Anadolu Üniversitesi Bilim ve Teknoloji Dergisi C – Yaşam Bilimleri ve Biyoteknoloji
Anadolu University Journal of Science and Technology C – Life Sciences and Biotechnology
Cilt: 4 Sayı: 1 - 2015
Sayfa: 1 - 11
DOI: 10.18036/btdc.55522

ARAŞTIRMA MAKALESİ / **RESEARCH ARTICLE**

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SYNTHESIS AND EVALUATION OF BENZODIOXOLE APPENDED PYRAZOLINE DERIVATIVES AS NEW ANTIMICROBIAL AGENTS

ABSTRACT

In the current work, new pyrazoline derivatives were synthesized *via* the reaction of 1-(chloroacetyl)-3-(2-thienyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline with sodium salts of *N*,*N*-disubstituted dithiocarbamic acids. The compounds were investigated for their inhibitory effects on pathogenic bacteria and yeasts using broth microdilution method. MTT assay was carried out to determine the cytotoxic effects of the compounds on NIH/3T3 mouse embryonic fibroblast cell line. Among the tested compounds, 1-[[(4-(4-fluorophenyl)piperazin-1-yl)thiocarbamoylthio]acetyl]-3-(2-thienyl)-5-(3,4-methylendioxyphenyl)-2-pyrazoline (**2**) was found to be the most promising antimicrobial agent due to its notable inhibitory effects on *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida parapsilosis* when compared with the reference agents. Compound **2** did not cause any toxicity against NIH/3T3 cell line. This outcome pointed out that the antimicrobial activity of compound **2** was selective.

Keywords: Benzodioxole, Dithiocarbamate, Pyrazoline, Antimicrobial activity, Cytotoxicity.

BENZODİOKSOL HALKASI TAŞIYAN PİRAZOLİN TÜREVLERİNİN SENTEZİ VE YENİ ANTİMİKROBİYAL AJANLAR OLARAK DEĞERLENDİRİLMESİ

ÖZ

Bu çalışmada, 1-(kloroasetil)-3-(2-tiyenil)-5-(3,4-metilendioksifenil)-2-pirazolinin *N*,*N*-disübstitüe ditiyokarbamik asitler ile reaksiyonuyla yeni pirazolin türevleri sentezlendi. Bileşikler, broth mikrodilüsyon yöntemi kullanılarak patojenik bakterilere ve mayalara karşı inhibitör etkileri için araştırıldı. Bileşiklerin NIH/3T3 fare embriyonik fibroblast hücre dizisine karşı sitotoksik etkilerini saptamak için MTT deneyi gerçekleştirildi. Referans maddeler ile kıyaslandığında test edilen bileşikler arasında, 1-[[(4-(4-florofenil)piperazin-1-il)tiyokarbamoiltiyo]asetil]-3-(2-tiyenil)-5-(3,4-metilendioksifenil)-2-pirazolin (**2**) *Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Escherichia coli* ve *Candida parapsilosis* mikroorganizmaları üzerindeki dikkate değer inhibitör etkilerine bağlı olarak en umut verici antimikrobiyal ajan olarak bulundu. Bileşik **2**, NIH/3T3 hücre dizisine karşı herhangi bir toksisiteye neden olmadı. Bu sonuç, bileşik **2**'nin antimikrobiyal etkisinin seçici olduğunu gösterdi.

Anahtar Kelimeler: Benzodioksol, Ditiyokarbamat, Pirazolin, Antimikrobiyal etki, Sitotoksisite.

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Received: 23 Aralık 2015 **Accepted:** 30 Aralık 2015

1. INTRODUCTION

The rapid emergence of resistance to existing antimicrobial drugs is a major threat to public health and life (Ramiz et al. 2010). Resistant bacteria are involved in majority of the infectious diseases including diarrhea, respiratory tract infections and nosocomial infections (Sivakumar et al. 2010).

Fungal infections pose a serious and enduring threat to present and future populations (Kathiravan et al. 2012). Besides fungal diseases have emerged as important public health problems contributing to high levels of morbidity and mortality (Rodrigues et al. 2014). Among these fungal diseases, infections caused by *Candida* species are the third most common cause of nosocomial infections in patients requiring deep care and represent the main cause of opportunistic fungal infections worldwide (Pierce and Lopez-Ribot 2013; Rodrigues et al. 2014).

A great number of studies have reported the fact that increasing problems about resistance to antimicrobial agents particularly occurred in bacterial pathogens, cause an obvious decline in new antibiotics being put on the market. The current antifungal therapy also suffers from drug resistance and additionally from toxicity and important drug-drug interactions. Correspondingly, there is a substantial need to develop new antimicrobial agents showing strong efficacy against resistant microorganisms (Moellering 2011; Malik et al. 2012).

