Marmara Pharmaceutical Journal 20: 157-163, 2016
DOI: 10.12991/mpj.20162093956

ORIGINAL RESEARCH

Antiproliferative activity of some tautomeric hydrazones derived from chalcones

Fatih TOK, Nagihan BEYHAN, Yalçın ERZURUMLU, Recep İLHAN, Petek BALLAR, Bedia KOCYİGİT-KAYMAKCİOGLU

ABSTRACT

A new series of hydrazones synthesized from chalcones. Synthesized compounds have been characterized by IR, ^{1}H-NMR and elemental analysis. Antiproliferative activity of compounds was investigated on Hela, A549, MCF7, HCC1937, MRC5 cells. All compounds exhibited cytotoxicity. Especially compounds **1b**, **1c**, **1f** and **1i** having 4-metylsulfonyl phenyl showed higher cytotoxicity against all of the cell lines compared to reference drug doxorubicin with low value of IC_{50}=5.56-21.93 μM. The most active compounds **1b**, **1c**, **1f** and **1i** were analyzed for their effect on autophagic processes. HCC1937 cells were treated with these compounds at IC_{50} concentration for 24 hours. An immunoblotting assay was performed to analysis autophagy markers and total polyubiquitinated protein levels. Compounds **1b**, **1c**, **1f** and **1i** significantly increased the conversion of LC3-I to LC3-II at IC_{50} concentration. None of the tested compounds changed the level of total polyubiquitinated proteins.

Keywords: chalcones, hydrazones, antiproliferative activity

Fatih TOK, Nagihan BEYHAN, Bedia KOCYİGİT-KAYMAKCİOGLU
Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, İstanbul, 34668, Turkey

Yalçın ERZURUMLU, Recep İLHAN, Petek BALLAR,
Ege University, Faculty of Pharmacy, Department of Biochemistry, İzmir, 35040, Turkey

Address for correspondence: Bedia KOCYİGİT-KAYMAKCİOGLU
Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 34668, Istanbul, Turkey
E-mail: bkaymakcioglu@marmara.edu.tr
Tel: +90-216-4142963/1023
Fax: +90-216-3452952

Submitted/Gönderilme: 10.03.2016 **Revised/Düzeltme:** 30.03.2016
Accepted/Kabul: 31.03.2016

INTRODUCTION

Cancer is one of the most important death causes of in the world. There are many problems for the control and treatment of cancer (1). Although numerous cancer treatment methods are available, the number of cancer diagnosis increase day by day. Therefore it is aimed to reach effective and selective treatment methods for cancer (2).

Chalcones consist of three carbon, α, β-unsaturated carbonyl system (3). Chalcone nucleus is associated several biological activities such as antibacterial, antifungal, antileishmanial, anticonvulsant (4), antimalarial, antiviral, anticancer, antiinflammatory, antioxidant (5, 6). Chalcone derivatives are known as xanthine oxidase inhibitors, aldose reductase inhibitors, epoxide hydrolase inhibitors, topoisomerase II inhibitors and aromatase inhibitors (7). The structure activity relationship shows us the presence of double bond with conjugation is responsible for the biological activities (8). Therefore hydrazone groups are synthesized from chalcones with maintaining this structure (9).

There are many studies about cytotoxicity of different

chalcone derivatives against cancer cell lines. Won and co-workers synthesized a new series of chalcones. They searched antiinflammatory and anticancer activity of compounds. 1-(2,5-dimethoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one was chosen as the lead molecule for treatment or prevention of MCF-7 cancer cell lines (10).

Kumar and co-workers synthesized some α-cyano bis(indolyl) chalcones and evaluated anticancer activity against three different cancer cell lines fluor and methoxy substitution on aromatic rings were increased tubulin polymerization (11).

Substitue-4-aryl-4-oxo-butenoic acid amides were synthesized by Todorovic *et al*, and all molecules showed antiproliferative activity. It was shown that unsubstitue molecule (4-oxo-N,4-diphenylbut-2-enamide) was the most potent inhibitor of tubulin (12).

A new series of isoxazolyl chalcones were synthesized and screened their activities against H1792, H157, A549 and Calu-1 cells. 1-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-3-(2-nitrophenyl)prop-2-en-1-one exhibited significant antitumor activity. The result of structure activity relationship was showed electron withdrawing group at only 2-position on aromatic ring provided more effective molecule than others (13).

