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SYNTHESIS AND CHARACTERIZATION OF *N*-HYDROXY-7-(3-SUBSTITUTEDUREIDO)-4-(4-((4-(MORPHOLINOMETHYL)PHENYL)ETHYNYL)PHENYL)-1,8-NAPHTHYRIDINE-2-CARBOXAMIDES:A NOVEL CLASS OF POTENTIAL ANTIBACTERIAL, ANTIFUNGAL AND ANTHELMINTHIC AGENTS

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ABSTRACT
N-hydroxy-7-(3-substitutedureido)-4-(4-((4-(morpholinomethyl)phenyl)ethynyl) phenyl)-1,8-naphthyridine-2-carboxamides were synthesized by treating Methyl 7-(3-substitutedureido)-4-(4-((4-(morpholinomethyl)phenyl)ethynyl) phenyl)-1,8-naphthyridine-2-carboxylate with hydroxyl amine in ethanol/THF. These newly synthesized 1,8-naphthyridine derivatives were screened for their antibacterial, antifungal and anthelmintic activities against the respective strains. The results showed that introduction of electron donating groups in the ureido moiety results in a significant decrease of antimicrobial activity and an increase in anthelmintic activity.

KEYWORDS
1,8-Naphthyridine, minimum inhibitory concentration, broth dilution, antibacterial activity, antifungal activity, anthelmintic activity.

INTRODUCTION
1,8-naphthyridine and its derivatives have attracted considerable attention because the 1,8-naphthyridine skeleton is present in many compounds that have been isolated from natural substances with various biological activities. A good deal of importance has been given to 1,8-naphthyridine derivatives due to their wide use in medicinal chemistry and some of them possess anti-tuberculosis, anti-neoplastic, anti-diabetic, anti-fertility, anti-hypothyroid and antibacterial activity. 1,8-Naphthyridine derivatives[i-ix] are reported to possess a wide spectrum of biological activities such as diuretic[x], antimalarial[xi], anti-inflammatory[xii], antitumor[xiii], antihypertensive[xiv] and antibacterial[xv,xvi] activities. In view of these observations, it appeared of interest to synthesize some novel 1,8-naphthyridine derivatives and evaluate their antimicrobial activities.

EXPERIMENTAL

MATERIALS AND METHODS

All chemicals and reagents were obtained from Merck India Limited. Melting points were determined in open capillary tubes and were uncorrected (in degree Celsius). The infrared spectra of the compounds were recorded as KBr discs on FT-IR (Spectrum ONE) spectrometer manufactured by Perkin-Elmer. The ^{1}H NMR spectra were recorded on a JOEL (300 MHz) spectrometer using TMS as an internal standard (chemical shifts in δ). The Mass spectra were recorded on a mass spectrometer JOEL sx-102 (FAB). Nutrient broth and nutrient agar were obtained from Hi-Media Laboratories Limited, India. The standard bacterial and fungal strains were procured from National Centre for Cell Science (NCCS), Pune, India.

BIOLOGICAL ACTIVITY

Determination of Minimum Inhibitory Concentration

Medium: Nutrient Agar medium

Method (Broth Dilution Method)

Standardized Inoculums (matched to McFarland $BaSO_4$ standard) of suspension of organisms were prepared. A series of glass tubes containing different concentration of test compounds dissolved in DMSO and spiller in Nutrient Broth were incubated with one drop of inoculum and mixed gently by shaking the rack. Two growth control tubes were also prepared without the addition of test compound and its optical density was determined as follows. 0.1 mL of control was mixed with 0.9 mL of Sterile Saline and with 0.2 µL loop, an agar plate was inoculated. The control should contain 1×10^{-5} colony forming units/mL = 20 colonies. The tubes were incubated for 24 hours at 37ºC. The turbidity produced in each tube was recorded by UV/Visible spectrophotometer. The turbidity produced by the broth (without inoculum) was considered as 100% transparency. The minimum inhibitory concentration (MIC) was noted as the concentration of the test substance, which completely inhibits the growth of the microorganism i.e. 100% transparency.

All synthesized compounds were screened for antibacterial activity against *Staphylococcus aureus* NCCS 2079, *Bacillus cereus* NCCS 2106, *Bacillus subtilis*, *Escherichia coli* NCCS2065 and *Pseudomonas aeruginosa* NCCS 2200 and antifungal activity against *Aspergillus niger* NCCS 1196 and *Candida albicans* NCCS 2106. The activity of novel compounds was expressed in terms of minimum inhibitory concentration (MIC). Broth dilution method was used to determine the minimum inhibitory concentration of an antimicrobial agent. It can be seen from Table-1 that introduction of electron donating groups has significantly decreased antimicrobial activity.

Anthelmintic activity studies

Anthelmintic activity studies were performed on *Pheretimaposthuma*. The selection of *Pheretimaposthuma for the* anthelmintic studies is owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[xvii-xxi].

Procedure for anthelmintic activity studies

The investigation was performed on adult earthworm, *Pheretimaposthuma*, collected from moist soil and washed with double distilled water. The selection of *P. posthuma* for the anthelmintic studies is owing to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. The earthworms of 5-6 cm in length were used in present investigations. Test samples were prepared at the concentrations of 25 mg/mL in dimethylformamide and six worms i.e. *Pheretimaposthuma,* were placed in petri dish containing 50 mL of test solution. Piperazine citrate (10 mg/mL) was used as reference

standard. Determination of time of paralysis and time of death of the worm were done. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^{o}C) followed with fading away of their body colors. It can be seen from the Table-1 that greater the electron donating nature of the substituent groups, the greater will be the anthelmintic activity.

Table-1: Antimicrobial activity & anthelmintic activity of novel substituted 1,8-napthyridine derivatives (Scheme-1) (10a-g)

Compound	R	Minimum Inhibitory Concentration(MIC) in g/mL Antibacterial Activity				Antifungal Activity		Anthelmintic Activity(min) 25 mg/mL	
		Staphylococcus aureus NCCS 2079	Bacillus cereus NCCS 2106	*Escherichia coli* NCCS 2065	*Pseudomonas aeruginosa* NCCS 2200	*Aspergillus niger* NCCS 1196	*Candida albicans* NCCS 2106	Paralysis	Death
10a	Phenyl	10.82	21.44	15.86	17.58	15.70	15.00	21	47
10b	4-Methoxy phenyl	9.46	13.9	13.32	14.78	10.14	11.1	21	52
10c	4-Chloro phenyl	4.48	10.52	7.18	8.76	5.18	6.68	26	54
10d	4-Nitro phenyl	3.42	6.46	5.92	7.42	4.08	6.64	28	60
10e	Ethyl	3.52	6.85	6.04	7.82	4.21	6.74	21	48
10g	Allyl	6.76	12.48	8.30	8.32	10.48	9.12	26	50

SYNTHESIS PROCEDURES AND SPECTRAL CHARACTERIZATION DATA OF COMPOUNDS

Synthesis of N-(6-aminopyridin-2-yl)acetamide (2)

To a solution of 2,6-diaminopyridine (25 g, 7.222 mmol) in THF (500 mL), cooled to 0°C was added triethylamine (22.9 g, 227.2 mmol) followed by acetyl chloride (24.7 mL, 227.2 mmol) over a period of 30 min and stirred at 0°C for 4 h. The reaction mixture was quenched with ice and extracted with (200 mL x 2) ethylacetate. The combined organic layer was washed with water (100 mL), brine (100 mL), dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to obtain **N-(6-aminopyridin-2-yl) acetamide (2).**

Grey solid, yield 64%, m.p. observed 158-160^{o}C (Literature value 158-162^{o}C).

