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An Eco-Friendly Synthesis of Heterocyclic Moieties Condensed with Pyrazole System under Green Conditions and Their Biological Activity

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Abstract

The efficient, facile and green synthesis of 4-bromo pyrazolone by using N-bromo saccharine as valuable green reagent encouraged us to prepare some new fused heterocycles as furopyrazole, pyranopyrazole, imidazopyrazole, pyrazolothiazole, pyrazol thiazolopyrimidine, pyrazolothiazine, oxathinopyrazole, pyrazolobenzooxazine, and pyrazoloquinoxaline. The synthesis was carried out by a basic condensation of bromo pyrazolone 2 and a suitable reagent in a one-pot reaction using chitosan as a green basic catalyst. The reactions were carried out by microwave irradiation technique as a green source of energy as well as the conventional heating. The antioxidant activity of the prepared compounds was studied using 1,1-phenyl-2-picrylhydrazyl (DPPH) assay and their antibacterial activity against Gram-positive, Gram-negative bacteria and antifungal activity was evaluated.

Keywords

4-Bromo Pyrazolone, N-Bromo Saccharine, Chitosan, Microwave Irradiation, DPPH

1. Introduction

The goals of green chemistry are to reduce and prohibit the pollution of nature, ensure perpetual life on earth and minimize the use and production of hazardous materials [1]-[3].

The chemistry of pyrazole system has attracted much attention and many methods for synthesis have been

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extended [4]. Pyrazoles exist in many compounds that are used as pharmaceuticals and agrochemicals [5]. Fused pyrazoles have fungicidal [6], herbicidal [7], veridical [8] and insecticidal activity [9] [10] and have been used for the treatment of rheumatoid arthritis [11] [12]. Beside the pharmaceutical importance pyrazoles are very important class of heterocycles due to their commercial uses in dyestuffs and food coloring agents [13].

In continuation to our program aiming at the synthesis of fused heterocyclic moities of anticipated biological activity, we report herein the eco-friendly synthesis of fused pyrazole derivatives [14]-[16].

2. Materials and Methods

2.1. Chemistry

Melting points were recorded on a Gallenkamp melting point apparatus and uncorrected. The infrared spectra were recorded on Perkin-Elmer FTIR 1430 spectrophotometer using the KBr disk technique. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC spectrometer (300 MHz) at 25˚C in DMSO-d_6 with TMS as internal standard and chemical shifts are reported in ppm as δ values. Reactions were conducted under microwave irradiation in closed vessels under magnetic stirring in a Synthos 3000 (Anton Paar) microwave with dual magnetrons system and with maximum power of 1000 W. Mass spectra were measured on a Finnigan MAT 8222 EX mass spectrometer at 70 eV. Microanalyses were performed on Perkin-Elemer 2400 Elemental Analyzer at microanalytical center at Cairo University. Reaction progress was monitored by thin layer chromatography (TLC) using benzene/acetone (2/1 by volume) as eluent. The strains for the biological activity were obtained from the Culture Collection of Bacteriology Laboratory, Microbiology Unit, Faculty of Science, Tanta University.

2.1.1. Preparation of 4-Bromo-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-5(4*H*)-one 2

1) Method A:

To a solution of 1-phenyl-3-pyridyl-5-pyrazolone **1** (4.74 g, 20 mmole) in chloroform (20 ml) add bromine (3.16 g, 20 mmole) dropwise at room temperature with stirring for 4 h. The formed precipitate was filtrated; washed with chloroform, dried and crystallized from ethanol to give compound **2**, m.p. 115˚C - 117˚C (68% yield).

2) Method B:

To a solution of 1-phenyl-3-pyridyl-5-pyrazolone **1** (4.74 g, 20 mmole) and acetonitrile (20 ml) add a solution of NBSc (N-bromo sacharrine) (5.2 g, 20 mmole) in acetonitrile (10 ml) dropwise at room temperature with stirring for 4 h. The formed precipitate was filtrated; washed with chloroform, dried and crystallized from ethanol to give compound **2**, (87% yield) with same melting and mixed m.p.

IR (KBr) υ/cm^{-1} = 2980 (CH_{aliph}), 1658 (C=O), 1558 (C=N), 748 (C-Br); ^{1}H NMR (DMSO): δ ppm = 7 - 7.64 (m, 5H, ph-H), 7.66 - 8.83 (m, 4H, H-pyridine); ^{13}C NMR (DMSO): δ ppm = 49 (C_4 pyridine), 120 - 132 (Ar-C), 124 - 152 (py-C), 155 (C_4 pyridine), 160 (C=O); Anal. Cald. For $C_{14}H_{10}BrN_3O$ (315.15); C, 53.17; H, 3.19; Br, 25.27; N, 13.29: Found: C, 53.74; H, 3.34; Br, 25.65; N, 13.88; MS *m/z* 315 (M^+), 317 (M^{+2}).

2.1.2. General Procedure for Synthesis of 4-Substituted 5-Amino-1-phenyl-3-(pyridin-3-yl)-1*H*-furo[2,3-*c*]pyrazole (3-5)

A mixture of compound **2** (0.7 g, 2.2 mmol), malononitrile, cyanoacetamide and/or ethyl cyanoacetate (2.2 mmol), chitosan (0.5 g) in dioxane (20 ml) was refluxed for 8 h. The reaction mixture was cooled, filtered and the filtrate was evaporated under reduced pressure, washed with diethyl ether and crystallized from ethanol to give compounds **3-5**.

1) 5-Amino-4-cyano-1-phenyl-3-(pyridin-3-yl)-1*H*-furo[2,3-*c*]pyrazole 3

m.p. 137˚C - 139˚C, yield 63%; IR (KBr) υ/cm^{-1} = 3345 (NH_2), 2219(CN), 1610 (C=N); ^{1}H NMR (DMSO): δ ppm = 4.47 (s, 2H, NH_2), 7.1 - 7.54 (m, 5H, HPh), 7.44 - 8.13 (dd, 4H, Hpyid); C^{13}. NMR (DMSO): δ ppm = 113 (CN), 99, 103 (2C, Fur), 122 - 135 (6C, Ph), 136 - 149 (5C, pyrid); Anal. Cald. For $C_{17}H_{11}N_5O$ (310.15); C, 67.77; H, 3.68; N, 23.24: Found: C, 68, 13; H, 4.12; N, 23.64; MS *m/z* 310 (M^+).

