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RESEARCH ARTICLE

Facile and Green Syntheses of Unsymmetrically Substituted 3-(1-Benzyl-1*H*-indol-3-yl)-2-(1-ethyl-1*H*-indole-3-carbonyl)-acrylonitrile and Study of their Antimicrobial Activities

G. THIRUPATHI*, M. VENKATANARAYANA,
P. K. DUBEY and Y. BHARATHI KUMARI

Department of Chemistry, Jawaharlal Nehru Technological University,
Hyderabad College of Engineering, Kukatpally, Hyderabad (A.P)-500085, India
thirupathigjntuh@gmail.com

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Abstract: Facile and green syntheses of unsymmetrically substituted-3-(1-benzyl-1*H*-indol-3-yl)-2-(1-ethyl-1*H*-indole-3-carbonyl)-acrylonitriles 5**(a-d)** is being reported. *L*-tryptophan has been utilized as an efficient and eco-friendly catalyst in the solvent free condition under the grind stone method at room temperature for Knoevenagel condensation of *N*-benzyl indole-3-carboxyaldehyde (**2**) with the active methylene compounds, 3-cyanoacetylindole **3(a-d)** at room temperature to afford substituted-3-(1-benzyl-1*H*-indol-3-yl)-2-(1*H*-indole-3-carbonyl)-acrylonitrile **4(a-d)** respectively. Subsequently these products were treated with DES and K_2CO_3 in the solvent free condition under the grind stone method at room temperature to afford the corresponding substituted-3-(1-benzyl-1*H*-indol-3-yl)-2-(1-ethyl-1*H*-indole-3-carbonyl)-acrylonitrile **5(a-d).** The antibacterial and antifungal activities of **4(a-d)** and **5(a-d)** have been studied.

Keywords: Indole-3-carboxyaldehyde, 3-Cyanoacetylindole, Knoevenagel reaction, *L*-tryptophan, Physical grinding

Introduction

Toda introduced a method called the grindstone method[1]. In this method, solids are ground together using a pestle and mortar to get the products. Recently, some solvent-free reaction conditions have been developed for oxidation[2], reduction[3], substitution[4], condensation[5] and the Michael addition[6]. And in many cases, these reactions are more efficient and selective than those executed in the corresponding solutions.

Developing green chemical methods is one of the most important purposes of organic synthesis. Organic synthesis in the absence of solvent is a powerful tool for the generation of structurally diverse molecules, due to their special selectivity, the ease of set-up and work-up, arousing great interest. Moreover, solvent-free reactions sometime are faster, taking just a few minutes rather than hours to complete because the reactants are close contact with each other. This aspect, coupled with the lower overall costs of running a reaction without solvent and no specially needed equipment, could become a decisive factor in industry.

Carbon–carbon bond formation reaction is the most important reaction in organic synthesis[7-8]**.** The Knoevenagel condensation is one such reaction which facilitates C-C bond formation and has been widely used in synthesis of olefins of biological significance[9-10]**.** These reactions are usually catalyzed by bases[11-13] such as ammonia, ethylenediamine, guanidine, dimethylamino pyridine, piperidine or corresponding piperidine salt, Lewis acids[13-15] in organic solvents and ionic liquids[16-18] have also been added to the existing list of substances that assisted Knoevenagel condensation in organic synthesis.

As environmental consciousness in chemical research and industry has increased, efficient, economic and clean procedures have received increased attention in recent years. Varies heterogeneous basic catalysts were used in Knoevenagel condensations under solvent-free conditions. More recently, a grinding method under solvent free conditions used in Knoevenagel condensation has rapidly increased. Many catalysts have been used in the synthetic procedure, for example, K_2O-Al_2O_3,[19] CaO,[20] $ZnCl_2$, NH_4OAC and triethyl benzyl ammonium chloride (TEBA). Knoevenagel reaction under solvent-free conditions carried out by microwave irradiation[21-24] and by grinding[25-28] has rapidly increased.But microwave irradiation processis difficult to apply in the industrial process until now and the grinding reaction is only suitable to some active solid reactants.

Experimental

Melting points were measured in open capillary tubes and are uncorrected. TLC was done on plates coated with silica gel-G and spotting was done using iodine or UV lamp. IR spectra were recorded using FT-IR in KBr phase. ^{1}H NMR spectra were recorded on a Gemini-200 and AV-400 instruments operating at 200 and 400 MHz respectively.

