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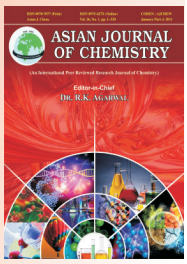

Synthesis, Crystal Structure, Spectral Characterization and Cytotoxic Activity of Novel Ethyl 2-Cyano-3-(quinolin-3-ylamino)acrylate

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The chemistry of quinoline derivatives has been of increasing interest, since many of these compounds exhibited several biological activities and useful application as antitumor. On the account of the reported anticancer activity of quinolines containing the different biologically active moieties, a novel ethyl 2-cyano-3-(quinolin-3-ylamino)acrylate (**2**) was synthesized using 3-aminoquinoline (**1**) as strategic starting material. Interaction of 3-aminoquinoline (**1**) with ethyl 2-cyano-3-ethoxyacrylate furnished the corresponding ethyl-2-cyano-3-(quinolin-3-ylamino)acrylate (**2**). The structure of th newly synthesized compound was confirmed by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR spectra and X-ray analysis. The target compound was subjected to *in vitro* cytotoxic activity against ehrlich ascites carcinoma cells. This compound showed higher activity with IC_{50} (25 µg/mL) when compared with doxorubicin [CAS 23214-92-8] as a reference drug (IC_{50} value 37.5 µg/mL).

Keywords: Novel quinoline, X-ray crystallographic study and cytotoxic activity.

INTRODUCTION

Quinoline derivatives were found to possess several pharmacological properties, including antibacterial and anticancer[1-4] activities. Also, the chemistry of quinoline derivatives has been of increasing interest, since many of these compounds exhibited several biological activities and useful application as antitumor[5,6] and antibacterial agents[7,8]. 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 there is a 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 sulfonamide carbonic anhydrase inhibitor was suggested by chegwidden and spencer[27]. 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 design, synthesis, cytotoxic activity and X-ray crystallographic study of novel ethyl 2-cyano-3-(quinolin-3-ylamino)acrylate.

EXPERIMENTAL

The strategic 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 dimethyl sulfoxide (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[36,37]. Crystallographic data of compound **2** has been deposited with the Cambridge Crystallographic Data Center (supplementary publication numbers CCDC-931068). 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 ethyl 2-cyano-3-(quinolin-3-ylamino) acrylate (2): A mixture of 3-aminoquinoline (**1**) (1.3 g, 0.01 mol) and ethyl 2-cyano-3-ethoxyacrylate (1.69 g, 0.01 mol) in absolute ethanol (30 mL) was refluxed for 8 h. The reaction mixture was cooled and the solid obtained was recrystallized from ethanol to give compound **2**. Yield % 89; m.p. 173.5 °C; IR (KBr, ν_{max}, cm^{-1}): 3307 (NH), 3088 (CH arom.), 2967, 2847 (CH aliph.), 2212 (C≡N), 1712 (C=O), 1610 (C=N). ^{1}H NMR spectrum in (DMSO-d_6): 1.2 [t, 3H, CH_3], 4.2 [q, 2H, CH_2], 7.1-7.9 [m, 5H, Ar-H], 8.2 [s, 1H, CH], 8.4 [s, 1H, N=CH quinoline], 8.9 [s, 1H, NH, D_2O-exchangeable]. ^{13}C NMR spectrum of compound **3** in (DMSO-d_6): 13.4, 62.6, 78.7, 114.6, 122.7, 124.8, 125.9, 126.5, 128.4, 130.6, 136.9, 141.3, 144.8, 158.7, 166.9. Anal. calcd. for $C_{15}H_{13}N_3O_2$ (267. 28): C, 67.40; H, 4.90; N, 15.72. Found: C, 67.77; H, 4.59; N, 15.48 %.

Antitumor activity (*in vitro* study): RPMI 1640 medium was purchased from Sigma (St. Louis, MO, USA). *Ehrlich ascites* carcinoma cells suspensions (2.5 × 10^6/mL) were obtained as described earlier. 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. The compound tested was compound **2**.