Hydrazones consist of –CO-NH-N=CH- group (14) and exhibit different biological activity such as antifungal (15), antimicrobial (16), antitubercular, anticancer (17) depending on different substituents. Tautomeric hydrazo groups act as donor and acceptor moieties. Therefore it facilitates binding to the receptor (18). Hydrazone scaffold are very important for pH-dependent release of drugs (19). Especially aroylhydrazide-hydrazone structures bearing hetero-ring such as pyridine, pyrazole, oxadiazole, triazole exhibit significant anticancer activity. 1-arylmethyl-3-aryl-1H-pyrazole-5-carbohydrazide hydrazone showed significant inhibitory effect against A549 cancer cell lines (20).

According to literature's data, benzothiazole hydrazones were synthesized and their anticancer activity against leukemia, breast, colon cells were evaluated (21). In another study, chalconephenyl hydrazones and their cyclized derivatives were synthesized and their effect on cathepsin B and H were evaluated. Two pharmacologically active group such as chalconephenyl hydrazones are beneficial for potential antitumor agent's effect (22).

On the basis of historical backgrounds of the anticancer activity of hydrazone derivatives, we synthesized different substituted tautomeric hydrazone derivatives and evaluated their antiproliferative activity.

Materials and Methods

Chemistry

Isoniazid was purchased from Sigma-Aldrich. The following reagent grade chemicals were purchased and used without further purification. Melting points were determined by Schmelzpunktbestimmer SMP II. IR spectra were recorded at Schimadzu FTIR-8400S. ^{1}H-NMR spectra were recorded on a Bruker instrument in DMSO-d_6 with TMS as internal standard for protons. All the compounds were isolated in satisfactory yields (40-65%) and purified by recrystallization from ethanol. The purity of the compounds was established by thin layer chromatography (TLC), High Pressure Liquid Chromatography (HPLC) and elemental analysis.

Synthesis of chalcone derivatives

The intermediated chalcones were prepared by reacting equimolar aldehyde and ketone in the presence of a base by conventional Claisen-Schmidt condensation. Physicochemical and spectroscopic characterization of the chalcones have been previously described (4) .

General synthesis methods of hydrazone derivatives (1a-1j)

The intermediated chalcones (0.001 mol) were dissolved in ethanol and stirred on magnetic stirrer. Then isoniazid (0.001 mol) was added, the mixture was refluxed at 90-100 ºC for 8 hours. The obtained hydrazones (1a-1j) were filtered, dried and crystallized from suitable solvent (23).

*N'-(1-(5-chlorothiophen-2-yl)-3-phenylallylidene) isonicotinohydrazide (**1a**)*

Pale yellow crystals from ethanol. (%60); m.p. 235-237 ; IR $\upsilon_{max.}$.(cm^{-1}): 3375, 3080, 1685, 1560, 1300, 894; ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) ppm: 7.20-7.33(d, 2H, -CH=), 8.02-8.24 (m, 10H, Ar-H and NH). For $C_{19}H_{14}ClN_3OS$ (M.W.: 367.85 g/mol) calculated: C: 62.04 H: 3.84 N: 11.42. Found: C: 62.08 H: 3.81 N: 11.38.

*N'-(1-(5-bromothiophen-2-yl)-3-(2,6-dichlorophenyl) allylidene)isonicotinohydrazide (**1b**)*

Pale yellow crystals from ethanol. (%45); m.p. 238-239 ; IR υ_{max}.(cm^{-1}): 3419, 3377, 3090, 1680, 1599,1315, 1261, 1066, 856; ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) ppm: 7.43-7.45(d, 2H, -CH=), 7.48-7.71(m, 9H, Ar-H), 8.05-8.06(s, 1H, NH). For $C_{19}H_{12}BrCl_2N_3OS$ (M.W.: 481.19 g/mol) calculated: C: 47.42 H: 2.51 N: 8.73. Found: C: 48.01 H: 2.49 N: 8.72.

*N'-(3-(2,6-dichlorophenyl)-1-(4-(methylsulfonyl)phenyl) allylidene)isonicotinohydrazide (**1c**)*

Pale yellow crystals from ethanol. (%55); m.p. 245-247 ; IR υ_{max}.(cm^{-1}): 3336, 3066, 2974, 2931, 1670, 1573, 1396, 1259, 1089, 837; ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) ppm:3.25(s, 1H, CH_3), 7.42-7.46(d, 2H, -CH=), 7.59-8.25(m, 12H, Ar-H and NH). For $C_{22}H_{17}Cl_2N_3O_3S$ (M.W.: 474.36 g/

mol) calculated: C: 55.70 H: 3.61 N: 8.86. Found: C: 55.71 H: 3.65 N: 8.84.

