Journal of Sciences, Islamic Republic of Iran 20(4): 325-329 (2009) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Synthesis and Anti Cancer Activity of Pyrido[2,3-*c*]Pyridazine Derivatives

V. Kumar,[1,3,*] V.K. Sanna,[2] A.T. Singh,[2] M. Jaggi,[2] P.K. Sharma,[3] R. Irchhaiya,[3] and A.C. Burman[1,2]

[1]*Chemical Research, Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad, 201010, UP, India*
[2]*Preclinical Research, Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad, 201010, UP, India*
[3]*Institute of Pharmacy, Bundelkhand University, Jhansi, UP, India*

Received: 8 March 2009 / Revised: 26 September 2009 / Accepted: 26 December 2009

Abstract

Cancer is not just one disease, but a large group of almost one hundred diseases. Its two main characteristics are uncontrolled growth of the cells in the human body and the ability of these cells to migrate from the original site and spread to distant sites. If the spread is not controlled, cancer can result in death. There has been continuous progress in the development of therapy for cancer. The successful treatment of cancer still remains a challenge. A new series of pyrido[2,3-*c*]pyridazine derivatives (**8-14**) were synthesized and screened for their anticancer activity. Amongst them few of the compounds have exhibited promising cytotoxicity along with good safety index. These compounds could be of use in designing new anti-cancer agents.