Procedure: *Ehrlich ascites* carcinoma cells were obtained by needle aspiration of the ascetic fluid from pre-inoculated 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, 25 and 10 µg/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 (Table-3).

$$\text{Non-viable cells} = \frac{\text{No. of non-viable cells}}{\text{Total non. of cells}}$$

Compounds producing more than 70 % non viable cells are considered active[41]. Doxorubicin (CAS 23214-92-8) is taken as a reference.

TABLE-1
CRYSTALLOGRAPHIC DATA OF
ETHYL 2-CYANO-3-(QUINOLIN-3-YLAMINO)ACRYLATE (**2**)

Empirical formula	$C_{15}H_{13}N_3O_2$
Formula weight	267.28
Temperature (K)	296
Crystal system	Monoclinic
Space group	P21/c
CuK_α radiation, λ	1.54178 Å
a(Å)	6.3092 (2)
b(Å)	10.0216 (3)
c(Å)	21.4856 (7)
α(º)	90.0
α(º)	95.834 (2)
α(º)	90.0
V(Å^3)	1351.46 (7)
Z	4
F(000)	560
Theta range for data collection (º)	4.1–69.1
μ(mm^{-1})	0.74
Density (calc.) (g/cm^3)	1.314
Crystal shape and color	Needle, colorless
Crystal size (mm^3)	0.68 × 0.21 × 0.16
h/k/l	–5.7/–11.12/–25.26
Measured reflections	8924
Independent reflections	2478 [R_{int} = 0.039]
Reflections with I > 2σ (I)	2478
Goodness-of-fit on F^2	1.11
R[F^2 > 2σ (F^2)]	0.061
wR(F^2)	0.208
$\Delta\rho_{max}$ (e Å^{-3})	0.46
$\Delta\rho_{min}$ (e Å^{-3})	–0.31

Compound **2** contains one molecule 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.

TABLE-2
HYDROGEN-BOND GEOMETRY (Å, °)

D–H···A	D–H	H···A	D···A	D–H···A
N2–H2N···O1	0.90 (3)	1.99 (3)	2.699 (3)	135 (2)

TABLE-3
in-vitro CYTOTOXIC ACTIVITY OF
ETHYL 2-CYANO-3-(QUINOLIN-3-YLAMINO) ACRYLATE (**2**)

Compd. No.	Non-viable cells (%) Concentration (µg/mL)				IC_{50} (µg/mL)
	100	50	25	10	
2	100	90	50	30	25
Doxorubicin (reference)	100	60	39	20	37.5

RESULTS AND DISCUSSION

Interaction of compound **1** with ethyl 2-cyano-3- ethoxyacrylate in refluxing absolute ethanol afforded the corresponding ethyl 2- cyano-3-(quinolin-3-yl-amino)acrylate (**2**)

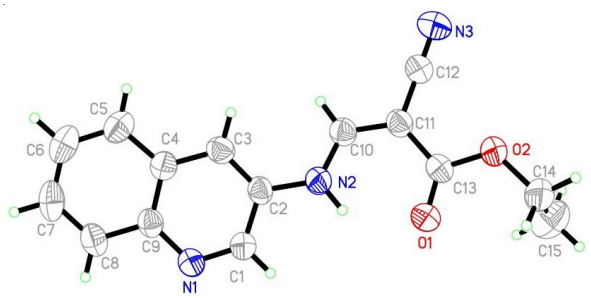


Fig. 1. ORTEP diagram of the title compound **2** drawn at 40 % ellipsoids for non-hydrogen atoms

