

Research Article

Synthesis of New Oxindoles and Determination of Their Antibacterial Properties

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A versatile method for the synthesis of new oxindoles was developed by the reaction between substituted isatins and 5-aminopyrazoles. The reaction was carried out at room temperature in ethanol using *p*-toluenesulfonic acid as the catalyst. The products were obtained with acceptable to excellent yields (44–96%). Structures of the new compounds were unambiguously established by spectroscopic and analytical techniques. The antibacterial activity was determined by microdilution assays. Compounds **3b**, **3e**, and **3g** showed antistaphylococcal activity, particularly compound **3e** displayed a potent activity against the vancomycin intermediate *Staphylococcus aureus* (VISA). Compounds **3i**, **3j**, and **3o** inhibited *Neisseria gonorrhoeae* growth.

1. Introduction

Nitrogen-containing heterocycles are present in many recognized drugs [1]; thus, synthetic chemists are increasingly motivated to discover new methods for the rapid construction of pharmacologically important nitrogen-based drug-like heterocyclic compounds [2]. Isatin- and pyrazole-based compounds are nowadays a huge nest of research that correlate with the biological activity that they show, such as antitumoral [3–6], antimicrobial [7–9], anti-inflammatory [8], and antimicrobial against multidrug-resistant cells [10], among others [11–16]. Previously, we have described different strategies to obtain pyrazole-based compounds and the evaluation of their biological activity as antifungal, antibacterial, and antitumor agents showing that some these pyrazole-derivatives were bioactive as antimicrobials [17–22]. Combination of isatin and pyrazole moieties could be a good alternative to trigger a better biological activity into a hybrid structure. Isatin-pyrazole Baylis-Hillman-type adducts have been obtained as intermediates in the synthesis of tricyclic-fused heterocycles [23–25]. Balamurugan et al. [26] described the synthesis of 3-(4-pyrazolyl)-oxindoles

via an InCl_3 -catalyzed tricomponent reaction between arylhydrazines, 3-aminocrotonitrile, and isatin under reflux in water at 100°C. Also, Yang et al. synthesized 3-(4-pyrazolyl)-oxindoles by a reaction of isatin derivatives and 5-aminopyrazoles under reflux in water [27].

The antimicrobial activity of isatin derivatives has also been reported [28]. They have been described as a versatile scaffold used as synthetic building blocks for diverse heterocyclic compounds, some of them with substantial antimicrobial activity. Isatin-*N*-Mannich bases of isatin-3-thiosemicarbazones have shown activity against several viruses and *M. tuberculosis* at the micromolar level [29]. Likewise, Ugale et al. reported that some derivatives of *N'*-(5 or 7 substituted-2-oxoindolin-3-ylidene) benzofuran-2-carbohydrazides exhibited antibacterial and antifungal activity. They found that combining benzofuran and isatin moieties may modulate the biological activity of these compounds [30]. Moreover, in an effort to fight against bacterial resistance, fluoroquinolone-isatin hybrids have been tested during the last decades showing potential anti-HIV, anti-bacterial, and anti-*M. tuberculosis* activity [31].

Recently, Thakur et al. described that isatin-based gluco-conjugated oxindoles had potent activity against *Plasmodium falciparum* with a good selectivity index [32]. Pervez et al. reported some isatin-derived bis-Schiff bases as promising cytotoxic agents and urease inhibitors. Although in other research they evaluated several isatin-3-hydrazone-thiazolines as urease inhibitors, despite some good results, substituents like 5-OCF₃ and 5-F over the isatin ring decreased the activity in various cases [33, 34]. In a more recent research, Pervez et al. reported several thiosemicarbazone-isatin derivatives with important activity as a urease inhibitors with lower IC₅₀ (0.87 to 11.23 μM) than thiourea (IC₅₀ = 22.3 μM) [35–37]. According to the abovementioned background about this kind of products, we have designed a series of isatin-pyrazole hybrids that could be effective as antimicrobials. In this sense, we examined the potential inhibitory activity of these novel compounds against several relevant drug-sensitive and -resistant bacteria.

2. Results and Discussion

2.1. Chemistry. In this work, we focused on the synthesis of highly substituted 3-(4-pyrazolyl)oxindoles **3** bearing lipophilic groups like hexyl and benzyl over the nitrogen atom of isatin, owing to several studies about the relationship of the lipophilic character of compounds and their biological activities [38, 39]. Another structural modification undertaken in order to improve the biological activity corresponded to the introduction of different halides such as chlorine and fluorine in the aryl ring, oriented to the known halogen bond effect [40–42], which is characterized for showing σ -hole interactions, that could increase the possibility for improving the activity of the studied compounds.

In a first attempt, we carried out the reaction between 3-methyl-1-phenyl-1H-pyrazol-5-amine (**1a**) and isatin (**2a**) using *p*-toluenesulfonic acid (PTSA) as the catalyst in ethanol at room temperature (Scheme 1). The reaction was monitored by thin layer chromatography (TLC). After 30 min, a white solid formed, it was isolated by filtration under vacuum, and washed with ethanol. The product **3a**, so obtained, did not require further purification with a yield of 69%. Spectroscopic and analytical data (NMR, FT-IR, EI-MS and elemental analysis) of compound **3a** matched with the information supplied for the same structure in references [24–27]. The main ¹H-NMR signals for **3a** correspond to the NH₂ protons at 5.28 ppm as a broad singlet, and the hydroxyl proton as a singlet at 6.61 ppm.

In order to evaluate the versatility and scope of this procedure, we performed the reaction, under the same reaction conditions, starting from substituted aminopyrazoles **1a-d** and isatins **2a-i** (Scheme 2), leading to the formation of diverse 3-(4-pyrazolyl)oxindole derivatives, **3a-p** (Scheme 2, Table 1).

Despite the acceptable-to-good reaction yields (44–96%) obtained in our approach, in comparison with the procedure described by Yang et al. [27], remarkably, our methodology required less reaction time and less consumption of energy.

A possible mechanistic route for the described reaction is outlined in Scheme 3. The procedure should involve the

TABLE 1: Synthesis of 3-(4-pyrazolyl)oxindoles **3**.

