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Synthesis and Evaluation of Some Substituted New Pyrazoline Derivatives of Biological Interest

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A B S T R A C T

The synthesis, structure and biological activity of Pyrazoline derivatives have long been the focus of research interests in the field of Medicinal Chemistry. A number of Pyrazoline derivatives have been reported to possess interesting biological activities such as Antimicrobial, and Anti-tubercular etc. In the present proposal, substituted Benzaldehyde was made to react with various Aromatic substituted ketones to yield different Chalcones. Chalcones so prepared were further allowed to react with Hydrazine Hydrate in the presence of Ethanol and Glacial acetic acid to get Pyrazoline derivatives, further Mannich reaction was carried out to give Mannich base **(A_1 - A_{16})** all synthesized compound were characterized by IR, H^1-NMR and CHN Analysis. All the compounds were evaluated for Antibacterial at the concentration of 200 µcg/mL by using cup-plate agar diffusion method. The activity was carried out on different micro-organisms (***E.coli, S. aureu,***) measured in terms of zone of inhibition and compared the standard drug Ciprofloxacin. The Antitubercular screening was carried out by Middle brook 7H9 agar medium against H_{37}Rv Strain. Middle brook 7H9 agar medium using Streptomycin as a standard. The Pyrazoline have shown considerable activity at high concentrations. These compounds with the suitable molecular modification may prove as a drug of choice in the treatment of microbial infectious disease in future.

Keywords: Pyrazoline, Anti-tubercular and Antimicrobial activity

A R T I C L E I N F O

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1. Introduction

Medicinal chemist have immeasurable quest for innovation, blending synthetic chemistry, molecular modeling, computational biology, structural genomics, drug repositioning and pharmacology to discovery and design new drugs and investigate their interaction on the substrate models and at the cellular level. Many efforts are being made in the design and development of novel drugs from synthetic origin. As it's well said "Need is the mother of inventions". Thus there is growing demand to discover and develop faster acting, affordable drugs to fight any disease.

Antimicrobial

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan's. Antimicrobial drugs either kill microbes or prevent the growth of microbes.[2] The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940's, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials. Broad screening program were instituted to find antibiotics that might be effective in the treatment of infectious resistant to existing chemotherapeutic as well as to provide safer and more effective chemotherapy. The discoveries of broad-spectrum antibacterial antibiotics such as fluroquinolones, chloramphenicol, tetracycline and antifungal antibiotics such as nystatin and griseofulvin and the ever increasing number of antibiotics that may be used to treat infectious agent that have developed resistance to some of the older antibiotics attest to the spectacular success of this approach as it has been applied in research programs throughout the world.[3]

The word "antibiotics" comes from the Greek anti ("against") and bios ("life"). Antibiotics are drugs that either destroy bacteria or prevent their reproduction. Antibiotics belong to the broader group of antimicrobial compounds, used to treat infections caused by microorganisms, including fungi and protozoa.[4] Paul Ehrlich, a German medical scientist in the late 1880s.[5] Scientific endeavors to understand the science behind what caused these diseases, the development of synthetic antibiotic chemotherapy, the isolation of the natural antibiotics marked milestones in antibiotic development.[6.] In 1928 Fleming made an important observation concerning the antibiosis by penicillin. Fleming postulated the effect was mediated by a yet unidentified antibiotic like compound which could be exploited a naturally occurring antibiotic although he initially characterized some of its antibiotic properties he didn't pursue its development.[7]

In the meantime, another synthetic antibacterial antibiotic Prontosil was developed and manufactured for commercial use by Domagk in 1932.[8] Florey credited Dubos with pioneering the approach of deliberately, systematically searching for antibacterial compounds. Such a methodology had led to the discovery of gramicidin, which revived Florey's research in penicillin.[9]

Antibiotics Resistance

Antibiotics are extremely important in medicine, but unfortunately bacteria are capable of developing resistance to them. Antibiotic-resistant bacteria are germs that are not killed by commonly used antibiotics. When bacteria are exposed to the same antibiotics over and over, the bacteria can change and are no longer affected by the drug. The problem of antibiotic resistance is worsened when antibiotics are used to treat disorders in which they have no efficacy (e.g. antibiotics are not effective against infections caused by viruses), and when they are used widely as prophylaxis rather than treatment. Resistance to antibiotics poses a serious and growing problem, because some infectious diseases are becoming more difficult to treat. Resistant bacteria do not respond to the antibiotics and continue to cause infection. Some of these resistant bacteria can be treated with more powerful medicines, but there some infections that are difficult to cure even with new or experimental drugs.[10]

Current status

Numerous semi synthetic and synthetic derivatives have been added to the total. Very few such compounds have found application in general medical practice, however, because in addition to the ability to combat infection or neoplastic disease, an antibiotic must possess other attributes.

