Asian Journal of Chemistry; Vol. 26, No. 21 (2014), 7389-7392

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.17050

Synthesis, Crystal Structure and Antitumor Activity of Novel 2-Cyano-*N*-(quinolin-3-yl)acetamide

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Received: 17 January 2014; *Accepted*: 25 April 2014; *Published online*: 30 September 2014; AJC-16144

Cancer has been ranked second after cardiovascular diseases. The plant-derived molecules have played an important role for the treatment of cancer. On the account of the reported anticancer activity of quinoline containing biologically active cyanoacetamide moiety, a novel 2-cyano-*N*-(quinolin-3-yl)acetamide (**2**) was synthesized using 3-aminoquinoline (**1**) as strategic starting materials. The corresponding 2-cyano-*N*-(quinoline-3-yl)-acetamide (**2**) was obtained in good yield *via* reaction of 3-aminoquinoline (**1**) with ethylcyanoacetate in dry toluene. The structure of the newly synthesized compound was confirmed on the basis of elemental analyses, IR, ^{1}H NMR, ^{13}C NMR spectra and X-ray analysis. Compound **2** was found to exert the most powerful effect on *Ehrlich ascites* carcinoma tumor cells (*in vitro*) compared with the positive control (doxorubicin) with IC_{50} of 2.14 µg/mL.

Keywords: Novel quinoline derivative, X-ray crystallography, Antitumor activity.

INTRODUCTION

Recently considerable attention has been devoted to the synthesis of new derivatives of quinoline on the account of their reported biological activities[1-8]. From the literature survey, several methods have been described for the elaboration of substituted quinolines[9-11], which as a class have been reported to have anticancer and antileukemic activity. Different mechanisms account for the cytotoxic effect of this class of compounds, the most prominent mechanism was the inhibition of carbonic anhydrase isozymes. Cancer is a top killer of human beings, thus great urgency to develop highly efficacious and minimally toxic treatments for cancer. Although tremendous progress has been achieved in the development of novel cancer treatments, most of the current cancer drugs usually exhibit high toxicity and are severely resisted by tumor cells in the clinic. This dilemma is particularly true for DNA-damaging agents, the mainstay of cancer treatment[12]. Quinolines were found to possess several pharmacological properties, including anticancer activity[13-17]. It was also found that the acetamide derivatives constitute an important class of drug, with several types of pharmacological agents possessing anticancer activity[18-21] among others. A large number of structurally novel quinolines have ultimately been reported to show substantial anticancer activity *in vitro* and *in vivo*[22]. Several mechanisms have been reported for anticancer activity of the quinoline sulfonamide compounds and the most prominent of these mechanisms was through the inhibition of the carbonic anhydrase[23-26]. The mechanism of tumor inhibition by quinoline sulfonamide carbonic anhydrase inhibitor was suggested by Chegwidden and Dodgson[26]. These compounds may reduce the provision of bicarbonate for the synthesis of nucleotides and other cell components such as membrane lipids. Based on the above information and as a continuation of a previous work on anticancer agents[28-35], we report the synthesis and X-ray crystallographic study of some novel quinoline derivative compound **2** to evaluate its cytotoxic activity against *Ehrlich ascites* carcinoma tumor cells (EAC).

EXPERIMENTAL

The starting material 3-aminoquinoline (**1**) was purchased from Sigma-Aldrich. Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, Stone) and were uncorrected. Precoated Silica gel plates (Kiesel gel 0.25 mm, 60 G F 254, Merck) were used for thin layer chromatography (TLC). The developing solvent system was chloroform / methanol (10: 3) and the spot were detected by ultraviolet light. Infrared (IR) spectra (KBr disc) were recorded on FT-IR spectrophotometer (Perkin Elmer) at the Research Center, College of Pharmacy, King Saud University, Saudi Arabia. ^{1}H NMR spectra were scanned in dimethylsulfoxide (DMSO-d_6) on a NMR spectrophotometer (Bruker AXS Inc.) operating at 500 MHz for ^{1}H and 125.76 MHz for ^{13}C at the aforementioned Research Center. Chemical shifts are expressed

in δ-values (ppm) relative to tetramethylsilane (TMS) as an internal standard. Exchangeable protons were confirmed by addition of drop of D_2O. Elemental analyses were done on a model 2400 CHNSO analyzer (Perkin Elmer). The X-ray measurement of compound **2** was performed using Bruker SMART APEXII CCD diffractometer. Crystallographic data of compound **2** has been deposited with the Cambridge Crystallographic Data Center (supplementary publication numbers CCDC-923299). Copy of the data may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (deposit@ccdc.cam.ac.uk).

