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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL 1,8-NAPHTHYRIDINES CONTAINING PYRAZOLINONE, PYRAZOLE, ISOXAZOLINONE, ISOXAZOLE AND PYRIMIDINE-2-ONES

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ABSTRACT: Diazonium salt of 2-(p-aminophenyl)-1,8-naphthyridine (I) was coupled with active methylene compounds to afford the hydrazono-1,8-napthyridinyl (III-V) and azo(VI) derivatives. Hydrazono ethyl acetoacetate (III) and ethyl cyanoacetate (IV) derivatives on treatment with hydrazine and substituted hydrazine's were later reacted with hydroxylamine HCl in the presence of ethanol followed by cyclization to afford pyrazolinone (IIIa-e) & (IVa-e) and isoxazolinones (VII&IX). Hydrazono acetyl acetone (V) and azo dibenzoyl methane (VI) derivatives on treatment with hydrazine and substituted hydrazines, hydroxylamine HCl and with urea in the presence of ethanol followed by cylization resulted in the formation of pyrazoles (Va-e) & (VIa-e), isoxazole (IX&XI) and substituted pyrimidine-2-ones (X&XII). All the newly synthesized compounds were screened for their in-vitro antibacterial activity & antifungal activity by Agar cup-plate method and Serial dilution methods. In Agar cup plate method the Compounds IIId, IVd, VId showed Impressive antibacterial and antifungal activity. In Serial dilution method the compounds Vd & VId showed excellent antibacterial activity against *B.Subtilis* with an MIC of 7.8 µg/ml where as the compounds IVd, Vd showed very good antifungal activity against *A.niger* and *C.albicans* with MIC value of 7.8 µg/ml & 15.6µg/ml. We found that the activity was due to presence of chloro group at the para position of phenyl ring of Pyrazolinone and pyrazole and also the presence of methyl group & amino group at the 3rd position, hydrazono and azo (N=N) group at 4th of the pyrazolinone (IIId, IVd) and pyrazole (Vd) ring are contributing to the antimicrobial activity.

INTRODUCTION: 1,8-Naphthyridine derivatives are reported to possess a wide spectrum of biological activities such as diuretic, [1] antimalarial, [2] anti-inflammatory,[3] antitumor,[4] antihypertensive, [5] and antibacterial activites.[6, 7] Pyrazolone and Isoxazolone compounds are associated with broad spectrum of biological activites.[8-11]

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Antipyrine-2,3-dimethyl-1-phenyl - 3 - pyrazolin-5-one, was the first pyrazolone derivative used in the management of pain and inflammation. The pyrazoles are the class of heterocyclic compounds and the pyrazole skeleton constitutes an important central template for a wide variety of biologically active compounds.

The pyrazole nucleus has been reported to possess a wide spectrum of biological activities such as anti-inflammatory,[12] antibacterial,[13] antifungal,[14] analgesic,[15] antiviral,[16] Hypoglycemic,[17] anticancer,[18] and anticonvulsant,[19] Isoxazole nucleus has been reported to possess a wide

spectrum of biological activities such as anti-inflammatory,[20] analgesic,[21] antituberculosis,[22] Hypoglycemic,[23] antcancer.[24] In the view of the above literature survey, planned to synthesize the following 1,8-naphthyridine containing pyrazolinone, pyrazole, isoxazolinone, isoxazole and pyrimidine-2-one derivatives, which have been found to possess an interesting profile of anti-inflammatory, along with analgesic and antimicrobial activities.

RESULTS AND DISCUSSION:

Chemistry:

Synthesis of Intermediate hydrazono (III-V) and Azo compounds (VI):

2-(p-aminophenyl)-1,8-naphyridine (I) was prepared according to the literature procedure.[25] The diazonium chloride (II) was prepared by diazotization of 2-(p-aminophenyl)-1,8-naphthyridine (I) with sodium nitrite and con HCl at 0-5℃. Coupling of diazonium salts (II) with active methylene compounds like ethyl aceto acetate, ethyl cyano acetate, acetyl acetone and dibenzoyl methane to affords the corresponding intermediate hydrazono(III-V) and azo (VI) compounds (**Scheme-1**).

The IR spectrum of the III shows an absorption bands at 3224.10cm[1], 3017.45cm,[-1]1734.16 cm^{-1} and 1606.41cm^{-1} corresponds to 2^o amine, aromatic C-H (stretch), ester and imine (C=N) groups respectively. The ^{1}H-NMR spectrum of compound III showed singlet peaks at δ 9.8 (1H, s, NH), 7.5-7.8 (4H,m, Ar-H), 2.45 (3H, s, CH_3), 8.4 (1H, m, C_3-H), 8.49 (1H, m, C_4-H), 8.63 (1H,m, C_5-H), 8.2 (1H, m, C_6-H) and 9.8 (1H, m, C_7-H) of 1,8-naphthyridine ring, 7.5-7.8 (4H, m, Ar-H),4.4 (2H,q,CH_2), 2.3(3H,s,$COCH_3$), 1.4 (3H, t,CH_3 for carboxylic ester). The presence of triplet (t) and quartet at delta value of 1.4 & 4.4 indicates presence of ethyl group of hydrazono compound.

Synthesis of 1,8 –naphthyridine linked with 3-methyl Pyrazolinone (IIIa-e) and Isoxazolinone (VII):

Hydrazono compound ethyl 2-{[4-(1,8-naphthyridin-2-yl) phenyl] hydrazono} – 3 - oxobutanoate (III) was treated with hydrazine hydrate and substituted hydrazines like phenyl hydrazine, thio semicarbazide, chlorophenyl hydrazine and isoniazide via cyclization gave the corresponding 1,8-naphthyridine linked with pyrazolinone (IIIa-e) and with hydroxylamine HCl afforded corresponding 1,8-naphthyridine linked with isoxazolinone (VII) (**Scheme-2**).

The structures of new compounds were confirmed by IR, NMR, and mass spectral data. Their IR spectra showed the disappearance of the characteristic bands of acetyl carbonyl group and carboxylic ester and the appearance of the strong bands in 3325-3458 cm^{-1} region, attributed to NH group stretching and the bands of the pyrazolinone ring C=O appearing at 1674 and 1680 cm^{-1}.The ^{1}H -NMR spectra of IIIa-e showed the absence of the signals for the ethyl group, while the pyrazolinone CH_3 signal appeared at δ 2.52-2.75 ppm.

Synthesis of 1,8 –Naphthyridine linked with 3-Amino Pyrazolinone (IVa-e) and Isoxazolinone (VIII):

Hydrazono compound ethyl cyano{[4-(1,8-naphthyridin-2-yl)phenyl]hydrazono}acetate (IV) was treated with hydrazine hydrate and substituted hydrazines like phenyl hydrazine, thiosemicarbazide, chlorophenyl hydrazine and Isoniazide via cyclization gave the corresponding 1,8-naphthyridine linked with pyrazolinone (IVa-e) and with hydroxylamine HCl afforded corresponding 1,8-naphthyridine linked with isoxazolinone (VIII) (**Scheme 3**).

The structures of new compounds were confirmed by IR, NMR, and mass spectral data. Their IR spectra showed the disappearance of the characteristic band CN band at 2360 cm^{-1} and carboxylic ester and the appearance of the strong bands in 3325-3458 cm^{-1} region, attributed to the vibration of NH_2 group. ^{1}H -NMR spectra of IVa-e showed the absence of the signals for the ethyl group and presence of NH_2 singlet appear at delta of 6.3-6.9 ppm.

