Research Article

Synthesis of Novel Pyridopyridazin-3(2H)-one Derivatives and Evaluation of Their Cytotoxic Activity against MCF-7 Cells

Periasamy Selvakumar,¹ Sathiah Thennarasu,¹ and Asit Baran Mandal²

¹ Organic Chemistry Division, CSIR-Central Leather Research Institute, Adyar, Chennai 600 020, India

² Chemical Laboratory, CSIR-Central Leather Research Institute, Adyar, Chennai 600 020, India

Correspondence should be addressed to Sathiah Thennarasu; thennarasu@gmail.com and Asit Baran Mandal; abmandal@hotmail.com

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A series of pyridopyridazin-3(2H)-one derivatives was synthesized in two facile steps. Mannich-type three-component condensation afforded the 2,6-diaryl piperidin-4-one derivatives, which underwent intramolecular cyclization in the presence of hydrazine or phenylhydrazine to yield the corresponding pyridopyridazin-3(2H)-one derivatives. All the derivatives of pyridopyridazin-3(2H)-one, except **3e** and **3f**, showed moderate activity against human breast adenocarcinoma (MCF-7) cells. The higher degree of inhibition of MCF-7 cell proliferation shown by **2a–2f** indicates the significance of the amide proton in pyridopyridazin-3(2H)-one derivatives.

1. Introduction

Among the nitrogen containing six-member heterocyclic compounds, the piperidine structural motif is often found in naturally occurring bioactive compounds such as alkaloids [1]. Piperidin-3-one derivatives are used as precursors for the synthesis of antimalarial agents, febrifugine, and isofebrifugine [2]. Piperidin-4-ones mostly display varied and potent biological properties such as antiviral, antitumour, analgesic, local anaesthetic, antimicrobial, fungicidal, herbicidal, insecticidal, antihistaminic, anti-inflammatory, anticancer, CNS stimulant, and depressant properties [3–9]. Recent reports suggest that compounds containing the piperidin-4-one moiety elicit excellent biological activities when aromatic substitutions are present at 2 and/or 6 positions [10].

Another pharmacologically important heterocyclic structural motif is the pyridazin-3(2H)-one unit, which has been found to inhibit the activities of cGMP-phosphodiesterase (PDE3) and cAMP-phosphodiesterase (PDE4) enzymes [11]. Also, pyridazin-3(2H)-one possesses different pharmacological activities like analgesic, anti-inflammatory, antibacterial, herbicidal, antifungal, antituberculosic, anti-AIDs, antitumour, antihypertensive, anticonvulsant, and antiviral

activities [12–16]. Besides these activities, the polyfunctional tetrahydro-2H-pyrano[3,2-c]pyridazin-3(6H)-one derivatives have been shown to act as potent anticancer agents [17]. Recently, a series of pyridazinone derivatives bearing benzenesulfonamide moiety have also been reported to act as anticancer agents [18].

Mannich-type condensation involving a ketone having two active methylene groups, an aromatic aldehyde, and ammonium acetate, resulting in the formation of 2,6-diarylpiperidin-4-one, was first reported by Noller and Baliah [19]. Formation of 2,6-diarylpiperidin-4-one derivatives from the condensation of aryl aldehyde, ammonia, and levulinic acid or its ethyl ester was also observed by Baliah and Ekambaram [20]. However, a mixture of 2,6-diarylpiperidin-4-one and 1,9-diazabicyclononane derivatives was obtained from the condensation of acetone, aryl aldehyde, and ammonia [21]. Later on, Baliah and Jeyaraman reported a similar condensation involving cyclic ketones resulting in the formation of different azabicyclononanes [22]. Recently, Thennarasu and Perumal have reported the formation of 3-pentyl-2,6-diphenylpiperidine-4-one in moderate yield via Mannich-type condensation of octan-2-one, benzaldehyde, and ammonium acetate in absolute ethanol [23].

We, herein, report an improved method for the synthesis and complete characterization of different 4-oxo-2,6-diphenylpiperidin-3-yl-acetates (Scheme 1). Subsequent cyclization of these derivatives into the corresponding pyrido[4,3-c]pyridazin-3(2H)-one derivatives using hydrazine and phenylhydrazine (Scheme 2) is also reported for the first time. In addition, the anticancer activity of these new pyrido[4,3-c]pyridazin-3(2H)-one derivatives is presented.

