Synthesis, Antimicrobial, DPPH Radical Scavenging Assays and Molecular Docking Study of New Series *N*-Phenylpyrazoline Derivatives

Media Noori Abdullah[1]

[1]Chemistry Department, College of Science, Salahaddin University, Erbil, Iraq
Correspondence: Media Noori Abdullah, Salahaddin University, Erbil, Iraq.
Email: media.abdullah@su.edu.krd

Doi: 10.23918/eajse.v7i1p1

Abstract: New candidates of *N*-phenylpyrazoline derivatives were designed combining the Indole moiety to explore its potential as antimicrobial and antioxidant activity. The starting material synthesis was achieved through One-pot three-component reaction; at that point, it was acylated and interacted with various substituted benzaldehydes to result in new chalcones that were later cyclized to *N*-phenylpyrazoline, through reaction with phenyl hydrazine. The structures of the synthesized compounds were reinforced by their spectral data. The final compounds were screened for antibacterial activity against resistant strains of *Staphylococcus aureus* and *Escherichia coli* microorganisms; as a result, it showed that the compounds with methoxy group at position *meta* and hydroxy at position *ortho* were the highly active derivatives. Meanwhile, the highly active compound with antifungal activity against *C. Albicans*, had nitro group at *para* position. During the evaluation of proton donating antioxidant activity, using DPPH radical scavenging assays method; it was exposed that, the derivatives with highly active antibacterial had the most antioxidant activity. In the final step, the molecular docking study exposed that the compound with highly active antibacterial and antioxidant activity was the most potent derivative. The resulting studies disclosed that the synthesized *N*-phenylpyrazoline derivatives displayed less specificity than the reference compounds.

Keywords: One Pot Reaction, Chalcone, N-Phenylpyrazoline, Antimicrobial, Dpph Scavenging Activity, Molecular Docking Study

1. Introduction

The discovery of new and effective drugs is still a top priority in the synthesis. Indoles is a scaffold that commonly appears in natural products and synthesized compounds (Singh & Singh, 2018). Acylated indoles are important structural motifs present in the natural products and serve as valuable intermediates in the synthesis of various dyes, pharmaceuticals (Grimster et al., 2005), alkaloids, indole derivatives, and several heterocycles. The general methods for the introduction of an acyl substituent at C-2 of pyrroles include reactions with acid chlorides (Katritzky et al., 2003, Ottoni et al., 2001).

Electrophilic substitution in indoles occurs predominantly in the β-position, and when this is blocked, either at the nitrogen or at the α-position (Ibrahim, 2007). 3-acylindoles was prepared regioselectivity and in high yields, without protection of the NH position of Indoles using a green method (Tran et al., 2015).

Received: January 13, 2021
Accepted: April 14, 2021
Media Noori Abdullah, M.N. (2021). Synthesis, Antimicrobial, DPPH Radical Scavenging Assays and Molecular Docking Study of New Series *N*-Phenylpyrazoline Derivatives. *Eurasian Journal of Science & Engineering*, 791), 1-24.

The chemistry of chalcones have generated intensive scientific studies in the world (Zhuang et al., 2017). Chalcones are formed from the reaction of ketone with aldehyde in the presence of alkaline or acid catalyst (Gomes et al., 2017). Chalcones are open chain flavonoids which include the reactive keto-ethylenic group –CO–CH=CH– (Gaonkar and Vignesh, 2017) that is active in synthesizing various heterocyclic compounds (Attarde et al., 2014). The chalcones that containing indole moiety are evaluated several biological activities such as induced methuosis in glioblastoma, anti-cancer (Robinson et al., 2012), anti-proliferative (Cong et al., 2018), anti-inflammatory, antioxidant (Özdemir et al., 2015), anti-bacterial (Subhashini, 2015, Sayed et al., 2018) and antifungal activities (Singh et al., 2013).

N-Phenylpyrazoline derivatives are prepared from the reaction of chalcones with phenyl hydrazine (Lévai and Jeko, 2007, Safaei-Ghomi et al., 2006). Indole-based chalcone derivatives are useful precursors for the synthesis of different heterocyclic compounds such as pyrazoline rings. They are a versatile lead compounds to design potent bioactive molecules for drug discovery and development (Rahman and Siddiqui, 2010) particularly in cancer therapy and these compounds which containing indole in their core of the skeleton have a broad spectrum of biological and pharmacological activity (Biswal et al., 2012) as antimicrobial and anti-cancer (Dawood et al., 2020, El-Sawy et al., 2012), antibacterial and antifungal (Mowlana and Nasser, 2015). Investigation showed that these types of heterocyclic compounds presented remarkable properties and have the highest antibacterial activity against all strains of bacteria with value higher than those of the corresponding reference antibiotics, ciprofloxacin and levofloxacin (Sayed et al., 2018). Consequently, various substituted *N*-phenylpyrazoline derivatives have been synthesized and investigated for their biological activities. Therefore, the objective of the current study is to investigate the *in vitro* antimicrobial and DPPH scavenging activity of new series of synthesized *N*-phenylpyrazoline, combined with indole nucleus, to show the consequence of the shared heterocyclic ring systems in one molecule, on the reactivity, along with study their molecular docking activity.

2. Experimental Section

Melting points were determined in open capillaries using an Electro thermal instrument. The progress of the reactions was monitored by TLC on aluminum plates coated with silica gel-G in a (55:25:20) n-hexanes: ethanol: ethyl acetate system. FT-IR ranges have been documented on a spectrometer (Thermo Fisher FT-IR Model: Nicolet iS 10. Ultrasonic cleaner (DIGITAL PRO+, 40 KHz and a normal power 180 W) and Spectrophotometer (JENWAY Model: 6705) were used. 1H- and 13C-NMR spectra are recorded on a Bruker AM 400 instruments (400 MHz) using DMSO-d6 as solvent, Chemical shifts were assessed in parts per million (δ ppm). Microanalyses were made on a Perkinelmer 2400 CHN analyzer. The GC-MS analyses were carried out in a Hewlett Packard 5890II chromatograph, using a TRB-I (100% methyl polysiloxane, 30 m x 0.25 mm x 0.25 μm) column. The obtained data are expressed in mass unit (m/z) and the values among the parenthesis correspond to the relative intensities regarding to the base peak (100%). The abbreviations were used s=singlet, d=doublet, t=triplet, m=multiplet, Ar=Aromatic, J= coupling constant and str. = Stretching.

2.1 General method for the Synthesis of N-((1H-indol-3-yl)(phenyl)methyl)-N-methylaniline (4) by one pot three components reaction (Devi et al., 2012).

In a typical experimentation, benzaldehyde (1) (5 mmol, 0.5 mL) was added to N-methyl aniline (2) (6 mmol, 0.64 mL) in a 20-mL round-bottomed flask; 5 mL of ethanol and a catalytic amount of ZnCl2 (10 mol %) were added. The reaction mixture was stirred at room temperature for 10 minutes. Then (5

mmol, 0.585 g) of indole (3) was added, kept stirred at room temperature for 1 hour. The progress of the reaction was monitored by TLC, (3x5 mL) of ethyl acetate was added to the reaction mixture after completion. The mixture was filtered; ZnCl2 was removed, and the solvent was evaporated under reduced pressure. The precipitate was purified through silica-gel column chromatography to produce pure product. Ethyl acetate: n-hexane (1:4) was used as eluents.

2.1.1 N-((1H-indol-3-yl) (phenyl)methyl)-N-methylaniline (4)

Chemical Formula= C22H20N2, brown color, 90% yield, m.p. =146-147 oC. FT-IR (cm-1): 3362 (NHstr.), 3022 (CHAr), 1614 (C=CAr). 1H-NMR (400MHz, DMSO-d6): δ 10.87 (1H, s, NH), 7.39-6.48 (m, 14HAr), 6.70 (1H, s, NHCH), 5.51 (1H, s, CH), 3.42 (3H, s, CH3). 13C-NMR (400MHz, DMSO-d6): δ 148.62, 137.19, 136.72, 129.51, 128.96, 128.54, 127.08, 126.22 (NHCH), 124.43, 121.43, 119.71, 119.19, 118.66, 112.97, 111.91, 70.93 (CH), 35.37 (CH3).

2.2 General Method for the Synthesis of 1-(3-((methyl(phenyl) amino)(phenyl)methyl)-1H-indol-2-yl)ethanone (5) (Katritzky et al., 2003; Ottoni et al., 2001) modified.

Compound (4) (0.1 mole, 0.312g) dissolved in 10 mL of acetonitrile, catalyzed by TiCl4 (10 mole %), then acetyl chloride (0.1 mol) was added dropwise at 0 °C with stirring for 30 minutes. The organic phase was extracted with ethyl acetate (15 mL), washed with water (5x15 mL) and dried over Na2SO4 and concentrated to give a crystalline solid compound (5). The product was purified through silica-gel column chromatography, using n-hexane: ethyl acetate mixture (7:3) as the mobile phase.

2.2.1 1-(3-((methyl(phenyl)amino) (phenyl)methyl)-1H-indol-2-yl) ethanone (5)

Chemical Formula = C24H22N2O, rusty color, 65% yield, m.p. =111-112 oC. FT-IR (cm-1): 3264 (NH str.), 3025 (CH Ar. str.), 1661 (CO sym. str.), 1602 (C=C Ar. str.). 1H-NMR (400MHz, DMSO-d6): δ 10.97 (1H, s, NH), 7.40 (2H, d, J=20 Hz, H4, H7), 7.32-7.27 (4H, m, H2a, H3a, H5a, H6a),7.26-7.21 (4H, m, H6, H4a, H5b, H3b), 7.15 (2H, d, H6b, H2b, J= 20 Hz), 7.07 (1H, t, H5, J= 37, 19 Hz), 6.88 (1H, t, H4b, J=37, 19 Hz), 5.78 (1H, s, CH), 3.21 (3H, s, NCH3), 1.78 (1H, s, CH3CO). 13C-NMR (400MHz, DMSO-d6): δ 169.56 (C=O), 144.52 (C1b), 143.93 (C7'), 142.79 (C1a), 137.17 (C2), 130.10 (C3'), 129.51, 129.05 (C5a, C3b, C5b), 128.80 (C2a, C6a, C3a), 127.31 (C3), 126.90 (C4b), 126.67 (C4a), 124.63 (C6), 121.64 (C5), 119.47 (C4), 118.89 (C6b), 118.03 (C2b), 112.05 (C7), 68.06 (CH), 36.96 (NCH3), 25.68 (COCH3).

2.3 General procedure for the synthesis of Chalcones (7a-g) (Ahmad et al., 2016)

Equimolar quantities (10 mmol) of compound (5) and substituted benzaldehyde (6a-g) (10 mmol) were dissolved in 15 mL of ethanol. Aliquot of a 4% ethanolic NaOH solution was added slowly to the reaction flask. The reaction mixture was allowed to stir at room temperature for approximately 24 hrs. Completion of the reaction was monitored by TLC plates. The mixture was poured into ice water and neutralized by HCl. The precipitate was filtered, dried and purified through silica-gel column chromatography to produce pure products (7a-g) using n-hexane: ethyl acetate mixture (3:2) as the mobile phase.

2.3.1 (E)-1-(3-((methyl(phenyl)amino) (phenyl)methyl)-1H-indol-2-yl)-3-phenylprop-2-en-1-one (7a)

Chemical Formula = C31H26N2O, deep purple color, 76 % yield, m.p. = 95-96oC. FT-IR (cm-1): 3258 (NHstr.), 1640 (C=Osym. str.), 1592 (C=Colefin), 1506, 1451(C=Car.str.). 1H-NMR (400MHz, DMSO-d6): δ 10.18 (1H, s, NH), 8.26 (1H, d, Hbeta, J=16 Hz) 8.12-7.18 (m, 19Har.), 5.85 (1H, d,

Halpha, J=16.4 Hz), 4.80 (1H, s, Ar-CH), 3.41 (1H, s, N-CH3). 13C-NMR (400MHz, DMSO-d6): δ 170.24 (CO), 154.50-115.75 (26Car.), 137.25 (Cbeta), 121.73 (Calpha), 70.65 (CH-N), 35.10 (N-CH3).

