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Araştırma Makalesi - Research Article

Yeni 1,3,4-Tiyadiazol-Piperazin Türevlerinin Sentezi, İn Vitro Antikolinesteraz ve Antimikrobiyal Değerlendirilmesi

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ÖZ

Bu çalışmada 2-((5-substitüe-1,3,4-tiyadiazol-2-il)amino)-2-oksoetil-4-substitüepiperazin-1-karboditiyat yapısında 8 yeni bileşiğin sentezi yapılarak, antimikrobiyal ve antikolinesteraz aktiviteleri incelenmiştir. Sentezlenen bileşiklerin yapıları IR, ^{1}H NMR, ^{13}C NMR ve kütle spektrumları kullanılarak kanıtlanmıştır. Sentezlenen bileşikler, sekiz bakteri suşuna karşı antibakteriyel aktivite ve dört mantar suşuna karşı antifungal aktivite açısından test edilmiştir. Bileşiklerin antibakteriyel aktiviteleri incelendiğinde **3c**, **3d**, **3g** ve **3h** kodlu bileşiklerin 25 µg/mL MİK değeri ile *E. fecalis* (ATCC 29212) ve *E. fecalis* (ATCC 51922) karşı etkili oldukları bulunmuştur. Piperazinin para konumunda bulunan karbon zincirinin aktiviteyi arttırdığı (**3c**, **3d**, **3g**, **3h**), ancak para konumuna benzil grubunun eklenmesinin (**3a**, **3b**, **3e**, **3f**) aktiviteyi etkilemediği belirlenmiştir. Ayrıca, sentezlenen bileşiklerin asetilkolinesteraz aktivitesi incelenmiş ancak bileşiklerden hiçbiri standart ilaç Donepezil kadar AChE inhibe edici aktivite göstermemiştir.

Anahtar Kelimeler- 1,3,4-Tiyadiazol, Antimikrobiyal, Antikolinesteraz, Piperazin

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Synthesis, In Vitro Anticholinesterase and Antimicrobial Evaluation of New 1,3,4-Thiadiazole-Piperazine Derivatives

ABSTRACT

In this study, 8 new compounds having 2-((5-substituted-1,3,4-thiadazol-2-yl)amino)-2-oxoethyl-4-substituepiperazine-1-carbodithioate structures were synthesized, antimicrobial and anticholinesterase activities were evaluated. The structures of synthesized compounds were proved by ^{1}H NMR, ^{13}C NMR and mass spectra. The synthesized compounds were tested for antibacterial activity against eight bacteria strains and antifungal activity against four fungus strains. When the antibacterial activities of the compounds were examined, it was found that the compounds **3c**, **3d**, **3g** and **3h** were effective against *E. faecalis* (ATCC 29212) and *E. faecalis* (ATCC 51922) with MIC value of 25 µg/mL. It was determined that the carbon chain at the para position of piperazine increased the activity but where as the addition of benzyl group to the para position (**3a**, **3b**, **3e**, **3f**) did not affect the activity. Furthermore, the acetylcholinesterase activity of the synthesized compounds was examined but none of the compounds showed AChE inhibitory activity as much as standard drug Donepezil.

Keywords- ***1,3,4-Thiadiazole, Antimicrobial, Anticholinesterase, Piperazine***

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I. INTRODUCTION

Today, there are many drugs on market for microbial infections, but the inappropriate and excessive use of antibiotics causes these microorganisms to develop resistance against these substances [1]. Hence, there is a great demand for the development of new antimicrobial drugs and drug candidates [2]. Very recent studies are confident that provide the design and synthesis of new heterocyclic compounds for desired applications as drugs with different biological activities, including the antimicrobial effects and anticholinesterase [3].

1,3,4-Thiadiazole moiety is connected to a broad spectrum of biological activities possibly due to the presence of pharmacophoric (N–C=S) moiety [4]. Thiadiazole by acting as "two-electron donor system" and "hydrogen binding domain" also made itself as a constrained pharmacophore. It can be bioisosterically replaced with oxazole, oxadiazole and benzene. The sulfur atom usually improves the ease of lipophilicity [5]. The core motif thiadiazole and its derivatives have been reported in the literature for wide varieties of pharmacological activities such as antitumoral, antimicrobial, antiviral, antifungal, anti-inflammatory, antiplatelet, anti-tubercular, and antidepressant activities [6]. Acetazolamide, methazolamide, and megazol are thiadiazole containing drugs available in the market [4].

