

STUDIES ON SOME NEW PYRAZOLO[3,4-c]PYRIDINE DERIVATIVES AS ANTIMICROBIAL AGENTS

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Abstract

The increasing clinical importance of drug-resistant bacterial and fungal pathogens has lent additional urgency to microbiological research and novel antimicrobial compounds development. For this aim, a series of *N*-[(Benzhydryl) / (1-phenylethyl)]-2-pyrazolo[3,4-*c*]pyridin-1-yl acetamide derivatives (3a-m) were synthesized by reacting 2-chloro-*N*-(benzhydryl)acetamide (1a) / 2-chloro-*N*-(1-phenylethyl)acetamide (1b) and pyrazolo[3,4-*c*]pyridine derivatives (2). The chemical structures of the compounds were elucidated by elemental analyses, IR, ¹H-NMR, FAB⁺-MS spectral data. Their antimicrobial activities against *E. coli* (ATCC 11229), *S. aureus* (ATCC 6538), *B. cereus* (ATCC 11778), *P. mirabilis* (ATCC 14153), *P. aeruginosa* (ATCC 1539), *K. pneumoniae* (ATCC 4352), *C. albicans* (ATCC 26555), *C. guilliermondii* (KUEN 998), *C. pseudotropicalis* (kefyr) (KUEN 1014), and *C. krusei* (ATCC 6258) were investigated. The results showed that some of the compounds have slight activity against *B. cereus* and *S. aureus*. Whereas, the same compounds did not show any significant antifungal activity against *Candida* species.

Key words: Pyrazolo[3,4-*c*]pyridine derivatives, Acetamide, Antimicrobial activity

Antimikrobiyal Ajan Olarak Bazı Yeni Pirazolo[3,4-*c*]piridin Türevleri Üzerine Çalışmalar

İlaçlara karşı direnç kazanmış patojen bakterilerin ve fungusların klinik önemindeki artış, yeni mikrobiyolojik araştırmalara ve yeni antimikrobiyal ilaçların geliştirilmesi çalışmalarına neden olmuştur. Bu amaçla, 2-kloro-*N*-(benzhidril)asetamid (1a) / 2-kloro-*N*-(1-feniletil)asetamid (1b) ve pirazolo[3,4-*c*]piridin türevleri (2) reaksiyona sokularak bir seri *N*-[(benzhidril)/(1-feniletil)]-2-pirazolo[3,4-*c*]piridin-1-il asetamid türevi (3a-m) sentezlenmiştir. Bileşiklerin kimyasal yapıları elemental analiz, IR, ¹H-NMR, FAB⁺-MS verileri ile aydınlatılmıştır. Bunların antimikrobiyal aktiviteleri *E. coli* (ATCC 11229), *S. aureus* (ATCC 6538), *B. cereus* (ATCC 11778), *P. mirabilis* (ATCC 14153), *P. aeruginosa* (ATCC 1539), *K. pneumoniae* (ATCC 4352), *C. albicans* (ATCC 26555), *C. guilliermondii* (KUEN 998), *C. pseudotropicalis* (kefyr) (KUEN 1014), and *C. krusei* (ATCC 6258) suşlarına karşı araştırılmıştır. *B. cereus* and *S. aureus* suşlarına karşı bileşiklerin bazıları zayıf derecede aktivite sonuçları gösterirken, aynı bileşikler *Candida* türlerine karşı herhangi bir aktivite göstermemiştir.

Anahtar kelimeler: Pirazolo[3,4-*c*]piridin türevleri, Asetamid, Antimikrobiyal aktivite

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INTRODUCTION

In recent years, the number of life-threatening infections caused by multi-drug resistant Gram-positive and Gram-negative pathogen bacteria have reached an alarming level in many countries around the world (1). The modern treatment of these infectious diseases involves administration of multi-drug regimen over a long period of time, which lead to patient noncompliance and rapid emergence of multi-drug resistance strain. The resistance problem demands to search for effective antimicrobial agents against pathogenic microorganisms resistant to current treatment. Moreover, treatment of infectious diseases is more difficult in immunodeficient patients such as those infected with human immunodeficiency virus (HIV). Recent studies showed that the application of appropriate dosage regimen with highly potent antimicrobial agents not only eradicates bacterial growth but also minimizes the probability of resistance constitution. The biochemical basis of both intrinsic and acquired resistance are now known and has contributed considerably towards the design of new entities by rational strategies that can be used to counteract the resistance. The development of new potential drugs, which will be devoid of side-effect profile of currently available drugs, will be one of the possible solutions to treat various infectious diseases with multi-drug treatment over a long period of time (2).