2.3.2 (Z)-3-(2-hydroxy-3-methoxyphenyl)-1-(3-((methyl(phenyl) amino) (phenyl)methyl)-1H-indol-2-yl) prop-2-en-1-one (7b)

Chemical Formula = C32H28N2O3, dark brown color, 80 % yield, m.p. = 89-90 oC. FT-IR (cm-1): 3384 (OH), 3204 (NH str.), 1635 (C=Osym. str.), 1602 (C=Colefin), 1506, 1494 (C=Car. str.), 1218 (C-O). 1H-NMR (400MHz, DMSO-d6): δ 10.99 (1H, s, NH), 10.33 (1H, s, OH), 7.43-6.79 (m, 17HAr.), 7.42 (1H, d, Hbeta, J=8 Hz), 5.75 (1H, d, Halpha, J=8 Hz), 4.87 (1H, s, Ar-CH), 3.97 (3H, s, O-CH3), 3.15 (3H, s, N-CH3).13C-NMR (400MHz, DMSO-d6): δ 176.57 (CO), 151.26-112.07 (26CAr.), 137.21 (Cbeta), 121.66 (Calpha), 65.53 (CH-N), 48.12 (O-CH3), 36.97 (N-CH3).

2.3.3 (Z)-3-(2-hydroxyphenyl)-1-(3-((methyl(phenyl)amino) (phenyl) methyl)-1H-indol-2-yl) prop-2-en-1-one (7c).

Chemical Formula = C31H26N2O2, deep brown color, 79% yield, m.p. = 69-70 oC. FT-IR (cm-1): 3401 (OH), 3259 (NHstr.), 1641 (C=Osym. str.), 1600 (C=Colefin), 1506, 1487 (C=Car.str.). 1H-NMR (400MHz, DMSO-d6): δ 10.01 (1H, s, NH), 9.15 (1H, s, OH), 7.95-7.14 (m, 18Har.), 7.57 (1H, d, Hbeta, J=8.4 Hz), 6.34 (1H, d, Halpha, J=8.8 Hz), 5.29 (1H, s, Ar-CH), 3.25 (3H, s, N-CH3). 13C-NMR (400MHz, DMSO-d6): δ ppm = 171.31 (CO), 150.78-111.95 (26 Car.), 137.70 (Cbeta), 121.73 (Calpha), 63.28 (CH-N), 33.34(N-CH3).

2.3.4 (E)-3-(4-methoxyphenyl)-1-(3-((methyl(phenyl)amino) (phenyl) methyl)-1H-indol-2-yl) prop-2-en-1-one (7d).

Chemical Formula = C32H28N2O2, reddish-brown color, 74% yield, m.p. = 85-86 oC. FT-IR (cm-1): 3256 (NHstr.), 1633 (C=Osym. str.), 1506, 1452 (C=Car. str.), 1598 (C=Colefin). 1H-NMR (400MHz, DMSO-d6): δ 11.00 (1H, s, NH), 7.91-6.89 (m, 18Har.), 7.42 (1H, d, Hbeta, J= 16 Hz), 6.97 (1H, d, Halpha, J= 16.4 Hz), 3.90 (1H, s, Ar-CH), 3.15 (3H, s, N-CH3), 1.78 (s, 3H, OCH3). 13C-NMR (400MHz, DMSO-d6): δ 174.56 (CO), 164.73 (26Car.), 143.95 (Cbeta), 121.64-112.07 (Calpha), 67.15 (CH-N), 48.09 (O-CH3), 35.96 (N-CH3).

2.3.5 (Z)-3-(2-chlorophenyl)-1-(3-((methyl(phenyl)amino) (phenyl) methyl)-1H-indol-2-yl) prop-2-en-1-one (7e).

Chemical Formula = C31H25ClN2O, orangish yellow color, 82% yield, m.p. 91-92 oC. FT-IR (cm-1): 3273 (NHstr.), 1634 (C=Osym. str.), 1602 (C=Colefin), 1506, 1494 (C=Car. str.). 1H-NMR (400MHz, DMSO-d6): δ 11.09 (1H, s, NH), 7.61-6.50 (m, 18Har.), 7.41 (1H, d, Hbeta, J=8 Hz), 5.74 (1H, d, Halpha, J=8.4Hz), 4.62 (1H, s, Ar-CH), 3.15 (3H, s, N-CH3). 13C-NMR (400MHz, DMSO-d6): δ 169.60 (CO), 148.62-111.96 (26Car.), 140.08 (Cbeta), 121.61 (Calpha), 60.72 (CH-N), 36.97 (N-CH3).

2.3.6 (Z)-3-(4-fluorophenyl)-1-(3-((methyl(phenyl)amino) (phenyl) methyl)-1H-indol-2-yl) prop-2-en-1-one (7f).

Chemical Formula = C31H25FN2O, reddish-brown color, 84 % yield, m.p. = 94-95 oC. FT-IR (cm-1): 3269 (NHstr.), 1634 (C=Osym. str.), 1506, 1450 (C=Car. str.), 1598 (C=Colefin), 741 (-F). 1H-NMR (400MHz, DMSO-d6): δ 10.98 (1H, s, NH), 7.88 (1H, d, Hbeta, J=8.4 Hz), 7.40-6.89 (m, 18Har.), 5.74 (1H, d, Halpha, J=7.6 Hz), 4.13 (1H, s, Ar-CH), 3.15 (3H, s, N-CH3). 13C-NMR

(400MHz, DMSO-d6): δ 171.95 (CO), 161.53-112.60 (26 Car.), 138.26 (Cbeta), 121.83 (Calpha), 71.11 (CH-N), 34.35 (N-CH3).

2.3.7. (E)-1-(3-((methyl(phenyl)amino) (phenyl)methyl)-1H-indol-2-yl)-3-(4-nitrophenyl) prop-2-en-1-one (7g)

Chemical Formula = C31H25N3O3, bloody color; 86 % yield, m.p. = 82-83 oC. FT-IR (cm-1): 3269 (NHstr.), 1633 (C=Osym. str.), 1601(C=Colefin), 1506, 1494 (C=Car. str.), 1341 (NO2). 1H-NMR (400MHz, DMSO-d6): δ 10.99 (1H, s, NH), 7.65 (1H, d, Hbeta, J=16.4 Hz), 7.42-6.88 (m, 18Har.), 5.75 (1H, d, Halpha, J=16 Hz), 4.70 (1H, s, Ar-CH), 3.15 (3H, s, N-CH3). 13C-NMR (400MHz, DMSO-d6): 172.56 (CO), 151.31-111.91 (26Car.), 141.11 (Cbeta), 121.44 (Calpha), 62.50 (CH-N), 34.96 (N-CH3).

2.4 General procedure for the synthesis of N-phenylpyrazoline derivatives (8a-g) (Gupta et al., 2010)

A chalcone derivatives (7a-g) (10 mmol), phenylhydrazine (10 mmol) dissolved in (10 mL) ethanol, and a catalytic amount of glacial acetic acid (10mol %) was added. Sonication of the mixture in ultrasound bath to get the maximum energy until the precipitate appeared and the reaction completed. Completion of the reactions monitored by TLC; the reaction mixture was poured into crushed ice. The precipitate was isolated; recrystallization with ethanol was done, then the products were purified by silica gel preparative thin layer chromatography (PTLC) using n-hexanes: ethyl acetate (4:1) as eluent to afford corresponding N-phenylpyrazoline derivatives (8a-g).

2.4.1 N-((2-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-indol-3-yl) (phenyl)methyl)-N-methylaniline (8a).

Dark brown color, 68% yield, m.p. = 84-85 oC. FT-IR (cm-1): 3270 (NH str.), 3056 (C=C-Hstr. ar.), 1602 (C=Nstr. pyrazoline ring), 1339 (CH2 Pyrazoline ring), 1249 (CN). 1H-NMR (400MHz, DMSO-d6): δ 10.90 (1H, s, NH), 8.52-6.66 (m, 24Har.), 5.55 (1H, s, Ar-CH), 4.67-4.59 (1H, dd, H5 pyrazoline ring, J=11.2 Hz, J=10.4 Hz), 3.70-3.55 (1H, dd, H4b pyrazoline ring, J= 8.8 Hz, J=9.6 Hz,), 3.27-3.13 (1H, dd, H4a pyrazoline ring, J= 7.6 Hz, J= 8.8 Hz), 2.91 (3H, s, N-CH3). 13C-NMR (400MHz, DMSO-d6): δ 145.22 (C3 pyrazoline ring), 161.90 -114.86 (32Car), 73.76 (Ar-CH), 57.13 (C5pyrazoline ring), 43.57 (N-CH3), 32.87 (C4 pyrazoline ring). MS: m/z Calculated for C37H32N4: 532.26, Found: 532.19. CHN-analysis: Calculated: %C=83.43, %H=6.06, %N=10.52; Found: %C=83.42, %H=6.05, %N=10.53.

2.4.2 Methoxy-6-(3-(3-((methyl(phenyl)amino) (phenyl)methyl)-1H-indol-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl) phenol (8b).

Dark coffee color, 60% yield, m.p. = 77-78 oC. FT-IR (cm-1): 3403(OH str.), 3271 (NH str.), 3056 (C=C-H str. Ar.), 1601 (C=N str. pyrazoline ring), 1339 (CH2 Pyrazoline ring), 1251(C-N). 1H-NMR (400MHz, DMSO-d6): δ 10.99 (1H, s, NH), 9.71 (1H, s, OH), 7.42-6.75 (m, 22HAr.), 5.75 (1H, s, Ar-CH), 4.04 (1H, t, H5 pyrazoline ring, J=6.8 Hz, J=14.4 Hz), 3.22-3.11 (1H, dd, H4b pyrazoline ring, J= 9.2 Hz, J=9.2 Hz), 3.01 (3H, s, O-CH3), 2.94-2.81(1H, dd, H4a pyrazoline ring, J= 8.4 Hz, J=8.4 Hz), 2.53 (3H, s, N-CH3). 13C-NMR (400MHz, DMSO-d6): δ 146.07 (C3 pyrazoline ring), 158.54-112.06 (32CAr), 70.86 (Ar-CH), 56.26 (C5 pyrazoline ring), 48.09 (O-CH3), 42.67 (N-CH3), 34.01 (C4 pyrazoline ring). MS: m/z Calculated for C38H34N4O2: 578.27, Found: 578.35. CHN-analysis: Calculated %C=78.87, %H=5.92, %N=9.68, Found: %C= 78.94, %H=5.89, %N=9.65.

2.4.3 2-(3-(3-((methyl(phenyl)amino) (phenyl)methyl)-1H-indol-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl) phenol (8c).

Milk coffee color, 80% yield, m.p. = 100-101 oC. FT-IR (cm-1): 3403 (OHstr.), 3274 (NHstr.), 3057 (C=C-Hstr. ar.), 1603 (C=N str. pyrazoline ring), 1340 (CH2 Pyrazoline ring), 1246 (C-N). 1H-NMR (400MHz, DMSO-d6): δ 10.95 (1H, s, NH), 10.02 (1H, s, OH), 8.21-6.66 (m, 23HAr.), 5.96 (1H, s, Ar-CH), 4.59-4.51 (1H, dd, H5 pyrazoline ring, J= 11.2 Hz, J=10.4 Hz), 4.09-4.01 (1H, H4b pyrazoline ring, dd, J= 9.2 Hz, J=9.6 Hz,), 3.95-3.82 (1H, dd, H4a pyrazoline ring, J=10.4 Hz, J=9.6 Hz), 2.52 (3H, s, N-CH3). 13C-NMR (400MHz, DMSO-d6): δ 146.07 (C3 pyrazoline ring), 156.29-111.16 (32CAr), 67.99 (Ar-CH), 57.12 (C5 pyrazoline ring), 45.12 (N-CH3), 33.58 (C4 pyrazoline ring). MS: m/z calculated for C37H32N4O: 548.26, Found: 548.15. CHN-analysis: Calculated %C=80.99, %H=5.88, %N=10.21, Found: %C=80.95, %H=5.92, %N=10.18.

