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Original Article

Synthesis, Characterization and Study Antibacterial Activity of Some New Fused Pyrazol Pyrimidin Derivatives

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ABSTRACT

New series of 2-heterocycle-substituted benzimidazole having a pyrazolo [3, 4-*d*] pyrimidine nucleus have been synthesized by reacting 2-(chloromethyl) benzimidazole with hydrazine hydrate to form compound (1). The vital compound in this synthesis 1-((benzoimidazol-2-yl)methyl)-3-methyl-pyrazol-5-one was obtained by reacting compound (1) with ethyl acetoacetate, and was used in the Biginelli multi-component cyclo condensation reaction with eight derivatives of benzaldehyde in presence of urea to form pyrimidine derivatives (3-10).

The products were characterized by C.H.N analyses, I.R spectra and ^{1}HNMR spectrum.

Antibacterial activity of some prepared compounds against two types of bacteria: *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative). Some the compounds showed high inhibition activity against bacteria.

Keywords: ^{1}*H*-benzimidazole; Pyrimidine; Pyrazol; Cyclocondensation.

INTRODUCTION

The benzimidazole nucleus is an important heterocyclic ring. Some benzimidazole derivatives with different Pharmacological effects, including antifungal[1], anathematic[2], anti-HIV[3], antihistaminic[4], antinuclear[5], cardio tonic[6], antihypertensive[7] and narcoleptic[8], So Compounds bearing benzimidazole moiety are reported to possess a number of interesting biological activities[9,10].

The benzimidazole structure is part of the nucleotide portion of vitamin B_{12} and the nucleus of some drugs, such as proton pump inhibitors and anathematic agents[11] Proton pump inhibitors (Pips) are substituted benzimidazole derivatives that selectively and irreversibly inhibit the gastric

hydrogen–potassium adenosine triphosphatase (H+K+-ATPase) pump mechanism, the antimicrobial activity of this class of compounds was investigated against *Helicobacter pylori*[12] and against oral streptococci[13].

Pyrazolo pyrimidines and related fused heterocycles have been identified as bioactive molecules. They are known to function as CNS (Central Nervous System) depressants[14], narcoleptic agents[15], and as tuberculostatic[16], Pyrazolo [3, 4-*d*] pyrimidines were identified as a general class of adenosine receptors[17].

Several synthetic strategies have been reported for the preparation of pyrimidine derivatives[18,19]. Most of these are based on modification of the classical one-pot Biginelli reaction[20], and in some cases on more complex multi-step processes [21]. New pyrimidine derivatives containing a benzimidazole nucleus were synthesized using Biginelli multi-component cyclocondensation reaction and characterized by CHN analyses, IR spectra and ^{1}HNMR technique as well as study anti bacterial activity.

EXPERIMENTAL

All chemicals used were supplied from Merck, BDH and Fluke chemicals company .Melting points were recorded using Electro thermal melting point apparatus, UK. The F.T.I.R spectra were recorded using Fourier transform infrared SHIMADZU FT.IR-8400S infrared spectrophotometer by KBr disc. The elemental analysis were recorded using E.A.G.E.R.-100, Carlo Erba, Italy. Thin layer chromatography (TLC) was performed on aluminum plates and coated with layer of silica gel, compounds were detected by iodine vapor. ^{1}H-NMR were recorded on Fourier transformation Bruker spectrometer, operating at (400 MHz) with (DMSO-d_6),

2-(Hydrazinylmethyl)-1H-benzoimidazole (1)

A mixture of 2-(chloromethyl) benzimidazole (0.01mol) and hydrazine hydrate (0.01mol) in (25ml) DMF was refluxed for (8) hours. The reaction mixture was poured in to ice cold water; the crude product was filtered, dried and recrystallized from 95% ethanol. Yield 82%, mp. 114ºC.

Anal. Calc. for $C_8H_{10}N_4$ C% 59.24 H% 6.21 N% 34.54
Found C% 58.87 H% 5.95 N% 34.22.

