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-naphthyridinyl (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 (IIId, IVd) and pyrazoles (Va, Vb) & (IVA, IVb) and isoxazoles (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 cyclization resulted in the formation of pyrazolones (Va-e) & (Vla-e), isoxazolones (VII&IX) and substituted pyrimidine-2-ones (X&XII). All the newly synthesized compounds were screened for their in-vitro antibacterial & 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 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 pyrazoline (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, antimalarial, anti-inflammatory, antitumor, antihypertensive, and antibacterial activities. Pyrazolone and Isoxazolone compounds are associated with broad spectrum of biological activities. 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, antibacterial, antifungal, analgesic, antiviral, Hypoglycemic, anticancer, and anticonvulsant. 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-naphthyridine (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°C. Coupling of diazonium salts (II) with active methylene compounds like ethyl acetoacetate, ethyl cyanoacetate, acetyl acetone and dibenzoyl methane to afford the corresponding intermediate hydrazono(III-V) and azo (VI) compounds (Scheme-1).

The IR spectrum of the III shows an absorption bands at 3224.10 cm⁻¹, 3017.45 cm⁻¹, 1734.16 cm⁻¹ and 1606.41 cm⁻¹ corresponds to ²° amine, aromatic C-H (stretch), ester and imine (C=N) groups respectively. The ¹H-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, CH₃), 8.4 (1H, m, C₃-H), 8.49 (1H, m, C₄-H), 8.63 (1H, m, C₅-H), 8.2 (1H, m, C₆-H) and 9.8 (1H, m, C₇-H) of 1,8-naphthyridine ring, 7.5-7.8 (4H, m, Ar-H), 4.4 (2H, q, CH₂), 2.3 (3H, s, CO₂CH₃), 1.4 (3H, t, CH₃ 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, thiosemicarbazide, 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 ¹H-NMR spectra of IIIa-e showed the absence of the signals for the ethyl group, while the pyrazolinone CH₃ 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 NH₂ group. ¹H-NMR spectra of IVa-e showed the absence of the signals for the ethyl group and presence of NH₂ 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 treated 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(VI-a, e), 3, 5-diphenyl isoxazole (XI) and 4,5 diphenyl pyrimidine-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-dimethyl pyrazole(VI-a-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-dimethyl 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 Nicolet 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 CCl₄/chloroform-d (1:1). Mass spectra 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-naphthyridine (I) was dissolved in a mixture of concentrated HCl and water (30ml) and then cooled to 0 - 5°C in ice-bath. A cold solution of aqueous sodium nitrite was added slowly maintaining the temperature at 0° C. The diazonium salt solution was filtered directly to a cold solution of active methylene compounds like ethyl acetocacetate, 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). ¹H-NMR δ (ppm): 10.1 (1H, s, NHN=C), 8.4 (1H, m, C₃-H), 8.49(1H, m, C₄-H), 8.63 (1H, m, C₅-H), 8.2 (1H, m, C₆-H) and 9.8 (1H, m, C₇-H) of 1,8-naphthyridine, 7.5-7.8 (4H, m, Ar-H), 2.45 (3H, s, COCH₃), 4.4 (2H, q, CH₂), 1.4 (3H, t, CH₃) 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). ¹H-NMR δ (ppm): 9.9 (1H, s, NHN=C ), 8.39 (1H, m, C₃-H), 8.41(1H, m, C₄-H), 8.62 (1H, m, C₅-H), 8.2 (1H, m, C₆-H) and 9.9 (1H, m, C₇-H) of 1,8-naphthyridine, 7.5-7.8 (4H, m, Ar-H),4.1(2H,q,CH₂), 2.2 (3H,s,CH₃)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). ¹H-NMR δ (ppm): 10.2 (1H, s, NHN=C ), 8.39 (1H, m, C₃-H), 8.41(1H, m, C₄-H), 8.62 (1H, m, C₅-H), 8.2 (1H, m, C₆-H) and 9.9 (1H, m, C₇-H) of 1,8-
naphthyridine, 7.5-7.8 (4H, m, Ar-H), 2.45(6H, s, 2xCOCH₃).

