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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antimicrobial and antioxidant activities of imidazotriazoles and new multicomponent reaction toward 5-amino-1-phenyl[1,2,4]triazole derivatives

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Abstract The Groebke-type multicomponent reaction between 5-amino-1,2,4-triazole derivatives, aromatic aldehydes and isocyanides has been studied from the viewpoint of convenient generation of combinatorial arrays of imidazo[2,1-*c*][1,2,4]triazoles. The reaction is considered to proceed via the formation of an iminium species followed by a [4 + 1] cyclo addition with the isocyanides using scandium triflate as a Lewis acid catalyst. The synthesized imidazo[2,1-*c*][1,2,4]triazole derivatives **4a**, **4b**, **4f** and **4g** were screened for antibacterial, antifungal and antioxidant activities. Among the tested compounds, **4b** followed by **4f** showed potent antibacterial and antifungal activities. All of these compounds were also screened in vitro for the antioxidant activity using DPPH assay. Most of them have shown very significant antioxidant activity.

Keywords 5-Amino-1-phenyl-1,2,4-triazole · MCR · Imidazo[2,1-*c*][1,2,4]triazole · Antioxidant · Antimicrobial

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Introduction

The chemistry of heterocyclic compounds has attracted much attention in recent times due to its increasing importance in the field of pharmaceuticals and industrial chemicals (Al-Tel and Al-Qawasmeh, 2010; Shukla *et al*., 2012; Bode *et al*., 2011). In fact, the development of simple, elegant and facile methodologies for the synthesis of heterocycles is one of the most important aspects in organic synthesis. Multicomponent reactions (MCRs), reactions involving at least three starting materials in a one-pot reaction, have brought some of the most efficient syntheses of heterocycles (Adib *et al*., 2011; Schwerkoske *et al*., 2005; Rousseau *et al*., 2007; Parchinsky *et al*., 2006). Isocyanide-based MCRs (IMCRs), such as the Passerini, the Ugi reactions and the Groebke–Blackburn–Bienaymé, are very useful for the diversity oriented synthesis of collections of compounds (El Kaim and Grimaud, 2009; Sadjadi and Heravi, 2011; Khan *et al*., 2012; Shaabani *et al*., 2001; Mandair *et al*., 2002; Guchhait and Madaan, 2011; Shukla *et al*., 2012). They allow a dramatic increase of structural complexity in just one step, introducing at the same time three or more diversity inputs with a high degree of atom economy (Agrebi *et al*., 2013; Xu *et al*., 2012; Shaw *et al*., 2012; El Kaim *et al*., 2010).

Materials and methods

Chemistry

Melting points were measured on an electrothermal apparatus. Reactions were checked with TLC using aluminum sheets with silica gel 60 F254 from Merck. Spectra IR were recorded on a Perkin-Elmer PARAGON FT-IR spectrometer covering field 400–4,000 cm^{-1}. The spectra of ^{1}H

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NMR and ^{13}C NMR were recorded in solution in $CDCl_3$ on a Bruker spectrometer (^1H at 400 MHz, ^{13}C at 100 MHz) and high-resolution mass spectra (HRMS). The chemical shifts are expressed in parts per million (ppm) by using tetramethylsilane (TMS) as internal reference. The multiplicities of the signals are indicated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quadruplet; and m, multiplet, and coupling constants are expressed in Hertz. The chemical reagents used in synthesis were purchased from Fluka, Sigma and Aldrich. The microwave-assisted reactions were carried out in synthetic microwave: Monowave 300 with a maximum power of 300 W.

General procedure for the synthesis and spectral data of 5-amino-1-phenyl[1,2,4]triazole derivatives **1a–c** using the one-step method

To a mixture of derivatives orthoester (1 mmol), phenyl hydrazine (1.2 mmol), acetic acid (5 mol%) and cyanamide (1 mmol) in 3 ml of methanol, it was heated with microwave radiation (200 W) for 40 min at 80 °C. After the completion of the reaction, as indicated by TLC (EtOAc-hexane, 90:10), the precipitated solid was filtered, washed with ether and was crystallized from a suitable solvent to obtain pure product.

*Amino-3-methyl-1-phenyl[1,2,4]triazole (**1a**)*

It was obtained as a yellow solid; yield: 95 %; mp: 131–134 °C; IR υ_{max}: 3,397, 3,477, 1,616, 1,537 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.53–7.44$ (4H, m, H-7, H-8, H-10, H-11), 7.35–7.31 (1H, m, H-9), 6.33 (2H, br, NH_2), 2.11 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 157.4$ (C, C-3), 154.6 (C, C-5), 137.3 (C, C-6), 129.2 (CH, C-8, C-10), 126.4 (CH, C-9), 122.2 (CH, C-7, C-11), 13.8 (CH_3); HRMS Calcd. for $C_9H_{10}N_4$: 174.0905, found 174.0907.

*Amino-3-ethyl-1-phenyl[1,2,4]triazole (**1b**)*

It was obtained as a yellow solid; yield: 94 %; mp: 120–124 °C; IR υ_{max} 3,396, 3,470, 1,622, 1,537 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.57–7.51$ (4H, m, H-7, H-8, H-10, H-11), 7.46–7.42 (1H, m, H-9), 4.88 (2H, br, NH_2), 2.59 (2H, q, $J = 7.7$ Hz, CH_2), 1.29 (3H, t, $J = 7.7$ Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 164.1$ (C, C-3), 156.3 (C, C-5), 138.1 (C, C-6), 130.7 (CH, C-8, C-10), 129.2 (CH, C-9), 124.9 (CH, C-7, C-11), 22.4 (CH_2), 12.8 (CH_3); HRMS Calcd. for $C_{10}H_{12}N_4$: 188.1062, found 188.1063.

*Amino-3-benzyl-1-phenyl[1,2,4]triazole (**1c**)*

It was obtained as a yellow solid; yield: 87 %; mp: 137–140 °C; IR υ_{max} 3,397, 3,477, 1,620, 1,539 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.54–7.53$ (4H, m), 7.45–7.40 (1H, m), 7.35–7.29 (4H, m), 7.21 (1H, t, $J = 7.0$ Hz, H-16), 4.88 (2H, s, NH_2), 3.90 (2H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 161.8$ (C, C-3), 156.5 (C, C-5), 139.1 (C, C-13), 138 (C, C-6), 130.7 (CH, C-7, C-11), 129.8 (CH, C-14, C-18), 129.5 (CH, C-8, C-10), 127.5 (CH, C-16), 129.2 (CH, C-9), 124.9 (CH, C-15, C-17), 35.2 (CH_2), HRMS Calcd. for $C_{15}H_{14}N_4$: 250.1218, found 250.1213.