IR (KBr)ν_{max} : 3340, 1454 and 1600 cm^{-1} due to NH of primary amine, -C=N of pyridine and aromatic -C=C stretch respectively.

^{1}H NMR (DMSO-d_6): δ 9.14 (s br, 1H,-N**H**-CO), 7.53-7.32 (m, 3H, Pyridine-**H**), 5.75 (s br, 2H, N**H**$_2$), 2.05 (s, 3H, COC**H**$_3$) ppm.

Synthesis of dimethyl 2-((6-acetamidopyridin-2-yl)amino) maleate (3)

To a stirred solution of N-(6-aminopyridin-2-yl)acetamide (30 g, 197.3 mmol) in methanol (600 mL) was added dimethyl acetylene dicarboxylate (26.5 mL, 217.1mmol) and heated at reflux for 15 h. The solvent was evaporated up to minimum and crude compound was

recrystallized in methanol to obtain **dimethyl 2-((6-acetamidopyridin-2-yl)amino)maleate (3).**

Yellow crystalline solid, yield 60%, m.p. 172-174 ^{o}C.

IR (KBr)ν_{max} : 3340, 1450, 1630, 1742, 1180, 1650 cm^{-1} due to amide N-H, Py –C=N, C=C of conjugated alkene, C=O of ester, sp^3 C-O of ester and amide C=O stretch respectively.

^{1}H NMR (DMSO-d$_6$): δ 9.14 (s br, 1H, CON**H**), 7.67-7.50 (d, 2H, Pyridine-**H**), 6.34 (d, 1H, Pyridine-**H**), 6.50 (s, 1H, =C**H**), 4.04 (s, 1H, N**H**), 3.78 (s, 6H, OC**H**$_3$), 2.05 (s, 3H, COC**H**$_3$) ppm.

Synthesis of methyl-7-acetamido-4-oxo-1,4-dihydro-1,8-naphthyridine-2-carboxylate (4)

A solution of Dimethyl 2-((6-acetamidopyridin-2-yl)amino) maleate (5.0g, 17.06 mmol) in dowtherm (100 mL) was heated at 180-190°C for 2 h. The reaction mixture was cooled to room temperature and diluted with pet ether, stirred for 15 min, filtered the precipitated solid, washed with pet ether to obtain the crude compound. The crude compound was purified by column chromatography (silica gel, 100- 200 mesh) using 3% methanol in chloroform as mobile phase to obtain **methyl 7-acetamido-4-oxo-1,4-dihydro-1,8-naphthyridine-2-carboxylate (4)**.

Yellow brown solid, yield 34%, m.p. 164-168 ^{o}C.

IR (KBr)ν_{max} : 3340, 1452, 1633, 1745, 1185, 1630, 1685 cm^{-1}, due to amide N-H, Py – C=N, C=C of conjugated alkene, C=O of ester, sp^3 C-O of ester, amide -C=O and conjugated –C=O stretch respectively.

^{1}H NMR (DMSO-d$_6$): δ 9.14 (bs, 1H, CON**H**), 7.65-7.90 (m, 2H, Pyridine-**H**), 4.09 (s, 1H, N**H**), 6.35 (s, 1H, =C**H**), 3.72 (s, 3H, OC**H**$_3$), 2.05 (s, 3H, COC**H**$_3$) ppm.

Synthesis of methyl-7-acetamido-4-bromo-1,8-naphthyridine-2-carboxylate (5)

To a suspension of **methyl 7-acetamido-4-oxo-1,4-dihydro-1,8-naphthyridine-2-carboxylate** (1.5 g, 5.72 mmol) in 1,2- dichloroethane (30 mL) at 0°C was added $POBr_3$ (4.92 g, 17.17 mmol). The reaction mixture was brought to room temperature and heated at reflux for 4 h. The reaction mixture was cooled to room temperature, added crushed ice, stirred for 5 min, basified with solid $NaHCO_3$ and extracted with dichloromethane (150 mL x 2). The combined organic layer was washed with water (100 mL), brine (50 mL), dried over anhydrous Na_2SO_4, evaporated to dryness, dried under vaccum to obtain **methyl-7-acetamido-4-bromo-1,8-naphthyridine-2-carboxylate (5)**.

Yellow brown solid, yield 41%, m.p. 182-184 ^{o}C.

IR (KBr)ν_{max}: 3340, 1452, 1745, 1185, 1630, 1080 cm^{-1} due to amide N-H, Pyridine C=N, , C=O of ester, sp^3 C-O of ester, amide -C=O and Ar-Br stretch respectively.

^{1}H NMR (DMSO-d$_6$): δ 9.14 (s br, 1H, CON**H**), 8.40-8.57 (m, 2H, Pyridine-**H**), 8.75 (d, 1H, Pyridine-**H**), 3.90 (s, 3H, OC**H**$_3$), 2.05 (s, 3H, COC**H**$_3$) ppm.

Synthesis of intermediate: (a)4-((trimethylsilyl) ethynyl) benzaldehyde (12)

To a degassed solution of 4-bromobenzaldehyde (50.0 g, 270.2 mmol) and triphenylphosphine (2.832 g, 10.1 mmol) in 500 mL of anhydrous triethylamine was added ethynyltrimethylsilane (39.43 g, 486.3 mmol) followed by palladium acetate (1.212 g, 5.40 mmol) at room temperature under argon atmosphere. The reaction mixture was heated at reflux for 2 h in sealed tube. The reaction mass was cooled to room temperature and the precipitated solid was filtered. The filtrate was concentrated to get crude compound. The crude compound was purified by column chromatography (silica gel, 100-200 mesh) using1% ethylacetate in pet-ether as mobile phase to get **4-((trimethylsilyl)ethynyl)benzaldehyde(12)**.

