Synthesis and Antiproliferative Activities of Chloropyridazine Derivatives Retain Alkylsulfonyl Moiety

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Some chloropyridazine derivatives have shown interesting pharmacodynamics properties in terms of antioxidant[1] and anti-human rotavirus (HRV) activities (Figure 1).[2,3] To date, however, no study has evaluated the antiproliferative effects of chloropyridazines in other types of human cancer cells.

Therefore, the replacement of the heterocycle of chloropyridazines by heteroatoms (or moieties) such as oxygen (O), sulfur (S), selenium (Se), sulfinyl (SO), and sulfonyl (SO_2) yields the target compounds (Figure 1). We designed substituted 3-chloropyridazine derivatives as possible antitumor candidates. These derivatives have three basic structures, namely chloropyridazine region, heteroatoms, and alkyl chain. We made an examination of antiproliferative activities for target chloropyridazines of five groups against breast cancer (MCF-7) and hepatocellular carcinoma (Hep3B) cells in Cell Counting Kit-8 (CCK-8) assays. As a result, we describe herein the preparation of some chloropyridazine derivatives and the refinement of potential proliferative inhibitors on human cancer cell lines.

The chloropyridazines were obtained mostly through the substitution reaction. Scheme 1 depicts the preparation of desired substituted chloropyridazine derivatives. We previously reported the synthesis of 3-allylthio-6-chloropyridazine in 95% yield through allylthiolation.[4] All 3-alkoxy-6-chloropyridazines **3b–3d** and 3-alkylthio-6-chloropyridazines **4b–4e** were prepared following the synthetic methods of existing literature.[5,6] The intermediate dichloropyridazinyl diselenide **2** was obtainable from the corresponding 3,6-dichloropyridazine **1** by reaction with sodium diselenide.[7] A synthetic pathway for dipyridyl diselenide has been developed [7,8] and the synthesis of substituted dipyridazinyl diselenide has been reported.[9] We applied a general method of preparing diaryl (or dialkyl) diselenide from diaryl (or dialkyl) halides and sodium diselenide.[10–12] A series of alkylselenylpyridazines **5b–5f** was prepared by Se–Se bond cleavage and Se-alkylation.

Treatment of alkylthiopyridazines **4a–4e** with 35% hydrogen peroxide afforded corresponding alkylsulfinylchloropyridazine **6a–6e** and alkylsulfonylchloropyridazine **7a–7e**. Target **6a–6e** and **7a–7e** were synthesized as illustrated in Scheme 1.

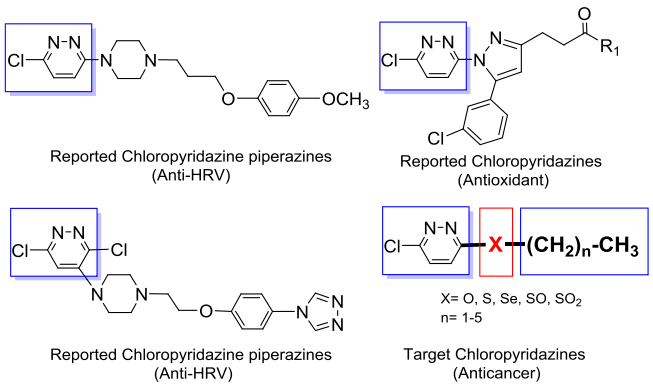
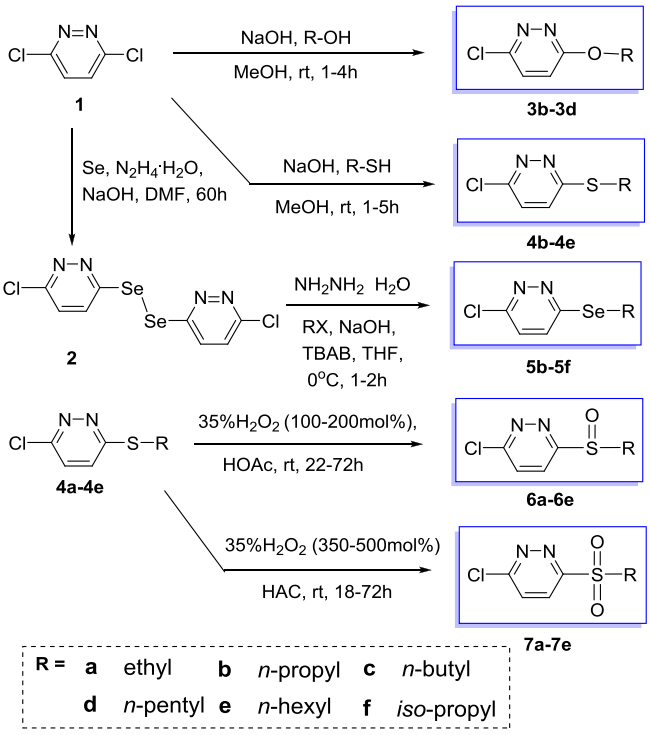
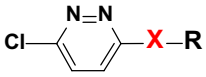


