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Synthesis and Cytotoxic Distinction of Benzo[*h*]naphtho[1,2-*b*][1,6] Naphthyridine and its Isomeric Benzo[*b*]naphtho[1,2-*h*][1,6] Naphthyridines

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Abstract

Benzo[*h*]naphtho[1,2-*b*][1,6]naphthyridine and its isomeric benzo[*b*]naphtho[1,2-*h*][1,6]naphthyridine with aliphatic, aromatic and hetero substitution were synthesized and screened for its antiproliferative activity against four human cancer cell lines. Among these, HeLa cells are more susceptible to compounds 3a, 3b, 9a and 9b with IC_{50} values of 3.62, 1.05, 6.21 and 1.41 μM respectively. Interestingly chloro substituted compound 9b showed IC_{50} values of 5.93, 7.01, and 6.81 μM against MCF7, K562 and Hep-G2 cancer cells, which is more active than the standard adriamycin. Furthermore chloro substituted compound 3b displayed good activity against MCF7 (IC_{50} 6.63 μM) and K562 (IC_{50} 7.23 μM) cancer cell lines. This study also revealed that, benzo[*h*]naphtho[1,2-*b*][1,6] naphthyridine series were more active than its isomeric benzo[*b*]naphtho[1,2-*h*][1,6] naphthyridines.

Keywords: Cholro quinolines; Cytotoxicity; SAR studies; Positional isomers

Introduction

The need of exploring novel synthetic strategies to make new heterocycles is still expanding owing to meet the challenges in identifying new lead compounds for various therapeutic areas. It is also quite evident from literature that closely related isomers/heterocycles behave quite differently to the biological target [1]. For instance, it was demonstrated that 6-isomers of 5, 8-O dimethyl acylshikonin derivatives exhibit higher inhibitory effects on DNA topoisomerase-I and also had upper hand *in vitro* IC_{50} values against L1210 cell than its corresponding 2-isomer (Figure 1). This triggers further interest to study one of the potent isomers which lead to potential candidate both *in vitro* and *in vivo* studies using KM mice model [2,3]. When screened a small collection of tricyclic 4-(phenylamino)furo[2,3-*b*] quinolone and its positional isomer, 2-(furan-2-yl)-4-(phenylamino) quinolone against 60 NCI cancer cells (Figure 1). Tzeng et al. found that one of the former isomer turns out to be more cytotoxic whereas its corresponding isomer is inactive [4]. A comparison of the biological activity of isomers with varying alkyl substitutions on the heterocyclic nitrogen of benzhydro[*f*]quinoline derivatives was made. The *trans*-isomer was effective rather than the cis-isomer in relaxing methacholine contracted guinea-pig trachea through a β-adrenergic mechanism since propranol blocked this response [5].

The continuous quest to develop nitrogen containing small molecules in the area of cancer is quite tremendous. Among them quinolones and naphthyridines were identified as one of the most promising scaffolds. As evident from the literature these compounds (EKB-569, HKI-272 and SNS-595) were in different phases of clinical trials [6]. Quinoline and its analogues were also known for its anti-tuberculosis [7,8], antiproliferative [9,10], anthelmintic [11], antibacterial [12], antiviral [13], Scr tyrosine kinase inhibitors [14], antioxidant activities [15] and metal chelating properties [16]. Various Naphthyridine derivatives exert their biological activity by inhibiting topoisomerase-I [17], Akt1 and Akt2 [18], HIV integrase [19,20], c-Met kinase inhibitors [21]. Certain naphthyridine derivatives also exhibit antitumour [22-24], anticonvulsive [25], CB_2 selective agonist properties [26].

These interesting facts coupled with our current interest in unraveling the interesting anticancer properties of various nitrogen heterocycles prompted us to explore the biological activities of two positional isomers in greater detail. A general strategy to obtain both benzo napthonaphthyridines and its isomer was developed and their *in vitro* cytotoxicity we studied systematically and the results are presented in this manuscript.

Experimental Protocols

General

Melting points (m.p.) were determined on Mettler FP 51 apparatus (Mettler Instruments) and are uncorrected. They are expressed in degree centigrade (°C). A Nicolet Avatar Model FT-IR spectrophotometer was used to record the IR spectrum (4000-400 cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AV 400 [400 MHz (^1H) and 100 MHz (^{13}C)] and AV 500 [500 MHz (^1H) and 125 MHz (^{13}C)] spectrometer using tetramethylsilane (TMS) as an internal reference. The chemical shifts are expressed in parts per million (ppm). Mass spectra (MS) were recorded on Auto Spec EI+ Shimadzu QP 2010 PLUS GC-MS mass spectrometer. Microanalyses were performed on a Vario EL III model CHNS analyzer (Vario, Germany). The solvent and reagents used for the preparations were of reagent grade and were purified by standard methods; petroleum ether used was of boiling range 60-80 °C. Anhydrous sodium sulphate was used to dry the solution of organic extracts. Thin layer chromatography (TLC) was performed using glass plates coated with silica gel-G containing 13% calcium sulphate as binder. Ethyl acetate and petroleum ether were used as developing solvents. A chamber containing iodine vapour was used to locate the spots. Separation and purification of the crude products was carried out using chromatographic columns packed with activated silica gel (60-120 mesh). In the case of mixture of solvents used for elution, the ratio of the mixture is given in brackets.

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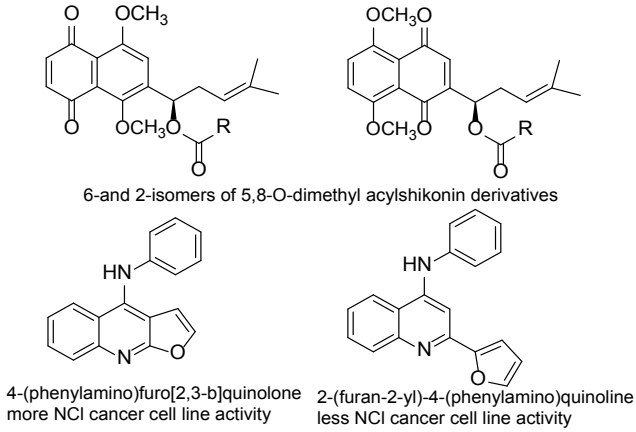


Figure 1: Structure of some isomer compounds.

Synthesis

Preparation of hetero substituted benzo[h]naphtho[1,2-b][1,6]naphthyridine (7, 8 and 9), general procedure: 2-Methyl-*N*-(1-naphthyl)quinolin-4-amine (3, 0.002 mol) and pyridine-3-carboxylic acid /thiophen-2-carboxylic acid/furan-2-carboxylic acid (0.0025 mol) were added to polyphosphoric acid (6 g of P_2O_5 in 3 mL of H_3PO_4) and heated at 110°C for 1 hour. The reaction was monitored by using TLC. After completion of the reaction, it was poured into ice water, neutralized with saturated sodium bicarbonate solution to remove excess of pyridine-3-carboxylic acid/thiophen-2-carboxylic acid/furan-2-carboxylic acid, extracted with ethyl acetate, purified by column chromatography using silica gel to get 7-9 which was recrystallised using methanol.

2,6-Dimethyl-7-(pyridin-3'-yl)benzo[*h*]naphtho[1,2-*b*][1,6]naphthyridine (7a): Pale yellow solid; mp: 242-244°C; Yield: (44%); IR (KBr, cm^{-1}) ν_{max}: 1634 (C=N), 1598, 1547; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 2.36 (s, 3H, C_4-CH_3), 2.92 (s, 3H, C_6-CH_3), 7.15 (d, 1H, C_3-H, *J*=9.00 Hz), 7.28 (t, 1H, C_5'-H, *J*=8.50 Hz, *J*=5.00 Hz), 7.38-7.73 (m, 4H, C_2, C_{12}, C_9 and C_{11}-H), 7.84 (dd, 1H, C_4'-H, *J*=1.50 Hz, *J*=6.00 Hz), 7.90 (d, 1H, C_8-H, *J*=8.50 Hz), 7.94 (s, 1H, C_2'-H), 7.99 (dd, 1H, C_6'-H, *J*=2.00 Hz, *J*=5.00 Hz), 8.14 (d, 1H, C_{10}-H, *J*=8.00 Hz), 9.29 (d, 1H, C_1-H, *J*=8.50 Hz), 9.68 (d, 1H, C_{13}-H, *J*=8.00 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (ppm) δ_C: 20.77 (C_6-CH_3), 28.75 (C_2-CH_3), 117.85 (C_{6a}), 123.03 (C_1), 124.67 (C_9), 125.31 (C_8), 125.68 (C_{7a}), 126.25 (C_{13}), 126.58 (C_{14b}), 127.41 (C_{12}), 127.82 (C_{10}), 128.33 (C_4), 128.84 (C_5'), 128.91 (C_3), 129.57 (C_{11}), 131.19 (C_2), 132.44 (C_{9a}), 133.92 (C_3'), 134.76 (C_{13a}), 135.04 (C_4'), 139.10 (C_7), 146.21 (C_{13b}), 147.68 (C_2'), 147.74 (C_{14a}), 149.11 (C_6'), 150.36 (C_{4a}), 158.32 (C_6); MS (EI) m/z (%) 385 (M^+, 100); Anal. Calcd. for $C_{27}H_{19}N_3$ (385): C, 84.13; H, 4.97; N, 10.90; Found : C 88.07, H 5.00, N 10.93%.

