

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SOME SUBSTITUTED-BENZOYL HYDRAZINO DERIVATIVES OF PYRIMIDINE

Darshitkumar M. Dave* and Dr. A. H. Bapodra¹

*Department of Chemistry, M. D. Science College, Porbandar-360575, Gujarat, India.

¹Bhakta Kavi Narsinh Mehta University, Khadia - 362263, Junagadh, Gujarat, India.

Article Received on
07 Feb. 2018,

Revised on 28 Feb. 2018,
Accepted on 20 March 2018,

DOI: 10.20959/wjpr20187-11689

*Corresponding Author

Darshitkumar M. Dave

Department of Chemistry,
M. D. Science College,
Porbandar-360575,
Gujarat, India.

ABSTRACT

Pyrimidine constitutes an important class of heterocycles in drug discovery and is very well known for their pharmacological activities. Pyrimidines and their derivatives possess various biological and pharmacological activities. Amides, aryl amides, heterocyclic aryl amides showed widely useful as pharmacologically activities. In vision of above conclusions, it appeared of concentration to synthesize Arylamide (Substituted-benzoyl hydrazino derivatives) of pyrimidine (KLB a-j) by the reaction of methyl-4-(4-fluorophenyl)-2-hydrazinyl-6-isopropylpyrimidine-5-carboxylate with various substituted benzoyl chloride. The structure elucidation of synthesized compounds has been

done on the basis of I.R. and ¹H N.M.R. and by Mass spectrometry. Purity of all compounds has been checked by T.L.C.

KEYWORDS: Pyrimidine, Benzoyl Chloride, Hydrazine Hydrate, Aryl amide, Antimicrobial activities.

INTRODUCTION

Pyrimidines, refers to a six membered heterocyclic system analogous to benzene having two nitrogen atoms at 1- and 3-position, are widely found in nature as they are vital components of nucleic acids, i.e., DNA and RNA. Pyrimidine and its derivatives demonstrate a diverse array of biological and pharmacological activities including anticonvulsant, antibacterial, antifungal, etc. properties. This broad spectrum of biochemical targets has been facilitated by the synthetic versatility of pyrimidine, which has allowed the generation of a large number of

structurally diverse derivatives including analogues derived from substitution of the aryl ring, and/or derivatisation of the pyrimidine nitrogen. Pyrimidines are synthetically versatile substrates, where they can be used for the synthesis of a large variety of heterocyclic compounds and as raw materials for drug synthesis.^[1-8] Hybrid molecules, an emerging trend containing two or more structural domains, acting on same or different targets have been reported to exhibit diverse pharmacological activities.^[9] The amide functionality is a common feature in small or complex synthetic or natural molecules. Amides also play a key role for medicinal chemists. An in-depth analysis of the Comprehensive Medicinal Chemistry database revealed that the carboxamide group appears in more than 25% of known drugs.^[10-13]

EXPERIMENTAL SECTION

All melting points were determined in an open capillary and are uncorrected. The IR spectra were recorded on a FTIR - 8400 spectrophotometer. ¹H NMR spectra recorded on a Bruker 300 MHz spectrometer with DMSO as a solvent and tetra methyl silane (TMS) as internal standard. The chemical shifts are expressed in parts per million (ppm) downfield from the internal standard and signals are quoted as *s* (singlet), *d* (doublet) and *m* (multiplet). Thin Layer Chromatography (TLC) analytical separation was conducted with Silica Gel 60 F-254 (Merck) plates of 0.25mm thickness eluted with Hexanes: Ethyl acetate (7: 3 v/v) and visualized with UV (254 nm) or iodine to check the purity of the synthesised compounds.

The antimicrobial activity was assayed by using the microtitre broth dilution method¹⁴ by measuring the zone of inhibition in mm. All the compounds were screened in vitro for their antimicrobial activities against bacterial strains such *Staphylococcus aureus*, *Escherichia coli*, *Bacillus megaterium*, *Pseudomonas aeruginosa* and fungi *Aspergillus niger* and *Aspergillus flavus* at various concentration. Standard drugs like Streptomycin, Ampicillin and Nystatin were used for comparison purpose. (Table-2, Chart-1).

