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Research Article

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Synthesis and evaluation of some pyrazoline derivatives as anticancer agents

Tawfeek Ahmed Ali Yahya[a]*, Jalal H. Abdullah[a], Mokhtar Abd Hafiz Al-Ghorafi[b] and Shada H. Yassin[a]

[a]*Medicinal Chemistry Department, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen*
[b]*Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen*

ABSTRACT

Pyrazolines are one of the heterocyclic compounds with very important biological activities. Pyrazoline carbaldehydes were synthesized using chalcones and hydrazine hydratein presence of formic acid. The structures of the synthesized compounds have been established on the basis of their spectral data. The synthesized selected compounds were evaluated for their anti-cancer activity.

Keywords: Chalcone, Pyrazoline, MCF7, anticancer.

INTRODUCTION

Breast cancer is expected to account for 203 500 new cancer cases and 39 600 deaths in 2002. Although major advances have been made in early detection, prevention, and treatment, the need for more effective therapy in the fight against late-stage breast cancer continues. Currently there is no curative treatment for women with metastatic breast cancer once they have failed adjuvant therapies. New, effective cytotoxic agents with novel mechanisms of action are therefore urgently needed for the treatment of women with metastatic breast cancer [1].

Chalcones or 1,3-diaryl-2-propen-1-ones are natural or synthetic compounds belonging to the flavonoid family. They exhibit different kinds of biological activities, such as antiproliferative [2], antioxidative [3], antibacterial [4], antifungal [5], antileishmanial [6], antinociceptive [7] and Antimicrobial [8] agents.

Many pyrazoline derivatives are acknowledged to possess a wide range of bioactivities. The pyrazoline motif makes up the core structure of numerous biologically active compounds. Thus, some representatives of this heterocycle exhibitantiviral/antitumor [11-13]antibacterial [14,15, 20, 21], antiinflammatory [16], analgesic [17], fungistatic [18], and antihyperglycemic activities [19]. The formyl group is introduced to pyrazoline aiming to enhance the anticancer activity.

EXPERIMENTAL SECTION

Chemicals and reagents

2-Bromo-1-(3-bromo-2-hydroxy-5-methylphenyl)ethanone, p-aminoacetophenonevarious benzaldehydes, hydrazine hydrate, sodium hydroxide, acetone, anhydrous potassium carbonate and formic acid.

Experimental procedures

2-[(4-acetylphenyl)amino]-1-(3-bromo-2-hydroxy-5-methylphenyl)ethanone (3)

A mixture of **(1)** (2.28 g, 10 mmol), p-aminoacetophenone**(2)** (1.35 g, 10 mmol) and anhydrous potassium carbonate(2 g) in dry acetone (50 ml) was refluxed for 4 h, then filtered while hot and the residue was washed

repeatedly with small portions of hot acetone. Evaporating the combined filtrate gave a pale yellow residue, which was crystallized from ethanol.

2-[(4-acetylphenyl)amino]-1-(3-bromo-2-hydroxy-5-methylphenyl)ethanone (3)
IR (ν_{max}): 3420 (-OH), 3230 (-NH), 3132 (Ar-H),1650 (Ar-C=C), 1693 (C=O), 1705 (). ^{1}HNMR (DMSO): 2.30 (s, 3H, CH_3), 2.51 (s, 3H, COCH3), 4.51 (s, 2H, CH_2), 6.66 (s, H, NH), 7.01 (d, 1H, phenyl H-4), 7.56 (d, 1H, phenyl H-6), 7,66 (d, 2H, phenyl H-2, H6), 7.68 (d, 2H phenyl H-3, H5), 11.92 (s, 1H, OH). Mass: m/z 349 (M^++2), 347 (M^+).

(1-(4-{[2-(3-bromo-2-hydroxy-5-methylphenyl)-2-oxoethyl]amino}phenyl)-3-(substituted phenyl)prop-2-en-1-one (4a-e)
To a mixture of **(3)** (3.468 g, 10 mmol) and 30% sodium hydroxide solution (20 ml); a solution of the appropriate aromatic aldehydes (12 mmol) in alcohol (30 ml) was added, the resulting red solution was allowed to stand for 24h at room temperature, diluted with water to 200 ml, then acidified with glacial acetic acid. The formed precipitate was collected by filtration, dried and recrystallized from ethanol. (121)

(1-(4-{[2-(3-bromo-2-hydroxy-5-methylphenyl)-2-oxoethyl]amino}phenyl)-3-(phenyl)prop-2-en-1-one (4a)
IR (ν_{max}): 3424 (-OH), 3236 (-NH), 3135 (Ar-H),1653 (Ar-C=C), 1695 (C=O), 1710 (). ^{1}HNMR (DMSO): 2.31 (s, 3H, CH_3), 4.54 (s, 2H, CH_2), 6.65 (s, H, NH), 6.70(d, 1H, α-olefinic proton), 6.97 (d, 1H, β-olefinic proton), 7.01-7.66 (m, 11 H, Ar-H), 11.96 (s, 1H, OH). Mass: m/z 451 (M^++2), 449 (M^+).