2) Ethyl 2-(5-amino-1-phenyl-3-(pyridin-3-yl)-1*H*-furo[2,3-*c*]pyrazol-4-yl)acetate 4

m.p. 165˚C - 167˚C, yield 58%; IR (KBr) υ/cm^{-1} = 3415 (NH_2), 2877 (CH_{aliph}), 1710 (C=O), 1597(C=N); ^{1}H NMR (DMSO): δ ppm = 1.23 (t, 3H, CH_3), 4.23 (q, 2H, CH_2), 4.65 (s, 2H, NH_2), 6.11 - 7.34 (m, 5H, HPh), 7.35 - 8.84 (m, 4H, Hpyid); ^{13}C. NMR (DMSO): δ ppm = 24 (CH_3), 66 (CH_2), 104, 123 (2C, Fur), 119 - 137 (6C, Ph),

138 - 152 (5C, pyrid), 168 (C=O); Anal. Cald. For $C_{18}H_{15}N_5O_2$ (362.15); C, 66.26; H, 5.04; N, 15.46: Found: C, 66.63; H, 5.26; N, 15.94; MS *m/z* 362 (M^+).

3) 2-(5-Amino-1-phenyl-3-(pyridin-3-yl)-1*H*-furo[2,3-*c*]pyrazol-4-yl)acetamide 5

m.p. 217˚C - 219˚C, yield 68%; IR (KBr) υ/cm^{-1} = 3324 (NH_2), 2232 ($CONH_2$), 1680 (C=O), 1610 (C=N); ^{1}H NMR (DMSO): δ ppm = 4.60 (s, 2H, NH_2), 5.27 (s, 2H, $CONH_2$), 6.97 - 7.44 (m, 5H, HPh), 7.33 - 8.54 (dd, 4H, Hpyid); ^{13}C. NMR (DMSO): δ ppm = 173 ($CONH_2$), 104, 123 (2C, Fur), 119 - 137 (6C, Ph), 138 - 152 (5C, pyrid); Anal. Cald. For $C_{18}H_{15}N_5O_2$ (333.05); C, 64.86; H, 4.54; N, 21.60: Found: C, 65.33; H, 4.96; N, 21.94; MS *m/z* 333 (M^+).

2.1.3. Cyclization of Compound 2 with p-Methoxy-Benzylidene Malononitrile

Synthesis of 6-amino-4-(4-methoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile 6.

A mixture of compound **2** (1 g, 3.17 mmole), p-methoxy-benzylidene malononitrile (0.56 g, 3.17 mmole), chitosan (0.5 g) in dioxane (20 ml) was refluxed for 8 h. The reaction mixture was cooled, filtered and the filtrate was evaporated under reduced pressure, washed with diethyl ether and crystallized from ethanol to give compound **6**.

m.p. 162˚C - 164˚C, yield 87%; IR (KBr) υ/cm^{-1} = 3414 (NH_2), 2954 ($CH_{3\ aliph}$), 2362 (CN), 1587 (C=N); ^{1}H NMR (DMSO): δ ppm = 3.75 (s, 3H, OCH_3), 4.61 (s, 2H, NH_2), 4.82 (s, H, Hpyran), 6.68 - 7.56 (m, 5H, Ph), 7.48 - 8.73 (m, 4H, pyid); Anal. Cald. For $C_{25}H_{19}N_5O_2$ (421.45); C, 71.25; H, 4.54; N, 16.62; Found: C, 71.74; H, 4.91; N, 16.94; MS *m/z* 421 (M^+).

2.1.4. General Procedure for Synthesis of Imidazo[4,5-*c*]pyrazole (7, 8)

A solution of compound **2** (0.7 g, 2.2 mmol), urea and/or guanidine HCl (2.2 mmole), chitosan (0.5 g) in dioxane (20 ml) was refluxed for 4 h. The reaction mixture was cooled, filtered and the filtrate was evaporated under reduced pressure, washed with diethyl ether and crystallized from ethyl acetate/ethanol to give compounds **7, 8**.

1) 1-Phenyl-3-(pyridin-3-yl)-1,4-dihydroimidazo[4,5-*c*]pyrazol-5-ol (7)

m.p. 214˚C - 216˚C, yield 63%; IR (KBr) υ/cm^{-1} = 3354 (NH, OH), 3100 (CH_{arom}) 1597 (C=N); ^{1}H NMR (DMSO): δ ppm = 5.23 (s, H, OH), 11.23 (s, H, NH), 6.23 - 7.34 (m, 5H, HPh), 7.35 - 8.84 (m, 4H, Hpyid); ^{13}C NMR (DMSO): δ ppm = 122 - 137 (6C, Ph), 138 - 152 (5C,$_{pyrid}$), 168 (C,$_{imid}$); Anal. Cald. For $C_{15}H_{12}N_5O$ (277.15); C, 64.96; H, 4.04; N, 25.46: Found: C, 65.23; H, 4.326; N, 25.84; MS *m/z* 277(M^+).

2) 1-Phenyl-3-(pyridin-3-yl)-1,4-dihydroimidazo[4,5-*c*]pyrazol-5-amine (8)

m.p. 234˚C - 236˚C, yield 63%; IR (KBr) υ_{max}/cm^{-1} = 3329 (NH_2), 3055 (CH_{arom}) 1587 (C=N); ^{1}H NMR (DMSO): δ ppm = 5.11 (s, 2H, NH), 10.23 (s, H, NH_2), 6.23 - 7.34 (m, 5H, HPh), 7.35 - 8.84 (m, 4H, Hpyid); ^{13}C NMR (DMSO): δ ppm = 122 - 137 (6C, Ph), 138 - 152 (5C,$_{pyrid}$), 168(C,$_{imid}$); Anal. Cald. For $C_{15}H_{12}N_6$ (277.15); C, 65.22; H, 4.38; N, 30.42: Found: C, 65.63; H, 4.66; N, 30.84; MS *m/z* 276 (M^+).

2.1.5. Synthesis of 1-Phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*d*]thiazol-5-amine (9)

A solution of compound **2** (0.7 g, 2.2 mmol), (0.17 g, 2.2 mmole) thiourea, chitosan (0.5 g) in dioxane (20 ml) was refluxed for 4 h. The reaction mixture was cooled, filtered and the filtrate was evaporated under reduced pressure, washed with diethyl ether and crystallized from ethylacetate/ethanol to give compound **9**; m.p. 242˚C - 244˚C, yield 68%; IR (KBr) υ/cm^{-1} = 3340 (NH_2), 3055 (CH_{arom}) 1587 (C=N), 1180 (C-S); ^{1}H NMR (DMSO): δ ppm = 10.23 (s, H, NH_2), 6.23 - 7.34 (m, 5H, HPh), 7.35 - 8.84 (m, 4H, Hpyid); Anal. Cald. For $C_{15}H_{11}N_5S$ (293.25); C, 61.42; H, 3.87; N, 23.77; S, 10.93: Found: C, 61.93; H, 4.21; N, 24.04; S, 11.14; MS *m/z* 293(M^+).

