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Green synthesized pyridazinone derivatives as promising biologically active and anticancer drugs

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Abstract

An efficient synthesis of substituted 4-acetyl-5,6-diphenylpyridazin-3(2H)-one derivative has been achieved using green chemistry tools such as grinding and microwave heating compared to conventional heating. In this work, 4-acetyl-5,6-diphenylpyridazin-3(2H)-one derivative attached to Schiff-bases, chalcones, pyridine, and pyrrole moieties have been prepared with the aim to possess engaging biological and pharmacological activities. The Schiff-base **2** was obtained by reacting 4-acetyl-5, 6-diphenylpyridazin-3(2H)-one 1 with p-amino acetophenone, and the corresponding chalcones **3a-d** were obtained by reacting the Schiff-base 2 with the relevant aldehydes. Novel pyridinyl-pyridazinones **5a-h** and pyrrole-pyridazinone derivatives **6a-h** were synthesized by the reaction of chalcones **3a-d** with different acetyls and aldehydes under grinding, microwave irradiations or under reflux conditions. The synthesized compounds were tested for both Gram-positive and Gram-negative antibacterial activity, antifungal, and anticancer activities. These substances were also evaluated using analytical and spectral data, such as ^{1}HNMR and mass spectrum analysis.

Keywords: Green chemistry; one-pot reactions; pyridazinone; pyridine pyrrole; chalcones; anticancer; antimicrobial

1. Introduction

The basis of green chemistry focuses on reducing hazardous generation during the synthesis process. One of the modern applied green chemistry methods is a one-pot reaction. Based on green chemistry principles, organic synthesis has been used through three mechanisms: grinding, microwave irradiations, and conventional method. The conventional method has different downsides, such as extended heating time, environmental pollution (due to the excessive use of solvents or reagents), and a higher estimated budget for complicated synthesis apparatus. Compared to the conventional synthesis method, grinding and microwave irradiation exhibit different advantages such as augmented reaction rates to reduce time, causes, significant yield, and improved pure product outputs via eco-friendly method[1–5].

Pyridazinone, Pyridine, and Pyrrole derivatives are promising compounds due to their cardiotonic , anti-inflammatory, antifungal antibacterial, anticancer besides their herbicidal properties[6–8]. Previously, we have reported the synthesis of different 3(2H)-pyridazinone derivatives bearing a linker of five and six heterocycles as promising pharmaceutical chemicals[9–15].

The pharmacological action of pyridazinones has been extensively investigated, and its cardiovascular effects are well recognized. At a concentration of 100 mM/mL, pyrrolo[3,4-d]pyridazinone derivatives(1,2) exhibit anti-inflammatory and cytokine (TNFa, IL-1b) inhibition effects among the pyridazinone compounds synthesized by Mogilski et al.[9]**(Figure 1)**.

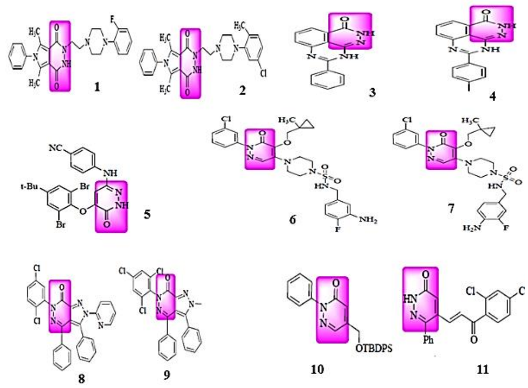

Figure 1: Some of pyridazinones derivatives[16]

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The neuroprotective impact of pyridazino [3,4,5-de]quinazolin-3(2H)-one derivatives was investigated by Wang et al. The synthesised compounds(3 ,4) exhibit neuroprotective effects in the PC12 cell model harmed by H2O2, and both of them demonstrated good activity with IC50 values of 0.148 mM and 0.152 mM, respectively[10].

In addition, pyridazinone inhibits HIV-1 in its wild type form. For example, Li et al. synthesized pyridazinone-based compounds (Compound 5) as the series' most powerful compound, with an EC50 of 0.21 0.03 mM [11].

Zych et al. synthesized and tested a range of substituted pyridazinones for antifungal activity. Compounds (6, 7) were produced to have significant antifungal efficacy after testing. Aspergillus fumigatus, and the isolated pathogenic clinical strains of Candida albicans (C. albicans) were tested [12].

