VERSITA

Chemical Papers 64 (5) 673–677 (2010)
DOI: 10.2478/s11696-010-0047-1

SHORT COMMUNICATION

Rapid synthesis and bioactivities of 3-(nitromethylene)indolin-2-one analogues

[a,b]Gang Chen, [b,c]Xiao-Jiang Hao*, [c]Qian-Yun Sun, [d]Jian Ding

[a] *College of Chemistry & Chemical Engineering, Xi'an Shiyou University, Xi'an, Shaanxi 710065, China*

[b] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, China*

[c] *Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, China*

[d] *State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*

Received 28 November 2009; Revised 23 February 2010; Accepted 2 March 2010

A new synthesis method of 3-(nitromethylene)indolin-2-one analogues is described, using the Henry reaction of isatin and *N*-substituted isatins with nitromethane followed by dehydration of the nitroaldol adduct with mesyl chloride. The use of diethylamine (rather than DBU) as the base catalyst in a solvent-free Henry reaction gave the nitroaldol adduct in sufficient purity as to allow its direct dehydration to nitroalkene. Overall yields for this two-step synthesis are satisfactory (typically 50–77 % after chromatographic purification). 3-(Nitromethylene)indolin-2-one analogues are valued protective agents against H_2O_2-induced apoptosis using PC12 cells, and for their cytotoxicity against the A549 and P388 lung cancer cell lines. One compound, (*E*)-1-benzyl-3-(nitromethylene)indolin-2-one (*VIII*), exhibited potent activity in the latter assay.

Keywords: 3-(nitromethylene)indolin-2-one, diethylamine, antitumor neuroprotection, catalyst

Isatin is an endogenous compound in mammalian tissues and body fluids (Glover et al., 1988) as well as in many plants. In addition, isatin is a versatile intermediate in the synthesis of potential drugs. Recently, 3-substituted indolin-2-ones have attracted attention due to their antitumor therapeutic activities (Sun et al., 1999, 2000). 3-(Methylene)indolin-2-one derivatives in particular have received extensive biological scrutiny (Laird et al., 2000; Fong et al., 1999; Mendel et al., 2000). One such analogue, SU11248 (product name: Sunitinib malate), was found to be clinically effective as an antiangiogenesis drug.

3-(Nitromethylene)indolin-2-one derivatives have been used as intermediates in the synthesis, by 1,3-dipolar cycloadditon reactions, of spiro[pyrrolidine-2,3′-oxindole] derivatives (Palmisano et al., 1996; Fejes et al., 2001; Long et al., 1978). For this purpose, the intermediate 3-hydroxy-3-(nitromethyl)indolin-2-one (*I*) (Fig. 1, R = H) was synthesized by the Henry nitroaldol reaction between isatin and nitromethane followed by a dehydration reaction to give 3-(nitromethylene)indolin-2-one (*II*) (Fig. 1, R = Me). Conn and Lindwall (1936) reported the synthesis of *I* in an ethanol solution using diethylamine as the catalyst; however, the reaction time was long (one day) and the yield was only moderate (71 %). The use of a stronger organic base, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), as the catalyst, did not improve this yield (Palmisano et al., 1996; Fejes et al., 2001; Long et al., 1978).

