Indian Journal of Chemistry
Vol. 48B, July 2009, pp. 1013-1018

Note

A new versatile strategy for design and synthesis of reduced risk fungicides: Bibenzyl core incorporated with modified as-indacene

Pravin K Singh* & Ibadur R Siddiqui

Laboratory of Green Technology, Department of Chemistry, University of Allahabad, Allahabad 211 002, India

E-mail: irspksjs@rediffmail.com

Received 22 November 2007; accepted (revised) 30 September 2008

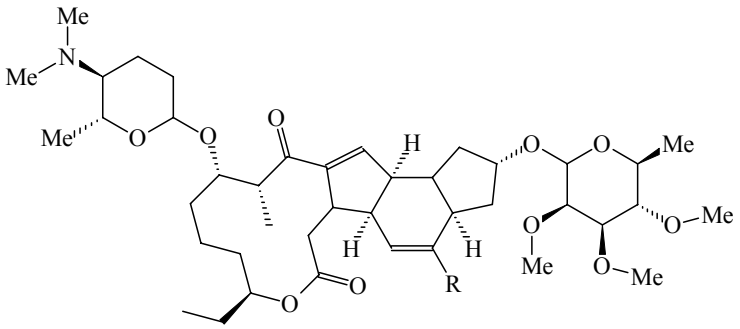
A novel montmorillonite K 10 clay catalyzed Michael addition followed by dehydrative cyclization in one-pot has been designed to synthesize 4,4′-bis(4″,8″-aryl-1″,4″-dihydro-bisthiazolo[3,2-a;5‴,4‴-e]pyrimidine-2″-thion-1″-yl)bibenzyls **6a-j** with excellent yield under microwave irradiation in solvent-free conditions. This eliminates a series of complex isolation procedures and often minimizes the use of large amount of expensive, toxic and hazardous solvents after each step. This procedure reduces reaction time, cost and enhances yield. It realizes integrated chemical process for library synthesis of bioactive compounds. Compounds **5a–j** and **6a–j** have been evaluated *in-vitro* for their fungitoxicities against *Fusarium oxysporum* and *Penicillium citrinum*. The fact that both of these fungus have developed resistance to several fungicide groups made them optimal candidates as target organisms for ongoing research about the potential application of as-indacene and analogue compounds as reduced-risk fungicides.

Keywords: Microwave induced, montmorillonite K 10 clay catalysed, cyclization, one-pot, bibenzyl.

