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Synthesis of novel spiro[chromeno[2,3-*d*][1,3,4]thiadiazolo[3,2-*a*] pyrimidines via an isatin-based three-component reaction

Maryam Khalili & Abbas Ali Esmaeili

This study presents an efficient and sustainable method for the synthesis of novel spiro[chromeno[2,3-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidine-10,3′-indoline]-2′,9,11-trione derivatives via a three-component condensation reaction is introduced. The reaction involves dimedone, 5*H*-[1,3,4]thiadiazolo[3,2-*a*] pyrimidine-5,7(6 H)-dione, and isatin derivatives, catalyzed by the economical and highly effective Keggin heteropolyacid ($H_3PW_{12}O_{40}$). This protocol achieves (70–90%) yields within relatively short reaction times under mild and solvent-free conditions. Using a green, cost-effective catalyst highlights the method's practicality and potential for broader applications in synthetic organic chemistry, offering an attractive route for spiro oxindole derivative synthesis.

Keywords Isatin derivatives, [1,3,4]Thiadiazolo[3,2-*a*]pyrimidine, Dimedone, Spiro-oxindole, Kegging hetero-polyacid

Multicomponent reactions (MCRs) have become a focal point in modern organic chemistry due to their exceptional advantages, including operational simplicity, mild reaction conditions, high efficiency, short reaction times, atom economy, and environmental friendliness. By significantly reducing chemical usage and synthetic steps, MCRs have revolutionized synthetic methodologies and played a pivotal role in the development of polycyclic and heterocyclic systems, a rapidly growing area of research[1–3]. Nitrogen heterocycles, a crucial class of cyclic organic compounds containing at least one nitrogen atom in their structure, are renowned for their extensive biological activities. These compounds are fundamental to natural products, fungicides, agrochemicals, advanced materials, and various biologically active substances[4].

Among them, spirooxindole derivatives a unique subgroup of nitrogen heterocycles have gained notable attention due to their presence in natural alkaloids and synthetically prepared molecules with significant biological properties[5]. These derivatives exhibit diverse biological activities, including antimicrobial[6], anticancer[7,8], antiviral[9,10], and antimalarial effects[11,12]. For example, two noteworthy spirooxindoles, spindomycins A and B, were isolated from the rhizosphere strain *Streptomyces sp.* XZQH-9 was identified as a promising tyrosine kinase BCR-ABL inhibitor (Fig. 1. A and B)[13]. Among naturally occurring spirooxindoles, NITD609 is a potent antibacterial agent (Fig. 1. C)[14]. Moreover, compound D exhibited significant IC50 values against human prostate cancer cell lines PC-3 and LNCaP[15], while compound E showed exceptional bioactivity against the human breast cancer cell line MBDMB-435 (Fig. 1. D and E)[16]. These examples underscore the therapeutic potential of spirooxindole derivatives as versatile agents in disease treatment.

Pyrimidines, play acrucial role in various fields, particularly in pharmaceuticals, due to their anticancer, antioxidant, and antiviral properties[17–21]. Of particular interest are 1,3,4-thiadiazolo[3,2-a]pyrimidines and their derivatives, members of the pseudo-purine family, which demonstrate a wide range of biological activities, including antiglycation[22], anticancer[23], antioxidant[24], antimicrobial[25,26], antitumor[27], and analgesic effects[28]. The chemistry of fused heterocycles, especially [1,3,4]thiadiazolo[3,2-*a*]pyrimidines, has emerged as a vital area of research in heterocyclic and medicinal chemistry[29–33]. Building on our ongoing efforts to synthesize novel heterocyclic molecules, we have designed an efficient method to develop a new spiro heterocyclic system that integrates spirooxindole and 1,3,4-thiadiazolo[3,2-*a*]pyrimidines frameworks. This approach aims to provide a

