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Synthesis of 2-amino-4*H*-chromene derivatives under microwave irradiation and their antimicrobial activity

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Abstract. Libraries of 2-amino-4*H*-chromenes, were efficiently synthesized via one-pot, three-component reactions of 5-chloro-3-methyl-1-aryl-4,5-dihydro-1*H*-pyrazole-4-carbaldehyde (**1a–c**), 2-naphthols (**2a–f**) and malononitrile in the presence of catalytic amount of ammonium acetate under microwave irradiation. The protocol offers rapid synthesis of structurally diverse 2-amino-4*H*-chromenes for biological screening. All the synthesized compounds were evaluated for their antimicrobial activity, and several compounds exhibited moderate to potent antimicrobial activity.

Keywords. Multicomponent reactions (MCRs); pyrazole; naphthols.

1. Introduction

Design of highly efficient chemical reaction sequences that provide maximum structural complexity and diversity with a minimum number of synthetic steps to assemble compounds with interesting properties is a major challenge of modern drug discovery.[1] Recently, multicomponent reactions have emerged as a highly valuable synthetic tool in the context of modern drug discovery. Atom economy and convergent character, simplicity of a one-pot procedure, possible structural variations, accessible complexity of the molecules, and very large number of accessible compounds are among the described advantages of multicomponent reactions.[2] Thus, they are perfectly amenable to automation for combinatorial synthesis.[3]

2-Aminochromenes are an important class of heterocyclic compounds having significant biological activities. During the last decade, such compounds have shown interesting pharmacological properties including, antimicrobial,[4] antiviral,[5,6] mutagenicity,[7] antiproliferative,[8] sex hormone,[9] antitumour,[10] cancer therapy,[11,12] and central nervous system activities.[13] 2-Aminochromenes were also used as biodegradable agrochemicals and components of many natural products.[14]

Pyrazole derivatives are well-established in the literature as important biologically effective heterocyclic compounds. These derivatives are the subject of many research studies due to their widespread potential pharmacological activities such as antiinflammatory, antipyretic, antimicrobial, antiviral, antitumour, anticonvulsant, antihistaminic and antidepressant activities.[15] Widely prescribed anti-inflammatory pyrazole derivatives, celecoxib[16] and deracoxib[17] are selective COX-2 inhibitors with reduced ulcerogenic side effects.

Microwave-assisted organic synthesis has rapidly gained popularity since it accelerates a variety of synthetic transformations,[18] and has prominent advantages of short reaction time and high yield.[19]

Thus, it goes without saying that the use of atom-economical multicomponent reactions (MCRs), together with the employment of energy-efficient microwave irradiation (MW), must be considered to be a facile and effective synthetic strategy of heterocyclic compounds with important bioactivities in the sense that the combination in itself offers greater potential than two parts in isolation.

With the aim to develop more efficient synthetic processes and minimized byproducts, and in continuation of our recent interest in the construction of heterocyclic scaffolds with antimicrobial activity,[20–22] we herein describe a practical, inexpensive and rapid MW-promoted method for synthesis of 2-amino-4*H*-chromenes derivatives via MCRs of 3-methyl-1-phenyl-5-chloro-4-pyrazolocarbaldehyde (**1a–c**), 2-naphthols (**2a–f**) and malononitrile in the presence of ammonium

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acetate in ethanol (EtOH). It is an efficient and promising method to construct the 2-amino-4*H*-chromene skeleton.

2. Experimental

2.1 *Materials and methods*

Solvents used were of analytical grade. All melting points were taken in open capillaries and are uncorrected. Thin-layer chromatography (TLC, on aluminium plates precoated with silica gel, 60F254, 0.25 mm thickness) (Merck, Darmstadt, Germany) was used for monitoring the progress of all reactions, purity and homogeneity of the synthesized compounds; eluent-hexane:ethyl acetate 6:4. UV radiation and/or iodine were used as visualizing agents. Elemental analysis (% C, H, N) was carried out with a Perkin-Elmer 2400 series-II elemental analyser (Perkin–Elmer, USA) and all compounds were within ±0.4 % of calculation. IR spectra were recorded in KBr pellet on a Perkin–Elmer Spectrum GX FT-IR Spectrophotometer (Perkin–Elmer, USA), and only characteristic peaks were reported in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 on a Bruker Avance 400F (MHz) Spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using solvent peak as internal standard at 400 and 100 MHz respectively. Chemical shifts were reported in parts per million (ppm).