Brown solid, yield 91%, m.p. observed 70-72^{o}C (Literature value 66-70^{o}C).

IR (KBr)ν_{max} : 2200, 1600, 1685, 2980 cm^{-1} due to alkyne C≡C, Ar C=C, conjugated aldehyde C=O and alkane C-H stretch respectively.

^{1}H NMR (DMSO-d_6): δ 9.82 (s, 1H, C**H**O), 7.85 (d, 2H, Ar-**H**), 7.73 (d, 2H, Ar-**H**), 0.10 (s,9H, (C**H**$_3$)$_3$) ppm.

(b) Synthesis of 4-ethynylbenzaldehyde (13)

To a solution of **4-((trimethylsilyl)ethynyl) benzaldehyde (12)** (50.0 g, 247.00 mmol) in methanol (500 mL) was added potassium carbonate (3.415 g, 24.47 mmol) at room temperature and the reaction mixture was stirred for 4 h. The solvent was removed under reduced pressure and the residue was diluted with dichloromethane (200 mL). The organic solution was washed with water (150 mL), brine solution (100 mL), dried over anhydrous magnesium sulfate and evaporated under reduced pressure to get **4-ethynylbenzaldehyde (13)**. Light brown solid, yield 94%, m.p. 90-92ºC.

IR (KBr)ν_{max} : 2200, 3300, 1600, 1685 cm^{-1} due to Alkyne C≡C, alkyne C-H, Ar C=C and conjugated aldehyde C=O stretch respectively.

^{1}H NMR (DMSO-d_6): δ 9.84 (s, 1H, CHO), 7.88 (m, 2H, Ar-H), 7.75 (d, 2H, Ar-H), 4.03 (s,1H, alkyne CH) ppm.

(c) Synthesis of 2-(4-iodophenyl)-5, 5-dimethyl-1, 3, 2-dioxaborinane (14)

To a solution of compound 4-iodophenylboronic acid (50.0 g, 202.17 mmol) in toluene (500 mL) was added 2,2-dimethyl-1,3-propanediol (23.1 g, 221.17 mmol) at room temperature. The reaction mixture was heated at reflux temperature for 18 h (**azeotroped)**. The solvent was evaporated under reduced pressure and the residue was washed with pentane to get **2-(4-iodophenyl)-5, 5-dimethyl-1, 3, 2-dioxaborinane (14)**.

Off-white solid, yield 86%, m.p. 130-132ºC.

IR (KBr)ν_{max} : 2980, 1600, 1100 cm^{-1} due to alkane C-H, Ar C=C and Ar-I stretch respectively.

^{1}H NMR (DMSO-d_6): δ 7.76 (d, 2H, Ar-**H**), 7.50 (d, 2H, Ar-**H**), 3.76 (s, 4H, C**H**$_2$), 0.97 (s,6H, C**H**$_3$) ppm.

(d)Synthesis of 4-((4-(5, 5-dimethyl-1, 3, 2-dioxaborinan-2-yl)phenyl)ethynyl) benzaldehyde (15)

To a argon purged solution of **2-(4-iodophenyl)-5,5-dimethyl-1,3,2-dioxaborinane**(50.0 g, 158.22 mmol) in acetonitrile (600 mL) was added Pd(PPh$_3$)$_2$Cl$_2$ (2.217 g, 3.164 mmol), CuI (0.300 g, 1.582 mmol) followed by triethylamine (66.49 mL, 474.2 mmol). The argon atmosphere was replaced by argon/hydrogen (~1:1). To this solution was added a solution of 4-ethynylbenzaldehyde (30.85 g, 237.33 mmol) in acetonitrile (100 mL) under argon/hydrogen atmosphere at room temperature. The reaction mixture was heated at 75ºC for 4 h. The solvent was evaporated under reduced pressure and the residue was diluted with water and filtered the precipitated solid, washed with water and dried under vacuum to get the crude compound. The crude compound was treated with charcoal in ethylacetate, filtered hot through a pad celite and the filtrate was evaporated under reduced pressure to obtain **4-((4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl)ethynyl)benzaldehyde (15)**.

Yellow solid, yield 99%, m.p. 132-134ºC.

IR (KBr)ν_{max} : 2980, 1600, 2200, 1685 cm^{-1} due to alkane C-H, Ar C=C, alkyne C≡C and C=O stretch respectively.

^{1}H NMR (DMSO-d_6): δ 9.86 (s, 1H, CHO), 7.20 (d, 2H, Ar-**H**), 7.45 (d, 2H, Ar-**H**), 7.31 (d,2H, Ar-**H**), 7.70 (d, 2H, Ar-**H**), 3.75 (t, 4H, O-C**H**$_2$), 1.02 (s, 6H, C**H**$_3$) ppm.

Synthesis of 4-(4-{[4-(5, 5-dimethyl-1, 3, 2-dioxa borinan-2-yl) phenyl] ethynyl} benzyl) morpholine(6)

To a solution of 4-((4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl)ethynyl) benzaldehyde (15) (10.0 g, 31.44 mmol) in 1,2-dichloroethane was added morpholine (8.218 g, 94.33 mmol) followed by acetic acid (0.180 mL, 3.14 mmol) and NaBH(OAc)$_3$ (16.655 g, 78.66 mmol) at 0°C. The reaction mixture was stirred at room temperature for 4 h. The reaction

mass was then quenched with ice cold water and extracted with chloroform (200 mL x 2). The combined organic layer was washed with water (100 mL x 2), brine solution (50 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get crude compound. The crude compound was purified by triturating with chilled methanol (10.00 mL) for ~10 min. and filtered to get **4-(4-{[4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl]ethynyl}benzyl)morpholine (6)** (**Scheme 2**).

Light yellow solid, yield 65%, m.p. 138-140ºC.

IR (KBr)ν_{max} : 2980, 1600, 2200, 1050, 1300 cm^{-1} due to alkane C-H, Ar C=C, Alkyne C≡C, C-O-C of cyclic ether and alkyl –C-N stretch respectively.

^{1}H NMR (DMSO-d_6): δ 7.17 (d, 2H, Ar-**H**), 7.45 (d, 2H, Ar-**H**), 7.33 (d, 2H, Ar-**H**), 7.70 (d,2H, Ar-**H**), 3.55 (s, 2H, N-C**H**$_2$), 3.68 (t, 4H, O-C**H**$_2$), 2.48 (t, 4H, N-C**H**$_2$ of ring), 3.76 (s,4H, OC**H**$_2$), 1.02 (s, 6H, C**H**$_3$) ppm.