**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. The following title compounds were prepared.

5-methyl-4-[(4-(1,8-naphthyridin - 2-yl)phenyl] hydrazono]-2,4-dihydro-3H-pyrazol-3-one (IIIa) IR(KBr)cm⁻¹: 3417.42 (NH), 3005.50 (Ar C-H), 1585.50 (imine),1667.20 (pyrazolonone). ¹H-NMR δ (ppm): 11.1 (1H, s, NH=N=C), 9.8 (1H,s, Pyrazolinone NH), 8.4 (1H, m, C₃-H), 8.49 (1H, m, C₄-H), 8.63 (1H, m, C₅-H), 8.2 (1H, m, C₆-H) and 9.1 (1H, m, C₇-H) of 1,8-naphthyridine, 7.5-7.8 (4H, m Ar-H), 2.45 (3H, s, CH₃) 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-3H-pyrazol-3-one (IIIb) IR (KBr) cm⁻¹: 3309.30(NH), 2917.70 (Ar C-H), 1590.90 (imine), 1656.70 (pyrazoinone ¹H-NMR δ (ppm): 9.9 (1H, s, NH=N=C), 8.32 (1H, m, C₃-H), 8.4 (1H, m, C₄-H), 8.52 (1H, m, C₅-H), 8.1 (1H, m, C₆-H) and 9.1 (1H, m, C₇-H) of 1,8-naphthyridine, 7.1-7.8 (9H, m, Ar-H), 2.5 (3H, s, CH₃). 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-1H-pyrazole-1-carboxioamide (IIIc): IR(KBr)cm⁻¹:3129.30 (NH), 2918.70 (Ar C-H), 1590.90 (imine), 1688.70 (pyrazolinone). ¹H-NMR ¹H-NMR δ (ppm): 10.0 (1H, s, NH=N=C), 8.32 (1H, m, C₃-H), 8.4 (1H, m, C₄-H), 8.52 (1H, m, C₅-H), 8.1 (1H,m, C₆-H) and 9.1 (1H, m, C₇-H) of 1,8-naphthyridine, 5.61 (2H, s, NH₂) 7.0-7.5 (4H, m, Ar-H), 2.5 (3H, s, CH₃).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-3H-pyrazol-3-one (IIId) IR(KBr)cm⁻¹: 3321.10 (NH), 2956.20 (Ar C-H), 1590.90 (imine), 1658.30 (pyrazolinone). ¹H-NMR δ (ppm): 10.1 (1H, s, NH=N=C), 8.22 (1H, m, C₃-H), 8.3 (1H, m, C₄-H), 8.51 (1H, m, C₅-H), 8.0 (1H,m, C₆-H) and 9.2 (1H, m, C₇-H) of 1,8-naphthyridine, 7.0-7.9 (8H, m, Ar-H), 2.6 (3H,s,CH₃), MS m/z: 435, 330, 307, 233, 202, (100%), 107, 79, 130.

3-methyl-4-[(4-(1,8-naphthyridin – 2-yl) phenyl] hydrazono]isoxazol-5(4H) one (VII): Ethyl 2-[4-(1,8-naphthyridin – 2-yl) phenyl] hydrazono]-3-butanote(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 (Isoxazinone ). ¹H-NMR δ (ppm): 10.1 (1H, s, NH=N=C), 8.41 (1H, m, C₃-H), 8.49 (1H, m, C₄-H), 8.63 (1H,m, C₅-H), 8.38 (1H, m, C₆-H) and 9.09 (1H, m, C₇-H) of 1,8-naphthyridine, 7.42-7.95 (4H, m, Ar-H), 2.42 (3H, s, CH₃). 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-3H-pyrazol-3-one (IVA): IR(KBr)cm⁻¹: 3483.10(NH), 2918.10 (Ar C-H), 1603.70 (imine), 1688.70 (pyrazolinone C=0). ¹H-NMR δ (ppm): 11.9 (1H, s, NH=N=C), 9.9 (1H,s, Pyrazolinone NH), 8.1 (1H, m, C₃-H), 8.2 (1H, m, C₄-H), 8.63 (1H, m, C₅-H), 8.38 (1H, m, C₆-H) and 9.09 (1H, m, C₇-H) of 1,8-naphthyridine, 5.62 (2H,s, NH₂), 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-3H-pyrazol-3-one (IVB): IR(KBr)cm⁻¹: 3217.10 & 3192 (NH₂),

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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$_7$-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-1H-pyrazole-1-carbothioamide (IVc) 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): 11.7 (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-naphthyridin-2yl)phenyl]hydrazono]isoxazol-5(4H)one (VIII)

Ethyl cyano[4-(1,8-naphthyridin-2-yl) phenyl] hydrazono]acetate (IV) (0.01 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=O). $^1$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) (0.01 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 - 1H - pyrazol-4-yl]diazenyl]phenyl]-1, 8-naphthyridine. (Va):