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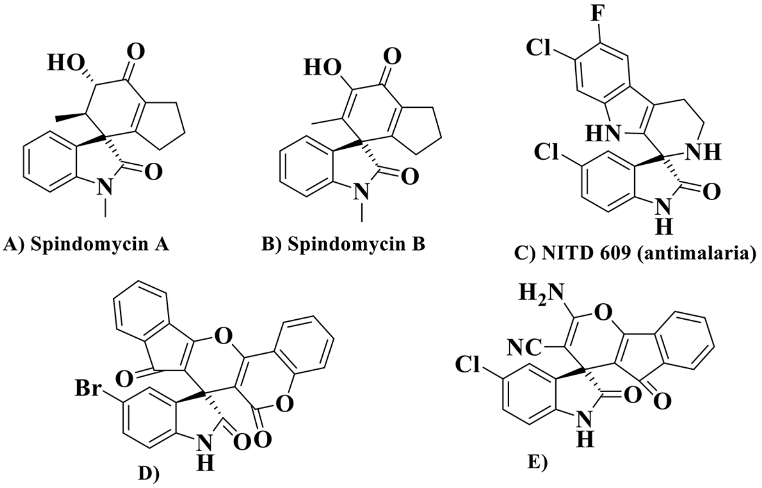
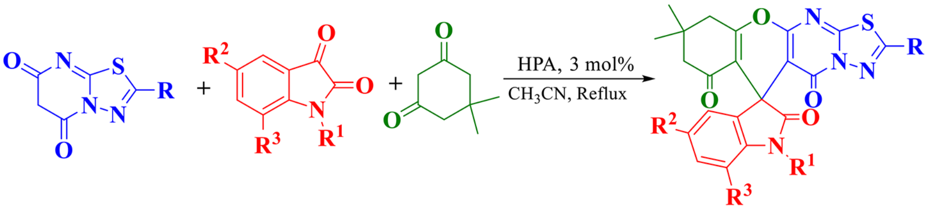


Fig. 1. Representative biologically active 3,3'-spiro oxindole molecules.



Scheme 1. Synthesis of spirooxindole derivatives.

unique system combining the properties of both spirooxindole and thiadiazolo[3,2-*a*]pyrimidine derivatives, potentially offering new opportunities in medicinal chemistry[29,34,35]. We have developed a highly efficient method for synthesizing a novel spiro heterocyclic system that combines spirooxindole and 1,3,4-thiadiazolo[3,2-*a*]pyrimidines moieties. In this work, we report the synthesis of this compound through a one-pot, three-component condensation reaction involving dimedone, 5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-5,7(6*H*)-dione, and isatin derivatives catalyzed by phosphotungstic acid, yielding the desired product in a straightforward approach (Scheme 1). This innovative system holds promise for exhibiting superior biological properties compared to previously reported compounds, positioning it as a valuable candidate for further exploration in medicinal chemistry.

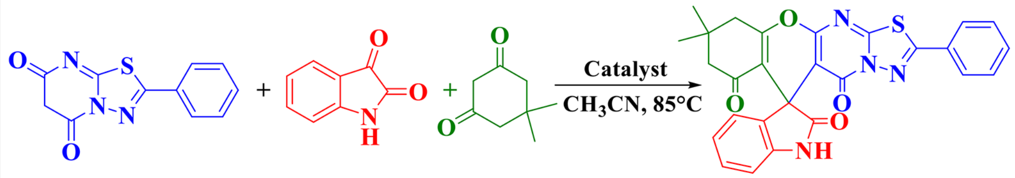
Results and discussion

The reaction between 2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-5,7(6 H)-dione, isatin, and dimedone was selected as a model reaction to determine the optimum reaction conditions. Initially, the reaction was conducted in acetonitrile without a catalyst for 24 h, yielding only a trace amount of the product **4a** (Table 1, entry 1). Subsequently, various acidic and basic catalysts, including *N*,*N*-diisopropylethylamine, DABCO, L-proline, p-toluenesulfonic acid, acetic acid, lactic acid, and phosphotungstic acid, were tested to evaluate their catalytic activities (Table 1, entries 2–8).

The results revealed that acidic catalysts (Table 1, entries 7 and 8) were more effective than basic ones (Table 1, entries 2–4). Notably, Keggin phosphotungstic acid ($H_3PW_{12}O_{40}$) exhibited the highest catalytic efficiency, achieving the best yield of **4a** (Table 1, entry 8). Furthermore, the potential of ionic liquids, including 1-(4-sulfonic acid)butyl-3-methylimidazolium hydrogen sulfate and 1-butyl-3-methylimidazolium bromide, was explored (Table 1, entries 9–10). Despite their recognized strength in promoting organic transformations, their catalytic performance was inferior to that of phosphotungstic acid. Finally, using molecular sieves as a catalyst resulted in only a slight amount of product under these conditions (Table 1, entry 11). These findings underscore the superior catalytic activity of Keggin phosphotungstic acid in this reaction, highlighting its potential for efficient organic synthesis.

