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One-pot, catalyst-free synthesis of novel spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3]benzothiazole] derivatives

Mahdieh Zangouei and Abbas Ali Esmaeili

Abstract

The present report describes one-pot three-component condensation of isatins, malononitrile, and 2-hydroxy-4*H*-pyrimido[2,1-*b*][1,3]benzothiazol-4-one in water–ethanol mixture at reflux to develop an efficient one-pot protocol for the synthesis of novel spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3]benzothiazole] derivatives. The significant features of this protocol are short reaction times, avoidance of toxic catalysts, and provision of excellent yields, no column chromatographic purification, and use of ethanol-water as an environmentally benign solvent. The molecular structure of **4a** has been supported by single-crystal X-ray diffraction.

Keywords

2-hydroxy-4*H*-pyrimido[2,1-*b*][1,3]benzothiazol-4-one, isatin, malononitrile, multicomponent reactions, spirooxindole

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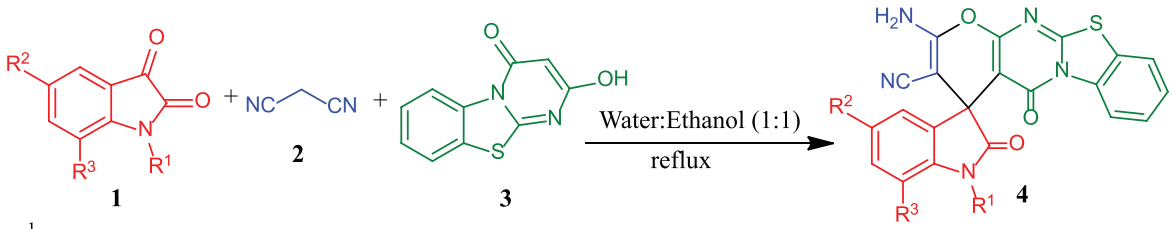

R^1 = H, Me, Et, $C_6H_5CH_2$, 4-$ClC_6H_4CH_2$
R^2 = H, Br, MeO, Cl, CH_3
R^3 = H, Me

13 examples (85-95%)

Introduction

As a major heterocycle, the spirooxindole system has been identified in a variety of pharmacological and natural products such as spirotryprostatin A, welwitindolinone A, horsfiline, elacomine, coerulescine, and alstonisine.[1,2] Spirotryprostatin A is a novel inhibitor of microtubule assembly[3] extracted from the fermentation broth of Aspergillus fumigatus. This natural alkaloid, along with pteropodine, positively modulate muscarinic 5-HT2 and M2 receptors.[4] Furthermore, owing to their beneficial pharmacological and biological properties, including antioxidant, antifungal,[5] anticoagulant,[6] anti-tumor,[7,8] anticancer,[9] anti-HIV,[10,11] and anti-malarial[12] activities, spirooxindole derivatives play a significant role in synthetic medicinal chemistry.[13] Some additional biologically active spirocyclic oxindoles have also been described in a broader-context review.[14–20] Spirooxindoles, especially those spiroannulated with heterocycles at the 3-position, have been reported to possess good biological activities.[21] For example, pyran-annulated oxindoles are widely spread in nature and exhibit various physiological activities.[22] The cytotoxic,[23] anticancer,[24] antimicrobial[25] (Figure 1), analgesic,[26] and herbicidal[27] activities of some synthetic spiroheterocyclic compounds containing both indole and pyran moieties have also been reported.

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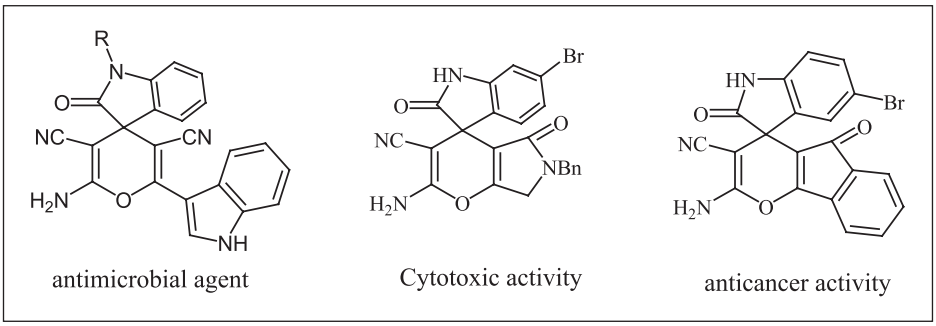


Figure 1. Selected bioactive molecules containing pyran-annulated oxindoles.

Therefore, intensive efforts have been made to design and develop new approaches for the construction of novel synthetic spirooxindole-fused heterocycles.

Fused-pyrimidine-containing heterocyclic skeletons are an important biologically active core unit in heterocyclic compounds and possess remarkable biological properties.[28–30] Among them, pyrimidobenzothiazole scaffolds have received considerable attention because of the synthetic challenges associated with their complex molecular architecture and their interesting biological properties, such as antihistaminic,[31] antibacterial,[32–34] anticancer,[35] antiviral,[36] and antifungal activities.[37] Recent studies focusing on the synthesis of novel benzo[4,5]thiazolo[1,2-*a*]pyrimidine-3-carboxylate derivatives have found these compounds to show cytotoxic activity against human breast adenocarcinoma MDA-MB-231 and MCF-7 tumor cell lines.[38] Moreover, pyranopyrimidine contains a diverse array of natural products, as well as pharmaceutically significant compounds, with a wide range of biological activities.[39,40] For example, pyrano[2,3-*d*]pyrimidine are known to exhibit antitumor,[41,42] hepatoprotective,[43] antibronchitic,[44] and pronounced antitubercular and antimicrobial activities.[45]

Based on the discussed facts and the significant biological properties of spirooxindoles, pyrano[2,3-*d*]pyrimidine, and pyrimidobenzothiazole, we assume that the synthesis of new substituted polyheterocyclic scaffolds containing spirooxindole, pyrano[2,3-*d*]pyrimidine, and pyrimidobenzothiazole may result in the finding of novel drug candidates. Hence, this paper describes an easy, highly efficient, environment-friendly, catalyst-free, one-pot procedure for the synthesis of novel spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3]benzothiazole] derivatives via the reaction of isatin **1**, malononitrile **2**, and 2-hydroxy-4*H*-pyrimido[2,1-*b*][1,3]benzothiazol-4-one **3**[46,47] as heterocyclic CH-acid systems.