IR(KBr) cm$^{-1}$: 3421.80 (NH), 3107.80 (Ar-C-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-1H-pyrazol-4-yl] diazenyl]phenyl]-1, 8-naphthyridine. (Vb):

IR (KBr) cm$^{-1}$: 3059.78(Ar-C-H), 2924.68 (Ar-C-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] - 1H – pyrazole - 1-carbothioamide (Ve) IR(KBr)cm$^{-1}$: 3412.81, 3247.33(NH$_2$), 3020 (Ar-C-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 (Ar-C-H),1602.52,imeine(C=N), 1458.15 azo (N=N). $^1$H-NMR δ (ppm): 2.4 (3H, s, C$_3$-CH$_3$) .29 (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, C6-H) and 8.21 (1H, m, C7-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(5H)-one (X):**

3-[[4-(1,8-naphthyridin-2-yl) phenyl] hydrazono] pentane-2,4-dione(V) (0.01 mole) and urea (0.6 gram) was heated under reflux for 5 hours. After cooling to room temperature, crushed ice was added and the mixture was stirred for 1 hour. 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).

1H-NMR δ (ppm): 11.1 (1H, s, NH-N=C), 7.41 (1H, m, C3-H), 7.52 (1H, m, C4-H), 7.72 (1H, m, C5-H), 7.31 (1H, m, C6-H) 8.1 (1H, m, C7-H) of 1,8-naphthyridine, 6.8-7.0(4H, m, Ar-H). 3.1 (6H, s, 2×CH3).

**SCHEME 1**

III R¹=COCH₃, R²= COOC₂H₅
IV R¹=CN, R²= COOC₂H₅
V R¹=COCH₃, R²= COCH₃

**SCHEME 2**

IIIa R = H  IIIb R= C₆H₅
IIIc R = CSNH₂, IIId R= Cl-C₆H₄
IIIe R=C₃H₄N CO

IR(KBr)cm⁻¹: 3424.79 (NH), 2924 (Ar C-H), 1601.51, Imine(C=N), 1681.51 (pyrimidine-2-one )(C=O).
SCHEME 3

IVa R = H, IVb R = C₆H₅, IVc R = CSNH₂, IVd R = ClC₆H₄,IVE R = C₅H₄NCO

(V)

R-NH-NH₂
NH₂-OH. HCl
NH₂-CO-NH₂

(Va-e)

(X)

(SCHEME 4)

Va R = H, Vb R = C₆H₅, Vc R = CSNH₂, Vd R = ClC₆H₄, Ve R = C₅H₄NCO

(VI)

R-NH-NH₂
NH₂-OH. HCl
NH₂-CO-NH₂

(VI a-e)

(XII)

(XI)
Anti-Microbial Activity:

**Determination of Zone of Inhibition**

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 Candida 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 into 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**

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 eighth 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, VII & IX) AND STANDARD (ZONE OF INHIBITION IN MM)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram positive</th>
<th>Gram negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B.subtilis</td>
<td>S.aureus</td>
</tr>
<tr>
<td>IIIa</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>IIIb</td>
<td>22</td>
<td>27</td>
</tr>
<tr>
<td>IIIc</td>
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<td>18</td>
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<tr>
<td>IIId</td>
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<td>31</td>
</tr>
<tr>
<td>IIle</td>
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<td>27</td>
</tr>
<tr>
<td>Iva</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td>IVb</td>
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<td>24</td>
</tr>
<tr>
<td>IVc</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>IVd</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>IVe</td>
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<td>28</td>
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<td>Va</td>
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<td>08</td>
</tr>
<tr>
<td>Vb</td>
<td>18</td>
<td>25</td>
</tr>
<tr>
<td>Vc</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Vd</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>Ve</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>Via</td>
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<td>12</td>
</tr>
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<td>Vlb</td>
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<td>30</td>
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<tr>
<td>Vlc</td>
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<td>14</td>
</tr>
<tr>
<td>VIId</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td>Vie</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>VII</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>IX</td>
<td>20</td>
<td>27</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>32</td>
<td>34</td>
</tr>
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### TABLE 2: ANTIFUNGAL ACTIVITY OF SYNTHESIZED COMPOUNDS (IIIa-e, IVa-e, Va-e, VIa-e, VII & IX) & STANDARD [ZONE OF INHIBITION (MM)]

