Res Chem Intermed
DOI 10.1007/s11164-016-2810-6

Choline chloride based thiourea catalyzed highly efficient, eco-friendly synthesis and anti-bacterial evaluation of some new 6-amino-4-aryl-2,4-dihydro-3-phenyl pyrano [2,3-c] pyrazole-5-carbonitrile derivatives

Masoumeh Gholami Dehbalaei[1] · Naser Foroughifar[1] · Hoda Pasdar[1] · Alireza Khajeh-Amiri[2] · Neda Foroughifar[3] · Mohammad Alikarami[4]

Received: 3 September 2016 / Accepted: 8 November 2016

Abstract In this study, a simple and highly efficient synthesis of some new 6-amino-4-aryl-2,4-dihydro-3-phenyl pyrano [2,3-c] pyrazole-5-carbonitrile derivatives by one-pot, four-component reaction with aryl aldehydes, hydrazine hydrate, ethyl benzoylacetate and malonitrile has been achieved. This method provides many advantages such as shorter reaction time with high yields, mild reaction conditions and environmental friendliness. Furthermore, all compounds were subsequently evaluated for their in-vitro antibacterial activity compared with cefazolin by minimum inhibitory concentration, all of these compounds were more active than cefazolin. The characterization of the synthesized compounds was established by melting point, IR, ^{1}H NMR and ^{13}C NMR spectra.

Keywords Pyranopyrazoles · Multicomponent reactions · Green chemistry · Deep eutectic solvents · MIC

✉ Naser Foroughifar
n_foroughifar@yahoo.com

[1] Department of Chemistry, Tehran North Branch, Islamic Azad University, Tehran, Iran

[2] Young Researchers and Elites Club, Yadegar-e Imam Khomeini (RAH) Branch, Islamic Azad University, Tehran, Iran

[3] Division of Diabetes, Endocrinology and Metabolism, Faculty of Medicine, Imperial College, London W12 ONN, UK

[4] Department of Chemistry, Ilam Branch, Islamic Azad University, Ilam, Iran

Published online: 18 November 2016

Introduction

Multicomponent reactions (MCRs) consist of two or more starting materials reacting simultaneously to give a single product without isolating the intermediates which are one-step processes [1]. These reactions are an efficient green tool for the synthesis molecular complexity and diversity coupled with shorter reaction time, atom economy, low cost and minimum waste production [2–4].

During the last fer years, pyrano pyrazoles have interested synthetic organic chemists and biochemists because of their biological [5] and pharmacological activities [6] such as antibacterial activity compared with cefazolin and ciprofloxacin [7, 8], biodegradable agrochemicals [8], antimicrobial [9], antifungal [10], antitumor, anticancer [11], analgesic and anti-inflammatory properties [12, 13], anti-Alzheimer's disease [14], anti-oxidant agents [15], and also as potential inhibitors of human Chk1 kinase [16].

The pyran derivatives can couple DNA through an intercalative mode wherein the planar polycyclic aromatic molecules insert and stack between the base pairs of DNA which are known to intercalate into DNA [17].

DNA intercalation with exogenous molecules leads to the making of DNA-specific drugs. The intercalation of small molecules into DNA is used in therapeutic approaches in which the suppression of DNA iteration and gene transcription is engaged to destroy tumor cells or infected tissue. Some of the studies have been performed to gain more intense insight into different aspects of the interaction of small molecules with DNA because of acquiring highly selective and efficient DNA intercalators [18]. A large number of drugs with a pyrazole structure that are affected in the treatment of trigeminal neuralgia, valproate, and topiramate are useful for migraine prophylaxis, for example, carbamazepine, phenytoin, lamotrigine and felbamate. This indicates a strong linkage between anti-epileptic activity and antinociception which is already proven for many established anticonvulsant drugs which have long been used in pain management, particularly in chronic neuropathic pain [19, 20], with gabapentin and carbamazepine as well as phenytoin being used in the treatment of post-herpetic neuralgia and diabetic neuropathy, respectively [21–23].

A number of methods have been reported for the synthesis of dihydro pyrano [2,3-c] pyrazoles employing different catalysts, such as organic bases [24, 25], magnetized water [26], γ-alumina [27], TrCl (trityl chloride) [28], DABCO [29, 30], [Dsim]$AlCl_4^-$ [31], ionic liquid Bmim-BF_4 [32], sodium bisulfite under ultrasound irradiation and solvent-free conditions [33], [DMDBSI].$2HSO_4$ as ionic liquid catalyst [34], isonicotinic acid [35], magnetic Fe_3O_4 nanoparticles [36], PS-PSTA [37], TEBAC (triethyl benzyl ammonium chloride) [38], catalyst-free [39], L-proline [40, 41], nano-TiO_2 [42], and bleaching earth clay (pH 12.5) [43, 44], using a one-pot four-component reaction in deep eutectic solvent, DES (choline chloride:urea).

Many chemical reactions have used ionic liquids such as green alternative media for volatile organic solvents because of their low vapor pressures, chemical and thermal stability, nonflammability, high ionic conductivity, and a wide

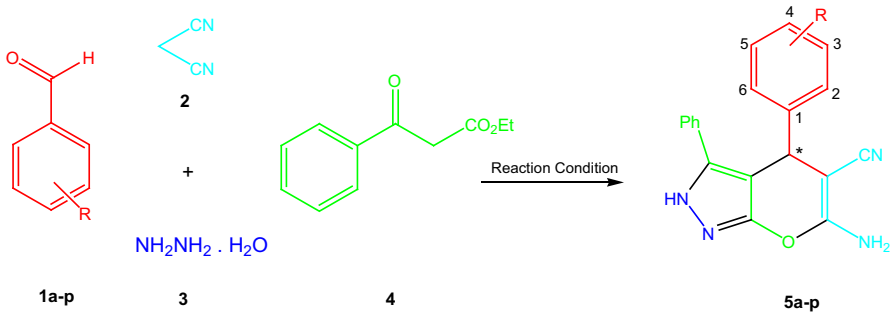
electrochemical potential window, but the high costs and toxicity of some aquatic species are the main disadvantages of these green solvents, which has limited their applications in the laboratory and industry [45, 46]. DESs, such as homogeneous catalysts, eutectic mixtures of an ammonium salt and a hydrogen-bond donor compound such as urea, acid, amine, and salts, developed by Abbott and co-workers, are alternatives to ionic liquids [47–49]. These eutectic mixtures are attractive alternatives to room-temperature ionic liquids, as DESs can be less expensive, more synthetically available, nontoxic, and biodegradable [47, 50–54].

