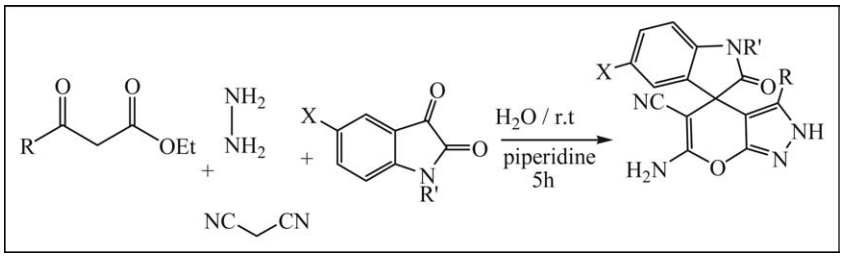
Somayeh Ahadi, Zahra Yasaei, and Ayoob Bazgir*

Department of Chemistry, Shahid Beheshti University, General Campus, Tehran 1983963113, Iran
*E-mail: a_bazgir@sbu.ac.ir

Received October 27, 2009
DOI 10.1002/jhet.437
Published online 13 July 2010 in Wiley Online Library (wileyonlinelibrary.com).



Piperidine catalyzes efficiently the one-pot, four-component reaction of β-ketoesters, hydrazine hydrate, malononitrile, and isatines in aqueous media. The reaction was done at room temperature and the spiro[indoline-3,4′-pyrano[2,3-*c*]pyrazole]-5′-carbonitriles were obtained with high yields and purity *via* an easy work-up procedure. These compounds were also investigated *in vitro* for antibacterial activities.

J. Heterocyclic Chem., **47**, 1090 (2010).

INTRODUCTION

Multicomponent reactions (MCRs) are of increasing importance in organic and medicinal chemistry because the strategies of MCR offer significant advantages over conventional linear-type syntheses. MCRs leading to interesting heterocyclic scaffolds are particularly useful for the creation of diverse chemical libraries of ''drug-like'' molecules for biological screening, as the combination of three or more small molecular weight building blocks in a single operation leads to a high combinatorial efficacy [1,2]. Designing of MCRs in water is another attractive area in chemistry [3] because water is a cheap, safe, and environmentally benign solvent. There is need for developing MCRs in water with a suitable catalyst and without the use of any harmful organic solvents.

The indole moiety is probably the most well-known heterocycle, a common and important feature of a variety of natural products and medicinal agents [4]. Furthermore, it has been reported that sharing of the indole 3-carbon atom in the formation of spiroindoline derivatives highly enhance biological activity [5–7]. The spirooxindole system is the core structure of many pharmacological agents and natural alkaloids [8–10]. Therefore, a number of methods have been reported for the preparation of spirooxindole-fused heterocycles [11–14].

Substituted amino-pyrans take a significant place among the six-membered oxygen-containing heterocycles. Some of them possess anticancer and antimicrobial activity [15,16]. Serotonin receptor modulators (pteropodine and its stereoisomers), natural alkaloids, containing both spiro-indole and pyran cycles, were isolated from stem bark of *Uncaria tomentosa* (Fig. 1) [8]. Several spiroheterocycles, containing both indole and pyran heterocycles, possess anticonvulsant and analgetic [17], herbicidal [18], and antibacterial activities [19]. Similarly, pyrano[2,3-*c*]pyrazoles play an essential role in biologically active compounds and therefore represent an interesting template for medicinal chemistry [20–22].

As part of our continuing efforts on the synthesis of biologically active heterocyclic compounds [23–32], especially spirooxindole derivatives [33–35], we report herein a novel and clean synthesis of spiro[indoline-3,4′-pyrano[2,3-c]pyrazole]-5′-carbonitriles 5 through a one-pot, four-component condensation reaction of β-ketoesters 1, hydrazine hydrate 2, malononitrile 3, and isatins 4 in water (Scheme 1).