*General procedure for the preparation of **4(a-d)***

A mixture of **2** (10 mmol), **3** (10 mmol) and *L*-tryptophan (2 mmol) were grinded under solvent free condition at room temperature for a specified period of time (Table 1). After completion of reaction (as shown by TLC checking), the mixture was poured into ice-cold water (50 mL). The separated solid was filtered, washed with water (100 mL) and dried to obtain crude **4**. The latter were then recrystallised from suitable solvent to afford pure **4(a-d).**

Table 1. Synthesis of novel Knoevenagel products by using *L*-tryptophan as eco-friendly catalyst in the solvent free condition under the grind stone method[a,b]

S.No	Reactants		Product	Time, h	Yield, %	M.P, °C
1	2(R^2=Ph-CH_2)	3a (R=H, R^1=H)	4a(R=H, R^1=H, R^2 = Ph-CH_2)	1	93	176-178
2	2(R^2= Ph-CH_2)	3b (R=H,R^1=OMe)	4b(R=H,R^1=OMe, R^2= Ph-CH_2)	1	92	258-259
3	2(R^2= Ph-CH_2)	3c (R=H,R^1=Br)	4c(R=H, R^1= Br, R^2 = Ph-CH_2)	1	94	277-278
4	2(R^2= Ph-CH_2)	3d (R=H, R^1=NO_2)	4d(R=H,R^1=NO_2, R^2 = Ph-CH_2)	1.1	95	164-167
5	4a(R,R^1=H, R^2= Ph-CH_2)	DES	5a(R= C_2H_5, R^1=H, R^2 = Ph-CH_2)	1	88	245-246

Contd…

6	4b(R=H, R^1=OMe, R^2=Ph-CH_2)	DES	5b(R=C_2H_5,R^1=OMe, R^2=Ph-CH_2)	1	87	228–229
7	4c(R=H, R^1=Br, R^2 = Ph-CH_2)	DES	5c(R= C_2H_5, R^1= Br, R^2 = Ph-CH_2)	1	89	281-282
8	4d(R=H, R^1=NO_2, R^2=Ph-CH_2)	DES	5d(R=C_2H_5,R^1=NO_2, R^2 = Ph-CH_2)	1	90	284-286

*a = Reaction Conditions for **4(a-d):** Indole-3-carboxyaldehyde, 3-cyanoacetylindole, L- tryptophan and room temperature. b = Reaction Conditions for **5(a-d)**: K_2CO_3, DES. Physical grinding*

*Characterization of **4a***

Yellow solid; Yield: 3.8 g (94.76%); m.p. 176–178 ^oC ; IR (KBr): 3242 cm^{-1} (due to –NH), 2212 cm^{-1} (due to –CN) and 1621 cm^{-1} (due to –CO); ^1H NMR spectrum (DMSO/d6/TMS): δ 5.61-5.62 (s, 2H, -N-CH_2), 7.22–8.26 (m, 13H, aryl protons of the two indole rings and aryl protons of the benzyl rings), δ 8.50–8.62 (m, 2H, *α*-protons of the two indole rings), δ 8.74 (vinylic proton of the indole ring), δ 12.18 (br s, 1H, D_2O exchangeable, −NH proton of the indole ring); Its ^{13}C NMR spectrum (DMSO/d6/TMS): δ 61.92, 110.1, 112.1, 113.9, 115.1, 118.5, 121.7, 121.56, 121.6, 121.99,122.1, 122.3, 123.3, 123.1,123.255,126.417, 126.5, 127.074,127.288, 127.7, 131.9, 134.4, 136.3, 136.7, 145.3, 183.7; MS *m/z*: 402 (M+1).

*Characterization of **4b***

Yellow solid; Yield: 3.95 g (91.64%); m.p: 258-259 °C; IR(KBr): 3231 cm^{-1} (medium, –NH stretching), 2208 cm^{-1} (sharp, –CN stretching) and 1624 cm^{-1} (very strong, carbonyl –CO); ^1H-NMR (DMSO/ d6/TMS): δ 3.23–3.26 (s, 3H, –OCH_3), 5.61-5.62 (s, 2H, -N-CH_2), 7.22–8.26 (m, 12H, aryl protons of the two indole rings and aryl protons of the benzyl rings), δ 8.50–8.62 (m, 2H, *α*-protons of the two indole rings), δ 8.74 (vinylic proton of the indole ring), δ 12.18 (br s, 1H, D_2O exchangeable, −NH proton of the indole ring); Its ^{13}C. NMR spectrum (DMSO/d6/TMS): δ, 53.1, 61.92, 110.1, 112.1, 113.9, 115.1, 118.5, 121.7, 121.56, 121.6,121.99,122.1, 122.3, 123.3, 123.1,123.255,126.417, 126.5, 127.074,127.288, 127.7, 131.9, 134.4, 136.3, 136.7, 145.3, 183.7; MS *m/z* = 432(M+1).