2.4.4 N-((2-(5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-indol-3-yl) (phenyl)methyl)-N-methylaniline (8d).

Light coffee color, 68% yield, m.p. = 81-82 oC. FT-IR (cm-1): 3269 (NH str.), 3056 (C=C-H str. ar.), 1601 (C=N str. pyrazoline ring), 1339 (CH2 pyrazoline ring), 1249 (C-N). 1H-NMR (400MHz, DMSO-d6): δ 10.96 (1H, s, NH), 7.62-6.86 (m, 23Har.), 5.74 (1H, s, Ar-CH), 3.89-3.80 (1H, dd, H5 pyrazoline ring J= 8 Hz, J=8.8 Hz), 3.38-3.23 (1H, dd, H4b pyrazoline ring, J= 8.4 Hz, J=8 Hz), 3.15 (3H, s, OCH3), 3.05-2.94 (1H, dd, H4a pyrazoline ring, J= 12 Hz, J=12.8 Hz), 2.52 (3H, s, NCH3). 13C-NMR (400MHz, DMSO-d6): δ 159.49-112.04 (32CAr), 146.07 (C3 pyrazoline ring), 69.32 (Ar-CH), 60.11 (C5 pyrazoline ring), 50.04 (O-CH3), 44.61 (N-CH3), 33.36 (C4 pyrazoline ring). MS: m/z Calculated for C38H34N4O: 562.27, Found: 562.30. CHN-analysis: Calculated %C=81.11, %H=6.09, %N=9.96, Found: %C=81.16, %H=6.08, %N=9.92.

2.4.5 N-((2-(5-(2-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-indol-3-yl) (phenyl)methyl)-N-methylaniline (8e).

Deep-brown color, 68% yield, m.p. = 78-79oC. FT-IR (cm-1): 3271 (NHstr.), 3058 (C=C-Hstr. ar.), 1600 (C=Nstr. pyrazoline ring), 1339 (CH2 pyrazoline ring), 1249 (C-N). 1H-NMR (400MHz, DMSO-d6): δ 10.91 (1H, s, NH), 8.26-6.63 (m, 23Har.), 5.56 (1H, s, Ar-CH), 4.59-4.47 (1H, dd, H5 pyrazoline ring, J=7.6 Hz, J=8.4 Hz), 4.11-3.90 (1H, dd, H4b pyrazoline ring, J= 8 Hz, J=9.6 Hz), 3.69-3.62 (1H, dd, H4a pyrazoline ring, J= 10.4 Hz, J=10 Hz), 3.08 (3H, S, N-CH3). 13C-NMR (400MHz, DMSO-d6): δ 153.02-111.53 (32Car.), 143.08 (C3 pyrazoline ring), 66.83 (Ar-CH), 51.23 (C5 pyrazoline ring), 42.30 (N-CH3), 35.35 (C4 pyrazoline ring). MS: m/z Calculated for C37H31ClN4: 566.22, Found: 566.23. CHN-analysis: Calculated %C=78.36, %H=5.51, %N=9.88, Found: %C=78.34, %H=5.53, %N=9.86.

2.4.6 N-((2-(5-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-indol-3-yl) (phenyl) methyl)-N-methylaniline (8f)

Chemical Formula = C37H31FN4, brown color, 70% yield, m.p. = 88-89 oC. FT-IR (cm-1): 3270 (NHstr.), 3057 (C=C-Hstr. ar.), 1602 (C=Nstr. pyrazoline ring), 1339 (CH2 pyrazoline ring), 1247 (C-N). 1H-NMR (400MHz, DMSO-d6): δ 10.91 (1H, s, NH), 8.17-6.73 (m, 23Har.), 5.83 (1H. s, Ar-CH), 4.26-4.15 (1H, dd, H5 pyrazoline ring, J= 12 Hz, J=11.2 Hz), 3.63-3.49 (1H, dd, H4b pyrazoline ring, J=9.2 Hz, J=9.2 Hz), 3.08-2.95 (1H, dd, H4a pyrazoline ring, J=9.2 Hz, J=8.4 Hz), 2.79 (3H, s, N-CH3). 13C-NMR (400MHz, DMSO-d6): δ 147.49 (C3 pyrazoline ring), 162.47-110.12 (32Car.), 69.45 (Ar-CH), 58.12 (C5 pyrazoline ring), 43.57 (N-CH3), 35.32 (C4 pyrazoline ring). MS: m/z

Calculated for C37H31FN4: 550.25, Found: 550.35. CHN-analysis: Calculated %C=80.70, %H=5.67, %N=10.17, Found: %C=80.51, %H=5.76, %N=10.26.

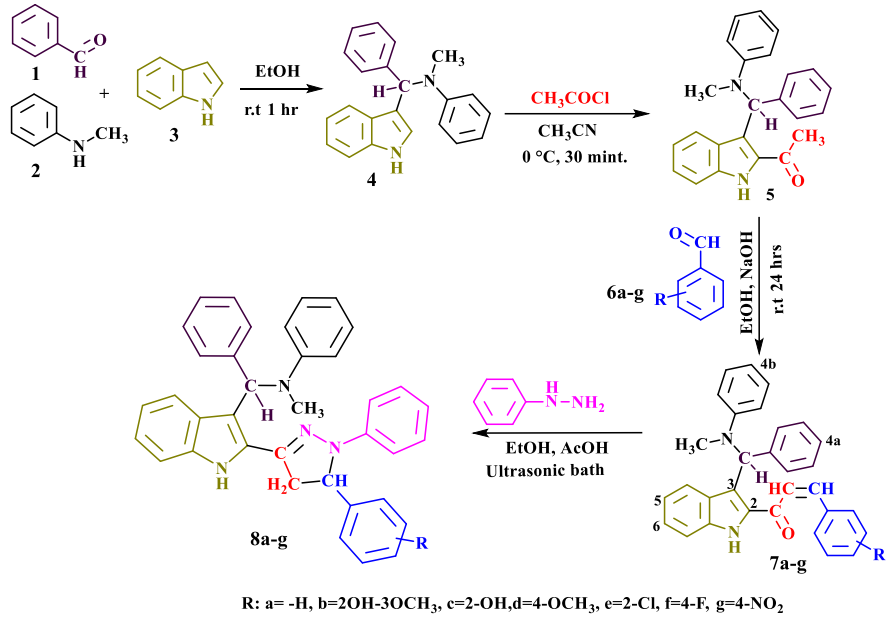
2.4.7 N-methyl-N-((2-(5-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-indol-3-yl) (phenyl)methyl) aniline (8g)

Chemical Formula = C37H31N5O2, light red color, 75% yield, m.p. = 78-79 oC. FT-IR (cm-1): 3244 (NHstr.), 3056 (C=C-Hstr. ar.), 1597 (C=Nstr. pyrazoline ring), 1333 (CH2 pyrazoline ring), 1263 (C-N). 1H-NMR (400MHz, DMSO-d6): δ 10.96 (1H, s, NH), 7.62-6.77 (m, 23Har.), 5.74 (1H, s, Ar-CH), 3.87-3.77 (1H, dd, H5 pyrazoline ring, J=12.8 Hz, J=10.8 Hz), 3.40-3.25 (1H, dd, H4b pyrazoline ring, J=9.2 Hz, J=8.8 Hz), 3.07-2.96 (1H, dd, H4a pyrazoline ring, J=8.8 Hz, J=8.8 Hz), 2.52 (3H, s, N-CH3). 13C-NMR (400MHz, DMSO-d6): δ 143.94 (C3 pyrazoline ring), 159.54-111.94 (32Car.), 72.03 (Ar-CH), 58.89 (C5pyrazoline ring), 42.61 (N-CH3), 33.23 (C4 pyrazoline ring). MS: m/z Calculated for C37H31N5O2: 577.25, Found: 577.20. CHN-analysis: Calculated %C=76.93, %H=5.41, %N=12.12, Found: %C=76.94, %H=5.39, %N=12.00.

3. Results and Discussion

3.1 Chemistry

The synthesized chalcones (7a-g) and N-phenylpyrazoline (8a-g) are presented in scheme (1).



Scheme 1: Synthetic steps for the formation of *N*-phenylpyrazoline derivatives (8a-g)

The FT-IR spectrum of compound (5) acylated indole showed the appearance of carbonyl group at 1661 cm-1 and -NH at 3264 cm-1 for indole. The 1H-NMR spectrum of compound (5) showed a singlet signal at (1.78) ppm that belongs to the three protons of (–CH3) attached to the carbonyl group and a singlet at (3.21) ppm for three protons of (-CH3) attached to the nitrogen group; in addition to, a multiplet signals at (7.40-6.88) ppm attributed to the (14) protons of aromatic rings, which were 15 in compound (4) and decreased by one proton for the acylation. Added to that, a singlet signal at (5.78) ppm for (ArCHN) proton and singlet signal at 10.97 for indole (NH), with the absence of a singlet band of (NH-CH), which confirms that the acetylation occurred in the indole ring at position two instead of position one. The 13C-NMR spectrum showed three peaks at (25.68, 36.96 and 48.06) ppm

that belong to the carbon atom of (-COCH3, -NCH3, and ArCHN), and peak at (169.56) ppm for carbonyl group, which confirms the acylation of compound (4) and formation of compound (5).

The FT-IR spectra of the chalcones (7a-g) showed the characteristic peaks of carbonyl functional groups in the region of (1641-1633) cm-1. The lowering of carbonyl frequency indicates the presence of (C=C) conjugated to the carbonyl groups (conjugated enones). The 1H-NMR spectrum of the chalcone derivatives showed characteristics of doublet signals at (8.27-7.40) ppm for beta protons of the chalcones. This deshielding refers to the effect of resonance of the phenyl ring that bonded to beta carbon atom; meanwhile, alpha hydrogen of the chalcones showed a doublet signal at (6.35-5.57) ppm. According to the coupling constant (J) value, compounds (7a, 7d and 7g) with the J value (16, 16.4), (16, 16.4) and (16.4, 16) have the characteristic of the E-configuration, because of the large value of J which clearly reveals trans geometry for the chalcone; whereas, compounds (7b, 7c, 7e, 7f) with the J value (8,8), (8.4, 8.8), (8, 8.4) and (8.4, 7.6), have the characteristic of Z-configuration of the α, β-double bond in the chalcone derivatives (Aksöz and Ertan, 2012). The 13C-NMR spectrum of chalcones (7a-g) showed a singlet at the region of (176.57-169.60) ppm for the carbonyl carbon of the chalcone, with 26 signals for aromatic carbons, these indications confirm the formation of chalcones (7a-g).

The FT-IR spectra of N-phenylpyrazoline derivatives (8a-g) showed a characteristic band in region (1603-1597) cm-1 for C=N stretching vibration of the pyrazoline ring moieties, band in the region (1263-1247) cm-1for (C-N) and a band at (1340-1333) cm-1 for (-CH2) pyrazoline ring. Besides the appearance of the above bands, the disappearance of carbonyl group peak at (1633-1641) cm-1for the chalcone moiety revealed the formation of pyrazoline ring. The 1H-NMR spectrum of N-phenylpyrazoline showed characteristic signals corresponding to protons of C4 and C5 of the pyrazoline ring. C4 (H4a, H4b) and C5 (H5) protons exhibited doublets of doublets at (3.95-2.81) ppm for H4a, (4.11-3.11) ppm for H4b and (4.67-3.77) ppm for H5, respectively. The 13C-NMR spectrum assignment of carbon atoms presented in the N-phenylpyrazoline showed signals at the region (35.35-32.87) ppm for C4, (60.11-51.23) ppm for C5, and (147.49-143.08) ppm for C3 of pyrazoline rings. Signals in the region (162.47-110.12) ppm for 32 aromatic carbons in the synthesized compounds (8a-g) which were 26 signals in the chalcone derivatives (7a-g). Mass spectrometry (MS): m/z Calculated for the pyrazoline derivatives gave convincing results. This evidence confirms the formation of N-phenylpyrazoline derivatives (8a-g).

The synthesis work is fruitless without performing biological activities, the newly synthesized pyrazoline derivatives, therefore, were screened for the antibacterial, antifungal and antioxidant activities.

3.2 Biological Activity

3.2.1 Assay of in Vitro Antimicrobial Activity

The in vitro antimicrobial activities of the synthesized N-phenylpyrazoline (8a-g) were observed against gram-positive strain, Staphylococcus aureus (ATCC 9144), and gram-negative strains, Escherichia coli (ATCC 8739), in addition to antifungal activity against Candida albicans (NCIM 3471) was evaluated. The Agar well diffusion method was used for each of antibacterial and antifungal assays. Two bacterial strains and a fungi strain were maintained on nutrient agar plates at 37 oC. Plates containing 20 ml of nutrient agar were spread with 100 µl of cultures (antibacterial and antifungal), the wells were made in the Agar Cork borer of width of 6mm, 100 µl of N-phenylpyrazoline compounds (1000 µg/mL) were loaded in the Agar well and DMSO used as control, incubated plates

for 24 hours. Standard antibiotic Amikacin for antibacterial activity and Nystatin for antifungal activity were used (Mathur et al., 2011, Landage et al., 2019).

