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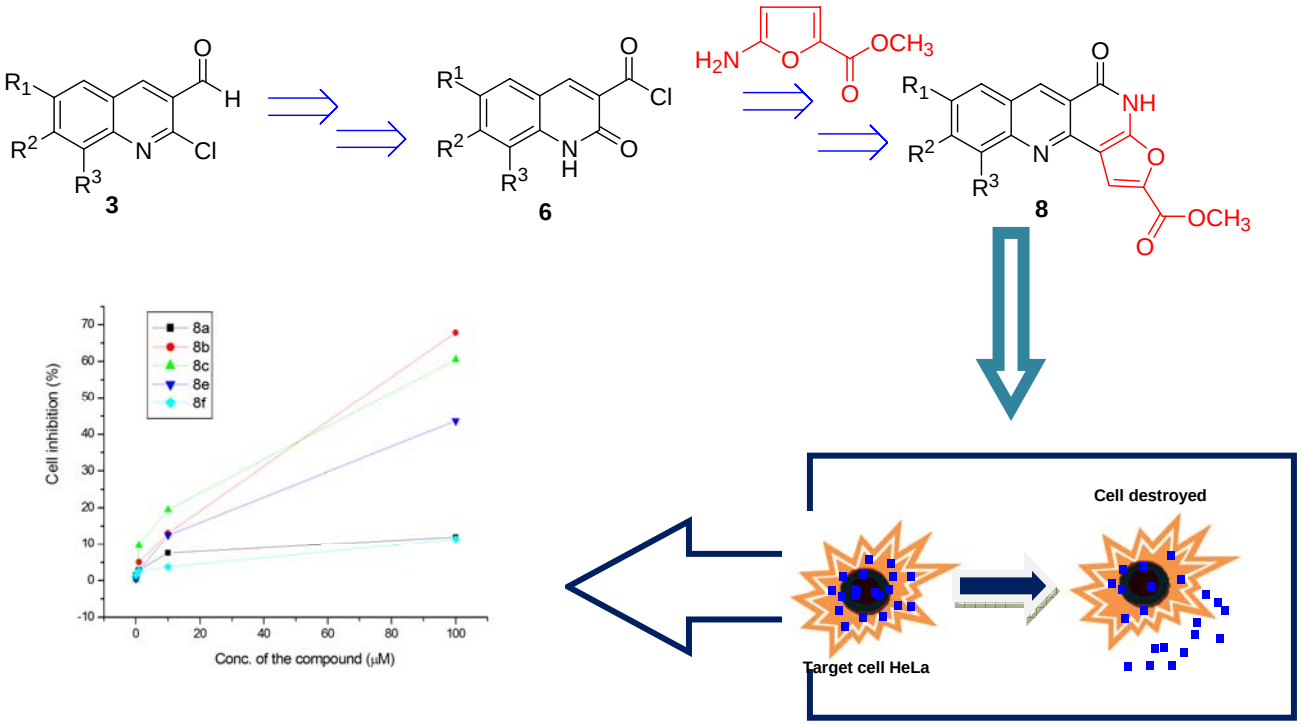
Research article

SYNTHESIS AND CELL LINE ACTIVITY OF ANGULAR BENZOFURO FUSED NAPHTHYRIDINES

Arumugam Murugesh, A. M. Magesh Selvakumar and Subramanian Parameswaran Rajendran*

School of Chemical Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India -641 046
E mail: rajendransp@yahoo.com

ABSTRACT: The present work describes the preparation of novel compounds based on the structure of 2-substituted benzo[*b*]-furo[2,3-*h*][1,6]napthyridin-5[4*H*]-one (**10**). Their methyl and methoxy derivatives were synthesized and their cytotoxicity evaluated against a cancer cell line HeLa. The analogue bearing methyl group on the system is three times more potent than the methoxy group. One could suggest that the methyl group activates the inhibitor's site. These results provide new information on the structure activity relationship of these naphthyridine based systems.