I.R spectra (C=N) benzimidazole ring (1540) cm^{-1}, (C-N) (1110) cm^{-1}, (N-H) (3250) cm^{-1}, (C=C) aromatic (1488) $cm^{-1}$and (713) cm^{-1} (C-H bending aromatic).

^{1}HNMR spectrum. (N-H) benzimi-dazole ring δ (12.2) ppm, (N-H) hydrazine δ (6.3) ppm, (NH_2) hydrazine δ (4.5) ppm, (C-H) δ (3.9) ppm and (C-H) aromatic δ (7.2-7.6) ppm.

1-((Benzoimidazol-2-yl) methyl)-3-methyl-pyrazol-5-one (2)

A mixture of (0.01mol) of compound (1) and ethyl acetoacetate (0.01mol) in abs. ethanol (20ml) was heated under reflux for (7) hours. After concentration and cooling, the solid product that forms was filtered off and recrystallized from ethanol, Yield 80%, m.p. 123ºC.

Anal. Calc. for $C_{12}H_{12}N_4$ **C%** 63.15 H% 5.30 N% 24.55.
Found C% 62.78 H% 5.12 N% 24.34.

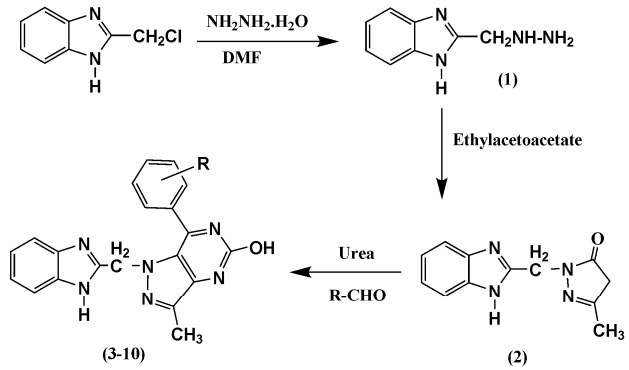
I.R spectra (C=N) benzimidazole ring (1550) cm^{-1}, (C=N) pyrazol ring (1575) cm^{-1} (C=O) (1650) cm^{-1}, (C-N) (1180) cm^{-1}, (N-H) (3270) cm^{-1}, (C=C) aromatic (1450) $cm^{-1}$and (710) cm^{-1} (C-H bending aromatic).

^{1}HNMR spectrum. (N-H) benzimi-dazole ring δ (12.0) ppm, (CH_2) pyrazol ring (2.3)

ppm, (CH) pyrazol ring (3.1) ppm (CH_2) δ (4.5) ppm, (CH_3) δ (1.9) ppm and (C-H) aromatic δ (7.3-7.7) ppm.

General procedure for synthesis of compounds (3–10)

A mixture of benzaldehyde or it is derivatives (0.01 mol), compound (2) (0.01 mol), urea (0.01 mol) and phosphorus pent oxide (200 mg) in 95 % ethanol (30 ml) was heated under refluxed condition for 5 hours. After cooling to room temperature, the crystalline product was filtered and recrystallized from a suitable solvent.



No.	3	4	5	6	7	8	9	10
R=	-H	2-Cl	4-Cl	2-OH	4-OH	2-NO_2	4-NO_2	4-OCH_3

1-((1H-benzoimidazol-2-yl) methyl)-3-methyl-7-phenyl-pyrazolo [4, 3-d] pyrimidin-5-ol (3)

Yield 73%, m.p. 165°C.

Anal. Calc. for ***$C_{20}H_{16}N_6O$*** C% 67.40 H% 4.53 N% 23.58
Found C% 67.11 H% 4.23 N% 23.22

I.R spectra (C=N) benzimidazole ring (1545)cm^{-1}, (C=N) pyrazol ring (1585)cm^{-1} ,(C-N) (1170) cm^{-1}, (N-H) (3260) cm^{-1}, (C=C) aromatic (1455) $cm^{-1}$and (715) cm^{-1} (C-H bending aromatic), (O-H) (3300-3450) cm^{-1} and disappear carbonyl group .