Entry	R ₁	R ₂	R ₃	Yield (%)
3a	H	H	H	69
3b	H	Bn	H	62
3c	H	H	5-F	51
3d	H	Hexyl	H	96
3e	H	Bn	5-Cl	70
3f	H	Me	5-Cl	48
3g	H	Hexyl	5-Cl	66
3h	4-Cl	Bn	5-Cl	45
3i	4-F	Bn	5-F	48
3j	4-Cl	Hexyl	5-Cl	62
3k	3,5-di-Cl	Bn	5-Cl	51
3l	3,5-di-Cl	Hexyl	5-Cl	55
3m	3,5-di-Cl	Bn	5-F	52
3n	4-Cl	Bn	5,7-di-Cl	44
3o	3,5-di-Cl	Bn	5,7-di-Cl	47
3p	H	Bn	5,7-di-Cl	72

protonation of isatin and formation of intermediate (**I**), and then the 5-aminopyrazole **1** performed the addition over C-3, affording the intermediate (**II**). Subsequently, a tautomeric process afforded the isolated products **3**.

2.2. Antibacterial Studies. All the isatin derivatives **3** were screened for the *in vitro* antibacterial activity against Gram-positive and Gram-negative bacteria. Wildtype and multi-resistant strains were included as follows: methicillin-susceptible *Staphylococcus aureus* ATCC 25923 (MSSA), methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRSA), vancomycin-intermediate *Staphylococcus aureus* (VISA), *Escherichia coli* ATCC 25922, carbapenemase-positive *Klebsiella pneumoniae* BAA 1705, *Klebsiella pneumoniae* ATCC 700603 (extended spectrum beta lactamase, ESBL positive), *Pseudomonas aeruginosa* ATCC 27853, and *Neisseria gonorrhoeae* ATCC 49226. *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* are part of the ESKAPE pathogens which are bacteria causing nosocomial infections. The minimum inhibitory concentration (MIC) is determined and reported in Table 2.

N. gonorrhoeae and VISA have been identified by the World Health Organization as priority pathogens as resistance has been developed against the first-line treatment and other commonly used antibiotics [43]. Three compounds (**3b**, **3e**, and **3f**) showed antistaphylococcal activity with MICs ranging from 3.9 μg/mL to 250 μg/mL. Compound **3e** (the benzyl-chloro-hydroxyl indolin) induced substantial growth inhibition of the VISA strain. This compound also showed discrete growth inhibition of the other two *Staphylococcus* strains (MRSA and MSSA). SAR analysis revealed that the 1-benzyl-5-chloro-substitution in the pyrazol-indolin seemed to confer to activity against the VISA strain whereas the 1-hexyl instead of the 1-benzyl improved growth inhibition of MSSA. Figure 1. VISA growth inhibition was improved 4 times in **3e** compared with **3b** analog suggesting that the activity is clearly enhanced by 5-chloro substitution at the isatin core. Two analogue compounds **3i** (the benzyl-fluoro-hydroxyl

TABLE 2: *In vitro* antibacterial activity (MIC values, $\mu\text{g/mL}$).

Compound	<i>S. aureus</i> ATCC 25923 $\mu\text{g/mL}$	<i>S. aureus</i> ATCC 43300 $\mu\text{g/mL}$	VISA $\mu\text{g/mL}$	<i>N. gonorrhoeae</i> ATCC 49226 $\mu\text{g/mL}$
3a	>1000	>1000	>1000	>1000
3b	1000	1000	62.5	>1000
3c	>1000	>1000	>1000	>1000
3d	>1000	>1000	>1000	>1000
3e	250	250	3.9	>1000
3f	>1000	>1000	>1000	>1000
3g	125	>1000	>1000	>1000
3h	1000	1000	1000	>1000
3i	>1000-	>1000	>1000	15.6
3j	>1000	>1000	>1000	15.6
3k	1000	1000	1000	>1000
3l	>1000	>1000	>1000	>1000
3m	>1000	>1000	>1000	>1000
3n	>1000	>1000	>1000	>1000
3o	>1000	>1000	>1000	>1000
3p	>1000	>1000	>1000	>1000
Gentamicin	0.4			
Penicillin				0.25
Tetracycline	0.2	0.05	0.1	

indolin) and **3j** (choro-hexyl-hydroxyl indolin with a chlorophenyl substitution in the pyrazole ring) were active against *N. gonorrhoeae* (MICs 15.6 $\mu\text{g/mL}$), and the benzyl-dichlorohydroxyindolin **3o** showed discrete inhibition of bacterial growth (MIC 250 $\mu\text{g/mL}$), Figure 1. The 4-fluoro or 4-chloro substituent at phenyl moiety of the pyrazole ring and the C-5 of the benzyl or hexyl-isatin seemed to be important for gonococcal inhibition. The presence of electron withdrawing substituents at C-5 of the isatin core has been previously associated with biological activity here, as well as moieties at N-1 may also modulate the activity [29].

The ability of the biologically active isatin-pyrazole compounds **3** to induce hemolysis in human red blood cells ranged from 0 to 7%. None of these compounds seemed to be active against *E. coli* and drug-resistant *K. pneumoniae* or *P. aeruginosa*.

The most active compounds are highlighted in bold. MIC values >1 mg/mL were considered as not relevant or not active.

3. Conclusions

In summary, we synthesized a series of 3-(4-pyrazolyl)-oxindoles **3** by the reaction of 5-aminopyrazoles **1** with highly substituted isatins **2** using PTSA as catalyst in mild conditions, room temperature, short reaction times, and in acceptable-to-excellent yields (44–96%). Some compounds of this series showed antistaphylococcal and antigonococcal activity; the benzyl substitution at N-1 and chloro substituent at C-5 of isatin seemed to promote activity against vancomycin-intermediate *Staphylococcus aureus* (VISA), whereas the hexyl at N-1 increased the activity against methicillin-susceptible *Staphylococcus aureus* (MSSA). The active compounds might be a starting point to design new series of analogs potentially active against VISA and *N. gonorrhoeae*. This finding is particularly relevant because of the rising of infections caused by drug-resistant *S. aureus*

and the limited therapeutic options for penicillin-resistant *N. gonorrhoeae*. None of the active compounds showed relevant hemolytic activity suggesting low membrane interactions and toxicity.

4. Experiment

4.1. General Information. Reagents and solvents used were obtained from commercial sources. The progress of the reactions was monitored by TLC with 0.2 μm precoated plates of silica gel 60GF254 (Merck). Melting points were measured using a Stuart SMP3 melting point apparatus. IR spectra were obtained with a Shimadzu IRAffinity-1 with ATR probe. The ^1H and ^{13}C -NMR spectra were recorded in a BRUKER DPX 400 spectrophotometer operating at 400 and 100 MHz, respectively, using dimethyl sulfoxide- d_6 as the solvent. The mass spectra were obtained on a SHIMADZU-GCMS-QP2010 spectrometer operating at 70 eV.