Synthesis of 1,8 –naphthyridine linked with 3,5-dimethyl pyrazole (Va-e) , 3,5-dimethyl Isoxazole (IX) and 4,5- dimethyl pyrimidin-2-one (X):

Hydrazono compound (V) was treatment with hydrazine hydrate and substituted hydrazines like

phenyl hydrazine, thiosemicarbazide, chlorophenylhydrazine and Isoniazide via cyclization gave the corresponding 1,8-naphthyridine linked with 3,5-dimethyl pyrazole (Va-e)and with treatment hydroxylamine HCl afforded corresponding 1,8-naphthyridine linked with 3, 5-dimethyl Isoxazole (IX) and was reacted with urea in the presence of ethanol to afforded 1,8-naphthyridine linked with 4, 5-dimethyl pyrimidine-2-one (X) (**Scheme - 4**).

The IR spectra of (Va-e) were characterized by the disappearance of the NH band and acetyl C=O absorption band and the presence of peak 1471 cm^{-1} and 1460 (N=N).

Synthesis of 1,8 –naphthyridine linked with 3,5-phenyl pyrazole(VIa-e), 3, 5-diphenyl isoxazole (XI) and 4,5 diphenyl pyrimidin-2-one (XII):

Hydrazono compound (VI) is treatment with hydrazine hydrate and substituted hydrazines like phenyl hydrazine, thiosemicarbazide, chlorophenylhydrazine and isoniazid via cyclization gave the corresponding 1,8-naphthyridine linked with 3,5-diphenyl pyrazole(VIa-e) and with treatment hydroxylamine HCl afforded corresponding 1,8-naphthyridine linked with 3, 5-diphenyl isoxazole(XI) and was reacted with urea in the presence of ethanol to afforded 1,8-naphthyridine linked with 4, 5-diphenyl pyrimidine-2-one (XII) (**Scheme - 5).**

Experimental:

Melting points of the newly synthesized compounds were determined by open capillary method and were uncorrected. Micro TLC was performed routinely to check purity of the synthesized compounds. Infrared spectra were recorded on Thermo Niclolet Nexus 670 spectrometer instruments and values are given in cm^{-1}. Proton magnetic resonance spectra were recorded on Varian Gemini-200, Varian unit-400 and Avance 300 MHz, Bruker Ux-NMR instrument. The samples were made in CCl_4/chloroform-d (1:1). Mass spectrums are recorded on VG Micromass 7070H (ESI and EI) and were given in mass units (m/z). Analytical thin layer chromatography (TLC) is performed on precoated silica gel-60 F254 (0.5mm) glass plates. Visualization of the spots on TLC plates is achieved either by exposure to iodine vapor or UV light.

Procedure for intermediate hydrazono (III-V) and Azo compounds (IV):

To 0.01 mole of compound 2- (P-aminophenyl)-1,8- napthyridine (I) was dissolved in a mixture of concentrated HCl and water (30ml) and then cooled to 0 - 5^oC in ice-bath. A cold solution of aqueous sodium nitrite was added slowly maintaining the temperature at 0^o C. The diazonium salt solution was filtered directly to a cold solution of active methylene compounds like ethyl acetoacetate, ethylcyanoacetate, acetyl acetone and sodium acetate in 50ml ethanol. The solid was filtered and washed with water and dried. The solid product was collected and recrystallized from ethanol to give the corresponding hydrazono derivative (III-V).

Ethyl-2-{[4-(1, 8-naphthyridin-2-yl) phenyl] hydrazono} -3-butanoate (III):

IR (KBr)cm^{-1} :3224.10(NH) ,3017.45(Ar C-H),1734.16 (ester), 1606.41(imine). ^{1}H-NMR δ (ppm): 10.1 (1H, s, NHN=C), 8.4 (1H, m, C_3-H), 8.49(1H, m, C_4-H), 8.63 (1H, m, C_5-H), 8.2 (1H, m, C_6-H) and 9.8 (1H, m, C_7-H) of 1,8-naphthyridine, 7.5-7.8 (4H, m, Ar-H), 2.45 (3H,s, $COCH_3$), 4.4 (2H,q, CH_2), 1.4 (3H,t, CH_3) for carboxylic ester.

Ethyl cyano {[4-(1,8-naphthyridin-2-l)phenyl] hydrazono} acetate (IV):

IR (KBr) cm^{-1} 3286.73(NH), 2990.33(Ar C-H), 1704.45(ester), 1585.50(imine). ^{1}H-NMR δ (ppm): : 9.9 (1H, s, NHN=C), 8.39 (1H, m, C_3-H), 8.41(1H, m, C_4-H), 8.62 (1H, m, C_5-H), 8.2 (1H, m, C_6-H) and 9.9 (1H, m, C_7-H) of 1,8-naphthyridine, 7.5-7.8 (4H, m, Ar-H),4.1(2H,q,CH_2), 2.2 (3H,s,CH_3)for carboxylic ester.

3-{[4-(1,8-naphthyridin-2-yl)phenyl] hydrazono} pentane-2,4-dione (V):

IR(KBr)cm^{-1}:3238.42(NH), 3026.50 (Ar C-H),1691.59(ester) 1585.50(imine). ^{1}H-NMR δ (ppm): : 10.2 (1H, s, NHN=C), 8.39 (1H, m, C_3-H), 8.41(1H, m, C_4-H), 8.62 (1H, m, C_5-H), 8.2 (1H, m, C_6-H) and 9.9 (1H, m, C_7-H) of 1,8-

naphthyridine, 7.5-7.8 (4H, m, Ar-H), 2.45(6H , s, 2×$COCH_3$).

General procedure for compound (IIIa-e) & VII (Scheme-2): A mixture of the appropriate hydrazono compounds (III) and hydrazine hydrate (0.01mol) in ethanol was heated under reflux for 4-6 hours. The solvent was concentrated and the reaction product was allowed to cool. The separated product was filtered off, washed with water, dried and recrystallized from ethanol. [25] The following title compounds were prepared.

5-methyl-4-{[4-(1,8- naphthyridin - 2- yl)phenyl] hydrazono}-2,4-dihydro-3*H*-pyrazol-3-one (IIIa) IR(KBr)cm^{-1} : 3417.42 (NH), 3005.50 (Ar C-H), 1585.50(imine),1667.20 (pyrazolonone). ^{1}H-NMR δ (ppm): 11.1 (1H, s, NHN=C), 9.8 (1H,s, Pyrazolinone NH), 8.4 (1H, m, C_3-H), 8.49 (1H, m, C_4-H), 8.63 (1H, m, C_5-H), 8.2 (1H, m, C_6-H) and 9.1 (1H, m, C_7-H) of 1,8-naphthyridine,7.5-7.8 (4H, m Ar-H).2.45 (3H, s, CH_3) MS m/z: 330, 248, 221(100%), 206, 130.

5-methyl-4-{[4-(1,8-naphthyridin - 2 - yl)phenyl] hydrazono}-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one(IIIb) IR (KBr) cm^{-1}: 3309.30(NH), 2917.70(ArC-H), 1590.90(imine), 1656.70 (pyrazoinone^{1}H-NMR δ (ppm): 9.9 (1H, s, NH-N=C), 8.32 (1H, m, C_3-H), 8.4 (1H, m, C_4-H), 8.52 (1H, m, C_5-H), 8.1 (1H, m, C_6-H) and 9.1 (1H, m, C_7-H) of 1,8-naphthyridine, 7.1-7.8 (9H, m, Ar-H), 2.5(3H, s, CH_3). MS m/z: 406, 278, 236, 202, 248(100%), 221, 206, 130.