^{1}HNMR spectrum. (N-H) benzimidazole ring δ(12.5)ppm, (CH_2) δ(5)ppm, (CH_3) δ(1.7)ppm, (O-H) δ(11.3)ppm, and (C-H)aromatic δ(7.2-7.8)ppm

1-((1H-benzoimidazol-2-yl)methyl)-7-(2-chlorophenyl)-3-methyl-pyrazolo[4,3-d]pyrimidin-5-ol (4)

Yield 75%, m.p. 178ºC.

Anal. Calc. for ***$C_{20}H_{15}N_6OCl$*** C% 61.46 H% 3.87 N% 21.50
Found C% 60.89 H% 3.65 N% 21.32

I.R spectra (C=N) benzimidazole ring (1550)cm^{-1}, (C=N) pyrazol ring (1595)cm^{-1} ,(C-N) (1180) cm^{-1}, (N-H) (3220) cm^{-1}, (C=C) aromatic (1450) $cm^{-1}$and (715) cm^{-1} (C-H bending aromatic), (O-H) (3280-3470) cm^{-1} and disappear carbonyl group .

^{1}HNMR spectrum. (N-H) benzimidazole ring δ(12.2)ppm , (CH_2) δ(4.9)ppm , (CH_3) δ(1.8)ppm ,(O-H) δ(11.4)ppm , and (C-H)aromatic δ(7.2-7.9)ppm and (C-H)aromatic

1-((1H-benzoimidazol-2-yl)methyl)-7-(4-chlorophenyl)-3-methyl-pyrazolo[4,3-d]pyrimidin-5-ol (5)

Yield 77%, m.p. 176ºC.

Anal. Calc. for ***$C_{20}H_{15}N_6OCl$*** C% 61.46 H% 3.87 N% 21.50
Found C% 60.82 H% 3.58 N% 21.27

I.R spectra (C=N) benzimidazole ring (1558)cm^{-1}, (C=N) pyrazol ring (1585)cm^{-1} ,(C-N) (1170) cm^{-1} , (N-H) (3200) cm^{-1}, (C=C) aromatic (1458) $cm^{-1}$and (712) cm^{-1} (C-H bending aromatic), (O-H) (3270-3460) cm^{-1} and disappear carbonyl group .

^{1}HNMR spectrum. (N-H) benzimidazole ring δ(12.3)ppm , (CH_2) δ(4.9)ppm, (CH_3) δ(1.7)ppm ,(O-H) δ(11.3)ppm , δ(7.3-7.8)ppm

1-((1H-benzoimidazol-2-yl)methyl)-7-(2-hydroxyphenyl)-3-methyl-pyrazolo[4,3-d]pyrimidin-5-ol (6)

Yield 72%, m.p. 181ºC.

Anal. Calc. for $C_{20}H_{16}N_6O_2$ C% 64.51 H% 4.33 N% 22.57
Found C% 64.34 H% 4.23 N% 22.41

I.R spectra (C=N) benzimidazole ring (1552)cm^{-1}, (C=N) pyrazol ring (1575)cm^{-1}, (C-N) (1177) cm^{-1} , (N-H) (3210) cm^{-1}, (C=C) aromatic (1460) $cm^{-1}$and (710) cm^{-1} (C-H bending aromatic), (O-H) (3280-3520) cm^{-1} and disappear carbonyl group .
^{1}HNMR spectrum. (N-H) benzimidazole ring δ(12.1)ppm , (CH_2) δ(4.8)ppm , (CH_3) δ(1.8)ppm ,(O-H) δ(11.3)ppm , ,(O-H) phenolic δ(7.1)ppm and (C-H)aromatic δ(7.3-8.1)ppm

1-((1H-benzoimidazol-2-yl)methyl)-7-(4-hydroxyphenyl)-3-methyl-pyrazolo[4,3-d]pyrimidin-5-ol (7)

Yield 72%, m.p. 185ºC.

