Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6660–6664
Bioorganic & Medicinal Chemistry Letters

Anticancer and anti-inflammatory activities of 1,8-naphthyridine-3-carboxamide derivatives

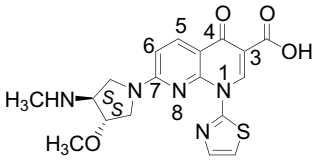
Sanjay K. Srivastava,[a] Manu Jaggi,[b,*] Anu T. Singh,[b] Alka Madan,[b] Nidhi Rani,[a] Manupriya Vishnoi,[b] Shiv K. Agarwal,[a,*] Rama Mukherjee[a,b] and Anand C. Burman[a,b]

[a]*Division of Medicinal Chemistry, Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad, 201 010 UP, India*
[b]*Division of Preclinical Research & Developmental Therapeutics, Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad, 201 010 UP, India*

Received 16 April 2007; revised 20 July 2007; accepted 3 August 2007
Available online 11 August 2007

Abstract—Several 1,8-naphthyridine-3-carboxamide derivatives (**8**–**23**) were synthesized and tested for in vitro cytotoxicity against eight cancer cell lines and a normal cell line. Compound **12** exhibited high cytotoxicity (IC_{50} = 1.37 μM) in HBL-100 (breast) cell line while compounds **17** (IC_{50} = 3.7 μM) and **22** (IC_{50} = 3.0 μM) have shown high cytotoxicity in KB (oral) and SW-620 (colon) cell lines, respectively. The synthesized 1,8-naphthyridine-3-carboxamides were also evaluated for anti-inflammatory and myeloprotective activities, indicated by modulation in cytokine and chemokine levels secreted by dendritic cells.

Recently, quinolones and naphthyridine class of compounds have been explored in cancer chemotherapy and one molecule of naphthyridine class, namely SNS-595, is presently in phase II clinical trial.[1] SNS-595 acts as a cell cycle modulator.[1,2] However, a limited information is available in the literature for the anticancer potential of naphthyridine class of compounds. Most of the chemical modifications were carried out at N-1, C-5, C-6, and C-7 positions in 1,8-naphthyridines.[1,3] The C-3 position has not been well exploited. In order to appreciate the actual utility of naphthyridines in cancer chemotherapy and to understand structure–activity relationship, modification at C-3 in 1,8-naphthyridines is required.



SNS-595

In addition, 1,8-naphthyridine class of molecules has been reported to exhibit potent anti-inflammatory activity.[4,5] 1,8-Naphthyridine-3-carboxamide derivatives were assessed for anti-inflammatory and myeloprotective activity using an in vitro screening assay based on murine bone marrow derived dendritic cells (DCs). The extent of modulation in pro-inflammatory cytokine and chemokine levels was taken as an indicator of anti-inflammatory and myeloprotective activity.

In the present paper, we have designed 1,8-naphthyridine-3-carboxamides where cyclic as well as open amino acids have been introduced at C-3 position. These amino acids may provide interaction with receptors and may lead to biological response. The propargyl group was introduced at N-1 position due to its hydrogen bonding capability. In order to have low molecular weight derivatives, the pyridine ring of 1,8-naphthyridine has been remained either unsubstituted or substituted with small groups such as halo or methyl group. Herein, we report the synthesis, cytotoxicity, anti-inflammatory, myeloprotective activity, and structure–activity relationship of 1,8-naphthyridine-3-carboxamides (**8**–**23**).

Synthesis of 1,8-naphthyridine-3-carboxamide derivatives (**8**–**22**) has been described in Scheme 1. The appropriate nicotinic acid **1** was treated with CDI in dry THF and the resulting imidazolide solution was reacted with

Keywords: Anticancer; Anti-inflammatory; Naphthyridine.
* Corresponding authors. Tel.: +91 120 4378610; fax: +91 120 4376902; e-mail: jaggim@dabur.com