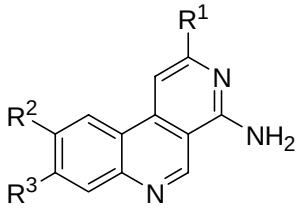


Key words*:* Naphthyridine; Cytotoxic activity; Benzo furo naphthyridine; 2-chloro-3-formyl quinoline.

INTRODUCTION

Cancer is a term encompassing a large assembly of diseases that can affect body organs. One describing feature of cancer is the rapid formation of abnormal cells that grow past their usual boundaries thus invading adjacent parts of the body and/or spreading to other distant organs through metastasis.(Anand *et al.,* 2008) (Moscow *et al.,* 2007) (Thun, 2007). Recently, most of the organic compounds have been explored against pathogenic bacteria and cancer cell lines. Especially nitrogen containing heterocyclic compounds increase the potency against the bacteria and cell lines. A number of important quinoline derivatives have been known to be good antimalarials, anticonvulsants (Wibbererley[a], 1975), anti bacterials (Wibbererley[b], 1975), antiamoebic agents(Lowe, 1984), antihelmintic agents (Albercht, 1921), anti virals (Crumplin *et al.,* 1980), leukotriene analog in antagonists(Nistigaki *et al.,* 1976) etc.

Our napthyridine systems namely benzo [*c*][2,7]naphthyridine (**1**) (Kyung-Hee Kim *et al.,* 2009) and dibenzo[*c,f*][2,7]naphthyridine (**2**) (Kobayashi *et al.,* 1962) also display enhanced the activity towards cancer cell lines and possess bacterial properties. These studies were further developed by introducing of new heterocyclic systems with the naphthyridine moiety which improve their power. Keeping all these pharmacological profiles in mind, we have identified new substituted hetero fused benzo [1,6] naphthyridines. A number of 1,6 naphthyridines that have been prepared in our laboratory were screened against several bacterial species, and they display moderate cytotoxic activity. This finding prompted us to develop analogues of 1,6-naphthyridine compounds because their basic structures were novel as antitumor agents. For this consideration, chemical modification was carried out on the C_7 and C_8 positions in addition to conversion of the benzo 1,6 naphthyridine ring into other fused benzo furo1,6 naphthyridine (**8**) ring system.



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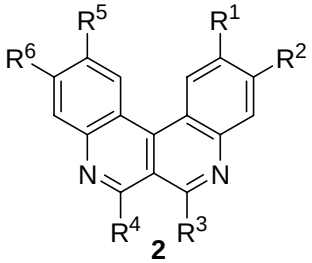

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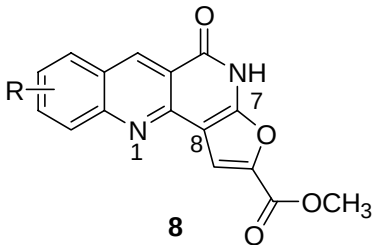

