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RESEARCH ARTICLE

Anticancer and immunomodulatory activities of novel 1,8-naphthyridine derivatives

Vivek Kumar[1,3], Alka Madaan[2], Vinod K. Sanna[2], Manupriya Vishnoi[2], Narendra Joshi[1], Anu T. Singh[2], Manu Jaggi[2], Pramod K. Sharma[3], Raghuveer Irchhaiya[3], and Anand C. Burman[1,2]

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Abstract

A number of 1,8-naphthyridine derivatives (**22–62**) have been synthesized and screened for their *in vitro* cytotoxicity against eight tumors and two non-tumor cell lines. Halogen substituted 1,8-naphthyridine-3-caboxamide derivatives showed potent activity with compound **47** having IC_{50} of 0.41 and 0.77 μM on MIAPaCa and K-562 cancer cell lines, respectively while, compound **36** had IC_{50} of 1.19 μM on PA-1 cancer cell line. However, one of the unsubstituted 1,8-naphthyridine-C-3′-heteroaryl derivative **29** showed potent cytotoxicity with IC_{50} of 0.41 and 1.4 μM on PA-1 and SW620 cancer cell lines, respectively. These compounds were also evaluated for anti-inflammatory activity as suggested by downregulation of proinflammaotory cytokines.

Keywords: *Anticancer; anti-inflammatory; naphthyridine*

Introduction

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Recently 1,8-naphthyridine is being exploited in cancer chemotherapy like SNS-595 (Figure 1), which is in second phase of clinical trial [1–4]. In our efforts to find out a potent molecule, we have modified the C-3 carboxylic acid of 1,8-naphthyridine with different non-conventional functionalized amino acids, which were synthesized "in house" to afford 1,8-naphthyridine-3-carboxamide derivatives (22–62) [5].

Mammalian Topoisomerase II is one of the known target for antitumor agents like doxorubicin, etoposide, ellipticine and amascrine [6]. 1,8-Naphtyridine derivatives were found to display moderate cytotoxic activity against murine P388 leukemia, when changes were carried out at N-1 and C-7 position [3,4]. We have carried out further changes at C-3 position and synthesized 1,8-naphthyridine-3-carboxamide derivatives (22–62), and tested them against different cancer cell lines. These compounds have shown promising anticancer activities and were further tested for their potential anti-inflammatory activity based on the molecular link between cancer and inflammation [7–9]. An *in vitro* septic shock assay based on murine bone marrow-DCs has been used to evaluate potential anti-inflammatory activity as indicated by resultant down regulation of various pro-inflammatory cytokines.

Materials and methods

All the solvents and reagents were purchased from Aldrich, Lancaster or Across & Rankem and were used as supplied. All TLC data (R_f values) were determined on aluminum sheets coated with silica gel 60 F_{254} (Merck) and visualization was achieved with UV light and iodine vapors. Column chromatography was performed using silica gel (100–200 mesh) and the synthesized compounds were characterized using ^{1}H NMR and mass spectroscopy. Proton Magnetic Resonance (^{1}H NMR) spectra were recorded on a Bruker 300 MHz instrument using tetramethylsilane (TMS) as an internal standard and mass spectra were recorded on a Micromass Quattro Micro™ instrument. The purity of the synthesized compounds was determined on a Shimadzu HPLC LC-2010 C HT instrument using a gradient system. Melting points were obtained

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in a capillary tube with a thermal scientific melting point apparatus Mettler Toledo and are uncorrected.

Chemistry

Commercially available, 2-chloro nicotinic acid **1** was reacted with 1,1′-carbonyldiimidazole (CDI), ethyl hydrogen malonate and methyl magnesium bromide in dry THF to afford nicotinoylacetate **2**. Compounds **2** upon treatment with triethyl orthoformate and acetic anhydride followed by reaction with propargyl amine, afforded ethyl nicotinoylacrylate **19**. Further cyclization of the compound **19** using K_2CO_3 in ethyl acetate provided ethyl 1,8-naphthyridine-3-carboxylate (**20**), Compound **20** on acidic hydrolysis resulted in 1,8-naphthyridine-3-carboxylic acids (**21**). The acid **21** was treated with different functionalized amino acids **3–18** (Table 1), prepared in-house, to afford 1,8-naphthyridine-3-carboxamide derivatives **22–62** (Table 2) as shown in Schemes 1 and 2.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopropylcarbamoyl-2-hydroxy-1-

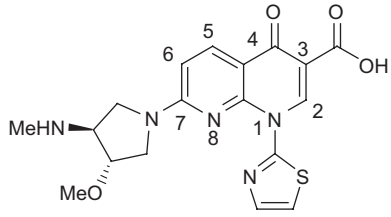
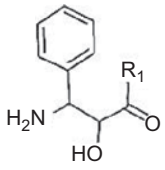


Figure 1. Structure of reference compound.

Table 1. Functionalised amino acid derivatives **(3–18)**.



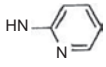
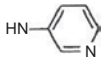
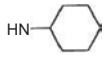
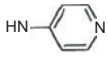
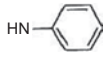
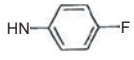
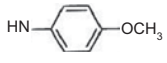
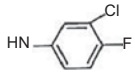
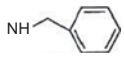
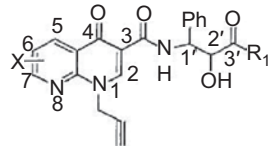
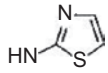
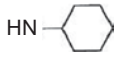
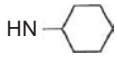
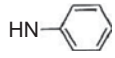
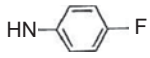
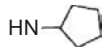
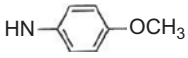
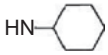
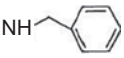
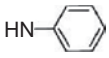
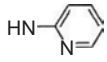
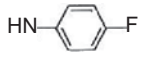
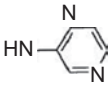
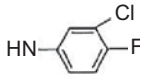
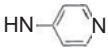
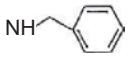
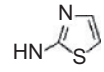
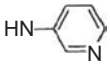
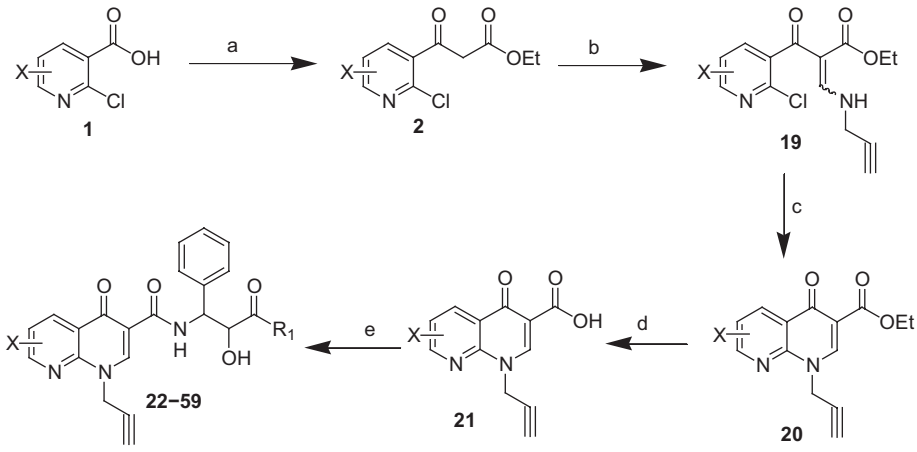
Compound No.	R_1	Compound No.	R_1
3		**11**	
4		**12**	
5		**13**	
6		**14**	
7		**15**	
8		**16**	
9		**17**	
10		**18**	OC_2H_5