2.2 *General procedure for the synthesis of 5-chloro-3-methyl-1-aryl-4,5-dihydro-1H-pyrazole-4-carbaldehyde (**1a**–**c**)*

5-Chloro-3-methyl-1-aryl-4,5-dihydro-1*H*-pyrazole-4-carbaldehyde was prepared, according to literature procedure,[23] by Vilsmeier–Haack reaction of 3-methyl-1-aryl-1*H*-pyrazol-5(4*H*)-one.

2.3 *General procedure for the synthesis of 2-amino-4H-chromenes derivatives (**3a**–**r**)*

A mixture of 5-chloro-3-methyl-1-aryl-4,5-dihydro-1*H*-pyrazole-4-carbaldehyde (1 mmol) (**1a**–**c**), 2-naphthols (1 mmol) (**2a**–**f**) and malononitrile (1 mmol) and ammonium acetate (0.2 mmol, 20 mol%) in EtOH (5 mL) was thoroughly mixed and irradiated at 300 W MW for 5–6 min (the reactions were monitored by TLC). After completion, the reaction mixture was cooled to room temperature, and the solid formed was collected by filtration and crystallized from the appropriate solvent. Physical, analytical and spectroscopic

Table 1. Antimicrobial activity of compounds (**3a**–**r**).

	Zone of inhibition (in mm)					
	Antibacterial activity			Antifungal activity		
Compound	*E. coli*	*B. substilis*	*S. aureus*	*F. oxysporum*	*A. niger*	*R. oryzae*
3a	19	**24**	19	15	18	**25**
3b	16	17	**24**	14	**24**	20
3c	20	**24**	20	**20**	19	17
3d	**24**	18	18	15	**23**	17
3e	**22**	20	20	15	16	**25**
3f	20	**25**	**23**	16	18	**22**
3g	17	**23**	16	13	**24**	20
3h	17	19	**25**	15	18	**25**
3i	**22**	19	18	**21**	**24**	20
3j	18	**24**	17	**20**	20	19
3k	**25**	20	20	17	19	**25**
3l	20	19	**24**	13	18	20
3m	**25**	18	19	14	19	**24**
3n	20	**23**	20	12	20	18
3o	19	17	**25**	15	**25**	18
3p	**24**	20	20	**20**	**24**	19
3q	20	**25**	17	12	20	**23**
3r	**25**	19	**24**	14	17	20
Ampicillin	28	30	30	–	–	–
Ciprofloxacin	35	34	33	–	–	–
Griseofulvin	–	–	–	26	28	30

characterization data of the synthesized compound **3a** is given below:

2.3a *3-Amino-1-(5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-yl)-2-cyano-N-phenyl-1H-benzo[f]chromene-5-carboxamide (**3a**)*: Yield 85%; m.p.: 225–226°C IR (KBr, ν, cm^{-1}): 3485 & 3290 (asym. & sym. stretching of NH_2), 2228 (CN str.), 1650 (C=O str.); ^1H nuclear magnetic resonance (NMR) (400 MHz, DMSO-d_6): δ 2.36 (s, 3H, CH_3), 5.24 (s, 1H, CH), 7.08 (s, 2H, NH_2), 7.12–8.21 (m, 15H, Ar–H), 8.45 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.50 (CH_3), 28.70 (chromene C4), 55.75 (C-CN), 114.12, 118.17, 122.78, 123.11, 123.25, 124.88, 124.97, 125.55, 126.15, 127.17, 128.71, 128.98, 129.16, 129.73, 130.25, 131.46, 132.81, 135.25, 137.12, 138.33, 145.02, 147.37, 158.81 (Ar–C, 23C), 159.25 (C=O); MS (m/z): 532 (M^+); Anal. Calcd. for $C_{31}H_{22}ClN_5O_2$ (531.99 g/mol): C, 69.99; H, 4.17; N, 13.16. Found: C, 69.91; H, 4.22; N, 13.12.

2.4 *Antibacterial activity*

In vitro antimicrobial activity was carried out against 24 h old cultures of three bacteria and three fungi by disc diffusion method.[24,25] Compounds (**3a**–**r**) have been tested for their antibacterial activity against *Escherichia coli* as Gram-negative bacteria and *Bacillus subtilis* and *Bacillus cereus* as Gram-positive bacteria and antifungal activity against *Aspergillus niger*, *Fusarium oxysporum* and *Rhizopus*. Nutrient agar and potato dextrose were used to culture the bacteria and fungus, respectively. The compounds were tested at 1000 ppm in Dimethylformamide (DMF) solution. Ciprofloxacin, Ampicillin and Griseofulvin were used as standards for comparison of antibacterial and antifungal activities, respectively. Inhibition was recorded by measuring the diameter of the inhibition zone at the end of 24 h for bacteria at 35°C and 48 h for fungus at 28°C. The results are summarized in table 1.