2-Chloro-6-methyl-7-(pyridin-3'-yl)benzo[*h*]naphtho[1,2-*b*][1,6]naphthyridine (7b): Pale yellow solid; mp: 240-242°C; Yield: (40%); IR (KBr, cm^{-1}) ν_{max}: 1655 (C=N), 1611, 1568; ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.85 (s, 3H, C_6-CH_3), 7.15 (d, 1H, C_3-H, *J*=9.00 Hz), 7.28 (dd, 1H, C_5'-H, *J*=8.50 Hz, *J*=5.00 Hz), 7.35-7.71 (m, 4H, C_4, C_9, C_{11} and C_{12}-H), 7.83 (dd, 1H, C_4'-H, *J*=1.50 Hz, *J*=6.00 Hz), 7.90 (d, 1H, C_8-H, *J*=8.50 Hz), 7.96 (s, 1H, C_2'-H), 7.81 (dd, 1H, C_6'-H, *J*=2.00 Hz, *J*=5.00 Hz), 8.18 (d, 1H, C_{10}-H, *J*=8.00 Hz), 9.36 (d, 1H, C_1-H, *J*=8.50 Hz), 9.72 (d, 1H, C_{13}-H, *J*=8.00 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (ppm) δ_C: 16.9 (C_6-CH_3), 24.4 (C_7-CH_3), 118.1 (C_{6a}), 122.2 (C_1), 124.1 (C_9), 125.2 (C_8), 125.4 (C_{7a}), 125.9 (C_{13}), 126.4 (C_{14b}), 127.3 (C_{12}), 127.6 (C_{10}), 128.0 (C_4), 128.6 (C_3), 128.9 (C_5'), 129.6 (C_{11}), 131.0 (C_2), 131.1 (C_{9a}), 133.5 (C_3'), 133.9 (C_{13a}), 135.9 (C_4'), 139.1 (C_7), 144.4 (C_{13b}), 146.8 (C_2'), 147.4 (C_{14a}), 148.8 (C_6'), 151.6 (C_{4a}), 158.6 (C_6); MS (EI) m/z (%) 405 (M^+, 100); Anal. Calcd. for $C_{26}H_{16}ClN_3$ (405): C, 76.94; H, 3.97; N, 10.35; Found: C, 76.98; H, 3.93; N, 10.31%.

2,6-Dimethyl-7-(furan-2'-yl)benzo[*h*]naphtho[1,2-*b*][1,6]naphthyridine (8a): Colourless prisms; mp: 262-264°C; Yield: (43%); IR (KBr, cm^{-1}) ν_{max}: 1620 (C=N), 1587; ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.29 (s, 3H, C_2-CH_3), 2.80 (s, 3H, C_6-CH_3), 6.65 (t, 1H, C_4'-H, *J*=3.50 Hz, *J*=1.50 Hz), 7.20 (d, 1H, C_3-H, *J*=2.00 Hz), 7.33 (dd, 1H, C_3'-H, *J*=3.00 Hz, *J*=1.00 Hz), 7.45-8.02 (m, 6H, C_4, C_8, C_7, C_9, C_{12}, C_5'-H), 8.27 (d, 1H, C_{10}-H, *J*=8.00 Hz), 9.29 (d, 1H, C_1-H *J*=1.50 Hz), 9.62 (d, 1H, C_{13}-H *J*=8.50 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (ppm) δ_C: 16.98 (C_6-CH_3), 27.75 (C_2-CH_3), 112.55 (C_4'), 117.90 (C_{6a}), 120. 44 (C_3'), 122.81 (C_1), 124.42 (C_9), 125.34 (C_8), 125.49 (C_{7a}), 125.90 (C_{13}), 126.38 (C_{14b}), 127.40 (C_{12}), 127.74 (C_{10}), 128.11 (C_4), 128.67 (C_3), 129.59 (C_{11}), 131.05 (C_2), 131.92 (C_{9a}), 133.83 (C_{13a}), 139.19 (C_7), 142.63 (C_5'), 146.33 (C_{13b}), 147.22 (C_{14a}), 147.21 (C_2'), 147.88 (C_{4a}), 157.91 (C_6); MS (EI) m/z (%) 374 (M^+, 100); Anal. Calcd. for $C_{26}H_{18}N_2O$ (374): C, 83.40; H, 4.85; N, 7.48; Found: C, 83.33; H, 4.91; N, 7.54%.

2-Chloro-7-(furan-2'-yl)-6-methylbenzo[*h*]naphtho[1,2-*b*][1,6]naphthyridine (8b): Colourless solid; mp: 255-257°C; Yield: (40%); IR (KBr, cm^{-1}) ν_{max}: 1619 (C=N), 1572; ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.77 (s, 3H, C_6-CH_3), 6.69 (dd, 1H, C_4'-H, *J*=3.50 Hz, *J*=1.5 Hz), 7.23 (d, 1H, C_3-H, *J*=2.00 Hz), 7.30 (dd, 1H, C_3'-H, *J*=3.00 Hz, *J*=1.00 Hz), 7.49-8.01 (m, 6H, C_4, C_8, C_7, C_9, C_{12}, C_5'-H), 8.29 (d, 1H, C_{10}-H, *J*=8.00 Hz), 9.31 (d, 1H, C_1-H, *J*=1.50 Hz), 9.65 (d, 1H, C_{13}-H *J*=8.50 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (ppm) δ_C: 17.24 (C_6-CH_3), 112.43 (C_4'), 118.52 (C_{6a}), 120.91 (C_3'), 122.65 (C_1), 124.27 (C_9), 125.22 (C_8), 125.29 (C_{7a}), 125.60 (C_{13}), 126.19 (C_{14b}), 127.35 (C_{12}), 127.77 (C_{10}), 128.34 (C_4), 128.71 (C_3), 129.60 (C_{11}), 130.99 (C_2), 131.43 (C_{9a}), 134.11 (C_{13a}), 139.35, (C_7), 142.56 (C_5'), 144.4 (C_{13b}), 147.4 (C_{14a}), 147.67 (C_{4a}), 148.59 (C_2') 158.09 (C_6); MS (EI) m/z (%) 396 (M+2, 35), 394 (M^+, 100); Anal. Calcd. for $C_{25}H_{15}ClN_2O$ (394): C, 76.05; H, 3.83; N, 7.09; Found : C 76.12, H 3.86, N 7.14%.

2,6-Dimethyl-7-(thiophen-2'-yl)benzo[*h*]naphtho[1,2-*b*][1,6]naphthyridine (9a): Colourless prisms; mp: 230-231°C; Yield: (33%); IR (KBr, cm^{-1}) ν_{max}: 1612 (C=N), 1555; ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.25 (s, 3H, C_2-CH_3), 2.86 (s, 3H, C_6-CH_3), 7.01 (d, 1H, C_3-H, *J*=8.50 Hz), 7.17 (t, 2H, C_4'-H, *J*=5.00 Hz) 7.64 (d, 1H, C_9-H, *J*=8.50 Hz), 7.69 (dd, 1H, C_5'-H, *J*=5.50 Hz, *J*=1.00 Hz), 7.73-7.89 (m, 4H, C_4, C_8, C_{11}, C_{12}-H), 7.95 (dd, 1H, C_3'-H *J*=4.50 Hz, *J*=1.50 Hz), 8.15 (d, 1H, C_{10}-H, *J*=7.50 Hz), 9.21 (d, 1H, C_1-H *J*=7.50 Hz), 9.50 (d, 1H, C_{13}-H, *J*=8.00 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (ppm) δ_C: 20.77 (C_6-CH_3), 28.75 (C_2-CH_3), 117.85 (C_{6a}), 123.03 (C_1), 124.67 (C_9), 125.31 (C_8), 125.68 (C_{7a}), 126.25 (C_{13}), 126.58 (C_{14b}), 127.41 (C_{12}), 127.82 (C_{10}), 128.33 (C_4), 128.74 (C_3'), 128.91 (C_3), 129.57 (C_{11}), 131.19 (C_2), 132.44 (C_{9a}), 133.92 (C_4'), 134.76 (C_{13a}), 135.04 (C_5'), 137.68 (C_2'), 139.10 (C_7), 146.21 (C_{13b}), 147.74 (C_{14a}), 150.36 (C_{4a}), 158.32 (C_6); MS (EI) m/z (%) 390 (M^+, 100); Anal. Calcd. for $C_{26}H_{18}N_2S$ (390): C, 79.97; H, 4.65; N, 7.17; S, 8.21; Found: C, 79.92; H, 4.62; N, 7.22; S, 8.24%.