2. Results and Discussion

2.1. Chemistry. Three-component condensation of benzaldehyde, ammonium acetate, and ethyl levulinate in methanol at $\sim 60^\circ\text{C}$ afforded ethyl 4-oxo-2,6-diphenylpiperidin-3-yl acetate in moderate yield (56%). After optimizing the reaction conditions (temperature, time, and amount of catalyst AcOH), various aryl aldehydes were allowed to react with ethyl levulinate and ammonium acetate in methanol-acetic acid medium at $\sim 60^\circ\text{C}$ to afford the corresponding ethyl 4-oxo-2,6-diarylpyridin-3-yl acetate derivatives in good yields. Typically, when benzaldehyde was used and the reaction was carried out in 70 mol% glacial acetic acid in methanol, the yield improved from 56% to 77%.

All the synthesized compounds were characterized using IR and NMR and ESI-MS techniques. For instance, the IR spectrum of compound **1a** displays the secondary amine NH stretching at 3425 cm^{-1} . The ketone and ester carbonyl vibrations occur, respectively, at 1723 cm^{-1} and 1654 cm^{-1} and indicate the formation of a cyclic ketone. The ^1H NMR spectrum of **1a** shows a broad peak at $\sim 2.09\text{ ppm}$ that can be ascribed to the NH proton of a secondary amine. D_2O induced proton exchange diminishes the intensity of the peak at $\sim 2.09\text{ ppm}$ confirming the exchangeability of the NH proton. All other protons were assigned with the aid of published literature [20]. The ketone and ester carbonyl resonances at ~ 207.8 and $\sim 172.3\text{ ppm}$, respectively, in the ^{13}C NMR spectrum of **1a** confirm the presence of the heterocyclic compound **1a**. The formation of **1a** was further confirmed using ESI-MS data. Similarly, the structures of 2,6-diarylpyridin-4-one derivatives **1b-f** were also confirmed using mass and spectroscopic data (see Supplementary Material available online at <http://dx.doi.org/10.1155/2014/410716>).

In view of the wide spectrum of biological activities documented for piperidine and pyridazinone pharmacophores, we reasoned that the combination of these two different pharmacophores into a single structural scaffold would confer synergistic properties on the new molecule. Accordingly, we synthesized pyrido[4,3-c]pyridazin-3(2H)-one derivatives using hydrazine and phenylhydrazine mediated cyclization as depicted in Scheme 2.

The reaction of hydrazine hydrate with ethyl 4-oxo-2,6-diarylpyridin-3-yl acetates in ethanol under reflux condition yielded in due course the corresponding 4,4a,5,6,7,8-hexahydro-5,7-diarylpyrido[4,3-c]pyridazin-3(2H)-one derivatives **2a-f**. Under identical reaction conditions, phenylhydrazine failed to yield the corresponding 4,4a,5,6,7,8-hexahydro-2,5,7-triarylpyrido[4,3-c]pyridazin-3(2H)-one derivatives **3a-f**. However, the reaction was accomplished

in dry toluene containing 20 mol% trifluoroacetic acid with moderate to good yields (Scheme 2) [24].

The structures of 4,4a,5,6,7,8-hexahydro-5,7-diarylpyrido[4,3-c]pyridazin-3(2H)-one derivatives **2a-f** were confirmed using spectroscopic data. The two bands at 3371 cm^{-1} and 3281 cm^{-1} in the IR spectrum of the compound **2a** could be assigned to the secondary amine NH and amide NH stretching vibrations, respectively. This observation is corroborated by the diminished intensity of amine NH peak at $\sim 2.1\text{ ppm}$ and the amide NH peak at $\sim 8.7\text{ ppm}$ in the ^1H NMR spectrum of **2a** obtained after proton exchange with D_2O . The strong amide carbonyl stretching at 1685 cm^{-1} substantiates the formation of pyrido[4,3-c]pyridazin-3(2H)-one structure. The appearance of amide carbon peak at $\sim 166\text{ ppm}$ and imine carbon peak at $\sim 152\text{ ppm}$ and the absence of parent-ketone peak at $\sim 207\text{ ppm}$ in the ^{13}C NMR spectrum provide complementary evidence to confirm the formation of compound **2a**.