General procedure for the synthesis and spectral data of substituted imidazotriazole (**4a–i**)

General procedure for MCR of 5-amino-1-phenyl-1,2,4-triazoles, aldehydes and isonitriles: A solution of 5-amino-1-phenyl-1,2,4-triazole (1 mmol), aldehyde (1 mmol) and 5 % $Sc(OTf)_3$ in 1 ml DMF was heated at 80 °C for 20 min, isonitrile (1.2 mmol) was added, and the mixture was brought to reflux. After 30 h, the crude product is isolated by flash chromatography on silica gel [PE/DCM (8:2)].

*N-(4-chlorobenzyl)-6-(4-chlorophenyl)-3-methyl-1-phenyl-1H-imidazo[2,1-c][1,2,4] triazol-5-amine (**4a**)*

It was obtained as a yellow solid; yield: 72 %; mp: 220–222 °C; IR υ_{max} 3,320, 3,065, 2,835, 1,597, 1,505, 1,489, 1,458, 1,433, 1,406 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 8.16$ (2H, d, $J = 8.0$ Hz, H-10, H-14), 7.94 (2H, d, $J = 7.5$ Hz, H-17, H-19), 7.52 (2H, t, $J = 7.5$ Hz, H-24, H-26), 7.42 (2H, d, $J = 7.5$ Hz, H-23, H-27), 7.32 (2H, d, $J = 8.8$ Hz, H-16, H-20), 7.25–7.22 (3H, m, H-11, H-12, H-13), 4.16 (2H, s, H-21), 3.2 (1H, br, NH), 2.58 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 145.8$ (C, C-3), 138.0 (C, C-9), 137.8 (C, C-22), 136.9 (C, C-8), 136.4 (C, C-6), 133.6 (C, C-18), 133.0 (C, C-25), 132.9 (C, C-15), 129.6 (CH, C-11, C-13), 129.3 (CH, C-17, C-19), 128.9 (CH, C-18, C-20), 128.6 (CH, C-24, C-26), 127.9 (CH, C-23, C-27), 124.4 (CH, C-12), 121.0 (C, C-5), 116.3 (CH, C-10, C-14), 54.1 (C, C-21, CH_2), 11.4 (C, CH_3); HRMS Calcd. for $C_{24}H_{19}Cl_2N_5$: 447.1018, found 447.1006.

*6-(4-chlorophenyl)-N-Cyclohexyl-3-methyl-1-phenyl-1H-imidazo[2,1-c][1,2,4]triazol-5-amine (**4b**)*

It was obtained as a yellow solid; yield: 57 %; mp: 220–223 °C; IR υ_{max} 3,294, 3,067, 2,925, 2,853, 1,609,

1,598, 1,505, 1,488, 1,459, 1,440 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 8.06 (2H, d, *J* = 8 Hz, H-11, H-13), 7.95 (2H, d, *J* = 8.8 Hz, H-16, H-20), 7.40 (2H, t, *J* = 8.0 Hz, H-10, H-14), 7.31 (2H, d, *J* = 8.0 Hz, H-17, H-19), 7.11 (1H, t, *J* = 7.5 Hz, H-12), 2.81 (1H, br, NH), 2.75 (2H, br, H-21), 2.62 (3H, s, CH_3), 1.76–1.35 (5H, m, H-22, H-23, H-24, H-25, H-26), 1.10–1.08 (5H, m, H-22, H-23, H-24, H-25, H-26); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 145.9 (C, C-3), 138.3 (C, C-9), 137.9 (C, C-8), 136.6 (C, C-6), 133.4 (C, C-18), 132.7 (C, C-15), 129.2 (CH, C-11, C-13), 128.4 (CH, C-17, C-19), 128.1 (CH, C-16, C-20), 124.3 (CH, C-12), 121 (C, C-5), 116.3 (CH, C-10, C-14), 58.6 (CH, C-21), 33.7 (CH, C-22, C-26), 25.7 (CH, C-24), 24.8 (CH, C-23, C-25), 11.7 (CH_3); HRMS Calcd. for $C_{23}H_{24}ClN_5$: 405.1720, found 405.1713.

*N-Cyclohexyl-6-(4-fluorophenyl)–3-methyl-1-phenyl-1H-imidazo[2,1-c][1,2,4]triazol-5-amine (**4c**)*

It was obtained as a yellow solid; yield: 60 %; mp: 182–185 °C; IR υ_{max} 3,219, 3,082, 2,933, 2,918, 2,851, 1,648, 1,593, 1,583, 1,501, 1,440 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 8.61 (2H, d, *J* = 7.8 Hz, H-10, H-14), 8.10 (2H, t, *J* = 7.8 Hz, H-16, H-20), 7.52 (2H, d, *J* = 7.62 Hz, H-11, H-13), 7.26–7.22 (3H, m, H-17, H-19, H-12), 3.30 (1H, br, NH), 2.82 (3H, s, CH_3), 2.05–1.32 (11H, m, H-21, H-22, H-23, H-24, H-25, H-26); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 144.2 (C, C-3), 137.7 (C, C-9), 137.6 (C, C-8), 136.2 (C, C-6), 133.2 (C, C-18), 133.1 (C, C-15), 129.7 (CH, C-11, C-13), 129.3 (CH, C-17, C-19), 124.4 (CH, C-16, C-20), 116.1 (CH, C-12), 115.1 (C, C-5), 114.9 (CH, C-10, C-14), 58.4 (CH, C-21), 33.8 (CH, C-22, C-26), 25.4 (CH, C-24), 24.8 (CH, C-23, C-25), 13.3 (CH_3); HRMS Calcd. for $C_{23}H_{24}FN_5$: 389.2016, found 389.2018.

