CHINESE JOURNAL OF MEDICAL GENETICS
ISSN: 1003-9406 Vol. 32 Iss. 4 2022

# SYNTHESIS OF NOVEL PYRAZOLINE DERIVATIVE AS A PROMISING CHEMOTHERAPEUTIC AGENT

**Dr. Sudarshan Nagarale[*1], Mr. Amit Pondkule[2], Dr. Vishal Babar[3], Mr. Avinash Choudhary[4], Priti Kale[5] & Mr. Ashish Jadhav[6]**

[1,3]Department of Pharmaceutical Chemistry
[2,4]Department of Pharmaceutics
[5,6]Department of Pharmaceutical Quality Assurance
[4]Dayanand Institute of Pharmacy
[1,2,3,5,6]Dattakala Collage of Pharmacy, Swami-Chincholi, Maharashtra

## ABSTRACT

Pyrazoline play a vital role in healthful chemistry, as a result of several of its derivatives have important biological activity during this study, chalcones and Schiffs bases of piperonal with completely different ketones With have 3,4-methylenedioxy moiety gift in piperonal was shown to be liable for numerous biological activities. Therefore, within the gift study we've got synthesized some novel three, 5-disubstituted and one, 3, 5-trisubstituted pyrazoline derivatives as potential antitubercular agents. The novel pyrazoline are synthesized via chalcone intermediates by reaction with substituted hydrazines. The intermediate chalcones were ready by Claisen-Schmidt condensation of piperonal with substituted acetophenone in presence of hydroxide. The cyclocondensation of chalcones with substituted hydrazines afforded substituted pyrazoline derivatives. The structures of the synthesized derivatives were confirmed by IR, 1HNMR and Mass spectral knowledge. The synthesized novel pyrazoline derivatives were screened for his or her In vitro antitubercular activity by Almar blue dye technique against M.tuberculosis H37 RV strains. on top of the quality medication. The synthesized pyrazolines have conjointly evaluated for cytotoxic activity by Tryphan blue exclusion assay on EAC cells and also the tested compounds have shown toxicity ranges from 15-59 nada. The results indicate that the synthesized pyrazolines will be helpful as effective therapy agents.

**Keywords:** Chalcone, Piperonal, Antitubercular, Almar blue Assay.

## 1. INTRODUCTION

Mycobacterium T.B., a chronic necrotizing communicable disease with a good type of manifestations caused by mycobacterium, is killing more or less 2 million individuals each year with quite eight million individuals developing active TB every year globally[1]. it's endemic in most developing countries and resurgent in developed countries with high rates of HIV infection. With the emergence of multi-drug resistant (MDR) TB, it became the world scourge by reaching epidemic proportions[2] related to poor treatment outcomes indicated by low cure rates of around hr and high return rates on the brink of half-hour when normal short-course TB treatment[3,4]. it absolutely was forecasted that one.3 million MDR-TB cases required to be treated between 2010 and 2015 in twenty seven countries with high MDR-TB burden[5]. in an exceedingly report from World Health Organisation (WHO) for 2011, India is quoted because the highest TB burden country with Associate in Nursing calculable incidence of two.2 million cases[6]. Poor primary health-care infrastructure in rural areas, unregulated personal health care resulting in widespread irrational use of first-line and second-line antiTB medication, spreading HIV infection, and impoverishment ar a number of the factors creating tough to regulate TB in India[7]. within the year 2005, 0.04% of the TB cases in India were diagnosed as MDR-TB, that rose to zero.15% (~4 times) within the year 2007[8]. This forbidding state of affairs incorporate an on the spot ought to develop newer, safer and potent antituberculosis medication for effective medical aid. The exploration of latest heterocyclics that may accommodate efficiency to multiple biological targets remains an eternal intriguing scientific endeavor. Pyrazolines are famed to play a vital role in healthful chemistry. multifariously substituted pyrazoline derivatives embedded with type of practical cluster ar necessary biological agents[9] and are found to exhibit various biological and medicine activities like antibacterial[10,11], antiamoebic[12], antidepressant[13,14],antioxidant[15,16], antiinflammatory[17], analgesic[18], hypotensive[19], anticoagulant[20], antifungal and insecticidal[21] actions. Some pyrazoline derivatives possessing thiourea, 3-chloro-4-fluoro aniline[22] and quinoline moieties[23]