The structural elucidation of 4,4a,5,6,7,8-hexahydro-2,5,7-triarylpyrido[4,3-c]pyridazin-3(2H)-one derivatives **3a-f** was achieved through spectral analysis. The appearance of amine NH band at $\sim 3320\text{ cm}^{-1}$ and absence of amide NH band at $\sim 3281\text{ cm}^{-1}$ in the IR spectrum of the compound **3b** are an indication of phenyl substitution at amide nitrogen of **3b**. This proposition is supported by the observation of only the amine NH peak at $\sim 2.08\text{ ppm}$ and the absence of any amide NH resonances in the ^1H NMR spectrum of **3b**. The strong amide carbonyl stretching at $\sim 1688\text{ cm}^{-1}$ is evidence to the formation of **3b**. The amide carbon resonance at $\sim 164\text{ ppm}$ and imine carbon resonance at $\sim 153\text{ ppm}$ in the ^{13}C NMR spectrum confirms the formation of compound **3b**.

Several conformational isomers are possible for **1a-f**, **2a-f**, and **3a-f** depending on the nature of the substituents and the reaction conditions used for their syntheses. Therefore, it was important to determine the preferred types of chair or boat conformations of **1a-f**, **2a-f**, and **3a-f**. The conformational assignments were investigated using ^{13}C NMR (chemical shifts) and ^1H NMR (vicinal coupling constants) spectra [25–28]. The dihedral angles derived from the vicinal coupling constant values obtained from the ^1H NMR spectrum of **3b** suggested a chair conformation to the piperidine ring and a distorted/half-chair conformation to the pyridazine ring. This conformation of **3b** was further confirmed through single crystal XRD analysis (Figure 1) [29].

2.2. Anticancer Activity. The newly synthesized pyridopyridazin-3(2H)-one derivatives **2a-f** and **3a-f** were evaluated for anticancer activity in vitro against MCF-7 breast cancer cells. Doxorubicin was used as the standard drug. MTT assay showed that all the pyridopyridazin-3(2H)-one derivatives **2a-f** and **3a-f** possessed moderate cytotoxic activity (Table 1) [30, 31]. Only the compounds **3e** ($\sim 810\text{ }\mu\text{M}$) and **3f** ($\sim 473\text{ }\mu\text{M}$) exhibited weak activity against the cancer cell line. A comparison of the anticancer activities of **2a-f** and **3a-f** reveals that only the three compounds **2d-f** bearing electron withdrawing substituents in the aromatic ring show the highest activity. It is also imperative to note that only the hydrazine

SCHEME 1: Synthesis of piperidin-4-one derivatives. Reagents and conditions: methanol-acetic acid (70 mol%), 60°C, 2 h.

SCHEME 2: Synthesis of pyridopyridazin-3(2H)-one derivatives. Reagents and conditions: (i) N₂H₄·H₂O, ethanol, reflux, 17 h; (ii) PhNHNH₂, dry toluene, TFA (20 mol%), reflux, 2 days.

FIGURE 1: ORTEP diagram of **3b** derived from single crystal XRD analysis.

TABLE 1: Inhibitory effects of compounds **2a–f**, **3a–f** on viability of breast cancer cells.

Compounds	R	MCF-7 IC ₅₀ ^a (μM)
2a	H	475.4 ± 27.8
2b	CH ₃	498.4 ± 30.0
2c	OCH ₃	344.1 ± 59.4
2d	Cl	61.3 ± 5.8
2e	Br	78.9 ± 14.7
2f	3-NO ₂	141.0 ± 22.5
3a	H	268.7 ± 15.4
3b	CH ₃	219.0 ± 11.0
3c	OCH ₃	248.1 ± 17.2
3d	Cl	364.1 ± 20.2
3e	Br	810.0 ± 35.7
3f	3-NO ₂	473.7 ± 26.1
Doxorubicin ^b	—	2.007 ± 0.05

^aInhibitory concentration (IC₅₀, μM) as obtained from MTT assay. All the values represent the average of three experimental samples. The cells were incubated for a period of 48 h.

^bDoxorubicin was used as the standard drug.

incorporated derivatives **2d–f** and not the phenylhydrazine incorporated derivatives **3d–f** show the high activity against MCF-7 breast cancer cell line. Thus, the aryl ring at position 3 seems unimportant and, in fact, reduces the activity against MCF-7 cells.

