



Faculty of Science - University of Benghazi

Science & its Applications

journal home page: www.sc.uob.edu.ly/pages/page/77

Synthesis and antimicrobial evaluation of a novel 1,3,5-trisubstituted Pyrazole derivative

H. Al-Difar, K.M. Darwish*, B.S. Baaiu

Department of Chemistry, University of Benghazi, Benghazi, Libya.

ARTICLE INFO

Article history:

Received 01 March 2017

Revised 26 March 2017

Accepted 31 May 2017

Available online 06 June 2017

Keywords:

Pyrazole, Dinitrophenyl hydrazine, Coupling, 2-Naphthol.

* Corresponding author:

E-mail address: kdarwish1962@gmail.com

K.M. Darwish

ABSTRACT

A novel 1,3,5-trisubstituted pyrazole derivative was synthesized through multistep reaction. Biological screening has been made for each new product of the multistep synthesis, using a gram-positive bacterium and one fungus. Most of the synthesized compounds exhibited comparatively moderate activity against the chosen species using variable concentrations.

© 2017 University of Benghazi. All rights reserved.

1. Introduction

In recent years there has been an increasing interest in the chemistry of pyrazoles because of their biological importance. Their long history of application in the pharmaceutical and agrochemical industries approved their anti-inflammatory (Gokhan-Kelekci et al., 2007), antipyretic (Behr et al., 1967), gastric secretion stimulatory (Rosiere et al., 1951), anti-depressant (Bailey et al., 1985), anti-rheumatoid arthritis (Kurowaski et al., 1987), antibacterial (Mahajan et al., 1991), anticonvulsant (Lepage et al., 1992), antitumor (Lin et al., 2007), antipsychotic (Barcelo et al., 2007), antimicrobial (Farag et al., 2008), antiviral (Larsen et al., 1999), antifungal and anti-filarial agents (Chauhan et al., 1993). Also, serve as herbicides (Dutra et al., 1992), fungicides (Natsume et al., 1992), pesticides (Londers, 1996), dyestuffs (Fahmy et al., 1980) and insecticides (Windholz, 1976). The recent synthetic approach for pyrazole derivatives includes trans-esterification of 1,3-diketoesters (Siddiqui et al., 2013), the use of catalysts such as P_2O_5 - SiO_2 and $Mg(ClO_4)$ (Bamoniri et al., 2012 and Mirjalili et al., 2010) and the use of microwave-assisted methods (Lee et al., 2012) and the use of chalcone intermediates in pyrazole synthesis (Alam et al., 2015 and Deshpande et al., 2015).

2. Experimental

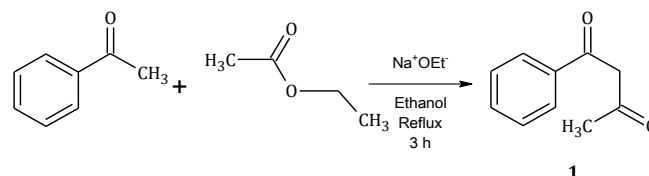
General

All melting points recorded are uncorrected. Chromatography was performed using precoated silica plates with chloroform: methanol (9:1) and the spots were checked under UV-lamp with different wavelengths. The IR spectra for all compounds were recorded using a Pye Unicam SP 1200 spectrophotometer using the KBr wafer technique. The 1H -NMR spectrum was determined for compound 5 on a Varian FT-200, or Bruker AC-200 MHz instrument using TMS as an internal standard. The chemical shifts (δ) are

expressed in ppm. The mass spectra were determined using MP model NS-5988 and Shimadzu single focusing mass spectrometer (70 eV). All solvents used were of HPLC/AnalaR grade. All reagents were used as received from Alfa Aesar.

Synthesis of the diketo derivative 1

Sodium ethoxide was synthesized by refluxing dry sodium (0.5 mol) with absolute ethanol (0.5 mol). Dry ethyl acetate (2 mol) and acetophenone (0.5 mol) were added to the synthesized sodium ethoxide (0.5 mol) with stirring for 2 h. The corresponding sodium salt of the diketo derivative 1.



Scheme 1: Synthesis of Diketo derivative 1

1-phenylbutane-1,3-dione 1

Yield 48%, m.p. 131-134°C. Anal. for $C_{10}H_{10}O_2$ (m.w. 162): found: C, 73.99; H, 6.17; Calcd: C, 74.07; H, 6.13; IR ν (cm^{-1}) (KBr pellet) (Aliphatic) C-H str, 1418 cm^{-1} ; (Ar) C-H str, 3010 cm^{-1} ; C=O str, 1610 cm^{-1} . MS m/z : 147 (M^+ peak).