Pyrazoline is a five-membered heterocyclic ring bearing two adjacent nitrogen atoms within the ring and pyrazolines are broadly used as useful synthons in organic synthesis (Rahman and Siddiqui 2010). The pyrazoline scaffold is quite stable and can be effectively utilized to synthesize a large number of new compounds possessing diverse pharmacological activities (Munawar 2008). Pyrazoline derivatives have been studied extensively owing to their wide range of biological activities including antibacterial, antifungal, anti-inflammatory, antidepressant, and antiviral activities (Holla et al. 2006; Samshuddin 2012). Additionally, 1,3-benzodioxole ring system is mainly a core structure in some compounds exhibiting a broad spectrum of biological activities (Kumar 2013). 1,3-Benzodioxole derivatives show several biological activities such as antimicrobial, anticancer, anticonvulsant, anti-inflammatory, antidepressant, antihypertensive, antiprotozoal, antioxidant and immunomodulator effects (Attia et al. 2014). 1,3-Benzodioxole ring system also takes part in various naturally occurring molecules like piperonal, sesamol, saffrole, myristicin etc. (Kumar 2013).

On the basis of afore-mentioned findings, herein we reported the synthesis and evaluation of a new series of benzodioxole appended pyrazoline derivatives as antimicrobial agents. Furthermore, all compounds were evaluated for their cytotoxicity against NIH/3T3 cell line.

2. MATERIALS and METHODS

2.1. Chemistry

All reagents were purchased from commercial suppliers and were used without further purification. Melting points (M.p.) were determined on an Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK) and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Mercury-400 FT-NMR spectrometer (Agilent, Palo Alto, CA, USA). Mass spectra were recorded on an Agilent LC-MSD-Trap-SL Mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyzer (Perkin-Elmer, Norwalk, CT, USA) and the results were within ±0.4% of the theoretical values. Thin Layer Chromatography (TLC) was performed on TLC Silica gel 60 F_{254} aluminium sheets (Merck, Darmstadt, Germany) to check the purity of the compounds.

2.1.1. General Procedure For The Synthesis of The Compounds

3-(3,4-Methylenedioxyphenyl)-1-(2-thienyl)-2-propen-1-one

A mixture of 2-acetylthiophene (0.06 mol), piperonal (0.06 mol) and 10% aqueous sodium hydroxide (10 mL) in ethanol (30 mL) was stirred at room temperature for 6 h. The resulting solid was washed, dried, and crystallized from ethanol (Özdemir et al. 2014).

5-(3,4-Methylenedioxyphenyl)-3-(2-thienyl)-2-pyrazoline

A mixture of the chalcone (0.03 mol) and 80% hydrazine hydrate (0.06 mol) in ethanol (30 mL) was refluxed for 3 h. The reaction mixture was cooled and kept at 0 °C overnight. The resulting solid was recrystallized from ethanol (Özdemir et al. 2014).

1-(Chloroacetyl)-3-(2-thienyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline

5-(3,4-Methylenedioxyphenyl)-3-(2-thienyl)-2-pyrazoline (0.02 mol) and triethylamine (0.02 mol) were dissolved in dry acetone (30 mL) with constant stirring. Later, the mixture was cooled in an ice bath, and chloroacetyl chloride (0.02 mol) was added dropwise with stirring. The reaction mixture thus obtained was further agitated for 2 h at room temperature. The precipitate was filtered, the solvent was evaporated to dryness under reduced pressure, and the products were recrystallized from ethanol (Özdemir et al. 2014).

Sodium salts of N,N-disubstituted dithiocarbamic acids

Sodium hydroxide (10 mmol) was dissolved in ethanol (80 mL) with constant stirring. After addition of the secondary amine (10 mmol) the mixture was cooled in an ice bath and carbon disulfide (100 mmol) was added dropwise with stirring. The reaction mixture was stirred for 1 h at room temperature. The solvent was evaporated under reduced pressure and then dry ether was added until precipitation. The products were afforded by filtration and recrystallised from ethanol (Altintop et al. 2013).

1-[(N,N-Disubstitutedthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazolines ***(1-10)***

A mixture of 1-(chloroacetyl)-3-(2-thienyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline (0.01 mol) and appropriate sodium salt of *N,N*-disubstituted dithiocarbamic acid (0.01 mol) was treated in acetone at room temperature for 3 h. The solvent was evaporated, the resulting solid was washed with water and recrystallized from ethanol.

1-[(4-Phenylpiperazin-1-yl)thiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(3,4-methylendioxyphenyl)-2-pyrazoline ***(1)***

Yield: 70%. M.p.: 67 ºC.