Table 2. 1,8-Naphthyridine derivatives **(22–62).**

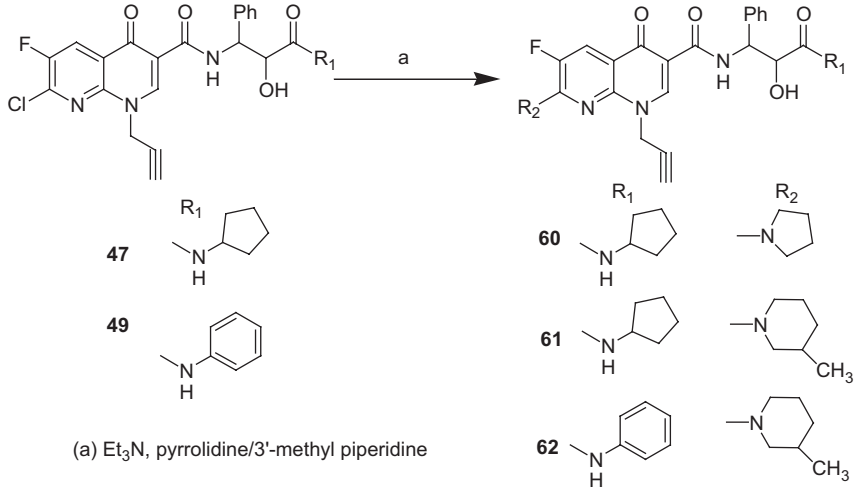


C. No.	R_1	X	C. No.	R_1	X
22		H	**43**		7-Cl
23		H	**44**		7-Cl
24		H	**45**		7-Cl
25		H	**46**		7-Cl
26		H	**47**		6-F, 7-Cl
27		H	**48**		6-F, 7-Cl
28		H	**49**		6-F, 7-Cl
29		H	**50**		6-F, 7-Cl
30		H	**51**		6-F, 7-Cl
31		H	**52**		6-F, 7-Cl
32		H	**53**		6-F, 7-Cl
33		H	**54**		6-F, 7-Cl
34		H	**55**		6-F, 7-Cl
35		7-Cl	**56**		6-F, 7-Cl
36		7-Cl	**57**		6-F, 7-Cl
37		7-Cl	**58**		7-CH_3
38		7-Cl	**59**	OCH_2CH_3	H
39		7-Cl	**60**		6-F, 7-pyrrolidine
40		7-Cl	**61**		6-F, 7-(3″-methylpiperidine)
41		7-Cl	**62**		6-F, 7-(3″-methylpiperidine)
42		7-Cl			



X = H/6-Cl/5-F, 6-Cl/6-CH_3
(a) (1) CDI; (2) $EtOCOCH_2COOH$, MeMgBr; (b) (1) $(EtO)_3CH$, Ac_2O (2) propargylamine; (c) K_2CO_3; (d) aq.HCl; (e) (1)$SOCl_2$ (2) **3–18**

Scheme 1. Synthesis of tested compounds (**22–59**).



Scheme 2. Synthesis of tested compounds (**60–62**).

*phenyl-ethyl)-amide**(22)***. Thionyl chloride (313 mg, 2.6 mmol) was added drop wise to a stirred solution of unsubstituted-1,8-naphthyridine-3-carboxylic acid (**21**, 500 mg, 2.2 mmol) in dichloromethane (30 mL) and catalytic amount of dimethyl formamide (2–4 drops). The stirring was continued for 4h at room temperature. The acyl chloride intermediate formed was dried under vacuum and again diluted with dichloromethane (30 mL). Functionalized amino acid (**3**) was added to it and stirred for 2h. The reaction mixture was diluted with water (30 mL) and extracted with dichloromethane (30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to provide the crude product. The crude product was purified over silica gel (mesh size 100–200) column using 2% MeOH/ DCM as eluent, to furnish compound (**22**).

R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$ + DMSO-d_6) δ 10.62 (d, 1H, *J* = 7.6 Hz), 9.14 (s, 1H), 8.86–8.81 (m, 2H), 7.62–7.44 (m, 4H), 7.33–7.19 (m, 2H), 5.88–5.86 (m, 1H), 5.65 (d, 1H, *J* = 8.5 Hz), 5.39–5.33 (m, 3H), 4.32 (d, 1H, *J* = 5.5 Hz), 2.73–2.72 (m, 1H), 2.66-2.61 (m, 1H, partially merged with DMSO peak), 0.63–0.59 (m, 2H), 0.43–0.41 (m, 1H), 0.34–0.33 (m, 1H); MS (ES+) 431 (M^++H), Yield 507 mg (53.6%), m.p. 152–154°C.

Compounds **23–59** were prepared in a similar to way to compound **22**.

*4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide **(23)***. R_f 0.5 (10 % MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.83 (d, 1H, *J* = 8.2 Hz), 9.14 (s, 1H), 8.84–8.81 (m, 2H), 7.52–7.45 (m, 3H), 7.34–7.22 (m, 3H), 6.69 (d, 1H, *J* = 7.6 Hz), 5.66 (dd, 1H, *J* = 2.8, 8.2 Hz), 5.27 (s, 2H), 4.52–4.49 (m, 1H), 4.31–4.30 (m, 1H), 4.21–4.12 (m, 1H), 2.52 (t, 1H, *J* = 2.2 Hz), 1.91–1.76 (m, 4H), 1.59–1.37 (m, 4H); MS (ES+) 459 (M^++H), Yield 850 mg (84.5%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclohexylcarbamoyl-2-hydroxy-1-phenyl-