Akbas and colleagues created a series of pyridazinone compounds and tested them for antibacterial activity. Compounds 8 and 9 were proven to have a significant antibacterial action against both Gram-negative and Gram-positive bacteria. The MIC values were observed to range between 0.31 and 0.0024 mg/mL[13].

Compound 10 was shown to be the most effective and potential antiplatelet compound among the derivatives produced by Costas et al., with an IC50 value of 0.55 0.08 mM[14]. Compound 11 was found to be an efficient antiplatelet compound by Maatougui et al., with an IC50 of 3.44 0.04 mM [15].

Hence, this paper aims to synthesize novel 3(2H)-pyridazinone derivatives bearing Schiff-bases and chalcones annulated with various five and six-membered heterocyclic moieties via conventional, grinding tools, and microwave irradiations and their biological activities[17–19]. Herein, we synthesized 4-acetyl-4, 5-diphenyl pyridazine-3(2H)-one **1** as a starting material in the presence of sodium ethoxide through the reaction of benzilmonohydrazone with ethyl acetoacetate in refluxing ethanol system. The synthesis approach was enhanced by combining a grinding process with the microwave-assisted method, which is growing rapidly in organic synthesis. The developed methodology is effective for the rapid synthesis of pyridazinone **1** with a high yield and promising biological activities[20–22].

The presence of the imine group in Schiff-base derivatives demonstrates a variety of biological applications, for instance, antifungal, antibacterial, antimalarial, anti-inflammatory, antiviral, and antipyretic properties[23–25]. Various strategies are employed to synthesize the Schiff-base **2** via conventional refluxing, grinding tool technique, and microwave-assisted methods[26]. It can be synthesized via the reaction of 4- acetyl 5,6-diphenyl 4,5-dihydro pyridazi-3(2H)-one **1** and *p* – aminoacetophenone with adding one drop of acetic acid in a short time which yields 82%. Compared with the conventional method, which yielded 55% of Schiff-base derivative **2** in 6 hours, the grinding tool technique gave 80% yield in 40 minutes, and the microwave radiations yielded 92% in 3 minutes.

Chalcones are considered as characteristic intermediates in organic synthesis via activated α, β-unsaturated carbonyl mechanism. They also exhibit a multimode of biological activities [27]. A series of novel chalcones **3a-d** were synthesized via the reaction of the Schiff-base **2** and appropriate aldehydes such as benzaldehyde ,p-chlorobenzaldehyde, p-nitrobenzaldehyde, and salicylaldehyde in 20 ml ethanol in the presence of potassium hydroxide at room temperature for 3-4 hours under stirring with 65-74% yields. Compared with the grinding method, which yields 83-86% of chalcone **3a-d** after 0.5-1hour, the microwave radiations yield 87-91% after 4 minutes (**Figure 2).**

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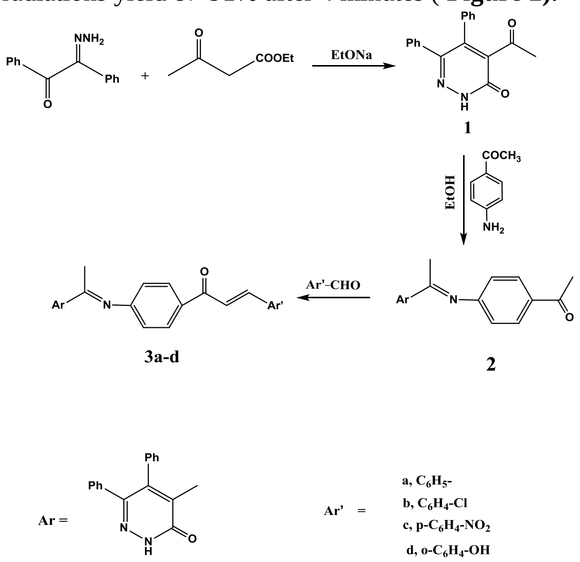

Figure 2 : Synthesis scheme of newly pyridazinones derivatives 3a-d

Pyridine is the parent ring system with several naturally occurring compounds, and this ring system is valuable in various industries, including industrial, medicinal, and agricultural chemicals[28]. A series of novel 3(2H)- pyridazinones bearing pyridine moieties were synthesized through the mixture of chalcones **3a-d,** and each of 2-acetyl thiophene or 3-aceyl pyridine in the presence of sodium hydroxide. The mixture was then crushed in a mortar for 20 minutes until the color changed, yielding the promised diketone intermediates **4a-h**. Finally, ammonium acetate was added, and the mixture was refluxed for 6 hours in acetic acid glacial, yielding the corresponding pyridyl pyridazinone derivatives **5a-h** in 55-71 percent yield, whereas the microwave method yielded 85-96 percent[29].