4.2. General Procedure for Preparation of 3-(4-Pyrazolyl)-oxindoles 3a-p. An equimolar mixture of 5-aminopyrazoles **1** and the corresponding isatin **2**, in the presence of 10 mol% of *p*-toluenesulfonic acid in 1 mL of ethanol, was stirred at room temperature for 1 h. The solid formed was filtered under vacuum and washed with ethanol. Products **3** did not require further purification.

4.3. Characterization Data of the Compounds 3

4.3.1. 3-(5-Amino-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-hydroxyindolin-2-one 3a. White solid, Yield: 69%. M.p.: 235–236°C (236°C [26], 218–220°C [27]). FT-IR (cm^{-1}): 3437, 3360, 3069, 1711. ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 1.45 (s, 3 H, 3-CH₃), 5.28 (br. s., 2 H, NH₂), 6.61 (s, 1 H, OH), 6.86 (d, J = 7.61 Hz, 1 H, Ar-H), 6.99 (t, J = 7.80 Hz, 1 H, Ar-H), 7.22–7.33 (m, 3 H, Ar-H), 7.46 (t, J = 7.90 Hz, 2 H, Ar-H),

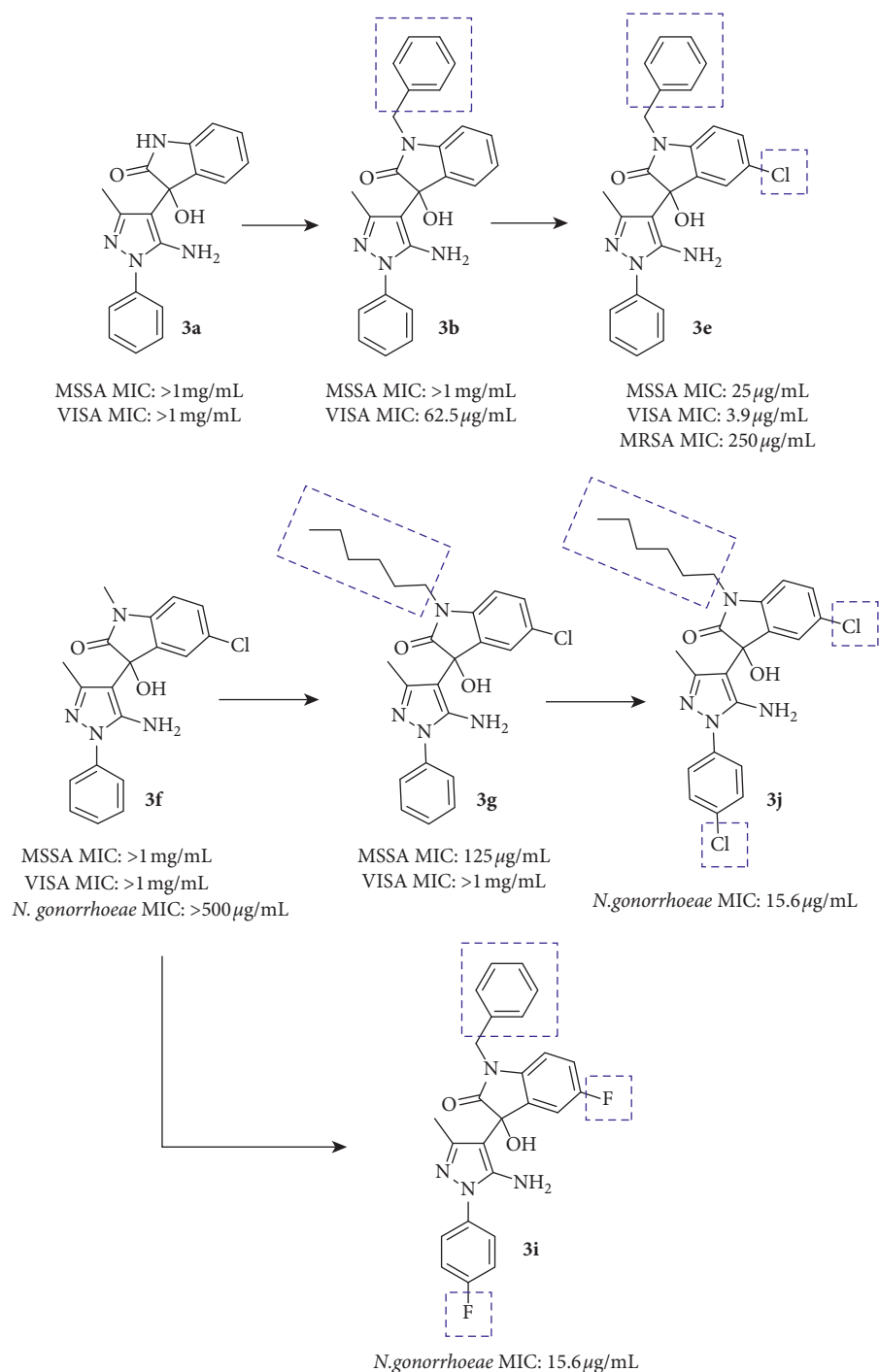
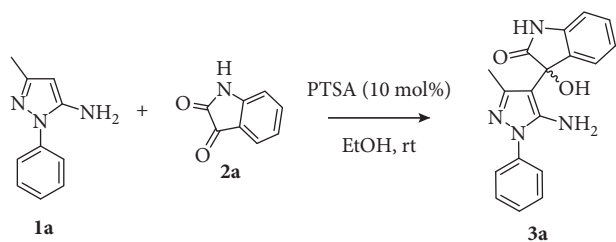
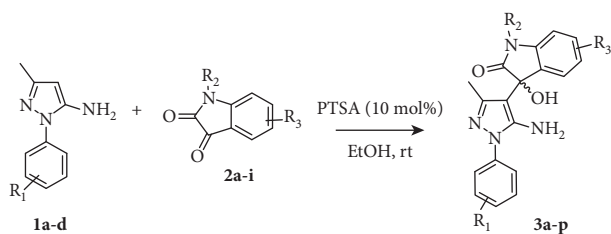


FIGURE 1: SAR of isatin-pyrazole compounds.

SCHEME 1: Synthesis of the 3-(4-pyrazolyl)oxindole **3a**.

7.57 (d, $J = 7.61$ Hz, 2 H, Ar-H), 10.34 (s, 1 H, NH). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ (ppm): 13.2 (3- CH_3), 75.0 (C), 100.1 (C), 110.2 (CH), 122.4 (C), 123.2 (CH), 125.5 (CH), 126.5 (CH), 129.6 (CH), 129.9 (C), 133.3 (C), 136.4 (C), 139.5 (C), 142.2 (C), 145.1 (C), 146.6 (C), 178.6 (C). EI-MS (20 eV) m/z : 320 (29, M^+), 304 (33), 291 (23), 275 (34), 173 (100), 120 (80), 92 (55), 69 (31). Anal. Calcd. For $\text{C}_{18}\text{H}_{16}\text{N}_4\text{O}_2$: C: 67.49; H: 5.03; N: 17.49. Found: C: 67.51; H: 5.01; N: 17.43.