ethyl)-amide ***(24)***. R_f 0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.88 (d, 1H, *J* = 8.4 Hz), 9.11 (s, 1H), 8.82 (d, 2H, *J* = 6.5 Hz), 7.50–7.44 (m, 3H), 7.30–7.18 (m, 3H), 6.72 (d, 1H, *J* = 8.5 Hz), 5.69 (dd, 1H, *J* = 2.8, 8.4 Hz), 5.27 (d, 2H, *J* = 2.4 Hz), 4.75 (d, 1H, *J* = 4.5 Hz), 4.51 (bs, 1H), 3.76–3.73 (m, 1H), 2.52 (t, 1H, *J* = 2.4 Hz), 2.10–2.09 (m, 1H), 1.83–1.49 (m, 5H), 1.27–1.08 (m, 4H); MS (ES+) 473 (M^++H) (100), Yield 810 mg (78.1%), m.p. 122–124°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-1-phenyl-2-phenylcarbamoyl-ethyl)-amide ***(25)***. R_f 0.6 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.63 (d, 1H, *J* = 8.8 Hz), 9.73 (s, 1H), 9.13 (s, 1H), 9.02–9.0 (m, 1H), 8.81 (d, 1H, *J* = 6.5 Hz), 7.77–7.66 (m, 3H), 7.51–7.36 (m, 4H), 7.34–7.27 (m, 3H), 7.11–7.06 (m, 1H), 6.46 (d, 1H, *J* = 5.7 Hz), 5.72 (d, 1H, *J* = 8.8 Hz), 5.43 (s, 2H), 4.46 (d, 1H, *J* = 5.7 Hz), 3.55 (1H, merged with water peak); MS (ES+) m/z 466 (M^++H), Yield 312 mg (30.5%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-(4-fluoro-phenylcarbamoyl)-2-hydroxy-1-phenyl-ethyl]-amide ***(26)***. R_f 0.6 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.54 (d, 1H, *J* = 8.9 Hz), 9.78 (s, 1H), 9.05 (s, 1H), 8.94–8.92 (m, 1H), 8.72 (dd, 1H, *J* = 1.7, 7.9 Hz), 7.68–6.61 (m, 3H), 7.42–7.24 (m, 5H), 7.09–7.03 (m, 2H), 6.38 (d, 1H, *J* = 5.7 Hz), 5.63 (d, 1H, *J* = 8.9 Hz), 5.36 (s, 2H), 4.39 (dd, 1H, *J* = 2.1, 5.7 Hz), 3.48 (t, 1H, *J* = 2.3 Hz); MS (ES+) m/z 485 (M^++H), Yield 325 mg (30.6%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-2-(4-methoxy-phenylcarbamoyl)-1-phenyl-ethyl]-amide ***(27)***. R_f 0.4 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.90 (d, 1H, *J* = 8.3 Hz), 9.09 (s, 1H), 8.80–8.76 (m, 2H), 8.67 (s, 1H), 7.49–7.39 (m, 5H), 7.30–7.16 (m, 3H), 6.73 (d, 2H, *J* = 8.8 Hz), 5.81 (dd, 1H, *J* = 2.3, 8.3 Hz), 5.22 (s, 2H), 5.13 (d, 1H, *J* = 5.2 Hz), 4.69–4.66 (m, 1H), 3.71 (s, 3H), 2.50 (t, 1H, *J* = 2.3 Hz); MS (ES+) m/z (relative intensity) 497 (M^++H), (100), Yield 510 mg (46.8%), m.p. 203–205°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-benzylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(28)***. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.56 (d, 1H, *J* = 8.7 Hz), 9.1 (s, 1H), 8.97 (d, 1H, *J* = 3.2 Hz), 8.70 (d, 1H, *J* = 6.9 Hz), 8.33–8.31 (m, 1H), 7.69–7.65 (m, 1H), 7.41–7.25 (m, 5H), 7.06–7.05 (m, 2H), 6.88–6.87 (m, 3H), 6.24 (d, 1H, *J* = 5.7 Hz), 5.62 (d, 1H, *J* = 8.7 Hz), 5.52–5.37 (m, 2H), 4.50–4.42 (m, 1H), 4.28 (d, 1H, *J* = 5.5 Hz), 4.10–4.04 (m, 1H), 3.54 (s, 1H); MS (ES+) m/z 481 (M^++H), Yield 716 mg (67.9%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide ***(29)***. R_f 0.5 (EtOAc); ^{1}HNMR (DMSO-d_6) δ 10.61 (d, 1H, *J* = 9.0 Hz), 9.7 (bs, 1H), 9.0 (s, 1H), 8.93 (d, 1H, *J* = 4.1 Hz), 8.74 (d, 1H, *J* = 7.4 Hz), 8.25 (d, 1H, *J* = 3.6 Hz), 8.07 (d, 1H, *J* = 8.4 Hz), 7.79–7.64 (m, 2H), 7.46–7.09 (m, 6H), 6.6 (d, 1H, *J* = 5.4 Hz), 5.7 (d, 1H, *J* = 8.8 Hz), 5.36 (s, 2H), 4.51 (d, 1H, *J* = 5.4 Hz), 3.48 (s, 1H); MS (ES+) m/z 468 (M^++H), Yield 158 mg (15.4%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-3-ylcarbamoyl)-ethyl]-amide ***(30)***. R_f 0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.94 (d, 1H, *J* = 8.3 Hz), 9.12 (s, 1H), 8.92 (s, 1H), 8.83–8.78 (m, 2H), 8.40 (s, 1H), 8.21–8.17 (m, 2H), 7.51–7.46 (m, 3H), 7.34–7.23 (m, 4H), 5.80 (dd, 1H, *J* = 2.3, 8.3 Hz), 5.73 (bs, 1H), 5.30–5.24 (m, 2H), 4.70 (d, 1H, *J* = 2.3 Hz), 2.53 (t, 1H, *J* = 2.4 Hz); MS (ES+) m/z 468 (M^++H), Yield 605 mg (58.9%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-4-ylcarbamoyl)-ethyl]-amide ***(31)***. R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.92 (d, 1H, *J* = 8.2 Hz), 9.13 (s, 1H), 8.96 (bs, 1H), 8.83–8.79 (m, 2H), 8.36–8.34 (m, 2H), 7.52–7.47 (m, 5H), 7.37–7.29 (m, 3H), 5.77 (d, 1H, *J* = 5.7 Hz), 5.26 (d, 2H, *J* = 2.1 Hz), 4.71 (d, 1H, *J* = 2.7 Hz), 2.52 (t, 1H, *J* = 2.4 Hz); MS (ES+) m/z 468 (M^++H), Yield 234 mg (22.8%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(thiazol-2-ylcarbamoyl)-ethyl]-amide ***(32)***. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.84 (d, 1H, *J* = 8.3 Hz), 10.69 (bs, 1H), 9.11 (s, 1H), 8.81–8.79 (m, 1H), 8.73–8.71 (m, 1H), 7.52–7.44 (m, 3H), 7.35–7.20 (m, 4H), 6.88 (d, 1H, *J* = 3.5 Hz), 6.33 (bs, 1H), 5.86 (d, 1H, *J* = 6.5 Hz). 5.24–5.21 (m, 2H), 4.80 (s, 1H), 2.51 (t, 1H, *J* = 2.4 Hz); MS (ES+) m/z 474 (M^++H), Yield 578 mg (55.6%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-piperidin-1-yl-propyl)-amide ***(33)***. R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.66 (d, 1H, *J* = 8.8 Hz), 9.10 (s, 1H), 8.9–8.8 (m, 2H), 7.54–7.46 (m, 3H), 7.39–7.26 (m, 3H), 5.57 (d, 1H, *J* = 8.2 Hz), 5.35–5.22 (m, 2H), 4.73 (d, 1H, *J* = 4.8 Hz), 4.49 (d, 1H, *J* = 6.4 Hz), 3.6-3.5 (m, 4H), 2.49 (t, 1H, *J* = 2.4 Hz), 1.65–1.35 (m, 6H); MS (ES+) m/z 459 (M^++H), Yield 608 mg (60.4%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-pyrrolidin-1-yl-propyl)-amide ***(34)***. R_f 0.4 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.72 (d, 1H, *J* = 8.6 Hz), 9.11 (s, 1H), 8.85–8.81 (m, 2H), 7.52–7.46 (m, 3H), 7.38–7.26 (m, 3H), 5.61 (dd, 1H, *J* = 2.5, 8.6 Hz), 5.26 (dd, 2H, *J* = 2.3, 14.8 Hz), 4.54 (dd, 1H, *J* = 2.5, 6.7 Hz), 4.28 (d, 1H, *J* = - 6.7 Hz), 3.57–3.42 (m, 4H), 2.5 (s, 1H), 1.97–1.85 (m, 4H); MS (ES+) m/z 445 (M^++H), Yield 292 mg (29.9%), m.p. 197-199°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(35)***. R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.76 (d, 1H, *J* = 7.8 Hz), 9.14 (s, 1H), 8.75 (d, 1H, *J* = 8.2 Hz), 7.47–7.21 (m, 6H), 6.62 (d, 1H, *J* = 7.3 Hz), 5.65 (d, 1H, *J* = 7.0 Hz), 5.21 (s, 2H), 4.49 (s, 1H), 4.19–4.07 (m, 2H), 2.55 (s, 1H), 1.91–1.74 (m, 2H), 1.54–1.20 (m, 6H); MS (ES+) m/z 493 (M^++H), Yield 621 mg (66.3%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclohexylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(36)***. R_f 0.4 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.76 (d, 1H, *J* = 8.1 Hz), 9.11 (s, 1H), 8.74 (d, 1H, *J* = 8.3 Hz), 7.46–7.23 (m, 6H), 6.57 (d, 1H, *J* = 8.4 Hz), 5.65 (dd, 1H, *J* = 2.6, 8.1 Hz), 5.20 (s, 2H), 4.50–4.48 (m, 1H), 4.16 (d, 1H, *J* = 4.9 Hz), 3.77–3.70 (m, 1H), 2.55 (t, 1H, *J* = 2.2 Hz), 1.84–1.81 (m, 1H), 1.55–1.51 (m, 2H), 1.37–0.88 (m, 7H); MS (ES+) m/z 507 (M^++H), Yield 598 mg (62.1%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-1-phenyl-2-phenylcarbamoyl-ethyl)-amide ***(37)***. R_f 0.4 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.45 (d, 1H, *J* = 8.8 Hz), 9.65 (s, 1H), 9.02 (s, 1H), 8.69 (d, 1H, *J* = 8.3 Hz), 7.70 (d, 1H, *J* = 8.3 Hz), 7.60 (d, 2H, *J* = 7.8 Hz), 7.42–7.20 (m, 7H), 7.02–6.97 (m, 1H), 6.38 (d, 1H, *J* = 5.5 Hz), 5.63 (d, 1H, *J* = 8.1 Hz), 5.26 (s, 2H), 4.38 (d, 1H, *J* = 3.8 Hz), 3.52 (s, 1H); MS (ES+) m/z 501 (M^++H), Yield 402 mg (42.2%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-(4-fluoro-phenylcarbamoyl)-2-hydroxy-1-phenyl-ethyl]-amide ***(38)***. R_f 0.7 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.46 (d, 1H, *J* = 9.0 Hz), 9.80 (bs, 1H), 9.04 (s, 1H), 8.71 (d, 1H, *J* = 8.1 Hz), 7.73–7.64 (m, 3H), 7.40-7.25 (m, 5H), 7.09–7.07 (m, 2H), 6.41 (bs, 1H), 5.65–5.63 (m, 1H), 5.28 (s, 2H), 4.39 (bs, 1H), 3.54 (s, 1H); MS (ES+) m/z 519 (M^++H), Yield 208 mg (21.1%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-2-(4-methoxy-phenylcarbamoyl)-1-phenyl-ethyl]-amide ***(39)***. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.81 (d, 1H, *J* = 7.9 Hz), 9.08 (s, 1H), 8.70 (d, 1H, *J* = 8.3 Hz), 8.57 (s, 1H), 7.49–7.20 (m, 8H), 6.75 (d, 2H, *J* = 8.7 Hz), 5.77 (d, 1H, *J* = 7.9 Hz), 5.17 (s, 2H), 4.77 (d, 1H, *J* = 5.2 Hz), 4.67 (s, 1H), 3.73 (s, 3H), 2.54 (s, 1H); MS (ES+) 531 (M^++H), Yield 446 mg (44.2%), m.p. > 250°C.