2.1.6. Cyclization of Compound 8 with *β*-Ketoester

1) Formation of substituted pyrazol[4,3-*b*]thiazolo[3,2-*a*]pyrimidine-7-one 10, 11

A mixture of compound **9** (1 g, 3.4 mmole), ethylacetoacetate and/or ethylbenzoylacetate (3.4 mmole), chitosan (0.5 g) in dioxane (20 ml) was refluxed for 6 h. The reaction mixture was cooled, filtered and the filtrate was evaporated under reduced pressure, washed with diethyl ether and crystallized from ethanol to give compounds **10, 11**.

2) 5-Methyl-pyrazol[4,3-*b*]thiazolo[3,2-*a*]pyrimidine-7-one 10

m.p. 185˚C - 187˚C, yield 87%; IR (KBr) υ/cm^{-1} = 2890 (CH_{3aliph}), 1690 (C=O), 1587 (C=N), 1460 (C=C),

1210 (C-S); ^{1}H NMR (DMSO): δ ppm = 2.11 (s, 3H, CH_3), 6.23 (s, H, Hpyrimid.), 7.12 - 7.34 (m, 5H, HPh), 7.45 - 8.84 (m, 4H, Hpyrid.); Anal. Cald. For $C_{19}H_{13}N_5OS$ (359.15); C, 63.42; H, 3.67; N, 19.47; S, 8.92: Found: C, 63.53; H, 3.91; N, 19.94; S, 9.32: MS *m/z* 359 (M^+).

3) 5-Phenyl-pyrazol[4,3-*b*]thiazolo[3,2-*a*]pyrimidine-7-one 11

205˚C - 207˚C, yield 68%; IR (KBr) υ/cm^{-1} = 3055 (CH_{arom}), 1677 (C=O), 1594 (C=N)), 1178 (C-S); ^{1}H NMR (DMSO): δ ppm = 6.08 (s, H, Hpyrimid.), 6.74 - 7.22 (m, 5H, HPh), 7.35 - 8.65 (m, 4H, Hpyid); Anal. Cald. For $C_{24}H_{15}N_5OS$ (421.05); C, 68.32; H, 3.57; N, 16.62; S, 7.61: Found: C, 68.73; H, 4.11; N, 16.94; S, 7.96; MS *m/z* 421 (M^+).

2.1.7. Cyclization of Compound 2 with *β*-Mercapto Derivatives (12-14)

A mixture of compound **2** (0.6 g, 20 mmol), appropriate reagent cysteine, ethyl mercapto acetate and/or 2-mercapto ethanol (20 mmole), and chitosan (0.5 g) in dry dioxane (10 ml), was refluxed for 4 h. The reaction mixture was cooled, filtered and the filtrate evaporated under reduced pressure; the residue was triturated with pet.ether 40˚C - 60˚C. The obtained products were refluxed in glacial acetic acid (10 ml) for 2 h. The reaction mixture was cooled and poured into ice water to give compounds **(12-14)**.

2.1.8. 1-Phenyl-3-(pyridin-3-yl)-1,5,6,7-tetrahydropyrazolo[4,3-*b*][1,4]thiazine-6-carboxylic Acid (12)

m.p. 182˚C - 194˚C, yield 90%; IR (KBr) υ/cm^{-1} = 3342 (OH, NH), 2885 (CH_{2aliph}), 1710 (C=O), 675 (C-S); ^{1}H NMR (DMSO): δ ppm = 3.5 (d, 2H, CH_2-S), 3.76 (t, H, CH-N), 4.52 (s, H, NH), 6.67 - 7.41 (m, 5H, HPh), 7.43 - 8.92 (m, 4H, Hpyid), 11.23 (s, H, COOH); Anal. Cald. For $C_{17}H_{14}N_4O_2S$ (338.08); C, 60.34; H, 4.17; N, 16.57; S, 9.48: Found: C, 60.83; H, 4.31; N, 16.94; S, 9.76; MS *m/z* 338 (M^+).

1) 1-Phenyl-3-(pyridin-3-yl)-5,6-dihydro-1*H*-[1,4]oxathiino[2,3-*c*]pyrazol-6-ol (13)

m.p. 211˚C - 213˚C, yield 85%; IR (KBr) υ/cm^{-1} = 3421 (OH), 2891 (CH_{2aliph}), 665 (C-S), 1156 (C-O); ^{1}H NMR (DMSO): δ ppm = 3.44 (d, 2H, CH_2-S), 4.2 (t, H, CH-O), 6.1 (s, H, OH), 7.21 - 7.54 (m, 5H, HPh), 7.55 - 8.67 (m, 4H, Hpyid); Anal. Cald. For $C_{16}H_{13}N_3O_2S$ (311.36); C, 61.72; H, 4.21; N, 13.50; S, 10.30; Found: C, 62.03; H, 4.62; N, 13.87; S, 10.56: MS *m/z* 312 (M^+).

2) 1-Phenyl-3-(pyridin-3-yl)-5,6-dihydro-1*H*-[1,4]oxathiino[2,3-*c*]pyrazole (14)

m.p. 165˚C - 168˚C, yield 82%; IR (KBr) υ/cm^{-1} = 2884 (CH_{2aliph}), 1574 (C=N), 670 (C-S), 1172 (C-O); ^{1}H NMR (DMSO): δ ppm = 3.36 (t, 2H, CH_2-S), 4.15 (t, 2H, CH_2-O), 7.32 - 7.44 (m, 5H, HPh), 7.45 - 8.56 (m, 4H, Hpyid); Anal. Cald. For $C_{16}H_{13}N_3OS$ (295.35); C, 65.06; H, 4.44; N, 14.23; S, 5.42; Found: C, 65.33; H, 4.72; N, 14.54; S, 11.16: MS *m/z* 295(M^+).