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Aspergillus niger</th>
<th>Candida albicans</th>
</tr>
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<tbody>
<tr>
<td>IIIa</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>IIIb</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
<td>IIIc</td>
<td>08</td>
<td>13</td>
</tr>
<tr>
<td>IIId</td>
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<td>30</td>
</tr>
<tr>
<td>IIle</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>Iva</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>IVb</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>IVc</td>
<td>22</td>
<td>19</td>
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<td>IVd</td>
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<td>IVe</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>Va</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>Vb</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Vc</td>
<td>19</td>
<td>20</td>
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<tr>
<td>Vd</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>Ve</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>VIa</td>
<td>18</td>
<td>06</td>
</tr>
<tr>
<td>VIb</td>
<td>25</td>
<td>10</td>
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<tr>
<td>VIc</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>VIId</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>VIe</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>VII</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>IX</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>Standard (Griseofulvin)</td>
<td>30</td>
<td>32</td>
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</tbody>
</table>
### TABLE 3: ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS (IIIa-e, IVa-e, Va-e, VIa-e, VII & IX) & STANDARD MIC (µg/ml).

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Minimum inhibitory concentrations (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. Subtilis</td>
</tr>
<tr>
<td>IIIa</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>IIIb</td>
<td>125</td>
</tr>
<tr>
<td>IIIc</td>
<td>62.5</td>
</tr>
<tr>
<td>IIId</td>
<td>62.5</td>
</tr>
<tr>
<td>IIIe</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>IVa</td>
<td>125</td>
</tr>
<tr>
<td>IVb</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>IVc</td>
<td>62.5</td>
</tr>
<tr>
<td>IVd</td>
<td>31.2</td>
</tr>
<tr>
<td>Ve</td>
<td>&gt; 500</td>
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<tr>
<td>Vb</td>
<td>15.6</td>
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<td>Vc</td>
<td>125</td>
</tr>
<tr>
<td>Vd</td>
<td>7.8</td>
</tr>
<tr>
<td>Ve</td>
<td>125</td>
</tr>
<tr>
<td>VIa</td>
<td>&gt; 1000</td>
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<tr>
<td>VIb</td>
<td>62.5</td>
</tr>
<tr>
<td>VIc</td>
<td>62.5</td>
</tr>
<tr>
<td>VIe</td>
<td>62.5</td>
</tr>
<tr>
<td>VII</td>
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<td>IX</td>
<td>250</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1.54</td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Minimum inhibitory concentrations (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>IIIa</td>
<td>250</td>
</tr>
<tr>
<td>IIIb</td>
<td>250</td>
</tr>
<tr>
<td>IIIc</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>IIId</td>
<td><strong>15.6</strong></td>
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<tr>
<td>IIIe</td>
<td>500</td>
</tr>
<tr>
<td>IVa</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>IVb</td>
<td><strong>62.5</strong></td>
</tr>
<tr>
<td>IVc</td>
<td>125</td>
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<tr>
<td>IVd</td>
<td><strong>7.8</strong></td>
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<tr>
<td>IVe</td>
<td>500</td>
</tr>
<tr>
<td>Ve</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>Vb</td>
<td>62.5</td>
</tr>
<tr>
<td>Vc</td>
<td>500</td>
</tr>
<tr>
<td>Vd</td>
<td>31.2</td>
</tr>
<tr>
<td>Ve</td>
<td>125</td>
</tr>
<tr>
<td>VIa</td>
<td>500</td>
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<tr>
<td>VIb</td>
<td>31.2</td>
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<tr>
<td>VIc</td>
<td><strong>15.6</strong></td>
</tr>
<tr>
<td>VIe</td>
<td>31.2</td>
</tr>
<tr>
<td>Vd</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>VII</td>
<td>125</td>
</tr>
<tr>
<td>IX</td>
<td>125</td>
</tr>
<tr>
<td>Standard (Griseofulvin)</td>
<td>3.41</td>
</tr>
</tbody>
</table>
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.
Results for anti-microbial activity: The results of cup plate method showed in Tables 1-2 and Fig.1 that all the synthesized compounds were showed potent to weak anti bacterial and antifungal activities. The compounds IIIId, 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 IIIId 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.coil 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.aureus 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, Vlb, VII & IX exhibit moderate antibacterial & antifungal activity against B.subtilis and P.aeruginosa and both the fungi the compounds IIIa, IVa, Va, Vla showed weak antibacterial and antifungal activity against different strains of bacteria and fungi. Compounds Va, Vla did not showed any activity against E.coli and P.aeruginosa respectively. The most active compounds IIIId, IVd, VId like N-(Cl-C6H5) pyrazolinone, & N-(Cl,C6H5) 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 & 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.coli 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 VIb, 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,
vila 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, VId showed very good antifungal activity against C.albicans with MIC value of 7.8 µg/ml.

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REFERENCES:


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