Synthesis of 2-cyano-*N*-(quinolin-3-yl)acetamide (2): A mixture of 3-aminoquinoline (**1**) (1.3 g, 0.01 mol) and ethylcyanoacetate (1.13 g, 0.01 mol) in dry toluene (30 mL) was refluxed for 5 h. The reaction mixture was cooled and the solid obtained was recrystallized from dioxane to give compound **2**. Yield % 92; m.p 220.4 °C; IR (KBr, ν_{max}, cm^{-1}): 3370 (NH), 3091 (CH arom.), 2977, 2863 (CH aliph.), 2189 (C≡N), 1682 (C=O), 1612 (C=N). ^{1}H NMR spectrum in (DMSO-d_6): 4.2 [s, 2H, CH_2], 7.1-8 [m, 5H, Ar-H], 8.5 [s, 1H, N=CH quinoline], 9.3 [s, 1H, NH, D_2O-exchangeable]. ^{13}C NMR spectrum in (DMSO-d_6): 25.4, 117.6, 125.8, 126.4, 127.9, 128.8, 129.7, 130.5, 138.7, 141.9, 145.1, 169.7. Anal. calcd. for $C_{12}H_{19}N_3O$ (211.22): C, 68.24; H, 4.29; N, 19.89. Found: C, 68.61; H, 3.93; N, 20.19 %.

Crystal structure determination: Slow evaporation of the pure compound **2** from ethanol yielded its single crystal. Single crystal of suitable size was selected for X-ray diffraction analysis. Data was collected on a Bruker APEX-II CCD area diffractometer equipped with graphite monochromatic CuK_α radiation (λ = 1.54178 Å) at 296 K. Cell refinement and data reduction were done by Bruker SAINT[36], program used to solve structure and refine structure is SHELXTL[37]. The final refinement was performed by full-matrix least-squares techniques with anisotropic thermal data for non hydrogen atoms on F^2. All the hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms. Multi-scan absorption correction was applied by use of SADABS software[36]. The crystallographic data and refinement information are summarized in Table-1.

Antitumor activity (*in vitro* study): RPMI 1640 medium waspursghased from Sigma (St. Louis, MO, USA). Ehrlich ascites carcinoma cells (EAC) suspension (2.5 × 106/mL) were obtained as described in 2.4.2. Trypan blue dye was prepared from a stock solution by dissolving 1 g of the dye in distilled water (100 mL). The working solution was then prepared by diluting 1 ml of the stock solution with 9 mL distilled water. The stain was used then for staining the dead ehrlich ascites carcinoma cells cells. The compound tested was compound **2**.

Procedure: *Ehrlich ascites* carcinoma cells were obtained by needle aspiration of the ascetic fluid from preinoculated mice under aseptic conditions[38]. The cells were tested for viability and contamination by staining certain cell volume of this fluid by an equal volume of the working solution of trypan blue dye[39,40]. The ascetic fluid was diluted with saline (1:10) to contain 2.5×10^6 cells on a hemocytometer. In a set of sterile test tubes 0.1 mL of tumor cells suspension, 0.8 mL RPMI 1640 media and 0.1 mL of each tested compound (corresponding to 100, 50 and 25 mg/mL) were mixed. The test tubes were incubated at 37 ºC for 2 h. Trypan blue exclusion test[39,40] was carried out to calculate the percentage of non-viable cells.

$$\text{Non-viable cells (\%)} = \frac{\text{No. of non-viable cells}}{\text{Total no. of cells}} \times 100$$

Compounds producing more than 70 % non viable cells are considered active[41].

