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Synthesis and antimicrobial evaluation of diversely substituted spirooxindole derivatives

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ABSTRACT

A series of novel spirooxindole derivatives have been synthesized from isatin, malononitrile, and cyclic 1,3-dicarbonylcompounds through a highly efficient and green one-pot multicomponent reaction using tetrabutylammonium bromide (TBAB) as catalyst in ethanol under reflux condition. The synthesized compounds were evaluated for their antimicrobial activity. Antimicrobial studies showed that all the target compounds processing good antibacterial and antifungal activities.

Keywords: isatin; spirooxindoles; tetrabutylammonium bromide; malononitrile; 1,3-dicarbonyl compounds; antimicrobial activity

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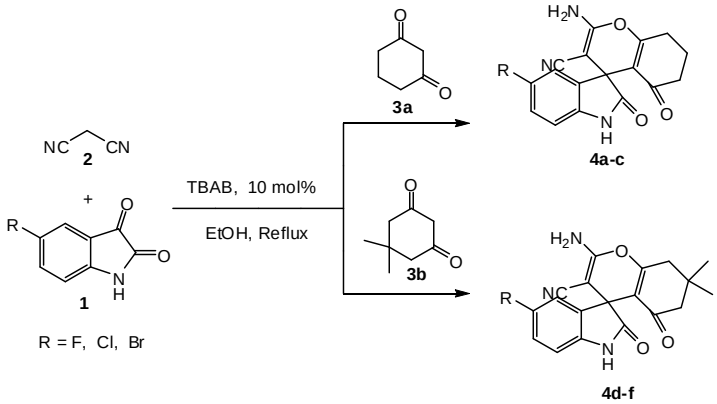
INTRODUCTION

The indole skeleton occurs in many important natural products, pharmaceuticals, and other synthetic materials exhibiting a variety of biological activities and other properties [1]. Spirocompounds represent an important class of naturally occurring substances characterized byhighly pronounced biological properties. The spirooxindole system is the core structure of many pharmacological agents and natural alkaloids [2-5]. Spirooxindoles with fused chromenes have been found to have a wide spectrum of activities such as antimicrobial [6], antiviral [7],anti-depressant[8], antiproliferative [9], sex pheromone [10], antitumor [11], and central nervous system activities [12].

One-pot multicomponent reactions (MCRs) by virtue of their convergence, productivity, facile execution and high yield have attracted considerable attention in recent years since they are performed without need to isolate the any intermediate during their processes and this reduces time saves both energy and raw materials [13].

A Three-component reaction of an isatin, malononitrile or cyanoacetic esters, and 1,3-dicarbonyl compounds provides an elegant procedure for the clean synthesis of these functionalized spirocyclicoxindoles in the presence of various catalysts. This protocol complies with the green chemistry philosophy that encourages the design of products and processes with the minimum use and generation of hazardous substances and ensures higher product yields, shorter reaction times, low cost, and convenience of operation. Significant recent advances in the synthesis of this fused heterocyclic system have led to an intense interest in the development of related compounds as potential medicinal agents or biological probes [14- 16].

Recently tetrabutylammonium bromide (TBAB)has emerged as mild, water-tolerant, inexpensive and environmentally compatible catalyst in various organic transformations [17–20]. Due to the biological importance of spirooxindoles [21-23], herein we report for TBAB catalyzed three-component reaction of a series of novel spirooxindole derivatives from isatin, malononitrile, and 1,3-dicarbonylcompounds in ethanol under reflux condition (Scheme 1).



Scheme 1Synthesis of various spirooxindole derivatives

MATERIALS AND METHODS

Apparatus and analysis

Chemicals were purchased from Merck, Fluka and Aldrich Chemical Companies. All yields refer to isolated products unless otherwise stated. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained using Bruker DRX- 500 Avance at ambient temperature, using TMS as internal standard. FT-IR spectra were obtained as KBr discs on Shimadzu spectrometer. Mass spectra were determined on a Varion - Saturn 2000 GC/MS instrument. Elemental analysis was measured by means of Perkin Elmer 2400 CHN elemental analyzer flowchart.