*7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-benzylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide****(40)***. R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.77 (d, 1H, *J* = 8.4 Hz), 9.03 (s, 1H), 8.68 (d, 1H, *J* = 8.3 Hz), 7.46–6.98 (m, 12H), 5.73 (dd, 1H, *J* = 2.4, 8.4 Hz), 5.20 (s, 2H), 4.61–4.52 (m, 2H), 4.40 (d, 1H, *J* = 5.3 Hz), 4.28–4.21 (m, 1H), 2.57 (t, 1H, *J* = 2.4 Hz); MS (ES+) m/z 515 (M^++H), Yield 566 mg (57.8%), m.p. 140-142°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide ***(41)***. R_f 0.4 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.50 (d, 1H, *J* = 9.0 Hz), 9.68 (s, 1H), 9.02 (s, 1H), 8.71 (d, 1H, *J* = 8.2 Hz), 8.24 (d, 1H, *J* = 4.3 Hz), 8.05 (d, 1H, *J* = 8.3 Hz), 7.78–7.69 (m, 2H), 7.43–7.21 (m, 5H), 7.10–7.06 (m, 1H), 6.57 (d, 1H, *J* = 5.5 Hz), 5.67 (d, 1H, *J* = 9.0 Hz), 5.26 (s, 2H), 4.48 (d, 1H, *J* = 5.5 Hz), 3.52 (s, 1H); MS (ES+) m/z 502 (M^++H), Yield 281 mg (29.4%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-3-ylcarbamoyl)-ethyl]-amide ***(42)***. R_f 0.4 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.49 (d, 1H, *J* = 8.9 Hz), 10.0 (s, 1H), 9.04 (s, 1H), 8.78 (bs 1H), 8.70 (d, 1H, *J* = 8.3 Hz), 8.21 (d, 1H, *J* = 4.0 Hz), 8.06–8.03 (m, 1H), 7.72 (d, 1H, *J* = 8.3Hz), 7.43–7.25 (m, 6H), 6.48 (d, 1H, *J* = 5.7 Hz), 5.65 (dd, 1H, *J* = 1.5, 8.9 Hz), 5.27 (s, 2H), 4.45–4.42 (m, 1H), 3.53 (t, 1H, *J* = 2.3 Hz); MS (ES+) m/z 502 (M^++H), Yield 381 mg (39.9%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(thiazol-2-ylcarbamoyl)-ethyl]-amide ***(43)***. R_f 0.4 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.76 (d, 1H, *J* = 8.2 Hz), 10.48 (bs, 1H), 9.1 (s, 1H), 8.67 (d, 1H, *J* = 8.3 Hz), 7.50–7.22 (m, 7H), 6.89 (d, 1H, *J* = 3.5 Hz), 5.99 (bs, 1H), 5.83–5.80 (m, 1H), 5.24–5.11 (m, 2H), 4.78 (s, 1H), 2.55 (t, 1H, *J* = 2.3 Hz); MS (ES+) m/z 508 (M^++H), Yield 295 mg (30.6%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-piperidin-1-yl-propyl)-amide ***(44)***. R_f 0.7 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.56 (d, 1H, *J* = 9.0 Hz), 9.07 (s, 1H), 8.74 (d, 1H, *J* = 8.3 Hz), 7.53–7.27 (m, 6H), 5.54 (d, 1H, *J* = 9.0 Hz), 5.29–5.10 (m, 2H), 4.74–4.72 (m, 1H), 4.47 (d, 1H, *J* = 6.4 Hz), 3.60–3.49 (m, 4H), 2.53 (t, 1H, *J* = 2.5 Hz), 1.71–1.57 (m, 6H); MS (ES+) m/z 493 (M^++H), Yield 381 mg (40.6%), m.p. 180-182°C.

*7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-3-oxo-1-phenyl-propyl)-amide****(45)***. R_f 0.6 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.37 (d, 1H, *J* = 8.4 Hz), 9.06 (s, 1H), 8.71 (d, 1H, *J* = 8.3 Hz), 7.69 (d, 1H, *J* = 8.3 Hz), 7.41–7.24 (m, 5H), 5.42 (d, 1H, *J* = 8.4 Hz), 5.30 (s, 2H), 5.20 (d, 1H, *J* = 6.2 Hz), 4.76 (s, 1H), 3.50–3.40 (m, 9H); MS (ES+) m/z 495 (M^++H), Yield 276 mg (29.3%), m.p. > 250°C.