Several marine-derived natural compounds, including heme, chlorophyll, bile pigments, vitamin B12, and pyrrole alkaloids, contain functionalized pyrroles[30]. Pyrrole derivatives are regarded to be one of the most fundamental heterocyclic chemical compounds. Novel pyrrolo pyridazinone derivatives can be easily produced by grinding chalcones **3a-d** with several aldehydes and ammonium acetate in one pot under microwave irradiation and repeating the process in the presence of sodium cyanide. The notable features are short reaction time, high yield, and high purification products compared to the classical condition[31]. The reaction of Schiff-bases with amines has been employed in the conventional Paal-Knorr method to synthesize these substituted pyrroles. A series of pyrrole derivatives were prepared via the Paal-Knorr method through a facile and efficient one-pot synthesis under grinding and microwave irradiation[32]. In the presence of sodium cyanide as a catalyst, chalcones **3a-d** and aldehydes such as 2-thiophene aldehyde and 3-pyridine aldehyde were mixed in Dimethylformamide (DMF) and smoothly converted into pyrrole derivatives **6a-h** in the existence of ammonium acetate under microwave irradiations in good yield and short reaction time **(Figure 3)**

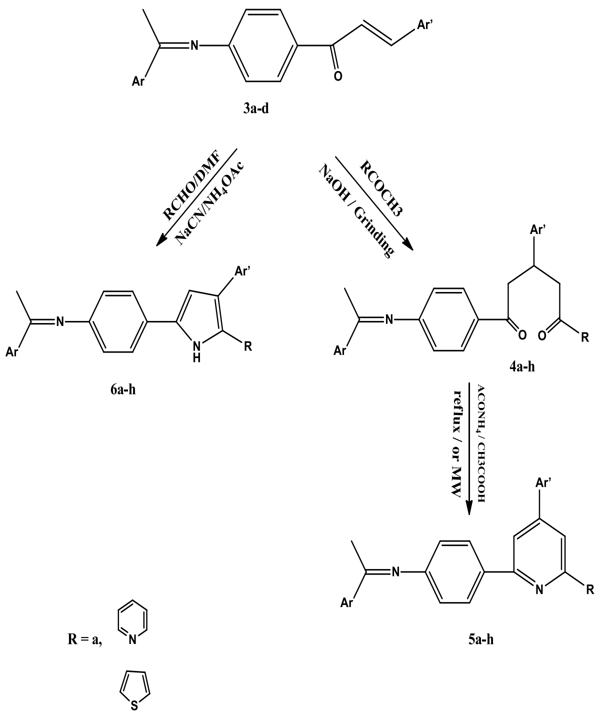

Figure 3 : Synthesis scheme of newly pyridazinones derivatives 5a-h and 6a-h

2. Experimental

2.1. Tests and analysis

All melting points were determined with a Gallenkamp melting point instrument and are uncorrected. The infrared spectra were recorded in potassium bromide discs on a pye Unicam SP 3300 and Shimadzu FT-IR 8101 PC infrared spectrophotometer. A Varian Mercury VXR-300 NMR spectrometer was used to record the NMR spectra. In dimethyl sulphoxide, ^{1}H spectra were conducted at 300 MHz, while 13C spectra were run at 75.46 MHz (DMSO-d6). Chemical shifts were proportional to the solvents. At 70 eV, mass spectra were acquired using a Shimadzu GCMS-QP 1000EX mass spectrometer.

2.2. Chemicals

Benzilmonohydrazone , ethanol , sodium ethoxide , ethyl acetoacetate , ammonium acetate , hydrochloride acid, acetic acid, benzaldehyde, p-chlorobenzaldehyde, p-nitrobenzaldehyde, and salicylaldehyde , potassium hydroxide , benzaldehyde .The chemicals and all solvents used in this study were purchased from Merck (Darmstadt, Germany) and Aldrich Chemical Co. (Steinheim, Germany).

2.3. Procedures

2.3.1. 4-Acetyl-5,6-diphenylpyridazin-3(2*H*)-one 1: Benzilmonohydrazone (0.02 mole) in 30 ml ethanol was added to a mixture of sodium ethoxide solution (0.02 mole) (0.46 gm sodium in 20 ml ethanol) and ethyl acetoacetate (0.02 mole) are refluxed for 6 hours. The pyridazinone derivative 1 was obtained by cooling the reaction mixture and pouring it up on ice/HCl. The precipitate was washed with water and recrystallized from ethanol to give a 65% yield.

