Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 5, 2011

Research Article

SYNTHESIS OF NOVEL PYRAZOLE ANALOGUES AS EFFICACIOUS ANTIMICROBIAL AGENTS

ALKA CHAUHAN[A]*, P. K. SHARMA[A], NIRANJAN KAUSHIK[A], NITIN KUMAR[A]

[a]Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, Meerut 250001; U.P. India.
Email: alka.chauhan86@gmail.com

Received: 4 July 2010, Revised and Accepted: 11 Sep 2011

ABSTRACT

In an attempt to find a new class of antimicrobial agents, a new series of substituted 3,5-diphenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide derivatives have been synthesized via series of intermediates; substituted (2*E*)-1,3-diphenylprop-2-en-1-one and phenyl(3-phenyloxiran-2-yl)methanone. Structures of compounds were confirmed by IR, ^{1}HNMR, Mass spectral data and elemental analysis. All synthesized compounds were investigated for their antibacterial and antifungal activity. Compound A15 displayed activity more than standard drug for bacteria *E.coli*. Six compounds (A3, A4, A5, A8, A9 and A10) exhibited more activity than standard at low concentration while only two compounds (A6 and A7) were found potent at higher concentrations for *S.aureus*. Three compounds (A5, A12 and A16) have shown good activity for fungus *A.niger* and two compounds (A3 and A7) for *P.chrysogenum*. Ofloxacin and Ketoconazole were used as standards drugs for antibacterial and antifungal activity, respectively.

Keywords: Antibacterial activity, Antifungal activity, Chalcone, Pyrazole

INTRODUCTION

There is real perceived need for the discovery of new compounds especially with antimicrobial activity, which are apparent from those of well-known classes of antibacterial agents to which many clinically relevant pathogens are now resistant. The modern era of antimicrobial chemotherapy began in 1929, with Fleming's discovery of the powerful bactericidal substance, penicillin, and Domagk's discovery in 1935 of synthetic chemicals (sulfonamides) with broad antimicrobial activity. Antimicrobials drugs are the greatest contribution of the present century to the therapeutics. Their importance is magnified in the developing countries, where infective disease predominates. This class was designed to inhibit or kill the infecting organism and to have no or minimal effects on receiver. This type of therapy is generally called *chemotherapy* [1].

Two groups of antimicrobial agents used in the treatment of infectious disease: *antibiotics*, which are natural substances produced by certain groups of microorganisms, and *chemotherapeutic agents*, which are chemically synthesized. A hybrid substance is a *semi synthetic antibiotic*, wherein a molecular version produced by the microbe is subsequently modified by the chemist to achieve desired properties. Antibacterials have many mechanisms of action (Fig 1), including inhibiting cell wall synthesis, activating enzymes that destroy the cell wall, increasing cell membrane permeability, and interfering with protein synthesis and nucleic acid metabolism.

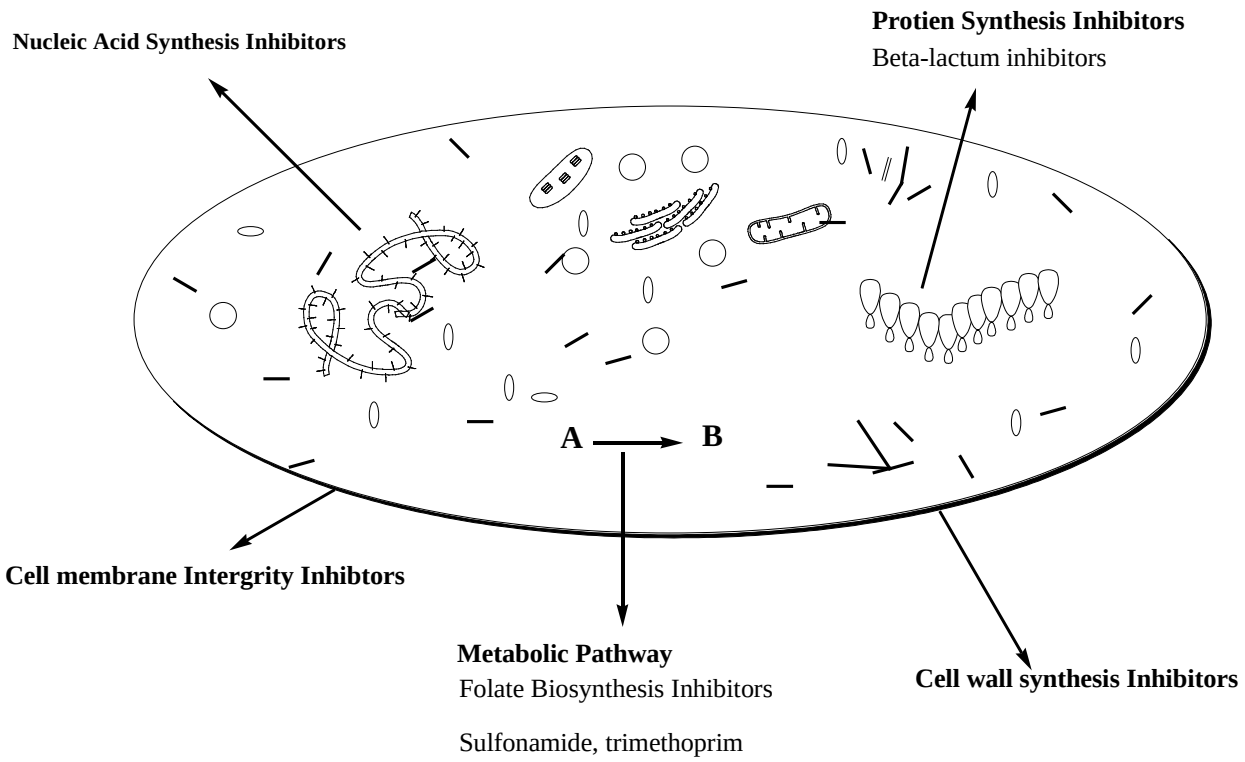

Fig. 1: Mechanism of action of antibacterial drugs

Infectious strains of *Escherichia coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In few cases, virulent strains also cause haemolytic-uremic syndrome, peritonitis, mastitis, septicemias and pneumonia [2]. *Staphylococcus aureus*, aerobic Gram-positive cocci, causes a number of illnesses from minor skin infections, like pimples, boils (furuncles), cellulitis folliculitis, scalded skin syndrome, and abscesses, to life-threatening diseases such as meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis [3]. Generally, the Gram-positive bacteria are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier [4], whereas the Gram-negative bacteria [5] possess an outer phospholipidic membrane carrying the structural lipo-polysaccharide components. This makes the cell wall impermeable to drug constituents [6].

Antifungal drugs are used for superficial and deep fungal infections. Antifungal medication is a medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others. Such drugs are usually obtained by a doctor's prescription or purchased over-the-counter [7]. A worrying trend after 1950s has been the emergence of more threatening type of fungal infections which are, to a large extent, iatrogenic. Because of breakdown of host defence mechanism, saprophytic type of fungus easily invades the living tissue. Types of fungal disease: *Skin infections*: e.g. foot fungus (usually smelly but not life threatening, sometimes becomes serious), ring worm; *Mucosal infections*: oral or vaginal (range from annoying to painful to very difficult, uncomfortable but rarely life threatening); *Systemic infections*: fungus in the blood and tissues (immunocompromised population, usually life threatening).

In addition, primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immunocompromised patients (AIDS, cancer and transplants). Like, when spores of fungus *Aspergillus niger* causes inhaled causes lung disease called *Aspergillosis*. Another major infection is otomycosis (ear infection). The airborne spores of *Penicillium chrysogenum* are important human allergens. Vacuolar and alkaline serine proteases have been implicated as the major allergenic proteins [8]. That is why; several reviews have appeared illustrating the problems encountered by today's infectious disease clinicians [9-11].

Pyrazofurin (pyrazomycin),containing pyrazole as its basic moiety is available in market as a broad spectrum antimicrobial drug. Often, itraconazole, ketoconazole, posaconazole, that are being used for the treatment of fungal infections, contains piperazine and one or more azole rings in their structures [12]. Antifungal drugs works by many different mechanisms (Fig 2), including azoles drugs targets the fungal-specific synthesis of membrane lipids, amphotericin inserts preferentially into fungal membranes and disrupts their function, echinocandins targets synthesis of β-glucan (a fungal cell specific cell wall molecule) and 5-fluorocytosine targets fungal-specific DNA replication, etc.