1. It must exhibit sufficient selective toxicity to be decisively effective against pathogenic microorganisms and without causing significant toxic effect.
2. It should be chemically stable enough to be isolated, processed and stored for a reasonable length of time without deterioration of potency.
3. The rates of biotransformation and elimination should be slow enough to allow a convenient dosing schedule.

The spectacular success of antibiotics in the treatment of human disease has prompted the expansion of their use into a number of related fields like veterinary medicine, fungal diseases of plant and in food preservation. With research activity stimulated to find new substances to treat viral infections that now are combated with only limited success and with the promising discovery that some antibiotics are active against cancer that may be viral in origin, the future development of more antibiotic and the increase in the amounts produced seem to be assured[11].

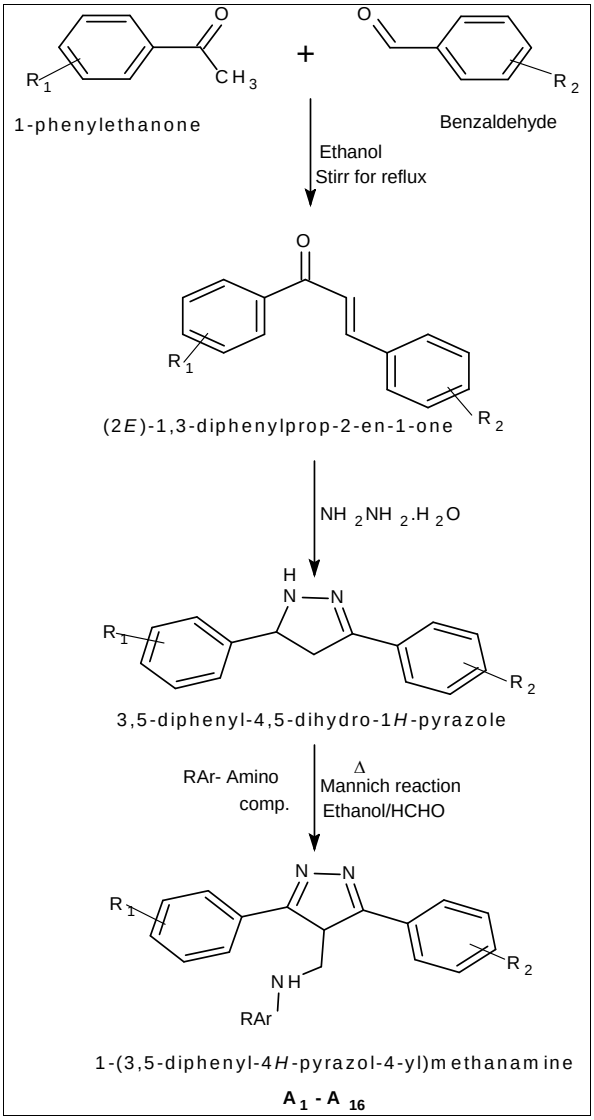
2. Experimental

Procedure for Scheme -

General procedure for the synthesis of chalcone derivatives: Place a solution of 11gm of NaOH in 100mL of distilled water & 60mL of rectified spirit was added in the round bottom flask .The flask was immersed in a bath of crushed ice & provided with mechanical stirrer . Pour in 16gm of (0.21 mole) of freshly distilled acetophenone, start the stirrer and then add 22ML(0.21) of substituted bezaldehydes**.** The temperature of the reaction mixture

maintained at 25°C & stir vigorously until the mixture is so thick that stirring is no longer effective (2-3 hrs.) .remove the stirrer & leave the reaction mixture in the ice chest overnight. Filter the product with the suction on Buchner funnel, wash with the cold water until the washing is neutral to the litmus .The crude chalcone, after dry in an air, the pale yellow solid M.P. 160°C, yield of chalcone is 85%.