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RESULTS AND DISCUSSION

2-substituted benzo[*b*]-furo[2,3-*h*][1,6]napthyridine 5[4*H*]-ones (8a-f) were synthesized from the corresponding 3-(2-substituted-5-furamido)quinolin-2[1*H*]- -ones (7a-f) which were prepared from the reaction of 2-oxo quinolin-3-carbonyl chlorides (6a-f) and 5-amino-2- methyl furoate (Bianco *et al.,* 2005). The 2-oxo quinolin-3-carbonyl chlorides (6a-f) were prepared by the following steps. First, the versatile starting material 2-chloro-3-formyl quinolines (3a-f) were converted into 2-oxo quinolin-3-carboxylic acids (5a-f) by oxidation using alkaline $KMnO_4$ through 2-oxo-3-formyl quinolines (4a-f) (Vijayalakshmi *et al*., 1994). Then, having obtained 2-oxo quinolin-3-carboxylic acids (5a-f) were converted to their acid chlorides (6a-f) by treating with thionyl chloride, which was outlined in the scheme. The obtained acid chloride (6b) was taken in dry benzene and refluxed with 5-amino -2- methyl furoate (350 mg, 2.41 mmol) for 2-4 h in water bath. The highly active 6-methyl, 2-oxo quinolin-3-carbonyl chloride (6b) was substituted by amine group of 5-amino -2- methyl furoate and leading to the formation of 3-(2-carbomethoxy-5-furamido)-6-methyl quinolin[1*H*]-2-one (7b) which was chromatographed over silica gel using petroleum ether: ethyl acetate (98:2) (v/v) as eluent which yielded yellow colored compound (150 mg, 43.6%), mp 275-276 °C. ν_{max} /cm^{-1} showed absorption peaks at 1732s for CO in ester, 1632w , 1628w for two -CONH- , 3136w for NH. In ^{1}H NMR (DMSO-d_6) showed at δ 2.4 for methyl, 3.8 for ester group, 7.0 (1H, d, C_4'-H, *J*=8Hz), 7.24 (1H, d, C_3'-H, *J*=8 Hz), 7.69 (1H, d, C_7-H, *J*=8 Hz), 7.9 (1H, d, C_8-H, *J*=8 Hz), 8.47 (1H, s, C_5-H), 8.92 (1H, s, C_4-H), 11.94 and 13.08 for two –NH. The elemental analysis also confirmed the molecular formula of the compound to be $C_{17}H_{14}N_2O_5$. Then, the of 3-(2-corbamethoxy) furamido 6-methyl quinolin 2[1*H*]-one (7b) (310 mg, 0.96 mmol) was heated with polyphosphoric acid (prepared by mixing 1.8 parts by weight of P_2O_5 and 1 part by weight of H_3PO_4) at 150 °C for five hours to provide targeted compound 2-carbamethoxy, 8-methyl benzo[*b*]-furo[2,3-*h*][1,6]napthyridine 5[4*H*]-one (8b) which was chromatographed over silica gel by using petroleum ether – ethyl acetate as eluent, gave a light brown crystals (150 mg, 43.6%), mp 145 °C.

The IR spectrum ν_{max} /cm^{-1} showed absorption peaks at1720 for $COOCH_3$, 1668 for NHCO , 3427 (-NH). In the ^{1}HNMR spectrum (DMSO-d_6, 500 MHz) showed peaks at 2.34 (3H, s, C_8-CH_3), 3.780 (3H, s, C_2-CO-OCH_3), 8.42(1H, s, C_6-H), 7.59 (1H, s, C_7-H), 7.23 (1H, d, C_9-H, *J* = 8.5 Hz), 7.43 (1H, d, C_{10}-H *J* = 8.5 Hz). The disappearance of two doublets appeared in 7b at δ 7.24 (C_3'-H) & δ 7.0 (C_4'-H) and appearance of a singlet at 7.23 for C_3'-H confirmed at the cyclisation occurred. The disappearance of one NH peak and the mass spectrum gave the peak at (m/z) 308 also confirmed the formation of targeted compound (8b).

