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Synthesis and anticancer activity of thiosemicarbazones

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Abstract—Twenty-six thiosemicarbazones (III-1–III-26) were synthesized via three steps starting from hydrazine hydrate and carbon disulfide. The testing of anticancer activity of these compounds in vitro against P-388, A-549, and SGC-7901 shows that compounds III-15 and III-16 possess a higher inhibitory ability for P-388 and SGC-7901. Further testing shows that the value of IC_{50} of compound III-16 against SGC-7901 reaches to 0.032 μM.

Thiosemicarbazones and its derivatives have attracted considerable pharmaceutical interest due to their antiviral,[1] antibacterial,[2–4] and antitumor activities.[5–11] The antitumor activity of these seems to be due to an inhibition of DNA synthesis produced by the modification in the reductive conversion of ribonucleotides to deoxyribonucleotides.[12] Thiosemicarbazones have drawn great interests for their high potential biological activity especially their antitumor activity. Recently, there have been a number of reports involving the preparation and biological activity of the complexes formed by transition metals coupled with thiosemicarbazones as ligands. Although much attention has been paid to the complexes and the biological activities thereof, our interests have been focused on the relationships between structures of thiosemicarbazones and their antitumor activities. In the present paper, we report the preparation of thiosemicarbazones, and their anticancer activities in vitro are also evaluated.

In our previous papers, we have reported that some thiosemicarbazones have effective antitumor activities, and the substituents in these compounds affect their antitumor activities strongly.[13–15] In a continuation of our work on the structure–activity relationship, twenty-six thiosemicarbazones were prepared[17] according to Wilson's method[16] as Scheme 1. In compound (III), the R^1 were substituted phenyl or heterocyclics, R^2 were H or Me, and R^3 were substituted aniline or piperazine. Since norfloxacin has good anti-microbial activity, piperazine substituents were chosen as R^3.

The preparations are summarized in Table 1. The structures of all compounds were identified by IR, ^{1}H NMR, and elemental analysis (Table 2).

The antitumor activities in vitro for these compounds were evaluated by the MTT method for P-388 cell, SRB for A-549, and SGC-7901. The results are summarized in Tables 3 and 4.

Comparing the structures of compounds III-12, III-14, III-15, III-16 with those of compounds III-1–III-7, they possess the same substituents R^2 and R^3, but the different R^1. From Table 3, it is obvious that compounds III-12, III-14, III-15, and III-16 possess a higher inhibitory activity against P-388 and A-549 than compounds III-1–III-7. So, it shows that a heterocyclic substituent such as 2-pyridyl, 2-furyl, 2-thiazolyl or 2-pyrimidinyl as R^1 is more preferable than phenyl or substituted phenyl when R^2 and R^3 are methyl and 2-pyridyl piperazine, respectively.

Compounds III-3 and III-8 have the same R^1 and R^3, but the different R^2. Table 3 shows both of them display a weak inhibitory activity against P-388 and A-549. When comparing the structures of compounds III-9 and III-10, they have the same R^1 (phenyl) and R^3 (piperazine substituted with norfloxacin), but the different R^2. Table 3 shows that the anticancer activity of compound III-10 is higher than that of III-9, which indicates that methyl as R^2 is better than hydrogen.

Keywords: Thiosemicarbazones; Anticancer activity; SAR.
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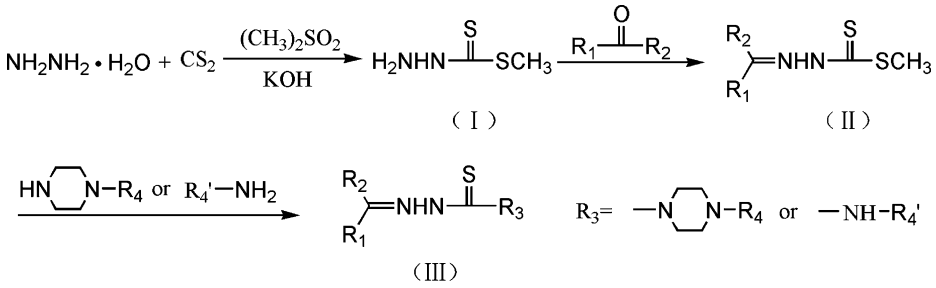


Scheme 1. Synthetic route of thiosemicarbazones.