^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 3.17 (1H, dd, J_{AX} = 4.80 Hz, J_{AB} = 18.00 Hz, pyrazoline C_4-H_A), 3.20-3.28 (4H, m, $C_{3,5}$-H piperazine), 3.88 (1H, dd, J_{BX} = 11.60 Hz, J_{BA} = 18.00 Hz, pyrazoline C_4-H_B), 4.08-4.14 (2H, m, $C_{2,6}$-H piperazine), 4.21-4.33 (2H, m, $C_{2,6}$-H piperazine), 4.61 (1H, d, *J* = 16.40 Hz, geminal proton, CO-C*H*), 4.74 (1H, d, *J* = 16.00 Hz, geminal proton, CO-C*H*), 5.51 (1H, dd, J_{AX} = 4.80 Hz, J_{BX} = 11.20 Hz, pyrazoline C_5-H_X), 5.97 (2H, d, *J* = 4.00 Hz, O-CH_2-O), 6.71-6.98 (6H, m, aromatic protons), 7.15-7.26 (3H, m, aromatic protons), 7.48 (1H, d, *J* = 2.80 Hz, aromatic proton), 7.76 (1H, dd, *J* = 0.40 Hz, 4.80 Hz, aromatic proton).

Anal. Calcd for $C_{27}H_{26}N_4O_3S_3$: C, 58.89; H, 4.76; N, 10.17. Found: C, 58.90; H, 4.74; N, 10.18.

MS (*ESI*) (*m/z*): $(M+H)^+$ 551

1-[[(4-(4-Fluorophenyl)piperazin-1-yl)thiocarbamoylthio]acetyl]-3-(2-thienyl)-5-(3,4-methylendioxyphenyl)-2-pyrazoline ***(2)***

Yield: 76%. M.p.: 66 ºC.

^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 3.14-3.23 (5H, m, $C_{3,5}$-H piperazine and pyrazoline C_4-H_A), 3.86 (1H, dd, J_{BX} = 11.60 Hz, J_{BA} = 18.00 Hz, pyrazoline C_4-H_B), 4.09-4.21 (2H, m, $C_{2,6}$-H piperazine), 4.28-4.46 (2H, m, $C_{2,6}$-H piperazine), 4.61 (1H, d, *J* = 16.00 Hz, geminal proton, CO-C*H*), 4.74 (1H, d, *J* = 16.00 Hz, geminal proton, CO-C*H*), 5.50 (1H, dd, J_{AX} = 4.40 Hz, J_{BX} = 11.60 Hz, pyrazoline C_5-H_X), 5.97 (2H, d, *J* = 4.00 Hz, O-CH_2-O), 6.72 (1H, dd, *J* = 1.60 Hz, 2.00 Hz, aromatic proton), 6.77 (1H, d, *J* = 2.00 Hz, aromatic proton), 6.84 (1H, d, *J* = 7.60 Hz, aromatic proton), 6.97-7.11 (5H, m, aromatic protons), 7.16 (1H, dd, *J* = 1.20 Hz, 3.60 Hz,

aromatic proton), 7.48 (1H, dd, *J* = 0.80 Hz, 3.20 Hz, aromatic proton), 7.76 (1H, dd, *J* = 0.80 Hz, 4.80 Hz, aromatic proton).

Anal. Calcd for $C_{27}H_{25}FN_4O_3S_3$: C, 57.02; H, 4.43; N, 9.85. Found: C, 57.02; H, 4.46; N, 9.82.

MS (*ESI*) (*m/z*): $(M+H)^+$ 569

*1-[[(4-(4-Methoxyphenyl)piperazin-1-yl)thiocarbamoylthio]acetyl]-3-(2-thienyl)-5-(3,4-methylendioxyphenyl)-2-pyrazoline (**3**)*

Yield: 59%. M.p.: 165 ºC.

^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 2.96-3.18 (5H, m, $C_{3,5}$-H piperazine and pyrazoline C_4-H_A), 3.69 (3H, s, OCH_3), 3.85 (1H, m, pyrazoline C_4-H_B), 4.09-4.44 (4H, m, $C_{2,6}$-H piperazine), 4.61 (1H, d, *J* = 16.00 Hz, geminal proton, CO-C*H*), 4.74 (1H, d, *J* = 16.00 Hz, geminal proton, CO-C*H*), 5.50 (1H, dd, J_{AX} = 4.40 Hz, J_{BX} = 11.20 Hz, pyrazoline C_5-H_X), 5.97 (2H, d, *J* = 4.00 Hz, O-CH_2-O), 6.71-7.17 (8H, m, aromatic protons), 7.47 (1H, d, *J* = 2.80 Hz, aromatic proton), 7.76 (1H, dd, *J* = 4.40 Hz, aromatic proton).

Anal. Calcd for $C_{28}H_{28}N_4O_4S_3$: C, 57.91; H, 4.86; N, 9.65. Found: C, 57.89; H, 4.86; N, 9.67.