A review of the literature showed that in the past few years, several pathways have been developed for the synthesis of spirooxindole from isatin, malononitrile, and various types of activated methylene group in one-pot multicomponent synthesis.[48,49] To the best of our knowledge, there is no report using 2-hydroxy-4*H*-pyrimido[2,1-*b*][1,3]benzothiazol-4-one **3** for the synthesis of spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3]benzothiazole] heterocyclic hybrids.

Results and discussion

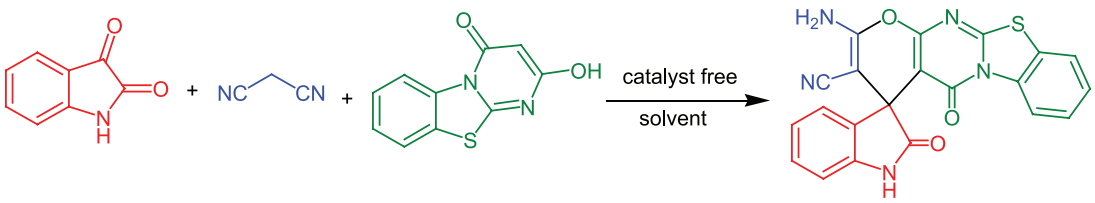
We first investigated the optimum conditions for the three-component reaction of isatin **1**, malononitrile **2**, and 2-hydroxy-4*H*-pyrimido[2,1-*b*][1,3]benzothiazol-4-one **3** as model reactions (Table 1). In the first step, the model reaction was performed under solvent-free conditions at room temperature (Table 1, entry 1). However, no conversion was detected after a long reaction time. We then conducted the model reaction at 60°C and 90°C under similar conditions and extracted the expected product at low yield (Table 1, entries 2–3).

In order to investigate the effects of solvent, we used a number of polar and non-polar reaction media to perform the test reactions. No conversion was detected in non-polar solvents, for example, toluene and xylene, even after long reaction times (Table 1, entries 4–5). On the other hand, moderate to good yields were obtained (60%–85%) in polar solvents like DMF, DMSO, THF, acetonitrile, and ethanol (Table 1, entries 6–10). Due to its low cost, availability, and environment-friendliness, H_2O was used as the reaction medium. However, no further improvement was observed in the yield due to the solubility problem of reactants (Table 1, entry 11). Subsequently, we decided to perform the one-pot reaction in a sequential manner using water-ethanol. As discussed earlier, ethanol–water (1:1) mixture was the best among other solvents in this study (Table 1, entry 12). Finally, examining the ethanol–water ratio failed to further improve the yield (Table 1, entries 13–16). We also used an acid (*p*-toluene sulfonic acid) and a base (diisopropylethylamine) as catalysts in this reaction, but the catalyst-free approach in H_2O/EtOH gave the best result.

Subsequently, a variety of isatins were reacted with malononitrile and 2-hydroxy-4*H*-pyrimido[2,1-*b*][1,3] benzothiazol-4-one. All reactions completed within 2 hours and resulted the formation of expected novel spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-b][1,3] benzothiazole] derivatives (4a -m, Table 2) in high to excellent yields (85% -95%).

The structures of compounds **4** were fully characterized by ^{1}H NMR, ^{13}C NMR, IR spectroscopy, and mass spectrometry (See Supporting information). For example, the ^{1}H NMR spectrum of **4b** contained a singlet for N-*Me* (δ 3.25), a triplet for CH_{Ar} (δ 7.01), a doublet for CH_{Ar} (δ 7.10), a doublet for CH_{Ar} (δ 7.18), a triplet for CH_{Ar} (δ 7.32), a multiplet for CH_{Ar} (δ 7.47–7.56), a broad singlet for NH_2 (δ 7.47–7.56), a doublet for CH_{Ar} (δ 8.02), and a doublet for CH_{Ar} (δ 8.60). The assignment was supported by IR absorptions at 3460, and 3333 cm^{-1} (NH_2), 2198 cm^{-1} (CN), 1716, 1649 (2 C=O). The 22

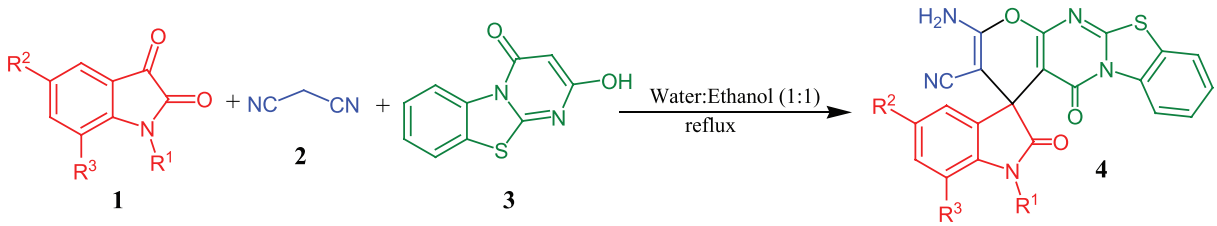
Table 1. Optimization of reaction conditions.



