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ORIGINAL PAPER

Synthesis and cyclization of β-keto-enol derivatives tethered indole and pyrazole as potential antimicrobial and anticancer activity

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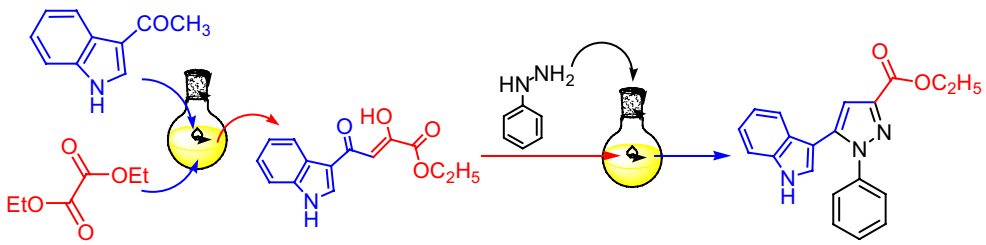
Abstract
Synthesis of biologically active new indole and pyrazole derivatives has earned a substantial position in the pharmaceutical industry. The study aims to synthesize and cyclize β-keto-enol derivatives tethered indole and pyrazole as potential antimicrobial and anticancer activity. Novel derivatives of enolic keto ester 1 have been obtained through Claisen condensation of 3-acetylindole with diethyl oxalate under basic conditions. Spectral data of newly produced compounds were characterized using Fourier transform infrared, hydrogen nuclear magnetic resonance, Carbon-13 nuclear magnetic resonance, and elemental analysis. Few compounds were assessed for their antimicrobial activity, contrary to gram-positive bacterial strains, gram-negative bacterial strain, and antifungal activity that have been approved against *Candida albicans*. However, compound 2 showed an excellent antimicrobial activity. In vitro antitumor action of few prepared compounds in contradiction to human breast carcinoma cell line, colon cell line, and normal retina cell line was evaluated.

Graphical Abstract



Keywords Antimicrobial · Anticancer · Cyclization · Derivatives · Pyrazole

Introduction

Heterocycle compounds with β-keto-enol moieties were recognized as significant biologically active compounds. Their medicinal chemistry advantages were steadfastly established [1, 2]. 1, 3-Diketones are highly important intermediates in organic synthesis [3, 4] that are widely represented in pharmaceuticals, natural products, and other biologically related compounds, or as key intermediates for synthesizing these species [5]. The synthesis of 1,3-diketones became highly essential for chemists to be used as a starting material to create different species of organic compounds [6].

Remarkable chemical properties of indole can motivate chemists to synthesize a variety of indole derivatives. Such derivatives represent many important classes in medicinal chemistry, such as anti-HIV antagonist, anticancer, antioxidant, antibacterial, antifungal, anti-allergic, and anti-inflammatory [6–9]. Pyrazoles and their derivatives display antimicrobial [10–12], anticancer [13], and anti-inflammatory activities [14]. Consequently, significant efforts were devoted to search for pyrazole and indole rings for decades

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[15]. Additionally, heterocycles were condensed to pyrazole ring as an essential source of bioactive molecules [16]. Concerning the hard work toward synthesizing biologically active heterocyclic compounds [17–19], researchers planned to synthesize new indole and pyrazole derivatives to study their biological activities, Therefore, described herein are the considered drugs, based on the β-keto-enol group embedded with heterocyclic moieties, such as pyrazole, pyridine, and pyrimidine using 3-acetylindole to find out novel biologically active compounds.

Experimental

The newly synthesized compounds melting points were determined on a melting point apparatus (Gallen Kamp electric) and were kept uncorrected. ATR-Alpha spectrophotometer was used to record IR spectra, using potassium bromide (KBr) pellets in the range of [400–4000] cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker AC-300 Hz instrument. Chemical shifts for ^{1}H and ^{13}C NMR spectra were expressed in δ (ppm), relative to the tetramethylsilane (TMS) as an internal standard; however, DMSO-d_6 [$(CH_3)_2S=O$)] was used as a solvent. Elemental analyses were determined at Micro-Analytical Center, Cairo University. Cytotoxic activity test (in vitro bioassay on human tumor cell lines) was conducted and determined by Bioassay-Cell Culture Laboratory, National Research Centre (NRC), Dokki, Giza, Egypt.