(1-(4-{[2-(3-bromo-2-hydroxy-5-methylphenyl)-2-oxoethyl]amino}phenyl)-3-(4-chlorophenyl)prop-2-en-1-one (4b)
IR (ν_{max}): 3425 (-OH), 3237 (-NH), 3138 (Ar-H),1656 (Ar-C=C), 1697 (C=O), 1709 (). ^{1}HNMR (DMSO): 2.34 (s, 3H, CH_3), 4.62 (s, 2H, CH_2), 6.68 (s, H, NH), 6.85 (d, 1H, α-olefinic proton), 7.04 (d, 1H, β-olefinic proton), 7.12-7.72 (m, 10 H, Ar-H), 12.01 (s, 1H, OH). Mass: m/z 485 (M^++2), 483 (M^+).

(1-(4-{[2-(3-bromo-2-hydroxy-5-methylphenyl)-2-oxoethyl]amino}phenyl)-3-(4-bromophenyl) prop-2-en-1-one (4c)
IR (ν_{max}): 3423 (-OH), 3234 (-NH), 3135 (Ar-H),1658 (Ar-C=C), 1695 (C=O), 1704 (). ^{1}HNMR (DMSO): 2.30 (s, 3H, CH_3), 4.60 (s, 2H, CH_2), 6.67 (s, H, NH), 6.84 (d, 1H, α-olefinic proton), 7.10 (d, 1H, β-olefinic proton), 7.23-7.80 (m, 10 H, Ar-H), 12.06 (s, 1H, OH). Mass: m/z 530 (M^++4), 528 (M^++2), 526 (M^+).

(1-(4-{[2-(3-bromo-2-hydroxy-5-methylphenyl)-2-oxoethyl]amino}phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (4d)
IR (ν_{max}): 3419 (-OH), 3228 (-NH), 3127 (Ar-H),1660 (Ar-C=C), 1691 (C=O), 1706 (). ^{1}HNMR (DMSO): 2.32 (s, 3H, CH_3), 2.58 (s, 3H, COCH3), 3.91 (s, 3H, OCH_3), 4.64 (s, 2H, CH_2), 6.69 (s, H, NH), 6.91 (d, 1H, α-olefinic proton), 7.23 (d, 1H, β-olefinic proton), 7.34- 7.96 (m, 10 H, Ar-H), 12.30 (s, 1H, OH). Mass: m/z 481 (M^++2), 479 (M^+).

(1-(4-{[2-(3-bromo-2-hydroxy-5-methylphenyl)-2-oxoethyl]amino}phenyl)-3-(4-dimethylaminophenyl)prop-2-en-1-one (4e)
IR (ν_{max}): 3413 (-OH), 3229 (-NH), 3127 (Ar-H),1663 (Ar-C=C), 1693 (C=O), 1706 (). ^{1}HNMR (DMSO): 2.25 (s, 3H, CH_3),2.64 (s, 6H, $N(CH_3)_2$, 4.64 (s, 2H, CH_2), 6.64 (s, H, NH), 6.93 (d, 1H, α-olefinic proton), 7.26 (d, 1H, β-olefinic proton), 7.36- 7.99 (m, 10 H, Ar-H), 12.25 (s, 1H, OH). Mass: m/z 494 (M^++2), 492 (M^+).

5-(substitutedphenyl)-3-(4-{[2-(2-hydroxy-3-bromo5-methylphenyl)-2-oxoethyl]amino}phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbaldehyde (5a-e)
Appropriate chalcone (**4a-e**) 0.01 mol was treated hydrazine hydrate 0.020 molin formic acid and refluxed for 6 hr. The hot reaction mixture was then poured into ice-cold water. The solid separated out was filtered, washed, dried and recrystallized from ethanol.

5-phenyl-3-(4-{[2-(2-hydroxy-3-bromo5-methylphenyl)-2-oxoethyl]amino}phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbaldehyde (5a)
IR (ν_{max}): 3418 (-OH), 3225 (-NH), 3129 (Ar-H),1666 (Ar-C=C), 1710 (HC=O). ^{1}HNMR (DMSO): 2.25 (s, 3H, CH_3), 3.10 (dd, 1H, axial-H of CH2-pyrazoline), 3.40 (dd, 1H, equatorial-H of CH2-pyrazoline), 4.67 (s, 2H, CH_2), 6.70 (s, H, NH), 7.56- 8.01 (m, 11 H, Ar-H), 9.31 (s, 1H, HC=O),12.30 (s, 1H, OH). Mass: m/z 491 (M^++2), 489 (M^+).