*Corresponding author, e-mail: gangchen@xsyu.edu.cn

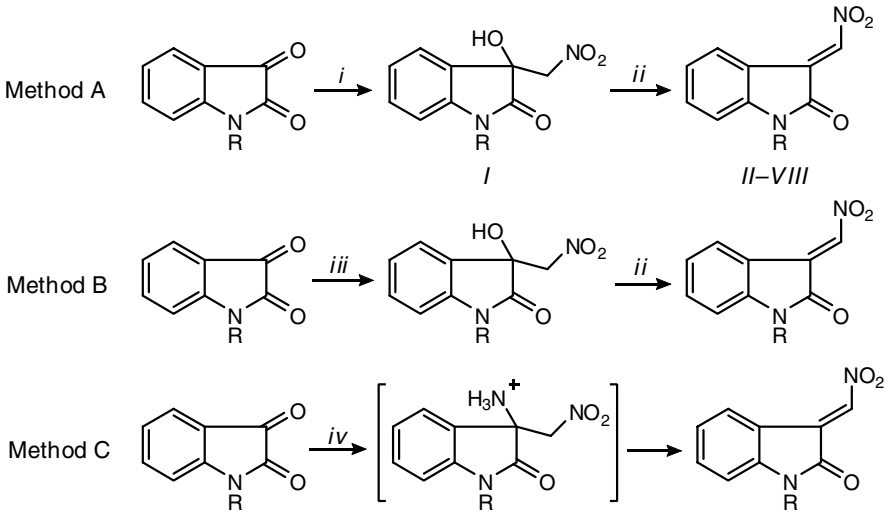

Fig. 1. Synthesis of (*E*)-1-alkyl-3-(nitromethylene)indolin-2-ones (*II*, R = H; *III*, R = methyl; *IV*, R = ethyl; *V*, R = propyl; *VI*, R = butyl; *VII*, R = pentyl; *VIII*, R = benzyl). Reaction conditions: *i*) CH_3NO_2, Et_2NH; *ii*) CH_3SO_2Cl, Et_3N; *iii*) CH_3NO_2, DBU; *iv*) CH_3NO_2, AcOH, NH_4OAc.

In order to increase the overall yield, a solvent-free synthesis of *N*-substituted analogues of *I* using diethylamine as the catalyst was developed. Without further purification of the nitroaldol intermediate, MsCl-dependent dehydration produced 3-(nitromethylene)indolin-2-one derivatives (Fig. 1, method A). A small series of these derivatives was prepared and their protective bioactivities on H_2O_2-induced apoptosis of PC12 cells and cytotoxicity against lung cancer A549 and P388 cell lines of these compounds were evaluated as a result of our continued interest in indole alkaloids (Chen et al., 2006, 2009; Di et al., 2007).

All starting materials and solvents (A.R. grade) were commercially available and were used without further purification. NMR spectra were recorded using a Bruker Drx-400 spectrometer operating at 400 MHz for ^1H and at 100 MHz for ^{13}C. Mass spectra were recorded on a Micromass Platform spectrometer using a direct-inlet system operating in the electron impact (EI) mode at 75 eV. Elemental analyses were obtained using a Carlo Erba 1106 elemental analyzer.

Spectral and analytical data of known (±)-3-hydroxy-3-(nitromethyl)indolin-2-one (*I*) (Fig. 1, R = H) and (*E*)-3-(nitromethylene)indolin-2-one (*II*) (Fig. 1, R = H) were in accordance with those already published (Elinson et al., 2008; Conn & Lindwall, 1936; Morales-Ríos et al., 2000).

New (*E*)-1-alkyl-3-(nitromethylene)indolin-2-ones (Fig. 1: *III*, R = methyl; *IV*, R = ethyl; *V*, R = propyl; *VI*, R = butyl; *VII*, R = pentyl; *VIII*, R = benzyl) were prepared according to the general procedure as follows: *N*-substituted isatin (0.294 g, 2 mmol) was dissolved in nitromethane (15 mL). One drop of diethylamine was added and the mixture was stirred at room temperature for a few minutes until the orange-colored solution turned colorless. The solvent was removed under reduced pressure to give the nitroaldol adduct as a white powder. This powder was dissolved in CH_2Cl_2 (20 mL), followed by an addition of triethylamine (3 mL) and methanesulfonyl chloride (0.5 mL). The reaction mixture was cooled to 0° and stirred at this temperature overnight. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate, gradient of $\varphi_r = 50 : 1$ to $\varphi_r = 20 : 1$) to give the target products. Analytical samples of red (or orange) crystals of 3-(nitromethylene)indolin-2-ones were obtained by crystallization from ethanol. *E*-stereochemistry was assigned to these products on the basis of literature data (Palmisano et al., 1996; Fejes et al., 2001). Analytical and spectral data are given in Tables 1 and 2.