1-(2,4-dinitrophenyl)-3-methyl-5-phenyl-1H-pyrazole 2

Yield 72%, m.p. 122-123 °C. Anal. for $C_{16}H_{12}N_4O_4$ (m.w. 324): found: C, 59.26; H, 3.70; N, 17.31; Calcd: C, 59.34; H, 3.67; N, 17.13; IR ν (cm^{-1}) (KBr pellet) (Aliph.) C-H str 1436 cm^{-1} ; (Ar) C-H str, 3016 cm^{-1} ; (Ar) C-H_{ortho}, 742 cm^{-1} ; (Ar) C-H_{meta}, 692, 782 cm^{-1} ; (Ar) N=O str 1536.37; MS m/z : 325 (M^+ peak).

4-(3-methyl-5-phenyl-1H-pyrazol-1-yl)benzene-1,3-diamine **3**

Yield 48%, m.p. 111-112 °C. Anal. for $C_{16}H_{16}N_4$ (m.w. 264): found: C, 72.73; H, 6.06; N, 21.22; Calcd: C, 73.01; H, 6.02; N, 21.20; IR ν (cm^{-1}) (KBr pellet) (Aliph.) C-H str 1432 cm^{-1} ; (Ar) C-H str, 3076 cm^{-1} ; (Ar) N-H, 1677, 3444 cm^{-1} ; MS m/z : 265 (M^+ peak).

Diazotization derivative of the 4-(3-methyl-5-phenyl-1H-pyrazol-1-yl)benzene-1,3-diamine **4**

Yield 56%, m.p. 80-83 °C. Anal. for $C_{16}H_{12}N_6Cl_2$ (m.w. 358): found: C, 53.63; H, 3.35; N, 23.47; Calcd: C, 53.69; H, 3.32; N, 24.01; IR ν (cm^{-1}) (KBr pellet) (Aliph.) C-H str 1424 cm^{-1} ; (Ar) C-H str, 3083 cm^{-1} ; (Ar) N $\equiv$ N str, 2255 cm^{-1} ; MS m/z : 232.28 (M^+ peak).

Coupling product compound **5**

Red crystals. Yield 80%, m.p. 90-93 °C. Anal. for $C_{36}H_{26}N_6O_2$ (m.w. 574): found: C, 75.26; H, 4.53, N, 14.63; Calcd: C, 75.06; H, 4.58; N, 14.66; IR ν (cm^{-1}) (KBr pellet) (Aliph.) C-H str 1427 cm^{-1} ; (Ar) C-H str, 3110 cm^{-1} ; O-H, 3310 cm^{-1} ; N=N, 1440 cm^{-1} ; C-N, 1320 cm^{-1} ; C=O, 1140 cm^{-1} . 1H NMR (DMSO- d_6) δ 11.56 (s, naphthyl-OH), 1.7 (s, 3H, CH_3); 7.14-7.80 (m, 21H; aromatic-H). MS m/z : 574 (M^+ peak).

3. Antimicrobial Activity

3.1 Antifungal Activity

A known weight of each compound **1-5** in dimethyl sulphoxide (DMSO) as solvent was taken and diluted suitably to give the resultant concentrations of 10, 50, 100 $\mu g/mL$. Whatmann No. 1 sterile paper discs (6 mm) were impregnated with solution and allowed to dry at room temperature. In vitro antifungal activity was determined by using Sabouraud Dextrose Agar obtained from Himedia Ltd/Mumbai. Twenty-four hours old culture of the selected fungus, *Pseudomonas* was mixed with physiological saline and the turbidity was corrected by adding sterile physiological saline and sub cultured on Sabouraud Dextrose and suspended in sterile

distilled water to an absorbance of 0.6 at 450 nm. Petri plates were prepared by pouring 10 mL Sabouraud Dextrose Agar for fungi containing microbial culture and was allowed to solidify. The discs were then applied and the plates were incubated at 28°C for 72-96 h (fungi) and the inhibition zone was measured in four directions and expressed as mean and the results were compared by using Kanamycin as antifungal standard.