*N-Cyclohexyl-6-(2,6-dichlorophenyl)-3-methyl-1-phenyl-1H-imidazo[2,1-c][1,2,4]triazol-5-amine (**4d**)*

It was obtained as a yellow solid; yield: 54 %; mp: 174–178 °C; IR υ_{max} 3,348, 3,065, 2,923, 2,852, 1,585, 1,504, 1,425 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.99 (2H, d, *J* = 8.5 Hz, H-17, H-19), 7.37–7.32 (4H, m, H-10, H-11, H-13, H-14), 7.18 (1H, t, *J* = 8.5 Hz, H-18), 7.06 (1H, t, *J* = 7.3 Hz, H-12), 2.64 (3H, s, CH_3), 2.59 (2H, br, NH, H-21), 1.72 (2H, d, *J* = 8.5 Hz, H-22, H-26), 1.52–1.41 (3H, m, H-23, H-25, H-24), 1.07–0.84 (5H, m, H-22, H-23, H-24, H-25, H-26); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 145.6 (C, C-3), 138.0 (C, C-9), 136.6 (C, C-8), 134.5 (C, C-16, C-20), 134.2 (C, C-6), 132.4 (C, C-15), 130.0 (CH, C-18), 129.1(CH, C-11, C-13), 128.8 (CH, C-10, C-14), 124.1 (CH, C-12), 123.0 (C, C-5), 116.5 (CH, C-17, C-19), 58.3 (CH, C-21), 33.5 (CH, C-22, C-26), 25.7 (CH, C-24), 24.6 (CH, C-23, C-25), 11.6 (CH_3); HRMS Calcd. for $C_{23}H_{23}Cl_2N_5$: 439.1331, found 439.1351.

*N-(4-methoxybenzyl)-6-(4-chlorophenyl)-3-methyl-1-phenyl-1H-imidazo[2,1-c][1,2,4] triazol-5-amine (**4e**)*

It was obtained as a white solid; yield: 72 %; mp: 170–175 °C; IR υ_{max} 3,322, 3,063, 2,999, 2,922, 2,834, 1,594, 1,541, 1,504, 1,488, 1,458 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 8.18 (2H, d, *J* = 7.0 Hz, H-16, H-20), 7.99 (2H, d, *J* = 8.0 Hz, H-17, H-19), 7.51 (2H, d, *J* = 7.5 Hz, H-23, H-26), 7.42 (2H, d, *J* = 8.4 Hz, H-24, H-25), 7.25–7.21 (3H, m, H-11, H-12, H-13), 6.89 (2H, d, *J* = 7.1 Hz, H-10, H-14), 4.15 (2H, s, H-21), 3.85 (3H, s, OCH_3), 3.16 (1H, br, NH), 2.58 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 159.2 (C, C-25), 155.2 (C, C-3), 145.8 (C, C-9), 137.9 (C, C-8), 136.6 (C, C-6), 133.1 (C, C-22), 132.6 (C, C-18), 130.7 (C, C-15), 129.5 (CH, C-11, C-13), 129.3 (C, C-17, C-19), 128.6 (C, C-16, C-20), 127.9 (CH, C-23, C-27), 124.3 (CH, C-12), 121.5 (C, C-5), 116.3 (CH, C-10, C-14), 114.1 (CH, C-24, C-26), 55.3 (CH_3, OCH_3), 54.4 (CH_2), 11.3 (CH_3); HRMS Calcd. for $C_{25}H_{22}ClN_5O$: 443.1513, found 443.1551.

*6-(4-chlorophenyl)-N-cyclohexyl-3-phenyl-1-phenyl-1H-imidazo[2,1-c][1,2,4]triazol-5-amine (**4f**)*

It was obtained as a white solid; yield: 60 %; mp: 194–196 °C; IR υ_{max} 3,299, 3,073, 2,974, 2,933, 2,852, 1,600, 1,587, 1,506, 1,460, 1,440, 1,409 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 8.20 (2H, d, *J* = 7.3 Hz, H-11, H-13), 8.05 (2H, d, *J* = 8.5 Hz, H-16, H-20), 7.53 (2H, t, *J* = 7.7 Hz, H-10, H-14), 7.42 (2H, d, *J* = 8.5 Hz, H-17, H-19), 7.28 (1H, t, *J* = 7.5 Hz, H-12), 3.10 (2H, q, *J* = 7.1 Hz, CH_2), 2.89 (1H, br, NH), 1.87 (1H, br, H-21), 1.73–1.64 (5H, m, H-22, H-23, H-24, H-25, H-26), 1.55 (3H, t, *J* = 7.4 Hz, CH_3), 1.2 (5H, m, H-22, H-23, H-24, H-25, H-26); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 146.0 (C, C-3), 141.5 (C, C-9), 138.3 (C, C-8), 138.0 (C, C-6), 133.5 (C, C-18), 132.5 (C, C-15), 129.2 (CH, C-11, C-13), 128.4 (CH, C-17, C-19), 128.2 (CH, C-16, C-20), 124.2 (CH, C-12), 120.6 (C, C-5), 116.4 (CH, C-10, C-14), 58.6 (CH, C-21), 33.8 (CH, C-22, C-26), 25.7 (CH, C-24), 24.9 (CH, C-23, C-25), 19.6 (CH_2), 11.4 (CH_3); HRMS Calcd. for $C_{24}H_{26}ClN_5$: 419.1876, found 419.1877.

*N-cyclohexyl-6-(2,6-dichlorophenyl)-3-ethyl-1H-imidazo[2,1-c][1,2,4]triazol-5-amine (**4g**)*

It was obtained as a white solid; yield: 70 %; mp: 138–141 °C; IR υ_{max} 3,345, 3,058, 2,933, 2,853, 1,671, 1,598, 1,579, 1,504, 1,455, 1,425 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 8.09 (2H, d, *J* = 8.8 Hz, H-17, H-19),

7.35–7.29 (4H, m, H-10, H-11, H-13, H-14), 7.14 (t, $J = 7.7$ Hz, 1H, H-18), 7.03 (t, $J = 7.7$ Hz, 1H, H-12), 2.99 (q, $J = 14.5$ Hz, 2H, CH_2), 2.58 (br, 2H, NH, H-21), 1.69 (d, $J = 12$ Hz, 2H, H-22, H-26), 1.49-1.41 (m, 6H, CH_3, H-23, H-25, H-24), 1.07–0.84 (m, 5H, H-22, H-23, H-24, H-25, H-26); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 145.7$ (C, C-3), 141.6 (C, C-9), 138.1 (C, C-8), 136.6 (C, C-16, C-20), 134.4 (C, C-6), 132.6 (C, C-15), 130.0 (CH, C-18), 129.2 (CH, C-11, C-13), 128.2 (CH, C-10, C-14), 124.1 (CH, C-12), 123.0 (C, C-5), 116.5 (CH, C-17, C-19), 58.3 (CH, C-21), 33.5 (CH, C-22, C-26), 25.7 (CH, C-24), 24.6 (CH, C-23, C-25), 19.65 (CH_2), 11.5 (CH_3); HRMS Calcd. for $C_{25}H_{25}Cl_2N_5$: 543.1487, found 543.1487.