*Characterization of **4c***

Yellow solid; Yield: 4.46 g (92.91%); m.p. 277-278 ^oC; IR (KBr): 3215 cm^{-1} (broad, –NH stretching), 2211 cm^{-1} (sharp, –CN stretching) and 1615 cm^{-1} (very strong, highly conjugated carbonyl –CO); ^1H-NMR (DMSO/ d6/TMS): δ 5.61-5.62 (s, 2H, -N-CH_2), 7.22–8.26 (m, 12H, aryl protons of the two indole rings and aryl protons of the benzyl rings), δ 8.50–8.62 (m, 2H, *α*-protons of the two indole rings), δ 8.74 (vinylic proton of the indole ring), δ 12.18 (br s, 1H, D_2O exchangeable, −NH proton of the indole ring); Its ^{13}C NMR spectrum (DMSO/d6/TMS): δ 61.92, 110.1, 112.1, 113.9, 115.1, 118.5, 121.7, 121.56, 121.6, 121.99,122.1, 122.3, 123.3, 123.1,123.255,126.417, 126.5, 127.074,127.288, 127.7, 131.9, 134.4, 136.3, 136.7, 145.3, 183.7; MS *m/z* = 481 (M+1).

*Characterization of **4d***

Yellow solid; Yield: 4.19 g (93.94%); m.p. 164-167 °C; IR (KBr): 3199 cm^{-1} (very broad, -NH stretching), 2211 cm^{-1} (sharp, –CN stretching) and 1621 cm^{-1} (very strong, highly conjugated carbonyl –CO); ^1H NMR (DMSO/ d6/TMS): δ 5.61-5.62 (s, 2H, -N- CH_2), 7.22–8.26

(m, 12H, aryl protons of the two indole rings and aryl protons of the benzyl rings), δ 8.50–8.62 (m, 2H, *α*-protons of the two indole rings), δ 8.74 (vinylic proton of the indole ring), δ 12.18 (br s, 1H, D_2O exchangeable, −NH proton of the indole ring); Its ^{13}C NMR spectrum (DMSO/d6/TMS): δ 61.92, 110.1, 112.1, 113.9, 115.1, 118.5, 121.7, 121.56, 121.6, 121.99,122.1, 122.3, 123.3, 123.1,123.255,126.417, 126.5, 127.074,127.288, 127.7, 131.9, 134.4, 136.3, 136.7, 145.3, 183.7. MS *m/z* = 447 (M+1).

*General procedure for the preparation of **5(a-d)** from **4(a-d)***

A mixture of **4** (10 mmole), diethyl sulphate (DES) (10 mmole), K_2CO_3 (20 mmole) were grinded under solvent free condition at room temperature for 15 min. At the end of this period, the mixture was poured into ice-cold water. The separated solid was filtered, washed with water and dried to obtain crude product. The latter were then recrystallized from suitable solvent to afford pure **5.**

*Characterization of **5a***

Yellow solid; Yield: 3.85 g (89.74%); m.p. 245–246 ^oC; IR(KBr): 2201 cm^{-1} (medium, due to –CN stretching), 1621 cm^{-1} (strong, due to –CO stretching); ^1H-NMR spectrum (DMSO/d6/TMS): δ 1.50–1.52 (s, 3H, N-CH_3 of indole ring*)*, 4.49–4.50 (s, 2H, N-CH_2 of indole ring*)*,7.22–8.26 (m, 12H, aryl protons of the two indole rings and aryl protons of the benzyl rings), δ 8.50–8.62 (m, 2H, α-protons of the two indole rings), δ 8.74 (vinylic proton of the indole ring), δ 12.18 (br s, 1H, D_2O exchangeable, −NH proton of the indole ring); ^{13}C NMR spectrum (DMSO/d6/TMS): δ 13.8,48.9, 34.8, 110.0, 111.2, 113.1, 114.1, 118.5, 121.7, 121.4, 122.8, 123.0, 123.3, 123.8, 126.5, 127.1, 131.6, 134.2, 136.1, 136.5, 145.2, 182.1 MS *m/z* = 430(M+1).