RESULTS AND DISCUSSION

We found that the one-pot, four-component condensation reaction of β-ketoesters **1a,b**, hydrazine hydrate **2**, malononitrile **3**, and isatins **4** proceeded rapidly in water at ambient temperature and were complete after 5 h to afford spiro[indoline-3,4′-pyrano[2,3-*c*]pyrazole]-5′-carbonitriles **5a–l**, in high yields (Table 1). ^{1}H and ^{13}C NMR spectra of the crude products clearly indicated the formation of spirooxindol-fused pyranopyrazole **5**. The nature of these compounds as 1:1:1:1 adducts was apparent from their mass spectra, which displayed, in each case, the molecular ion peak at appropriate m/z values. Compounds **5a–l** are stable solids whose structures were

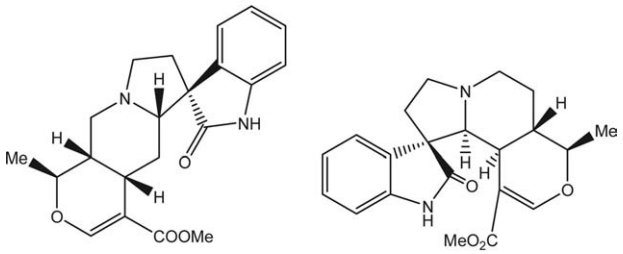


Figure 1. Spirooxindole natural alkaloid.

established by IR, ^{1}H and ^{13}C NMR spectroscopy, and elemental analysis. The structures of **5j** were confirmed by a single-crystal X-ray analysis [36] (Fig. 2).

The results were good in terms of yields and product purity in the presence of piperidine, whereas without piperidine the yields of products were very low (<40%) even after 24 h.

To the best of our knowledge, this new procedure provides the first example of an efficient and four-component method for the synthesis of spiro[indoline-3,4′-pyrano[2,3-*c*]pyrazole]-5′-carbonitriles. This method, based on four-component piperidine-catalyzed reaction in water, is the most simple and convenient and would be applicable for the synthesis of different types of spiroindoline-pyranopyrazoles.

For the investigation of the reaction mechanism, it is notable that when the ethyl acetoacetate **1a**, hydrazine hydrate **2**, malononitrile **3**, and isatin **4a** were reacted for 1 h, the intermediate **6** and **7** were isolated and characterized by spectroscopic methods. When intermediate **6** and **7** were isolated and reacted in the presence of piperidine under the same reaction conditions, the product **5a** was obtained in 75% yield (Scheme 2).

According to the results, the formation of products **5** can be rationalized by initial formation of pyrazol-5-ol **6** *via* condensation of **1** and **2**. Subsequent Michael-type addition of **6** to the intermediate **7** (formed *in situ* by reaction of the malononitrile **3** and isatin **4**), followed by cyclization and tautomerization afforded the corresponding products **5** (Scheme 3).

To further explore the potential of this protocol for spiro-heterocyclic synthesis, we investigated reaction of acenaphthylene-1,2-dione **8** instead of isatin **4** and

Scheme 1

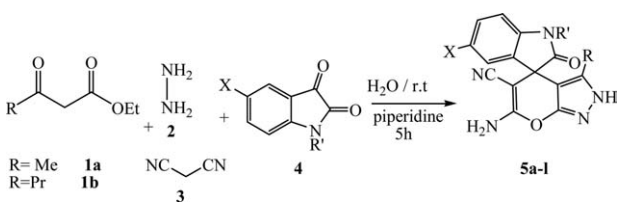


Table 1

Synthesis of spiroindoline-pyranopyrazoles **5**.

Product **5**	R	R′	X	Yield (%)
A	Me	H	H	89
B	Me	Me	H	85
C	Me	Et	H	80
D	Me	$PhCH_2$	H	85
E	Me	H	Br	95
F	Me	Me	Br	93
G	Me	Et	Br	90
H	Me	H	NO_2	94
I	n-Pr	H	H	92
J	n-Pr	Me	H	90
K	n-pr	H	Br	97
L	n-Pr	H	NO_2	95

obtained spiro[acenaphthylene-1,4′-pyrano[2,3-*c*]pyrazole]-5′-carbonitrile **9** in 87% yield (Scheme 4).