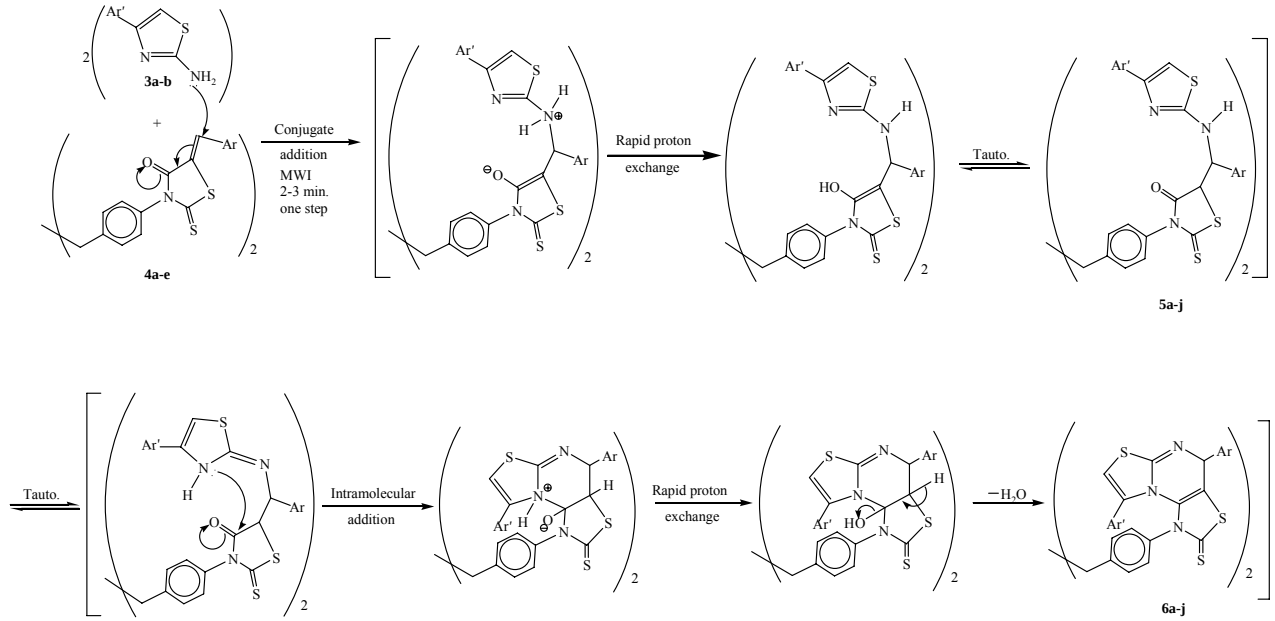
Large number of chemical crop protectants are being used for the management and control of the fungal diseases in the modern agriculture. Control of fungal diseases is essential in maintaining high agriculture productivity and to minimize monetary losses. Further, agricultural industry has successfully developed a wide array of fungicides with various chemical structures and mode of action. However, an inevitable problem associated with the use of fungicides is the occurrence of the increased resistance to commercially available agrochemicals as well as the stricter environmental and toxicological regulations now being introduced world-wide and need to be replaced by safer and more effective agrochemicals with reduced environmental and / or mammalian toxicity remains important.

The trade name of mixture of spinosyn A **1** [a.k.a.(-)-lepicidin A] and spinosyn D **2** is Spinosad™ (a.k.a. Tracer™), which is commercially produced by fermentation method and is used for pest control management. The core unit of Spinosad™ consist of as-indacene. Further, it displays relatively low toxicity in mammals as well as birds and, as such, has been designated a reduced-risk agent by the environmental protection in the USA[1].

Thiazole[2,3], rhodanine[4,5] and bibenzyl[6-8], analogues are of interest because of their biological activities. Furthermore, some fused-ring systems derived from the fussion of a rhodanine nucleus with other biolabile heterocycles have been reported to display appreciable antifungal activity[9,10]. In continuation of our study on structure-activity relationship showed that sometimes, minor changes in heterocyclic nuclei enhance the pharmacological profile many folds than



1 R = H
2 R = CH_3



Compd.	Ar	Ar′	Compd.	Ar	Ar′
6a.	C_6H_5	C_6H_5	**6f.**	C_6H_5	4-$CH_3O.C_6H_4$
6b.	4-$CH_3O.C_6H_4$	C_6H_5	**6g.**	4-$CH_3O.C_6H_4$	4-$CH_3O.C_6H_4$
6c.	4-$Cl.C_6H_4$	C_6H_5	**6h.**	4-$Cl.C_6H_4$	4-$CH_3O.C_6H_4$
6d.	3,4-$(CH_3O)_2$-C_6H_3	C_6H_5	**6i.**	3,4-$(CH_3O)_2$-C_6H_3	4-$CH_3O.C_6H_4$
6e.	4-$NO_2.C_6H_4$	C_6H_5	**6j.**	4-$NO_2.C_6H_4$	4-$CH_3O.C_6H_4$

Scheme I

parent nuclei. Further search for new, effective and safer nuclei has led to an improvement in the existing drugs by increasing their potency, duration of action and decreasing their toxic effects. Essential to these efforts is the identification of new lead candidates possessing high levels of desirable biological activities, reduced unwanted toxicities, new structural types, and perhaps different modes of action, thereby providing protection from cross-resistance to currently used agrochemicals. An additional benefit is their ability to decompose rapidly thereby reducing risk to the environment.