3. Results and discussion

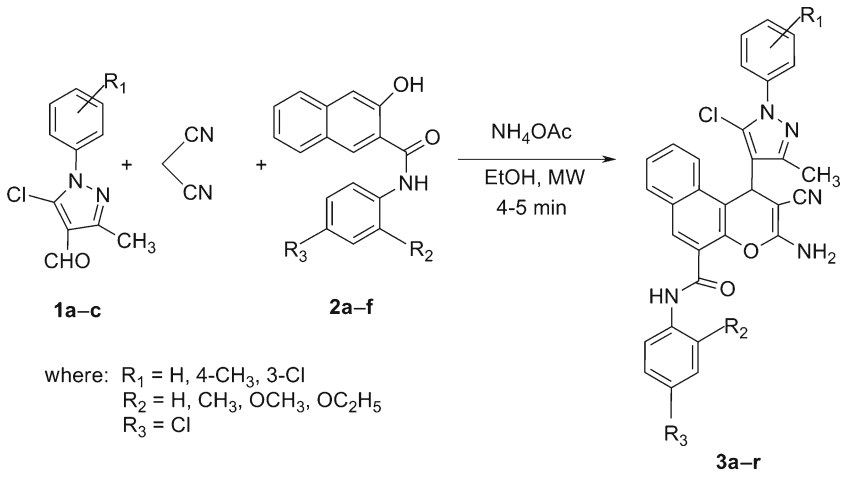
Synthesis of the target compounds is outlined in scheme 1. The required starting material, 5-chloro-3-methyl-1-aryl-4,5-dihydro-1*H*-pyrazole-4-carbaldehyde was prepared by Vilsmeier–Haack reaction of 1-aryl-3-methyl-1*H*-pyrazol-5(4*H*)-one which leads to chloroformylation to afford 5-chloro-3-methyl-1-aryl-4,5-dihydro-1*H*-pyrazole-4-carbaldehyde according to literature procedure.[23] The one-pot three-component condensation reaction of 5-chloro-3-methyl-1-aryl-4,5-dihydro-1*H*-pyrazole-4-carbaldehyde (**1a**–**c**), 2-naphthols (**2a**–**f**) and malononitrile proceeded smoothly in ethanol under MW irradiation in the presence of ammonium acetate as a catalyst to give the corresponding 2-amino-4*H*-chromene (**3a**–**r**) derivatives in good to excellent yields.

To choose the most appropriate medium in this heterocyclization reaction, the MW-assisted reaction of 5-chloro-3-methyl-1-aryl-4,5-dihydro-1*H*-pyrazole-4-carbaldehyde (**1a**–**c**), 2-naphthols (**2a**–**f**) and malononitrile, was examined in Acetic acid (HOAc), glycol, tetrahydrofuran (THF), DMF and EtOH as solvents, respectively under MW at the maximum power of 350 W. The reaction in EtOH resulted in higher yields and shorter reaction time than others. Therefore, EtOH was chosen as the solvent of this reaction. Moreover, to further improve the reaction yields, different solvents such as ammonium acetate, NaOH, K_2CO_3, dimethyl amino pyridine (DMAP), Et_3N and piperidine were examined in ethanol. Ammonium acetate afforded the target product **3a** in 85% yield. So, ammonium acetate was chosen for all further MW assisted reactions. The base optimization for yields is listed in table 2.

We propose a mechanism of the ammonium acetate-catalysed condensation as shown in scheme 2. Condensation of 5-chloro-3-methyl-1-aryl-4,5-dihydro-1*H*-pyrazole-4-carbaldehyde (**1a**–**c**), 2-naphthols (**2a**–**f**) and malononitrile may occur by a mechanism of Knoevenagel condensation, Michael addition, intramolecular cyclization and isomerization. Initially, Knoevenagel condensation of 5-chloro-3-methyl-1-aryl-4,5-dihydro-1*H*-pyrazole-4-carbaldehyde (**1a**–**c**) and malononitrile by the action of ammonium acetate to afford a cyanocinnamonitrile derivative **4**. Then, the proton of 2-naphthols (**3a**–**f**) is abstracted by ammonium acetate to form intermediate **5**. Michael addition of intermediate **5** on **4** leads to the formation of **6**, followed by cyclization and isomerization, affords the corresponding products (**3a**–**r**) (scheme 2).