*N'-(3-(4-hydroxyphenyl)-1-(thiophen-2-yl)allylidene) isonicotinohydrazide (**1d**)*

Pale yellow crystals from ethanol. (%45); m.p. 238-240 ; IR υ_{max} (cm^{-1}): 3450, 3360, 3085, 1685, 1570, 1378, 845. ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) ppm: 6.80-6.83(d, 2H, -CH=), 7.25-8.24(m, 12H, Ar-H and NH), 10.3(s, 1H, OH). For $C_{19}H_{15}N_3O_2S$ (M.W.: 349.41 g/mol) calculated: C: 65.31 H: 4.33 N: 12.03. Found: C: 65.30 H: 4.31 N: 12.06.

*N'-(3-(4-methoxyphenyl)-1-(4-(trifluoromethyl)phenyl) allylidene)isonicotinohydrazide (**1e**)*

Pale yellow crystals from ethanol. (%50); m.p. 232-233 ; IR $\upsilon_{max.}$ (cm^{-1}): 3456, 3046, 2937, 2837,1678, 1512, 1319, 1249, 1066, 831; ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) ppm: 3.35(s, 3H, OCH_3), 6.90-6.98(d, 2H, -CH=), 7.34-8.15(m, 13H, Ar-H and NH). For $C_{23}H_{18}F_3N_3O_2$ (M.W.: 425.40 g/mol) calculated: C: 64.94 H: 4.26 N: 9.88.Found: C: 65.01 H: 4.25 N: 9.90.

*Methyl 4-(3-(2-isonicotinoylhydrazono)-3-(4-(methylsulfonyl) phenyl)prop-1-enyl)benzoate (**1f**)*

Pale yellow crystals from ethanol. (%55); m.p. 250-252 ; IR $\upsilon_{max.}$ (cm^{-1}): 3419, 3010, 2920, 1662, 1309,1215, 1087, 831; ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) ppm: 3.32(s, 3H, CH3), 3.88(s, 3H, OCH3), 7.82-7.86(d, 2H,—CH=), 8.02-8.40(m, 13H, Ar-H and NH). For $C_{24}H_{21}N_3O_5S$ (M.W.: 463.51 g/mol) calculated: C: 62.19 H: 4.57 N: 9.07. Found: C: 62.04 H: 4.56 N: 9.08.

*Methyl 4-(3-(5-bromothiophen-2-yl)-3-(2-isonicotinoylhydrazono)prop-1-enyl)benzoate (**1g**)*

Pale yellow crystals from ethanol. (%54); m.p. 210-212 ; IR υ_{max}.(cm^{-1}): 3228, 3093, 3003, 2955, 2847, 1647, 1568, 1315, 1213, 1076, 846; ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) ppm: 3.88(s, 3H, OCH3), 7.33-7.45(d, 2H, -CH=), 7.51-8.26(m, 11H, Ar-H and NH). For $C_{21}H_{16}BrN_3O_3S$ (M.W.: 470.34 g/mol) calculated: C: 53.63 H: 3.43 N: 8.93. Found: C: 53.04 H: 3.42 N: 8.91.

*N'-(3-(4-(dimethylamino)phenyl)-1-(thiophen-2-yl) allylidene)isonicotinohydrazide (**1h**)*

Pale red crystals from ethanol. (%57); m.p. 248-250 ; IR $\upsilon_{max.}$ (cm^{-1}):3255, 3163, 3084, 2895, 2818, 1629, 1554, 1317, 1228, 1080, 856; ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) ppm: 3.01(s, 6H, CH_3), 6.74-6.76(d, 2H, -CH=), 7.27-8.23(m, 12H, Ar-H and NH). For $C_{21}H_{20}N_4OS$ (M.W.: 376.47 g/mol) calculated: C: 67.00 H: 5.35 N: 14.88. Found: C: 67.04 H: 5.36 N: 14.90.

*N'-(3-(2,6-dichlorophenyl)-1-(thiophen-2-yl)allylidene) isonicotinohydrazide (**1i**)*

Pale yellow crystals from ethanol. (%48); m.p. 270-271 ; IR $\upsilon_{max.}$.(cm^{-1}): 3219, 3078, 2962, 2816, 1668, 1556, 1356, 1217, 1045, 869; ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) ppm: 6.88-6.96 (d, 2H, -CH=), 7.35-8.33(m, 11H, Ar-H and NH). For $C_{19}H_{13}Cl_2N_3OS$ (M.W.: 402.30 g/mol) calculated: C: 56.73 H: 3.26 N: 10.45. Found: C: 56.74 H: 3.25 N: 10.40.