*6-(4-benzonitrile)-N-cyclohexyl-3-ethyl-1-phenyl-1-H-imidazo[2,1-c][1,2,4]triazol-5-amine (**4h**)*

It was obtained as a white solid; yield: 57 %; mp: 170–173 °C; IR υ_{max} 3,466, 3,062, 2,928, 2,853, 2,226, 1,644, 1,601, 1,537, 1,505, 1,450, 1,404 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 8.11$ (2H, d, $J = 7.0$ Hz, H-17, H-19), 8.03 (2H, d, $J = 7.0$ Hz, H-11, H-13), 7.54 (2H, d, $J = 7.0$ Hz, H-16, H-20), 7.37 (2H, t, $J = 7.5$ Hz, H-10, H-14), 7.09 (1H, t, $J = 7.3$ Hz, H-12), 2.89 (2H, q, $J = 7.8$ Hz, CH_2), 2.70 (2H, br, NH, H-21), 1.70 (2H, s, H-22, H-26), 1.59–1.51 (3H, m, H-23, H-24, H-25), 1.39 (3H, t, $J = 7.5$ Hz, CH_3), 1.08–1.04 (5H, m, H-22, H-23, H-24, H-25, H-26); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 146.1$ (C, C-3), 141.3 (C, C-9), 139.6 (C, C-8), 137.9 (C, C-15), 137.4 (C, C-6), 132.0 (CH, C-17, C-19), 129.2 (CH, C-11, C-13), 127.0 (CH, C-16, C-20), 124.4 (CH, C-2), 121.9 (C, C-5), 119.4 (C, CN), 116.6 (CH, C-10, C-14), 109.6 (C, C-18), 58.7 (CH, C-21), 33.7 (CH, C-22, C-26), 25.7 (CH, C-24), 24.8 (CH, C-23, C-25), 19.6 (CH_2), 11.1 (CH_3); HRMS Calcd. for $C_{25}H_{26}N_6$: 410.2219, found 410.2211.

*3-Benzyl-6-(4-chlorophenyl)-N-cyclohexyl-1-phenyl-1H-imidazo[2,1-c][1,2,4]triazol-5-amine (**4i**)*

It was obtained as a white solid; yield: 62 %; mp: 179–182 °C; IR υ_{max} 3,294, 3,067, 2,926, 2,853, 1,662, 1,609, 1,597, 1,539, 1,505, 1,488, 1,458, 1,440, 1,405 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 8.03$ (2H, d, $J = 8.0$ Hz, H-17, H-19), 7.35–7.10 (10H, m, H-10, H-11, H-12, H-13, H-14, H-29, H-30, H-31, H-32, H-33), 7.05 (1H, t, $J = 7.5$ Hz, H-12), 4.35 (2H, s, H-27), 2.40 (1H, br, NH), 2.35 (1H, br, H-21), 1.59 (2H, d, $J = 12.0$ Hz, H-22, H-26), 1.41–1.34 (3H, m, H-23, H-24, H-25), 0.87–0.69 (5H, m, H-22, H-23, H-24, H-25, H-26); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 145.7$ (C, C-3), 138.7 (C, C-9), 138.0 (C, C-28), 136.6 (C, C-16, C-20), 135.5 (C, C-8), 143.7 (C, C-6), 132.6 (C, C-15), 130.0 (CH, C-18), 129.3 (CH, C-11, C-13), 128.8 (CH, C-29, C-33), 128.7 (CH, C-30, C-32), 128.2 (CH, C-10, C-14), 127.2 (CH, C-31), 124.4 (CH, C-12), 123.0 (C, C-5), 116.7 (CH, C-17, C-19), 55.7 (CH, C-21), 33.4 (CH, C-22, C-26), 31.7 (CH_2), 25.7 (CH, C-24), 24.6 (CH, C-23, C-25); HRMS Calcd. for $C_{29}H_{27}Cl_2N_5$: 515.1644, found 515.1649(−).

Antimicrobial activity

Authentic pure cultures of bacteria were obtained from international culture collections (ATCC) and the local culture collection of the Centre of Biotechnology of Sfax, Tunisia. They included Gram-positive bacteria: *B. cereus* ATCC 14579, *S. aureus* ATCC 25923, and Gram-negative bacteria: *S. enteritidis* (food isolate), *E. coli ATCC 25922* and *P. aeruginosa* ATCC 9027. The fungi tested in the antifungal test were *Aspergillus niger* CTM 10099, *Fusarium oxysporum* CTM10402, *Fusarium culmorum* ISPAVE 21w and *Rhizopus Nigerians* CTM 10150. The bacterial strains were grown on Mueller–Hinton broth (Bio-Rad, France) at 37 °C for 12–14 h and on potato dextrose agar (PDA) (1.5 % agar) at 28 °C for 4 days for fungus. Inocula were prepared from an overnight broth culture by their dilution in saline solution to approximately 10^7 colony-forming units CFU/ml for bacteria and 10^6 spores/ml for fungus. The compounds were dissolved in DMSO.

Antibacterial and antifungal tests were performed by agar well diffusion method as described by Tagg and McGiven (1971) and broth microdilution assay using sterile Mueller–Hinton media (Bio-Rad, France) for bacterial strains and potato dextrose agar (Bio-Rad, France) for antifungal tests. A freshly cell suspension (100 µl) adjusted to 10^7 CFU/ml for bacteria and 10^5 spores/ml for fungus were inoculated onto the surface of agar plates. Thereafter, wells with 6 mm in diameter were punched in the inoculated agar medium with sterile Pasteur pipettes and the extracts were added to each well. Negative controls consisted of DMSO, used to dissolve the synthesized compounds. Gentamicin (15 µg/well) and amphotericin B (20 µg/well) were used as positive control to determine the sensitivity of each bacterial and fungal strain, respectively. The plate was allowed to stand for 2 h at 4 °C to permit the diffusion of the extracts followed by incubation at 37 °C for 24 h for bacterial strains and 72 h for fungi at 28 °C. The antibacterial activity was evaluated by measuring the zones of inhibition (clear zone around the well) against the test microorganisms. All tests were repeated three times.

