An easy protocol for the domino synthesis of diversely functionalized spirocarbocycles and their biological evaluation

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Abstract. A base-catalysed domino reaction for the synthesis of spirocarbocycles in excellent yields using chalcones and vinyl malononitriles is reported. This consists of a series of steps including Knoevenagel condensation followed by intermolecular vinylogous Michael addition and intramolecular cyclization. The synthesized compounds were evaluated for their antimicrobial activity and the compounds exhibited moderate to excellent activities.

Keywords. Spirocarbocycles; L-proline; vinyl malononitriles; chalcones; antimicrobial activity.

1. Introduction

Carbon-carbon and carbon-heteroatom bond forming reactions play a crucial role in organic synthesis. Synthesis of diversely functionalized molecules from simple starting materials which fulfils both economic and environmental aspects together is a great challenge to organic chemists.^{1a} Multicomponent reactions (MCRs) which involve domino processes assist in achieving this ideal goal.¹ MCRs often do not require the usage of expensive catalysts, tedious protection and deprotection processes, purification and isolation procedures.² Hence, MCRs are of growing importance in the construction of diversely functionalized heterocycles.³

Development of efficient methods to construct spiro compounds has been a topic of great relevance in organic synthesis due to their pronounced biological importance as they are used as anticancer agents,⁴ antibacterial agents,⁵ anticonvulsant agents,⁶ anti-tuberculosis agents,^{7a} etc.⁷ Methodologies for the synthesis of spirocyclic compounds range from the classical approaches involving [3+2] cycloadditions⁸ to the recently emerging transition metal-based processes.⁹ Their synthesis also includes other protocols such as ring closure methodologies,¹⁰ radical cyclizations,¹¹ cleavage of bridged ring systems, etc.¹² Such diverse spirocycles are characterized by a spiro ring fusion with varied heterocyclic motifs.¹³ Among the various heterocyclic motifs, isoxazol-5-ones and oxindole moieties

have proven to be valuable intermediates for the construction of spirocarbocycles due to their importance in both synthesis and pharmacology.^{14,15}

Biological importance of these privileged medicinal scaffolds possessing a spiro ring fusion led us to the synthesis of spirocarbocycles. As a continuation of our research work devoted to the development of multicomponent domino reactions,¹⁶ we would herein like to report the synthesis of spirocarbocycles utilizing base-catalysed multicomponent domino reactions involving chalcones and vinyl malononitriles along with biological evaluation of the synthesized compounds.

2. Experimental

2.1 Materials, methods and instruments

Melting points were determined on Gallenkamp melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer FTIR spectrophotometer as KBr pellets. ¹H and ¹³C NMR spectra were obtained in DMSO-*d*₆ on a JEOL spectrometer at 500 and 125 MHz, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in parts per million. The number of protons (*n*) for a given resonance was indicated as *n*H. Coupling constants (*J*) are given in hertz. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet). Mass spectra were recorded on a Thermo Finnigan LCQ Advantage MAX 6000

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ESI mass spectrometer and Perkin-Elmer GC-MS. Elemental analyses were recorded using a Thermo Finnigan FLASH EA 1112CHN analyser. All the compounds gave C, H and N analysis within $\pm 0.5\%$ of the theoretical values. Analytical TLC was performed on pre-coated plastic sheets of silica gel G/UV-254 of 0.2 mm thickness (Macherey-Nagel, Germany) using analytical grade solvents and visualizing with UV light ($\lambda = 254$ and 365 nm).

2.2 Experimental procedure for the synthesis of **4a–l**

3-Phenyl-5-isoxazolone **1** (1 mmol), aldehydes **2a–k** were stirred in a mixture of EtOH:H₂O (2:1) in the presence of L-proline (15 mol%) for 10 min followed by the addition of alkylidene malononitrile **3a–c** (1 mmol) at room temperature for 1 h. The solid precipitated out was filtered off and purified by recrystallization from methanol to afford product **4a–l** as white crystalline solid.