According to chemistry of sulphur donor ligands such as dithiocarbamates are becoming very wide spreading in pharmaceutical and industrial application and also it is well known that, *N*-mono and *N*,*N*-di substituted dithiocarbamate derivatives show antibacterial, antiviral and antifungal activities. The structure-activity relationship study revealed that activity on thiocarbonyl aromatic compounds was significantly affected by the lipophilicity, that is obtained by thiocarbonyl moiety, especially the calculated log P value and the balance between hydrophilic substituent and hydrophobic substituent on the aromatic compounds [7].

Thus, in present study we designed and synthesized eight new piperazine based dithiocarbamate-1,3,4 thiadiazole derivatives to investigate their inhibitory profile on acetylcholinesterase and antimicrobial activities.

II. MATERIAL AND METHOD

All of the chemicals used in the study were purchased either from Merck (Merck KGaA, Darmstadt, Germany) or Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, MO, USA) and used without further chemical purifications. Chemical purities of the compounds were checked by classical TLC applications performed on silica gel 60 F254 (Merck KGaA, Darmstadt, Germany). Melting points of the synthesized compounds were determined by using a MP90 series automatic melting point determination system (Mettler-Toledo, OH, USA) and were presented as uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 by a Bruker digital FT-NMR spectrometer (Bruker Bioscience, MA, USA) at 300 MHz and 75 MHz, respectively (splitting patterns in the NMR spectra were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet; coupling constants (*J*) were reported in Hertz). Mass spectra were determinated on a Shimadzu, 8040 LC-MS-MS spectrophotometer (Kyoto, Japan).

A. *Chemistry*

General procedure for 2-Chloro-N-[(5-substituted thiadiazol-2-yl)]acetamide (1a-1b): 5-Substituted thiadiazole-2-amine (0.022 mol) and triethylamine (0.06 mol) were dissolved in THF (100 mL). This mixture was allowed to stir on an ice bath. Chloroacetyl chloride (0.022 mol) in THF (10 ml) was added drop by drop. After this stage, the content was stirred for 1h at room temperature. THF was evaporated and the product was recrystallized from ethanol [9].

Synthesis of the sodium salts of dithiocarbamic acids (2a-2d): Sodium hydroxide (10 mmol) was dissolved in ethanol (80 mL) with constant stirring. After addition of the secondary amine (10 mmol) to this solution, carbon disulphide (100 mmol) was added dropwise in ice bath. The reaction mixture was stirred for 1 h in room temperature. The solvent was evaporated under reduced pressure and then the residue was washed with diethyl eter to obtain pure product [10].

General synthesis procedure for 2-[(5-substituted thiadiazole-2-yl)amino]-2-oxoethyl 4-substituted piperazine-1-carbodithioate derivatives (3a-3h): Compounds (1a-1b) (10 mmol) were stirred with appropriate sodium salts of dithiocarbamic acids (2a-2d) (10 mmol) in acetone for 7 h. After TLC screening, the mixture was filtered and the obtained crude product was washed with water, then crystallized from ethanol.

2-((1,3,4-thiadazol-2-yl)amino)-2-oxoethyl-4-(4-methylbenzyl)piperazine-1-carbodithioate (3a): Yields: 72 %. M.P: 194.8. FTIR (ATR, cm^{-1}): 3252 (N-H), 1663 (C=O), 1224 (C=S), 852 (1,4-disubstituebenzen). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.27 (3H, s, -CH_3), 2.36-2.45 (4H, m, piperazine CH_2), 3.44 (2H, s, -CH_2), 3.93 (2H, br.s., piperazine CH_2), 4.18 (2H, br.s., piperazine CH_2), 4.42 (2H, s, -CH_2), 7.13 (2H, d, *J*=7.92 Hz, 1,4-disubstituebenzen), 7.19 (2H, d, *J*=7.95 Hz, 1,4-disubstituebenzen), 9.15 (1H, s, thiadizole C-H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 21.17, 45.27, 50.41, 51.71, 52.30, 61.48, 67.28, 129.41, 134.83, 136.66, 149.11, 159.30, 166.95, 186.70, 194.32. ESI-MS $[M+H]^+$: 408.