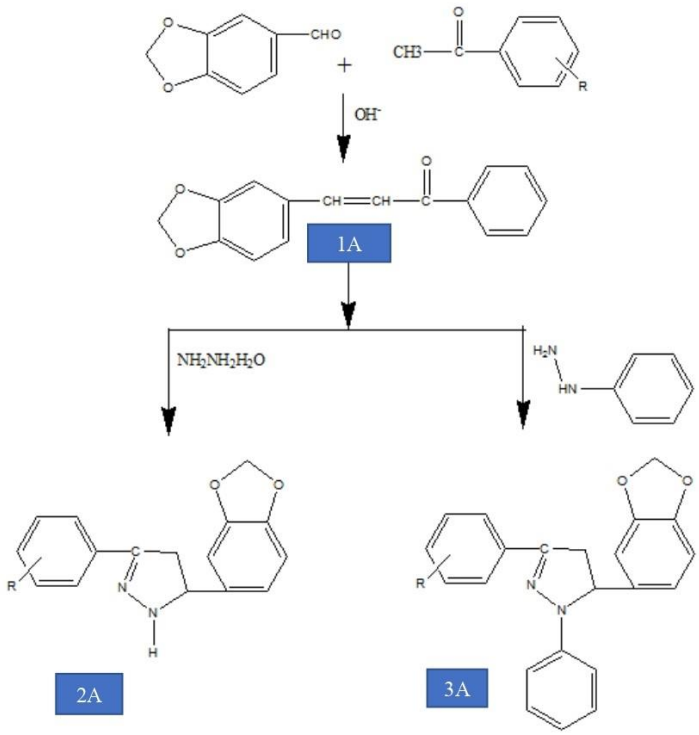
## 2. MATERIALS AND METHODS

The chemicals and reagents utilized in this project were of AR grade, piperonal purchased from letter of the alphabet Aldrich (St.Louis (USA) and substituted acetophenone SRL Ltd (Mumbai).

The techniques for the characterisation of the synthesized compounds were ultraviolet light spectra (UV 1601Shimadzu mass spectrometer, Bagalkot) IR spectra (Fourier transforms IR mass spectrometer model shimadzu mistreatment KBr pellets. Model FTIR 8400S, Bijapur) 1H magnetic resonance (Bruker Avance II

four hundred megacycle per second magnetic resonance mass spectrometer, Chandigarh) mistreatment DMSO-d6 solvent and TMS as internal standard) spectrometer. (AB sciex, ESI technique, model API 2000)
EAC cell lines, Tryphan Blue and HBBS were procured from Hi media laboratories, Mumbai. Metzer medicine analysis magnifier, Haemocytometer, C.O.D apparatus (Thermlab, Mumbai) and icebox centrifuge (MPW-350R), analysis centrifuge (Remi industries, Mumbai). All the opposite chemicals and solvents used were AR grade.

In vitro short-run toxicity was assayed by deciding the proportion viability of EAC cells mistreatment Tryphan blue exclusion technique.




**Synthesis of Chalcone (1A)**
In to a clean dry cone-shaped flask substituted acetophenone [0.06 mol] and piperonal [0.06 mol] and ethyl alcohol [15-20ml] was introduced and cooled to 10-15°c in ice bath. To the cooled solution NaOH [30ml] was additional by drop wise with continuous stirring for four hours exploitation magnetic stirrer and so left long. The reaction mixture was poured into crushed ice and if necessary, it had been neutralised with acid. obtained was filtered and washed in turn 3 times with cold water. The ensuing chalcone were sublimate by recrystallization with acceptable solvent. The purity of the compounds was checked by TLC.