Figure 1. Bioactive chloropyridazines and target chloropyridazines.



Scheme 1. Synthetic routes for target chloropyridazine analogues.

Table 1. Inhibitory results for five groups of synthesized chloropyridazines against MCF-7, Hep3B.



Comp. No.	R	X	IC_{50} (μM) MCF-7	IC_{50} (μM) Hep3B
3b[a]	*n*-propyl	O	1756.3	1517.6
3c[a]	*n*-butyl	O	>4000	2143.2
3d[a]	*n*-pentyl	O	2491.7	1397.1
4b[b]	*n*-propyl	S	>4000	3812.1
4c[b]	*n*-butyl	S	1404.6	1321.4
4d[b]	*n*-pentyl	S	>4000	>5000
4e	*n*-hexyl	S	39.9	695.8
5b[c]	*n*-propyl	Se	2436.6	>5000
5c[c]	*n*-butyl	Se	>4000	>5000
5d[c]	*n*-pentyl	Se	2552.4	1223.8
5e	*n*-hexyl	Se	>4000	1896.2
5f	*iso*-propyl	Se	>4000	1805.0
6a[d]	ethyl	SO	106.7	389.8
6b[d]	*n*-propyl	SO	1890.2	4601.0
6c[d]	*n*-butyl	SO	1737.5	1768.6
6d	*n*-pentyl	SO	2185.3	912.2
6e	*n*-hexyl	SO	2830.6	915.3
7a	ethyl	SO_2	550.3	418.5
7b	*n*-propyl	SO_2	419.0	316.9
7c	*n*-butyl	SO_2	125.7	115.0
7d	*n*-pentyl	SO_2	130.5	127.4
7e	*n*-hexyl	SO_2	250.3	268.3

[a] **3b–3d** were reported.[5]
[b] **4b–4d** were reported.[6]
[c] **5b–5d** were reported.[10]
[d] **6a–6c** were reported.[16]

A series of compound **4a–4e** was prepared from **1** using alkylthiolation with alkylmercaptan. Sulfides **4a–4e** were oxidized to **6a–6e** using 1–2 equiv of 35% hydrogen peroxide as an oxidant.[13–16] Sulfones **7a–7e** were synthesized using 3.5–5 equiv of oxidants. The oxidation of **4a–4e** to **7a–7e** was also accomplished with aqueous hydrogen peroxide at room temperature in acetic acid for 18–72 h.

We applied a common method for the CCK-8 assays[17] of synthetic compounds. In Table 1, we summarize the antiproliferative activities for targets. Among 22, four compounds (**4e**, **6a**, **7c** and **7d**) that had IC_{50} values below 200 μM inhibited the growth of MCF-7 at standard concentrations (6.25, 25, 100, and 400 μg/mL). Two compounds (**7c**, **7d**) that had IC_{50} values below 200 μM inhibited the growth of Hep3B. The antiproliferative activities of derivatives were not good as shown in Figures 2 and 3.

Compound **4e** inhibited MCF-7 cell growth and **7c** resisted Hep3B cell growth at IC_{50} in a dose-dependent manner at a low concentration (6.25 μg/mL). Both compounds **7c** (3-*n*-butylsulfonyl-6-chloropyridazine) and **7d** (3-*n*-pentylsulfonyl-6-chloropyridazine) had potentially antiproliferative activity against MCF-7 and Hep3B cell lines. CCK-8 assays indicated that compounds **7c**, **7d** inhibited cancer cells proliferation by triggering cell death.