MS (*ESI*) (*m/z*): $(M+H)^+$ 581

*1-[[(4-(4-Nitrophenyl)piperazin-1-yl)thiocarbamoylthio]acetyl]-3-(2-thienyl)-5-(3,4-methylendioxyphenyl)-2-pyrazoline (**4**)*

Yield: 74%. M.p.: 102 ºC.

^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 3.17 (1H, dd, J_{AX} = 4.80 Hz, J_{AB} = 18.00 Hz, pyrazoline C_4-H_A), 3.37-3.71 (4H, m, $C_{3,5}$-H piperazine), 3.88 (1H, dd, J_{BX} = 11.60 Hz, J_{BA} = 18.00 Hz, pyrazoline C_4-H_B), 4.11-4.47 (4H, m, $C_{2,6}$-H piperazine), 4.62 (1H, d, *J* = 16.00 Hz, geminal proton, CO-C*H*), 4.75 (1H, d, *J* = 16.00 Hz, geminal proton, CO-C*H*), 5.50 (1H, dd, J_{AX} = 4.40 Hz, J_{BX} = 11.60 Hz, pyrazoline C_5-H_X), 5.98 (2H, d, *J* = 4.40 Hz, O-CH_2-O), 6.72 (1H, dd, *J* = 1.60 Hz, 2.00 Hz, aromatic proton), 6.77 (1H, d, *J* = 2.00 Hz, aromatic proton), 6.84 (1H, d, *J* = 7.60 Hz, aromatic proton), 6.93-7.06 (2H, m, aromatic protons), 7.17 (1H, dd, *J* = 1.60 Hz, 3.60 Hz, aromatic proton), 7.48 (1H, dd, *J* = 0.80 Hz, 3.20 Hz, aromatic proton), 7.76 (1H, dd, *J* = 0.80 Hz, 4.80 Hz, aromatic proton), 8.03-8.11 (2H, m, aromatic protons).

Anal. Calcd for $C_{27}H_{25}N_5O_5S_3$: C, 54.44; H, 4.23; N, 11.76. Found: C, 54.41; H, 4.25; N, 11.77.

MS (*ESI*) (*m/z*): $(M+H)^+$ 596

*1-[[(4-(Pyrimidin-2-yl)piperazin-1-yl)thiocarbamoylthio]acetyl]-3-(2-thienyl)-5-(3,4-methylendioxyphenyl)-2-pyrazoline (**5**)*

Yield: 75%. M.p.: 65 ºC.

^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 3.17 (1H, dd, J_{AX} = 4.80 Hz, J_{AB} = 18.00 Hz, pyrazoline C_4-H_A), 3.79-3.88 (4H, m, $C_{3,5}$-H piperazine), 4.05-4.49 (5H, m, $C_{2,6}$-H piperazine and pyrazoline C_4-H_B), 4.61 (1H, d, *J* = 16.40 Hz, geminal proton, CO-C*H*), 4.75 (1H, d, *J* = 16.00 Hz, geminal proton, CO-C*H*), 5.50 (1H, dd, J_{AX} = 4.40 Hz, J_{BX} = 11.60 Hz, pyrazoline C_5-H_X), 5.98 (2H, d, *J* = 5.60 Hz, O-CH_2-O), 6.67-6.86 (3H, m, aromatic protons), 7.16 (1H, t, *J* = 1.60 Hz, 4.80 Hz, aromatic proton), 7.48 (1H, d, *J* = 4.40 Hz, aromatic proton), 7.76 (1H, d, *J* = 4.40 Hz, aromatic proton), 8.39-8.41 (3H, m, aromatic protons).

Anal. Calcd for $C_{25}H_{24}N_6O_3S_3$: C, 54.33; H, 4.38; N, 15.21. Found: C, 54.34; H, 4.35; N, 15.23.

MS (*ESI*) (*m/z*): $(M+H)^+$ 553

*1-[[(4-(2-Hydroxyethyl)piperazin-1-yl)thiocarbamoylthio]acetyl]-3-(2-thienyl)-5-(3,4-methylendioxyphenyl)-2-pyrazoline (**6**)*

Yield: 67%. M.p.: 85 ºC.