2-Chloro-6-methyl-7-(thiophen-2'-yl)benzo[*h*]naphtho[1,2-*b*][1,6]naphthyridine (9b): Colourless prisms; mp: 231-233°C; Yield: (32%); IR (KBr, cm^{-1}) ν_{max}: 1600 (C=N), 1581; ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.75 (s, 3H, C_6-CH_3), 7.00 (d, 1H, C_3-H, *J*=8.50 Hz), 7.20 (t, 1H, C_4'-H, *J*=4.50 Hz), 7.60 (d, 1H, C_9-H, *J*=8.00 Hz), 7.65 (dd, 1H, C_5'-H, *J*=5.00 Hz, *J*=1.00 Hz), 7.71-7.86 (m, 4H, C_4, C_8, C_{11}, C_{12}-H), 7.93 (dd, 1H, C_3'-H, *J*=4.00 Hz, *J*=1.50 Hz), 8.12 (d, 1H, C_{10}-H, *J*=7.50 Hz), 9.25 (d, 1H, C_1-H, *J*=8.00 Hz), 9.56 (d, 1H, C_{13}-H *J*=8.50 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (ppm) δ_C: 22.27 (C_6-CH_3), 118.03 (C_{6a}), 122.43 (C_1), 124.47 (C_9), 125.28 (C_8), 125.54 (C_{7a}), 126.16 (C_{13}), 126.32 (C_{14b}), 127.28 (C_{12}), 127.75 (C_{10}), 128.01 (C_4), 128.56 (C_3'), 128.98 (C_3), 129.91 (C_{11}), 130.86 (C_2), 132.44 (C_{9a}), 133.92 (C_4'), 134.45 (C_{13a}), 134.99

(C_5'), 137.89 (C_2'), 138.93 (C_7), 146.46 (C_{13b}), 147.62 (C_{14a}), 149.37 (C_{4a}), 159.43 (C_6); MS (EI) m/z (%) 410 (M^+, 100); Anal. Calcd. For $C_{25}H_{15}ClN_2S$ (410): C, 73.07; H, 3.68; N, 6.82; S, 7.80; Found: C, 73.10; H, 3.62; N, 6.85; S, 7.77%.

Preparation of 2-methyl-N-phenylbenzo[h]quinolin-4-amine (15) general procedure: 4-Chloro-2-methylbenzo[*h*]quinoline (10, 0.004 mol) was reacted with *p*-toluidine and *p*-chloroaniline (0.004 mol) under neat condition at 190°C for half an hour. The product was washed with water, dried, adsorbed and purified using silica gel column chromatography and eluted with ethylacetate: methanol (95:5) mixture to get 12 which was then recrystallised using methanol.

2-Methyl-*N*-*p*-tolylbenzo[*h*]quinolin-4-amine (12a): Brown solid; mp: 295-297°C; Yield: (73%); IR (KBr, cm^{-1}) ν_{max} : 3371 (NH), 1628 (C=N), 1138; ^{1}H NMR (400 MHz, DMSO-d_6) (ppm) δ_H: 2.37 (s, 3H, C_4'-CH_3), 2.70 (s, 3H, C_2-CH_3), 6.63 (s, 1H, C_3-H), 7.62-8.52 (m, 9H, C_5, C_6, C_7, C_8, C_9, C_2', C_3', C_5', C_6'-H), 9.27 (dd, 1H, J_o=8.80 Hz, J_m=2.00 Hz, C_{10}-H), 10.35 (s, 1H, C_4-NH amino form), 13.56 (s, 1H, C_1-NH imino form, ratio of amino form : imino form is 1 : 1); ^{13}C NMR (100 MHz, DMSO-d_6) (ppm) δ_C: 19.2 (C_4'-CH_3), 20.5 (C_2-CH_3) 102.6 (C_3), 119.1 (C_6' and C_2'), 119.2 (C_{4a}), 124.0 (C_5), 128.1 (C_{10}), 128.2 (C_6), 128.6 (C_9), 128.7 (C_8), 129.1 (C_7), 130.6 (C_3' and C_5'), 131.9 (C_4'), 134.6 (C_{10a}), 135.8 (C_{6a}), 139.0 (C_1'), 140.7 (C_{10b}), 154.0 (C_4), 154.2 (C_2); Anal. Calcd. for $C_{21}H_{18}N_2$ (298): C 84.53; H, 6.08, N 9.39; Found : C 84.64, H 5.99, N 9.37%.

***N*-(4'-chlorophenyl)-2-methylbenzo[*h*]quinolin-4-amine (12b):** Brown solid; mp: 294-296°C; Yield: (72%); IR (KBr, cm^{-1}) ν_{max} : 3401 (NH), 1647 (C=N), 1197; ^{1}H NMR (400 MHz, DMSO-d_6) (ppm) δ_H: 2.76 (s, 3H, C_2-CH_3), 6.97 (s, 1H, C_3-H), 7.51-8.54 (m, 9H, C_5, C_6, C_7, C_8, C_9, C_2', C_3', C_5', C_6'-H), 9.25 (dd, 1H, J_o=8.40 Hz, J_m=1.50 Hz, C_{10}-H), 10.69 (s, 1H, C_4-NH amino form), 13.59 (s, 1H, C_1-NH imino form, ratio of amino form : imino form is 1 : 1); ^{13}C NMR (100 MHz, DMSO-d_6) (ppm) δ_C: 20.5 (C_2-CH_3), 102.6 (C_3), 119.2 (C_{4a}), 121.3 (C_6' and C_2'), 124.0 (C_5), 128.1 (C_{10}), 128.2 (C_6), 128.6 (C_9), 128.7 (C_8), 129.1 (C_7), 129.9 (C_4'), 130.4 (C_3' and C_5'), 134.6 (C_{10a}), 135.8 (C_{6a}), 140.7 (C_{10b}), 141.5 (C_1'), 154.0 (C_4), 154.2 (C_2); Anal. Calcd. for $C_{20}H_{15}ClN_2$ (318): C 75.35, H 4.74, N 8.79; Found : C 75.38, H 4.83, N 8.70%.

General procedure for the synthesis of compound (13,14,15)

2-methyl-*N*-phenylbenzo[*h*]quinolin-4-amine (12) (0.002 mol) and benzoic acid/acetic acid/1-naphthoic acid (0.0025 mol) were added to polyphosphoric acid (6 g of P_2O_5 in 3 mL of H_3PO_4) and heated at 160°C for 3 hours. The reaction was monitored by using TLC. After the completion of the reaction, it was poured into ice water, neutralized with saturated sodium bicarbonate solution to remove excess of benzoic acid/acetic acid/1-naphthoic acid, extracted with ethyl acetate, purified by column chromatography using silica gel and product eluted with petroleum ether: ethyl acetate (99 : 1) mixture to get 13, 14, 15 which was recrystallised using methanol.

6,9-Dimethyl-7-phenylbenzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine (13a): Yellow prisms; mp: 202-204°C; Yield: (54%). IR (KBr, cm^{-1}) ν_{max} : 1624 (C=N), 1561, 1536 and 1484; ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.38 (s, 3H, C_6-CH_3), 3.15 (s, 3H, C_9-CH_3), 7.35-7.98 (m, 10H, C_2, C_3, C_8, C_9, C_{10}, C_{11}, C_2', C_3', C_5', C_6' -H), 8.01 (d, 1H, C_1-H, *J*=9.20 Hz), 8.25 (d, 1H, C_{14}-H, *J*=8.08 Hz), 9.39 (d, 1H, C_4-H, J_o=8.00 Hz, J_m=2.00 Hz), 9.45 (d, 1H, C_{13}-H, *J*=9.20 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (ppm) δ_C: 21.7 (C_9-CH_3), 29.7 (C_6-CH_3), 121.0 (C_{6a}), 122.1 (C_{13}), 122.4 (C_{7a}), 124.7 (C_4), 126.0 (C_{12b}), 126.5 (C_3), 127.0 (C_{14}), 127.4 (C_2), 127.8 (C_1), 128.3 (C_3', C_4', C_5'), 128.5 (C_2' and C_6'), 129.1 (C_8), 130.0 (C_{4a}), 130.7 (C_{14a}), 130.9 (C_{11}), 132.6 (C_{10}), 133.4 (C_{11a}), 134.9 (C_9), 135.1 (C_1'), 137.5 (C_7) 148.4 (C_{4b}), 149.0 (C_{12a}), 159.4 (C_6); Anal. Calcd. for $C_{28}H_{20}N_2$ (384) C, 87.47; H, 5.24; N, 7.29. Found : C, 87.41, H 5.27, N 7.32%.