Entry	Solvent (°C)	Temp (°C)	Time (h)	Yield (%)
1	Solvent free	RT	48	—
2	Solvent free	60	48	25
3	Solvent free	90	48	30
4	Toluene	reflux	48	—
5	Xylene	100	48	—
6	DMF	100	6	65
7	DMSO	100	6	60
8	THF	reflux	8	70
9	CH_3CN	reflux	5	85
10	EtOH	reflux	5	85
11	Water	reflux	48	45
12	Water:ethanol (1:1)	reflux	2	95
13	Water:ethanol (1:2)	reflux	2	90
14	Water:ethanol (1:3)	reflux	2	90
15	Water:ethanol (2:1)	reflux	2	70
16	Water:ethanol (3:1)	reflux	2	65

RT: room temperature.

Table 2. Reaction of isatin **1**, malononitrile **2**, and 2-hydroxy-4*H*-pyrimido[2,1-*b*][1,3]benzothiazol-4-one **3**.



Product	R^1	R^2	R^3	Yield (%)[a]
4a	H	H	H	93
4b	CH_3	H	H	95
4c	CH_3CH_2	H	H	91
4d	$C_6H_5CH_2$	H	H	93
4e	4-$ClC_6H_4CH_2$	H	H	94
4f	H	Br	H	90
4g	H	OCH_3	H	85
4h	H	Cl	H	91
4i	CH_3	Cl	H	94
4j	CH_3CH_2	Cl	H	92
4k	H	CH_3	H	90
4l	CH_3	CH_3	H	92
4m	H	H	CH_3	88

[a]Isolated yield.

distinct resonances indicated by the 1H decoupled ^{13}C NMR spectrum of **4b** confirmed the proposed structure. A characteristic ^{13}C NMR signal due to the cyano carbons was observed at $\delta = 117.65$ ppm, and the carbonyl carbons appeared at $\delta = 158.91$ and 176.47 ppm. The mass spectrometry of all products showed molecular ion peaks at relevant *m*/*z* values.

The structure of compound **4a** was further confirmed by single-crystal X-ray analysis which supported our speculations regarding the structures of the products (Figure 2).

(Selected X-ray crystallographic data for compound **4a**: (CCDC 1527746): $C_{27}H_{25}N_7O_5S$: MW = 559.6, Triclinic, space group P–1, cell dimensions a = 11.076 (2) Å, b = 11.486 (2) Å, c = 12.435 (3) Å, α = 66.76 (3)°, β = 75.03 (3)°, γ = 63.07 (3)°, V = 1289.9 (6) Å^3, Z = 2, Dx = 1.441 Mg m^{-3}, F000 = 584, crystal size 0.240 × 0.120 × 0.120 mm. Crystallographic data were collected on a Bruker APEX area-detector diffractometer, with graphite monochromated Mo Kα radiation (λ = 0.71073 Å). A total of 4522 reflections ($2.6 \leqslant \theta \leqslant 25.0$) were collected at a temperature of 298 K in a series of ω scans in 1° oscillations, and integrated using the Stoe X-AREA software package (3438) reflections were unique with I> 2σ(I). The structure was solved by direct method and subsequent different Fourier map and then refined on F2 by a full-matrix least-square procedure using anisotropic displacement parameters. All non-H atoms were refined anisotropically, and H atoms were placed in the ideal positions. Final residuals were R = 0.0664 and Rw = 0.1717 for 3906 parameters.) A crystal suitable for X-ray diffraction analysis was obtained by evaporation from a mixed solvent of methanol and DMF (v/v = 4:1) at room temperature.

A plausible mechanism is presented in Scheme 1. As seen, during a typical cascade reaction, the first step was a fast Knoevenagel condensation in which isatin **1** was condensed with malononitrile **2** to afford isatylidene malononitrile derivative **5**. In the next step, **5** was attacked through Michael addition of 2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*] pyrimidine-5,7(6*H*)-dione **3** affording intermediate **6** which underwent 6-exo-dig-cyclization to the desired product **4** (Scheme 1).

