Synthesis and biological activity of new 1-[4-(substituted)-piperazin-1-ylmethyl]-1H-benzotriazole

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A new kind of benzotriazole derivatives has been designed and synthesised. The structures of all of the title compounds were characterised by ^{1}H NMR, IR, MS and elemental analyses. Herbicidal activities of the benzotrizole derivatives were evaluated with barnyard grass and rape cup and KARI tests. The results showed that compounds exhibited weak herbicidal activities against barnyardgrass and rape and KARI enzyme.

Keywords: synthesis, benzotriazole derivatives, herbicidal activity, KARI activity

In recent reports, it has been shown that benzotriazole are very useful starting materials for the synthesis of various bioactive molecules.[1] Substituted benzotriazole derivatives are also widely applied in medicine and agriculture.[2]

Our research group has synthesised many heterocyclic compounds, such as sulfonylureas.[3] These compounds contain pyrimidine ring and can successfully inhibit acetohydroxyacid synthase (ALS or AHAS; EC2.2.2.6), a key enzyme catalysing the biosynthesis of branched-chain amino acids.[3] Plants and microorganisms contain numerous enzymes that are potential targets for bioactive compounds such as herbicides and antibiotics. This has stimulated our interests in looking for novel inhibitors of other enzymes, including the second enzyme in the common metabolism pathway,[4] ketol-acid reductoisomerase (KARI; EC 1.1.1.86) by design and synthesis of heterocyclic compounds.[5]

In our early study, based on the reported 1.65 Å high-resolution crystal structure of spinach KARI (ketol-acid reductoisomerase) complex,[6] we obtained 279 molecules with low binding energy toward KARI from MDL/ACD 3D database searching, using program DOCK 4.0.[7] These potential structures provide further information for design of new KARI inhibitors, of which the title compound is one that has been synthesised. And we also synthesised its derivatives. Herbicidal activities of these benzotriazole compounds synthesised were evaluated through barnyard grass and rape cup tests and KARI tests. The results showed that these compounds exhibited weak herbicidal activities against barnyardgrass and rape and KARI enzyme.

Experimental

Instruments

Melting points were determined using a Yanaco MP-241 apparatus and were uncorrected. IR spectra were recorded on a Bruker Equinox55 spectrophotometer as potassium bromide tablets. ^{1}H NMR spectra were measured on a Bruker AC-P500 instrument (300 MHz) using tetramethylsilane as an internal standard and deuterochloroform as solvent. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage LC/mass detector instrument. Elemental analyses were performed on a Yanaco MT-3CHN elemental analyser.

Synthesis of compounds

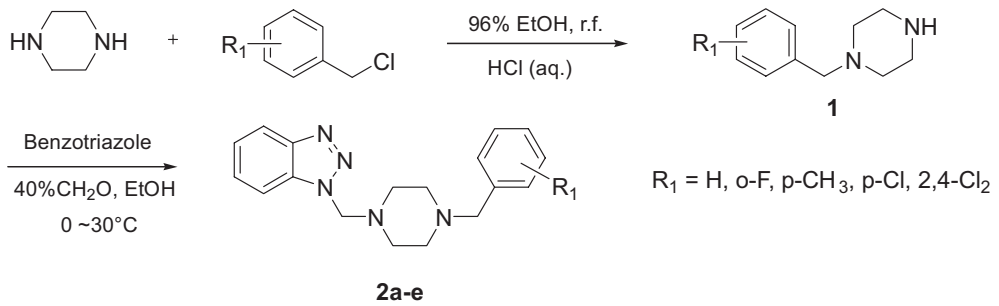
The title compounds were synthesised according to the route shown in Schemes 1 and 2.

General procecure

Piperazine (50 mmol), dissolved in 20 ml 96% of ethanol, was added dropwise to the stirred solution of substituted benzyl chlorine (25 mmol) at reflux. The mixture was stirred for 1–8 hours at reflux, TLC monitored. The mixture was stirred overnight at room temperature, evaporated in vacuum and the residue was dissolved in 30 ml of ethanol. Then, 30 ml of sat. K_2CO_3 was added and the mixture was stirred for an additional 1 h. It was partly evaporated in vacuum, extracted three times with chloroform, dried (Na_2SO_4), evaporated in vacuum and recrystallised.[8] Then compounds **2a–e** were prepared by treating compound 1(0.01 mol) with benzotriazole (0.01 mol), 40% formalin (0.012 mol) and methanol (15 ml). The crude solid was purified by recrystallisation from ethanol to give the title compound.