Antimicrobial activity was screened against Gram-positive Staphylococcus aureus and Gram-negative bacteria Escherichia coli. N-phenylpyrazoline compounds were active except (8b, and 8e) that screened highly effective against Staphylococcus aureus bacteria, compounds 8b and 8f screened highly active against Escherichia coli. Thus, the N-phenylpyrazoline could be used as a broad spectrum of antibacterial agents. Another important feature of synthesized compounds is their activity against antifungal agent yeast (Candida Albicans).

Table 1: The antibacterial and antifungal activity of *N*-phenylpyrazoline derivatives (8a-g) with inhibition zone diameters in (mm) scale against two strains of bacteria and fungi

	Antibacterial Activity		Antifungal
Compounds	S. aureus	E. coli	C. albicans
8a	29	28	16
8b	32	31	22
8c	27	26	21
8d	26	27	20
8e	31	30	17
8f	30	31	18
8g	28	28	23
Amikacin	34		NT
Nystatin	NT		24
control	0		

S. aureus= Staphylococcus aureus, E. coli= Escherichia coli, C. albicans= Candida albicans

NT: not test.

For antibacterial: Highly active (inhibition zone > 30 mm); active (inhibition zone 23-30 mm); moderately active (inhibition Zone 16--23 mm); slightly active (inhibition zone 9-16 mm); inactive (inhibition zone < 9 mm)

For antifungal: Highly active (inhibition zone > 20 mm); active (inhibition zone 15-20 mm); moderately active (inhibition Zone 10-15 mm); slightly active (inhibition zone 5-10 mm); inactive (inhibition zone < 5 mm) ion zone < 5 mm)

3.2.2 Assay of in Vitro DPPH Radical Scavenging Activity (Hassan, 2019)

The antioxidant activity of N-phenylpyrazoline derivatives were observed through the DPPH free radical scavenging assay. A solution of 1, 25 and 50 µg/ml was prepared from the N-phenylpyrazoline derivatives. An equal volume of sample solution was added to an equal volume of 0.1 mM ethanolic DPPH, the mixture was mixed and vortexed thoroughly and kept at the dark place for 30 minutes. The absorbance was read against a blank at 517 nm with UV/Visible spectrophotometer. Radical

scavenging activity was measured as the inhibition percentage and was calculated using the following equation: -

$$DPPH\ scavenged\ \% = \frac{Ac - As}{Ac} \times 100$$

Ac = absorbance of control (DPPH radical+ ethanol).

As= absorbance of N-phenylpyrazoline (N-phenylpyrazoline + ethanol + DPPH radical).

The DPPH scavenging activity of N-phenylpyrazoline derivatives (8a-g) were evaluated and compared to standard ascorbic acid, which have IC50 equal to 33.04µg/ml. Half maximal Inhibitory Concentration IC50 value is the concentration of the sample that scavenge 50% of DPPH free radical in DPPH free radical scavenging method that generally used for evaluation of proton donating antioxidant from phenolic compounds. As a result, the DPPH scavenging activity of Ascorbic acid › 8b › 8c › 8d› 8a› 8e› 8f and 8g, which shown in Table (2).

Table 2: DPPH scavenging for *N*-phenylpyrazoline derivatives (8a-g)

Sample	Concentration µg/ml	Absorbance	%SCV	IC_{50}(µg/ml)
8a	1	0.03	14.28	45.37
	25	0.025	28.57	
	50	0.019	45.71	
8b	1	0.031	12.9	37.16
	25	0.024	31.42	
	50	0.016	66.66	
8c	1	0.028	20	38.37
	25	0.02	32.85	
	50	0.013	62.85	
8d	1	0.028	20	43.71
	25	0.022	37.14	
	50	0.016	54.28	
8e	1	0.029	17.14	56.2
	25	0.021	40	
	50	0.016	54.28	
8f	1	0.027	22.85	57.91
	25	0.019	45.71	
	50	0.016	54.28	
8g	1	0.032	8.57	87.74
	25	0.027	22.85	
	50	0.024	31.42	
Ascorbic acid (standard)	1	0.029	17.142	33.04
	25	0.021	40	
	50	0.011	68.57	

Note: absorbance of the blank (ethanol with DPPH without synthesized compounds) is equal to 0.035

The compounds displayed a structure–activity relationship (SAR) because the activity of compounds varies with substituent on the aromatic ring. Compound 8b containing hydroxyl and methoxy groups at position 2 and 3 showed most potent antioxidant activity, while compound 8c with hydroxy group at position two showed less activity compared to the standard ascorbic acid, on the hand compound 8e, 8f, 8g that contain halogens and nitro on the phenyl group were least active. These results made us infer that the methoxy group at meta position and Indole-NH might play a significant role in the redox reaction process.

4. Molecular Docking Study

Molecular docking technique is routinely used in modern drug discovery for understanding the drug-receptor interaction. The study was completed in order to determine the exact interactions of the newly synthesized phenylpyrazoline derivatives with the target molecule and to get the model binding affinity and orientation of small drug molecules at the target site (Patel et al., 2019). For this reason, penicillin-binding protein 2a (PDB ID: 1VQQ) from a methicillin-resistant S. aureus strain was chosen as a possible molecular docking target (Fishovitz et al., 2014). The synthesized compounds (8a-g) showed in table (3) were docked into the active binding site of the protein, and predicted binding energies that have lower than Amikacin, and in the most cases, hydrogen bonding interactions were observed between the compounds and GLU:379, GLU:294, GLU:378, ASP:509, SER:476, GLU:511, LEU:472, LEU:508 of penicillin-binding protein 2a (PDB ID: IVQQ), besides pi-cation, pi-alkyl, pi-sulfur, pi-sigma and pi-pi T-shaped were also observed with the protein .

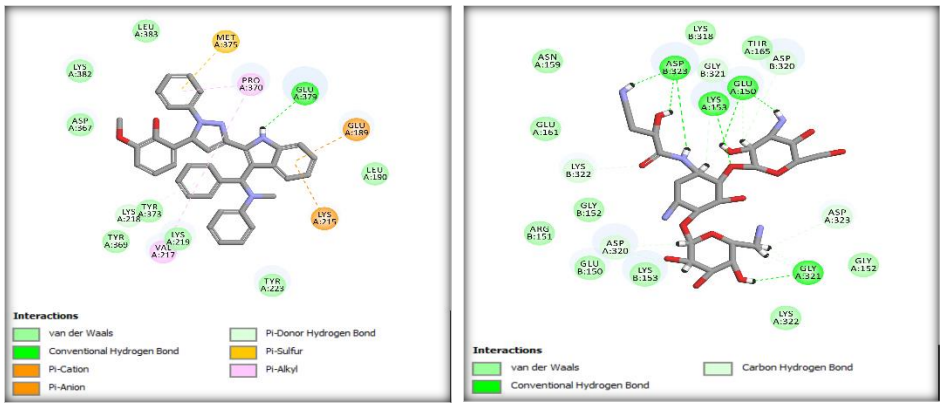


Figure 1: The two-dimension interaction plot of compound 8b (left) and the standard (right) docked into the active site of PDB ID: IVQQ

A molecular docking study was performed to show how compounds interacted with the receptor. As shown in table (3) compound 8b was the most potent derivative while compound 8c and 8d were the least potent one. These results are in agreement with the observed antibacterial results. The three-dimensional (3D) diagrams of the compounds 8b and the standard in the binding site of IVQQ are shown in Figure (2). The docking score, binding site interaction analysis revealed that N-phenylpyrazoline exhibit less specificity than known antibiotic amikacin.

Table 3: Docking score and binding energy of compounds (8a-g) docked into the penicillin-binding protein 2a (PDB ID: IVQQ)

Entry	Binding Energy (kcal/mol)	Interactions
8a	-8.9	Conventional H-bond GLU379, Pi-sigma LYS:219, Pi-Alkyl VAL:217, PRO:370, Pi-Anion GLU:189
8b	-8.5	Conventional H-bond GLU:379, Pi-Cation LYS:215,Pi-Anion GLU:189, Pi-Sulfer MET:375, Pi-Alkyl PRO:370, VAL217
8c	-9.3	Conventional H-bond GLU:294, Unfavorable Doner-Doner LYS:322, Pi-cation LYS:319, Pi-Sigma LYS:290, Pi-Pi T-shaped TYR:272, Pi-Alkyl LYS:273, LYS:289
8d	-9.3	Conventional H-bond GLU:379, CHB GLU:378, Pi-Cation LYS:215, Pi-Anion GLU:189, Pi-Sulfer MET:375, Pi-Alkyl PRO:370, VAL:217
8e	-8.9	Conventional H-bond ASP:509, SER:476, Carbon H-Bond GLU:11, LEU:472, LEU:508, Pi-Alkyl LYS:477, LYS:478, LEU:514
8f	-8.9	Conventional H-bond LYS:273, Pi-Alkyl LYS:289, LYS:319, Pi-Cation LYS:322, Pi-Pi Stacked TYR:272
8g	-9.1	Conventional H-bond GLU379, LYS:382, Pi-Alkyl PRO:370, VAL:217, Pi-Anion GLU:189, LYS:215
Amikacin standard	-7.2	Conventional H-bond GLU150, GLY321, LYS153, ASP323

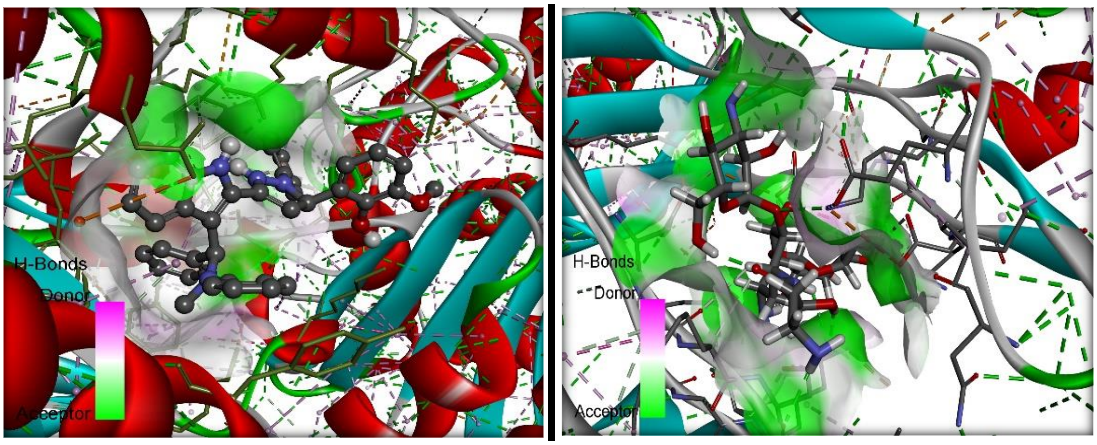


Figure 2: 3D binding model of compound 8b (left) and the standard (right) docked into the active site of PDB ID: IVQQ

5. Conclusion

New method was used to combine indole core with pyrazoline ring to enhance the biological activity of the synthesized compounds (8a-g). The generality and operational simplicity of this method make it attractive for alternative construction of 2-acylindoles that formed in appropriate yield at room temperature without competing N-arylation or any further undesirable side reactions. The results showed that the antibacterial, antifungal and antioxidant activities of the synthesized N-phenylpyrazoline molecules (8a-g) are less in comparison to that of reference standard. This synthesis can be modified using substituted indole in the first step along with the use of hydrazine hydrate, which will determine a more potential key point to properly tune the biological performance of the pyrazoline derivatives.

References

Ahmad, M. R., Sastry, V. G., Bano, N., & Anwar, S. (2016). Synthesis of novel chalcone derivatives by conventional and microwave irradiation methods and their pharmacological activities. *Arabian Journal of Chemistry*, 9**,** S931-S935.

Aksöz, B. E., & Ertan, R. (2012). Spectral properties of chalcones II. *Fabad J. Pharm. Sci*, *37*(4), 205-216.

Attarde, M., Vora, A., Varghese, A., & Kachwala, Y. (2014). Synthesis and evaluation of chalcone derivatives for its alpha amylase inhibitory activity. *Organic Chemistry: An Indian Journal*, 10**,** 192-204.