*Characterization of **5b***

Yellow solid; Yield: 3.99 g (86.92%); m.p. 228-229 °C; IR (KBr): 2167 cm^{-1} (sharp, –CN stretching) and 1616 cm^{-1} (very strong, highly conjugated carbonyl –C=O); ^1H NMR (DMSO/ d6/TMS): δ 3.24–3.25 (s, 3H, -O-CH_3*)*, 1.50–1.52 (s, 3H, N-CH_3 of indole ring*)*, 4.49–4.50 (s, 2H, N-CH_2 of indole ring*)*,7.22–8.26 (m, 12H, aryl protons of the two indole rings and aryl protons of the benzyl rings), δ 8.50–8.62 (m, 2H, α-protons of the two indole rings), δ 8.74 (vinylic proton of the indole ring), δ 12.18 (br s, 1H, D_2O exchangeable, −NH proton of the indole ring); ^{13}C NMR spectrum (DMSO/d6/TMS): δ 13.8,48.9, 34.8, 61.92, 110.1, 112.1, 113.9, 115.1, 118.5, 121.7, 121.56, 121.6 ,121.99,122.1, 122.3,123.3, 123.1,123.255,126.417, 126.5, 127.074,127.288, 127.7, 131.9, 134.4, 136.3, 136.7, 145.3, 183.7; MS *m/z* = 460 (M+1).

*Characterization of **5c***

Yellow solid; Yield: 4.47 g (87.99%); m.p. 281-282 ^oC; IR (KBr): 2212 cm^{-1} (sharp, –CN stretching) and 1616 cm^{-1} (very strong, highly conjugated carbonyl –C=O); ^1H NMR (DMSO/ d6/TMS): δ 1.50–1.52 (s, 3H, N-CH_3 of indole ring*)*, 4.49–4.50 (s, 2H, N-CH_2 of indole ring*)*,7.22–8.26 (m, 12H, aryl protons of the two indole rings and aryl protons of the benzyl rings), δ 8.50–8.62 (m, 2H, α-protons of the two indole rings), δ 8.74 (vinylic proton of the indole ring), δ 12.18 (br s, 1H, D_2O exchangeable, −NH proton of the indole ring); Its ^{13}C-NMR spectrum (DMSO/d6/TMS): δ 13.8,48.9, 34.8, 61.92, 110.1, 112.1, 113.9, 115.1, 118.5, 121.7, 121.56, 121.6 ,121.99,122.1, 122.3, 123.3, 123.1,123.255, 126.417, 126.5, 127.074,127.288, 127.7, 131.9, 134.4, 136.3, 136.7, 145.3, 183.7; MS *m/z* = 509 (M+1).

*Characterization of **5d***

Yellow solid; Yield: 4.26 g (89.87%); m.p. 284-286°C; IR (KBr): 2222 cm^{-1} (sharp, –CN stretching) and 1618 cm^{-1}(very strong, highly conjugated carbonyl –C=O); ^1H NMR (DMSO/d6/TMS): δ 1.50–1.52 (s, 3H, N-CH_3 of indole ring*)*, 4.49–4.50 (s, 2H, N-CH_2 of indole ring*)*,7.22–8.26 (m, 12H, aryl protons of the two indole rings and aryl protons of the benzyl rings), δ 8.50–8.62 (m, 2H, *α*-protons of the two indole rings), δ 8.74 (vinylic proton of the indole ring), δ 12.18 (br s, 1H, D_2O exchangeable, −NH proton of the indole ring); Its ^{13}C NMR spectrum (DMSO/d6/TMS): δ13.8,48.9, 34.8, 61.92, 110.1, 112.1, 113.9, 115.1, 118.5, 121.7, 121.56, 121.6, 121.99,122.1, 122.3, 123.3, 123.1,123.255,126.417, 126.5, 127.074, 127.288, 127.7, 131.9, 134.4, 136.3, 136.7, 145.3, 183.7; MS *m/z* = 475 (M+1).