3-methyl-4-{[4-(1,8-naphthyridin – 2 - yl)phenyl] hydrazono}-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (IIIc): IR(KBr)cm^{-1}:3129.30 (NH), 2918.70 (Ar C-H), 1590.90 (imine), 1688.70(pyrazolinone). ^{1}H-NMR ^{1}H-NMR δ (ppm): 10.0 (1H, s, NH-N=C), 8.32 (1H, m, C_3-H), 8.4 (1H, m, C_4-H), 8.52 (1H, m, C_5-H), 8.1 (1H,m, C_6-H) and 9.1 (1H, m, C_7-H) of 1,8-naphthyridine, 5.61 (2H. s, NH_2) 7.0-7.5 (4H, m, Ar-H), 2.5(3H,s,CH_3).MS m/z: 389, 245, 233 (100%),130.

5-methyl-4-{[4-(1,8-naphthyridin – 2 - yl)phenyl] hydrazono}-2-(pyridine - 4 - ylcarbonyl) -2, 4-dihydro-3*H*-pyrazol-3-one (IIIe) IR(KBr)cm^{-1}: 3321.10 (NH), 2956.20 (Ar C-H), 1590.90 (imine),1658.30 (pyrazolinone). ^{1}H-NMR δ (ppm): 10.1 (1H, s, NH-N=C), 8.22 (1H, m, C_3-H), 8.3 (1H, m, C_4-H), 8.51 (1H, m, C_5-H), 8.0 (1H,m, C_6-H) and 9.2 (1H, m, C_7-H) of 1,8-naphthyridine, 7.0-7.9 (8H, m, Ar-H), 2.6 (3H,s,CH_3), MS m/z: 435, 330, 307, 233, 202, (100%), 107, 79, 130.

3-methyl-4-{[4-(1,8-napthyridin - 2yl) phenyl] hydrazono}isoxazol-5(4*H*) one (VII):
Ethyl 2-{[4-(1,8-naphthyridin – 2 - yl) phenyl] hydrazono}-3-butanoate(III) (0.01mole) was dissolved in ethanol. A solution of sodium acetate (2 g) and hydroxyl amine hydrochloride (0.01) in water was added. Then it was refluxed for 3 hrs. The resulting solution then poured onto crushed ice to get solid product.

IR(KBr)cm^{-1}: 3426.50(NH), 2917.70 (Ar-C-H), 1594.50(imine),1698.30(Isoxazolinone). ^{1}H-NMR δ (ppm): 10.1 (1H, s, NH-N=C), 8.41 (1H, m, C_3-H), 8.49 (1H, m, C_4-H), 8.63 (1H,m, C_5-H), 8.38 (1H, m, C_6-H) and 9.09 (1H, m, C_7-H) of 1,8-naphthyridine, 7.42-7.95 (4H, m, Ar-H), 2.42 (3H,s, CH_3). MS m/z: 331, 248, 221(100%) 206, 221, 130.

General procedure for compounds (IVa-e & VIII) (scheme-3):
Ethyl cyano{[4-(1,8-naphthyridin-2-l)phenyl] hydrazono}acetate (IV) (0.01) and hydrazine hydrate(0.01mol) in ethanol was heated under reflux for 4-6 hours. The solvent was concentrated and the reaction product was allowed to cool. The separated product was filtered off, washed with water, dried and recrystallized from methanol.

5-Amino-4-{[4-(1,8-naphthyridin-2-yl) phenyl] hydrazono}-2,4-dihydro-3*H*-pyrazol-3-one(IVa): IR(KBr)cm^{-1}: 3483.10(NH), 2918.10 (Ar C-H), 1603.70.(imine),1688.70 (pyrazolinone C=0). ^{1}H-NMR δ (ppm): 11.9 (1H, s, NH-N=C),9.9(1H,s, Pyrazolinone NH), 8. 1 (1H, m, C_3-H), 8.2(1H, m, C_4-H), 8.63 (1H, m, C_5-H), 8.38 (1H, m, C_6-H) and 9.09 (1H, m, C_7-H) of 1,8-naphthyridine, ,5.62 (2H,s. NH_2), 7.42-7.95 (4H, m, Ar-H), MS m/z : 331, 248, 221(100%) 206, 203, 130.

5-Amino-4-{[4-(1,8 - naphthyridin-2-yl) phenyl] hydrazono}-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (IVb): IR(KBr)cm^{-1}: 3217.10 & 3192 (NH_2),

2918.10 (Ar C-H), 1604.70. (imine), 1689.40 (pyrazolinoneC=O). ^{1}H-NMR δ (ppm): 11.4 (1H, s, NH-N=C), 6.3 (3H, s, NH_2), 8.41(1H, m, C_3-H), 8.49(1H, m, C_4-H), 8.63(1H, m, C_5-H), 8.38(1H, m C_6-H) and 9.09 (1H, m, C_7-H) of 1,8-naphthyridine, 7.2-7.9(9H, m, Ar-H) .MS m/z : 407, 279 (100%),248,206, 130.

3-Amino-4-{[4-(1,8-naphthyridin - 2 - yl) phenyl] hydrazono}-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (IVc) IR(KBr)cm^{-1}: 3217.10 &3192 (NH_2), 2918.10(Ar C-H), 1604.70 (imine),1689.40 (pyrazolinoneC=0). ^{1}H-NMR δ (ppm): 11.7 (1H, s, NH-N=C),6.3 (2H, s, CS NH_2),5.21(2H, s, NH_2), 8.41(1H, m, C_3-H), 8.49 (1H, m, C_4-H), 8.63(1H, m, C_5-H), 8.38(1H, m, C_6-H) and 9.09 (1H,m, C_7-H) of 1,8-naphthyridine, 7.2-7.5(4H, m, Ar-H). MS m/z : 390, 236, 233, 217(100%),130.

Synthesis of 3-amino-4-{[4-(1,8-napthyridin-2yl)phenyl]hydrazono}isoxazol-5(4*H*)one (VIII)
Ethyl cyano{[4-(1,8-naphthyridin-2-l) phenyl] hydrazono}acetate (IV) (.01 mole) was dissolved in ethanol. A solution of sodium acetate (2 g) and hydroxyl amine hydrochloride (0.01 mole) in water was added. Then it was refluxed for 3 hrs. The resulting solution then poured in to crushed ice to get solid product.

IR(KBr)cm^{-1}: 3410(NH),2924.17 (Ar C-H), 1634.26 (imine),1694.40 (Isoxazolinone C=0). ^{1}H-NMR δ (ppm): 10.1 (1H, s, NH-N=C),4.4 (3H, s, NH_2), 8.40(1H, m, C_3-H), 8.48 (1H, m, C_4-H), 8.64(1H, m, C_5-H), 8.37(1H, m, C_6-H) and 9.8 (1H,m, C_7-H) of 1,8-naphthyridine,7.1-7.6 (4H, m, Ar-H) MS m/z : 332, 248, 205, 204 (100%),130.

General Procedure for compounds (Va-e, IX & X) (scheme-4):
3-{[4-(1,8-naphthyridin-2-yl) phenyl] hydrazono} pentane-2,4-dione (V) (.01 mole) and substituted hydrazine hydrate (0.01mol) in ethanol was heated under reflux for 4-6 hours. The solvent was concentrated and the reaction product was allowed to cool. The separated product was filtered off, washed with water, dried and recrystallized from water & methanol.

2-{4-[(3, 5-dimethyl - 1*H* - pyrazol-4-yl)diazenyl]phenyl}-1, 8-naphthyridine. (Va):
IR(KBr) cm^{-1}: 3421.80 (NH), 3107.80 (ArC-H), 1602.30 (imine),1471(azo)(N=N). ^{1}H-NMR δ (ppm): 10.0 (1H, s, N-H), 2.8 (3H, s,C_5-CH_3), 2.2 (3H, s,C_3-CH_3)(pyrazole), 7.5(1H, m, C_3-H), 7.72 (1H, m, C_4-H), 7.92(1H, m, C_5-H), 7.42(1H, m, C_6-H) and 8.12(1H, m, C_7-H) of 1,8-naphthyridine, 7.2-7.5(4H, m, Ar-H). MS m/z: 328, 221(100%), 235, 205, 130.