2.3.2. (A)-4-(1-((4-acetyl phenyl)imino)ethyl)-5,6-diphenylpyridazin-3(2*H*)-one 2:

2.3.2.1.Conventional method: 4-Acetyl-5,6-diphenylpyridazin-3(2H)-one 1 (0.02 mole) was refluxed with p- amino acetophenone (0.02 mole) in 50 ml acetic acid for 6 hours aimed at producing buff precipitate, which was filtered, washed and recrystallized from ethanol to give Schiff-base 2 in a 88% yield.

2.3.2.2.Green method: In the presence of a few drops of acetic acid, A mixture of 4-acetyl-5,6-diphenylpyridazin-3(2H)-one and p- amino acetophenone (0.02 mole) was ground in a mortar for 1 hour until it turned a buff color, yielding a buff precipitate. This preciptate was filtered, washed, and recrystallized from acetone to yield a product that was identical in every way (M.P, mixed M.P. and TLC). When the same reactant was combined in dimethylformamide)DMF (in a 10 ml glass vial and microwave irradiated for 3 minutes, the generated solid was purified by recrystallization from ethanol/DMF (1:1), yielding a product that was identical in all respects (M.P, mixed M.P, TLC).

2.3.3. 4-((A)- 1-((4-((A)-3-(4-substitutedphenyl)acryloyl) phenyl)imino)ethyl)-5,6-diphenylpyridazin-3(2H)-one 3a-d: (Chalcones)

2.3.3.1.Conventional method:

To obtain the corresponding chalcone derivatives **3a-d**, a mixture of (0.02 mole) Schiff-base 2 and appropriate aldehydes such as benzaldehyde, p-chlorobenzaldehyde, p-nitrobenzaldehyde, and salicylaldehyde (0.02 mole) was stirred at room temperature in 20 ml ethanol in the presence of 0.01g potassium hydroxide for 6 hours.

2.3.3.2.Green method: In the presence of 0.01g potassium hydroxide, a mixture of (0.02 mole) Schiff-base 2 and appropriate aldehydes such as benzaldehyde, p- chlorobenzaldehyde, p-nitrobenzaldehyde, and salicylaldehyde (0.02 mole) was ground in mortar for the appropriate time until color change to yield (**3a-d**) products that were identical in all respects.

2.3.4.(6)-4-(l-((4-(6-(2-oxo-2-(thiophen-2-yl)ethyl)-4-phenylpyridin-2-yl)phen1) imino)ethyl)-5,6-diphenylpyridazin-3(2H)-one 5a-h:

2.3.4.1.Conventional method: In the presence of sodium hydroxide, a mixture of chalcones (0.02 mole) and 2-acetyl thiophene or 3-aceyl pyridine (0.02 mole) was refluxed until color change to obtain diketone **4a-h** as intermediate, then add ammonium acetate (1 mmol) and the mixture was refluxed in glacial acetic acid for 6 hours until the reaction was completed (monitored by TLC) to provide the corresponding derivatives **5a-h**.

2.3.4.2. Green method: A mixture of chalcones (0.02 mole) and 2-acetyl thiophene or 3-aceylpyridine (0.02 mole) was ground in a mortar for 20 minutes until color change occurred to attain diketone **4a-h** as an intermediate stage, then added ammonium acetate (1mmol) and one drop of glacial acetic acid in a 10 ml glass vial followed by microwave irradiation for 1-2 minutes. Finally, we acquire the formed solid (M.P, mixed M.P)

(ñ)-4-(1-((4-(5-(2-oxo-2-(pyridin-3-y1)ethyl)-4 substitutedphenylpyrol-2-yl)pheny1)imino)ethy1)-5,6 diphenylpyridazin-3(2//)-one 6a-h:

Conventional method: In the presence of sodium cyanide (0.18 mmol) and ammonium acetate (1 mmol), a mixture of chalcones **3(a-d)** (0.02 mole) and appropriate aldehydes, namely 2-thiophene aldehyde and 3-pyridine aldehyde (0.02 mole) were refluxed in DMF for 10 hours until the reaction was completed (monitored by TLC) to give precipitates.

Green method: A mixture of chalcones **1(a-e)** (1 mmol), and appropriate aldehydes such as 2-thiophene aldehyde and 3-pyridine aldehyde (1 mmol) in the presence of sodium cyanide (0.18 mmol) was ground in mortar for 20 minutes until color change occurred, then ammonium acetate (1 mmol) was added, along with 3 ml DMF, and microwave irradiation for 3 minutes.