Anal. Calc. for $C_{20}H_{16}N_6O_2$ C% 64.51 H% 4.33 N% 22.57
Found C% 64.11 H% 4.16 N% 22.28

I.R spectra (C=N) benzimidazole ring (1555)cm^{-1}, (C=N) pyrazol ring (1585)cm^{-1} ,(C-N) (1170) cm^{-1}, (N-H) (3230) cm^{-1}, (C=C) aromatic (1450) $cm^{-1}$and (715) cm^{-1} (C-H bending aromatic), (O-H) (3250-3500) cm^{-1} and disappear carbonyl group .

^{1}HNMR spectrum. (N-H) benzimidazole ring δ(12.2)ppm , (CH_2) δ(4.9)ppm, (CH_3) δ(1.7)ppm ,(O-H) δ(11.2)ppm, (O-H) phenolic δ(9.3)ppm and (C-H)aromatic δ(6.8-7.6)ppm

1-((1H-benzoimidazol-2-yl)methyl)-3-methyl-7-(2-nitrophenyl)- pyrazolo[4,3-d]pyrimidin-5-ol (8)

Yield 72%, m.p. 187ºC.

Anal. Calc. for $C_{20}H_{15}N_7O_3$ C% 59.85 H% 3.77 N% 24.43
Found C% 59.64 H% 3.55 N% 23.95

I.R spectra (C=N) benzimidazole ring (1545)cm^{-1}, (C=N) pyrazol ring (1575)cm^{-1}, (C-N) (1173) cm^{-1}, (N-H) (3210) cm^{-1}, (C=C) aromatic (1458) $cm^{-1}$and (718) cm^{-1} (C-H bending aromatic), (O-H) (3280-3490) cm^{-1} and disappear carbonyl group .

^{1}HNMR spectrum. (N-H) benzimidazole ring δ(12.1)ppm, (CH_2) δ(5.0)ppm, (CH_3) δ(1.7)ppm, (O-H) δ(11.4)ppm, and (C-H)aromatic δ(7.2-8.0)ppm

1-((1H-benzoimidazol-2-yl)methyl)-3-methyl-7-(4-nitrophenyl)- pyrazolo[4,3-d]pyrimidin-5-ol (9)

Yield 75%, m.p. 183ºC.

Anal. Calc. for $C_{20}H_{15}N_7O_3$ C% 59.85 H% 3.77 N% 24.43
Found C% 59.47 H% 3.22 N% 23.88

I.R spectra (C=N) benzimidazole ring (1555)cm^{-1}, (C=N) pyrazol ring (1585)cm^{-1}, (C-N) (1175) cm^{-1}, (N-H) (3230) cm^{-1}, (C=C) aromatic (1470) $cm^{-1}$and (715) cm^{-1} (C-H bending aromatic), (O-H) (3250-3500) cm^{-1} and disappear carbonyl group .

^{1}HNMR spectrum. (N-H) benzimidazole ring δ(12.2)ppm, (CH_2) δ(4.9)ppm, (CH_3) δ(1.8)ppm, (O-H) δ(11.3)ppm, and (C-H)aromatic δ(7.2-8.3)ppm

1-((1H-benzoimidazol-2-yl)methyl)-7-(4-methoxyphenyl)-3-methyl-pyrazolo[4,3-d]pyrimidin-5-ol (10)

Yield 73%, m.p. 177ºC.
Anal. Calc. for $C_{21}H_{18}N_6O_2$ C% 65.27 H% 4.70 N% 21.75
Found C% 64.83 H% 4.68 N% 21.45

I.R spectra (C=N) benzimidazole ring (1545)cm^{-1}, (C=N) pyrazol ring (1575)cm^{-1} ,(C-N) (1170) cm^{-1}, (C-O) (1185) cm^{-1} (N-H) (3215) cm^{-1}, (C=C) aromatic (1480) $cm^{-1}$and (718) cm^{-1} (C-H bending aromatic), (O-H) (3260-3510) cm^{-1} and disappear carbonyl group.