9-Chloro-6-methyl-7-phenylbenzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine (13b): Pale yellow solid; mp: 208-210°C; Yield: (52%). IR (KBr, cm^{-1}) ν_{max} : 1633 (C=N), 1598, 1538 and 1469; ^{1}H NMR (400 MHz, $CDCl_3$) (ppm) δ_H: 2.45 (s, 3H, C_6-CH_3), 7.49-8.02 (m, 10H, C_2, C_3, C_8, C_9, C_{10}, C_{11}, C_2', C_3', C_5', C_6' -H), 8.10 (d, 1H, C_1-H, *J*=8.80 Hz), 8.37 (d, 1H, C_{14}-H, *J*=8.08 Hz), 9.36 (d, 1H, C_4-H, J_o=8.80 Hz, J_m=2.00 Hz), 9.40 (d, 1H, C_{13}-H, *J*=9.20 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) (ppm) δ_C: 29.7 (C_6-CH_3), 121.0 (C_{6a}), 122.1 (C_{13}), 122.3 (C_{7a}), 124.7 (C_4), 126.0 (C_{12b}), 126.5 (C_3), 127.0 (C_{14}), 127.4 (C_2), 127.8 (C_1), 128.0 (C_8), 128.3 (C_3', C_4', C_5'), 128.5 (C_2' and C_6'), 129.3 (C_9), 130.0 (C_{4a}), 130.7 (C_{14a}), 131.1 (C_{11}), 132.8 (C_{10}), 133.1 (C_{11a}), 135.1 (C_1'), 137.5 (C_7) 148.4 (C_{4b}), 149.0 (C_{12a}), 159.4 (C_6); Anal. Calcd. for $C_{27}H_{17}ClN_2$ (404): C 80.09, H 4.23, N 6.91; Found : C 80.11, H 4.27, N 6.98%.

6,7,9-Trimethylbenzo[*b*]naphtho[1,2-*h*][1,6]naphthyridine (14a): Pale yellow prisms; mp: 172-174°C; Yield: (54%); IR (KBr, cm^{-1}) ν_{max} : 1620 (C=N), 1558, 1521; ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.64 (s, 3H, C_6-CH_3), 3.36 (s, 3H, C_9-CH_3), 3.39 (s, 3H, C_7-CH_3), 7.70-8.05 (m, 5H, C_1, C_2, C_3, C_{10}, C_{11}-H), 8.09 (s, 1H, C_8-H), 8.30 (d, 1H, C_{14}-H, *J*=8.00 Hz), 9.33 (d, 1H, C_4-H, J_o=8.50 Hz, J_m=2.00 Hz), 9.37 (d, 1H, C_{13}-H, *J*=9.00 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (ppm) δ_C: 21.7 (C_9-CH_3), 24.56 (C_7-CH_3), 29.7 (C_6-CH_3), 120.76 (C_{6a}), 122.87 (C_{13}), 123.02 (C_{7a}), 124.32 (C_4), 126.15 (C_{12b}), 126.93 (C_3), 127.21 (C_{14}), 127.52 (C_2), 127.89 (C_1), 129.55 (C_8), 130.34 (C_{4a}), 130.97 (C_{14a}), 131.09 (C_{11}), 132.76 (C_{10}), 133.39 (C_{11a}), 134.77 (C_9), 138.04 (C_7) 147.95 (C_{4b}), 149.53 (C_{12a}), 159.31 (C_6); MS (EI) m/z (%) 322 (M^+, 100); Anal. Calcd. for $C_{23}H_{18}N_2$ (322): C, 85.68; H, 5.63; N, 8.69; Found: C, 85.70; H, 5.64; N, 8.5%.

9-Chloro-6,7-dimethylbenzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine (14b): Pale yellow prisms; mp: 170-172°C; Yield: (57%); IR (KBr, cm^{-1}) ν_{max}: 1612 (C=N), 1540, 1513; ^{1}H NMR (400 MHz, $CDCl_3$) (ppm) δ_H: 2.50 (s, 3H, C_6-CH_3), 3.29 (s, 3H, C_7-CH_3), 7.71-8.17 (m, 6H, C_{14}, C_2, C_3, C_8, C_{10}, C_{11}-H), 8.21 (d, 1H, C_{14}-H, *J*=8.08 Hz), 9.39 (d, 1H, C_4-H, J_o=8.50 Hz, J_m=1.50 Hz), 9.48 (d, 1H, C_{13}-H, *J*=9.20 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) (ppm) δ_C: 23.89 (C_7-CH_3), 28.63 (C_6-CH_3), 120.87 (C_{6a}), 122.54 (C_{13}), 123.36 (C_{7a}), 124.63 (C_4), 126.39 (C_{12b}), 126.71 (C_3), 127.42 (C_{14}), 127.87 (C_2), 127.96 (C_1), 128.98 (C_8), 129.62 (C_9), 130.71 (C_{4a}), 130.64 (C_{14a}), 131.37 (C_{11}), 132.49 (C_{10}), 133.57 (C_{11a}), 137.89 (C_7), 148.11 (C_{4b}), 149.23 (C_{12a}), 158.65 (C_6); MS (EI) m/z (%) 342 (M^+, 100), 344 (M+2, 32); Anal. Calcd. for $C_{22}H_{15}ClN_2$ (342): C, 77.08; H, 4.41; N, 8.17; Found: C, 77.14; H, 4.38; N, 8.24%.

6,9-Dimethyl-7-(naphthalen-1'-yl)benzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine (15a): Yellow prisms; mp: 239-240°C; Yield: (54%). IR (KBr, cm^{-1}) ν_{max} : 1598, 1540 (C=N); ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.32 (s, 3H, C_6-CH_3), 3.16 (s, 3H, C_9-CH_3), 7.39-8.09 (m, 13H, C_1, C_2, C_3, C_8, C_{10}, C_{11}, C_2', C_3', C_4', C_5', C_6', C_7', C_8',-H), 8.19 (d, 1H, C_{14}-H, *J*=8.00 Hz), 9.33 (d, 1H, C_4-H, J_o=8.50 Hz, J_m=2.50 Hz), 9.46 (d, 1H, C_{13}-H, *J*=9.00 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (ppm) δ_C: 21.7 (C_9-CH_3), 29.7 (C_6-CH_3), 119.57 (C_2'), 120.79 (C_{6a}), 122.67 (C_{13}), 123.20 (C_{7a}), 124.86 (C_4), 125.89 (C_{12b}), 126.68 (C_3), 127.02 (C_{14}), 127.31 (C_2), 127.56 (C_1), 127.77 (C_8'), 127.91 (C_3') 128.22 (C_4'), 128.39 (C_5'), 128.51 (C_6'), 128.73 (C_7'), 129.24 (C_8), 130.35 (C_{4a}), 130.88 (C_{14a}), 131.01 (C_{11}), 132.36 (C_{10}), 132.66 (C_{8a}'), 133.65 (C_{11a}), 134.27 (C_{4a}'), 134.86 (C_9), 136.86 (C_1'), 138.00 (C_7), 148.27 (C_{4b}), 149.31 (C_{12a}), 158.73 (C_6); Anal. Calcd. for $C_{32}H_{22}N_2$ (434): C, 88.45; H, 5.10; N, 6.45; Found : C, 88.41, H 5.17, N 6.50%.