In continuation of our work on the synthesis of biologically important compounds using simple, efficient, nontoxic and readily available catalysts, we have used catalysts such as alumina, DABCO, L-proline, benzyl triphenyl phosphonium chloride (BTPPC) and choline chloride:thiourea (DES) and choline chloride:urea as ionic liquid catalysts for the synthesis of new pyranopyrazole derivatives from aryl aldehydes, malonitrile, hydrazine hydrate, and ethyl benzoyl acetate under reflux and ultrasonic irradiation conditions in short reaction times with high yields (Scheme 1). In this work, the best results according to the reaction conditions in the presence of catalyst choline were with chloride:thiourea.

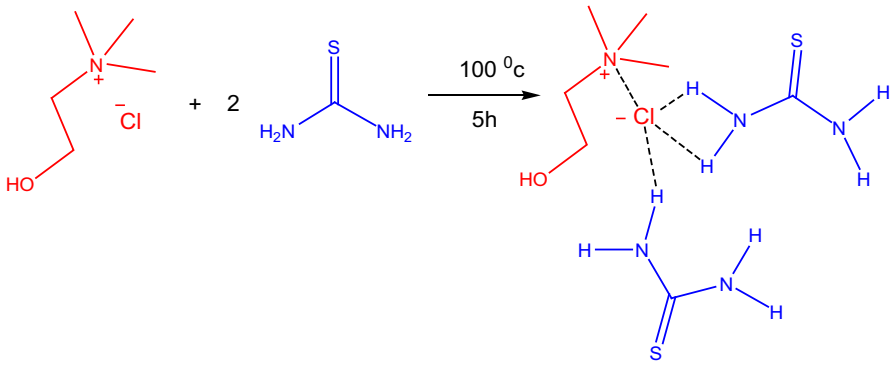
Experimental

General

All melting points were uncorrected and measured using capillary tubes on an electrothermal digital apparatus. IR spectra were recorded on a Shimadzo (FT)-IR 300 spectrophotometer in KBr. NMR spectra were recorded on Brucker 500 and 400 MHz spectrometers in DMSO-d_6 as solvent with TMS as an internal standard. Chemical shifts (δ) for ^{1}H and ^{13}C NMR was reported in parts per million (ppm) and was referenced to the solvent peak; DMSO-d_6 (2.50 ppm for ^{1}H and 39.70 ppm for ^{13}C). Multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). The progress of the reaction was monitored by TLC (thin-layer chromatography) using n–hexane/EtOAc (1:1) as an eluent.



Scheme 1 Synthesis of compounds **5a–p**



Scheme 2 Synthesis of DES based on choline chloride and thiourea

Synthesis of deep eutectic solvent

According to Ref. [44], we have synthesized choline chloride:thiourea ionic liquid catalyst. A mixture of choline chloride (14 g) and thiourea (16 g) was heated in thae flask at 100 °C with stirring for 5 h (Scheme 2). The resulting eutectic solvent was then allowed to cool to room temperature and was used for the synthesis of new pyranopyrazoles derivatives.

General procedure for the synthesis of pyranopyrazole derivatives 5a–p

To a mixture of aryl aldehyde (1 mmol), malonitrile (1 mmol) and ethanol (10 mL) was added a catalyst (10% mol), and the reaction mixture was stirred magnetically for 5 min at room temperature. After 5 min of stirring, hydrazine hydrate (1.5 mmol) and ethyl benzoyl acetate (1 mmol) were added. The mixture was ground under reflux conditions at 80 °C or ultrasonic irradiation until completion of the reaction. The progress of the reaction was followed by TLC (thin-layer chromatography). After completion of the reaction, the mixture was cooled to room temperature and then water was added to it for washing, and filtered through the filtration flask to afford the pure product without further purification.

Spectroscopic data for selected products

*6-amino-2,4-dihydro-3-phenyl-4-p-tolylpyrano [2,3-c] pyrazole-5-carbonitrile (**5b**)*

Yellow solid; m.p: 237–238 °C; Yield 98%; IR (KBr, cm^{-1}) ν_{max}: 3480 (NH), 3212 and 3123 (NH_2), 2196 (C≡N). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 2.18 (s, 3H, CH_3); 4.94 (s, 1H, CH); 6.90 (brs, 2H, NH_2, D_2O-exchangable); 6.98–7.024 (dd, J = 7.7 Hz, 4H, C_6H_4): 6.98–7.00 (d_1, 2H, $^3J_{H2,3}$ = 8.0 Hz, $H_{2,3}$); 7.02–7.00 (d_2, 2H, $^3J_{H4,5}$ = 8.0 Hz, $H_{4,5}$, C_6H_4); 7.24–7.48 (m, 5H, C_6H_5); 12.88 (s, 1H, NH, D_2O-exchangable). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 160.4, 156.5, 142.2, 138.3,

136.1, 129.4, 129.2, 129.1, 128.7, 127.6, 126.6, 121.1, 97.9, 58.9, 36.9, and 21.3. Anal.Calc for $C_{20}H_{16}N_4O$ (328.4); C, 73.17; H, 4.87; N, 17.07%.