Keywords: Pyrido[2,3-c]pyridazine; Anti cancer; Cytotoxic

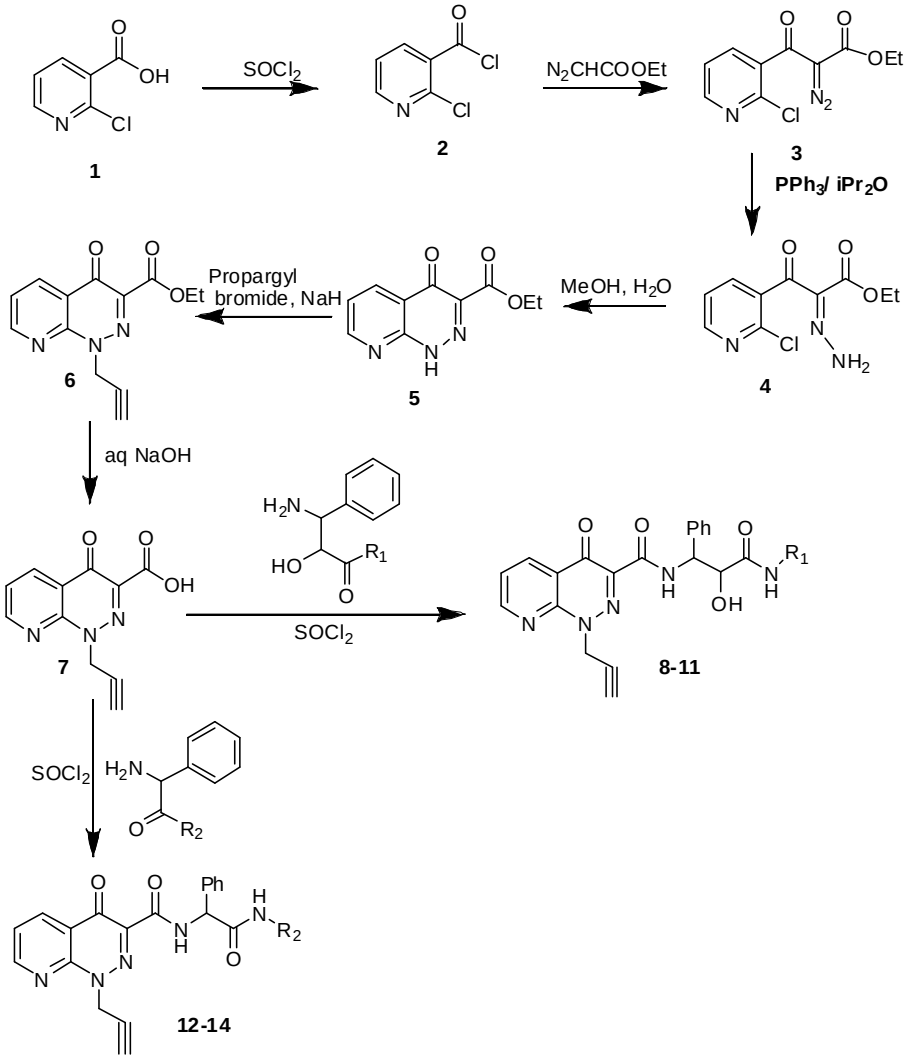
Introduction

There has been continuous progress in the development of therapy for cancer. This disease remains the second major cause of death in the US. But due to the lack of specificity for tumors, drugs that are presently administered often lead to systemic toxicity and to undesirable side effects [1]. The successful treatment of cancer still remains a challenge in the 21st century, and there is a need to search for newer specific and safer anticancer agents. Mammalian Topoisomerase II is one of the known target for antitumor agents like doxorubicin, etoposide, ellipticine and amascrine [2]. Earlier, Tomita *et al.* has tried for pyrido[2,3-*c*]pyridazine derivative as an anticancer but were unsuccessful [3]. We had further carried out work on pyrido[2,3-*c*]pyridazine ring system at C-3 position and synthesized few highly cytotoxic molecules with selective cytotoxicity and good safety index.

Materials and Methods

All the solvents and reagents were purchased from different companies such as Aldrich, Lancaster, 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). Visualization was achieved with UV light and iodine vapor. Column chromatography was performed using silica gel (100-200 mesh). Proton Magnetic Resonance (PMR) spectra were recorded on a Bruker 300 MHz instrument using tetramethylsilane (TMS) as an internal standard. Mass

* Corresponding author, Tel.: +911204378501, Fax: +911204376905, E-mail: vivek_niper@yahoo.co.in



Scheme 1.

spectra were recorded on a Micromass Quattro Micro™ instrument. The purity of the synthesized compounds was determined on Shimadzu HPLC LC-2010 C HT instrument using gradient system.

To synthesize pyrido[2,3-*c*]pyridazine heterocyclic system 2-chloro nicotinic acid **1** was refluxed with thionyl chloride to afford 2-chloronicotinoyl chloride (**2**) as shown in Scheme 1. Compound **2** was allowed to react with ethyl diazoacetate to provide 2-diazo nicotinoylacetate (**3**). Compound **3** was treated with triphenylphosphine in isopropyl ether and resulting 2-hydrazono nicotinoylacetate (**4**) was refluxed in a mixture of methanol and water to afford ethyl pyrido[2,3-*c*]pyridazine-3-carboxylate (**5**) [4]. Ethyl-*N*-1-propargyl pyrido[2,3-*c*]pyridazine-3-carboxylate (**6**) was prepared by the *N*-alkylation of compound **5** in presence of sodium hydride, which upon basic hydrolysis afforded *N*-1-propargyl pyrido[2,3-*c*]pyridazine-3-carboxylic acid (**7**). Compound **7** was coupled with different "in house" synthesized functionalized amino acids to afford desired *N*-1-propargyl pyrido[2,3-*c*]pyridazine-3-carboxamide derivatives (**8-14**) as shown in Table 1 [5].

*4-Oxo-1-prop-2-ynyl-1,4-dihydro-pyrido[2,3-c]pyridazine-3-carboxylic acid ethyl ester **(6)***. To a suspension of sodium hydride (95%, 0.14 g, 5.54 mmol) in DMF (20ml) was added dropwise, a solution of compound **5** (0.50 g, 2.28 mmol) in DMF (5 ml) [4]. The reaction mixture was heated at 60°C for 1h. Reaction mixture was brought to rt, propargyl bromide (0.31 g, 5.6 mmol) was added dropwise and reaction mixture was again heated at 60°C for 4h. The reaction

mixture was brought to rt and diluted with cold water. Organic layer was extracted with ethyl acetate (2×50 ml), dried over anhydrous Na_2SO_4 and concentrated to dryness to afford a crude product. The crude product was chromatographed on silica gel with 5% EtOAc/hexane to afford compound **6** (0.34 g, 58%).

R_f 0.5 (5% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ: 1.40-1.45 (m, 3H, CH_3), 2.38 (t, 1H, *J* = 2.4 Hz, ≡CH), 4.47 (t, 2H, *J* = 7.1 Hz, -OCH_2), 5.43 (d, 2H, *J* = 2.4 Hz, -NCH_2), 7.48 (dd, 1H, *J* = 4.5, 8.0 Hz, -Ar), 8.68 (dd, 1H, *J* = 1.7, 8.0 Hz, -Ar), 8.90 (dd, 1H, *J* = 1.7, 4.38 Hz, -Ar); m.p 187-189 °C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-pyrido[2,3-c]pyridazine-3-carboxylic acid (**7**).