*N'-(3-(4-methoxyphenyl)-1-(4-(methylsulfonyl)phenyl) allylidene)isonicotinohydrazide (**1j**)*

Pale yellow crystals from ethanol. (%52); m.p. 262-263 ; IR υ_{max}.(cm^{-1}):3402, 3010, 2928, 2841, 1658, 1566, 1307, 1215, 1026, 819; ^{1}H-NMR (400 MHz), (DMSO-d_6/TMS) ppm: 3.31(s, 3H, OCH_3), 3.83(s, 3H, OCH3), 7.02-7.06(d, 2H, -CH=), 7.75-8.35(m, 11H, Ar-H and NH). For $C_{23}H_{21}N_3O_4S$ (M.W.: 435.50 g/mol) calculated: C: 63.43 H: 4.86 N: 9.65. Found: C: 63.64 H: 4.85 N: 9.66.

Anticancer activity

Cell lines and cell culture conditions

Four cancer cell lines, A549 (human lung cancer cell line), HeLA (human cervical cancer cell line), MCF7 (human breast adenocarcinoma cell line), HCC1937 (human breast adenocarcinoma cell line with BRCA1 mutation) and one non-cancer cell line, MRC5 (human fetal lung fibroblast) were obtained from ATCC (Rockville, MD, USA). DMEM (used for A549, MCF7, HeLA), RPMI 1640 (used for HCC1937) and EMEM (used for MRC5) were purchased from Lonza (Switzerland). All cell culture media were supplemented with L-glutamine (2 mmol/L), and 10% fetal bovine serum. Cells were cultured under 95% air-5% CO_2 at 37°C.

Antiproliferative activity assay

The antiproliferative activities of compounds were evaluated using WST-1 reagent as previously described (24). Briefly, the cells (5.000) were seeded and grown in 96-well plates. After 24 hours, cells were treated with test compounds at various concentrations (30, 15, 10, 7.5, 5μM). DMSO is used as negative solvent control and Doxorubicin is used as positive control. The viability of cells was quantitatively assessed by WST-1 assay at 48 h post-exposure with test compounds. The assay was carried out according to the manufacturer's instructions. Briefly, at the end of 48 h treatment, WST-1 cell proliferation reagent (Roche Applied Sciences, Indianapolis, IN, USA) was added to the cells in 1:10 dilution. Three hours after addition of WST-1 reagent, the optical density of each well was determined by a spectrophotometric reader at 450 nm (Versamax, Molecular Devices, California, USA). 690 nm wavelength was used as reference. All measurements were performed in triplicates. IC_{50} values were calculated using non-linear curve fitting with GraphPad Prism (version 6 GraphPad Software). All measurements were performed in triplets.

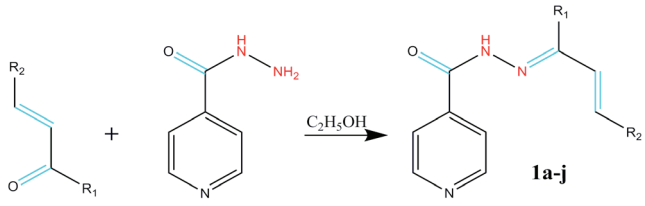
Immunoblotting

HCC1937 (human breast adenocarcinoma cell line) cells were treated with the compounds or DMSO control. Immunoblotting studies have been performed as previously described (25). Cells were lysed in RIPA buffer (150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS in 50 mM Tris-HCl pH 7.5) supplemented with protease inhibitor cocktail. After determining the protein concentration of cell lysates using BCA Protein Assay kit (PIERCE, Rockford, IL, USA), equal amounts of proteins were loaded to the gels for SDS-PAGE. Following electrophoresis, proteins were transferred to PVDF membrane (Millipore, Bedford, MA, USA). Immunoblotting was performed by using LC3 antibody (Cell Signaling, Danvers, Massachusetts, USA), p62 antibody (Cell Signaling, Danvers, Massachusetts, USA), and actin antibody (Sigma Aldrich, USA). For visualization of the results, chemiluminescence procedure was performed with ECL substrate kit (BioRAD, USA) according to the manufacturer's protocol. The chemiluminescence light was captured using Fusion-FX7 imaging system (Vilber Lourmat, France). Image J software was used for quantification of band intensity. n=6 used in these processes.