9-Chloro-6-methyl-7-(naphthalen-1'-yl)benzo[*b*]naphtho[1,2-*h*][1,6]naphthyridine (15b): Pale yellow solid; mp: 236-238°C; Yield: (52%). IR (KBr, cm^{-1}) ν_{max} : 1624 (C=N), 1589, 1543; ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.40 (s, 3H, C_6-CH_3), 7.35-8.02 (m,

12H, C_1, C_2, C_3, C_8, C_{10}, C_{11}, C_2', C_3', C_5', C_4' C_6', C_7', C_8'-H), 8.11 (d, 1H, C_{14}-H, *J*=8.00 Hz), 9.29 (d, 1H, C_4-H, J_o=8.50 Hz, J_m=2.50 Hz), 9.41 (d, 1H, C_{13}-H, *J*=9.00 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (ppm) δ_C: 21.7 (C_9-CH_3), 29.7 (C_6-CH_3), 119.64 (C_2'), 120.87 (C_{6a}), 122.91 (C_{13}), 122.46 (C_{7a}), 124.77 (C_4), 126.01 (C_{12b}), 126.84 (C_3), 127.09 (C_{14}), 127.41(C_2), 127.60 (C_1), 127.76 (C_8'), 127.89 (C_3'), 128.05 (C_8), 128.25 (C_4'), 128.41 (C_5'), 128.72 (C_6'), 128.90 (C_7'), 129.85 (C_9), 130.74 (C_{4a}), 130.91 (C_{14a}), 131.32 (C_{11}), 132.56 (C_{10}), 132.96 (C_{8a}'), 133.77 (C_{11a}), 135.98 (C_1'), 137.69 (C_7), 147.55 (C_{4b}), 149.12 (C_{12a}), 158.77 (C_6); Anal. Calcd. for $C_{31}H_{19}ClN_2$ (404): C, 81.84; H, 4.21; N, 6.16; Found : C 81.78, H 4.27, N 6.22%.

General procedure for the synthesis of compound (16-18)

2-methyl-*N*-phenylbenzo[*h*]quinolin-4-amine (12) (0.002 mol) and various hetero aromatic carboxylic acids (0.0025 mol) were added to polyphosphoric acid (6 g of P_2O_5 in 3 mL of H_3PO_4). The reaction time, temperature maintained and various acid used for the synthesis of respective product are mentioned in the Table 2. The reaction was monitored by using TLC. After the completion of the reaction, it was poured into ice water, neutralized with saturated sodium bicarbonate solution to remove excess of carboxylic acids, extracted with ethyl acetate, purified by column chromatography using silica gel and product eluted with petroleum ether:ethyl acetate (99:1) mixture to get 16-18 which was then recrystallised using methanol.

6,9-Dimethyl-7-(pyridin-3'-yl)benzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine (16a): Yellow prisms; mp: 239-240°C; Yield: (41%). IR (KBr, cm^{-1}) ν_{max} : 1610 (C=N), 1588, 1555; ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.40 (s, 3H, C_6-CH_3), 3.11 (s, 3H, C_9-CH_3), 7.20 (t, 1H, C_5'-H, *J*=8.50 Hz, *J*=4.00 Hz) 7.45-7.71 (m, 5H, C_2 C_3, C_8, C_{10}, C_{11}-H), 7.81 (dd, 1H, C_6'-H, *J*=2.00 Hz, *J*=5.50 Hz), 7.87 (dd, 1H, C_4'-H, *J*=2.00 Hz, *J*=5.00 Hz), 7.95 (s, 1H, C_2'-H), 8.02 (d, 1H, C_1-H, *J*=9.50 Hz), 8.10 (d, 1H, C_{14}-H, *J*=7.50 Hz), 9.41 (d, 1H, C_4-H, *J*=8.50 Hz, 9.59 (d, 1H, C_{13}-H, *J*=8.00 Hz); Anal. Calcd. for $C_{27}H_{19}N_3$ (385) C, 84.13; H, 4.97; N, 10.90; Found : C, 84.09, H 5.00, N 10.93%.

9-Chloro-6-methyl-7-(pyridin-3'-yl)benzo[*b*]naphtho[1,2-*h*] [1,6]naphthyridine (16b): Yellow prisms; mp: 233-235°C; Yield: (41%). IR (KBr, cm^{-1}) ν_{max} : 1608 (C=N), 1572, 1550; ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.44 (s, 3H, C_6-CH_3), 7.23 (t, 1H, C_5'-H *J*=8.50 Hz, *J*=4.00 Hz) 7.43-7.75 (m, 5H, C_2 C_3, C_8, C_{10}, C_{11}-H), 7.85 (dd, 1H, C_6'-H, *J*=2.00 Hz, *J*=5.50 Hz), 7.86 (dd, 1H, C_4'-H, *J*=2.00 Hz, *J*=5.00 Hz), 7.99 (s, 1H, C_2'-H), 8.06 (d, 1H, C_1-H, *J*=9.50 Hz), 8.15 (d, 1H, C_{14}-H, *J*=7.50 Hz), 9.47 (d, 1H, C_4-H, *J*=8.50 Hz), 9.61 (d, 1H, C_{13}-H, *J*=8.00 Hz); Anal. Calcd. for $C_{26}H_{16}ClN_3$ (405): C, 76.94; H, 3.97; N, 10.35; Found : C, 77.00; H, 3.99; N, 10.33%.

6,9-dimethyl-7-(furan-2'-yl)benzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine (17a): Colourless prisms; mp: 243-245°C; Yield: (54%); IR (KBr, cm^{-1}) ν_{max} : 1613 (C=N), 1566, 1511; ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.55 (s, 3H, C_6-CH_3), 3.21 (s, 3H, C_9-CH_3), 6.70 (t, 1H, C_4'-H, *J*=4.50 Hz), 7.36 (d, 1H, C_3'-H, *J*=5.50 Hz), 7.51-8.05 (m, 6H, C_1, C_2, C_3, C_{10}, C_{11}, C_5'-H), 8.13 (d, 1H, C_8-H, *J*=1.50 Hz), 8.44 (d, 1H, C_{14}-H, *J*=8.50 Hz), 9.37 (d, 1H, C_4-H, J_o=8.50 Hz, J_m=2.00 Hz), 9.41 (d, 1H, C_{13}-H, *J*=9.50 Hz); Anal. Calcd. for $C_{26}H_{18}N_2O$ (374): C, 83.40; H, 4.85; N, 7.48; Found : C, 83.44, H, 4.81, N, 7.51%

9-Chloro-6-methyl-7-(furan-2'-yl)benzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine (17b): Colourless prisms; mp: 235-237°C; Yield: (54%); IR (KBr, cm^{-1}) ν_{max} : 1613 (C=N), 1566, 1511; ^{1}H NMR (500 MHz, $CDCl_3$) (ppm) δ_H: 2.49 (s, 3H, C_6-CH_3), 6.72 (t, 1H, C_4'-H, *J*=5.50 Hz), 7.40 (d, 1H, C_3'-H, *J*=5.00 Hz), 7.54-8.07 (m, 6H, C_1, C_2, C_3, C_{10}, C_{11}, C_5'-H), 8.17 (d, 1H, C_8-H, *J*=2.00 Hz), 8.51 (d, 1H, C_{14}-H, *J*=8.50 Hz), 9.40 (d, 1H, C_4-H, J_o=8.50 Hz, J_m=2.00 Hz), 9.49 (d, 1H, C_{13}-H, *J*=9.50 Hz); Anal. Calcd. for $C_{25}H_{15}ClN_2O$ (394): C, 76.05; H, 3.83; N, 7.09; Found : C, 76.10, H, 3.79, N, 7.13%

6,9-Dimethyl-7-(thiophen-2'-yl)benzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine (18a): Colourless prisms; mp: 249-250°C; Yield: (54%); IR (KBr, cm^{-1}) ν_{max} : 1613 (C=N), 1566, 1511; NMR ($CDCl_3$) δ_H: 2.53 (s, 3H, C_6-CH_3), 3.20 (s, 3H, C_9-CH_3), 6.71 (t, 1H, C_4'-H, *J*=4.50 Hz), 7.39 (d, 1H, C_3'-H, *J*=5.00 Hz), 7.52-8.00 (m, 6H, C_1, C_2, C_3, C_{10}, C_{11}, C_5'-H), 8.15 (d, 1H, C_8-H, *J*=1.50 Hz), 8.49 (d, 1H, C_{14}-H, *J*=8.00 Hz), 9.34 (d, 1H, C_4-H, J_o=9.00 Hz, J_m=2.00 Hz), 9.50 (d, 1H, C_{13}-H, *J*=9.00 Hz); Anal. Calcd. for $C_{26}H_{18}N_2S$ (390): C, 79.97; H, 4.65; N, 7.17; S, 8.21; Found : C, 79.94, H, 7.19, N, 8.24%

9-Dhloro-6-methyl-7-(thiophen-2'-yl)benzo[*b*]naphtho[1,2-*h*] [1,6]naphthyridine (18b): Colourless prisms; mp: 258-250°C; Yield: (35%); IR (KBr, cm^{-1}) ν_{max} : 1603 (C=N), 1576, 1511; NMR ($CDCl_3$) δ_H: 2.49 (s, 3H, C_6-CH_3), 6.72 (t, 1H, C_4'-H, *J*=5.50 Hz), 7.40 (d, 1H, C_3'-H, *J*=5.00 Hz), 7.54-8.07 (m, 5H, C_1, C_2, C_3, C_{10}, C_{11}, C_5'-H), 8.17 (d, 1H, C_8-H, *J*=2.00 Hz), 8.51 (d, 1H, C_{14}-H, *J*=8.50 Hz), 9.40 (d, 1H, C_4-H, J_o=8.50 Hz, J_m=2.00 Hz), 9.49 (d, 1H, C_{13}-H, *J*=9.50 Hz); Anal. Calcd. for $C_{25}H_{15}ClN_2S$ (410): C, 73.07; H, 3.68; N, 6.82; S, 7.80; Found: C, 73.12, H, 3.72, N, 6.79; S, 7.77%.