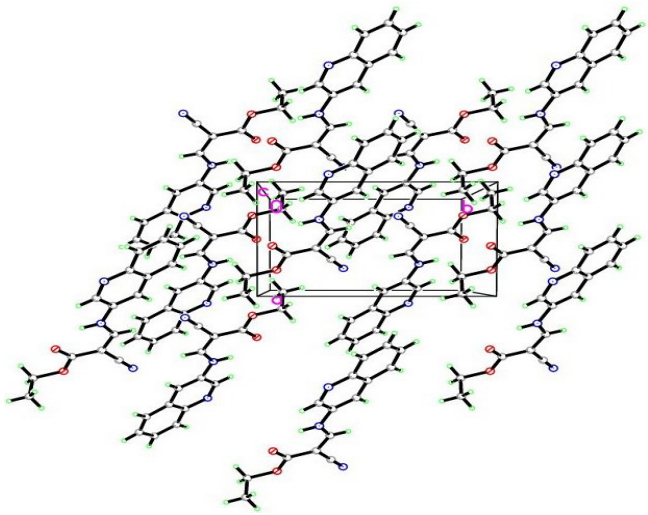
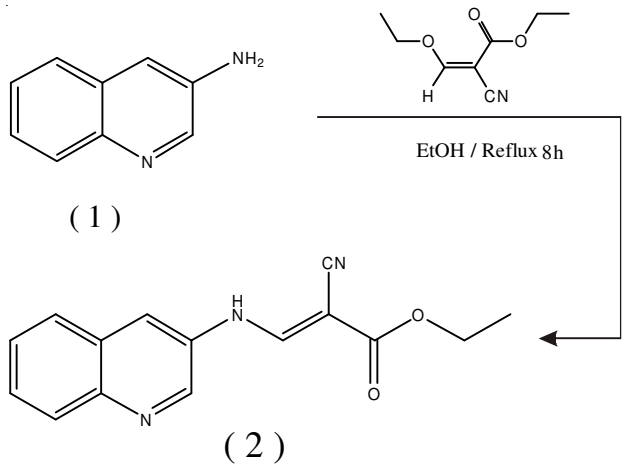


Fig. 2. Crystal packing of ethyl 2-cyano-3-(quinolin-3-ylamino) acrylate (**2**)

in good yield (**Scheme-I**). The structure of **2** was confirmed from its microanalysis, IR, ^{1}H NMR, ^{13}C NMR and X-ray data. Thus, IR spectrum of **2** exhibited the presence of characteristic bands for (NH) at 3307 cm^{-1}, (CH aromatic) at 3088 cm^{-1}, (CH aliphatic) at 2967, 2847 cm^{-1}, (C≡N) at 2212 cm^{-1}, (C=O) at 1712 cm^{-1} and (C=N) at 1610 cm^{-1}. ^{1}H NMR spectrum of **2** in (DMSO-d_6) revealed a triplet at 1.2 ppm corresponding to methyl group, a quartet at 4.3 ppm due to CH_2 group, singlet at 8.2 ppm for CH group, 8.4 ppm for N=CH of quinoline and 8.9 ppm for NH group. ^{13}C NMR spectrum of **2** in (DMSO-d_6) showed signals at 13.4, 62.6, 78.7, 114.6, 122.7, 124.8, 125.9, 126.5, 128.4, 130.6, 136.9, 141.3, 144.8, 158.7, 166.9.



Scheme-I: Formation of ethyl 2-cyano-3-(quinolin-3-ylamino)acrylate (**2**)

X-ray crystallographic data: 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; program used to solve structure and refine structure is SHELXTL. 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. The crystallographic data and refinement information are summarized in Tables 1 and 2.

Cytotoxic activity: The relationship between surviving fraction and compounds concentration was plotted to obtain the survival curve of EAC 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*) with IC_{50} of 25 µg/mL compared with the positive control (doxorubicin) with IC_{50} of 37.5 µg/mL (Table-3).

Conclusion

It is concluded that administration of the tested compound **2** on *Ehrlich ascites* carcinoma tumor cells (*in vitro*) showed promising anticancer activity with (IC_{50} value = 25 µg/mL) compared with doxorubicin with (IC_{50} = 37.5 µg/mL) as positive control. Results and analysis of the X-ray crystal structure of compound **2** are also reported.

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