3.2 Antibacterial Activity

As the sensitivity was not observed at conc. <0.01 mg/mL, the antibacterial activity of the test compounds has been screened at concentration more than 0.1 mg/mL using dimethyl sulphoxide (DMSO) as solvent and chloramphenicol (100 $\mu g/mL$) as standard for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* in Mueller Hinton Agar by using cup plate agar diffusion method (Kawangh, 1963 and British Pharmacopeia, 1998). 10 mL of this sterilized agar media were poured into Petri-dishes and allowed to solidify. On the surface of media microbial suspension were spread with the help of sterilized triangular loop. A stainless steel cylinder of 10 mm diameter (pre-sterilized) was used to bore the cavity. Into these wells were added 0.1 mL portion of the test compound in the solvent. The drug solution was allowed to diffuse for about an hour into the medium. The plates were incubated at 37°C for 24 h. Zone of inhibition observed around the cup after respective incubation was measured with the help of Vernier Calipers. The results of antifungal and antibacterial activities are given in the Table 1.

Concentrations less than 10 $\mu g/mL$ (0.1 mg/ml) have shown poor activity towards all the species used. Increasing the concentration to 50 $\mu g/mL$, using DMSO as a solvent and both Kanamycin and Chloramphenicol as standards, have shown moderately acceptable results as antifungal derivatives and were highly active as antibacterial.

Table 1: Results of Antimicrobial activity of the Tested compounds

Compound $\mu g/mL$	Concentration	Diameter of zone of inhibition, mm		
		Gram +ve Bacteria	Gram -ve Bacteria	Fungus
		<i>S. aureus</i>	<i>E. coli</i>	<i>Pseudomonas</i>
1	10	+++	+++	++
	50	—	—	—
2	10	+++	+++	++
	50	—	—	—
3	10	+++	+++	++
	50	—	—	—
4	10	+++	+++	++
	50	—	—	—
5	10	+++	+++	++
	50	—	—	—
Kanamycin	10	+++	+++	++
Chloramphenicol	10	—	—	—
Solvent (DMSO)	10% v/v	—	—	—

Highly active = +++ (zone of inhibition >12 mm), moderately active = ++ (zone of inhibition > 9-12 mm), slightly active = + (zone of inhibition > 7-9 mm), inactive = - (zone of inhibition < 7 mm).

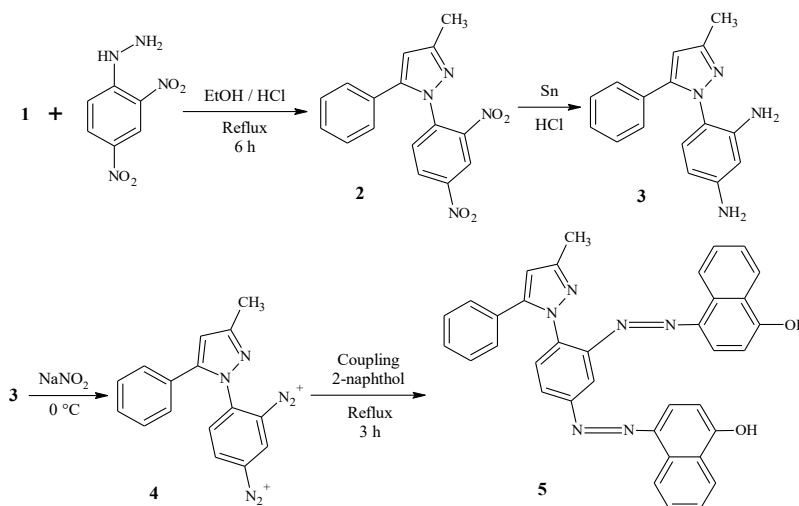
4. Results and Discussion

Identification of the isolated compounds **1-5** was mostly supported by the IR spectra. For example, the IR spectrum (in KBr) of compound **1** revealed the presence of an absorption band at ν 1610 cm^{-1} due to the two C=O groups whereas the IR spectrum of compound **2** showed the absence of this band and the presence of another band at 1536 cm^{-1} due to the stretching

vibration of the two aromatic nitro groups. Also the mass spectra of compound **1** showed an absorption peak at m/z : 147 whereas compound **2** mass spectra showed a peak at m/z : 325. On the other hand, the IR spectrum of compound **3** showed two absorptions at 1677 and 3444 cm^{-1} relative to the presence of two amino groups. These readings disappeared upon checking the IR spectrum of the diazotization product (compound **4**) which showed an absorption at 2255 cm^{-1} due to the N $\equiv$ N

stretching vibration. The mass spectra for compounds **3** and **4** showed absorption peaks at m/z : 265 and m/z : 232.28 respectively. Compound **5** was identified first by IR spectrum which revealed the presence of the two O-H and N=N absorption bands at 3310 and 1440 cm^{-1} respectively. In addition, this was supported by the ^1H NMR spectra (in $\text{DMSO-}d_6$) which showed

one singlet-peak at $\delta = 11.56$ ppm relative to the naphthyl-OH group, another singlet-peak at $\delta = 1.7$ ppm relative to the 3-H atoms of CH_3 group and multiplet-peak at $\delta = 7.14\text{--}7.80$ ppm for 21 aromatic-H. In addition, the identification of these derivatives was supported by the mass spectra showing an absorption peak at m/z : 574.



