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Published in:
Biomedical Research

Publication date:
2016

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Document Version
Publisher's PDF, also known as Version of record

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Ghorab, M. M., Shahat, A. A., Alsaid, M. S., Higgins, M., Dinkova-Kostova, A. T., & Naser, A. A. (2016). Synthesis and NAD(P)H: Quinone oxidoreductase 1 inducer activity of acetamide and pyridine-3-carbonitrile derivatives. *Biomedical Research*, 27(4), 1045-1048. <http://www.alliedacademies.org/articles/synthesis-and-nadph-quinone-oxidoreductase-1-inducer-activity-of-acetamide-and-pyridine3carbonitrile-derivatives.html>

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Synthesis and NAD(P)H: quinone oxidoreductase 1 inducer activity of acetamide and pyridine-3-carbonitrile derivatives.

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Abstract

Acetamide derivatives and one pyridone derivative were prepared. The structure of the synthesized compounds was verified through elemental analyses, ¹H -NMR and ¹³C- NMR, IR spectra. The NQO1 inducer activity of the synthesized compounds was evaluated using a quantitative bioassay in Hepa1c1c7 murine hepatoma cells. The acetamide derivatives showed weak activity, with the 3-ethylphenyl being more potent than the 2-ethylphenyl derivative. The pyridone derivative was inactive.

Keywords Acetamides, Pyridone, NQO1, Electrophilicity.

Accepted on March 23, 2016

Introduction

The antioxidant activity is related with compounds competent of protecting a biological system against the potential harmful effects of oxidative processes. The antioxidant components in the last years have received enlarged care from medical researchers and nutritionists for their potential activities in stopping cancer, cardiovascular disorders, as well as aging [1]. Acetamide derivatives have been reported to possess a wide variety of important biological properties such as anti-malarial [2], antimicrobial [3-5], anti-proliferative [6], inhibitors of Pim-1 kinase [7], anti-inflammatory [8], anticonvulsant [9], antidyslipidemic, antioxidant [10], anti-HIV [11]. A large number of acetamide derivatives have been reported to show potential anticancer activity *in vitro* and *in vivo* [12-14]. In addition compounds containing heteroaromatic rings regularly are playing very important role as supports of bioactive substances. It is known that the pyridone and its derivatives are among the most popular N-heteroaromatic compounds integrated into the structures of many pharmaceutical compounds and their structural units occur in several

molecules showing diverse biological activities [15-20]. Recently, a series of pyridine derivatives were reported to possess antioxidant, anticancer and angiotensin-I-converting enzyme (ACE-I) inhibitory activity [21-23]. As part of our continuing interest in this area, in the present work we synthesised acetamide derivatives (3, 5), and, dihydropyridine derivative (6) to evaluated their cytoprotective activity. We used induction of the cytoprotective enzyme NAD(P)H: quinone oxidoreductase 1 (NQO1) as a measure of cytoprotective activity.

Experimental

The melting points (°C, uncorrected) are determined in open capillaries on a Gallenkemp melting point apparatus (Sanyo Gallenkemp, Southborough, UK). Pre-coated with Silica gel plates (silica gel, 60 G F 254, 0.25 mm, Merck, Germany) are used for TLC, methanol/dichloromethane (0.5: 9.5 mL) mixture was used as a developing solvent system. IR spectra were recorded in KBr discs using IR-Shimadzu spectrometer (Shimadzu, Tokyo, Japan). NMR spectra in (DMSO-d₆) were

recorded on Bruker Ac-500 ultra-shield NMR spectrometer (Bruker, Flawil, Switzerland, δ ppm) at 500 MHz, using TMS as internal standard. Elemental analyses were performed on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany). All compounds were within $\pm 0.4\%$ of the theoretical values.

2-Cyano-N-(2-ethylphenyl) acetamide (3)

A mixture of 2-ethyl aniline 1 (1.21 g, 0.01 mol) and ethyl cyanoacetate 2 (1.13 g, 0.01 mol) was refluxed for 2 h, concentrated and cooled. The obtained solid was filtered and recrystallized from benzene/ether (3:2) give compound 3 [24].