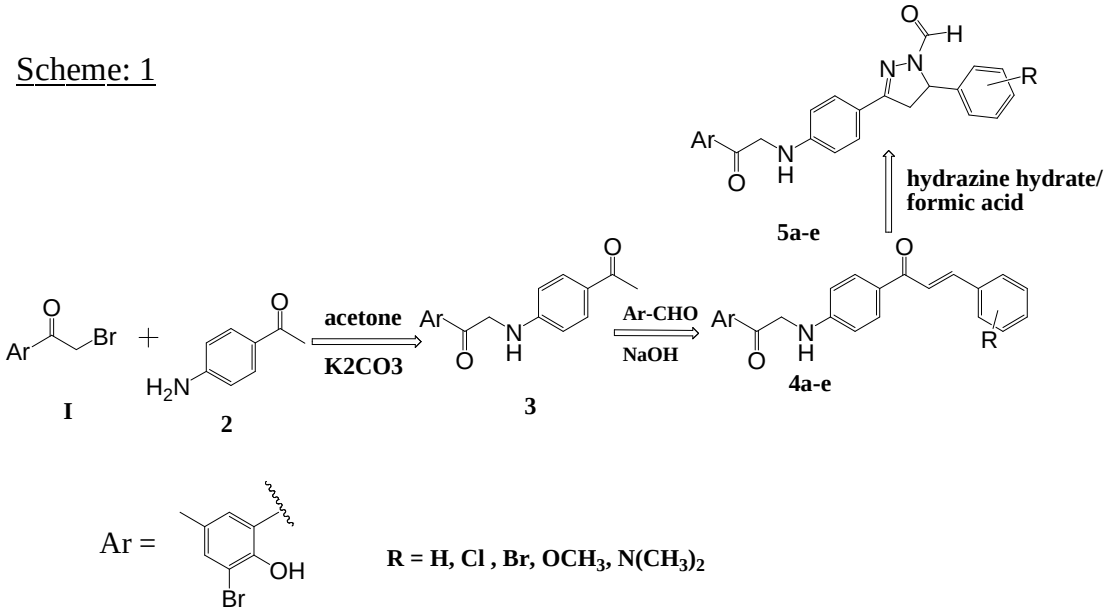
5-(4-chlorophenyl)-3-(4-{[2-(2-hydroxy-3-bromo5-methylphenyl)-2-oxoethyl]amino}phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbaldehyde (5b)
IR (ν_{max}): 3421 (-OH), 3227 (-NH), 3132 (Ar-H),1670 (Ar-C=C), 1712 (HC=O), (). ^{1}HNMR (DMSO): 2.27 (s, 3H, CH_3), 3.21 (dd, 1H, axial-H of CH2-pyrazoline), 3.46 (dd, 1H, equatorial-H of CH2-pyrazoline), 4.69 (s, 2H, CH_2), 6.75 (s, H, NH), 7.61- 8.11 (m, 10 H, Ar-H), 9.33 (s, 1H, HC=O),12.35 (s, 1H, OH). Mass: m/z 525 (M^++2), 523 (M^+).

5-(4-bromophenyl)-3-(4-{[2-(2-hydroxy-3-bromo-5-methylphenyl)-2-oxoethyl]amino}phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbaldehyde (5c)
IR (ν_{max}): 3420 (-OH), 3225 (-NH), 3130 (Ar-H),1671 (Ar-C=C), 1710 (HC=O), (). ^{1}HNMR (DMSO): 2.25 (s, 3H, CH_3), 3.22 (dd, 1H, axial-H of CH2-pyrazoline), 3.45 (dd, 1H, equatorial-H of CH2-pyrazoline), 4.67 (s, 2H, CH_2), 6.73 (s, H, NH), 7.60- 8.03 (m, 10 H, Ar-H), 9.30 (s, 1H, HC=O),12.30 (s, 1H, OH). Mass: m/z 570 (M^++4), 568 (M^++2), 566 (M^+).