3. Conclusion

In conclusion, three efficient methods for the syntheses of different derivatives of ethyl 4-oxo-2,6-diarylpiperidin-3-yl acetate, 4,4a,5,6,7,8-hexahydro-5,7-diarylpyrido[4,3-c]pyridazin-3(2H)-one, and 4,4a,5,6,7,8-hexahydro-2,5,7-triarylpyrido[4,3-c]pyridazin-3(2H)-one are reported. The yields of ethyl 4-oxo-2,6-diarylpiperidin-3-yl acetates (**1a–f**) are improved significantly when glacial acetic acid is used as the catalyst. The requirement of a strongly acidic 20% TFA/toluene medium for the formation of cyclic products **3a–f** is also demonstrated. Further, the inhibitory activity of the test compounds against MCF-7 human breast adenocarcinoma cells is provided. A comparative account explaining the importance of functional groups for the higher levels of activity shown by **2d**, **2e**, and **2f** is also presented.

4. Experimental Section

4.1. General. All the chemicals used in this study were purchased from Sigma Aldrich without further purification. Melting points were determined in open capillary tubes and are uncorrected. IR spectra were taken as KBr pellets for solids on a Perkin Elmer Spectrum RXI FT-IR. ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) spectra were recorded in CDCl₃ solutions with TMS as an internal standard on a JEOL instrument and Bruker Avance instrument. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage MAX 6000 ESI spectrometer. Column chromatography was

performed using neutral aluminium oxide, 150–200 mesh, and eluent, 7% ethyl acetate in hexane.

4.2. General Procedure for the Synthesis of 4-Oxo-2,6-diphenylpiperidin-3-yl-acetate Derivatives (1a–f). 4-Oxo-2,6-diphenylpiperidin-3-yl-acetate (**1a**) was synthesized as described elsewhere[23] with a slight modification. Methanol was used as the solvent and glacial acetic acid (70 mol%) was used as the catalyst. In a typical experiment, ammonium acetate (3.85 g, 50 mmol) was dissolved in methanol (60 mL) and then benzaldehyde (9.0 mL, 100 mmol) was added and warmed over a water-bath for 5 min. Ethyl levulinate (7.0 mL, 50 mmol) and glacial acetic acid (2.0 mL, 35 mmol) were added and heated at ~60°C over a water-bath for 2 h and then left aside at room temperature. The pale-yellow precipitate formed was crystallized from methanol.

4.3. Synthesis of 4,4a,5,6,7,8-Hexahydro-5,7-diphenylpyrido[4,3-c]pyridazin-3(2H)-ones (2a–2f). To a solution of appropriate ethyl 4-oxo-2,6-diphenylpiperidin-3-yl-acetates (337 mg, 1.0 mmol) in ethanol was added hydrazine hydrate (0.053 mL, 1.2 mmol) and the resultant solution was refluxed for 17 h. The solvent was evaporated and the residue obtained was extracted with chloroform (3 × 20 mL). The combined organic layer was dried over sodium sulphate, concentrated under reduced pressure and the solid obtained was purified by crystallization using methanol.

4.3.1. 4,4a,5,6,7,8-Hexahydro-5,7-diphenylpyrido[4,3-c]pyridazin-3(2H)-one (2a). Specifications are as follows: colourless solid; mp 153–154°C; yield 82%; IR (KBr cm⁻¹): 3371, 3281, 3025, 2925, 2848, 1685, 1514, 1416, 1334, 1106, 1038, 823, 759; ¹H NMR (500 MHz, CDCl₃): δ ppm 2.10 (bs, 1H, amine NH), 2.17–2.28 (m, 2H), 2.51–2.56 (m, 1H), 2.71–2.76 (m, 2H merged), 3.63 (d, 1H, J = 9.7 Hz), 3.9 (d, 1H, J = 11.4 Hz), 7.26–7.35 (m, 6H Ar protons), 7.41–7.44 (m, 4H Ar protons), 8.77 (bs, 1H, amide NH); ¹³C NMR (125 MHz, CDCl₃): δ ppm 29.7, 40.5, 41.7, 60.9, 68.3, 126.7, 127.6, 127.9, 128.6, 128.7, 128.9, 140.5, 142.9, 152.8, 166.3; MS m/z = 306 [MH]⁺.