3. Results and discussion

3.1. physical properties of prepared pridazinone derivatives by green and conventional technique

Table 1 shows the physical properties such as characteristic melting point and the product yield with different synthesis methods . Moreover it exhibits the chemical formulas and elementary analysis of carbon, hydrogen, nitrogen and sulphur.

The structure of **1** was confirmed by spectral data such as IR, ^{1}HNMR, and Mass spectral data. IR spectra explained the presence of NH group, C=O band, and OH group due to strong absorption band at 3196 cm^{-1}, 1692 cm^{-1}, and 3330, respectively. ^{1}HNMR spectra revealed the presence of a singlet signal at δ 2.82 ppm due to $COCH_3$. Singlet signals at δ 7.14-8.5 ppm and 11.50 ppm indicate the presence of aromatic protons and OH exchangeable groups, respectively, that proved the presence of Lactam-Lactim tautomerism in 4-acetyl-4,5-diphenylpyridazin-3(2H)-one **1**[33].

The structure of the Schiff-base **2** was elucidated by its spectroscopic characterization data, where its IR spectrum showed C-N, C=C, C-N), C=O, N≡H bands due to the presence of distinguishable bands at 1179 cm^{-1}, 1537 cm^{-1}, 1590 cm^{-1}, 1674 cm^{-1}, 3263 cm^{-1}, 3296 cm^{-1} respectively. The ^{1}H NMR displayed a characteristic singlet signal at δ 2.39 (s, 3H, CH_3-C=N-), 2.84 (s, 3H, CH_3C=O), 6.55-7.93 (m, 12H, Ar-H), 11.28 (s, 1H, NH proton). Its ^{13}C NMR spectrum showed a δ 24.26-24.77 ppm (CH_3C=N), 169.09- 169.67 ppm (CH_3C=O).

Table 1: Physical and chemical structure data of the newly synthesized compound

CH3-C=N-), and disappeared a signal due to

omp No.	Formula /M.Wt	M.p.°	Conven. Yield/Time	Mic.wave Yield/Time	Grind. Yield/Time	Analysis C	H	N	S
1	$C_{18}H_{14}N_2O_2$ 290.56	275	65/6h	87/3min	85/60min	74.49 74.43	4.82 4.79	9.65 9.60	
2	$C_{26}H_{21}N_3O_2$ 407.66	222	55/6h	82/3min	80/40min	76.65 76.62	5.16 5.12	10.32 10.30	
3a	$C_{33}H_{25}N_3O_2$ 495.98	168	69/5h	89/2min	88/30min	9.98 79.65	5.08 5.00	8.48 8.42	
3b	$C_{33}H_{24}N_3O_2Cl$ 530.02	195	72/5h	90/2min	89/30min	74.78 74.66	4.56 4.50	7.93 7.89	
3c	*p*-$C_{33}H_{24}N_4O_3$ 540.57	205	77/5h	92/3min	85/30min	73.32 73.27	4.48 4.43	10.36 10.29	
3d	*o*-$C_{33}H_{25}N_2O_3$ 511.57	188	66/5h	88/3min	87/30min	77.48 77.40	4.93 4.90	8.21 8.18	
5a	$C_{39}H_{28}N_4OS$ 642.77	280	69/6h	87/3min	86/30min	76.61 76.55	4.70 4.66	8.72 8.69	6.92 6.89
5b	$C_{40}H_{29}N_5O$ 637.73	156	73/6h	85/3min	82/30min	79.10 79.00	4.90 4.86	10.98 10.92	
5c	$C_{39}H_{27}ClN_4OS$ 677.21	263	66/6h	90/3min	87/30min	72.72 72.70	4.32 4.30	8.27 8.25	4.73 4.70
5d	$C_{40}H_{28}ClN_5O$ 672.17	144	66/6h	92/3min	80/30min	75.05 75.00	4.50 4.95	10.40 10.32	
5e	$C_{39}H_{27}N_5O_3S$ 687.77	255	73/6h	85/3min	87/30min	71.60 71.55	4.25 4.20	10.18 10.02	4.68 4.62
5f	*p*-$C_{40}H_{28}N_5O_3$ 682.73	211	70/6h	87/3min	80/30min	73.89 73.82	4.43 4.40	12.31 42.26	
5g	$C_{39}H_{29}N_4O_2S$ 658.77	276	77/6h	88/3min	82/30min	74.75 74.70	4.54 4.50	8.50 8.46	4.86 4.82
5h	*o*-$C_{40}H_{29}N_4O_2$ 653.73	233	66/6h	89/3min	83/30min	77.10 77.00	4.78 4.72	10.71 10.63	
6a	$C_{38}H_{27}N_4OS$ 630.76	265	58/10h	85/3min	88/30min	76.17 76.05	4.79 4.73	8.88 8.83	5.07 5.00
6b	$C_{39}H_{28}N_5O$ 625.72	180	72/10h	82/3min	87/30min	78.70 78.62	4.99 4.95	11.19 11.09	
6c	$C_{38}H_{26}ClN_4OS$ 665.20	233	62/10h	88/3min	88/30min	72.22 72.14	4.39 4.30	8.42 8.39	4.85 4.80
6d	$C_{39}H_{27}ClN_5O$ 660.16	206	72/10h	91/3min	87/30min	74.59 74.53	4.58 4.53	10.61 10.52	
6e	$C_{38}H_{26}N_5O_3S$ 675.75	254	65/10h	86/3min	79/30min	71.10 71.00	4.33 4.90	10.36 10.30	4.75 4.70
6f	*p*-$C_{39}H_{27}N_5O_3$ 670.71	231	72/10h	87/3min	85/30min	73.42 73.38	4.51 4.47	12.53 10.48	
6g	$C_{39}H_{29}N_4O_2S$ 646.72	277	65/10h	88/3min	87/30min	74.28 74.20	4.68 4.64	8.66 8.62	4.95 4.90
6h	*o*-$C_{39}H_{28}N_4O_2$ 641.72	195	55/10h	86/3min	81/30min	77.74 77.70	4.87 4.81	10.91 1088	