^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 2.41-2.45 (4H, m, CH_2-CH_2-OH and CH_2 piperazine), 3.16 (1H, dd, J_{AX} = 4.40 Hz, J_{AB} = 18.00 Hz, pyrazoline C_4-H_A), 3.51 (2H, q, *J* =

5.60 Hz, CH_2 piperazine), 3.86 (1H, dd, J_{BX} = 11.60 Hz, J_{BA} = 18.00 Hz, pyrazoline C_4-H_B), 3.92-4.18 (5H, m, $C_{2,6}$-H piperazine and O-H), 4.46 (2H, t, J = 5.20 Hz, CH_2-$\underline{CH_2}$-OH), 4.58 (1H, d, J = 16.40 Hz, geminal proton, CO-CH), 4.71 (1H, d, J = 16.00 Hz, geminal proton, CO-C$\underline{H}$), 5.49 (1H, dd, J_{AX} = 4.40 Hz, J_{BX} = 12.00 Hz, pyrazoline C_5-H_X), 5.98 (2H, d, J = 5.20 Hz, O-CH_2-O), 6.71 (1H, dd, J = 1.20 Hz, 2.00 Hz, aromatic proton), 6.76 (1H, d, J = 2.00 Hz, aromatic proton), 6.84 (1H, d, J = 7.60 Hz, aromatic proton), 7.15-7.17 (1H, m, aromatic protons), 7.48 (1H, dd, J = 0.80 Hz, 1.20 Hz, aromatic proton), 7.76 (1H, d, J = 4.00 Hz, aromatic proton).

Anal. Calcd for $C_{23}H_{26}N_4O_4S_3$: C, 53.26; H, 5.05; N, 10.80. Found: C, 53.28; H, 5.02; N, 10.81.

MS (*ESI*) (*m/z*): $(M+H)^+$ 519

*1-[[(4-Ethylpiperazin-1-yl)thiocarbamoylthio]acetyl]-3-(2-thienyl)-5-(3,4-methylendioxyphenyl)-2-pyrazoline (**7**)*

Yield: 55%. M.p.: 90 ºC.

^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 1.01 (3H, t, J = 7.20 Hz, CH_2-$\underline{CH_3}$), 2.35-2.45 (6H, m, $\underline{CH_2}$-CH_3 and CH_2 piperazine), 3.16 (1H, dd, J_{AX} = 4.40 Hz, J_{AB} = 18.00 Hz, pyrazoline C_4-H_A), 3.86 (1H, dd, J_{BX} = 11.60 Hz, J_{BA} = 18.00 Hz, pyrazoline C_4-H_B), 3.92-4.18 (4H, m, $C_{2,6}$-H piperazine), 4.59 (1H, d, J = 16.00 Hz, geminal proton, CO-CH), 4.70 (1H, d, J = 16.00 Hz, geminal proton, CO-C$\underline{H}$), 5.50 (1H, dd, J_{AX} = 4.40 Hz, J_{BX} = 12.00 Hz, pyrazoline C_5-H_X), 5.98 (2H, d, J = 5.60 Hz, O-CH_2-O), 6.70- 6.76 (2H, m, aromatic protons), 6.84 (1H, d, J = 7.60 Hz, aromatic proton), 7.15-7.17 (1H, m, aromatic proton), 7.47 (1H, m, aromatic proton), 7.76 (1H, d, J = 4.00 Hz, aromatic proton).

Anal. Calcd for $C_{23}H_{26}N_4O_3S_3$: C, 54.96; H, 5.21; N, 11.15. Found: C, 54.94; H, 5.20; N, 11.18.

MS (*ESI*) (*m/z*): $(M+H)^+$ 503

*1-[[(4-(2-(Dimethylamino)ethyl)piperazin-1-yl)thiocarbamoylthio]acetyl]-3-(2-thienyl)-5-(3,4-methylendioxyphenyl)-2-pyrazoline (**8**)*

Yield: 68%. M.p.: 79 ºC.

^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 2.14 (6H, s, N($\underline{CH_3}$)$_2$), 2.35-2.42 (4H, m, $\underline{CH_2}$-$\underline{CH_2}$), 2.50 (4H, s, $C_{3,5}$-H piperazine), 3.17 (1H, dd, J_{AX} = 4.60 Hz, J_{AB} = 18.00 Hz, pyrazoline C_4-H_A), 3.87 (1H, dd, J_{BX} = 11.60 Hz, J_{BA} = 18.00 Hz, pyrazoline C_4-H_B), 3.90-4.18 (4H, m, $C_{2,6}$-H piperazine), 4.60 (1H, d, J = 16.00 Hz, geminal proton, CO-CH), 4.69 (1H, d, J = 16.00 Hz, geminal proton, CO-C$\underline{H}$), 5.51 (1H, dd, J_{AX} = 4.40 Hz, J_{BX} = 11.20 Hz, pyrazoline C_5-H_X), 5.98 (2H, d, J = 5.60 Hz, O-CH_2-O), 6.71 (1H, d, J = 7.60 Hz, aromatic proton), 6.76 (1H, m, aromatic proton), 6.84 (1H, d, J = 7.60 Hz, aromatic proton), 7.15-7.17 (1H, m, aromatic protons), 7.48 (1H, d, J = 3.20 Hz, aromatic proton), 7.76 (1H, d, J = 4.80 Hz, aromatic proton).