Yield, 80 %, m.p. 130-132 °C; IR (KBr, max, cm^{-1}): 3180 (NH), 2255 (CN), 1703 (CO). $^1\text{H-NMR}$ spectrum of 3 in (DMSO- d_6) δ : 1.09 [t, ^3H , CH_3], 2.23 [q, ^2H , CH_2], 3.85 [s, ^2H , CH_2CN], 7.3-7.5 [m, ^4H , Ar-H], 9.45 [s, ^1H , NH, exchangeable with D_2O], "Anal. Calculated; for $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}$ C, 70.21; H, 6.38, 14.89; found: Carbon, 70.50; Hydrogen, 6.10; Nitrogen, 14.60."

2-Cyano-N-(3-ethylphenyl) acetamide (5)

A mixture of 3-ethylaniline 4 (1.21 g, 0.01 mol) and ethyl cyanoacetate 2 (1.13 g, 0.01 mol) was fused at 220 °C (for 3 h). The concentrated reaction mixture was cooled. The gotten product was crystallized from the ethanol to afford compound 5 [25] (Scheme 1). The yield as 88 %, m. p. 86-88 °C; IR (KBr, cm^{-1}): 3317 (NH), 3100 (CH. arom.), 2960, 2870 (CH aliph.), 2260 ($\text{C}\equiv\text{N}$), 1670 ($\text{C}=\text{O}$). $^1\text{H NMR}$ spectrum of 3 in (DMSO- d_6) δ : 1.2 [t, ^3H , CH_3], 2.6 [q, ^2H , CH_2], 3.6 [s, ^2H , CH_2], 4.2 [s, ^1H , NH, exchangeable with D_2O], 7.03-7.7 [m, ^4H , Ar-H]. "Anal. Calcd; for $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}$: C, 70.21; H, 6.38, 14.89; found: C, 70.50; H, 6.10; N, 14.60.

1-(3-Ethylphenyl)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (6)

Interaction of component 3 (1.88 g, 0.01 mol) with acetylacetone (1.00 g, 0.01 mol) in ethyl alcohol (50 mL), piperidine (0.5 mL) was refluxed for 5 h. The reaction mixture was triturated with ethyl alcohol and the obtained solid was recrystallized from dioxane to yield compound 6 [26] (Scheme 1). Yield: 86%. M.p.: 198-200 °C. IR (KBr, cm^{-1}): 3100 (CH arom.), 2931, 2872 (CH aliph.), 2210 ($\text{C}\equiv\text{N}$), 1660 ($\text{C}=\text{O}$). $^1\text{H NMR}$ (300 MHz, DMSO- d_6 , δ , ppm): 1.2 (t, 3H, CH_3 , $J=0.66$ Hz), 1.5 (s, 6H, 2CH_3), 2.6 (q, 2H, CH_2 , $J=0.66$ Hz), 5.6 (s, 1H, CH pyridine), 7.27-7.7 (m, 4H, ArH). Anal. calcd. for $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}$: C, 76.16; H, 6.39; N, 11.10. Found: C, 76.50; H, 6.70; N, 11.40%".

Biological assay

The NQO1 inducer activity was determined using a quantitative microtiter plate bioassay [27,28]. Compounds were prepared as stock solutions in DMSO, and then were diluted in the cell culture medium 1:1000. The final concentration of the solvent in the medium of the growing cells

was 0.1% (v/v). Each compound was tested at eight replicates of 8 serial dilutions. The murine Hepa1c1c7 cell line was maintained at 37°C, 5% CO_2 , in α -MEM supplemented with 10% (v/v) fetal bovine serum that had been heat-and charcoal-inactivated. Cells (10^4 per well) were plated in 96-well plates, and 24 h later, they were exposed to the compounds in fresh medium for a further 48 h. The cell culture medium was then removed; cells were washed 3 times with PBS, and lysed for 30 min at 25°C in digitonin (0.1 g/L, pH 7.8). To measure the enzyme activity of NQO1 the quinone menadione was used as a substrate. Protein concentrations were determined by the BCA assay (Thermo Scientific), and the values were used to calculate the specific NQO1 activity (Table 1 and Figure 1).