2.1.9. Condensation of 2 with *o*-Aminopheno and/or *o*-Phenylene Diamine

1) Formation of pyrazolo[3,4-*b*]benzoxazine and pyrazolo[3,4-*b*]quinoxaline (15,16)

A mixture of compound **2** (0.6 g, 20 mmol), o-aminophenol and/or o-phenylene diamine (20 mmole) and chitosan (0.5 g) in dry dioxane (20 ml) was heated under reflux for 6 h. The reaction mixture was cooled and filtered. The filtrate evaporated under reduced pressure and the residue was triturated with pet.ether 40˚C - 60˚C. The obtained products were refluxed in 10 ml glacial acetic acid for 2 h. The reaction mixture was cooled and poured into ice water to give compounds **15, 16**.

2) 1-Phenyl-3-(pyridin-3-yl)-4,9-dihydro-1*H*-pyrazolo[3,4-*b*]benzoxazine (15)

m.p. 226˚C - 228˚C, yield 80%; IR (KBr) υ/cm^{-1} = 3346 (NH), 1170 (C-N), 1155 (C-O); ^{1}H NMR (DMSO): δ ppm = 4.56 (s, H, NH), 6.5 - 7.44 (10H,$_{aromatic}$), 7.53 - 8.71 (m, 4H,$_{pyid}$); Anal. Cald. For $C_{20}H_{14}N_4O$ (326.13); C, 73.61; H, 4.34; N, 17.17; Found: C, 73.92; H, 4.75; N, 17.54; MS *m/z* 326 (M^+).

3) 1-Phenyl-3-(pyridin-3-yl)-4,9-dihydro-1*H*-pyrazolo[3,4-*b*]quinoxaline (16)

m.p. 226˚C - 228˚C, yield 80%; IR (KBr) υ/cm^{-1} = 3353 (NH), 1240 (C-N), 1206 (C-O); ^{1}H NMR (DMSO): δ ppm = 4.44 (s, 2H, NH), 7.32 - 7.44 (m, 5H, Ph), 7.45 - 8.56 (m, 4H, pyid); Anal. Cald. For $C_{20}H_{15}N_5$ (325.34); C, 73.83; H, 4.65; N, 21.52; Found: C, 74.02; H, 4.83; N, 21.75: MS *m/z* 325 (M^+).

2.2. Antimicrobial Assay

An aliquot of 0.1 ml of each bacterial strain was inoculated and spread on nutrient agar while 0.1 ml of the yeast was spread on sabaroud agar slopes. Antimicrobial activity of the synthesized compounds was tested in *vitro*

against different types of bacteria and one fungal strain by the cut plug method [17]. The assay plates were inoculated with 100 ml containing the diluted inoculums (107 CFU/ml) of each tested organism that were spread on the corresponding media. After solidification, the wells were made and 10 mg of the synthesized chemicals were dissolved in 1 ml DMSO and inserted in the wells. Nutrient agar plated was incubated at 37˚C for 24 h while plates were incubated at 25˚C for 48 h. The zones of inhibition around the wells were measured and the average based on three replies was recorded.

2.3. DPPH Radical Scavenging Assay

The antioxidant activities of the tested compounds were measured by using DPPH radical scavenging assay with L-ascorbic acid as drug reference [18]. Each tested sample and L-ascorbic acid (50 µg) was dissolved in 1 ml DMSO. The dissolved sample (250 ml) was added to 1 ml DPPH/DMSO solution (6 µg/50ml) and the total volume was adjusted to 3 ml with DMSO. An equal amount of DMSO was used as a control. After vortexing the mixture was incubated for 30 min in dark at room temperature. Absorbance was measured using a spectrophotometer at 517 nm). DPPH radical scavenging % = 1/(A sample/A control) × 100. Serial dilutions (5 - 50 µg/ml) of each compound were measured by the same assay to obtain the IC50 according to Brand-Williams *et al*. [19].

3. Results and Discussion

3.1. Chemistry

The synthetic routes for the synthesis of compounds **2-16** are outlined in (**Schemes 1-5**).

The key intermediate 4-bromo-1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-5(4H)-one **2** is the building block for the synthesis of fused pyrazolo moieties provide synergistic cytotoxic activity. The presence of a pyridyl group in position 3 gave a slightly basic effect; beside the pyridine moiety is ubitiquitous in natural products having tremendous physiological properties [20]-[22].

The key intermediate **2** was easily prepared from 1-phenyl-3-pyridyl-5-pyrazolone **1** [23] by bromination with N-bromosaccharine (sodium salt of saccharine with potassium bromide and oxone in water at room temperature) in 90% yield (**Scheme 1**). It is worthy mentioning that the classical bromination methods (bromine and/or N-bromo-succinamide in chloroform) the yield did not exceed 68%.

The I.R spectrum of compound **2** shows a strong absorption band at ν 1558 cm^{-1} (C=O) and a band at ν 748 cm^{-1} (C-Br). The ^{1}H NMR and mass spectrum of the prepared compound confirm the structure.