The use of diethylamine as the base catalyst in the solvent-free reaction of isatin and nitromethane resulted in an effective and fast reaction, proceeding within a few minutes. Removal of the solvent from this reaction mixture gave 3-hydroxy-3-(nitromethyl)indolin-2-one (*I*, R = H) in a nearly quantitative yield. The yield and purity of this intermediate allowed its direct dehydration, without its isolation (Fig. 1, method A), with methanesulfonyl chloride to give 3-(nitromethylene)indolin-2-one (*II*, R = H). The overall yield of these two steps reached 77 % after chromatographic purification. (*E*)-1-Methyl-3-(nitromethylene)indolin-2-one (*III*) was obtained by this method in the 61 % yield. For comparison, previous syntheses (Fig. 1, method B) of two compounds (*II* and *III*), as well as classical synthesis of (*E*)-β-nitrostyrene (Fig. 1, method C), were repeated. The results showed that method A consistently provided improved yields compared to methods B and C. Moreover, method A offers short reaction times for the first step.

Table 1. Characterization data of newly prepared (*E*)-1-alkyl-3-(nitromethylene)indolin-2-ones

Compound	Formula	M_r	w_i(calc.)/mass % w_i(found)/mass %			Yield	M.p.
			C	H	N	%	℃
III	$C_{10}H_8N_2O_3$	204.18	58.82	3.95	13.72	61	137–138
			58.80	3.95	13.71		
IV	$C_{11}H_{10}N_2O_3$	218.21	60.55	4.62	12.84	55	142–143
			60.56	4.60	12.80		
V	$C_{12}H_{12}N_2O_3$	232.24	62.06	5.21	12.06	53	147–148
			62.01	5.22	12.07		
VI	$C_{13}H_{14}N_2O_3$	246.26	63.40	5.73	11.38	53	150–151
			63.44	5.71	11.33		
VII	$C_{14}H_{16}N_2O_3$	260.29	64.60	6.20	10.76	51	151–153
			64.62	6.21	10.75		
VIII	$C_{16}H_{12}N_2O_3$	280.29	68.56	4.32	9.99	62	165–167
			68.59	4.33	10.02		

Table 2. Spectral data of newly prepared (*E*)-1-alkyl-3-(nitromethylene)indolin-2-ones