In conclusion, we designed and synthesized a total of five groups of alkoxy-(or alkylthio-, alkylselenyl-, alkylsufinyl-, alkylsulfonyl-)chloropyridazines, and their antiproliferative activity was evaluated in the human cancer cell lines. IC_{50} values showed that the alkylsulfonylchloropyridazine compounds exhibited more active than the other four groups having alkoxy, alkylthio, alkylselenyl, alkylsulfinyl moieties against MCF-7 and Hep2B Cells.

Experimental

Materials and Methods for Bioassays: Cell Line Culture Conditions. MCF-7 and Hep3B cancer cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA).

Antiproliferative CCK-8 Assays. The cytotoxic activity of compounds was determined in vitro using the CCK-8 assay kit (Dojindo, Kumamoto, Japan).

General Synthetic Procedure for Alkoxypyridazines 3b–3d **and Alkylthiopyridazines** 4b–4e**.** The alkoxypyridazines **3b–3d** and alkylthiopyridazines **4b–4d** were synthesized from **1** through alkoxylation (or alkylthiolation) using a modification on previously reported methods.[4–6,18]

3-(*n*-Hexylthio)-6-chloropyridazine (4e). Yield: 54%. m.p. 62–64°C. ^{1}H NMR ($CDCl_3$) δ 8.15 (d, J = 8.8 Hz,

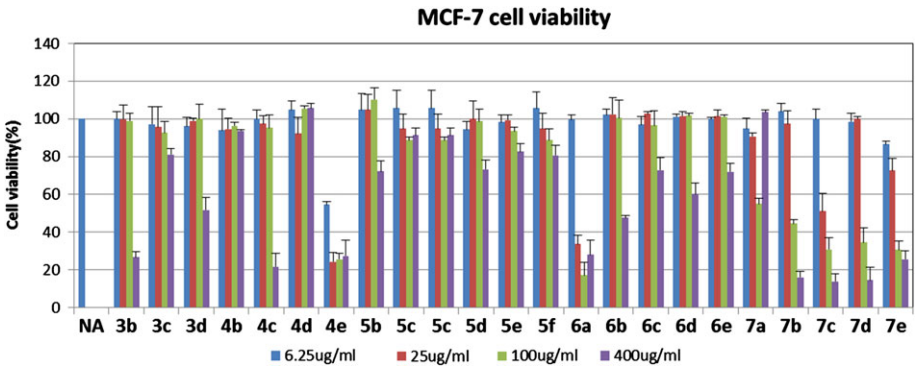


Figure 2. Antiproliferative activity of chloropyridazines in MCF-7 cancer cells.

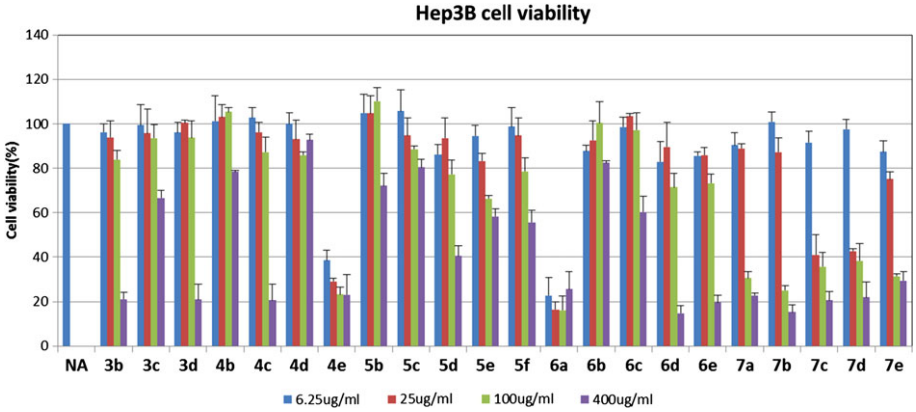


Figure 3. Antiproliferative activity of chloropyridazines in Hep3B cancer cells.

