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Novel 1,2,4-triazole derivatives as antitumor agents against hepatocellular carcinoma

Rasha R. Radwan*, Nashwa H. Zaher, Marwa G. El-Gazzar

Drug Radiation Research Department, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), P.O. Box 29, Nasr City, Cairo, Egypt

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ABSTRACT

Fifteen novel 1,2,3-triazole derivatives were prepared in series of synthetic steps starting from 4-amino-5-hydrazino-4H-1,2,4-triazole-3-thiol **1**. The structures of the obtained compounds were verified through micoanalytical and spectral data. All the compounds were screened for their anticancer activity against liver human cancer cell lines (HEPG2) using Doxorubicin as standard. The most promising triazolothiadiazine derivative **12** was further tested for its degree of toxicity by estimating the median lethal dose (LD 50) and its antitumor activity through inhibiting the angiogenesis and progression of tumor against diethylnitrosamine (DENA)/CCl_4 induced hepatocellular carcinoma (HCC) in rats. To elucidate its mechanism of action, the following parameters were determined including: vascular endothelial growth factor (VEGF) as a marker of angiogenesis; hepatic tyrosine kinase (HTK) as a marker for tumor growth; serum alpha fetoprotein (AFP) as a marker for hepatocarcinoma; aspartate and alanine aminotransferases (AST & ALT) as liver function test; malondialdehyde (MDA) and glutathione (GSH) as markers of antioxidant activity. Liver histopathological analysis was also evaluated. Carcinogenic rats showed drastic elevation in all investigated parameters accompanied by reduction in hepatic glutathione. Administration of compound **12** into rats after induction of experimental HCC, improved the biochemical changes induced by DENA/CCl_4. These observations were supported by histopathological study of liver sections. It was concluded that triazolothiadiazine compound **12** could be promising anti HCC agent after more investigations on higher animals.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth common tumor and the second prominent cause of cancer mortality worldwide [1]. The main risk aspects for HCC are hepatitis B or C virus infection, alcohol consumption, diabetes, obesity, exposure to environmental carcinogens such as aflatoxins and nitrosamines [2]. It is well known that diethylnitrosamine (DENA) is a potent hepatocarcinogenic agent present in tobacco smoke, agricultural chemicals, cosmetics, and pharmaceutical products [3]. DENA metabolised in the liver generating reactive oxygen species (ROS) and causing oxidative stress. In experimental animal models, HCC mediated by DENA induction, seems to resemble the important features of human diseases, allowing the screening of potential antitumor compounds [4].

A promising strategy for cancer prevention today is chemotherapy. Therefore, it is vital to develop and evaluate new treatment options against HCC as well as to elucidate their mechanisms of action. Interestingly, 1,2,4-triazole and 1,3,4-thiadiazole derivatives have drawn the attention of many investigators owing to their potential applications. They have a wide scope of pharmacological activities including anti-inflammatory [5], anticonvulsant [6], antibacterial [7] and antitumor activities against different cancer cell lines [8]. Furthermore, it was reported that triazole and thiadiazole derivatives show free radical scavenging and antioxidative activity by inhibiting lipid peroxidation [9]. Recently, **Li** and co-workers [10] synthesized 1,2,4 triazole complex, reported to have potential anticancer properties against breast cancer. In addition, fused 1,2,4 triazole moieties displayed potent activity against breast cancer cell lines [11]. Moreover, thiadiazine moiety was reported to possess anticancer activity as a novel heterocyclic ring system [12]. A selective cytotoxic effect toward tumor cells was achieved by thiadiazine derivative and used as lead compound [13]. Fused

* Corresponding author.
E-mail address: rasha_radwan33@yahoo.com (R.R. Radwan).

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triazolothiadiazine system was reported as promising drug candidate against liver cancer [14].

It is well established that HCC is a hypervascular tumor. HCC growth and progression is critically dependent on the angiogenesis. The vascular endothelial growth factor (VEGF) is specifically involved in HCC pathogenesis [15]. **Gershtein** and coworkers detected high VEGF levels in the serum and cancerous tissue of HCC patients [16]. VEGFR exists as three subtypes, VEGFR-1, VEGFR-2, as well as VEGFR-3. VEGFR-2 is the main target for antiangiogenic therapy [17]. Based on these findings, considerable efforts have been made to develop compounds that suppress the angiogenesis in cancer therapy [18].