The structure of compounds **3a-d** was confirmed by its elemental analysis and spectral data. IR spectra showed C-N, C=C, C=N, and N≡H due to the presence of distinguishable bands at 1176-1179

cm^{-1}, 1531-1539 cm^{-1},1589-1594 cm^{-1}, 3057, and 3354 cm^{-1}. ^{1}H NMR spectra displayed a singlet signal at δ 2.09 ppm due to methyl protons (s, 3H,

proton (CH=CH), in addition to aromatic multiplin the region δ 6.55-8.23 ppm, while -NH-proton (s, 1H, NH) revealed singlet signals at δ 10.30-11.22 ppm, its ^{13}C NMR spectra showed a δ 24.52 (CH3C=N), 100.24-156.61(aromatics), 169.34 (CH3C=N) [34].

The structure of compounds **5a-h** was confirmed by its elemental analysis and spectral data. IR spectra of showed: 29200- 2936 (C-H, aliphatic), 1677-1675

(C=O), 1590-1601 (C=N), 1534-1520 (C=C),3330-3200 (OH) ,[1]HNMR δppm: 1.93 (s, 3H, CH_3C=), 2.51 (s, 3H, CH_3C=O), 7.37-8.01 (m, 19-18 H, ArH), 10.51-10.21 (s,1H,NH pyridazine)and 9.11-9.52(br.1H, OH) .[13]CNMR δppm: 26.55-25.88(CH_3C=O), 46.60-47.67, 140.21-143.55, 168.32-170.21, 175.33-178.55 and 192.65-200.56 aromatics and MS (*m/z*): MS (*m/z*): 642,637,677 and 672 (M^+)[35].

The IR spectrum of the compounds **6a-h** confirmed C-N bands in the range of 1172 to 1184 cm^{-1}, C-O bands at 1235 and 1263 cm^{-1} for compounds **6b, d, f, and h**, C-S bands in the range of 1314 to 1327 cm^{-1} for compounds **6a, c, e, and g**, and C-S bands in the range of 1314 to 1327 cm^{-1} for compounds 6a, c, e, and C=C bands in the range 1513-1538 cm^{-1}, C=N bands in the range 1587-1599 cm^{-1}, NH bands in the range 3055-3115 cm^{-1}, and two symmetric and asymmetric bands due to the OH group in the range 3112-3305 cm^{-1}. In addition, the ^{1}H NMR spectrum of these compounds revealed a characteristic singlet signal due to methyl protons 1.91(s, 3H, CH3-C=N-) at 2.09 ppm in all compounds, and a signal due to proton (CH=CH) disappeared, while a signal due to proton 10.51(-NH-pyridazine) appeared at 4.70-6.04, in addition to singlet signals due to –(NH- proton Pyrrole)[36].