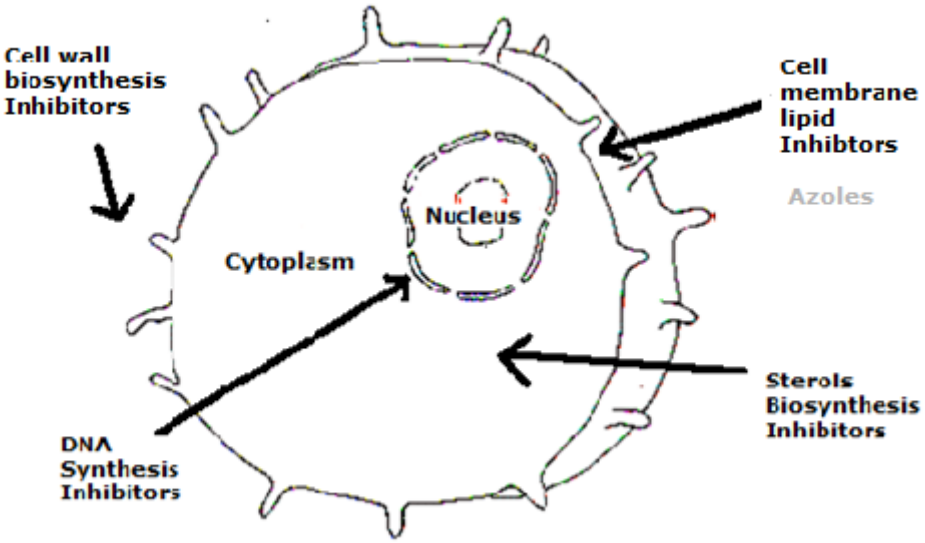

Fig 2: Mechanism of action of antifungal drugs

Electron-rich nitrogen containing heterocycles play an important role in diverse biological activities. Sometimes, introducing different substitutions in pyrazolidinone ring [13, 14] results in enhanced activity. Second nitrogen in the five-membered ring also influences the antibacterial or pharmacokinetic properties [15, 16].

In medicine, derivatives of pyrazoles (diazoles) are used for their antioxidant, anti-inflammatory, antibacterial, antifungal, anticonvulsant activities, antihyperglycemic, analgesic, antipyretic, hypoglycemic and sedativehypnotic activities [17, 18]. The treatment of pain continues to be the subject of considerable pharmaceutical and clinical research [19, 20]. A systematic investigation of this class of heterocyclic lead revealed that pyrazole containing pharmacoactive agents play important role in medicinal chemistry. The prevalence of pyrazole cores in biologically active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic lead.

Pyrazole derivatives have been proposed as potential treatments for neurodegenerative disorders like Alzheimer's disease. Pyrazoles are also active antitumour [21], anticonvulsant [22] and antimicrobial agents [23]. Sometimes useful as insecticides [24] and are used in dyeing [25].

The aim of the present work is to evaluate the antibacterial and antifungal activity of pyrazole derivatives synthesized via new series of substituted chalcone (Fig 3). The derivatives of pyrazole have proven to be of great importance in exhibiting and enhancing great antibacterial as well as antifungal activities.

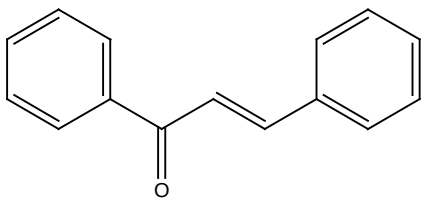

Fig. 3: Chalcone

Pyrazoles (Fig 4) are important nitrogen containing 5-membered heterocyclic compounds and different methods have been produced for their synthesis. Pyrazole constitute interesting group of

heterocycles because of their synthetic versatility and effective biological activities.

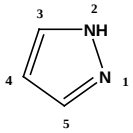

Fig. 4: Pyrazole

In our search novel pyrazole derivatives (**A1-A19**) were synthesized via chalcone derivatives (**AC1-AC19**) and evaluated for antibacterial and antifungal activity. We have contemplated a research focusing to synthesize pharmaceutically important novel antibacterial and antifungal agents.

Chemistry

Reaction of substituted acetophenone with substituted aromatic aldehyde in presence catalyst alkaline conditions (NaOH and ethanol, acting as Lewis base). The catalyst abstracts α-H from terminal methyl group of acetophenone (I) and resulting it to become electron rich species (in turns behaves as nucleophile) then further nucelophilic acetophenone species donates extra electron pair to aromatic aldehyde which is acting in reaction as electron acceptor moiety. Then by leaving one molecule of water it will form a newly substituted chalcone intermediate molecule (II) (Fig 5, Step 1). In Step 2, hydrogen peroxide is doing epoxidation (Peroxidation) reaction in acetone and methanolic solution in presence of again alkaline conditions and donates its one oxygen by simply reducing itself (Fig 6). Thiosemicarbazide brings nucleophilic addition and cyclisation in NaOH and ethanol (acting as catalyst). Thiosemicarbazide functioning as nucleophilic moiety and donates lone pair of electrons from it's both the N-atoms on adjacent position to form new five member heterocyclic ring but having extra hydroxyl group on ring (Fig 7**,** Step 3). Then in step 4; dehydration of hydroxyl group is required from pyrazole ring to form the desired compound by adding H_2SO_4 drop wise that will removing one molecule of water and HSO_4^- ion on (Fig 8).

Stepwise Mechanism of Reaction

Step 1: Cross aldol condensation

Reaction of substituted acetophenone with substituted aromatic aldehyde in presence NaOH and ethanol (alkaline conditions, acting as Lewis base) abstracts α-H from terminal methyl group of acetophenone (I) and because of this acetophenone will become electron rich and in turns behaves as nucleophile and it will donates extra electron pair to aromatic aldehyde molecule (acting here as electron acceptor species). Then by leaving one molecule of water it will form a new substituted chalcone intermediate molecule (II) (Fig 5, Step 1).

Step 2: Peroxidation reaction

In Step 2, hydrogen peroxide is doing epoxidation reaction in acetone and methanolic solution in again alkaline conditions and donates its one oxygen by simply reducing itself (Fig 6).

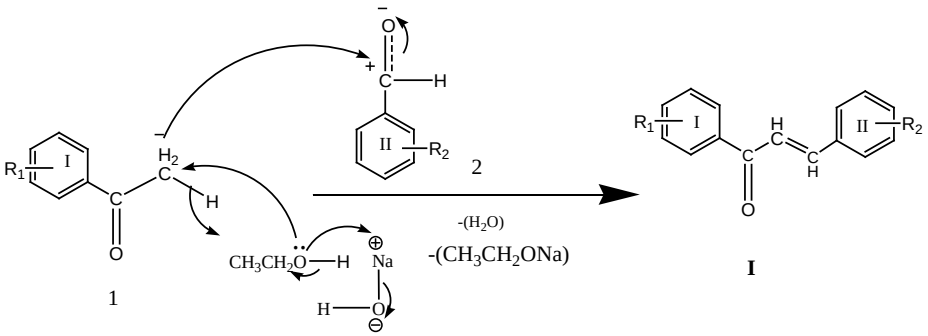

Fig. 5: Cross aldol condensation

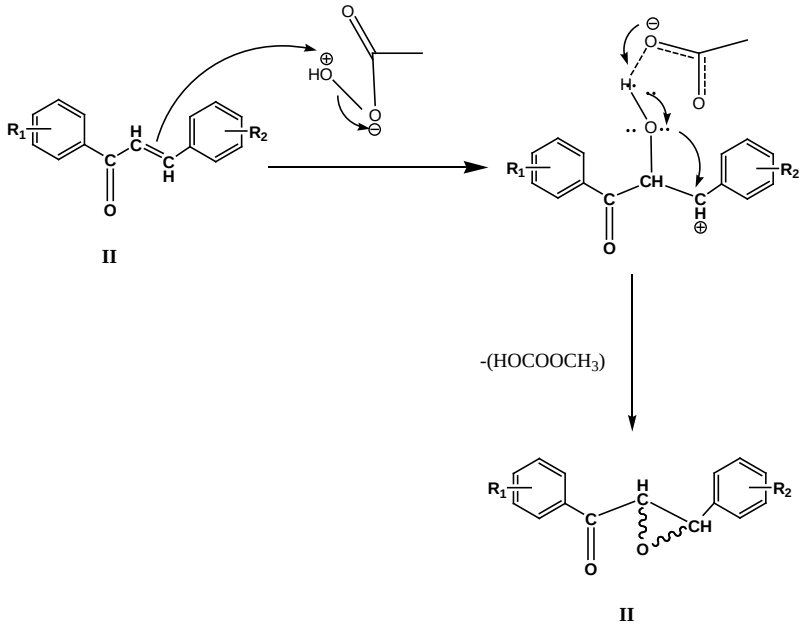

Fig. 6: Peroxidation reaction

Step 3: Nucleophilic addition and cyclisation reaction

Thiosemicarbazide brings nucleophilic addition in presence NaOH and ethanol (acting as catalyst). Thiosemicarbazide functioning as nucleophillic moiety and donating lone pair of electrons from it's both the N-atoms on adjacent position to form new five member heterocyclic ring but having extra hydroxyl group (Fig 7**,** Step 3).