The biological and commercial significance of the spinosyns together with their challenging molecular architectures have prompted a number of synthetic studies[11,12]. The pivotal feature associated with our approach to develop efficacious agricultural fungicidal lead compound, promoted us to devise a convenient synthesis of the hitherto unknown title compound **6a-j** incorporating the biolabile thiozole, rhodamine and bibenzyl moieties together. The reaction sequence leading to the formation of **6a-j** and proposed mechnism is outlined in **Scheme I**.

After some preliminary experimentation, it was found that the envisaged one-pot three-step (OPTS) reactions (Michael addition, intermolecular nucleophilic addition followed by elimination) was successful. Michael addition of 2-amino-4-arylthiazole **3a,b** to 4,4′-bis(5″-arylidine rhodanin-3″-yl)bibenzyl **4a-e** under microwave irradiation in solvent-free conditions on solid support, montmorillonite K 10 followed by dehydrative cyclocondensation in one-pot leads to the formation of 4,4′-bis(4″,8″-aryl-1″,4″-dihydrobisthiazolo[3,2-a;5‴,4‴-e]pyrimidine-2″-thion-1″-yl)-bibenzyls **6a-j** with excellent yield. Results obtained showed considerable enhancement of yields (yield: 81-92%) and reduction in time (2-3 min.). This may be attributed to the efficiency in generation of **5a-j** as well as in its use for intramolecular nucleophilic addition followed by elimination reactions. Furthermore integrated process minimizes the mechanical loss of the intermediate during process of isolation. Coexistence of acidic and basic sites on surface of montmorillonite K 10 accelerated the organic reactions synergistically. It was also ascertained that other mineral support viz., basic alumina, silica gel or neutral alumina was less effective resulting in either no reaction with basic alumina or relatively low yield (12-40%) with neutral alumina and silica gels.

Table I — Antifungal screening results of compounds **5a-j** and **6a-j**.

Compd	Av % inhibition after 96 hr against					
	F. oxysporum			*P. citrinum*		
	1000 ppm	100 ppm	10 ppm	1000 ppm	100 ppm	10 ppm
5a	40	32	15	38	30	12
5b	50	42	16	47	32	17
5c	55	34	17	51	42	21
5d	54	29	14	53	39	22
5e	65	54	30	62	41	24
5f	45	32	25	42	32	23
5g	65	25	17	64	35	24
5h	45	35	26	42	31	17
5i	54	26	15	51	35	16
5j	53	27	16	51	34	18
6a	81	63	50	89	42	35
6b	82	69	55	81	49	39
6c	100	89	62	100	78	69
6d	80	72	44	78	65	36
6e	82	48	35	87	59	37
6f	78	42	33	82	74	65
6g	100	91	65	99	48	42
6h	100	81	61	100	80	67
6i	80	56	32	78	65	31
6j	84	60	41	88	63	33
Griseofulvin	100	95	91	100	94	90
Dithane M-45	100	91	86	100	95	89

For comparison purpose, the final temperature was recorded immediately after the MW irradiation for two min and was found to reach about 90°C from 27°C (room temperature). The same reaction was also carried out using a thermostated water-bath at the same bulk of temperature (90°C) as for the microwave activated method but for a longer (optimized) period of time to accertain whether the MW method improves the yield or simply increases conversion rates. In addition to this one most important advantage of MW accelerated reactions is that when the same reaction is carried out in conventional thermal method, only first step is completed and for the remaining steps the intermediate is isolated purified and refluxed with H_2SO_4 to obtain final product. While in microwave accelerated reaction these two steps (TS) are completed in one-pot (OP). So organic synthesis involving three-step one-pot (TSOP) reactions under solvent free conditions is a basic protocol because multi-step synthesis (MSS) produces considerable waste, mainly due to a series of complex isolation procedures often involving expensive, toxic and hazardous solvents after each step. Thus, TSOP perfectly useful for library synthesis, and are finding increasing use in discovery processes for new drugs and agrochemicals.