Minimum inhibitory concentrations of the synthesized compounds were determined according to Eloff (1998) in sterile 96-well microplates with a final volume in each

microplate well of 200 µl. A stock solution of the extract (50 mg/ml) was prepared in 20 % DMSO. Thereafter, a twofold serial dilution of the extract was prepared in the microplate wells over the range 0.039–10 mg/ml. To each test, well was added 10 µl of cell suspension to a final inoculum concentrations of 10^6 CFU/ml for bacteria and 10^5 spores/ml for fungus. Positive growth control wells consisted of bacteria or fungi only in their adequate medium. DMSO was used as negative control. The plates were then covered with the sterile plate covers and incubated at 37 °C for 24 h for bacterial strains and 72 h for fungi at 28 °C. The MIC was defined as the lowest concentration of the extract at which the microorganism does not demonstrate visible growth after incubation. As an indicator of microorganism growth, 25 µl of *p*-iodonitrotetrazolium violet (INT) (0.5 mg/ml) dissolved in sterile water were added to the wells and incubated at 37 °C for 30 min. Following the determination of the MIC, the MBC was determined by transferring a 10 µl aliquot from each of the wells at the concentration corresponding to the MIC and those concentrations above into agar plate and were incubated under the same conditions. The MBC was considered to be the lowest concentration of the compound at which no growth occurred. All experiments were performed in triplicate.

Determination of DPPH radical-scavenging capacity

The radical-scavenging activity of each synthesized compounds was evaluated on the basis of its activity in scavenging the stable DPPH radical, according to the method described by Kirby and Schmidt (1997). In this procedure, 1 ml of a 4 % solution of DPPH radical in methanol (w/v) was mixed with 500 µl of sample solutions at different concentrations. The scavenging capacity was determined spectrophotometrically after 20 min of incubation by monitoring the decrease of the absorbance at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. Ascorbic acid was used as standard. The percent DPPH scavenging effect was calculated using the following equation: DPPH scavenging effect (%) = (Acontrol − Asample/Acontrol) × 100. A control is the absorbance of the control reaction where the sample is replaced by 500 µl of methanol. Tests were carried out in triplicates.

Results and discussion

Chemistry

*The synthesis of 5-amino-1-phenyl-1,2,4-triazole derivatives **1a–c** in one-step method*

Due to the convenience and high degree of atom economy, MCRs have become one of the most efficient tools for rapid scaffold construction and introduction of molecular diversity (Chen *et al*., 2014). In our search for operationally simple, resource and cost-effective processes, we have been investigating 5-amino-1-phenyl-1,2,4-triazole-based MCRs.

The advent of single-mode microwave reactors, which enable precise control of reaction conditions, has opened the way for the exploration of microwave-assisted synthetic methods in combinatorial and parallel synthesis. As part of an ongoing program of research, we decided to investigate the possibility of using microwaves to accelerate the synthesis of a range of fused 5-aminotriazoles via a three-component reaction. The synthesis of this latter was obtained after using both classical and microwave heating. Their classical synthesis in two steps was well described in the literature (Chihaoui *et al*., 1981).

The one-pot method in the multicomponent reaction of orthoester, phenylhydrazine and cyanamide under microwave irradiation led to the synthesis of aminotriazole with high yield (40 min, 200 W, 80 °C) but in classical conditions need 48 h (Table 1). In this case, we obtained essentially a convenient and rapid method that gives better yields and higher purity of the products than the conventional method for an appropriate time (Scheme 1).

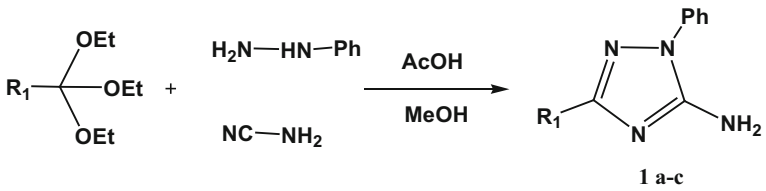
*The synthesis of imidazo[2,1-c][1,2,4]triazole derivatives **(4a–i)***

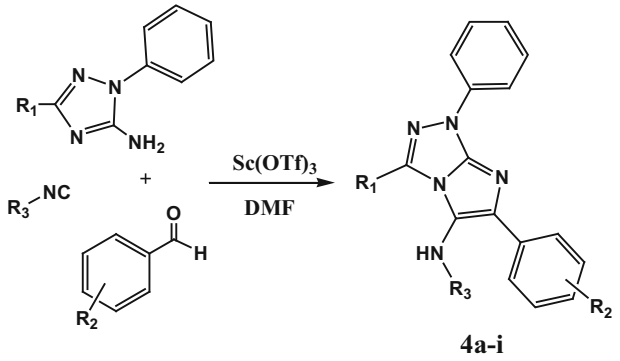
The reaction of 5-amino-1,2,4-triazoles **1a–c** with aldehydes aromatic and isocyanides using scandium triflate as catalyst was carried out in DMF as solvent and has been reported to produce the expected imidazo[2,1-*c*][1,2,4]triazoles **4a–i** into a reliable process that would enable the production of compound arrays in a combinatorial fashion. Herein, we present the results of these studies (Scheme 2).

Table 1 Microwave-assisted synthesis of compounds **1a–c**

Compounds	R_1	Classical condition		Microwave	
		Yields (%)	Time (h)	Yields (%)	Time (min)
1a	CH_3	85	48	95	40
1b	C_2H_5	88	48	94	40
1c	Ph-CH_2	65	48	87	40

Scheme1 Synthesis of 5-amino-1-phenyl-1,2,4-triazoles derivatives **1a–c**





Scheme 2 Synthesis of imidazo[2,1-*c*][1,2,4]triazoles derivatives **4a–i**

A number of isocyanides were combined in dimethylformamide with 5-amino-1,2,4-triazoles and a variety of heterocyclic aldehydes (Table 2) and heated at reflux for 30 h in the presence of scandium triflate $Sc(OTf)_3$.

A mechanistic rationalization for this scandium triflate triggered Groebke–Bienaymé–Blackburn reaction is provided in (Scheme 3).

In these cases, the condensation of amine group with aromatic aldehyde, gives an alternative imine. The trapping of the more electrophilic iminium derivatives allows a far more efficient coupling with a wide range of aldehydes aromatics, which is then trapped by the isocyanide to give imidazo[2,1-*c*][1,2,4] triazoles in modest yields. The crude products were purified by column chromatography on silica gel using appropriate gradients of petroleum ether in dichloromethane (8:2 PE/DCM) as eluent to provide moderate to excellent yields of **4a–i** (Table 2).