1-phenyl- 2-{4-[(3, 5-dimethyl-1*H*-pyrazol-4-yl)diazenyl]phenyl}-1, 8-naphthyridine. (Vb):
IR (KBr) cm^{-1}: 3059.78(ArC-H), 2924.68 (ArC-H), 1601.08(imine)(C=N) 1458.37 azo (N=N). ^{1}H-NMR δ (ppm): 2.5 cm^{-1}(3H, s,C_5-CH_3), 2.9 (3H, s,C_3-CH_3)(pyrazole) , 7.42(1H,m, C_3-H), 7.52(1H, m, C_4-H), 7.81(1H, m, C_5-H), 7.30(1H, m, C_6-H) and 8.42(1H, m, C_7-H) of 1, 8-naphthyridine, 7.2-7.8(9H, m, Ar-H). MS m/z: 404, 248, 189 (100%), 130.

3,5-dimethyl-4-{[4-(1,8-naphthyridin – 2 - yl) phenyl] diazenyl} - 1*H* – pyrazole - 1-carbothioamide (Vc) IR(KBr)cm^{-1}:
3412.81, 3247.33(NH_2), 3020 (ArC-H), 1608.23, imine(C=N), 1464.14 azo (N=N). ^{1}H-NMR δ (ppm): 4.3 (2H,s,NH_2) 2.9 (3H, s,C_5-CH_3), 2.62 (3H, s,C_3-CH_3)(pyrazole), 7.49 (1H, m, C_3-H), 7.82(1H, m, C_4-H), 8.12(1H, m, C_5-H), 7.32 (1H, m, C_6-H) ,8.56 (1H, m, C_7-H) of 1,8-naphthyridine, 6.8-7.1(4H, m, Ar-H). MS m/z: 387, 251(100%), 219, 130.

Preparation of 2-{4-[3,5-dimethylisoxazol-4-yl)diazenyl}-1,8-naphthyridine (IX):
3-{[4-(1,8-naphthyridin-2-yl) phenyl] hydrazono} pentane-2,4-dione(V) (0.01 mole was dissolved in glacial acetic acid and is treated with hydroxyl amine (0.02 mole) in round bottom flask. The mixture was refluxed for 4 hrs. The mixture then was cooled by pouring in 100ml of chilled water and then allowed to stand overnight. The solid was filtered, dried and recrystallized with ethanol.

IR(KBr)cm^{-1}: 3058 (ArC-H),1602.52,imine(C=N), 1458.15 azo (N=N). ^{1}H-NMR δ (ppm): 2.4 (3H, s, C_3-CH_3) ,2.9 (3H, s,C_5-CH_3) (isoxazole). 7.52 (1H, m, C_3-H), 7.72 (1H, m, C_4-H), 7.92(1H, m, C_5-H),

7.48 (1H, m, C_6-H) and 8.21 (1H, m, C_7-H) of 1,8-naphthyridine ,6.8-7.0 (4H, m, Ar-H).

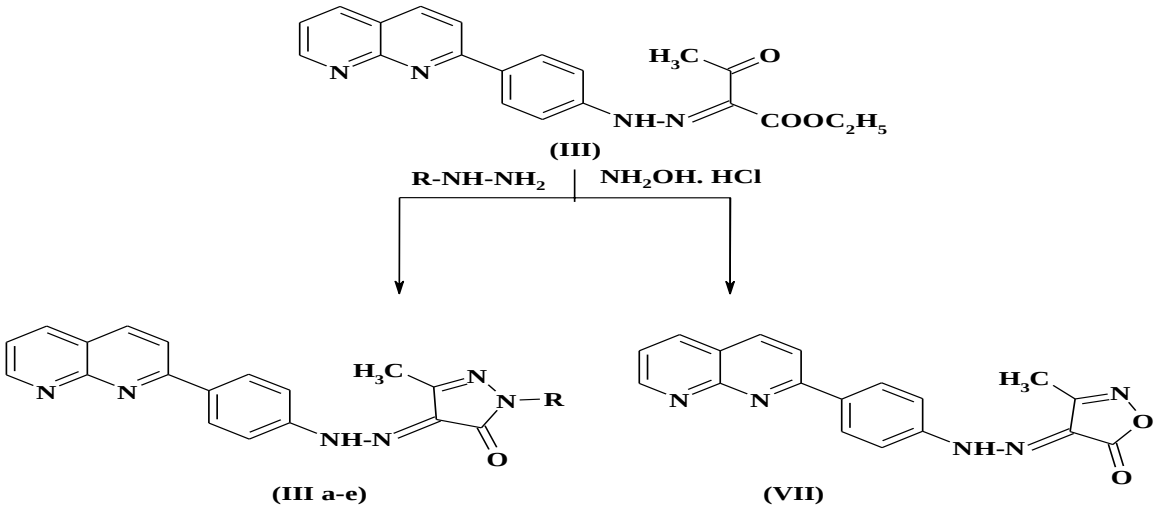
Preparation of 4,6-dimethyl-5-{[4-(1,8-naphthyridin -2-yl) phenyl] hydrazono} pyrimidin - 2(5*H*)-one (X):

3-{[4-(1,8-naphthyridin-2-yl) phenyl] hydrazono} pentane-2,4-dione(V) (0.01 mole) and urea (0.6gram) was heated under reflux for 5 hours. After cooling to room temperature, crushed ice was added and the mixture was stirred for 1 hours. The separated product was collected by filtration and recrystallized from aqueous ethanol.

IR(KBr)cm^{-1}: 3424.79 (NH), 2924 (Ar C-H),1601.51,Imine(C=N), 1681.51 (pyrimidine-2-one)(C=O). ^{1}H-NMR δ (ppm): 11.1 (1H, s, NH-N=C),7.41(1H, m, C_3-H), 7.52(1H, m, C_4-H), 7.72 (1H, m, C_5-H), 7.31(1H, m, C_6-H) 8.1(1H, m, C_7-H) of 1,8-naphthyridine, 6.8-7.0(4H, m, Ar-H).3.1(6H,s,2×CH_3).

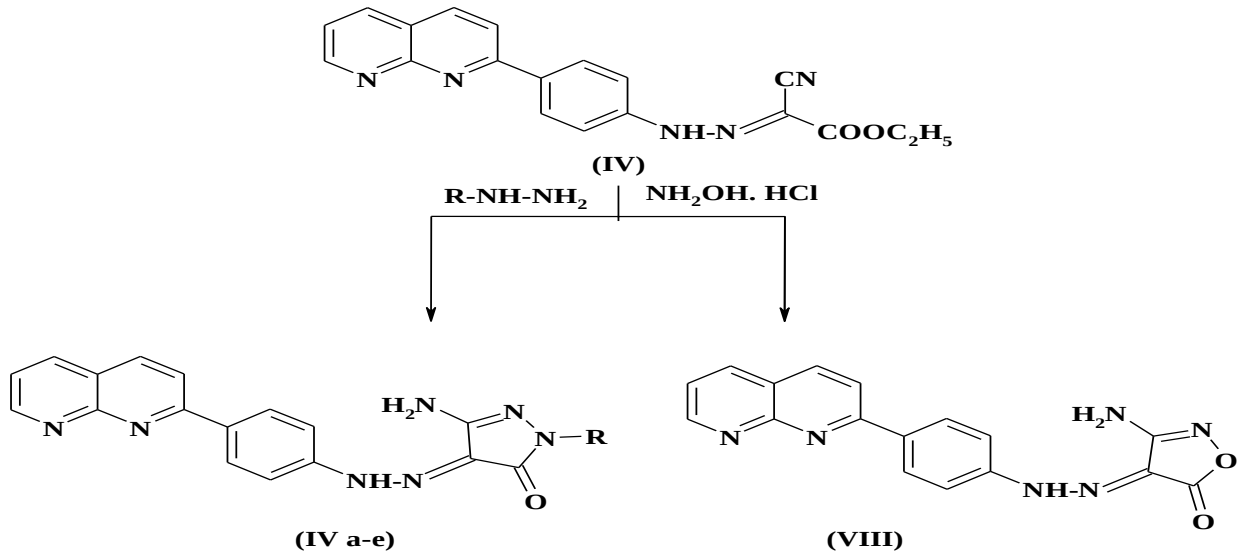
SCHEME-1

III $\mathbf{R^1}$=$COCH_3$, $\mathbf{R^2}$= $COOC_2H_5$
IV $\mathbf{R^1}$=CN, $\mathbf{R^2}$= $COOC_2H_5$
V $\mathbf{R^1}$=$COCH_3$, $\mathbf{R^2}$= $COCH_3$