Biswal, S., Sahoo, U., Sethy, S., Kumar, H., & Banerjee, M. (2012). Indole: the molecule of diverse biological activities. *Asian J. Pharm. Clin. Res*, 5**,** 1-6.

Cong, H., Zhao, X., Castle, B. T., Pomeroy, E. J., Zhou, B., Lee, J., Wang, Y., Bian, T., Miao, Z., & Zhang, W. (2018). An Indole–Chalcone Inhibits Multidrug-Resistant Cancer Cell Growth by Targeting Microtubules. *Molecular Pharmaceutics*, 15**,** 3892-3900.

Dawood, D. H., Nossier, E. S., Ali, M. M. & Mahmoud, A. E. (2020). Synthesis and molecular docking study of new pyrazole derivatives as potent anti-breast cancer agents targeting VEGFR-2 kinase. *Bioorganic Chemistry***,** 103916.

Devi, C. L., Rao, V. J., & Palaniappan, S. (2012). PANI-HBF4: a reusable polymer-based solid acid catalyst for three-component, one-pot synthesis of 3-substituted amino methyl indoles under solvent-free conditions. *Synthetic Communications*, 42**,** 1593-1603.

El-Sawy, E., Mandour, A., Mahmoud, K., Islam, I., & Abo-Salem, H. (2012). Synthesis, antimicrobial and anti-cancer activities of some new N-ethyl, N-benzyl and N-benzoyl-3-indolyl heterocycles. *Acta Pharmaceutica*, 62**,** 157-179.

Fishovitz, J., Hermoso, J. A., Chang, M., & Mobashery, S. (2014). Penicillin-binding protein 2a of methicillin-resistant Staphylococcus aureus. *IUBMB Life*, 66**,** 572-577.

Gaonkar, S. L. & Vignesh, U. (2017). Synthesis and pharmacological properties of chalcones: a review. *Research on chemical intermediates*, 43**,** 6043-6077.

Gomes, M. N., Muratov, E. N., Pereira, M., Peixoto, J. C., Rosseto, L. P., Cravo, P. V., Andrade, C. H., & Neves, B. J. (2017). Chalcone derivatives: promising starting points for drug design. *Molecules*, 22**,** 1210.

Grimster, N. P., Gauntlett, C., Godfrey, C. R., & Gaunt, M. J. (2005). Palladium-Catalyzed Intermolecular Alkenylation of Indoles by Solvent-Controlled Regioselective C□ H Functionalization. *Angewandte Chemie International Edition*, 44**,** 3125-3129.

Gupta, R., Gupta, N., & Jain, A. (2010). Improved synthesis of chalcones and pyrazolines under ultrasonic irradiation.

Hassan, S. A. (2019). Synthesis, Spectroscopic study, and biological activity of some New Heterocyclic compounds derived from Sulfadiazine. *ZANCO Journal of Pure and Applied Sciences*, 31**,** 92-109.

Ibrahim, M. N. (2007). Studies on Acetylation of Indoles. *Journal of Chemistry*, 4**,** 415-418.

Katritzky, A. R., Suzuki, K., Singh, S. K., & He, H.Y. (2003). Regiospecific C-acylation of pyrroles and indoles using N-acylbenzotriazoles. *The Journal of organic chemistry*, 68**,** 5720-5723.

Landage, V., Thube, D., & Karale, B. (2019). Synthesis, characterisation and antimicrobial screening of some new thiazolyl chromones and pyrazoles.

Lévai, A., & Jeko, J. (2000). Synthesis of carboxylic acid derivatives of 2-pyrazolines. *Arkivoc*, 1**,** 134-45.

Mathur, A., Singh, R., Yousuf, S., Bhardwaj, A., Verma, S. K., Babu, P., Gupta, V., Prasad, G. & Dua, V. (2011). Antifungal activity of some plant extracts against clinical pathogens. *Advances in applied science Research*, 2**,** 260-264.

Mowlana, M. Y. & Nasser, A. (2015). Synthesis and Molecular Docking studies of Heterocyclic Chalcone Derivatives as BRCA1 inhibitors. *International Journal of Pharmaceutical Chemistry*, 3**,** 196-200.

Ottoni, O., Neder, A. D. V., Dias, A. K., Cruz, R. P., & Aquino, L. B. (2001). Acylation of Indole under Friedel− Crafts Conditions an Improved Method To Obtain 3-Acylindoles Regioselectively. *Organic Letters*, 3**,** 1005-1007.

Özdemir, A., Altıntop, M. D., Turan-Zitouni, G., Çiftçi, G. A., Ertorun, İ., Alataş, Ö., & Kaplancıklı, Z. A. (2015). Synthesis and evaluation of new indole-based chalcones as potential antiinflammatory agents. *European journal of medicinal chemistry*, 89**,** 304-309.

Patel, H., Rajani, D., Sharma, M., & Bhatt, H. (2019). Synthesis, molecular docking and biological evaluation of mannich products based on thiophene nucleus using ionic liquid. *Letters in Drug Design & Discovery*, 16**,** 119-126.

Rahman, M. A., & Siddiqui, A. A. (2010). Pyrazoline derivatives: a worthy insight into the recent advances and potential pharmacological activities. *Int J Pharm Sci Drug Res*, 2**,** 165-175.

Robinson, M. W., Overmeyer, J. H., Young, A. M., Erhardt, P. W., & Maltese, W. A. (2012). Synthesis and evaluation of indole-based chalcones as inducers of methuosis, a novel type of nonapoptotic cell death. *Journal of medicinal chemistry*, 55**,** 1940-1956.

Safaei-Ghomi, J., Bamoniri, A., & Soltanian-Telkabadi, M. (2006). A modified and convenient method for the preparation of N-phenylpyrazoline derivatives. *Chemistry of Heterocyclic Compounds*, 42**,** 892-896.

Sayed, M., Kamal El-Dean, A. M., Ahmed, M., & Hassanien, R. (2018). Synthesis, Characterization, and Screening for Anti-inflammatory and Antimicrobial Activity of Novel Indolyl Chalcone Derivatives. *Journal of Heterocyclic Chemistry*, 55**,** 1166-1175.

Singh, A. K., Prasad, R. K., & Singh, C. S. (2013). Synthesis, characterization and pharmacological evaluation of some novel 3-indole derivatives. *Der Pharma Chemica*, 5**,** 311-319.

Singh, T. P., & Singh, O. M. (2018). Recent progress in biological activities of indole and indole alkaloids. *Mini Reviews in Medicinal Chemistry*, 18**,** 9-25.

Subhashini, N. (2015). TT 1 and Shivaraj. *Synthesis characterization and anti-bacterial activity of novel chalcone derivatives of indole***,** 38-45.

Tran, P. H., Tran, H. N., Hansen, P. E., Do, M. H. N., & Le, T. N. (2015). A simple, effective, green method for the regioselective 3-acylation of unprotected indoles. *Molecules*, 20**,** 19605-19619.

Zhuang, C., Zhang, W., Sheng, C., Zhang, W., Xing, C., & Miao, Z. (2017). Chalcone: a privileged structure in medicinal chemistry. *Chemical reviews*, 117**,** 7762-7810.

Appendix:

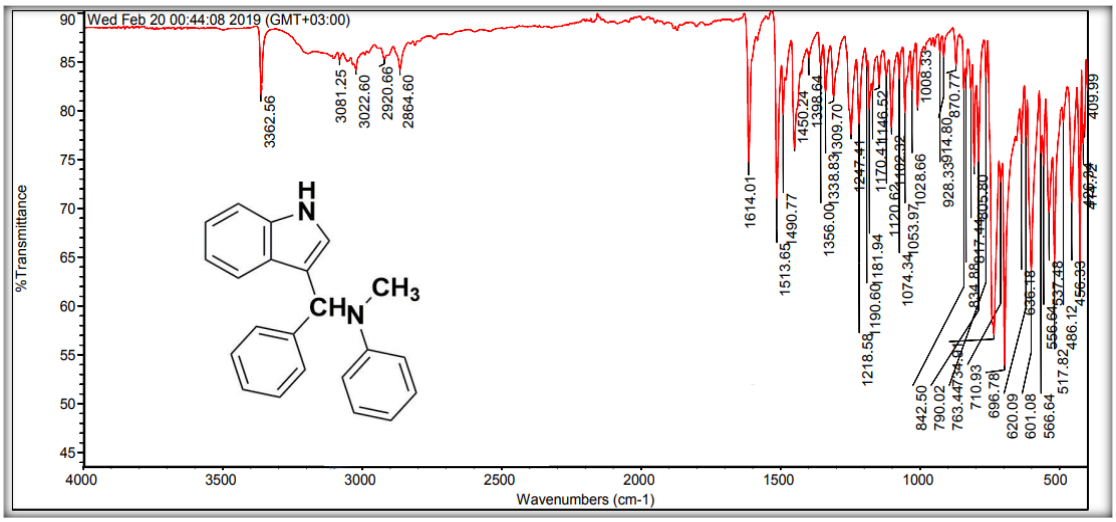


Figure 3: FT-IR Spectrum of *N*-((1*H*-indol-3-yl) (phenyl)methyl)-*N*-methylaniline (4)

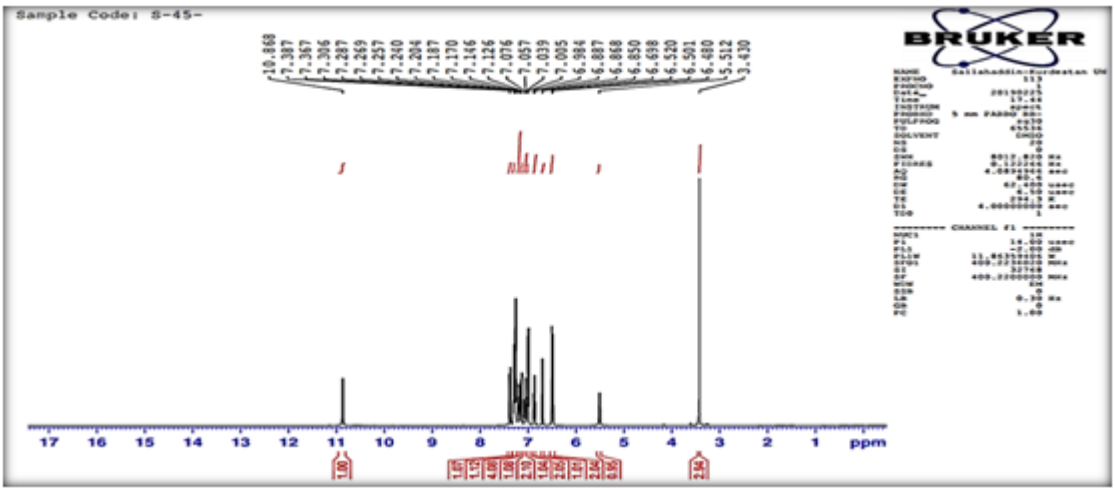


Figure 4: ^{1}H-NMR Spectrum of *N*-((1*H*-indol-3-yl) (phenyl)methyl)-*N*-methylaniline (4)

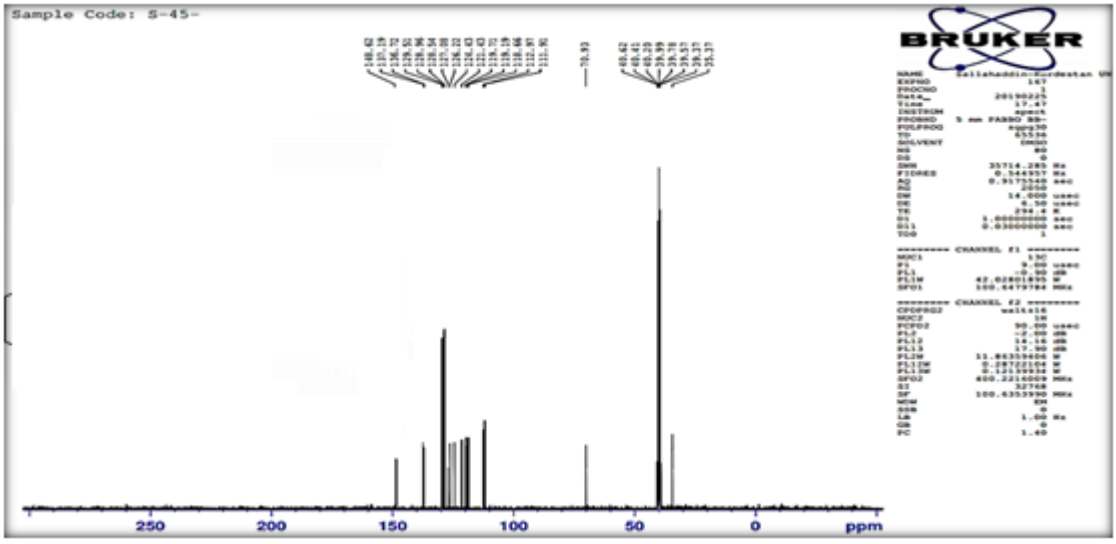


Figure 5: ^{13}C-NMR Spectrum of *N*-((1*H*-indol-3-yl) (phenyl)methyl)-*N*-methylaniline (4)