1H, CH, pyridazine), 7.80 (d, J = 8.8 Hz, 1H, CH, pyridazine), 3.59 (t, J = 8.0 Hz, 2H, SCH_2, hexyl), 1.85–1.74 (m, 2H, CH_2, hexyl), 1.48–1.39 (m, 2H, CH_2, hexyl), 1.30–1.27 (m, 1H, CH_2, hexyl), 0.86 (t, J = 6.7 Hz, 3H, CH_3, hexyl). ^{13}C NMR ($CDCl_3$) δ 161.11, 159.73, 130.00, 126.63 (pyridazine), 52.39, 31.02, 27.91, 22.19, 21.99, 13.81 (hexyl).

General Synthetic Procedure for Alkylselenylpyridazines 5b–5f. To a stirred mixture of dipyridazyinyl diselenide **2** (5.2 mmol), powdered sodium hydroxide (26 mmol), and tetrabutylammonium bromide (1.04 mmol) in absolute tetrahydrofuran (THF) (60 mL), a solution of hydrazine monohydrate (1.73 mmol) was added at room temperature (RT). After 1 h of stirring, the color of the reaction mixture turned to orange red from pale yellow. The reaction mixture was then cooled to 0°C, alkyl halide (10.4 mmol) with the same amount of THF was added slowly dropwise, and stirring continued for 1 h at 0°C. After the reaction was complete, the solvent THF was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (50 mL). The organic layer was washed with water (50 mL × 3) and subsequently dried over anhydrous sodium sulfate (Na_2SO_4). After solvent evaporation, the residue was purified by column chromatography (hexanes/EtOAc 3:1, 2:1) on silica gel to afford **5b–5f**.

3-(*n*-Hexylselenyl)-6-chloropyridazine (5e). Yield: 38%. m.p. 45–48°C. ^{1}H NMR ($CDCl_3$) δ 7.49 (d, J = 8.8 Hz, 1H, CH, pyridazine), 7.30 (d, J = 8.8 Hz, 1H, CH, pyridazine), 3.33 (t, J = 7.4 Hz, 2H, $SeCH_2$, hexyl), 1.87–1.77 (m, 2H, CH_2, hexyl), 1.32–1.327 (m, 6H, CH_2, hexyl), 0.87 (t, J = 6.9 Hz, 3H, CH_3, hexyl). ^{13}C NMR ($CDCl_3$) δ 159.04, 155.25, 129.95, 129.16 (pyridazine), 31.26, 29.66, 29.57, 26.57, 22.49, 13.95 (hexyl).

3-(*iso*-Propylselenyl)-6-chloropyridazine (5f). Yield: 59%. m.p. 53–54°C. ^{1}H NMR ($CDCl_3$) δ 7.18 (d, J = 9.0 Hz, 1H, CH, pyridazine), 6.80 (d, J = 9.0 Hz, 1H, CH, pyridazine), 3.83 (m, 1H, CH, isopropyl), 1.58 (d, J = 7.6 Hz, 6H, CH_3, isopropyl). ^{13}C NMR ($CDCl_3$) δ 159.74, 154.17, 131.42, 127.33 (pyridazine), 34.80 (isopropyl CH), 24.36 (isopropyl CH_3). FT-IR (KBr) cm^{-1} 3059 (aromatic), 1542(N═N), 1492 (CH_2), 1451 (CH_3), 757 (C─Se), 697 (C─Cl); GC-MS m/z (%) 236 (M+), 194.0 (100.0), 192.0 (54.11), 196.0 (50.52), 236 (45.16), 155.10 (41.53).

General Synthetic Procedure for Alkylsulfinylpyridazines 6a–6e. The compound **6a–6e** was prepared from 3-chloroalkylthiopyridazine **4a–4e** as a white solid using a modification of a previously reported method. To a solution of **4a–4e** (7 mmol) in 15 mL acetic acid, 35% hydrogen peroxide (100–200 mol%) was added at RT. The resulting mixture was stirred for 22–72 h at RT. After completion of the reaction, the mixture was diluted with 30 mL ether and 15 mL water, and it was partitioned between ether and water layers in a separating funnel. The ether layer was extracted with 15 mL sodium hydroxide (1 N-NaOH) solution three times. The combined water layer was reextracted several times with 50 mL dichloromethane. The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, and concentrated. The crude product was further purified by silica gel column chromatography (Hexanes/EtOAc 1:1) to provide the target compound as a white solid. The compound **6a–6c** was prepared using the reported synthetic procedure, and these spectra data were identified with the literature.[16]

