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A RAPID, GREEN, AND EFFICIENT MICROWAVE-ASSISTED SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SPIROINDENO[1,2-*b*] PYRIDO[2,3-*d*]PYRIMIDINE-5,3'-INDOLINE DERIVATIVES

Ghodsi MOHAMMADI ZIARANI,[a,b,*] Zahra HASSANZADEH,[a] Parisa GHOLAMZADEH[a] and Ali ABOLHASSANI SOORKI[c]

[a] Department of Chemistry, Alzahra University, Vanak Square, P.O. Box 1993893973, Tehran, Iran
[b] National Laboratory of Pharmaceutical Research, Alzahra University, Tehran, Iran
[c] ACECR- Research Institute of Applied Sciences, Shahid Beheshti University,Tehran, Iran

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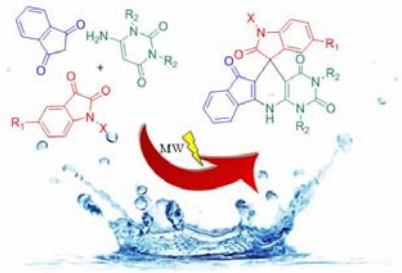
A highly efficient microwave-assisted synthesis of spiroindeno[1,2-*b*]pyrido[2,3-*d*]pyrimidine-5,3'-indolines is reported in water media and under catalyst-free condition. Synthesis of these products was developed through one-pot condensation reaction of isatin derivatives, 6-aminouracil and 1,3-indandione compounds under microwave (MW) irradiation. This methodology suggests several benefits including short reaction time, excellent yields of the products, and environmentally benign mild reaction conditions. Additionally, antimicrobial and antifungal properties of the products were investigated.



INTRODUCTION

In the past decades, industrial microwave radiation has become an efficient alternative for thermal heating in organic synthesis. The use of microwave irradiation generates faster reactions, easy scale-up, better yield and reproducibility of the products.[1] One-pot multicomponent reaction is a simple and flexible strategy to synthesize heterocyclic compounds by reducing the consumption of solvent and raw materials. In addition, it is not necessary to isolate the intermediate in this method, which saves time and accelerates the reaction.[2]

Pyridopyrimidine scaffold exhibits a wide spectrum of pharmacological potencies and biological activities including antibacterial,[3] analgesic,[4] calcium channel antagonist,[5] anticonvulsants,[6] tyrosine kinase,[7] and antihypertensive properties.[8] Encouraged by the above potencies of pyridopyrimidine scaffold and advantages of using MW irradiation, developing an efficient and rapid method for the synthesis of spiroindeno[1,2-*b*]pyrido[2,3-*d*]pyrimidine-5,3'-indoline derivatives will be an interesting issue.

Pyridopyrimidine can be obtained by different synthetic methods. In 2011, Imani Shakibaei *et al.* established the first method to achieve pyridopyrimidine derivatives *via* the three-component reaction of 1,3-indandione, amino uracils and isatins in refluxing ethanol.[11] These compounds may be also synthesized through a three-component reaction in presence of a catalyst such as PEG-OSO_3H[10] and/or *N*,*N*,*N*,*N*-tetramethylguanidinium triflate ($TMGT_f$)[13]. Khandelwal *et al.* reported the synthesis of spiroindeno[1,2-*b*]pyrido[2,3-*d*]pyrimidine-5,3'-indoline

* Corresponding author: gmziarani@hotmail.com, gmohammadi@alzahra.ac.ir; tel.: +98-21-88041344

using choline chloride-oxalic acid mixture as a catalyst/solvent system.[12] These methods show some disadvantages such as a long reaction time and difficulties in the isolation of the products.