*6-amino-2,4-dihydro-4-(3-nitrophenyl)-3-phenylpyrano [2,3-c] pyrazole-5-carbonitrile (**5c**)*

Yellow solid; m.p: 254–255 °C; Yield 93%; IR (KBr, cm^{-1}) ν_{max}: 3472 (NH), 3247 and 3096 (NH_2), 2190 (C≡N), 1530 and 1349 (NO_2). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 5.33 (s, 1H, CH); 7.11 (brs, 2H, NH_2, D_2O-exchangable); 7.23–7.48 (m, 5H, C_6H_5); 7.492–7.514 (dd, 1H, $^3J_{H6,5} = 1.2$ Hz, H_6, C_6H_4); 7.576–7.602 (dt, H_5): 7.576–7.579 (dt_1, 1H, $^3J_{H5,4} = 1.2$ Hz, H_5, C_6H_4); 7.599–7.602 (dt_2, 1H, $^3J_{H5,6} = 1.2$ Hz, H_5, C_6H_4); 7.96–7.969 (dd, 1H, $^4J_{H2,4} = 1.6$ Hz, H_2, C_6H_4); 7.978–7.986 (dd): 7.978–7.980 (d_1, 1H, $^3J_{H4,5} = 0.8$ Hz, H_4, C_6H_4); 7.984–7.986 (d_2, 1H, $^4J_{H4,2} = 0.8$ Hz, H_4, C_6H_4); 12.96 (s, 1H, NH, D_2O-exchangable). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 160.9, 156.1, 147.9, 147.1, 138.9, 134.7, 130.4, 129.0, 128.9, 128.7, 126.9, 122.3, 122.2, 120.8, 96.9, 57.5, and 36.5. Anal. Calc for $C_{19}H_{13}N_5O_3$ (359.4): C, 63.51; H, 3.62; N, 19.50%.

*6-amino-2,4-dihydro-4-(3-hydroxyphenyl)-3-phenylpyrano [2,3-c] pyrazole-5-carbonitrile (**5d**)*

White solid; m.p: 258–260 °C; Yield 94%; IR (KBr, cm^{-1}) ν_{max}: 3833 (OH), 3472(NH), 3331 and 3205 (NH_2), 2193 (C≡N). ^{1}H-NMR (DMSO-d_6,400 MHz) δ ppm: 4.85 (s, 1H, CH); 6.91 (brs, 2H, NH_2, D_2O-exchangable); 6.473–6.482 (dd, 1H, $^4J_{H2,4} = 2$ Hz, H_2, C_6H_4); 6.5–6.528 (dd, 1H, J = 7.7 Hz); 6.5–6.502 (d_1, 1H, $^3J_{H4,5} = 0.8$ Hz, H_4); 6.526–6.528 (d_2, 1H, $^4J_{H4,6} = 0.8$ Hz, H_4); 6.571–6.59 (dd, 1H, $^3J_{H6,5} = 7.6$ Hz, H_6); 6.98–7.02 (t, 1H, $^3J_{H5,6} = 7.6$ Hz, H_5); 7.25–7.48 (m, 5H, C_6H_5); 9.26 (1H, s, OH, D_2O-exchangable); 12.89 (s, 1H, NH, D_2O-exchangable). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 160.5, 157.8, 156.5, 146.7, 138.3, 129.7, 129.2, 129.1, 128.8, 126.6, 121.1, 118.5, 114.4, 114.3, 97.9, 58.9 and 37.2. Anal. Calc for $C_{19}H_{14}N_4O_2$ (330.37): C, 69.09; H, 4.24; N, 16.97%.

*6-amino-2,4-dihydro-4-(4-methoxyphenyl)-3-phenylpyrano[2,3-c]pyrazole-5-carbonitrile (**5i**)*

White solid; m.p: 241–243 °C; Yield 98%; ^{1}H-NMR (DMSO-d_6,400 MHz) δ ppm: 3.34 (s, 3H, OCH_3); 5.05 (s, 1H, CH); 6.98 (brs, 2H, NH_2, D_2O-exchangable); 7.116–7.267 (dd, 4H, C_6H_4, J = 7.7 Hz): 7.116–7.137 (d_1, 2H, $^3J_{H2,3} = 8.4$ Hz, $H_{2,3}$): 7.240–7.261 (d_2, 2H, $^3J_{H5,6} = 8.4$ Hz, $H_{5,6}$); 7.274–7.466 (m, 5H, C_6H_5); 12.90 (s, 1H, NH, D_2O-exchangable).^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 161.06, 160.6, 156.3, 144.04, 138.5, 136.5, 133.07, 131.5, 130.5, 129.6, 129.5, 129.06, 128.9, 128.8, 128.7, 126.7, 120.9, 97.4, 58.2, 36.5. Anal. Calc for $C_{20}H_{16}N_4O_2$ (344.6): C, 69.64; H, 4.64, N, 16.25%.

*6-amino-4-(4-bromophenyl)-2,4-dihydro-3-phenylpyrano [2,3-c] pyrazole-5-carbonitrile (**5k**)*

White solid; m.p: 255–256 °C; Yield 89%; IR (KBr, cm^{-1}) ν_{max}: 3431 (NH), 3287 and 3134 (NH_2), 2185 (C≡N), 1064 (Br). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 5.05 (s, 1H, CH); 6.99 (brs, 2H, NH_2, D_2O-exchangable); 7.052v7.085 (dd, 4H, C_6H_4, J = 7.7 Hz): 7.052–7.063 (d_1, 1H, $^3J_{H3,2}$ = 2.4 Hz, H_3); 7.075–7.085 (d_2, 1H, $^3J_{H2,3}$ = 2.4 Hz, H_2); 7.25–7.40 (m, 5H, C_6H_5); 7.449–7.472 (d_1, 1H, $^3J_{H5,6}$ = 1.6 Hz, H_5); 7.075–7.085 (d_2, 1H, $^3J_{H6,5}$ = 1.6 Hz, H_6); 12.93 (s, 1H, NH, D_2O-exchangable). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 160.6, 156.3, 144.5, 138.5, 131.7, 130.1, 129.0, 128.9, 126.7, 121.0, 120.1, 97.3, 58.2 and 36.6. Anal. Calc for $C_{19}H_{13}BrN_4O$ (393.3); C, 58.01; H, 3.31; N, 14.25%.