Synthesis of methyl 7-acetamido-4-(4-(2-(4-(morpholinomethyl)phenyl)ethynyl)phenyl)-1,8-naphthyridine-2-carboxylate (7)

To a degassed solution of Methyl-7-acetamido-4-bromo-1,8-naphthyridine-2-carboxylate (5) (0.75 g, 0.6172 mmol) in 1,4-dioxane (20 mL) was added 4-(4-(2-(4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl)ethynyl)benzyl)morpholine**6**(0.9 g, 2.31 mmol), Pd (dppf)Cl_2 (0.17 g, 0.23 mmol) followed by KOAc (0.68 g, 6.94 mmol) and purged the reaction mixture with argon for 10 min. The reaction mixture was then heated at 80°C for 4 h. The solvent was evaporated under reduced pressure to get the crude compound. The crude compound was purified by column chromatography (silica gel, 100- 200 mesh) using 3% methanol in chloroform as mobile phase to obtain **methyl 7-acetamido-4-(4-(2-(4-(morpholinomethyl)phenyl)ethynyl)phenyl)-1,8-naphthyridine-2-carboxylate (7).**

Brown solid, yield 51%, m.p. 142-144ºC.

IR (KBr)ν_{max}: 3340, 1650, 1454, 1740, 1140, 1600, 2200, 1030, 1245, 2980 cm^{-1} due to amide N-H, amide -C=O, Py C=N, C=O of ester, sp^3 C-O of ester, Ar C=C, alkyne C≡C, C-O-C of cyclic ether, alkyl –C-N and alkane C-H stretch respectively.

^{1}H NMR (DMSO-d_6): δ 9.80 (s br, 1H, CONH), 8.26 (d, 1H, Naphthyl-H), 8.46 (d, 1H, Naphthyl-H), 8.59 (s, 1H, Naphthyl-**H**), 7.64 (d, 2H, Ar-**H**), 7.74 (d, 2H, Ar-**H**), 7.42 (d, 2H, Ar-**H**), 7.22 (d, 2H, Ar-**H**), 3.85 (s, 3H, COOC**H**$_3$), 2.35 (t, 4H, N-C**H**$_2$ of ring), 3.82 (m, 4H, O-C**H**$_2$), 3.54 (s, 2H, N-C**H**$_2$), 2.16 (s, 3 H, COC**H**$_3$) ppm.

Synthesis of methyl-7-amino-4-(4-((4-(morpholinomethyl)phenyl)ethynyl)phenyl)-1,8-naphthyridine-2-carboxylate (8)

To a suspension of Methyl-7-acetamido-4-(4-((4-morpholinomethyl) phenyl) ethynyl)phenyl)-1,8-naphthyridine-2-carboxylate (7) (100 mg, 0.19 mmol) in methanol (2.0 mL) was added thionyl chloride (114 mg, 0.96 mmol) and heated to reflux for 1 h. The solvent was evaporated under reduced pressure and added crushed ice, diluted with water (10 mL) and extracted with dichloromethane (25 mL x 2). The combined organic layer was washed with saturated $NaHCO_3$ (5 mL), brine (5 mL), dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum to obtain **methyl-7-amino-4-(4-((4-(morpholinomethyl) phenyl)ethynyl)phenyl) -1,8-naphthyridine-2-carboxylate (8)**.

Brown solid, yield 60%, m.p. 146-148ºC.

IR (KBr)ν_{max} : 3400, 1445, 1745, 1160, 1602, 2200, 1035, 1255, 2985 cm^{-1} due to 1° N-H, pyridine –C=N, C=O of ester, sp^3 C-O of ester, Ar C=C, alkyne C≡C, C-O-C of cyclic ether, alkyl –C-N and alkane C-H stretch respectively.

^{1}H NMR (DMSO-d_6): δ 6.72 (d, 1H, Naphthyl-H), 7.88 (d, 1H, Naphthyl-H), 8.69 (s, 1H, Naphthyl-**H**), 7.54 (d, 4H, Ar-**H**), 7.68 (d, 2H, Ar-**H**), 7.29 (s br, 2H, NH_2), 7.22 (d, 2H, Ar-**H**), 3.82 (s, 3H, COOC**H**$_3$), 2.30 (t, 4H, N-C**H**$_2$ of ring), 3.80 (t, 4H, O-C**H**$_2$ of ring), 3.56 (s, 2H, N-C**H**$_2$) ppm.

Synthesis of methyl 4-(4-((4-(morpholinomethyl) phenyl) ethynyl)phenyl)-7-(3-phenylureido)-1,8-naphthyridine-2-carboxylate (9a)

To a solution of Methyl-7-amino-4-(4-((4-(morpholinomethyl) phenyl) ethynyl)phenyl)-1,8-naphthyridine-2-carboxylate (8) (0.2 g, 0.42 mmol) in dry dimethyformamide (4.0 mL) was added diisopropylisocyanate (71.22 mg, 0.84 mmol), dibutyltindiacetate (0.1 mL) and pyridine (67 mg, 0.84 mmol). The reaction mixture was irradiated at 70°C in microwave for 30 min. The reaction mixture was cooled to room temperature, stirred for 5 min, filtered the precipitated solid, washed with chilled water and dried under vacuum to get the crude product. The crude compound was purified by column chromatography (silica gel, 100-200 mesh) using 3% methanol in chloroform as mobile phase to obtain **methyl 4-(4-((4-(morpholinomethyl) phenyl) ethynyl)phenyl)-7-(3-phenylureido)-1,8-naphthyridine-2-carboxylate (9a). C**ompounds 9b-9g were prepared on the same lines.

Yellow brown solid, yield 51%, m.p. 154-156ºC.

IR (KBr)ν_{max} : 3340, 1450, 1730, 1181, 1560, 2155, 2925, 1042, 1206 cm^{-1} due to amide N-H, pyridine C=N, C=O of ester, sp^3 C-O of ester, Ar C=C, alkyne C≡C, alkane C-H, C-O-C of cyclic ether and alkyl C-N respectively.

^{1}H NMR (DMSO-d_6): δ 9.01 (m, 2H, CONH), 6.73 (d, 1H, Naphthyl-H), 7.90 (d, 1H, Naphthyl-H), 8.68 (s, 1H, Naphthyl-H), 7.56 (d, 2H, Ar-H), 7.70 (d, 2H, Ar-H), 7.38 (d, 2H, Ar-H), 7.12 (d, 2H, Ar-H), 3.84 (s, 3H, $COOCH_3$), 2.32 (t, 4H, N-CH_2), 3.84 (t, 4H, O-CH_2), 3.56 (s, 2H, N-CH_2), 7.60 (d, 2H, Ar-H), 7.49 (d, 2H, Ar-H), 7.21(t, 1H, Ar-H) ppm.