^{1}HNMR spectrum. (N-H) benzimidazole ring δ(12.1)ppm , (CH_2) δ(4.8)ppm, (CH_3) δ(1.7)ppm, (O-CH_3) δ(3.8)ppm, (O-H) δ(11.4)ppm, and (C-H)aromatic δ(7.0-7.6)ppm

Antibacterial activity test

Eight compounds [3-10] were used for their antibacterial activity against the *Staphylococcus aureous* (Gram-positive) and *Pseudomonase aeroginosa* (Gram-negative) by Well diffusion method[22]. Each bacteria isolate was inoculated on to the Muller-Hinton Agar [sterilize in autoclave] by dipping a cotton swab in to the suspension and streaking over the surface of the agar plates. Then, in the solidified medium, four holes were made (6 mm). These holes were filled with (0.5 ml) of the prepared compounds (10 mg of the compound dissolved in 1ml of DMSO solvent). These plates were incubated at 37^0C and measured of zone inhibition after 24 hours.

RESULTS

Standardization of Extract

Several pyrimidine derivatives containing a benzimidazole nucleus were synthesized at reflux temperature. compound(1) 2-(hydrazinylmethyl)-1H-benzoimidazole was synthesized first by reaction 2-(chloromethyl)benzimidazole with hydrazine hydrate , The structure of which was assigned from the I.R spectrum which showed strong absorption for benzimidazole ring (1540)cm^{-1}, (C-N) (1110) cm^{-1}, (N-H) (3250) cm^{-1}, (C=C) aromatic (1488) $cm^{-1}$and (713) cm^{-1} (C-H bending aromatic), Elementary analysis showed good agreement of the calculated and found percentages and ^{1}HNMR singles showed (N-H) benzimidazole ring δ(12.2)ppm , (N-H)hydrazine δ(2.1)ppm, (C-H) δ(3.9)ppm and (C-H)aromatic δ(7.2-7.6)ppm.

The vital compound in this synthesis 1-((benzoimidazol-2-yl)methyl)-3-methyl-pyrazol-5-one compound(2) was obtained by reacting compound(1) with ethyl acetoacetate, This compound were characterized by C.H.N analysis (the percentage of found agreement with calculated) I.R spectrum show (C=N) benzimidazole ring (1550)cm^{-1}, (C=N) pyrazol ring (1575)cm^{-1} (C=O) (1650) cm^{-1}, (C-N) (1180) cm^{-1}, (N-H) (3270) cm^{-1}, (C=C) aromatic (1450) $cm^{-1}$and (710) cm^{-1} (C-H bending aromatic) and ^{1}HNMR singles gives (N-H) benzimidazole ring δ(12.0)ppm, (C-H) pyrazol ring (2.3)ppm, (CH_2) δ(4.5)ppm, (CH_3) δ(1.9)ppm and (C-H)aromatic δ(7.3-7.7)ppm.

Pyrimidine derivatives (3), (4), (5), (6), (7), (8), (9) and (10) are prepared by addition compound(2) to benzaldehyde or it is derivatives in presence of urea according to the Biginelli multi-component cyclocondensation reaction, also These derivatives are identifying by I.R spectra, C.H.N. analysis and ^{1}HNMR (all results showed in experimental part).

The prepared compounds (3-10) were examined for antibacterial activity against *Staphylococcus aureou* (Gram-positive) and *Pseudomonase aeroginosa* (Gram-negative) by Well diffusion method in Mueller-Hinton agar medium. After 24 hours were measured for zone of inhibition around each disc. The test results presented in Table (1) showed that compound (7) was exhibited slightly active against these bacterial while compounds (3) and (4) were exhibited inactive against S. aureous and P. aeroginosa. Compounds [6, 8, 9 and 10] were exhibited highly active against S. aureous while were exhibited moderately

active against P. aeroginosa. The compound (5) was exhibited moderately active against S. aureous while it was exhibited inactive against P. aeroginosa.

