European Journal of Medicinal Chemistry 44 (2009) 3356–3362

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Preliminary communication

1,8-Naphthyridine-3-carboxamide derivatives with anticancer and anti-inflammatory activity

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ARTICLE INFO

Article history:
Received 3 December 2008
Received in revised form
13 February 2009
Accepted 12 March 2009
Available online 24 March 2009

Keywords:
1,8-Naphthyridine
Anti-tumor
Anti-inflammatory

ABSTRACT

A number of 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**15**–**35**) have been synthesized and screened for their in vitro cytotoxicity and anti-inflammatory activity. Compounds **22**, **31** and **34** have shown high cytotoxicity against a number of cancer cell lines, while compound **24** showed significant anti-inflammatory activity.

1. Introduction

Cancer, a disease of worldwide importance, according to the American Cancer Society, is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Recently, quinolines and 1,8-naphthyridine are being exploited in cancer chemotherapy and one of the molecules SNS-595 is in second phase of clinical trials [1,2]. Mammalian Topoisomerase II is one of the known targets for anti-tumor agents like doxorubicin, etoposide, ellipticine and amsacrine [3]. 1,8-Naphthyridine derivatives were found to display moderate cytotoxic activity against murine P388 leukemia, when changes were carried out at N-1 and C-7 positions [4,5]. However, further structural exploitations in 1,8-naphthyridine skeleton are required to establish a meaningful structure–activity relationship. Earlier, we have synthesized C-3 carboxamide derivatives with a spacer, which have shown good cytotoxicity along with anti-inflammatory activity [6]. Based on these observations and SAR we have further modified the C-3 carboxamide acid with different amino acid derivatives (**4a**–**d** and **8a**–**c**) to afford 1,8-naphthyridine-3-carboxamide derivatives (**15**–**35**). The latter being not only cytotoxic but also safer on normal cell lines vs. tumor cells. The C-3 amide linkage in 1,8-naphthyridine-3-carboxamide derivatives may provide hydrophilic interaction, while functionalized amino acids may interact with the receptors, and as a consequence, it could trigger physiological response.

Compounds (**15**–**35**) 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].

2. Chemistry

The synthesis of *N*-substituted 1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives **15**–**35** was carried out using functionalized amino acid derivatives **4a**–**d** and **8a**–**c**. The synthesis of the racemic **4a**–**d** and **8a**–**c** are described in Scheme 1. The amino group of DL-3-amino-3-phenyl propionic acid (**1**) was protected with Boc anhydride to furnish Boc substituted amino acid **2**. The coupling of **2** with appropriate amines, using EDCI–HOBt provided the respective propionamide **3a**–**d**. The Boc groups of **3a**–**d** were removed by its treatment with 50% TFA/DCM to yield the corresponding DL-*N*-substituted 3-amino-3-phenyl propionamide (**4a**–**d**). Similarly, DL-*N*-substituted phenyl glycinamide derivatives (**8a**–**d**) were prepared starting from DL-phenyl glycine (**5**).

The synthesis of compounds **15**–**34** is shown in Scheme 2. Commercially available 2-chloro nicotinic acid **9** was reacted with

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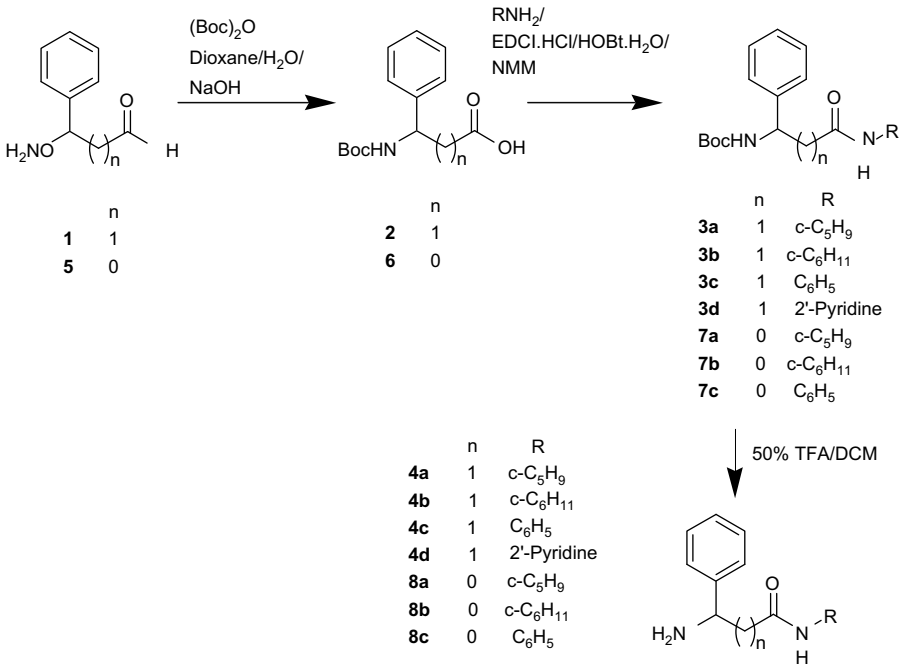
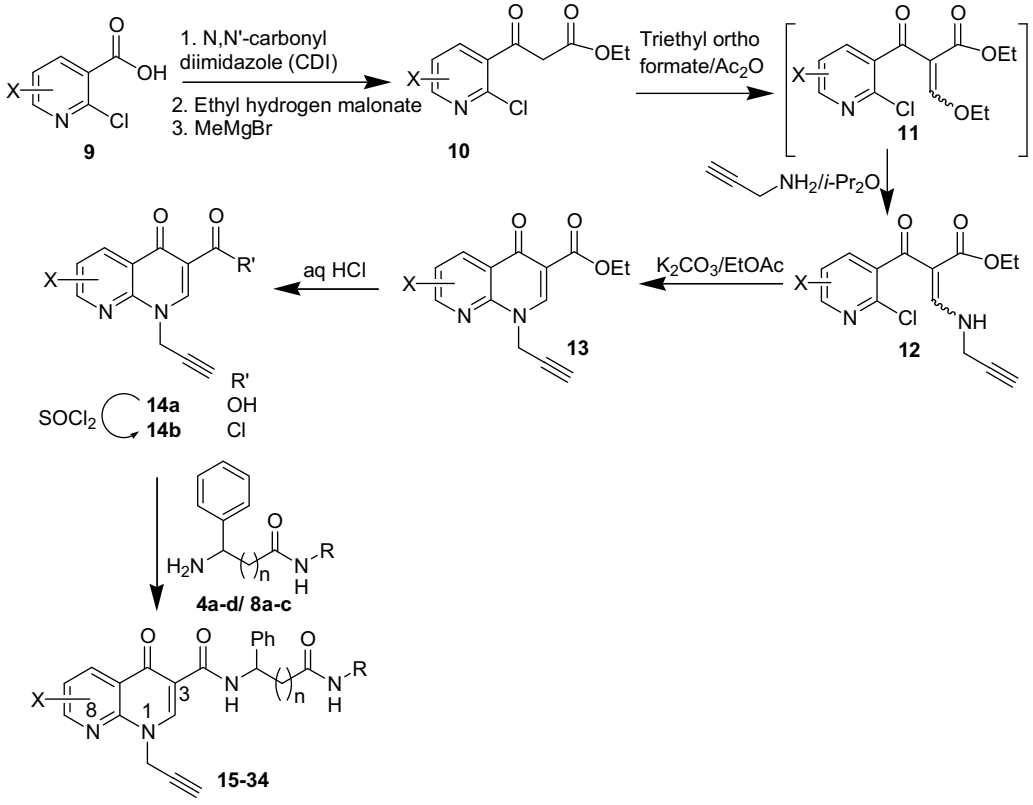
doi:10.1016/j.ejmech.2009.03.015