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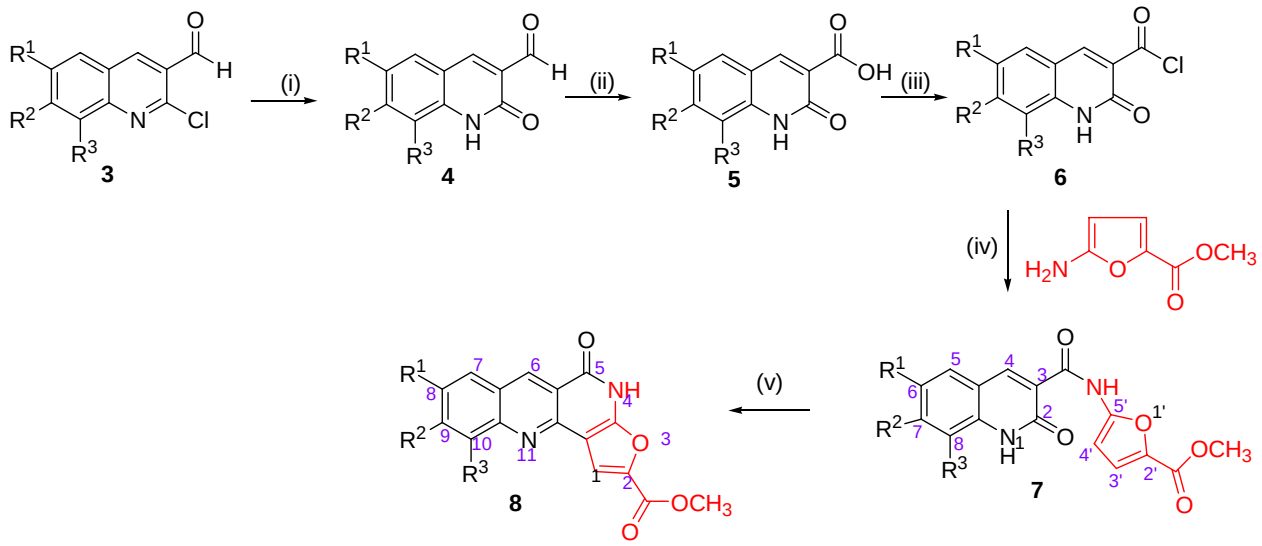
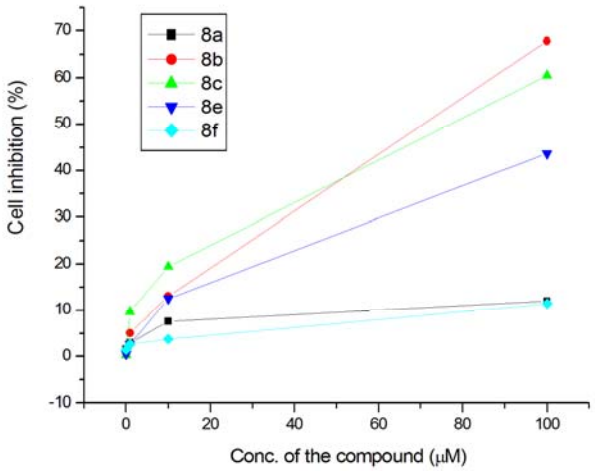


Scheme : (i) HCl (ii) alk. $KMnO_4$ iii) $SOCl_2$ (iv) 10 ml dry benzene (v)PPA

a) R^1 = H, R^2 = H, R^3 = H ; b) R^1 = CH_3, R^2 = H, R^3 = H ; c) R^1 = H, R^2 = CH_3, R^3 = H ; d) R^1 = H, R^2 = H, R^3 = CH_3 ; e) R^1 = OCH_3, R^2 = H, R^3 = H ; f) R^1 = H, R^2 = H, R^3 = OCH_3 ;

Pharmacology

Cytotoxicity studies of the compounds (8a-c, e and f) were carried out on human cervical cancer cells (HeLa) which were obtained from National Centre for Cell Science, Pune, India. Cell viability was carried out using the MTT assay method (Lown *et al.,* 1982). All the cancer cells were grown in Eagles minimum essential medium containing 10% fetal bovine serum (FBS). For the screening experiment, the cells were seeded into 96-well plates in 100 µL of the respective medium containing 10% FBS, at a plating density of 10000 cells/well, and incubated at 37 °C, under conditions of 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to the addition of compounds. The compounds were dissolved in DMSO and diluted in the respective medium containing 1% FBS. After 24 hrs, the medium was replaced with the respective medium with 1% FBS containing the compounds at various concentrations and incubated at 37 °C under conditions of 5% CO_2, 95% air, and 100% relative humidity for 48 hrs. Triplication was maintained, and the medium not containing the compounds served as the control. After 48 h, 10 µL of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4 hrs. The medium with MTT was then flicked off, and the formed formazan crystals were dissolved in 100 µL of DMSO. The absorbance was then measured at 570 nm using a microplate reader. The percentage of cell inhibition was determined using the formula, % inhibition = [mean OD of untreated cells (control)/mean OD of treated cells (control)] × 100 and a graph was plotted with the percentage of cell inhibition versus concentration. From this, the IC_{50} value was calculated.