Table 1. Preparation of thiosemicarbazones

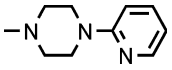
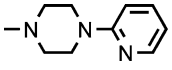
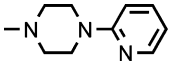
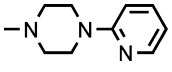
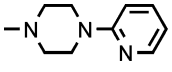
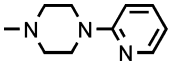
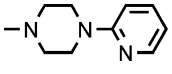
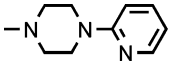
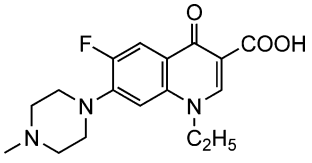
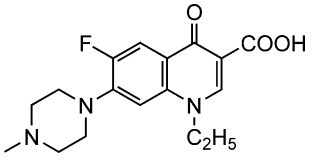
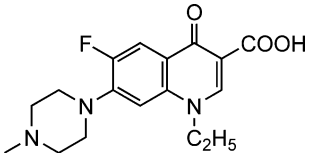
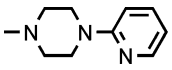
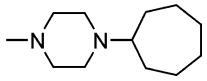
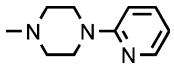
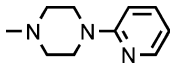
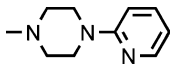
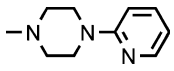
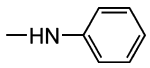
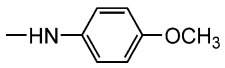
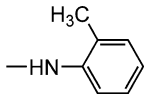
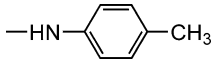
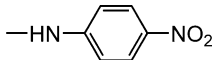
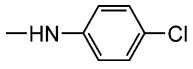
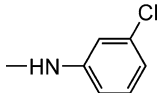
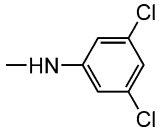
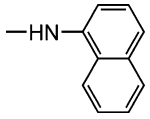
Entry	R^1	R^2	R^3	mp (°C)	Yield (%)
III-1	4-$NO_2C_6H_4$	–CH_3		180–181	80.7
III-2	4-Cl C_6H_4	–CH_3		142–143	82.8
III-3	C_6H_5	–CH_3		139–140	63.0
III-4	4-$CH_3C_6H_4$	–CH_3		159–161	85.7
III-5	4-$CH_3OC_6H_4$	–CH_3		134–135	83.8
III-6	4-HOC_6H_4	–CH_3		192–193	55.7
III-7	4-$NH_2C_6H_4$	–CH_3		169–171	61.2
III-8	C_6H_5	–H		162–164	61.5
III-9	C_6H_5	–H		226–227	46.2
III-10	C_6H_5	–CH_3		228–229	68.7
III-11	2-Pyridyl	–CH_3		241–242	48.5
III-12	2-Pyridyl	–CH_3		174–176	73.5

Table 1 (*continued*)

Entry	R^1	R^2	R^3	mp (°C)	Yield (%)
III-13	2-Pyridyl	$-CH_3$		147–148	67.0
III-14	2-Furyl	$-CH_3$		135–137	27.3
III-15	2-Thiazolyl	$-CH_3$		138–139	58.0
III-16	2-Pyrimidinyl	$-CH_3$		195–196	66.0
III-17	2-Thienyl	$-CH_3$		154–156	84.3
III-18	2-Thienyl	$-CH_3$		176–178	60.3
III-19	2-Thienyl	$-CH_3$		189–192	68.4
III-20	2-Thienyl	$-CH_3$		168–170	75.5
III-21	2-Thienyl	$-CH_3$		188–190	82.6
III-22	2-Thienyl	$-CH_3$		218–220	43.5
III-23	2-Thienyl	$-CH_3$		190–193	62.5
III-24	2-Thienyl	$-CH_3$		155–157	60.4
III-25	2-Thienyl	$-CH_3$		166–169	59.4
III-26	2-Thienyl	$-CH_3$		198–200	74.1

