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Short communication

"On water" expedient synthesis of 3-indolyl-3-hydroxy oxindole derivatives and their anticancer activity *in vitro*

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ABSTRACT

A series of 3-indolyl-3-hydroxy oxindole derivatives ($n = 41$) were synthesized by the green amino-catalytic method with excellent yields under mild reaction conditions. All the newly synthesized derivatives were subjected to evaluate their cytotoxic properties against different human cancer cell lines. Results indicated that about 73% of the derivatives exhibited significant anti-proliferative activities against leukemia (U937, THP-1), lung (A549) and breast cancer (MCF7) cell lines. Among them a few of the derivatives exhibited the most potent and effective cytotoxic activities on U937 (**34**, **36**, **38** and **41**) and MCF7 (**12**, **35**, **40** and **41**) cell lines, and their anti-proliferation activities are better than the positive control, Etoposide.

1. Introduction

C3 functionalised oxindole is featured heterocyclic nucleus in a number of natural products as well as medicinally relevant compounds [1]. In particular 3-substituted-3-hydroxyoxindole is an emerging new scaffold for drug discovery with a broad spectrum of biological activities including antiviral, antibacterial, antitubercular, anti-inflammatory, antiangiogenic, antifungal, anticonvulsant and new targets for cancer chemotherapy [2]. Notably these derivatives have been served as potential synthons for complex natural product synthesis [3]. Several pharmacologically active alkaloids such as Maremycin A and B, Flustramnol, Arundaphine, Donaxaridine, CPC-1, Welwitindoline C in addition to several others contain 3-hydroxyoxindole moiety as shown in (Fig. 1) [4]. Recently 3-hydroxy-3,3′-bisindolin-2-one **1** has been reported as potential synthon for the enantioselective total synthesis of optically pure(+)-folicanthine [5].

Isatins are well-known biological manifolds with a reactive keto-carbonyl group that readily undergoes condensations, resulting in C-3 functionalised oxindole derivatives. Increasing demands for more efficient and scalable synthetic processes, which are both economical and environmentally responsible in terms of feedstock, energy consumption and waste production, require the use of "green" solvents especially water.

Owing to the significance of 3-substituted-3-hydroxy-2-oxindoles and intense research activity of medicinal chemists in the construction of small bioactive chemical entities, we envisioned the combination of both indole and oxindole motif with a hydroxy-bearing C3 substitution to generate biologically attractive architectures (Fig. 2). The use of readily available isatins **1a** and indoles **1b** through a Friedal–Crafts type reaction represents an attractive one-step entry to the valuable targets (Scheme 1) [6]. The literature survey reveals that direct Friedal–Crafts reactions of isatins for the selective synthesis of 3-indolyl-3-hydroxy oxindole derivatives are less explored compared to the methods for synthesis of 3,3′-di(indolyl)oxindoles [7]. Of the developed strategies for the synthesis of target **1** organocatalysis provides an efficient protocol due to its ease of operation, tolerance of wide array of functional groups [8]. As part of our current studies on the design of new routes for C-

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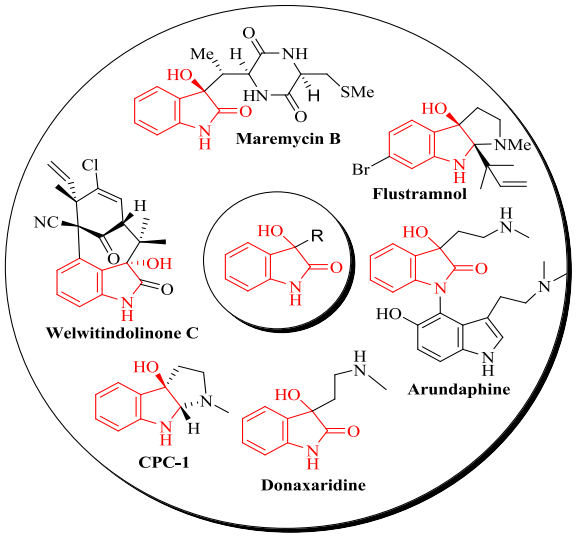


Fig. 1. Bioactive natural products built with 3-substituted-3-hydroxy-2-oxindole core scaffold.