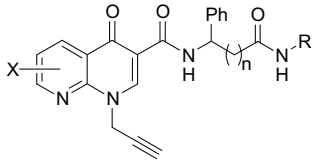
Scheme 1. Synthesis of functionalized amino acids (**4a–d** and **8a–c**).

1,1′-carbonyldiimidazole (CDI) in dry THF to afford the imidazolide solution, which was allowed to react with ethyl hydrogen malonate and methyl magnesium bromide to afford nicotinoylacetate **10**. Compound **10** on treatment with triethyl orthoformate and acetic anhydride (**11**) followed by the addition of propargyl amine afforded ethyl nicotinoylacrylate **12**. Ethyl 1,8-naphthyridine-3-carboxylate (**13**) was prepared by base-assisted (K_2CO_3) cyclization of acrylate **12** in ethyl acetate, upon acidic hydrolysis **13**; provided 1,8-naphthyridine-3-carboxylic acid **14a**, which was treated with thionyl chloride to afford **14b**. The 1-propargyl-1,8-naphthyridine-



Scheme 2. Synthesis of 1,8-naphthyridine-3-carboxamide derivatives (**15–34**).

Table 1
List of 1-propargyl-1,8-naphthyridine derivatives (**15**–**35**).



15-35

Compound no.	X	*n*	R	Compound no.	X	*n*	R
15	H	1	c-C_5H_9	**26**	H	0	c-C_5H_9
16	H	1	c-C_6H_{11}	**27**	H	0	c-C_6H_{11}
17	H	1	C_6H_5	**28**	H	0	C_6H_5
18	H	1	2′-Pyridine	**29**	7-Cl	0	c-C_5H_9
19	7-Cl	1	c-C_5H_9	**30**	7-Cl	0	c-C_6H_{11}
20	7-Cl	1	C_6H_5	**31**	7-Cl	0	C_6H_5
21	7-Cl	1	2′-Pyridine	**32**	6-F,7-Cl	0	C_5H_9
22	6-F,7-Cl	1	c-C_5H_9	**33**	6-F,7-Cl	0	C_6H_{11}
23	6-F,7-Cl	1	c-C_6H_{11}	**34**	6-F,7-Cl	0	C_6H_5
24	6-F,7-Cl	1	C_6H_5	**35**	6-F, 7-pyrrolidine	0	C_6H_5
25	6-F,7-Cl	1	2′-Pyridine				

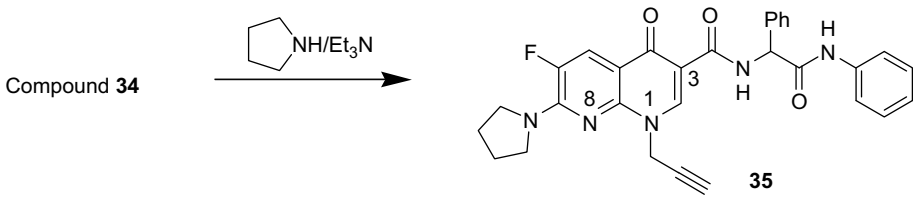
3-carboxamide derivatives **15**–**34** were prepared by coupling of appropriate 1,8-naphthyridine-3-carbonyl chloride (**14b**) with functionalized amino acids **4a**–**d** and **8a**–**c**, respectively. The *N*-substituted 1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives **15**–**34** are listed in Table 1. Compound **34** on treatment with pyrrolidine in the presence of triethylamine yielded compound **35** as shown in Scheme 3.

3. Results and discussion

The synthesized compounds (**15**–**35**) are divided into two classes based on the substitution of phenyl propionamide (**4a**–**d**) and phenyl glycinamide (**8a**–**c**) functionalized amino acid into *N*-(2-*N*-substituted carbomyl-1-phenylethyl)-1,4-dihydro-4-oxo -1-propargyl-1,8-naphthyridine-3-carboxamide (**15**–**25**) and *N*-(2-*N*-substituted carbomyl-1-phenylmethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**26**–**35**) derivatives, respectively. These carboxamides are further divided into three categories based on the substitution pattern at C-6 and C-7 (unsubstituted: compounds without any substitution at C-6 and C-7, monohalo substituted: C-7 chloro substituted and dihalo substituted: C-6 fluoro-C-7 chloro substituted compounds).