From Table 1, comparing the structures of compounds III-3 and III-10, they possess the same R^1 (phenyl), and the same R^2 (methyl), but the different R^3. The difference of their anticancer activity in Table 3 shows that piperazine substituent bearing norfloxacin as R^3 (III-10) is preferable to that bearing piperidine (III-3). However, interestingly, when their R^1 substituent (phenyl) was changed into 2-pyridyl, such as in compounds III-11 and III-12, the reverse result was given, that is, compound III-12, which bears the piperidine in the piperazine substituent as R^3, possesses an anticancer activity higher than that of compound III-11 which bears norfloxacin in piperazine as R^3 (Table 3). According to this, so we can conclude that the substituents R^1 and R^3 should match each other, and they should be considered as a whole, not be considered separately.

From Tables 3 and 4, it has been found that compounds III-15 and III-16 possess an excellent inhibitory activity against P-388 and SGC-7901. More accurate two tests (a and b) of IC_{50} of compound III-16 against SGC-7901 are listed in Table 5. It shows that the value of IC_{50} reaches to 0.032 μM. So this kind of thiosemicarbazone is valuable for being further studied.

Table 2. Elemental analysis (calcd data in parentheses), IR, and ^{1}H NMR data (III-1–III-26)

Compound	Elemental analysis (%)			IR (cm^{-1})	^{1}H NMR (δ)
	C	H	N		
III-1	56.22 (56.23)	5.29 (5.24)	21.83 (21.86)	1598, 1520, 1490, 1443, 1345, 1293, 1180, 981, 856, 778	8.56–6.64 (m, 8H), 4.23 (t, 4H), 3.76 (t, 4H), 2.34 (s, 3H)
III-2	57.50 (57.82)	5.41 (5.39)	18.70 (18.73)	1600, 1492, 1473, 1464, 1363, 1265, 1236, 948, 835, 770	8.23–6.60 (m, 8H), 4.20 (t, 4H), 3.80 (t, 4H), 2.44-2.68 (m, 3H)
III-3	63.45 (63.68)	6.01 (6.24)	20.89 (20.63)	1600, 1485, 1440, 1360, 1297, 1236, 1030, 982, 756	8.22 (d, 1H), 7.44–7.26 (m, 6H), 6.24 (t, 2H), 4.23 (t, 4H), 3.63 (t, 4H), 2.25–2.62 (m, 3H)
III-4	65.00 (64.66)	6.53 (6.56)	19.94 (19.81)	1598, 1488, 1479, 1467, 1360, 1265, 1232, 976, 827, 763	8.23–6.60 (m, 8H), 4.20 (t, 4H), 3.65 (t, 4H), 2.38 (s, 3H), 2.67 (m, 3H)
III-5	61.77 (61.76)	6.04 (6.27)	18.96 (18.96)	1605, 1445, 1360, 1303, 1260, 1231, 1188, 983, 830, 770	8.23–6.70 (m, 8H), 4.18 (t, 4H), 3.85 (t, 4H), 3.64 (s, 3H), 2.52 (s, 3H)
III-6	60.77 (60.82)	5.91 (5.96)	19.89 (19.70)	1604, 1495, 1424, 1355, 1315, 1225, 1142, 989, 830, 783	8.16–6.58 (m, 3H), 4.40 (t, 4H), 3.60 (t, 4H), 2.42 (s, 3H)
III-7	60.75 (60.99)	6.55 (6.26)	23.83 (23.71)	1630, 1598, 1480, 1344, 1270, 1190, 1055, 950, 840, 775	8.12–6.64 (m, 8H), 5.64 (b, 2H), 4.40 (t, 4H), 3.85 (t, 4H), 2.54 (s, 3H)