There are two principle approaches to develop a new antimicrobial drug: (i) synthesis of analogues, modifications or derivatives of existing compounds for shortening and improving microbial infection treatment and, (ii) searching new structures, that the pathogen organism has never been presented with before, for the treatment of multi-drug resistant pathogen (3).

To pursue this goal, our research efforts are directed to finding new chemical classes of antimicrobially active agents. The methods of investigation using structure-activity relationships (SAR) enabled us to find some new pharmacophores of the above mentioned activity. Many studies have been performed on heterocyclic systems bearing an acetamide group as a pharmacophore (4-7) and a number of pyrazolopyridine derivatives have been claimed to possess interesting antimicrobial activity properties (8).

In view of these observations, we aimed at the synthesis and antimicrobial evaluation of new pyrazolo[3,4-c]pyridine derivatives.

EXPERIMENTAL

Chemistry

All melting points (m.p.) were determined in open capillaries on a Gallenkamp apparatus (Weiss-Gallenkamp, Loughborough-United Kingdom) and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck, Darmstadt-Germany). Spectroscopic data were recorded with the following instruments: IR; Shimadzu IR-435 spectrophotometer (Shimadzu, Tokyo, Japan); ¹H-NMR: Bruker 250 MHz spectrometer (Bruker, Billerica, Massachusetts, USA) in DMSO-*d*₆ using TMS as internal standard; and FAB⁺-MS: VG Quattro Mass spectrometer (Agilent, Minnesota, USA). Elemental analyses were performed with a Leco CHNS-932 (LECO Corporation, Michigan, USA) instrument.

General procedure for 2-chloro-N-(benzhydryl)acetamide (1a) and 2-chloro-N-(1-phenylethyl)acetamide (1b)

The amine (diphenylmethylamine or 1-phenylethylamine) (0.01 mmol) and triethylamine (0.01 mmol) were dissolved in benzene (30 ml) with constant stirring. Later, the mixture was cooled in an ice bath, and chloroacetylchloride (0.01 mmol) was added dropwise with stirring. The reaction mixture thus obtained was further agitated for 1 h at room temperature. The

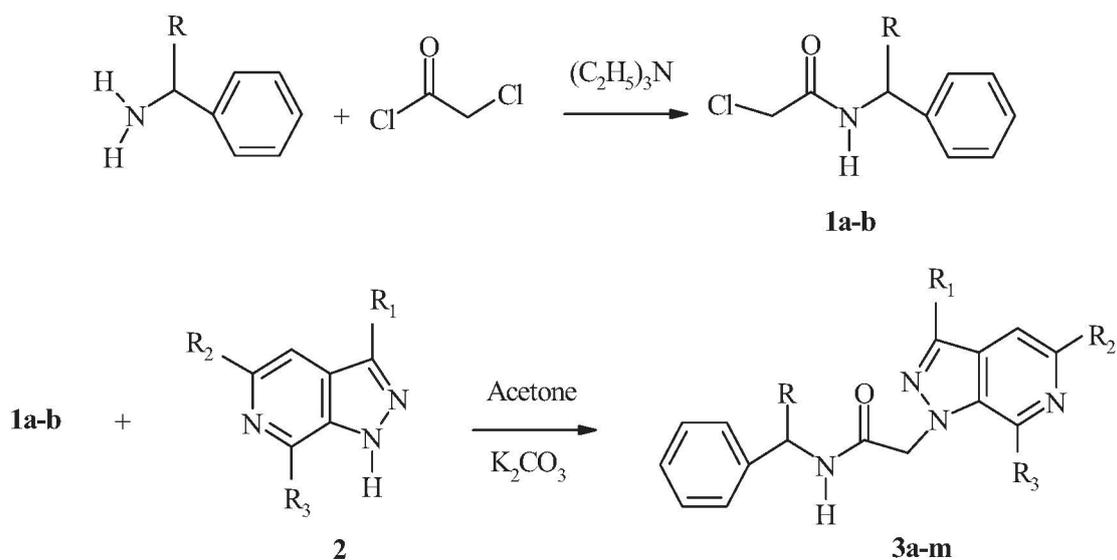
precipitate which is triethylamine ammonium chloride was filtrated, the solvent was evaporated to dryness under reduced pressure and the products were recrystallized from ethanol.

General procedure of 1H-pyrazolo[3,4-c]pyridine derivatives (2)

1H-pyrazolo[3,4-c]pyridine derivatives (**2**) used in the synthesis were prepared according to the methods reported in the literature (9).