In this study, we investigated the cytotoxic properties of the selected test compounds (8a-c, e, and f), there was a strong activity observed against HeLa for 2-carbamethoxy-8-methyl benzo [*b*]-furo [2,3-*h*][1,6]naphthyridine-5[4*H]*-one (8b) and 2-carbamethoxy-9-methyl benzo [*b*]-furo [2,3-*h*][1,6]naphthyridine-5[4*H]*-one (8c). 2-carbamethoxy-8-methoxy benzo [*b*]-furo [2,3-*h*][1,6]naphthyridine-5[4*H]*-one (8e) was moderate activity against HeLa. The 2-carbamethoxy benzo [*b*]–furo[2,3-*h*] [1,6]naphthyridine-5[4*H*]-one (8a) and 2-carbamethoxy-10-methoxy benzo [*b*]-furo [2,3-*h*][1,6]naphthyridine-5[4*H]*-one (8f) were no significant activity observed against the HeLa cell line. From these results, methyl derivatives were more cell inhibitions against the HeLa cell line than methoxy and parent derivatives.

Experimental Section

Preparation of 3-(2-Carbamethoxy furamido quinoline) 2[2H]-one compound (7a-f)

General procedure

The 2-oxo quinolin-3-carbonyl chloride (**6**) was taken in 10 mL dry benzene and of 5-amino-2-mehtyl furoate (2.41 mmol) was added and refluxed for 2-4 hrs on a water bath. After the completion of reaction, the reaction mixture was poured into crushed ice, filtered, dried and chromatographed over silica gel using petroleum ether: ethyl acetate (98:2) (v/v) as eluent, which yielded yellow colored compound. It was recrystallised from absolute methanol.

3-(2-carbamethoxy furamido quinoline) 2[1*H*]-one (7a).Yield 42%; mp 295 ^{o}C; found C, 61.00; H, 3.25; N, 8.68. $C_{16}H_{12}N_2O_5$ requires C, 61.54; H 3.87; N, 8.97%; υ_{max}/cm^{-1} 1732s, 1631w, 1628w, 3426w; δ_H (500 MHz, DMSO-d_6) 3.8 (3H, s, OCH_3 ester), 7-8 (m, 6H, C_4, C_5, C_7, C_8, C_3',C_4'-H), 8.95 (1H, s, C_4-H), 11.90 (1H, s, -NH), 12.65 (1H, s, -NH);

3-(2- carbamethoxy furamido) 6-methlyl quinoline 2[1*H*]-one (7b).Yield 43.33%; mp 275 ^{o}C; found C, 62.00; H, 4.15; N, 8.01. $C_{17}H_{14}N_2O_5$ requires. C 62.57, H 4.32, N 8.59; υ_{max}/cm^{-1}1732s, 1631w, 1628w, 3426w; δ_H (500 MHz, DMSO-d_6) 2.35 (3H,s, C_6-CH_3), 3.80 (3H,s,C_2'-OCH_3, ester), 7.0 (1H,d,C_4'-H, *J*=8 Hz), 7.24 (1H,d,C_3'-H,*J*=8 Hz), 7.69 (1H,d C_7-H, *J*=8 Hz), 7.9 (1H,d,C_8-H, *J*=8 Hz), 8.47 (1H,s,C_5-H), 8.92 (1H,s,C_4-H), 11.94 (1H,s, -NH), 13.08 (1H,s,-NH).

3-(2- carbamethoxy furamido) 7-methlyl quinoline 2[1*H*]-one (7c). Yield 43 %; mp 307 ^{o}C; found C, 62.23; H, 4.19; N, 8.21. $C_{17}H_{14}N_2O_5$ requires . C, 62.57; H, 4.32; N, 8.59. υ_{max}/cm^{-1} 1712s, 1650w, 1242w, 3415w. δ_H (500 MHz, DMSO-d_6) 2.5 (3H,s, C_7-CH_3), 3.8 (3H,s,C_2'-OCH_3, ester), 7.2 (1H,d,C_4'-H, *J*=7.8 Hz), 7.28 (1H,d,C_3'-H,*J*=8 Hz), 7.60 (1H,d C_5-H, *J*=7.5 Hz), 7.85 (1H,d,C_5-H, *J*=8 Hz), 8.55 (1H,s,C_8-H), 8.90 (1H,s,C_4-H), 11.60 (1H,s, -NH), 13.20 (1H,s,-NH).