4.3.2. 4,4a,5,6,7,8-Hexahydro-5,7-bis(4-methylphenyl)pyrido[4,3-c]pyridazin-3(2H)-one (2b). Specifications are as follows: colourless solid; mp 183–185°C; yield 72%; IR (KBr cm⁻¹): 3303, 3210, 3087, 2944, 2831, 1665, 1611, 1511, 1458, 1303, 1367, 1241, 1175, 1107, 1031, 834; ¹H NMR (500 MHz, CDCl₃): δ ppm 2.07 (bs, 1H, amine NH), 2.18–2.34 (m, 2H), 2.36 (s, 3H), 2.37 (s, 3H), 2.54 (dd, 1H, J = 15, 12 Hz), 2.72–2.78 (m, 2H merged), 3.62 (d, 1H, J = 9.5 Hz), 3.96 (dd, 1H, J = 12, 3 Hz), 7.18 (d, 4H, J = 6.5 Hz Ar protons), 7.32–7.35 (m, 4H Ar protons), 8.66 (bs, 1H, amide NH); ¹³C NMR (125 MHz, CDCl₃): δ ppm 21.12, 21.14, 29.6, 40.4, 41.7, 60.6, 68.0, 126.4, 127.4, 129.3, 129.4, 137.51, 137.55, 138.2, 140.0, 152.9, 166.2; MS m/z = 334 [MH]⁺.

4.3.3. 4,4a,5,6,7,8-Hexahydro-5,7-bis(4-methoxyphenyl)pyrido[4,3-c]pyridazin-3(2H)-one (2c). Specifications are as follows: colourless solid; mp 188–190°C; yield 76.7%; IR (KBr cm⁻¹): 3318, 3222, 3090, 2920, 2849, 1664, 1439, 1414, 1370,

1305, 1230, 1174, 1091, 1014, 828; ^1H NMR (500 MHz, CDCl_3): δ ppm 2.04 (bs, 1H, amine NH), 2.17–2.33 (m, 2H), 2.52 (dd, 1H, $J = 15.5, 11.5$ Hz), 2.69–2.75 (m, 2H merged), 3.59 (d, 1H, $J = 9.5$ Hz), 3.81 (s, 3H), 3.82 (s, 3H), 3.93 (dd, 1H, $J = 11.5, 3$ Hz), 6.88–6.90 (m, 4H Ar protons), 7.34–7.38 (m, 4H Ar protons), 8.66 (bs, 1H, amide NH); ^{13}C NMR (125 MHz, CDCl_3): δ ppm 29.6, 40.6, 41.7, 55.30, 55.34, 60.3, 67.7, 114.0, 114.1, 127.6, 128.6, 132.6, 135.1, 152.9, 159.1, 159.6, 166.2; MS $m/z = 366$ [MH] $^+$.

4.3.4. 4,4a,5,6,7,8-Hexahydro-5,7-bis(4-chlorophenyl)pyrido[4,3-c]pyridazin-3(2H)-one (**2d**). Specifications are as follows: colourless solid, mp 185–187°C, yield 79%, IR (KBr cm^{-1}): 3425, 3322, 3225, 3086, 2931, 2367, 2339, 1663, 1525, 1478, 1353, 1211, 1166; ^1H NMR (500 MHz, CDCl_3): δ ppm 2.07 (bs, 1H, amine NH), 2.16–2.30 (m, 2H), 2.48 (dd, 1H, $J = 15, 11.5$ Hz), 2.67–2.75 (m, 2H merged), 3.63 (d, 1H, $J = 9.5$ Hz), 3.96 (dd, 1H, $J = 11.5, 3$ Hz), 7.32–7.39 (m, 8H Ar protons), 8.75 (bs, 1H, amide NH); ^{13}C NMR (125 MHz, CDCl_3): δ ppm 29.5, 40.4, 41.6, 60.1, 67.4, 127.9, 128.9, 129.0, 133.6, 134.4, 138.8, 141.1, 151.6, 165.8; MS $m/z = 374$ [MH] $^+$.

4.3.5. 4,4a,5,6,7,8-Hexahydro-5,7-bis(4-bromophenyl)pyrido[4,3-c]pyridazin-3(2H)-one (**2e**). Specifications are as follows: brown solid, mp 133–135°C, yield 75%, IR (KBr cm^{-1}): 3236, 3097, 2922, 2854, 1674, 1485, 1439, 1412, 1345, 1305, 1230, 1102, 1070, 1008, 825; ^1H NMR (500 MHz, CDCl_3): δ ppm 2.07 (bs, 1H, amine NH), 2.17–2.31 (m, 2H), 2.48 (dd, 1H, $J = 15, 12$ Hz), 2.67–2.75 (m, 2H merged), 3.62 (d, 1H, $J = 10$ Hz), 3.95 (dd, 1H, $J = 11.5, 3$ Hz), 7.31 (d, 4H, $J = 8.5$ Hz Ar protons), 7.48–7.53 (m, 4H Ar protons), 8.67 (bs, 1H, amide NH); ^{13}C NMR (125 MHz, CDCl_3): δ ppm 29.5, 40.4, 41.5, 60.2, 67.5, 121.7, 122.5, 128.2, 129.2, 131.8, 132.0, 139.2, 141.6, 151.5, 165.7; MS $m/z = 463$ [MH] $^+$.