*7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopropylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide****(46)***. R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.62 (d, 1H, *J* = 8.0 Hz), 9.12 (s, 1H), 8.51 (d, 1H, *J* = 7.2 Hz), 7.44–7.26 (m, 5H), 6.79 (bs, 1H), 5.65–5.62 (m, 1H), 5.21 (s, 2H), 4.49–4.43 (m, 1H), 3.85 (bs, 1H), 2.69–2.57 (m, 2H), 0.74–0.65 (m, 2H), 0.48–0.33 (m, 2H); MS (ES+) m/z 483 (M^++H), Yield 328 mg (38.2%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(47)***. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.62 (d, 1H, *J* = 8.1 Hz), 9.04 (s, 1H), 8.43 (d, 1H, *J* = 7.2 Hz), 7.38–7.35 (m, 2H), 7.27–7.15 (m, 3H), 6.57 (d, 1H, *J* = 7.6 Hz), 5.58 (d, 1H, *J* = 6.0 Hz), 5.13 (s, 2H), 4.41 (s, 1H), 4.14–4.07 (m, 1H), 4.02 (d, 1H, *J* = 4.8 Hz), 2.49 (s, 1H), 1.65–1.6 (m, 1H), 1.55–1.26 (m, 4H), 1.18–1.10 (m, 3H); MS (ES+) m/z 511 (M^++H), Yield 456 mg (50.2%), m.p. 214–216°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclohexylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(48)***. R_f 0.4 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.34 (d, 1H, J = 8.8 Hz), 9.07 (s, 1H), 8.65 (d, 1H, J = 7.7 Hz), 7.37–7.20 (m, 6H), 6.06 (d, 1H, J = 5.4 Hz), 5.48 (d, 1H, J = 8.8 Hz), 5.31 (d, 2H, J = 2.0 Hz), 4.16 (dd, 1H, J = 2.0, 5.4 Hz), 3.56–3.40 (m, 2H), 1.60–1.45 (m, 5H), 1.22–0.99 (m, 5H); MS (ES+) m/z 525 (M^++H), Yield 325 mg (34.8%), m.p. 215–217°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-1-phenyl-2-phenylcarbamoyl-ethyl)-amide ***(49)***. R_f 0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.42 (d, 1H, *J* = 8.8 Hz), 9.63 (s, 1H), 9.06 (s, 1H), 8.64 (d, 1H, *J* = 7.8 Hz), 7.61 (d, 2H, *J* = 7.8 Hz), 7.44–7.23 (m, 7H), 7.04–7.02 (m, 1H), 6.39 (d, 1H, *J* = 5.2 Hz), 5.65 (d, 1H, *J* = 8.8 Hz), 5.29 (s, 2H), 4.40 (s, 1H), 3.54 (s, 1H); MS (ES+) m/z 519 (M^++H), Yield 301 mg (32.6%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-(4-fluoro-phenylcarbamoyl)-2-hydroxy-1-phenyl-ethyl]-amide ***(50)***. R_f0.5 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.42 (d, 1H, *J*=8.5 Hz), 9.78 (s, 1H), 9.06 (s, 1H), 8.63 (m, 1H), 7.66–7.63 (m, 2H), 7.42–7.24 (m, 5H), 7.11–7.05 (m, 2H), 6.41 (d, 1H, *J*=5.4 Hz), 5.65–5.63 (m, 1H), 5.29 (s, 2H), 4.40 (s, 1H), 3.55 (s, 1H); MS (ES+) m/z 537 (M^++H), Yield 275 mg (28.8%), m.p. > 250°C.

*7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid [2-(3-chloro-4-fluoro-phenylcarbamoyl)-2-hydroxy-1-phenyl-ethyl]-amide****(51)***. R_f0.7 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.42 (d, 1H, *J*=9.0 Hz), 9.97 (s, 1H), 9.05 (s, 1H), 8.62 (d, 1H, *J*=7.7 Hz), 7.96–7.92 (m, 1H), 7.63–7.58 (m, 1H), 7.42–7.22 (m, 6H), 6.49 (d, 1H, *J*=5.7 Hz), 5.65–5.62 (m, 1H), 5.29–5.28 (m, 2H), 4.40 (dd, 1H, *J*=2.2, 5.7 Hz), 3.54 (t, 1H, *J*=2.3 Hz); MS (ES+) m/z 571 (M^++H), Yield 270 mg (26.6%), m.p. 202-204°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid (2-benzylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(52)***. R_f0.3 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.41 (d, 1H, *J*=8.6 Hz), 9.09 (s, 1H), 8.57 (d, 1H, *J*=7.8 Hz), 8.32 (s, 1H), 7.50–7.24 (m, 5H), 7.05–6.93 (m, 5H), 6.24 (s, 1H), 5.60–5.58 (m, 1H), 5.30 (s, 2H), 4.44–4.39 (m, 1H), 4.26 (s, 1H), 4.08–4.05 (m, 1H), 3.54 (s, 1H); MS (ES+) m/z 533 (M^++H), Yield 312 mg (32.9%), m.p.236–238°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide ***(53)***. R_f0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.46 (d, 1H, *J*=9.0 Hz), 9.67 (s, 1H), 9.07 (s, 1H), 8.67 (d, 1H, *J*=7.8 Hz), 8.25 (d, 1H, *J*=3.7 Hz), 8.04 (d, 1H, *J*=8.3 Hz), 7.79–7.38 (m, 1H), 7.43–7.22 (m, 5H), 7.11–7.07 (m, 1H), 6.60 (d, 1H, *J*=5.6 Hz), 5.68–5.65 (m, 1H), 5.28–5.27 (m, 2H), 4.48 (dd, 1H, *J*=1.9, 5.6 Hz), 3.54 (t, 1H, *J*=2.3 Hz); MS (ES+) m/z 542 (M^++H), Yield 225 mg (23.3%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-3-ylcarbamoyl)-ethyl]-amide ***(54)***. R_f0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.42 (d, 1H, *J*=8.6 Hz), 9.98 (s, 1H), 9.05 (s, 1H), 8.78 (bs, 1H), 8.62 (d, 1H, *J*=7.7 Hz), 8.21–8.22 (m, 1H), 8.05–8.02 (m, 1H), 7.42–7.24 (m, 6H), 6.48 (d, 1H, *J*=5.3 Hz), 5.64 (d, 1H, *J*=8.6 Hz), 5.27 (s, 2H), 4.42 (d, 1H, *J*=5.3 Hz), 3.54 (s, 1H); MS (ES+) m/z 520 (M^++H), Yield 298 mg (30.9%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-4-ylcarbamoyl)-ethyl]-amide ***(55)***. R_f0.6 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.41 (d, 1H, *J*=9.0 Hz), 10.10 (s, 1H), 9.04 (s, 1H), 8.62 (d, 1H, *J*=7.7 Hz), 8.36 (d, 2H, *J*=5.7 Hz), 7.67 (d, 2H, *J*=6.2 Hz), 7.42–7.21 (m, 5H), 6.48 (d, 1H, *J*=5.7 Hz), 5.66–5.63 (m, 1H), 5.27 (d, 2H, *J*=1.8 Hz), 4.43 (dd, 1H, *J*=2.1, 5.7 Hz), 3.54 (s, 1H); MS (ES+) m/z 520 (M^++H), Yield 237 mg (25.6%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(thiazol-2-ylcarbamoyl)-ethyl]-amide ***(56)***. R_f0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.61 (d, 1H, *J*=8.4 Hz), 9.03 (s, 1H), 8.38 (d, 1H, *J*=7.2 Hz), 7.41–7.15 (m, 5H), 7.01 (d, 1H, *J*=3.5 Hz), 6.80 (d, 1H, *J*=3.5 Hz), 6.27 (bs, 1H), 5.82–5.79 (m, 1H), 5.22-5.19 (m, 2H), 4.67 (s, 1H), 2.49–2.48 (m, 1H); MS (ES+) m/z 526 (M^++H), Yield 318 mg (34.0%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-piperidin-1-yl-propyl)-amide ***(57)***. R_f0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.47 (d, 1H, *J*=9.0 Hz), 9.06 (s, 1H), 8.50 (d, 1H, *J*=7.2 Hz), 7.51–7.24 (m, 5H), 5.54–5.51 (m, 1H), 5.24–5.08 (m, 2H), 4.73–4.72 (m, 1H), 4.48 (d, 1H, *J*=6.0 Hz), 3.61–3.49 (m, 4H), 2.55 (s, 1H), 1.98–1.99 (m, 1H), 1.78–1.44 (m, 5H); MS (ES+) m/z 511 (M^++H), Yield 377 mg (41.5%), m.p. 217-219°C.

7-Methyl-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide ***(58)***. R_f0.3 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.62 (d, 1H, *J*=9.0 Hz), 9.67 (s, 1H), 8.97 (s, 1H), 8.59 (d, 1H, *J*=8.1 Hz), 8.24 (d, 1H, *J*=4.3 Hz), 8.04 (d, 1H, *J*=8.2 Hz), 7.78–7.73 (m, 1H), 7.51 (d, 1H, *J*=8.1 Hz), 7.43–7.21 (m, 5H), 7.09–7.05 (m, 1H), 6.56 (d, 1H, *J*=5.6 Hz), 5.67–5.64 (m, 1H), 5.33 (s, 2H), 4.48–4.47 (m, 1H), 3.48–3.47 (m, 1H), 2.65 (s, 3H); MS (ES+) m/z 504 (M^++H), Yield 457 mg (43.9%), m.p. > 250°C.

