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Bioorganic & Medicinal Chemistry Letters 17 (2007) 4924–4928
Bioorganic & Medicinal Chemistry Letters

Eco-friendly synthesis and study of new plant growth promoters: 3,3′-Diindolylmethane and its derivatives

Churala Pal,[a] Sumit Dey,[a] Sanjit Kumar Mahato,[a] Jayaraman Vinayagam,[a] Prasun K. Pradhan,[a] Venkatachalam Sesha Giri,[a] Parasuraman Jaisankar,[a,*] Tanvir Hossain,[a,†] Shikhi Baruri,[a,†] Debjit Ray[a,†] and Suparna Mandal Biswas[b]

[a]*Department of Medicinal Chemistry, Indian Institute of Chemical Biology (Unit of C.S.I.R), 4, Raja S.C. Mullick Road, Jadavpur, Calcutta 700 032, India*
[b]*Agricultural and Ecology Research Unit, Indian Statistical Institute 203, B.T. Road, Calcutta 700 108, India*

Received 4 May 2007; revised 4 June 2007; accepted 7 June 2007
Available online 10 June 2007

Abstract—3,3′-Diindolylmethane (DIM) derivatives **3a**–**k**, prepared in one-pot from indoles **1a**–**k** and hexamethylenetetramine (**2**) using ionic liquid [Bmim]BF_4 as eco-friendly recyclable solvent as well as catalyst, showed good plant growth promoting activity on *Oryza sativa*. Among the DIM derivatives synthesized **3c** shows potent auxin like growth promoting activity.

Natural auxins constitute a group of phytohormones which play an important role in plant growth activity.[1–4] Their best applications are in their ability to promote cell division and root formation.[4,5] The most widely occurring natural auxin, indole-3-acetic acid (IAA), is found in both free and conjugated state in different plants and in their seeds. The labile nature in aqueous solution of this auxin prevents its widespread use. This has resulted in the search for alternative auxin analogues.[4,6,7]

3,3′-Diindolylmethane (DIM) and its derivatives are used as dietary supplements,[8,9] which promote healthy estrogen metabolism in humans by converting both estrone and estradiol to their respective 2-hydroxy derivatives (non-carcinogenic metabolites). There are numerous reports, which show that DIM is an effective inhibitor of human prostate cancer cells.[10] Recent study[11] shows that DIM and 5-methoxy-diindolylmethane possess potential radical scavenging activities associated with cancer cells. Besides, a highly absorbable patented formulation of diindolylmethane is also under study for cervical dysplasia, breast pain, and *human papilloma virus* (HPV) related conditions.[9,12]

Room temperature ionic liquids (RTILs) are recognized as green recyclable alternatives to the traditional volatile organic solvents because of their unique chemical and physical properties.[13] As a result of their green credentials and potential to enhance rates and selectivities, ionic liquids find increasing applications in organic synthesis.[14] Recent reports have shown that they can also promote and catalyze many transformations of commercial importance under mild conditions without the need for any additional acid catalyst.[15,16] Earlier, we have reported[17] the synthesis of DIMs using $InCl_3$-HMTA. We now report herein the use of ionic liquid [Bmim]BF_4 for the synthesis of plant growth promoter diindolylmethane (DIM) and its derivatives in mild condition without the requirement for additional catalyst and solvent. Their plant growth promoting activity was tested on *Oryza sativa* over a wide concentration range. The results revealed that DIMs significantly increase germination and plant growth in comparison with **IAA**.

In the course of developing simple and efficient synthetic methodology for the preparation of bioactive DIM derivatives, it was observed that treatment of 12 equiv

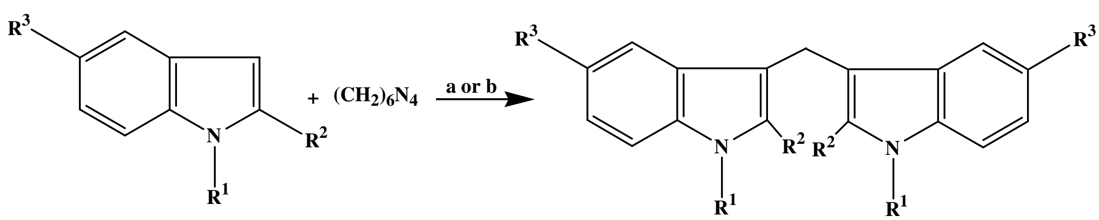
Keywords: Ionic liquid; Diindolylmethane; Plant growth promoter; Auxin; *Oryza sativa*.
* Corresponding author. Tel.: +91 33 24733491/0492; fax: +91 33 24735197; e-mail: jaisankar@iicb.res.in
† Summer trainee from Bengal College of Engineering and Technology, Durgapur 713212, India.