Results and Discussion

Chemistry

The synthetic route to the target compounds is outlined in Scheme 1. The intermediate chalcones were prepared by reacting equimolar aldehyde and ketone in presence of a base by conventional Claisen-Schmidt condensation. Physicochemical and spectroscopic characterization of the chalcone derivatives have been previously described (4). Tautomeric hydrazone derivatives were synthesized by the reaction of substituted chalcones and isoniazid. The purities of the synthesized compounds were checked by reversed phase HPLC (Chromasil C_{18} 3.6 x150 mm column using acetonitrile and water (50:50 v/v) as the eluent). The structures of the hydrazone compounds (**1a-j**) were confirmed by IR, ^{1}H-NMR and elemental analysis. IR spectra of the compounds (**1a-j**) afforded hydrazone C=N streching (1512-1573), N-H streching (3219-3456) and C=O streching (1647-1685) bands and aromatic rings C=C streching (1560-1599), C-H streching (3003-3085) bands. The NH protons of hydrazone groups resonated as a singlet or two different singlet peak because of *E/Z* isomer at 8.24-8.38 ppm. The CH protons (methylsulfonyl) appeared as a singlet at 3.35-3.88 ppm. The CH=CH protons (chalcones) were seen as multiplet at 7.25-8.40 ppm with aromatic protons. The elemental analysis of hydrazone compounds were in agreement with the proposed structures of the compounds.



Scheme 1. Synthetic pathway for targed compounds.

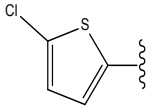
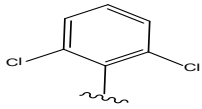
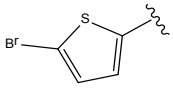
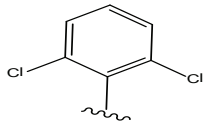
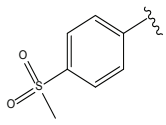
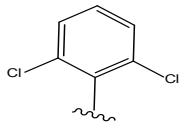
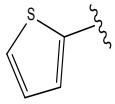
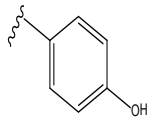
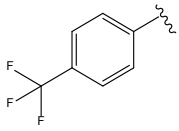
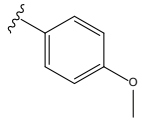
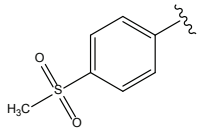
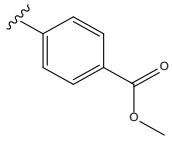
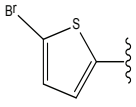
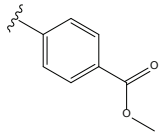
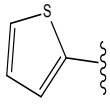
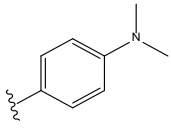
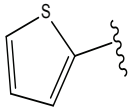
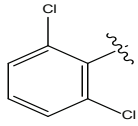
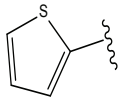
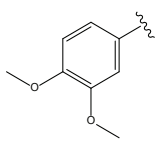
Antiproliferative activity

Antiproliferative activity of compounds was investigated on four cancer cell lines, A549 (human lung cancer cell line), HeLA (human cervical cancer cell line), MCF7 (human breast adenocarcinoma cell line), HCC1937 (human breast adenocarcinoma cell line with BRCA1 mutation) and one non-cancer cell line, MRC5 (human fetal lung fibroblast). Antiproliferative activity results are presented in Table 1. As seen in Table 1, **1a, 1b, 1c, 1f** and **1i** showed cytotoxicity below 30 μM (except A549 of 1a). While **1c** is the most cytotoxic compound for HeLA, A549 and MRC5 cells; 1f and 1i are the most active compounds in MCF7 and HCC1937, respectively. The IC_{50} values of **1d**, **1e**, **1g**, **1h** and **1j** could not be calculated using tested concentrations (above 30 μM).

The most active compounds **1b**, **1c**, **1f** and **1i** were analyzed for their effect on autophagic processes. HCC1937 cells were treated with these compounds at IC_{50} concentration for 24 hours. An immunoblotting assay was performed to analysis autophagy markers and total polyubiquitinated protein levels. Our results show that **1b**, **1c**, **1f** and **1i** at IC_{50} concentration significantly increased the conversion of LC3-I to LC3-II. LC3 is a classical autophagosome marker and conversion of LC3-I to its lipidated form LC3-II reflects maturation autophagosome. Since both autophagy inhibitors and inducers may increase the LC3-II/LC3-1 ratio, we analyzed the level of p62 protein, which is a known autophagy substrate and marker of the stream of the autophagic process (26). We noticed that all tested compounds caused a significant increase on p62 levels ($p<0.005$) compared with DMSO treated control samples suggesting that autophagy is inhibited. Bafilomycin A1, inhibits lysosomal degradation by hampering acidification of lysosomes by inhibiting vacuolar type H^+ ATPase (V-ATPase) (27). As a known autophagy inhibitor, Bafilomycin A1 is used as positive control at 100 ng/ml for 4 hours.