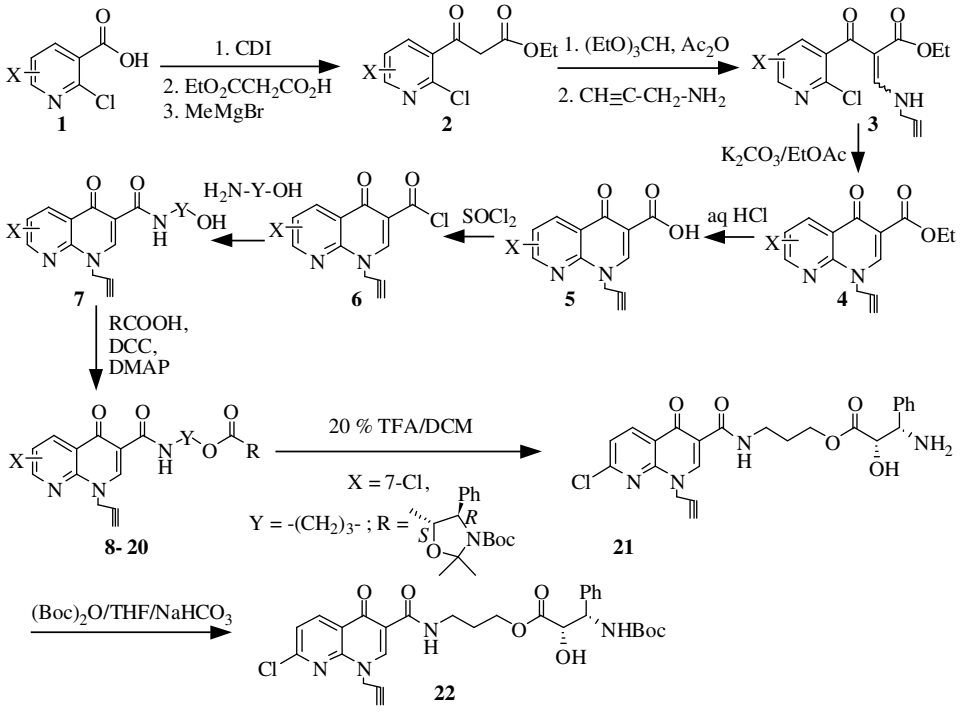
0960-894X/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2007.08.006

ethyl hydrogen malonate and methyl magnesium bromide to give ketoester **2**. Treatment of **2** with triethyl orthoformate and acetic anhydride followed by the addition of propargyl amine afforded the corresponding ethyl nicotinoylacrylate **3**. Compound **3** was cyclized by K_2CO_3 in ethyl acetate to afford 1,8-naphthyridine-3-carboxylate **4**. The acidic hydrolysis of **4** provided the corresponding acid derivative **5**, which was converted to carbonyl chloride derivative **6** with thionyl chloride.[3] Compound **6** was reacted with suitable amino alcohol to give compound **7**. The 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives, **8** and **12**, were prepared by coupling of suitable derivative of **7** with 4*R*,5*S*-1-*N*-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-phenyl-5-oxazolidine carboxylic acid[6] in the presence of DCC and DMAP.[7] Using the same method as described for **8** and **12**, compounds **9**–**11** and **13**–**20** were synthesized from the reaction of **7** with commercially available Boc-protected D-amino acids. The oxazolidine ring of compound **12** was opened with 20% TFA/DCM to provide compound **21**, which was then converted to **22** with di-*tert*-butylpyrocarbonate. Compound **23** was prepared in two steps starting from **7** as shown in Scheme 2. The displacement of 7-Cl in compound **7** by 3-methylpiperidine was performed by using K_2CO_3 as a base to provide compound **7a**, which was coupled with Boc-D-proline, as described for **8**–**20**, to give compound **23**. The 1,8-naphthyridine-3-carboxamide derivatives **8**–**20** are listed in Table 1.