General Procedure for the synthesis of compounds (3) and KLB b are as under. (Fig.-1).

Preparation of methyl 4-(4-fluorophenyl)-2-hydrazinyl-6-isopropylpyrimidine-5-carboxylate (3)

Methyl 4-(4-fluorophenyl)-2-hydroxy-6-isopropylpyrimidine-5-carboxylate (0.01 mol, 2.9 gm) was taken and added POCl₃ (10 ml) at 0-5°C, heated this mixture at 70-80°C on water bath for 5-6 hrs. The completion of reaction was monitored by TLC. After completion of

reaction the reaction mass was cooled at room temperature and poured slowly in ice water with stirring. The product was filtered, washed with sodium bicarbonate solution and dried. Taking this product (2) (methyl-2-chloro-4-(4-fluorophenyl)-6-isopropylpyrimidine-5-carboxylate) (0.01 mol, 3.08 gm), added neat hydrazine hydrate (12 ml) and heated at 80-85°C on water bath for 7-8 hrs. After completion of the reaction, the reaction mass was cooled at room temperature and poured into ice water. The product was filtered and washed with water and dried. Yield: 75%, m.p.-120°C.

Preparation of Methyl-4-(4-fluorophenyl)-6-isopropyl-2-[2-(4-methylbenzoyl)hydrazino]-pyrimidine-5-carboxylate (KLB b)

4-(4-fluorophenyl)-2-hydrazinyl-6-isopropylpyrimidine-5-carboxylate (0.01 mol, 3.04 gm) and 4-methylbenzoyl chloride (0.015 mol) were taken separately in pyridine and cooled to 0-5°C. Then 4-methylbenzoyl chloride was added to the solution of 4-(4-fluorophenyl)-2-hydrazinyl-6-isopropylpyrimidine-5-carboxylate drop-wise and warmed the mixture at 60°C for 2 hrs. After completion of the reaction, the reaction mass was stayed for a night at room temperature and poured into HCl and ice water (1:1), collected the product, washed with dil. HCl and sodium bicarbonate solution and dried the product. The product was purified by recrystallization from methanol.

Similarly, other Methyl-4-(4-fluorophenyl)-6-isopropyl-2-[2-(substitutedbenzoyl)hydrazino]pyrimidine-5-carboxylate were prepared. The physical constants are recorded in Table-1.

Methyl-4-(4-fluorophenyl)-6-isopropyl-2-[2-(4-methylbenzoyl)hydrazino]-pyrimidine-5-carboxylate (KLB b)

Yield 46 %, m. p. 77°C; **IR(KBr):** ν Sec. Amine N-H Str. 3304, Amide N-H Str. 3246, Alkane C-H str. (asym.) 2962, -CH₃ C-H str. (sym.) 2868, Ester C=O 1732, Pyrimidine C=C 1552, -CH₃ def. (Asym.) 1427, -CH₃ def. (sym.) 1321, Ester C-O Str. 1261, Aromatic C-H i-p def. 1220 & 1159, C-H o-o-p def. 831, C-F Str. 1072; **¹H NMR (DMSO – d₆, δ, ppm):** 1.0-1.2 (d, 6H, HC-(CH₃)₂), 2.5 (s, 3H, Ar-CH₃), 3.0-3.1 (m, 1H, -CH(CH₃)₂), 3.6 (s, 3H, -COOCH₃), 7.3-7.8 (d, 8H, Ar-H), 9.4 (s, 1H, Pyrimidine-NH-), 10.3 (s, 1H, Amide CO-NH-)
Mass m/z: 422, M.F.: C₂₂H₂₃FN₄O₃