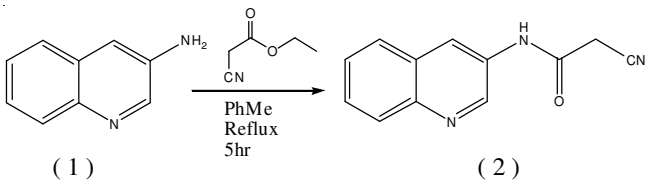
Doxorubicin (CAS 23214-92-8) is taken as a reference drug.

TABLE-1
CRYSTALLOGRAPHIC DATA OF
2-CYANO-*N*-(QUINOLIN-3-YL)ACETAMIDE (**2**)

Empirical formula	$C_{12}H_9N_3O$
Formula weight	211.22
Temperature (K)	296
Crystal system	Monoclinic
Space group	P21/c
CuKα radiation, λ	1.54178 Å
a(Å)	18.3511 (5)
b(Å)	11.1409 (4)
c(Å)	10.1411 (3)
α(º)	90.0
β(º)	93.908 (2)
γ(º)	90.0
V(Å^3)	2068.50 (12)
Z	8
F(000)	880
Theta range for data collection (º)	2.4–66.8
μ(mm^{-1})	0.74
Density (calc.) (g/cm^3)	1.357
Crystal shape and color	Plate, pink
Crystal size (mm^3)	0.49 × 0.35 × 0.08
h/k/*l*	–19.22/–13.13/–11.90
Measured reflections	13888
Independent reflections	3774 [R_{int} = 0.038]
Reflections with I > 2σ (I)	2503
Goodness-of-fit on F^2	1.10
R[F^2 > 2σ (F^2)]	0.065
w$R(F^2)$	0.195
$\Delta\rho_{max}$(e Å^{-3})	0.22
$\Delta\rho_{min}$(e Å^{-3})	–0.26

RESULTS AND DISCUSSION

The present work was devoted to design, synthesis, antitumor and X-ray crystallographic study of novel 2-cyano-*N*-(quinoline-3-yl)-acetamide (**2**) (**Scheme-I**). Interaction of 3-ami-noquinoline (**1**) with ethylcyanoacetate gave the 2-cyano-*N*-(quinolin-3-yl)-acetamide (**2**) in good yield. The structure of compound **2** was supported on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR spectra and X-ray data. IR spectrum of compound **2** revealed the presence of characteristic bands for (NH) at 3370 cm^{-1}, (CH aromatic) at 3091 cm^{-1}, (CH aliphatic) at 2977, 2863 cm^{-1}, 2189 (C≡N), (C=O) at 1682 cm^{-1} and (C=N) at 1612 cm^{-1}. Also, ^{1}H NMR spectrum in (DMSO-d_6) indicated the presence of a signals at 4.2 ppm which could be assigned to CH_2 group, 8.5 ppm due to N=CH of quinoline and 9.3 ppm for NH group. ^{13}C NMR spectrum of compound **2** in (DMSO-d_6) showed signals at 25.4, 117.6, 125.8, 126.4, 127.9, 128.8, 129.7, 130.5, 138.7, 141.9, 145.1, 169.7.



Scheme-I: Formation of novel 2-cyano-*N*-(quinolin-3-yl)acetamide (**2**)

Crystal structure of 2-cyano-*N*-(quinolin-3-yl)acetamide (2): The crystal structure of the compound **2** contains two molecules in the asymmetric unit. The labeled displacement ellipsoid plot of this molecule is shown in (Fig. 1). The hydrogen-bonding interactions are listed in (Table-2), (Fig. 2) depicts the packing of the molecules in the crystal structure. The crystal structure is stabilized by C-H···N and C-H···O hydrogen bonds into a three-dimensional framework structure.

TABLE-2
HYDROGEN-BOND GEOMETRY (Å,) FOR
2-CYANO-*N*-(QUINOLIN-3-YL)ACETAMIDE (**2**)

D–H···A	D–H	H···A	D···A	D–H···A
N2B–H2NB···N1A^{i}	0.88 (2)	2.14 (2)	3.012 (3)	177 (2)
N2A–H2NA···N3A^{ii}	0.86 (2)	2.14 (2)	2.997 (3)	170 (2)
C3A–H3AA···O1A	0.9300	2.3300	2.907 (3)	120.00
C5A–H5AA···O1B^{i}	0.9300	2.5600	3.454 (3)	160.00
C1B–H1BA···O1B	0.9300	2.2400	2.867 (4)	124.00
C11A–H11A···N3B^{iii}	0.9700	2.6000	3.434 (3)	144.00
C11B–H11D···O1A^{iv}	0.9700	2.6000	3.103 (3)	113.00