Table 1. NQO1 inducer activity of acetamide and pyridine-3-carbonitrile derivatives.

Compounds	Induction Magnitude (Fold)
"2-Cyano-N-(2-ethylphenyl) acetamide (3)"	1.2
"2-Cyano-N-(3-ethylphenyl) acetamide (5)"	1.65
"1-(3-Ethylphenyl)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (6)"	Inactive

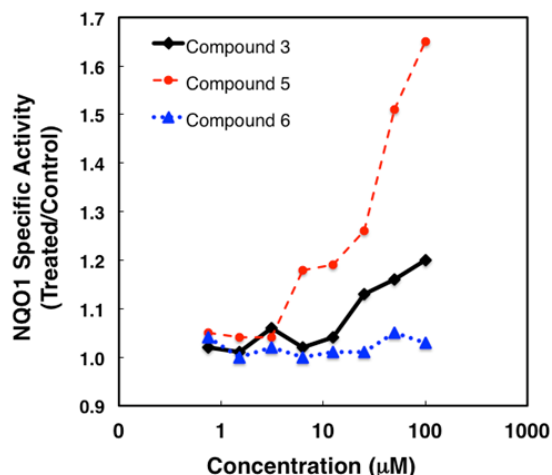


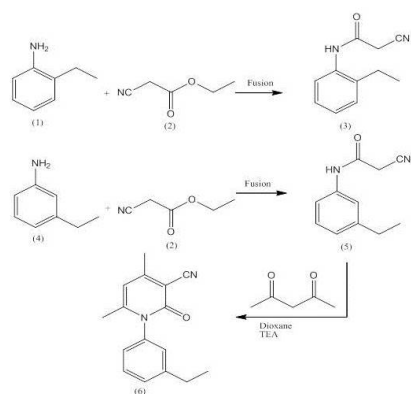
Figure 1. Dose-response of NQO1 inducer activity of acetamide and pyridine-3-carbonitrile derivatives.

Results and Discussion

The aim of this work was to design and synthesis of a series of acetamides (3, 5) and pyridine (6) (Scheme 1) and evaluation of their potential NQO1 inducer activity. Interaction of 2-ethyl 1 or 3-ethylaniline 4 with ethyl cyanoacetate 2 furnished the corresponding acetamide derivatives 3 and 5, respectively. The structure of compounds 3 and 5 was proved in the basis of elemental analysis and spectral data. The corresponding pyridine derivative 6 was obtained *via* reaction of compound 5 with acetylacetone in the presence of a catalytic amount of piperidine. The structure of compound 6 was established by elemental analysis, spectral data.

Synthesis and NAD(P)H: quinone oxidoreductase 1 inducer activity of acetamide and pyridine-3-carbonitrile derivatives

NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1) is a cytoprotective enzyme which is activated by electrophilic compounds via the Keap1/Nrf2 pathway [29]. Numerous compounds which have been shown to induce NQO1 have been subsequently found to be broadly cytoprotective and to effectively inhibit tumor formation in animal models [30,31]. We found that the acetamide derivatives have weak NQO1 inducer activity. The 3-ethylphenyl (5) is more potent than the 2-ethylphenyl (3) derivative. The pyridone derivative (6) is inactive.



Scheme 1. Formation of acetamide and pyridone derivatives 3,5,6.

Conclusion

The present study objective to synthesize and evaluate the potential anticancer activities of some acetamide and pyridine derivatives. The acetamide derivative (5) was the most active.

Acknowledgements

Mostafa M. Ghorab¹, Abdelaaty A. Shahat and Mansour S. Alsaied extend their truthful appreciation to the Deanship of Scientific Research at King Saud University for funding of this research through the Research Group Project no. RGP-VPP-262. Maureen Higgins and Albena T. Dinkova-Kostova are also grateful to Cancer Research UK (C20953/A10270) for financial support.

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