We have also compared the effect of **1b**, **1c**, **1f** and **1i** on ubiquitin mediated proteasomal degradation. While proteasome inhibitor Mg132 significantly increased the level of total polyubiquitinated proteins, none of the tested compounds changed the level of total polyubiquitinated proteins compared with DMSO treatment (data not shown).

Table 1. IC_{50} values (μM) of all compounds.

Comp	R_1	R_2	Hela	A549	MCF7	HCC1937	MRC5
1a			19.78±0.98	>30	14.39±0.93	14.21±0.27	12.88±0.53
1b			13.74±0.65	22.58±0.97	10.23±0.62	12.22±0.49	8.45±0.87
1c			8.35±0.51	21.93±1.15	19.24±1.93	9.92±0.54	5.56±0.75
1d			>30	>30	>30	>30	>30
1e			>30	>30	>30	>30	>30
1f			12.35±0.69	28.6±1.91	7.24±0.47	12.62±0.66	8.45±0.36
1g			>30	>30	>30	>30	>30
1h			>30	>30	>30	>30	>30
1i			11.33±0.81	26.12±1.21	8.75±0.97	9.09±0.76	8.39±0.31
1j			>30	>30	>30	>30	>30
	-	-	0.87±0.05	1.58±0.08	0.79±0.05	0.68±0.06	0.92±0.03

*Reference drug is doxorubicin

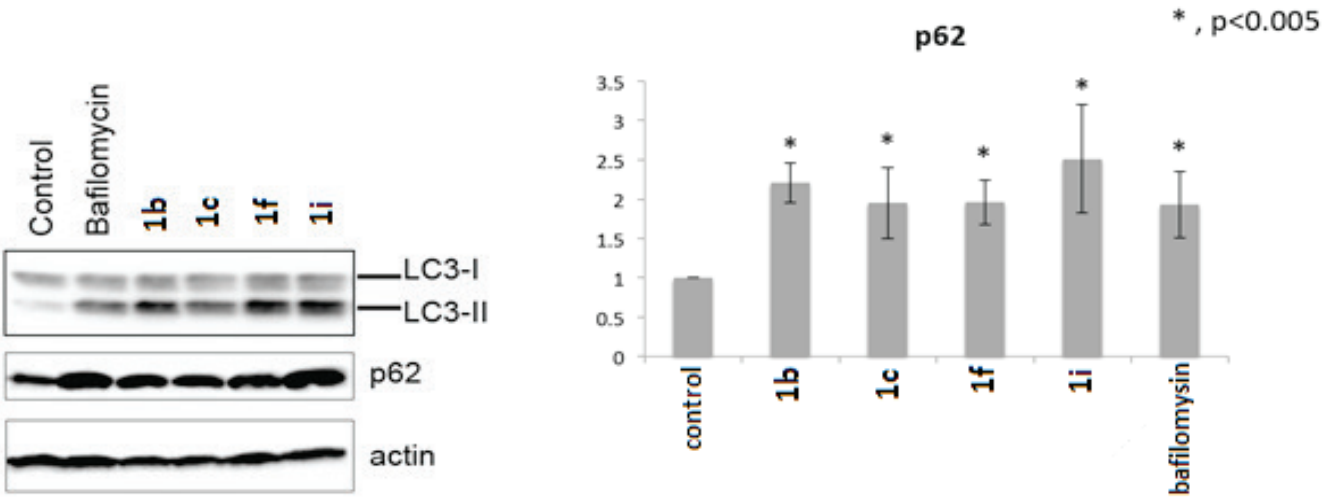


Figure 1. The effect of indicated compounds on autophagy was analysed at IC_{50} doses in HCC1937 cells. Conversion of LC3-1 to LC3-II was determined by immunoblotting with the antibody against LC3. p62 levels were examined by immunoblotting with anti- SQSTM1/p62 antibody. Actin was used as loading control.

