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RESEARCH ARTICLE

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SYNTHESIS AND CYTOTOXICITY EVALUATION OF DIARYLPYRAZINE AND DIHYDROPYRAZINE DERIVATIVES

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ABSTRACT

Some 2,3-diaryl pyrazines and 5,6-diaryl-2,3-dihydropyrazine were synthesized and evaluated for their cytotoxicity study. 2,3-Diarylpyrazines were prepared from oxidation of 5,6-diarly-2,3-dihydropyrazine by sulfur. The synthesized compounds were tested for their cytotoxicity effects toward (MT-29) cancer cell line using MTT-assay. The results showed compounds 4a and 4b were two most active compounds.

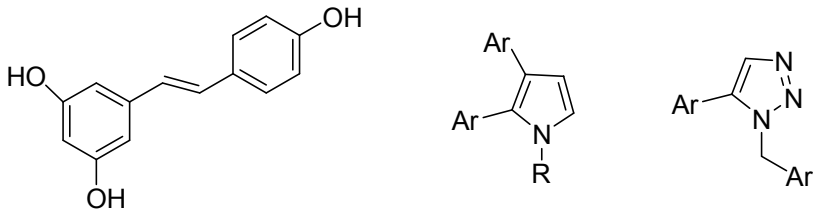
KEYWORDS

Diarylpyrazine, Diaryldihydropyrazine, Cytotoxicity, MT-29,

INTRODUCTION

In recent decades, much research has been focused on the cytotoxicity of stilbenes analogous or rigid derivatives of stilbenes such as diaryl heterocyclic compounds.
Several cis and trans-stilbenes, related to known reveratrol (a, fig1) have been synthesized and tested for anticancer effect on HL-60 leukemia cell line[1].
Their cytotoxicity evaluation reveal that cis-isomers was more potent that trans-isomers. These results conducted to prepare rigid analogous of cis-stilbenes that is constructed by using a 1,2-diaryl heterocyclic system.

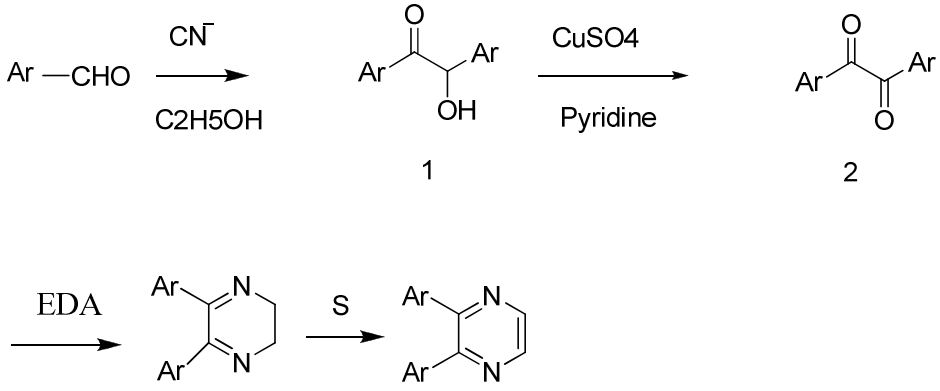
The biological evaluation of a wide range of diaryl heterocyclic compounds has been studied. For example diaryl 1,2,3-triazole substituents and 4,5-diaryl-1H-pyrrole-2-carboxylate (b and c fig1) analogous showed a potential microtubule-binding and antimitotic activity respectively[2,3].
In the present study, a series of 5,6-diaryl-2,3-dihydro pyrazine and 2,3-diaryl pyrazine derivatives were synthesized and evaluated for cytotoxicity effects.



a) Resveratrol b c

Figure 1
Structure of Resveratrol, diarylpyrole and triazole derivatives

Sheme 1
Synthesis pathway of 2,3-diarylpyrazines



1 2 3 4

Ar = a; phenyl, b; 4-fluoro phenyl, c; 4-chloro phenyl, d; 4-methoxy phenyl.

MATERIALS AND METHODS

Instrumentations and Chemicals

Melting points were determined with a Reichert-Jung hot-stage microscope and are uncorrected. ^{1}HNMR (500 MHz) spectra were recorded on a Brucker spectrometer using $CDCl_3$ as solvent. Chemical shifts (δ) are reported in ppm related to TMS as internal standard. Infra red spectra were acquired on a Nicolet Magna 550-FT spectrometer. All solvent and chemical reagents were purchase from Merck (Darmstadt, Germany). MTT reagent was obtained from Sigma-Aldrich (St. Louis, MO, USA). DMEM, RPMI, FBS and penicillin-streptomycin were purchased from Gibco (Gibco BRL life technologies, UK). All other chemicals were obtained from Merck (Darmstadt, Germany).

Synthesis

The synthetic pathway for the preparation of diarylpyrazine (4) and diaryldihydropyrazine (3) derivatives is outlined in scheme1. 2,3-dihydro-5,6-diaryl pyrazines were prepared by reaction of ethylene diamine with related benzils (2). The oxidation of dihydropyrazine ring by sulfur gave 2,3-diaryl pyrazine derivatives as reported previously[4]. The method for the preparation of benzil[5] was used for the preparation of compounds (2). Compound 2 was prepared from related benzoin as reported[6].

General procedures for preparation of 2,3 dihydro-5,6-diarylpyrazine (3)

To a stirring solution of compound 2 (23.7 mmole) in ethyl alchole, ethylene diamine (28.8 mmole) were added and refluxed for 1hr. The solvent was removed under reduced pressure to 50% of initial volume. After cooling, yellow crystals were collected and recrystalized from ethanol to yield 2,3-dihydro-4,5-diaryl pyrazine.