SCHEME 2: Established approach for synthesis of diverse 3-(4-pyrazolyl)oxindoles **3a-p**.

4.3.2. 3-(5-Amino-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-benzyl-3-hydroxyindolin-2-one **3b**. Yellow pale solid, Yield: 62%. M. p.: 191–193°C. FT-IR (cm^{-1}): 3451, 3366, 3067, 1715. ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 1.29 (s, 3 H, 3- CH_3), 4.85 (d, J : 15.80 Hz, 1 H, CHH), 4.93 (d, J : 15.80 Hz, 1 H, CHH), 5.36 (s, 2 H, NH_2), 6.88 (s, 1 H, OH), 6.95 (d, J : 7.80 Hz, 1 H, Ar-H) 7.06 (t, J : 7.80 Hz, 1 H, Ar-H), 7.24–7.42 (m, 8 H, Ar-H), 7.47 (t, J : 7.90 Hz, 2 H, Ar-H), 7.57 (d, J : 7.80 Hz, 2 H, Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 13.2 (3- CH_3), 43.3 (CH_2), 74.8 (C), 99.7 (C), 109.8 (CH), 123.3 (CH), 125.3 (CH), 126.7 (CH), 128.0 (CH), 129.1 (CH), 129.6 (CH), 130.0 (CH), 132.5 (C), 136.7 (C), 139.3 (C), 142.7 (C), 145.1 (C), 146.8 (C), 177.0 (C). EI-MS (20 eV) m/z : 410 (15, M^+), 394 (22), 237 (64), 173 (100), 146 (95), 91 (53). Anal. Calcd. For $\text{C}_{25}\text{H}_{22}\text{N}_4\text{O}_2$: C: 73.15; H: 5.40; N: 13.65. Found: C: 73.10; H: 5.45; N: 13.73.

4.3.3. 3-(5-Amino-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-fluoro-3-hydroxyindolin-2-one **3c**. White solid, Yield: 51%. M. p.: 227–229°C (212–214°C [27]). FT-IR (cm^{-1}): 3391, 3308, 3073, 2359, 1709. ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 1.49 (s, 3 H, 3- CH_3), 5.30 (s, 2 H, NH_2), 6.76 (s, 1 H, OH), 6.87 (dd, J : 8.39, 4.29 Hz, 1 H, Ar-H), 7.06–7.16 (m, 2 H, Ar-H), 7.27–7.33 (m, 1 H, Ar-H), 7.43–7.50 (m, 2 H, Ar-H), 7.54–7.59 (m, 2 H, Ar-H), 10.39 (s, 1 H, NH). ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 13.1 (3- CH_3), 75.2 (C), 99.6 (C), 123.3 (CH), 126.7 (CH), 129.6 (CH), 138.2 (C), 139.3 (C), 144.9 (C), 146.7 (C), 160.5 (C), 178.6 (C). EI-MS (20 eV) m/z : 338 (43, M^+), 322 (29), 310 (22), 296 (33), 293 (54), 236 (32), 173 (100), 138 (72), 82 (69), 57 (88). Anal. Calcd. For $\text{C}_{18}\text{H}_{15}\text{FN}_4\text{O}_2$: C: 63.90; H: 4.47; N: 16.56. Found: C: 63.85; H: 4.38; N: 16.50.

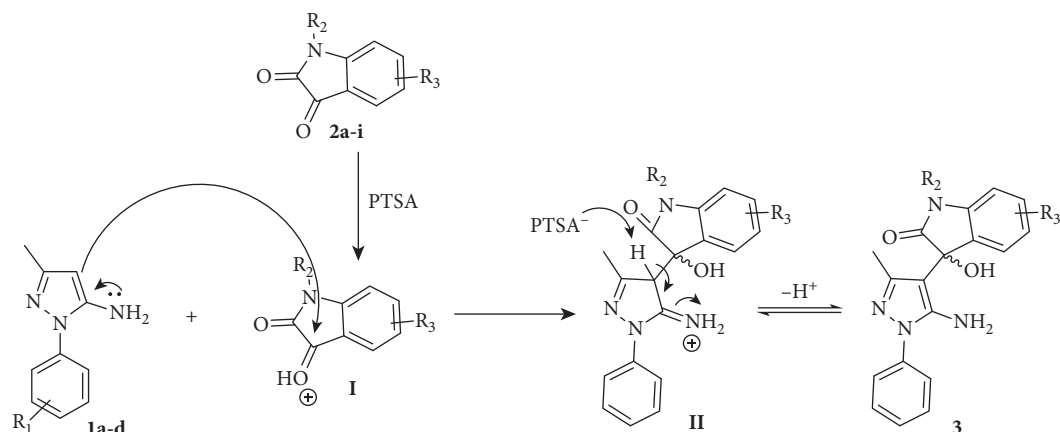
4.3.4. 3-(5-Amino-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-hexyl-3-hydroxyindolin-2-one **3d**. Yellow pale solid, Yield: 96%. M. p.: 159–161°C. FT-IR (cm^{-1}): 3451, 3356, 2928, 2359, 1719. ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 0.83 (t, J : 6.94 Hz, 3 H, CH_3), 1.19–1.34 (m, 6 H, 3 \times CH_2), 1.36 (s, 3 H, 3- CH_3), 1.53–1.65 (m, 2 H, CH_2), 3.57–3.73 (m, 2 H, CH_2), 5.33 (s, 2 H, NH_2), 6.71 (s, 1 H, OH), 7.02–7.11 (m, 2 H, Ar-H), 7.26–7.38 (m, 3 H, Ar-H), 7.47 (t, J : 7.81 Hz, 2 H, Ar-H), 7.55 (d, J : 7.61 Hz, 2 H, Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 13.1 (3- CH_3), 14.4 (CH_3), 22.5 (CH_2), 26.5 (CH_2), 27.5 (CH_2), 31.4 (CH_2), 39.7 (CH_2), 74.7 (C), 99.8 (C), 109.3 (CH), 123.0 (CH), 123.2 (CH), 125.3 (CH), 126.6 (CH), 129.6 (CH), 130.1 (CH), 132.6 (C), 139.3 (C), 143.0 (C), 145.0 (C), 146.8 (C), 176.7 (C). EI-MS (20 eV) m/z :

404 (23, M^+), 388 (24), 310 (22), 173 (100), 161 (62), 132 (98), 119 (41), 69 (36). Anal. Calcd. For $\text{C}_{24}\text{H}_{28}\text{N}_4\text{O}_2$: C: 71.26; H: 6.98; N: 13.85. Found: C: 71.35; H: 6.91; N: 13.72.