Ethyl 2-hydroxy-4-(1H-indol-3-yl)-4-oxobut-2-enoate

30 ml of diethyl oxalate (2.92 g, 0.02 mol) was added to a mixture of 3-acetylindole (3.2 g, 0.02 mol) and sodium ethoxide (0.46 g, 0.02 mol) in ethanol. The mixture was heated under reflux for 3 h. The solvent was removed under vacuum after cooling; then, the residue was taken up in water (100 ml) and acidified using concentrated HCl (1 ml). Aqueous mixture was extracted using diethyl ether (3 × 50 ml). The combined extracts were washed and dried. In order to provide compound 1, the obtained solid was recrystallized from ethanol. Yield 75%, m.p. 217–218 °C. IR (KBr, *v*, cm^{-1}): 3443 (OH), 3172 (NH), 3083 (CH arom.), 2939, 2836 (CH aliph.), and 1751–1685 (2C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta=1.29$ (t, 3H, J = 7.6 Hz CH_3), 4.20 (q, 2H, J = 7.6 Hz, CH_2), 7.53 (s, 1H, olefinic (CH=C), 7.12–7.76 (m, 4H, Ar–H), 8.54 (s, 1H, 2H indole), 11.49 (s, 1H, NH), 11.91 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta=14.56$ (CH_3), 61.47 (CH_2), 104.33, 111.1, 113.9, 119.01, 122.8, 126.1, 130.9, 149.3, 154.1, 162.4, 167.2, and 189.7; elemental analysis calcd (%) for $C_{14}H_{13}NO_4$ (259.26): C, 64.86; H, 5.05; N, 5.40; found: C, 64.73; H, 4.93; N, 5.52.

3-[2-(1H-indol-3-yl)-2-oxoethylidene]-3, 4, 4a, 8a-tetrahydro-2H-1, 4-benzoxazin-2-one

A mixture of both ester 1 (0.39 g, 1.5 mmol) and 2-aminophenol (1.7 g, 1.5 mmol) in toluene (10 ml) was refluxed for 3 h. Subsequently, the resulting precipitate was recrystallized from ethanol to provide compound 2. Yield 45%, m.p. 120–122 °C. IR (KBr, *v*, cm^{-1}): 3172–3150 (2NH), 1692–1662 (2C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta=7.22$ (s, 1H, CH=C), 6.81–8.02 (m, 8H, Ar–H), 8.57 (s, 1H, 2H indole), 10.65 (s, 1H, NH), 11.62 (s, 1H, NH); elemental analysis calcd (%) for $C_{18}H_{14}N_2O_3$ (306.1): C, 70.58; H, 4.61; N, 9.15; found: C, 70.50; H, 4.51; N, 9.08%.

Ethyl 5-(1H-indol-3-yl)-1-phenyl-1H-pyrazole-3-carboxylate

A mixture of compound 1 (0.26 g, 1 mmol) and phenylhydrazine (0.11 g, 1 mmol) in (20 ml) ethanol was refluxed for 8 h. The mixture was poured into iced water to cool. The obtained solid was recrystallized from toluene. Yield 75%, m.p. 250–252 °C. IR (KBr, *v*, cm^{-1}): 3276 (NH), 1739 (C=O), and 1620 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta=1.30$ (t, 3H, J = 7.6 Hz, CH_3), 4.40 (q, 2H, J = 7.5 Hz, CH_2), 7.11 (s, 1H, pyrazole-H), 7.18–8.02 (m, 9 H, Ar–H), 8.54 (s, 1H, 2H indole), 10.60 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta=14.25$, 56.82, 62.4, 111.6, 112.6, 114.6, 118.3, 119.7, 122.1, 126.3, 127.5, 128.4, 139.7, 139.8, 144.6, 151.5, 153.1, 160.2; elemental analysis calcd (%) for $C_{20}H_{17}N_3O_2$ (331.37): C, 72.49; H, 5.17; N, 12.68; found: C, 72.40; H, 5.15; N, 12.59.

3-(1H-indol-3-yl)-2-phenyl-2H-pyridazino[3,4-b][1, 4]benzoxazine

A mixture of compound 2 (0.31 g, 1 mmol) and phenylhydrazine (0.11 g, 1 mmol) in ethanol (30 ml) was refluxed for 8 h. The mixture was cooled by pouring into iced water. The obtained solid was recrystallized from ethanol. Yield 38%, m.p. 278–280 °C. IR (KBr, *v*, cm^{-1}): 3380 (NH), 3100 (CH arom.), and 1618 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta=5.5$ (s, 1H, pyridazine), 7.06–8.18 (m, 14 H, Ar–H), 8.50 (s, 1H, 2H indole), 10.64 (s, 1H, NH); elemental analysis calcd (%) for $C_{24}H_{16}N_4O$ (376.41): C, 76.58; H, 4.28; N, 14.88; found: C, 76.49; H, 4.24; N, 14.86.