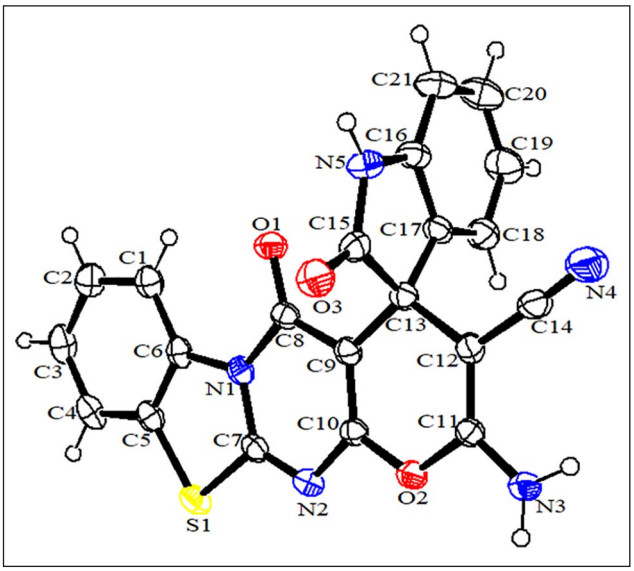


Figure 2. ORTEP diagram of compound **4a.**

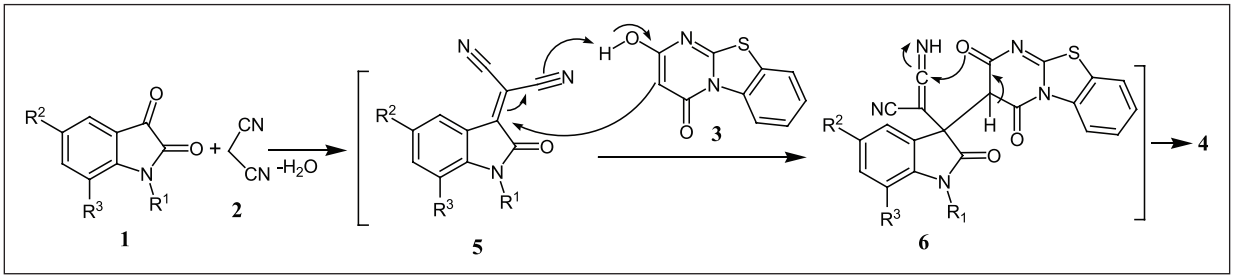
Overall, this paper described a modified environment-friendly one-pot catalyst-free protocol for the synthesis of novel spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3]benzothiazole] derivatives from a three-component reaction between isatin, malononitrile, and 2-hydroxy-4*H*-pyrimido[2,1-*b*][1,3]benzothiazol-4-one. We put forward a method in which the target products could be isolated by a simple filtration method due to a difference in the solubility of product and reactant materials. The obtained products were crystallized from MeOH/DMF (4:1) to afford the pure products. This prevented multiple extraction steps and separation by chromatography. Considering the availability of the starting materials, the green and one-pot procedure, easy workup, and high to excellent yields of the products, the products synthesized in the present study may find useful applications in synthetic organic and medicinal chemistry.

Melting points were recorded on an Electrothermal-type 9100 melting point apparatus and are uncorrected. The IR spectra were obtained on an Avatar 370 FT-IR Thermo-Nicolet spectrometer. ^{1}H and ^{13}C NMR spectra were run on BRUKER DRX-300 AVANCE spectrometer at 300 for ^{1}H NMR, and 75 MHz for ^{13}C NMR DMSO-d_6 was used as solvent. The mass spectra were scanned on a Varian Mat CH-7 at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyser. X-ray crystal structure data were collected on a Bruker D8 VENTURE PHOTON 100 CMOS diffractometer with graphite monochromated Cu Kα radiation at 296(2) K.

General procedure for the synthesis of 4

A mixture of isatin **1** (1 mmol), malononitrile **2** (1.2 mmol), and 2-hydroxy-4*H*-pyrimido[2,1-b][1,3]benzothiazol-4-one **3** (1 mmol) in water-ethanol (1:1, 5 mL) was refluxed for 2 h in an oil bath. After the completion of the reaction (monitored by TLC (thin layer chromatography)), the mixture was cooled to room temperature, and the precipitate was filtered off and washed with cold ethanol and then crystallized from MeOH/DMF (4:1) to afford pure product **4** (yield 85%–95%) as white solid.

*2′-Amino-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3]*



Scheme 1. A plausible mechanism.

benzothiazole]-3′-carbonitrile (**4a**): White solid, 384 mg (93%), m.p.>300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.50; IR (KBr, cm^{-1}): υ = 3436, 3336 (NH_2), 2197 (CN), 1713, 1661 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.90 (br s, 2H, H_{Ar}), 7.11–7.21 (m, 2H, H_{Ar}), 7.48 (br s, 1H, H_{Ar}), 7.48 (br s, 2H, NH_2), 8.04–8.05 (m, 1H, H_{Ar}), 8.61–8.63 (m, 1H, H_{Ar}). ^{13}C NMR (75 MHz, $CDCl_3$): δ 48.5 (Cspiro), 57.3 (=*C*–CN), 95.5 (*C*–C=O), 109.8 (CN), 117.7 (C_{Ar}–S), 118.8, 122.3, 123.6, 124.3, 124.5, 127.5, 127.7, 129.0 (8CH_{Ar}), 133.8 (C_{Ar}), 135.6 (C_{Ar}–N), 142.5 (C_{Ar}–N), 142.6 (C=O), 158.9 (*C*–NH_2), 159.8 (N–*C*–O), 162.5 (S–*C*=N), 177.9 (C=O_{isatin}). MS: (*m*/*z*, %), 413 (M^+, 48), 412 (60), 383 (72), 355 (48), 216 (29), 207 (74), 193 (51), 176 (70), 149 (49), 69 (32), 28 (75). Anal. Calcd for $C_{21}H_{11}N_5O_3S$: C, 61.01; H, 2.68; N, 16.94; S, 7.76; found: C, 60.88; H, 3.01; N, 16.44; S, 7.45.