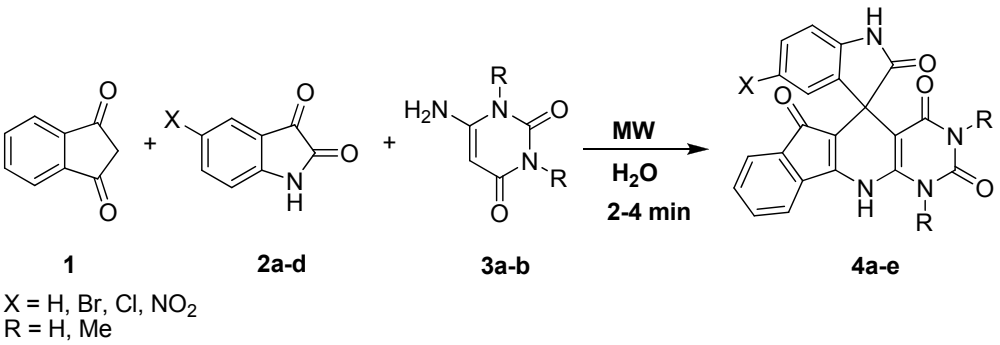
In continuation of our researches,[9] we have developed an efficient and rapid method for the synthesis of spiroindeno[1,2-*b*]pyrido[2,3-*d*]pyrimidine-5,3'-indoline derivatives, associated by biological activities. In this paper, we want to describe the effect of MW irradiation in the synthesis of pyridopyrimidine derivatives in water and under green conditions. The results revealed that the reaction is completed within 4 minutes, in a very good yield, with an easy work-up and under environmentally friendly conditions.

RESULTS AND DISCUSSION

A green, rapid and highly efficient procedure for the synthesis of spiroindeno[1,2-*b*]pyrido[2,3-*d*]pyrimidine-5,3'-indoline compounds **4a-e** was performed through condensation reaction of 1,3-indandione **1**, isatin derivatives **2a-d** and 6-aminouracil **3a** (or its methylated derivative **3b**) under MW irradiation for 2-4 min in water (Scheme 1). As shown in Table 1, the products were obtained in high yields, and their melting points (m.p.) were compared with the literature data. The new compound **4c** was analyzed by FT-IR, Mass, ^{1}H NMR and ^{13}C NMR techniques.

According to the proposed mechanism by Paul and Das,[10] here in, a similar mechanism is described for the synthesis of spiroindeno[1,2-*b*]pyrido[2,3-*d*]pyrimidine-5,3′-indoline **4** (Scheme 2). First, nucleophilic addition of 1,3-indandione **1** (in its enolic form **5**) to the carbonyl group of isatin **2** gives intermediate **6**. Next, 6-aminouracil **3** is added to the latter through a Michael reaction to gain intermediate **7**. Intramolecular addition of amine functional group of uracil moiety to the carbonyl group of indandione moiety follow by a dehydration gives the product **4**.

As far as we know, there are only four previous reports for the synthesis of spiroindeno[1,2-*b*]pyrido[2,3-*d*]pyrimidine-5,3'-indolines in literature (Table 2). In the present work, the products were synthesized without the use of any catalyst with excellent yields (83-97%) and in a shorter reaction time (2 to 4 minutes). Additionally, the current inexpensive procedure is completely green and easy procedure.