The optimization of the model reaction was conducted using various solvents, including EtOH, H_2O, MeOH, a 1:1 mixture of EtOH/H_2O, DMF, EG, PEG, MeCN, and solvent-free conditions. Among these, the highest yield of **4a** was achieved under reflux in acetonitrile (Table 2, entry 9). MeOH (Table 2, entry 4) and EtOH (Table 2, entry 2) also delivered relatively high yields compared to the other solvents.

Further investigation of catalyst loading revealed that 3.0 mol% of the catalyst provided the best results, producing the highest yield of **4a** under optimized conditions (Table 2, entry 9). Reducing the catalyst loading led to a decline in yield (Table 2, entry 10), while increasing the amount beyond 3.0 mol% did not result in any



Entry	Catalyst (mol%)	Time (h)	Yield (%)
1	–	24	Trace
2	DIPEA (10)	24	10
3	DABCO (10)	24	40
4	L-Proline (10)	24	30
5	*P*-toluene sulphonic acid (10)	24	10
6	Acetic Acid (10)	24	20
7	Lactic acid (10)	24	40
8	**$H_3PW_{12}O_{40}$(3)**	**10**	**78**
9	[bmim] Br	24	40
10	[bmim][HSO_4]	24	60
11	Molecular sieve 4° A°	24	30

Table 1. The effects of different catalysts on the model reaction[a]. [a]Reaction condition: 5,5-dimethylcyclohexanedione (1 mmol), 2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-5,7(6*H*)-dione (1.0 mmol) and isatin (1.0 mmol) in CH_3CN (5.0 ml) at reflux condition.

Entry	Catalyst (mol%)	Solvent	Temperature (℃)	Time (h)	Yield (%)
1	3.0	–	100	24	30
2	3.0	EtOH	Reflux	24	40
3	3.0	H_2O	Reflux	24	25
4	3.0	MeOH	Reflux	24	50
5	3.0	EtOH/H_2O	90	24	30
6	3.0	DMF	110	24	30
7	3.0	EG	110	24	10
8	3.0	PEG	110	24	10
9	**3.0**	**CH_3CN**	**Reflux**	**10**	**78**
10	2.0	CH_3CN	Reflux	10	55
11	4.0	CH_3CN	Reflux	10	78
12	5.0	CH_3CN	Reflux	10	78

Table 2. Optimization of the reaction condition.a. [a]Reaction condition: 5,5-dimethylcyclohexanedione (1.0 mmol), 2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidine-5,7(6*H*)-dione (1.0 mmol) isatin (1.0 mmol) and acetonitrile (5.0 ml).

improvement (Table 2, entries 11 and 12). Therefore, the optimal conditions for the reaction were identified as 3.0 mol% phosphotungstic acid in refluxing acetonitrile (Table 2, entry 9).

The scope of the reaction was explored for the efficient synthesis ofspiro-pyrano-thiadiazolo-pyrimidine derivatives (**4a-o**) via a one-pot, three-component condensation of various heterocyclic 1,3-dicarbonyl compounds, diverse isatin derivatives, and Dimedone under solvent-free reflux conditions (Table 3). Remarkably, all isatin derivatives and heterocyclic 1,3-diones, regardless of the presence of electron-withdrawing or electron-donating substituents, produced the desired products in yields, ranging from 70 to 90% (Table 3). The structures of all synthesized compounds were unambiguously confirmed using a combination of IR, ^1H NMR, ^{13}C NMR, mass spectrometry, and CHN elemental analysis along with two-dimensional NMR (HMBC and HSQC) for product **4n** (see Supplementary Data). For instance, the IR spectrum of compound **4a** exhibited a broad absorption band at 3317 cm^{-1}, corresponding to NH stretching of the lactam group. Sharp peaks at 1728, 1707, 1656, and 1618 cm^{-1} were attributed to three C = O groups and one C = N group, respectively. In the ^1H NMR spectrum of **4a**, two singlets at $\delta = 0.94$ and 1.00 ppm (6 H) correspond to diastereotopic CH_3 protons. Additionally, two AB-quartet systems at $\delta = 2.01–2.22$ ppm ($J_{AB} = 15.0$ Hz) and $\delta = 2.55–2.71$ ppm ($J_{AB} = 18.0$ Hz), integrating for 4 protons, were assigned to the diastereotopic methylene groups of Dimedone moeity. These findings confirmed the successful synthesis and detailed characterization of the spiro derivatives, showcasing the versatility and efficiency of this synthetic methodology. The ^1H NMR spectrum of compound **4a** displayed characteristic signals for nine aromatic hydrogens, corresponding to the two aromatic heterocyclic dione rings