Step 4: Dehydration reaction

Dehydration of hydroxyl group is required on pyrazole ring in step 4 to form the desired compounds by removing one molecule of water and HSO_4^- ion on addition of H_2SO_4 (Fig 8).

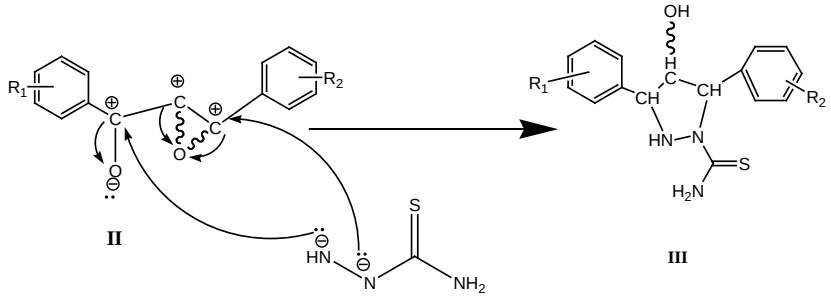

Fig. 7: Nucleophilic addition reaction

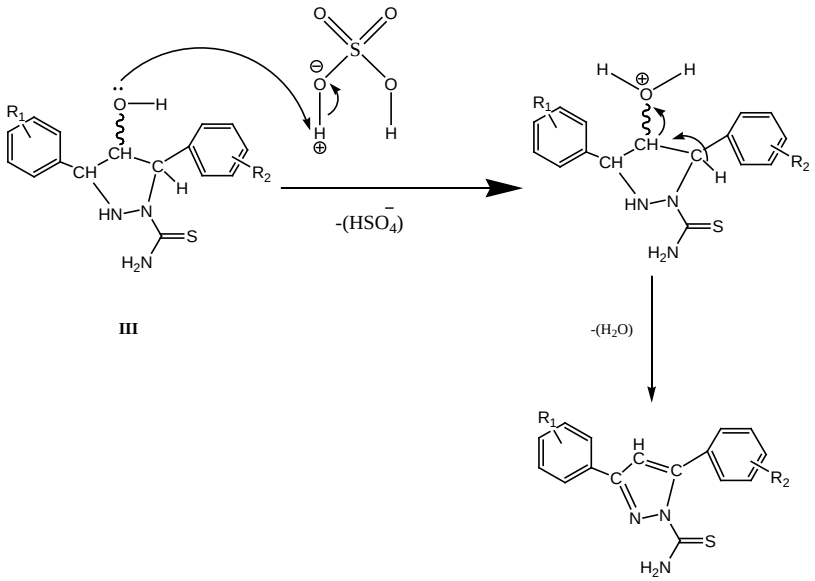

Fig. 8: Dehydration reaction

MATERIAL AND METHODS

The purified pyrazole derivatives were obtained in yields of 45-95%. The synthetic scheme is illustrated in Fig 9. Thin layer chromatography was used to reach completion of reaction and purity of compounds synthesized, using silica gel as stationary phase and Toulene:ethyl acetate:formic acid as solvent system (4:2:1) and visualized by U.V. visualizing cabinet.

All solvents used were analytical grade. The chemicals used were obtained from sigma –Aldrich (St. Louis Mossuri, USA). The structures of compounds were identified using infrared spectroscopy, Mass spectroscopy and proton nuclear magnetic resonance studies. Melting points were determined by digital melting point apparatus and were uncorrected. Elemental proportions for 'C', 'N' and 'S' were determined by instrument Elementar vario EL III-C, Elementar vario EL III-N, Elementar vario EL III-S, Respectively. IR Spectra were recorded by KBR pellet technique using FTIR-84005 shimadzu spectrophotometer. ^{1}HNMR Spectra were obtained on Bruker model DRX (300MHzNMR) Spectrometer in DMSO-d6/$CDCl_3$ as solvent and using tetramethylsilane as internal standard. Mass were recorded on API 2000 triple quadrapole mass spectrophotometer. The purity of the synthesized compounds was analyzed by thin-layer chromatography. MAC Autoclave vertical Cat No MX-101 (DX) was used for autoclaving for antimicrobial activity. Bacteriological incubator Cat No- MSW: 231 was used for incubating the microbes. Biological safety Cabinet (Cat No- MSW 164) or laminar flow bench was used for pouring and preparing the agar plates.

General Synthetic procedure

General Synthesis of chalcone compounds (Step 1)[19]

Aldolic condensation (clasien-schmidt condensation reaction) of equimolar quantities of substituted acetophenone (1) with appropriate quantity of substituted aromatic aldehyde (2) in presence of aqueous alcoholic alkali was used for formation of α, β – unsaturated ketones (i.e. chalcones). Equimolar portions of the substituted acetophenone (10mmol, 1 equiv) and substituted benzaldehyde (10mmol, 1 equiv) were dissolved in 15mL of ethanol. The mixture was allowed to stir for several minutes at 5-10 ºC. Aliquot of a 40% aqueous NaOH solution was then slowly added to the reaction flask. The reaction mixture was allowed to stir at room temperature for approximately 4-6hrs in ice bath.

All the crude products were washed first with cold water until washings were neutral to pH paper and precipitate formed was then collected and recrystallized from ethanol to give chalcone derivatives (I).

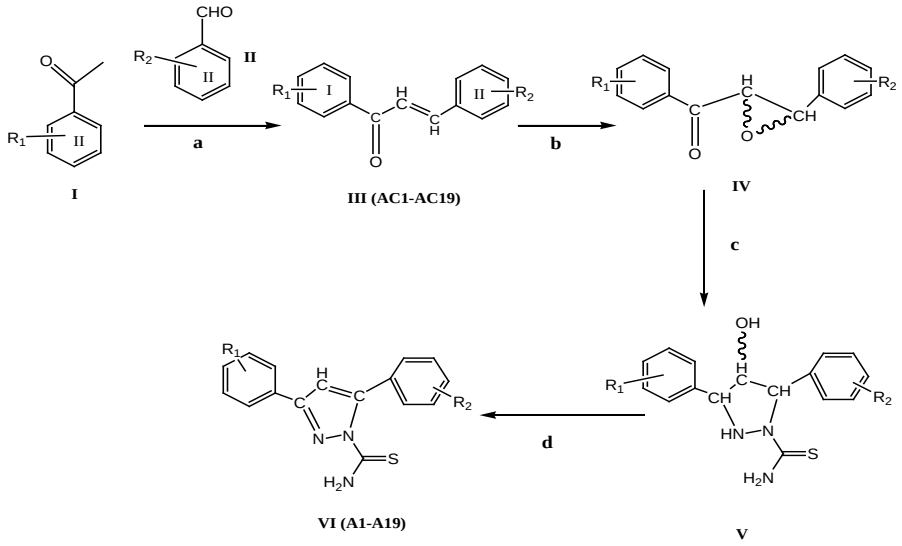

Fig. 9: Complete synthetic scheme (Step1 - Step4): a; 40%NaOH, EtOH; b; H_2O_2, 5%NaOH, CH_3COCH_3: MeOH (3:2); c; Thiosemicarbazide, 10% NaOH, EtOH; d; H_2SO_4:AcOH (1:1)

Table 1: Physio-chemical data of synthesized derivatives (A1-A19)