A suspension of compound **6** (0.50 g, 1.94 mmol) in 20 ml of dioxane/water (1:1) was treated with 40% NaOH (15 ml) solution and refluxed for 6h. The solution was cooled and acidified to pH 2 with conc. HCl. Solid thus obtained was filtered and dried under vacuum to provide compound **7** (0.11 g, 24.7%).

R_f 0.3 (15% MeOH/DCM); ^{1}H NMR (DMSO)δ: 3.34 (bs, 1H, ≡CH), 5.45 (d, 2H, *J* = 2.3 Hz, -NCH_2), 7.70 (dd, 1H, *J* = 4.5, 8.01 Hz, -Ar), 8.62 (dd, 1H, *J* = 1.8, 8.0 Hz, -Ar), 9.06 (dd, 1H, *J* = 1.8, 4.5 Hz, -Ar); m.p >250 °C.

N-(3-(cyclopentylamino)-2-hydroxy-3-oxo-1-phenyl-propyl)-4-oxo-1-(prop-2-ynyl)-1,4-dihydropyrido[2,3-c]pyridazine-3-carboxamide (**8**).

Thionyl chloride (2 ml) was added to a suspension of compound **7** (0.50 g, 2.18 mmol) in DMF (20 ml) and the mixture was stirred at room temperature for 4h. To reaction mixture functionalized amino acid (3-Amino-N-cyclopentyl-2-hydroxy-3-phenyl-propionamide) (0.82 g, 3.3 mmol) was added after diluting with DMF (5 ml). Reaction mixture was stirred for 2h and diluted with water (50 ml). Product was extracted with EtOAc (2×50 ml) and dried over anhydrous sodium sulfate. The organic layer was concentrated to dryness to provide crude product. The crude product obtained was purified by column chromatography using silica gel and 2% MeOH/DCM as eluent, to furnish compound **8** (0.328 g, 32.7%).

R_f 0.4 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ: 1.16-1.71 (m, 8H, -cylopentyl CH_2), 3.93-4.00 (m, 1H, ≡CH), 4.17 (d, 1H, *J* = 3.12 Hz, -NHCH of cylopentyl), 5.45 (d, 2H, *J* = 7.9 Hz, -NCH_2), 5.74 (s, 1H, -NHCH), 6.10 (d, 1H, *J* = 5.5 Hz, -CHOH), 7.18-7.48 (m, 5H, -Ar), 7.75 (dd, 1H, *J* = 8.1, 4.5 Hz, -Ar), 8.68 (dd, 1H, *J* = 8.1, 1.6 Hz, -Ar), 9.08 (dd, 1H, *J* = 4.3, 1.6 Hz, -Ar), 10.14 (d, 1H, *J* = 8.6 Hz, -NH); MS (ES+) 460 (M+H), m.p. > 250°C.

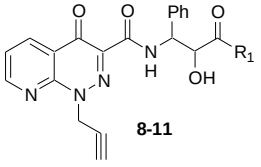
Compounds **9-14** were prepared similarly **8**.

N-(3-(cyclohexylamino)-2-hydroxy-3-oxo-1-phenylpropyl)-4-oxo-1-(prop-2-ynyl)-1,4-dihydropyrido [2,3-c]pyridazine-3-carboxamide **(9).** R_f 0.3 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ: 0.95-1.81 (m, 10H, -cyclohexyl CH_2), 2.37 (s, 1H, NHCH of cyclohexyl), 3.74 (bs, 1H, ≡CH), 4.54 (s, 2H, -NCH_2), 5.54 (s, 1H, -NHCH), 5.72 (d, 1H, J = 7.6 Hz, -CHOH), 6.57 (d, 1H, J = 7.4 Hz, -NH), 7.29-7.56 (m, 6H, -Ar), 8.78 (d, 1H, J = 7.4 Hz, -Ar), 8.97 (s, 1H, -Ar), 10.81 (d, 1H, J = 7.4 Hz, -NH); MS (ES+) 474 (M+H), Yield 0.352 g (34.1%), m.p. > 250°C.

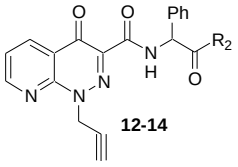
N-(3-(anilino)-2-hydroxy-3-oxo-1-phenylpropyl)-4-oxo-1-(prop-2-ynyl)-1,4-dihydropyrido[2,3-c]pyridazine -3-carboxamide **(10).** R_f 0.3 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ: 2.35 (s, 1H, ≡CH), 4.74 (d, 1H, J = 4.1 Hz, -NH), 5.10 (d, 1H, J = 5.8 Hz, -NHCH), 5.51 (d, 2H, J = 1.8 Hz, -NCH_2), 5.80 (d, 1H, J = 4.8 Hz, -CHOH), 7.05-7.08 (m, 1H, -Ar), 7.29-7.59 (m, 9H, 2H partially merged with $CDCl_3$ peak -Ar), 8.74 (d, 2H, J = 6.4 Hz, -Ar), 8.97 (d, 1H, J = 2.8 Hz, -Ar), 10.83 (d, 1H, J = 7.9 Hz, -NH); MS (ES+) 468 (M+H), Yield 0.189 g (18.5%), m.p. > 250°C.