3.1 *Spectroscopic analysis*

The structures of newly synthesized compounds were elucidated by combined use of IR, ^1H and ^{13}C NMR, mass spectral data and elemental analysis. Absorption bands for compounds (**3a**–**r**) in IR-spectra were observed in the range of 2200–2236 cm^{-1}, corresponding to C≡N. The NH_2 stretching and C=O stretching vibrations for all the compounds were observed in range of 3280–3495 cm^{-1} and 1635–1675 cm^{-1}, respectively. The ^1H NMR spectrum of compounds (**3a**–**r**) indicated the presence of one singlet in the range δ 5.24–5.56 ppm



Compound	R_1	R_2	R_3	Compound	R_1	R_2	R_3
3a	H	H	H	**3j**	4-CH_3	OC_2H_5	H
3b	H	CH_3	H	**3k**	4-CH_3	Cl	H
3c	H	OCH_3	H	**3l**	4-CH_3	CH_3	Cl
3d	H	OC_2H_5	H	**3m**	3-Cl	H	H
3e	H	Cl	H	**3n**	3-Cl	CH_3	H
3f	H	CH_3	Cl	**3o**	3-Cl	OCH_3	H
3g	4-CH_3	H	H	**3p**	3-Cl	OC_2H_5	H
3h	4-CH_3	CH_3	H	**3q**	3-Cl	Cl	H
3i	4-CH_3	OCH_3	H	**3r**	3-Cl	CH_3	Cl

Scheme 1. Synthetic pathway for the 2-amino-4*H*-chromenes.

of C_4H proton. Moreover, the ^{1}H NMR spectrum of all the compounds showed broad singlet in the range of δ 7.04–7.13 ppm due to the NH_2 protons and one singlet in the range δ 8.43–9.41 ppm of NH proton. In the ^{13}C NMR spectra of (**3a**–**r**), the signals assigned to C4 δ 28.14–28.93 ppm and to the carbonyl group δ 195.45–195.65 were the most relevant features. The signal at around δ 55.16–55.93 ppm is assigned to carbon attached to carbonitrile. The obtained elemental analysis values are in good agreement with theoretical data. Mass spectra of the title compounds gave $[M+H]^+$ peaks in agreement with their exact mass or molecular weight.

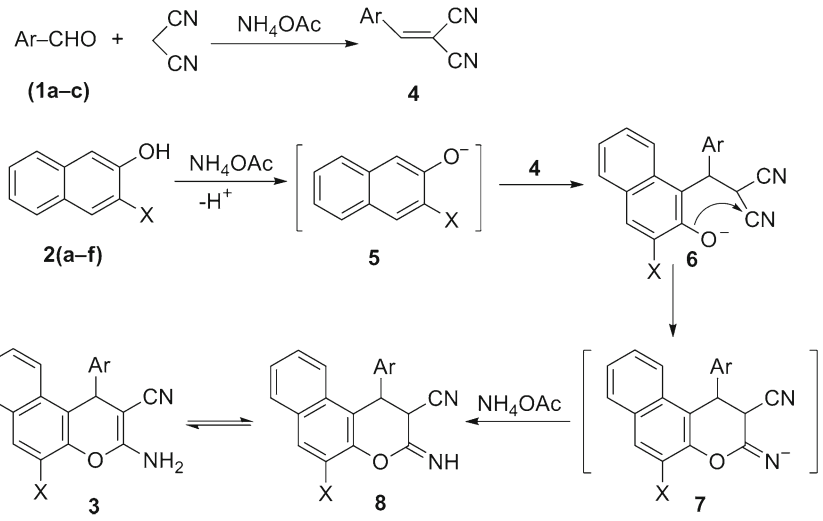
Table 2. Base optimization for the synthesis of **3a** under microwave irradiation.