In vitro cytotoxicity

Experimental procedure for SRB assay: The cell lines (K562, MCF7, Hep-G2, and HeLa) were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 90 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, one plate of each cell line was fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in appropriate solvent at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with complete medium containing test compound at a concentration of 10^{-3}. Additional three, 10-fold serial dilutions were made to provide a total of four drug concentrations plus control. Aliquots of 10 µl of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 µL of medium, resulting in the required final drug concentrations.

Endpoint measurement: After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB)[28,29] solution (50 µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength. The results were expressed as the concentration at which there was 50% inhibition (IC_{50}).

Results and Discussion

Chemistry

In the present work the synthesis of benzo[*h*]naphtho[1,2-*b*][1,6] naphthyridine and benzo[*b*]naphtho[1,2-*h*][1,6]naphthyridine isomers was achieved from 4-chloro-2-methylquinolines (1a,b) and 4-chloro-2-methylbenzo[*h*]quinolines (10) through the key intermediates 2,8-dimethyl-*N*-(1-naphthyl)quinoline-4-amine (3a,b) and 2-methyl-*N*-*o*-tolylbenzo[*h*]quinolin-4-amine (12a,b) respectively.

Recently we have reported the synthesis of compounds (3a,b-6a,b) [27]. Now we report the preparation of novel hetero benzo[*h*] naphtho[1,2-*b*][1,6]naphthyridines (7a,b-9a,b) using the similar protocol which is depicted in Scheme 1.

Our aim is to introduce hetero ring in benzonaphthonaphthyridine analogues. In order to achieve this the potential intermediate (3a) was reacted with pyridine-3-carboxylic acid in the presence of PPA (Polyphosphoric acid) at 110°C for an hour. IR spectrum of compound 4a showed three sharp bands at 1634 cm^{-1}, 1598 cm^{-1}, 1547 cm^{-1} confirms the presence of three C=N groups. In its ^{1}H NMR spectrum two singlets at δ 2.36 and 2.92 accounts for C_4 and C_8-CH_3 respectively. All other aromatic protons resonated in the region at δ 7.15-9.68. Its ^{13}C NMR spectrum clearly showed the presence of 27 carbons. All the spectral and analytical details attest the structure of the compound as 2,6-dimethyl-7-(pyridin-3'-yl)benzo[*h*]naphtho[1,2-*b*] [1,6]naphthyridine (7a). The same reaction was carried out with other hetero substituted carboxylic acids like furan-2-carboxylic acid and thiophen-2-carboxylic acid, the reaction conditions (including time and temperature) is represented in Table 1. The structures of all the compounds (7a,b-9a,b) were established by elemental and spectral analysis (Refer experimental section).

We envisaged the synthesis of benzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine isomer, the second isomer by treating 4-Chloro-2-methylbenzo[*h*]quinoline [28] (10) with *p*-toluidine (11a) under neat condition at 190°C (Scheme 2). As expected compound 12a was obtained as a brown solid in 73% yield. In IR spectrum the absorption bands at 3371 cm^{-1} and 1628 cm^{-1} confirms the presence of NH and C=N functional groups. Its ^{1}H NMR spectrum showed the presence of methyl groups at δ 2.37 and 2.70 for C_4' and C_2-CH_3. The peculiar C_3-H appeared as a singlet at δ 6.63. All the 10 aromatic protons appeared at δ 7.62-9.27 while two broad singlets each for one proton integration observed at δ 10.35 and δ 13.56 were assigned for C_4-NH amino form and N_1-H imino form respectively. The ratio of amino and imino form was found to be 1:1. Its ^{13}C NMR spectrum confirmed the presence of 21 carbons.

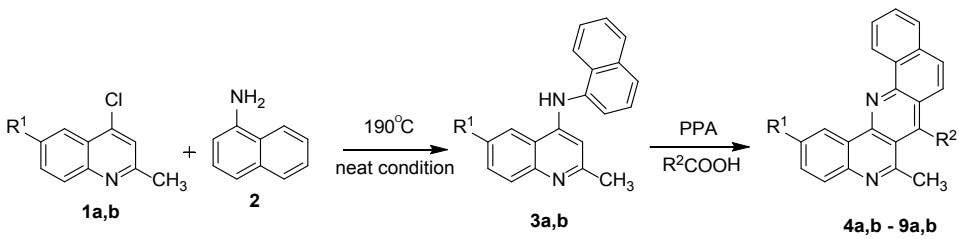
Finally the cyclisation of 2-methyl-*N*-*p*-tolylbenzo[*h*]quinolin-4-amine (12a) with benzoic acid in presence of polyphosphoric acid as catalyst afforded 6,9-dimethyl-7-phenylbenzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine (13a) (Scheme 3).

The IR Spectrum of 13a showed the absorption bands at 1624 cm^{-1} and 1561 cm^{-1} which were due to two C=N functional groups. The ^{1}H NMR spectrum of 13a exhibited two singlets each at δ 2.38 and 3.15 for C_6-CH_3 and C_{11}-CH_3 respectively. All the aromatic protons resonated at δ 7.35-8.25 except for two proton doublets which were very much deshielded at δ 9.39 (*J*=8.00 Hz) and δ 9.45 (*J*=9.00 Hz). With the help of 2D NMR studies (H,H-COSY, C,H-COSY, HSQC and HMBC) the deshielded proton at δ 9.39 was assigned for C_4-H while the proton at δ 9.45 for C_{13}-H. Its ^{13}C NMR spectrum showed the appearance of 28 carbon signals and the mass spectrum identified the molecular ion peak at *m/z* 384. From its elemental analysis the molecular formula was deduced as $C_{28}H_{20}N_2$. All the above spectral and analytical details attest the structure of the compound as 6,9-dimethyl-7-phenylbenzo[*b*] naphtho[1,2-*h*][1,6]naphthyridine (13a). The generality of the reaction was tested with 4-chloroaniline (12b) in order to get the corresponding benzonaphthonaphthyridines (13b). The similar set of reaction was also extended to other carboxylic acids *i.e.*, acetic acid and 1-naphthoic acid to get 6,7 -dimethylbenzo[*b*]naphtho[1,2-*h*][1,6]naphthyridines 14 and 6-methyl-7-(naphthalen-1-yl) benzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine 15 respectively (Scheme 3). In all cases the C_4-H and C_{13}-H were deshielded. The reason for the two protons to get deshielded very much could be due to the interaction of these protons with the nitrogen atom at 5th and 12th position.

Further substrate scope of the reaction was examined using pyridine-3-carboxylic acid, furan-2-carboxylic acid and thiophen-2-carboxylic acid the reaction conditions (including time and temperature) are represented in Table 2. The structures of all compounds were confirmed by elemental and spectral analysis (Refer experimental section).

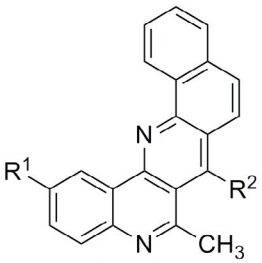
Biological activity

Cytotoxicity: Various substituted benzo[*h*]naphtho[1,2-*b*][1,6] naphthyridine (4a,b-9a,b) and its isomeric benzo[*b*]naphtho[1,2-*h*] [1,6]naphthyridine derivatives (13a,b-18a,b) were synthesized from appropriate starting materials using two step synthetic steps which includes the condensation followed by subsequent cyclization with various aliphatic, aromatic and heteroaromatic carboxylic acids (Schemes 1 and 2). Positional isomers could show different biological response(s) during the *in vitro* assay, so we screened these isomeric compounds against four cancer cell lines namely, K562 (human leukaemia cancer cell line), MCF7 (human breast cancer cell line), Hep-G2 (human liver cancer cell line) and HeLa (human cervical cancer cell line) by SRB method [29,30]. The results for benzo[*h*] naphtho[1,2-*b*][1,6]naphthyridines (4a,b-9a,b) is presented in Table 3 and those for benzo[*b*]naphtho[1,2-*h*][1,6]naphthyridine derivatives (13a,b-18a,b) are depicted in Table 4. Adriamycin (ADR), one of the