Compound	Spectral data
III	^{1}H NMR ($CDCl_3$), δ: 11.40 (s, 1H, $HCNO_2$), 8.10 (d, 1H, J = 7.2 Hz, H_{aryl}), 7.26 (s, 1H, H_{aryl}), 7.09 (t, 1H, J = 7.6 Hz, H_{aryl}), 6.83 (t, 1H, J = 7.2 Hz, H_{aryl}), 3.28 (s, 3H, CH_3) ^{13}C NMR ($CDCl_3$), δ: 26.2 (CH_3), 121.2, 122.5, 123.9, 126.6, 128.1, 138.2 ($CHNO_2$), 141.5, 144.5, 169.1 (C═O) MS, m/z (I_r/%): 204 (24) (M^+)
IV	^{1}H NMR ($CDCl_3$), δ: 11.60 (s, 1H, $HCNO_2$), 8.12 (d, 1H, J = 7.6 Hz, H_{aryl}), 7.37 (t, 1H, J = 7.6 Hz, H_{aryl}), 7.07 (t, 1H, J = 7.6 Hz, H_{aryl}), 6.85 (d, 1H, J = 7.6 Hz, H_{aryl}), 3.81 (q, 2H, J = 7.2 Hz, NCH_2), 1.29 (t, 3H, J = 7.2 Hz, CH_3) ^{13}C NMR ($CDCl_3$), δ: 14.6 (CH_3), 31.2 (CH_2), 121.1, 123.2, 123.7, 126.7, 128.4, 138.8 ($CHNO_2$), 141.7, 146.6, 169.1(C═O) MS, m/z (I_r/%): 218 (18) (M^+)
V	^{1}H NMR ($CDCl_3$), δ: 11.30 (s, 1H, $HCNO_2$), 8.11 (d, 1H, J = 7.2 Hz, H_{aryl}), 7.39 (t, 1H, J = 8.0 Hz, H_{aryl}), 7.08 (t, 1H, J = 8.0 Hz, H_{aryl}), 6.86 (d, 1H, J = 8.0 Hz, H_{aryl}), 3.72 (t, 2H, J = 7.2 Hz, NCH_2), 1.73 (m, 2H, CH_2), 0.98 (t, 3H, J = 7.2 Hz, CH_3) ^{13}C NMR ($CDCl_3$), δ: 22.1 (CH_3), 26.8 (CH_2), 40.1 (CH_2), 121.2, 122.3, 124.0, 125.9, 128.7, 137.9 ($CHNO_2$), 142.0, 146.5, 169.1 (C═O) MS, m/z (I_r/%): 232 (21) (M^+)
VI	^{1}H NMR ($CDCl_3$), δ: 11.20 (s, 1H, $HCNO_2$), 8.12 (d, 1H, J = 7.6 Hz, H_{aryl}), 7.37 (m, 1H, H_{aryl}), 7.07 (m, 1H, H_{aryl}), 6.86 (d, 1H, J = 8.0 Hz, H_{aryl}), 3.76 (t, 2H, J = 7.2 Hz, NCH_2), 1.68 (m, 2H, CH_2), 1.41 (m, 2H, CH_2), 0.96 (t, 3H, J = 7.6 Hz, CH_3) ^{13}C NMR ($CDCl_3$), δ: 22.4 (CH_3), 26.9 (CH_2), 28.8 (CH_2), 40.2 (CH_2), 121.4, 122.5, 123.5, 126.9, 128.0, 138.7 ($CHNO_2$), 141.8, 146.2, 169.0 (C═O) MS, m/z (I_r/%): 246 (27) (M^+)
VII	^{1}H NMR ($CDCl_3$), δ: 11.18 (s, 1H, $HCNO_2$), 8.12 (d, 1H, J = 7.2 Hz, H_{aryl}), 7.39 (t, 1H, J = 8.0 Hz, H_{aryl}), 7.08 (t, 1H, J = 7.6 Hz, H_{aryl}), 6.86 (d, 1H, J = 8.0 Hz, H_{aryl}), 3.75 (t, 2H, J = 7.2 Hz, NCH_2), 1.70 (m, 2H, CH_2), 1.37 (m, 4H, CH_2), 0.90 (m, 3H, CH_3) ^{13}C NMR ($CDCl_3$), δ: 14.2 (CH_3), 22.5 (CH_2), 26.9 (CH_2), 28.9 (CH_2), 40.2 (CH_2), 121.5, 122.6, 123.5, 126.9, 128.0, 138.7 ($CHNO_2$), 141.8, 146.2, 169.0 (C═O) MS, m/z (I_r/%): 260 (33) (M^+)
VIII	^{1}H NMR ($CDCl_3$), δ: 11.40 (s, 1H, $HCNO_2$), 8.13 (d, 1H, J = 7.6 Hz, H_{aryl}), 7.20–7.41 (m, 6H, H_{aryl}), 7.10 (m, 1H, H_{aryl}), 6.76 (d, 1H, J = 7.6 Hz, H_{aryl}), 4.97 (s, 2H, CH_2) ^{13}C NMR ($CDCl_3$), δ: 48.7 (CH_2), 121.4, 122.5, 123.1, 123.9, 126.6, 127.2, 127.6, 128.1, 128.8, 138.2 ($CHNO_2$), 146.5, 169.1 (C═O) MS, m/z (I_r/%) 280 (12) (M^+)

Compared with the yield of *III* (61 %), the yields of the other *N*-substituted isatin derivatives were slightly lower (51–62 %, after chromatographic purification).

Compounds *III*–*VII* as well as isatin were screened for their protective effect on the apoptosis of PC12 cells induced by H_2O_2 and for their cytotoxicity

Table 3. Inhibitory and protective effects of (*E*)-1-alkyl-3-(nitromethylene)indolin-2-ones

Compound	Inhibitory effect/%[a]			Protective effect/%[b]		
	200 μM	20 μM	2 μM	200 μM	20 μM	2 μM
Isatin	92	0	3	–	20	40
III	62	14	22	–	0	0
IV	78	6	7	–	28	52
V	92	16	17	44	63	66
VI	66	27	14	–	–	0
VII	83	19	19	–	0	0
VE	–	–	–	–	–	22

a) Inhibition of PC12 cell growth; *b*) protective effect on the apoptosis of PC12 cells induced by H_2O_2.