4.3.6. 4,4a,5,6,7,8-Hexahydro-5,7-bis(3-nitrophenyl)pyrido[4,3-c]pyridazin-3(2H)-one (**2f**). Specifications are as follows: pale brown solid, mp 248–250°C, yield 69%, IR (KBr cm^{-1}): 3417, 3322, 3227, 3091, 2926, 2853, 1665, 1523, 1353, 1453, 808, 735, 688; ^1H NMR (500 MHz, CDCl_3): δ ppm 1.89 (dd, 1H, $J = 9, 17.5$ Hz), 2.32 (dd, 1H, $J = 13.5, 17.5$ Hz), 2.42–2.48 (m, 1H), 2.70 (dd, 1H, $J = 14.5, 3$ Hz), 2.81–2.87 (m, 1H), 3.90 (d, 1H, $J = 10$ Hz), 4.11 (d, 1H, $J = 10.5$ Hz), 7.64–7.70 (m, 2H Ar protons), 7.93–7.97 (m, 2H Ar protons), 8.16 (2 set of dd, 2H, $J = 8, 1.5$ Hz 8.5, 2 Hz Ar protons), 8.34–8.39 (m, 2H Ar protons), 10.6 (bs, 1H, amide NH); ^{13}C NMR (125 MHz, CDCl_3): δ ppm 29.7, 39.3, 40.8, 59.0, 66.0, 121.8, 122.6, 123.1, 123.2, 130.3, 134.2, 135.2, 144.0, 146.1, 148.2, 148.3, 150.6, 165.8. Ms $m/z = 395.34$ [M] $^+$.

4.4. Synthesis of 2-Phenyl-5,7-diarylpyrido[4,3-c]pyridazin-3(2H)-one Derivatives (**3a–3f**). A mixture of ethyl 4-oxo-2,6-diphenylpiperidin-3-yl-acetates (337 mg, 1.0 mmol) in dry toluene were added to phenyl hydrazine (0.118 mL, 1.2 mmol) and trifluoroacetic acid (0.0155 mL, 0.20 mmol), and the solution was refluxed under nitrogen atmosphere for 2 days. After the reaction was complete the solvent was evaporated under reduced pressure, and the residue obtained was extracted

with chloroform (3×20 mL). The combined organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure and the residue obtained was purified using column chromatography (neutral aluminium oxide, 150–200 mesh; eluent: 7% ethyl acetate in hexane).

4.4.1. 4,4a,5,6,7,8-Hexahydro-2,5,7-triphenylpyrido[4,3-c]pyridazin-3(2H)-one (**3a**). Specifications are as follows: pale brown solid; mp 140–143°C; yield 79%; IR (KBr cm^{-1}): 3304, 3030, 2960, 2887, 2806, 1680, 1595, 1492, 1454, 1326, 1305, 1227, 1160, 1107, 752; ^1H NMR (500 MHz, CDCl_3): δ ppm 2.18 (bs, 1H, amine NH), 2.43–2.54 (m, 2H), 2.66 (dd, 1H, $J = 15, 11.5$ Hz), 2.90–2.96 (m, 2H merged), 3.73 (d, 1H, $J = 9.5$ Hz), 4.07 (dd, 1H, $J = 11.5, 2.5$ Hz), 7.27–7.33 (m, 2H Ar protons), 7.34–7.44 (m, 7H Ar protons), 7.48–7.52 (m, 6H Ar protons); ^{13}C NMR (125 MHz, CDCl_3): δ ppm 31.3, 41.0, 41.7, 60.8, 68.4, 124.9, 126.5, 126.7, 127.6, 127.9, 128.6, 128.7, 128.8, 140.6, 140.9, 142.9, 153.6, 164.3; MS $m/z = 382$ [MH] $^+$.