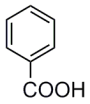
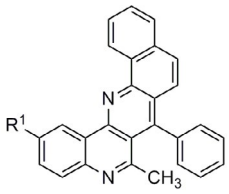
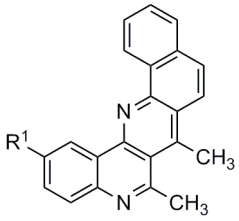
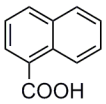
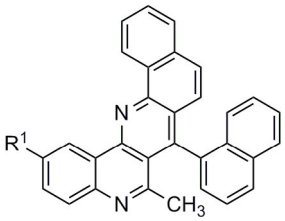
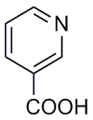
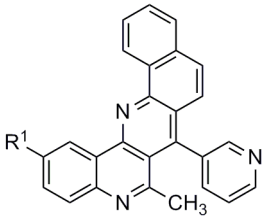
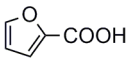
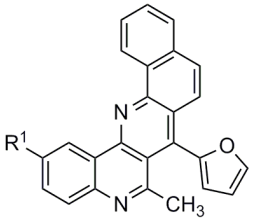
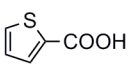
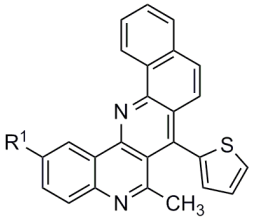
1,3, 4 - 9a) R^1 = CH_3;b) R^1 =Cl
R^2= CH_3*, C_6H_5*, $C_{10}H_7$* C_5H_4N, C_4H_3S, C_4H_3O
*= Reported earlier from our group[26]

Scheme 1: Synthesis of benzo[h]naphtho[1,2-b][1,6]naphthyridines 4a-b - 9a,b.



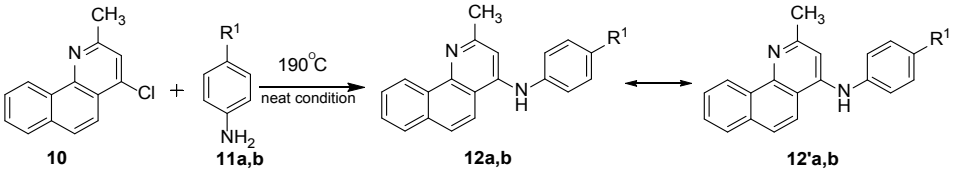
4a,b-9a,b

4 - 9a) $R^1 = CH_3$,
b) $R^1 = Cl$
$R^2 = CH_3$*, C_6H_5*, $C_{10}H_7$*, C_5H_4N, C_4H_3O, C_4H_3S
*** = Reported**

Compound	Acid	Product[a]	t (h)	T (°C)
*4a,b			3	160
*5a,b	CH_3COOH		3	160
*6a,b			3	160
7a,b			1	110
8a,b			1	110
9a,b			1	110

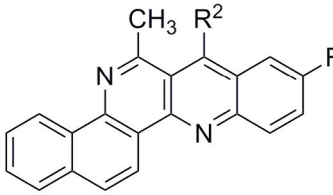
[a]-The products were characterized by IR, NMR, mass and elemental analysis

Table 1: Reaction conditions for the preparation of benzo[*h*]naphtho[1,2-*b*][1,6]naphthyridines 7-9a,b.



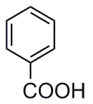
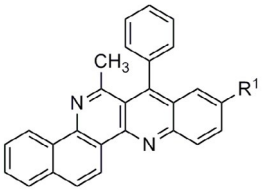
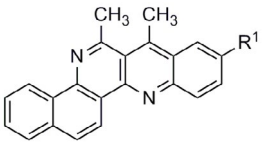
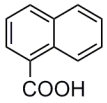
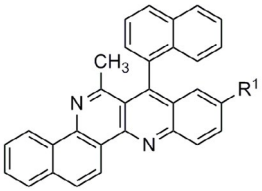
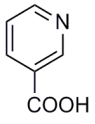
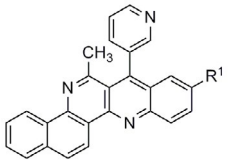
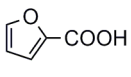
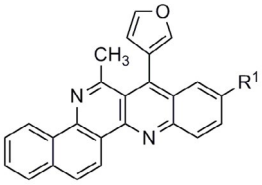
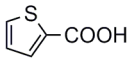
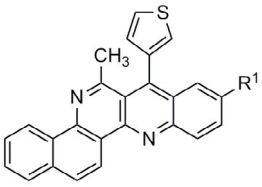
11, 12a) $R^1 = CH_3$; b) $R^1 = Cl$

Scheme 2: Synthesis of the intermediate compounds (12).



13 - 18a) $R^1 = CH_3$,
b) $R^1 = Cl$,
$R^2 = CH_3, C_6H_5, C_{10}H_7, C_5H_4N, C_4H_3O, C_4H_3S$

13a,b - 18a,b

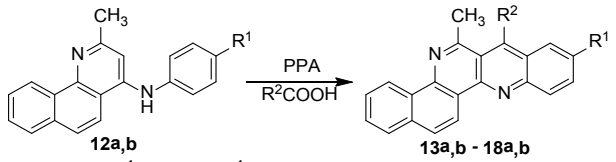
Compound	Acid	Product[a]	t (h)	T (°C)
13a,b			3	160
14a,b	CH_3COOH		3	160
15a,b			3	160
16a,b			1	110
17a,b			1	110
18a,b			1	110

[a]-The products were characterized by IR, NMR, mass and elemental analysis

Table 2: Reaction conditions for the preparation of benzo[*b*]naphtho[1,2-*h*][1,6]naphthyridines 13a,b-18a,b.

effective anticancer drug was taken as a reference to compare the cytotoxicity of the synthesized molecules. After careful examination of the results obtained, it is interesting to that both the isomers showed different activities in different cell lines as anticipated. Furthermore it also evident from the Tables 3 and 4 that in all the cases the chlorine derivative is more active than its methyl counterpart which clearly depicts the importance of electron withdrawing groups at 2nd position of benzo[*h*]naphtho[1,2-*b*][1,6]naphthyridine and 9th position of benzo[*b*]naphtho[1,2-*h*][1,6]naphthyridine for its cytotoxicity. The results from *in vitro* activities of both the isomers were compared (Tables 3 and 4).

The precursors for the cyclization 3a (quinoline moiety and its 4th position was substituted by naphthyl amine) and 12a (benzoquinoline core moiety and its 4th position was substituted with aniline derivatives) showed good activity against all the four cell lines with IC_{50} range of 3.62-17.80 μM and 28.77-49.20 μM (Entry 1 in Tables 3 and 4) respectively. Very interestingly, incorporation of chlorine in 3a *i.e.,* compound 3b (Entry 2 in Table 3) showed very good activity with IC_{50} values of 1.05, 11.65 μM against HeLa and Hep-G2 cell lines which are 10 and 2 fold more active than adriyamycin (11.52 and 21.73 μM) whereas for K562 and MCF7 cell lines its IC_{50} values are 7.23 and 6.63 μM respectively, which is comparable with positive control ADR (8.71



12a,b → **13a,b - 18a,b**

12, 13-18a) $R^1 = CH_3$; b) $R^1 = Cl$
$R^2 = CH_3, C_6H_5, C_{10}H_7, C_5H_4N, C_4H_3S, C_4H_3O$

Scheme 3: Benzo[b]naphtho[1,2-h][1,6]naphthyridines13a,b - 18a,b.

Entry	Cpds	K562[a]	MCF7[b]	Hep-G2[c]	HeLa[d]
1	3a	17.20	9.17	17.80	3.62
2	3b	7.23	6.63	11.65	1.05
3	4a	>100	>100	>100	>100
4	4b	>100	>100	>100	>100
5	5a	>100	>100	>100	>100
6	5b	>100	95.48	90.68	>100
7	6a	74.94	77.29	>100	>100
8	6b	66.42	70.56	68.23	>100
9	7a	49.01	18.06	29.09	41.25
10	7b	42.18	12.33	24.66	32.11
11	8a	29.66	14.22	14.41	20.53
12	8b	21.86	9.03	10.76	13.82
13	9a	15.85	11.44	7.93	6.21
14	9b	7.01	5.93	6.86	1.41
15	ADR	8.71	9.93	21.73	11.52

[a] **K562** (human leukaemia cancer cell line), [b] **MCF7** (human breast cancer cell line),
[c] **Hep-G2** (human liver cancer cell line), [d] **HeLa** (human cervical cancer cell line)

Table 3: Cytotoxicity of compounds **3-9** (IC_{50} in μM).