4.3.5. 3-(5-Amino-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-benzyl-5-chloro-3-hydroxyindolin-2-one **3e**. Yellow pale solid, Yield: 70%. M. p.: 208–210°C. FT-IR (cm^{-1}): 3447, 3362, 3065, 2359, 1720. ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 1.33 (s, 3 H, 3- CH_3), 4.85 (d, J : 15.61 Hz, 1 H, N- CHH), 4.94 (d, J : 15.80 Hz, 1 H, N- CHH), 5.37 (s, 2 H, NH_2), 6.99 (d, J : 8.59 Hz, 1 H, Ar-H), 7.06 (s, 1 H, OH), 7.25–7.39 (m, 8 H, Ar-H), 7.47 (t, J : 8.00 Hz, 2 H, Ar-H), 7.53–7.57 (m, 2 H, Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 13.2 (3- CH_3), 43.4 (CH_2), 74.7 (C), 99.1 (C), 100.0 (C), 111.5 (C), 123.4 (CH), 125.2 (CH), 126.8 (CH), 127.5 (C), 128.0 (CH), 128.1 (CH), 129.1 (CH), 129.7 (CH), 129.8 (CH), 134.6 (C), 136.3 (C), 139.1 (C), 141.4 (C), 144.7 (C), 146.9 (C), 176.6 (C). EI-MS (40 eV) m/z : 444 (35, M^+), 391 (31), 271 (21), 173 (71), 91 (100), 77 (44). Anal. Calcd. For $\text{C}_{25}\text{H}_{21}\text{ClN}_4\text{O}_2$: C: 67.49; H: 4.76; N: 12.59. Found: C: 67.41; H: 4.84; N: 12.48.

4.3.6. 3-(5-Amino-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-chloro-3-hydroxy-1-methylindolin-2-one **3f**. White solid, Yield: 48%. M. p.: 199–200°C. FT-IR (cm^{-1}): 3399, 3308, 3063, 2785, 2359, 1721. ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 1.41 (s, 3 H, 3- CH_3), 3.15 (s, 3 H, N- CH_3), 5.34 (s, 2 H, NH_2), 6.89 (s, 1 H, OH), 7.10 (d, J : 8.39 Hz, 1 H, Ar-H), 7.27–7.37 (m, 2 H, Ar-H), 7.39–7.51 (m, 3 H, Ar-H), 7.54 (d, J : 7.80 Hz, 2 H, Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 13.1 (3- CH_3), 26.6 (N- CH_3), 74.7 (C), 99.3 (C), 110.9 (CH), 123.4 (CH), 124.8 (CH), 126.8 (CH), 127.3 (C), 129.7 (CH), 129.8 (C), 134.7 (C), 139.2 (C), 142.2 (C), 144.7 (C), 146.8 (C), 174.8 (C), 176.4 (C). EI-MS (20 eV) m/z : 368 (32, M^+), 352 (16), 322 (24), 195 (29), 173 (100), 138 (48), 81 (39), 69 (45). Anal. Calcd. For $\text{C}_{19}\text{H}_{17}\text{ClN}_4\text{O}_2$: C: 61.88; H: 4.65; N: 15.19. Found: C: 61.81; H: 4.77; N: 15.27.

4.3.7. 3-(5-Amino-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-chloro-1-hexyl-3-hydroxyindolin-2-one **3g**. Brown pale solid, Yield: 66%. M. p.: 184–187°C. FT-IR (cm^{-1}): 3451, 3360, 2916, 2361, 1728. ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 0.83 (t, J : 6.92 Hz, 3 H, CH_3), 1.18–1.35 (m, 6 H, 3 \times CH_2), 1.41 (s, 3 H, 3- CH_3), 1.51–1.63 (m, 2 H, CH_2), 3.59–3.73 (m, 2 H, CH_2), 5.34 (s, 2 H, NH_2), 6.89 (s, 1 H, OH), 7.12 (d, J : 8.39 Hz, 1 H, Ar-H), 7.28–7.35 (m, 2 H, Ar-H), 7.41 (dd, J : 8.39, 2.15 Hz, 1 H, Ar-H), 7.47 (t, J : 7.80 Hz, 2 H, Ar-H), 7.55 (d, J : 7.80 Hz, 2 H, Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm): 13.2 (3- CH_3), 14.4 (CH_3), 22.5 (CH_2), 26.4 (CH_2), 27.3 (CH_2), 31.4 (CH_2), 39.9 (CH_2), 74.6 (C), 99.3 (C), 111.1 (CH), 123.4 (CH), 125.1 (CH), 126.8 (CH), 127.1 (C), 129.6 (CH), 129.9 (CH), 134.7 (C), 139.2 (C), 141.8 (C), 144.6 (C), 146.9 (C), 176.3 (C). EI-MS (45 eV) m/z : 438 (8, M^+), 422 (8), 385 (8), 265 (7), 195 (19), 173 (52), 166 (39), 86 (60), 43 (100). Anal. Calcd. For $\text{C}_{24}\text{H}_{27}\text{ClN}_4\text{O}_2$: C: 65.67; H: 6.20; N: 12.76. Found: C: 65.57; H: 6.22; N: 12.64.



SCHEME 3: Suggested mechanistic pathway for the synthesis of 3-(4-pyrazolyl)-oxindoles **3**.

4.3.8. 3-(5-Amino-1-(4-chlorophenyl)-3-methyl-1H-pyrazol-4-yl)-1-benzyl-5-chloro-3-hydroxy-indolin-2-one **3h**. White solid, Yield: 45%. M. p.: 207–209°C. FT-IR (cm^{-1}): 3466, 3435, 3358, 3067, 2922, 2359, 1717. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 1.33 (s, 3 H, 3- CH_3), 4.85 (d, $J=15.61$ Hz, 1 H, CHH), 4.94 (d, $J=15.61$ Hz, 1 H, CHH), 5.43 (s, 2 H, NH_2), 7.00 (d, $J=8.98$ Hz, 1 H, Ar-H), 7.04 (s, 1 H, OH), 7.25–7.41 (m, 7 H, Ar-H), 7.52 (d, $J=8.78$ Hz, 2 H, Ar-H), 7.60 (d, $J=8.78$ Hz, 2 H, Ar-H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ (ppm): 13.2 (3- CH_3), 43.4 (CH_2), 74.7 (C), 99.6 (C), 111.5 (CH), 124.9 (CH), 125.2 (CH), 127.5 (C), 128.0 (CH), 128.1 (CH), 129.1 (CH), 129.6 (CH), 129.8 (CH), 130.9 (C), 134.5 (C), 136.3 (C), 138.1 (C), 141.5 (C), 145.2 (C), 147.1 (C), 176.5 (C). EI-MS (45 eV) m/z : 478 (4, M^+), 271 (17), 207 (31), 180 (54), 91 (100). Anal. Calcd. For $\text{C}_{25}\text{H}_{20}\text{Cl}_2\text{N}_4\text{O}_2$: C: 62.64; H: 4.21; N: 11.69. Found: C: 62.71; H: 4.24; N: 11.77.