Antifungal Screening

The *in-vitro* antifungal screening of the compounds **5a-j** and **6a-j** was carried out against *Fusarium oxysporum* and *Penicillium citrinum* by agar growth technique[15] at 10, 100 and 1000 ppm concentration using commercial fungicide, Griseofulvin and Dithane M-45 as standards. The number of replicate assays in each were three, and six replicate controls were used. No remarkable morphological change was observed in the developing fungi. The test fungi were inoculated in the centre of the petridishes and incubated at 28±1°C for 96 hr. After this time, the percent inhibition of the mycelial growth compared with that in control dishes was recorded. Most of the screened compounds showed promising fungicidal activity at 1000 ppm concentration with both the test fungi *Fusarium oxysporum* and *Penicillium citrinum*

Table II — Physical and spectral data of compounds **5a-j** and **6a-j**

Compd	Mp ºC	Yield[a] % (sec)	Yield[b] % (sec)	Yield[c] % (sec)	Yield[d] % (sec)	Yield[e] % (hr)	Mol. Formula[f]	M^+ m/z	^{1}H NMR (δ ppm) ($CDCl_3$)
5a	233	—	—	—	—	50 (7.30)	$C_{52}H_{40}N_6O_2S_6$	972	2.88 (4H, s, acyclic CH_2CH_2), 4.00 (2H, d, NH), 4.10 (2H, d, S-CH-C=O), 4.40 (2H, dd, Ar-CH-N), 6.80-7.48 (30H, m, ArH)
5b	210	—	—	—	—	65 (7.00)	$C_{54}H_{44}N_6O_4S_6$	1032	2.88 (4H, s, acyclic CH_2CH_2), 3.73 (6H, s, OCH_3), 4.00 (2H, d, NH), 4.10 (2H, d, S-CH-C=O), 4.40 (2H, dd, Ar-CH-N), 6.80-7.48 (28H, m, ArH)
5c	195	—	—	—	—	61 (7.30)	$C_{52}H_{38}Cl_2N_6O_2S_6$	1040	2.88 (4H, s, acyclic CH_2CH_2), 4.00 (2H, d, NH), 4.10 (2H, d, S-CH-C=O), 4.40 (2H, dd, Ar-CH-N), 6.80-7.48 (28H, m, ArH)
5d	180	—	—	—	—	52 (7.45)	$C_{56}H_{48}N_6O_6S_6$	1092	2.88 (4H, s, acyclic CH_2CH_2), 3.73 (12H, s, OCH_3), 4.00 (2H, d, NH), 4.10 (2H, d, S-CH-C=O), 4.40 (2H, dd, Ar-CH-N), 6.80-7.48 (26H, m, ArH)
5e	184	—	—	—	—	52 (6.45)	$C_{52}H_{38}N_8O_6S_6$	1062	2.88 (4H, s, acyclic CH_2CH_2), 4.00 (2H, d, NH), 4.10 (2H, d, S-CH-C=O), 4.40 (2H, dd, Ar-CH-N), 6.80-8.14 (28H, m, ArH)
5f	190	—	—	—	—	57 (6.30)	$C_{54}H_{44}N_6O_4S_6$	1032	2.88 (4H, s, acyclic CH_2CH_2), 3.73 (6H, s, OCH_3), 4.00 (2H, d, NH), 4.10 (2H, d, S-CH-C=O), 4.40 (2H, dd, Ar-CH-N), 6.80-7.48 (28H, m, ArH)
5g	195	—	—	—	—	54 (7.00)	$C_{58}H_{50}N_4O_6S_6$	1092	2.88 (4H, s, acyclic CH_2CH_2), 3.73 (12H, s, OCH_3), 4.00 (2H, d, NH), 4.10 (2H, d, S-CH-C=O), 4.40 (2H, dd, Ar-CH-N), 6.80-7.48 (26H, m, ArH)
5h	198	—	—	—	—	58 (6.00)	$C_{54}H_{42}Cl_2N_6O_4S_6$	1100	2.88 (4H, s, acyclic CH_2CH_2), 3.73 (6H, s, OCH_3), 4.00 (2H, d, NH), 4.10 (2H, d, S-CH-C=O), 4.40 (2H, dd, Ar-CH-N), 6.80-7.48 (26H, m, ArH)
5i	194	—	—	—	—	49 (8.00)	$C_{58}H_{52}N_6O_8S_6$	1152	2.88 (4H, s, acyclic CH_2CH_2), 3.73 (18H, s, OCH_3), 4.00 (2H, d, NH), 4.10 (2H, d, S-CH-C=O), 4.40 (2H, dd, Ar-CH-N), 6.80-7.48 (24H, m, ArH)
5j	213	—	—	—	—	55 (6.30)	$C_{54}H_{42}N_8O_8S_6$	1122	2.88 (4H, s, acyclic CH_2CH_2), 3.73 (6H, s, OCH_3), 4.00 (2H, d, NH), 4.10 (2H, d, S-CH-C=O), 4.40 (2H, dd, Ar-CH-N), 6.80-8.14 (26H, m, ArH)
6a	248	84 (180)	—	20 (170)	10 (150)	50 (11.45)	$C_{52}H_{36}N_6S_6$	936	2.88 (4H, s, acyclic CH_2CH_2), 3.50 (2H, s, Ar-CH), 5.73 (2H, s, S-CH), 7.00-7.30 (28H, m, ArH)
6b	260	86 (190)	—	22 (190)	08 (170)	51 (12.30)	$C_{54}H_{40}N_6O_2S_6$	996	2.88 (4H, s, acyclic CH_2CH_2), 3.50 (2H, s, Ar-CH), 3.73 (6H, s, OCH_3), 5.73 (2H, s, S-CH), 6.65-7.30 (26H, m, ArH)
6c	288	90 (202)	—	24 (208)	14 (200)	54 (11.45)	$C_{52}H_{34}Cl_2N_6S_6$	1004	2.88 (4H, s, acyclic CH_2CH_2), 3.50 (2H, s, Ar-CH), 5.73 (2H, s, S-CH), 7.00-7.30 (26H, m, ArH)