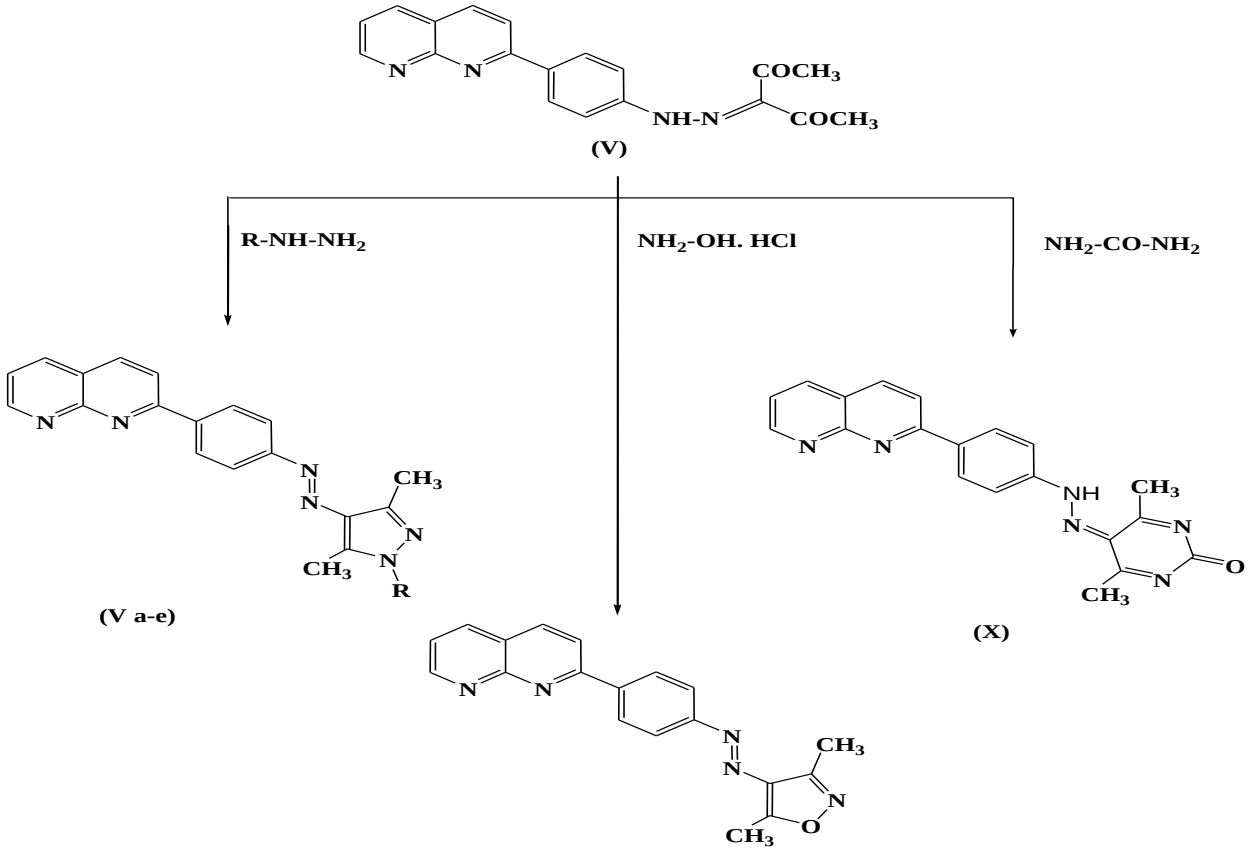
SCHEME - 2

IIIa R= H IIIb R= C_6H_5,
IIIc R = $CSNH_2$, IIId R= Cl-C_6H_4,
IIIe R=C_5H_4N CO



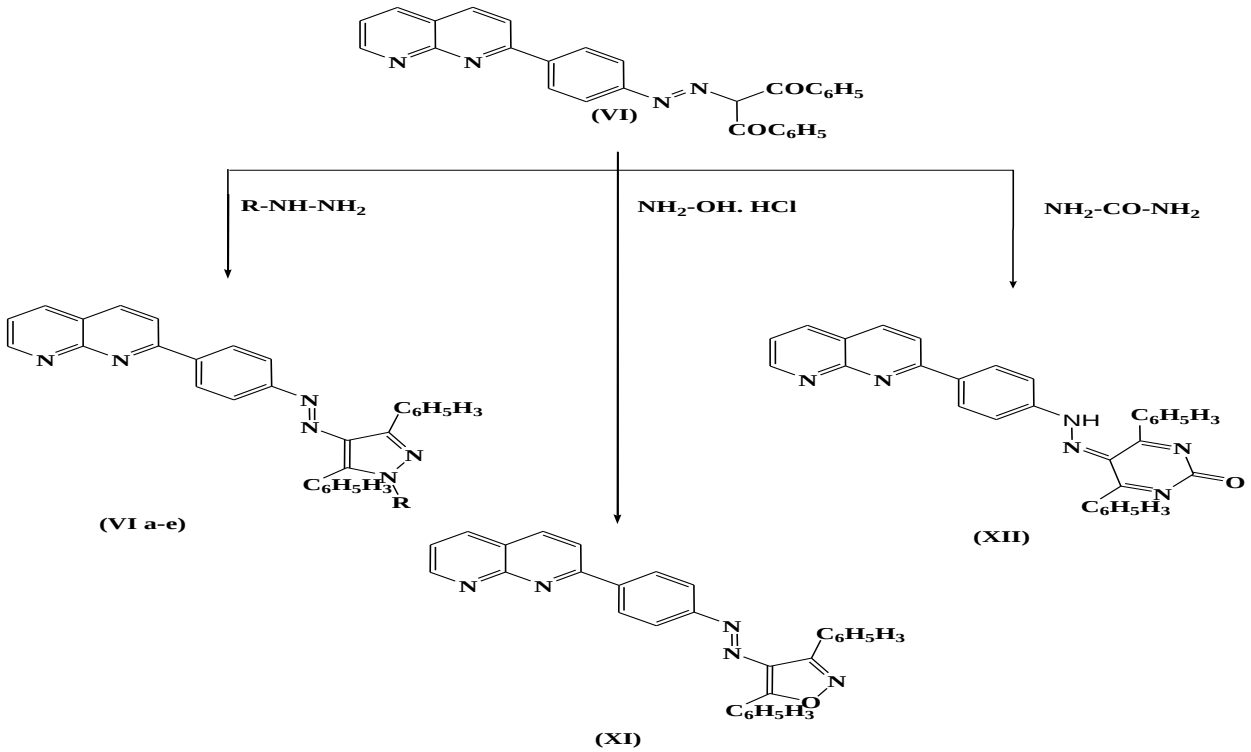
SCHEME-3

IVa R= H, IVb R= C_6H_5, IVc R = $CSNH_2$, IVd R= Cl-C_6H_4, IVe R=C_5H_4N CO



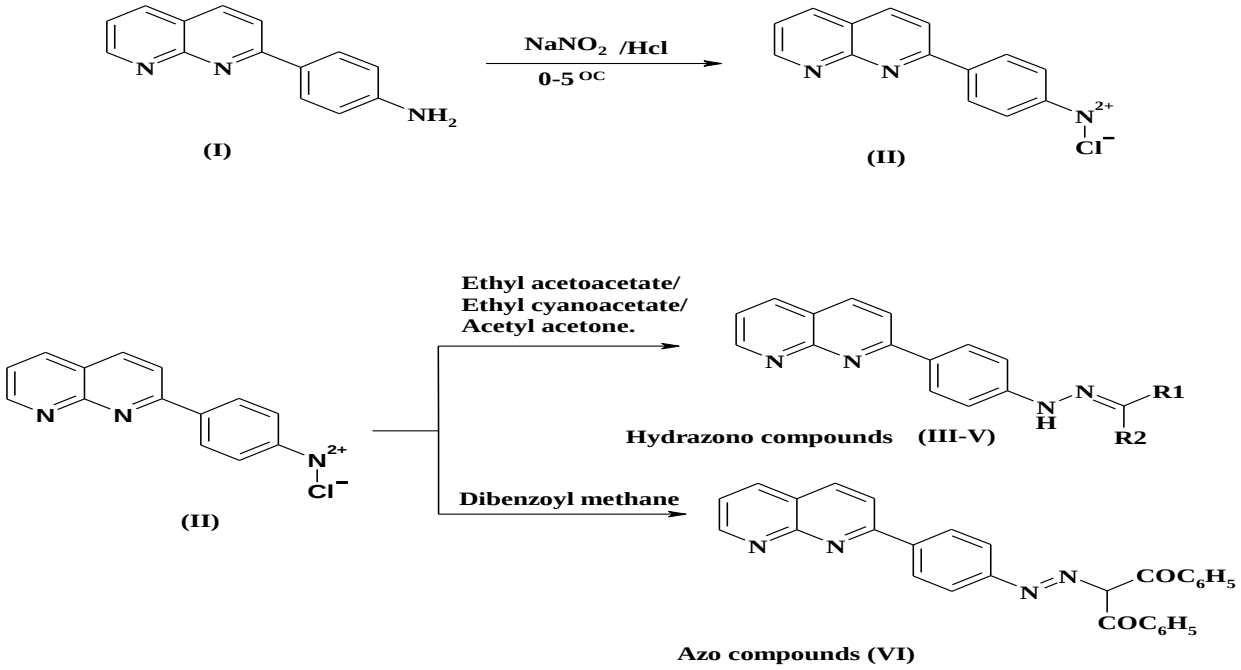
SCHEME-4

Va R= H, Vb R= C_6H_5, Vc R = $CSNH_2$, Vd R= ClC_6H_4, Ve R=C_5H_4N CO



SCHEME - 5

VI a R= H VI b R= C_6H_5,
VI c R = $CSNH_2$, VI d R= ClC_6H_4,
VI e R= C_5H_4N CO



Anti-Microbial Activity:

Determination of Zone of Inhibition [26]:

The synthesized compounds were screened for their in-vitro antibacterial activity against two gram positive organism and gram negative organism. Further antifungal activity was carried out compounds against the organism like *C*andida *albicans* and *Aspergillus niger*. A suspension of the test organism was well mixed with 25ml of sterile liquid nutrient agar media, at a temperature between 40-50°C and poured immediately in to a pre-sterilized petridishes. A sterile borer was used to prepare 4 cups of 8mm diameter in the agar media. Test solutions of the synthesized compounds were prepared at a concentration of 500mg/ml with DMSO. A solution of standard drug ampicillin was prepared at the same concentration. Test and standard solutions were added to the cups with a micropipette. After adding all petridishes were incubated at 37±1°C for 24 hours. The solvent DMSO was used as blank. The diameter of zone of inhibition was measured in mm.

Determination of MIC [27]:

All the synthesized compounds were dissolved separately to prepare a stock solution containing 1000µg/ml of DMSO. Different synthesized compounds (20mg) (IIIa-e, IVa-e, Va-e, VII,) were dissolved in 2 ml of the DMSO and 1 ml of this solution was aseptically transferred to the sterile nutrient broth medium and made up to 16 ml with sterile nutrient media, thus 1 ml of the resulted solution gives 1000 µg/ml. One ml (1ml) of the above solution was transferred to 1 ml of DMSO to give half the concentration of first. Successive concentrations like 250, 125, and 62.5 and so were prepared in a similar manner up to 8 dilutions from eigth one ml of the solution is discarded. The tubes were mixed well after each addition. All the tubes were inoculated with one loop full of one of the test organism. The process was