Anal. Calcd for $C_{25}H_{31}N_5O_3S_3$: C, 55.02; H, 5.73; N, 12.83. Found: C, 55.05; H, 5.71; N, 12.82.

MS (*ESI*) (*m/z*): $(M+H)^+$ 546

*1-[[(4-(3-(Dimethylamino)propyl)piperazin-1-yl)thiocarbamoylthio]acetyl]-3-(2-thienyl)-5-(3,4-methylendioxyphenyl)-2-pyrazoline (**9**)*

Yield: 69%. M.p.: 90 ºC.

^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 1.57 (2H, p, J = 5.20 Hz, CH_2-$\underline{CH_2}$-CH_2), 2.16 (6H, s, N($\underline{CH_3}$)$_2$), 2.26-2.34 (4H, m, $\underline{CH_2}$-CH_2-$\underline{CH_2}$), 2.44 (4H, brs, $C_{3,5}$-H piperazine), 3.16 (1H, dd, J_{AX} = 4.80 Hz, J_{AB} = 18.00 Hz, pyrazoline C_4-H_A), 3.82-4.19 (5H, m, $C_{2,6}$-H piperazine and pyrazoline C_4-H_B), 4.58 (1H, d, J = 16.40 Hz, geminal proton, CO-C$\underline{H}$), 4.71 (1H, d, J = 16.00 Hz, geminal proton, CO-C$\underline{H}$), 5.49 (1H, dd, J_{AX} = 4.40 Hz, J_{BX} = 11.60 Hz, pyrazoline C_5-H_X), 5.98 (2H, d, J = 5.20 Hz, O-CH_2-O), 6.69-7.17 (4H, m, aromatic protons), 7.48 (1H, d, J = 2.40 Hz, aromatic proton), 7.76 (1H, d, J = 4.80 Hz, aromatic proton).

Anal. Calcd for $C_{26}H_{33}N_5O_3S_3$: C, 55.79; H, 5.94; N, 12.51. Found: C, 55.81; H, 5.93; N, 12.50.

MS (*ESI*) (*m/z*): $(M+H)^+$ 560

*1-[[(4-Benzylpiperazin-1-yl)thiocarbamoylthio]acetyl]-3-(2-thienyl)-5-(3,4-methylendioxyphenyl)-2-pyrazoline (**10**)*

Yield: 65%. M.p.: 76 ºC.

^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 2.46 (4H, brs, $C_{3,5}$-H piperazine), 3.17 (1H, dd, J_{AX} = 4.80 Hz, J_{AB} = 18.00 Hz, pyrazoline C_4-H_A), 3.52 (2H, s, N-CH_2-phenyl), 3.81-4.21 (5H, m, $C_{2,6}$-H piperazine and pyrazoline C_4-H_B), 4.57 (1H, d, *J* = 16.40 Hz, geminal proton, CO-C*H*), 4.72 (1H, d, *J* = 16.00 Hz, geminal proton, CO-C*H*), 5.50 (1H, dd, J_{AX} = 4.80 Hz, J_{BX} = 11.20 Hz, pyrazoline C_5-H_X), 5.98 (2H, d, *J* = 5.20 Hz, O-CH_2-O), 6.77-6.88 (2H, m, aromatic protons), 7.14-7.34 (7H, m, aromatic protons), 7.47 (1H, d, *J* = 2.80 Hz, aromatic proton), 7.78 (1H, d, *J* = 4.80 Hz, aromatic proton).

Anal. Calcd for $C_{28}H_{28}N_4O_3S_3$: C, 59.55; H, 5.00; N, 9.92. Found: C, 59.56; H, 5.01; N, 9.90.

MS (*ESI*) (*m/z*): $(M+H)^+$ 565

2.2. Microbiology

The microbiological assay was carried out according to the CLSI reference M7-A7 broth microdilution method. Compounds **1-10** were investigated for their *in vitro* growth inhibitory activity against pathogenic bacteria such as *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 51922), *Listeria monocytogenes* (ATCC 1911), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35218) and yeasts such as *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258), *Candida parapsilosis* (ATCC 22019). Chloramphenicol and ketoconazole were used as reference agents.