0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2007.06.025

of 2-phenyl-indole (**1h**) with 1 equiv of hexamethylenetetramine (HMTA) (**2**) in ionic liquid [Bmim]BF_4 at 60 °C for 7 h. resulted[18] in 2,2′-Diphenyl-3,3′-diindolylmethane (**3h**) in 82% yield (Scheme 1). This efficient method was applied to prepare a number of DIMs **3a**–**k** in very good yield (Table 1). This is the first report of the synthesis of DIMs using HMTA as methylene donor in presence of ionic liquid [Bmim]BF_4. The reaction condition requires a catalytic amount of ionic liquid (20 mol%) in *i*-PrOH at 28 °C for 7–27 h. leading to the corresponding DIMs in very good yield (Table 1). The ionic liquid [Bmim]BF_4 could be recycled three times without affecting the yield of DIMs.

The formation of DIMs from HMTA (**2**) and indoles **1a**–**k** could be explained on the basis of the earlier proposed mechanism[17] except for the fact that in this case ionic liquid [Bmim]BF_4 facilitates the formation of the ammonium complex with indole and HMTA. All the compounds **3a**–**k** have been characterized from their spectral data mainly NMR and mass spectra and further compared with the authentic samples available[17] with us.

The study of growth promoting activity[19] of DIM and its derivatives **3a**–**k** as shown in Table 2 on *O. sativa* indicates that they have good root and shoot growth



1a-k **2** **3a-k**

Scheme 1. Reagents and conditions: (a) i—[Bmim]BF_4, 60 °C, 5–20 h; ii—H_2O, 80 °C, 30 min.; (b) i—[Bmim]BF_4 (20 mol%), *i*-PrOH, 28 °C, 7–27 h; ii—H_2O, 80 °C, 30 min.

Table 1. Formation of DIM derivatives **3a**–**k** under Methods A and B

Entry	R^1	R^2	R^3	Time (h)		Isolated yield (%)	
				Method A	Method B	Method A	Method B
a	H	H	H	5	7	78	73
b	Me	H	H	7	8	71	68
c	H	Me	H	8	10	67	61
d	H	H	OMe	9	12	80	72
e	Me	H	OMe	8	10	72	67
f	H	H	Br	14	17	65	60
g	Me	H	Br	16	27	61	58
h	H	Ph	H	7	10	82	77
i	Me	Ph	H	9	13	73	67
j	Me	Me	H	10	15	66	60
k	Me	H	NO_2	20	25	59	54

Method A: (i) [Bmim]BF_4, 60 °C, 5–20 h; (ii) H_2O, 80 °C, 30 min.
Method B: (i) [Bmim]BF_4 (20 mol%), *i*-PrOH, 28 °C, 7–27 h; (ii) H_2O, 80 °C, 30 min.

Table 2. Effect of DIM derivatives in the shoot and root growth of *Oryza sativa*

Compound	31.25 ppm		62.5 ppm		125 ppm		250 ppm		500 ppm		1000 ppm	
	SL	RL	SL	RL	SL	RL	SL	RL	SL	RL	SL	RL
IAA	8.72	15.61	9.81	18.73	10.6	20.26	9.25	17.62	7.66	14.45	5.28	11.72
3a	11.24	24.56	13.41	26.33	15.67	29.50	13.01	25.87	10.95	22.37	8.45	19.85
3b	9.20	20.42	10.70	23.47	12.23	25.41	10.30	22.52	8.37	19.66	6.1	16.72
3c	16.43	40.20	19.60	42.78	21.05	46.50	18.57	40.66	16.32	38.52	13.34	35.12
3d	13.40	28.35	15.26	30.57	17.62	33.78	15.23	29.42	13.40	26.5	11.62	23.62
3e	9.78	25.70	11.53	28.25	13.56	30.45	11.42	26.72	9.66	23.26	7.80	20.78
3f	9.50	18.57	10.97	21.05	12.73	23.24	10.70	19.72	8.53	16.56	6.70	13.80
3g	9.05	16.82	10.12	19.57	11.41	22.64	9.72	18.56	8.12	15.92	5.92	12.90
3h	15.23	33.62	17.43	35.87	19.47	38.74	17.32	34.20	15.5	32.62	12.90	29.76
3i	13.80	29.56	15.44	31.24	17.23	34.46	15.01	30.27	13.62	28.24	10.80	25.60
3j	14.67	34.17	16.37	36.42	18.74	39.37	16.80	35.42	14.72	33.36	12.02	30.72
3k	8.87	16.24	9.96	18.92	10.96	21.40	9.56	18.16	7.82	15.21	5.76	12.12

SL, Shoot length in mm; RL, Root Length in mm.

promoting activities. It could be seen from Figure 1 that all the eleven derivatives of DIM, **3a**–**k**, at concentration of 125 ppm showed better growth promoting activities of both the roots and shoots of *O. sativa* compared to that of **IAA**. But, maximum growth was observed in presence of compound **3c** at the same concentration.