**1-(4-aminophenyl)-3-(benzo[d]dioxol-4-yl) prop-2-en-1one (1A)**
Yellow needles; $R_f$: 0.40, Chloroform: xylene (1:9), % yield: 67.41; m.p.: 212°; UV ($\lambda_{max}$, nm, DMSO): 360.0; IR, $cm^{-1}$ 2903.98( Ar-CH), 1641.97(C=O), 1028.50(sym C-O-C), 1241.56(asym C-O-C), 1590.18(Ar C=C), $^1H$ NMR: δ 7.10-7.86(m, 7H, Ar-H), 6.03(s, 2H $CH_2$), 7.60, 7.89(dd, 2H, $CH_2$)

**General procedure for synthesis of pyrazolines (2b-i) and (3b-i)**
In to a clean dry spherical bottom flask chalcone [0.02 mol] and reducing agent hydrate [0.03mol] / phenyl hydrazine agent [0.024mol] were introduced and dissolved in [15-20ml] glacial ethanoic acid and refluxed for eight hours. The progress of reaction was monitored by TLC. The ensuing mixture was allowed to face long and distilled to get rid of way over solvent. The resultant mixture was poured into cold water thus} it had been neutral with bicarbonate resolution The solid so obtained was filtered and washed in turn thrice with cold water the ensuing pyrazolines were sublimate by recrystallization with applicable solvent. The purity of the compounds was checked by tender loving care. The Physico-chemical constant and therefore the spectral knowledge of pyrazolines and N-(substituted phenyl) pyrazolines square measure conferred in table no. 3, 4 and 5, six severally.

**4-(5-benzo[d][1,3]dioxol-4-yl)-4,5-dihydro-1-H-pyrazol-3-yl)benzeneamine (2A)**
Creamish white; $R_f$: 0.81, Butanol: Pet.Ether(2:8), % yield: 18.82; m.p.: 244°; UV ($\lambda_{max}$, nm, DMSO): 307.0; IR, $cm^{-1}$ 2974.23(Ar-CH),1521.06(C=N), 1036.74(sym C-O-C), 1242.30 (asym C-O-C), $^1$H NMR: δ 6.67-7.66(m, 7H, Ar-H) 5.93($CH_2$Pyraz) 3.08, 3.74(dd, 2H, $CH_2$Pyraz) 5.45(t, 1H, CH) 2.31(s, 2H, $NH_2$)

**5-(Benzo[d][1,3]dioxol-4-yl]-1phenyl-4,5-dihydro-1H-pyrazoline-3-yl)benzenamine (3A)**
Dull yellow amorphous; $R_f$: 0.83, Butanol: Pet.Ether (3:7), % yield: 51.89; m.p.: 201°; UV ($\lambda_{max}$, nm, DMSO): 370.0; IR, $cm^{-1}$ $^{2915.50}$(Ar-CH),1599.04(C=N), 1036.77(sym C-O-C), 1244.13(asym C-O-C) $^1$H NMR: δ 5.97-7.38(m, 12H, Ar-H)5.91(s,2H, $CH_2$Pyraz)3.75, 3.10(2H $CH_2$Pyraz)5.15(t, 1H, CH)2.16(dd, $NH_2$)

**Antitubercular activity**
The antitubercular activity of compounds was assessed against M. T.B. H37Rv by microplate alamar blue assay [24]. First, two hundred two hundred of sterile deionised water was side to any or all outer perimeter wells of sterile ninety six wells plate to reduce the evaporation of medium within the take a look at wells throughout incubation. The wells received 100 µl of the Middle brook 7H9 broth and serial dilution of compounds dissolved in DMSO were created directly on plate from a hundred to zero.2 µg/ml. The higher than same wells were inoculated with 100 µl of 2000 cfu/ml of organisms in Middle brook 7H9 broth.. Plates were coated and sealed with parafilm and incubated at 37º for 5 days. After this, twenty five twenty five of freshly ready 1:1 mixture of almar blue chemical agent and 100 percent tween eighty was side to the plate and incubated for twenty-four hours. a similar technique was followed for management (DMSO) and normal medication (pyrazinamide and isoniazid). A blue colorize the well was taken as no microorganism growth, and pink color was scored as growth. The MIC was outlined as lowest drug concentration that prevented the color amendment from blue to pink.