3-Hydroxy-1-[5-(1H-indol-3-yl)-1-phenyl-1H-pyrazol-3-yl]-3-pyridin-3-ylprop-2-en-1-one

10 ml of toluene was added to a suspension of sodium (0.34 g, 1.5 mmol) and to pyrazole-3-carboxylate 3 (3.97 g, 1.2 mmol), and 20 ml of toluene was slowly added; then, 3-acetylpyridine (1.45 g, 1.2 mmol) was added at 0 °C in 10 ml of toluene. The attained mixture was stirred at room temperature for 2 days. Formed precipitate was filtered, washed with toluene, and dissolved in water; afterward, it was neutralized using acetic acid. After extraction with CH_2Cl_2, the organic layer was dried and then concentrated under vacuum. The gotten residue was filtered and recrystallized from methanol. Yield 35%, m.p. 132–134 °C. IR (KBr, *v,* cm-1): 3415 (OH), 3386 (NH), 1640 (C=O) 1618 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): $\delta = 6.67$ (s, 1H, pyrazole-H), 6.98 (s, 1H, olefinic CH), 7.00–8.63 (m, 14 H, Ar–H), 11.60 (s, 1H, NH), 15.32 (s, 1H, OH); elemental analysis calcd (%) for $C_{25}H_{18}N_4O_2$ (406.44): C, 73.88; H, 4.46; N, 13.78; found: C, 73.81; H, 4.43; N, 13.69.

3-[1-Phenyl-3-(3-pyridin-3-ylisoxazol-5-yl)-1H-pyrazol-5-yl]-1H-indole

A mixture of diketone 5 (0.41 g, 1 mmol), hydroxylamine hydrochloride (0.07 g, 1 mmol), and anhydrous potassium carbonate (0.14 g, 1 mmol), in 20 ml ethanol, was refluxed for 8 h. The mixture was poured into cold water, and the solid product was filtered off, washed with water, dried, and crystallized from ethanol to afford isoxazole derivative 6. Yield 64%, m.p. 140–142 °C. IR (KBr, *v,* cm-1): 3386 (NH), 1620 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): $\delta = 6.32$ (s, 1H, CH pyrazole), 7.00–8.63 (m, 15 H, Ar–H), 11.61 (s, 1H, NH). Anal. Calcd for $C_{25}H_{17}N_5O$ (403.44): C, 74.43; H, 4.25; N, 17.36; found: C, 74.35; H, 4.20; N, 17.29%.

5-(1H-indol-3-yl)-1-phenyl-5′-pyridin-3-yl-1H, 2′H-3, 3′-bipyrazole

Hydrazine hydrate (0.01 g, 2 mmol) has been added to a solution of diketone 5 (0.82 g, 2 mmol) in 30 ml of ethanol. The mixture was refluxed for 6 h and left to cool; the formed solid product was filtered off, dried, and then crystallized from ethanol to provide compound 7. Yield 62%, m.p. 246–248 °C. IR (KBr, *v,* cm^{-1}): 3281–3221 (2NH), 1619 (C=N) cm-1; ^1H NMR (DMSO-d_6, 300 MHz): $\delta = 6.32$ (s, 1H, CH pyrazole), 6.63 (s, 1H, CH pyrazole). 7.00–8.63 (m, 14 H, Ar–H), 11.61 (s, 1H, NH), 13.70 (s, 1H, pyrazole NH). Anal. Calcd for $C_{25}H_{18}N_6$ (402.45): C, 74.61; H, 4.51; N, 20.88; found: C, 74.53; H, 4.48; N, 20.86%.

General procedure for synthesis of pyrimidines 8, 9

A diketone 5 (0.82 g, 2 mmol) was added to sodium ethoxide solution prepared from sodium metal (0.046 g, 2 mmol) and 25 ml of absolute ethanol; then, urea or thiourea [$SC(NH_2)_2$ (2 mmol)] was added. The reaction mixture was refluxed for 16 h and left to cool. The solution was poured into crushed ice and neutralized with diluted hydrochloric acid (HCl). The precipitated product was collected by filtration, washed with ethanol, and dried. Crystallization from ethanol afforded the pyrimidine derivatives 8, 9, respectively.

4-[5-(1H-indol-3-yl)-1-phenyl-1H-pyrazol-3-yl]-6-pyridin-3-ylpyrimidine-2(1H)-one

Yield 68%, m.p. 218–220 °C. IR (KBr, *v*, cm^{-1}): 3238, 3170 (2NH), 1669 (C=O), 1633 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): $\delta = 5.46$ [s, 1H, pyrimidine C(5)], 6.63 (s, 1H, CH pyrazole), 7.00–8.63 (m, 14 H, Ar–H), 9.94 (s, 1H, pyrimidine NH), 12.07 (s, 1H, indole NH) ppm. Anal. Calcd for $C_{26}H_{18}N_6O$ (430.46): C, 72.55; H, 4.21; N, 19.52; found: C, 72.54; H, 4.13; N, 19.44%.