Amongst compounds (**15**–**25**), substitution in 1,8-naphthyridine ring had played crucial role in eliciting cytotoxicity. Unsubstituted 1,8-naphthyridine derivatives (**15**–**18**) were found inactive except compound **17**, which resulted in slight cytotoxicity on prostate cancer cell line. While, halo substituted derivatives (**19**–**25**) were found better than unsubstituted ones (**15**–**18**). The monohalo substituted cycloalkyl derivative (**19**) and its dihalo substituted analog **22** have shown high cytotoxicity on ovary cancer cell line with IC_{50} of 1.1 and 0.68 μM, respectively. Compound **22** was found as most cytotoxic molecule against breast cancer cell line with IC_{50} of 2.0 μM in this series. Upon expansion of cyclopentyl ring in compound **22** to cyclohexyl ring (**23**), cytotoxicity was lowered. The monohalo aryl substituted derivative (**20**) and its dihalo substituted analog **24** exhibited high cytotoxicity on ovarian cancer cell line. In addition, compound **20** has also shown good cytotoxicity on colon and pancreas cancer cell lines. The monohalo substituted heteroaryl derivative **21** exhibited high cytotoxicity on ovarian and colon cancer cell lines but its dihalo substituted analog **25** was found inactive against ovarian cancer cell line. However, compound **25** has resulted in high cytotoxicity against prostate, oral and colon cancer cell lines with $IC_{50} \leq 2.3$ μM. These results indicated that the halo substituted 1-propargyl-1,8-naphthyridine with 3-phenyl propionamide functionalized amino acid substitution has shown high cytotoxicity.

In second series *N*-(2-*N*-substituted carbomyl-1-phenylmethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide **26**–**35**, compounds **26** and **28** were found relatively better than earlier series derivatives (**15**–**17**). However, the monohalo substituted cycloalkyl derivatives (**29** and **30**) were found inactive. The monohalo substituted aryl derivative **31** exhibited high cytotoxicity on prostate, oral and leukemia cancer cell lines with IC_{50} of 1.7, 2.1 and 3.3 μM in this series. Similar to the earlier series dihalo derivatives (**22**–**24**), compounds **32**–**34** resulted in high cytotoxicity against a number of cancer cell lines. Compound **32** has shown broad-spectrum cytotoxicity with IC_{50} of 1.7 and 3.2 against pancreas and endothelial cancer cell lines but also exhibited cytotoxicity on normal cancer cell line. Compound **33** has shown selective and high cytotoxicity against ovarian cancer cell line. Aryl substituted phenyl derivative **34** has shown high and broad spectrum of cytotoxicity with IC_{50} of 0.5, 0.6, 1.1 and 1.4 μM against



Scheme 3. Synthesis of compound **35**.

ovarian, prostate, oral and colon cancer cell lines along with good safety index. Further, replacement of the C-7 chloro group in compound **34** with pyrrolidine (**35**) leads to complete loss of activity.

It indicated that both phenyl propionamide and phenyl glycinamide functionalized amino acid substituted 1-propargyl-1,8-naphthyridine-3-carboxamides have shown high cytotoxicity on a number of cancer cell lines particularly on ovary cancer cell line. The C-6/C-7 halo substituent in the 1,8-naphthyridine played a crucial role in eliciting cytotoxicity.

Compounds **15**, **17**, **18**, **20**, **28** & **34** exhibit >50% inhibition of IL-1-β at 1 μg/ml. >50% inhibition of IL-6 was observed by **18**, **19**, **20**, **28** & **34** at both 1 and 0.1 μg/ml. However, compounds **21**, **22**, **24**, **25** & **31** demonstrated >50% down regulation of both IL-1-β and IL-6 at 1 and 0.1 μg/ml and suggest promising anti-inflammatory activity. Compound **24** was found to be most active as it demonstrated a significant down regulation of TNF-α and IP-10 also in addition to IL-1-β & IL-6.

4. Conclusions

A number of 1,8-naphthyridine-3-carboxamide derivatives (**15**–**35**) have been synthesized and evaluated for their in vitro cytotoxicity and anti-inflammatory activity. Amongst them compound **34** has shown a high and broad spectrum of cytotoxicity with IC_{50} of 0.5, 0.6, 1.1 and 1.4 μM against ovarian, prostate, oral and colon cancer cell lines. Compounds **22** and **31** show significant cytotoxicity against a number of cancer cell lines, while compound **24** showed significant down regulation of TNF-α and IP-10 also in addition to IL-1-β and IL-6.

5. Experimental protocols

5.1. Chemistry

All the solvents and reagents were purchased from companies such as Aldrich, Lancaster, Acros & 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). Visualization was achieved with UV light and iodine vapor. Column chromatography was performed using silica gel (100–200 mesh). ^{1}H NMR spectra were recorded on a Bruker 300 MHz instrument using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a Micromass Quattro Micro™ instrument. Elemental analyses (C, H, N) were undertaken using an elementer analyzer and were within 0.4% of the calculated values. Melting points were determined in a capillary tube with a thermal scientific melting point apparatus Mettler Toledo and are uncorrected.

5.1.1. 3-tert-*Butoxycarbonylamino-3-phenyl-propionic acid* (***2***)

Di-*tert*-butyl pyrocarbonate (Boc_2O, 2.4 g, 11 mmol) was added in portions to the stirred solution of (DL)-2-amino phenyl propionic acid **1** (1.65 g, 10 mmol) in dioxane (20 ml), water (10 ml) and 1 N NaOH (10 ml) at 0–5 °C. The reaction mixture was stirred at ambient temperature for 30 min and concentrated in vacuum to a volume of 10–15 ml. The resulting mixture was cooled to 0–5 °C, ethyl acetate (30 ml) was added, and the mixture was acidified with dilute hydrochloric acid to pH 2–3. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (2 × 15 ml). The ethyl acetate extracts were combined, washed with water (2 × 30 ml), dried over anhydrous Na_2SO_4 and concentrated in vacuum to furnish the titled compound, m.p. 143–145 °C.