**Cytotoxic activity**
The synthesized compounds were tested for in vitro toxicity by tryphan blue exclusion assay technique [25] against EAC cells. On the fifteenth day, the EAC cells were aspirated aseptically from the bodily cavity of the mice and washed with HBBS and centrifuged for fifteen min at 1500 rpm in an exceedingly cold centrifuge. The pellet was resuspended with HBBS and also the method was recurrent 3 times. Finally, the cells were suspended in an exceedingly notable amount of HBBS and also the cell count was adjusted to 1×106 cells/ millilitre. This suspension mensuration zero.1 millilitre was taken in eppendoRf tubes and zero.1 millilitre of various concentration of sample and 5-fluorouracil in DMSO or plane DMSO was side and incubated at three7º for 3 h. The viable cells were counted on a hemocytometer victimisation tryphan blue dye exclusion technique. Tdead is that the range of dead cells within the treated cluster, Cdead is that within the management cluster, and Ttotal is that the total range of cells within the treated cluster.

***Table 1: Antitubercular and Cytotoxicity activity of compounds 2A and 3A***

| **Compund** | **Antitubercular Activity MIC/Ug/Ml)** | **Cytotoxic activity % Inhibition (µg/ml)** | | |
|---|---|---|---|---|
| | | **50** | **100** | **200** |
| 2A | 0.8 | - | - | - |
| 3A | 0.8 | 39.16 | 30.83 | 39.83 |
| Pyrazinamide | 3.12 | - | - | - |
| Isoniazide | 0.5 | - | - | - |
| 5-Fluorouracil | | 50.83 | 57.38 | 66.19 |

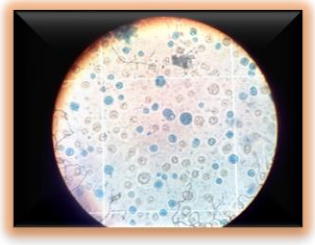


***Figure no. 01 Cytotoxic activity of 1, 3, 5-trisubstituted pyrazolines (3A) by Tryphan blue exclusion assay***

## 3. RESULT AND DISCUSSION

The compounds 2A and 3A were screened for in vitro antitubercular activity by almar blue dye technique against the simplest studied virulent laboratory strain tubercle bacillus H37Rv ATCC 25618. This technique uses a thermally stable chemical agent and shows sensible correlation with proportional and BACTEC radiometric ways. The activity information is shown in Table a pair of. Generally, all the tested compounds exhibited efficiency over pyrazinamide the second line antitubercular drug and fewer than bactericide (INH), the first-line antitubercular drug. All the compounds i.e 2A & 3A exhibited the MIC at zero.8 µg/ml. whereas pyrazinamide and antibacterial exhibited MIC at three.125 and 0.5 µg/ml, severally.

The toxicity of one, 3, 5-trisubstituted pyrazolines 3A was assessed by tryphan blue exclusion assay mistreatment Earlich pathology Cells (EAC). The results urged that activity failed to follow a selected pattern and it absolutely was found to be nonlinear with relation to compounds 3A(Table1) but, the quality drug 5-fluorouracil exhibited cytotoxic activity that was found to be linear with compounds. The toxicity of three, 5-disubstituted pyrazolines 3A wasn't concluding as a result of the precipitation of DMSO answer of those compounds once it comes to bear with liquid medium of the take a look at.

## 4. CONCLUSION

A brand new series of three, 5-disubstituted and one, 3, 5-trisubstituted pyrazoline derivatives were synthesized with sensible yield and their structures were confirmed by IR, 1HNMR and spectroscopy.

The purity of the compounds was checked by their TLC and temperature that gave sharp temperature.
3, 5-disubstituted and one, 3, 5-trisubstituted pyrazolines square measure therapeutically necessary category of heterocyclic compounds. {the technique|the tactic|the strategy} employed in this study is one in every of the simplest method for introducing substitution at three and five position of the pyrazoline ring.

The cyclocondensation of chalcones with reducing agent hydrate or phenyl reducing agent in presence of carboxylic acid afforded three, 5-disubstituted pyrazoline 2A and one, 3, 5-trisubstituted pyrazolines 3A severally in moderate to sensible yield. The compounds 3A might solely exhibited toxicity up to thirty six.83 that when tested against Earlisch pathology Cells (EAC) by tryphan blue exclusion assay. Compounds 2A and 3A exhibited vital antitubercular activity against M.tuberculosis H37 RV strains once studied by Almar blue technique. The activity of those compounds was found to be more than the quality medicine like pyrazinamide and antibiotic drug.