3-(2- carbamethoxy furamido) 8-methlyl quinoline 2[1*H*]-one (7d). Yield 43.5 %; mp 317 ^{o}C; found C, 62.12; H, 4.09; N, 8.01. $C_{17}H_{14}N_2O_5$ requires C, 62.57; H, 4.32; N, 8.59. υ_{max}/cm^{-1} 1708s, 1657w, 1240w, 3405w; δ_H (500 MHz, DMSO-d_6) 2.5 (3H,s, C_8-CH_3), 3.7 (3H,s,-OCH_3, ester), 7.32-7.60 (4H, m, C_5, C_6, C_7, C_3'-H), 7.80 (1H,d,C_2'-H, *J*= 7.6 Hz), 8.91 (1H,s,C_4-H), 11.65 (1H,s, -NH), 13.30 (1H,s,-NH).

3-(2- carbamethoxy furamido) 6-methoxy quinoline 2[1*H*]-one (7e). Yield 60 %; mp 315 ^{o}C; found C, 59.00; H, 4.09; N, 8.00. $C_{17}H_{14}N_2O_6$ requires C, 59.65; H, 4.12; N, 8.18. υ_{max}/cm^{-1} 1698w, 1655w, 1202s, 3505w; δ_H (500 MHz, DMSO-d_6) 3.4 (3H,s, C_6-OCH_3), 4.2 (3H,s,-OCH_3, ester), 7.20 (1H,d,C_4'-H, *J*=7.8 Hz), 7.24 (1H,d, C_3'-H, *J*= 8 Hz), 7.65 (1H,d C_7-H, *J*= 7.5 Hz), 7.94 (1H,d,C_8-H, *J*=7.9 Hz), 8.37 (1H,s,C_5-H), 8.79 (1H,s,C_4-H), 11.25 (1H,s, -NH), 12.80 (1H,s,-NH).

3-(2- carbamethoxy furamido) 8-methoxy quinoline 2[1*H*]-one (7f). Yield 61 %; mp 290 °C; found C, 59.00; H, 4.00; N, 8.00. $C_{17}H_{14}N_2O_6$ requires C, 59.65; H, 4.12; N, 8.18. υ_{max}/cm^{-1} 1732s, 1631w, 3512w; δ_H (500 MHz, DMSO-d_6) 3.5 (3H,s, C_8-OCH_3), 4.0 (3H,s,-OCH_3, ester), 7.32-7.60 (4H, m, C_5, C_6, C_7, C_3'-H), 7.72 (1H,d,C_2'-H, *J*=8 Hz), 8.90 (1H,s,C_4-H), 11.20 (1H,s, -NH), 13.20 (1H,s,-NH).

Preparation of 2-Carbamethoxy benzo [b]furo [2,3-h] [1,6] napthyridine 5[4H] –one (8)

General procedure

310 mg (0.96 mmol) of 3-(2-carbamethoxy furamido quinoline 2[1*H*]-one compound was heated with polyphosphoric acid at 150 °C for 5 hours. After the completion of the reaction, the reaction mixture was poured in to crushed ice, filtered and chromatographed over silica gel by using petroleum ether – ethyl acetate as eluent. It was recrystallised from ethyl acetate gave a light brown color crystals.

2-Carbamethoxy benzo [*b*]–furo[2,3-*h*] [1,6]naphthyridine-5[4*H*]-one (8a). Yield 40%; mp 195°C; found C, 65.05; H, 3.11; N, 9.50. $C_{16}H_{10}N_2O_4$ requires C, 65.31; H, 3.43; N, 9.52. υ_{max}/cm^{-1} 1720s, 1652w, 3173w; δ_H (500 MHz, DMSO-d_6) 3.9 (3H,s,C_2-C=O-OCH_3), 8.53 (1H, s, C_6-H), 7.2- 7.5 (4H, m, C_7, C_8, C_9, C_{10}), 7.63 (1H,s, C_1-H), 12.06 (1H, s, -NH).