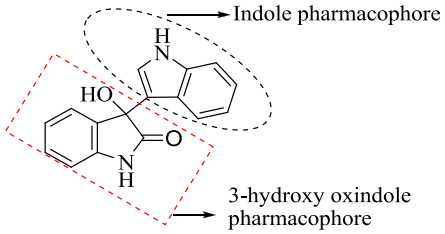
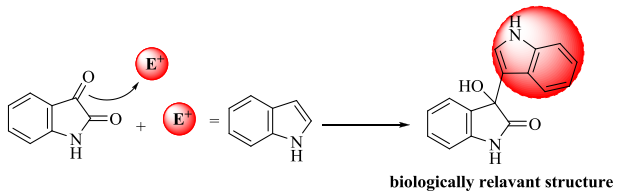


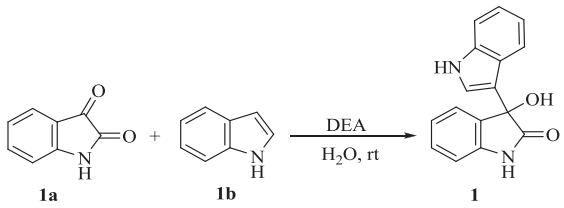
Fig. 2. Oxindole motif bearing hydroxy-group and indole at C3 position.



Scheme 1. Electrophilic addition of indoles to isatins.

3 functionalization of isatins [9] to biologically active heterocyclic compounds, we herein disclose a simple, green aminocatalytic method for the efficient synthesis of 3-indolyl-3-hydroxy oxindoles in water and their evaluation for anticancer activity.

Initially, we examined the reaction of isatin **1a** and indole **1b** for the synthesis of 3-indolyl-3-hydroxy oxindole **1** with various amine based catalysts, including triethylamine, pyrrolidine, ethylene



Scheme 2. Synthesis of 3-indolyl-3-hydroxy oxindoles from isatin.

Table 1
Synthesis of 3-indolyl-3-hydroxy oxindole derivatives[a].

Compds	R	R^1	R^2	R^3	R^4	Yield [%][b]
1	H	H	H	H	H	98
2	H	Cl	H	H	H	92
3	H	Br	H	H	H	91
4	H	I	H	H	H	83
5	H	F	H	H	H	86
6	H	Me	H	H	H	88
7	H	H	H	Cl	H	89
8	H	OCF_3	H	H	H	94
9	$PhCH_2$	H	H	H	H	81
10	Me	H	H	H	H	84
11	H	H	Br	H	H	81
12	H	OCF_3	Br	H	H	91
13	H	H	Me	H	H	89
14	H	Cl	Br	H	H	91
15	H	Br	Br	H	H	90
16	H	I	Me	H	H	81
17	H	F	Me	H	H	92
18	H	Me	Me	H	H	84
19	$PhCH_2$	H	Br	H	H	81
20	H	H	H	H	Me	83
21	H	H	OMe	H	H	91
22	H	F	OMe	H	H	94
23	H	Cl	OMe	H	H	92
24	H	I	OMe	H	H	92
25	H	F	Br	H	H	91
26	H	I	F	H	H	90
27	H	Br	OMe	H	H	92
28	H	OCF_3	Me	H	H	81
29	H	F	F	H	H	91
30	H	H	F	H	H	93
31	4-Br-$PhCH_2$	Cl	Br	H	H	89
32	2-Br-$PhCH_2$	H	Br	H	H	81
33	2-Cl-$PhCH_2$	H	Br	H	H	80
34	2-Cl-$PhCH_2$	Cl	Br	H	H	84
35	$PhCH_2$	Cl	Br	H	H	80
36	$PhCH_2$	Cl	H	H	H	88
37	2-Cl-$PhCH_2$	H	H	H	H	84
38	2-Cl-$PhCH_2$	Cl	H	H	H	88
39	2-Br-$PhCH_2$	H	OMe	H	H	86
40	2-Cl-$PhCH_2$	H	OMe	H	H	89
41	2-Cl-$PhCH_2$	Cl	OMe	H	H	85

[a] Reaction conditions: Isatin (0.5 mmol), Indole (0.5 mmol), diethanolamine (0.02 mmol) and water (2 mL) at room temperature.
[b] Overall yield of the product.