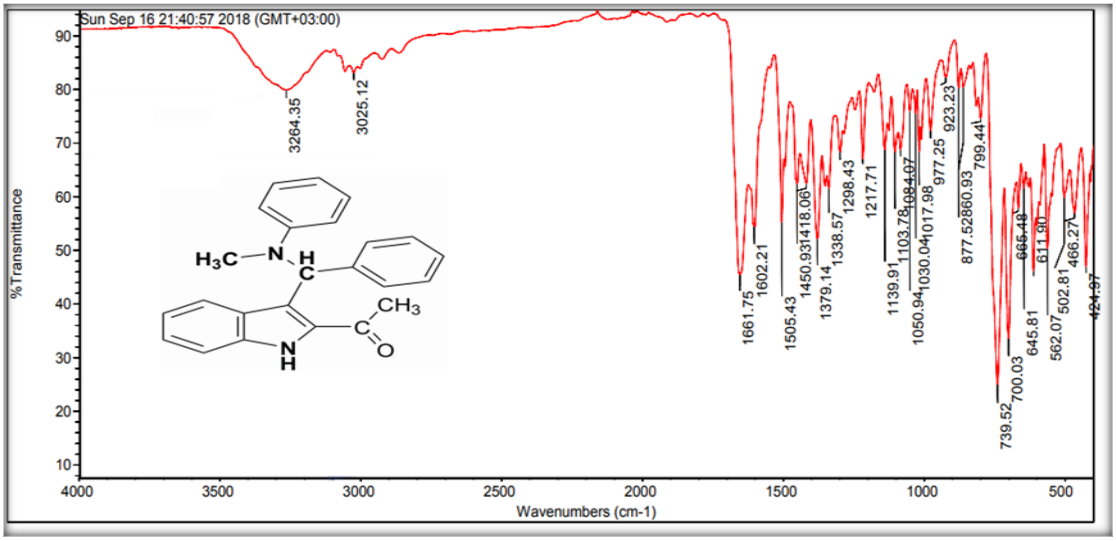


Figure 6: FT-IR Spectrum of 1-(3-((methyl(phenyl)amino) (phenyl)methyl)-1*H*-indol-2-yl)ethanone (5)

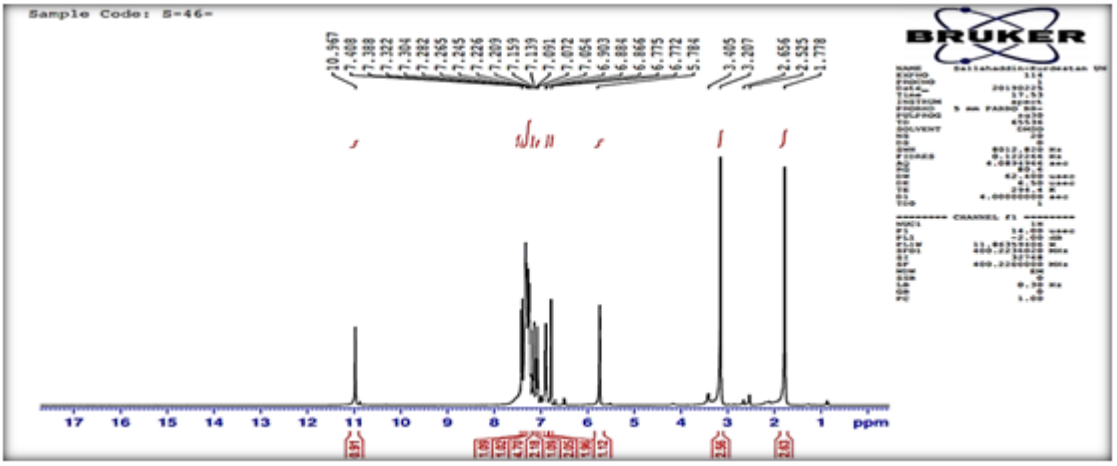


Figure 7: ^{1}H-NMR Spectrum of 1-(3-((methyl(phenyl)amino) (phenyl)methyl)-1*H*-indol-2-yl)ethanone (5)

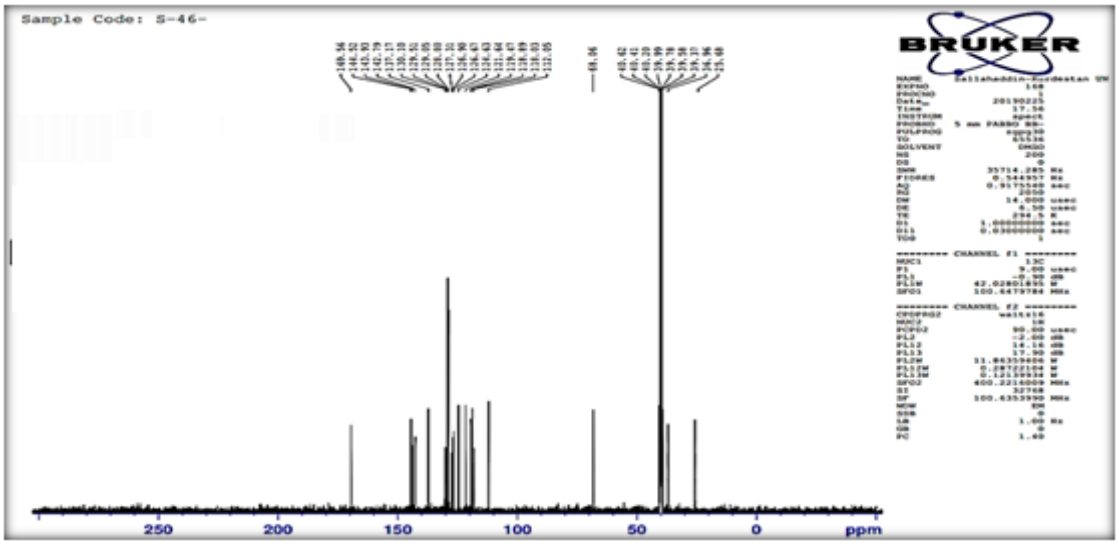


Figure 8: ^{13}C-NMR Spectrum of 1-(3-((methyl(phenyl)amino) (phenyl)methyl)-1*H*-indol-2-yl) ethanone (5)

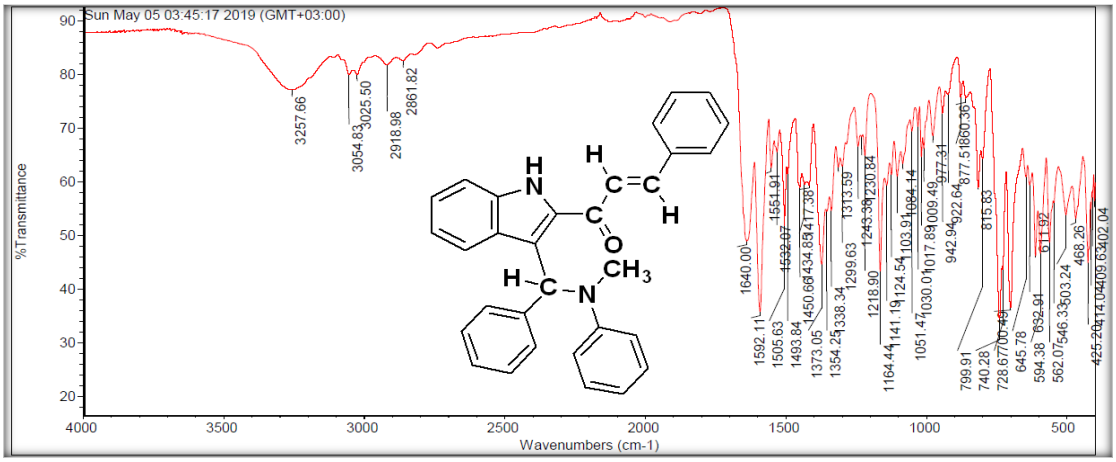


Figure 9: FT-IR Spectrum of (*E*)-1-(3-((methyl(phenyl)amino) (phenyl)methyl)-1*H*-indol-2-yl)-3-phenylprop-2-en-1-one (7a)

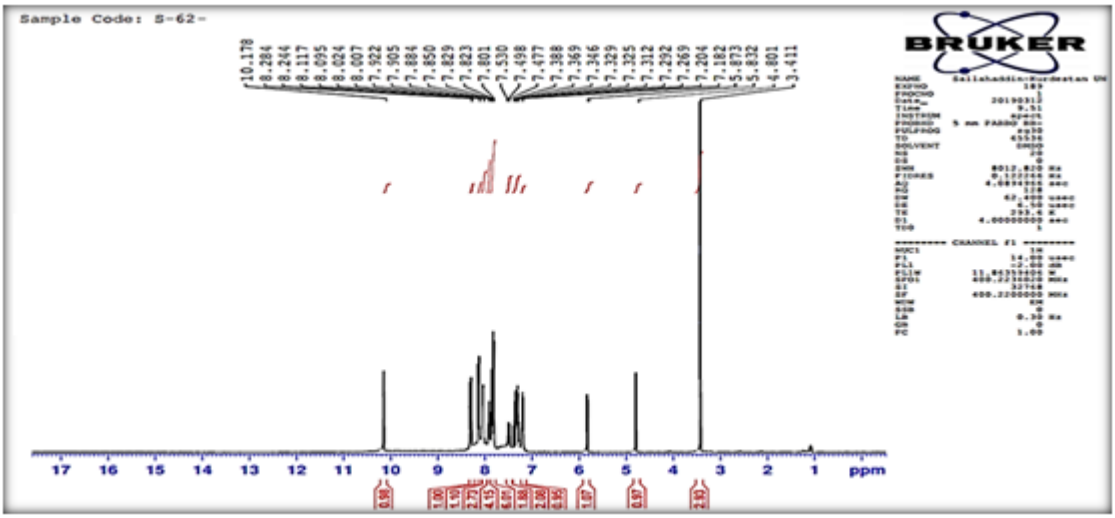


Figure 10: ^{1}H-NMR Spectrum of (*E*)-1-(3-((methyl(phenyl)amino) (phenyl)methyl)-1*H*-indol-2-yl)-3-phenylprop-2-en-1-one (7a)

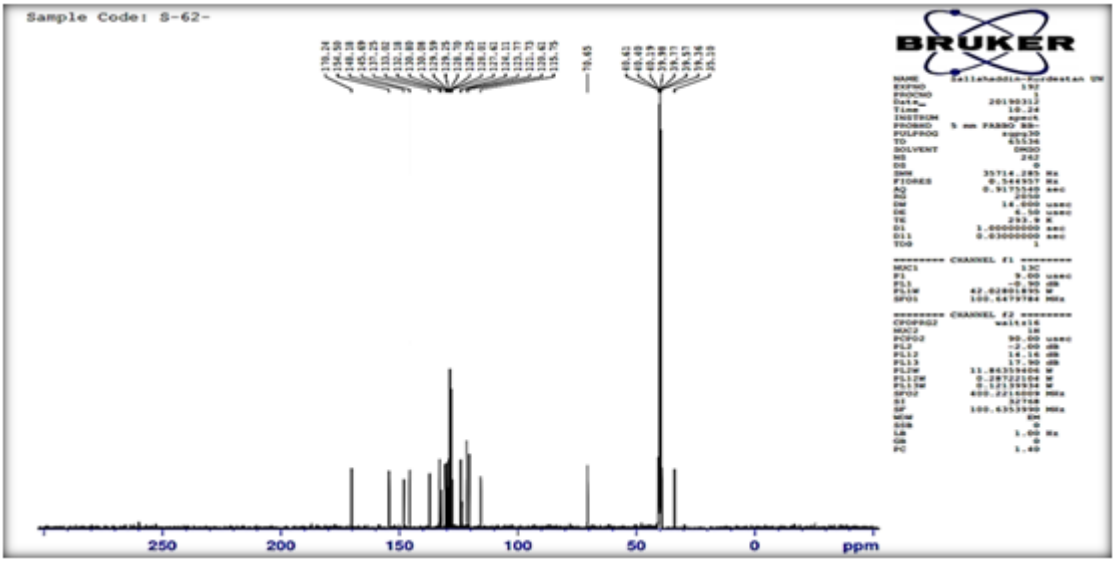


Figure 11: ^{13}C-NMR Spectrum of (*E*)-1-(3-((methyl(phenyl)amino) (phenyl)methyl)-1*H*-indol-2-yl)-3-phenylprop-2-en-1-one (7a)