General procedure of N-[(benzhydryl) / (1-phenylethyl)]-2-pyrazolo[3,4-c]pyridin-1-yl acetamide derivatives (3a-m)

A mixture of (**1a-b**) (0.01 mol), pyrazolo[3,4-c]pyridine derivatives (**2**) (0.01 mol), and K_2CO_3 (0.01 mol) were treated in acetone (50 ml) at room temperature for 8-10 h. The solvent was evaporated until dryness. The residue was washed with water (200 mL) and recrystallized from ethanol (**Scheme 1**). Some characteristics of the synthesized compounds are shown in **Table 1**.



Scheme 1.

2-(5-Chloropyrazolo[3,4-c]pyridin-1-yl)-N-(1-phenylethyl)acetamide (3a)

IR (KBr) 3398, 3062, 2965, 2920, 1670, 1596, 1564, 1503, 1420 cm^{-1} ; 1H -NMR (DMSO- d_6 , 250 MHz): 1.45 (d, 3H, CH_3), 4.90-5.00 (m, 1H, N-CH), 5.35 (s, 2H, $COCH_2$), 7.20-7.40 (m, 5H, Ph), 7.80 and 7.90 (two s, 1H, H-4), 8.20 and 8.50 (two s, 1H, H-3), 8.80 and 8.90 (two d, 1H, NH), 8.95 and 9.05 (two s, 1H, H-7); FAB⁺-MS: m/z: 314 [M], 315 [M+1], 316 [M+2]; For $C_{16}H_{15}ClN_4O$, calculated: C, 61.05; H, 4.80; N, 17.80. found: C, 61.12; H, 4.94; N, 17.63%.

Table 1. Some characteristics of the compounds

Comp.	R	R ₁	R ₂	R ₃	M.P. (°C)	Yield (%)	Mol. For.	MW
3a	CH ₃	H	Cl	H	214-216	64	C ₁₆ H ₁₅ ClN ₄ O	314.5
3b	CH ₃	H	H	Cl	104-106	60	C ₁₆ H ₁₅ ClN ₄ O	314.5
3c	CH ₃	H	H	OCH ₃	250-252	62	C ₁₇ H ₁₈ N ₄ O ₂	310
3d	CH ₃	Cl	Cl	H	226-228	63	C ₁₆ H ₁₄ Cl ₂ N ₄ O	349
3e	CH ₃	Cl	H	Cl	192-194	59	C ₁₆ H ₁₄ Cl ₂ N ₄ O	349
3f	CH ₃	NO ₂	Cl	H	208-210	65	C ₁₆ H ₁₄ ClN ₅ O ₃	359.5
3g	Phenyl	H	Cl	H	176-178	58	C ₂₁ H ₁₇ ClN ₄ O	376.5
3h	Phenyl	H	H	Cl	166-168	61	C ₂₁ H ₁₇ ClN ₄ O	376.5
3i	Phenyl	H	H	Br	150-152	60	C ₂₁ H ₁₇ BrN ₄ O	421
3j	Phenyl	Cl	Cl	H	206-207	63	C ₂₁ H ₁₆ Cl ₂ N ₄ O	411
3k	Phenyl	Cl	H	Cl	210-212	64	C ₂₁ H ₁₆ Cl ₂ N ₄ O	411
3l	Phenyl	Br	H	Br	202-204	57	C ₂₁ H ₁₆ Br ₂ N ₄ O	500
3m	Phenyl	NO ₂	H	Cl	122-124	62	C ₂₁ H ₁₆ ClN ₅ O ₃	421.5

2-(7-Chloropyrazolo[3,4-c]pyridin-1-yl)-N-(1-phenylethyl)acetamide (3b)

IR (KBr) 3382, 3065, 2955, 2905, 1665, 1576, 1560, 1500, 1410 cm⁻¹; ¹H-NMR (DMSO-*d*₆, 250 MHz): 1.43 (d, 3H, CH₃), 4.80-5.95 (m, 1H, N-CH), 5.30 (s, 2H, COCH₂), 7.15-7.35 (m, 5H, Ph), 7.75 and 7.85 (two s, 1H, H-4), 8.15 and 8.40 (two s, 1H, H-3), 8.70 and 8.80 (two d, 1H, NH), 8.95 and 9.00 (two s, 1H, H-7); FAB⁺-MS: m/z: 314 [M], 315 [M+1], 316 [M+2], 317 [M+3]; For C₁₆H₁₅ClN₄O, calculated: C, 61.05; H, 4.80; N, 17.80. found: C, 61.14; H, 4.87; N, 17.73%.

