Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(1):94-98
(http://derpharmachemica.com/archive.html)

Synthesis and biological evaluation of some novel isatin derivatives as antimicrobial agents

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ABSTRACT

A new and convenient procedure has been developed for the one-pot synthesis of different types of isatin derivatives from isatin, malononitrile, and cyclic 1,3-dicarbonyl compounds using tetrabutylammonium bromide (TBAB) as an inexpensive, non-toxic, non-metallic and readily available catalyst in ethanol under reflux condition. The compounds were screened 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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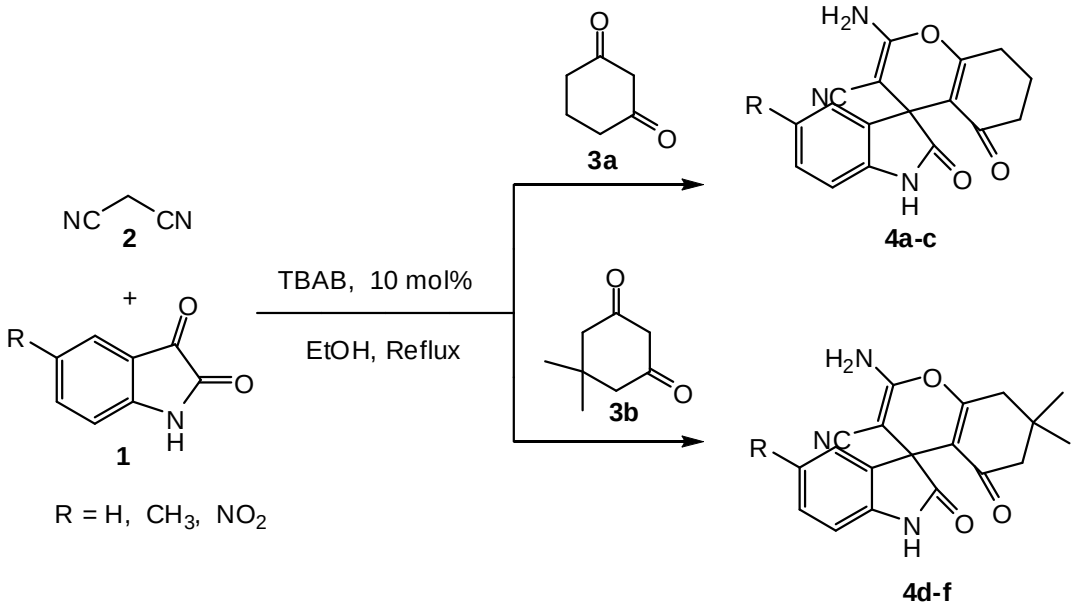
INTRODUCTION

Isatins (1*H*-indole-2,3-dione) are synthetically versatile substrates, where they can be used for the synthesis of a large variety of heterocyclic compounds, such as indoles and quinolines, and as raw material for drug synthesis. Isatins have also been found in mammalian tissue and their function as a modulator of biochemical processes has been the subject of several discussions. The advances in the use of isatins for organic synthesis during the last twenty-five years, as well as asurvey of its biological and pharmacological properties are extensively reported[1].

The various biological activities of spirooxindole derivatives have attracted much attention from organic chemists, and as a consequence, a number of methods have been reported for the preparation of spirooxindole-fused heterocycles [2-6]. Isatin and its derivatives may be the most useful starting materials or precursors in the synthesis of a wide number of spirocyclicoxindoles [7,8].

Due to its simple process, easy operation, efficiency and high atomic economy, the multicomponent reaction based on isatin and its derivatives have become an efficient method for the synthesis of various spirooxindoles in recent years [9,10]. It is known that the multicomponent reactions of isatins with in situ formed azomethineylides have become the efficient synthetic procedure for constructing versatile spirooxindole systems [11-16].

Recently tetrabutylammonium bromide (TBAB)has emerged as mild, water-tolerant, inexpensive and environmentally compatible homogenous catalyst in various organic transformations [17–20]. Considering the biological importance of spirooxindoles [21-23], and as part of our program aimed at developing new multicomponent reactions for the construction of complex heterocyclic compounds, we wish in this work to report the TBAB catalysed efficient three-component synthesis of a series of novel spirooxindole derivatives from isatin, malononitrile, and 1,3-dicarbonylcompounds in ethanol under reflux condition (Scheme 1).