4-[5-(1H-indol-3-yl)-1-phenyl-1H-pyrazol-3-yl]-6-pyridin-3-ylpyrimidine-2(1H)-thione

Yield 64%, m.p. 214–216 °C. IR (KBr, *v*, cm^{-1}): 3238–3170 (2NH), 1623 (C=N),1256 (C=S) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): $\delta = 5.60$ [s, 1H, pyrimidine C(5)], 6.60 (s, 1H, CH pyrazole), 7.00–8.63 (m, 14 H, Ar–H), 9.90 (s, 1H, pyrimidine NH), 11.27 (s, 1H, indole NH) ppm. Anal. Calcd for $C_{26}H_{18}N_6S$ (446.53): C, 69.94; H, 4.06; N, 18.82; S, 7.18; found: C, 69.90; H, 4.05; N, 18.71; S, 7.10%.

Antimicrobial activity

A modified Kirby-Bauer disc diffusion method was used to determine antimicrobial activity of some prepared compounds [21]. 100 µl of the test bacteria/fungi was grown in a 10 ml of fresh media, until the cell concentration was standardized to approximately 1×10^8 (cells/ml) for bacteria and 1×10^5 (cells/ml) for fungi [22]. The antimicrobial resistance intimidates the activities of science and medicine, as it deactivates the conventional antimicrobial therapeutics [23]. The microbial suspension of 100 µl was spread onto the agar plates, corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role were selected from primary agar plates and tested for susceptibility by disc diffusion method [24–28].

Powder samples coded 1, 2, and 3 were dissolved in dimethyl sulfoxide [DMSO, $(CH_3)_2SO$] to reach a final concentration of 10 (mg/ml). On the other hand, liquid samples coded

4 and 5 were dissolved in methanol (CH_3OH). 50 ml of the bioassay medium (nutrient agar) seeded with 100 µl of cell suspension contained [1.5×10^8 CFU/ml; 0.5 mac fir land] of the 24-hour-old test organisms. 5 ml well diameter was cut into the agar medium using a sterilized cork borer, and then 50 µl of the samples was poured into the wells, while filter discs were cut for the samples coded 4 and 5; however, filter discs (doublet) were saturated with 20 and 50 µl of each sample separately and then placed onto the surface of each seeded plate with the tested organisms. It was then left in the refrigerator for 1 h, followed by incubation at 37 °C for 24 h. Subsequently, the diameter of inhibition zones was measured in mm.

Anticancer activity

Cell culture

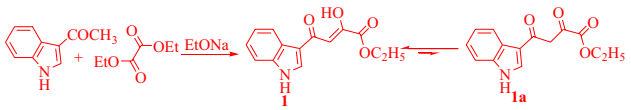
Cells were obtained from Bioassay-Cell Culture Laboratory, National Research Centre, Dokki, Giza 12622, Egypt. Cells were grown in RPMI-1640 medium [Roswell Park Memorial Institute-1640 medium]. The samples were improved using 10% heat, inactivated fetal bovine serum (FBS), 50 units/ml of penicillin, and 50 mg/ml of streptomycin. It was preserved in a humidified atmosphere encompassing 5% CO_2. The cells were sustained as monolayer culture by serial subculturing. Cell culture reagents were obtained from Lonza (Basel, Switzerland). The anticancer activity of tested compounds was evaluated against MCF-7 cells (breast cancer), HCT116 [colon cell line], and RPE1 [normal retina cell line] [27]. The half maximal inhibitory concentration (IC_{50}) values were obtained by the analysis of obtained data using the IBM SPSS software [28].

Results and discussion

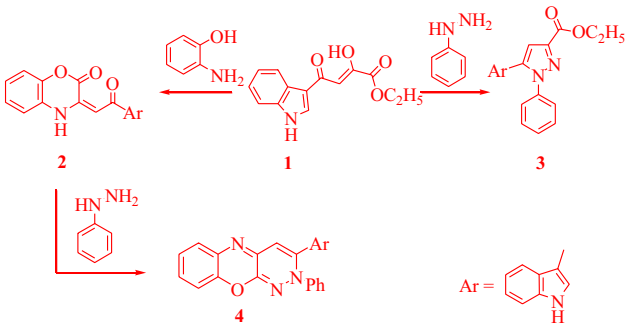
Chemistry

Claisen reaction between 3-acetylindole and diethyl oxalate furnished β-diketones 1 that mainly presents thermodynamic enol form (stabilized by conjugation addition to intramolecular hydrogen bond to enol). Spectral data of compound 1 were confirmed by elemental analyses, IR, ^{1}H NMR, and ^{13}C NMR. The IR spectra showed the existence of characteristic bands for OH at 3443 (cm^{-1}) and 2C=O in the range of 1751–1685 (cm^{-1}) (Scheme 1).