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Table 1. Antibacterial activity of some synthesized compounds

Comp. No.	*Staphylococcus aureous* (Gram-positive)	*Pseudomonase aeroginosa* (Gram-negative)
3	-	-
4	-	-
5	++	-
6	+++	++
7	+	-
8	+++	++
9	+++	++
10	+++	++

Key of symbols
Highly active = +++ (inhibition zone > 15 mm)
Moderately active = ++ (inhibition zone 11-15 mm)
Slightly active = + (inhibition zone 5-10 mm)
Inactive = - (inhibition zone < 5 mm)

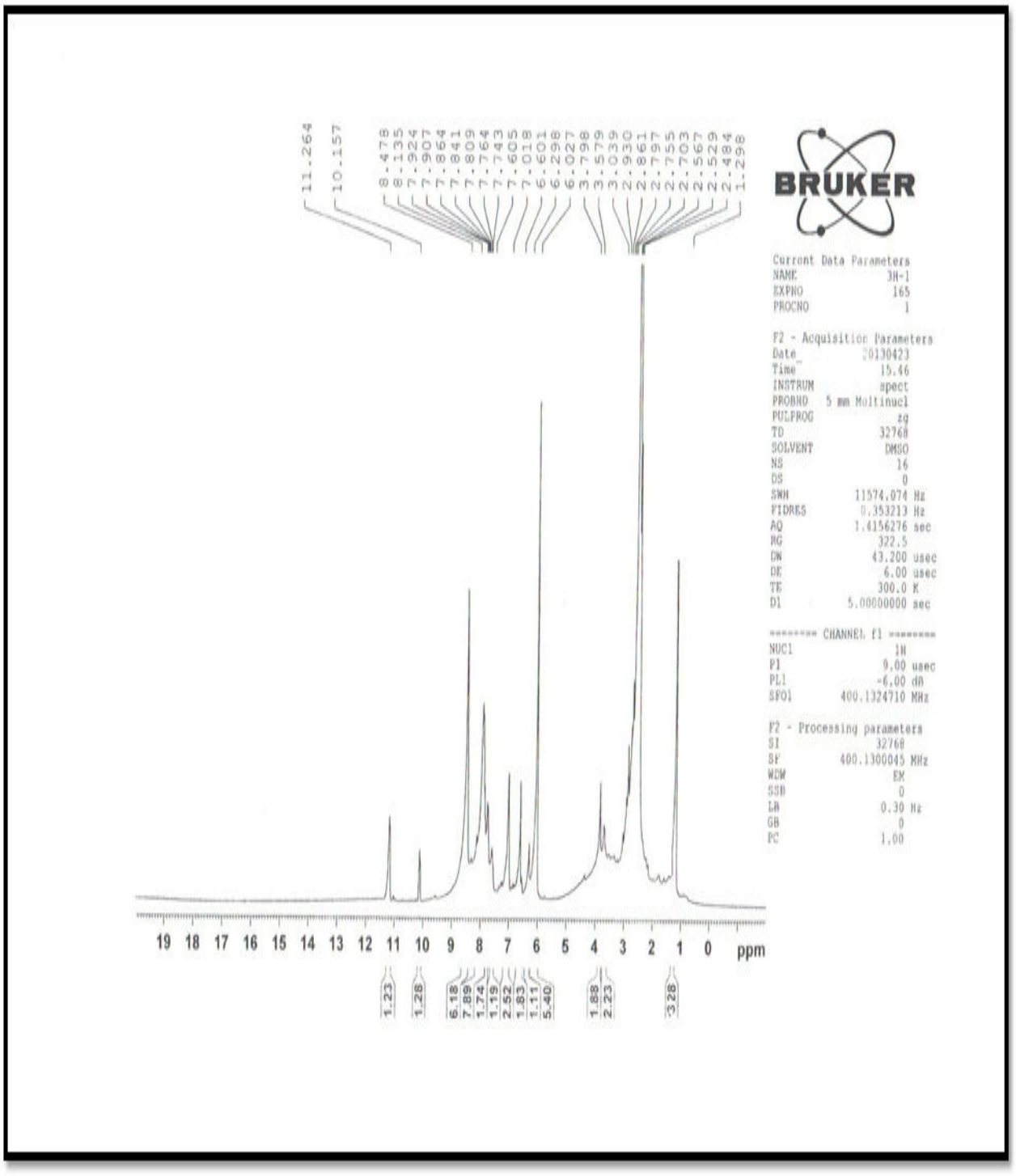

Figure 1. ^{1}H-NMR spectrum of the compound (5)