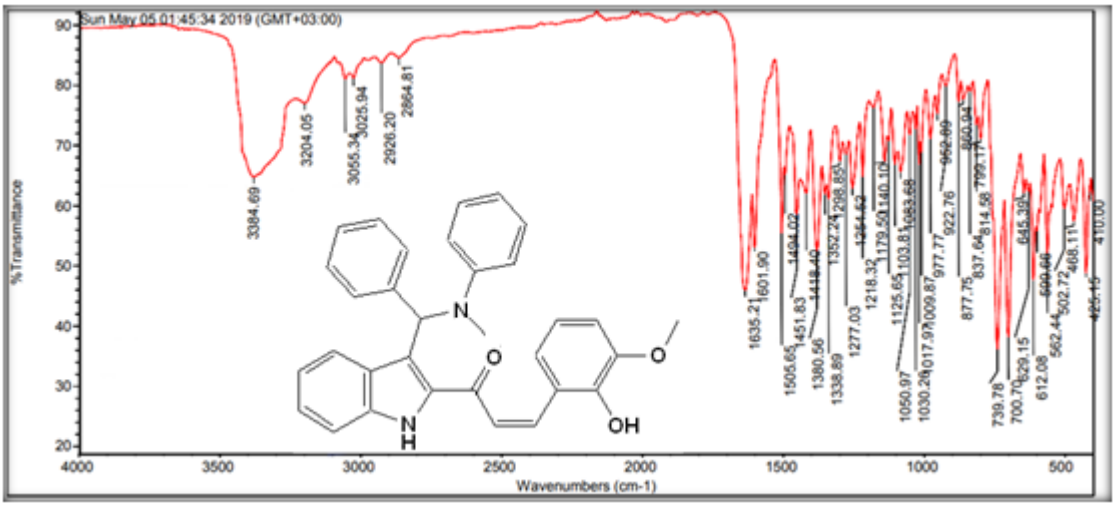


Figure 12: FT-IR Spectrum of (Z)-3-(2-hydroxy-3-methoxyphenyl)-1-(3-((methyl(phenyl) amino) (phenyl)methyl)-1H-indol-2-yl) prop-2-en-1-one (7b)

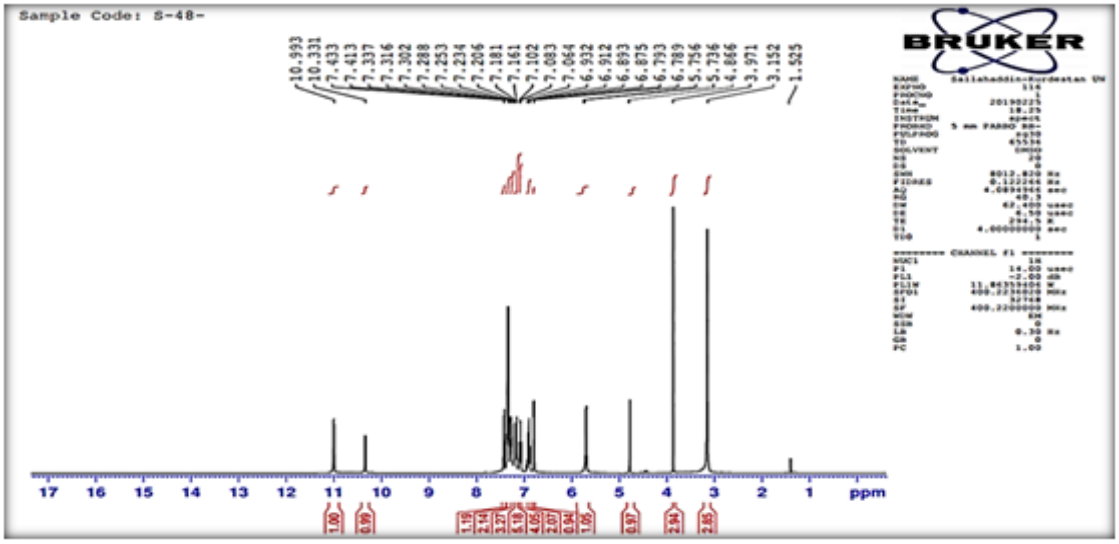


Figure 13: 1H-NMR Spectrum of (Z)-3-(2-hydroxy-3-methoxyphenyl)-1-(3-((methyl(phenyl) amino) (phenyl)methyl)-1H-indol-2-yl) prop-2-en-1-one (7b)

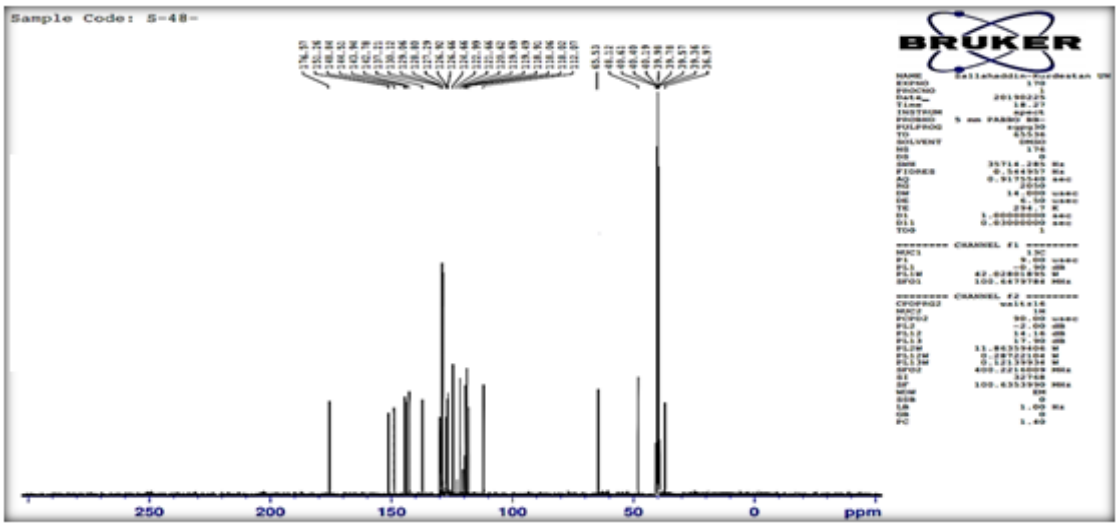


Figure 14: 13C-NMR Spectrum of (Z)-3-(2-hydroxy-3-methoxyphenyl)-1-(3-((methyl(phenyl) amino) (phenyl)methyl)-1H-indol-2-yl) prop-2-en-1-one (7b)

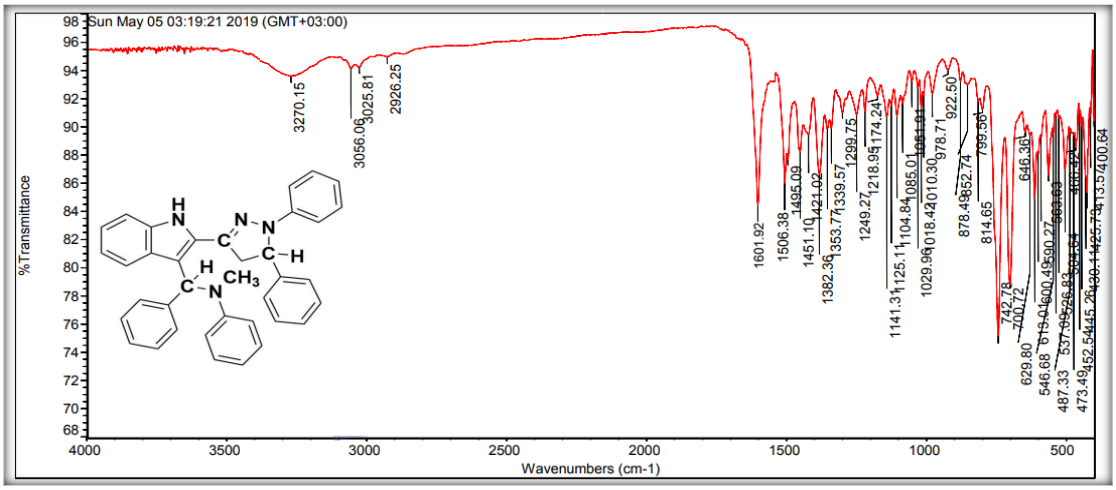


Figure 15: FT-IR Spectrum of N-((2-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-indol-3-yl) (phenyl)methyl)-N-methylaniline (8a)

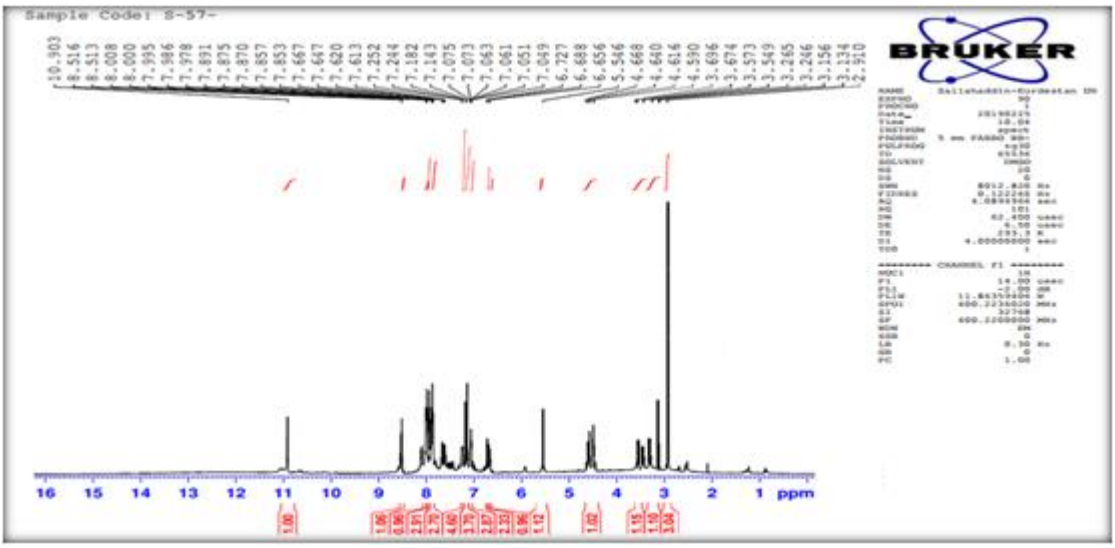


Figure 16: 1H-NMR Spectrum of N-((2-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-indol-3-yl) (phenyl)methyl)-N-methylaniline (8a)