*2′-Amino-1-methyl-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3] benzothiazole]-3′-carbonitrile* (**4b**): White solid, 405 mg (95%), m.p. >300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.55, IR (KBr, cm^{-1}): υ = 3460, 3333 (NH_2), 2198 (CN), 1716, 1649 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.25 (s, 3H, CH_3), 7.01 (t, 1H, *J* = 7.5 Hz, H_{Ar}), 7.10 (d, 1H, *J* = 7.8 Hz, H_{Ar}), 7.18 (d, 1H, *J* = 6.3 Hz, H_{Ar}), 7.32 (t, 1H, *J* = 7.8 Hz, H_{Ar}), 7.47–7.56 (m, 2H, H_{Ar}), 7.47–7.56 (m, 2H, NH_2), 8.02 (d, 1H, *J* = 7.8 Hz, H_{Ar}), 8.60 (d, 1H, *J* = 7.3 Hz, H_{Ar}). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.0 (CH_3), 48.1 (Cspiro), 56.9 (=*C*–CN), 95.4 (*C*–C=O), 108.7 (CN), 117.6 (C_{Ar}–S), 118.8, 123.0, 123.6, 124.1, 124.5, 127.6, 127.7, 129.2 (8CH_{Ar}), 133.0 (C_{Ar}), 135.6 (C_{Ar}–N), 144.1 (C_{Ar}–N), 158.9 (C=O), 158.9 (*C*–NH_2), 159.9 (N–*C*–O), 162.6 (S–*C*=N), 176.4 (C=O_{isatin}). MS: (*m*/*z*, %), 427 (M^+, 98), 396 (96), 370 (80), 221 (100), 207 (92), 176 (96), 153 (76), 139 (30), 69 (68), 29 (95). Anal. Calcd for $C_{22}H_{13}N_5O_3S$: C, 61.82; H, 3.07; N, 16.38; S, 7.50; found: C, 61.21; H, 2.76; N, 16.50; S, 7.28.

*2′-Amino-1-ethyl-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3] benzothiazole]-3′-carbonitrile* (**4c**): White solid, 401 mg (91%), m.p. >300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.58, IR (KBr, cm^{-1}): υ = 3460, 3334 (NH_2), 2196 (CN), 1713, 1670 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.25 (t, 3H, *J* = 6.9 Hz, CH_3), 3.73–3.90 (m, 2H, CH_2), 6.99 (t, 1H, *J* = 7.5 Hz, H_{Ar}), 7.12 (d, 1H, *J* = 7.8 Hz, H_{Ar}), 7.18 (d, 1H, *J* = 6.3 Hz, H_{Ar}), 7.30 (t, 1H, *J* = 6.3 Hz, H_{Ar}), 7.49–7.57 (m, 2H, H_{Ar}), 7.49–7.57 (m, 2H, NH_2), 8.07 (d, 1H, *J* = 7.8 Hz, H_{Ar}), 8.61 (d, 1H, *J* = 7.8 Hz, H_{Ar}). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.8 (CH_3), 35.0 (CH_2), 47.9 (Cspiro), 57.1 (=*C*–CN), 95.3 (*C*–C=O), 108.7 (CN), 117.5 (C_{Ar}–S), 118.8, 123.0, 123.7, 124.2, 124.5, 127.5, 127.7, 129.2 (8CH_{Ar}), 133.0 (C_{Ar}), 135.6 (C_{Ar}–N), 143.0 (C_{Ar}–N), 158.9 (C=O), 158.9 (*C*–NH_2), 159.9 (N–*C*–O), 162.6 (S–*C*=N), 175.9 (C=O_{isatin}). MS: (*m*/*z*, %), 441 (M^+, 98), 411 (100), 396 (24), 382 (92), 366 (18), 234 (63), 206 (90), 176 (92), 152 (66), 125 (39), 69 (35), 43 (20), 28 (82). Anal. Calcd for $C_{23}H_{13}N_5O_3S$: C, 61.82; H, 3.07; N, 16.38; S, 7.50; found: C, 62.02; H, 3.07; N, 16.41; S, 7.39.

*2′-Amino-1-benzyl-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3] benzothiazole]-3′-carbonitrile* (**4d**): White solid, 468 mg (93%), m.p. >300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.61, IR (KBr, cm^{-1}): υ = 3375, 3319 (NH_2), 2204 (CN), 1726, 1656 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.97 (d, 1H, ^2J = 16.2 Hz, Ph-C$H_A$$H_B$), 5.09 (d, 1H, ^2J = 16.0 Hz, Ph-$CH_A$$H_B$), 6.83 (d, 1H, *J* = 7.8 Hz, H_{Ar}), 7.00 (t, 1H, *J* = 7.5 Hz, H_{Ar}), 7.18–7.24 (m, 2H, H_{Ar}), 7.29–7.39 (m, 3H, H_{Ar}), 7.51–7.58 (m, 4H, H_{Ar}), 7.51–7.58 (m, 2H, NH_2), 8.04–8.07 (m, 1H, H_{Ar}), 8.61–8.64 (m, 1H, H_{Ar}). ^{13}C NMR (75 MHz, $CDCl_3$): δ 43.9 (CH_2), 48.3 (C_{spiro}), 56.9 (=*C*–CN), 95.3 (*C*–C=O), 109.3 (CN), 117.8 (C_{Ar}–S), 118.7, 123.1, 123.7, 124.3, 124.5 (5CH_{Ar}), 127.6 (2CH_{Ar}), 127.7 (2CH_{Ar}), 128.8, 129.1 (2CH_{Ar}), 133.1, 135.6 (2C_{Ar}), 136.5 (C_{Ar}–N), 143.3 (C_{Ar}–N), 158.9 (C=O), 159.0 (*C*–NH_2), 160.0 (N–*C*–O), 162.7 (S–*C*=N), 176.7 (C=O_{isatin}). MS: (*m*/*z*, %), 504 (M^+, 66), 474,(21), 410 (71), 337 (8), 282 (88), 254 (41), 216 (67), 176 (67), 149 (66), 91 (100), 65 (72), 28 (68). Anal. Calcd for $C_{28}H_{17}N_5O_3S$: C, 66.79; H, 3.40; N, 13.91; S, 6.37; found: C, 66.94; H, 3.13; N, 13.75; S, 6.21.