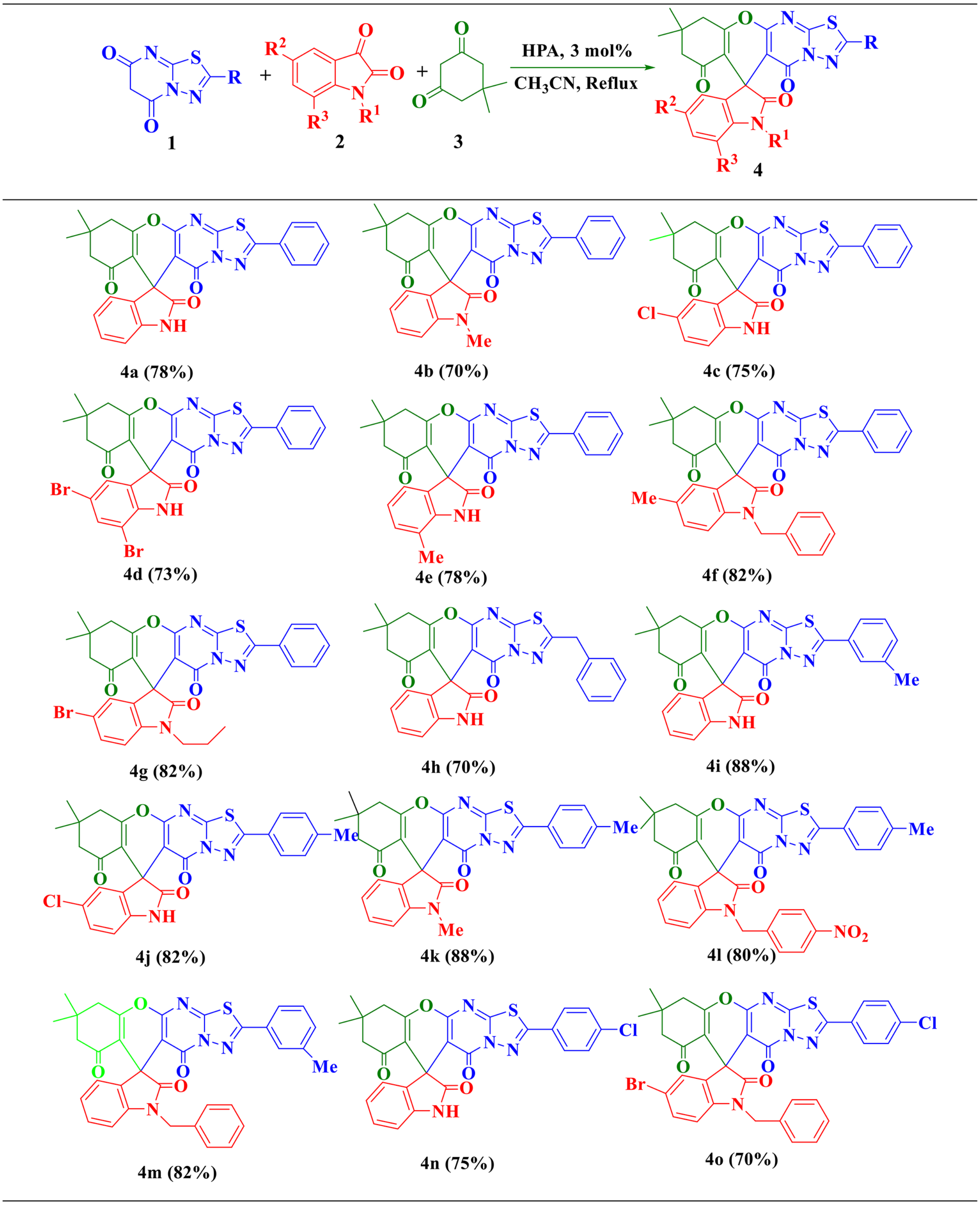
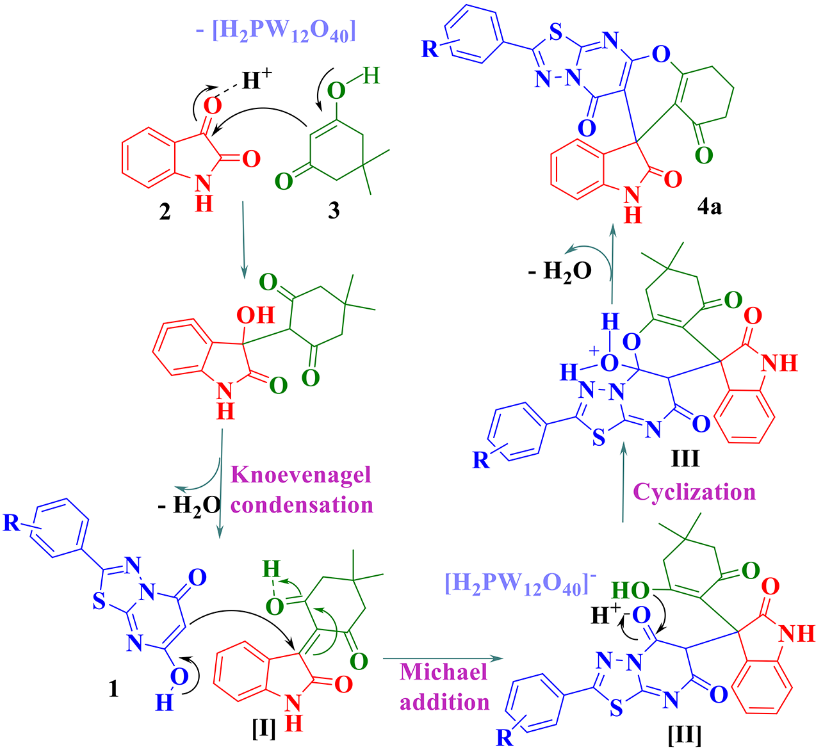