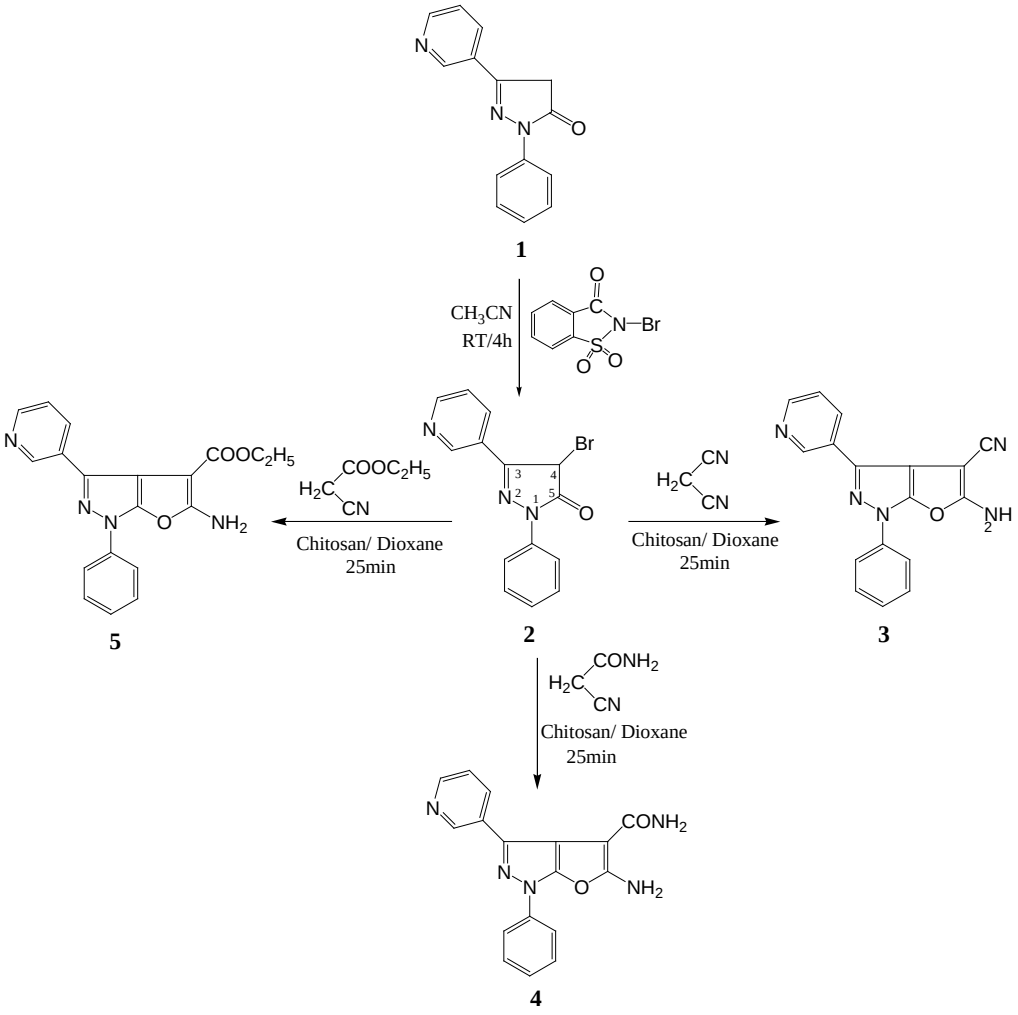
The base catalyzed reaction of compound **2** with some active methylene reagents as malononitrile, ethyl cynoacetate and ethyl cyanoacetamide was carried out by using the heterogeneous basic catalyst chitosan in dioxane or diphenyl ether to give the corresponding furopyrazole moieties 5-amino-4-cyano-1-phenyl-3-(pyridin-3-yl)-1*H*-furo[2,3-*c*]pyrazole **3**, ethyl-5-amino-1-phenyl-3-(pyridin-3-yl)-1*H*-furo[2,3-*c*]pyrazole-4-car-boylate **4** and 5-amino-1-phenyl-3-(pyridin-3-yl)-1*H*-furo[2,3-*c*]pyrazole-4-carboxamide **5** respectively (**Scheme 1**).

The structure of compounds **3-5** was proved on the basis of analytical and spectral data. Thus IR spectrum for compound **3** shows bands at ν 3345 cm^{-1} (NH_2), ν 2219 cm^{-1} (CN) and the disappearance of ν C=O band. The ^{1}H NMR spectrum for compound **3** showed the presence of a singlet for NH_2 protons at δ 4.47 ppm which disappeared by mixing with D_2O. The IR spectrum for compound **4** shows bands at ν 3324 cm^{-1} (NH_2), ν 3350 cm^{-1} (NH_{2amide}) and ν 1680 cm^{-1} (C=O). The ^{1}H NMR spectrum of compound **4** shows the presence of a singlet for NH_2 protons at δ 5.27 ppm. All spectroscopic and analytical data are given in experimental part.

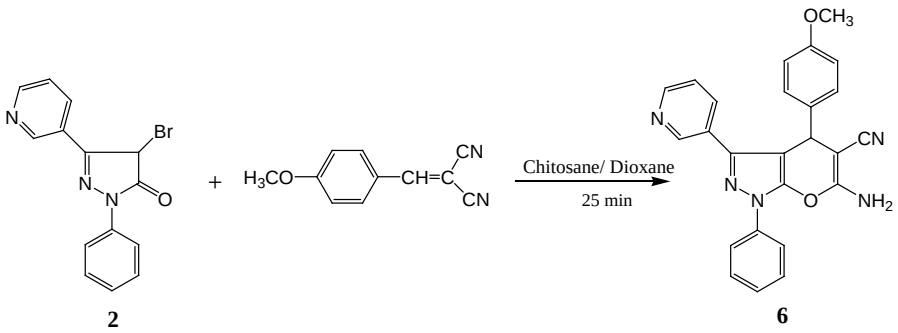
The IR spectrum for compound **5** shows bands at ν 3415 cm^{-1} (NH_2), ν 1710 cm^{-1} (C=O ester). The ^{1}H NMR spectrum of compound **5** showed triplet for CH_3 at δ 1.23 ppm, quartet for CH_2 at δ 4.23 ppm for ester protons and a singlet for NH_2 protons at δ 4.65 ppm. All spectroscopic and analytical data are given in experimental part.

The reaction of compound **2** with 2-(4-methoxybenzylidene) malononitrile in dioxane in the presence of chitosan as a green catalyst gave the corresponding 6-imino-4-(4-methoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1,4,5,6-tetrahydro- pyrano[2,3-*c*]pyrazole-5-carbonitrile **6** (**Scheme 2**).

The structure of compound **6** was proved by spectral data, IR spectrum showed bands at ν 3414 cm^{-1} (NH_2), ν 2954 cm^{-1} (CH_{3aliph}) and ν 2362 cm^{-1} (CN). The ^{1}H NMR spectrum of compound **6** showed a singlet for OCH_3 at δ 3.75 ppm, a singlet for NH_2 protons at δ 4.61 ppm and a singlet proton of pyrane ring at δ 4.82 ppm. All spectroscopic and analytical data are given in experimental part.