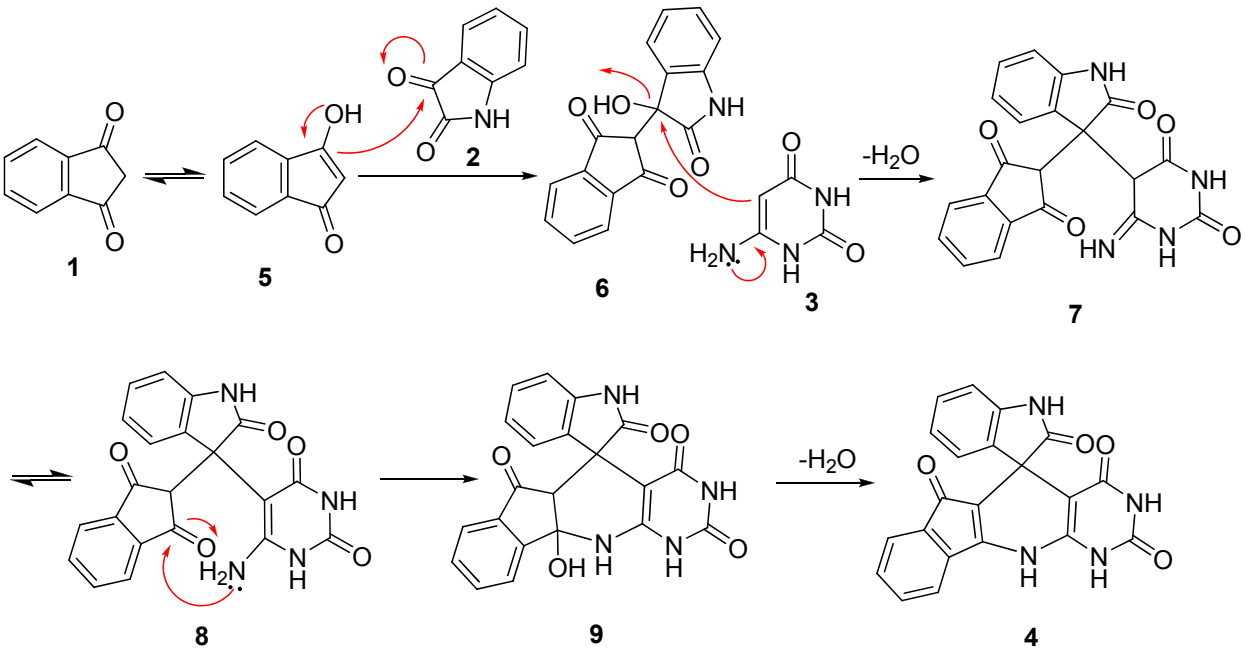


Scheme 1 – Synthesis of spiroindeno[1,2-b]pyrido[2,3-d]pyrimidine-5,30-indoline **4a-g** subjected to MW irradiation.

Table 1

MW-assisted synthesis of spiroindeno[1,2-b]pyrido[2,3-d]pyrimidine-5,30-indoline derivatives **4a-g**

Entry	Product	X	R	Time (min)	Yield (%)	m.p. (°C)	m.p. (Lit)
1	**4a**	H	H	2	94	>300	295-298[11,12]
2	**4b**	Br	H	2	96	>300	>300[10,11]
3	**4c**	Cl	H	2	97	>300	New
4	**4d**	NO_2	H	2	94	>300	>300[11]
5	**4e**	H	Me	4	83	>300	294-296[13]



Scheme 2 – Proposed mechanism for the synthesis of spiroindeno[1,2-*b*]pyrido[2,3-*d*]pyrimidine-5,3'-indoline **4**.

Table 2

Comparison of different conditions in synthesis of spiroindeno[1,2-*b*]pyrido[2,3-*d*]pyrimidine-5,3'-indolines derivatives

Entry	Catalyst	Solvent	Reaction conditions	Time (h)	Yield (%)	Year
1	PEG-OSO_3H*	H_2O	70 °C	2.5	81-94	2013[10]
2	-	EtOH	Reflux	3	80-93	2011[11]
3	DES**	H_2O	70 °C	35-50 min	89-94	2014[12]
4	$TMGT_f$***	-	80 °C	5 min	91-93	2012[13]
5	-	H_2O	MW	2-4 min	83-97	Present work

*Polyethylene glycol functionalized sulfonic acid
**Deep eutectic solvent (choline chloride-oxalic acid)
****N*,*N*,*N*,*N*-tetramethylguanidinium triflate

1. Antimicrobial activity

Investigating the antimicrobial activity of the synthesized compounds was performed through a disk diffusion method (IZ) (Table 3) and the minimum inhibitory concentration technique (MIC), as well (Table 4). Their antibacterial efficacy were studied against gram positive bacteria *Bacillus subtilis* (ATCC 465) and *Staphylococcus aureus* (ATCC 25923), gram negative bacteria *Escherichia coli* (ATCC 25922) as well as *Pseudomonas aeruginosa* (ATCC 85327). Moreover, their antifungal properties were investigated against *Candida albicans* (ATCC 10231). Microdilution method was used to determine the MIC of the compounds. In the first step, a suspensions of the microorganisms (10^9 cells/ml, 100 µl) were disseminated on the sterile Muller–Hilton agar plates and then the compounds were dissolved in DMSO (100 µg/ml) and 25 µl of the solutions was applied to 6-mm paper disks which were then placed on the surface of the culture plates. Compound **4b** displayed the best results among the other compounds against *B. subtilis* and *S. aureus* with 22 and 20 mm of IZ, and 16 and 32 µg/ml of MIC, respectively. These results were compared to the commercial antibiotics such as Chloramphenicol, Gentamicin and Nystatin. Compounds **4c** showed poor activity against *B. subtilis* and *S. Aureus* and compound **4e** displayed only a limited activity against *B. subtilis*. None of the synthesized compounds showed activity against *E. coli*, *P. aeruginosa* and *C. albicans*.