General procedure for the synthesis of spirooxindole

A mixture of substituted isatins (1 mmol),cyclic 1,3-diketone (1 mmol), malononitrile (1 mmol)and TBAB (10 mol%) in EtOH (10mL) was stirred at reflux temperature. Uponcompletion, monitored by TLC(*n*-hexane/ethyl acetate: 2/1), the reaction mixture was allowed to cool to room temperature. The catalyst was separated by filtration of this solution. The solution was concentrated under vacuum to afford the product, which was purified by recrystallization in the ethanol. All the products were analysed by FT-IR, ^{1}H NMR and ^{13}C NMR spectra and elemental analysis.

Spectral data for selected compounds

*2-Amino-5'-chloro-2',5-dioxo-5,6,7,8-tetrahydrospiro [chromene-4,3'-indoline]-3-carbonitrile (**4b**)*

IR (KBr, cm^{-1}): 3274 and 3152(NH and NH_2), 2192 (CN), 1718(CO) 1656(CO); ^{1}H NMR (500 MHz, DMSO-d_6): 2.08-2.78 (m, 6H, 3xCH_2), 6.78-7.15 (m, 3H, Ar-H), 7.22 (s, 2H, NH_2), 10.39 (s, 1H, NH) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 32.40, 47.28, 50.47, 57.98, 109.70, 111.26, 117.80, 122.14, 123.48, 128.63, 134.88, 142.52, 159.24, 164.60, 178.49, 195.33 ppm. MS (ESI): *m/z* 342 $(M+H)^+$. Anal. Calcd. for $C_{17}H_{12}ClN_3O_3$: C, 59.75; H, 3.54; N, 12.30 %. Found: C, 59.71; H, 3.50; N, 12.26%.

*2-Amino-5'-fluoro -7,7-dimethyl-2',5-dioxo-5,6,7,8-tetrahydrospiro [chromene-4,3'-indoline]-3-carbonitrile (**4d**)*

IR (KBr, cm^{-1}): 3252 and 3132 (NH and NH_2), 2184 (CN), 1710 (CO) 1664 (CO); ^{1}H NMR (500 MHz, DMSO-d_6): 1.00 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 2.12 - 2.54 (m, 4H, 2xCH_2), 6.92-7.19 (m, 3H, Ar-H), 7.15 (s, 2H, NH_2), 10.62 (s, 1H, NH) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ22.14, 24.18, 32.55, 47.33, 50.61, 56.15, 108.96, 110.88, 117.16, 123.24, 124.66, 128.60, 133.72, 142.44, 158.38, 164.60, 176.12, 194.16ppm. MS (ESI): *m/z* 354 $(M+H)^+$. Anal. Calcd. for $C_{19}H_{16}FN_3O_3$: C, 64.58; H, 4.56; N, 11.89 %. Found: C, 64.52; H, 4.54; N, 11.82%.

RESULTS AND DISCUSSION

We have developed an efficient synthesis of a series of spirooxindole derivatives from isatin, malononitrile, and 1,3-dicarbonylcompoundsin the presence of TBAB as an efficient catalyst in ethanol under reflux. Herein, we report our results.

To realize the reaction shown in Scheme **1**, isatin **1**,malononitrile **2**, 1,3-dicarbonylcompounds**3** and TBAB (10mol%) were refluxed for 40-60 min to afford the corresponding spirooxindole product. The reaction was examined in different solvents including acetonitrile, methanol, ethylacetate and ethanol. The best results were obtained in ethanol medium.

In order to investigate the scope of these conditions, several examples were studied and are summarized in Table**1**. In all cases, the three component reaction proceeded smoothly to give the corresponding spirooxindoles in good

yields. As shown in Table **1**, it was found that this method works with wide scope of substrates. A variety of varioushalo substituted isatins and different 1,3-cyclohexanedionewere subjected to this reaction.