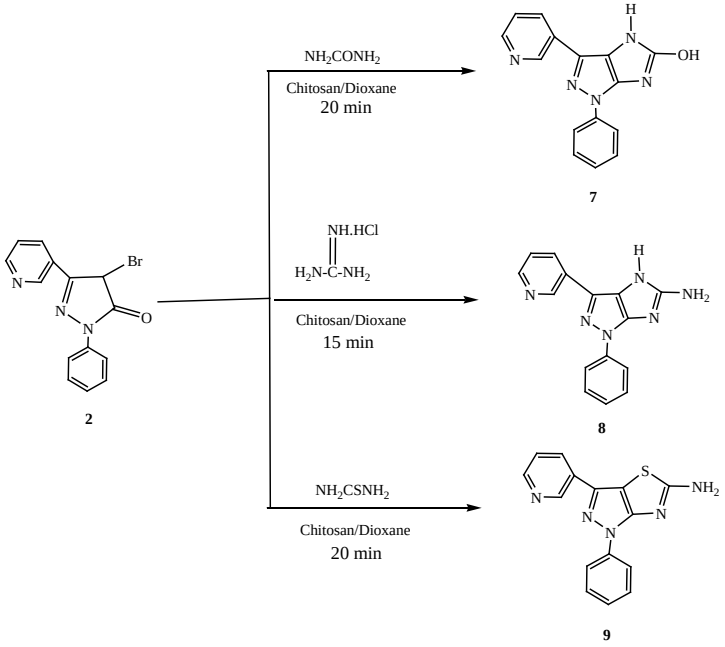
Scheme 1 Preparation of 4-substituted 5-amino-1-phenyl-3-(pyridin-3-yl)-1H-furo[2,3-c]pyrazole **2-5**



Scheme 2 Preparation of 6-imino-4-(4-methoxyphenyl)-1-phenyl-3-(pyridin-3-yl)-1,4,5,6-tetrahydro- pyrano[2,3-c]pyrazole-5-carbonitrile **6**

The reaction of compound **2** with urea, guanidine hydrochloride and thiourea in dioxane in the presence of chitosan gave the corresponding imidazopyrazoles **7** and **8** or pyrazolothiazole **9** respectively (**Scheme 3**).

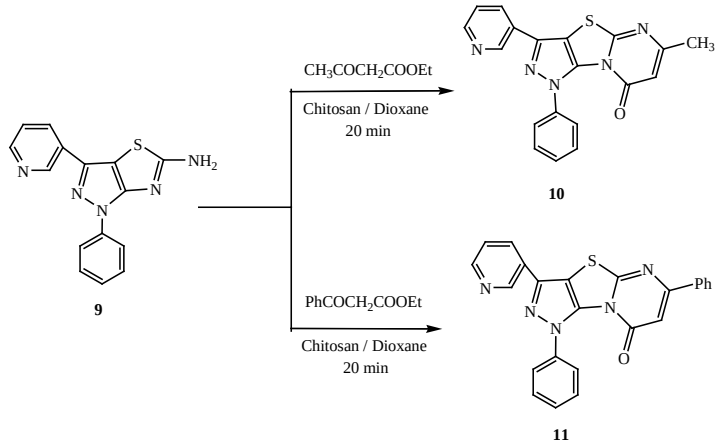
The structure of compounds **7-9** was confirmed by analytical and spectral data. The IR spectram showed bands at ν 3329 - 3354 cm^{-1} (OH, NH_2) and disappearance of *ν* C-Br and ν C=O bands. ^{1}H NMR spectram for compounds **7** and **8** showed a singlet NH proton of imidazole moiety at *δ* 11.23 ppm. All spectroscopic and analytical data are given in experimental part.



Scheme 3 Preparation of 1-phenyl-3-(pyridin-3-yl)-1,4-dihydroimidazo[4,5-*c*]pyrazol-5-ol **7,** 1-phenyl-3-(pyridin-3-yl)-1,4-dihydroimidazo[4,5-c]pyrazol-5-amine **8** and 1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d]thiazol-5-amine **9**

On the other hand, the reaction of compound **9** with some ketoesters named ethyl acetoacetate and ethyl benzoylacetate in dioxane in the presence of chitosan gave the corresponding 5-substituted pyrazol[4,3-*b*] thiazolo[3,2-*a*]pyrimidine-7-ones **10** and **11** respectively (**Scheme 4**).

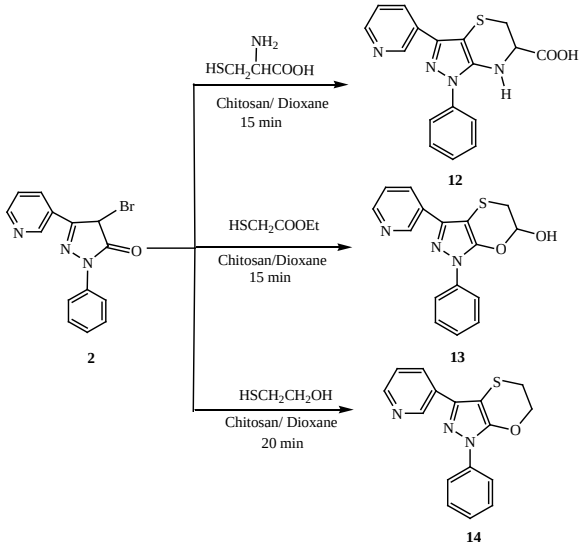
The structure of compounds **10** and **11** was proved on the basis of analytical and spectral data. The IR spectrum showed bands at ν 1677 - 1690 cm^{-1} (C=O), and disappearance of ν (NH_2) group. ^1H NMR spectrum showed a singlet pyrimidine ring proton at δ 6.08 - 6.23 ppm and a singlet for CH_3 protons at δ 2.11 ppm for compound **10**. All spectroscopic and analytical data are given in experimental part.



Scheme 4 Preparation of 5-substituted pyrazol[4,3-b]thiazolo[3,2-a]pyrimidine-7-one **10** and **11**

Also, the reaction of compound **2** with some mercapto derivatives as cysteine, ethyl mercaptoacetate and mercaptoethanol in dioxane in the presence of chitosan as a green catalyst gave the corresponding fused pyrazolo derivatives 1-phenyl-3-(pyridin-3-yl)-1,5,6,7-tetrahydropyrazolo[4,3-*b*][1,4]thiazine-6-carboxylic acid **12**, 1-phenyl-3-(pyridin-3-yl)-5,6-dihydro-1*H*-[1,4]oxathiino[2,3-*c*]pyrazol-6-ol **13** and 1-phenyl-3-(pyridin-3-yl)-5,6-dihydro-1*H*-[1,4]oxathiino[2,3-*c*]pyrazole **14** respectively (**Scheme 5**).