2-(7-Methoxypyrazolo[3,4-c]pyridin-1-yl)-N-(1-phenylethyl)acetamide (3c)

IR (KBr) 3392, 3075, 2971, 2928, 1681, 1586, 1554, 1523, 1429 cm⁻¹; ¹H-NMR (DMSO-*d*₆, 250 MHz): 1.35 (d, 3H, CH₃), 3.95 (s, 3H, O CH₃), 4.80-4.90 (m, 1H, N-CH), 5.20 (s, 2H, COCH₂), 7.15-7.35 (m, 5H, Ph), 7.60 (d, 1H, H-4 J_{4,5}= 5.81 Hz), 7.70 (d, 1H, H-5 J_{5,4}= 5.77 Hz), 8.70 (d, 1H, NH), 8.05 (two s, 1H, H-3); FAB⁺-MS: m/z: 311 [M+1]; For C₁₇H₁₈N₄O₂, calculated: C, 65.79; H, 5.85; N, 18.05. found: C, 65.94; H, 5.75; N, 17.84%.

2-(3,5-Dichloropyrazolo[3,4-c]pyridin-1-yl)-N-(1-phenylethyl)acetamide (3d)

IR (KBr) 3410, 3082, 2988, 2918, 1674, 1581, 1544, 1523, 1408 cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , 250 MHz): 1.40 (d, 3H, CH_3), 4.85-4.95 (m, 1H, N- $\underline{\text{CH}}$), 5.30 (s, 2H, COCH_2), 7.20-7.40 (m, 5H, Ph), 7.85 (s, 1H, H-4), 8.85 (d, 1H, NH), 9.10 (s, 1H, H-7); $\text{FAB}^+\text{-MS}$: m/z: 350 [M+1], 351 [M+2], 353 [M+4]; For $\text{C}_{16}\text{H}_{14}\text{Cl}_2\text{N}_4\text{O}$, calculated: C, 55.03; H, 4.04; N, 16.04. found: C, 55.23; H, 4.10; N, 15.94%.

2-(3,7-Dichloropyrazolo[3,4-c]pyridin-1-yl)-N-(1-phenylethyl)acetamide (3e)

IR (KBr) 3402, 3072, 2961, 2911, 1677, 1553, 1528, 1512, 1413 cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , 250 MHz): 1.45 (d, 3H, CH_3), 4.85-4.95 (m, 1H, N- $\underline{\text{CH}}$), 5.45 (s, 2H, COCH_2), 7.20-7.40 (m, 5H, Ph), 7.70 and 7.80 (two s, 1H, H-4), 8.00 and 8.15 (two s, 1H, H-5), 8.80 and 9.00 (two d, 1H, NH); $\text{FAB}^+\text{-MS}$: m/z: 350 [M+1], 351 [M+2], 353 [M+4]; For $\text{C}_{16}\text{H}_{14}\text{Cl}_2\text{N}_4\text{O}$, calculated: C, 55.03; H, 4.04; N, 16.04. found: C, 55.14; H, 3.97; N, 16.17%.

2-(5-Chloro-3-nitropyrazolo[3,4-c]pyridin-1-yl)-N-(1-phenylethyl)acetamide (3f)

IR (KBr) 3415, 3098, 2965, 2930, 1671, 1596, 1514, 1500, 1425 cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , 250 MHz): 1.40 (d, 3H, CH_3), 4.90-5.00 (m, 1H, N- $\underline{\text{CH}}$), 5.65 (s, 2H, COCH_2), 7.20-8.50 (m, 7H, aromatic protons), 8.95 (d, 1H, NH); $\text{FAB}^+\text{-MS}$: m/z: 359 [M], 360 [M+1], 361 [M+2]; For $\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}_3$, calculated: C, 53.42; H, 3.92; N, 19.47. found: C, 53.38; H, 3.94; N, 19.42%.

N-Benzhydryl-2-(5-chloropyrazolo[3,4-c]pyridin-1-yl)acetamide (3g)

IR (KBr) 3425, 3034, 2995, 2957, 1678, 1592, 1574, 1515, 1416 cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , 250 MHz): 5.45 (s, 2H, COCH_2), 6.10-6.20 (m, 1H, N- $\underline{\text{CH}}$), 7.20-7.40 (m, 10H, Ph), 7.85 and 7.90 (two s, 1H, H-4), 8.25 and 8.55 (two s, 1H, H-3), 9.00 and 9.15 (two d, 1H, NH), 9.35 and 9.45 (two s, 1H, H-7); $\text{FAB}^+\text{-MS}$: m/z: 376 [M], 377 [M+1], 378 [M+2]; For $\text{C}_{21}\text{H}_{17}\text{ClN}_4\text{O}$, calculated: C, 66.93; H, 4.55; N, 14.87. found: C, 66.82; H, 4.67; N, 14.69%.