2-((1,3,4-thiadazol-2-yl)amino)-2-oxoethyl-4-(4-methoxybenzyl)piperazine-1-carbodithioate (3b): Yields: 77 %. M.P: 202.5. FTIR (ATR, cm^{-1}): 3256 (N-H), 1662 (C=O), 1222 (C=S), 854 (1,4-disubstituebenzen). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.32-2.44 (4H, m, piperazine CH_2), 3.42 (2H, s, -CH_2), 3.82 (3H, s, -OCH_3), 3.92 (2H, br.s., piperazine CH_2), 4.18 (2H, br.s., piperazine CH_2), 4.42 (2H, s, -CH_2), 7.13 (2H, d, *J*=7.92 Hz, 1,4-disubstituebenzen), 7.19 (2H, d, *J*=7.95 Hz, 1,4-disubstituebenzen), 9.14 (1H, s, thiadizole C-H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 45.84, 51.14, 51.74, 53.62, 55.86, 61.44, 66.28, 129.44, 134.56, 137.85, 147.66, 158.32, 165.95, 186.71, 193.18. ESI-MS $[M+H]^+$: 424.

2-((1,3,4-thiadazol-2-yl)amino)-2-oxoethyl-4-(2-(dimethylamino)ethyl)piperazine-1-carbodithioate (3c): Yields: 82 %. M.P: 206.5. FTIR (ATR, cm^{-1}): 3254 (N-H), 1664 (C=O), 1226 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.52 (2H, q, -CH_2), 2.18 (6H, s, -CH_3), 2.22-2.38 (6H, m, -CH_2, piperazine CH_2), 3.92 (2H, br.s., piperazine CH_2), 4.17 (2H, br.s., piperazine CH_2), 4.34 (2H, s, -CH_2). 9.14 (1H, s, thiadizole C-H). ^{13}C NMR (75 MHz, DMSO-d_6): δ =, 25.88, 46.47, 48.35, 49.54, 50.55, 51.74, 52.68, 55.70, 158.24, 159.72, 166.48, 194.32. ESI-MS $[M+H]^+$: 375.

2-((1,3,4-thiadazol-2-yl)amino)-2-oxoethyl-4-(2-(dimethylamino)propyl)piperazine-1-carbodithioate (3d): Yields: 80 %. M.P: 186.9. FTIR (ATR, cm^{-1}): 3251 (N-H), 1666 (C=O), 1224 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.55 (2H, q, -CH_2), 2.14 (6H, s, -CH_3), 2.24-2.36 (8H, m, -CH_2, piperazine CH_2), 3.92 (2H, br.s., piperazine CH_2), 4.17 (2H, br.s., piperazine CH_2), 4.36 (2H, s, -CH_2). 9.14 (1H, s, thiadizole C-H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 18.30, 25.44, 46.41, 48.47, 49.62, 50.46, 51.66, 52.74, 55.70, 157.14, 159.69, 165.74, 194.48. ESI-MS $[M+H]^+$: 389.

2-((5-methyl-1,3,4-thiadazol-2-yl)amino)-2-oxoethyl-4-(4-methylbenzyl)piperazine-1-carbodithioate (3e): Yields: 71 %. M.P: 221.4. FTIR (ATR, cm^{-1}): 3252 (N-H), 1660 (C=O), 1224 (C=S), 852 (1,4-disubstituebenzen). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.21 (3H, s, -CH_3), 2.34 (3H, s, -CH_3), 2.50-2.66 (4H, m, piperazine CH_2), 3.47 (2H, s, -CH_2), 3.93 (2H, br.s., piperazine CH_2), 4.18 (2H, br.s., piperazine CH_2), 4.42 (2H, s, -CH_2), 7.13 (2H, d, *J*=7.92 Hz, 1,4-disubstituebenzen), 7.19 (2H, d, *J*=7.92 Hz, 1,4-disubstituebenzen). ^{13}C NMR (75 MHz, DMSO-d_6): δ =15.27, 21.17, 45.26, 50.37, 51.69, 52.30, 61.48, 67.28, 129.28, 134.83, 136.58, 158.99, 159.76, 166.60, 186.71, 194.30. ESI-MS $[M+H]^+$: 422.