Since compound **3c** showed maximum growth at concentration of 125 ppm, it prompted us to study the effect of different concentration of compound **3c** on the growth of root and shoot against **IAA**. This studies (Fig. 2 and 3) clearly showed that a concentration of ~125 ppm of **3c** is the desired one as any concentration

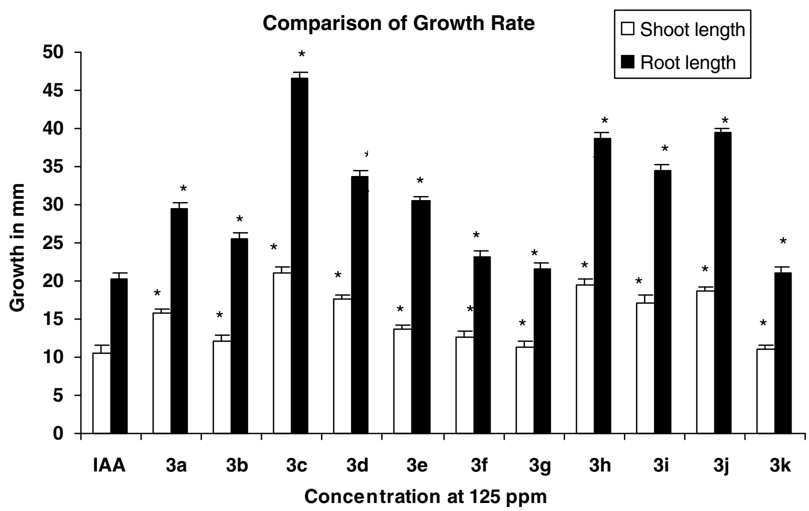


Figure 1. Data were analyzed by ANOVA and Dunnett's test. *Denotes significant promotion as compared to control ($p < 0.05$).

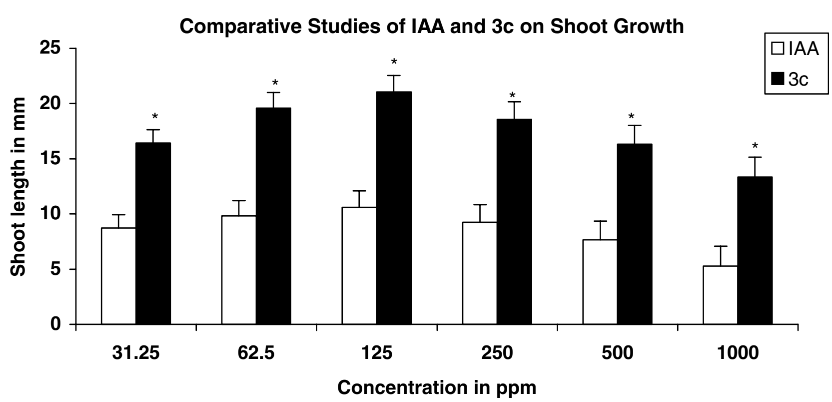


Figure 2. Data were analyzed by ANOVA and Dunnett's test. *Denotes significant promotion as compared to control ($p < 0.05$).

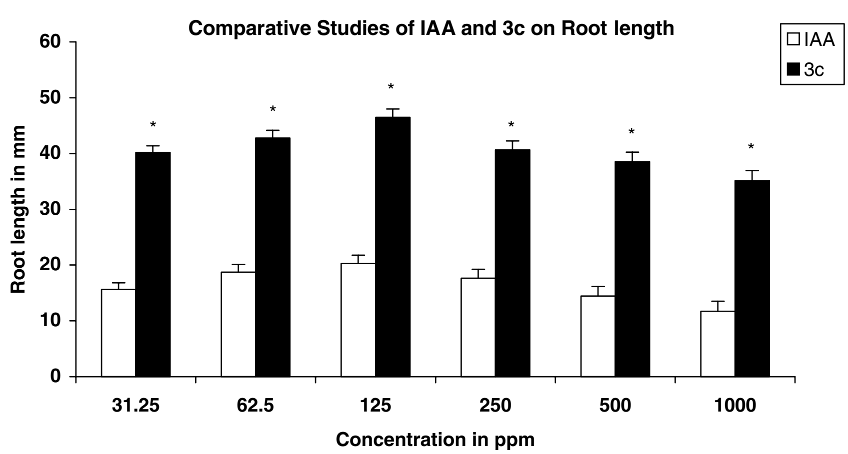


Figure 3. Data were analyzed by ANOVA and Dunnett's test. *Denotes significant promotion as compared to control ($p < 0.05$).

above or below showed slow growth which is more alike natural auxin **IAA**.