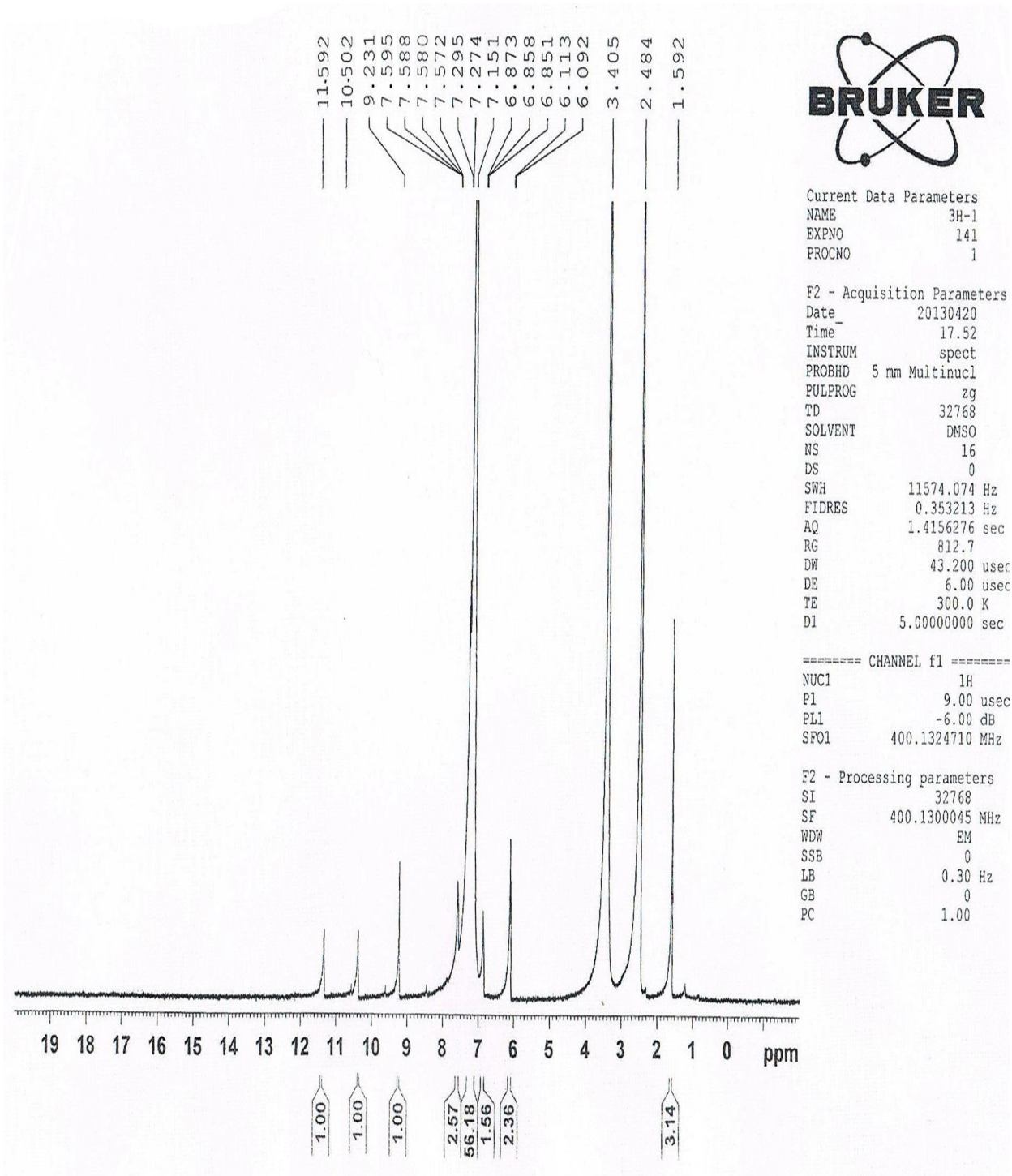

Figure 2. ^{1}H-NMR spectrum of the compound (7)