Entry	Cpds	K562[a]	MCF7[b]	Hep-G2[c]	HeLa[d]
1	12a	49.20	28.77	30.33	37.3
2	12b	36.81	25.18	24.17	28.77
3	13a	>100	>100	>100	>100
4	13b	>100	>100	>100	>100
5	14a	>100	>100	>100	>100
6	14b	>100	>100	>100	>100
7	15a	72.22	58.45	67.05	NA
8	15b	53.55	50.54	50.47	NA
9	16a	52.52	45.12	40.54	40.11
10	16b	48.24	43.19	35.54	36.42
11	17a	40.09	35.37	27.77	24.22
12	17b	37.88	34.19	22.22	20.77
13	18a	33.11	18.09	21.56	17.87
14	18b	30.8	13.68	17.85	14.91
15	ADR	8.71	9.93	21.73	11.52

[a] **K562** (human leukaemia cancer cell line), [b] **MCF7** (human breast cancer cell line),
[c] **Hep-G2** (human liver cancer cell line), [d] **HeLa** (human cervical cancer cell line)

Table 4: Cytotoxicity of compounds **12-18** (IC_{50} in μM).

and 9.93 μM). Similarly, 12b (chlorine derivative) is more active than its methyl derivative compound 12a, (Entries 1 and 2 in Table 4). The overall comparision of intermediates depicts that 3b is more potential than 12b which is pictorially represented in Figure 2.

Substitution of methyl, phenyl substituents at 7th position (Entries 3-6 in Tables 3 and 4) did not give beneficial results in both the isomers (4a,b-5a,b and 13a,b-14a,b), whereas increasing the hydrophobicity from phenyl to naphthyl, increases the activity marginally in both isomers (Entries 7, 8 in Tables 3 and 4).

As evident from the Tables 3 and 4 (Entries 9-14), a clear trend was found in cytotoxicity when the substitution at 7th position containing pyridine moiety (7a,b and 16a,b) was replaced by furan ring (8a,b and 17a,b) which in turn is replaced by thiophene moiety (9a,b and 18a,b). Compound 7a derived from pyridine carboxylic acid showed moderate anticancer activity (IC_{50} value range 18.06-49.01 μM) and the activity further increases marginally by a chlorine substitution at 2nd position (7b). Compound 7b showed better activity against MCF7 with IC_{50} value of 12.33 μM, moderate activity against Hep-G_2 and HeLa cell lines with IC_{50} values 24.66 and 32.11 μM respectively, displayed least active against K562 with IC_{50} value of 42.18 μM. In case of its isomeric compounds *i.e.,* compound (16a,b) showed moderate activity towards all the four cell lines with the IC_{50} values in the range of 35.54 to 52.52 μM.

Interestingly when pyridine carboxylic acid is replaced by furan and thiophene carboxylic acids the activity shoots up steeply. For compounds 8a,b similar pattern was observed that chloro substituted compound 8b was more active than methyl substituted compound 8a as mentioned earlier. Compound 8b displayed stronger cytotoxicity against MCF7 and Hep-G2 cell lines with IC_{50} values 9.03 and 10.76 μM. It showed almost equipotent activity with the control against MCF7 and tenfold more active against Hep-G2 cancer cell. For K562 and HeLa cancer lines 8b showed moderate activity. Its isomeric compound 17b showed moderate activity against all the four cell lines. To our delight, thiophene substituted isomers, (9a,b and 18a,b) showed the best anti-proliferative activity among the compounds screened in this study. Among them 9b is the most outstanding compound which showed highest range of activity in this series with IC_{50} values 7.01, 5.93, 6.86 and 1.41 μM against K562, MCF7, Hep-G2 and HeLa cell lines which were 15 fold active than standard against Hep-G2 and 10 fold more active against HeLa cancer cell lines. Its isomeric compound 18b also showed excellent activity against Hep-G2 cancer cell line with an IC_{50} value 17.85 which was four fold active when compared to standard and showed significant activity against MCF7 and HeLa cell lines with IC_{50} 13.68 and 14.91 and least activity against K562 cell line. This observation strongly support that the presence of thiophene moiety [31,32] enhance the cytotoxic activity. By comparing the two isomers, various substituted benzo[*h*]naphtho[1,2-*b*][1,6] naphthyridine series (4-9) were more active than its isomeric various substituted benzo[*b*] naphtho[1,2-*h*][1,6] naphthyridines (13-18) and is pictorially depicted in Figure 3.

From the present study, it is clear that substitution at 7th position of benzo[*h*]naphtho[1,2-*b*][1,6] naphthyridine and its isomeric benzo[*b*]naphtho[1,2-*h*][1,6]naphthyridine derivatives is one of the potential sites to derivatize and could be instrumental in achieving good cytotoxicity. In this study, hetero substituted compounds showed excellent activity than aliphatic and aromatic substituted compounds. Among the hetero substituted compounds, compound containing thiophene moiety displayed highest activity than furan and pyridine substituted benzo[*h*]naphtho[1,2-*b*][1,6] naphthyridines and benzo[*b*] naphtho[1,2-*h*][1,6]naphthyridines (Figure 4). In general electron withdrawing [33] chloro group enhances the anticancer activity of all the tested compounds when compared to electron donating group methyl group.

Conclusion

In conclusion, We have synthesized 7-substituted benzo[*h*] naphtho[1,2-*b*][1,6]naphthyridines 4-9 and its isomeric benzo[*b*] naphtho[1,2-*h*][1,6]naphthyridines 13-18 where the substituents hold alkyl, aryl and hetero moieties and successful screened for anticancer against four cancer cell lines (K562, MCF7, Hep-G2 and HeLa). The structure-activity relationship study revealed that benzo[*h*]naphtho[1,2-*b*][1,6]naphthyridine 4-9 series showed good cytotoxicity compared to its isomeric benzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine derivatives 13-18. The intermediate compound 3b bearing chloro group and the compound 14 holds chloro group and thiophene moiety in naphthyridine nucleus turns out to be the best candidate in the series screened. All the above results indicates that these new compounds represent useful templates for development of new anticancer agents.

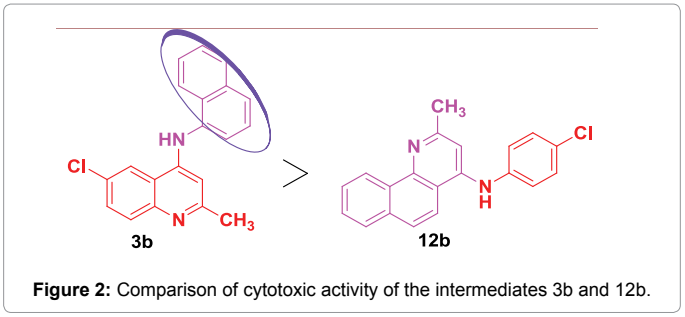


Figure 2: Comparison of cytotoxic activity of the intermediates 3b and 12b.

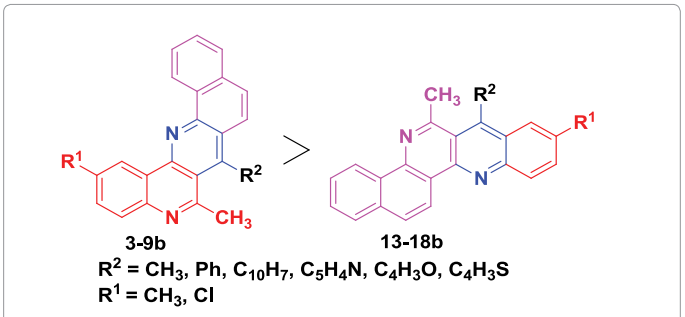


Figure 3: Benzo[*h*]naphtho[1,2-*b*][1,6] naphthyridine showing better cytotoxicity than its isomer benzo[*b*]naphtho[1,2-*h*][1,6] naphthyridine.

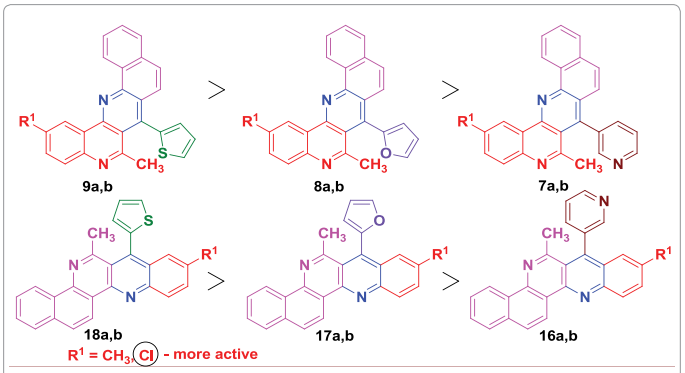


Figure 4: Influence of hetero substituents on cytotoxicity.

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