Antimicrobial activity

Antibacterial activity

All the compounds substituted-3-(1-benzyl-1*H*-indol-3-yl)-2-(1*H*-indole-3-carbonyl)-acrylonitrile **4(a-d)** and substituted-3-(1-benzyl-1*H*-indol-3-yl)-2-(1-ethyl-1*H*-indole-3-carbonyl)-acrylonitrile **5(a-d)** were screened for their antibacterial activities[38] against gram-positive bacteria such as *Bacillus subtilis, Staphylococcus aureus* (ATCC6538) and also against gram-negative bacteria such as *Klebsiella pneumonia, Escherichia coli* (ATCC8739) bacterial strains[39] at concentrations of 50, 100, 200, 300 and 500 µg/mL. Streptomycin was used as a reference standard**.** Petri plates and necessary glassware were sterilized in hot air oven at 190 °C for 45 min. The muelier hinton agar and saline (0.82% Nacl) media were sterilized in autoclave (121 °C, 15psi, 20 min). Inoculum was prepared in sterile saline (0.82% Nacl) and the optical density of all pathogens was adjusted to 0.10 at 625 nm on a chemito spectra scan UV 2600 spectrophotometer that is equivalent to 0.5Mc Farland standards[40]. The muelier hinton agar plates were prepared by the pour plate method**.** The activity of the compounds was tested by agar disc diffusion method**.** All the bacterial cells were cultured in muelier hinton agar plates and the compounds to be tested were dissolved in *N*,*N*-dimethyl-formamide(DMF) and were soaked in agar disc and the Petri plates incubated at 37 °C for 24 h. The diameter (mm) of the zone of inhibition around each agar disc was measured and results were recorded in Table 4**. 4(a-d)** and **5(a-d)** compounds tested were found to have excellent anti-bacterial activity against *klebsiella pneumoniae* and *Escherichia coli*. However, they were found to have moderate activity against *Staphylococcus aureus* and *Bacillus subtilis.*

Table 4. Antibacterial activity of **4(a-d)** and **5(a-d)** against *Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*

S.No	Compound No.	Types of bacteria	Inhibition zone in mm for concentration of				
			50 µg/mL	100 µg/mL	200 µg/mL	300 µg/mL	500 µg/mL
1	**4a**	*Klebsiella pneumonia*	9	14	18	23	31
		Escherichia coli	8	13	16	20	30
		Staphylococcus aureus	6	10	14	18	25
		Bacillus subtilis	4	9	12	15	23

Contd…

2	**4b**	*Klebsiella pneumonia*	9	14	18	23	32
		Escherichia coli	8	13	15	20	31
		Staphylococcus aureus	6	10	13	16	26
		Bacillus subtilis	4	9	11	14	22
3	**4c**	*Klebsiella pneumonia*	9	14	18	23	32
		Escherichia coli	8.4	13.5	14	21	30
		Staphylococcus aureus	6	10	13	17	25
		Bacillus subtilis	5	9.5	11	15	23
4	**4d**	*Klebsiella pneumonia*	9	14	18	23	32
		Escherichia coli	8	13	15	20	31
		Staphylococcus aureus	6	10	13	16	26
		Bacillus subtilis	4	9	11	14	22
5	**5a**	*Klebsiella pneumonia*	9	14	18	23	31
		Escherichia coli	8	13	16	20	30
		Staphylococcus aureus	6	10	14	18	25
		Bacillus subtilis	4	9	12	15	23
6	**5b**	*Klebsiella pneumonia*	9	14	18	23	32
		Escherichia coli	8	13	15	20	31
		Staphylococcus aureus	6	10	13	16	26
		Bacillus subtilis	4	9	11	14	22
7	**5c**	*Klebsiella pneumonia*	9	4	18	23	32
		Escherichia coli	8.4	13.5	14	21	30
		Staphylococcus aureus	6	10	13	17	25
		Bacillus subtilis	5	9.5	11	15	23
8	**5d**	*Klebsiella pneumonia*	9	14	18	23	31
		Escherichia coli	8	13	16	20	30
		Staphylococcus aureus	6	10	14	18	25
		Bacillus subtilis	4	9	12	15	23
9	Strepto mycin	*Klebsiella pneumonia*	*Klebsiella pneumonia*	14	18	23	32
		Escherichia coli	*Escherichia coli*	13	15	20	31
		Staphylococcus aureus	*Staphylococcus aureus*	10	13	16	26
		Bacillus subtilis	*Bacillus subtilis*	9	11	14	22