MS m/*z*: found 598 $(M+H)^+$; calcd. 597. Anal. $C_{36}H_{31}N_5O_4$.

Synthesis of N-hydroxy-4-(4-((4-(morpholinomethyl)phenyl)ethynyl) phenyl)-7-(3-phenylureido)-1,8-naphthyridine-2-carboxamide (10a)

To a solution of Methyl 4-(4-((4-(morpholinomethyl) phenyl) ethynyl)phenyl)-7-(3-phenylureido)-1,8-naphthyridine-2-carboxylate (9a) (100 mg, 0.17 mmol) in 2 mL Methanol/THF (1:1) was added aqueous 50% hydroxylamine (1.0 mL) followed by catalytic amount of KCN (~2 mg) and the resulting solution was stirred at room temperature for 4 h. The reaction mixture was cooled to 0°C and quenched with 10% aqueous citric acid solution (1 mL), stirred for 15 min, filtered, the precipitated solid was washed with water and dried under vacuum to obtain **N-hydroxy-4-(4-((4-(morpholinomethyl)phenyl) ethynyl) phenyl)-7-(3-phenylureido)-1,8-naphthyridine-2-carboxamide (10a).** Compounds 10b -10g were prepared on the same lines (**Scheme 1**).

Pale yellow solid, yield 28%, m.p. 152-154ºC.

IR (KBr)ν_{max} :3250, 1665, 1452, 1610, 2155, 2925, 1042, 1231, 3610 cm^{-1} due to amide N-H, amide C=O, pyridine C=N, Ar C=C, alkyne C≡C, alkane C-H, C-O-C of cyclic ether, alkyl C-N and O-H stretch respectively.

^{1}H NMR (DMSO-d_6): δ 8.01 (s br, 2H, CONH), 6.75 (d, 1H, Naphthyl-H), 7.92 (d, 1H, Naphthyl-H), 8.69 (s, 1H, Naphthyl-H), 7.58 (d, 2H, Ar-H), 7.69 (d, 2H, Ar-H), 7.42 (d, 2H, Ar-H), 7.15 (d, 2H, Ar-H), 8.50 (s br, 1H, N**H**-OH), 2.16 (sb, 1H, NH-O**H**), 2.35 (t, 4H, N-CH_2), 3.82 (t, 4H, O-CH_2), 3.56 (s, 2H, N-CH_2), 7.60 (d, 2H, Ar-H), 7.48 (d, 2H, Ar-H), 7.22 (t, 1H, Ar-H) ppm.

^{13}C NMR (DMSO-d_6): δ 65.2(2), 55.8(2), 64.5, 137.8, 128.1(2), 131.8(2), 120.6, 89.2(2),122.9, 132.1(2), 127.0(2), 140.4, 152.0, 114.8, 150.8, 155.4, 156.2, 111.2, 128.6, 121.3,159.2, 150.5, 138.1, 120.2(2), 129.3(2), 127.6 ppm.

MS m/*z*: found 599 $(M+H)^+$; calcd. 598. Anal. $C_{35}H_{30}N_6O_4$.

N-hydroxy-7-(3-(4-methoxyphenyl)ureido)-4-(4-((4-(morpholinomethyl)phenyl) ethynyl) phenyl)-1,8-naphthyridine-2-carboxamide (10b)

Yield 68%, m.p. 163-165ºC.

IR (KBr)ν_{max}: 3612, 2929, 1454 cm^{-1} due to OH, alkane C-H, pyridine C=N stretch respectively.
^{1}H NMR (DMSO-d_6): δ 8.20 (sb, 2H, CONH), 6.79 (d, 1H, Naphthyl-H), 8.12 (d, 1H, Naphthyl-H), 8.75 (s, 1H, Naphthyl-H), 7.65 (d, 2H, Ar-H), 7.75 (d, 2H, Ar-H), 7.49 (d, 2H, Ar-H), 7.22 (d, 2H, Ar-H), 8.55 (sb, 1H, N**H**-OH), 2.20 (sb, 1H, NH-O**H**), 2.52 (t, 4H, N-CH_2), 3.67 (t, 4H, O-CH_2), 3.53 (s, 2H, N-CH_2), 7.54 (d, 2H, Ar-H), 7.12 (d, 2H, Ar-H), 3.86 (s, 3H, Ar-O-CH_3) ppm.
MS m/z: found 630 $(M+H)^+$; calcd. 629. Anal. $C_{36}H_{32}N_6O_5$. Found C 68.58 (68.78), H 4.97 (5.13), N 13.16 (13.37).

7-(3-(4-chlorophenyl)ureido)-N-hydroxy-4-(4-((4-(morpholinomethyl)phenyl)ethynyl) phenyl)-1,8-naphthyridine-2-carboxamide (10c)

Yield 73%, m.p. 149-151ºC.
IR (KBr)ν_{max}: 3615, 2930, 1456 cm^{-1} due to OH, alkane C-H, pyridine C=N stretch respectively.
^{1}H NMR (DMSO-d_6): δ 8.19 (sb, 2H, CONH), 6.78 (d, 1H, Naphthyl-H), 8.11 (d, 1H, Naphthyl-H), 8.74 (s, 1H, Naphthyl-H), 7.63 (d, 2H, Ar-H), 7.72 (d, 2H, Ar-H), 7.49 (d, 2H, Ar-H), 7.22 (d, 2H, Ar-H), 8.51 (sb, 1H, N**H**-OH), 2.18 (s br, 1H, NH-O**H**), 2.58 (t, 4H, N-CH_2), 3.60 (t, 4H, O-CH_2), 3.50 (s, 2H, N-CH_2), 7.75 (d, 2H, Ar-H), 7.47 (d, 2H, Ar-H) ppm.
MS m/z: found 634 $(M+H)^+$; calcd. 633. Anal. $C_{35}H_{29}N_6O_4Cl$. Found C 66.20 (66.40), H 4.41 (4.62), N 13.40 (13.27).

N-hydroxy-4-(4-((4-(morpholinomethyl)phenyl)ethynyl)phenyl)-7-(3-(4-nitrophenyl) ureido)-1,8-naphthyridine-2-carboxamide (10d)

Yield 63%, m.p. 154-156ºC.
IR (KBr)ν_{max}: 3617, 2975, 1458 cm^{-1} due to OH, alkane C-H, pyridine C=N stretch respectively.
^{1}H NMR (DMSO-d_6): δ 7.80 (sb, 2H, CONH), 6.72 (d, 1H, Naphthyl-H), 8.10 (d, 1H, Naphthyl-H), 8.70 (s, 1H, Naphthyl-H), 7.60 (d, 2H, Ar-H), 7.70 (d, 2H, Ar-H), 7.50 (d, 2H, Ar-H), 7.20 (d, 2H, Ar-H), 8.10 (s br, 1H, N**H**-OH), 2.18 (sb, 1H, NH-O**H**), 2.48 (t, 4H, N-CH_2), 3.58 (t, 4H, O-CH_2), 3.48 (s, 2H, N-CH_2), 7.80 (d, 2H, Ar-H), 8.25 (d, 2H, Ar-H) ppm.
MS m/z: found 645 $(M+H)^+$; calcd. 644. Anal. $C_{35}H_{29}N_7O_6$. Found C 65.11 (65.31), H 4.33 (4.54), N 15.03 (15.23).