*6-amino-2,4-dihydro-4-(4-isopropylphenyl)-3-phenylpyrano [2,3-c] pyrazole-5-carbonitrile (**5m**)*

White solid; m.p: 250–251 °C; Yield 97%; IR (KBr, cm^{-1}) ν_{max}: 3481 (NH), 3222 and 3108 (NH_2), 2196 (C≡N). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ ppm:1.094–1.09 (d, 3H, $^3J_{H(CH3,\ CH)\ Isopropyl}$ = 2.4 Hz, CH_3); 1.1084–1.1132 (d, 3H, $^3J_{H(CH3,\ CH)\ Isopropyl}$ = 2.4 Hz, CH_3); 2.48–3.34 (m, 1H, $CH_{Isopropyl}$); 4.92 (s, 1H, CH); 6.89 (brs, 2H, NH_2, D_2O-exchangable); 7.0102–7.0822 (dd, 4H, C_6H_4): 7.0102–7.0265 (d_1, 2H, $^3J_{H2,3}$ = 8.15 Hz, $H_{2,3}$); 7.0659–7.0822 (d_2, 2H, $^3J_{H5,6}$ = 8.15 Hz, $H_{5,6}$); 7.228–7.455 (m, 5H, C_6H_5); 12.87 (s, 1H, NH, D_2O-exchangable). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ ppm: 160.1, 156.1, 146.5, 142.2, 137.6, 128.7, 128.6, 128.5, 128.2, 127.1, 126.9, 126.3, 126.2, 126.1, 126.1, 120.7, 97.6, 58.5, 36.4, 32.8, 23.8 and 23.6. Anal. Calc for $C_{22}H_{20}N_4O$ (356.46); C, 74.12; H, 5.67; N, 15.72%.

*6-amino-4-(2-chlorophenyl)-2,4-dihydro-3-phenylpyrano [2,3-c]pyrazole-5-carbonitrile (**5o**)*

White solid; m.p: 271–273 °C; Yield 91%; IR (KBr, cm^{-1}) ν_{max}: 3442 (NH), 3268 and 3120 (NH_2), 2190 (C≡N), 1058 (Cl). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 5.43 (s, 1H, CH); 6.99 (brs, 2H, NH_2, D_2O-exchangable); 7.086–7.274 (m, 5H, C_6H_5); 7.294–7.298 (dd, 1H, $^3J_{H6,5}$ = 1.6 Hz, H_6, C_6H_4); 7.305–7.314 (dt, 1H, J_{H4} = 3.6 Hz, $H_{4(5)}$, C_6H_4); 7.361–7.385(dd, 1H, $^3J_{H3,4}$ = 1.6 Hz, H_3, C_6H_4); 12.86 (s, 1H, NH, D_2O-exchangable). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 160.9, 156.6, 141.5, 138.5, 132.5, 131.1, 129.8, 128.9, 128.8, 128.0, 126.5, 120.5, 97.1, 56.9 and 34.5. Anal. Calc for $C_{19}H_{13}ClN_4O$ (348.81); C, 65.36; H, 3.73; N, 16.05%.

Results and discussion

Chemistry

First, we began our study with the optimization of the four-component reaction in ethanol between arylaldehyde, malonitrile, hydrazine hydrate and ethylbenzylacetate.

This reaction was initially carried out in the presence of the choline chloride/thiourea catalyst (10% mol DES) under reflux for 40 min or under ultrasonic irradiation for 8 min and afforded 6-amino-2,4-dihydro-3-phenyl-4-p-tolylpyrano[2,3-c]pyrazole-5-carbonitrile, **5a** (Fig. 1).

It was also studied under several conditions. Several catalysts (choline chloride/urea, choline chloride/thiourea, DABCO, Al_2O_3, L-proline, and BTPPC) and various environmentally benign solvents (water, water–EtOH and EtOH) were investigated to find the best conditions (Scheme 3). After optimization, the best results in terms of yield were achieved by using 10% mol choline chloride/thiourea (DES) in EtOH under reflux or ultrasonic irradiation conditions (isolated yields were, respectively, 98 and 95%). The results of this comparative experiment are shown in Table 1. The recovery and reusability of the catalyst are very important for commercial and industrial applications as well as green process considerations. Thus, after completion of the reaction, the ChCl.2thiourea ionic liquid catalyst was recycled by simple extraction of the product with diethyl ether from the reaction mixture. The viscous ionic liquid that remains in the reaction test tube was thoroughly washed with ether and reused in subsequent reactions without further purifications. Moreover, the catalyst is reusable for the next catalytic cycles after activating the ionic liquid at 80 °C under vacuum in each cycle. To optimize the reaction conditions, we also verified the amount of catalyst needed for the preparation of **5a–p**, and the best result was obtained using 10% mol ChCl.2thiourea catalyst (Table 2). The results compare the effect of the catalyst on the reaction conditions shown in the Tables 3 and 4.

The structure of compounds (**5a–p**) was established via IR, ^{1}HNMR, and ^{13}CNMR spectroscopic data for compound **5b** as a representative example. The ^{1}HNMR spectrum of **5b** has a singlet at 2.19 ppm, ascribable to methyl hydrogens; a singlet at 4.94 ppm, ascribable to hydrogen attached to the pyran ring, a broad singlet at 6.90 ppm for NH_2 hydrogens, and a part of the aromatic has a doublet of doublet for C_6H_4, a multiplet for C_6H_5, and a singlet at 12.88 ppm, for N–H hydrogen.

We proposed a possible mechanism for the synthesis of pyrano [2,3-c] pyrazole, in a one-pot reaction in the presence of a catalyst. Firstly, there is Knoevenagel condensation between the aryl aldehyde and the malonitrile to from an alkene intermediate in situ. The intermediate undergoes Michael addition with pyrazolone

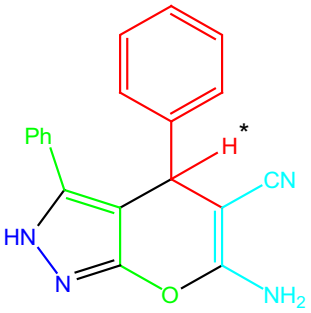
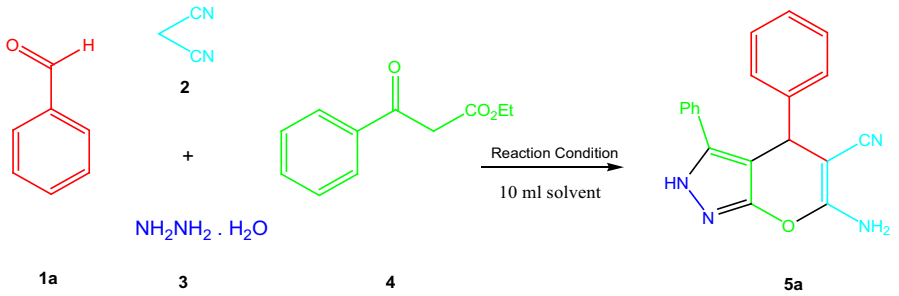