*2′-Amino-1-(4-chlorobenzyl)-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3]benzothiazole]-3′-carbonitrile* (**4e**): White solid, 505 mg (94%), m.p. >300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.45, IR (KBr, cm^{-1}): υ = 3444, 3284 (NH_2), 2193 (CN), 1701, 1682 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.02 (br s, 2H), 6.86 (d, 1H, *J* = 7.8 Hz, H_{Ar}), 7.00 (t, 1H, *J* = 7.5 Hz, H_{Ar}), 7.21 (t, 2H, *J* = 7.2 Hz, H_{Ar}), 7.42 (t, 2H, *J* = 7.9 Hz, H_{Ar}), 7.52–7.60 (m, 4H, H_{Ar}), 7.52–7.60 (m, 2H, NH_2), 8.05–8.08 (m, 1H, H_{Ar}), 8.61–8.64 (m, 1H, H_{Ar}). ^{13}C NMR (75 MHz, $CDCl_3$): δ 43.2 (CH_2), 48.2 (C_{spiro}), 56.8 (=*C*–CN), 95.2 (*C*–C=O), 109.3 (CN), 117.8 (C_{Ar}–S), 118.8, 123.3, 123.7, 124.3, 124.3 (5CH_{Ar}), 127.7, 128.7 (2CH_{Ar}), 129.1 (2CH_{Ar}), 129.7 (2CH_{Ar}), 133.1 (C–Cl), 135.6 (C_{Ar}), 136.5 (C_{Ar}–N), 143.0 (C_{Ar}–N), 158.9 (C=O), 159.0 (*C*–NH_2), 160.0 (N–*C*–O), 162.8 (S–*C*=N), 176.7 (C=O_{isatin}). MS: (*m*/*z*, %), 538 (M^+, 47), 509,(25), 410 (75), 316 (82), 288 (59), 216 (73), 176 (72), 149 (72), 124 (100), 89 (73), 69 (72), 63 (47), 39 (33), 29 (73). Anal. Calcd for $C_{28}H_{16}ClN_5O_3S$: C, 62.51; H, 3.00; N, 13.02; S, 5.96; found: C, 62.88; H, 2.84; N, 12.73; S, 5.89.

*2′-Amino-5-bromo-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3] benzothiazole]-3′-carbonitrile* (**4f**): White solid, 442 mg (90%), m.p. >300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.55, IR (KBr, cm^{-1}): υ = 3448, 3327 (NH_2), 2198 (CN), 1725, 1674 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.86 (d, 1H, *J* = 8.1 Hz, H_{Ar}), 7.36–7.40 (m, 2H, H_{Ar}), 7.50–7.59 (m, 2H, H_{Ar}), 7.50–7.59 (m, 2H, NH_2), 8.08 (d, 1H, *J* = 7.8 Hz, H_{Ar}), 8.66 (d, 1H, *J* = 7.8 Hz, H_{Ar}), 10.79 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 48.7 (Cspiro), 56.5 (=*C*–CN), 94.9 (*C*–C=O), 111.7 (CN), 114.0 (C_{Ar}–Br), 117.7 (C_{Ar}–S), 118.8, 123.7, 124.5, 127.3, 127.6, 127.7, 131.7 (7CH_{Ar}), 135.6 (C_{Ar}), 136.2 (C_{Ar}–N), 142.0 (C_{Ar}–N), 159.0 (C=O), 159.0 (*C*–NH_2), 160.0 (N–*C*–O), 162.7 (S–*C*=N), 177.7 (C=O_{isatin}). MS: (*m*/*z*, %), 492 (M^+, 8), 463,(20), 436 (9), 284 (23), 269 (62), 245 (28), 216 (38), 175 (50), 148 (44), 138 (29), 69 (50), 44 (42), 28 (100). Anal. Calcd for $C_{21}H_{10}BrN_5O_4S$: C, 51.23; H, 2.05; N, 14.23; S, 6.51; found: C, 51.46; H, 1.85; N, 13.90; S, 6.36.

*2′-Amino-5-methoxy-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3] benzothiazole]-3′-carbonitrile* (**4g**): White solid, 376 mg (85%), m.p. >300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.52, IR (KBr, cm^{-1}): υ = 3437, 3366 (NH_2), 2194 (CN), 1719, 1676 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.65 (s, 3H, OCH_3), 6.78 (br s, 3H, H_{Ar}), 7.46–7.56 (m, 2H, H_{Ar}), 7.46–7.56 (m, 2H, NH_2), 8.08 (d, 1H, *J* = 7.5 Hz, H_{Ar}), 8.66 (d, 1H, *J* = 8.1 Hz, H_{Ar}), 10.46 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.0 (Cspiro), 55.8 (OCH_3), 57.4 (=*C*–CN), 95.5 (*C*–C=O), 110.1 (CN), 111.2 (C_{Ar}–S), 113.7 (C_{Ar}–OCH_3), 117.8, 118.8, 123.7, 124.5, 127.5, 127.7, 135.1 (7CH_{Ar}), 135.6 (C_{Ar}), 135.9 (C_{Ar}–N), 155.5 (C_{Ar}–N), 158.9 (C=O), 158.9 (*C*–NH_2), 159.8 (N–*C*–O), 162.5 (S–*C*=N), 177.8 (C=O_{isatin}). MS: (*m*/*z*, %), 443 (M^+, 67), 415 (54), 398 (65), 386 (31), 236 (65), 208 (89), 176 (78), 149 (83), 126 (67), 69 (67), 44 (68), 28 (69). Anal. Calcd for $C_{22}H_{13}N_5O_4S$: C, 59.59; H, 2.95; N, 15.79; S, 7.23; found: C, 59.43; H, 2.68; N, 15.25; S, 7.60.