2-Hydroxy-3-[(4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carbonyl)-amino]-3-phenyl-propionic acid ethyl ester ***(59)***. R_f0.6 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.65 (d, 1H, *J*=8.6 Hz), 9.08 (s, 1H), 8.77–8.74 (m, 2H), 7.43–7.19 (m, 6H), 5.66–5.63 (m, 1H), 5.19 (dd, 2H, *J*=2.1, 7.4 Hz), 4.47 (d, 1H, *J*=2.1 Hz), 4.23-4.16 (m, 2H), 3.44 (d, 1H, *J*=5.2 Hz), 2.44 (s, 1H), 1.23 (t, 3H, *J*=7.1 Hz); MS (ES+) m/z 420 (M^++H), Yield 427 mg (46.4%), m.p. > 250°C.

6-Fluoro-4-oxo-1-prop-2-ynyl-7-pyrrolidin-1-yl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(60)***. To a solution of **47** (500 mg, 0.9 mmol) and triethylamine (290 mg, 2.8 mmol) in acetonitrile (20 mL) was added pyrrolidine (0.10 g, 1.4 mmol). The resulting mixture was refluxed for 3h, concentrated, diluted with water and extracted in dichloromethane (50 mL). The organic layer was dried over sodium sulphate and concentrated to dryness to afford a crude product, which was chromatographed on silica gel (mesh size 100-200) column with 2% MeOH/DCM as eluent to afford compound **60** (0.41 g, 76.9%).

R_f0.4 (8% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 11.09 (d, 1H, *J*=8.1 Hz), 8.81 (s, 1H), 8.03 (d, 1H, *J*=12.8 Hz), 7.47–7.22 (m, 5H), 6.71 (d, 1H, *J*=7.8 Hz), 5.61 (dd, 1H, *J*=2.6, 8.1 Hz), 5.05-5.04 (m, 2H), 4.50–4.49 (m, 1H), 4.19–4.16 (m, 1H), 3.81–3.80 (m, 4H), 2.45 (t, 1H, *J*=2.4 Hz), 2.03-1.24 (m, 12H); MS (ES+) m/z 546 (M^++H), Yield 408 mg (76.40%), m.p. > 250°C.

Compounds **61** and **62** were prepared in a similar way to compound **60**.

6-Fluoro-7-(3-methyl-piperidin-1-yl)-4-oxo-1-prop-2-ynyl-1,4-dihydro-1,8] naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(61)***. R_f0.5 (8% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 11.03 (d,

1H, *J* = 8.1 Hz), 8.82 (s, 1H), 8.06 (d, 1H, *J* = 13.7 Hz), 7.47–7.22 (m, 5H), 6.70 (d, 1H, *J* = 7.8 Hz), 5.62 (dd, 1H, *J* = 2.0, 7.8 Hz), 5.02 (s, 2H), 4.49–4.36 (m, 4H), 4.21–4.14 (m, 1H), 3.14–3.06 (m, 1H), 2.82–2.74 (m, 1H), 2.46–2.45 (m, 1H), 1.91–1.23 (m, 13H), 0.97 (d, 3H, *J* = 6.5 Hz); MS (ES+) m/z 574 (M^++H), Yield 497 mg (88.6%), m.p. 177–179°C.

6-Fluoro-7-(3-methyl-piperidin-1-yl)-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid (2-hydroxy-1-phenyl-2-phenylcarbamoyl-ethyl)-amide ***(62)***. R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 11.15 (d, 1H, *J* = 7.5 Hz), 8.80–8.73 (m, 2H), 8.05 (d, 1H, *J* = 13.7 Hz), 7.55–7.49 (m, 4H), 7.34–7.04 (m, 6H), 5.72 (d, 1H, *J* = 3.9 Hz), 5.24 (s, 1H), 4.99 (s 2H), 4.69 (s, 1H), 4.45–4.35 (m, 2H), 3.14–3.05 (m, 1H), 2.82-2.74 (m, 1H), 2.44 (s, 1H), 1.92–1.70 (m, 3H), 1.26–1.24 (m, 2H), 0.96 (d, 3H, *J* = 6.2 Hz); MS (ES+) m/z 582 (M^++H), Yield 472 mg (84.3%), m.p. > 250°C.

Cytotoxicity

All the synthesized naphthyridine derivatives (**22–62**) were tested for *in vitro* cytotoxicity on eight tumors as well as on two non-tumorous cell lines and IC_{50} values were calculated in micro molar (μM). The human tumor cell lines used in the study are ovary (PA1), prostate (DU145), oral (KB), colon (SW620), breast (HBL100), lung (A-549), pancreas (MIAPaCa2) and leukemia (K562). All the 1,8-naphthyridine **22–62** and assay standard Doxorubicin HCl (data not shown) were also tested against normal mouse fibroblast (NIH3T3) and normal ovary (CHO) cell line to evaluate their cancer cell specificity (safety index). Derivatives of 1,8-naphthyridine **22-62** were screened for cytotoxic activity at the highest soluble concentration of 10 μM and on four lower concentrations on eight human tumor and two non-tumorous cell lines. Briefly, a three day MTT *in vitro* cytotoxicity assay was performed, which is based on the principle of uptake of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), a tetrazolium salt, by the metabolically active cells where it is metabolized by active mitochondria into a blue colored formazan product that is read spectrophotometrically [10]. MTT was dissolved in phosphate buffer saline with a pH of 7.4 to obtain an MTT concentration of 5 mg/mL; the resulting mixture was filtered through a 0.22-micron filter to sterilize and remove a small amount of insoluble residue. For each type of tumor and normal cell, 5000 to 10000 cells were seeded in a 96-well culture plate and incubated with various concentrations of 1,8-naphthyridine derivatives (**22–62**) in a CO_2 incubator for 72 h. Control cells not treated with 1,8-naphthyridine-3-carboxamide derivatives (**22–62**) were similarly incubated. The assay was terminated after 72 h by adding 125 μg (25 μL) MTT to each well, then incubating for 3 h, and finally adding 50 μL of 10% SDS-0.01N HCl to each well to lyse the cells and dissolve formazan. After incubating for 1 h, the plate was read spectrophotometrically at 540 nm and the cytotoxicity percentage calculated using the following formula: Cytotoxicity percentage = (1-(X/R_1)* 100, where X = (absorbance of treated sample at 540 nm)-(absorbance of blank at 540 nm), R_1 = absorbance of control sample at 540 nm)-(absorbance of blank at 540 nm). The cytotoxicity data is summarized in Table 3 and the compounds, which were inactive at 10 μM, are not listed.

Anti-inflammatory activity

1,8-Naphthyridine-3-carboxamide derivatives (22–62) were also able to down regulate the levels of LPS stimulated TNF-α, IL-1β, IP-10 and MIP-1-α secreted by DCs and identified to have potential anti-inflammatory activity. The down regulation of cytokine and chemokine levels by >25% was considered as significant.

BMDC based ex-vivo septic shock assay to evaluate potential anti-inflammatory activity: Primary DC cultures were generated from femoral bone marrow of 8–12 weeks old C57BL/6 mice [11]. Bone marrow progenitors were cultured in RPMI-1640 supplemented with 10% FBS (Hyclone) and rmGMCSF (20 ng/mL) at 37°C, 5% CO_2. Immature DCs were stimulated with lipopolysaccharide (LPS; 10 ng/mL,) and incubated with the naphthyridine carboxamide derivatives at various concentrations ranging from 0.001 to 10 μg/mL, preferably between 0.1 and 1 μg/mL for 24 h. The IL-1-β, TNF-α, MIP-1-α, and IP-10 secreted by the DCs were measured in culture supernatants by Enzyme Linked Immunosorbent Assays (R&D Systems Inc, MN, USA). Percentage change in cytokine/chemokine = {(B-A)/A*100, where B = concentration of cytokine/chemokine (pg/mL) secreted by LPS stimulated DCs when incubated with test molecule, A = concentration of cytokine/chemokine (pg/mL) secreted by LPS stimulated DCs alone. LPS treated DCs were used as positive control.