[a]Compounds	R_1	R_2	Mol. Formula (Mol.Wt.)	[b]R_f value	[c]Yield (%)	[d]m.p. (ºC)
A1	-H	-H	$C_{16}H_{15}N_3S$ (281.37)	0.90	45.00	66-68
A2	-H	-2,OCH_3	$C_{17}H_{17}N_3OS$ (311.40)	0.83[1]	84.15	123-125
A3	-H	-4,OCH_3	$C_{17}H_{15}N_3OS$ (309.38)	0.68[1]	41.66	146-148
A4	-2,4,di-OCH_3	-H	$C_{18}H_{19}N_3O_2S$ (341.42)	0.84[1]	47.34	144-146
A5	-H	-3,4,5,tri-OCH_3	$C_{19}H_{19}N_3O_3S$ (369.43)	0.78[1]	81.25	92-94
A6	2,4,di-Cl	-3,4,5,tri-OCH_3	$C_{19}H_{17}Cl_2N_3O_3S$ (438.32)	0.73[1]	93.50	113-115
A7	2-OCH_3	-2,4,di-Cl	$C_{17}H_{13}Cl_2N_3OS$ (378.27)	0.66[2]	45.40	185-187
A8	-2,5,di-OCH_3	-2,NO_2	$C_{18}H_{16}N_4O_4S$ (384.40)	0.75[2]	77.00	98-100
A9	-H	-2,NO_2	$C_{16}H_{12}N_4O_2S$ (324.35)	0.87[3]	83.74	125-127
A10	-4Cl	-H	$C_{16}H_{12}ClN_3S$ (313.80)	0.87[3]	75.90	128-130
A11	-2,4,di-Cl	-H	$C_{16}H_{11}Cl_2N_3S$ (348.24)	0.83[1]	95.07	180-182
A12	-H	-2,5,di-Cl	$C_{16}H_{11}Cl_2N_3S$ (348.24)	0.79[1]	90.30	84-86
A13	-4,Br	-H	$C_{16}H_{12}BrN_3S$ (358.25)	0.86[1]	84.74	112-114
A14	-3,NH_2	-H	$C_{16}H_{14}N_4S$ (294.37)	0.73[2]	70.50	120-122
A15	-H	-3,NH_2	$C_{16}H_{14}N_4S$ (294.37)	0.87[1]	76.92	102-104
A16	-3,NH_2	-2,5,di-Cl	$C_{16}H_{12}Cl_2N_4S$ (363.26)	0.94[1]	79.540	130-132
A17	-3,NH_2	-3,NO_2	$C_{16}H_{13}N_5O_2S$ (339.37)	0.73[3]	75.60	118-120
A18	-2, OH	-H	$C_{16}H_{13}N_3OS$ (295.35)	0.65[1]	66.60	102-104
A19	-2, OH	-2,4,di-Cl	$C_{16}H_{11}Cl_2N_3OS$ (364.24)	0.80[1]	83.70	149-151

[a] Products were characterized by IR, NMR, MS and elemental analysis

[b] 1; Toulene : EthylAcetate : Formic Acid (4:2:1), 2; EthylAcetate : n-Hexane (3:7), 3; Pet. Ether : EthylAcetate (2:1)

[c] Isolated yield

[d] Melting points are uncorrected.

General synthesis of epoxide chalcone (Step 2)

To chalcone (10mmol) obtained from step 1, a solution mixture of acetone and methanol (3:2) and 5%NaOH was added along with drop wise addition of hydrogen peroxide (6mL) at 0-4 ºC. The reaction mixture was stirred for 15min until hydrogen peroxide was added completely and then for next 3hrs afforded for the synthesis of corresponding α, β-epoxide derivatives.

The mixture was left overnight at -50ºC. The precipitate formed washed with water until it turns neutral to pH paper, filtered and recrystallized from ethanol to give epoxide derivatives (II).

General synthetic procedure of pyrazole compounds (Step 3,A1-19)

To mixture of epoxide chalcone derivatives II (10mmol), thiosemicarbazide (10mmol), and 10 % NaOH (0.025 mol, 10 ml) was refluxed in ethanol (25 mL) for 8-12 h. The resulting mixture (III) was poured into ice-water and stirred.

Dehydration of extra hydroxyl group on pyrazole ring (Step 4)

A solution of sulphuric acid and glacial acetic acid (1:1) was added drop wise to aliquot obtained from step 3 reaction, until it turns pH paper blue to green (neutral). The crude product (IV) were separated and recrystallized from Acetone.

Experimental Section

Pyrazole derivatives were synthesized in four steps, respectively, as shown Fig 9. Physico-chemical data of all synthesized compounds are reported in Table 1.

RESULT AND DISCUSSION

In the present communication aromatic aldehyde and acetophenone has been chosen to develop pyrazole derivatives. A novel series of substituted 3,5-diphenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (A1-A19) have been synthesized by treating substituted (2*E*)-1,3-diphenylprop-2-en-1-one derivatives (AC1-AC19) with thiosemicarbazide. The synthesized compounds were investigated for antimicrobial activity. The assignment of the structures of intermediates I (AC1-AC19) and final compounds VI (A1-A19) scheme (Figure 10) was based on their correct elemental analyses and spectroscopic data. IR spectra of intermediate compounds (AC1-AC19) showed characteristic peaks of functional group C=O str between 1600-1700 cm^{-1}, Armatic ring C=C str between 1450-1600 cm^{-1}. C-H def peaks were found between 750-900. For final derivatives (A1-A19) characteristic bands was there in each spectra in between wavenumber 3000-2500 cm^{-1} and peaks between 1400-1600 for C=C Aromatic ring str. Presence of pyrazole moiety peaks can be confirmed by looking over characteristic peaks in range of 1250-1300 cm^{-1}, 1230-1250 cm^{-1} and 1600-1650 cm^{-1} for N-N=C, C-N and C=N stretches, respectively. Pyrazole ring posses a thiourea skeleton and was established because of appearance of peaks between 1000-1100 cm^{-1}, 1630-1700, 2950-300 cm^{-1} for C=S str, NH_2 scissor, N=C-S str, respectively. Molecular ion [M]+ peak was observed for compounds A13, A15 and A17. [M+1] peak for compounds A1, A2, A4, A9, A11, A12, A14, A16, A18 and A19 at different intensities in positive and negative ionization mode and confirmed the molecular weights. For intermediate series, [M]+ peak was observed for compounds AC1, AC5, AC15 and AC17. Aryl proton on both the rings were found as multiplets in between δ 6.82 ~ 7.68 in most of the compounds, OCH_3 on aryl moieties was confirmed because of presence of singlet between δ 3.4 ~ 3.7. CH of pyrazole showed multiplet peaks in between δ 6.2 ~ 6.8. Proton attached with amine group on pyrazole ring was clarified by peaks in between δ 1.8 ~ 2.2. The rest of the aromatic protons appeared in the usual region. Structures of compounds were confirmed by IR, ^{1}H NMR, Mass spectral data and elemental analysis.

Spectral and Elemental analysis: General structure for Pyrazole derivative (Fig 9)

(A1): 3,5-diphenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide; Light yellow; IR KBr ν (cm^1): NH_2scissor (1677.95), C=C str Ar ring (1591.16, 1533.30, 1487.01, 1446.51), N-N=C str (1288.36), C-N str (1226.64), C=S str (1099.35); ^{1}HNMR ($CDCl_3$) δ in ppm: 7.1 ~ 6.9 (m, 5H, CH ring I), 6.08 ~ 7.11 (m, 5H, CH ring II), 3.2 (t, CH), 1.6 (m, NH_2); MS (ESI) (m/z): (M+1 = 282.1), 209.1, 265.1, 248.2, 221.1, 148.1; Elemental Anal. for $C_{16}H_{15}N_3S$: Found C, 68.28; N, 14.87); S, 11.39; Calc. C, 68.30; N, 14.93; S, 11.40

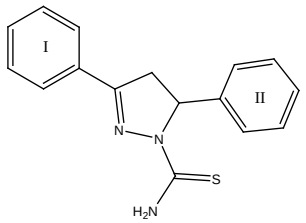

Fig. 10: General structure for Pyrazole derivative

(A2): 5-(2-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide; Yellow; IR KBr ν (cm^1): C=N str (1589.23), C=C Ar ring (1531.37, 1419.51, 1487.01, 1446.51), Ar-O-C str (1272.93), C-N str (1209.28), C=S str (1027.99); ^{1}HNMR ($CDCl_3$) δ in ppm: 7.1 ~ 7.6 (m, 5H, CH ring I), 6.8, 6.97, 7.02 & 7.1 (m, 5H, CH ring II), 3.4 (t, CH of pyrazole ring), 2.8 (d, $OCH_3$of ring II), 2.2 (d, CH_2 pyrazole ring); MS (ESI) (m/z): (M+1 = 310.1), 240.2, 209.1, 223.1, 164.1, 145.1,120; Elemental Anal. for $C_{17}H_{17}N_3OS$: Found: C, 65.52; N, 13.45; S, 10.25; Calc. C, 65.57; N, 13.49; S, 10.30

(A3): 5-(4-methoxyphenyl)-3-phenyl-1*H*-pyrazole-1-carbothioamide; Light yellow;

IR KBr ν (cm^1): NH_2scissor (1606.59), C=C str Ar ring (1535.23, 1512.09, 1473.51), Ar-C-O str (1384.79), N-N=C str (1272.93), C-N str (1245.93), C=S str (1085.85); ^{1}HNMR ($CDCl_3$) δ in ppm: 7.1 ~ 7.44 (m, 5H, CH ring I), 6.9 ~ 7.4 (m, 5H, ring II), 6.2 (t, CH, pyrazole), 3.4 (s, CH_3 of OCH_3), 1.8 (d, NH_2); MS (ESI) (m/z): (M+1 = 308.05), 237.03, 254.02, 192.02, 116.03; Elemental Anal. for $C_{17}H_{17}N_3OS$: Found: C, 55.46; N, 13.53; S, 10.31; Calc. C, 66.00; N, 13.58; S, 10.36