The structures of the products **4a–i** were deduced from their IR, ^{1}H NMR, ^{13}C NMR spectra and HRMS.

Biological activities

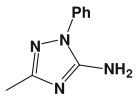
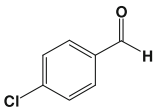
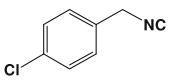
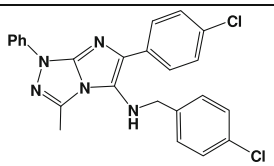
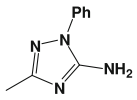
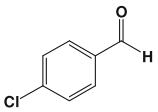
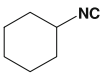
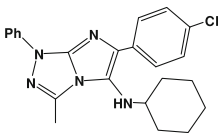
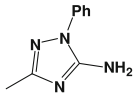
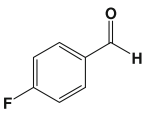
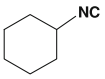
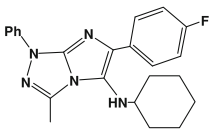
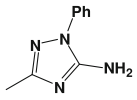
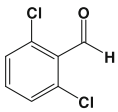
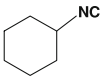
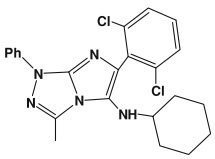
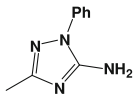
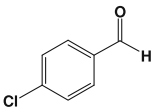
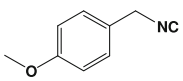
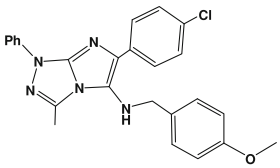
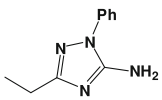
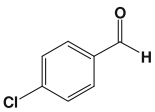
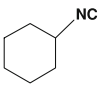
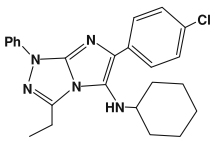
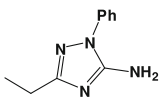
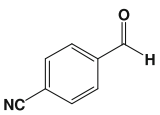
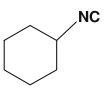
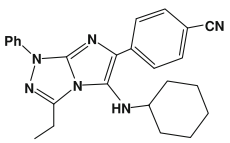
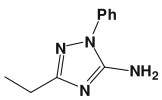
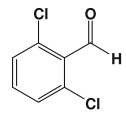
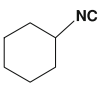
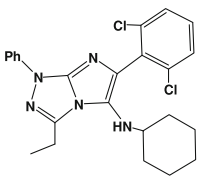
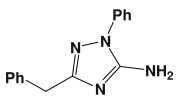
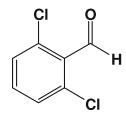
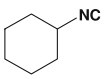
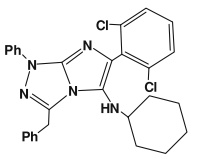
Antimicrobial activity

The new synthesized compounds were screened for their antibacterial activity against two Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus* species and three Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enteritidis*. This activity was evaluated by means of the diameter of inhibition zones (in mm) around wells, minimum inhibitory concentration (MIC), as determined by the dilution method and minimum bactericidal concentration (MBC) for bacteria and minimum fungicidal concentration (MFC) for fungi. The results are summarized in (Table 3). The antibacterial activity was observed with **4a**, **4b** and **4f** against only Gram-positive bacteria *B. cereus* and *S. aureus* one of the most common Gram-positive bacteria causing food poisoning. However, these compounds are inactive against Gram-negative bacteria. Particularly, *para*-chloro substituted compound **4b** exhibited excellent inhibition of the Gram-positive bacteria with inhibition zone diameter ranged from 29 to 20 mm and MIC values of 78 and 312 μg/ml against *B. cereus* and *S. aureus*, respectively. Unlike compound **4g** was inactive in our bacterial growth-inhibiting assay due to the cyano substituents. As with the antibacterial activity, we evaluated antifungal activity by means of inhibition zone, MIC and MFC observations, and the results are summarized in (Table 3). Antifungal activity was seen in three of the derivatives **4a**, **4b** and **4f**. The compound **4b** demonstrated an excellent in vitro activity and a wide spectrum of antifungal activity with inhibition zones diameters varied from 9 to 30 mm and MIC range from 0.156 to 0.625 μg/ml. The MBC determination showed a bactericidal effect of the compound **4b** with MBC/MIC ratio less than or equal to 4 with *A. niger*, *F. culmorum*, and *R. nigricans* (Fig. 1).

Antioxidant activity

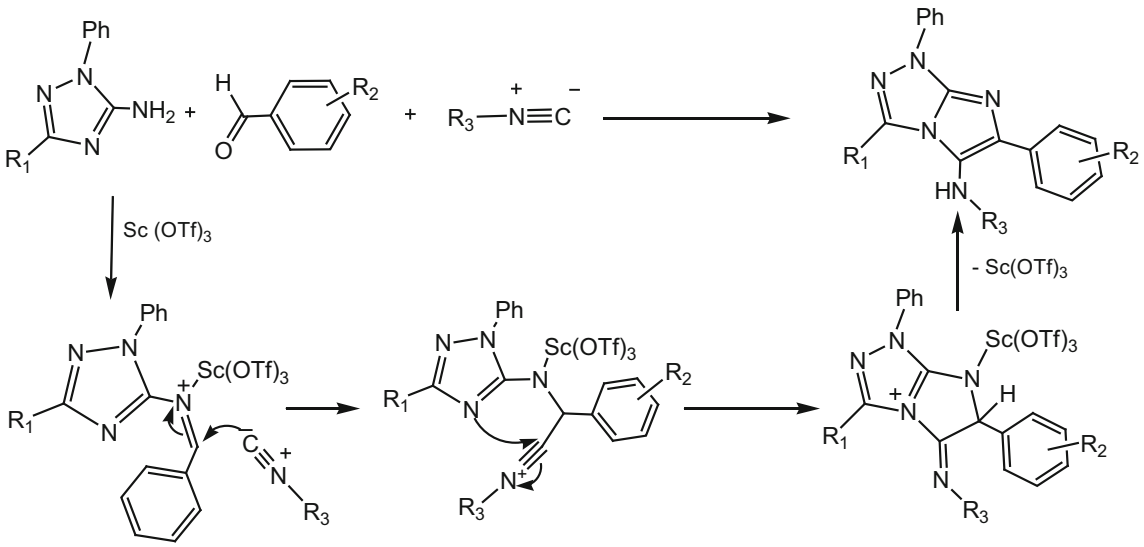
The synthesized compounds **4a**, **4b**, **4g** and **4f** were tested for their antioxidant properties by using the free stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). This assay was used to test the capacity of the antioxidative compounds as proton radical scavengers or hydrogen donors. The DPPH radical reacts with suitable reducing agents; then, electrons become paired off, and the solution loses color, observed by the decrease in absorbance at 517 nm, stoichiometrically with the number of electrons taken up. The antioxidant efficiency can be also evaluated by the determination of the EC_{50} value corresponding to the amount of the compound required to scavenge 50 % of DPPH radicals present in the reaction mixture. High IC_{50} values indicated low antioxidant activity. As shown in Fig. 2, the DPPH radical scavenger capacities of compounds were in the following order: **4g** > **4a** > **4b** > **4f**.