N-Benzhydryl-2-(7-chloropyrazolo[3,4-c]pyridin-1-yl)acetamide (3h)

IR (KBr) 3431, 3024, 2985, 2953, 1672, 1584, 1572, 1505, 1409 cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , 250 MHz): 5.35 (s, 2H, COCH_2), 6.05-6.15 (m, 1H, N- $\underline{\text{CH}}$), 7.15-7.35 (m, 10H, Ph), 7.80 and 7.85 (two s, 1H, H-4), 8.15 and 8.45 (two s, 1H, H-3), 9.05 and 9.15 (two d, 1H, NH), 9.30 and 9.40 (two s, 1H, H-7); $\text{FAB}^+\text{-MS}$: m/z: 376 [M], 377 [M+1], 378 [M+2], 379 [M+3]; For $\text{C}_{21}\text{H}_{17}\text{ClN}_4\text{O}$, calculated: C, 66.93; H, 4.55; N, 14.87. found: C, 66.89; H, 4.65; N, 14.67%.

N-Benzhydryl-2-(7-bromopyrazolo[3,4-c]pyridin-1-yl)acetamide (3i)

IR (KBr) 3428, 3048, 3007, 2955, 1675, 1591, 1534, 1505, 1426 cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , 250 MHz): 5.50 (s, 2H, COCH_2), 6.15-6.20 (m, 1H, N- $\underline{\text{CH}}$), 7.25-8.70 (m, 13H, aromatic protons), 9.40 (d, 1H, NH); $\text{FAB}^+\text{-MS}$: m/z: 422 [M+1], 423 [M+2]; For $\text{C}_{21}\text{H}_{17}\text{BrN}_4\text{O}$, calculated: C, 59.87; H, 4.07; N, 13.30. found: C, 59.89; H, 4.01; N, 13.26%.

N-Benzhydryl-2-(3,5-dichloropyrazolo[3,4-c]pyridin-1-yl)acetamide (3j)

IR (KBr) 3419, 3059, 2988, 2932, 1674, 1597, 1571, 1502, 1436 cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , 250 MHz): 5.40 (s, 2H, COCH_2), 6.10-6.15 (m, 1H, N- $\underline{\text{CH}}$), 7.25-7.40 (m, 10H, Ph), 7.90 (s, 1H, H-4), 9.10 (s, 1H, H-7), 9.35 (d, 1H, NH); $\text{FAB}^+\text{-MS}$: m/z: 411 [M], 412 [M+1], 413 [M+2], 415 [M+4]; For $\text{C}_{21}\text{H}_{16}\text{Cl}_2\text{N}_4\text{O}$, calculated: C, 61.33; H, 3.92; N, 13.62. found: C, 61.12; H, 3.82; N, 13.83%.

N-Benzhydryl-2-(3,7-dichloropyrazolo[3,4-c]pyridin-1-yl)acetamide (3k)

IR (KBr) 3442, 3049, 2958, 2922, 1670, 1598, 1574, 1508, 1433 cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , 250 MHz): 5.50 (s, 2H, COCH_2), 6.10-6.20 (m, 1H, N- $\underline{\text{CH}}$), 7.20-7.45 (m, 10H, Ph), 7.65 and 7.80 (two d, 1H, H-4 $J_{4,5} = 5.75$ Hz), 8.00 and 8.20 (two d, 1H, H-5 $J_{5,4} = 5.80$ Hz), 9.15, 9.30,

and 9.45 (three d, 1H, NH); FAB⁺-MS: m/z: 411 [M], 412 [M+1], 413 [M+2], 415 [M+4]; For C₂₁H₁₆Cl₂N₄O, calculated: C, 61.33; H, 3.92; N, 13.62. found: C, 61.12; H, 3.82; N, 13.83%.

N-Benzhydryl-2-(3,7-dibromopyrazolo[3,4-c]pyridin-1-yl)acetamide (3l)

IR (KBr) 3426, 3058, 3018, 2965, 1672, 1579, 1573, 1509, 1416 cm⁻¹; ¹H-NMR (DMSO-*d*₆, 250 MHz): 5.65 (s, 2H, COCH₂), 6.05-6.15 (m, 1H, N-CH), 7.20-7.50 (m, 10H, Ph), 7.60 and 7.70 (two d, 1H, H-4 J_{4,5}= 5.73 Hz), 7.95 and 8.15 (two d, 1H, H-5 J_{5,4}= 5.68 Hz), 9.15, 9.35, and 9.40 (three d, 1H, NH); FAB⁺-MS: m/z: 501 [M+1], 502 [M+2], 504 [M+4]; For C₂₁H₁₆Br₂N₄O, calculated: C, 50.43; H, 3.22; N, 11.20. found: C, 50.68; H, 3.27; N, 11.32%.