2-Carbamethoxy8-methyl benzo [*b*]–furo[2,3-*h*][1,6]naphthyridine-5[4*H*]-one (8b). Yield 43.6%, mp 145 °C; found C, 66.05; H, 3.11; N, 9.00. $C_{17}H_{12}N_2O_4$ requires C, 66.23; H, 3.92; N, 9.09; υ_{max}/cm^{-1} 1720s, 1668w, 3426w; δ_H (500 MHz, DMSO-d_6) 2.35 (3H, s, C_8-CH_3), 3.80 (3H,s,C_2-C=O-OCH_3), 8.42 (1H, s, C_6-H), 7.59(1H,s, C_7-H), 7.24 (1H,d, C_9-H *J*=8.5 Hz), 7.44 (1H,d, C_{10}-H, *J* 6.5 H_Z), 11.96 (1H, s, -NH); Mass (IE^+) M^+ : 308.

2-Carbamethoxy9-methyl benzo [*b*]-furo [2,3-*h*][1,6]naphthyridine-5[4*H*]-one (8c). Yield 44 % ; mp 196 °C; found C, 66.00; H, 3.05; N, 9.00. $C_{17}H_{12}N_2O_4$ requires C, 66.23; H, 3.92; N, 9.09; υ_{max}/cm^{-1} 1732, 1631, 3431; δ_H (500 MHz, DMSO-d_6) 2.30 (3H, s, C_9-CH_3), 3.78 (3H,s,C_2- OCH_3, ester), 8.6 (1H, s, C_6-H), 7.52(1H,d, C_7-H, *J*=8 Hz), 7.30 (1H,d, C_8-H, *J*=8 Hz), 7.45 (1H,s, C_{10}-H) 11.92 (1H, s, -NH).

2-Carbamethoxy10-methyl benzo [*b*]-furo[2,3-*h*][1,6]naphthyridine-5[4*H*]-one (8d). Yield 44 %; mp 210 °C; found C, 66.07; H, 3.55; N, 8.91. $C_{17}H_{12}N_2O_4$ requires C, 66.23; H, 3.92; N, 9.09; υ_{max}/cm^{-1} 1735s, 1643w, 1196w, 3178w; δ_H (500 MHz, DMSO-d_6) 2.30 (3H, s, C_{10}-CH_3), 3.80 (3H,s,C_2- OCH_3, ester), 8.54 (1H, s, C_6-H), 7.30-7.52(3H,m, C_7-H, C_8-H, C_9-H), 11.86 (1H, s, -NH).

2-Carbamethoxy8-methoxy benzo [*b*]-furo[2,3-*h*][1,6]naphthyridine-5[4*H*]-one (8e). Yield 42 %; mp 252 °C; found C, 62.45; H, 3.35; N, 8.40. $C_{17}H_{12}N_2O_5$ requires C, 62.96; H, 3.73; N, 8.64. υ_{max}/cm^{-1} 1720s, 1668w, 1213s,1505w, 3437w; δ_H (500 MHz, DMSO-d_6) 3.62 (3H, s, C_8-OCH_3), 3.80 (3H,s,C_2-OCH_3,ester), 8.45 (1H, s, C_6-H), 7.64(1H,s, C_7-H), 7.28 (1H,d, C_9-H *J*=8.5 Hz), 7.45 (1H,d, C_{10}-H *J*=6.5 H_Z), 11.98 (1H, s, -NH).

2-Carbamethoxy10-methoxy benzo [*b*]-furo [2,3-*h*][1,6]naphthyridine-5[4*H*]-one (8f). Yield 42 %; mp 275°C; found C, 62.25; H, 3.15; N, 8.00. $C_{17}H_{12}N_2O_5$ requires C, 62.96; H, 3.73; N ,8.64; υ_{max}/cm^{-1} 1720s, 1668w, 1213s, 1505w, 3437w; δ_H (500 MHz, DMSO-d_6) 2.25 (3H, s, C_{10}-CH_3), 3.60 (3H,s,C_2- OCH_3, ester), 8.50 (1H, s, C_6-H), 7.30-7.50(3H,m, C_7-H, C_8-H, C_9-H), 11.24 (1H, s, -NH).

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