2.2a Spectral data for a representative compound **4a**:

White solid. mp: 152–154°C. $\nu_{\max}$ (KBr): 3345, 3224, 2926, 2860, 2207, 1790, 1640, 1593, 1514, 1446, 1393, 1151, 908, 881, 764, 691, 640 cm⁻¹. ¹H NMR (DMSO-*d*₆, 500 MHz): δ 0.74 (q, 1H, *J* = 9 Hz), 1.25 (m, 1H), 1.40 (m, 1H), 1.62 (m, 1H), 2.07 (m, 1H), 2.17 (m, 1H), 2.22 (s, 3H), 3.06 (d, 1H, *J* = 12.5 Hz), 3.18 (m, 1H), 5.76 (m, 1H), 6.14 (d, 1H, *J* = 8.5 Hz), 6.60 (bs, 2H, -NH₂, D₂O exchangeable), 6.86 (d, 1H, *J* = 7.5 Hz), 7.05 (d, 1H, *J* = 8 Hz), 7.16 (d, 1H, *J* = 8.5 Hz), 7.59 (m, 5H). ¹³C NMR (DMSO-*d*₆, 125 MHz): δ 20.6, 21.5, 25.0, 27.4, 31.3, 50.1, 59.1, 82.1, 117.1, 119.2, 125.6, 126.4, 126.7, 128.6, 129.5, 129.6, 130.3, 130.7, 131.5, 132.3, 137.6, 147.6, 164.3, 176.1 MS (*m/z*): 410 (M+H)⁺. Anal. Calcd. for C₂₆H₂₃N₃O₂: C, 76.26; H, 5.66; N, 10.26. Found: C, 76.24; H, 5.67; N, 10.27.

2.3 Experimental procedure for the synthesis of **6a–e**

3-(2-Oxo-2-phenylethylidene)indolin-2-one **5** (1 mmol) and alkylidene malononitrile **3a–e** (1 mmol) were stirred in a mixture of EtOH:H₂O (2:1) in the presence of L-proline (15 mol%) at room temperature for 3 h. The solid precipitated out was filtered off and recrystallized from methanol to afford product **6a–e** as a white solid.

2.3a Spectral data for a representative compound **6b**:

White solid. mp: 212–214°C. $\nu_{\max}$ (KBr): 3433, 3351, 3305, 3059, 2930, 2828, 2200, 1708, 1669, 1633, 1575,

1473, 1395, 1212, 755, 626 cm⁻¹. ¹H NMR (DMSO-*d*₆, 500 MHz): δ 0.96 (q, 1H, *J* = 12.0 Hz), 1.21 (m, 1H), 1.14 (m, 1H), 1.33 (m, 1H), 1.63 (m, 1H), 2.07 (m, 1H), 3.41 (m, 2H), 4.40 (d, 1H, *J* = 12.5 Hz), 5.42 (s, 2H, -NH₂, D₂O exchangeable), 5.56 (m, 1H), 6.71 (m, 2H), 7.03 (t, 1H, *J* = 7.5 Hz), 7.36 (t, 3H, *J* = 7.5 Hz), 7.50 (t, 1H, *J* = 8 Hz), 7.80 (d, 1H, *J* = 8.5 Hz), 10.57 (s, 1H, -NH, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 125 MHz): δ 14.3, 21.8, 24.9, 27.5, 32.7, 52.0, 54.1, 60.0, 81.0, 110.0, 116.7, 118.2, 121.8, 124.9, 127.3, 128.3, 128.8, 129.6, 131.7, 133.7, 137.7, 143.2, 154.4, 176.0, 200.5. MS (*m/z*): 396 (M+H)⁺. Anal. Calcd. for C₂₅H₂₁N₃O₂: C, 75.93; H, 5.35; N, 10.63. Found: C, 75.91; H, 5.36; N, 10.64.

2.4 Biological evaluation

2.4a Antimicrobial activity: Antimicrobial activities were carried out using disc diffusion method.¹⁷ Petri plates were prepared with 20 ml of sterile Mueller Hinton agar (MHA) (Hi-media, Mumbai). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min and a specific amount of synthesized compound at 1mg/disc was added to each disc separately. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvents. Streptomycin was used as positive control against bacteria. Ketoconazole was used as positive control for fungi. The plates were incubated for 24 h at 37°C for bacteria and for 48 h at 28°C for fungi. Zones of inhibition were recorded in millimetres and the experiment was repeated twice. Bacterial inocula were prepared by growing cells in Mueller Hinton broth (MHB) (Himedia) for 24 h at 37°C. Filamentous fungi were grown on Sabouraud dextrose agar (SDA) slants at 28°C for 10 days and the spores were collected using sterile doubled distilled water and homogenized. Yeast was grown on Sabouraud dextrose broth (SDB) at 28°C for 48 h.