diamine, piperidine, pyridine, imidazole and diethanolamine using water as solvent. However the reaction proceeded spontaneously at ambient temperature with diethanolamine in water and the corresponding 3-indolyl-3-hydroxy oxindole **1** was obtained in 98%

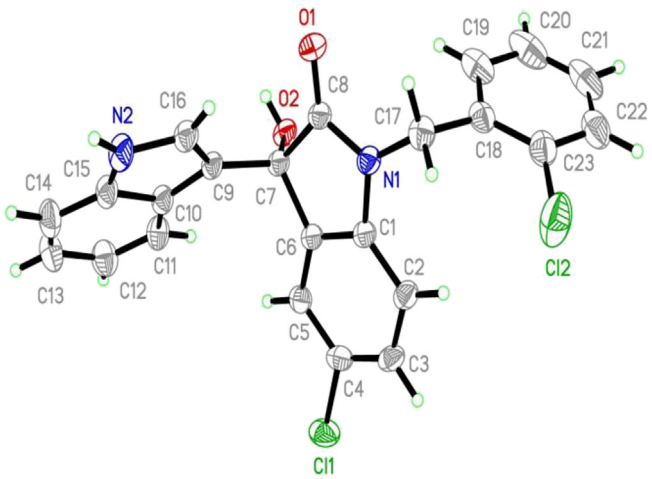


Fig. 3. ORTEP drawing of **38** (30% thermal ellipsoids).

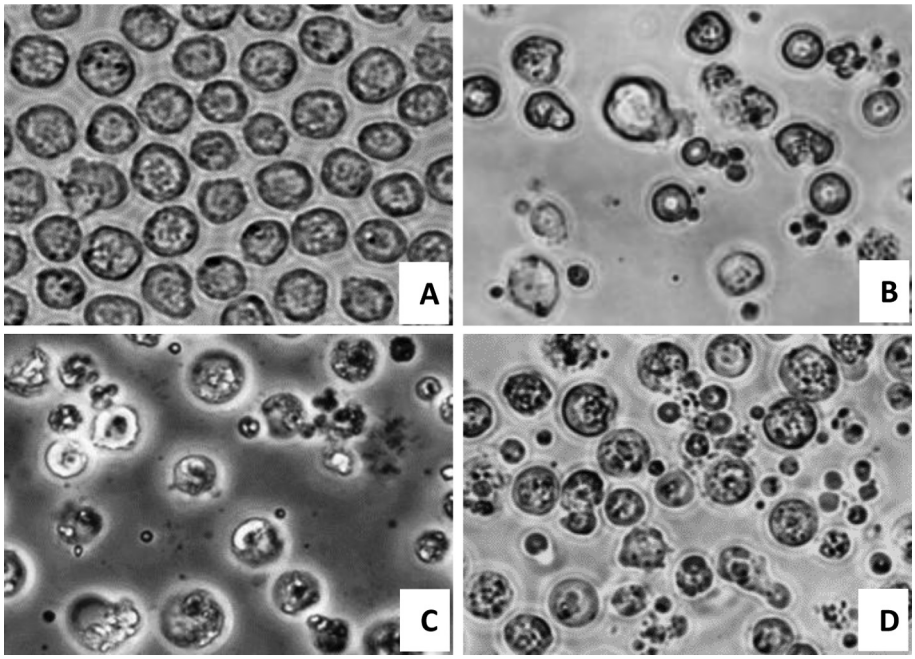


Fig. 4. Images showing morphological changes in U937 cells after exposure to IC_{50} concentrations of different test derivatives (400X). (A) Controls with intact cell structure, (B, C and D) U937 Treated cells (4.33, 6.07 and 8.07 μg/ml of derivatives 36, 41 and 35, respectively) exhibited shrinkage in cell size, nuclear condensation and formation of apoptotic bodies.

Table 2
In vitro cytotoxicity of oxindole derivatives (structural analogues) against U937, THP-1, A549 & MCF7 human cancer cells by MTT assay.