4,6-Disubstituted pyrimidine was prepared by reaction of the diazonium salts with concentrated hydrochloric acid and zinc chloride,[9] then reacted with piperazine in Na_2CO_3 solvent at 50–65°C. Similar benzotriazole compounds containing substituted-pyrimidine group in place of the substituted benzyl group (**5a–e**) were obtained in the same route. The crude solid was purified by recrystallisation from ethanol to give the title compounds.

1-[(4-benzylpiperazin-1-yl)methyl]-1H-benzo[d][1,2,3]triazole (**2a**): The compound was obtained in 85.3% yield as a white crystal; m.p. 115–116°C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 8.06(d, J = 8.3 Hz), benzo-H, 1H), 7.59(d, J = 8.3 Hz, benzo-H, 1H), 7.48(t, J = 7.5 Hz, benzo-H, 1H), 7.18–7.40(m, benzo-H and ArH, 6H), 5.56(s, benzo-CH_2, 2H), 3.45(s, benzyl-CH_2, 2H), 2.69(s, piperazine, 4H), 2.47 (s, piperazine, 4H) IR (KBr), ν/cm^{-1}: 3058, 3022, 2942, 1494, 1451, 1174, 1160. MS (ESI), *m/z*: 306(M-1). Elemental anal. (%), calculated for $C_{18}H_{21}N_5$: C, 70.33; H, 6.89; N, 22.78; found: C, 70.38; H, 6.68; N, 22.65.



Scheme 1 Synthesis route for compounds **2a–e.**

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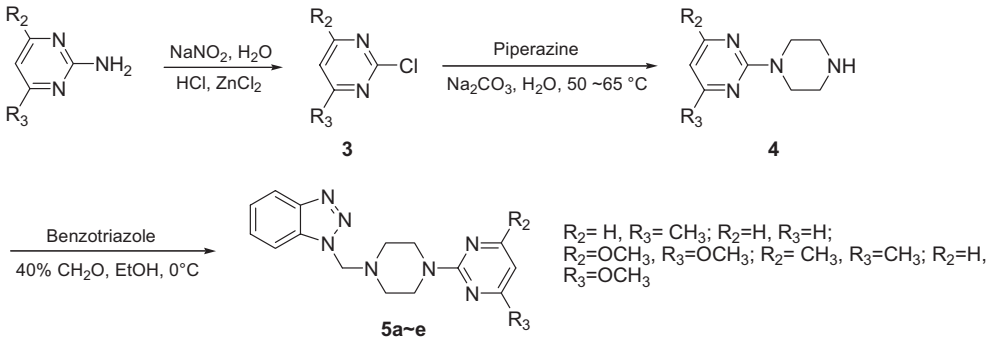

Scheme 2 Synthesis route for compounds **5a–e**.

1-{[4-(2-fluorobenzyl)piperazin-1-]methyl}-1H-benzo[d][1,2,3] triazole (**2b**): The compound was obtained in 66.0% yield as a white crystal; m.p. 90–92°C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 8.07(d, *J* = 8.3 Hz, benzo-H, 1H), 7.59(d, *J* = 8.3 Hz, benzo-H, 1H), 7.49 (t, *J* = 7.5 Hz, benzo-H, 1H), 7.38(t, *J* = 7.5 Hz, benzo-H, 1H), 7.18–7.26(m, ArH, 4H), 5.46(s, benzo-CH_2, 2H), 3.47(s, benzyl-CH_2, 2H), 2.73(s, piperazine, 4H), 2.51(s, piperazine, 4H); IR (KBr), ν/cm^{-1}: 3064, 2948, 1612, 1583, 1489,1452, 1169, 1133. MS (ESI), *m/z*: 324 (M -1). Elemental anal. (%), calculated for $C_{18}H_{20}FN_5$: C, 66.44; H, 6.20; N, 21.52; found: C, 66.20; H, 6.49; N, 21.66.