7-(3-(4-ethylphenyl)ureido)-N-hydroxy-4-(4-((4-(morpholinomethyl)phenyl)ethynyl) phenyl)-1,8-naphthyridine-2-carboxamide (10e)

Yield 76%, m.p. 140-142ºC.
IR (KBr)ν_{max} : 3610, 2925, 1455 cm^{-1}
^{1}H NMR (DMSO-d_6): δ 7.00 (s br, 2H, CONH), 6.52(d, 1H, Naphthyl-H), 8.00 (d, 1H, Naphthyl-H), 8.50 (s, 1H, Naphthyl-H), 7.40 (d, 2H, Ar-H), 7.60 (d, 2H, Ar-H), 7.50 (d, 2H, Ar-H), 7.20 (d, 2H, Ar-H), 8.51 (s br, 1H, N**H**-OH), 2.16 (s br, 1H, NH-O**H**), 2.45 (t, 4H, N-CH_2), 3.58 (t, 4H, O-CH_2), 3.48 (s, 2H, N-CH_2), 3.20 (q, 2H, N-C**H**$_2$-CH_3), 1.12 (t, 3H, N-CH_2-C**H**$_3$) ppm.
MS m/z: found 552 $(M+H)^+$; calcd. 551. Anal. $C_{31}H_{30}N_6O_4$. Found C 67.42 (67.62), H 5.28 (5.49), N 15.06 (15.26).

N-hydroxy-7-(3-(4-isopropylphenyl)ureido)-4-(4-((4-morpholinomethyl)phenyl)ethynyl) phenyl)-1,8-naphthyridine-2-carboxamide (10f)

Yield 62%, m.p. 133-135ºC.
IR (KBr)ν_{max}: 3612, 2928, 1454 cm^{-1} due to OH, alkane C-H, pyridine C=N stretch respectively.
^{1}H NMR (DMSO-d_6): δ 7.00 (s br, 2H, CONH), 6.50 (d, 1H, Naphthyl-H), 8.05 (d, 1H, Naphthyl-H), 8.51 (s, 1H, Naphthyl-H), 7.42 (d, 2H, Ar-H), 7.62 (d, 2H, Ar-H), 7.52 (d, 2H,

Ar-H), 7.22 (d, 2H, Ar-H), 8.20 (s br, 1H, N**H**-OH), 2.16 (s br, 1H, NH-O**H**), 2.45 (t, 4H, N-CH_2), 3.58 (t, 4H, O-CH_2), 3.48 (s, 2H, N-CH_2), 1.24 (d, 6H, isopropyl terminal -CH), 4.18 (m, 1H, isopropyl -CH) ppm.

MS m/z: found 566 $(M+H)^+$; calcd. 565. Anal. $C_{32}H_{32}N_6O_4$. Found C 67.82 (68.07), H 5.50 (5.71), N 14.68 (14.88).

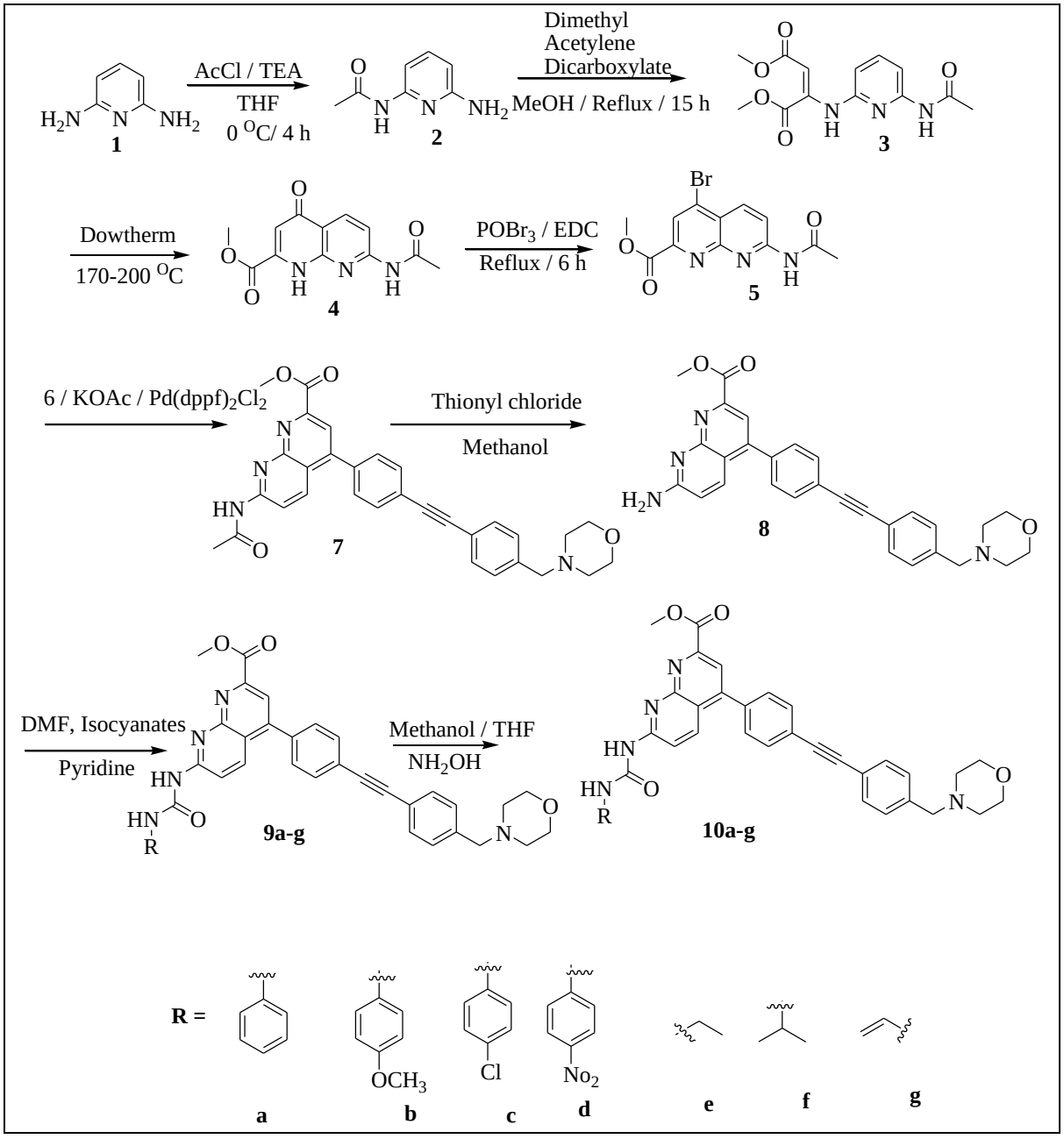
7-(3-allylureido)-*N*-hydroxy-4-(4-((4-(morpholinomethyl)phenyl)ethynyl)phenyl)-1,8-naphthyridine-2-carboxamide (10g)

Yield 69%, m.p. 138-140ºC.