Repeated with different test organisms. A positive control and a negative control were also prepared to confirm the nutritive property and sterility, respectively of the prepared medium. The tubes were incubated at 37°C for 24 hours. The presence or absence of growth of organism was observed after incubation compared with that of standard drug (ampicillin). Similar procedure was carried out for the evaluation of antifungal activity using

Sabourauds dextrose agar medium by standard drug (griseofulvin).

TABLE 1: ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS (IIIa-e, IVa-e, Va-e, VIae, VII & IX) AND STANDARD (ZONE OF INHIBITION IN MM)

Compounds	Gram positive		Gram negative	
	B.subtilis	***S.aureus***	***E.coli***	***P.aeruginosa***
IIIa	18	20	18	10
IIIb	22	27	29	22
IIIc	25	18	10	20
IIId	30	**31**	25	19
IIIe	**25**	**27**	**27**	**23**
Iva	23	18	21	15
IVb	21	24	23	21
IVc	25	27	20	18
IVd	**28**	**26**	**29**	**22**
IVe	25	28	22	19
Va	21	08	__	12
Vb	18	25	27	20
Vc	22	23	17	21
Vd	24	26	25	27
Ve	25	18	17	22
Via	08	12	06	__
VIb	25	30	25	20
Vic	16	14	28	17
VId	**28**	**22**	**30**	**26**
Vie	18	21	24	16
VII	16	18	21	17
IX	20	27	22	21
Ampicillin	32	34	32	29

TABLE 2: ANTIFUNGAL ACTIVITY OF SYNTHESIZED COMPOUNDS (IIIa-e, IVa-e, Va-e, VIa-e, VII & IX) &STANDARD [ZONE OF INHIBITION (MM)]

Compounds	Organism	
	Aspergillus niger	***Candida albicans***
IIIa	15	18
IIIb	**27**	**29**
IIIc	08	13
IIId	**28**	**30**
IIIe	**25**	**22**
IVa	12	16
IVb	**16**	**21**
IVc	22	19
IVd	**27**	**28**
IVe	18	21
Va	12	18
Vb	17	14
Vc	19	20
Vd	25	26
Ve	21	18
VIa	18	06
VIb	25	10
VIc	**26**	**24**
VId	**28**	**30**
VIe	14	12
VII	22	24
IX	24	18
Standard (Griseofulvin)	**30**	**32**

TABLE 3: ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS (IIIa-e, IVa-e, Va-e, VIa- e, VII & IX) &STANDARD MIC (µg/ml).