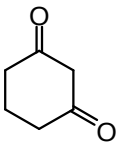
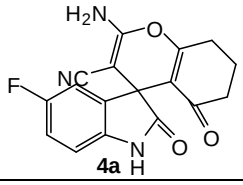
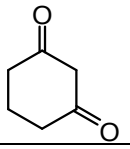
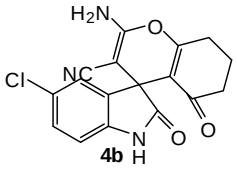
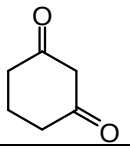
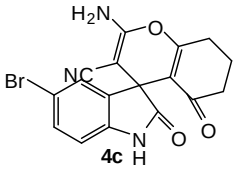
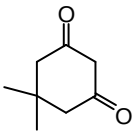
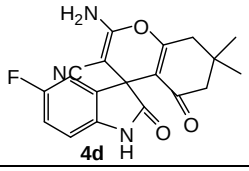
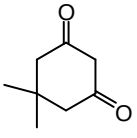
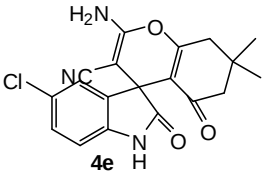
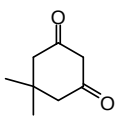
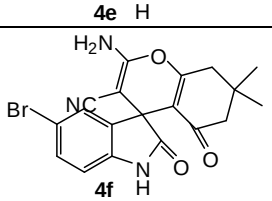
Biological evaluations

The synthesized spirooxindole derivatives were evaluated for their antimicrobial activity. They were tested for their antibacterial and antifungal activity at different concentrations in DMSO. *Ciprofloxacin* and *Amphoterecin-B* were used as the positive control drugs for antibacterial and antifungal tests, respectively. Inoculums of the bacterial and fungal cultures were also prepared. The minimum concentration at which no growth was observed was taken as the minimum inhibitory concentration (MIC) value [24-26].

Antibacterial activity

The newly synthesized compounds were screened for their *in vitro* antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* bacterial strains by serial plate dilution method. Serial dilutions of the drug in Muller Hinton broth were taken in tubes and their *pH* was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16-18 h at 37 ^{o}C. The MIC is the lowest concentration of the drug for which no growth is detected. The results are summarized in Table 2. The MIC values were evaluated at concentration range, 12.5-25 µg/mL. Upon exploration of the antibacterial activity data (Table 2), it has been observed that all compounds were found to have antibacterial activity against *E. coli*, *P. aeruginosa* and *K. pneumoniae*, and *S. aureus* when compared with the employed standard drug.

Table 1TBAB catalyzed one-pot multi component synthesis of spirooxindoles[a]

S.No	R	1,3-Dicarbonyl Compound	Product	Time (min)	Yield (%)[b]
1	F		 **4a**	40	93
2	Cl		 **4b**	50	90
3	Br		 **4c**	60	88
4	F		 **4d**	45	92
5	Cl		 **4e**	55	91
6	Br		 **4f**	60	87

[a]*Reaction conditions: isatins (1 mmol),malononitrile (1 mmol) and cyclic 1,3-diketone (1 mmol) in the presence of TBAB (10 mol %) in EtOHat reflux.*
[b]*Isolated yield.*

Antifungal activity

Newly prepared compounds were also screened for their antifungal activity against *Aspergillus flavus, Rhizopus schipperae* and *Aspergillus niger* in DMSO by serial plate dilution method. Sabourauds agar media was prepared by dissolving peptone (1 g), D glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the *pH* to 5.7. Normal saline was used to make a suspension of sore of fungal strains for lawning. Activity of each compound was compared with *Amphoterecin-B* as standard. The results are summarized in Table 2. The MIC values were evaluated at concentration range, 25-50 µg/mL. The results given in Table 4 shows that all compounds exhibited antifungal activity with MIC against *A. flavus*, *R. schipperae* and *A. niger* compared with *Amphoterecin-B* as standard drug.

Table 2*In vitro* antibacterial and antifungal activities of compounds 4a–f

Compound	Minimum inhibitory concentration (MIC) in µg/mL					
	Antibacterial activity			Antifungal activity		
	E. coli	*P. aeruginosa*	*K. pneumonia*	*A. flavus*	*R. schipperae*	*A. niger*
4a	75	75	75	100	75	100
4b	150	75	150	150	75	150
4c	125	75	125	100	75	100
4d	100	75	75	100	75	100
4e	125	50	125	100	75	100
4f	100	75	100	100	100	100
Ciprofloxacin	25	12.5	25	-	-	-
Amphoterecin-B	-	-	-	50	25	50

CONCLUSION

In conclusion, we have described a simple one-pot three component reaction involving isatin, malononitrile and cyclic 1,3-diketo compound to yield a series of spirooxindole derivatives using TBAB as a novel and inexpensive catalyst in ethanol under reflux condition. Furthermore, this methodology conveniently afforded the desired products in good yields under mild conditions with operational simplicity.

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