Table 3. One-pot synthesis of spiro-chromeno thiadiazole pyrimidine-indoline derivatives.a.

and the isatin moiety. Specifically, a multiplet at δ = 6.70–6.76 (2 H) was assigned to CH-Ar protons, a doublet of doublets at δ = 6.88 (J = 7.3, 1.3 Hz, 1 H) to another CH-Ar, and a triplet of doublets at δ = 7.05 (J = 7.7, 1.3 Hz, 1 H) to CH-Ar protons on the isatin ring. Additionally, a multiplet at δ = 7.52–7.62 (3 H) and another multiplet at δ = 7.83–7.86 (2 H) were attributed to the aromatic protons of the phenyl ring in the thiadiazolopyrimidine moiety. The N-H proton of the oxindole ring appeared as a distinct singlet at δ = 10.45 ppm.



Scheme 2. Proposed mechanism for the synthesis of spirooxindole derivatives.

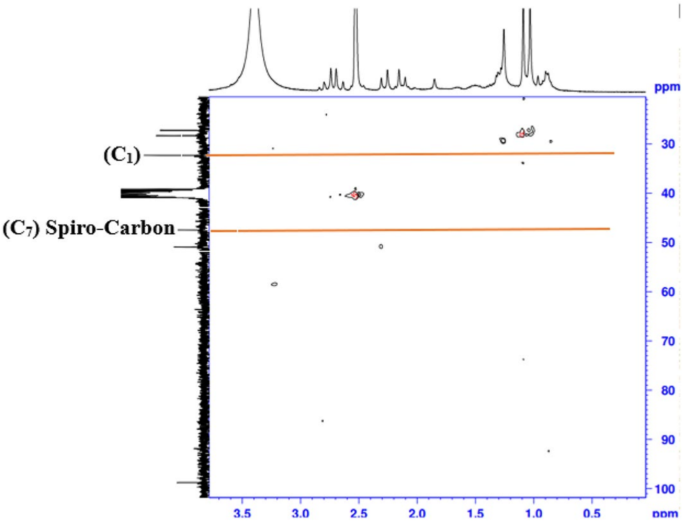


Fig. 2. HSQC correlations of **4n**.

The ^{13}C NMR spectrum confirmed the structure of **4a**, showing 25 distinct carbon signals consistent with the proposed framework. Notable signals included the spiro carbon at $\delta = 47.4$ ppm and two carbonyl carbons at $\delta = 178.0$ and 182.0 ppm. The molecular ion peak in the mass spectrum at $m/z = 496$ matched the calculated molecular weight, supporting the formation of the expected 1:1:1 adduct product.