Symmetry codes: (i) –x, y–1/2, –z–1/2; (ii) –x+1, y+1/2, –z+1/2; (iii) x+1, y, z; (iv) –x, –y+1, –z

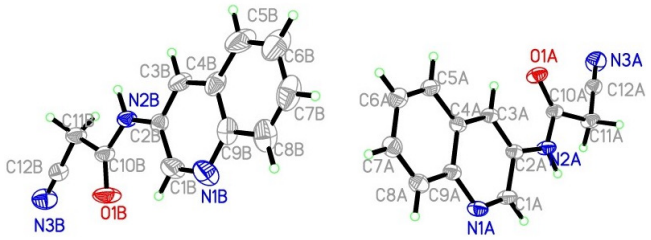


Fig. 1. ORTEP diagram of the title 2-cyano-*N*-(quinolin-3-yl)acetamide (**2**) drawn at 40 % ellipsoids for non- hydrogen atoms

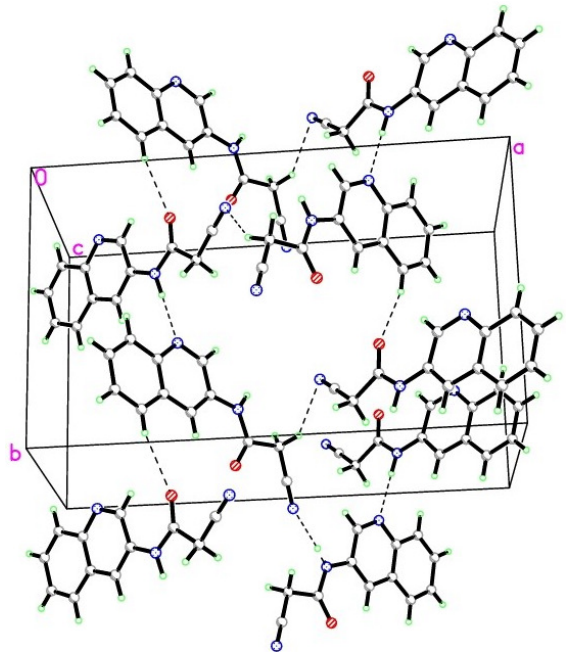


Fig. 2. Crystal packing of 2-cyano-*N*-(quinolin-3-yl)acetamide (**2**) showing intermolecular hydrogen bonds as dashed lines

Cytotoxic activity: The relationship between surviving fraction and compounds concentration was plotted to obtain the survival curve of *Ehrlich ascites* carcinoma cell. The response parameter calculated was IC_{50} value which corresponds to the compound concentration causing 50 % mortality in net cells. The results of cytotoxic activity indicated that compound (**2**) having the quinoline nucleus with cyano and acetamide moieties was found to exert the most powerful effect ehrlich ascites carcinoma tumor cells (*in vitro*) compared with the positive control (doxorubicin) with IC_{50} of 2.14 µg/mL (Table-3).

TABLE-3
in vitro CYTOTOXIC ACTIVITY OF
2-CYANO-*N*-(QUINOLIN-3-YL)ACETAMIDE (**2**)

Compd. No.	Non-viable cells (%) Concentration (µg/mL)			IC_{50} (µg/mL)
	100	50	25	
2	100	100	95	2.14
Doxorubicin (Reference drug)	100	55	20	43.6

Conclusion

From the above results, it is concluded that administration of the tested compound **2** on *Ehrlich ascites* carcinoma tumor cells (*in vitro*) showed promising anticancer activity. Compound **2** with (IC_{50} = 2.14.2 µg) was found to be more potent than doxorubicin with (IC_{50} = 43.6 µg) as positive control. Results and analysis of the X-ray crystal structure of compound **2** are also reported.

Conflict of interests: The authors have declared that there is no conflict of interests.

ACKNOWLEDGEMENTS

The authors extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project No. RGP-VPP- 302.

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