5.1.2. *General procedure for the synthesis of substituted* N-tert-*butoxycarbonylamino-3-phenyl-propionamides* (***3a–d***)

Appropriate amine (10 mmol) was added, to a stirred solution of (DL)-3-*tert*-butoxycarbonylamino-3-phenyl-propionic acid **2** (2.65 g, 10 mmol) in dry dichloromethane (50 ml). The resulting solution was placed in an ice bath for 15 min and 1-hydroxybenzotriazole hydrate ($HOBt \cdot H_2O$, 1.84 g, 12 mmol) and *N*-methylmorpholine (NMM, 1.01 g, 10 mmol) were added. The stirring was continued for 30 min at 0 °C and 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl, 1.92 g, 10 mmol) was added. The reaction mixture was stirred for 3 h at 0 °C and at rt for 5 h. Then, water (50 ml) was added to the mixture, which was extracted with dichloromethane (100 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to afford the crude residue. The crude product was used in the next step without further characterization.

5.1.3. *General procedure for the synthesis of substituted of 3-amino-3,*N-*phenyl-propionamide* (***4a–d***)

50% TFA/DCM (trifluoro acetic acid/dichloromethane, 50 ml) was added to substituted *N-tert*-butoxycarbonylamino-3-phenyl-propionamides (**3a**–**d**) (10 mmol) at 0 °C. The reaction mixture was stirred for 4 h at ambient temperature and then left overnight. The resulting mixture was neutralized with aqueous $NaHCO_3$ saturated solution. DCM layer was separated, dried over Na_2SO_4 and evaporated to afford the crude product. The obtained product was purified by column chromatography using 2% MeOH/DCM as eluent.

*5.1.3.1. 3-Amino-*N*-cyclopentyl-3-phenyl-propionamide* (***4a***). Yield: 65.4%; m.p. 113–116 °C; R_f 0.4 (10% MeOH/DCM); ^{1}H NMR (DMSO) δ 7.83 (d, 1H, $J = 6.99$ Hz), 7.34–7.15 (m, 5H), 4.17 (t, 1H, $J = 6.80$ Hz), 3.95–3.90 (m, 1H), 2.29 (d, 1H, $J = 6.87$ Hz), 1.72–1.22 (m, 8H); MS (ES+) 233 (M + H).

*5.1.3.2. 3-Amino-*N*-cyclohexyl-3-phenyl-propionamide* (***4b***). Yield: 72.4%; m.p. 137–139 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 7.40–7.26 (m, 5H), 6.67 (d, 1H, $J = 7.1$ Hz), 4.53 (s, 1H), 3.79–3.76 (m, 1H), 2.75–2.73 (m, 2H), 2.32 (bs, 2H), 1.84–1.59 (m, 5H), 1.42–1.05 (m, 5H); MS (ES+) 247 (M + H).

*5.1.3.3. 3-Amino-3,*N*-diphenyl-propionamide* (***4c***). Yield: 61.3%; m.p. 113–116 °C; R_f 0.5 (10% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.05 (s, 1H), 7.54 (d, 2H, $J = 7.64$ Hz), 7.38 (d, 2H, $J = 7.26$ Hz), 7.30–7.16 (m, 5H), 7.02–6.97 (m, 1H), 4.30–4.26 (m, 1H), 2.56–2.45 (m, 2H); MS (ES+) 241 (M + H).

*5.1.3.4. 3-Amino-3-phenyl-*N*-pyridin-2-yl-propionamide* (***4d***). Yield: 50.4%; m.p. 108–110 °C; R_f 0.2 (10% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 8.24 (d, 1H, $J = 8.25$ Hz), 7.85 (d, 1H, $J = 4.44$ Hz), 7.70 (t, 1H, $J = 8.0$ Hz), 7.45 (m, 2H), 7.44–7.31 (m, 3H), 4.30 (s, 1H), 2.73–2.71 (m, 2H), 1.66 (bs, 1H); MS (ES+) 242 (M + H).

5.1.4. N-tert-*Butoxycarbonylamino-2-phenylglycine* (***6***)

It was prepared in a similar manner as described for the synthesis of **2** except (DL)-2-phenylglycine (1.51 g, 10 mmol) was used in place of (DL)-3-aminophenylpropionic acid, m.p. 90–92 °C.

5.1.5. *General procedure for synthesis of 2-*N-tert-*butoxycarbonylamino-2-phenyl acetamides* (***7a–c***)

These compounds were similarly prepared as described for **3a**–**d**, from (DL)-*N-tert*-butoxycarbonylamino-2-phenylglycine (**6**) in place of **2**. Compounds were used in the next step without further characterization.

*5.1.6. General procedure for the synthesis of 2-amino-2-phenyl acetamides (**8a–c**)*

These compounds were similarly prepared as described for **4a–d**, from 2-*N-tert*-butoxycarbonylamino-2-phenyl acetamides (**7a–c**) in place of **3a–d**.

*5.1.6.1. 2-Amino-*N*-cyclopentyl-2-phenyl acetamide (**8a**).* Yield: 68.9%; m.p. 178–180 °C; R_f 0.6 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 8.62 (s, 1H), 7.42–7.26 (m, 5H), 5.55 (d, 1H, J = 7.96 Hz), 5.11 (s, 1H), 3.78–3.69 (m, 1H), 1.76–1.00 (m, 8H); MS (ES+) 219 (M + H).

*5.1.6.2. 2-Amino-*N*-cyclohexyl-2-phenyl acetamide (**8b**).* Yield: 65.3%; m.p. 91–93 °C; R_f 0.4 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 7.86 (d, 1H, J = 7.86 Hz), 7.36–7.17 (m, 5H), 4.27 (s, 1H), 2.18 (s, 1H), 1.72–1.10 (m, 10H); MS (ES+) 233 (M + H).

*5.1.6.3. 2-Amino-2,*N*-diphenyl acetamide (**8c**).* Yield: 53.2%; m.p. 112–114 °C; R_f 0.4 (7% MeOH/DCM); ^{1}H NMR (DMSO) δ 9.99 (bs, 1H), 7.62–7.21 (m, 9H), 7.02 (t, 1H, J = 7.3 Hz), 4.51 (s, 1H); MS (ES+) 225 (M + H).