N-Benzhydryl-2-(7-chloro-3-nitropyrazolo[3,4-c]pyridin-1-yl)acetamide (3m)

IR (KBr) 3438, 3099, 2970, 2945, 1678, 1586, 1534, 1520, 1445 cm⁻¹; ¹H-NMR (DMSO-*d*₆, 250 MHz): 5.55 (s, 2H, COCH₂), 6.15-6.25 (m, 1H, N-CH), 7.35-7.45 (m, 10H, Ph), 7.95 (s, 1H, H-4), 9.15 (s, 1H, H-7), 9.25 (d, 1H, NH); FAB⁺-MS: m/z: 422 [M+1], 423 [M+2], 425 [M+4]; For C₂₁H₁₆ClN₅O₃, calculated: C, 59.79; H, 3.82; N, 16.60. found: C, 59.68; H, 3.78; N, 16.73%.

Microbiology

Agar well diffusion method was used in the antimicrobial activity tests. Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) and the cation-adjusted Mueller Hinton Broth (CAMHB) for the bacteria, Sabouraud Dextrose Broth (SDB), Potato Dextrose Agar (PDA) for the yeasts were used as media. The bacteria and the yeasts used in this research are shown in Table 2.

Table 2. Used microorganisms.

Bacteria	Yeasts
<i>Staphylococcus aureus</i> ATCC 6538	<i>Candida albicans</i> ATCC 26555
<i>Escherichia coli</i> ATCC 11229	<i>Candida guilliermondii</i> KUEN 998
<i>Pseudomonas aeruginosa</i> ATCC 1539	<i>Candida pseudotropicalis</i> (kefyr) KUEN 1014
<i>Proteus mirabilis</i> ATCC 14153	<i>Candida krusei</i> ATCC 6258
<i>Klebsiella pneumoniae</i> ATCC 4352	
<i>Bacillus cereus</i> ATCC 11778	

Agar Well Diffusion Method

The compounds were dissolved in DMSO (500 µl). Bacteria suspensions were prepared equal to the turbulence of Mc Farland 0.5 standart (1-2 x 10⁸ cfu/ml) and yeast suspensions were prepared equal to the turbulence of Mc Farland 1 standart (3 x 10⁸ cfu/ml). 0.1 ml (100 µl) microorganism suspensions were cultivated on agar medium. After that, 6 mm in diameter were cut in agar plate. Then, 0.05 ml (50 µl) was taken from the stock suspensions of compounds and put into the wells. Thus, the final concentrations of compounds suspensions were decreased in 1/10 ratio.

The solvent used for the compounds is dimethylsulphoxide (DMSO). The standart antibacterial agent meropenem (10µg/50µl) and the standart antifungal agent fluconazole (100µg/50 µl) were tested in the same manner as control. All the culture plates were incubated at 35⁰C, 16-18 hours for bacteria and 18-24 hours for *Candida* species. After the incubation, the diameter of zone inhibition was measured in millimeters. Each test was performed twice and the average of the results was taken (10-11). DMSO neither affected the growth of any of the microorganisms nor *Candida* species. The standart antibacterial agent (meropenem) inhibited

the growth of the bacteria. On the other hand, the standart antifungal agent (fluconazole) inhibited the growth of *Candida* species.

Macrodilution Tube Broth Method

Steril test tubes used to conduct the test. A control tube containing broth without antimicrobial agent used for each organism tested. The compounds were dissolved in (DMSO) to a final concentration of 100 mg/ml and sterilized by filtration through a membran filter with 0.45 μm diameter. The final two fold dilutions of compounds prepared in the cation-adjusted Mueller Hinton Broth (CAMHB) and the concentrations of the compounds were prepared in 10 tubes (12-13).

A standardized inoculum for the macrodilution broth method prepared by growing microorganisms to the turbulence of the 0.5 Mc Farland standart in broth. Then the 0.5 Mc Farland suspension (1×10^8 cfu/ml) diluted 1:10 to yield 10^7 cfu/ml. Within 15 minutes 0.5 ml of the adjusted inoculum added to each tube already containing 0.5 ml of extract in the dilution series and a positive control tube containing only broth and each tube was mixed. The inoculated macrodilution tubes incubated at 35°C for 16 to 20 hours in the incubator. After incubation the amount of growth in the tubes containing the compounds and antibiotic compared with the amount of growth in the control tubes (no compound and antibiotics) used in each set of tests when determining the growth end points. Meropenem (512 $\mu\text{g}/\text{ml}$) were used as positive reference standarts to determine the sensitivity of one strain in each microbial species was tested. Each assay in this experiment was repeated twice (12-13). The MIC for each compound was read at the lowest concentration at which there was no detectable growth. The results are shown in Table 3.