2.4b Minimum inhibitory concentration: Minimum inhibitory concentration (MIC) studies of the synthesised compounds were performed according to the standard reference methods for bacteria,¹⁸ for filamentous fungi¹⁹ and yeasts.²⁰ Required concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.62 µg/mL and 7.81 µg/mL) of the compound were dissolved in DMSO (2%), and diluted to give serial two-fold dilutions that were added to each medium in 96 well plates. An inoculum of 100 µL from each well was inoculated. Antifungal agents such as Ketoconazole for fungi and

Scheme 1. Synthesis and screening of reaction conditions for **4a**.

Streptomycin for bacteria were included in the assays as positive controls. For fungi, the plates were incubated for 48 to 72 h at 28°C and for bacteria the plates were incubated for 24 h at 37°C. The MIC for fungi was defined as the lowest extract concentration, showing no visible fungal growth after incubation time. 5 µL of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperatures. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting visual growth of the test cultures on the agar plate.

3. Results and discussions

3.1 Chemistry

In our investigatory experiment, 3-phenyl-5-isoxazolone **1**, 4-methyl benzaldehyde **2a** and cyclohexylidene malononitrile **3a** were stirred to afford the product **4a** under various reaction conditions viz. solvents,

temperature, base catalysts and adding sequence of substrates (scheme 1, table 1).

The onset of the uncatalysed model reaction in ethanol to afford the product **4a** in a low yield of 45% (table 1, entry 1) prompted us to explore the optimum base catalyst for the reaction by using a range of base catalyst promoters (pyrrolidine, L-proline, DABCO, triethylamine, etc.) (table 1, entries 2–8). The reaction afforded comparatively better yields when L-proline was used as a base catalyst (table 1, entry 8). With the best catalyst L-proline, the reaction was performed in various solvents which included THF, DMF, MeOH, water, etc. (table 1, entries 9–15). The reaction was also found to proceed under neat conditions (table 1, entry 14), but the completion was observed only after prolonged hours with a diminished yield of 40%. In case of L-proline as the base catalyst, a slight increase in the stoichiometry altered the yield from 85% to 87% (table 1, entry 16). Further increase had no impact on the yield of the reaction (table 1, entry 17). The adding sequence of substrates also influenced the product

Table 1. Screening of solvents and base catalysts for the synthesis of **4a**.

Entry	Solvent	Base Catalyst ^a	Conditions	Time (h)	Yield (%) ^b
1	EtOH	–	rt	10	45
2	EtOH	Pyrrolidine	rt	5	67
3	EtOH	DBU	Reflux	24	58
4	EtOH	NEt ₃	rt	10	59
5	EtOH	K ₂ CO ₃	rt	2.5	62
6	EtOH	NEt ₃	rt	4	60
7	EtOH	DABCO	Reflux	8	66
8	EtOH	L-Proline	rt	3	75
9	MeOH	L-Proline	rt	3	60
10	THF	L-Proline	rt	8	65
11	H ₂ O	L-Proline	rt	9	75
12	EtOH: H ₂ O	L-Proline	rt	1	85
13	CH ₃ CN	L-Proline	rt	5	68
14	–	–	rt	48	40
15	DMF	L-Proline	rt	6	55
16	EtOH: H₂O	L-Proline	rt	1	87^c
17	EtOH: H ₂ O	L-Proline	rt	1	86 ^d

^a All the reactions were performed using 10 mol% of the base catalyst

^b Isolated yield of **4a** after recrystallization

^c The reaction was performed using 15 mol% of the catalyst

^d The reaction was performed using 20 mol% of the catalyst

Scheme 2. Plausible mechanism for the formation of spirocarbocycle **4a**.

formation because the addition of 4-methyl benzaldehyde **2a** to cyclohexylidene malononitrile **3a** resulted in the formation of a pyran which reduced the yield of **4a**. Hence, the best result (table 1, entry 16) was observed when 3-phenyl-5-isoxazolone **1** and 4-methyl benzaldehyde **2a** were stirred for 10 min in the presence of 15 mol% of L-proline in ethanol: water mixture²¹ at room temperature followed by the addition of cyclohexylidene malononitrile **3a** to afford a precipitate which was filtered off and recrystallized from methanol to provide the product **4a** in 87% yield.