(A4): 5-(2,4-dimethoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole -1-carbothioamide; Cream; IR KBr ν (cm^1): C=N str (1616.24), C=C str Ar ring str (1575.73, 1504.37, 1416.58), N-N=C str (1288.36), C-N str (1226.64), C=S str (1099.35), Ar-C-O (1328.86); ^{1}HNMR ($CDCl_3$) δ in ppm: 7.2 ~ 7.8 (m, 5H, CH ring I), 6.3 (m, CH, ring II), 6.26 (m, CH of pyrazole ring), 3.0 (s, CH of pyrazole ring), 3.63 & 3.56 (d, 2H, OCH_3 ring II), 2.1 (NH_2); MS (ESI) (m/z): (M+1 = 342.02), 264.08, 204.06, 220.1, 67.01; Elemental **Anal.** for $C_{18}H_{19}N_3O_2S$: Found C, 63.; N, 12.; S, 9.39; Calc. C, 63.32; N, 12.31; S, 9.39

(A5): 3-phenyl-5-(3,4,5-trimethoxyphenyl)-1*H*-pyrazole-1-carbothioamide; Creamish yellow; IR KBr ν (cm^1): C=N str (1616.24), C=C Ar ring str (1575.73, 1504.37, 1461.94), Ar-C-O (1328.21), C-N str (1238.21), C=S str (1126.35); ^{1}HNMR ($CDCl_3$) δ in ppm: 7.22 ~ 7.48 (m, 5H, CH of ring I), 6.44 & 6.44 (s, 2H, ring II CH), 3.73, 3.73, 3.73 (s, 3H, OCH_3 of ring II), 2.0 (m, NH_2); MS (ESI) (m/z): (M + 1 = 370.2), 309.1, 268.1, 255.4, 234.0, 191.9; Elemental Anal. for $C_{19}H_{19}N_3O_3S$: Found C, 61.70; N, 11.31; S, 8.61; Calc. C, 61.77; N, 11.37; S, 8.68

(A6): 3-(2,4-dichlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-pyrazole -1-carbothioamide; Yellow; IR KBr ν (cm^1): =C-H str (3166.83), N=C-S str (2937.38), NH_2 scissor (1670.24), C=N str (1616.17), C=C Ar ring str (1577.66, 1507.66, 1458.08), Ar-C-O (1355.86), C-N str (1238.21), C=S str (1126.35), C-Cl (997.13); ^{1}HNMR ($CDCl_3$) δ in ppm: 7.26 ~ 7.42 (m, 3H, CH of ring I), 6.44 & 6.44 (s, 2H, CH of ring), 3.82 (s, OCH_3 of ring II); MS (ESI) (m/z): (M + 1 = 440.0), 423.0, 270.0, 262.0, 294.1, 212.9; Elemental Anal. for $C_{19}H_{17}Cl_2N_3O_3S$: Found C, 52.01; N, 9.55; S, 7.29; Calc. C, 52.06; N, 9.59; S, 7.32

(A7): 5-(2,4-dichlorophenyl)-3-(2-methoxyphenyl)-1*H*-pyrazole -1-carbothioamide; Cream; IR KBr ν (cm^1): C=C Ar ring str (1596.95, 1490.80, 1411.80), Ar-C-O str (1355.86), C-N str (1278.72), O-C str (1220.86), C=S str (1039.56), C-Cl (997.13); ^{1}HNMR ($CDCl_3$) δ in ppm: 7.03 ~ 6.46 (m, 3H, CH of ring I), 7.38 ~ 7.12 (m, 3H, CH of ring II), 6.4 (s, CH pyrazole), 3.03 (s, OCH_3 of ring I), 2.2 (m, NH_2); MS (ESI) (m/z): (M + 1 = 374.01), 271.12, 150.1, 242.12; Elemental Anal. for $C_{17}H_{13}Cl_2N_3OS$: Found C, 53.95; N, 11.05; S, 8.43; Calc. C, 53.98; N, 11.11; S, 8.48

(A8): 3-(2,4-dimethoxyphenyl)-5-(2-nitrophenyl)-1*H*-pyrazole-1-carbothioamide; Dark Brown; IR KBr ν (cm¹): Ar-NO_2 (1530.12), C=C Ar ring str (1534.45, 1423.65, 1485.34), Ar-C-O str (1357.98), C-N str (1268.72), O-C str (1254.86), C=S str (1089.52); ¹HNMR ($CDCl_3$) δ in ppm: 6.24 ~ 7.48 (m, 3H, CH of ring I), 7.68 ~ 8.04 (m, 5H, CH of ring II), 6.8 (s, CH of pyrazole ring), 3.64 (s, OCH_3 of ring I); MS (ESI) (m/z): (M + 1 = 375.0), 319.9, 317.8, 304.9, 216.9, 255.2, 189.0, 192.0; Elemental Anal. for $C_{18}H_{16}N_4O_4S$: Found C, 56.18; N, 14.52; S, 8.28; Calc. C, 56.24; N, 14.57; S, 8.34

(A9): 5-(2-nitrophenyl)-3-phenyl-1*H*-pyrazole-1-carbothioamide; Dark Brown; IR KBr ν (cm¹): Ar-NO_2 (1513.02), C=C Ar ring str (1590.01, 1491.04, 1404.04), C-N str (1284.75), C=S str (1044.30); ¹HNMR ($CDCl_3$) δ in ppm: 7.22 ~ 7.46 (m, 5H, CH of ring I), 7.61 ~ 8.24 (m,5H,CH of ring II), 6.8 (s, CH of pyrazole ring), 2.1 (m, NH_2); MS (ESI) (m/z): (M + 1 = 324.1), 305.0, 283.2, 264.1, 255.2, 48.0, 221.8; Elemental Anal. for $C_{16}H_{12}N_4O_2S$: Found C, 59.20; N, 17.22; S, 9.83; Calc. C, 59.25; N, 17.27; S, 9.89

(A10): 5-(4-chlorophenyl)-3-phenyl-1*H*-pyrazole-1-carbothioamide; Light yellow; IR KBr ν (cm¹): N=C-S str (2935.90), NH_2 scissor (1673.24), C=N str (1616.17), C=C Ar ring str (1577.66, 1507.66, 1476.98), C-N str (1205.02), C=S str (1175.94), C-Cl (934.95); ¹HNMR ($CDCl_3$) δ in ppm: 7.12 ~ 7.45 (m, 5H, CH of ring I), 7.31 ~ 7.32 (m, 4H, CH of ring II), 6.8 (s, CH of pyrazole ring), 2.2 (m, NH_2); MS (ESI) (m/z): (M + 1 = 316.9), 299.0, 116.01; Elemental Anal. for $C_{16}H_{12}ClN_3S$: Found C, 61.19; N, 13.35; S, 10.08; Calc. C, 61.24; N, 13.39; S, 10.22

(A11): 5-(2,4-dichlorophenyl)-3-phenyl-1*H*-pyrazole-1-carbothioamide; Yellow; IR KBr ν (cm¹): N=C-S str (2935.90), NH_2 scissor (1670.24), C=N str (1616.17), C=C Ar ring str (1577.66, 1507.66, 1476.98), C-N str (1205.02), C=S str (1175.94), C-Cl (934.95); ¹HNMR ($CDCl_3$) δ in ppm: 7.72 ~ 7.88 (m, 5H, CH of ring I), 6.81 ~ 7.23 (m, 3H, CH of ring II), 5.8 (s, CH of pyrazole ring); MS (ESI) (m/z): (M + 1 = 340.11), 269.09, 176.02, 237.02; Elemental Anal. for $C_{16}H_{11}Cl_2N_3S$: Found C, 55.12; N, 12.01; S, 9.16; Calc. C, 55.18; N, 12.07; S, 9.21

(A12): 3-(2,5-dichlorophenyl)-5-phenyl-1*H*-pyrazole-1-carbothioamide; Off white; IR KBr ν (cm¹): C=C Ar ring str (1560.03, 1492.07, 1403.01), C-N str (1204.70), C=S str (1102.00), C-Cl (867.97); ¹HNMR ($CDCl_3$) δ in ppm: 7.12 ~ 7.53 (m, 5H, CH of ring I), 6.9 ~ 7.48 (m, 5H,CH of ring II), 5.5 (s, CH of pyrazole ring), 2.5 (m, NH_2); MS (ESI) (m/z): (M + 1 = 346.01), 303.28, 272.09; Elemental Anal. for $C_{16}H_{11}Cl_2N_3S$: Found C, 55.04; N, 12.02; S, 9.17; C, 55.18; N, 12.07; S, 9.21