S. no/Test code	IC_{50} values[a] (μg/ml)			
	U937	THP-1	A549	MCF7
1	NA	*NA*	*NA*	*NA*
2	19.82 ± 2.22	*NA*	*NA*	*NA*
3	19.27 ± 0.22	*NA*	72.47 ± 1.68	*NA*
4	21.56 ± 0.46	*NA*	*NA*	*NA*
5	43.74 ± 2.13	*NA*	*NA*	*NA*
6	83.36 ± 4.05	*NA*	*NA*	*NA*
7	*NA*	86.14 ± 4.20	*NA*	*NA*
8	16.79 ± 2.29	13.72 ± 2.9	*NA*	71.55 ± 7.71
9	42.98 ± 0.33	64.52 ± 3.91	22.75 ± 0.75	*NA*
10	*NA*	*NA*	*NA*	48.96 ± 2.94
11	*NA*	*NA*	*NA*	*NA*
12	*NA*	*NA*	12.99 ± 2.98	5.47 ± 0.22
13	*NA*	*NA*	*NA*	*NA*
14	25.31 ± 0.38	98.52 ± 5.76	19.81 ± 0.55	95.49 ± 1.59
15	43.32 ± 0.67	86.11 ± 1.40	25.13 ± 3.04	68.70 ± 1.79
16	23.15 ± 1.31	87.11 ± 2.83	25.20 ± 1.56	84.49 ± 2.95
17	35.51 ± 0.94	*NA*	*NA*	*NA*
18	*NA*	*NA*	*NA*	*NA*
19	17.07 ± 1.6	14.17 ± 1.3	25.1 ± 3.78	31.22 ± 1.74
20	*NA*	*NA*	*NA*	*NA*
21	*NA*	*NA*	*NA*	*NA*
22	55.86 ± 1.85	*NA*	*NA*	*NA*
23	39.81 ± 0.52	*NA*	*NA*	*NA*
24	*NA*	69.18 ± 0.01	*NA*	*NA*
25	*NA*	75.95 ± 4.69	*NA*	*NA*
26	*NA*	62.4 ± 3.04	43.71 ± 1.74	*NA*
27	*NA*	*NA*	*NA*	*NA*
28	*NA*	*NA*	*NA*	*NA*
29	*NA*	*NA*	*NA*	*NA*
30	*NA*	*NA*	*NA*	*NA*
31	12.16 ± 2.27	6.06 ± 1.29	18.37 ± 1.61	20.37 ± 4.35
32	14.93 ± 0.83	6.93 ± 0.38	29.76 ± 0.61	24.73 ± 2.03
33	7.16 ± 0.17	7.37 ± 0.11	16.16 ± 0.22	22.31 ± 0.69
34	5.31 ± 2.83	6.12 ± 0.87	16.52 ± 2.31	21.26 ± 0.72
35	8.02 ± 0.91	7.12 ± 1.91	13.32 ± 1.34	8.64 ± 0.34
36	4.33 ± 0.64	5.03 ± 0.56	18.32 ± 3.23	24.03 ± 0.91
37	14.99 ± 2.65	6.97 ± 2.65	28.14 ± 1.27	39.27 ± 2.24
38	4.89 ± 2.39	7.01 ± 2.39	15.76 ± 0.78	20.62 ± 1.24
39	*NA*	*NA*	*NA*	*NA*
40	7.6 ± 0.47	5.6 ± 1.76	33.09 ± 1.67	13.44 ± 0.56
41	6.07 ± 0.35	4.27 ± 0.92	14.09 ± 0.16	10.82 ± 0.34
Etoposide[b]	6.14 ± 1.19	2.16 ± 0.15	10.71 ± 1.26	17.76 ± 1.31

Exponentially growing cells were treated with different concentrations of oxindole derivatives for 24 h and cell growth inhibition was analyzed through MTT assay.

[a] IC_{50} is defined as the concentration, which results in a 50% decrease in cell number as compared with that of the control cultures in the absence of an inhibitor and were calculated using the respective regression analysis. The values represent the mean ± SE of three individual observations.

[b] Etoposide was employed as positive control, *NA* indicates that the derivatives are not active at 100 μg/ml concentration.

yield (Scheme 2). These results provided the incentive for further study of reactions with various other isatin derivatives and substituted indoles to furnish the corresponding 3-indolyl-3-hydroxy oxindoles [12]. Diverse functionalities of isatin as well as indoles gave excellent yields (80–98%) of the desired product with diethanolamine under mild reaction conditions (Table 1).