— Contd

Table II — Physical and spectral data of compounds **5a-j** and **6a-j —** *Contd*

Compd	Mp ºC	Yield[a] % (sec)	Yield[b] % (sec)	Yield[c] % (sec)	Yield[d] % (sec)	Yield[e] % (hr)	Mol. Formula[f]	M^+ m/z	^{1}H NMR (δ ppm) ($CDCl_3$)
6d	226	86 (204)	—	21 (212)	14 (220)	64 (12.00)	$C_{56}H_{44}N_6O_4S_6$	1056	2.88 (4H, s, acyclic CH_2CH_2), 3.50 (2H, s, Ar-CH), 3.73 (12H, s, OCH_3), 5.73 (2H, s, S-CH), 6.46-7.30 (24H, m, ArH)
6e	240	84 (198)	—	24 (210)	08 (210)	62 (12.00)	$C_{52}H_{34}N_8O_4S_6$	1026	2.88 (4H, s, acyclic CH_2CH_2), 3.50 (2H, s, Ar-CH), 5.73 (2H, s, S-CH), 7.00-8.07 (26H, m, ArH)
6f	250	92 (132)	—	26 (130)	18 (122)	63 (10.32)	$C_{54}H_{40}N_6O_2S_6$	996	2.88 (4H, s, acyclic CH_2CH_2), 3.50 (2H, s, Ar-CH), 3.73 (6H, s, OCH_3), 5.73 (2H, s, S-CH), 6.72-7.19 (26H, m, ArH)
6g	223	84 (186)	—	25 (180)	12 (170)	62 (12.20)	$C_{56}H_{44}N_6O_4S_6$	1056	2.88 (4H, s, acyclic CH_2CH_2), 3.50 (2H, s, Ar-CH), 3.73 (12H, s, OCH_3), 5.73 (2H, s, S-CH), 6.65-7.19 (24H, m, ArH)
6h	243	82 (240)	—	20 (240)	14 (240)	55 (13.00)	$C_{54}H_{38}Cl_2N_6O_2S_6$	1064	2.88 (4H, s, acyclic CH_2CH_2), 3.50 (2H, s, Ar-CH), 3.73 (6H, s, OCH_3), 5.73 (2H, s, S-CH), 6.72-7.19 (24H, m, ArH)
6i	200	94 (184)	—	25 (170)	8 (182)	56 (12.00)	$C_{58}H_{48}N_6O_6S_6$	1116	2.88 (4H, s, acyclic CH_2CH_2), 3.50 (2H, s, Ar-CH), 3.73 (18H, s, OCH_3), 5.73 (2H, s, S-CH), 6.51-7.19 (22H, m, ArH)
6j	265	89 (132)	—	24 (120)	16 (126)	54 (10.30)	$C_{54}H_{38}N_8O_6S_6$	1086	2.88 (4H, s, acyclic CH_2CH_2), 3.50 (2H, s, Ar-CH), 3.73 (6H, s, OCH_3), 5.73 (2H, s, S-CH), 6.72-8.07 (24H, m, ArH)