*2′-Amino-5-chloro-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3] benzothiazole]-3′-carbonitrile* (**4h**): White solid, 407 mg (91%), m.p. >300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.58, IR (KBr, cm^{-1}): υ = 3452, 3327 (NH_2), 2200 (CN), 1724, 1676 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.89 (d, 1H, *J* = 6.9 Hz, H_{Ar}), 7.24–7.27 (m, 2H, H_{Ar}), 7.48–7.56 (m, 2H, H_{Ar}), 7.48–7.56 (m, 2H, NH_2), 8.08 (d, 1H, *J* = 7.5 Hz, H_{Ar}), 8.66 (d, 1H, *J* = 7.8 Hz, H_{Ar}), 10.78 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 48.8 (Cspiro), 56.5 (=*C*–CN), 94.9 (*C*–C=O), 111.1 (CN), 117.6 (C_{Ar}–S), 118.8, 123.7, 124.5, 124.6, 126.3, 127.6, 127.7 (7CH_{Ar}), 128.9 (C–Cl), 135.6 (C_{Ar}), 135.8 (C_{Ar}–N), 141.6 (C_{Ar}–N), 159.0 (C=O), 160.0 (*C*–NH_2), 160.0 (N–*C*–O), 162.7 (S–*C*=N), 177.8 (C=O_{isatin}). MS: (*m*/*z*, %), 448 (M^+, 30), 419 (65), 393 (26), 242 (99), 177 (55), 150 (28), 29 (100). Anal. Calcd for $C_{21}H_{10}ClN_5O_3S$: C, 56.32; H, 2.25; N, 15.64; S, 7.16; found: C, 56.81; H, 2.33; N, 15.55; S, 7.53.

*2′-Amino-5-chloro-1-methyl-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3]benzothiazole]-3′-carbonitrile* (**4i**): White solid, 434 mg (94%), m.p. >300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.54, IR (KBr, cm^{-1}): υ = 3456, 3322 (NH_2), 2199 (CN), 1708, 1670 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.24 (s, 3H, CH_3), 7.13 (d, 1H, *J* = 8.4 Hz, H_{Ar}), 7.32–7.40 (m, 2H, H_{Ar}), 7.47–7.61 (m, 2H, H_{Ar}), 7.47–7.61 (m, 2H, NH_2), 8.08 (d, 1H, *J* = 7.5 Hz, H_{Ar}), 8.63 (d, 1H, *J* = 8.0 Hz, H_{Ar}). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.1 (CH_3), 48.3 (Cspiro), 56.1 (=*C*–CN), 94.8 (*C*–C=O), 110.2 (CN), 117.5 (C_{Ar}–S), 118.8, 123.7, 124.4, 124.5, 127.1, 127.7, 129.0 (7CH_{Ar}), 135.0 (C–Cl), 135.6 (C_{Ar}), 143.0 (C_{Ar}–N), 159.0 (C_{Ar}–N), 159.0 (C=O), 160.1 (*C*–NH_2), 160.0 (N–*C*–O), 162.8 (S–*C*=N), 176.2 (C=O_{isatin}). MS: (*m*/*z*, %), 462 (M^+, 4), 431,(3), 406 (3), 254 (27), 241 (98), 216 (63), 186 (34), 176 (65), 149 (60), 122 (20), 95 (19), 69 (60), 44 (34), 29 (100). Anal. Calcd for $C_{22}H_{12}ClN_5O_3S$: C, 57.21; H, 2.62; N, 15.16; S, 6.94; found: C, 57.39; H, 2.48; N, 15.09; S, 6.61.

*2′-Amino-5-chloro-1-ethyl-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3] benzothiazole]-3′-carbonitrile* (**4j**): White solid, 437 mg (92%), m.p. >300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.63, IR (KBr, cm^{-1}): υ = 3458, 3331 (NH_2), 2196 (CN), 1715, 1670 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.23 (t, 3H, *J* = 6.9 Hz, CH_3), 3.40–3.90 (m, 2H, CH_2), 7.16 (d, 1H, *J* = 8.1 Hz, H_{Ar}), 7.32–7.37 (m, 2H, H_{Ar}), 7.47–7.59 (m, 2H, H_{Ar}), 7.47–7.59 (m, 2H, NH_2), 8.06 (d, 1H, *J* = 7.5 Hz, H_{Ar}), 8.61 (d, 1H, *J* = 8.1 Hz, H_{Ar}). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.7 (CH_3), 35.2 (CH_2), 48.2 (Cspiro), 56.3 (=*C*–CN), 94.8 (*C*–C=O), 110.2 (CN), 117.4 (C_{Ar}–S), 118.8, 123.7, 124.5, 124.6, 126.9, 127.6, 127.7 (7CH_{Ar}), 135.2 (C–Cl), 135.6 (C_{Ar}), 142.0 (C_{Ar}–N), 159.0 (C_{Ar}–N), 159.0 (C=O), 160.0 (*C*–NH_2), 160.0 (N–*C*–O), 162.8 (S–*C*=N), 175.8 (C=O_{isatin}). MS: (*m*/*z*, %), 476 (M^+, 62), 447,(50), 419 (41), 254 (20), 240 (25), 216 (24), 176 (62), 149 (17), 69 (10), 28 (100). Anal. Calcd for $C_{23}H_{14}ClN_5O_3S$: C, 58.05; H, 2.97; N, 14.72; S, 6.74; found: C, 58.13; H, 2.70; N, 14.87; S, 6.22.