Conclusion

Clinical studies have proven anticancer activity of tautomeric hydrazone groups. They have also less side effect. Because chalcones are not foreign to human body structures of chalcone is available in natural product. Therefore chalcones which have demonstrated anticancer activity are very important for cancer treatment. In this study, we reported the synthesis of a new series of hydrazones synthesized from chalcones, which were evaluated for their antiproliferative activity on on Hela, A549, MCF7, HCC1937, MRC5 cells. The in vitro assays revealed that all compounds showed antiproliferative activity. The replacement of metylsulfonyl groups on aromatic rings is beneficial for activity. This study may be helpful for finding molecules which are associated with cancer treatment.

Şalkonlardan hareketle elde edilen bazı tautomerik hidrazonların antiproliferatif aktiviteleri

ÖZ

Yeni bir grup hidrazon bileşiği şalkonlardan hareketle sentez edilmiştir. Sentez edilen bu bileşiklerin yapıları IR, ^{1}H-NMR ve elementel analiz ile aydınlatılmıştır. Bileşiklerin antiproliferatif aktiviteleri Hela, A549, MICF7, HCC1937, MRC5 hücre hatlarında araştırılmıştır. Bütün bileşikler sitotoksik etki göstermiştir. Özellikle 4-(metilsülfonil)fenil yapısı taşıyan **1b, 1c, 1f ve 1i** kodlu maddeler çok daha yüksek sitotoksik etki göstermiş ve referans ilaç doksorubisin ile karşılaştırıldığında IC_{50}=5,56-21,93 µM değerine sahip olduğu tespit edilmiştir. En aktif bileşikler olan **1b**, **1c**, **1f** ve **1i**'nin otofaj üzerine etkileri incelenmiştir. Bu bileşikler, HCC1937 hücreleri üzerinde 24 saatte etki göstermiştir. İmminoblotting deneyleri, otofaji belirteçleri ve total protein düzeyleri incelenmiştir. **1b**, **1c**, **1f** ve **1i** kodlu maddeler, IC50 konsantrasyonlarında LC3-I'in LC3-II'ye dönüşümünü anlamlı olarak artırmışlardır. Hiçbir bileşik total protein düzeylerinde değişiklik oluşturmamıştır.

Anahtar kelimeler: Şalkonlar, hidrazonlar, antiproliferatif aktivite

REFERENCES

1. Abdel HA, Elsaman T, Dhfyan A, Attia M, Rashood KA, Obaid AR. Synthesis and anticancer potential of certain novel 2-oxo-N'-(2-oxoindolin-3-ylidene)-2H-chromene-3-carbohydrazides. Eur J Med Chem 2013; 70: 358-63.
2. Sashidhara KV, Kumar A, Kumar M, Sarkar J, Sinha S. Synthesis and in vitro evaluation of novel coumarin-chalcone hybrids as potential anticancer agents. Bioorg Med Chem Lett 2010; 20: 7205-11.
3. Nowakowska Z. A review of anti-infective and anti-inflammatory chalcones. Eur J Med Chem 2007; 42: 125-37.
4. Beyhan N, Kaymakçıoglu B, Gümrü S, Arıcıoglu F. Synthesis and anticonvulsant activity of some 2-pyrazolines derived from chalcones. ARABJC. 2013; 1027: 1-10.
5. Mathew B, Suresh J, Anbazghagan S, Paulraj J, Krishnan GK. Heteroaryl chalcones: Mini review about their therapeutic

voyage. Biomed Prev Nutr 2014; 4: 451-8.