Finally, all synthesized compounds were screened for antimicrobial activity. The microorganisms used in this study were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginusa* ATCC 85327, (Gram-negative bacteria), *Enterococcus faecalis* ATCC 29737, *Bacillus subtilis* ATCC 465, *Bacillus pumilus* PTCC 1114, *Micrococcus luteus PTCC* 1110, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Sterptococcus mutans* PTCC 1601 (Gram-positive bacteria). The minimum inhibitory concentration of the synthesized compounds determined by microdilution method [37] and compared to two commercial antibiotics (Table 2).

As can be seen from Table 2, good to improved antibacterial activity was observed for most of the compounds against all species of Gram positive and Gram negative bacteria used in the study. Almost, all of the

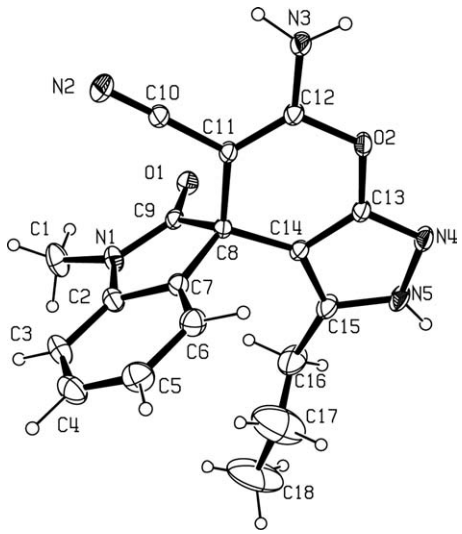
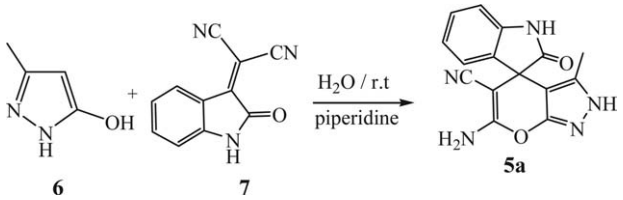
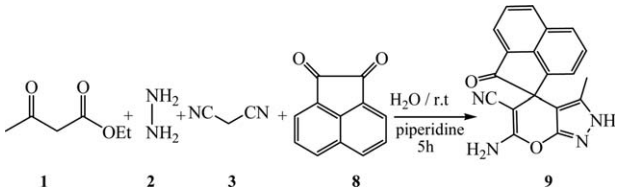


Figure 2. X-ray crystal structure of **5j**.

Scheme 2



Scheme 4



compounds were found to be more active than Gentamicin against all tested strains.

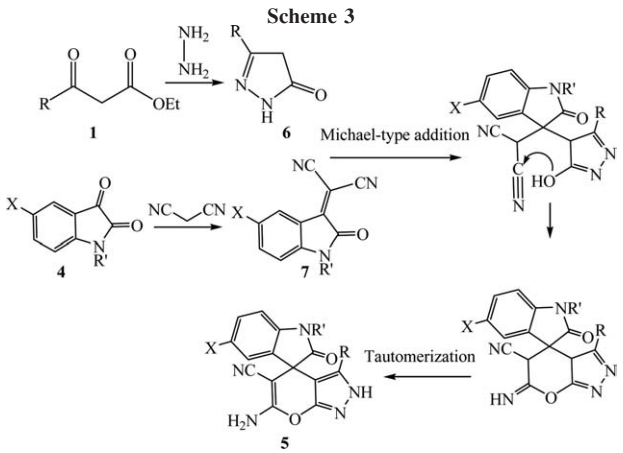
In conclusion, we have developed a facile, one-pot and four-component procedure for the preparation of 1′*H*-spiro[indoline-3,4′-pyrano[2,3-*c*]pyrazole]-5′-carbonitriles of potential synthetic and biological interest. The method is simple, starts from readily accessible commercial reagents, and provides biologically interesting spirooxindol derivatives in good yields. Almost most of the compounds exhibited good to excellent antibacterial activity against all the tested strains.