(A13): 5-(4-bromophenyl)-3-phenyl-1*H*-pyrazole-1-carbothioamide; Off White; IR KBr ν (cm¹): NH_2scissor (1665.90), C=C str Ar ring (1505.85, 1465.40, 1385.21), N-N=C str (1245.06), C-N str (1232.94), C=S str (1085.31), Ar-C-Br 1030.65; ¹HNMR ($CDCl_3$) δ in ppm: 7.12 ~ 7.58 (m, 5H, CH of ring I), 7.47 ~ 7.59 (m, 5H, CH of ring II), 6.5 (s, CH of pyrazole ring); MS (ESI) (m/z): (M+ = 360.0), (M + 1 = 358.0), 343.0, 225.9, 177.1, 243.0; Elemental Anal. for $C_{16}H_{12}BrN_3S$: Found C, 53.59; N, 11.68; S, 8.90; Calc. C, 53.64; N, 11.73; S, 8.95

(A14): 5-(3-aminophenyl)-3-phenyl-1*H*-pyrazole-1-carbothioamide; Light Brown; IR KBr ν (cm¹): N=C-S str (2076.93), C=N str (1543.94), C=C str (1538.33, 1498.23, 1404.20, 1409.55), C-N str (1275.33), C=S str (1095.64); ¹HNMR ($CDCl_3$) δ in ppm: 6.82 ~ 7.22 (m, 4H, CH of ring I), 7.32 ~ 7.01 (m, 5H, CH of ring II), 6.2 (s, CH of pyrazole ring), 5.6 (m, NH_2 of ring II), 2.7 (m, NH_2); MS (ESI) (m/z): (M + 1 = 293.01), 254.0, 239.2, 176.1; Elemental Anal. for $C_{16}H_{14}N_4S$: Found C, 65.22; N, 18.99; S, 10.84; Calc. C, 65.28; N, 19.03; S, 10.89

(A15): 3-(3-aminophenyl)-5-phenyl-1*H*-pyrazole-1-carbothioamide; Dull Brown; IR KBr ν (cm¹): N=C-S str (2054.43), C=N str (1502.31), C=C str (1504.94, 1467.65), C-N str (1292.04), C=S str (1150.63); ¹HNMR ($CDCl_3$) δ in ppm: 6.52 ~ 7.12 (m, 4H, CH of ring I), 7.02 ~ 7.38 (m, 5H, CH of ring I), 6.1 (s, CH of pyrazole ring), 3.7 (m, NH_2 of ring I), 1.6 (m, NH_2); MS (ESI) (m/z): (M + = 294.09), (M + 1 = 299.09), 216.9, 234.0, 255.1, 203.9, 192.0, 188.0, 139.0; Elemental Anal. for $C_{16}H_{14}N_4S$: Found: C, 65.22; N, 18.98; S, 10.82; Calc. C, 65.28; N, 19.03; S, 10.89

(A16): 5-(3-aminophenyl)-3-(2,5-dichlorophenyl)-1*H*-pyrazole-1-carbothioamide; Dark Yellow; IR KBr ν (cm¹): Ar-C-H str (3037.68), N=C-S str (2860.24), C=N str (1668.31), NH_2 scissor (1608.52), C=C Ar ring str (1583.45, 1529.45, 1463.87), C-N str (1251.72), C=S str (1126.35), C-Cl (727.11); ¹HNMR ($CDCl_3$) δ in ppm: 7.13 ~ 7.41 (m, 3H, CH of ring I), 6.37 ~ 7.32 (m, 4H, CH of ring II), 6.2 (s, CH of pyrazole ring), 4.1 (m, NH_2 of ring II), 2.1 (m, NH_2); MS (ESI) (m/z): (M + 1 = 360.01), 322.0, 299.1, 209.0, 131.1, 105.0; Elemental Anal. for $C_{16}H_{12}Cl_2N_4S$: Found C, 52.86; N, 15.38; S, 8.78; Calc. C, 52.90; N, 15.42; S, 8.83

(A17): 3-(3-aminophenyl)-5-(3-nitrophenyl)-1*H*-pyrazole-1-carbothioamide; Dark Brown; IR KBr ν (cm¹): C=C Ar ring str (1521.73, 1458.08), Ar-NO_2 (1348.08), C=S str (1099.35), C-H def (688.54, 617.18); ¹HNMR ($CDCl_3$) δ in ppm: 6.41 ~ 7.07 (m, 4H, CH of ring I), 7.58 ~ 8.41 (m, 4H, CH of ring II), 6.11 (s, CH of pyrazole ring), 4.2 (m, NH_2 of ring I), 2.6 (m, NH_2); MS (ESI) (m/z): (M + = 339.08), 325.1, 311.1, 298.052, 283.2, 269.1, 251.0, 105.1; Elemental Anal. for $C_{16}H_{13}N_5O_2S$: Found C, 56.60; N, 20.59; S, 9.40; Calc. C, 56.63; N, 20.64; S, 9.45

(A18): 5-(3-hydroxyphenyl)-3-phenyl-1*H*-pyrazole-1-carbothioamide; Yellowish brown; IR KBr ν (cm¹): Ar-O-H str (3245.42), N=C-S str (2044.93), C=N str (1554.20), C=C str (1502.53, 1489.31, 1455.52, 1424.43), C-N str (1286.32), C=S str (1054.13); ¹HNMR ($CDCl_3$) δ in ppm: 7.21 ~ 7.71 (m, 5H, CH of ring I), 6.59 ~ 7.11 (m, 4H, CH of ring II), 5.8 (s, CH of pyrazole ring), 4.92 (d, OH of ring II), 2.7 (m, NH_2); MS (ESI) (m/z): (M + 1 = 290.01), 256.1, 190.03, 252.2; Elemental Anal. for $C_{16}H_{13}N_3OS$: Found: C, 65.01; N, 14.18; S, 10.81; Calc. C, 65.06; N, 14.23; S, 10.86

(A19): 3-(2,4-dichlorophenyl)-5-(3-hydroxyphenyl)-1*H*-pyrazole-1-carbothioamide; Off Cream; IR KBr ν (cm¹): Ar-O-H sstr (3201.61), N=C-S str (2848.67), C=N str (1595.02), C=C str (1577.66, 1560.30, 1527.52, 1427.23), C-N str (1296.08), C=S str (1105.14), C-Cl (827.41); ¹HNMR ($CDCl_3$) δ in ppm: 7.41 ~ 7.74 (m, 3H, CH of ring I), 6.39 ~ 7.73 (m, 4H, CH of ring II), 6.6 (s, CH of pyrazole ring), 4.2 (d, OH of ring II), 2.1 (m, NH_2); MS (ESI) (m/z): (M + 1 = 364.0), 216.9, 245.9, 302.9, 325.0; Elemental Anal. for $C_{16}H_{11}Cl_2N_3OS$: Found C, 52.71; N, 11.50; S, 8.76; Calc. C, 52.76; N, 11.54; S, 8.80

BIOLOGICAL ACTIVITY

(Antibacterial activity and antifungal activity)

In vitro agar diffusion cup plate method [26-28] was used for the assessment of antibacterial and antifungal activity of the newly synthesized compounds for bacterial and fungal strains.

Microbiological assay

Antibacterial activity of the test compounds was evaluated against two stains, Gram-positive bacteria, *Staphylococcus aureus* and Gram –negative bacteria, *E.coli*. Ofloxacin was used as standard drug. Antifungal activity was evaluated for *Aspergillus niger* and *Penicillium chrysogenum*. Ketoconazole was used as standard drug.

Nutrient agar Medium (composition, peptic digest 5 g/l, beef extract 1 g/l, yeast extract 1.50 g/l, sodium chloride 5 g/l, agar 15 g/l, pH at 25 º C, 7.4 ± 0.2) was used for growth of bacterial microbes. Nutrient agar media (NAM) was melted on a water bath and cooled to 45 ºC with gentle shaking to bring about uniform cooling. Potato dextrose agar (PDA) (Composition per litre, potato Infusion of 300 g potato in 500ml, Dextrose 20 g, Agar 15 g, Final volume upto 1 litre, pH: 5.6 ± 0.2 at 25°C) [29] is used for the cultivation of fungi. Conforms to Harmonized USP/EP/JP Requirements [30-32]. Formula may be adjusted and/or supplemented as required to meet performance specifications. Fungus was incubated at period of 48 hrs, till sporulation. Spore of strains were then further utilized. Drug diffuses through the well in agar plates and produces a clear zone of inhibition. The diameter of zone of inhibition was measured and an estimation of the degree of activity of the antimicrobial substance was obtained.