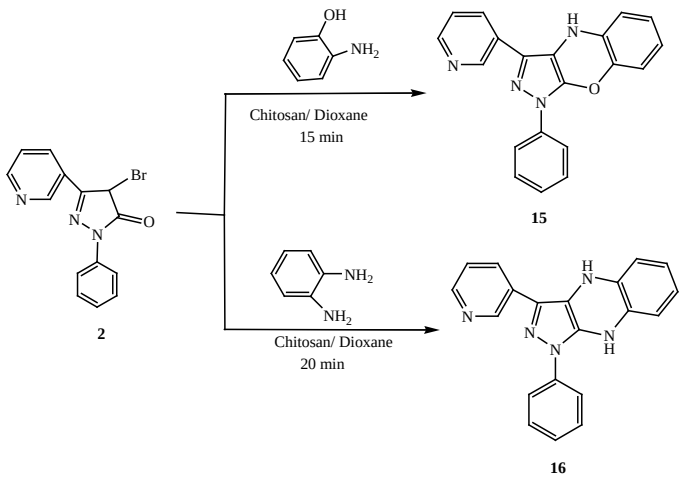
The structure of compounds **12-14** was proved on the basis analytical and spectral data. The IR spectrum of compound **12** showed bands at ν 3342 cm^{-1} (OH), ν 1710 cm^{-1} (C=O) of carboxylic group and ν 675 cm^{-1} (C-S). Also, ^{1}H NMR spectrum showed doublet and triplet for CH_2 and CH of thiazine ring protons at δ 3.44 and 4.20 ppm respectively and a singlet of carboxylic proton at δ 11.23 ppm. The IR spectram of compounds **13** and **14** showed bands at ν 2891, 2884 cm^{-1} (CH_2 aliph. of oxazine ring) respectively. Also, ^{1}H NMR spectrum of compound **13** showed doublet and triplet CH_2 and CH of oxazine ring protons at δ 3.44 and 4.20 ppm respectively, while, The ^{1}H NMR spectrum of compound **14** showed two triplet signals for two CH_2 protons of oxazine ring at δ 3.36 and 4.15 ppm respectively. All spectroscopic and analytical data are given in experimental part.



Scheme 5 Preparation of1-phenyl-3-(pyridin-3-yl)-1,5,6,7-tetrahydropyrazolo[4,3-*b*][1,4]thiazine-6-carboxylic acid **12**, 1-phenyl-3-(pyridin-3-yl)-5,6-dihydro-1*H*-[1,4]oxathiino[2,3-*c*]pyrazol-6-ol **13** and 1-phenyl-3-(pyridin-3-yl)-5,6-dihydro-1H-[1,4]oxathiino[2,3-*c*]pyrazole **14**

Finally, the reaction of compound **2** with *o*-amino phenol and *o*-phenylenediamine in the presence of dioxane gave the pyrazolo[3,4-*b*]benzo(*e*)[1,4] oxazine **15** and pyrazolo[3,4-*b*]quinoxaline **16** respectively (**Scheme 6**).

The structure of compounds **15** and **16** was proved by analytical and spectral data. The IR spectrum showed bands at ν 3346, 3352 cm^{-1} (NH) and disappearance of ν C=O and ν C-Br bands. The ^{1}H NMR spectrum showed multiplet at δ 6.50 - 8.71 and 7.32 - 8.56 ppm respectively corresponding to aromatic and heteroaromatic protons. All spectroscopic and analytical data are given in experimental part.



Scheme 6 Preparation of pyrazolo[3,4-b]benzo(e)[1,4]oxazine **15** and pyrazolo[3,4-b]quinoxaline **16**

The microwave irradiation technique as a source of energy was used. Under this technique interesting results were obtained in which the reaction time was reduced from 4-8 hours to only few minutes (15 - 25 min.) and the yields were increased from 50% - 82% to 80% - 92%. Also, the products obtained are more pure than that obtained by conventional heating procedure (**Table 1**).

3.2. Pharmacology

3.2.1. Antimicrobial Evaluation

Throughout history, there has been a continual battle between humans and the multitude of microorganisms that cause infection and disease. Diseases caused by microbial infection are a serious menace to the health of human beings and often have connection to some other diseases whenever the body system gets debilitated. During the 20th century, vaccines for bacterial toxins and many other common acute viral infections were developed and made widely available.

The antimicrobial properties of the synthesized compounds **7-16** were tested against Gram-negative bacteria (*Klibsella*, *E. coli*, *Serratia* and *Citrobacter*), Gram-positive bacteria (*Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas vulgarus* and *Staphylococcus aureus*) along with the non-filamentous fungus (*Candida albicans*) along with the non-filamentous fungus (*Candida albicans*) as pathogenic bacterial strains. Three different broadly used antibiotics (Amoxycilline, Chlormphenicol and Tetracycline) were used as references.

Reviewing the antimicrobial activity data (**Table 2**), it is concluded that compound **9** and compound **16** is the most active among all the synthesized compounds against most of the tested organisms, while compounds **7**, **8** and **12-15** were found to have slight or moderate activity. It is worthy mentioning that minor change in molecular configuration of these compounds profoundly influences the biological activities.

Minimum inhibitory concentration (MIC) is important in diagnostic laboratories to confirm resistance microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. An MIC is generally regarded as the most basic measurement of the activity of an antimicrobial agent against organism. The present data shows that the most sensitive organism to the tested compound **9** is *Candida albicans*, *Staphylo coccusaures*, *Citrobacter*, *Bacillus cereus* and *E. coli* which showed (MIC) value of 250, 180, 250, 250 and 250 respectively. While, the most sensitive organism to the tested compound **16** is *Candida albicans*, *Bacillus subtilis* and *Pseudonas vulgarus* which showed (MIC) value of 250, 180 and 250 respectively compared with other (MIC) for the other organisms (**Table 3**).

Table 1. Experimental data for the synthesis of compounds **2-8** by traditional methods and microwave assisted methods.

Comp. no	Time		Yield (%)			
	Micro. Irr. (120˚C - 130˚C)	Conv. H. (reflux)	Microwave irradiation		Conventional heating	
3	25 min	8 h	90[a]	87[c]	67[a]	60[c]
4	25 min	8 h	89 - 92[a]	87[c]	65[a]	63[c]
5	25 min	8 h	92[a]	89[c]	62[a]	64[c]
6	25 min	8 h	93 - 95[a]	91[c]	80[a]	77[c]
7	20 min	4 h	90 - 92[b]	88[c]	66[b]	64[c]
8	15 min	4 h	84 - 86[b]	88[c]	58[b]	55[c]
9	20 min	4 h	90 - 92[b]	88[c]	63[b]	66[c]
10	20 min	6 h	92 - 94[a]	92[c]	85[a]	82[c]
11	20 min	6 h	88[a]	85[c]	76[a]	73[c]
12	15 min	4 h	91 - 92[b]	90[c]	63[b]	59[c]
13	15 min	4 h	86 - 88[b]	85[c]	69[b]	66[c]
14	20 min	4 h	82[b]	84[c]	80[b]	76[c]
15	15 min	6 h	75 - 77[b]	80[c]	56[b]	50[c]
16	20 min	6 h	79[b]	82[c]	57[b]	50[c]

[*]Micro. Irr.; microwave irradiation, Conv. H.; conventional heating. [a]piperidine in ethanol, [b]sodium carbonate in diphenyl ether, [c]chitosan in dioxane (green medium).