General procedures for preparation of 2,3-diaryl pyrazine (4)

To a stirring solution of compound 3 (20mmole), 40mmole of sulfur were added and heated at 140°C on a oil bath for 1h. After cooling, the residue was transferred on a silica-gel (60-80 mesh size) column chromatography and purified using a mixture of petroleum ether and chloroform (4:1) as mobile phase. The pure compounds were crystallized from petroleum ether and chloroform to yield 2,3-diarylpyrazine.

Cytotoxixity study

Cell culture

The human cancer cells HT-29 (colon adenocarcinoma) were obtained from the cell bank of Pasture Institute of Iran (NCBI). Cells were cultured in RPMI 1640 or DMEM supplemented with 10% fetal bovine serum (FBS), 2mmol/L L-glutamin, 1% penicillin (100U/mL) and streptomycin (100µg/mL), and maintained at 37 ^oC with 5 % CO2 in a humidified atmosphere.

In vitro cytotoxicity assay

All synthetic compounds were tested for their cytotoxicity effects towards cancer cell lines using MTT (3, 4, 5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) assay. Cells were added in 96-wells plates to make 10^4 cells/well. After 24 h, they were exposed to 50 µM of different test compounds for 24 h. Cells grown in media containing equivalent amount of DMSO served as positive control and cells in medium without any supplementation were used as negative control. After the treatment, media containing compound were carefully removed and treated cells were incubated with MTT (0.5 mg/mL in phosphate buffered saline) for 4 h at 37 °C. The medium was removed and dye crystal formazan was solublized in DMSO. The absorbance was measured at 545 nm using an ELISA plate reader. Cell viability was measured as the percentage of absorbance compared to control. Three independent experiments performed in triplicate were used for these calculations.

RESULTS AND DISCUSSION

Chemistry

The yield, melting point and proton NMR data of the synthesized compounds are as the following:

5,6-Bis phenyl-2,3-dihydropyrazine (3a)

This compound was obtained in a yield of 90%, mp; 145-149°C. ^{1}HNMR ($CDCl_3$) δ: 3.7(S, 4H, CH_2), 7.23-7.42(m, 10H, phenyl).

5,6-Bis-(4-fluorophenyl)-2,3-dihydropyrazine (3b)

This compound was obtained in a yield of 78%, mp; 89-95°C. ^{1}HNMR($CDCl_3$) δ: 3.69 (s, 4H, CH2) , 6.93 – 7 (m,4H,H3,5-phenyl), 7.36 – 7.41 (m,4H,H2,6-phenyl) .

5,6-Bis-(4-chlorophenyl)-2,3-dihydropyrazine (3c)

This compound was obtained in a yield of 76%, mp; 151-157°C. ^{1}HNMR ($CDCl_3$) δ: 3.69 (s, 4H, CH2), 7.25 (d, J=8.8Hz, 4H), 7.32 (d, J=8.8Hz, 4H).

5,6-Bis-(4-methoxyphenyl)-2,3-dihydropyrazine (3d)

This compound was obtained in a yield of 83%, mp; 124-127°C. ^{1}HNMR ($CDCl_3$) δ: 3.65(s, 4H, CH2) , 3.8(S,6H,OCH3) , 6.78(d, J=8.5Hz, 4H) , 7.37(d, J=8.5Hz, 4H).

2,3-Bis-phenylpyrazine (4a)

This compound was obtained in a yield of 81.7% , mp;118-123°C, ^{1}HNMR ($CDCl_3$) δ : 7.29 – 7.36 (m, 6H, $H_{3,4,5}$ phenyl) , 7.43 – 7.49 (m, 4H, $H_{2,6}$ phenyl) , 8.6(s, 2H, 2xCH-pyrazine).

2,3-Bis-(4-fluorophenyl)pyrazine(4b)

This compound was obtained in a yield of 80% , mp;100-104°C, ^{1}HNMR ($CDCl_3$) δ: 6.9 – 7.08 (m, 4H, $H_{3,5}$ phenyl) , 7.4 – 7.48 (m, 4H, $H_{2,6}$ phenyl) , 8.6(s, 2H, 2xCH-pyrazine) .

2,3-Bis-(4-chlorophenyl)pyrazine(4c)

This compound was obtained in a yield of 53.4%, mp; 112-118°C, ^{1}HNMR ($CDCl_3$) δ : 7.31(d, J=8.8Hz, 4H), 7.39(d, J=8.8Hz, 4H) , 8.6(s, 2H, 2xCH-pyrazine).

2,3-Bis-(4-methoxyphenyl)pyrazine(4d)

This compound was obtained in a yield of 60%, mp;126-128°C, ^{1}HNMR ($CDCl_3$) δ : 3.85(s,6H,OCH3) , 6.85(d, J=8.5Hz ,4H), 7.43(d, J=8.5Hz, 4H), 8.53(s, 2H,2xCH-pyrazine).

Biological evaluation

The compounds 4a-d was evaluated for their ability to inhibit the growth of HT-29 colon cancer cell using the MTT assay. The results (table 1) demonstrated that some 2,3-diarylpyrazine derivatives, 4a and 4b, had greater cytotoxic activity than the others. All experiments was done triplicate and the result in table 1 is as mean value.

The authors suggest synthesis and evaluation of cytotoxicity for more analogs in pyrazine class.

Table 1
Cytotoxiciy by 4a-d

Compound Number	% Cytotoxicity (% of cell death at 50μM concentration in HT-29 cell line)
4a	54.4
4b	46.3
4c	28.1
4d	40.2

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