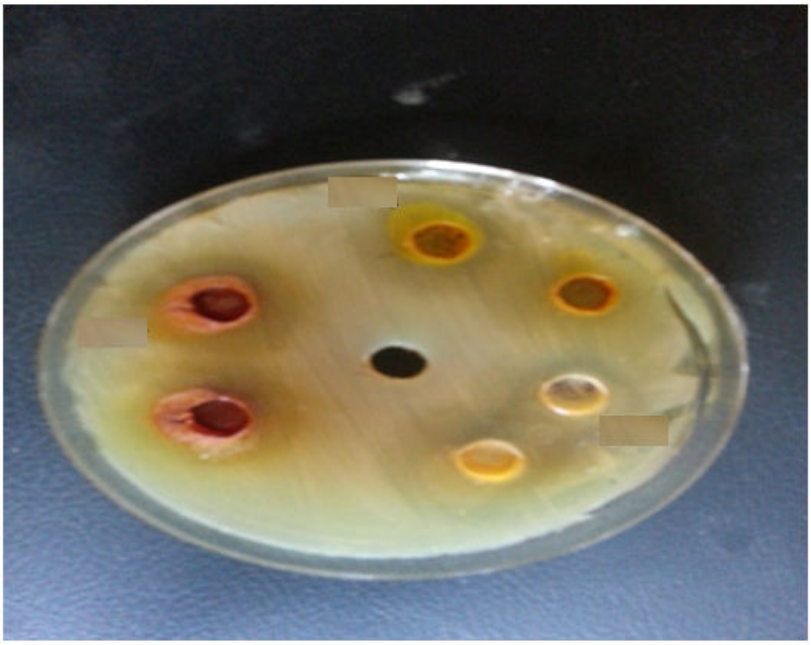

Figure 3. Antibacterial activity of some prepared compounds against *Staphylococcus aureous*.

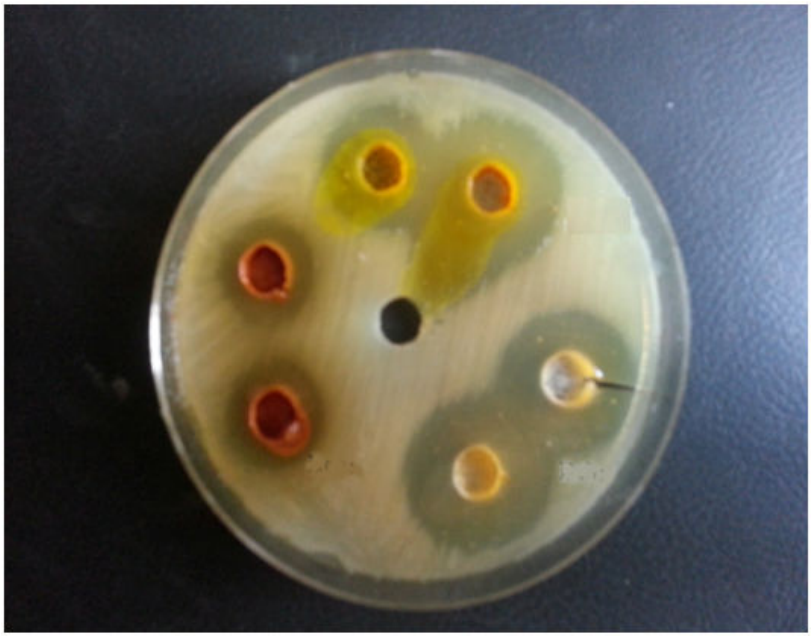

Figure 4. Antibacterial activity of some prepared compounds against *E. Coli*