4.3.9. 3-(5-Amino-1-(4-fluorophenyl)-3-methyl-1H-pyrazol-4-yl)-1-benzyl-5-fluoro-3-hydroxy-indolin-2-one **3i**. White solid, Yield: 48%. M. p.: 224–226°C. FT-IR (cm^{-1}): 3431, 3352, 3090, 2359, 1711. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 1.33 (s, 3 H, 3- CH_3), 4.85 (d, $J=15.80$ Hz, 1 H, CHH), 4.92 (d, $J=15.60$ Hz, 1 H, CHH), 5.34 (s, 2 H, NH_2), 6.96 (dd, $J=8.49, 4.19$ Hz, 1 H, Ar-H), 6.99 (s, 1 H, OH), 7.13 (td, $J=9.02, 2.63$ Hz, 1 H, Ar-H), 7.21 (dd, $J=7.80, 2.54$ Hz, 1 H, Ar-H), 7.25–7.40 (m, 7 H, Ar-H), 7.57 (dd, $J=8.88, 4.98$ Hz, 2 H, Ar-H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ (ppm): 13.2 (3- CH_3), 43.4 (CH_2), 74.9 (C), 99.3 (C), 110.9 (CH), 113.1 (CH), 116.1 (CH), 116.2 (CH), 116.5 (CH), 125.6 (CH), 125.7 (CH), 128.0 (CH), 129.1 (CH), 134.3 (C), 135.6 (C), 136.5 (C), 138.8 (C), 144.8 (C), 147.0 (C), 158.0 (C), 159.5 (C), 160.4 (C), 161.9 (C), 176.9 (C). EI-MS (20 eV) m/z : 446 (64, M^+), 430 (18), 400 (25), 255 (51), 198 (69), 191 (97), 164 (100), 91 (94). Anal. Calcd. For $\text{C}_{25}\text{H}_{20}\text{F}_2\text{N}_4\text{O}_2$: C: 67.26; H: 4.52; N: 12.55. Found: C: 67.33; H: 4.50; N: 12.51.

4.3.10. 3-(5-Amino-1-(4-chlorophenyl)-3-methyl-1H-pyrazol-4-yl)-5-chloro-1-hexyl-3-hydroxyindolin-2-one **3j**. White solid,

Yield: 62%. M. p.: 169–170°C. FT-IR (cm^{-1}): 3464, 3437, 3360, 2960, 2360, 1715. ^1H NMR (400 MHz, CDCl_3-d) δ (ppm): 0.87 (t, $J=6.92$ Hz, 3 H, CH_3), 1.22–1.41 (m, 6 H, 3 \times CH_2), 1.61 (s, 3 H, 3- CH_3), 1.63–1.70 (m, 2 H, CH_2), 3.52–3.62 (m, 1 H, N- CHH), 3.67–3.76 (m, 1 H, N- CHH), 4.04 (br. s., 1 H, OH) 4.62 (br. s., 2 H, NH_2), 6.80 (d, $J=8.20$ Hz, 1 H, Ar-H), 7.31 (dd, $J=8.39, 2.15$ Hz, 1 H, Ar-H), 7.40–7.44 (m, 3 H, Ar-H), 7.44–7.48 (m, 2 H, Ar-H). ^{13}C NMR (100 MHz, CDCl_3-d) δ (ppm): 13.2 (3- CH_3), 14.0 (CH_3), 22.5 (CH_2), 26.6 (CH_2), 27.2 (CH_2), 31.4 (CH_2), 40.5 (N- CH_2), 74.9 (C), 99.5 (C), 109.9 (CH), 124.9 (C), 125.3 (CH), 125.9 (CH), 128.7 (C), 129.5 (C), 129.7 (CH), 130.0 (CH), 132.5 (C), 133.1 (C), 136.6 (C), 141.1 (C), 145.5 (C), 145.6 (C), 176.5 (C). EI-MS (20 eV) m/z : 472 (4, M^+), 456 (7), 265 (24), 207 (100), 195 (49), 166 (57), 153 (34), 43 (28). Anal. Calcd. For $\text{C}_{24}\text{H}_{26}\text{Cl}_2\text{N}_4\text{O}_2$: C: 60.89; H: 5.54; N: 11.84. Found: C: 60.77; H: 5.66; N: 11.75.

4.3.11. 3-(5-Amino-1-(3,5-dichlorophenyl)-3-methyl-1H-pyrazol-4-yl)-1-benzyl-5-chloro-3-hydroxy-indolin-2-one **3k**. White solid, Yield: 51%. M. p.: 214–216°C. FT-IR (cm^{-1}): 3474, 3374, 3069, 2361, 1748. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 1.33 (s, 3 H, 3- CH_3), 4.85 (d, $J=15.61$ Hz, 1 H, N- CHH), 4.94 (d, $J=15.61$ Hz, 1 H, N- CHH), 5.63 (s, 2 H, NH_2), 7.01 (d, $J=8.59$ Hz, 1 H, Ar-H), 7.10 (s, 1 H, OH), 7.25–7.40 (m, 7 H, Ar-H), 7.51 (t, $J=1.76$ Hz, 1 H, Ar-H), 7.67 (d, $J=1.95$ Hz, 2 H, Ar-H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ (ppm): 13.2 (3- CH_3), 43.5 (CH_2), 74.6 (C), 100.4 (C), 111.6 (CH), 121.1 (CH), 125.3 (CH), 125.7 (CH), 127.5 (C), 128.1 (CH), 129.1 (CH), 129.9 (CH), 134.3 (C), 134.9 (C), 136.3 (C), 141.4 (C), 141.5 (C), 146.3 (C), 147.7 (C), 176.4 (C). EI-MS (20 eV) m/z : 514 (2, M^+), 498 (11), 368 (14), 271 (46), 241 (45), 180 (71), 91 (100). Anal. Calcd. For $\text{C}_{25}\text{H}_{19}\text{Cl}_3\text{N}_4\text{O}_2$: C: 58.44; H: 3.73; N: 10.90. Found: C: 58.36; H: 3.81; N: 10.83.