1-{[4-(4-methylbenzyl)piperazin-1-]methyl}-1H-benzo[d][1,2,3] triazole (**2c**): The compound was obtained in 86.9% yield as a white crystal; m.p. 108–110°C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 8.07(d, *J* = 8.3 Hz, benzo-H, 1H), 7.59(d, *J* = 8.3 Hz, benzo-H, 1H), 7.48 (t, *J* = 7.6 Hz, benzo-H, 1H), 7.37(t, *J* = 7.6 Hz, benzo-H, 1H), 7.08–7.24(m, ArH, 4H), 5.46(s, benzo-CH_2, 2H), 3.42(s, benzyl-CH_2, 2H), 2.67(s, piperazine, 4H), 2.48(s, piperazine, 4H), 2.26(s, Ar-CH_3, 3H); IR (KBr), ν/cm^{-1}: 3062, 3027, 2941, 1610, 1574, 1488, 1159, 1123. MS (ESI), *m/z*: 320 (M -1). Elemental anal. (%), calculated for $C_{19}H_{23}N_5$: C, 71.00; H, 7.21; N, 21.79; found: C, 70.92; H, 7.27; N, 21.95.

1-{[4-(4-chlorobenzyl)piperazin-1-]methyl}-1H-benzo[d][1,2,3] triazole (**2d**): The compound was obtained in 86.4% yield as a white crystal; m.p. 104–106°C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 8.07(d, *J* = 8.3 Hz, benzo-H, 1H), 7.59(d, *J* = 8.3 Hz, benzo-H, 1H), 7.49 (t, *J* = 7.5 Hz, benzo-H, 1H), 7.38(t, *J* = 7.5 Hz, benzo-H, 1H), 7.19–7.26(m, ArH, 4H), 5.46(s, benzo-CH_2, 2H), 3.49(s, benzyl-CH_2, 2H), 2.74(s, piperazine, 4H), 2.52(s, piperazine, 4H); IR (KBr), ν/cm^{-1}: 2962, 2934, 1617, 1581, 1495, 1452, 1151, 1123. MS (ESI), *m/z*: 340 (M -1). Elemental anal. (%), calculated for $C_{18}H_{20}ClN_5$: C, 63.24; H, 5.85; N, 20.17; found: C, 63.24; H, 5.85; N, 20.17.

1-{[4-(2,4-dichlorobenzyl)piperazin-1-]methyl}-1H-benzo[d] [1,2,3]triazole (**2e**): The compound was obtained in 76.6% yield as a white crystal; m.p. 131–132°C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 8.08(d, *J* = 8.3 Hz, benzo-H, 1H), 7.60(d, *J* = 8.3 Hz, benzo-H, 1H), 7.50(t, *J* = 7.5 Hz, benzo-H, 1H), 7.32–7.42(m, ArH and benzo-H, 3H), 7.16(d, *J* = 7.8 Hz, ArH(6), 1H), 5.47(s, benzo-CH_2, 2H), 3.58(s, benzyl-CH_2, 2H), 2.73(s, piperazine, 4H), 2.57(s, piperazine, 4H); IR (KBr), ν/cm^{-1}: 3062, 2941, 1617, 1581, 1502, 1474, 1187, 1159. MS (ESI), *m/z*: 375 (M -1). Elemental anal. (%), calculated for $C_{18}H_{19}Cl_2N_5$: C, 57.45; H, 5.09; N, 18.61; found: C, 57.62; H, 5.22; N, 18.52.