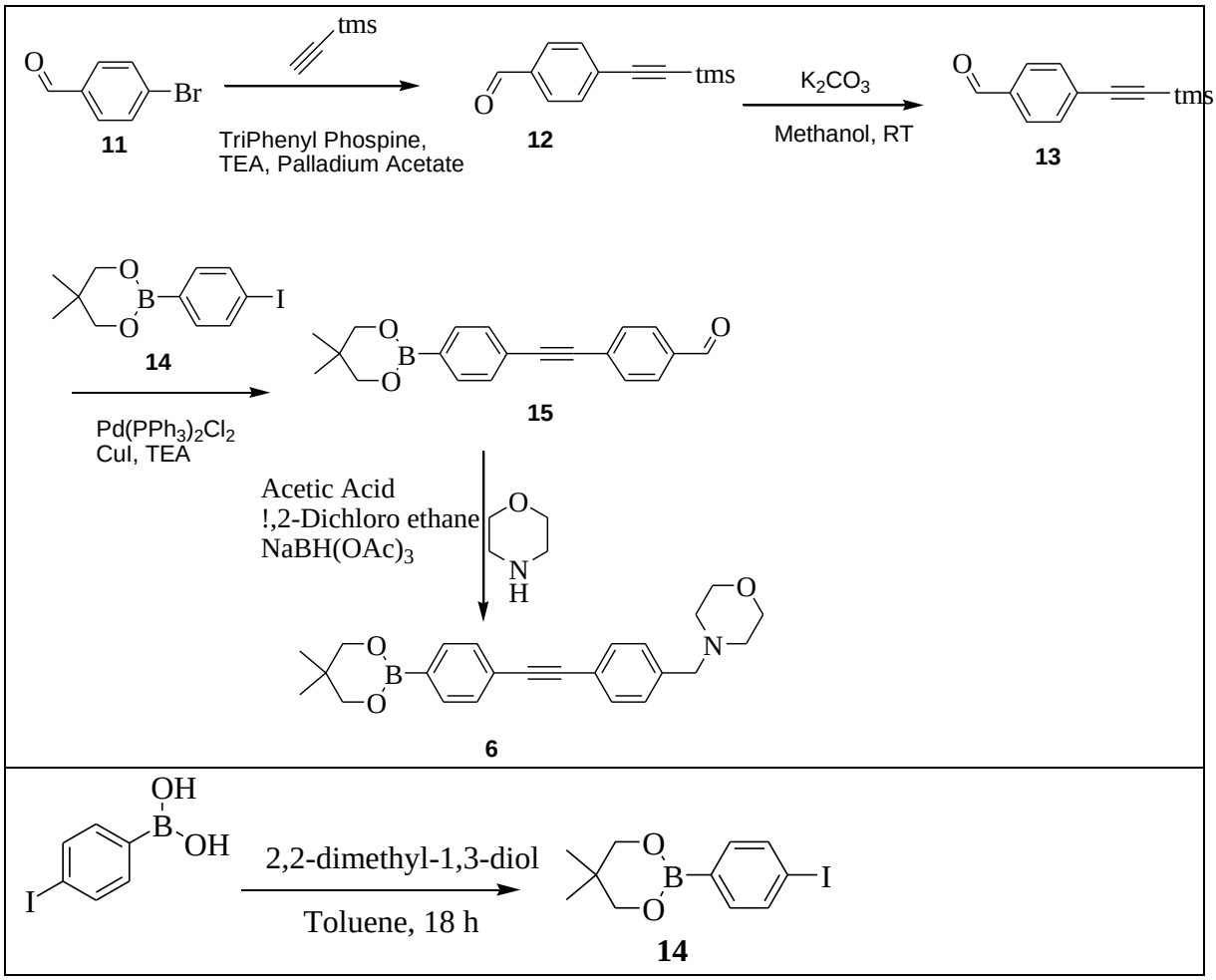
IR (KBr)ν_{max}: 3615, 2924, 1452 cm^{-1} due to OH, alkane C-H, pyridine C=N stretch respectively.

^{1}H NMR (DMSO-d_6): δ 7.00 (s br, 2H, CONH), 6.50 (d, 1H, Naphthyl-H), 8.05 (d, 1H, Naphthyl-H), 8.51 (s, 1H, Naphthyl-H), 7.42 (d, 2H, Ar-H), 7.62 (d, 2H, Ar-H), 7.52 (d, 2H, Ar-H), 7.22 (d, 2H, Ar-H), 8.52 (s br, 1H, N**H**-OH), 2.20 (s br, 1H, NH-O**H**), 2.45 (t, 4H, N-CH_2), 3.58 (t, 4H, O-CH_2), 3.48 (s, 2H, N-CH_2), 4.16 (d, 2H, allyl –CH_2), 5.90 (m, 1H, allyl =CH), 5.15 (d, 1H, allyl terminal –CH_2), 5.20 (d,1H, allyl terminal =CH_2) ppm.

MS m/z: found 564 $(M+H)^+$; calcd. 563. Anal. $C_{32}H_{30}N_6O_4$. Found C 68.11 (68.31), H 5.17 (5.37), N 14.74 (14.94).



Scheme-1: Synthesis of Compounds **10a-g**



Scheme-2: Intermediate Preparation

RESULTS AND DISCUSSION

A solution of 2,6-diaminopyridine in THF, at 0°C was treated with triethylamine followed by acetyl chloride and stirred at 0°C for 4 h to obtain **N-(6-aminopyridin-2-yl)acetamide (2)** as a grey solid. The reaction that results in monoacylation was confirmed by the appearance of signals for NH and NH_2 protons at δ 9.14 and 5.75 respectively along with a signal for CH_3 protons at 2.05 in ^{1}H NMR.