2.3. Cytotoxicity

2.3.1. Cell Culture And Drug Treatment

NIH/3T3 mouse embryonic fibroblast cells were obtained from the American Type Culture Collection (ATCC, USA). The cells were incubated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Life Technologies, UK), 100 IU/mL penicillin (Gibco, Paisley, Scotland) and 100 mg/mL streptomycin (Gibco) at 37 ºC in a humidified atmosphere of 95 % air and 5 % CO_2. Exponentially growing cells were plated at $2x10^4$ cells/mL into 96-well microtiter tissue culture plates (Nunc, Denmark) and incubated for 24 h before the addition of the drugs (the optimum cell number for cytotoxicity assays was determined in preliminary experiments). The stock solutions of compounds were prepared in dimethyl sulphoxide (DMSO; Sigma-Aldrich, Poole, UK) and further dilutions were made with fresh culture medium (the concentration of DMSO in the final culture medium was <0.1% which had no effect on the cell viability).

2.3.2. MTT assay

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed to determine the proliferation of the cells (Mosmann 1983; Altıntop et al. 2011).

After 24 h of preincubation, the tested compounds were added to give final concentration in the range 0.5-500 µg/mL and the cells were incubated for 24 h. At the end of this period, MTT was added to a final concentration of 0.5 mg/mL and the cells were incubated for 4 h at 37 ºC. After the medium was removed, the formazan crystals formed by MTT metabolism were solubilized by addition of 200 µL DMSO to each well and absorbance was read at 540 nm with a microtitre plate spectrophotometer (Bio-Tek plate reader). Each concentration was repeated in three wells and IC_{50} values were defined as the drug concentrations that reduced absorbance to 50% of control values.

3. RESULTS and DISCUSSION

The synthesis of new pyrazoline derivatives (**1-10**) was carried out according to the steps shown in Scheme 1. In the initial step, 3-(3,4-methylenedioxyphenyl)-1-(2-thienyl)-2-propen-1-one was synthesized *via* the base-catalyzed Claisen-Schmidt condensation of 2-acetylthiophene with piperonal. The ring closure reaction of the chalcone with hydrazine hydrate afforded 5-(3,4-methylenedioxyphenyl)-3-(2-thienyl)-2-pyrazoline. 1-(Chloroacetyl)-3-(2-

thienyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline was obtained by the reaction of 5-(3,4-methylenedioxyphenyl)-3-(2-thienyl)-2-pyrazoline with chloroacetyl chloride in the presence of triethylamine. Sodium salts of *N*,*N*-disubstituted dithiocarbamic acids were prepared by the reaction of secondary amine with carbon disulfide in the presence of sodium hydroxide. The reaction of 1-(chloroacetyl)-3-(2-thienyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline with sodium salts of *N*,*N*-disubstituted dithiocarbamic acids afforded 1-[(*N*,*N*-disubstitutedthiocarbamoylthio)acetyl]-3-(2-thienyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline derivatives (**1-10**). The structures of compounds **1-10** were confirmed by ^{1}H NMR, mass spectral data and elemental analyses.

Compounds **1-10** were tested *in vitro* against a number of Gram-positive and Gram-negative bacteria and yeasts using broth microdilution method. Based on this assay, the minimum inhibitory concentrations (MICs) of the compounds were determined.

As shown in Table 1, compounds **2**, **3**, **6**, **7**, **8** and **9** showed notable antibacterial activity against *K. pneumoniae* with a MIC value of 200 µg/mL when compared with chloramphenicol (MIC= 200 µg/mL). Furthermore, Compounds **8**, **9** and chloramphenicol exhibited the same level of antibacterial activity against *E. faecalis* with a MIC value of 100 µg/mL.

All compounds exhibited remarkable antibacterial activity against *P. aeruginosa* with a MIC value of 200 µg/mL when compared with chloramphenicol (MIC= 200 µg/mL). The results demonstrated that the antibacterial effects of these compounds on *P. aeruginosa* did not depend on the substituents.

Compounds **2** and **8** were the most potent antibacterial agents against *E. coli* with a MIC value of 200 µg/mL when compared with chloramphenicol (MIC= 200 µg/mL).

Among the pathogenic fungi species, *C. parapsilosis* was the most susceptible yeast to the tested compounds (Table 2). All compounds exhibited remarkable antifungal activity against *C. parapsilosis* with a MIC value of 200 µg/mL when compared with ketoconazole (MIC= 200 µg/mL). The microbiological results revealed that the antifungal effects of the compounds on *C. parapsilosis* did not depend on the substituents.

In order to evaluate the selectivity, MTT assay was carried out to determine the cytotoxic effects of the compounds on NIH/3T3 mouse embryonic fibroblast (healthy) cell line (Table 3). Compounds **1**, **2**, **3**, **4** and **5** were found to be non-toxic, whereas compounds **6**, **7**, **8** and **9** showed high cytotoxicity against NIH/3T3 mouse embryonic fibroblast (healthy) cells. This outcome indicated that antimicrobial effects of compounds **6**, **7**, **8** and **9** were not selective.