Scheme-1



General procedure for synthesis of 3, 5-diphenyl-4, 5-dihydro-1H-pyrazole:

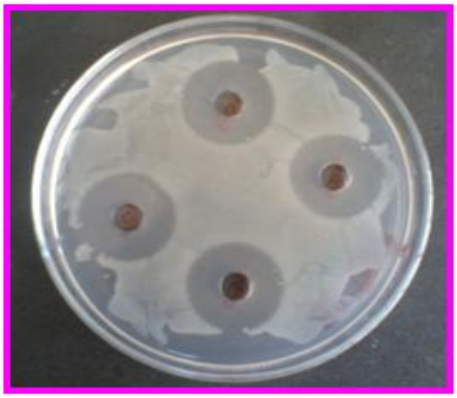
Equimolar concentration of chalcone derivative (0.01mole) is reacted with 0.01 mole of hydrazine hydrate. This refluxed for 45 min., allowed to cool & the residue is removed from alcohol drop by drop & recrystallized. M.P & TLC checked.

General procedure for synthesis 1-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl) methanamine:

0.01mole 3,5-diphenyl-4,5-dihydro-1H-pyrazole& 0.01M primary amine was dissolved in ethanol, to above mixture. 0.01 M formaldehyde was added and refluxed for 2 hours. The reaction mixture cooled and poured over crushed ice and kept in refrigerator for overnight .The product was filtered and dried and recrystallized. M.P and percentage yield was reported

3. Biological Screening

Antibacterial Activity of Synthesized Compounds on S.aureus and E.coli.



Staphylococcus aureus

Escherichia coli

Table 2: Antibacterial activity of the synthesized compounds (A_1-A_{16})

Compd.	Zone of inhibition at 200µcg/mL (in mm.)	
	E. coli	S. aureus
A_1	19	17
A_2	15	13
A_3	16	18
A_4	18	13
A_5	12	13
A_6	11	16
A_7	10	12
A_8	14	13
A_9	18	19
A_{10}	16	15
A_{11}	21	13
A_{12}	17	15
A_{13}	15	14
A_{14}	12	10
A_{15}	14	18
A_{16}	16	14
Ciprofloxacin	15	15

Compounds **A_1**, **A_2**, **A_3**, **A_4**, **A_6**, **A_8**, **A_9**, **A_{1O}**, **A_{11}**, **A_{12}**, **A_{13}**, **A_{15}** **&** **A_{16}** have shown maximum antibacterial activity. Ciprofloxacin was used as standard drug. The antitubercular screening was carried out by Middle brook 7H9 agar medium against H_{37}Rv Strain. Middle brook 7H9 agar medium containing different derivatives **(A_1-A_{12})**, standard drug as well as control, Middle brook 7H9 agar medium was inoculated with Mycobacterium tuberculosis of H_{37}Rv Strain. The inoculated bottles were incubated for 37°C for 4 weeks. At the end of 4 weeks they were checked for growth.

4. Results and Discussion

The synthesized compounds were subjected to various anti-bacterial and anti-tubercular activities by using standard methods.

Anti-bacterial activity:

All the synthesized pyrazoline derivatives were screened for anti-bacterial activity at 200 µg/ml concentration by using cup-plate agar diffusion method and the standard drug used was Ciprofloxacin. Compounds **A_1**, **A_2**, **A_3**, **A_4**, **A_8**, **A_9**, **A_{1O}**, **A_{11}**, **A_{12}**, **A_{13}** and**A_{16}** shows maximum. Activity against Escherichia coli (ATCC 25922). Compounds **A_1**, **A_2**, **A_3**, **A_6**, **A_9**, **A_{1O}**, **A_{12}**, **A_{13}** and**A_{15}** showed maximal activity against Staphylococcus aureus(ATCC 25923

Anti-tubercular activity:

CompoundsA_1, A_2, A_3, A_4, A_5, A_6 ,A_7 and A_8 have shown promising antitubercular activity at both the concentration. H_{37} Rv strain was used as standard tubercular organism. Streptomycin was used as standard drug. However, Streptomycin have shown antitubercular activity at 25 µcg /ml.