A solution of N-(6-aminopyridin-2-yl)acetamide and dimethylacetylene dicarboxylate on heating in methanol at reflux for about 15 h results in **Dimethyl 2-((6-acetamidopyridin-2-yl)amino) maleate (3)** as yellow crystalline solid whose structure was confirmed by the spectral analysis. The disappearance of signal for NH_2 group of compound (2) in proton NMR compound (3) confirms its formation.

A solution of Dimethyl 2-((6-acetamidopyridin-2-yl)amino) maleate (3) in dowtherm was heated at 180-190°C for about 2 h to obtain **methyl-7-acetamido-4-oxo-1,4-dihydro-1,8-naphthyridine-2-carboxylate (4)** (34%) as yellow brown solid.

When a suspension of Methyl-7-acetamido-4-oxo-1,4-dihydro-1,8-naphthyridine-2-carboxylate (4) in 1,2- dichloroethane at 0°C was treated with $POBr_3$ and heated at reflux for 4 h. **methyl-7-acetamido-4-bromo-1,8-naphthyridine-2-carboxylate (5)** (40.5%) was obtained as yellow brown solid. The structure of compound (5) was confirmed by ^{1}H NMR and IR spectral analysis. The occurrence of an absorption band for C-Br stretching at 1080 cm^{-1} in IR spectrum confirms the successful of bromination of compound (5).

The intermediate, **4-(4-{[4-(5, 5-dimethyl-1, 3, 2-dioxa borinan-2-yl) phenyl] ethynyl} benzyl) morpholine (6),** was prepared starting from 4-bromobenzaldehyde. To a solution of 4-bromobezaldehyde and triphenylphosphine in anhydrous triethylamine was added ethynyltrimethylsilane followed by palladium acetate at room temperature under argon atmosphere to get 4-((trimethylsilyl)ethynyl)benzaldehyde (12) (91%) as a brown solid. 4-((trimethylsilyl)ethynyl)benzaldehyde (12) when stirred with potassium carbonate in methanol gave 4-ethynylbenzaldehyde (13) (93.7%) as light brown solid. To an argon purged solution of 2-(4-iodophenyl)-5,5-dimethyl-1,3,2-dioxaborinane (14) in acetonitrile was added $Pd(PPh_3)_2Cl_2$, CuI followed by triethylamine. The argon atmosphere was replaced by argon/hydrogen. To this solution was added a solution of 4-ethynylbenzaldehyde (13) in acetonitrile. The reaction mixture was heated at 75^oC for 4 h and the solvent evaporated under reduced pressure and to obtain 4-((4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl)ethynyl)benzaldehyde (15) (99%) as a yellow solid after purification. A solution of 4-((4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl)ethynyl)benzaldehyde (15) in 1,2-dichloroethane was stirred with morpholine, acetic acid and $NaBH(OAc)_3$ to get **4-(4-{[4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl]ethynyl}benzyl) morpholine (6)** (65.41%)as light yellow solid.

To a solution of Methyl-7-acetamido-4-bromo-1,8-naphthyridine-2-carboxylate (5) in 1,4 dioxane was added 4-(4-((4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl)ethynyl) benzyl)morpholine (6), $Pd(dppf)Cl_2$ followed by KOAc and purged the reaction mixture with argon for 10 min. The reaction mixture was then heated at 80°C for 4 h. The solvent was evaporated under reduced pressure to get the crude product of compound (7). The crude compound was purified to obtain **methyl-7-acetamido-4-(4-((4-morpholinomethyl) phenyl) ethynyl) phenyl)-1,8-naphthyridine-2-carboxylate (7)** (51.5 %) as brown solid.

When a suspension of Methyl-7-acetamido-4-(4-((4-morpholino methyl)phenyl)ethynyl)phenyl)-1,8-naphthyridine-2-carboxylate (7)in methanol was heated under reflux withthionylchloride,**methyl-7-amino-4-(4-((4 (morpholinomethyl)phenyl)ethynyl)phenyl)-1,8-naphthyridine-2-carboxylate(8)**(60.4%) was obtained as brown solid. A broad two proton singlet in the ^1H NMR spectrum corresponding to primary amino group (NH_2) at δ 7.29 confirms the formation of compound**methyl-7-amino-4-(4-((4-(morpholinomethyl)phenyl) ethynyl)phenyl)-1,8-naphthyridine-2-carboxylate (8)**.

To a solution of Methyl 7-amino-4-(4-((4-(morpholinomethyl)phenyl)ethynyl)phenyl)-1,8-naphthyridine-2-carboxylate(8) in dry dimethyformamide was added diisopropylisocyanate, dibutyltindiacetate and pyridine. The reaction mixture was irradiatedat 70°C in microwave for 30 min. The crude product of compound **methyl 4-(4-((4-(morpholinomethyl) phenyl)ethynyl)phenyl)-7-(3-phenylureido)-1,8-naphthyridine-2-carboxylate (9a)** obtained was purified by column chromatography.

Compounds 9b-g were prepared using the appropriate isocyanates by a similar procedure. The spectral data of compounds 9a-g confirm the structure of these compounds. An absorption band in IR at around 3340 cm^{-1} corresponds to NH stretching in amide. The appearance of a two proton singlet at around δ 9.00 corresponding to NH protons confirms the structure of compounds (9a-g).

Methyl 4-(4-((4-(morpholinomethyl)phenyl) ethynyl)phenyl)-7-(3-phenylureido)-1,8-naphthyridine-2-carboxylate (9a) on reaction with aqueous 50% hydroxylamine in presence of catalytic amount of KCN gave **N-hydroxy-4-(4-((4-(morpholinomethyl)phenyl)ethynyl)phenyl)-7-(3-phenylureido)-1,8-naphthyridine-2-carboxamide (10a)** (28%) as pale yellow solid.

In similar reactions compounds 9b-g react with hydroxylamine to furnish 10b-g.

The ^{1}H NMR spectra of compounds **10a-10g** showed two broad singlet's, one at around δ 8.00 and another at around 2.20 corresponding to NH and OH protons respectively in NH-OH group, which confirm the structure of the compounds. The other spectral data are consistent with the structures of the compounds for 10a-g.

CONCLUSION

A series of new class of substituted napthyridine derivatives have been prepared. The compounds were characterized by elemental analysis, IR and ^{1}H NMR spectral data. The novel heterocycles were evaluated for antimicrobial activity and anthelmintic activity. The compounds demonstrated moderate antimicrobial activity against selected fungal and bacterial strains and considerable anthelmintic activity against *P. posthuma*. From the results obtained it is concluded that increasing the number of electron donating groups results in significant decrease of antimicrobial activity. Similarly, increasing the number of electron donating substituents results an increase in anthelmintic activity.

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