EXPERIMENTAL

Melting points were taken on an Electrothermal 9100 apparatus and left uncorrected. IR spectra were obtained on a Shimadzu IR-470 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER DRX-300 AVANCE spectrometer at 300.13 and 75.47 MHz. NMR spectra were obtained on solutions in DMSO using TMS as internal standard. All of the chemicals were purchased from Fluka, Merck, and Aldrich and used without purification.

Typical procedure for the preparation of 6′-amino-3′-methyl-2-oxo-2′*H*-spiro[indoline-3,4′-pyrano[2,3-*c*]pyrazole]-5′-carbonitrile (5a). To a magnetically stirred solution of ethyl acetoacetate **1a** (1 mmol) and hydrazine hydrate 96% **2** (1 mmol) in water (5 mL) for 0.5 h were added isatin **4a** (1 mmol), malononitril **3** (1 mmol), and piperidine (0.3 mmol) at room temperature. The mixture was finally stirred for 4.5 h. After completion of the reaction (TLC), the reaction mixture was filtered off and the residue was washed with water (10 mL) and then residue recrystallized from EtOH to afford the pure product **5a** as brown powder (89%). m.p. 224°C (dec). IR (KBr) (ν_{max}/cm^{-1}): 3383, 3332, 2182, 1714. MS (EI, 70 eV) *m*/*z*: 293 (M$^+$). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.52 (3H, s, CH_3), 6.88–7.25 (6H, m, H-Ar, and NH_2), 10.60 (1H, s, NH), 12.28 (1H, s, NH). ^{13}C NMR (DMSO-d$_6$): δ_c = 9.4, 47.7, 55.5, 95.8, 110.1, 119.2, 122.9, 124.9, 129.3, 133.1, 135.1, 141.9, 155.7, 162.9, 178.4. Anal. Calcd. for $C_{15}H_{11}N_5O_2$:C, 61.43; H, 3.78; N, 23.88. Found: C, 61.38; H, 3.74; N, 23.80%.

Scheme 3



6′-Amino-1,3′-dimethyl-2-oxo-2′*H*-spiro[indoline-3,4′-pyrano[2,3-*c*]pyrazole]-5′-carbonitrile (5b). Cream powder (85%); m.p. 262°C (dec). IR (KBr) (ν_{max}/cm^{-1}): 3374, 3329, 2188, 1709. MS (EI, 70 eV) *m*/*z*: 307 (M$^+$). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.44 (3H, s, CH_3), 3.19 (3H, s, CH_3), 7.08–7.33 (6H, m, H-Ar, and NH_2), 12.30 (1H, s, NH). ^{13}C NMR (DMSO-d$_6$): δ_c = 9.3, 26.7, 47.4, 55.1, 95.6, 109.1, 119.1, 123.6, 124.6, 129.5, 132.3, 135.2, 143.3, 155.6, 163.0, 176.7. Anal. Calcd for $C_{16}H_{13}N_5O_2$: C, 62.53; H, 4.26; N, 22.79%. Found: C, 62.59; H, 4.21; N, 22.73%.

6′-Amino-5-bromo-3′-methyl-2-oxo-2′*H*-spiro[indoline-3,4′-pyrano[2,3-*c*]pyrazole]-5′-carbonitrile (5e). Cream powder (95%); m.p. 243°C (dec). IR (KBr) (ν_{max}/cm^{-1}): 3343, 3132, 2182, 1709. MS (EI, 70 eV) *m*/*z*: 373 (M$^+$), 371 (M$^+$). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.58 (3H, s, CH3), 6.87–7.44 (5H, m, H-Ar, and NH2), 10.76 (1H, s, NH), 12.34 (1H, s, NH). ^{13}C NMR (DMSO-d$_6$): δ_c = 9.5, 47.9, 54.9, 95.1, 112.2, 114.6, 119.1, 127.7, 132.2, 135.2, 135.5, 141.2, 155.6, 162.9, 178.0. Anal. Calcd. for $C_{15}H_{10}BrN_5O_2$: C, 48.41; H, 2.71; N, 18.82%. Found: C, 48.45; H, 2.64; N, 18.74%.