Table 2: Zone of inhibition (Antibacterial activity)

Compound code	Zone of inhibition(mm) (*E.coli*)		Zone of inhibition(mm) (*S.aureus*)	
	100 µg/ml	200 µg/ml	100 µg/ml	200 µg/ml
Std (ofloxacin)	22	28	34	36
A1	14	15	24	28
A2	16	18	13	27
A3	15	18	35	38
A4	16	20	37	40
A5	14	16	36	42
A6	17	18	32	42
A7	---	10	33	37
A8	16	19	42	44
A9	17	19	37	41
A10	17	18	35	38
A11	20	21	26	27
A12	18	20	32	34
A13	17	19	21	26
A14	14	19	24	27
A15	23	29	---	---
A16	20	22	26	30
A17	11	13	23	27
A18	19	21	33	35
A19	13	20	25	27

--- No activity

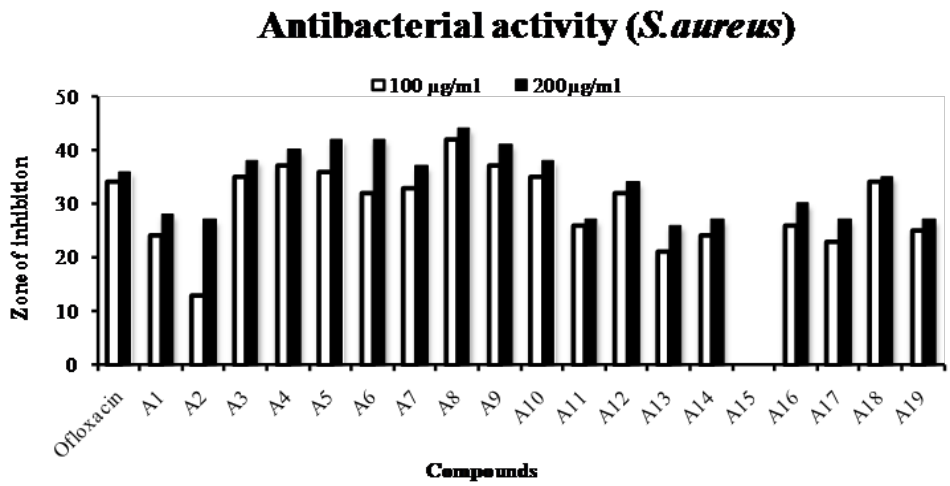

Fig. 11: Zone of inhibition for *S.aureus*

Fig. 12: Zone of inhibition for *E.coli*

Table 3: Zone of inhibition (Antifungal activity)

Compound code	Zone of inhibition (*A.niger*)		Zone of inhibition (*P.chrysogenum*)	
	100 µg/ml	200 µg/ml	100 µg/ml	200 µg/ml
Std (Ketoconazole)	23	25	24	26
A1	17	19	15	13
A2	16	19	17	20
A3	12	17	26	28
A4	22	24	17	19
A5	24	28	---	11
A6	16	22	21	18
A7	16	19	25	28
A8	19	21	18	19
A9	18	21	---	13
A10	13	18	10	15
A11	19	21	13	16
A12	23	27	13	14
A13	20	19	18	20
A14	13	19	17	20
A15	21	24	---	16
A16	24	28	14	18
A17	15	17	12	16
A18	19	23	19	20
A19	18	21	16	21

--- No activity

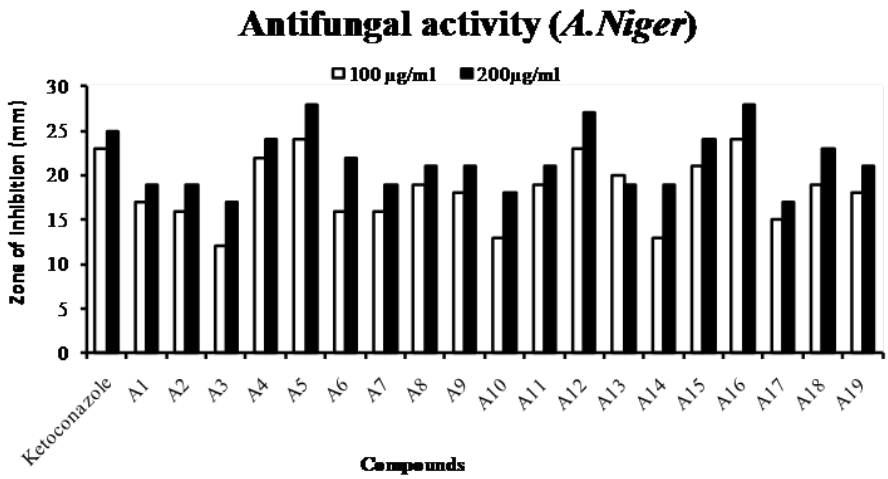

Fig. 13: Zone of inhibition for *A.niger*

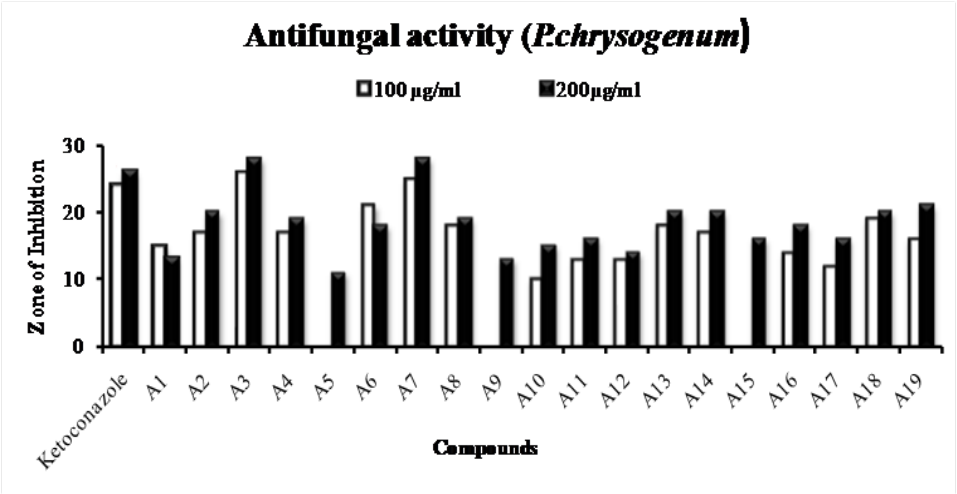

Fig. 14: Zone of inhibition for *P.chrysogenum*

Method of Assay

NAM and PDA were used for growing the bacterial and fungal microbes. Respectively. Mixture was heated with frequent agitation and boiled for one minute to completely dissolve the medium. Autoclaved at 121°C for 20 minutes was done. Media was poured 40ml into each sterilized Petri dishes (90 mm diameter approx).

Preliminary screening was conducted for all the compounds at 100 and 200 µg/ml concentration against the following strains: *Staphylococcus aureus, Escherichia coli* (Bacterial strains) and *Aspergillus niger* and *Penicillium chrysogenum* (fungal stains).

Media was allowed to settle in plates under aseptic conditions and different solutions of microbes (0.1ml) were spread over each Petri plate. Two wells were made by punching the agar surface with a sterile borer (size 8mm). Two dilutions of 100 and 200 µg/ml test and standard drug (0.1ml) were added to each well. Culture strains of bacteria were maintained on nutrient agar slant at 37± 2°C for 24 h and for fungal on PDA at 28 ± 2°C for 48 hrs. Dimethyl sulfoxide (DMSO, 1%) was used to prepare the stock and dilutions of compounds. The test was carried out in duplicate. The drug was allowed to diffuse for 24 h and 48 hrs through the media for bacterial and fungal strains, respectively. Positive controls (with DMSO) Ofloxacin and Ketoconazole were also included in the test. At the end of the incubation period, inhibition zones formed on the medium are evaluated in millimeters and compared with standard. 1 % DMSO was as negative control. Drug diffuses through the well in agar plates and produces a clear zone of inhibition. The diameter of zone of inhibition was measured and an estimation of the degree of activity of the antimicrobial substance was obtained. Results are reported in table2 and 3 for antibacterial and antifungal activity, respectively.

CONCLUSION

The purpose of the present study was to examine whether molecular modification might result in detection of new potential antimicrobial agents. Generally, the Gram-positive bacteria are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier [33]. Whereas the Gram-negative bacteria [34] possess an outer phospholipidic membrane carrying the structural lipo-polysaccharide components. This makes the cell wall impermeable to drug constituents [35]. So the maximum inhibitory activity is observed in Gram-positive bacteria *Staphylococcus aureus*. Compounds **A3, A4, A5, A8, A9 and A10** has shown best activity due to presence of p-methoxy, dimethoxy, trimethoxy, o-nitro and p-chloro. While **A7** and **A6** were only effective at higher concentrations, indicated that dichloro, dimethoxy and trimethoxy groups were solely responsible for activity. In the case of Gram-negative *E.coli* the zone of inhibitory activity was not much significant because of a multilayered phospholipidic membrane carrying the structural lipo-polysaccharide components [36] and among all the compounds only **A15** was found to be most active when compared with std drug (Ofloxacin) at both the concentrations because of possession of amino group on ring I. Antibacterial activity was compared with standard drug ofloxacin.