5-(4-methoxyphenyl)-3-(4-{[2-(2-hydroxy-3-bromo5-methylphenyl)-2-oxoethyl]amino}phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbaldehyde (5d)
IR (ν_{max}): 3425 (-OH), 3222 (-NH), 3122 (Ar-H),1674 (Ar-C=C), 1711 (HC=O), (). ^{1}HNMR (DMSO): 2.25 (s, 3H, CH_3, 3.22 (dd, 1H, axial-H of CH2-pyrazoline), 3.45 (dd, 1H, equatorial-H of CH2-pyrazoline), 3.97 (s, 3H, OCH_3), 4.62 (s, 2H, CH_2), 6.70 (s, H, NH), 7.61- 8.10 (m, 10 H, Ar-H), 9.28 (s, 1H, HC=O),12.29 (s, 1H, OH). Mass: m/z 521 (M^++2), 519 (M^+).

5-(4-dimethylaminophenyl)-3-(4-{[2-(2-hydroxy-3-bromo5-methylphenyl)-2-oxoethyl]amino}phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbaldehyde (5e)
IR (ν_{max}): 34259 (-OH), 32225 (-NH), 3126 (Ar-H),1676 (Ar-C=C), 1713 (HC=O), (). ^{1}HNMR (DMSO): 2.24 (s, 3H, CH_3, 2.69 (s, 6H, $N(CH_3)_2$, 3.26 (dd, 1H, axial-H of CH2-pyrazoline), 3.48 (dd, 1H, equatorial-H of CH2-pyrazoline), 3.99 (s, 3H, OCH_3), 4.64 (s, 2H, CH_2), 6.72 (s, H, NH), 7.69- 8.20 (m, 10 H, Ar-H), 9.29 (s, 1H, HC=O),12.31 (s, 1H, OH). Mass: m/z 534 (M^++2), 532 (M^+).

Scheme: 1



Chemistry
The compound **(3)** was synthesized by reaction of 2-bromo-1-(3-bromo-2-hydroxy-5-methylphenyl)ethanone**(1)** with p-aminoacetophenone**(2)** in refluxing acetone as shown in **Scheme 1**. Chalcone derivatives **(4a-e)**were prepared through Claisen–Schmidt condensation of acetophenone derivative **(3)** with appropriate benzaldehydes.Compounds**(5a-e)**were synthesized bythe reaction of hydrazine hydrate with Chalcone derivatives **(4a-e)** in formic acid (**Scheme 1)**.The physical data of the synthesized compounds was given in**Table 1**.

General procedures
Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded using a PerkinElmer FT-IR 1650 spectrophotometer. ^{1}H NMR spectra were recorded on Bruker AM 400 instrument (at 400 MHz) using tetramethylsilane (TMS) as an internal standard and DMSO-d6 as a solvent. Chemical shifts are given inparts per million (ppm). Splitting patterns are designated asfollows: s- singlet, d- doublet, t- triplet, q-quartet and m-multiplet. Mass spectra (MS) were recorded using finnigan SSQ 7000 Gas Chromatograph–Mass. All the

synthesized compounds were purified by recrystallization. The reactions were followed up and the purity of compounds was monitored on pre-coated TLC plates and visualizing the spots in ultraviolet light**.**

Anticancer activities[20]

The synthesized compounds have been tested for cytotoxic activity against human mammary carcinoma cell line (MCF7) in the National Cancer Institute, Cairo University. The screening involves calculation of the percentage growth or surviving fraction of the drug treated cell lines compared by untreated control using Sulforhodamine B (SRB) colorimetric assay. Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 hours before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentrations of compound under test (0.0, 1.0, 2.5, 5.0 and 10.0 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hours at 37ºC and in atmosphere of 5 % CO_2. After 48 hours, cells were fixed, washed and stained with Sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with tri EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of the tumor cell line after the specified compound. The results were described in the TABLE 2.

RESULTS AND DISCUSSION

In our study, a series of chalcones**(4a-e)**and pyrazolines**(5a-e)** showed moderate to significant anticancer activity when compared with standard drugs. However it is less than standard drugs like Doxorubicin but compound**(4e)** Showed significant anticancer activity when compared to standard drug.Data were presented in **TABLE**2 revealed the IC_{50} of the synthesized compounds.