Cyclization of O-aminophenol with 2-hydroxy-4-(1H-indol-3-yl)-4-oxobut-2-enoic acid 1 gave the benzoxazinone 2. Compound 1 underwent pyrazole cyclization by nucleophilic attack of phenyl hydrazine directed to the electrophilic center of compound 1. This process was followed by intramolecular cyclodehydration to give ethyl



Scheme 1 Synthesis of Enolic Keto Ester 1



Scheme 2 Synthesis of Benzoxain and Pyrazole derivatives

5-(1H-indol-3-yl)-1-phenyl-1H-pyrazole-3-carboxylate 3. Spectral data of compound 2 were confirmed by IR and ^{1}H NMR. The IR data revealed the disappearance of the OH group band, accompanied with the appearance of new band in the range of 3172–3150 (cm^{-1}) due to the benzoxazinone NH. These outcomes were confirmed by the data given from the ^{1}H NMR that elucidated the fading of triplet and quartet of ethyl group (C_2H_5) accompanied with the presence of the amine (NH) signal for benzoxazinone NH at 10.65 ppm. IR spectra of compound 3 showed the existence of bands at 1739 cm^{-1}, targeting the C=O and at 1620 cm^{-1} for C=N; In contrast, no band of OH group was identified. ^{1}H NMR spectra of similar compound revealed the presence of triplet at 1.30 ppm, assigned to CH_3 ester and a quartet at 4.40 ppm owing to the CH_2 ester. The heterocyclization of benzoxazinone 2 using phenyl hydrazine as bidentate nucleophile ensued 2-phenylpyridazino[3,4-b] benzoxazine 4 [1, 4]. The structure of compound 4 was confirmed via IR spectra, which exhibited the disappearance of the C=O absorption bands, and in ^{1}H NMR, the proton at position 4 in dihydropyridazine ring appeared in olefinic region 5.5 ppm (Scheme 2).

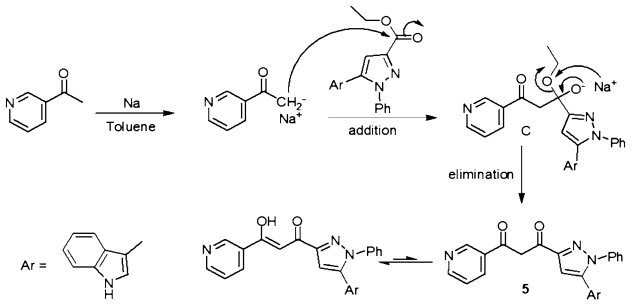
Base-mediated intermolecular condensation of 3-acetylpyridine and ethyl heterocyclic-3-carboxylate 3 produced the stabilized enolic form 3-hydroxy-1-[5-(1H-indol-3-yl)-1-phenyl-1H-pyrazol-3-yl]-3-pyridin-3-ylprop-2-en-1-one 5. Interconversion of β-keto-enol was determined using ^{1}H NMR integration of signals from the enol = C–H and the ketone CH_2. Certainly, the parent β-diketones occur entirely in the enol form, and only a trace of the keto form was seen about 4 ppm. Synthesis started with the formation of a ketone enolate nucleophile in cool conditions at zero

celsius after the addition of proper heterocyclic carboxylate. Obtained mixture was continuously stirred for 2 days at room temperature. The formed enolate initially experienced nucleophilic attack at the ester carbonyl to yield tetrahedral intermediate. In order to produce a stabilized enolate ion product (C) as a precipitated salt, the expulsion of ethoxide ion from the unstable tetrahedral intermediate of the initial Claisen yielded a β-diketone. Acidic alpha proton from the β-diketone was consequently removed. The precipitate was filtered, washed with toluene, dissolved in water, and then neutralized using acetic acid (CH_3COOH) to give the title products with an acceptable yield (Schemes 3 and 4).

