin leading to hyperglycemia. On the contrary, the synthesized compounds were to be tested against *Type 2 diabetes*. Moreover, attempts to induce *Type 2* diabetes in rats- dosing females with alcohol (36 % of body weight) during pregnancy and also during nursing- failed to induce the disorder in the off-springs, as was conclusive from the results of OGTT which was carried out when the animals were 16-weeks old. Hence, it became imperative to rely only on the results of OGTT carried on blood of normal animals. Based on the pharmacological studies carried out in our laboratory, *i.e.* OGTT, it can roughly be inferred that compound **3d** effectively lowers the elevated blood-glucose levels; the effects shown is near to the effect exerted by the Pioglitazone and Rosiglitazone (seen below).

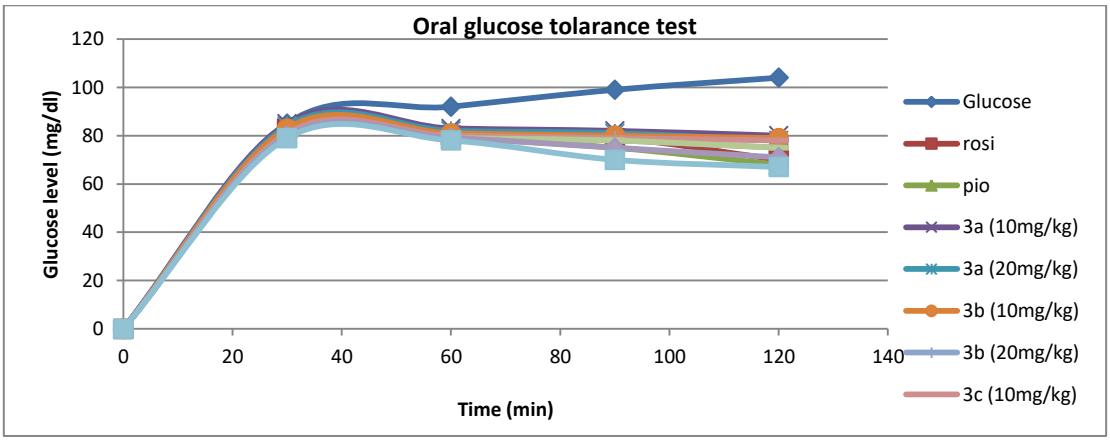


Figure 2: Oral glucose tolerance test

CONCULSION:

On the contrary, the synthesized compounds were supposed to be tested against *Type 2 diabetes*; the anti-hyperglycemic ability of synthesized compounds was envisaged to be established by their ability to increase sensitivity of peripheral tissues to circulating insulin in the animals. Hence, it was thought worth to validate and use a reported ethanol-induced diabetic model in rats, which would definitely examine the anti-hyperglycemic activity of the synthesized compounds, and also their anti-diabetic potential, especially for treating *Type 2 diabetes*. Based on the pharmacological studies carried out in our laboratory, *i.e.* OGTT, it can roughly be inferred that compound **3d** effectively lowers the elevated blood-glucose levels; the effects shown is near to the effect exerted by the Pioglitazone and Rosiglitazone.

REFERENCES:

1. Wilson, T.M., Brown, P.J., Sternbach, D.D.; et al., J. Med. Chem.; 2000, 43(4):527-47 https://doi.org/10.1021/jm990554g

2. Alberti, K.G.M.M.; Diabetes. Obes. Metab., 2001, 3(1):S3-S10

3. Tilbaldi, J.; Clinical Practice, 2007, 61(4):633-44

4. Waine, C.; British Nutrition Foundation Nutrition Bulletin, 2006, 31:111-14 https://doi.org/10.1111/j.1467-3010.2006.00563.x

5. Setter, S.M., White, J.R., Campbell, R.K.; et al., Textbook of Therapeutic Drug and Disease Management,8th ed., Lippincott Williams and a Wolter Kluwer Business's, 2020, 1033-43

6. Ostenson, C.G.; ActaPhysiolScand, 2001, 171:241-47

7. Newman, D.G.; Australian Transport Safety Bureau, 2005, 1-29 https://doi.org/10.1080/14443050509388018

8. Fowler, M.J.; Clinical Diabetes, 2018, 26(2):72-82

9. Lythgoe, B.; Rayner, L. S. "Substitution Reactions of Pyrimidine and its 2- and 4-Phenyl Derivatives". Journal of the Chemical Society: 1951; 2323-2329. https://doi.org/10.1039/jr9510002323

10. Brown, D. J.; Evans, R.F.; Cowden, W. B.; Fenn, M. D. The Pyrimidines. New York: John Wiley & Sons. 1994; 5-6. ISBN 0-471-50656-7.

11. Albert, Adrien (1968). Heterocyclic Chemistry, an Introduction. London: Athlone Press. pp. 56-62.

12. Nuevo M, Milam SN, Sandford SA, Elsila JE, Dworkin JP (2021). "Formation of uracil from the ultraviolet photo-irradiation of pyrimidine in pure H2O ices". Astrobiology 2009 ; 9(7): 683-695. Bibcode: As Bio...9683N. https://doi.org/10.1089/ast.2008.0324

13. Ameen Ali Abu-hashem Design and synthesis of novel thiophenecarbohydrazide,thienopyrazole and thienopyrimidine derivatives as antioxidant and antitumor agents Acta Pharm. 2010; 60:311-323 https://doi.org/10.2478/v10007-010-0027-6

14. Acta Pharm. 60 (2010) 311-323Synthesis and Analgesic Activity of Novel Pyrimidine Derivatives ofCoumarin Moiety ActsPolonaisePharmaceutical n Drug Research, 2020; 68(5):785-793,

15. Ebubekir Septioglu Synthesis and Anticonvulsant Activity of Some New Hexahydropyrimidine-2,4-dione Derivatives CNS-active Drugs - Hypnotics - Psychotropic - Sedatives Li Sun Synthesis and Antifungal Activities of Some Novel Pyrimidine DerivativesMolecules. 2021; 16:5618-5628; \ https://doi.org/10.3390/molecules16075618

16. Salah abdel-muttalib abdel-aziz1design, synthesis and antidiabetic activity of some new 4-amino (or 6-oxo)-2-methyl/benzylthio (or substituted amino) pyrimidine derivatives bull. pharm. sci., assiut university, 2011; 34(2):149-158 https://doi.org/10.21608/bfsa.2011.63262

17. Santaram G.K. synthesis and pharmacological evaluation of some new pyrimidine derivatives containing 1,2,4- triazole APB 2020; 2(2):213-22.

18. Rajgopal, G.;Toora, B.D.; Practical Biochemistry, 6th ed., 2004, 75-81

19. Mukherjee, K.L.; Medical Laboratory Technology, 4th ed., 1998, 985-96