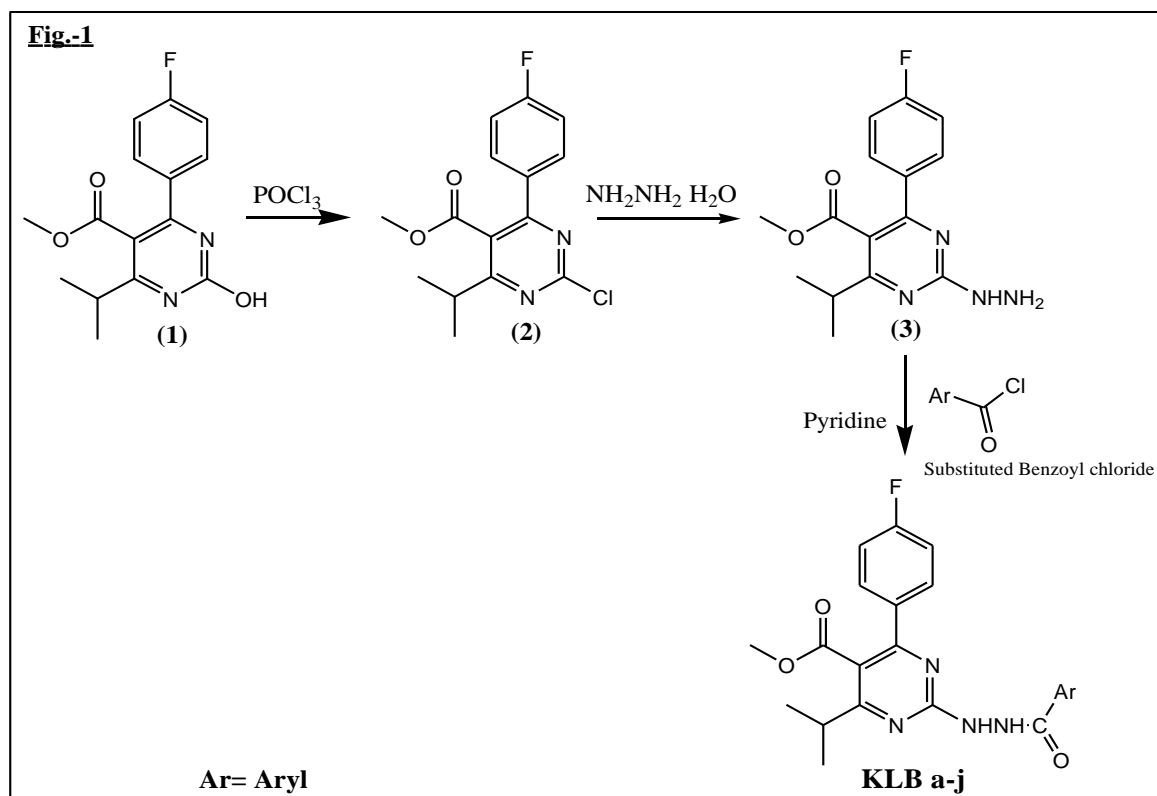


Fig.-1.

Table 1: Characterisation data of compound (KLB a-j).

| No | Substituent Ar | Molecular Formula | M.P. °C | % Yield |
|-------|---|--|---------|---------|
| KLB c | 3,4-di(OCH ₃) ₂ -C ₆ H ₃ | C ₂₄ H ₂₅ FN ₄ O ₅ | 218 | 51 |
| KLB d | 2-Cl-C ₆ H ₄ | C ₂₂ H ₂₀ ClFN ₄ O ₃ | 77-80 | 43 |
| KLB e | 4-Cl-C ₆ H ₄ | C ₂₂ H ₂₀ ClFN ₄ O ₃ | 233-235 | 50 |
| KLB f | 3-Cl-C ₆ H ₄ | C ₂₂ H ₂₀ ClFN ₄ O ₃ | 170 | 49 |
| KLB g | 3-CH ₃ -C ₆ H ₄ | C ₂₃ H ₂₃ FN ₄ O ₃ | 168-170 | 52 |
| KLB h | 4-NO ₂ -C ₆ H ₄ | C ₂₂ H ₂₀ FN ₅ O ₅ | 225-226 | 42 |
| KLB i | 3-NO ₂ -C ₆ H ₄ | C ₂₂ H ₂₀ FN ₅ O ₅ | 149-150 | 53 |
| KLB j | C ₆ H ₅ | C ₂₂ H ₂₁ FN ₄ O ₃ | 223-225 | 43 |