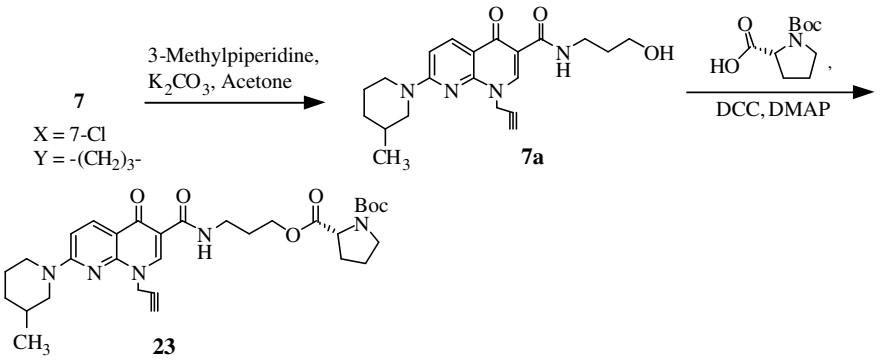
Results and discussion. The 1,8-naphthyridine-3-carboxamide derivatives (**8**–**23**) were tested for in vitro cytotoxicity in eight tumor cell lines and IC_{50} values were calculated in micromole (μM).[8,9] The human tumor cell lines used in the study were ovary (PA-1), prostate (DU145), oral (KB), colon (SW-620), breast (HBL-100), lung (A-549), pancreas (MiAPaCa2), and leukemia (K562) cancers. Compounds **8**–**23** were also screened against normal mouse fibroblast (NIH3T3) cell line to evaluate their cancer cell specificity (safety index).[8] The cytotoxicity data are given in Table 2.

Except compound **10**, none of the unsubstituted 1,8-naphthyridine-3-carboxamides (**8**–**11**) have shown cytotoxicity. Compound **10** exhibited cytotoxicity in PA-1 and KB cell lines. The 7-chloro naphthyridines (**12**–**16**) were found to be better than unsubstituted naphthyridines (**8**–**11**). Compound **12**, containing oxazolidine ring, was found to be the most potent derivative as it exhibited high cytotoxicity (IC_{50} = 1.37 μM) in HBL-100 cell line and was also found to be active in SW-620 and PA-1 cell lines. Compounds having proline (**13**) and alanine (**16**) substituent showed cytotoxicity in HBL-100 cell line. Compound **13** also showed cytotoxicity in MIAPaCa 2 cell line. Based on the above results, several analogs, related to active compounds **12**, **13**, and **16**, were further designed and synthesized to improve the cytotoxicity.

Upon replacing the 7-chloro group in compound **13** with methyl group (compound **17**), activity in HBL-100 cell line was lost but was found to be most potent in KB cell line (IC_{50} = 3.7 μM). It seems that nature of group present in pyridine ring in naphthyridine-3-carboxamides plays a vital role in eliciting cytotoxicity profile. On the other hand, by replacing propyl spacer with isopropyl (compound **18**) in compound **13**, cytotoxicity profile has been changed. Compound **18** was found to be more than 2-fold less cytotoxic in HBL-100 than compound **13** but exhibited activities in PA-1, KB, and SW-620 cell lines. It indi-

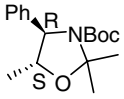
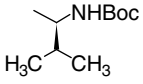
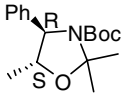
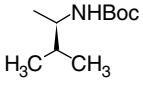
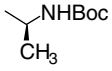
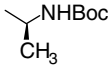
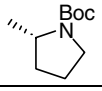


Scheme 1.



Scheme 2.

Table 1. 1,8-Naphthyridine-3-carboxamide derivatives (**8**–**20**)

Compound	X	Y	R
8	H	–$(CH_2)_3$–	
9	H	–$(CH_2)_3$–	
10	H	–$(CH_2)_3$–	–CH_2–NHBoc
11	H	–$(CH_2)_3$–	
12	7-Cl	–$(CH_2)_3$–	
13	7-Cl	–$(CH_2)_3$–	
14	7-Cl	–$(CH_2)_3$–	–CH_2–NHBoc
15	7-Cl	–$(CH_2)_3$–	
16	7-Cl	–$(CH_2)_3$–	
17	7-CH_3	–$(CH_2)_3$–	
18	7-Cl		
19	7-Cl		
20	7-CH_3		

cates that spacer has also played an important role in determining cytotoxic profile. When the 7-chloro group in compound **13** was replaced by 3-methylpiperidine (compound **23**), cytotoxicity was lost. As described in the activity profile for compound **18**, upon replacing the propyl spacer with isopropyl (compound **19**) in compound **16**, a similar cytotoxic profile was obtained except in SW-620 cell line. Compound **19** was 2-fold less cytotoxic than **16** in HBL-100 but exhibited cytotoxicity in PA-1 and KB cell lines. On the other hand, when oxazolidine ring in compound **12** was opened (compound **21**), activity was lost. Further, upon converting amine group in compound **21** to its Boc derivative **22**, activity HBL-100 was lost but was found to be highly potent in SW-620 cell lines (IC_{50} = 3.0 μM).