In summary, the present methodology developed by us offers an efficient one-pot synthesis of biologically important DIM and its derivatives in very good yield under eco-friendly recyclable ionic liquid [Bmim]BF_4 medium. The study of plant growth promoting activities of DIMs on *O. sativa* revealed that among the ones studied compound **3c** is the best choice for growth promoting activity than all the other ones including natural auxin **IAA**.

Acknowledgments

Authors C.P., and S.D. thankfully acknowledge CSIR, New Delhi, India, and UGC, New Delhi, India, respectively, for the financial support in the form of Research Fellowships.

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18. *General procedure for synthesis of 2,2′-diphenyl-3,3′-diindolylmethane* (**3h**): Method A: A solution of 2-phenyl indole (**1h**) (193 mg, 1 mmol) and hexamethylenetetramine (**2**) (11.76 mg, 0.084 mmol) in [Bmim]BF_4 (5 mL) was stirred at 60 °C for 7 h. After completion of the reaction, water (7 mL) was added to the reaction mixture followed by heating at 80 °C for 30 min. The reaction mixture was then extracted with diethyl ether (3× 25 mL). The organic layer was dried over anhydrous Na_2SO_4, and the solvent removed to give a solid mass. Column chromatography of the residue over silica gel using increasing concentration of chloroform in petroleum ether yielded **3h** as white solid (164 mg, 82%).
Recycling of ionic liquid [Bmim]BF_4:The ionic liquid [Bmim]BF_4 was recovered by extracting the aqueous phase of the above reaction with dichloromethane (3 × 25 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and the solvent removed to give the ionic liquid. The ionic liquid thus obtained was further dried at 80 °C under reduced pressure for subsequent runs. Method B: Ionic liquid (45.2 mg, 0.2 mmol, 20 mol%) was added to a solution of 2-phenyl indole (1 h) (193 mg, 1 mmol) and hexamethylenetetramine (2) (11.76 mg, 0.084 mmol) in dry *i*-PrOH (7 mL). The reaction mixture was then stirred at room temperature for 10 h. Water (7 mL) was added to the reaction mixture followed by heating at 80 °C for 30 min. It was then extracted with $CHCl_3$ (3× 25 mL). The organic layer was dried over anhydrous Na_2SO_4, and the solvent removed to give a solid mass. Column chromatography of the residue over silica gel using increasing concentration of chloroform in petroleum ether yielded **3h** as white solid (154 mg, 77%).
19. Comparative studies of plant growth activities with **IAA** and DIMs on Rice seeds (*Oryza sativa*):Fresh solutions of both **IAA** and DIMs in the concentration of 1000 ppm were prepared in acetone. From this stock solution further dilutions were made in the following manner:500 ppm—10 mL stock solution + 10 mL acetone; 250 ppm—10 mL of 500 ppm + 10 mL acetone; 125 ppm—10 mL of 250 ppm + 10 mL acetone; 62.5 ppm—10 mL of 125 ppm + 10 mL acetone; 31.25 ppm—10 mL of 62.5 ppm + 10 mL acetone, 15.62 ppm—10 mL of 31.25 ppm + 10 mL acetone.10 mL solutions of each concentration of both **IAA** and DIM were soaked in Whatman I filter papers, which were placed in the lower lid of a 15 mL Petri dish (Borosil glass). Petri dishes were wrapped with black electrician's tape. Both distilled water and acetone were soaked separately with same type of filter papers as control. After drying of filter papers, 15 mL of distilled water added to all Petri dishes including the control one. Seeds

were surface sterilized by soaking with 0.1% $HgCl_2$ solution followed by washing with distilled water. Seeds (42) of the same type were placed on each of the above Petri dishes in horizontal manner; otherwise their growth could be affected. The Petri dishes were placed in greenhouse in which the temperature varied from ~15 °C (night) to ~30 °C (day). Level of water was maintained at the 15 mL mark of each Petri dish before start of the days experiment to compensate the water loss. Readings of the shoot and root growths were measured in millimeter scale on day 5.