RESULTS AND DISCUSSION

Antibacterial activity

The newly synthesized entities (**KLB a-j**) were screened for their antibacterial assay against a broad panel of gram-positive bacteria *S. aureus*, *B. subtilis*, gram-negative bacteria *E. coli*, *P. aeruginosa*. When the synthesized derivatives were tested against *B. subtilis*, compound KLB g [Ar= 3-CH₃-C₆H₄] was found to exhibit excellent activity as compared to the standard drug ampicillin. Other compounds, except KLB g, were found to possess poor activity. On testing the synthesized compound against *S. aureus*, compound KLB j [Ar= C₆H₅] was found to

exhibit maximum activity as compared to the standard drug. However compounds KLB g and KLB i were found to possess moderate activity as compared to the standard drug ampicillin. The antibacterial property of the synthesized derivatives was found to be more effective on the gram-negative bacteria as compared to the gram-positive bacteria. On testing the compounds against *E. coli*, KLB g [Ar= 3-CH₃-C₆H₄] and KLB h [Ar= 4-NO₂-C₆H₄] were found to exhibit maximum activity as compared to the standard drug streptomycin. However compounds KLB d and KLB f were found to possess good activity as compared to the standard drug. Compound KLB h [Ar= 4-NO₂-C₆H₄] was found to exhibit excellent activity on testing against *P. aeruginosa* as compared to the standard drug streptomycin. On other hand, compound KLB a, KLB b and KLB d were found to have moderate activity against the same bacterial strain.

Antifungal activity

The antifungal tests were performed against two different fungal strains *A. niger* and *A. flavus*, where nystatin was used as a standard drug. Antifungal property of the synthesized derivatives was found to be more effective on *A. niger* as compared to *A. flavus*. On testing the synthesized derivatives against *A. niger*, compound KLB g [Ar= 3-CH₃-C₆H₄] and KLB h [Ar= 4-NO₂-C₆H₄] were found to exhibit excellent activity as compared to the standard drug nystatin. However compounds KLB a, KLB b, KLB f and KLB i were found to possess significant activity. Compounds KLB f [Ar= 3-Cl-C₆H₄] and KLB g [Ar= 3-CH₃-C₆H₄] were found to exhibit maximum activity on testing against *A. flavus* as compared to the standard drug. However compound KLB a was found to possess good activity against the same fungal strain.

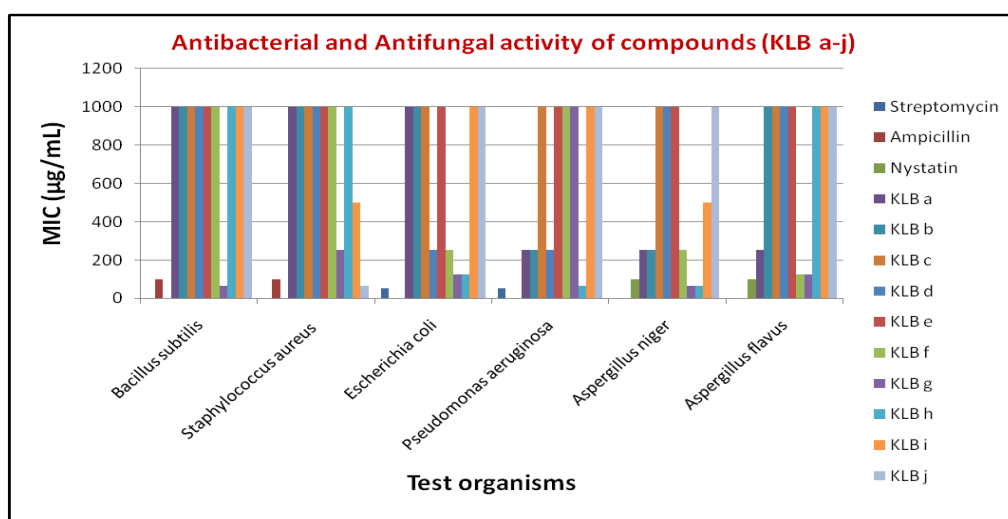


Chart-1: Antibacterial and antifungal activity data of compounds (KLB a-j).