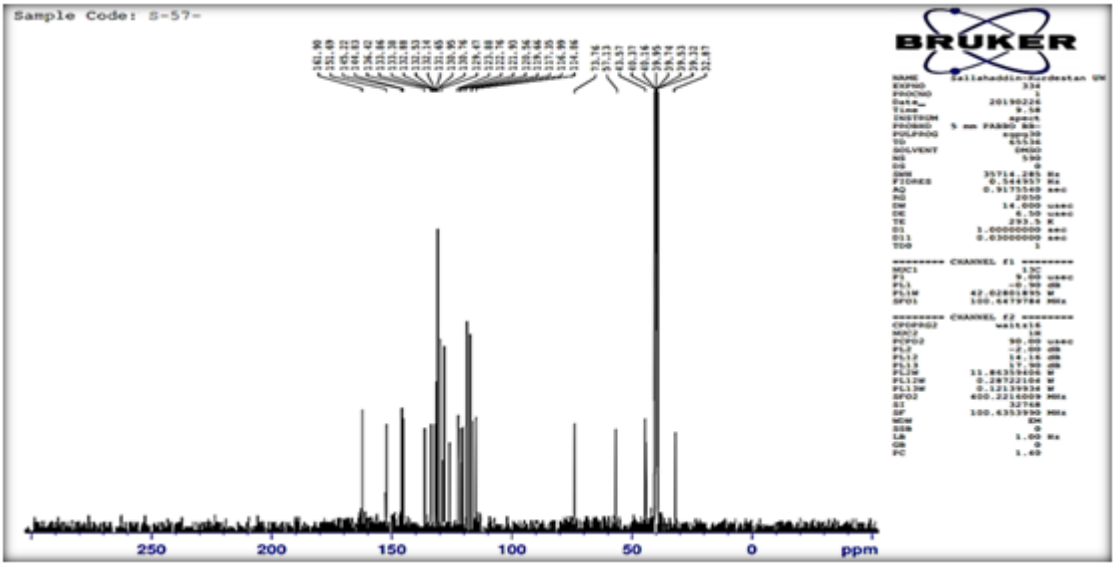


Figure 17: 13C-NMR Spectrum of N-((2-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-indol-3-yl) (phenyl)methyl)-N-methylaniline (8a)

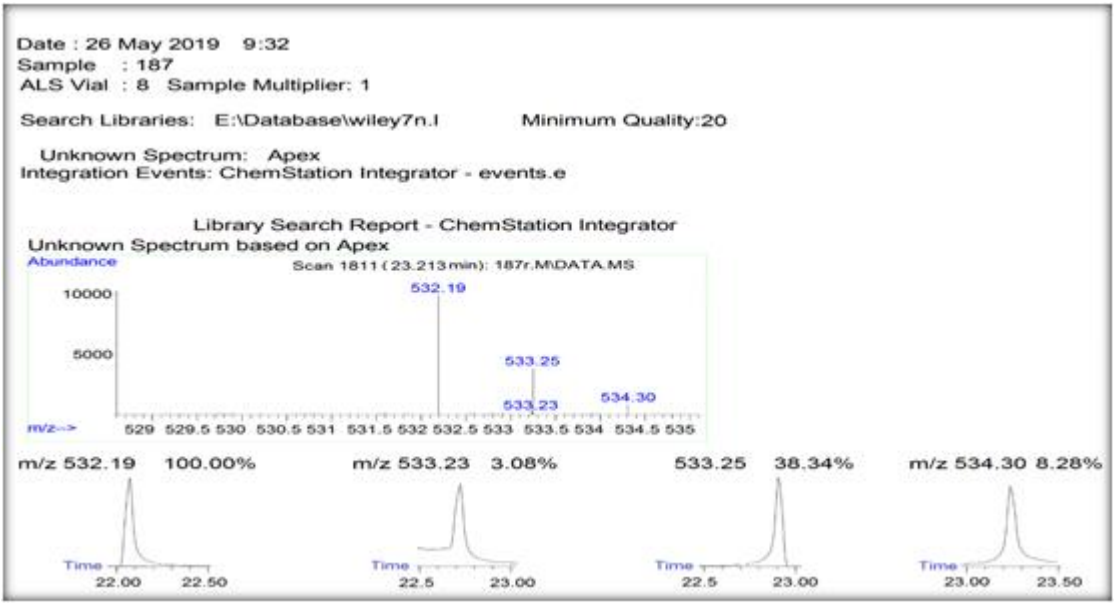


Figure 18: GC-Mass Spectrum of N-((2-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-indol-3-yl) (phenyl)methyl)-N-methylaniline (8a)

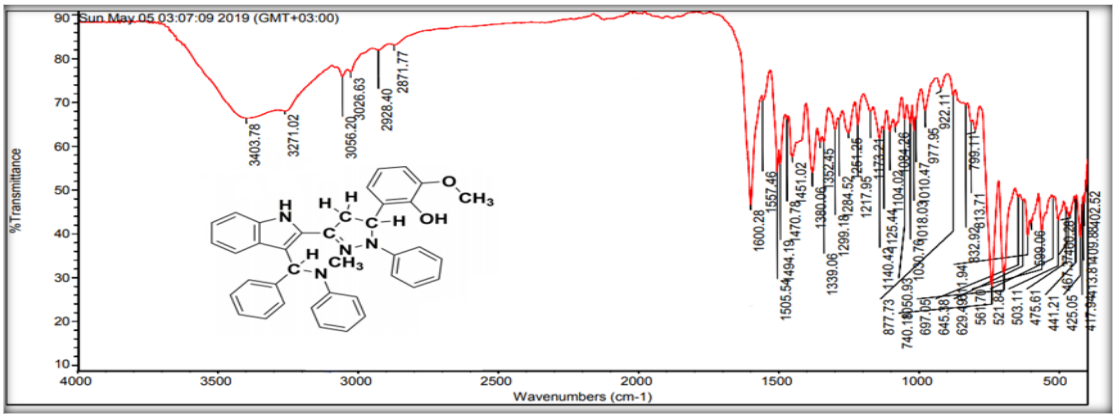


Figure 19: FT-IR Spectrum of Methoxy-6-(3-(3-((methyl(phenyl)amino) (phenyl)methyl)-1H-indol-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl) phenol (8b)

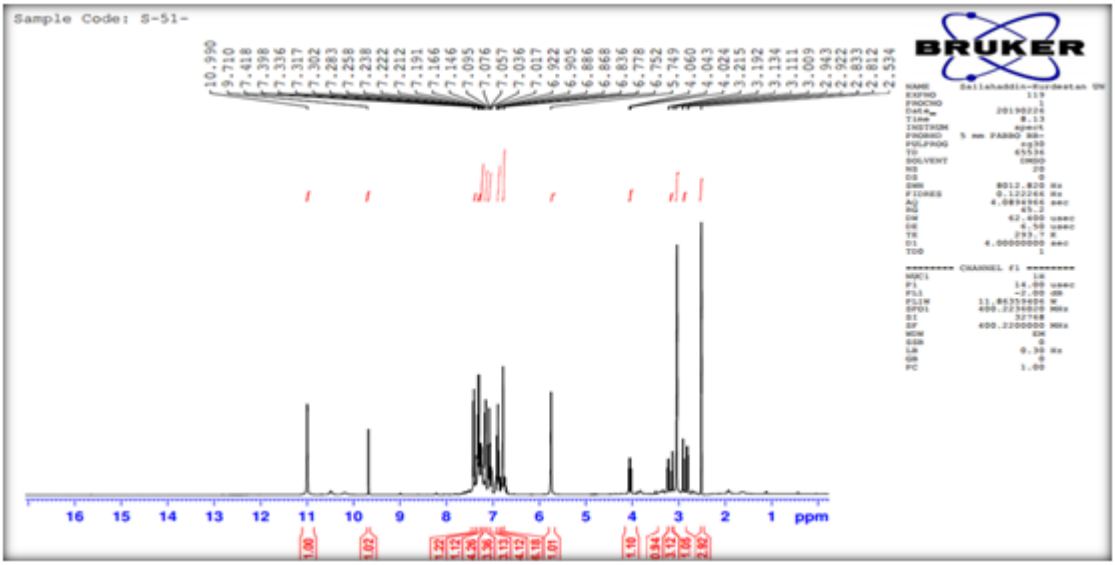


Figure 20: 1H-NMR Spectrum of Methoxy-6-(3-(3-((methyl(phenyl)amino) (phenyl)methyl)-1H-indol-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl) phenol (8b)

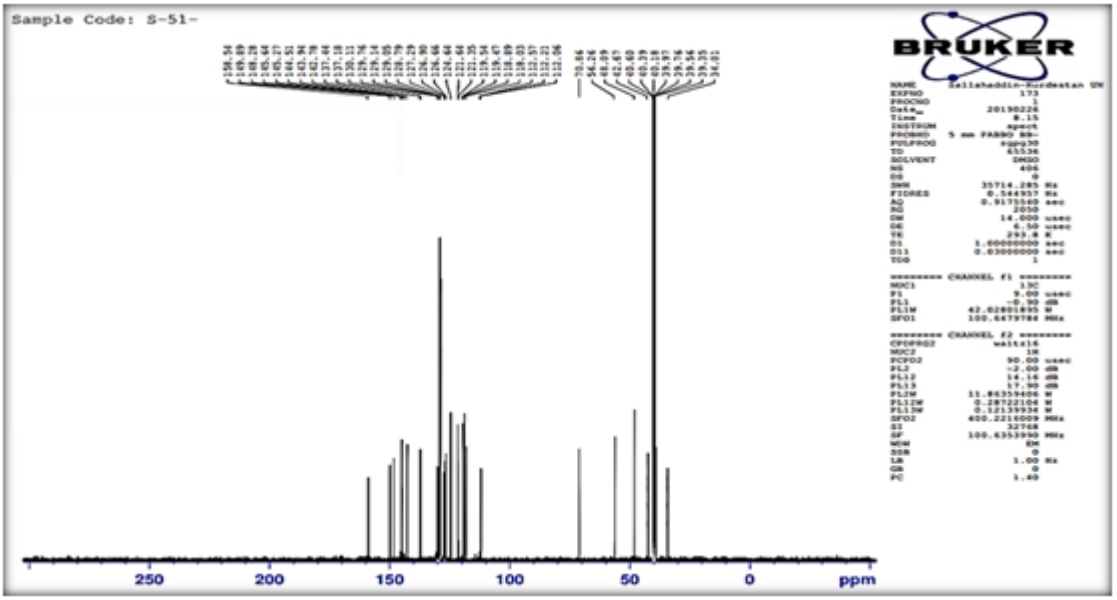


Figure 21: 13C-NMR Spectrum of Methoxy-6-(3-(3-((methyl(phenyl)amino) (phenyl)methyl)-1H-indol-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl) phenol (8b)

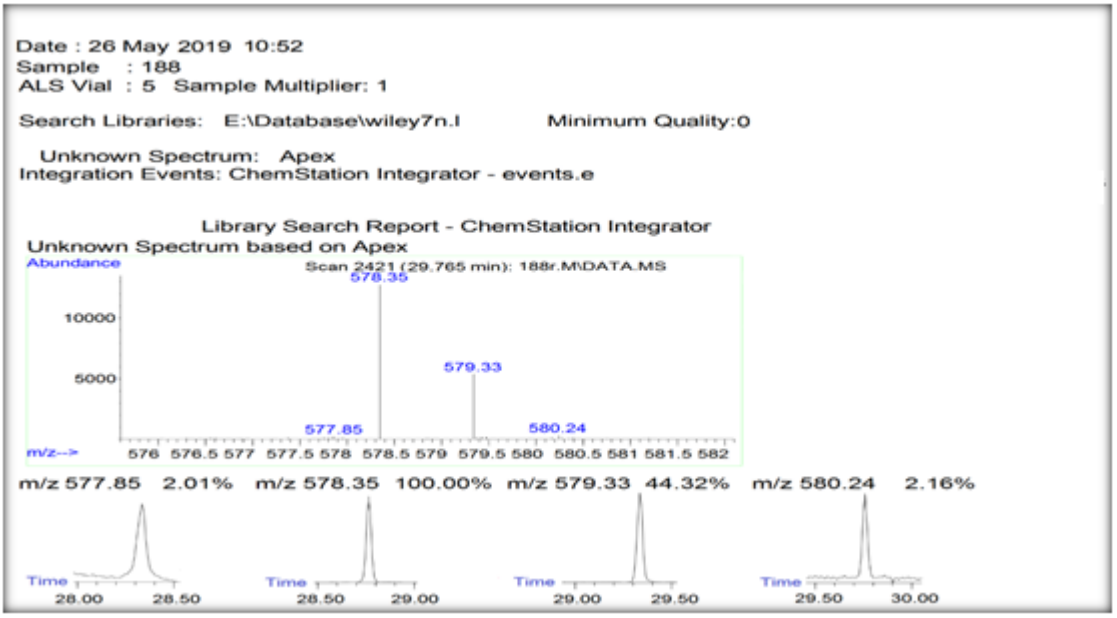


Figure 22: GC-Mass Spectrum of Methoxy-6-(3-(3-((methyl(phenyl)amino) (phenyl)methyl)-1H-indol-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl) phenol (8b)