Fig. 1 Structure of compound **5a**, synthesized during the optimization studies



Scheme 3 Synthesized compound **5a** during the optimization studies

Table 1 Comparison the effect of catalyst for the optimization of reaction conditions (**5a**)

Entry	Catalyst (10% mol)	Solvent (10 ml)	Reflux (80 °C)		Sonication (r.t.)	
			Time	Yield[a] (%)	Time	Yield[a] (%)
1	–	EtOH	12 h	20	1 h	25
2	ChCl.2thiourea	EtOH	40 min	98	8 min	95
3	ChCl.2urea	EtOH	1 h	87	12 min	80
4	ChCl.2thiourea	Water	70 min	80		
5	DABCO	EtOH	3 h	50		
6	Al_2O_3	EtOH–water	4 h	45		
7	L-Proline	EtOH	1 h	90	10 min	88
8	BTPPC (20% mol)	–	4 h	78	1 h	25

[a] Isolated yield

Table 2 Optimization of amount of catalyst (% mol of ChCl.2thiourea)

Catalyst (%mol)	Reflux		Sonication (r.t.)	
	Time (min)	Yield[a] (%)	Time (min)	Yield[a] (%)
2	55	35	20	40
5	40	70	15	60
7	30	96	12	93
10	30	98	10	96

[a] Isolated yield

(formed in situ from hydrazine hydrate and ethylbenzoylacetate) followed by an intra-molecular cyclization to give polyfunctionalized pyrano [2,3-c] pyrazoles. The possible mechanism is shown in Scheme 4.

Table 3 Effect of catalyst for the reaction conditions (**5a–p**)

Entry		Benzaldehyde (R)	Catalyst (10% mol)	Reflux (80 °C)		Sonication (r.t.)	
				Time	Yield[a] (%)	Time	Yield[a] (%)
1	**5a**	H	ChCl.2thiourea	40 min	98	8 min	95
2	**5b**	4-Me	ChCl.2thiourea	40 min	94	11 min	90
3	**5b**	4-Me	ChCl.2urea	1 h	80	15 min	76
4	**5c**	3-NO_2	ChCl.2thiourea	30 min	93	10 min	92
5	**5c**	3-NO_2	ChCl.2urea	40 min	83	17 min	80
6	**5d**	3-OH	ChCl.2thiourea	50 min	94		
7	**5d**	3-OH	ChCl.2urea	30 min	89		
8	**5e**	2-Me	ChCl.2thiourea	45 min	97		
9	**5f**	4-OH	ChCl.2thiourea	35 min	97		
10	**5f**	4-OH	ChCl.2urea	1 h	88		
11	**5g**	2-NO_2	ChCl.2thiourea	50 min	93		
12	**5h**	2-OH	ChCl.2thiourea	1 h	88		
13	**5h**	2-OH	ChCl.2urea	2 h	69		
14	**5i**	4-OMe	ChCl.2thiourea	30 min	98		
15	**5i**	4-OMe	ChCl.2urea	45 min	73		
16	**5j**	4-Cl	ChCl.2thiourea	20 min	91	4 min	90
17	**5j**	4-Cl	ChCl.2urea	25 min	80	11 min	82
18	**5k**	4-Br	ChCl.2thiourea	15 min	89		
19	**5l**	4-$N(Me)_2$	ChCl.2thiourea	5 min	96		
20	**5l**	4-$N(Me)_2$	ChCl.2urea	12 min	75		
21	**5m**	4-$CH(Me)_2$	ChCl.2thiourea	10 min	89		
22	**5m**	4-$CH(Me)_2$	ChCl.2urea	30 min	80		
23	**5n**	4-NO_2	ChCl.2thiourea	23 min	91		
24	**5n**	4-NO_2	ChCl.2urea	90 min	89		
25	**5o**	2-Cl	ChCl.2thiourea	20 min	89		
26	**5p**	3-Br	ChCl.2thiourea	15 min	96		

[a] Isolated yield

Antibacterial studies

All the synthesized compounds were assessed for their antibacterial activity against two Gram-positive bacteria (*Staphylococcus saprophyticus* and *S. aureus*) and two Gram-negative bacteria (*Esherichia coli* and *Pseudomonas aeruginosa*). Normal saline was used for preparation of inoculants having 0.5 McFarland standards. The compounds were dissolved in dimethylsolfuxide (DMSO) for bioassay. The microplates were incubated at 37 °C for 24 h. Values of minimum inhibitor concentration (MIC) were recorded as the lowest concentration of substance, which gives no growth of inoculated bacteria. All the compounds showed antibacterial activity against both Gram-positive and Gram-negative standard strains and their MICs

Table 4 The preparation of pyranopyrazole (**5a–p**) derivatives using ChCl.2thiourea as catalyst, under both ultrasonic irradiation and reflux conditions

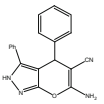
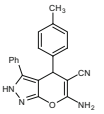
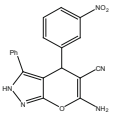
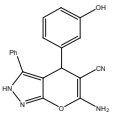
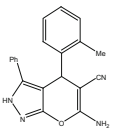
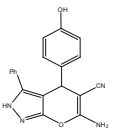
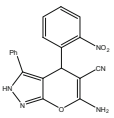
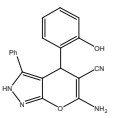
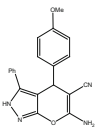
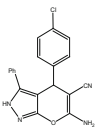
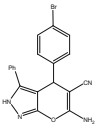
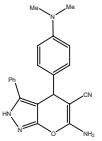
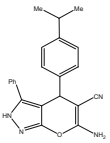
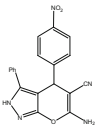
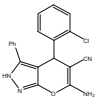
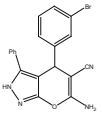
Entry	Product no.	Product	M.P (°C) found	M.P (°C) reported (Ref.)	Yield[a] (%)	Yield[a] (%) (Ref.)
1	**5a**		242–244	–	98	–
2	**5b**		237–238	–	94	–
3	**5c**		254–255	255–256 [7]	96	95 [7]
4	**5d**		258–260	–	94	–
5	**5e**		238–240	–	97	–
6	**5f**		240–242	–	97	–
7	**5g**		255–257	–	93	–
8	**5h**		208–210	–	88	–
9	**5i**		241–243	–	98	–
10	**5j**		244–245	244–246 [7]	91	90 [7]