2-((5-methyl-1,3,4-thiadazol-2-yl)amino)-2-oxoethyl-4-(4-methoxybenzyl)piperazine-1-carbodithioate (3f): Yields 78 %. M.P: 196.7. FTIR (ATR, cm^{-1}): 3254 (N-H), 1660 (C=O), 1224 (C=S), 850 (1,4-disubstituebenzen). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.24 (3H, s, -CH_3), 2.32-2.45 (4H, m, piperazine CH_2), 3.44 (2H, s, -CH_2), 3.81 (3H, s, -OCH_3), 3.93 (2H, br.s., piperazine CH_2), 4.18 (2H, br.s., piperazine CH_2), 4.42 (2H, s, -CH_2), 7.13 (2H, d, *J*=7.92 Hz, 1,4-disubstituebenzen), 7.19 (2H, d, *J*=7.95 Hz, 1,4-disubstituebenzen). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 21.14, 42.54, 50.22, 51.18, 52.84, 55.49, 62.38, 66.24, 128.34, 132.71, 136.62, 148.11, 157.64, 166.75, 185.71, 193.46. ESI-MS $[M+H]^+$: 438.

2-((5-methyl-1,3,4-thiadazol-2-yl)amino)-2-oxoethyl-4-(2-(dimethylamino)ethyl)piperazine-1-carbodithioate (3g): Yields: 68 %. M.P: 216.7. FTIR (ATR, cm^{-1}): 3254 (N-H), 1662 (C=O), 1226 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.50 (2H, q, -CH_2), 2.16 (6H, s, -CH_3), 2.21 (3H, s, -CH_3), 2.28-2.42 (6H, m, -CH_2, piperazine CH_2), 3.92 (2H, br.s., piperazine CH_2), 4.17 (2H, br.s., piperazine CH_2), 4.34 (2H, s, -CH_2). ^{13}C

NMR (75 MHz, DMSO-d_6): δ = 16.30, 25.18, 45.44, 46.74, 50.32, 51.67, 52.82, 55.74, 66.18, 158.19, 159.46, 167.18, 193.56. ESI-MS $[M+H]^+$: 389.

2-((5-methyl-1,3,4-thiadazol-2-yl)amino)-2-oxoethyl-4-(2-(dimethylamino)propyl) piperazine-1-carbodithioate (3h): Yields: 66 %. M.P: 214.9. FTIR (ATR, cm^{-1}): 3256 (N-H), 1664 (C=O), 1224 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.56 (2H, q, -CH_2), 2.14 (6H, s, -CH_3), 2.20 (3H, s, -CH_3), 2.23-2.34 (8H, m, -CH_2, piperazine CH_2), 3.92 (2H, br.s., piperazine CH_2), 4.17 (2H, br.s., piperazine CH_2), 4.36 (2H, s, -CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 15.30, 24.68, 45.45, 45.47, 46.62, 50.33, 51.65, 52.65, 55.70, 65.47, 157.48, 159.69, 166.88, 194.42. ESI-MS $[M+H]^+$: 403.

B. Anticholinesterase assay

All compounds were subjected to a slightly modified method of Ellman's test [8] in order to evaluate their potency to inhibit the AChE.

C. Antimicrobial assay

The study was designed to compare MICs obtained by the CLSI reference M7–A7 broth microdilution method [8]. MIC readings were performed twice for each chemical agent. Final products were tested for their *in vitro* growth inhibitory activity against human pathogenic *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 1911), *Enterococcus faecalis* (ATCC 29212), *E. faecalis* (ATCC 51922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 35218), *E. coli* (ATCC 25923) and yeast as *Candida albicans* (90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019). Chloramphenicol and ketoconazole were used as control drugs.