Table 2: Antibacterial and antifungal activity data of compounds (KLB a-j).

| Compounds | Minimum Inhibitory Concentration (MIC) in µg/ml | | | | | |
|--------------|---|------------------|------------------------|---------------------|-----------------|------------------|
| | Gram-positive bacteria | | Gram-negative bacteria | | Fungi | |
| | <i>B. subtilis</i> | <i>S. aureus</i> | <i>E. coli</i> | <i>P.aeruginosa</i> | <i>A. niger</i> | <i>A. flavus</i> |
| Streptomycin | | | 50 | 50 | | |
| Ampicillin | 100 | 100 | | | | |
| Nystatin | | | | | 100 | 100 |
| KLB a | 1000 | 1000 | 1000 | 250 | 250 | 250 |
| KLB b | 1000 | 1000 | 1000 | 250 | 250 | 1000 |
| KLB c | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| KLB d | 1000 | 1000 | 250 | 250 | 1000 | 1000 |
| KLB e | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| KLB f | 1000 | 1000 | 250 | 1000 | 250 | 125 |
| KLB g | 62.5 | 250 | 125 | 1000 | 62.5 | 125 |
| KLB h | 1000 | 1000 | 125 | 62.5 | 62.5 | 1000 |
| KLB i | 1000 | 500 | 1000 | 1000 | 500 | 1000 |
| KLB j | 1000 | 62.5 | 1000 | 1000 | 1000 | 1000 |

CONCLUSION

The present study leads to a convenient synthetic method for the synthesis of new compounds. Which show significant antibacterial and antifungal activity. Further investigation with appropriate structural modification of the above compounds may result in therapeutically useful products.

ACKNOWLEDGMENT

The authors are thankful to authorities of M. D. Science College, Porbandar, Gujarat, India for providing research facilities. The authors are very thankful to Dr. K. D. Ladva, Principal, Shree M & N Virani Science college (autonomous), Rajkot for constant guidance and support during the research and we are also thankful to Department of Chemistry Saurashtra University, Rajkot for NMR spectral analysis. The authors are also thankful to UGC for financial support under the scheme of innovative PG Programme and also to Shree M. & N. Virani Science college (autonomous), Rajkot for I.R., Mass spectral & elemental analysis and Atmiya in-vitro Testing Laboratory, Yogidham, for antimicrobial screening.

REFERENCES

1. S Futterman. *J Biol Chem.*, 1957; 228: 1031.
2. L Kartik; MS Vadodaria; US Dave; P Hansa. *Org Chem An Indian J.*, 2014; 10(5): 188-191.

3. D Pansuriya; K Menpara; N Kachhadiya; J Menpara; K Ladva. *Der Pharma Chemica*, 2015; 7(10): 212-218.
4. NM Thumar; AA Kaneria; M Vadodaria; K Ladva. *Int Lett Chem Phy Astr*, 2016; 65: 11-26.
5. TP Selvam; CR James; PV Dniandev; SK Valzita. *Res Pharm*, 2012; 2: 1-9.
6. AK Gupta; Sanjay; HP Kayath; A Singh; G Sharma; KC Mishra. *Indian J Pharm*, 1994; 26: 227-228.
7. V Sharma; N Chitranshi; A Agarwal. *Int J Med Chem.*, 2014; 31-64.
8. KS Jain; TS Chitre; PB Miniyar; MK Kathiravam. *Curr Sci.*, 2006; 90: 793-798.
9. MR Ali; G Verma; S Mohammad; M Akhter; MA Alam. *J Taibah Uni Med Sci.*, 2015; 1-7.
10. Menpara K, Pansuriya D, Kachhadiya N, Menpara J, Ladva K, *J. Applicable Chem.*, 2014; 3(2): 523-40.
11. Gadhiya B, Rajput M, Bapodra A, Ladva K, *Rasayan J. Chem.*, 2016; 9(3): 355-72.
12. Gadhiya B, Rajput M, Bapodra A, Ladva K, *Der Pharma Chemica.*, 2016; 8(16): 40-7.
13. K. T. Raut, *Der Pharma Chemica*, 2012; 4(4): 1435-42.
14. Rattan, A., *Antimicrobials in Laboratory Medicine*, B I Churchill Livingstone Pvt. Ltd, New Delhi. India, 2000.