4.3.12. 3-(5-Amino-1-(3,5-dichlorophenyl)-3-methyl-1H-pyrazol-4-yl)-5-chloro-1-hexyl-3-hydroxy-indolin-2-one **3l**. White solid, Yield: 55%. M. p.: 181–183°C. FT-IR (cm^{-1}): 3466, 3362, 2916, 2360, 1728. ^1H NMR (400 MHz, $\text{DMSO}-d_6$)

δ (ppm): 0.83 (t, $J = 7.02$ Hz, 3 H, CH₃), 1.20–1.35 (m, 6 H, 3 \times CH₂), 1.41 (s, 3 H, CH₃), 1.52–1.63 (m, 2 H, CH₂), 3.56–3.73 (m, 2 H, CH₂), 5.59 (s, 2 H, NH₂), 6.94 (s, 1 H, OH), 7.13 (d, $J = 8.39$ Hz, 1 H, Ar-H), 7.32 (d, $J = 2.15$ Hz, 1 H, Ar-H), 7.41 (dd, $J = 8.29, 2.24$ Hz, 1 H, Ar-H), 7.51 (t, $J = 1.85$ Hz, 1 H, Ar-H), 7.66 (d, $J = 1.76$ Hz, 2 H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 13.2 (3-CH₃), 14.3 (CH₃), 22.5 (CH₂), 26.4 (CH₂), 27.3 (CH₂), 31.4 (CH₂), 40.0 (CH₂), 74.5 (C), 100.5 (C), 111.1 (CH), 121.1 (CH), 125.1 (CH), 125.7 (CH), 127.2 (C), 130.0 (CH), 134.3 (C), 134.9 (C), 141.4 (C), 141.8 (C), 146.2 (C), 147.6 (C), 176.1 (C). EI-MS (45 eV) *m/z*: 508 (6, M⁺), 492 (8), 265 (16), 241 (50), 195 (46), 166 (95), 111 (47), 43 (100). Anal. Calcd. For C₂₄H₂₅Cl₃N₄O₂: C: 56.76; H: 4.96; N: 11.03. Found: C: 56.81; H: 4.88; N: 11.14.

4.3.13. 3-(5-Amino-1-(3,5-dichlorophenyl)-3-methyl-1H-pyrazol-4-yl)-1-benzyl-5-fluoro-3-hydroxy-indolin-2-one **3m**. Brown pale solid, Yield: 52%. M. p.: 231–233°C. FT-IR (cm⁻¹): 3215, 2361, 1722. ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 1.32 (s, 3 H, CH₃), 4.85 (d, $J = 15.60$ Hz, 1 H, N-CHH), 4.92 (d, $J = 15.60$ Hz, 1 H, N-CHH), 6.98 (dd, $J = 8.59, 4.10$ Hz, 1 H, Ar-H), 7.11 (s, 1 H, OH), 7.13–7.17 (m, 1 H, Ar-H), 7.21 (dd, $J = 7.80, 2.54$ Hz, 1 H, Ar-H), 7.23–7.30 (m, 2 H, Ar-H), 7.31–7.40 (m, 5 H, Ar-H, NH₂), 7.50–7.54 (m, 1 H, Ar-H), 7.67 (d, $J = 1.76$ Hz, 2 H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 13.1 (3-CH₃), 43.5 (CH₂), 74.8 (C), 100.6 (C), 111.0 (CH), 113.2 (CH), 116.3 (CH), 121.2 (CH), 125.8 (CH), 128.1 (CH), 129.1 (CH), 133.9 (C), 134.9 (C), 136.4 (C), 138.8 (C), 141.2 (C), 146.3 (C), 147.8 (C), 176.6 (C). EI-MS (45 eV) *m/z*: 496 (2, M⁺), 480 (5), 255 (21), 241 (16), 198 (21), 164 (45), 108 (22), 91 (100). Anal. Calcd. For C₂₅H₁₉Cl₂FN₄O₂: C: 60.37; H: 3.85; N: 11.27. Found: C: 60.43; H: 3.77; N: 11.20.

4.3.14. 3-(5-Amino-1-(4-chlorophenyl)-3-methyl-1H-pyrazol-4-yl)-1-benzyl-5,7-dichloro-3-hydroxy-indolin-2-one **3n**. White solid, Yield: 44%. M. p.: 205–207°C. FT-IR (cm⁻¹): 3447, 3331, 3065, 2359, 1726. ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 1.45 (s, 3 H, 3-CH₃), 5.18 (d, $J = 16.56$ Hz, 1 H, N-CHH), 5.26 (d, $J = 16.56$ Hz, 1 H, N-CHH), 5.44 (s, 2 H, NH₂), 7.22 (s, 1 H, OH), 7.24–7.30 (m, 3 H, Ar-H), 7.30–7.37 (m, 2 H, Ar-H), 7.42 (d, $J = 1.95$ Hz, 1 H, Ar-H), 7.50 (d, $J = 2.15$ Hz, 1 H, Ar-H), 7.52 (d, $J = 8.78$ Hz, 2 H, Ar-H), 7.60 (d, $J = 8.78$ Hz, 2 H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 13.5 (3-CH₃), 44.8 (CH₂), 74.2 (C), 99.1 (C), 115.7 (C), 124.6 (CH), 125.1 (CH), 126.7 (CH), 127.7 (CH), 128.2 (C), 129.0 (CH), 129.6 (CH), 131.0 (C), 131.3 (CH), 137.2 (C), 137.7 (2 \times C), 138.0 (C), 145.1 (C), 147.2 (C), 177.3 (C). EI-MS (45 eV) *m/z*: 515 (3, M⁺), 496 (4), 305 (16), 214 (34), 207 (29), 91 (100), 65 (20). Anal. Calcd. For C₂₅H₁₉Cl₃N₄O₂: C: 58.44; H: 3.73; N: 10.90. Found: C: 58.37; H: 3.66; N: 10.82.