Table 3

Inhibition zones of synthesized compounds against some gram-positive bacteria, gram-negative bacteria and fungi by disc diffusion method

Compound	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
4a	0	0	0	0	0
4b	22	20	0	0	0
4c	17	15	0	0	0
4d	0	0	0	0	0
4e	15	0	0	0	0
Chloramphenicol	26	22	24	8	-
Gentamicin	28	20	18	18	-
Nystatin	-	-	-	-	18

Table 4

Minimum inhibitory concentration (µg/ml) of synthesized compounds against some gram -positive bacteria, gram-negative bacteria and fungi

Compound	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
4a	-	-	-	-	-
4b	16	32	-	-	-
4c	64	128	-	-	-
4d	-	-	-	-	-
4e	128	-	-	-	-
Chloramphenicol	4	8	4	256	-
Gentamicin	0.125	0.5	0.5	1	-
Nystatin	-	-	-	-	8

EXPERIMENTAL

1. Materials and equipment

All chemicals were procured from Merck Company and were used as purchased. Melting points were determined by the use of a Barnstead Electrothermal 9200 apparatus. Fourier transform infrared (FT-IR) spectra were recorded on KBr pellets with a FT-IR Bruker Tensor 27 instrument. A Milestone MicroSYNTH (Microwave Synthesis Labstation) apparatus was used to irradiate the reaction mixture. Mass spectra data were obtained using Network Mass selective detector (Agilent) 6890/5973 instrument. ^{1}HNMR was run on a Bruker DPX, 300 MHz and ^{13}CNMR on Bruker DPX, 75 MHz in the TMS as an internal standard and in DMSO-d_6 as the solvent.

2. General procedure for the synthesis of spiroindeno[1,2-*b*]pyrido[2,3-*d*]pyrimidine-5, 3'-indolines (4a-g)

The condensation reaction of 1,3-indandione **1** (0.147g, 1mmol), isatin **2** (0.146g, 1mmol) and 6-aminouracil **3** (0.127g, 1mmol) was accomplished through a catalyst-free condition under MW irradiation (500 W, 95 °C) for the synthesis of pyrido[2,3-*d*]pyrimidine compounds. After completion of the reaction, which was traced by TLC, the reaction mixture was completely dissolved in a minimum volume of hot ethanol and then cooled to give the pure product precipitate. The new compound **4c** was characterized by Mass, FT-IR and NMR spectroscopy techniques. The melting points of the products were compared with those reported in the literature.

The physical and spectral data (FT-IR, ^{1}HNMR, ^{13}CNMR and Mass) for the new compound are given below:

5'-Chloro-1H-spiro[indeno[1,2-b]pyrido[2,3-d] pyrimidine-5,3'-indoline]-2,2',4,6(3'H,10'H)-tetraone (**4c**)

Mp > 300 °C. FT-IR (KBr): υmax = 3350, 3472, 3157, 3100, 1710, 1654 cm^{-1}. ^{1}HNMR (300 MHz, DMSO-d_6): δ_H (ppm) 6.76 (1H, d, $^{3}J_{HH}$ =7.8), 7.14 (1H, m, H-Ar), 7.22 (1H, d, $^{3}J_{HH}$ =6.84), 7.37 (1H, m, H-Ar), 7.49 (2H, d), 7.93 (1H, s, H-Ar), 10.53 (3H, s, NH), 10.96 (1H, s, NH).^{13}CNMR (75 MHz, DMSO-d_6): δ_C (ppm) 46.3, 90.1, 106.8, 109.9, 119.6, 121.0, 123.6, 125.1, 127.6, 130.8, 132.1, 132.4, 135.3, 137.0, 141.6, 145.7, 149.6, 154.2; MS (*m*/*z*) = 545 (M+), 418, 265, 144, 105, 91, 69, 57 and 42.

CONCLUSIONS

In this paper, we demonstrated a highly efficient three-component reaction of isatin derivatives, 1,3-indandione and 6-aminouracil and/or its methylated derivative for the synthesis of pyridopyrimidine compounds under MW irradiation in an aqueous media and catalyst-free conditions. The advantages of this method were the simplicity of reaction, eco-friendly solvent, very rapid reaction and high yields of the products. Additionally, investigating the antibacterial activities of the products, which were synthesized through this procedure, showed that the compounds **4b** and **4c** have antibacterial activities against *B. subtilis* and *S. aureus* and **4e** has activities only against *B. subtilis*.

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