Antifungal activity

All the compounds **4(a-d)** and **5(a-d)** synthesized compound were screened for antifungal activity against *Rhizoctonia solani, Fusarium oxysporum, Aspergillus Niger* and *Aspergillus flavus* at concentrations of 50, 100, 200, 300 and 500 μg/mL. Mycostatin was used as a reference standard. Potato dextrose agar (PDA) was used as basal medium for test fungi. Glass petridishes used were sterilized. Sterilized melted PDA medium (~ 45 ^{o}C) was poured at the rate of 15 mL into each petridish (90 mm). After solidification of the medium, small portions of the mycelium of each fungus were spread carefully over the centre of each PDA plate with the help of sterilized needles. Thus, each fungus was transferred to a number of PDA plates, which were then incubated at (25±2) ^{o}C and ready for use after five days of incubation. Prepared discs of samples were placed gently on solidified agar plates, freshly seeded with the test organisms with sterile forceps. A control disc was also placed on the test plates to compare the effect of the test samples and to nullify the effect of solvent respectively. The plates were then kept in a refrigerator at 4 ^{o}C for 24 h so that the materials had sufficient time to diffuse over a considerable

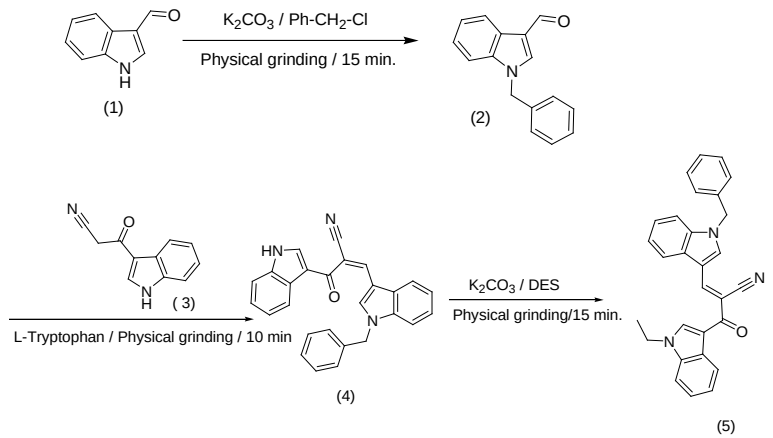
area of the plates. After this, the plates were incubated at 37 ^{o}C for 72 h. *N, N*-dimethylformamide (DMF) was used as solvent to prepare desired solutions of the compounds and also to maintain proper control. The diameter (mm) of the zone of inhibition around each agar disc was measured and results were recorded in Table 5. **4(a-d)** and **5(a-d)** compounds tested were found to have very good antifungal activity against *Rhizoctonia solani* and *Fusarium oxysporum.* However, they were found to good activity against *Aspergillus niger* and *Aspergillus flavus.*

Table 5. Antifungal activity of **4(a-d)** and **5(a-d)** against *Rhizoctonia solani*, *Fusarium oxysporum*, *Aspergillus niger* and *Aspergillus flavus*

S.No	Compound No.	Types of fungus	Zone of inhibition in mm for concentration of				
			50 µg/mL	100 µg/mL	200 µg/mL	300 µg/mL	500 µg/mL
1	**4a**	*Rhizoctonia solani*	9	14	18	23	31
		Fusarium oxysporum	9	13.8	17	22.6	30
		Aspergillus niger	6	10	14	18	25
		Aspergillus flavus	6.5	11	13.6	18.3	25.2
2	**4b**	*Rhizoctonia solani*	8	13	15	20	31
		Fusarium oxysporum	8	13	14	19	30
		Aspergillus niger	4	9	11	14	22
		Aspergillus flavus	4.3	8.9	11.1	13.9	22.1
3	**4c**	*Rhizoctonia solani*	8.4	13.5	14	21	30
		Fusarium oxysporum	8	13	13	20	29
		Aspergillus niger	5	9.5	11	15	23
		Aspergillus flavus	5	9	10.8	14.8	23
4	**4d**	*Rhizoctonia solani*	9	14	18	23	32
		Fusarium oxysporum	8	13	15	20	31
		Aspergillus niger	6	10	12	15	23
		Aspergillus flavus	4	9	11	14	22
5	**5a**	*Rhizoctonia solani*	9	14	18	23	31
		Fusarium oxysporum	8	13	16	20	30
		Aspergillus niger	6	10	14	18	25
		Aspergillus flavus	4	9	12	15	23
6	**5b**	*Rhizoctonia solani*	9	14	18	23	32
		Fusarium oxysporum	8	13	15	20	31
		Aspergillus niger	6	10	13	16	26
		Aspergillus flavus	4	9	11	14	22
7	**5c**	*Rhizoctonia solani*	9	14	18	23	32
		Fusarium oxysporum	8.4	13.5	14	21	30
		Aspergillus niger	6	10	13	17	25
		Aspergillus flavus	5	9.5	11	15	23
8	**5d**	*Rhizoctonia solani*	9	14	18	23	32
		Fusarium oxysporum	8	13	15	20	31
		Aspergillus niger	6	10	13	16	26
		Aspergillus flavus	4	9	11	14	22
13	Mycostanin	*Rhizoctonia solani*	13	16	20	28	35
		Fusarium oxysporum	13	16	19	27	35
		Aspergillus niger	9.2	11	14.6	21	30
		Aspergillus flavus	9	11	14	20	29