[a]One-pot isolated yield with montmorillonite K 10 clay,
[b]One-pot isolated yield with basic alumina,
[c]One-pot isolated yield with neutral alumina,
[d]One-pot isolated yield with silica gel,
[e]Overall yield for the corresponding stepwise process,
[f]satisfactory elemental microanalysis obtained C±0.07, H±0.09, N±0.06

(**Table I**). Among the tested compounds, **6c**, **6g** and **6h** displayed fungicidal action comparable with Griseofulvin and Dithane M-45 at 1000 ppm concentration and inhibited 42-67% mycelial growth of both fungal species even at the lowest concentration. This demonstrates that the presence of as-indacene nucleus with the bibenzyl core resulted in appreciable enhancement of fungitoxicity of these compounds.

For the most active compounds **6c**, **6g** and **6h** it was ascertained whether they are fungistatic or fungicidal. Thus, following the procedure[16] of Garber and Houston, it was found that compounds **6c**, **6g** and **6h** caused complete inhibition of mycelial growth of the test fungi in treated as well as reinoculated dishes and hence were fungicidal.

The present study indicates that bibenzyl frame work incorporated with modified as-indacene nucleus reported herein might be useful for developing efficacious fungicides by a suitable combination of heterocyclic moiety and substituent present on the as-indacene nucleus.

Experimental Section

All aromatic aldehydes, silica gel and neutral alumina were obtained from Aldrich and Fluka Chemicals and used as such without further purification. Melting points were determined on to an open glass capillary method and are uncorrected. The structural assignments of the synthesized products were based on elemental analysis (C, H, N), ^{1}H NMR spectra and mass spectra **(Table II)**. ^{1}H NMR spectra were recorded on a Bruker 40ºC (400 MHz) FT spectrometer in $CDCl_3$ using TMS as internal reference. Mass spectra were recorded on a JEOL D-300 mass spectrometer at 70 eV. Elemental analyses were carried out using a Coleman automatic carbon, hydrogen and nitrogen analyzer. An unmodified domestic household microwave oven (Padmini Essentia, Model Brownie) operating at 2450 MHz was used at a power output of 100 W for all the experiments. Completion of the reaction was monitored by TLC (silica gel). The final products were purified by column chromatography using silica gel (100 mesh) with increasing percentage of MeOH in benzene.

2-Amino-4-arylthiazole 3a,b. A standard procedure[13] was followed to furnish analytically pure **3a,b** and is in agreement with the analytical data already reported in literature.

4,4′-Bis(5′′-arylidine rhodanin-3″-yl)bibenzyl 4a-e. A standard procedure[13] was followed to furnish analytically pure **4a-e** and is in agreement with the analytical data already reported in literature.

4,4′-Bis[2″-{phenyl-(4″′-phenyl-thiazol-2″′-ylamino)-methyl}rhodanin-2″-yl]bibenzyls 5a-j (stepwise manner). A mixture of 2-amino-4-arylthiazole **3a,b** (100 mmole) and 4,4′-bis (5″-arylidine rhodanin-3″-yl)bibenzyl **4a-e** (100 mmole) was refluxed in dry ethanol for 6-8 hr. After the completion of the reaction (checked by TLC), excess of the solvent was evaporated under reduced pressure. The residue obtained was purified by flash chromatography and crystallized with benzene:MeOH (8 : 2 v/v) to give pure **5a-j**.