Several N-alkylated isatins with substituents (methyl, benzyl, allyl, halo substituted benzyl) at N-1 position are synthesized by adapting the reported procedure and subjected to functionalization at C-3 position [10]. The reaction is fast with high yields with unsubstituted isatin and indoles when compared to substituted ones (Table 1). In case of Cl, Br and OCF3, at C-5 position the reaction proceeded faster with good yields for compounds **2**, **3**, and **8**. In case of benzyl and methyl substituent at the N-1 position, the reaction observed to be slow with 81 and 84 % yields respectively for compounds **9**, and **10**. The reaction of isatins with electron donating groups is rapid with high yields compared to electron withdrawing groups. All examples of isatins **1**–**41** with electron

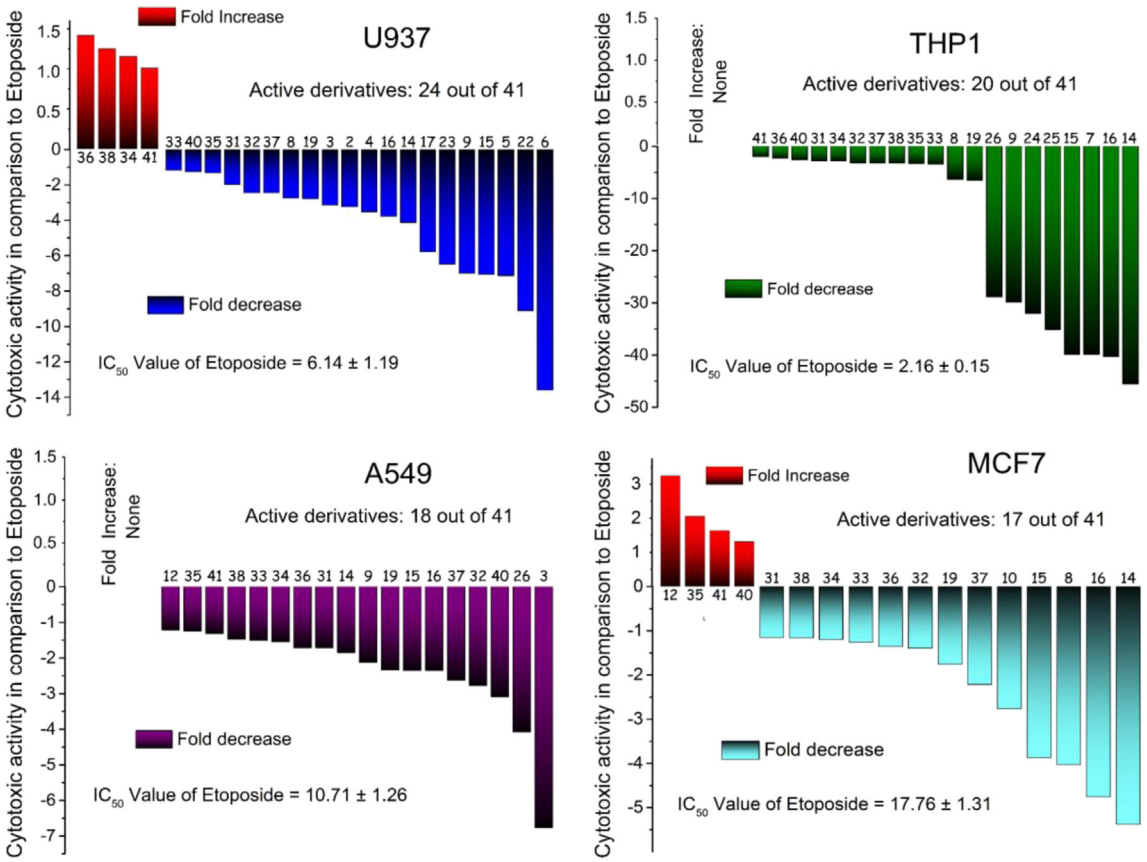
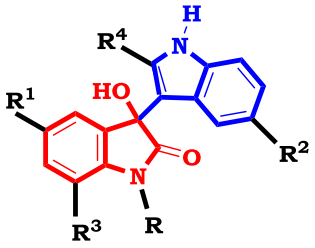


Fig. 5. Comparison of activity of oxindole derivatives on U937, THP1, A549 and on MCF7 cancer cell lines.

withdrawing groups at C-5 position, C-7 position and substituents at N-1 position and substitutions at C-5 position of indoles lead to equally satisfying results, which were then evaluated for their potential biological activity. The general structure of the obtained products i.e. 3-indolyl-3-hydroxy oxindole derivatives (**1**–**41**) are represented as follows.