Table 3. MIC values $\mu\text{g}/\text{mL}$ of compounds.

Comp.	3b	3d	3g	3h	3i	3j	3k	Ref.
A	-	>50000	>50000	-	6250	>50000	>50000	<0.0625
B	6250	-	-	>50000	3125	-	-	<0.0625

A: *Staphylococcus aureus* ATCC 6538, **B:** *Bacillus cereus* ATCC 11778;

Ref: Meropenem

RESULTS AND DISCUSSION

In the present work, N-(benzhydryl)-2-chloroacetamide (**1a**) and 2-chloro-N-(1-phenylethyl)acetamide (**1b**) was prepared by reacting diphenylmethylaniline and 1-phenylethylamine with chloroacetyl chloride in accordance with the method described in the literature (14-15).

N-[(Benzhydryl) / (1-phenylethyl)]-2-pyrazolo[3,4-c]pyridin-1-yl acetamide derivatives (**3a-m**) were synthesized by reacting (**1a-b**) and pyrazolo[3,4-c]pyridine derivatives (**2**). The chemical structures of the compounds **3a-m** were confirmed by elemental analyses, IR, $^1\text{H-NMR}$, FAB^+ -MS spectral data.

IR data were very informative. In the IR spectra, some significant stretching bands due to, N-H, C=O, C=N and C=C were at $3382\text{-}3442\text{ cm}^{-1}$, $1670\text{-}1681\text{ cm}^{-1}$, and $1408\text{-}1598\text{ cm}^{-1}$, respectively. In the $^1\text{H NMR}$ spectra, the signal due to COCH_2 methylene protons, present in all compounds, appeared at 5.20-5.65 ppm as singlets. The signals due to N-CH proton appeared at 4.80-6.20 ppm as multiplets. The NH proton was observed at 8.70-9.45 ppm as a doublet, two doublet, and three doublet. It could be commented that because of the different structural conformations the NH protons appeared as a doublet, two doublet, and three doublet. All the

other aromatic and aliphatic protons were observed at expected regions. The mass spectra (FAB⁺-MS) of compounds showed [M+1] peaks, in agreement with their molecular formula.

All of the compounds were evaluated for their antimicrobial properties. While none of the compounds showed antimicrobial activity against to *Escherichia coli* (ATCC 11229), *Proteus mirabilis* (ATCC 14153), *Pseudomonas aeruginosa* (ATCC 1539), *Klebsiella pneumoniae* (ATCC 4352), *Candida albicans* (ATCC 26555), *Candida guilliermondii* (KUEN 998), *Candida pseudotropicalis* (kefyr) (KUEN 1014), and *Candida krusei* (ATCC 6258), some of (**3b** and **3i**) them showed poor antimicrobial activities against to *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 11778). In the series the compounds **3b**, **3h**, and **3i** showed antibacterial activity against *Bacillus cereus* (**3b** zone diameter: 6 mm, MIC value: 6250 µg/ml, **3h** zone diameter: 2 mm, MIC value: > 50000 µg/ml, and **3i** zone diameter: 9 mm, MIC value: 3125 µg/ml). The compounds **3d**, **3g**, **3i**, **3j**, and **3k** also showed antibacterial activity against *Staphylococcus aureus* (**3d** zone diameter: 4 mm, MIC value > 50 000 µg/ml, **3g** zone diameter 3 mm, MIC value > 50 000 µg/ml, **3i** zone diameter: 9 mm, MIC value: 6250 µg/ml, **3j** zone diameter : 8 mm, MIC value > 50 000 µg/ml, **3k** zone diameter: 3 mm, MIC value > 50 000 µg/ml), Whereas, the same compounds did not show any significant antifungal activity against *Candida* species. In comparison with meropenem is found to be more effective than the compounds against *Bacillus cereus* (zone diameter: 28 mm, MIC value <0.0625 µg/ml) and *Staphylococcus aureus* (zone diameter: 28 mm, MIC value <0.0625 µg/ml) Table 3-4.

Table 4. Zone diameter (mm) of compounds.