Base	Yield[a]%
NaOH	58
K_2CO_3	70
DMAP	56
Et_3N	76
Piperidine	80
Ammonium acetate	85

[a]Isolated yields

3.2 *Antimicrobial activity*

A reviewing antibacterial activities of 4*H*-chromenes (**3a**–**r**) (table 1) assay indicated that compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains. In this view, against *E. coli* analogues **3k**, **3m**, **3r** (Zone of inhibition 25), **3d**, **3p** (Zone of inhibition 24), **3e** and **3i** (Zone of inhibition 22) exhibited good antibacterial activity compared to the standard ampicillin (Zone of inhibition 28). With regard to activity against *B. subtilis*, good inhibitory effects was displayed by compounds **3f**, **3q** (Zone of inhibition 25), **3a**, **3c**, **3j** (Zone of inhibition 24), **3g** and **3n** (Zone of inhibition 23) relative to ampicillin (Zone of inhibition 30). Inhibiting Gram-positive bacteria *Staphylococcus*



Scheme 2. Plausible mechanism for the formation of 2-amino-4*H*-chromene derivatives (**3a**–**r**).

aureus compounds **3h**, **3o**, **3r** (Zone of inhibition 25), **3b**, **3l** (Zone of inhibition 24) and **3f** (Zone of inhibition 23) displayed good potency relative to ampicillin (Zone of inhibition 30).

Concerning the antifungal activity of tested compounds, compound **3i** showed highest activity against *F. oxysporum*, i.e., Zone of inhibition 21, while three compounds namely **3c**, **3j** and **3p** (Zone of inhibition 20) exhibited good growth inhibitory action on *F. oxysporum* compared to the standard Griseofulvin (Zone of inhibition 26).

Analogue **3o** (Zone of inhibition 25) showed chief inhibitory activity whereas **3b**, **3g**, **3i**, **3p** (Zone of inhibition 24) and **3d** (Zone of inhibition 23) produce good growth inhibitory activity against *A. niger* compared to the standard griseofulvin (Zone of inhibition 28). With regard to activity against *Rhizopus oryzae*, compounds **3a**, **3e**, **3h**, **3k** (Zone of inhibition 25), **3m** (Zone of inhibition 24), **3q** (Zone of inhibition 23) and **3f** (Zone of inhibition 22) showed good activity compared to the standard griseofulvin (Zone of inhibition 30). Remaining compounds showed mild to moderate antifungal activity.

The data indicate that a change in the substituent might also affect the antibacterial activity of title compounds **3a**–**r**. A close examination of the structures of the active compounds in table 1 revealed that, their antimicrobial activity is strongly bound to the nature of the substituent at the pyrazole together with the substituent linked to the naphthols part of the structure. It could be clearly recognized that potential antibacterial activity against *Escherichia* was encountered with **3k**, **3m** and **3r**. However, replacement of R_1 = 4-CH_3 in **3k** with H (Compound **3e**) or 3-Cl (Compound **3q**) resulted in decrease in antibacterial activity. Replacement of R_2 = Cl in **3k**, i.e., electron-withdrawing group with electron-releasing group resulted in remarkable reduction in antibacterial spectrum. Against *B. substilis*, potential antibacterial activity was encountered with **3f** and **3q**. However, replacement of R_1 = H in **3f** with 4-CH_3 (Compound **3l**) or 3-Cl (Compound **3r**) showed marked decrease in antibacterial activity. Against *S. aureus* analogues **3h** and **3o** exhibited chief activity however replacement of R_1 = 4-CH_3 in **3h** with 3-Cl (Compound **3n**) showed marked decrease in antibacterial activity. It also appears from antifungal activity data that compounds were more active towards *R. oryzae* and less active against *F. oxysporum*. Compounds which exhibited excellent activity towards *R. oryzae* compared to griseofulvin include **3a**, **3e**, **3h** and **3k**. Replacement of R_2 with electron donating group in **3a** (Compound **3b**, **3c** and **3d**) resulted in marked decrease in antifungal activity.

4. Conclusion

In summary, we have successfully combined the advantages of microwave technology with multicomponent reactions to facilitate a competitive synthesis of 2-amino-4*H*-chromenes in ethanol. Particularly, valuable features of this method included the good to excellent yields and operational simplicity as well as increased safety for small-scale high-speed synthesis. This synthetic strategy allows the construction of relatively

complicated nitrogen and oxygen containing fused heterocyclic system as well as introduction of various (hetero) aromatic substitutions into 4-position of chromene system. Most of the compounds showed better antibacterial activity. Further optimization and development is needed in designing more potent antibacterial and antifungal agents for therapeutic use. It is worth mentioning that minor change in molecular configuration of these compounds profoundly influences the activity.

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Supplementary information

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

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