The structure in the crystalline solid state of compound **38** was determined by single-crystal X-ray diffraction analysis. Suitable crystals of it for structural determination were obtained from slow evaporation of an ethyl acetate solution. Molecular structure of product **38** is shown in Fig. 3 [11].

2. *In vitro* cytotoxic activity

The biological activities of 3-indolyl-3-hydroxy oxindole derivatives (n = 41) were evaluated to investigate their anti-proliferative/cytotoxic activities on four different types of human cancer cell lines such as leukemia (U937, THP-1), lung and breast cancer cell lines (A549 and MCF7, respectively) using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay, according to the method of Mossman [13] and the exposed cells with different IC_{50} concentrations of derivatives showed the characteristic nuclear and cytological alterations (morphological changes) under light microscopy (Fig. 4). It is evident from the results that most of the test compounds have shown significant cytotoxic activity on all tested cell lines in a concentration-dependent manner (Table 2).

The oxindoles derivatives are found to be effective on U937 cells (58%) followed by THP-1 (49%), A549 (44%) and MCF7 cells (41%) at below 100 μg/ml concentration. It is apparent from the results that these derivatives are found to be more potent on U937, MCF7 cell lines followed by THP1 and A549. Among the derivatives, **3**, **12** and **26** are effective only against any of the two cell lines followed by **8** and **9** on any of the three cell lines tested. From the screened compounds, 11 derivatives were found to be active on any one of the cell lines used. The compounds **35** and **41** have exhibited an excellent cytotoxic activity against all the four cell lines.

The relative fold activity (increase/decrease) of the active derivatives in comparison to Etoposide is presented in Fig. 5. Comparatively, few of the derivatives (**34**, **36**, **38** and **41** on U937 cell lines; **12**, **35**, **40** and **41** against MCF7 cell lines) exhibited most potent activity, which are better than the activity of positive control, Etoposide, *i.e.*, 0.5–1.5 fold and 1–3 fold, respectively. The cytotoxic activities of many of these derivatives (≅12 derivatives) are pretty close to the inhibitory activity of Etoposide.

3. Conclusion

In view of synthetic and biological significance of oxindole architecture with a 3,3-disubstituted framework, we have developed

a facile protocol involving diethanolamine as catalyst for Friedal–Crafts reaction of isatin with indole for the synthesis of 3-indolyl-3-hydroxy oxindoles in water.

It can be concluded from the results that a few of the derivatives like **34**, **36**, **38**, **41** on U937 cell lines and **12**, **35**, **40**, **41** against MCF7 cell lines exhibited high potent activity than the positive control, Etoposide. The order of sensitivity of human cancer cell lines towards oxindole derivatives is MCF7 > U937 > A549 > THP1. Furthermore, these derivatives could be developed as potent anticancerous drug molecules after evaluating on other cancer cell lines and *in vivo*.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.07.004.