Scheme 1One-pot synthesis of various isatin 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 isatin derivatives

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. Upon completion, 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-2',5-dioxo-5,6,7,8-tetrahydrospiro [chromene-4,3'-indoline]-3-carbonitrile (**4a**)*

IR (KBr, cm^{-1}): 3282 and 3166 (NH and NH_2), 2173 (CN), 1703 (CO) 1634 (CO); ^{1}H NMR (500 MHz, DMSO-d_6): 2.18 - 2.63 (m, 6H, 3xCH_2), 6.92 -7.22(m, 4H, Ar-H), 7.31 (s, 2H, NH_2), 10.51 (s, 1H, NH) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 31.55, 48.08, 51.57, 58.08, 108.88, 111.06, 118.26, 123.72, 123.82, 127.53, 135.08, 143.58, 160.04, 166.02, 177.97, 194.22ppm. MS (ESI): *m/z* 308 $(M+H)^+$. Anal. Calcd. for $C_{17}H_{13}N_3O_3$: C, 66.44; H, 4.26; N, 13.67 %. Found: C, 66.40; H, 4.22; N, 13.61%.

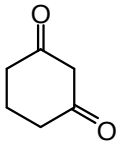
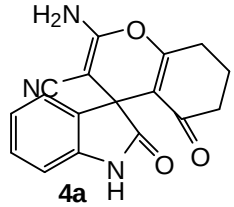
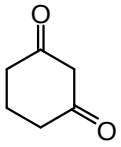
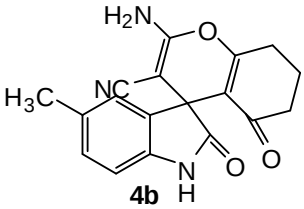
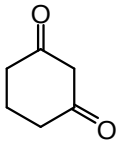
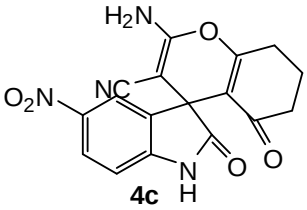
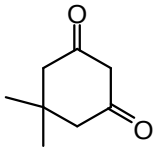
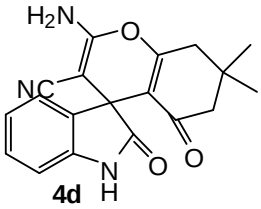
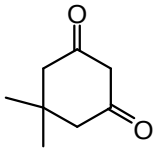
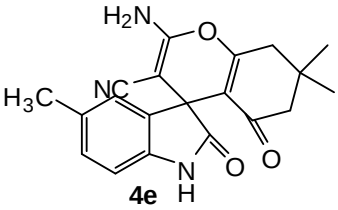
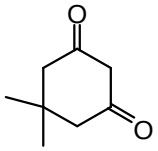
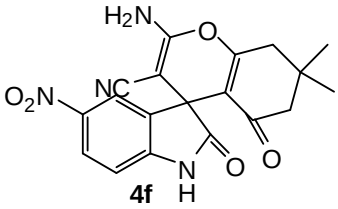
*2-Amino-7,7-dimethyl-2',5-dioxo-5,6,7,8-tetrahydrospiro [chromene-4,3'-indoline]-3-carbonitrile (**4d**)*

IR (KBr, cm^{-1}): 3261 and 3144 (NH and NH_2), 2199 (CN), 1700 (CO) 1653 (CO); ^{1}H NMR (500 MHz, DMSO-d_6): 1.07 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 2.06 - 2.66 (m, 4H, 2xCH_2), 6.85-7.27 (m, 4H, Ar-H), 7.22 (s, 2H, NH_2), 10.41 (s, 1H, NH) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ19.77, 22.27, 33.05, 48.44, 51.00, 55.65, 108.96, 110.88, 118.00, 123.24, 124.35, 128.71, 133.72, 142.12, 157.38, 165.34, 177.33, 192.88ppm. MS (ESI): *m/z* 336 $(M+H)^+$. Anal. Calcd. for $C_{19}H_{17}N_3O_3$: C, 68.05; H, 5.11; N, 12.53 %. Found: C, 68.00; H, 5.05; N, 12.48%.

RESULTS AND DISCUSSION

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

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

S.No	R	1,3-dicarbonyl compound	Product	Time (min)	Yield (%)[b]
1	H		 **4a**	45	90
2	CH_3		 **4b**	50	88
3	NO_2		 **4c**	60	89
4	H		 **4d**	40	91
5	CH_3		 **4e**	50	86
6	NO_2		 **4f**	55	93

[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.*

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 various 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. 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.

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 results given in Table 2 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	50	50	50	100	50	100
4b	100	50	100	150	75	150
4c	75	50	100	100	75	100
4d	75	50	75	100	50	100
4e	125	50	125	100	75	150
4f	75	50	75	100	50	150
Ciprofloxacin	25	12.5	25	-	-	-
Amphoterecin-B	-	-	-	50	25	50

CONCLUSION

In summary, spirooxindole derivatives were synthesized by the one-pot three component reaction involving isatin, malononitrile and cyclic 1,3-diketo compound using TBAB as a novel and inexpensive catalyst in ethanol under reflux condition. It represents a straightforward protocol for the eco-friendly and efficient synthesis of spirooxindole derivatives with potential biological activities.

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