## References

World Health Organization. Treatment of TB: Guidelines for National Programmes. Geneva: WHO; 2003.

du Toit LC, Pillay V, Danckwerts MP. Tuberculosis chemotherapy: Current drug delivery approaches. Respir Res 2006;7:118-35.

Espinal MA, Kim SJ, Suarez PG, Kam KM, Khomenko AG, Migliori GB, et al. Standard short-course chemotherapy for drug-resistant tuberculosis: Treatment outcomes in 6 countries. J Am Med Assn 2000;283:2537-45.

Migliori GB, Espinal M, Danilova ID, Punga VV, Grzemska M, Raviglione MC. Frequency of recurrence among MDR-tB cases ‘successfully’ treated with standardised short-course chemotherapy. Int J Tuberc Lung Dis 2002;6:858-64.

World Health Organization. Global tuberculosis control: A short update to the 2009 report. Geneva: 2009. Available from: http://www.who.int/ tb/publications/global_report/2009/update/en/index.html [Last accessed on 2012 Dec 21].

World Health Organization. Global Tuberculosis Control 2012. Geneva: WHO; 2012. Available from: Http://www.who.int/tb/publications/ global_report/[Last accessed on 2012 Dec 23].

Sandhu GK. Tuberculosis: Current Situation, Challenges and Overview of its Control Programs in India. J Glob Infect Dis 2011;3:143-50.

World Health Organization. Global Tuberculosis Report. Geneva: WHO; 2009.

Rahman MA, Siddiqui AA. Pyrazoline derivatives: A worthy insight into the recent advances and potential pharmacological activities. Int J Pharma Sci Drug Res 2010;2:165-75. 10. Kolhe SV, Doshi AG, Raut AW. Synthesis of new pyrazolines and their derivatives. Indian J Heterocycl Chem 2002;11:281-2.

Holla SB, Veerendra B, Shivananda MK, Latha KP, Vaidya VP. Antimicrobial, analgesic and anthelmentic activity of some arylfurylpyrazolines. Indian J Heterocycl Chem 2003;12:385-6.

Mbarki S. Dguigui K, Hallaoui ME. Construction of 3D-QSAR models to predict antiamoebic activities of pyrazoline and dioxazoles derivatives. J Mater Environ Sci 2011;2:61-70.

Palaskaa E, Aytemira M, Uzbay IT, Erola D. Synthesis and antidepressant activities of some 3,5-diphenyl-2-pyrazolines. Eur J Med Chem 2001;36:539-43.

Prasad YR, Rao AL, Prasoona L, Murali K, Kumar PR. Synthesis and antidepressant activities of some 1,3,5-triphenyl-2-pyrazolines and 3-(2-hydroxynapthalen-1-yl)-1,5-diphenyl-2-pyrazolines. Bioorg Med Chem Lett 2005;15:5030-4.

Hazra K, Nargund LV, Rashmi P, Sharadchandra JNN, Nanda B. Synthesis and antioxidant activity of fluorobenzothiazolo pyrazoline. Der Chem Sin 2011;2:149-57.

Mondal P, Jana S, Mahanti B, Kanthal LK, Banerjee. Synthesis and evaluation of some novel pyrazoline derivatives of indol 2,3-dione as a potential antioxidant and antibacterial agents. Int J Pharm Sci Tech 2009;3:8-11.

Shoman ME, Abdelaziz M, Aly OM, Farag HH, Morsy MA. Synthesis and investigation of antiinflammatory activity and gastric ulcerogenicity of novel nitric oxide donating-pyrazoline derivatives. Eur J Med Chem 2008;22:1-9.

Amir M, Kumar H, Khan SA. Synthesis and pharmacological evaluation of pyrazoline derivatives as new antiinflammatory and analgesic agents. Bioorg Med Chem Lett 2008;18:918-22.

Zitounia GT, Chevallet P, Kilic FS, Erolc K. Synthesis of some thiazolyl-pyrazoline derivatives and preliminary investigation of their hypotensive activity. Eur J Med Chem 2000;35:635-41.