It reveals that 1,8-naphthyridine-3-carboxamides, in general, exhibited cytotoxicity in oral, colon, and breast cancers and the nature of group present in pyridine ring and the spacer, in particular, determined cytotoxicity profile. It was also interesting to note that 1,8-naphthyridine-3-carboxamides, in general, have shown good safety index as well. However, compounds **12**, **18**, and **22** are under further biological evaluation.

Anti-inflammatory activity. 1,8-Naphthyridine-3-carboxamide derivatives (**8**–**23**) were able to downregulate the levels of LPS stimulated TNF-α, IL-1β and IP-10 secreted by DCs that were identified to have potential anti-inflammatory activity. The downregulation of cytokine and chemokine levels by >25% was considered as significant.[10,11]

Figure 1 shows the downregulation of a key pro-inflammatory cytokine TNF-α by selected molecules. Compounds **12**, **13**, **14**, and **22** exhibit >50% TNF-α inhibition at 1 μg/ml, reflecting significant anti-inflammatory activity. Compound **13** shows a remarkable downregulation of TNF-α activity even at 0.1 μg/ml. Out of these few selected compounds, **13**, **14**, and **22** demonstrate a significant inhibition of IP-10 activity, as shown in Figure 2. In addition, compounds **8**, **12**, and **21** exhibited >50% IL-1β inhibitory activity (Fig. 3).

The downregulation of TNF-α, IL-1β, and IP-10 levels by compounds **8**, **12**, **13**, **14**, **16**, **21**, and **22** suggests potential anti-inflammatory activity.

1,8-Naphthyridine-3-carboxamides (**8**–**23**) with potential myeloprotective activity were identified by evaluating modulation in MIP-1-α, CCL-22, and TNF-α from basal levels secreted by DCs, when incubated with these molecules.[12]

Table 2. In vitro cytotoxicity of 1,8-naphthyridine-3-carboxamide derivatives (**8**–**23**)

Compound	IC_{50} (μM)								
	PA-1 (ovary)	DU-145 (prostate)	KB (oral)	SW-620 (colon)	HBL-100 (breast)	A-549 (lung)	Miapaca (pancreas)	K-562 (leukemia)	NIH3T3 (normal fibroblast)
10	9.0	>10	7.0	>10	>10	>10	>10	>10	>10
12	6.0	>10	>10	3.96	1.37	>10	>10	>10	>10
13	>10	>10	>10	>10	3.7	>10	7.6	>10	8.4
16	>10	>10	>10	>10	5.4	>10	>10	>10	9.2
17	>10	>10	3.7	>10	>10	>10	>10	>10	>10
18	5.2	>10	6.6	3.3	8.4	>10	>10	>10	>10
19	4.9	>10	8.3	>10	>10	>10	>10	>10	>10
22	>10	>10	>10	3.0	>10	>10	>10	>10	7.84

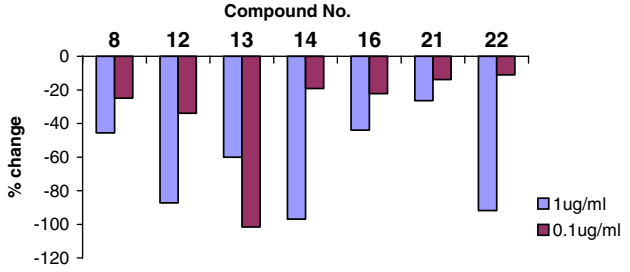


Figure 1. Anti-inflammatory activity of selected 1,8-naphthyridine-3-carboxamide derivatives as a measure of TNF-α downregulation. % change in TNF-α was calculated with reference to LPS stimulated levels secreted by murine DCs.

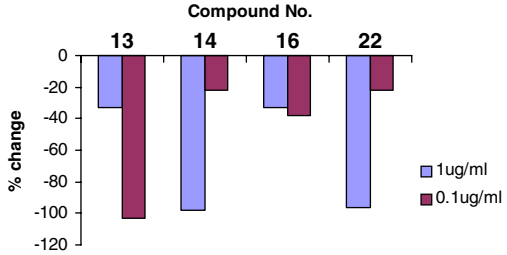


Figure 2. IP-10 downregulation (% change calculated with reference to LPS stimulated levels secreted by DCs) by selected 1,8-naphthyridine-3-carboxamide derivatives.