Results and discussion

1,8-Naphthyridine-3-carboxamide derivatives are divided into three categories based on the substitution pattern at C-6 and C-7 position, unsubstituted: compounds without any substitution at C-6 and C-7 position; monohalo substituted: C-7 chloro substituted; and dihalo substituted: C-6-fluoro-C-7-chloro substituted compounds.

In C-3′ cycloalkyl derived unsubstituted 1,8-naphthyridine derivatives (**22–24**) were inactive on different cancer cell lines. While, monohalo substituted derivatives (**35** and **36**) have resulted in improved cytotoxicity compared to unsubstituted derivatives (**22–24**). The C-3′ cyclohexyl substituted derivative **36** exhibited potent cytotoxicity on ovarian (PA-1) cancer cell line with IC_{50} of 1.19 μM and safety index of ~7 against normal ovary (CHO) and ~4 on NIH3T3 (normal fibroblast) cell lines. The dihalo-substituted 1,8-naphthyridine derivatives (**47–48**) showed potent to moderate cytotoxicity on ovarian and other cancer cell lines. The cyclopentyl-substituted derivative **47** has showed potent cytotoxicity with IC_{50} of 0.41, 0.77 and 1.5 μM on pancreas (MIAPaCa), leukemia (K-652) and lung (A549) cancer cell lines, respectively in this series. While, expansion of cycloalkyl ring from cyclopentyl (**47**) to cyclohexyl (**48**) leads to slight decrease in cytotoxicity.

In C-3′ aryl substituted 1,8-naphthyridine derivatives (**25–27**), compound **25** has showed potent cytotoxicity

Table 3. *In vitro* cytotoxicity of 1,8-Naphthyridine derivatives (**22–62**).

		IC_{50} (μ_M)									
S. No.	Compound No	PA-1 (Ovary)	DU-145(Prostate)	KB (Oral)	SW620 (Colon)	HBL100 (Breast)	A549 (Lung)	MIAPaCa (Pancreas)	K-562 (Leukemia)	NIH3T3 (Normal fibroblast)	CHO (Normal ovary)
1.	Doxorubicin	0.63	0.10	3.0	0.08	0.24	0.08	0.15	0.10	0.39	1.0
2.	**25***	9.1	2.9	>10	>10	5.9	6.09	9.81	>10	4.79	NA
3.	**29****	0.41	>10	3.7	1.4	4.1	3.06	>10	4.4	2.2	NA
4.	**35**	3.12	>10	>10	6.2	8.99	>10	8.26	6.34	9.76	NA
5.	**36**	1.19	>10	>10	4.62	4.7	9	8	9.4	5	8.7
6.	**38**	1.2	6.1	2.6	3.2	6.9	>10	>10	>10	NA	NA
7.	**39**	3.22	>10	>10	5.27	8.8	>10	8.2	>10	7.3	NA
8.	**40**	8.1	1.6	>10	>10	>10	>10	>10	>10	NA	NA
9.	**41**	7.6	>10	>10	>10	>10	>10	9.0	>10	NA	NA
10.	**42**	2.0	>10	>10	7.8	7.5	>10	7.3	>10	5.5	NA
10.	**43**	3.49	>10	>10	7.13	4	9	7.6	9.2	1.8	9.7
11.	**44**	3.33	>10	>10	>10	>10	>10	>10	>10	NA	NA
12.	**45**	3.95	>10	>10	>10	9.1	>10	9.1	>10	NA	NA
13.	**46**	2.54	8.56	9.6	4.11	6.60	>10	>10	8.60	5.86	NA
14.	**47**	3.55	>10	>10	2.79	>10	1.5	0.41	0.77	1.05	NA
15.	**48**	1.7	>10	7.2	4.4	5.0	6.8	3.8	9.9	3.4	2.2
16.	**49**	>10	>10	>10	>10	4.33	4.82	1.28	2.50	2.24	NA
17.	**51**	3.6	6.1	3.5	2.8	8.2	9.9	6.7	>10	7.9	2.4
18.	**52**	>10	>10	>10	>10	9.26	6.99	3.17	5.39	2.85	NA
19.	**53**	2.62	3.45	9.12	3.79	3.17	9.53	2.83	8.20	4.86	NA
20.	**54**	3.1	>10	>10	7.9	8.7	>10	3.3	8.4	4.1	NA
21.	**55**	4.7	>10	8.42	3.35	5.5	>10	8.1	7	7.6	NA
22.	**56**	3.1	3.6	7.9	6.3	3.20	2.58	3.50	4.16	5.31	NA
23.	**57**	>10	>10	>10	>10	5.22	7.49	1.78	4.99	1.69	NA

*** Salt*

** Precipitation observed during aqueous dilution*

Cytotoxicity was assessed by MTT assay as described in Methods. Data shown are IC_{50} of single independent experiments done in triplicates. If IC_{50} was not achieved even at the highest concentration tested i.e. 10 μM, it was represented as NA.

with IC_{50} of 2.9 μM on prostate (DU-145) cancer cell line. While, the other aryl substituted derivatives with electron withdrawing or donating groups (**26** and **27**) were inactive. Benzyl substituted derivative **28** has shown no activity. However, in mono halo substituted 1,8-naphthyridine derivatives, compound **38** with electron withdrawing group has showed potent cytotoxicity on oral (KB) cancer cell line with IC_{50} of 2.6 μM. *N'*-Benzyl substituted derivative **40** has resulted in selective potent cytotoxicity on prostate (DU145) cancer cell line with IC_{50} of 1.6 μM. In dihalo substituted 1,8-naphthyridine derivatives (**49–51**), compound **49** has showed good activity on pancreas (MIAPaCa) and leukemia (K562) cancer cell lines. Benzyl substituted derivative **52** has shown moderate cytotoxicity on pancreas cancer cell line.

In C-3' heteroaryl substituted 1,8-naphthyridine derivatives, amongst unsubstituted 1,8-naphthyridine derivatives (**29–32**), only compound **29** has showed potent cytotoxicity with IC_{50} of 0.41 and 1.4 μM on ovary (PA-1) and colon (SW620) cancer cell lines, respectively. Reversing the position of the nitrogen in pyridine ring from second position leads to complete loss of activity (**30** and **31**). In mono halo substituted 1,8-naphthyridine derivatives (**41–43**), improvement in cytotoxicity was observed in 3-amino pyridyl derivative (**42**) and thiazole (**43**) substituted derivatives but 2-amino pyridyl derivative (**41**) has showed very slight activity. In dihalo substituted 1,8-naphthyridine derivatives (**53–55**), compound **53** has showed broad spectrum of activity with IC_{50} < 4 μM on five cancer cell lines. While, compounds **54** and **55** have resulted in moderate cytotoxicity.

In C-3'-tertiary amine substituted 1,8-naphthyridine derivatives, unsubstituted compounds (**33** and **34**) were found to be inactive. Whereas, mono halo substituted derivatives piperidine (**44**) and morpholino (**45**) showed selective cytotoxicity on ovary (PA-1) cancer cell line. In dihalo substituted 1,8-naphthyridine derivative, compound **57** has shown potent activity on pancreas (MIAPaCa) cell line with IC_{50} 1.78 μM and modest to low activities on other cell lines. Further we have studied that substitution of C-7 position with group having inductive effect like methyl (**58**) leads to the complete loss of activity and replacement of the C-3' amide group by ester (**59**) linkage caused complete loss of activity.