*2′-Amino-5-methyl-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3] benzothiazole]-3′-carbonitrile* (**4k**): White solid, 384 mg (90%), m.p. >300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.55, IR (KBr, cm^{-1}): υ = 3444, 3324 (NH_2), 2198 (CN), 1721, 1675 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.19 (s, 3H, CH_3), 6.76 (d, 1H, *J* = 7.8 Hz, H_{Ar}), 6.93 (s, 1H, H_{Ar}) 7.01 (d, 1H, *J* = 8.4 Hz, H_{Ar}), 7.45–7.57 (m, 2H, H_{Ar}), 7.45–7.57 (m, 2H, NH_2), 8.08 (d, 1H, *J* = 7.5 Hz, H_{Ar}), 8.66 (d, 1H, *J* = 8.1 Hz, H_{Ar}), 10.53 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.0 (CH_3), 48.5 (Cspiro), 57.5 (=*C*–CN), 95.6 (*C*–C=O), 109.5 (CN), 117.8 (C_{Ar}–S), 118.8, 123.7, 124.5, 124.9, 127.5, 127.7, 129.2 (7CH_{Ar}), 131.1, 133.9 (2C_{Ar}), 135.6 (C_{Ar}–N), 140.2 (C_{Ar}–N), 158.9 (C=O), 159.7 (*C*–NH_2), 159.8 (N–*C*–O), 162.5 (S–*C*=N), 177.9 (C=O_{isatin}). MS: (*m*/*z*, %), 427 (M^+, 30), 398 (53), 371 (15), 221 (64), 207 (11), 176 (22), 149 (12), 29 (100). Anal. Calcd for $C_{22}H_{13}N_5O_3S$: C, 61.82; H, 3.07; N, 16.38; S, 7.50; found: C, 61.42; H, 3.04; N, 16.08; S, 7.30.

*2′-Amino-1,5-dimethyl-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3] benzothiazole]-3′-carbonitrile* (**4l**): White solid, 405 mg (92%), m.p. >300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.57, IR (KBr, cm^{-1}): υ = 3444, 3326 (NH_2), 2196 (CN), 1712, 1672 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.25 (s, 3H, CH_3), 3.35 (s, 3H, CH_3), 7.13 (d, 1H, *J* = 8.4 Hz, H_{Ar}), 7.32–7.39 (m, 2H, H_{Ar}), 7.47–7.60 (m, 2H, H_{Ar}), 7.47–7.60 (m, 2H, NH_2), 8.08 (d, 1H, *J* = 7.5 Hz, H_{Ar}), 8.62 (d, 1H, *J* = 7.5 Hz, H_{Ar}). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.1 (2CH_3), 48.3 (Cspiro), 56.1 (=*C*–CN), 94.8 (*C*–C=O), 110.2 (CN), 117.5 (C_{Ar}–S), 118.8, 123.7, 124.4, 124.5, 127.1, 127.6, 127.7 (7CH_{Ar}), 129.0, 135.0 (2C_{Ar}), 135.6 (C_{Ar}–N), 143.0 (C_{Ar}–N), 158.9 (C=O), 159.0 (*C*–NH_2), 160.1 (N–*C*–O), 162.8 (S–*C*=N), 176.2 (C=O_{isatin}). MS: (*m*/*z*, %), 441 (M^+, 45), 396 (14), 382 (55), 366 (28), 234 (63), 206 (72), 176 (92), 152 (66), 125 (39), 69 (35), 43 (20), 28 (82). Anal. Calcd for $C_{23}H_{15}N_5O_3S$: C, 62.58; H, 3.42; N, 15.86; S, 7.26; found: C, 62.17; H, 3.73; N, 15.99; S, 7.86.

*2′-Amino-7-methyl-2,5′-dioxo-1,2-dihydro-5′H-spiro[indole-3,4′-pyrano[2′,3′:4,5]pyrimido[2,1-*b*][1,3] benzothiazole]-3′-carbonitrile* (**4m**): White solid, 375 mg (88%), m.p. >300 °C, R_f (1:5 *n*-hexane/EtOAc) 0.52, IR

(KBr, cm^{-1}): υ = 3436, 3334 (NH_2), 2200 (CN), 1705, 1660 (2 C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.29 (s, 3H, CH_3), 6.83 (d, 1H, J = 7.5 Hz, H_{Ar}), 6.92 (d, 1H, J = 7.2 Hz, H_{Ar}), 7.03 (d, 1H, J = 7.5 Hz, H_{Ar}), 7.45–7.54 (m, 2H, H_{Ar}), 7.45–7.54 (m, 2H, NH_2), 8.06 (d, 1H, J = 7.8 Hz, H_{Ar}), 8.65 (d, 1H, J = 8.1 Hz, H_{Ar}), 10.69 (s, 1H, NH).). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.9 (CH_3), 48.7 (Cspiro), 57.5 (=*C*–CN), 95.7 (*C*–C=O), 99.9 (CN), 117.8 (C_{Ar}–S), 118.8, 121.7, 122.2, 123.7, 124.5, 127.5, 127.7 (7CH_{Ar}), 130.4, 133.6 (2C_{Ar}), 135.6 (C_{Ar}–N), 141.2 (C_{Ar}–N), 158.9 (C=O), 159.7 (*C*–NH_2), 159.8 (N–*C*–O), 162.5 (S–*C*=N), 178.4 (C=O_{isatin}). MS: (*m*/*z*, %), 427 (M^+, 23), 426, (49), 396 (67), 382 (20), 369 (23), 220 (100), 207 (30), 176 (44), 149 (30), 69 (9), 28 (82). Anal. Calcd for $C_{22}H_{13}N_5O_3S$: C, 61.82; H, 3.07; N, 16.38; S, 7.50; found: C, 61.51; H, 3.37; N, 16.66; S, 7.44.

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Supplemental Material

Supplemental material for this article is available online. Experimental procedures and characterization of synthesized products are accessible in supplemental information. Copies of the NMR, IR, and mass spectra are available.

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