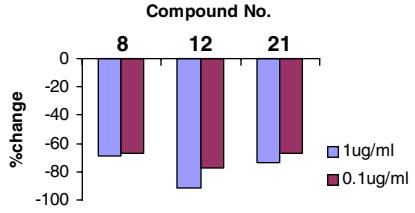


Figure 3. IL-1-β downregulation (% change calculated with reference to LPS stimulated levels secreted by DCs) by selected 1,8-naphthyridine-3-carboxamide derivatives.

Figure 4 demonstrates MIP-1-α upregulation by selected compounds **13**, **14**, and **16**, suggesting significant myeloprotective activity. Also, compounds **13**, **14**, and **16** were able to downregulate CCL-22 and TNF-α as shown in Figures 5 and 6, respectively. Endogenous upregulation of MIP-1-α under the influence of proposed 1,8-naphthyridine-3-carboxamide derivatives is also likely to exert similar myeloprotective effects.

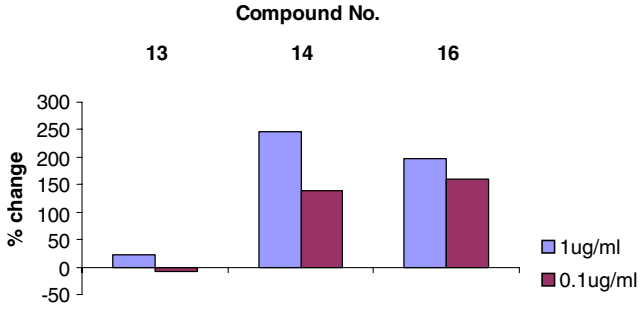


Figure 4. Upregulation of MIP-1-α levels (% change calculated with reference to basal levels secreted by DCs) by selected 1,8-naphthyridine-3-carboxamide derivatives with potential myeloprotective activity.

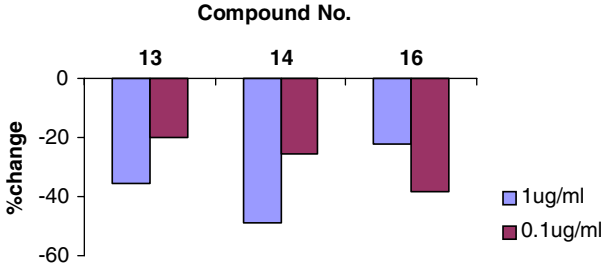


Figure 5. CCL-22 downregulation (% change calculated with reference to basal levels secreted by DCs) by selected 1,8-naphthyridine-3-carboxamide derivatives.

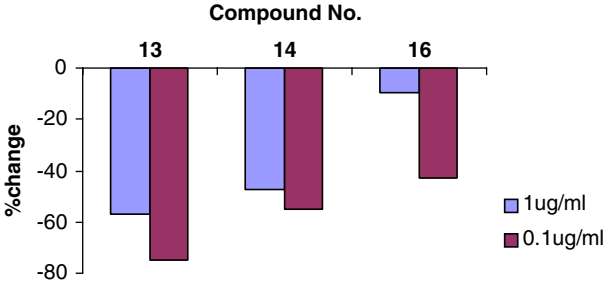


Figure 6. TNF-α downregulation of selected 1,8-naphthyridine-3-carboxamide derivatives with potential myeloprotective activity. % change in TNF-α was calculated with reference to basal levels secreted by DCs.

In addition, these molecules showing a downregulation of TNF-α from basal levels (Fig. 6) were

predicted to have lower tissue toxicity due to reduced probability of TNF-α induced inflammation leading to tissue damage.