Results and Discussion

Treatment of *N*-benzyl indole-3-carboxyaldehyde (**2)** with 3-cyanoacetylindoles **3(a-d)** in the presence of *L*-tryptophan in the solvent free condition under the grind stone method at RT for 10 min. resulted in the formation of substituted 3-(1-benzyl-1*H*-indol-3-yl)-2-(1*H*-indole-3-carbonyl)acrylonitriles **5(a**–**d)** in 92–95% yields (Table 1, Scheme 1). This method is very facile and convenient for the preparation of large amount of Knoevenagel adducts with high yields in less time. *L*-tryptophan acts as a base to induce the reaction.



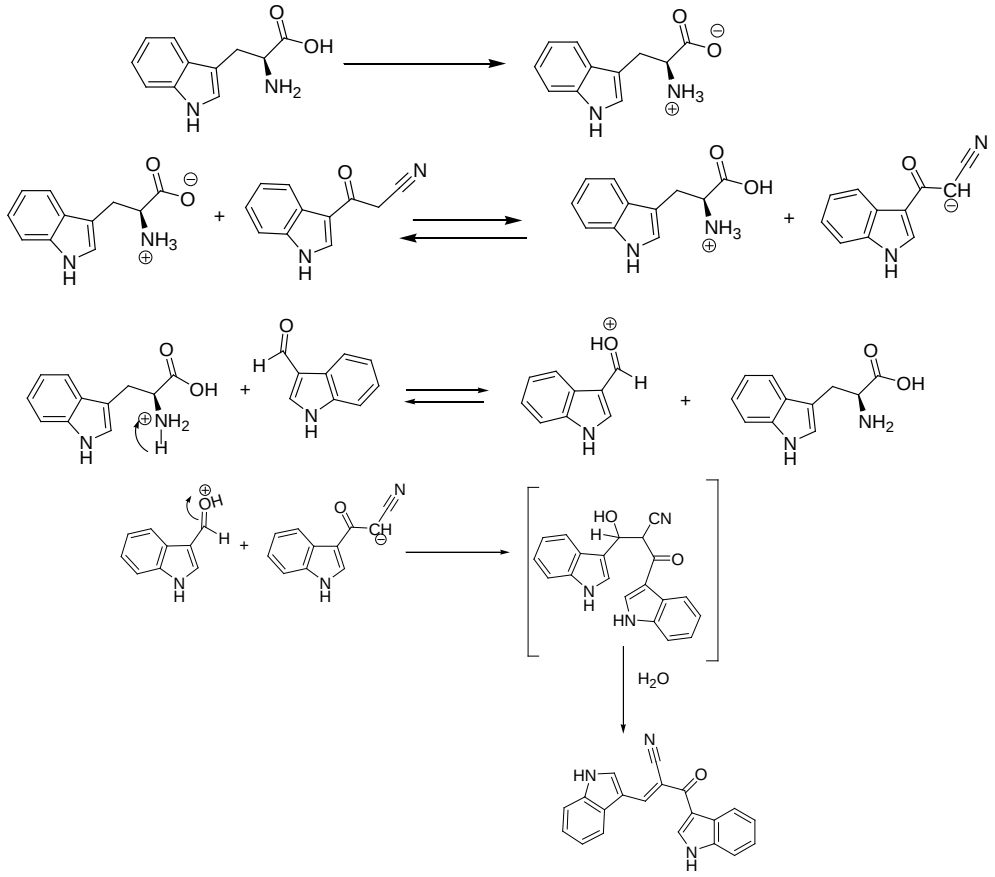
Scheme 1. Knoevenagel condensation of *N*-benzyl indole-3-carboxy aldehyde with 3-cyanoacetylindole in presense of *L*-tryptophan under solvent free condition