Comp.	Minimum inhibitory concentrations (µg/ml)			
	B. Subtilis	***S. Aureus***	***E. Coli***	***P.Aeruginosa***
IIIa	> 500	250	>500	250
IIIb	125	31.2	15.6	125
IIIc	62.5	500	> 500	250
IIId	62.5	125	250	500
IIIe	>500	>500	250	>500
IVa	125	500	250	> 500
IVb	> 500	62.5	500	125
IVc	62.5	31.2	250	500
IVd	31.2	250	31.2	125
IVe	62.5	31.2	125	62.5
Va	> 500	> 500	250	> 500
Vb	15.6	62.5	15.6	125
Vc	125	125	> 500	250
Vd	7.8	31.2	15.6	31.2
Ve	125	31.2	62.5	125
VIa	> 1000	250	> 1000	> 500
VIb	62.5	15.6	62.5	250
VIc	62.5	> 500	15.6	> 500
VId	**7.8**	**15.6**	**15.6**	**31.2**
VIe	500	62.5	62.5	> 500
VII	**62.5**	**62.5**	**15.6**	**125**
IX	250	31.2	15.6	250
Ampicillin	1.54	1.54	3.12	3.12

TABLE 4: ANTIFUNGAL ACTIVITY OF SYNTHESIZED COMPOUNDS (IIIa-e, IVa-e, Va-e, VIa-e, VII & IX) &STANDARD MIC (µg/ml).

Compounds	Minimum inhibitory concentrations (µg/ml)	
	Aspergillus niger	***Caninda albicans***
IIIa	250	500
IIIb	250	125
IIIc	> 500	62.5
IIId	**15.6**	**31.2**
IIIe	500	**62.5**
IVa	> 500	> 500
IVb	**62.5**	**125**
IVc	125	500
IVd	**7.8**	**15.6**
IVe	500	31.2
Va	> 500	500
Vb	62.5	125
Vc	500	62.5
Vd	31.2	15.6
Ve	125	500
VIa	500	> 500
VIb	31.2	> 500
VIc	**15.6**	**31.2**
VId	31.2	62.5
VIe	> 500	250
VII	125	62.5
IX	125	31.2
Standard (Griseofulvin)	3.41	1.23

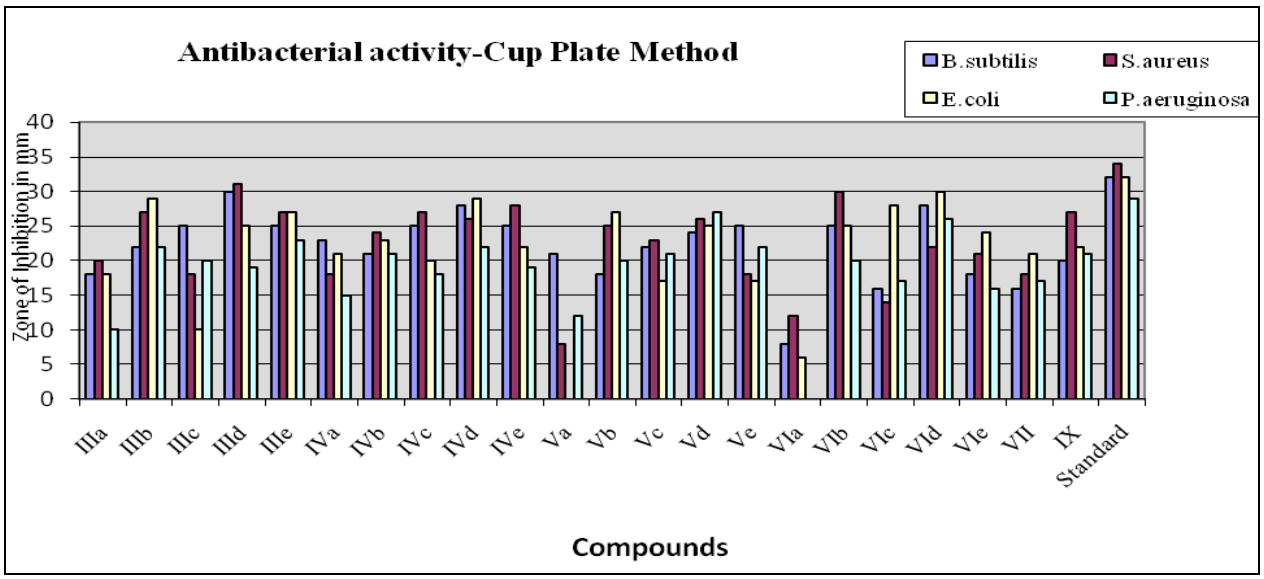


FIG.1: ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS (IIIa-e, IVa-e, Va-e, VIa-e, VII & IX) AND STANDARD (Zone of Inhibition in mm)

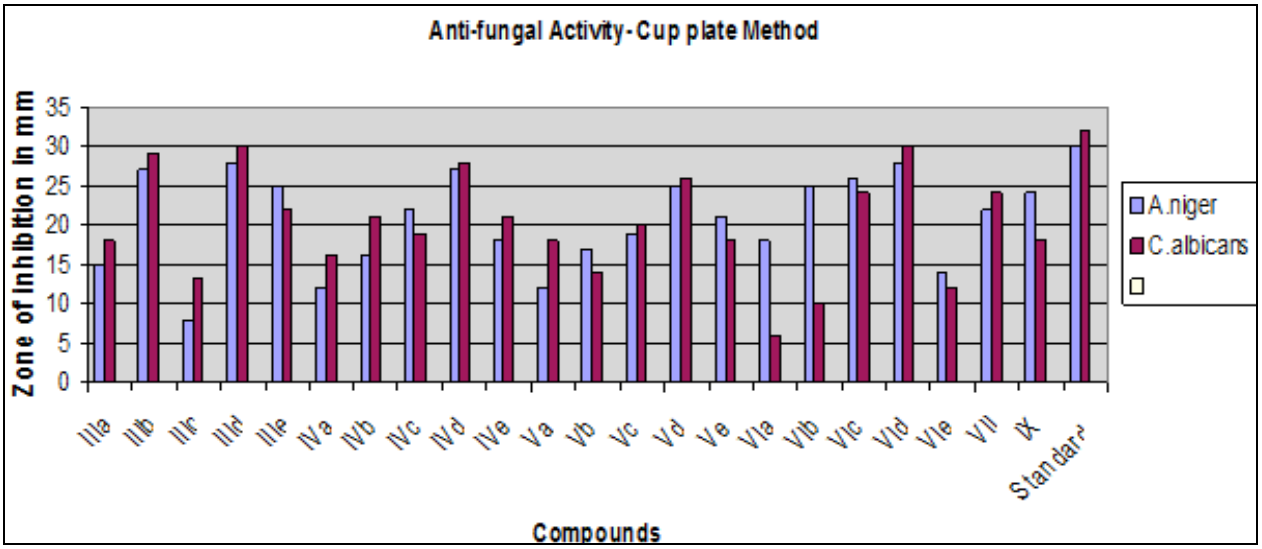


FIG.2: ANTIFUNGAL ACTIVITY OF SYNTHESIZED COMPOUNDS (IIIa-e, IVa-e, Va-e, VIa-e, VII & IX) AND STANDARD (ZONE OF INHIBITION IN MM)