References and notes

1. FDAnews Drug Pipeline Alert, March 21, **2006**; Vol. *4*, No. 56.
2. Tsuzuki, Y.; Tomita, K.; Shibamori, K.-i.; Sato, Y.; Kashimoto, S.; Chiba, K. *J. Med. Chem.* **2004**, *47*, 2097.
3. Tomita, K.; Tsuzuki, Y.; Shibamori, K.-i.; Tashima, M.; Kajikawa, F.; Sato, Y.; Kashimoto, S.; Chiba, K.; Hino, K. *J. Med. Chem.* **2002**, *45*, 5564.
4. Grossi, G.; Di Braccio, M.; Roma, G.; Ballabeni, V.; Tognolini, M.; Barocelli, E. *Eur. J. Med. Chem.* **2005**, *40*, 155.
5. Dianzani, C.; Collino, M.; Gallicchio, M.; Di Braccio, M.; Roma, G.; Fantozzi, R. *J. Inflamm.* **2006**, *3*, 4. doi:10.1186/1476-9255-3-4.
6. 4*R*,5*S*-*N*-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4-phenyl-5-oxazolidine carboxylic acid was purchased from Dabur Pharma Ltd, Kalyani, WB, India.
7. Compound **8**: R_f 0.7 (5% MeOH/DCM); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.76 (br s, 1H), 9.1 (s, 1H), 8.75–8.66 (m, 2H), 7.43–7.39 (m, 1H), 7.25–7.19 (m, 5H), 5.21 (s, 2H), 5.01 (br s, 1H), 4.48 (d, 1H, J = 5.2 Hz), 4.26–4.24 (m, 2H), 3.45–3.43 (m, 2H), 2.46 (s, 1H), 1.96–1.92 (m, 2H), 1.70 (s, 3H), 1.63 (s, 3H) 1.18–1.05 (m, 9H); MS (ES+) *m*/*z* (% relative intensity) 589 (M+H) (20), 611 (M+H+Na) (100).
8. Derivatives of 1,8-naphthyridine-3-carboxamide (**8**–**23**) were screened for cytotoxic activity at the highest soluble concentration of 10 μM and on four lower concentrations on eight human tumors and one non-tumorous cell lines. Briefly, a 3-day MTT in vitro cytotoxicity assay was performed, which was 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 was metabolized by active mitochondria into a blue colored formazan product that was read spectrophotometrically.[9] MTT was dissolved in phosphate-buffered saline with a pH of 7.4 to obtain a MTT concentration of 5 mg/ml; the resulting mixture was filtered through a 0.22-μm filter to sterilize and remove a small amount of insoluble residue. For each type of tumor and normal cell, 5000–10,000 cells were seeded in a 96-well culture plate and incubated with various concentrations of 1,8-naphthyridine-3-carboxamide derivatives (**8**–**23**) in a CO_2 incubator for 72 h. Control cells not treated with 1,8-naphthyridine-3-carboxamide derivatives (**8**–**23**) were similarly incubated. The assay was terminated after 72 h by adding 125 μg (25 μL) MTT to each well, then incubating for three hours, and finally adding 50 μL of 10% SDS–0.01 N HCl to each well to lyse the cells and dissolve formazan. After incubating for 1 h, the plate was read spectrophotometrically at 540 mm and the cytotoxicity percentage calculated using the following formula: cytotoxicity percentage = $(1 - X/R1) * 100$, where X = (absorbance of treated sample at 540 nm) – (absorbance of blank at 540 nm), R_1 = absorbance of control sample at 540 nm.
9. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
10. *BMDC based ex-vivo septic shock assay to evaluate potential anti-inflammatory activity:* Primary DC cultures were generated from femoral bone marrow of 8- to 12-week-old C57BL/6 mice.[11] Bone marrow progenitors were cultured in RPMI-1640 supplemented with 10% FBS and rmGMCSF (20ng/ml) at 37 °C, 5% CO_2. Immature DCs were stimulated with lipopolysaccharide (LPS; 100 ng/ml) and incubated with the 1,8-naphthyridine-3-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 TNF-α, IL-1-β-, and IP-10 secreted by 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.
11. Lutz, M. B.; Kukutsch, N.; Ogilvie, A. L.; Rossner, S.; Koch, F.; Romani, N.; Schuler, G. *J. Immunol. Methods* **1999**, *223*, 77.
12. *BMDC based assay for myeloprotective activity:* The procedure employed to analyze changes in chemokine (MIP-1-α, CCL-22) and cytokine (TNF-α) levels expressed by DCs under the influence of 1,8-naphthyridine-3-carboxamide derivatives is described by below. Murine bone marrow derived dendritic cells[10] were incubated with test compounds at 0.1 and 1 μg/ml. The amounts of MIP-1-α, CCL-22, and TNF-α secreted by DCs after 24 h incubation were measured in the culture supernatants by respective 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 DCs when incubated with test molecule, A = concentration of cytokine/chemokine (pg/ml) secreted by untreated DCs.