Table 2 Synthesis of imidazo[2,1-*c*][1,2,4]triazoles derivatives **4a–i**

Exp.No	Triazole	Aldehyde	Isonitrile	Product	Yield (%)
4a					72
4b					57
4c					60
4d					54
4e					72
4f					60
4g					57
4h					70
4i					62

The compounds **4a–i** were obtained by a mixture of triazole (1 eq), aldehyde (1 eq), isonitrile (1,2 eq) and 5 % $Sc(OTf)_3$ in DMF of 80 °C in 30 h

These compounds exhibited significantly high DPPH radical-scavenging activity when compared to the standard BHA and α-tocopherol. The synthesized 5-amino-3-methyl-1-phenyl-1,2,4-triazole and the 5-amino-3-ethyl-1-benzyl-1,2,4-triazole used in the preparation of **4a**, **4b**, **4d**, **4f** and **4g** showed a low inhibition capacities of 5 and 7 %, respectively, at 1.2 mg/ml and therefore a low antioxidant activity.



Scheme 3 Mechanism for the synthesis of imidazo[2,1-*c*][1,2,4]triazoles derivatives **4a–i**

Table 3 Antimicrobial activities of **4a**, **4b**, **4f** and **4g** against fungi, foodborne and spoiling bacteria and the determination of the MIC, MBC and MFC

	Synthesized compounds									
	4a		**4b**		**4f**		**4g**		Genta	
	IZ	MIC (MBC)	IZ	MIC (MBC)	IZ	MIC (MBC)	IZ	MIC	I Z	MIC
Bacterial strains										
Gram positive										
B. cereus ATCC 14579	10	0.312 (0.972)	29	0.078 (0.087)	18	0.624 (1.248)	0	–	20	0.001
S. aureus ATCC 25923	0	–	20	0.312 (0.624)	09	1.248 (>5)	0	–	25	0.002
Gram negative										
E. coli ATCC 25922	0	–	0	–	0	–	0	–	21	0.004
P. aeruginosa ATCC 27853	0	–	0	–	0	–	0	–	18	0.004
S. enteritidis (food isolate)	0	–	0	–	0	–	0	–	16	0.007

	IZ	MIC (MFC)	IZ	MIC (MFC)	IZ	MIC (MFC)	IZ	Ampho B	
								IZ	MIC
Fungal strains									
Aspergillus niger CTM 10099	0		09	0.625 (1.25)	0		0	15	0.002
Fusarium oxysporum CTM10402	0		12	0.312 (2.5)	0		0	12.5	0.008
Fusarium culmorum ISPAVE 21w	0		30	0.156 (0.156)	13	5 (>5)	0	14	0.004
Rhizopus nigricans CTM 10150	10	2.5 (>5)	18	0.625 (2.5)	14	2.5 (5)	0	12	0.016

Diameter of inhibition zones of various extracts including the diameter of the disk (6 mm)

MIC minimal inhibition concentration, *MBC* minimum bactericidal concentration, *Genta* Gentamicin was used as a standard antibiotic at 15 μg/ml, Amph: Amphotericine B was used as antifungal standard at 20 μg/ml, *MFC* minimum fungicidal concentration

–: activity not detected

Conclusion

In conclusion, We have developed a rapid and efficient microwave-assisted method for the synthesis of a range of fused 5-amino-1-phenyl-1,2,4-triazoles in good to excellent yields via MCRs. This methodology should prove useful for the synthesis of libraries of such derivatives with improved efficiency using high-throughput synthesis methods. The

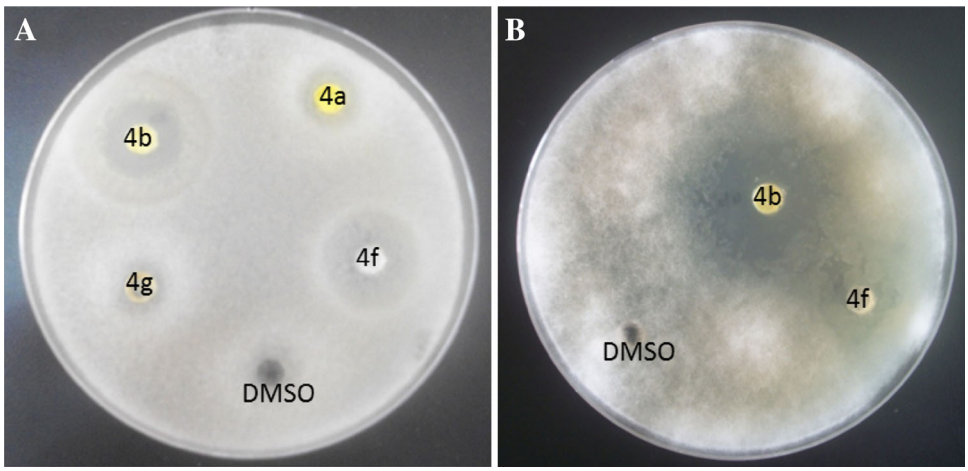


Fig. 1 Plate showing antifungal activity of **4a** and **4b** and **4f** against *R. nigricans* and *F. culmorum*