In particular, compound **2** was found to be the most promising antimicrobial agent in the series due to its selective antimicrobial activity against *K. pneumoniae*, *P. aeruginosa*, *E. coli* and *C. parapsilosis.*

4. CONCLUSIONS

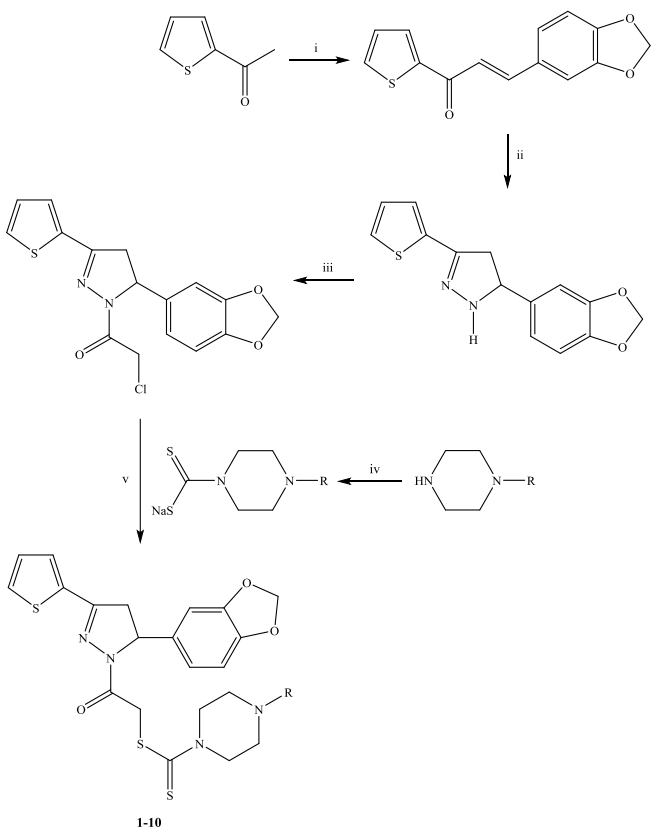
In the present study, new benzodioxole-based pyrazoline derivatives were synthesized and investigated for their antimicrobial activity and cytotoxicity against NIH/3T3 cell line.

An ideal drug is expected to exhibit high therapeutic effect and minimum toxicity. For this reason, the cytotoxic effects of all compounds were also investigated on NIH/3T3 cell lines.

Among the tested compounds, compound **2** can be identified as the most promising antimicrobial derivative against *K. pneumoniae, P. aeruginosa, E. coli* and *C. parapsilosis* with a MIC value of 200 µg/mL when compared with the reference agents. In addition, this agent did not show any cytotoxicity against NIH/3T3 cell line. Further studies are required to elucidate the mechanism of action for the antimicrobial activity of compound **2**.

ACKNOWLEDGEMENTS

The authors would like to thank Anadolu University Medicinal Plants, Drugs and Scientific Research Center for biological activity tests.



Scheme 1. The synthetic route for the preparation of new pyrazoline derivatives (**1-10**). Reagents and conditions: (i) Piperonal, 10% aqueous sodium hydroxide solution, ethanol, rt, 6 h; (ii) 80% $NH_2NH_2.H_2O$, ethanol, reflux, 3 h; (iii) $ClCOCH_2Cl$, TEA, dry acetone, rt, 2 h; (iv) CS_2, NaOH, rt, 1 h; (v) Acetone, rt, 3 h.

Table 1. Antibacterial activity of compounds **1-10**

Compound	**MIC value (µg/mL)**					
	S.aureus	*E.faecalis*	*L.monocytogenes*	*K.pneumoniae*	*P.aeruginosa*	*E.coli*
1	400	200	50	400	200	400
2	400	200	50	200	200	200
3	400	400	50	200	200	400
4	400	400	50	400	200	400
5	400	400	100	400	200	400
6	400	400	100	200	200	400
7	400	200	100	200	200	400
8	100	100	50	200	200	200
9	400	100	100	200	200	400
10	400	200	100	400	200	400
Chloramphenicol	12.5	100	1.625	200	200	200

Table 2. Anticandidal activity of compounds **1-10**

Compound	**MIC value (µg/mL)**			
	C.albicans	*C.glabrata*	*C.krusei*	*C. parapsilosis*
1	200	200	100	200
2	100	400	100	200
3	100	400	100	200
4	200	200	200	200
5	100	200	200	200
6	100	200	100	200
7	100	200	100	200
8	100	100	100	200
9	200	200	200	200
10	400	200	200	200
Ketoconazole	50	50	1.625	200

Table 3. The cytotoxic effects of compounds **1-10** against NIH/3T3 cells

Compound	**IC_{50}** (µg/mL)
1	> 500
2	> 500
3	> 500
4	> 500
5	> 500
6	49.30
7	< 10
8	31.70
9	36.20
10	261

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