3-(*n*-Pentylsulfinyl)-6-chloropyridazine (6d). Yield: 54%. m.p. 57–59°C. ^{1}H NMR ($CDCl_3$) δ 8.13 (d, J = 8.8 Hz, 1H, CH, pyridazine), 7.78 (d, J = 8.8 Hz, 1H, CH, pyridazine), 3.30–3.20 (m, 1H, SCH_2, pentyl), 3.08–2.99 (m, 1H, SCH_2, pentyl), 1.95–1.69 (m, 1H, CH_2, pentyl), 1.60–1.54 (m, 1H, CH_2, pentyl), 1.49–1.31 (m, 4H, CH_2, pentyl), 0.90 (t, J = 7.2 Hz, 3H, CH_3, pentyl). ^{13}C NMR ($CDCl_3$) δ 168.63, 157.89, 129.82, 125.70 (pyridazine), 55.30, 30.60, 22.15, 21.31, 13.69 (pentyl). FT-IR (KBr) cm^{-1} 3025 (aromatic), 1543 (N═N), 1492, 1451 (CH_2), 1028 (SO), 540 (CCl). GC-MS m/z (%) 232.73 (M+) 145.90 (base peak).

3-(*n*-Hexylsulfinyl)-6-chloropyridazine (6e). Yield: 48%. m.p. 62–65°C. ^{1}H NMR ($CDCl_3$) δ 8.12 (d, J = 8.7 Hz, 1H, CH, pyridazine), 7.76 (d, J = 8.7 Hz, 1H, CH, pyridazine), 3.28–3.19 (m, 1H, SCH_2, hexyl), 3.08–2.97 (m, 1H, SCH_2, hexyl), 1.91–1.86 (m, 1H, CH_2,

hexyl), 1.60–1.54 (m, 1H, CH_2, hexyl), 1.53–1.30 (m, 2H, CH_2, hexyl), 1.28–1.24 (m, 4H, CH_2, hexyl), 0.86 (t, J = 6.9 Hz, 3H, CH_3, hexyl). ^{13}C NMR ($CDCl_3$) δ 168.62, 157.89, 129.82, 125.70 (pyridazine), 55.32, 31.21, 28.17, 22.30, 21.57, 13.85 (hexyl). FT-IR (KBr) cm^{-1} 3025 (aromatic), 1543 (N═N), 1492, 1452 (CH_2), 1028 (SO), 540 (CCl). GC-MS *m/z* (%) 246.76 (M+) 123.00 (base peak).

General Synthetic Procedure for Alkylsulfonylpyridazines 7a–7e**.** The compound **7a–7e** was prepared from 3-chloroalkylthiopyridazine **4a–4e** as a white solid using a modification of a previously reported oxidation method.[16]

3-(Ethylsulfonyl)-6-chloropyridazine (7a). Yield: 51%. m.p. 68–70°C. ^{1}H NMR ($CDCl_3$) δ 8.16 (d, J = 8.9 Hz, 1H, CH, pyridazine), 7.81 (d, J = 8.9 Hz, 1H, CH, pyridazine), 3.67 (q, J = 14.9 Hz, 2H, SCH_2, ethyl), 1.40 (t, J = 7.4 Hz, 3H, CH_3, ethyl). ^{13}C NMR ($CDCl_3$) δ 160.68, 159.79, 130.03, 126.79 (pyridazine), 47.00, 6.78 (ethyl). FT-IR (KBr) cm^{-1} 3025 (aromatic), 1545 (N═N), 1491, 1452 (CH_2), 1325, 1154 (SO_2), 1028 (SO), 540 (CCl). GC-MS *m/z* (%) 206.69 (M+) 78.90 (100.0), 113.90 (62.9), 115.90 (20.0), 51.90 (15.9), 140.90 (13.6).

3-(*n*-Propylsulfonyl)-6-chloropyridazine (7b). Yield: 71%. m.p. 66–67°C. ^{1}H NMR ($CDCl_3$) δ 8.15 (d, J = 8.9 Hz, 1H, CH, pyridazine), 7.80 (d, J = 8.8 Hz, 1H, CH, pyridazine), 3.59 (m, 2H, SCH_2, propyl), 1.86 (m, 2H, CH_2, propyl), 1.08 (t, J = 7.2 Hz, 3H, CH_3, propyl). ^{13}C NMR ($CDCl_3$) δ 161.25, 159.77, 129.99, 126.59 (pyridazine), 54.07, 16.03, 12.95 (propyl). FT-IR (KBr) cm^{-1} 3025 (aromatic), 1545 (N═N), 1491, 1452 (CH_2), 1320 (SO_2), 1028 (SO), 540 (CCl). GC-MS *m/z* (%) 220.69 (M+) 78.90 (100.0), 113.90 (68.4), 127.90 (26.1), 115.90 (21.7), 51.90 (12.3).