6. Perdana F, Eryanti Y, Zamri A. Synthesis and toxicity assessments some para-methoxy chalcones derivatives. Procedia Chem 2015; 16: 129-33.
7. Mahapatra DK, Bharti SK, Asati V. Anti-cancer chalcones: Structural and molecular target perspectives. Eur J Med Chem 2015; 98: 69-114.
8. Singh P, Anand A, Kumar V. Recent developments in biological activities of chalcones: A mini review. Eur J Med Chem 2014; 85: 758-77.
9. Millan D, Dominguez M, Rezende MC. Solvatochromic hydrazone anions derived from chalcones. Dyes and Pigments 2008; 77: 441-5.
10. Won SJ, Liu CT, Tsao LT, Weng JR, Ko HH, Wang JP, Lin CN. Synthetic chalcones as potential anti-inflammatory and cancer chemopreventive agents. Eur J Med Chem 2005; 40: 103-12.
11. Kumar D, Kumar NM, Tantak MP, Ogura M, Kusaka E, Ito T. Synthesis and identification of α-cyano bis(indolyl)chalcones as novel anticancer agents. Bioorg Med Chem Lett 2014; 24: 5170-4.
12. Todorovic MD, Nikolic AE, Kolundzija B, Hamel E, Ristic S, Juranic IO, Drakulic BJ. (E)-4-Aryl-4-oxo-2-butenoic acid amides, chalcone-aroylacrylic acid chimeras: Design, antiproliferative activity and inhibition of tubulin polymerization. Eur J Med Chem 2013; 62: 40-50.
13. Wan M, Xu L, Hua L, Li A, Li S, Lu W, Pang Y, Cao C, Liu X, Jiao P. Synthesis and evaluation of novel isoxazolyl chalcones as potential anticancer agents. Bioorg Chem 2014; 54: 38-43.
14. Lindgren EB, Yoneda JD, Leal KZ, Nogueira AF, Vasconcelos T, Wardell JL, Wardell S. Structure of hydrazones, (E)-2-(1,3-benzothiazolyl)-NH-N=CH-Ar, [Ar=4-(pyridin-2-yl) phenyl, pyrrol-2-yl, thien-2-yl and furan-2-yl]: Difference in conformations and intermolecular hydrogen bonding. J Mol Struct 2013; 1036: 19-27.
15. Altıntop MD, Özdemir A, Zitouni G, Ilgın S, Atlı Ö, İşcan G, Kaplancıklı ZA. Synthesis and biological evaluation of some hydrazine derivatives as new anticandidal and anticancer agents. Eur J Med Chem 2012; 58: 299-307.
16. Metwally KA, Abdel LM, Lashine ES, Husseiny MI, Badawy RH. Hydrazones of 2-aryl-quinoline-4-carboxylic acid hydrazides: Synthesis and preliminary evaluation as antimicrobial agents. Bioorg Med Chem. 2006; 14: 8675-82.
17. Yu X, Shi L, Ke S. Acylhydrazone derivatives as potential anticancer agents: Synthesis, bio-evaluation and mechanism of action. Bioorg Med Chem Lett. 2015; 25: 5772-6.
18. Vicini P, Incerti M, Doytchinova IA, Colla P, Busonera B, Loddo R. Synthesis and antiproliferative activity of benzo[d] isothiazole hydrazones. Eur J Med Chem. 2006; 41: 624-32.
19. Machakanur SS, Patil BR, Badiger DS, Bakale RP, Gudasi KB, Bligh SA. Synthesis, characterization and anticancer evaluation of novel tri-arm star shaped 1,3,5-triazine hydrazones. J Mol Struct 2012; 1011: 121-7.
20. Xia Y, Fan CD, Zhao BX, Zhao J, Shin DS, Miao JY. Synthesis and structure-activity relationships of novel 1-arylmethyl-3-aryl-1H-pyrazole-5-carbohydrazide hydrazone derivatives as potential agents against A549 lung cancer cells. Eur J Med Chem 2008; 43: 2347-53.
21. Lindgren EB, Brito MA, Vasconcelos TR, Moraes M, Montenegro RC, Yoneda JD, Leal KZ. Synthesis and anticancer activity of (E)-2-benzothiazole hydrazones. Eur J Med Chem 2014; 86: 12-6.
22. Raghav N, Singh M. SAR studies of differently functionalized chalcones based hydrazones and their cyclized derivatives as inhibitors of mammalian cathepsin B and cathepsin H. Bioorg Med Chem 2014; 22: 4233-45.
23. Vogel S, Kaufman D, Pojarova M, Müller C, Pfaller T, Kühne S, Bednarski PJ, Angerer EV. Aroyl hydrazones of 2-phenylindole-3-carbaldehydes as novel antimitoticagents. Bioorg Med Chem 2008; 16: 6436-47.
24. Istanbullu H, Erzurumlu Y, Ballar Kirmizibayrak P, Erciyas E. Evaluation of alkylating and intercalating properties of mannich bases for cytotoxic activity. Lett Drug Des Discov 2014; 11: 1096-1106.
25. Erzurumlu Y, Aydin-Kose F, Gozen O, Gozuacik D, Toth EA, Ballar P. A Unique IBMPFD-related p97/VCP mutation with differential binding pattern and subcellular localizatio. Int J Biochem Cell Biol 2013; 45: 773-82.
26. Pankiv T, Clausen H, Lamark T, Brech A, Bruun JA, Outzen H, Øvervatn A, Bjørkøy G, Johansen T. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 2007; 282: 24131-45.
27. Lee S, Kim E, Park SB. Discovery of autophagy modulators through the construction of a high-content screening platform via monitoring of lipid droplets. Chem Sci 2013; 4: 3282-7.