6′-Amino-3′-methyl-5-nitro-2-oxo-2′*H*-spiro[indoline-3,4′-pyrano[2,3-c]pyrazole]-5′-carbonitrile (5h). Dark red powder (80%); m.p. 270°C (dec). IR (KBr) (ν_{max}/cm^{-1}): 3048, 1706, 1666, 1607. MS (EI, 70 eV) *m*/*z*: 492 (M$^+$). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 5.00 (bs, 2H, NCH_2), 6.72–7.81 (m, 17H, ArH), 11.68 (s, 1H, NH). Anal. Calcd for $C_{33}H_{20}N_2O_3$: C, 80.47; H, 4.09; N, 5.69%. Found: C, 80.38; H, 4.01; N, 5.58%.

6′-Amino-3′-propyl-2-oxo-2′*H*-spiro[indoline-3,4′-pyrano[2,3-*c*]pyrazole]-5′-carbonitrile (5i). Cream powder (92%); m.p. 230°C (dec). IR (KBr) (ν_{max}/cm^{-1}): 3322, 3183, 2192, 1716. MS (EI, 70 eV) *m*/*z*: 321 (M$^+$). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 0.52 (3H, t, 3JHH= 7.2 Hz, CH3), 0.94–1.16 (2H, m, CH2), 1.85 (2H, t, 3JHH = 7.5 Hz, CH2), 6.88–7.26 (6H, m, H-Ar and NH2), 10.61 (1H, s, NH), 12.29 (1H, s, NH). ^{13}C NMR (DMSO-d$_6$): δ_c = 13.8, 21.4, 26.3, 47.8, 55.7, 95.5, 110.0, 119.2, 122.9, 125.1, 129.4, 133.5, 139.6, 141.9, 155.5, 162.8, 178.8. Anal. Calcd for $C_{17}H_{15}N_5O_2$: C, 63.54; H, 4.71; N, 21.79%. Found: C, 63.50; H, 4.76; N, 21.70%.

6′-Amino-1-methyl-2-oxo-3′-propyl-2′*H*-spiro[indoline-3,4′-pyrano[2,3-*c*]pyrazole]-5′-carbonitrile (5j). Cream powder (90%); m.p. 251°C (dec). IR (KBr) (ν_{max}/cm^{-1}): 3388, 3316, 2196, 1710. MS (EI, 70 eV) *m*/*z*: 335 (M$^+$). ^{1}H NMR (300

Table 2

MIC (μg/mL) values of products **5** and **9**.

	Products													Standard	
	5a	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	**5j**	**5k**	**5l**	**9**	Tetracycline	Gentamicin
Bacillus subtilis	16	8	4	4	*	32	16	16	32	24	4	8	16	4	*
Bacillus pumilus	6	4	2	<2	<2	8	4	2	64	32	16	32	32	8	*
Micrococcus luteus	2	2	2	<2	8	16	4	2	16	16	8	64	4	4	*
Staphylococcus aureus	8	6	4	4	8	2	16	32	2	2	*	*	16	4	*
Staphylococcus epidermidis	6	2	2	2	8	<2	2	8	64	32	*	*	8	<2	*
Sterptococcus mutans	6	4	2	<2	128	16	2	64	4	4	6	8	32	2	*
Escherichia coli	4	2	2	2	4	2	4	4	4	2	4	4	4	*	4
Enterococcus faecalis	6	4	2	2	8	16	4	4	32	16	2	4	2	8	*
Pseudomonas aeruginosa	8	4	2	4	2	2	2	<2	<2	<2	4	4	8	*	8

* Not active.