3-(*n*-Butylsulfonyl)-6-chloropyridazine (7c). Yield: 64 %. m.p. 65–70°C. ^{1}H NMR ($CDCl_3$) δ 8.15 (d, J = 8.9 Hz, 1H, CH, pyridazine), 7.80 (d, J = 8.9 Hz, 1H, CH, pyridazine), 3.60 (t, J = 1.7 Hz, 2H, SCH_2, butyl), 1.84–1.73 (m, 2H, CH_2, butyl), 1.53–1.41 (m, 2H, CH_2, butyl), 0.95 (t, J = 4.5 Hz, 3H, CH_3, butyl). ^{13}C NMR ($CDCl_3$) δ 161.25, 159.77, 129.93, 126.54 (pyridazine), 52.14, 23.09, 21.53, 13.32 (butyl). FT-IR (KBr) cm^{-1} 3025 (aromatic), 1545 (N═N), 1491, 1452 (CH_2), 1319, 1181, 1154 (SO_2), 1028 (SO), 702, 540 (CCl). GC-MS *m/z* (%) 234.69 (M+) 78.90 (100.0), 113.90 (68.5), 127.90 (26.2), 115.90 (22.0), 51.90 (12.3).

3-(*n*-Pentylsulfonyl)-6-chloropyridazine (7d). Yield: 79%. m.p. 77–82°C. ^{1}H NMR ($CDCl_3$) δ 8.14 (d, J = 8.8 Hz, 1H, CH, pyridazine), 7.79 (d, J = 8.8 Hz, 1H, CH, pyridazine), 3.61 (t, J = 2.9 Hz, 2H, SCH_2, pentyl), 1.86–1.78 (m, 2H, CH_2, pentyl), 1.47–1.27 (m, 4H, CH_2, pentyl), 0.89 (t, J = 4.6 Hz, 3H, CH_3, pentyl). ^{13}C NMR ($CDCl_3$) δ 161.12, 159.73, 130.00, 126.62 (pyridazine), 52.35, 30.33, 21.72, 21.68, 13.59 (pentyl). FT-IR (KBr) cm^{-1} 3025 (aromatic), 1545 (N═ N), 1491, 1452 (CH_2), 1319, 1180, 1153 (SO_2), 1028 (SO), 702, 540 (CCl). GC-MS *m/z* (%) 248.69 (M+) 78.90 (100.0), 113.90 (97.3), 140.90 (76.3), 115.90 (31.9), 155.90 (29.5).

3-(*n*-Hexylsulfonyl)-6-chloropyridazine (7e). Yield: 78%. m.p. 88–90°C. ^{1}H NMR ($CDCl_3$) δ 8.15 (d, J = 8.8 Hz, 1H, CH, pyridazine), 7.80 (d, J = 8.8 Hz, 1H, CH, pyridazine), 3.59 (t, J = 8.0 Hz, 2H, SCH_2, hexyl), 1.85–1.75 (m, 2H, CH_2, hexyl), 1.48–1.39 (m, 2H, CH_2, hexyl), 1.30–1.27 (m, 4H, CH_2, hexyl), 0.86 (t, J = 6.7 Hz, 3H, CH_3, hexyl). ^{13}C NMR ($CDCl_3$) δ 161.11, 159.74, 130.00, 126.63 (pyridazine), 52.39, 31.02, 27.92, 22.19, 21.99, 13.81 (hexyl). FT-IR (KBr) cm^{-1} 3025 (aromatic), 1545 (N═N), 1491, 1452 (CH_2), 1320 (SO_2), 1028 (SO), 702, 540 (CCl). GC-MS *m/z* (%) 262.69 (M+) 140.90 (100.0), 113.90 (93.6), 78.90 (82.3), 142.90 (32.3), 115.90 (31.1).

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