In the absence of *L*-tryptophan, the reaction does not proceed the reactants in the solvent free condition under grind stone method at room temperature for 4 h. The use of *L*-tryptophan as a catalyst helps to avoid the use of environmentally unfavourable organic solvents (DMF, C6H6, Toluene, DMSO, *etc*....,) as reaction medium. It is inexpensive, readily available and found to retain its activity even in the presence of water and other active functional groups such as CHO, −CO, NO2 and CN present in the substrates. In all cases, the reaction proceeded smoothly with catalytic amount of *L*-tryptophan to give products of good purity. In the above reaction, the product has been assigned *E*-configuration (first and second priority groups *i.e*., indolyl and 3-cyanoacetylindol respectively are trans to each other) on the basis of the assumption that the groups with maximum stereo chemical bulk would be more stable in a trans configuration.

The above reactions of *N*-benzyl indole-3-carboxyaldehyde **(2)** with 3-cyano-acetylindoles **3(a-d)** were attempted in the presence of various bases like NaOH, KOH were too strong bases to result in more by products. K_2CO_3, ammonium acetate can not catalyze effectively this reaction under same conditions. Low yield was obtained and long reaction time is needed using piperidine and triethylamine as catalyst for condensation of *N*-benzyl aldehyde-3-carboxyaldehyde with 3-cyanoacetylindole in the solvent free condition under the grind stone method at room temperature.

From Table 1, it was shown that the condensation of 3-cyanoacetylindole with electron withdrawing group such as –Br and $–NO_2$ at 5-th position of indole ring with *N*-benzyl indole -3-carboxyaldehyde can be carried out in relatively shorter time and higher yield than with electron donating group such as $–OCH_3$ in the solvent free condition under the grind stone method at room temperature.

A plausible mechanism for the formation of **4** from **2** and **3** in the presence of *L*-tryptophan as catalyst is shown in the Scheme 2. In the mechanism shown in Scheme 2, *L*-tryptophan, in its zwitterionic form **(Ib),** abstracts a proton from 3-cyanoacetylindole **(3)** forming the carbanion of 3-cyanoacetylindole *i.e*. **(3^{1})** which then attacks the protonated indole-3-carboxyaldehyde**(1^{1})** forming the corresponding intermediate **(1^{II})** that loses water to form the end product **4.**which on alkylation results the title compound **5.**



Scheme 2. Plausible mechanism for the formation of 4 from 2 & 3 in the presence of *L* -tryptophan under solvent free condition at room temperature

Treatment of **4(a–d)** each with DES independently, K_2CO_3 as base in the solvent free condition under the grind stone method at room temperature for 15 min. resulted unsymmetrically substituted 3-(1-benzyl-1*H*-indol-3-yl)-2-(1-ethyl-1*H*-indole-3-carbonyl) acrylonitriles **5(a–d)** respectively in 87-90% yields (Scheme 1). Treatment of indole-3-carboxyldehyde with benzyl chloride, K_2CO_3 as base in the solvent free condition under the grind stone method at room temperature for 15 min. resulted *N*-benzyl indole-3-carboxyaldehyde (2). All the above reactions are summarized in Scheme 1.

It is obvious from the above results that K_2CO_3 as base in the solvent free condition under the grind stone method at room temperature for alkylation of **2** and **4(a–d)** resulting **5(a–d)** respectively.

Conclusion

L-Tryptophan has been employed as an efficient catalyst for the preparation of indolo olefinic compounds by a Knoevenagel reaction in the solvent free condition under the grind stone method at room temperature. This method is applicable to a wide range of *N*-substituted indole-3-carboxyldehydes (**2**) and active methylene compounds. The attractive features of this procedure are the mild reaction conditions, high conversions, operational simplicity and inexpensive and ready availability of the catalyst, all of which make it a useful and attractive strategy for the preparation of olefins.

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