1-{[4-(4-methylpyrimidin-2-yl)piperazin-1-yl]methyl}-1H-benzo[d][1,2,3]triazole (**5a**): The compound was obtained in 76.8% yield as a white crystal; m.p. 164–165°C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 8.04–8.08(m, benzo-H and PyH(5), 2H), 7.62(d, *J* = 8.3 Hz, benzo-H, 1H), 7.49(t, *J* = 7.2 Hz, benzo-H, 1H), 7.36(t, *J* = 7.6 Hz, benzo-H, 1H), 6.31(d, *J* = 5.0 Hz, PyH, 1H), 5.51(s, benzo-CH_2, 2H), 3.84(t, *J* = 4.8 Hz, piperazine, 4H), 2.69(t, *J* = 4.8 Hz, piperazine, 4H), 2.25(s, $PyCH_3$, 3H); IR (KBr), ν/cm^{-1}: 3091, 3062, 3034, 1574, 1488, 1151, 1116. MS (ESI), *m/z*: 307 (M -1). Elemental anal. (%), calculated for $C_{16}H_{19}N_7$: C, 62.12; H, 6.19; N, 31.69; found: C, 62.34; H, 6.21; N, 31.89.

1-{[4-(pyrimidin-2-yl)piperazin-1yl]methyl}-1H-benzo[d][1,2,3] triazole (**5b**): The compound was obtained in 86.7% yield as a white crystal; m.p. 149–150°C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 8.24(d, *J* = 4.7 Hz, PyH, 2H), 8.06(d, *J* = 8.4 Hz, benzo-H, 1H), 7.64 (d, *J* = 8.3 Hz, benzo-H, 1H), 7.51(t, *J* = 7.5 Hz, benzo-H, 1H), 7.37(t, *J* = 7.5 Hz, benzo-H, 1H), 6.45(t, *J* = 4.8 Hz, PyH, 1H), 5.53(s, benzo-CH_2, 2H), 3.86(t, *J* = 5.0 Hz, Piperazine, 4H), 2.69 (t, *J* = 5.0, piperazine, 4H); IR (KBr), ν/cm^{-1}: 3019, 2984, 2948, 1588, 1545, 1481, 1159, 1123. MS (ESI), *m/z*: 294 (M -1). Elemental anal. (%), calculated for $C_{15}H_{17}N_7$: C, 61.00; H, 5.80; N, 33.20; found: C, 60.81; H, 5.72; N, 33.11.

1-{[4-(4,6-dimethoxypyrimidin-2-yl)piperazin-1-yl]methyl}-1H-benzo[d][1,2,3]triazole (**5c**): The compound was obtained in 63.9% yield as a white crystal; m.p. 144–145°C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 8.07(d, *J* = 8.3 Hz, benzo-H, 1H), 7.63(d, *J* = 8.3 Hz, benzo-H, 1H), 7.52(t, *J* = 7.5 Hz, benzo-H, 1H), 7.38(t, *J* = 7.5 Hz, benzo-H, 1H), 5.53(s, benzo-CH_2, 2H), 5.32(s, PyH, 1H), 3.80–3.85 (m, piperazine and O-CH_3, 10H), 2.69(t, *J* = 5.1, piperazine, 4H); IR (KBr), ν/cm^{-1}: 2998, 2948, 1581, 1509, 1445, 1159. MS (ESI), *m/z*: 354 (M -1). Elemental anal. (%), calculated for $C_{17}H_{21}N_7O_2$: C, 57.45; H, 5.96; N, 27.59; found: C, 56.96; H, 5.87; N, 27.66.

1-{[4-(4,6-dimethylpyrimidin-2-yl)piperazin-1-yl]methyl}-1H-benzo[d][1,2,3]triazole (**5d**): The compound was obtained in 80.9% yield as a white crystal; m.p. 190–191°C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 8.06(d, *J* = 8.3 Hz, benzo-H, 1H), 7.63(d, *J* = 8.3 Hz, benzo-H, 1H), 7.51(t, *J* = 7.5 Hz, benzo-H, 1H), 7.37(t, *J* = 7.5 Hz, benzo-H, 1H), 6.22(s, PyH, 1H), 5.53(s, benzo-CH_2, 2H), 3.88(s, piperazine, 4H), 2.71(t, *J* = 4.8 Hz, piperazine, 4H), 2.26(s, Py-CH_3, 6H); IR (KBr), ν/cm^{-1}: 3062, 3034, 3005, 1567, 1495, 1438, 1159. MS (ESI), *m/z*: 322 (M -1). Elemental anal. (%), calculated for $C_{17}H_{21}N_7$: C, 63.14; H, 6.55; N, 30.32; found: C, 62.67; H, 6.76; N, 30.14.