Table 3: Antitubercular activity of the synthesized compounds

S. No.	Compounds	25 mcg/mL	50 mcg/mL	100 mcg/mL
1.	A_1	S	S	S
2.	A_2	S	S	S
3.	A_3	S	S	S
4.	A_4	R	S	S
5.	A_5	R	S	S
6.	A_6	S	S	R
7.	A_7	S	S	S
8.	A_8	R	S	S
9.	A_9	R	R	S
10.	A_{10}	R	R	**S**
11.	A_{11}	R	R	**S**
12.	A_{12}	R	R	**S**
13.	A_{13}	S	R	R
14.	A_{14}	S	R	R
15.	A_{15}	S	R	R
16.	A_{16}	S	R	R
STD.	**Streptomycin**	**S**	**S**	**S**

R denotes Resistance and **S** denotes Sensitive.

Compounds**A_1**, **A_2**, **A_3**, **A_4**, **A_5**, **A_6**, **A_7** and **A_8**have shown promising ant tubercular activity at both the concentration. H_{37} Rvstrain was used as standard tubercular organism. Streptomycin was used as standard drug. However, Streptomycin have shown antitubercular activity at **25 µcg /ml.**

5. Conclusion

- Around 16 new Pyrazoline derivatives were synthesized, with the standard chemicals and well established procedures.
- The synthesized compounds were tested for their Preliminary Tests, Physical Constants, and TLC.
- The structures of the final compounds were confirmed by IR, ^{1}H-NMR Spectra and CHN analysis.
- The proposed compounds were screened for their Antimicrobial and Antitubercular activities with the standard drugs in the well-equipped microbiology and pharmacology lab by using standard methods.
- Compounds**A_1**, **A_2**, **A_3, A_4, A6, A8**, **A_9**, **A_{1O}, A_{11}**, **A_{12}**, **A_{13}**, **A_{15}** and **A_{16}** were found to possess maximum activity against both Escherichia coli, staphylococcus aureus.
- All the compounds were screened for anti-tubercular activity, Compounds **A_1**, **A_2**, **A_3**, **A_4**, **A_5**, **A_6**, **A_7** and **A_8** have showed excellent antitubercular activity &compounds **A_9, A_{10}, A_{11}**, **A_{12}**, **A_{13}, A_{14}, A_{15}** and **A_{16}**have showed moderate activity.
- The proposed work has given out many active Antibacterial and Antitubercular agents. Some of the compounds have showed excellent activities. These compounds with suitable modification can be explored better for their therapeutic activities in the future.

6. References

[1] John HK, John MB. Wilson and Griswold's Textbook of Organic medicinal and Pharmaceutical Chemistry. 11th Edition, Philadelphia: Lippincott William and Wilkins; 2004:14.

[2] Antimicrobial - Definition from the Merriam-Webster Online Dictionary". http://www.merriam-webster.com/dictionary/Antimicrobial. Retrieved 2009-05-02.

[3] Essentials of Medical Pharmacology by K D Tripathi, Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, 5th edition**;** 2004, 698-708.

[4] Davey PG."Antimicrobial chemotherapy". In Ledingham JGG, Warrell D A. Concise Oxford Textbook of Medicine. Oxford: Oxford University Press. 2000,1475.

[5] Calderon CB, Sabundayo BP. Antimicrobial Classifications: Drugs for Bugs. In Schwalbe R, Steele-Moore L, Goodwin AC. Antimicrobial Susceptibility Testing Protocols. CRC Press. Taylor & Frances group. 2007;564

[6] Foster W, Raoult A "Early descriptions of antibiosis". J R Coll Gen. Pact.24 (149), 12, 1974; 889–94.

[7] Sykes R. "Penicillin: from discovery to product". Bull. World Health Organization.79, 8, 2001; 778–9.

[8] Bosch F, Rosich L."The contributions of Paul Ehrlich to pharmacology: a tribute on the occasion of the centenary of his Nobel Prize". Pharmacology82, 3, 2008; 171–9.

[9] Van Epps HL, "Rene Dubos: unearthing antibiotics". J. Exp. Med.203, 2, 2006: 259.

[10] Pearson, Carol. "Antibiotic Resistance Fast-Growing Problem Worldwide". Voice of America. 2,2007,28

[11] The Pharmacological Basis of Therapeutics by Alfred Goodman Gilman, McGraw-Hill Medical Publishing Division 10th edition, 2001, 1284.

[12] Kulkarni SK. Handbook of experimental pharmacology. 3rd ed. Delhi: Vallabh Prakashan; 1999:127-30.

[13] Mark H. Beers., The Merck Manual of Medical Information., 2nd Home Ed. Whitehouse Station, NJ: Merck; 2003.20