Table 4 continued

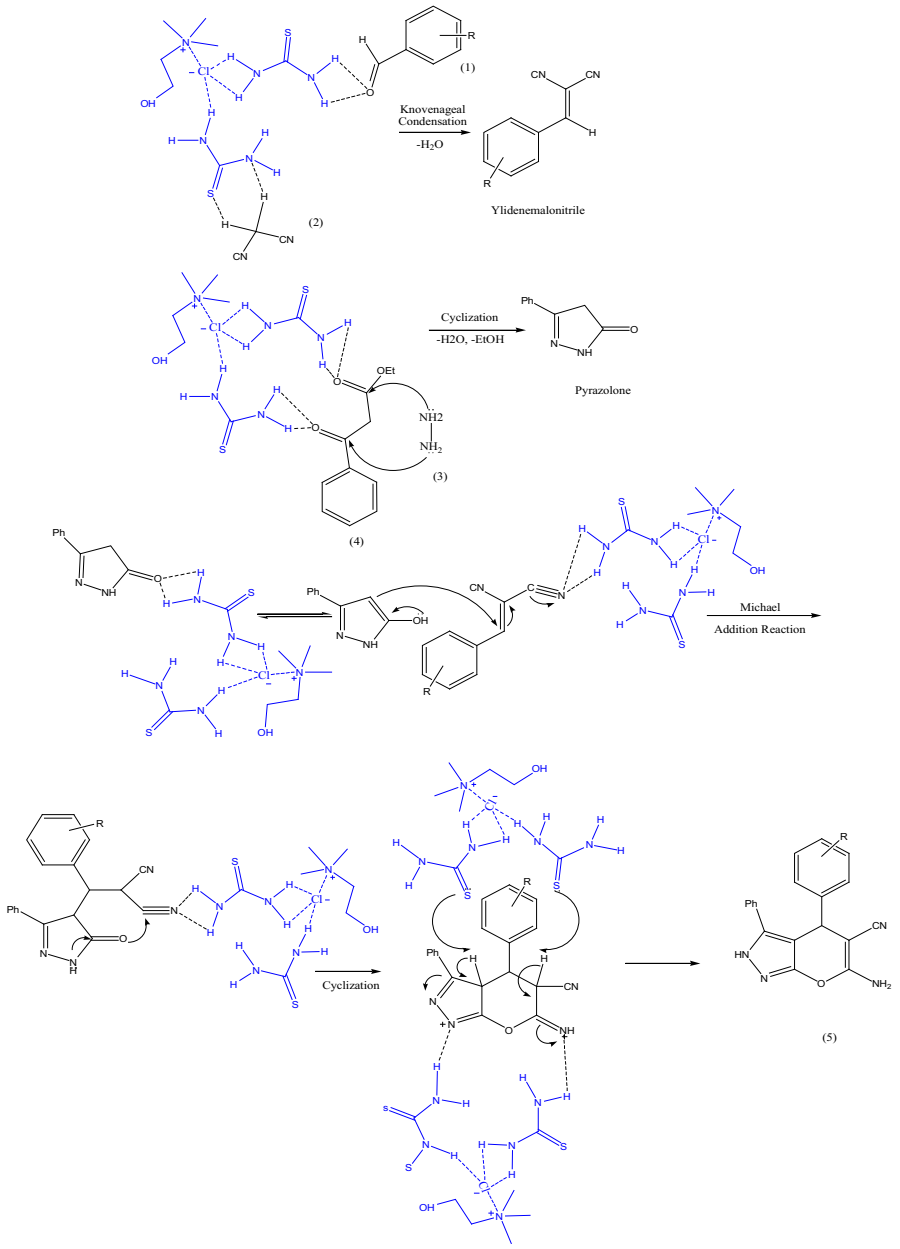
Entry	Product no.	Product	M.P (°C) found	M.P (°C) reported (Ref.)	Yield[a] (%)	Yield[a] (%) (Ref.)
11	**5k**		255–256	–	89	–
12	**5l**		233–234	–	96	–
13	**5m**		250–251	–	89	–
14	**5n**		249–250	–	91	–
15	**5o**		271–273	–	89	–
16	**5p**		261–262	262–263 [7]	96	94 [7]

[a] Isolated yield

ranged between 15 and 45 μg/ml. The MICs of these compounds and cefazolin were determined by using the standard protocol of the NCCLS Broth Microdilution MIC method [55] and the results are presented in Tables 5 and 6.

Conclusion

The structures of some new 6-amino-4-aryl-2,4-dihydro-3-phenyl pyrano [2,3-c] pyrazole-5-carbonitrile derivatives were assigned on the basis on their spectral data and reported compounds by comparing them with earlier literature. This one-pot green chemistry protocol is advantageous in requiring shorter reaction times with high yields, mild reaction conditions, easily available and cheap catalyst, and a simple and practical procedure without any special handing technique and requiring routine reagents.



Scheme 4 Plausible mechanism for the synthesis of substituted pyrano [2,3-c] pyrazole

Screening of the compounds for their antibacterial activity compared with cefazolin were performed at different concentrations: 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 μg/ml by using the Broth Microdilution MIC method.

Table 5 MIC values of compounds (**5a–p**)

Comp no.	MIC (μg ml^{-1})			
	E. coli	*P. aeruginosa*	*S. saprophyticus*	*S. aureus*
5a	25	20	30	35
5b	30	40	35	30
5c	30	40	25	40
5d	35	20	35	35
5e	35	20	30	40
5f	25	40	30	25
5g	30	20	35	30
5h	40	20	40	35
5i	40	45	30	30
5j	30	35	35	35
5k	35	30	30	30
5l	30	25	25	35
5m	30	45	35	35
5n	30	45	15	35
5o	25	30	35	15
5p	15	20	35	25

Table 6 Antibacterial activity of different compounds by the Broth Microdilution MIC method