5.1.7. General procedure for the synthesis of N*-substituted 1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**15–34**)*

Thionyl chloride (15 mmol) was added dropwise to a stirred solution of 1-propargyl-1,8-naphthyridine-3-carboxylic acid (**14a**, 10 mmol) in dichloromethane (50 ml). The stirring was continued for 4 h at room temperature and dried under vacuum to provide acid chloride intermediate **14b**. Compound **14b** was diluted with dichloromethane (50 ml) and appropriate amine (15 mmol) was added to it and stirred for 2 h. To the reaction mixture was added water (50 ml), which was extracted with dichloromethane (100 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness to provide crude product. The obtained crude product was purified over silica column using MeOH/DCM as eluent, to furnish the desired pure compound.

5.1.7.1. N*-(2-*N*-Cyclopentylcarbomyl-1-phenylethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**15**).* Yield: 39%; m.p. 193–195 °C; R_f 0.5 (7% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 10.61 (d, 1H, J = 8.1 Hz), 9.18 (s, 1H), 8.82–8.79 (2H, m), 7.50–7.24 (m, 6H), 5.88 (d, 1H, J = 7.1 Hz), 5.61 (dd, 1H, J = 6.63 Hz), 5.28 (s, 2H), 4.18 (m, 1H), 2.81–2.78 (m, 2H), 2.53 (m, 1H), 1.87–1.49 (m, 6H), 1.28–1.23 (m, 2H); MS (ES+) 443 (M + H).

5.1.7.2. N*-(2-*N*-Cyclohexylcarbomyl-1-phenylethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**16**).* Yield: 62.7%; m.p. 208–210 °C; R_f 0.3 (7% Acetone/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.64 (d, 1H, J = 8.1 Hz), 9.17 (s, 1H), 8.83–8.81 (m, 2H), 7.50–7.22 (m, 6H), 5.70 (m, 2H), 5.28 (s, 2H), 3.75–3.64 (m, 1H), 2.80 (m, 2H), 2.52 (m, 1H), 1.77–0.87 (m, 10H); MS (ES+) 457 (M + H).

5.1.7.3. N*-(2-*N*-Phenylcarbomyl-1-phenylethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**17**).* Yield: 44.1%; m.p. 185–187 °C; R_f 0.4 (5% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 10.68 (d, 1H, J = 7.65 Hz), 9.20 (s, 1H), 8.82–8.78 (m, 2H), 8.24 (s, 1H), 7.60–7.18 (m, 10H), 7.07–7.03 (m, 1H), 5.72 (q, 1H, J = 7.9, 13.6 Hz), 5.35–5.22 (m, 2H), 3.14–2.97 (m, 2H), 2.53 (s, 1H); MS (ES+) 451 (M + H).

5.1.7.4. N*-(2-*N*-Pyridin-2′-ylcarbomyl-1-phenylethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**18**).* Yield: 38.4%; m.p. 228–230 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.56 (s, 1H), 10.35 (d, 1H, J = 8.37), 9.12 (1H, s), 8.96–8.94 (m, 1H), 8.70 (dd, 1H, J = 1.86 Hz), 8.26 (d, 1H, J = 3.84 Hz), 8.02 (d, 1H, J = 8.4 Hz), 7.74–7.64 (m, 2H), 7.43–7.32 (m, 4H), 7.26–7.21 (m, 1H), 7.05–7.03 (m, 1H), 5.60 (q, 1H, J = 7.59, 14.8), 5.40 (d, 2H, J = 2.37 Hz), 3.51–3.49 (m, 1H), 3.09–2.97 (m, 2H); MS (ES+) 452 (M + H).

5.1.7.5. N*-(2-*N*-Cyclopentylcarbomyl-1-phenylethyl)-7-chloro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**19**).* Yield: 55.4%; m.p. 208–210 °C; R_f 0.6 (5% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 10.57 (d, 1H, J = 8.01 Hz), 9.15 (s, 1H), 8.73 (d, 1H, J = 8.3), 7.46–7.22 (m, 5H), 5.75 (d, 1H, J = 7.37), 5.60 (q, 1H, J = 6.7, 14.5 Hz), 5.22–5.21 (m, 2H), 4.17 (q, 1H, J = 6.7, 13.5), 2.78 (d, 2H, J = 6.61), 2.56–2.54 (m, 1H), 1.87–1.79 (m, 2H), 1.59–1.51 (m, 4H), 1.26–1.20 (m, 2H); MS (ES+) 477 (M + H).

5.1.7.6. N*-(2-*N*-Phenylcarbomyl-1-phenylethyl)-7-chloro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**20**).* Yield: 50.4%; m.p. 220–222 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 10.59 (d, 1H, J = 8.0 Hz), 9.17 (s, 1H), 8.70 (d, 1H, J = 8.3), 8.16 (s, 1H), 7.51–7.02 (m, 11H), 5.71 (d, 1H, J = 6.1), 5.21 (s, 2H) 3.07–3.00 (m, 2H), 2.56 (s, 1H); MS (ES+) 485 (M + H).

5.1.7.7. N*-(2-*N*-Pyridin-2′-ylcarbomyl-1-phenylethyl)-7-chloro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**21**).* Yield: 52.7%; m.p. 208–210 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.58 (s, 1H), 10.27 (d, 1H, J = 8.34 Hz), 9.10 (s, 1H), 9.08 (s, 1H), 8.67 (d, 1H, J = 8.31 Hz), 8.27 (d, 1H, J = 3.6), 8.01 (d, 1H, J = 8.34 Hz), 7.74–7.69 (m, 2H), 7.42–7.24 (m, 5H), 7.05 (m, 1H), 5.60 (q, 1H, J = 7.3, 14.7), 5.30–5.31 (m, 1H), 3.57 (t, 1H, J = 2.3 Hz), 3.12–2.96 (m, 2H); MS (ES+) 486 (M + H).