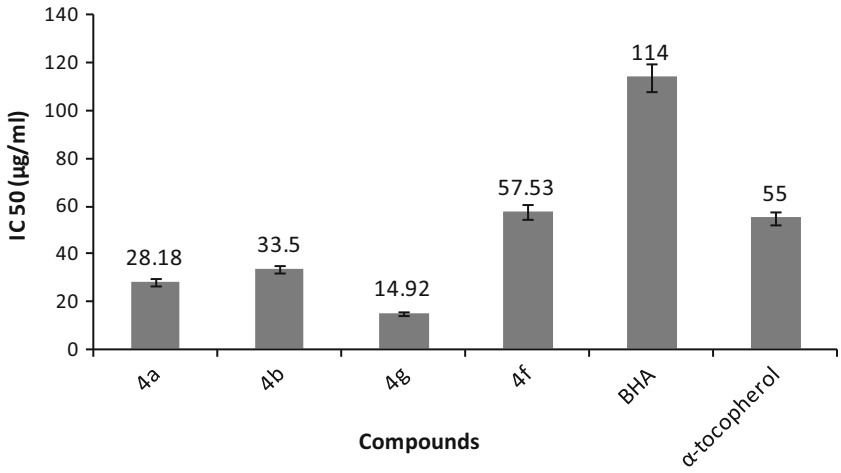


Fig. 2 Scavenger effect of **4a**, **4b**, **4g** and **4f** fractions at different concentrations on the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Results are expressed as percentage decrement of absorbance at 517 nm with respect to control. BHA and α-tocopherol were used as a standard. Each value represents the mean ± SD of three experiments

diversity offered by the process have been further enhanced by the use of these compounds in a Groebke three-component forming fused imidazo[2,1-*c*][1,2,4]triazoles **4a**–**i** in good yields. Derivatives were tested for antifungal, antibacterial and antioxidant activity. The compound **4b** showed very good antifungal and antioxidant activity, which suggests a possible clinical significance.

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References

Adib M, Sheikhi E, Rezaei N (2011) One-pot synthesis of imidazo[1,2-*a*]pyridines from benzyl halides or benzyltosylates, 2-aminopyridinesandisocyanides. Tetrahedron Lett 52:3191–3194

Agrebi A, Allouche F, Chabchoub F, El-Kaim L, Alves S, Baleizão C, Farinha JP (2013) $Sc(OTf)_3$ promoted multicomponent synthesis of fluorescent imidazo[1,2-*c*]pyrazolo [3,4-*d*] pyrimidine. Tetrahedron Lett 54:4781–4784

Al-Tel TH, Al-Qawasmeh RA (2010) Post Groebke–Blackburn multicomponent protocol: synthesis of new polyfunctional imidazo[1,2-*a*]pyridine and imidazo[1,2-*a*]pyrimidine derivatives as potential antimicrobial agents. Eur J Med Chem 45:5848–5855

Bode ML, Gravestock D, Moleele SS, Van Der Westhuyzen CW, Pelly SC, Steenkamp PA, Hoppe HC, Khan T, Nkabinde LA (2011) Imidazo[1,2-*a*]pyridin-3-amines as potential HIV-1 non-nucleoside reverse transcriptase inhibitors. Bioorg Med Chem 19:4227–4237

Chen ZP, Wang HB, Wang YQ, Zhu QH, Xie Y, Liu SW (2014) Synthesis of fused pyrrolo [3,4-*d*] tetrahydropyrimidine derivatives by proline-catalyzed multicomponent reaction. Tetrahedron 70:4379–4385

Chihaoui M, Baccar B, Mathis R (1981) Préparation de *N,N* aminotriazolones et de *N* aminotriazoles à partir d'hydrazonate. C R Acad Sci Paris t:293–573

El Kaim L, Grimaud L (2009) Beyond the Ugi reaction: less conventional interactions between isocyanides and iminium species. Tetrahedron 65:2153–2171

El Kaim L, Grimaud L, Purumandla SR (2010) Ugi–smiles couplings in water. Tetrahedron Lett 51:4962–4964

Eloff JN (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 64:711–713

Guchhait SK, Madaan C (2011) Groebke–Blackburn–Bienaymé multicomponent reaction in scaffold-modification of adenine, guanine and cytosine: synthesis of aminoimidazole-condensed nucleobases. Tetrahedron Lett 52:56–58

Khan A, Sidick Basha R, Lal M (2012) Bromodimethylsulfonium bromide (BDMS) catalyzed synthesis of imidazo[1,2-*a*]pyridine derivatives and their fluorescence properties. Tetrahedron Lett 53:2211–2217

Kirby AJ, Schmidt RJ (1997) The antioxidant activity of Chinese herbs for eczema and of placebo herbs-1. J Ethnopharmacol 56:103–108

Mandair GS, Light M, Russell A, Hursthouse M, Bradley M (2002) Re-evaluation of the outcome of a multiple component reaction-2- and 3-amino-imidazo[1,2-*a*]pyrimidines? Tetrahedron Lett 43:4267–4269

Parchinsky VZ, Koleda VV, Shuvalova O, Kravchenko DV, Krasavin M (2006) Air-oxidized products of multi-component reactions between 3-amino-1,2,4-triazole, aromatic aldehydes and isonitriles. Tetrahedron Lett 47:6891–6894

Rousseau AL, Matlaba P, Parkinson CJ (2007) Multicomponent synthesis of imidazo[1,2-a] pyridines using catalytic zinc chloride. Tetrahedron Lett 48:4079–4082

Sadjadi S, Heravi MM (2011) Recent application of isocyanides in synthesis of heterocycles. Tetrahedron 67:2707–2752

Schwerkoske J, Masquelin T, Perun T, Hulme C (2005) New multicomponent reaction accessing 3-aminoimidazo[1,2-*a*]pyridines. Tetrahedron Lett 46:8355–8357

Shaabani A, Yavari I, Teimouri MB, Bazgir A, Bijanzadeh HR (2001) New and efficient synthesis of dialkyl-2-[1-p-nitrophenyl-2-(alkylamino)-2-oxoethyl]malonates. Tetrahedron 57:1375–1378

Shaw AY, Xu Z, Hulme C (2012) Ugi/Robinson–Gabriel reactions directed toward the synthesis of 2,4,5-trisubstituted oxazoles. Tetrahedron Lett 53:1998–2000

Shukla NM, Salunke DB, Yoo E, Mutz CA, Balakrishna R, David SA (2012) Antibacterial activities of Groebke–Blackburn–Bienaymé-derived imidazo[1,2-*a*]pyridin-3-amines. Bioorg Med Chem 20:5850–5863

Tagg JohnR, McGiven AR (1971) Assay system for bacteriocins. Appl Microbiol 21:943

Xu Z, Shaw AY, Dietrich J, Cappelli AP, Nichol G, Hulme C (2012) Facile, novel two-step syntheses of benzimidazoles, bis-benzimidazoles, and bis benzimidazole-dihydro quinoxalines. Mol Divers 16:73–79