3.2. Biological Activity

3.2.1. Antibacterial activity

Using the agar well diffusion method and measuring the zone of inhibition in millimeters, all of the synthesized derivatives were tested for antimicrobial activity *in vitro* against Escherichia coli (E.Coli), Klebsiella, Staphylococcus epidermidis, Bacillus cereus, Micrococcus luteus, and Staphylococcus aureus. The compounds were tested at a concentration of 200 ppm in a 5% DMF solution. The solution was poured into the cup/well of bacteria seeded agar plates. The plates for E. Coli were incubated for 24 hours at 37°C, while the plates for the other three bacteria were incubated for 24 hours at 272°C[37]. The compounds were examined at 200 ppm concentration in a 5% DMF solution. The solution was put into the cup/well of bacteria seeded agar plates. The plates for E. Coli were incubated for 24 hours at 37°C, whereas the plates for the other three bacteria were incubated for 24 hours at 27°C. The activity is measured in millimeters by the diameter of the inhibitory zone. Ciprofloxacin was designated as a standard drug for antibacterial action. Nutrient agar was employed as a culture medium, while DMSO was used as a solvent.

Pyridazinone was found to be inactive towards the six microorganisms, while the Schiff-base **2** was found to possess moderate activities. Chalcones **3a-d** exhibit high antibacterial activities, especially **3b** and **3c,** more than **3a** and **3d**. The structure action relationship (SAR) has appeared from the result; it is evident that the compounds **5b, 5c,5g, 6b,** and **6e** exhibit significant antibacterial activity against all microorganisms due to the presence of chloro and nitro groups as electron attracting groups also, a sulphur atom in thiophene derivatives enhance antimicrobial activities more than nitrogen atoms present in pyridine derivatives. The presence of five and six hetero rings increase the reactivity, while the existence of electron-electron repelling groups in **3d, 5h,** and **6h** inhibits the biological activities.

3.1.2. Antifungal Activity

Antifungal activity was tested on all of the newly synthesized substances. Using the cup plate method, the preliminary antifungal activity of Candida Albicans and Aspergillus Nigar (As. Nigar) was studied. Each test chemical was dissolved in 5 ml of Dimethyl Sulfoxide at 1000 µg/ml. A volume of and 1mg/ml of each compound was used for testing. As a standard drug, ketoconazole (50 and 100 µg/ml) was used, whereas dimethyl sulfoxide was used as a control. The observed inhibitory zone was measured in mm[38].

Compared to standards, most of the produced compounds are active against all bacteria, as demonstrated in **Table 3**. The structure action relationship (SAR) was exposed from the results, and it is apparent that the compounds **2, 3b, 3c, 5b, 5f, and 6f** have noteworthy antifungal activity against Candida Albicans due to the presence of electron attracting groups and hetero five and six-membered rings attached to the compounds. In contrast, all newly synthesized compounds had a moderate effect against As. Nigar.

Table 2 : Antibacterial activity

Sample No.	Escherichia Coli	Klebsiella	Staphylococcus Epidermidis	Bacillus Cereus	Micrococcus Luteus	Staphylococcus Aureus
Ciproflaxacin	**20**	**20**	**22**	**20**	**24**	**22**
1	**-**	**18**	**-**	**18**	**-**	**20**
2	**-**	**18**	**15**	**17**	**-**	**-**
3a	**15**	**-**	**20**	**15**	**-**	**12**
3b	**19**	**18**	**17**	**20**	**22**	**18**
3c	**20**	**20**	**20**	**17**	**18**	**18**
3d	**15**	**-**	**15**	**18**	**15**	**-**
5a	**15**	**20**	**15**	**20**	**18**	**18**
5b	**18**	**22**	**20**	**18**	**20**	**22**
5c	**20**	**20**	**18**	**20**	**18**	**20**
5d	**20**	**24**	**-**	**22**	**18**	**15**
5e	**16**	**18**	**22**	**18**	**16**	**-**
5f	**20**	**20**	**22**	**15**	**16**	**15**
5g	**20**	**16**	**22**	**18**	**22**	**-**
5h	**16**	**15**	**20**	**22**	**14**	**14**
6a	**-**	**18**	**15**	**-**	**-**	**-**
6b	**18**	**20**	**20**	**-**	**20**	**-**
6c	**22**	**20**	**19**	**18**	**-**	**18**
6d	**20**	**20**	**18**	**-**	**18**	**18**
6e	**18**	**20**	**-**	**18**	**19**	**-**
6f	**20**	**18**	**17**	**20**	**-**	**18**
6g	**18**	**20**	**20**	**22**	**-**	**18**
6h	**17**	**15**	**22**	**16**	**-**	**-**

*Sample 1 refer to 4- acetyl 5,6-diphenyl 4,5-dihydro pyridazi-3(2H)-one ,while sample 2 refer to (E)-4-(1-(4-acetylphenyl)iminoethyl)-5,6-diphenylpyridazin-3(2H)-one
*All of the synthesized derivatives were tested for antimicrobial activity *in vitro* against Escherichia coli (E.Coli), Klebsiella, Staphylococcus epidermidis, Bacillus cereus, Micrococcus luteus, and Staphylococcus aureus. while ciproflaxacin is the standard drug for antibacterial action.