4.4.2. 4,4a,5,6,7,8-Hexahydro-5,7-bis(4-methylphenyl)-2-phenylpyrido[4,3-c]pyridazin-3(2H)-one (**3b**). Specifications are as follows: pale brown solid; mp 166–167°C; yield 72%; IR (KBr cm^{-1}): 3320, 3021, 2919, 2878, 2815, 1688, 1594, 1492 1321, 1299, 1228, 1155, 1105, 821, 756; ^1H NMR (500 MHz, CDCl_3): δ ppm 2.08 (bs, 1H, amine NH), 2.38 (s, 3H), 2.39 (s, 3H), 2.41–2.51 (m, 2H), 2.64 (dd, 1H, $J = 15, 12$ Hz), 2.87–2.91 (m, 2H merged), 3.69 (d, 1H, $J = 9.5$ Hz), 4.03 (dd, 1H, $J = 11.5, 3$ Hz), 7.19–7.22 (m, 4H, Ar protons), 7.28–7.29 (m, 1H, Ar protons), 7.36–7.38 (m, 4H, Ar protons), 7.40–7.43 (m, 2H, Ar protons), 7.51 (d, 2H, $J = 8$ Hz Ar protons); ^{13}C NMR (125 MHz, CDCl_3): δ ppm 21.14, 21.16, 31.3, 41.0, 41.8, 60.6, 68.1, 124.9, 126.4, 126.6, 127.4, 128.6, 129.3, 129.5, 137.5, 137.6, 140.0, 141.0, 153.9, 164.4; MS $m/z = 410$ [MH] $^+$.

4.4.3. 4,4a,5,6,7,8-Hexahydro-5,7-bis(4-methoxyphenyl)-2-phenylpyrido[4,3-c]pyridazin-3(2H)-one (**3c**). Specifications are as follows: pink solid, mp 128–130°C; yield 74%; IR (KBr cm^{-1}): 3415, 3306, 2954, 2832, 1682, 1606, 1506, 1449, 1302, 1242, 1175, 1105, 927, 833, 752; ^1H NMR (500 MHz, CDCl_3): δ ppm 1.99 (bs, 1H, amine NH), 2.39–2.54 (m, 2H), 2.62 (dd, 1H, $J = 14.5, 12$ Hz), 2.84–2.88 (m, 2H merged), 3.66 (d, 1H, $J = 9.5$ Hz), 3.82 (s, 3H), 3.84 (s, 3H), 4.00 (dd, 1H, $J = 12, 3$ Hz), 6.90–6.93 (m, 4H Ar protons), 7.27–7.28 (m, 1H, Ar proton), 7.38–7.43 (m, 6H, Ar protons), 7.50 (d, 2H, $J = 8$ Hz Ar protons); ^{13}C NMR (125 MHz, CDCl_3): δ ppm 31.1, 41.0, 41.7, 55.20, 55.27, 60.1, 67.2, 113.9, 114.0, 124.9, 126.6, 127.5, 128.5, 132.6, 135.0, 140.8, 153.9, 159.0, 159.6, 164.3; MS $m/z = 442$ [MH] $^+$.

4.4.4. 4,4a,5,6,7,8-Hexahydro-5,7-bis(4-chlorophenyl)-2-phenylpyrido[4,3-c]pyridazin-3(2H)-one (**3d**). Specifications are as follows: pale brown solid; mp 113–115°C; yield 77%; IR (KBr cm^{-1}): 3315, 3061, 2853, 2810, 1676, 1593, 1491, 1442, 1413, 1359, 1329, 1304, 1227, 1202, 1158, 1093, 1014, 830; ^1H NMR (500 MHz, CDCl_3): δ ppm 2.07 (bs, 1H, amine NH), 2.39–2.51 (m, 2H), 2.57 (dd, 1H, $J = 15, 12$ Hz), 2.81–2.88 (m, 2H merged), 3.69 (d, 1H, $J = 10$ Hz), 4.02 (dd, 1H, $J = 12, 3$ Hz), 7.27–7.29 (m, 1H Ar protons), 7.34–7.44 (m, 10H Ar protons),

7.48 (d, 2H, $J = 8.5$ Hz Ar protons); ^{13}C NMR (125 MHz, CDCl_3): δ ppm 31.3, 40.9, 41.7, 60.1, 67.5, 124.9, 126.8, 127.9, 128.6, 128.9, 128.9, 129.1, 133.6, 134.4, 138.9, 140.8, 141.2, 152.6, 163.9; MS $m/z = 450$ $[\text{MH}]^+$.