As dihalo substituted 1,8-naphthyridine derivatives **47** and **49** have shown potent cytotoxicity, the C-7 chloro group of **47** and **49** was replaced with different secondary amine as shown in Scheme 2. But it leads to complete loss of activity (**60–62**). This indicates that C-7 halo group is essential for the activity.

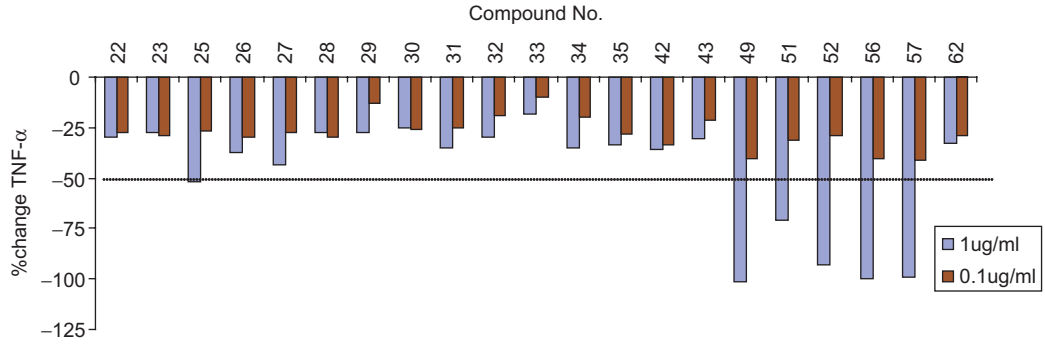


Figure 2. Anti-inflammatory activity as a measure of TNF-α downregulation.

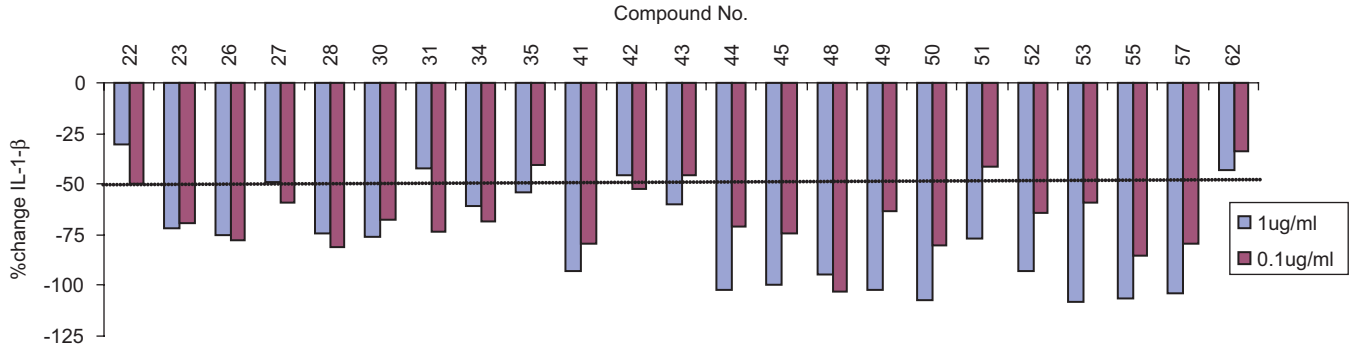


Figure 3. IL-1-β modulation of selected molecules. Dotted line shows IC_{50} (concentration at which 50% inhibition occurs).

The overall results indicated that halo substituted 1,8-naphthyridine derivatives with five and six membered cycloalkyl ring substituent have shown maximum cytotoxicity. Halo substituted compound **47** has shown IC_{50} of 0.41 and 0.77 μM on MIAPaCa and K-562 cancer cell lines, respectively. While, compound **36** has resulted in IC_{50} of 1.19 μM on PA-1 cancer cell line. However, one of the unsubstituted 1,8-naphthyridine 3'-heteroaryl derivative **29** has showed potent cytotoxicity with IC_{50} of 0.41 and 1.4 μM on ovary (PA-1) and colon (SW620) cancer cell lines, respectively. Replacement of C-7 halo group with secondary amine leads to loss of the activity.

Figure 2 shows the down regulation of TNF-α (primary mediator of tissue damage and pain in inflammatory disorders) by selected 1,8-naphthyridine-3-carboxamide derivatives. Compounds **49**, **51**, **52**, **56** and **57** exhibited a very high TNF-α inhibition at 1 μg/mL. Table 4 demonstrates IC_{50} value for TNF-α inhibition by selected molecules screened at various concentrations ranging from 0.001 to 10 μg/mL.

Compounds showing high TNF-α down regulation **49**, **51**, **52** and **57** were also found to be potent inhibitors of IL-1-β secretion by LPS-stimulated DCs (Figure 3).

Inhibition of MIP-1-α and IP-10 (pro-inflammatory chemokines) activity is suggestive of anti-inflammatory activity of 1,8-naphthyridine-3-carboxamide derivatives. Compounds **44**, **45**, **49**, **50**, **53** and **55** showed >50% down regulation of MIP-1-α in addition to TNF-α and IL-1-β inhibition (Figure 4). Compounds **45**, **48**, **54**, **55** and **56** have demonstrated high IP-10 inhibitory activity as shown in Table 5 & Figure 5.

Table 4. IC_{50} values for TNF-α modulation by selected 1,8-naphthyridine carboxamide derivatives. Molecules were subjected to screening over a multiple dose concentration range of 0.001 μg/mL to 10 μg /mL.

Molecule Numbers	IC_{50} value (μg)
41	<0.001
44	<0.001
45	0.31
48	<0.001
50	~0.001
53	<0.001
54	1.1
55	0.59

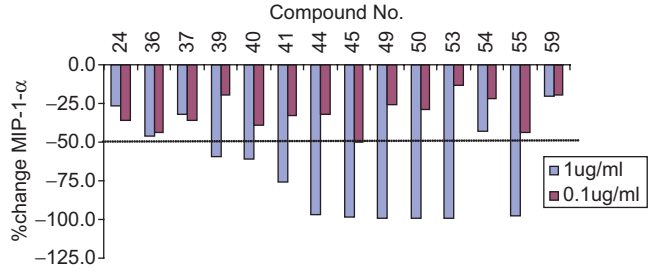


Figure 4. MIP-1-α modulation of selected molecules. Dotted line shows IC_{50} (concentration at which 50% inhibition occurs).

Compounds **45** and **55** were able to induce remarkable down regulation of TNF-α, IL-1-β, MIP-1-α and IP-10 activity and hence were found to be most active anti-inflammatory compounds among 1,8-naphthyridine-3-carboxamide derivatives.

Table 5. Downregulation of IP-10 levels to 50% (IC_{50}) of selected 1,8-naphthyridine derivatives. Molecules were subjected to screening over a multiple dose concentration range of 0.001 μg/mL to 10 μg/mL.

Molecule Number	IC_{50} value (μg)
55	0.62
45	0.32
54	1.1

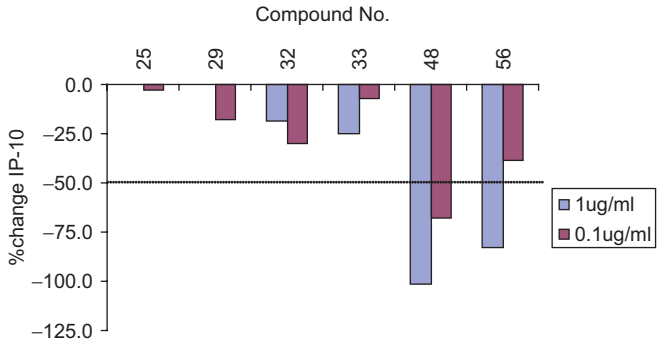


Figure 5. IP-10 modulation of selected molecules. Dotted line shows IC_{50} (concentration at which 50% inhibition occurs).

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