III. RESULTS AND DISCUSSION

A. Chemistry

The synthesized compounds were obtained in three steps. In the first step, the 2-amino-1,3,4-thiadiazole derivatives were acetylated with chloroacetyl chloride in THF. In the second step, dithiocarbamate salts of secondary amines were prepared with carbon disulfide in ethanol. In the last step, acetylated thiadiazole derivatives and dithiocarbamate salts were reacted in acetone to give 8 novel compounds. The synthesis scheme for obtaining the target compounds was given in **Scheme 1**. The structures of synthesized compounds were proved by ^{1}H NMR, ^{13}C NMR and mass spectra.

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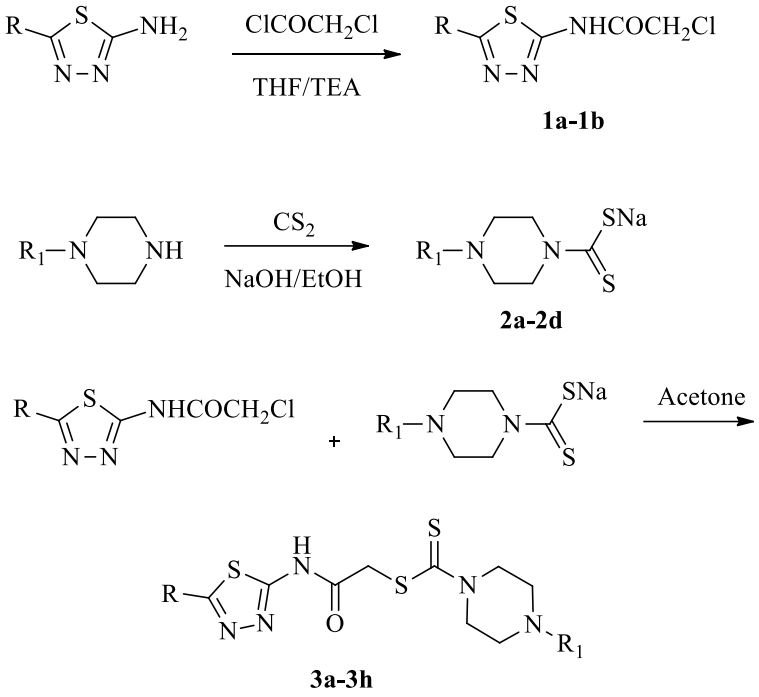

Comp	R	R1
3a	-H	4-methylbenzyl
3b	-H	4-methoxybenzyl
3c	-H	*N*,*N*- dimethylaminoethyl
3d	-H	*N*,*N*- dimethylaminopropyl
3e	-CH_3	4-methylbenzyl
3f	-CH_3	4-methoxybenzyl
3g	-CH_3	*N*,*N*- dimethylaminoethyl
3h	-CH_3	*N*,*N*- dimethylaminopropyl

Scheme 1. The Synthetic Protocol of The Compounds.

B. Anticholinesterase assay

Inhibition activities of compounds (3a-3h) against AChE were measured using Ellman's modified colorimetric method. Donepezil was used as the reference drug in the experiment.

The inhibition rate (%) was calculated by the following equation:

Inhibition % = [(AC-AB) – (AI-AB)] / (AC-AB) x 100

Where AI is the absorbance in the presence of the inhibitor, AC is the absorbance of the control and AB is the absorbance of blank reading. Both of the values are corrected with blank-reading value. SPSS for Windows 15.0 was used for statistical analysis. Student's t- test was used for all statistical calculations. Data were expressed as Mean ± SD inactive in culture medium.

Results of inhibitory activity of the synthesized compounds on AChE enzyme are summarized in **Table 1.** None of the compounds showed AChE inhibitory activity as much as standard drug Donepezil.

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Table 1. % Inhibition of Compounds **3a-3h** and Donepezil Against AChE.