For antifungal activity, among all compounds three compounds (**A5**, **A12** and **A16)** exhibited appreciable activity for strain *A.niger* when compared with standard drug (Ketoconazole), because of presence of methoxy, amino and dichloro groups.

For fungus strain *P.chrysogenum*, compound **A3** and **A7** was effective in controlling the growth of pathogenic strains to good extent due to presence of methoxy and chloro substitutions at both concentrations.

ACKNOWLEDGEMENT

The authors would like to express their gratitude and thanks to the Head, CDRI lucknow and Department of pharmaceutical technology, M.I.E.T., Meerut for providing necessary facilities for this research work.

REFERENCES

1. Tripathi KD. Essentials of medical pharmacology. First ed.. Jaypee brothers publication (P), LTD, New Delhi; 226-238.
2. Todar K. Pathogenic *E.coli*. Online Textbook of Bacteriology. University of Wisconsin–Madison Department of Bacteriology. 2007; 11-30.
3. http://en.wikipedia.org/wiki/Staphylococcus_aureus
4. Arias ME, Gomez JD, Cudmani NM, Vattuone MA and Isla MI. Antibacterial activity of ethanolic and aqueous extracts of *Acacia aroma* Gill Ex Hook et. Arn. Life Sci. 2004; 75: 191-202.
5. Sharath N, Halehatty S, Bhojya VN, Kumar V and Hoskeri J. Antibacterial, Molecular Docking, DNA Binding and Photocleavage Studies on Novel Heterocyclic Pyrazoles. British J. Pharm. Res. 2011; 1(2): 46-65, 57.
6. Betoni JEC, Mantovani RP and Barbosa, LN. Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Memo´ rias do Instituto Oswaldo, Rio de Janeiro. 2006; 101: 2387–390.
7. http://www.pillwatch.com/info/otc-antifungal-medications.html
8. Shen HD, Chou H, Tam MF, Chang CY, Lai HY and Wang SR. Molecular and immunological characterization of Pen ch 18, the vacuolar serine protease major allergen of *Penicillium chrysogenum*. Allergy. 2003; 58(10): 993–1002.
9. Sharma A, Patel VK, Rawat S, Ramteke P and Verma R. Identification of the antibacterial component of some Indian medicinal Plants against *Klebsiella Pneumoniae*. Int J Pharmacy and Pharm Sci. 2010; (2)3: 123-127.
10. Qureshi AA, Omer S, Kumar KE and Bhajipale NS. Probiotics In Diarrhea: Myths And Facts. 2010; 2(3): 24.
11. Neu HC. The crisis in antibiotic resistance. Science. 1992; 257: 1064.
12. http://www.can-r.com/mediaResources/Azoles.pdf
13. Patel VM and Desai KR. Eco-Friendly Synthesis Of Fluorine-Containing Pyrazoline Derivatives Over Potassium Carbonate. KIVOC. 2004; I: 123-129.
14. Abunada NM, Hassaneen HM, Kandile NG and Miqdad OA. Synthesis and Antimicrobial Activity of Some New Pyrazole Fused Pyrazolo[3,4-d]-pyrimidine and Pyrazolo[4,3-e][1,2,4]-triazolo[1,5-c]pyrimidine Derivatives. Molecules. 2008; 13: 1501-1517.
15. Jungheim LN, Holmes RE, Ott JL, Ternansky RJ, Draheim SE, Neel DA, Stepherd TA and Sigmund SK. Antimicrobial Agents and Chemotherapy, Presented at 26th Interscience conference, New Orleans, LA. 1998; Sept.28 - Oct.1: Paper 601.
16. Jungheim LN, Holmes RE, Ternansky RJ, Stepherd TA, Neel DA, Draheim, SE, Pike AJ and Wu CYE. Antimicrobial Agents and chemotherapy, Presented at 28th Interscience conference Los Angels, CA, 1998; Oct 23 – 26: Paper 240. 12.
17. Gomes A, Fernandes E, Garacia MB, Silva AM, Pinto DC, Santo CM, Cavalerio JA and Lima JL. Cyclic voltametric analysis of 2-styrylchromenes; relationship with antioxidant activity. Bioorg. Med. Chem. 2008; 16: 7939-7943.
18. Schmidt JG. Ber. Dtsch Chem. Ges. 1880; 13: 2342.
19. Claisen L, Claparede A. Ber. Dtsch. Chem. Ges. 1881; 14: 349.
20. Kohler EP, Chadwell HM. Org. Synth. 1922; 2: 1.
21. Pereira CMP, Quina FH, Silva FAN, Emmerich DJ and Machulek A. Synthesis Of 4-Iodopyrazoles: A Brief Review. Bentham Science Publishers Ltd. 2008; 5: 331-335.
22. Tripathi KD. Essentials of medical pharmacology. 1st ed.; Jaypee brothers publication (P), LTD, New Delhi, 226-238.
23. Fraley ME, Hoffman WF, Rubino RS, Hungate RW, Tebbon AJ, Rutledge RZ, McFall RC, Huckle WR, Kendall RL, Coll KE and Thomas KA. Bioorg. Med. Chem. Lett. 2002; 12: 2767.
24. Huang Y, Hu X, Shen D, Chen Y and Xu P. Mol. Divers. 2007; 11: 73.
25. Wilson WL and Bottiglieri NG. Cancer Chemother. Rep.1962; Part 1: 137.
26. Aziz MA, El-Din A G. Abuo-Rahma and Alaa A. Hassan. Synthesis of novel pyrazole derivatives and evaluation of their antidepressant and anticonvulsant activities. Euro J Med. Chem. 2009; 44(9): 3480-3487.
27. Dawane BS, Konda SG, Shaikh BM, Chobe SS, Khandare NT, Kamble VT and Bhosale RB. Synthesis and In Vitro Antimicrobial Activity Of Some New 1-Thiazolyl-2-Pyrazoline Derivatives. 2010; 1(2): 342.

28. Hu H, Ge C, Ding L and Zhang A. Synthesis of Novel 1-[(2,6-Dichloro-4-trifluoromethyl)phenyl]-3-aryl-1H-pyrazole-4-carbaldehydes. Molecules. 2010; 15: 7472-7481.
29. http://www.chemyq.com/patentfmen/pt5/46017_4D602.htm
30. Lamani RS, Kumar N, Shetty S, Ravindra R, Kamble and Khazi M IA. Synthesis and antimicrobial studies of novel methylene bridged bezisoxazolyl imidazol[2,1-b] [1,3,4]thiadiazole derivatives. Eur. J. Med. Chem. 2009; 44: 2823-2833.
31. Kulkarni SK. Handbook of Experimental Pharmacology, Vallabh Prakashan, 1999; 3: 123-131.
32. Collins AH. Microbiological Methods, London Butterworth, 1976; 3: 2.
33. http://en.wikipedia.org/wiki/Potato_dextrose_agar
34. The United States pharmacopeia, 31st ed., Amended Chapters 61, 62, 111. The United States Pharmacopeial Convention. 2007. Rockville, MD.
35. Directorate for the Quality of Medicines of the Council of Europe (EDQM). 2007. The European Pharmacopoeia, Amended Chapters 2.6.12, 2.6.13, 5.1.4, Council of Europe, 67075 Strasbourg Cedex, France.
36. The Minister of Health, Labor, and Welfare. Japanese Pharmacopoeia 2007. Society of Japanese Pharmacopoeia. Amended Chapters 35.1, 35.2, 7.
37. Arias ME, Gomez JD, Cudmani NM, Vattuone MA and Isla MI. Antibacterial activity of ethanolic and aqueous extracts of *Acacia aroma* Gill. Ex Hook et Arn. *Life Sci*, 2004; *75*: 191-202.
38. Sharath N, Halehatty S, Bhojya VN, Kumar V and Hoskeri J. Antibacterial, Molecular Docking, DNA Binding and Photocleavage Studies on Novel Heterocyclic Pyrazoles. British J. Pharm. Res. 2011; 1(2): 46-65,57.
39. Betoni JEC, Mantovani RP and Barbosa LN. Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Memo´ rias do Instituto Oswaldo, Rio de Janeiro. 2006; 101: 387–390.
40. Nikaido V. Molecular basis of bacterial outer membrane permeability. Microbiol. 1985; 1: 1–32.