4.3.15. 3-(5-Amino-1-(3,5-dichlorophenyl)-3-methyl-1H-pyrazol-4-yl)-1-benzyl-5,7-dichloro-3-hydroxyindolin-2-one **3o**. White solid, Yield: 47%. M. p.: 208–210°C. FT-IR (cm⁻¹): 3474, 3374, 3067, 2920, 2359, 1732. ¹H NMR (400 MHz,

DMSO-*d*₆) δ (ppm): 1.45 (s, 3 H, 3-CH₃), 5.18 (d, $J = 16.70$ Hz, 1 H, N-CHH), 5.26 (d, $J = 16.70$ Hz, 1 H, N-CHH), 5.63 (s, 2 H, NH₂), 7.23–7.29 (m, 3 H, Ar-H), 7.30 (s, 1 H, OH), 7.31–7.36 (m, 2 H, Ar-H), 7.40 (d, $J = 2.15$ Hz, 1 H, Ar-H), 7.50 (d, $J = 1.95$ Hz, 1 H, Ar-H), 7.52 (t, $J = 1.76$ Hz, 1 H, Ar-H), 7.66 (d, $J = 1.76$ Hz, 2 H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 13.4 (3-CH₃), 44.8 (CH₂), 74.1 (C), 99.9 (C), 115.7 (C), 121.3 (CH), 124.6 (CH), 125.9 (CH), 126.7 (CH), 127.7 (CH), 128.3 (C), 129.0 (CH), 131.4 (CH), 134.9 (C), 136.9 (C), 137.7 (C), 141.3 (C), 146.1 (C), 147.8 (C), 177.2 (C). EI-MS (20 eV) *m/z*: 548 (1, M⁺), 532 (3), 305 (19), 241 (30), 214 (35), 91 (100). Anal. Calcd. For C₂₅H₁₈Cl₄N₄O₂: C: 54.77; H: 3.31; N: 10.22. Found: C: 54.70; H: 3.26; N: 10.17.

4.3.16. 3-(5-Amino-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-benzyl-5,7-dichloro-3-hydroxyindolin-2-one **3p**. White solid, Yield: 72%. M. p.: 211–213°C. FT-IR (cm⁻¹): 3429, 3341, 3071, 2818, 1730. ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 1.46 (s, 3 H, 3-CH₃), 5.19 (d, $J = 16, 76$ Hz, 1 H, N-CHH), 5.27 (d, $J = 16, 76$ Hz, 1 H, N-CHH), 5.36 (s, 2 H, NH₂), 7.20 (s, 1 H, OH), 7.23–7.29 (m, 3 H, Ar-H), 7.30–7.37 (m, 3 H, Ar-H), 7.43 (d, $J = 2.15$ Hz, 1 H, Ar-H), 7.45–7.51 (m, 3 H, Ar-H), 7.53–7.57 (m, 2 H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 13.5 (3-CH₃), 44.8 (CH₂), 74.2 (C), 98.2 (C), 115.6 (C), 123.5 (CH), 124.6 (CH), 126.7 (CH), 126.9 (CH), 127.7 (CH), 128.2 (C), 129.0 (CH), 129.7 (CH), 131.3 (CH), 137.7 (C), 137.8 (C), 144.6 (C), 147.0 (C), 177.4 (C). EI-MS (20 eV) *m/z*: 548 (1, M⁺), 532 (3), 305 (19), 241 (30), 214 (35), 91 (100). Anal. Calcd. For C₂₅H₂₀Cl₂N₄O₂: C: 62.64; H: 4.21; N: 11.69. Found: C: 62.59; H: 4.27; N: 11.63.

4.4. Antibacterial Activity. Stock solutions (100 mg/mL) of compounds **3** were prepared in dimethyl sulfoxide (DMSO) and diluted to a final screening concentration of 500 μ g/mL. An initial screening of bacterial inhibition was performed by the agar diffusion method. In brief, sterile Mueller Hinton agar (MHA, BBL) was prepared in Petri dishes and inoculated with a bacterial suspension prepared in trypticase soy broth and adjusted to 1.5×10^8 colony-forming unit CFU/mL (i.e., 0.08–0.1 OD at 600 nm) [44]. Wells (6 mm in diameter) were punched in the agar, and 10 μ L of each compound (stock solution) were filled into each well. Dimethyl sulfoxide and trypticase soy broth were included as negative controls (i.e., no inhibition of bacterial growth). Gentamicin (Sigma-Aldrich) and trimethoprim sulfamethoxazole were included as positive controls of growth inhibition. Derivatives **3** showing growth inhibition were tested at least twice before being selected for microdilution testing. For *N. gonorrhoeae*, the agar diffusion method was also used in the screening process with some modifications. For this method, 200 μ L of a bacterial suspension (1.5×10^8 CFU/mL) was inoculated in gonococcal (GC) agar (BBL) supplemented with 1% isovitalax (BBL), and then the compounds were added to the wells as mentioned above and incubated at 35–36.5°C in 5% CO₂ atmosphere for 48 h. Penicillin and ceftriaxone (BBL) were used as controls [45].

4.4.1. Microdilution Test. Minimum inhibitory concentration (MIC) was determined in those compounds with visible and reproducible antibacterial inhibition by the screening test. Bacterial suspensions were adjusted with Mueller Hinton broth (MHB) to a concentration of 5×10^5 – 8×10^5 [44]. Stock solutions of the novel compounds were diluted in MHB containing 5% DMSO and 0.1% Tween 80 [46] and added to 90 μ L of the bacterial inoculum. The microplates were incubated for 24 h at 35–37°C. MICs defined the lowest concentration with visible inhibition of bacterial growth [44] and/or detection using resazurin (125 μ g/mL). Gentamicin and tetracycline (Sigma-Aldrich) were included as control of inhibition growth; MHB and DMSO were used as a negative control. Experiments were performed in duplicate.

For *N. gonorrhoeae*, those compounds showing growth inhibition in the screening test were further tested for MIC on agar plates as described by the Centers for Disease Control and Prevention [47] and the Clinical and Laboratory Standards Institute [45] with modifications. Briefly, GC agar supplemented with 1% isovitalex was prepared containing increasing concentrations of the novel compounds and inoculated with 10 μ L of the bacterial suspension (i.e., 1×10^4 CFU). The lowest concentration of the compound that inhibited bacterial growth was determined as the MIC. Bacterial growth was examined and verified using the oxidase test. Experiments were performed in duplicate.

4.5. Hemolytic Activity. Human red blood cells (huRBC) were adjusted to 5% hematocrit in phosphate buffered saline (PBS). 240 μ L was placed into each well of 96-well plate and subsequently exposed against 200 μ g/mL of each compound. Free hemoglobin was measured after 24 h incubation at 37°C, by spectrophotometry (420 nm Cytation 3M, Biotek). Nonspecific absorbance was subtracted from a blank. Determinations were performed by triplicate in at least two independent experiments [48].

Data Availability

The data used to support the findings of this study are provided in Section 4 and in the supplementary materials. Additional inquiries may be directed to the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supplementary Materials

^1H NMR and ^{13}C NMR spectra of compounds **3a-p** are gathered in the supplementary file. (*Supplementary Materials*)

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