N-(3-(pyridin-2-yl)-2-hydroxy-3-oxo-1-phenylpropyl)-4-oxo-1-(prop-2-ynyl)-1,4-dihydropyrido[2,3-c]pyridazine-3-carboxamide **(11)**. R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ: 2.35 (s, 1H, ≡CH), 3.47 (m, 1H, -NH), 4.56 (s, 1H, -NHCH), 5.51 (s, 2H, -NCH_2), 5.98 (d, 1H, J = 8.3 Hz, -CHOH), 6.86-7.01 (m, 2H, -Ar), 7.26-7.65 (m, 7H, -Ar), 8.23 (d, 1H, J = 8.2 Hz, -Ar), 8.78 (d, 1H, J = 7.4 Hz, -Ar), 8.94

Table 1. 1-Propargyl pyrido[2,3-c]pyridazine-3-carboxamide derivatives **(8-14)**



8-11



12-14

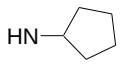
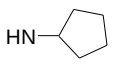
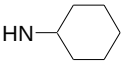
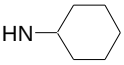
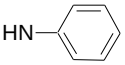
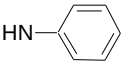
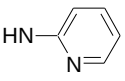
Compound No.	R1	Compound No.	R2
8		**12**	
9		**13**	
10		**14**	
11			

Table 2. *In vitro* cytotoxicity of 1-propargyl pyrido[2,3-c]pyridazine-3-carboxamide derivatives **(8-14)**

S. No.	C. No.	IC_{50} (μM)									
		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	**11**	>10	>10	>10	>10	>10	6.0	8.9	>10	NA	NA
3	**13**	>10	>10	9.0	>10	>10	>10	>10	>10	NA	NA
4	**14**	>10	>10	8.3	>10	>10	>10	>10	>10	NA	NA

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.

(s, 1H, -Ar), 9.59 (s, 1H, -Ar), 10.67 (d, 1H, J = 7.9 Hz, -NH); MS (ES+) 469 (M+H), Yield 0.409 g (40.1%), m.p. > 250°C.

N-(2-(cyclopentylamino)-2-oxo-1-phenylethyl)-4-oxo-1-(prop-2-ynyl)-1,4-dihydro pyrdo[2,3-c]pyridazine -3-carboxamide ***(12).*** R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ: 1.25-1.84 (m, 8H, -CH_2 of cylopentyl), 3.44 (s, 1H, ≡CH), 3.94-4.02 (m, 1H, -NHCH), 5.47 (d, 2H, *J* = 2.1 Hz, -NCH_2), 5.68 (d, 1H, *J* = 7.7 Hz, -NHCH), 7.25-7.51 (m, 5H, -Ar+-NH), 7.73 (dd, 1H, *J* = 8.0, 4.4 Hz, -Ar), 8.41 (d, 1H, *J* = 7.08 Hz, -Ar), 8.68 (dd, 1H, *J* = 8.0, 1.7 Hz, -Ar), 9.07 (dd, 1H, *J* = 4.35, 1.68 Hz, -Ar), 10.31(d, 1H, *J* = 7.8 Hz, -NH); MS (ES+) 430 (M+H), Yield 0.325 g (34.7%), m.p. 221-223°C.

N-(2-(cyclohexylamino)-2-oxo-1-phenylethyl)-4-oxo-1-(prop-2-ynyl)-1,4-dihydro pyrdo[2,3-c]pyridazine -3-carboxamide **(13).** R_f 0.5 (5% MeOH/ DCM); ^{1}HNMR

(DMSO-d_6) δ: 1.06-1.79 (m, 10H, -CH_2 of cyclohexyl), 3.43-3.49 (m, 2H, ≡CH + NHCH), 5.46 (d, 2H, *J* = 2.2 Hz, NCH_2), 5.68 (d, 1H, *J* = 7.8 Hz, NHCH), 7.24-7.46 (m, 5H, -Ar + -NH), 7.73 (dd, 1H, *J* = 8.0, 4.4 Hz, -Ar), 8.33 (d, 1H, *J* = 7.7 Hz, -Ar), 8.68 (dd, 1H, *J* = 8.0, 1.7 Hz, Ar), 9.07 (dd, 1H, *J* = 4.4, 1.8 Hz, -Ar), 10.31 (d, 1H, *J* = 7.8 Hz, -NH); MS (ES+) 444 (M+H), Yield 0.207 g (21.4%), m.p. 243-245°C.