5.1.7.8. N*-(2-*N*-Cyclopentylcarbomyl-1-phenylethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**22**).* Yield: 67.6%; m.p. 208–210 °C; R_f 0.6 (5% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 10.52 (d, 1H, J = 8.07 Hz), 9.15 (s, 1H), 8.50 (d, 1H, J = 7.32 Hz), 7.42–7.22 (m, 5H), 5.69 (d, 1H, J = 7.26 Hz), 5.58 (q, 1H, J = 6.7 Hz, 14.31 Hz), 5.20 (s, 2H), 4.16 (q, 1H, J = 6.9 Hz, 13.6 Hz), 2.78–2.76 (m, 2H), 2.56 (d, 1H, J = 2.5 Hz), 1.87–1.19 (m, 8H); MS (ES+) 495 (M + H).

5.1.7.9. N*-(2-*N*-Cyclohexylcarbomyl-1-phenylethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**23**).* Yield: 67.5%; m.p. 179–181 °C; R_f 0.3 (7% Acetone/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.57 (d, 1H, J = 8.1 Hz), 9.14 (s, 1H), 8.51 (d, 1H, J = 7.32 Hz), 7.42–7.22 (m, 5H), 5.60–5.51 (m, 2H), 5.20 (d, 2H, J = 2.5 Hz), 3.75–3.72 (m, 1H), 2.77–2.75 (m, 2H), 2.5 (q, 1H, J = 2.5 Hz), 1.73–1.5 (m, 4H), 1.30–0.94 (m, 6H); MS (ES+) 509 (M + H).

5.1.7.10. N*-(2-*N*-Phenylcarbomyl-1-phenylethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**24**).* Yield: 50.7%; m.p. 171–173 °C; R_f 0.6 (7% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.28 (d, 1H, J = 8.2 Hz), 9.98 (s, 1H), 9.1 (1H, s), 8.62 (d, 1H, J = 7.8 Hz), 7.50 (d, 2H, J = 7.8 Hz), 7.40–7.23 (m, 5H), 7.13–7.11 (m, 3H), 6.98 (t, 1H, J = 7.2 Hz), 5.59 (q, 1H, J = 7.1, 14.2 Hz), 5.30 (s, 1H), 3.56 (s, 1H), 3.01–2.85 (m, 2H); MS (ES+) 503 (M + H).

5.1.7.11. N*-(2-*N*-Pyridin-2′-ylcarbomyl-1-phenylethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**25**).* Yield: 37.0%; m.p. 196–198 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.56 (s, 1H), 10.23 (d, 1H, J = 8.34 Hz), 9.10 (s, 1H), 8.60 (d, 1H, J = 7.8 Hz), 8.25 (d, 1H, J = 4.14 Hz), 8.0 (d, 1H, J = 8.34 Hz), 7.69 (m, 1H), 7.40–7.30 (m, 4H), 7.22 (t, 1H, J = 7.14 Hz), 7.04 (q, 1H, J = 5.2, 6.8 Hz), 5.58 (q, 1H, J = 7.4, 14.2 Hz), 5.31–5.30 (d, 2H, J = 2.1 Hz), 3.56 (t, 1H, J = 2.14 Hz), 3.10–2.95 (m, 2H); MS (ES+) 504 (M + H).

5.1.7.12. N-*(2*-N-*Cyclopentylcarbomyl-1-phenylmethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (***26***). Yield: 52.5%; m.p. 215–217 °C; R_f 0.4 (2% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 10.77 (d, 1H, $J = 6.5$ Hz), 9.16 (s, 1H), 8.81 (d, 1H, $J = 6.6$ Hz), 7.51–7.30 (m, 5H), 5.83 (d, 1H, $J = 6.7$ Hz), 5.61 (d, 1H, $J = 6.9$ Hz), 5.27 (q, 2H, $J = 2.4$, 4.8 Hz), 4.22 (d, 2H, $J = 6.9$ Hz), 2.52 (d, 2H, $J = 2.3$ Hz), 1.97–1.92 (m, 2H), 1.60–1.25 (m, 6H); MS (ES+) 429 (M + H).

5.1.7.13. N-*(2*-N-*Cyclohexylcarbomyl-1-phenylmethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (***27***). Yield: 65.6%; m.p. 206–208 °C; R_f 0.4 (7% Acetone/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.66 (d, 1H, $J = 6.21$ Hz), 9.16 (s, 1H), 8.81 (d, 2H, $J = 6.7$ Hz), 7.51–7.26 (m, 6H), 5.70 (d, 2H, $J = 8.3$ Hz), 5.61 (d, 1H, $J = 7.09$ Hz), 5.28–5.20 (m, 2H), 3.81–3.75 (m, 1H), 2.80–2.78 (m, 1H), 2.51 (d, 1H, $J = 2.5$ Hz), 2.02–1.63 (m, 3H) 1.63–1.02 (m, 7H); MS (ES+) 443 (M + H).

5.1.7.14. N-*(2*-N-*Phenylcarbomyl-1-phenylmethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (***28***). Yield: 66.0%; m.p. 187–189 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.73 (d, 1H, $J = 5.82$ Hz), 10.51 (s, 1H), 9.15 (s, 1H), 8.96 (s, 1H), 8.73 (d, 1H, $J = 7.35$ Hz), 7.68–7.29 (m, 10H), 7.04 (s, 1H), 5.89 (d, 1H, $J = 7.4$ Hz), 5.41 (bs, 2H), 3.51 (s, 1H); MS (ES+) 437 (M + H).

5.1.7.15. N-*(2*-N-*Cyclopentylcarbomyl-1-phenylmethyl)-7-chloro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (***29***). Yield: 75.5%; m.p. 191–193 °C; R_f 0.3 (2% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.52 (s, 1H), 9.09 (s, 1H), 8.69 (d, 1H, $J = 7.02$ Hz), 8.38 (d, 1H, $J = 9.36$ Hz), 7.70 (d, 1H, $J = 9.75$ Hz), 7.40–7.27 (m, 5H), 5.65 (s, 1H), 5.34–5.27 (m, 2H), 3.93 (s, 1H), 3.54–3.52 (m, 1H), 1.82–1.11 (m, 8H); MS (ES+) 463 (M + H).