Antibacterial activity against standard strain-compounds	Concentration (μg/ml)									
	5	10	15	20	25	30	35	40	45	50
5a										
1	+	+	+	+	−	−	−	−	−	−
2	+	+	+	−	−	−	−	−	−	−
3	+	+	+	+	+	−	−	−	−	−
4	+	+	+	+	+	+	−	−	−	−
5b										
1	+	+	+	+	+	−	−	−	−	−
2	+	+	+	+	+	+	+	−	−	−
3	+	+	+	+	+	+	−	−	−	−
4	+	+	+	+	+	−	−	−	−	−
5c										
1	+	+	+	+	+	−	−	−	−	−
2	+	+	+	+	+	+	+	−	−	−
3	+	+	+	+	−	−	−	−	−	−
4	+	+	+	+	+	+	+	−	−	−
5d										
1	+	+	+	+	+	+	−	−	−	−
2	+	+	+	−	−	−	−	−	−	−
3	+	+	+	+	+	+	−	−	−	−

Table 6 continued

Antibacterial activity against standard strain-compounds	Concentration (μg/ml)									
	5	10	15	20	25	30	35	40	45	50
4	+	+	+	+	+	+	−	−	−	−
5e										
1	+	+	+	+	+	+	−	−	−	−
2	+	+	+	−	−	−	−	−	−	−
3	+	+	+	+	+	−	−	−	−	−
4	+	+	+	+	+	+	+	−	−	−
5f										
1	+	+	+	+	−	−	−	−	−	−
2	+	+	+	+	+	+	+	−	−	−
3	+	+	+	+	+	−	−	−	−	−
4	+	+	+	+	−	−	−	−	−	−
5g										
1	+	+	+	+	+	−	−	−	−	−
2	+	+	+	−	−	−	−	−	−	−
3	+	+	+	+	+	+	−	−	−	−
4	+	+	+	+	+	−	−	−	−	−
5h										
1	+	+	+	+	+	+	+	−	−	−
2	+	+	+	−	−	−	−	−	−	−
3	+	+	+	+	+	+	+	−	−	−
4	+	+	+	+	+	+	−	−	−	−
5i										
1	+	+	+	+	+	+	+	−	−	−
2	+	+	+	+	+	+	+	+	−	−
3	+	+	+	+	+	−	−	−	−	−
4	+	+	+	+	+	−	−	−	−	−
5j										
1	+	+	+	+	+	−	−	−	−	−
2	+	+	+	+	+	+	−	−	−	−
3	+	+	+	+	+	+	−	−	−	−
4	+	+	+	+	+	+	−	−	−	−
5k										
1	+	+	+	+	+	+	−	−	−	−
2	+	+	+	+	+	−	−	−	−	−
3	+	+	+	+	+	−	−	−	−	−
4	+	+	+	+	+	−	−	−	−	−
5l										
1	+	+	+	+	+	−	−	−	−	−
2	+	+	+	+	−	−	−	−	−	−
3	+	+	+	+	−	−	−	−	−	−

Table 6 continued

Antibacterial activity against standard strain-compounds	Concentration (μg/ml)									
	5	10	15	20	25	30	35	40	45	50
4	+	+	+	+	+	+	−	−	−	−
5m										
1	+	+	+	+	+	−	−	−	−	−
2	+	+	+	+	+	+	+	+	−	−
3	+	+	+	+	+	+	−	−	−	−
4	+	+	+	+	+	+	−	−	−	−
5n										
1	+	+	+	+	+	−	−	−	−	−
2	+	+	+	+	+	+	+	+	−	−
3	+	+	−	−	−	−	−	−	−	−
4	+	+	+	+	+	+	−	−	−	−
5o										
1	+	+	+	+	−	−	−	−	−	−
2	+	+	+	+	+	−	−	−	−	−
3	+	+	+	+	+	+	−	−	−	−
4	+	+	−	−	−	−	−	−	−	−
5p										
1	+	+	−	−	−	−	−	−	−	−
2	+	+	+	−	−	−	−	−	−	−
3	+	+	+	+	+	+	−	−	−	−
4	+	+	+	+	−	−	−	−	−	−

Cefazolin is taken as a standard drug and its MIC is >35 μg/mL against all the four strains

1 Esherichia coli, *2 Pseudomonas aeruginosa*, *3 Staphylococcus saprophyticus*, *4 Staphylococcus aureus*

+ Resistant, − susceptible

In this work, the catalytic activity was greater for choline chloride:thiourea compared with choline chloride:urea because of the acidic strength of thiourea compared with urea. All the synthesized compounds showed antibacterial activity against both Gram-positive and Gram-negative standard strains, showing that the antibacterial activity of these compounds is greater than cefazolin.

Acknowledgements We gratefully acknowledge the financial support from the Research Council of Islamic Azad University-Tehran North Branch.

References

1. R.M. Armstrong, A.P. Combs, P.A. Tempest, S.D. Brown, T.A. Keating, Acc. Chem. Res. **29**, 123 (1996)
2. I. Ugi, Pure Appl. Chem. **73**, 187 (2001)
3. Y. Gu, Green Chem. **14**, 2091 (2012)