MHz, DMSO-d_6): δ_H 0.50 (3H, t, 3JHH = 7.2 Hz, CH3), 0.93–1.03 (2H, m, CH2), 1.66–1.82 (2H, m, CH2), 3.18 (3H, s, CH3), 7.04–7.37 (4H, m, H-Ar), 7.26 (2H, s, NH2), 12.31 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ_c = 13.8, 21.4, 26.3, 26.7, 47.4, 55.2, 95.3, 109.1, 119.0, 123.7, 124.7, 129.5, 132.6, 139.5, 143.3, 155.4, 162.9, 177.0. Anal. Calcd. for $C_{18}H_{17}N_5O_2$: C, 64.47; H, 5.11; N, 20.88%. Found: C, 64.43; H, 5.16; N, 20.94%.

4-Methyl-1*H*-pyrrol-2-ol (6). White powder; m.p. 219–222°C. IR (KBr) (ν_{max}/cm^{-1}): 3314, 3324, 2199, 1687. MS (EI, 70 eV) *m*/*z*: 98 (M^+). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.35 (3H, s, CH3), 5.81 (1H, s, CH),12.86 (1H, s, NH).

2-(2-Oxoindolin-3-ylidene)malononitrile (7). Brick-red powder; m.p. 215–217°C. IR (KBr) (ν_{max}/cm^{-1}): 3236, 3016, 2232, 1611. MS (EI, 70 eV) *m*/*z*: 3195 (M^+). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 6.91–7.86 (4H, m, H-Ar), 11.20 (1H, s, NH).

6′-Amino-3′-methyl-2-oxo-2*H*,2′*H*-spiro[acenaphthylene-1,4′-pyrano[2,3-*c*]pyrazole]-5′-carbonitrile (9). Brown powder (87%); m.p. 246°C (dec). IR (KBr) (ν_{max}/cm^{-1}): 3414, 3320, 2187, 1714. MS (EI, 70 eV) *m*/*z*: 328 (M^+). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.05 (3H, s, CH3), 7.31 (2H, s, NH2), 7.45–8.40 (6H, m, H-Ar), 12.26 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ_c = 9.5, 52.1, 56.2, 96.7, 119.3, 121.7, 123.0, 125.4, 129.4, 129.9, 130.4, 131.0, 133.1, 135.1, 141.4, 141.5, 155.7, 162.9, 204.3. Anal. Calcd. for $C_{19}H_{12}N_4O_2$: C, 69.51; H, 3.68; N, 17.06.%. Found: C, 69.57; H, 3.64; N, 17.11%.

Acknowledgments. The authors gratefully acknowledge financial support from the Research Council of Shahid Beheshti University.

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[36] X-Ray data for **5j**: $C_{18}H_{17}N_5O_2$, $M = 335.37$ g/mol, monoclinic system, space group C2/c, $a = 18.0812(12)$, $b = 8.0724(7)$, $c = 23.3904(16)$ Å, $\beta = 95.122(5)^\circ$, $V = 3400.4(4)$ Å^3, $Z = 8$, Dc $= 1.31$ g cm^{-3}, μ(Mo-Kα) $= 0.090$ mm^{-1}, crystal dimention of 0.30 mm $\times$ 0.21 mm $\times$ 0.12 mm. The structure was solved by using SHELXS. The structure refinement and data reduction were carried out with SHELXL of the X-Step32 suite of programs [38]. The nonhydrogen atoms were refined anisotropically by full matrix least-squares on F^2 values to final $R1 = 0.0853$, wR2 $= 0.1987$, and $S = 1.092$ with 256 parameters using 4600 independent reflection (θ range $= 1.75$–29.32°). Hydrogen atoms were located from expected geometry and were not refined. Crystallographic data for **5j** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to The Director, CCDC 732154, Union Road, Cambridge CB2 1EZ, UK. Fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk.

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