The reactivity of diketone 5 toward nitrogen nucleophiles is a suitable functionality for further heterocyclization. Therefore, compound 5 reaction with hydroxylamine hydrochloride in refluxing ethanol furnished isoxazole derivative 6. Meanwhile, the reaction of 5 with hydrazine hydrate gave rise to the pyrazole derivatives 7 in an upright yield. IR spectra of compounds 6 and 7 revealed the disappearance of the two carbonyl groups with the appearance of the NH band in case of compound 7 within the range of 3281–3221 (cm^{-1}), while ^{1}H NMR spectra was recorded for isoxazole ring 6 signal at aromatic region with the appearance of a new NH signal due to pyrazole ring of compound 7 at 13.70 ppm. An essential principle for the synthesis of pyrimidines is the reaction of 1, 3-dicarbonyl compound with urea and/or thiour [20]. Consequently, pyrimidine cyclization of dicarbonyl compound 5 was accomplished by the reaction with urea and/or thiourea in the presence of sodium ethoxide (C_2H_5ONa) that provided the pyrimidine derivatives 8, 9, respectively (Scheme 5). The structure of compound 8 was confirmed by IR. It revealed bands at 3238, 3170 cm^{-1} for the 2NH groups and a band at 1669 cm^{-1} typical for the C=O, respectively. ^{1}H NMR displayed two singlets at δ 9.94 for pyrimidine NH and δ 12.07 for indole NH by ppm. On the other hand, the IR of pyrimidine derivatives 9 exhibited a band at 1256 cm^{-1} owing to C=S while ^{1}H NMR revealed a signal of pyrimidine NH at δ 9.90.

Screening of antimicrobial activity

Five synthesized compounds were assessed by screening the in vitro growth inhibitory activity contrary to the variety



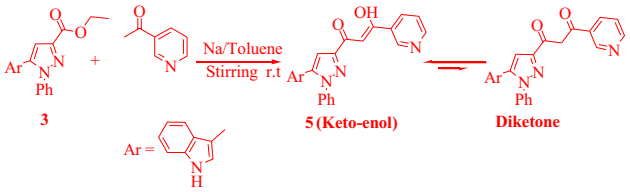
Scheme 4 A plausible mechanistc pathway to explain the formation of Diketone 5

of strains of bacteria and fungi. This approach used gram-positive strains (*Staphylococcus aureus* and *Streptococcus pyogenes*) and gram-negative strains (*Salmonella enterica* and *Klebsiella pneumonia*). Besides, *Candida albicans* was used as dimorphic fungal strain. Results of antimicrobial activity are listed in Table 1.

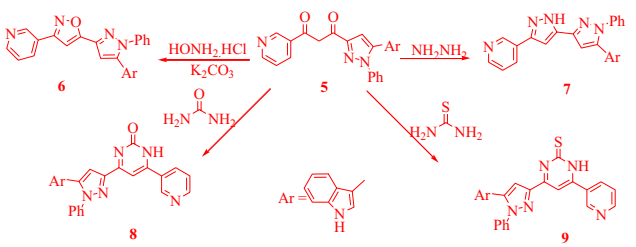
All of the tested compounds showed no antimicrobial effect on *S. pyogenes*. Low antimicrobial activity was exhibited on the *S. enterica* and *K. pneumonia* with inhibition zone diameter (IZD) in the range of (11–23 mm). On the other hand, the unassertive effect on *C. albicans* verified an inhibition zone diameter range (6–34 mm). Efficient antimicrobial activity presented gram-positive bacteria (*S. aureus*) with inhibition zone of a diameter ranging from (12 to 60 mm). Accordingly, it could be concluded that compound 2 possessed good antibacterial activity as well as antifungal activity in comparison with other compounds (Figs. 1, 2). Thus, compound 2 can be used as a comprehensive spectrum antibacterial agent.

Anticancer activity test

Anticancer activity test was held against human breast carcinoma cell line (MCF7), HCT116 [colon cell line], and RPE1 [normal retina cell line], using positive control [adriamycin (doxorubicin)]. Compound 2 being the most potent compound concerning the antibacterial and antifungal activity has been chosen in parallel with compounds 1 and 5.



Scheme 3 Formation of Diketone 5



Scheme 5 Synthesis of isoxazole, pyrazole and pyrimidine derivatives

Table 1 Outcomes of antimicrobial activity

Sample code	*Salmonella enterica*	*Klebsiella pneumoniae*	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Candida albicans*
Compound 1	Nil	11	Nil	12	Nil
Compound 2	23	Nil	Nil	60	34
Compound 3	Nil	Nil	Nil	16	Nil
Compound 4					
20 µL	Nil	Nil	Nil	19	6
50 µL	Nil	Nil	Nil	26	12
Compound 5					
20 µL	Nil	Nil	Nil	12	Nil
50 µL	Nil	Nil	Nil	17	Nil
DMSO (blank)	Nil	Nil	Nil	Nil	Nil