Table 4. In vitro inhibitory activities against P388 and A549 cell line

Compound	Inhibitory activity/%	
	P388	A549
Isatin	28	49
III	17	63
IV	53	53
V	51	62
VI	53	58
VIII	88	90
R-XK469	90	85

against A549 and P388 cell lines by reported methods (Chen et al., 2009; Mosmann, 1983). The results are given in Table 3.

Compounds *IV* and *V* as well as isatin showed potent activity, and were more effective than vitamin E (VE, (±)-α-tocopherol), with percentages of 52 %, 66 %, and 40 % at concentrations of 2 μM, respectively. Besides, the three compounds were weakly cytotoxic to PC12 cells at the concentrations of 2–20 μM, while the other compounds were either inactive or cytotoxic to PC12 cells.

The in vitro cytotoxicity of compounds *III*–*VI*, *VIII*, and isatin against A549 and P388 cell lines at the concentration of 100 μM are given in Table 4. Almost all compounds inhibited both cancer cell lines at the concentration of 100 μM (≥ 50 %), and were more potent than isatin. Only isatin and *III* were inactive against P388. Compound *VIII* was the most potent of the nitromethylene series showing 88 % inhibition of P388 and 90 % inhibition of A549, and also more potent than (*R*)-2-(4-(7-chloro-2-quinoxalinyloxy)phenoxy)propanoic acid (R-XK469).

Acknowledgements. This work was financially supported by the grants from the Chinese Academy of Sciences (29O702441 2P1, O7023112S1) and the Natural Science Research Plan Projects of the Shaanxi Science and Technology Department (SJ08B20).

References

Chen, G., Wang, Y., Gao, S., He, H.-P., Li, S.-L., Zhang, J.-X., Ding, J., & Hao, X.-J. (2009). Synthesis and bioactivity evaluation of 3-hydroxy-3-(phenylethynyl)indol-2-one analogues. *Journal of Heterocyclic Chemistry*, *46*, 217–220. DOI: 10.1002/jhet.58.

Chen, G., Wang, Y., He, H., Gao, S., Yang, X., & Hao, X. (2006). L-Proline-catalyzed asymmetric aldol condensation of *N*-substituted isatins with acetone. *Heterocycles*, *68*, 2327–2333. DOI: 10.3987/COM-06-10856.

Conn, W. R., & Lindwall, H. G. (1936). Oxindole amines from isatin. *Journal of the American Chemical Society*, *58*, 1236–1239. DOI: 10.1021/ja01298a042.

Di, Y.-T., He, H.-P., Wang, Y.-S., Li, L.-B., Lu, Y., Gong, J.-B., Fang, X., Kong, N.-C., Li, S.-L., Zhu, H.-J., & Hao, X.-J. (2007). Isolation, X-ray crystallography, and computational studies of calydaphninone, a new alkaloid from *Daphniphyllum calycillum*. *Organic Letters*, *9*, 1355–1358. DOI: 10.1021/ol070218r.

Elinson, M. N., Ilovaisky, A. I., Merkulova, V. M., Barba, F., & Batanero, B. (2008). Electrochemically induced Henry reaction of nitromethane and carbonyl compounds. *Tetrahedron*, *64*, 5915–5919. DOI: 10.1016/j.tet.2008.04.039.

Fejes, I., Nyerges, M., Szöllösy, A., Blaskó, G., & Töke, L. (2001). 2-Oxoindolin-3-ylidene derivatives as 2π components in 1,3-dipolar cycloadditions of azomethine ylides. *Tetrahedron*, *57*, 1129–1137. DOI: 10.1016/S0040-4020(00)01085-1.