Nexavar® (Sorafenib) is the only approved drug for HCC acting by inhibition of VEGFR-2 [19]. Sunitinib is another drug evaluated in phase II clinical studies in patients with advanced HCC [20].

The current study aimed at evaluating newly synthesized 1,2,4-triazole derivatives, *in vitro* for their cytotoxic properties against liver human cancer cell lines (HEPG2). The most promising one is to be subjected for further investigation against its degree of toxicity and antitumor activity *in vivo* progression of HCC rat model. Accompanied with interpretation of the possible underlying mechanism(s); particularly, its impact on the angiogenesis, tumor growth, tyrosine kinase inhibition, liver function and oxidative stress.

2. Material and methods

2.1. Chemistry

2.1.1. Instruments & chemicals

Melting points were taken in an open capillary tube on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK) and were uncorrected. The IR spectra of the compounds were recorded on FT-IR Shimadzu spectrometer (Shimadzu, Tokyo, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury Plus Oxford 400 MHz spectrometer (Varian Inc., Palo Alto, CA) using TMS as an internal Standard and DMSO-d_6 as solvent. Mass spectra were run on HP Model MS-5988 (Hewlett Packard, Palo, Alto, California, USA). Microanalyses were obtained on a Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany), all values were within±0.4% of the theoretical values. Purity of the compounds was checked by TLC on pre-coated SiO_2 gel (HF_{254}, 200 mesh) aluminum plates (Merk, Darmstadt, Germany). A developing solvent system of chloroform/methanol (8:2) was used and the spots were visualized under UV light. IR, ^{1}H NMR, ^{13}C NMR, Mass and elemental analysis were consistent with the assigned structures. Starting triazole (1) & all reagents used were of analytical grade and were purchased from Sigma (St. Louis, MO).

2.1.2. General experimental protocol for preparation of compounds ***(2–4)***

A mixture of **1** (1 g, 0.007 mol) and different solvents 20 ml (formic acid, acetic anhydride and triethylorthoformate) was stirred at room temperature for 3hs. Excess solvent was removed under reduced pressure. A sticky mass was formed which was triturated using diethyl ether. The solid formed was recrystallized from ethanol to give **2**–**4**, respectively.

*2.1.2.1. N'-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)formohydrazide **(2)**.* Yield, 88%; mp, 189–190 °C; IR (KBr, cm^{-1}): 3256, 3204, (NH, NH_2), 2950, 2876 (CH-aliph.), 1672 (C=O); ^{1}H NMR (DMSO-d_6, ppm): 2.4 (s, 2H, 2NH, exchangeable with D_2O),5.40 (s, 2H, NH_2, exchangeable with D_2O), 8.04 (s, 1H, CHO), 11.12 (hump, 1H, SH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, ppm): 151.36 (C-NH), 160.71 (CHO), 165.81 (C-SH); MS *m*/*z*: 174 (M^+); Analysis calculated for $C_3H_6N_6OS$: C, 20.69; H, 3.47; N, 48.25, found: C, 20.83; H, 3.77; N, 48.33.

*2.1.2.2. N'-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)acetohydrazide **(3)**.* Yield, 92%; mp, 169–170 °C; IR (KBr, cm^{-1}): 3363, 3344, (NH, NH_2), 2944, 2870 (CH-aliph.), 1677 (C=O); ^{1}H NMR (DMSO-d_6, ppm): 2.47 (s, 3H, CH_3), 5.37 (s, 2H, NH_2, exchangeable with D_2O), 8.71 (s, 1H, NH, exchangeable with D_2O), 9.76 (s, 1H, NH, exchangeable with D_2O), 10.89 (hump, 1H, SH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, ppm): 21.03 (CH_3), 152.02 (C-NH), 165.37 (C-SH), 169.29 (C=O); MS *m*/*z*: 188 (M^+); Analysis calculated for $C_4H_8N_6OS$: C, 25.53; H, 4.28; N, 44.65, found: C, 25.59; H, 4.12; N, 44.43.