5.1.7.16. N-*(2*-N-*Cyclohexylcarbomyl-1-phenylmethyl)-7-chloro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (***30***). Yield: 64.4%; m.p. >250 °C; R_f 0.4 (7% Acetone/$CHCl_3$); ^{1}H NMR (DMSO) δ 10.52 (d, 1H, $J = 7.8$ Hz), 9.09 (s, 1H), 8.69 (d, 1H, $J = 8.4$ Hz), 8.32 (d, 1H, $J = 7.74$ Hz), 7.71 (d, 1H, $J = 8.2$ Hz), 7.43–7.16 (m, 5H), 5.68 (d, 1H, $J = 7.8$ Hz), 5.30 (d, 2H, $J = 1.83$ Hz), 3.55 (s, 2H), 1.78–1.49 (m, 5H), 1.26–0.83 (m, 5H); MS (ES+) 477 (M + H).

5.1.7.17. N-*(2*-N-*Phenylcarbomyl-1-phenylmethyl)-7-chloro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (***31***). Yield: 65.1%; m.p. >250 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.66 (d, 1H, $J = 7.5$ Hz), 10.50 (s, 1H), 9.14 (s, 1H), 8.72 (d, 1H, $J = 8.37$ Hz), 7.73 (d, 1H, $J = 8.34$ Hz), 7.61–7.54 (m, 4H), 7.43–7.28 (m, 5H), 7.08–7.03 (m, 1H), 5.89 (d, 2H, $J = 7.4$ Hz), 5.33 (d, 1H, $J = 1.58$ Hz), 3.58 (s, 1H); MS (ES+) 471 (M + H).

5.1.7.18. N-*(2*-N-*Cyclopentylcarbomyl-1-phenylmethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (***32***). Yield: 65.0%; m.p. 238–240 °C; R_f 0.4 (7% Acetone/$CHCl_3$); ^{1}H NMR (DMSO) δ 10.41 (d, 1H, $J = 7.83$ Hz), 9.04 (s, 1H), 8.57 (d, 1H, $J = 7.83$ Hz), 8.32 (d, 1H, $J = 7.17$ Hz), 7.34 (d, 2H, $J = 7.35$ Hz), 7.28–7.16 (m, 3H), 5.58 (d, 1H, $J = 7.8$ Hz), 5.25 (d, 2H, $J = 2.3$ Hz), 3.93–3.84 (m, 1H), 3.50 (t, 1H, $J = 2.3$ Hz), 1.63–1.17 (m, 8H); MS (ES+) 481 (M + H).

5.1.7.19. N-*(2*-N-*Cyclohexylcarbomyl-1-phenylmethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (***33***). Yield: 57.1%; m.p. >250 °C; R_f 0.5 (7% Acetone/$CHCl_3$); ^{1}H NMR (DMSO) δ 10.47 (d, 1H, $J = 8.31$ Hz), 9.11 (s, 1H), 8.64 (d, 1H, $J = 6.18$ Hz), 8.31 (d, 1H, $J = 7.89$ Hz), 7.43–7.25 (m, 5H), 5.67 (d, 1H, $J = 7.65$ Hz), 5.32 (d, 2H, $J = 2.3$ Hz), 3.56–3.48 (m, 2H), 1.77–1.04 (m, 10H); MS (ES+) 495 (M + H).

5.1.7.20. N-*(2*-N-*Phenylcarbomyl-1-phenylmethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (***34***). Yield: 52.8%; m.p. >250 °C; R_f 0.4 (7% Acetone/$CHCl_3$); ^{1}H NMR (DMSO) δ 10.61 (d, 1H, $J = 7.5$ Hz), 10.49 (s, 1H), 9.1 (s, 1H), 8.66 (d, 1H, $J = 7.7$ Hz), 7.59–7.45 (m, 4H), 7.41–7.26 (m, 5H), 7.07–7.02 (m, 1H), 5.87 (d, 1H, $J = 7.5$ Hz), 5.32 (d, 2H, $J = 2.3$ Hz), 3.57 (d, 1H, $J = 2.38$ Hz); MS (ES+) 489 (M + H).

5.1.7.21. N-*(2*-N-*Phenylcarbomyl-1-phenylmethyl)-6-fluoro-7-pyrrolidinyl-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (***35***). Triethylamine (1.1 g, 11 mmol) and pyrrolidine (2.13 g, 30 mmol) were added to a suspension of compound **34** (4.89 g, 10 mmol) in acetonitrile (50 ml) and refluxed for 3 h. The reaction mixture was cooled; the precipitate thus separated was collected by filtration, washed with acetonitrile and dried to give compound **35**. Yield 4.52 g (86.3 %). m.p. >250 °C; R_f 0.4 (2% Methanol/DCM); ^{1}H NMR (DMSO) δ 10.98 (d, 1H, $J = 7.4$ Hz), 10.45 (s, 1H), 8.8 (s, 1H), 7.98–7.94 (m, 1H), 7.59–7.26 (m, 9H), 7.04 (s, 1H), 5.85 (d, 1H, $J = 7.9$ Hz), 5.23 (s, 2H), 3.75 (bs, 4H), 3.45 (s, 1H), 1.94 (s, 4H); MS (ES+) 523 (M + H).

5.2. Biological activity

5.2.1. Cytotoxicity

All the synthesized derivatives **15**–**35** were tested for in vitro cytotoxicity on nine cancerous as well as a non-cancerous cell lines and IC_{50} values were calculated in micromole (μM) [10]. The human cancer cell lines used in the study are ovary (PA1), prostate (DU145), oral (KB), colon (SW620), breast (HBL100), lung (A549), pancreas (MIAPaCa2), leukemia (K562) and endothelial (ECV304) cancer. All the 1,8-naphthyridine **15**–**35** and assay standard Doxorubicin HCl were also tested against normal mouse fibroblast (NIH3T3) cell line to evaluate their cancer cell specificity (safety index). The cytotoxicity data are summarized in Table 2. Compounds, which were found inactive, are not listed in Table 2. Derivatives of 1,8-naphthyridine-3-carboxamide (**15**–**35**) were screened for cytotoxic activity at the highest soluble concentration of 10 μM and on four lower concentrations on nine human tumor and one non-tumorous cell lines.