Table 1 Physical data of the synthesized compounds

Compound No.	Melting point(º)	Yield (%)	Molecular Formula
3	113	72	$C_{17}H_{16}BrNO_3$
4a	123	73	$C_{24}H_{20}BrNO_3$
4b	137	76	$C_{24}H_{19}BrClNO_3$
4c	167	77	$C_{24}H_{19}Br_2NO_3$
4d	141	74	$C_{25}H_{22}BrNO_4$
4e	163	60	$C_{26}H_{25}BrN_2O_3$
5a	135	67	$C_{25}H_{22}BrN_3O_3$
5b	178	63	$C_{25}H_{21}BrClN_3O_3$
5c	207	64	$C_{25}H_{21}Br_2N_3O_3$
5d	181	63	$C_{26}H_{24}BrN_3O_4$
5e	203	53	$C_{27}H_{27}BrN_4O_3$

TABLE 2 Anticancer activity of the synthesized compounds (IC_{50}) (against MCF7 cell line)

Compound No.	IC_{50} µg/ml
4a	2.34
4b	4.72
4c	3.61
4d	2.34
4e	0.86
5a	2.3
5b	4.72
5c	4.69
5d	3.46
5e	1.84
Doxorubicin	0.7

REFERENCES

[1] A Jemal;A Thomas;T Murray;M Thun, Ca-*Cancer J. Clin*.,**2002**, 52,23-47.
[2] ML Go; X Wu;XL Liu,*Curr. Med. Chem*.,**2005**, 12, 499.
[3] CA Calliste; JCL Bail; P Trouillas; C Pouget; et al,*Anticancer Res*.,**2001**,21, 3949-3956.
[4] S Iwata; T Nishino; N Nagata; Y Satomi; et al,*Biol.Pharm. Bull.*,**1995**,18, 1710-1713.
[5] K Bowden; A Dalpozzo; CKDuah,*J.Chem. Res*., **1990**,12, 377.
[6] PBoeck; PC Leal; RA Yunes; VCFilho; et al,*Arch.Pharm*. (Weinheim)., **2005**,338, 87-95.
[7] SF Nielsen; SB Christensen; G Cruciani; A Kharazmi;T Liljefors.,*J. Med. Chem*.,**1998**,41, 4819-4832.
[8] F de Campos-Buzzi; J Pereira de Campos; P PozzaTonini; R Correa, et al, *Arch. Pharm*. (Weinheim).,**2006**,339, 361- 365.
[9] S Manfredini; R Bazzanini; PGBaraldi; M Guarneri; D Simoni;ME Marongiu; A Pani; ETramontano; P La Colla, *J. Med. Chem*.,**1992**, 35, 917-924.
[10] S Manfredini; RBazzanini; PG Baraldi; M Bonora; M Marangoni; D Simoni; A Pani; F Scintu; E Pinna, Anti-*cancer Drug Des*., **1996**, 11, 193-204.
[11] HA Park; K Lee; SJ Park; B Ahn; JC Lee; HY Cho; KI Lee,*Bioorg. Med.Chem. Lett*.,**2005**, 15, 3307-3312.
[12] A Tanitame; Y Oyamada; K Ofuji; M Fujimoto; N Iwai; Y Hiyama; K Suzuki;H Ito; M Wachi; JYamagishi, J. *Med. Chem*., **2004**, 47, 3693-3696.
[13] SG Kucukguzel; S Rollas; H Erdeniz; MKiraz; AC Ekinci; A Vidin, *Eur. J. Med.Chem.*,**2000**, 35, 761-771.
[14] TD Penning; JJ Talley; SR Bertenshaw; JS Carter; PW Collins; SDocter;MJ Graneto; LF Lee; JWMalecha; JM Miyashiro; RS Rogers; DJRogier;SS Yu; GD Anderson; EG Burton; JNCogburn; SA Gregory; CMKoboldt;WE Perkins; K Seibert; AWVeenhuizen; YY Zhang; PC Isakson, *J. Med.Chem*.,**1997**, 40, 1347-1365.
[15] G Menozzi; L Mosti; P Fossa; F Mattioli; M Ghia,J. *Heterocycl. Chem*., **1997**, 34, 963-968.
[16] R Sridhar; PT Perumal; S Etti; G Shanmugam; MNPonnuswamy;VR Prabavathy; N Mathivanan,*Bioorg. Med. Chem. Lett*.,**2004**, 14, 6035-6040.
[17] GR Bebernitz; G Argentieri; B Battle; C Brennan; B Balkan; BFBurkey; M Eckhardt; J Gao; PKapa; RJ Strohschein;HF Schuster; M Wilson; DD Xu, *J. Med. Chem*.,**2001**, 44, 2601-2611.
[18] NN Shah;HM Ziauddin; M Zameer; SS Hingole; MA Baseer, *J. Chem. Pharm. Res*., **2010**, 2(6), 441-445.
[19] KA Pramer;JR Vihol; YM Dabhi; SD Sutariya, *J. Chem. Pharm. Res*., **2012**, 4(3),1584-1588.
[20] P Skehan;R Storeng;D Scudiero;A Monks;J McMahon;D Visitica;JT Warren; H Bokesch;S Kenney; RM Boyd, *J. Natl. Cancer Inst*., **1990**, 82, 1107-1112.