Scheme 2: Synthesis of the Coupling Product **5**

5. Conclusion

The structure of the newly synthesized dye (compound **5**) was confirmed by ^1H NMR spectra. The biological screenings of all the synthesized derivatives were carried out against two bacteria and one fungus and it was concluded that the tested compounds revealed the antibacterial activity much better than their antifungal activities at comparatively higher concentrations.

References

- Alam, F. and Dey, B. K. (2015) *J. of Glob. Trends in Pharm. Sci.*, 6 (1), pp. 2411-2416.
- Bailey, D. M., Hansen, P. E., Hlavac, A. G., et al., (1985) *J. Med. Chem.*, 28, pp. 256.
- Bamoniri, A. and Mirjalili, B. F. (2012) *Iranian J. of Catalysis*, 2 (2), pp. 75-78.
- Barcelo, M., et al., (2007) *Bioorg. Med. Chem. Lett.*, 17, pp. 4873.
- Behr, L. C., Fusco, R., Jarboe, C. H. (1967) In: *The Chemistry of Heterocyclic Compounds, Pyrazoles, Pyrazolines, Pyrazolidines, Indazoles and Condensed Rings*; Weissberger, A., Ed.; Interscience Publishers: New York, p 1.
- British Pharmacopeia – II (1998) *Biological Assay and Tests*. The Stationary Office Ltd.: London; p A-205.
- Chauhan, P. M. S., et al., (1993) *Indian J. Chem. B.*, 32, pp. 858.
- Deshpande, S. R. and Nagrale, S. N. (2015) *Indian J. Pharm. Sci.*, 77 (1), pp. 24-33.
- Dutra, G. A., et al., (1992) *PCT Int. Appl.*, WO 8206, 962; *Chem. Abstr.*, 117, 69859.
- Fahmy, B. S. M. and Elnagdi, M. H. (1980) *J. Chem Tech. B: Technol.*, 30.
- Farag, A. M., et al., (2008) *Bioorg. Med. Chem.*, 16, pp. 4569.
- Gokhan-Kelekci, N., Yabanoglu, S., Kupeli, E., et al., (2007) *Bioorg. Med. Chem.*, 15, pp. 5775.
- Kawangh, F. (1963) *Analytical Microbiol.*, Academic Press: New York.
- Kurowaski, M., Dunky, A., and Geddawi, M. (1987) *Eur. J. Clin. Pharmacol.*, 307, pp. 31.
- Larsen, J. S., et al., (1999) *Monatsh. Chem.*, 130, pp. 1167.
- Lee, J. and Hong, M. (2012) *Tetrahedron*, 68, pp. 2045-2051.
- Lepage, F. and Hubiot, B. (1992) *Eur. Pat. Appl. EP*, 459, 887 *Chem. Abstr.*, 116, 128914.
- Lin, R., et al., (2007) *Bioorg. Med. Chem. Lett.*, 17, pp. 4557.
- Londershausen, M. (1996) *Pestic. Sci.*, 48, pp. 269-292.
- Mahajan, N., Havaladar, F. H., and Fernandes, P. S. (1991) *J. Indian Chem. Soc.*, 68, pp. 245.
- Mirjalili, B. F. and Bamoniri, A. (2010) *Digest J. of Nanomaterials and Biostructures*, 5 (4), pp. 897-902.
- Natsume, B., et al., (1992) *Eur. Pat. Appl. EP*, 462, 573; *Chem. Abstr.*, 116, pp. 128.
- Rosiere, C.E. and Grossman, M. I. (1951) *Science*, 113, pp. 651.
- Siddiqui, N.J. and Idrees, M. (2013) *Bull. Chem. Soc. Ethiop.*, 27 (1), pp. 85-94.
- Windholz, M. (1976) *The Merck Index*, 9th ed.; Merck and Co., Rahway: New Jersey, 8851.