Briefly, a three days 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. MTT is metabolized by active mitochondria into a blue colored formazan product that is read spectrophotometrically at 540 nm. MTT solution (5 mg/ml) was prepared in phosphate buffered saline, pH 7.4 and filtered through a 0.22-μm filter. For each type of tumor and normal cell, 5000–10,000 cells were seeded in a 96-well culture plate and treated with various concentrations of 1,8-naphthyridine-3-carboxamide derivatives (**15**–**35**) for an incubation period of 72 h in CO_2 incubator. Control cells were not treated with 1,8-naphthyridine-3-carboxamide derivatives. The assay was terminated after 72 h by adding 125 μg (25 μl) MTT to each well. After incubation for 3 h, 50 μl of 10% SDS–0.01 N HCl was added to each well to lyse the cells and dissolve formazan. Plate was read spectrophotometrically at 540 nm after 1 h. Cytotoxicity percentage was calculated using the following formula: Cytotoxicity percentage $= (1 - (X/R_1)) \times 100$, where $X =$ (absorbance of treated sample at 540 nm) − (absorbance of blank at 540 nm), $R_1 =$ absorbance of control sample at 540 nm.

5.2.2. Anti-inflammatory activity

Dendritic cells (DCs) are central to an immune response and function as the best antigen-presenting cells. DCs have been identified as the cellular target for understanding pharmacological role of various immunomodulatory agents [11,12]. Pro-inflammatory

Table 2
In vitro cytotoxicity of 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**15**–**35**).

Compound no.	IC_{50} (μM)									
	PA1 (ovary)	DU145 (prostate)	KB (oral)	SW620 (colon)	HBL100 (breast)	A549 (lung)	MIAPaCa2 (pancreas)	K562 (leukemia)	ECV304 (endothelial)	NIH3T3 (normal fibroblast)
Doxorubicin	0.63	0.10	3.0	0.08	0.24	0.08	0.15	0.10	NA	0.39
17	>10	7.2	>10	>10	>10	>10	>10	>10	>10	NA
19	1.1	>10	>10	2.7	3.2	9.5	2.4	6.8	7.8	4.4
20	0.54	>10	>10	2.9	4.0	>10	3.0	7.7	4.0	4.4
21	1.7	4.9	5.2	2.9	>10	>10	>10	5.9	>10	NA
22	0.68	>10	4.9	2.1	2.0	6.1	4.4	7.3	5.1	2.4
23	2.3	8.03	>10	>10	>10	>10	4.9	6.2	9.8	NA
24	2.1	5.9	>10	5.7	9.3	>10	6.9	6.6	>10	9.7
25	>10	2.1	2.3	2.3	>10	>10	>10	>10	>10	4.2
26	>10	>10	4.5	>10	>10	>10	>10	>10	>10	NA
28	8.9	8.8	3.0	>10	>10	>10	>10	>10	>10	NA
31	>10	1.7	2.1	2.2	7.8	>10	9.7	3.3	>10	NA
32	1.8	3.2	3.5	3.4	5.1	>10	1.7	5.9	3.2	0.4
33	1.8	>10	>10	>10	>10	>10	>10	>10	>10	7.4
34	0.5	0.6	1.1	1.4	>10	>10	>10	5.0	9.1	NA

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

Table 3
Down regulation of IL-1-β and IL-6 activity by 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (% change calculated with reference to LPS stimulated levels secreted by DCs).

Compound no.	IL-1-β (% inhibition)		IL-6 (% inhibition)	
	1 μg/ml	0.1 μg/ml	1 μg/ml	0.1 μg/ml
15	−52.1	−62.4	–	–
17	−78.5	−75.1	–	–
18	−50.1	−34.1	−74.9	−74.4
19	−18.1	−82.0	−81.7	−79.8
20	−99.4	−28.3	−75.4	−69.5
21	−82.0	−57.3	−82.9	−78.2
22	−102.0	−83.4	−100.0	−80.1
24	−106.8	−84.8	−96.0	−60.0
25	−109.6	−83.4	−99.1	−75.5
28	−64.6	−39.9	−79.3	−79.3
31	−86.3	−82.0	−91.7	−76.5
34	−97.0	−44.3	−99.9	−84.4

Table 4
Down regulation of TNF-α and IP-10 activity by 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (% change calculated with reference to LPS stimulated levels secreted by DCs).

Compound no.	TNF-α (% inhibition)		IP-10 (% inhibition)	
	1 μg/ml	0.1 μg/ml	1 μg/ml	0.1 μg/ml
15	−34.7	−19.3	−25.0	−38.8
17	−29.7	−19.1	−33.1	−31.7
24	−99.0	−80.0	−102.4	−17.3

Cytokine levels were estimated by ELISA as described in Methods. Data shown are percent inhibition of cytokine/chemokine in duplicate.

cytokines are being explored as potential targets in therapeutic interventions for various inflammatory disorders such as Rheumatoid Arthritis [13]. In the present study, various derivatives were evaluated for down regulation of IL-1-β, IL-6, TNF-α and IP-10 levels secreted by LPS stimulated DCs.

Primary DC cultures were generated from femoral bone marrow of 8–12 weeks old C57BL/6 mice [14]. Percent change in cytokine/chemokine $= \{(B - A)/A \times 100$, 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. Bone marrow progenitors were cultured in RPMI-1640 supplemented with 10% FBS (Hyclone) and 20 ng/ml rmGMCSF (R&D Systems, MN, USA) at 37 °C, 5% CO_2. Immature DCs at day 6 were stimulated with 10 ng/ml lipopolysaccharide (LPS; SIGMA) and incubated with the 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives at two concentrations, 0.1 and 1 μg/ml for 24 h. The IL-1-β and IL-6, TNF-α and IP-10 secreted by the DCs were measured in culture supernatants by Enzyme Linked Immunosorbent Assays (R&D Systems Inc, MN, USA). Compounds showing down regulation of one or more of the cytokines or chemokines by >25% were considered to have potential anti-inflammatory activity as shown in Tables 3 and 4.

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