3.1.3.Anticancer activity

The Skehan *et al*. method[39] was used to assess the potential cytotoxicity of the obtained pyridazinone derivatives *in vitro*. Some of the newly synthesized chemicals that have been investigated are **1, 2, 3c, 5b, 5d, 5h, 6c**, and **6e**. The majority of the chemicals examined were active against the MCF7 breast cancer cell line as well as the HCT-116 colon carcinoma cell line. Doxorubicin (DOX) was used as a positive control in this work. The inhibitory activities were presented as micromolar doses of compounds that exhibited 50% inhibition per unit of the enzyme under the assay conditions (IC50)[40].

Compounds **2 and 3c** displayed the best activity against both cell lines (3.52 g/ml and 5.60 g/ml for compound 2) (5.22 g/ml and 5.52 g/ml for compound **3c**). Conversely, a group of chemicals (IC50=6.71-13.60g/ml) presented moderate efficacy against a colon carcinoma cell line. Furthermore, SAR found in pyridazinones connected to Schiff-base derivative 2 and chalcone derivative **3c**, has a high cytotoxic activity against two cell lines, viewing the importance of the imino and electron-withdrawing groups on the reactivity of pyridazinones as potential anticancer medicines[41]

Table 3 : Antifungal activity

Sample no.	Candida Albicans	Aspergillus Nigar
Ketoconazole	8.25	7.25
1	-	5.75
2	7.50	6.00
3a	6.15	-
3b	8.00	7.15
3c	7.00	5.25
3d	6.15	-
5a	5.15	-
5b	8.25	5.40
5c	7.35	-
5d	7.33	-
5e	6.8	-
5f	8.25	5.40
5g	7.15	-
5h	-	-
6a	-	5.50
6b	7.55	7.00
6c	8.25	7.55
6d	6.75	5.55
6e	6.75	6.40
6f	7.55	-
6g	-	6.55
6h	5.55	-

*Sample 1 refer to 4- acetyl 5,6-diphenyl 4,5-dihydro pyridazi-3(2H)-one ,while sample 2 refer to (E)-4-(1-(4-acetylphenyl)iminoethyl)-5,6-diphenylpyridazin-3(2H)-one

*Using the cup plate method, the preliminary antifungal activity of Candida Albicans and Aspergillus Nigar was studied. Ketoconazole (50 and 100 µg/ml) was used as standard drug for antifungal action.

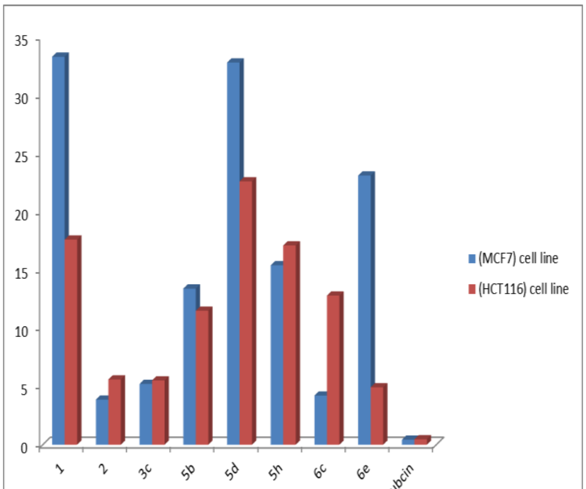

Figure 4: Cytotoxicity of compounds against breast (MCF7) and colon carcinoma(H1lllines). Doxorubicin is the standard drug for cancer activity

4. Conclusion

In conclusion, pyridazinone derivatives were synthesized using conventional and environmentally friendly methods, such as microwave and grinding, resulting in faster reaction rates, improved yields of pure products, and environmental benefits. The newly synthesized chemicals were evaluated for antibacterial, antifungal, and anticancer activities. The majority of the newly synthesized compounds were active, and structural changes to the molecules above resulted in therapeutically useful products.

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Conflict of interest

The authors declare the absence of conflict of interest.

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