A plausible mechanism to account for the formation of spirocarbocycle **4a** is explained in scheme 2. 3-Phenyl-5-isoxazolone **1** adds to 4-methyl benzaldehyde **2a** with concomitant elimination of water leading to the formation of chalcone **1a**. Alkylidene malononitrile **3a** in the presence of L-proline undergoes intermolecular vinylogous Michael addition onto the activated double bond of chalcone **1a** forming intermediate **1b**. Among the two transition states **TS I** and **TS II**, the former suffers from unwanted steric interactions and congestions. Hence **TS II** of **1b** undergoes intramolecular addition to the nitrile carbon resulting in the formation of a six membered spirocyclic intermediate **1c**. The latter undergoes isomerization to provide the spirocarbocyclic diastereomer **4a**.

Having optimized the conditions, we expanded the scope of the reaction by varying the aldehyde and alkylidene malononitrile substrates. The reaction proceeded smoothly with aldehydes possessing electron-donating groups. Halogen substituents on the aldehyde (table 2, entries 2–4) did not have any pronounced effect on the yield of the products, but took longer duration for the completion of reaction than its counterparts having electron-rich substituents such as 4-methyl benzaldehyde and 3,4-dimethoxy benzaldehyde (table 2, entries 1 and 6). Various aldehydes including heterocyclic aldehydes were also explored (table 2, entries

7–12). Varying the ring size of the vinylogous alkylidene substrates (table 2, entries 1, 2 and 4) afforded the products in good yields. Thus, the reaction was found to be general and has a broad scope due to its applicability to a variety of substrates and the products **4a–I** were isolated in excellent yields (75–87%) under milder reaction conditions. The results are summarized in table 2.

Structures of compounds **4a–I** were consistent with IR, ¹H and ¹³C NMR, mass spectroscopy and elemental analysis data. The IR spectrum of **4a** exhibited peaks at 2207 and 1790 cm^{−1} for the nitrile and lactone ester carbonyl groups, respectively. The ¹H NMR spectra of **4a** showed a singlet at δ 6.60 for the −NH₂ protons (D₂O exchangeable). The signal at δ 82.1 indicated the presence of spiro carbon in the ¹³C NMR spectra. The mass spectra also exhibited a distinguishing peak at *m/z* 410 [M+H]⁺. Thus the above data confirms the formation of **4a**. The structure was further confirmed by single crystal X-ray analysis performed for a representative compound **4g** (figure 1).²² The stereochemistry was assigned based on ¹H NMR spectroscopy and X-ray single crystal analysis. The ¹H NMR spectra for **4a** exhibits a doublet for the H_a proton which possesses coupling constant of *J* = 12.5 Hz characteristic of a *trans* relation between H_a and H_b protons. The H_a and H_b protons show *trans* relation with respect to each other which is clearly observed from the single crystal X-ray analysis of compound **4g** which further supports the ¹H NMR spectroscopy. This shows that the reaction of 3-phenyl-5-isoxazolone **1** with aldehydes **2a–I** and alkylidene malononitriles **3a–c** is completely diastereoselective in affording only the *trans* diastereomer.

To extend the utility of this multicomponent reaction, studies were extended to other chalcones such as isatin chalcones under the same protocol which results in the formation of spiroxindoles. The reaction provided

Table 2. Synthesis of spirocarbocycles (**4a–l**).

Entry	Aldehyde	Alkylidene malononitriles	Product	Time (h)	Yield (%) ^[a]
1				1	87
2				7	82
3				3	81
4				10	80
5				6.5	85
6				1	84
7				1.5	82

Table 2. (continued)

Entry	Aldehyde	Alkylidene malononitriles	Product	Time (h)	Yield (%) ^[a]
8				8	83
9				3	77
10				4.5	75
11				4	83
12				3.5	80

[^a] Isolated yield after recrystallization from methanol **Figure 1.** ORTEP diagram of **4g**.

spiroxindoles in good yields (79–83%) and the results are tabulated in table 3.

Structures of compounds **6a–d** were consistent with IR, ^1H and ^{13}C NMR, mass spectroscopy and elemental analysis data. The IR spectrum of **6b** exhibited peaks at 2200, 1708 and 1669 cm^{-1} for the nitrile, keto and amide carbonyls, respectively. The ^1H NMR spectra of **6b** showed two singlets at δ 5.42 and 10.57 for the

$-\text{NH}_2$ and $-\text{NH}$ protons (D_2O exchangeable), respectively, clearly indicating the incorporation of both the moieties in the product. The doublet appearing for the H_a proton of the product **6b** shows a coupling constant value of 12.5 Hz indicative of the *trans* stereochemistry between the H_a and H_b protons. In ^{13}C NMR spectra, the spiro carbon atom displayed a signal at δ 81.0 and the amide and keto carbonyl carbon atoms

Table 3. Synthesis of spirooxindole derivatives (**6a–e**).