Compounds	3b	3d	3g	3h	3i	3j	3k	Meropenem
A	-	4	3	-	9	8	3	30
B	6	-	-	2	9	-	-	28

A: *Staphylococcus aureus* ATCC 6538, **B:** *Bacillus cereus* ATCC 11778
MIC: Minimal Inhibitory Concentration ; -: No inhibition

CONCLUSION

The present study describes the synthesis of thirteen N-[(benzhydryl) / (1-phenylethyl)]-2-pyrazolo[3,4-c]pyridin-1-yl acetamide derivatives. Their antimicrobial activities have been evaluated. The results showed that some of the compounds have slight activity against *Bacillus cereus* and *Staphylococcus aureus*. Whereas, the same compounds did not show any significant antifungal activity against *Candida* species.

REFERENCES

1. Mitscher, L.A., Pillai, S.P., Gentry, E.J., Shankel, D.M., "Multiple drug resistance" *Med. Res. Rev.* 39, 477-496, 1999.
2. Chandrakant, G.B., Naresh, J.G., "Synthesis and preliminary evaluation of some pyrazine containing thiazolines and thiazolidinones as antimicrobial agents" *Bioorganic Medicinal Chemistry* 12, 2151-2161, 2004.
3. Crabb, C., "Global Alliance at full steam for new TB drugs" *Bull World Health Organ.* 80, 517, 2002.
4. Turan-Zitouni G., Kaplancıklı Z.A., Uçucu Ü., Özdemir A., Chevallet P., Tunalı Y., "Synthesis of some 2-[(benzazole-2-yl)thio]diphenylmethylacetamide derivatives and their antimicrobial activity" *Phosphorus, Sulfur, and Silicon and the Related Elements* 179, 2183-2188, 2004.

5. **Turan-Zitouni, G., Demirayak, Ş., Özdemir, A., Kaplancıklı, Z.A., Yıldız, M.T.**, “Synthesis of some 2-[(benzazole-2-yl)thioacetyl amino]thiazole derivatives and their antimicrobial activity and toxicity” *Eur. J. Med. Chem.* 39, 267-272, **2004**.
6. **Turan-Zitouni, G., Kaplancıklı, Z.A., Yıldız, M.T., Chevallet, P., Kaya, D.**, “Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[N-(2-thiazolyl)acetamido]thio-4H-1,2,4-triazole derivatives” *Eur. J. Med. Chem.* 40, 607-613, **2005**.
7. **Turan-Zitouni, G., Kaplancıklı, Z.A., Özdemir, A., Revial, G., Güven, K.**, “Synthesis and antimicrobial activity of some 2-(benzo[d]oxazol/benzo[d]imidazol-2-ylthio)-N-(9H-fluoren-9-yl)acetamide derivatives” *Phosphorus, Sulfur, and Silicon and the Related Elements*; 182, 639-646, **2007**.
8. **Zou, X., Tu, S., Shi, F., Xu, J.**, “An efficient synthesis of pyrazolo[3,4-b]pyridine derivatives under microwave irradiation” *Arkivoc*, ii, 130-135, **2006**.
9. **Zhu, G., Gong, J., Gandhi, V.B., Woods, K., Luo, Y., Liu, X., Guan, R., Klinghofer, V., Johnson, E.F., Stoll, V.S., Mamo, M., Li, Q., Rosenberg, S.H., Giranda, V.L.**, “Design and synthesis of pyridine-pyrazolopyridine-based inhibitors of protein kinase B/Akt” *Bioorg. Med. Chem.* 15, 2441-2452, **2007**.
10. National Committee for Clinical Laboratory Standards. Performance standards for Antimicrobial Disk susceptibility Tests; Approved Standard Eight Edition. NCCLS document M2-A8. NCCLS, Pennsylvania USA, (2003).
11. **May, J.L., King, A., Warren, C.A.** “Fluconazole disc diffusion testing for the routine laboratory” *Journal of Antimicrobial Chemotherapy*; 40, 511-516, **1997**.
12. Clinical and Laboratory Standards Institute; Performance Standards for Antimicrobial Susceptibility Testing. CLSI Document M100-S15. Wayne, 2005; PA:CLSI.
13. National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Sixth Edition. NCCLS document M100-S13 (M7-A6). Wayne, PA USA 2003.
14. **Kaplancıklı, Z.A., Turan-Zitouni, G., Revial, G., Işcan, G.**, “Synthesis of some dithiocarbamate derivatives and their antimicrobial activity” *Phosphorus, Sulfur, and Silicon, and the Related Elements* 179, 1449-1454, **2004**.
15. **Ateş, Ö., Cesur, N., Güner, H., Uzun, M., Kiraz, M., Kaya, D.** “Synthesis of some N,N-disubstituted carbamodithioic acid esters tested for antifungal activity” *Farmaco* 50, 361-364, **1995**.

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