Table 2. Diameters of inhibition zones (mm) of newly synthesized compounds against different test bacteria and fungi on nutrient agar at 30˚C after 24.

Comp. no.	*Candida albicans*	*Staphylococcus aures*	*Citrobacter*	*Bacillus subtilus*	*Bacillus cereus*	*Klibsella*	*Serratia*	*E. coli*	*Pseudomonas vulgarus*
7	10	−ve	−ve	5	−ve	7	11	−ve	−ve
8	8	−ve	8	−ve	11	−ve	9	−ve	8
9	15	13	15	20	19	20	17	20	9
12	14	−ve	−ve	14	13	15	−ve	−ve	−ve
13	17	−ve	−ve	12	18	17	22	9	−ve
14	20	−ve	12	15	20	10	17	−ve	−ve
15	27	−ve	−ve	16	15	6	14	19	−ve
16	30	17	16	−ve	24	19	16	10	16
Amoxycillin	−ve	−ve	−ve	−ve	−ve	−ve	12	−ve	10
Chlormphe-nicol	21	18	10	16	17	−ve	25	20	−ve
Tetracycline	10	18	−ve	−ve	14	10	−ve	18	−ve

The sensitivity of microorganisms to the tested compounds is identified in the following manner *: Highly sensitive = Inhibition zone: 15 - 20 mm; moderately sensitive = Inhibition zone: 10 - 15 mm; slightly sensitive = Inhibition zone: 1 - 10 mm; Not sensitive = Inhibition zone: 0 mm; *each result represents the average of triplicate readings.

Table 3. Minimal inhibitory concentration (MIC) of the provided samples against test microorganisms (MIC) µg/ml.

Comp. no.	*Candida albicans*	*Staphylo coccusaures*	*Citrobacter*	*Bacillus subtilus*	*Bacillus cereus*	*Klibsella*	*Serratia*	*E. coli*	*Pseudonas vulgarus*
9	250	180	250	500	250	500	1000	250	500
16	250	500	1000	1000	180	500	1000	500	250
Chlorm-phenicol	31.25	62.5	62.5	31.25	31.25	-	31.25	62.5	-

All the dilutions of both samples and standards were performed by double fold dilution.

3.2.2. Antioxidant Screening

Since antioxidants are gaining attention as a potential means of treating a large number of life style diseases like cancer, it is immense significance via a convenient synthetic methodology. The DPPH radical has been widely used to the ability of compounds to behave as been radical scavengers or hydrogen donors. Briefly the assay measures the decrease in absorbance of the DPPH radicals at a characteristic wave length after 60 min incubation of the DPPH radical with different concentrations (from 5 µg/ml to 50 µg/ml) of the antioxidant compounds according to the method of Brand-Williams *et al*. [19]. The absorbance of the reaction mixture was recorded by bcc using a UV visible spectrometer (Genway 6305). L-ascorbic acid (vitamin C) was used as standard antioxidant (positive control). Results are expressed as the percentage of the DPPH free radical scavenging at (five concentrations), each value is expressed as the average of three experiments per concentration ±SD.

Radical of the tested measured and the results were depicted in (**Table 4**) and (**Figure 1**). From DPPH method the range of IC_{50} for compounds (**2-16**) is from 3.2 - 6.6 µg/ml. The highest IC_{50} (6.6 µg/ml) and lowest activity showed in compound **2**. The fused furane, pyrane, imidazole, oxathiine and benzooxine rings with pyrazole ring showed decrease in IC_{50} (4.1 - 5.5) and increase in antioxidant activity. The fused pyrazole ring with thiazole, thiazolopyrimidine and quinoxaline rings showed more decrease in IC_{50} (3.3 - 3.6) and more increase in antioxidant activity. Thiazine carboxylic acid fused with pyrazole ring **12** showed much closed antioxidant reactivity IC_{50} (3.2 µg/ml) compared with standard ascorbic acid due to the free carboxylic group in thiazine ring.

Table 4. Percentage of free radical scavenging activity (DPPH radical) obtained for the tested compounds.

Comp. No.	IC_{50} (μg/ml)
2	6.6
3	4.2
4	5.4
5	5.2
6	5.5
7	5.1
8	5.0
9	3.6
10	4.5
11	3.4
12	3.2
13	4.6
14	4.2
15	4.1
16	3.3
Ascorbic acid	3.2

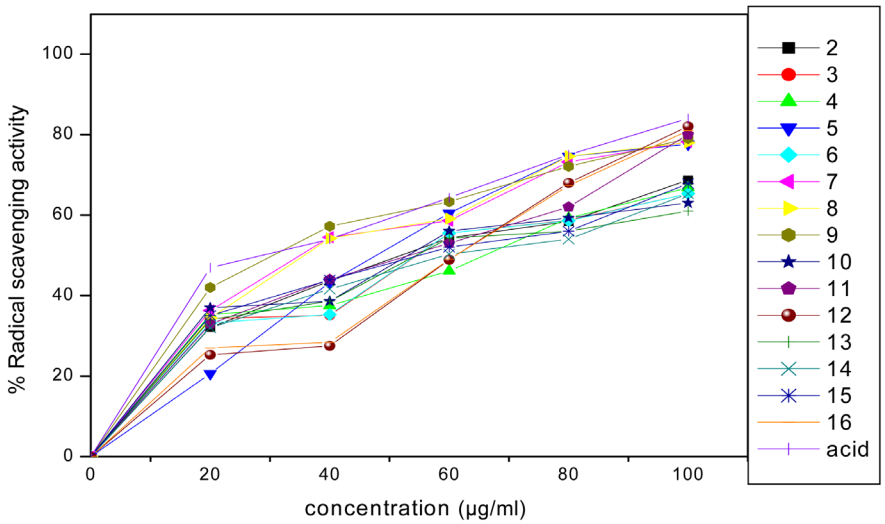


Figure 1. Scavenging antioxidant percentage of the tested compounds.

4. Conclusion

In the present work we could manage to report a new, simple eco-friendly technique for the synthesis of heterocyclic moieties fused with pyrazole system. The antimicrobial screening and antioxidant activity of some synthesized compounds have shown promising activities.

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