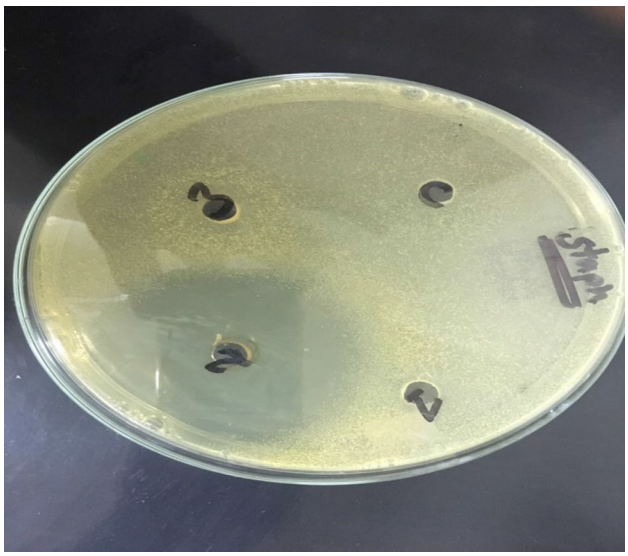


Fig. 1 Antibacterial activity

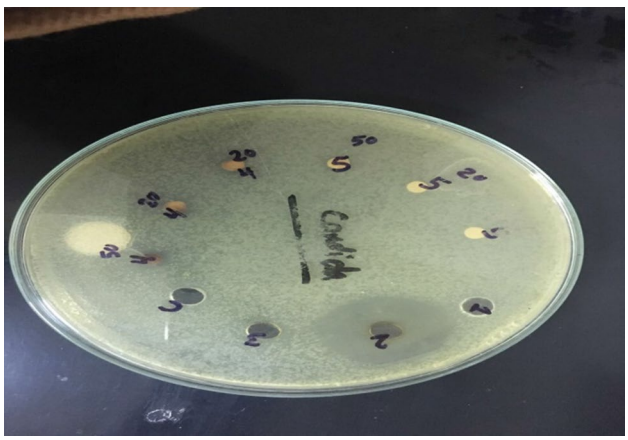


Fig. 2 Antifungal activity

Table 2 In vitro cytotoxic activity IC_{50} at 100 ppm

Sample code	MCF7 IC_{50} (µg/ml) (%)	HCT116 IC_{50} (µg/ml) (%)	RPE1 IC_{50} (µg/ml) (%)
1	59.4	5.6	83.3
2	100	100	100
5	11.2	2.8	27.5
DMSO	3	1	5
Negative control	0	0	0

Sample concentration ranged between 100 and 0.78 (µg/ml), using a colorimetric assay for assessing cell metabolic activity (MTT assays). IC_{50} is the half maximal inhibitory concentration as compared with the control structures in the absence of an inhibitor (Table 2).

Conclusion

This study presented a comprehensive assessment to provide the description of Claisen reaction taking place between 3-acetylindole and diethyl oxalate. Our study highlights the effect of 1, 3-dicarbonyl compound derivatives, linked to the indole moiety. It has been delineated that these compounds, particularly compound 2, have a promising influence on antitumor and anticancer impact.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