N-(2-(anilino)-2-oxo-1-phenylethyl)-4-oxo-1-(prop-2-ynyl)-1,4-dihydro pyrdo[2,3-c]pyridazine-3-carbox-amide ***(14).*** R_f 0.5 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ: 3.44 (s, 1H, =CH), 5.47 (s, 2H, -NCH_2), 5.89 (d, 1H, *J* = 6.3 Hz, -NHCH), 7.05 (m, 1H, -Ar), 7.29-7.72 (m, 10H, -Ar), 8.69 (d, 1H, *J* = 7.3 Hz, -Ar), 9.07 (s, 1H, -Ar), 10.48 (m, 2H, NH); MS (ES+) 438 (M+H), Yield 0.278 g (29.1%), m.p. > 250°C.

Results and Discussion

Cytotoxicity

All the synthesized *N*-1-propargyl pyrido[2,3-*c*]pyridazine-3-carboxamide derivatives (**8-14**) 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 *N*-1-propargyl pyrido[2,3-*c*]pyridazine-3-carboxamide derivatives (**8-14**) and assay standard Doxorubicin HCl were also tested against normal mouse fibroblast (NIH3T3) and normal ovary (CHO) cell line to evaluate their cancer cell specificity (safety index) [6]. Derivatives of *N*-1-propargyl pyrido[2,3-*c*]pyridazine-3-carboxamide (**8-14**) were screened for cytotoxic activity at the highest soluble concentration of 10 μM. The cytotoxicity data is summarized in Table 2 and the compounds, which were inactive at 10 μM, are not listed. 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 [7].

In substituted-2-hydroxy-3-phenyl-propionamide derivatives (**8-11**), C-3 position of pyrido[2,3-*c*]pyridazine carboxylic acid was substituted with different cycloalkyl based functionalized amino acids. The resulting cyclopentyl substituted derivative (**8**) showed no cytotoxicity. On further expansion of cycloalkyl ring from cyclopentyl to cyclohexyl (**9**) resulted in no improvement in the cytotoxicity. Similarly, replacement

of cycloalkyl ring with aryl leads to inactive compound (**10**), However, heteroaryl (2-pyridyl) substituted derivative (**11**) showed IC_{50} of 6.0 and 8.9 μM on A549 and MIAPaCa cancer cell lines, respectively.

In substituted-2-phenyl-acetamide derivatives (**12-14**), cycloalkyl based derivative (**12**) was inactive, similar to the earlier cycloalkyl substituted-2-hydroxy-3-phenyl-propionamide derivative. However, compound **13** showed cytotoxicity with IC_{50} of 9.0 μM on KB cell line. In addition, heteroaryl substituted compound **14** also showed selective cytotoxicity with IC_{50} of 8.3 on KB cell line. All the compounds have also showed high safety index through out the series.

References

1. FDA news Drug Pipeline Alert, **4**(56): (2006).
2. Burden D.A., Osheroff N. Mechanism of action of eukaryotic topoisomerase II and drugs targeted to the enzyme. *Biochim. Biophys. Acta*, **1400**: 139-154 (1998).
3. Tomita K., Tsuzuki Y., Shibamori K., Tashima M., Kajikawa F., Sato Y., Kashimoto S., Chiba K., Hino K. Synthesis and structure-activity relationships of novel 7-substituted 1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acids as antitumor agents. Part 1. J. Med. Chem., 45(25): 5564-5575 (2002).
4. Heterocyclic mutilin esters and their use as antibacterials WO 02/12199.
5. Kumar V., Mudgal M.M., Rani N., Jha A., Jaggi M., Singh A.T, Sanna V.K., Burman, A.C. Synthesis of functionalised amino acid derivatives as new pharmacophores for designing anticancer agents. *J. Enz. Inhib. Med. Chem.*, **24** (3): 769-776 (2009).
6. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **65**: 55-63 (1983).
7. Lutz M.B., Ukutsch N., Oilvie A.L. Rossner S., Koch F., Romani N., Schuler G. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J. Immunol. Methods; 223: 77-92 (1999).