Fejes, I., Töke, L., Nyerges, M., & Pak, C. S. (2000). Tandem in situ generation of azomethine ylides and base sensitive nitroethylene dipolarophiles. *Tetrahedron*, *56*, 639–644. DOI: 10.1016/S0040-4020(99)01028-5.

Fong, T. A. T., Shawver, L. K., Sun, L., Tang, C., App, H., Powell, T. J., Kim, Y. H., Schreck, R., Wang, X., Risau, W., Ullrich, A., Hirth, K. P., & McMahon, G. (1999). SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. *Cancer Research*, *59*, 99–106.

Glover, V., Halket, J. M., Watkins, P. J., Clow, A., Goodwin, B. L. & Sandler, M. (1988). Isatin: identity with the purified endogenous monoamine oxidase inhibitor tribulin. *Journal of Neurochemistry*, *51*, 656–659. DOI: 10.1111/j.1471-4159.1988.tb01089.x.

Laird, A. D., Vajkoczy, P., Shawver, L. K., Thurnher, A., Liang, C., Mohammadi, M., Schlessinger, J., Ullrich, A., Hubbard, S. R., Blake, R. A., Fong, T. A. T., Strawn, L. M., Sun, L., Tang, C., Hawtin, R., Tang, F., Shenoy, N., Hirth, K. P., McMahon, G., & Cherrington, J. M. (2000). SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. *Cancer Research*, *60*, 4152–4160.

Long, D. R., Richards, C. G., & Ross, M. S. F. (1978). The stereochemistry of 2-oxoindolin-3-ylidene derivatives. *Journal of*

Heterocyclic Chemistry, *15*, 633–636. DOI: 10.1002/jhet.5570150421.

Mendel, D. B., Laird, A. D., Smolich, B. D., Blake, R. A., Liang, C., Hannah, A. L., Shaheen, R. M., Ellis, L., Weitman, M., S., Shawver, L. K., & Cherrington, J. M. (2000). Development of SU5416, a selective small molecule inhibitor of VEGF receptor tyrosine kinase activity, as an anti-angiogenesis agent. *Anti-Cancer Drug Design*, *15*, 29–41.

Morales-Ríos, M. S., García-Velgara, M., Cervantes-Cuevas, H., Alvarez-Cisneros, C., & Joseph-Nathan, P. (2000). Push–pull and pull–push effects in isatylidenes *Magnetic Resonance in Chemistry*, *38*, 172–176. DOI: 10.1002/(SICI)1097-458X(200003)38:3<172::AID-MRC618>3.0.CO;2-D.

Mosmann, T. (1983). Rapid colorimetric assay for celluar growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunology Methods*, *65*, 55–63. DOI: 10.1016/0022-1759(83)90303-4.

Palmisano, G., Annunziata, R., Papeo, G., & Sisti, M. (1996). Oxindole alkaloids. A novel non-biomimetic entry to (-)-horsfiline. *Tetrahedron: Asymmetry*, *7*, 1–4. DOI: 10.1016/0957-4166(95)00406-8.

Sun, L., Tran, N., Liang, C., Hubbard, S., Tang, F., Lipson, K., Schreck, R., Zhou, Y., McMahon, G., & Tang, C. (2000). Identification of substituted 3-[(4,5,6,7-tetrahydro-1*H*-indol-2-yl)methylene]-1,3-dihydroindol-2-ones as growth factor receptor inhibitors for VEGF-R2 (Flk-1/KDR), FGF-R1, and PDGF-Rβ tyrosine kinases. *Journal of Medicinal Chemistry*, *43*, 2655–2663. DOI: 10.1021/jm9906116.

Sun, L., Tran, N., Liang, C., Tang, F., Rice, A., Schreck, R., Waltz, K., Shawver, L. K., McMahon, G., & Tang, C. (1999). Design, synthesis, and evaluations of substituted 3-[(3- or 4-carboxyethylpyrrol-2-yl)methylidenyl]indolin-2-ones as inhibitors of VEGF, FGF, and PDGF receptor tyrosine kinases. *Journal of Medicinal Chemistry*, *42*, 5120–5130. DOI: 10.1021/jm9904295.