Two-dimensional NMR techniques, including HSQC and HMBC, were instrumental in signal assignments and structural elucidation (Figs. 2 and 3) of **4n**. HSQC correlations revealed direct hydrogen-carbon connectivity, confirming that the peaks at $\delta = 32.27$ ppm (C-1) and $\delta = 47.4$ ppm (C-7) lacked cross-peaks, classifying them as quaternary carbons. HMBC analysis provided insights into long-range ^1H–^{13}C connectivity. Key cross-peaks demonstrated that the N-H proton of the oxindole ring (H-25) correlated with C-24 (^2J, $\delta = 144.4$ ppm), the spiro carbon (^3J, $\delta = 47.4$ ppm), and C-23 (^3J, $\delta = 133.5$ ppm). These correlations confirmed the presence of the spiro carbon and the indolin-2-one core, further validating the molecular structure of compound **4n**. While single-

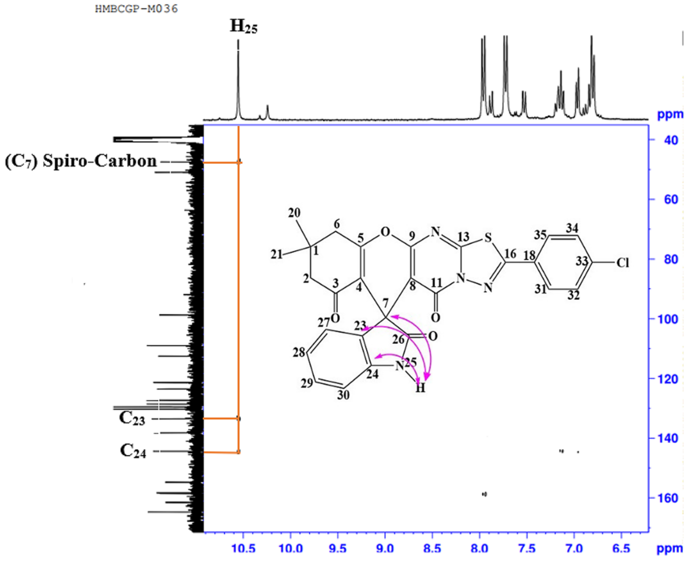


Fig. 3. HMBC correlations of **4n**.

crystal X-ray diffraction is the gold standard for determining the precise structure and absolute stereochemistry of compound **4a-o**, obtaining suitable crystals for this analysis was unfortunately not possible in this case, as none of the 15 products could be grown into crystals of sufficient quality for X-ray diffraction studies.

This section outlines the proposed reaction mechanism for the synthesis of spiro derivative **4** (Scheme 1). The process begins with isatin (2) reacting with Dimedone (3) in the presence of the acidic catalyst $H_3PW_{12}O_{40}$. This reaction forms intermediate [I] through water elimination and Knoevenagel condensation. Subsequently, intermediate [I] undergoes a Michael addition with 2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-5,7(6 H)-dione (1), producing intermediate [II]. Intermediate [II] then undergoes intramolecular cyclization to yield intermediate [III]. Finally, the cyclization step involves the elimination of water from [III], resulting in the formation of the desired product, **4**. Throughout this mechanism, the acidic catalyst plays a crucial role in facilitating each step, ensuring high efficiency and selectivity in the reaction.

Conclusion

In summary, we report a successful procedure for synthesizing novel spirooxindole derivatives by establishing a three-component condensation reaction between different derivatives of isatin, 5,5-dimethylcyclohexane-1,3-dione and 2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-5,7(6*H*)-dione derivatives in acetonitrile and the presence of phosphotungstic acid. The moderate-to-high yields and using phosphotungstic acid as an effective catalyst in preparing the final product make this procedure efficient for modern synthetic chemistry.

Experimental

The starting materials needed in this research were supplied by Merck and Sigma-Aldrich and used as received. Also, isatin derivatives were synthesized according to the literature[36–39].

Typical procedure to synthesize 4a

A mixture of 5,5-dimethylcyclohexane-1,3-dione (0.14 g, 1 mmol), isatin (0.14 g, 1 mmol), 2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-5,7(6*H*)-dione(0.245 g, 1 mmol), and catalyst (0.06 g, 3 mol%) in dry acetonitrile (5 mL) was refluxed for the times mentioned in Table 3. The reaction progress was monitored by TLC (ethyl acetate/hexane (2:1)). Once the reaction was finalized, the mixture was cooled to room temperature. Afterward, the precipitated products were filtered and washed with CH_3CN (3 × 5 mL) followed by column chromatography (silica gel, chloroform/ethylacetate (30:1)) and further purification by plate chromatography (silica gel, n-hexane/ethylacetate (1:3)) to give the desired product **4**.

Spectroscopy data on products are provided in the “supplementary data” section.

Data availability

The data underlying this study, are available in the published article and its Supplementary data.

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Author contributions

Maryam khalili: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration. Abbas Ali Esmaeili: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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