References

1. Y. Pommier, A.A. Johnson, C. Marchand, Integrase inhibitors to treat HIV/AIDS. Nat. Rev. Drug Discov. **4**(3), 236–248 (2005)
2. M.S. Egbertson, N.J. Anthony, V. Summa, HIV integrase inhibitors: from diketo acids to heterocyclic templates: history of HIV integrase medicinal chemistry at Merck West Point and Merck Rome (IRBM) leading to discovery of raltegravir, in *HIV-1 Integrase: Mechanism and Inhibitor Design* (2011), pp. 197–229
3. A.V. Kel'in, Recent advances in the synthesis of 1, 3-diketones. Curr. Org. Chem. **7**(16), 1691–1711 (2003)
4. A.V. Kel'in, A. Maioli, Recent advances in the chemistry of 1, 3-diketones: structural modifications and synthetic applications. Curr. Org. Chem. **7**(18), 1855–1886 (2003)
5. D. Lim, F. Fang, G. Zhou, D.M. Coltart, Direct Carbon–Carbon bond formation via soft enolization: a facile and efficient synthesis of 1, 3-diketones. Org. Lett. **9**(21), 4139–4142 (2007)
6. D. Kumar, N. Kumar, S. Kumar, T. Singh, C.P. Singh, Synthesis of pharmacologically active 2-phenyl sulpha/substituted Indoles. Int. J. Eng. Sci. Technol. **2**(7), 2553–2557 (2010)
7. C. Karaaslan, S. Suzen, Electrochemical behavior of biologically important indole derivatives. Int. J. Electrochem. **2011**, 10 (2011), Article ID 154804
8. V.F. Ximenes, A. Campa, L.H. Catalani, The oxidation of indole derivatives catalyzed by horseradish peroxidase is highly chemiluminescent. Arch. Biochem. Biophys. **387**(2), 173–179 (2001)
9. R.P. Singh, D.V. Singh, C.R. Singh, S. Singh, Synthesis and fungitoxicity of 1-[N-benzoyl-3-(2^1-substituted-3^1-sulphonyl-5^1-methoxyindol-3^1-Yl)-2-pyrazolines and 1-(N-phenylsulphonyl)-3-(2^1-substituted-3^1-sulphonyl)-5^1-methoxyindol-3^1-YL)-2-pyrazolines. Acta Chimica and Pharmaceutica Indica **2**(3), 143–150 (2012)
10. N.D. Gaikwad, S.V. Patil, V.D. Bobade, Synthesis and antimicrobial activity of novel thiazole substituted pyrazole derivatives. J. Heterocycl. Chem. **50**(3), 519–527 (2013)
11. E.M. Sharshira, N.M.M. Hamada, Synthesis and antimicrobial evaluation of some pyrazole derivatives. Molecules **17**(5), 4962–4971 (2012)
12. C.Y. Zhang, X.H. Liu, B.L. Wang, S.H. Wang, Z.M. Li, Synthesis and antifungal activities of new pyrazole derivatives via 1, 3-dipolar cycloaddition reaction. Chem. Biol. Drug Des. **75**(5), 489–493 (2010)
13. E.M. Flefel, W.A. Tantawy, W.A. El-Sayed, H.H. Sayed, N.M. Fathy, Synthesis and anticancer activity of new substituted pyrazoles and their derived 1, 2, 4-triazoles and sugar derivatives. J. Heterocycl. Chem. **50**(2), 344–350 (2013)
14. S.M. El-Moghazy, F.F. Barsoum, H.M. Abdel-Rahman, A.A. Marzouk, Synthesis and anti-inflammatory activity of some pyrazole derivatives. Med. Chem. Res. **21**(8), 1722–1733 (2012)
15. M. Li, B.-X. Zhao, Progress of the synthesis of condensed pyrazole derivatives (from 2010 to mid-2013). Eur. J. Med. Chem. **85**, 311–340 (2014)
16. D. Raffa, B. Maggio, M.V. Raimondi, S. Cascioferro, F. Plescia, G. Cancemi, G. Daidone, Recent advanced in bioactive systems containing pyrazole fused with a five membered heterocycle. Eur. J. Med. Chem. **97**, 732–746 (2015)
17. W.S. Shehab, S.M. Mouneir, Design, synthesis, antimicrobial activity and anticancer screening of some new 1, 3-thiazolidin-4-ones derivatives. Eur. J. Chem. **6**(2), 157–162 (2015)
18. W.S. Shehab, M.H. Sherif, M.G. Assy, Synthesis of some 3-indolyl heterocycles and evaluation of its antimicrobial activity. Iran. J. Org. Chem. **8**(3), 1851–1858 (2016)
19. W.S. Shehab, H.A. Saad, S.M. Mouneir, Synthesis and antitumor/antiviral evaluation of 6-thienyl-5-cyano-2-thiouracil derivatives and their thiogalactosides analogs. Curr. Org. Synth. **14**(2), 291–298 (2017)
20. S. Radi, S. Tighadouini, O. Feron, O. Riant, M. Bouakka, R. Benabbes, Y.N. Mabkhot, Synthesis of novel β-keto-enol derivatives tethered pyrazole, pyridine and furan as new potential antifungal and anti-breast cancer agents. Molecules **20**(11), 20186–20194 (2015)
21. A.W. Bauer, W.M. Kirby, J.C. Sherris, M. Turck, Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. **45**(4), 493–496 (1966)
22. X. Niu, Y. Liu, Y. Song, J. Han, H. Pan, Rosin modified cellulose nanofiber as a reinforcing and co-antimicrobial agents in polylactic acid/chitosan composite film for food packaging. Carbohydr. Polym. **183**, 102–109 (2018)
23. A.R. Katritzky, T.I. Yousaf, A C-13 nuclear magnetic resonance study of the pyrimidine synthesis by the reactions of 1, 3-dicarbonyl compounds with amidines and ureas. Can. J. Chem. **64**(10), 2087–2093 (1986)
24. A.S. Abd-El-Aziz, C. Agatemor, N. Etkin, Antimicrobial resistance challenged with metal-based antimicrobial macromolecules. Biomaterials **118**, 27–50 (2017)
25. National Committee for Clinical Laboratory Standards, Performance Vol. 41, Antimicrobial susceptibility of Flavobacteria (1997)
26. National Committee for Clinical Laboratory Standards, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, PA, USA (1997)
27. National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilu
28. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drug screening. JNCI J. Natl. Cancer Inst. **82**(13), 1107–1112 (1990)