4. P. Prasanna, S. Perumal, Menéndez JC. Green Chem **15**, 1292–1299 (2013)
5. A. Mobinikhaledi, N. Foroughifar, T. Mosleh, A. Hamta, Iranian J of Pharm Res **13**, 873 (2013)
6. N. J. Parmar, B. R. Pansuriya, B. D. Parmar, H. A. Barad, Med. Chem. Res., doi:10.1007/s00044-013-0608-2
7. S.R. Mandha, S. Siliveri, M. Alla, V.R. Bommena, M.R. Bommineni, S. Balasubramanian, Bioorg. Med. Chem. Lett. **22**, 5272 (2012)
8. M. Shahi, N. Foroughifar, A. Mobinikhaledi, Iran J Pharm Res. **14**, 757 (2014)
9. H. Junek, H. Aigner, Chem. Ber. **106**, 914 (1973)
10. A.H. Abdel-Rahman, E.M. Keshk, M.A. Hanna, S.H.M. El-Bady, Bioorg Med Chem. **12**, 2483 (2004)
11. S.M. Bensaber, H.A. Allafe, N.B. Ermeli, S.B. Mohamed, A.A. Zetrini, S.G. Alsabri, M. Erhuma, A. Hermann, M.I. Jaeda, A.M. Gbaj, Med. Chem. Res. **23**, 5120 (2014)
12. D. Kaushik, R. Kumar, S. Ahmed Khan, G. Chawla, Med. Chem. Res. **21**, 3646 (2011)
13. M.E.A. Zaki, H.A. Soliman, O.A. Hiekal, A.E. Rashad, Naturforsch. **61C**, 1 (2006)
14. N.R. Dighore, P. Anandgaonker, S.T. Gaikwad, A.S. Rajbhoj, Green Process. Synth. **5**, 139 (2016)
15. A. Anshu Dandia, D. Saini, S. Bhaskaran, D.K. Saini, Med. Chem. Res. (2013). doi:10.1007/s00044-013-0671-8
16. N. Foloppe, L.M. Fisher, R. Howes, A. Potter, A.G.S. Robertson, A.E. Surgenor, Bioorg Med Chem. **14**, 4792 (2006)
17. J.K. Barton, Science **233**, 727 (1986)
18. R.D. Snyder, Mutat. Res. **623**, 72 (2007)
19. M.A. Rogawski, Epilepsy Res. **68**, 22 (2006)
20. M.A. Rogawski, W. Loscher, Nat. Med. **10**, 685 (2004)
21. P. Wiffen, S. Collins, H. McQuary, D. Carroll, A. Jadad, Cochrane Database Syst. Rev. **3**:CD001133 (2005)
22. C.P. Taylor, Curr. Pharm. Des. **2**, 375 (1996)
23. S.L. Collins, R.A. Moore, H.J. McQuary, P. Wiffen, J Pain Symp Manage. **20**, 449 (2000)
24. G. Vasuki, K. Kumaravel, Tetrahedron Lett. **49**, 5636 (2008)
25. S.H.S. Azzam, M.A. Pasha, Tetrahedron Lett. **53**, 6834 (2012)
26. M. Bakherad, A. Keivanloo, M. Gholizadeh, R. Doosti, M. Javanmardi, Res. Chem. Intermed. (2016). doi:10.1007/s11164-016-2680-y
27. H. Mecadon, M.D.R. Rohman, M. Rajbangshi, B. Myrboh, Tetrahedron Lett. **52**, 2523 (2011)
28. A.R. Moosavi-Zare, M.A. Zolfigol, A. Mousavi-Tashar, Res. Chem. Intermed. (2016). doi:10.1007/s11164-016-2537-4
29. A. Hasaninejad, M. Shekouhy, N. Golzar, A. Zare, M. M. Doroodmand, Appl. Catal, A: General. **402**, 11 (2011)
30. Z. Li, H. Zheng, Green Process Synth. **3**, 447 (2014)
31. A. R. Moosavi-Zare, M. A. Zolfigol, E. Noroozizadeh, M. Tavasoli, V. Khakyzadeh, A. Zare, New J Chem. 4089 (2013)
32. J.M. Khurana, B. Nand, S. Kymar, Synthetic Commun. **41**, 405 (2011)
33. S.N. Darandale, J.N. Sangshetti, D.B. Shinde, J of the Korean Chem Soc. **56**, 2512 (2012)
34. M. Zakeri, M.M. Nasef, T. Kargaran, A. Ahmad, E. Abouzari-Lotf, J. Asadi, Res. Chem. Intermed. (2016). doi:10.1007/s11164-016-2648-y
35. M.A. Zolfigol, M. Tavasoli, A.R. Moosavi-Zare, P. Moosavi, H.G. Kruger, M. Shiri, V. Khakyzadeh, RSC Adv. **3**, 25681 (2013)
36. M.A.E.A.A. El-Remaily, Tetrahedron **70**, 2971 (2014)
37. M.A. Chaudhari, J.B. Guijar, D.S. Kawade, N.R. Jogdand, M.S. Shingare, Cogent Chemistry (2015). doi:10.1080/23312009.1063830
38. A. Mobinikhaledi, T. Mosleh, N. Foroughifar, Res. Chem. Intermed. **41**, 2985 (2015)
39. A. Mobinikhaledi, N. Foroughifar, H. Moghanian, R. Mozaffari, Res. Chem. Intermed. **41**, 6523 (2015)
40. H. Mecadon, M.D.R. Rohman, I. Kharbangar, B.M. Laloo, I. Kharkongor, M. Rajbangshi, B. Myrboh, Tetrahedron Lett. **52**, 3228 (2011)
41. S. Ambethkar, V. Padmini, N. Bhuvanesh, J Adv Res. **6**, 975 (2015)
42. H.R. Shaterian, K. Azizi, Res. Chem. Intermed. **40**, 661 (2014)
43. R.D. Kamble, B.S. Dawane, O.S. Yemul, A.B. Kale, S.D. Patil, Res. Chem. Intermed. **39**, 3859 (2013)

44. M.R. Bhosle, L.D. Khillare, S.T. Dhumal, R.A. Mane, Chin. Chem. Lett. (2015). doi:10.1016/j.cclet.12.005
45. N.V. Plechkova, K.R. Seddon, Chem. Soc. Rev. **37**, 123 (2008)
46. N.V. Plechkova, K.R. Seddon, P. Tundo, A. Perosa, F. Zecchini, Methods Reag. Green Chem. **105**, 853 (2007)
47. C. Burda, X.B. Chen, R. Narayanan, M.A. El-Sayed, Chem. Rev. **105**, 1025 (2005)
48. A.P. Abbott, R.C. Harris, K.S. Ryder, C. D'Agostino, L.F. Gladden, M.D. Mantle, Green Chem. **13**, 82 (2011)
49. I.T. Horvath, Green Chem. **10**, 1024 (2008)
50. A.P. Abbott, G. Capper, D.L. Davies, R.K. Rasheed, V. Tambyrajah, Chem. Commun. **7**, 70 (2003)
51. B. Singh, H. Lobo, G. Shankarling, Catal. Lett. **141**, 178 (2011)
52. I. Mamajanov, A.E. Engelhart, H.D. Bean, N.V. Hud, Angew. Chem. Int. Ed. **36**, 6310 (2010)
53. A. Khajeh-Amiri, A. Mobimikhaledi, Res. Chem. Intermed. **39**, 1491 (2013)
54. A. Mobimikhaledi, A. Khajeh-Amiri, Res. Chem. Intermed. **41**, 2063 (2015)
55. P. A. Wayne, NCCLS, Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Information Supplement M, In National Committee for Clinical Laboratory Standards, 100–512 (2002)