4,4′-Bis(4″,8″-aryl-1″,4″-dihydro-bisthiazolo-[3,2-a;5′″,4′″-e]pyrimidine-2″-thion-1″-yl)bibenzyls 6a-j (step-wise manner). Compound **5a-j** were refluxed with 70% H_2SO_4 for 4-5 hr. cooled the reaction mixture and poured into ice-cold water. The crude product obtained was purified by flash-chromatography and crystallized with ethanol to give pure **6a-j**.

4,4′-Bis(4″,8″-aryl-1″,4″-dihydro-bisthiazolo-[3,2-a;5′″,4′″-e]pyrimidine-2″-thion-1″-yl)bibenzyls 6a-j (One-pot manner). 2-Amino-4-arylthiazole **3a,b** (10 mmole) and 4,4′-bis (5″-arylidine rhodanin-3″-yl)bibenzyl **4a-e** (10 mmole) were taken in a flame dried 100 mL pyrex beaker and adsorbed on montmorillonite K 10 clay. This reaction mixture was placed in a microwave oven and irradiated for the 2-3 min. at an interval of 10 sec. After every 30 sec. the completion of reaction monitored by TLC (silica gel, benzene: ethyl acetate, 7:3 v/v), the reaction mixture was cooled to room temperature and eluted with acetone (3 × 20 mL). The eluate was evaporated under reduced pressure to obtained crude product. The residue on purification with flash chromatography gave analytically pure **6a-j**.

The reaction was also carried out with other solid adsorbent such as neutral alumina, basic alumina or silica gel in place of montmorillonite K 10 clay by same method to give **6a-j**.

Acknowledgement

The authors express their sincere thanks to RSIC, CDRI, Lucknow, India and FEAT Lab IIT, Kanpur, India, for providing microanalyses and spectra. One of the authors is also thankful to CSIR, New Delhi, for financial assistance.

References

1 Anastas P, Kirchchoff M & Williamson T, *Green Chem*, 1, **1999**, G174.
2 Patil L S, Chaudhari D T & Sengupta S R, *J Indian Chem Soc*, 71, **1994**, 693.
3 Naithani P K, Srivastava V K, Gupta T K & Shanker K, *J Indian Chem Soc*, 68, **1991**, 422.
4 Singh J S, Khan M H, Tiwari N & Nizamuddin, *Indian J Chem*, 33B, **1994**, 350.
5 Yadav L D S, Sharma, Sangeeta S & Vaish A, *J Agric Food Chem*, 40, **1992**, 1214.
6 Pavletic Z & Stilionic B, *Acta Bot. Croatica*, 22, **1963**, 133.
7 Gorham J, *Phytochemistry*, 16, **1977**, 249.
8 Pryce R J, *Phytochemistry*, 11, **1972**, 1355.
9 Siddiqui I R, Singh J, Singh P K, Dwivedi S, Shukla P K & Singh J, *Indian J Heterocyclic Chem*, 14, **2005**, 231.
10 Siddiqui I R, Singh P K, Singh J & Singh J, *Indian J Chem*, 44B, **2005**, 2102.
11 Paquette L A, Gao Z N & Smith G. F, *Tetrahedron Lett*, 38, **1997**, 1271.
12 Roush W R & Works A B, *Tetrahedron Lett*, 37, **1996**, 8065.
13 Siddiqui I R, Singh P K, Singh J & Singh J, *J Chem Res*, **2004**, 554.
14 Siddiqui I R, Singh J, Singh P K & Singh J, *Indian J Hetero Chem*, 14, **2005**, 231.
15 Siddiqui I R, Singh P K, Singh J & Singh J, *J Agric Food Chem*, 51(24), **2003**, 7062.
16 Garber R H & Houston B R, *Phytophthology*, 49, **1959**, 449.