References

[1] (a) C.V. Galliford, K.A. Scheidt, Angew. Chem. Int. Ed. 46 (2007) 8748–8758;
(b) C. Marti, E.M. Carreira, Eur. J. Org. Chem. (2003) 2209–2219.
[2] (a) S. Peddibhotla, Curr. Bioact. Compd. 5 (2009) 20–38;
(b) C.V. Galliford, K.A. Scheidt, Angew. Chem. 119 (2007) 8902–8912.
[3] (a) T. Ueda, M. Inada, I. Okamoto, N. Morita, O. Tamura, Org. Lett. 10 (2008) 2043–2046;
(b) T. Itoh, H. Ishikawa, Y. Hayashi, Org. Lett. 11 (2009) 3854–3857;
(c) S. Lin, Z. Yang, B.H.B. Kwok, M. Koldobskiy, C.M. Crews, J. Am. Chem. Soc. 126 (2004) 6347–6355.
[4] (a) Y.Q. Tang, I. Sattler, R. Thiericke, S. Grabley, Eur. J. Org. Chem. (2001) 261–267;
(b) J.I. Jimenez, U. Huber, R.E. Moore, G.M.L. Patterson, J. Nat. Prod. 62 (1999) 569–572;
(c) V.U. Khuzhaev, I. Zhalolov, K.K. Turgunov, B. Tashkhodzhaev, M.G. Levkovich, S.F. Aripova, A.S. Shashkov, Chem. Nat. Compd. 40 (2004) 269–273;
(d) H.B. Rasmussen, J.K. MacLeod, J. Nat. Prod. 60 (1997) 1152–1154.
[5] C. Guo, J. Song, J.Z. Huang, P.H. Chen, S.W. Luo, L.Z. Gong, Angew. Chem. Int. Ed. 51 (2012) 1046–1050.
[6] (a) V.P. Kumar, R. Sridhar, B. Srinivas, M. Narender, K. Rama Rao, J. Org. Chem. 73 (2008) 1646–1648;
(b) Z.R. Musabekova, P.A. Gurevich, T.V. Zykova, Zhurnal Obshchei Khimii 64 (1994) 254;
(c) G. Shanti, N.V. Lakshmi, P.T. Perumal, ARKIVOC 29 (2009) 121–130;
(d) H.M. Meshram, D. Aravind Kumar, P.R. Goud, B.C. Reddy, Synth. Commun. 40 (2010) 39–45;
(e) J. Deng, S. Zhang, P. Ding, H. Jiang, W. Wang, J. Li, Adv. Synth. Catal. 352 (2010) 833–838;
(f) N.V. Hanhan, A.H. Sahin, T.W. Chang, J.C. Fettinger, A.K. Franz, Angew. Chem. Int. Ed. 49 (2010) 744–747;
(g) M. Hosseini-Sarvari, M. Tavakolian, Appl. Catal. A – Gen. 441 (2012) 65–71.
[7] (a) S.Y. Wang, S.J. Ji, Tetrahedron 62 (2006) 1527–1535;
(b) K. Rad-Moghadam, M. Sharifi-Kiasaraie, H. Taheri-Amlashi, Tetrahedron 66 (2010) 2316–2321.
[8] (a) P.I. Dalko, L. Moisan, Angew. Chem. Int. Ed. 43 (2004) 5138–5175;
(b) P.I. Dalko, L. Moisan, Angew. Chem. Int. Ed. 40 (2001) 3726–3748;
(c) H. Groger, J. Wilken, Angew. Chem. Int. Ed. 40 (2001) 529–532.
[9] (a) P. Sai Prathima, K. Srinivas, K. Balaswamy, R. Arundhathi, G.N. Reddy, B. Sridhar, M.M. Rao, P.R. Likhar, Tetrahedron – Asymmetry 22 (2011) 2099–2103;
(b) P. Sai Prathima, K. Srinivas, B. Sridhar, M. Mohan Rao, RSC Adv. 3 (2013) 7708–7712.
[10] (a) S.J. Garden, J.C. Torres, L.E. da Silva, A.C. Pinto, Synth. Commun. 28 (1998) 1679–1689;
(b) K.L. Vine, J.M. Locke, M. Ranson, S.G. Pyne, J.B. Bremner, J. Med. Chem. 50 (2007) 5109–5117;
(c) A.N.C. Lötter, R. Pathak, T.S. Sello, M.A. Fernandes, W.A.L. van Otterlo, C.B. de Koning, Tetrahedron 63 (2007) 2263–2274;
(d) T.M. Bridges, J.E. Marlo, C.M. Niswender, C.K. Jones, S.B. Jadhav, P.R. Gentry, H.C. Plumley, C.D. Weaver, P.J. Conn, C.W. Lindsley, J. Med. Chem. 52 (2009) 3445–3448.
[11] Supplementary crystallographic data (CIF File) for the compound with CCDC-891982 have been provided in the Supporting information. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre (CCDC), www.ccdc.cam.ac.uk/data_request/cif.
[12] *Typical experimental procedure for Friedal–Crafts reaction of isatins*: To the reaction mixture containing isatin (0.5 mmol) in water (2 mL), indole (0.5 mmol), diethanolamine (20 mol %) was slowly added at room temperature. After thecompletion of reaction as monitored by TLC, the reaction mixture was washed with brine solution and then extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and the product was purified by flash chromatography on the silica gel column using a gradient of petroleum ether/ethyl acetate, as eluent to afford pure products **1**–**41**.
[13] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.