Comp.	AChE Inhibition (%)	
	10^{-3} M	10^{-4} M
3a	20.12±0.95	11.80±0.64
3b	15.19±0.21	8.74±0.14
3c	25.48±0.19	11.28±0.14
3d	16.28±0.74	8.20±0.46
3e	37.51±0.76	16.59±0.54
3f	29.74±0.64	18.16±0.32
3g	13.04±0.52	7.08±0.38
3h	19.49±0.48	9.40±0.36
Donepezil	99.48±1.92	98.24±1.88

C. Antimicrobial assay

Antibacterial activity was investigated by finding minimum inhibitory concentration (MIC) of the synthesised compounds **(3a-3h)** against *S. aureus* (ATCC 25923), *E. fecalis* (ATCC 29212), *E. fecalis* (ATCC 51922), *L. monocytogenes* (ATCC 1911), *K. pneumoniae* (ATCC 700603), *P. aeruginosa* (ATCC 27853), *E.coli* (ATCC 35218), *E.coli* (ATCC 25923) comparing with chloramphenicol as standard drug. The MIC value (µg/ml) of the compounds and control drug are summarized in **Table 2.**

Table 2. Antibacterial Activity Results of Compounds **3a-3h**

Comp.	A	B	C	D	E	F	G	H
3a	800	800	400	800	800	800	800	800
3b	400	400	400	400	400	400	400	400
3c	200	25	25	200	200	200	100	200
3d	200	25	25	100	200	100	200	200
3e	400	200	200	200	400	400	400	400
3f	200	200	200	200	200	200	400	400
3g	200	25	25	200	200	200	200	200
3h	200	25	25	200	200	200	200	200
Chlor.	25	3.125	25	12.5	12.5	25	25	25

A: S. aureus (ATCC 25923), B: E. fecalis (ATCC 29212), C: E. fecalis (ATCC 51922), D: L. monocytogenes (ATCC 1911), E: K. pneumoniae (ATCC 700603), F: P. aeruginosa (ATCC 27853), G: E. coli (ATCC 35218), H: E. coli (ATCC 25923

The synthesized compounds were found to have interesting antibacterial activity. When the result of antibacterial activity is examined, compounds **3c, 3d, 3g** and **3h** showed significant activity especially against both of *E. faecalis*. When the structures of these compounds are examined, it is seen that they carry carbon chain in the 4th position of piperazine. When the structures of the compounds are examined, the presence of the carbon chain at position 4th of the piperazine increases the activity, while the presence of aromatic groups decreases the activity.

Antifungal activity results are summarized in **Table 3.** Antifungal activity was investigated by finding minimum inhibitory concentration (MIC) of the synthesised compounds (**3a-3h**) against *C. glabrata* (ATCC 90030), *C. krusei* (ATCC 6258), *C. parapsilosis* (ATCC 22019) and *C. albicans* (ATCC 24433) comparing with ketoconazole as standard drug.

Table 3. Antifungal Activity Results of Compounds **3a-3h**

Comp.	I	J	K	L
3a	800	800	800	400
3b	400	400	800	400
3c	200	200	400	200
3d	200	200	200	200
3e	800	200	800	200
3f	200	200	200	200
3g	200	200	100	200
3h	200	200	200	200
Ketoco.	25	25	12.5	12.5

I: C. albicans (ATCC 90028), J: C. glabrata (ATCC 90030), K: C. krusei (ATCC 6258), L: C. parapsilopsis (ATCC 22019).

The minimal inhibitory concentration (MIC) values of the compounds range between 100-800 µg/ml. The tested compounds possessed moderate or weak antifungal activities**.**

IV. CONCLUSIONS

The antimicrobial activity potential of the synthesized compounds (**3a-3h**) against eight bacteria and four fungus strains at various concentrations (800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.75, 1.875 µg/ml) was evaluated. MIC values for the bacteria and fungus strains of the compounds were presented in Table **2-3**. Compounds 3c, 3d, 3g and 3h were found to have the highest activity against *E. faecalis* (ATCC 51922). These compounds showed activity similar to reference drug against the *E. faecalis* (ATCC 51922) with a MIC value (25 µg/ml). I was determined that the carbon chain at the para position of piperazine increased the activity but where as the addition of benzyl group to the para position (**3a**, **3b**, **3e**, **3f**) did not affect the activity. Furthermore, the acetylcholinesterase activity of the synthesized compounds was examined but none of the compounds showed AChE inhibitory activity as much as standard drug Donepezil.

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