Entry	Vinylogous malononitrile	Product	Time (h)	Yield (%) ^[a]
1			3	83
2			2.5	80
3			2	79
4			3.5	82
5			4	80

^[a] Isolated yield after recrystallization from methanol

resonated at δ 176.0 and 200.5, respectively. The mass spectra also exhibited a distinguishing peak at m/z 396 $[M+H]^+$. The reaction was also performed using 2-(2,3-dihydronaphthalen-4(1H)-ylidene)malononitrile **3e** as vinyl malononitrile to afford **6e** in 80% yield.

3.2 Pharmacology

3.2a Antimicrobial activity: In the present study, antimicrobial activities of synthesized compounds were screened against bacteria (*Bacillus subtilis* MTCC 441, *Micrococcus luteus* MTCC 106, *Enterobacter aerogenes* MTCC 111, *Staphylococcus aureus* MTCC 96, *Klebsiella pneumoniae* MTCC 109, *Proteus vulgaris* MTCC 1771, *Salmonella typhimurium* MTCC 1251 and *Staphylococcus aureus* (MRSA-methicillin resistant) and fungi (*Malassezia pachydermatis* and *Candida albicans*) using *in vitro* disc diffusion method. Streptomycin was used as a positive control against bacteria and Ketoconazole was used as a positive control against fungi.

Results revealed that most of the synthesised compounds exhibited antimicrobial activities against *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Salmonella typhimurium*, *Staphylococcus aureus* (MRSA-methicillin resistant), *Malassezia pachydermatis* and *Candida albicans* (see [Supplementary information](#)). Compounds **4b**, **4c**, **4e**, **4h**, **6a**, **6b**, **6c** and **6d** have shown considerable antimicrobial activities. Compounds **4d**, **4e**, **4h**, **6c**, **6d** show excellent activities more than the standard drug against the Gram-positive bacteria *S. aureus*. Among the compounds synthesized and tested for antimicrobial activity, **4c**, **4h** and **6b** exhibit significant antimicrobial activities and compound **4h** has shown promising activity against both Gram-positive and Gram-negative bacteria.

3.2b Minimum inhibitory concentration: Minimum inhibitory concentration studies of the synthesized compounds were performed according to the standard reference methods for bacteria, filamentous fungi and yeasts. MIC values of active compounds against bacteria and fungi are given in [Supplementary information](#). Significant MIC values were observed against Gram-positive, Gram-negative bacteria and fungi. Compounds **4b**, **4c**, **4d**, **4e**, **4h**, **6a**, **6b**, **6c**, and **6d** showed good antibacterial activity against bacteria at 1 mg/disc (see [Supplementary information](#)). Interestingly compounds **4e** and **4h** show promising antibacterial activity against *S. aureus*. Among all the compounds tested for minimum inhibitory concentration, **6b** has shown significant MIC values against *Enterobacter aerogenes*, *Proteus vulgaris* and *Salmonella typhimurium*.

4. Conclusion

Thus, we have successfully established a new multi-component domino reaction for the synthesis of poly-functionalized spirocarbocycles from activated double bonds such as chalcones and vinylogous malononitriles. The spirocarbocycles were produced in a single step in excellent yields without involving tedious extraction and isolation procedures. The reaction brings forth a green protocol involving the usage of environmentally benign organocatalyst L-proline in ecofriendly solvent medium such as ethanol–water mixture. It also eliminates the use of hazardous solvents and expensive catalysts. The compounds were also screened for antimicrobial activities against four Gram-positive bacteria and Gram-negative bacteria and also against two fungi. Most of the compounds have found to exhibit significant antimicrobial activity of which **4e**, **4h** and **6b** have shown good activity and hence are promising candidates as antibacterial and antifungal agents.

Supplementary information

The electronic Supplementary information can be seen at www.ias.ac.in/chemsci.

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21. The EtOH: H₂O mixture was tried in order to effect easier precipitation of the product
22. Crystallographic data for compound **4g** in this paper have been deposited with the Cambridge Crystallographic Data centre as supplemental publication no. CCDC- 905942. Copies of the data can be obtained, free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 01223 336033 or email: deposit@ccdc.cam.ac.uk)