4.4.5. 4,4a,5,6,7,8-Hexahydro-5,7-bis(4-bromophenyl)-2-phenylpyrido[4,3-c]pyridazin-3(2H)-one (3e). Specifications are as follows: pale brown solid; mp 101–102°C; yield 71.6%; IR (KBr cm^{-1}): 3300, 3041, 2923, 2814, 1679, 1593, 1488, 1410, 1328, 1305, 1227, 1201, 1159, 1106, 1069, 827; ^1H NMR (500 MHz, CDCl_3): δ ppm 2.16 (bs, 1H, amine NH), 2.36–2.48 (m, 2H), 2.53 (dd, 1H, $J = 15.5, 11.5$ Hz), 2.78–2.84 (m, 2H merged), 3.65 (d, 1H, $J = 10$ Hz), 3.98 (dd, 1H, $J = 12.5, 3$ Hz), 7.23–7.26 (m, 1H Ar protons), 7.31–7.39 (m, 6H Ar protons), 7.43–7.52 (m, 6H Ar protons); ^{13}C NMR (125 MHz, CDCl_3): δ ppm 31.2, 41.0, 41.7, 60.2, 67.6, 121.8, 122.6, 125.0, 126.9, 128.3, 131.9, 132.1, 139.4, 140.8, 141.7, 152.6, 164.0; MS $m/z = 539$ $[\text{MH}]^+$.

4.4.6. 4,4a,5,6,7,8-Hexahydro-5,7-bis(3-nitrophenyl)-2-phenylpyrido[4,3-c]pyridazin-3(2H)-one (3f). Specifications are as follows: brown solid; m.p 190–191°C; yield 67%; IR (KBr cm^{-1}): 3418, 3085, 2922, 2852, 1679, 1596, 1522, 1345, 1103, 908, 810; ^1H NMR (500 MHz, CDCl_3): δ ppm 2.04 (bs, amine NH), 2.42–2.52 (m, 2H), 2.64 (dd, 1H, $J = 11.5, 15.5$ Hz), 2.91–2.97 (m, 2H merged), 3.88 (d, 1H, $J = 10$ Hz), 4.19 (dd, 1H, $J = 12.5, 3$ Hz), 7.24–7.27 (m, 1H Ar protons), 7.35–7.40 (m, 2H Ar protons), 7.44 (d, 2H, $J = 8$ Hz Ar protons), 7.55–7.62 (m, 2H Ar protons), 7.81–7.85 (m, 2H Ar protons), 8.18 (d, 1H, $J = 8$ Hz Ar protons), 8.23 (d, 1H, $J = 9$ Hz Ar protons), 8.35–8.39 (m, 2H Ar protons); ^{13}C NMR (125 MHz, CDCl_3): δ ppm 31.1, 40.8, 41.4, 60.1, 67.3, 121.8, 122.7, 123.3, 123.9, 125.0, 127.0, 128.8, 130.0, 130.2, 132.9, 133.9, 140.7, 142.3, 144.4, 148.6, 148.7, 151.3, 163.5. Ms $m/z = 471.42$ $[\text{MH}]^+$.

4.5. Pharmacological Study: Chemicals and Reagents. Human breast adenocarcinoma (MCF-7) cell culture was procured from the National Centre for Cell Sciences (NCCS), Pune, India. The cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS), penicillin (100 IU/mL), streptomycin (100 $\mu\text{g/mL}$), and amphotericin-B (5 $\mu\text{g/mL}$) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were trypsinized with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 flat bottles and the studies were carried out in 96-well microtiter plates.

4.5.1. Cell Culture and In Vitro Cytotoxicity Assay. Cells were plated in 96-well flat bottom microtiter plate at a density of 1×10^4 cells per well and cultured for 24 h at 37°C in 5% CO_2 atmosphere to allow cell adhesion. After 24 h, when partial monolayer was formed, medium was removed and cells were treated with different concentrations of standard drug (doxorubicin) and test compounds. Microscopic examination was carried out and observations recorded every 24 h. After the treatment, the solutions in the wells were discarded and 50 μL of the freshly prepared MTT solution (2 mg/mL PBS) was added to each well. The plates were gently shaken and

incubated for 3 h at 37°C in 5% CO_2 atmosphere. After 3 h, the supernatant was removed and the formazan crystals formed in the cells were solubilized by adding isopropanol (50 μL). Finally, the amount of formazan formed in different wells was measured from the absorbance at 540 nm using a microplate reader (Bio-Tek, EL X-800 MS). The concentration of drug required to kill 50% of cells in exponentially growing cultures after a 48 h exposure to the drug (IC_{50} values) was calculated from the plot of A_{540} versus concentration of test sample. The cytotoxicity of the complex was measured from the spectrophotometric data by means of this equation: % cell cytotoxicity = $[\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}} / \text{Abs}_{\text{control}}] \times 100$.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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