1-{[4-(4-methoxypyrimidin-2-yl)piperazin-1-yl]methyl}-1H-benzo[d][1,2,3]triazole (**5e**): The compound was obtained in 61.5% yield as a white crystal; m.p. 114–115°C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 8.07(d, *J* = 8.4 Hz, benzo-H, 1H), 7.97(d, *J* = 5.7 Hz, PyH(5), 1H), 7.63(d, *J* = 8.3 Hz, benzo-H, 1H), 7.51(t, *J* = 7.5 Hz, benzo-H, 1H), 7.38(t, *J* = 6.9, benzo-H, 1H), 5.94(d, *J* = 5.7 Hz, PyH, 1H), 5.52(s, benzo-CH_2, 2H), 3.84–3.87(m, piperazine and O-CH_3, 7H), 2.71(t, *J* = 5.1 Hz, piperazine, 4H); IR (KBr), ν/cm^{-1}: 3077, 3019, 3984, 1588, 1559, 1502, 1466, 1151, 1123. MS (ESI), *m/z*: 324 (M -1). Elemental anal. (%), calculated for $C_{16}H_{19}N_7O$: C, 59.06; H, 5.89; N, 30.13; found: C, 59.04; H, 6.06; N, 29.93.

The herbicidal activity tests

Inhibition of the root-growth of rape (Brassica campestris)

The compounds to be tested were made into an emulsion to aid dissolution. Rape seeds were soaked in distilled water for 4 hours before being placed on a filter paper in a 6-cm Petri plate, to which 2 ml of inhibitor solution had been added in advance. As usual, 15 seeds were used on each plate. The plate was placed in a dark room and allowed to germinate for 65 hours at 28 ± 1°C. The lengths of 10 rape roots selected from each plate were measured and the means were calculated. The check test was carried out in distilled water only. The percentage of the inhibition was calculated.

Inhibition of the seedling-growth of barnyard grass (Echinochloa frumentacea)

The compounds evaluated were made into an emulsion to aid dissolution.[10] Barnyard grass seeds were placed into a 50 ml cup covered with a layer of glass beads and a piece of filter paper at the bottom, to which 5 ml of inhibitor solution had been added in advance. The cup was placed in a bright room and allowed to germinate for 65 hours at 28 ± 1°C. The heights of seedlings of above-ground plant parts from each cup were measured and the means were calculated. The check test was carried out in distilled water only. The percentage of the inhibition was calculated.

KARI activity tests

Gerwick *et al.*[11] reported that the inhibition of *E. coli* KARI is time-dependent. To characterise the steady-state inhibition constant, Escherichia coli KARI was preincubated 10 min in advance, with

Table 1 Herbicidal activity data of title compounds (% inhibition)

No.	R_2	R_3	Rape root test 100μg/ml	Barnyard grass cup test 100μg/ml	KARI 200 μg/ml
2a			0	10.5	25.9
2b			6.2	4.3	0
2c			24	15.9	8.0
2d			0	5.1	-
2e			18.3	17.5	33.9
5a	H	Me	9.7	11.8	13.3
5b	H	H	0	1.3	0
5c	OMe	OMe	0	0	19.1
5d	Me	Me	0	0	0
5e	H	OMe	0	4.6	11.5

Indicates the compound can not be dissolved in our test system, so no data obtained.

NADPH, Mg^{2+} and the title compounds, then the reaction was initiated with hydroxypyruvate. Under these conditions, the change in A_{340} was found to be linear with time.

Results and discussion

Synthesis

The work described here starts by *N*-substitute piperazine and reaction of the corresponding formaldehyde with benzotriazole to give target compounds **2a–e** and **5a–e**. Compounds **2a–e** and **5a–e** were identified by ^{1}H NMR. The measured elemental analyses are also consistent with the corresponding calculated ones.

Herbicidal and KARI acitivity

Herbicidal activities of these benzotriazole compounds synthesised were evaluated through barnyardgrass and rape cup tests and KARI tests. The results (Table 1) showed that compounds exhibited weak herbicidal activities against barnyard grass and rape and KARI enzyme.

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