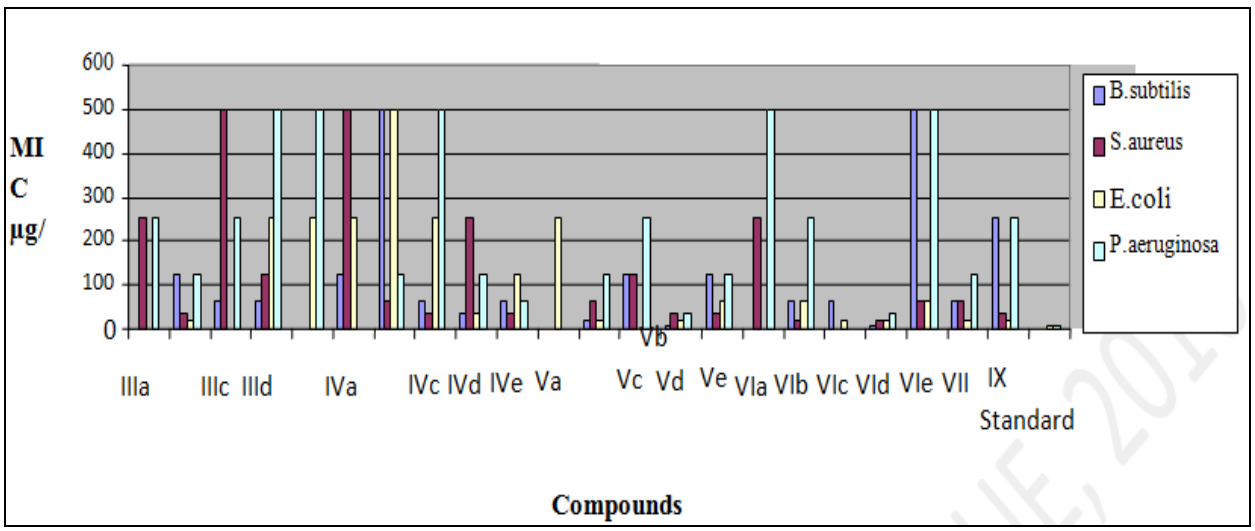


FIG.3: ANTIBACTERIAL ACTIVITY- SERIAL DILUTION METHOD, GRAPHICAL REPRESENTATION OF ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS.

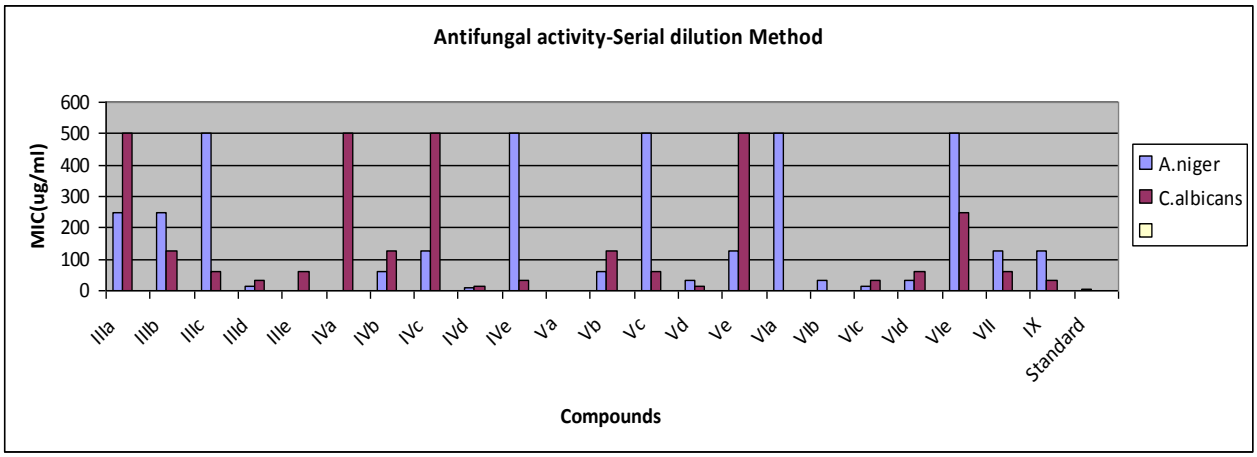


FIG.4: GRAPHICAL REPRESENTATION OF ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS (IIIa-e,IVa-e, Va-e, VIa-e, VII & IX) & STANDARD MIC (µg/ml)

Results for anti-microbial activity:

The results of cup plate method showed in **Tables 1-2** and **Fig.1-2** that all the synthesized compounds were showed potent to weak anti bacterial and antifungal activities. The compounds IIId, IVd, VId showed impressive antibacterial and antifungal activities. The activity was due to presence of chloro group at the para position of phenyl ring of Pyrazolinone and pyrazole nucleus. Compound IIId showed very potent antibacterial & antifungal activity against *S.Aureus* and *C.albicans* with zone of inhibition of 31mm& 30 mm respectively. Whereas compounds IVd & VId showed very potent antibacterial and antifungal activities against *E.coli* and *A.niger* with zone of inhibition of 29 & 30mm with compared to the standard.

The activity was increased due to presence of chloro group at the para position of phenyl ring of Pyrazolinone and pyrazole. Compounds IIIb, IVb displayed moderate antibacterial activity against S.auresus with zone of inhibition of 27 and 24mm where as compound IIIb showed good anfungal activity against *Caninda albicans* with zone of inhibition as 29mm.The compounds Vb, VIb, VII & IX exhibit moderate antibacterial & antifungal activity against *B.subtilis* and *P.aeruginosa* and both the fungi the compounds IIIa, IVa, Va, VIa showed weak antibacterial and antifungal activity against different strains of bacteria and fungi. Compounds Va, VIa did not showed any activity against *E.c*oli and *P.aeruginosa* respectively. The most active compounds IIId, IVd, VId like N-(Cl-C_6H_5) pyrazolinone, & N-(Cl-C_6H_5) pyrazoles linked 1,8-naphthyridine shows better antimicrobial activities. The activity was due to presence of chloro group at the para position of phenyl ring of Pyrazolinone and pyrazole nucleus. The presence of methyl group& amino group at the 3rd position, hydrazono and azo (N=N) group at 4th of the pyrazolinone (IIId, IVd) and pyrazole(Vd) ring may be contributing to the antimicrobial activity. The compounds having hydrazono & azo groups shows significant antimicrobial activity.

Serial dilution method:

The results of cup plate method showed in tables 3-4& figures 3-4, the synthesized compounds IIIa-e, IVa-e, Va-e, VIa-e, VII and IX were tested for antibacterial and antifungal activities against various strains by the serial dilution method for the determination of minimum Inhibitory concentration (MIC).Compounds Vd &VId showed excellent antibacterial activity against *B.subtilis* the MIC value of 7.8 µg/ml where as the compounds IVd , Vd showed very good antifungal activity against *A.niger* and *C.albicans* with MIC value of 7.8 µg/ml & 15.6µg/ml. Compound IIIb was found to be active against *E.c*oli at concentration level of 15.6µg/ml, compound IIIc showed active against *B.subtilis* with concentration of 62.5 µg/ml. Whereas compounds IVb, Vb, VII exhibit moderate antibacterial against *S.aureus* at a concentration level of 62.5 µg/ml , compound Vb, IX showed potent activity against *B.subtilis* and *E. coli* at concentration level of 15.6 µg/ml. Compound IVb, Vb exhibit moderate antifungal activity against *A.niger* at a concentration level of 62.5 µg/ml and compound VII showed potent antifungal activity against *C.albicans.* The compounds IIIa, Iva, VA,

vIa showed weak antibacterial and antifungal activity against different strains of bacteria and fungi.

CONCLUSION: An agar cup plate method result indicates that the compounds IIId, IVd, VId showed impressive antibacterial and antifungal activities. The activity was due to presence of chloro group at the para position of phenyl ring of pyrazolinone and pyrazole nucleus and also the presence of methyl & amino groups at the 3rd position, hydrazono and azo (N=N) group at 4th of the Pyrazolinone (IIId, IVd) and pyrazole(Vd) ring may be contributing to the antimicrobial activity. The compounds having hydrazono & azo groups shows significant antimicrobial activity. In serial dilution method the compounds Vd &VId showed excellent antibacterial activity against *B.subtilis* the MIC value of 7.8 µg/ml where as the compounds IVd, Vd showed very good antifungal activity against *A.niger* and *C.albicans* with MIC value of 7.8 µg/ml & 15.6 µg/ml. The compounds IIId, IVd, Vd, showed potent antimicrobial activity that have a chloro substitution at a para position of phenyl ring.

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