*2.1.2.3. 4-Amino-5-(2-(diethoxymethyl)hydrazinyl)-4H-1,2,4-triazole-3-thiol **(4)**.* Yield, 90%, mp, 206–207 °C; IR (KBr, cm^{-1}): 3310, 3300, (NH, NH_2), 2920, 2866 (CH-aliph.); ^{1}H NMR (DMSO-d_6, ppm): 1.01–1.04 (t, 6H, $2CH_3$, J = 6.8 Hz), 4.09–4.11 (q, 4H, $2CH_2$), 5.25 (s, 1H, CH), 5.45 (s, 1H, NH, exchangeable with D_2O), 5.77 (s, 2H, NH_2, exchangeable with D_2O), 5.99 (s, 1H, NH, exchangeable with D_2O),, 10.98 (s, 1H, SH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, ppm): 18.5 ($2CH_3$), 59.5 ($2CH_2$), 115.0 (CH), 147.0 (C-NH), 163.61 (C-SH); MS *m*/*z*: 248 (M^+); Analysis calculated for $C_7H_{16}N_6O_2S$: C, 33.86; H, 6.49; N, 33.85, found: C, 33.63; H, 6.36; N, 33.90.

*2.1.2.4. 2-(2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)hydrazinyl)-1-phenylethanone **(5)**.* A mixture of **1** (1 g, 0.007 mol) and phenacyl bromide (1.4 g, 0.007 mol) was reacted under reflux in anhydrous ethanol for 5hs. A yellow needle shaped crystals was formed on hot, which was filtered and allowed to dry to give 5.

Yield, 85%; mp, 190–192 °C; IR (KBr, cm^{-1}): 3429, 3310, (NH, NH_2), 3049 (CH-arom.), 2969, 2852 (CH-aliph.), 1690 (C=O); ^{1}H NMR (DMSO-d_6, ppm): 3.72 (s, 1H, NH, exchangeable with D_2O), 3.92 (s, 2H, CH_2), 4.60 (s, 1H, NH, exchangeable with D_2O), 4.76 (s, 2H, NH_2, exchangeable with D_2O), 7.42–8.16 (m, 5H, ArH), 10.79 (s, 1H, SH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, ppm): 56.45 (CH_2), 128.15–133.56, (Aromatic Cs), 158.93 (C-NH), 167.1 (C-SH), 195.3 (C=O); MS *m*/*z*: 264 (M^+); Analysis calculated for $C_{10}H_{12}N_6OS$: C, 45.44; H, 4.58; N, 31.80, found: C, 45.52; H, 4.61; N, 31.77.

2.1.3. General experimental protocol for preparation of compounds ***(6–8)***

A mixture of **1** (1 g, 0.007 mol) and different solvents 20 ml (formic acid, acetic anhydride and triethylorthoformate) was reacted under reflux for 5 hs. Excess solvent was removed under reduced pressure. A sticky mass was formed which was triturated using diethyl ether. The solid formed was recrystallized from ethanol to give **6**–**8**, respectively.

*2.1.3.1. N-(3-(2-formylhydrazinyl)-5-mercapto-4H-1,2,4-triazol-4-yl) formamide**(6)**.* Yield, 89%; mp, 200–201 °C; IR (KBr, cm^{-1}): 3277 (NH), 2922, 2892 (CH-aliph.) 1691 (C=O); ^{1}H NMR (DMSO-d_6, ppm): 2.1 (s, 2H, 2NH, exchangeable with D_2O), 4.2 (s, 2H, 2NH, exchangeable with D_2O), 8.0 (s, 2H, 2 CH), 11.79 (s, 1H, SH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, ppm): 147.4 (C-N-triazole), 160.1 (C-formyl), 162.9 (C-formyl), 167.2 (C-SH); MS *m*/*z*: 202 (M^+); Analysis calculated for $C_4H_6N_6O_2S$: C, 23.76; H, 2.99; N, 41.56, found: C, 23.64; H, 3.03; N, 41.39.

*2.1.3.2. N-(3-(2-acetylhydrazinyl)-5-mercapto-4H-1,2,4-triazol-4-yl) acetamide**(7)**.* Yield, 87%; mp, 190–192 °C; IR (KBr, cm^{-1}): 3277 (NH), 2992, 2918 (CH-aliph.), 1634 (C=O). ^{1}H NMR (DMSO-d_6, ppm): 1.85 (s, 6H, $2CH_3$), 7.25 (s, 1H, NH, exchangeable with D_2O),