III-8	63.06 (62.74)	5.86 (5.88)	21.63 (21.52)	1598, 1557, 1488, 1430, 1329, 1237, 1044, 947, 890, 750	9.63 (s, 1H), 8.24–6.64 (m, 9H), 6.58 (s, 1H), 4.20 (t, 4H), 3.72 (t, 3H)
III-9	60.09 (59.86)	5.22 (5.02)	14.55 (14.55)	1738, 1630, 1479, 1440, 1384, 1290, 1260, 1029, 850, 755	8.80 (s, 1H), 8.20 (s, 1H), 7.88–7.05 (m, 7H), 4.52 (q, 2H), 4.10 (t, 4H), 3.52 (t, 4H), 1.42 (s, 3H)
III-10	60.75 (60.59)	5.39 (5.29)	14.05 (14.13)	1744, 1633, 1480, 1437, 1358, 1255, 1235, 1030, 810, 762	8.80–7.11 (m, 8H), 4.52 (q, 2H), 4.16 (t, 4H), 3.48 (t, 4H), 2.40 (s, 3H), 1.42 (s, 3H)
III-11	57.71 (58.05)	5.28 (5.07)	16.95 (16.93)	1730, 1634, 1473, 1430, 1387, 1239, 1150, 1025, 980, 800	8.80 (s, 1H), 8.70–7.05 (m, 7H), 4.56 (q, 2H), 4.18 (t, 4H), 3.50 (t, 4H), 2.54 (s, 3H), 1.43 (t, 3H)
III-12	60.28 (59.97)	5.89 (5.92)	25.08 (24.69)	1600, 1490, 1471, 1433, 1368, 1264, 1231, 1154, 980, 784	8.68–6.58 (m, 8H), 4.20 (t, 4H), 3.75 (t, 4H), 2.44 (s, 3H)
III-13	63.54 (63.47)	8.06 (8.13)	19.44 (19.48)	1610, 1580, 1563, 1430, 1364, 1293, 1002, 979, 776	8.75–7.25 (m, 4H), 4.09 (t, 4H), 2.66 (s, 3H), 2.38–2.61 (m, 5H), 1.81–1.39 (m, 12H)
III-14	58.45 (58.34)	6.25 (5.81)	21.74 (21.27)	1598, 1479, 1435, 1359, 1300, 1268, 1229, 1158, 980, 750	8.24–6.64 (m, 7H), 4.23 (t, 4H), 3.68 (t, 4H), 2.28 (s, 3H)
III-15	51.71 (52.00)	5.13 (5.24)	24.30 (24.26)	1596, 1570, 1482, 1438, 1397, 1213, 981, 936, 781, 735	8.60–6.60 (m, 6H), 4.22 (t, 4H), 3.77 (m, 4H), 2.80–2.40 (m, 3H)
III-16	55.90 (56.28)	5.47 (5.61)	28.46 (28.72)	1593, 1561, 1479, 1364, 1297, 1213, 981, 936, 781, 735	8.83–6.66 (m, 7H), 4.25 (t, 4H), 3.66 (t, 4H), 2.70 (s, 3H)
III-17	58.45 (58.34)	5.53 (5.54)	20.20 (20.26)	1592, 1559, 1478, 1417, 1337, 1312, 1264, 1232, 1173, 1157	8.22–6.63 (m,7H), 4.20 (t, 4H), 3.70 (t, 4H), 2.70 (s, 3H)
III-18	56.71 (56.69)	4.82 (4.75)	15.38 (15.25)	3299, 3227, 1530, 1496, 1362, 1291, 1196, 704, 688, 538	9.31 (s, 1H), 8.66 (s, 1H), 7.71–7.05 (m, 8H), 2.35 (s, 3H)
III-19	54.68 (55.05)	4.93 (4.95)	13.53 (13.75)	3292, 3217, 1521, 1487, 1297, 1242, 1192, 1027, 836, 715	9.07 (s, 1H), 8.64 (s, 1H), 7.48 (d, 2H), 7.32–7.01 (m, 3H), 6.90 (d, 2H), 3.78 (s, 3H), 2.29 (s, 3H)
III-20	57.81 (58.10)	5.45 (5.22)	14.90 (14.52)	3295, 3218, 1518, 1488, 1292, 1258, 1191, 1046, 726,711	9.13 (s, 1H), 8.68 (s, 1H), 7.87–7.01 (m, 7H), 2.32 (s, 3H), 2.30 (s, 3H)
III-21	58.09 (58.10)	5.22 (5.22)	14.51 (14.52)	3294, 3217, 1522, 1486, 1293, 1259, 1192, 1048, 776, 709	9.51 (s, 1H), 9.07 (s, 1H), 7.84 (d, 2H), 7.82–7.36 (m, 3H), 7.52 (d, 2H), 2.66 (s, 3H), 2.64 (s, 3H)
III-22	48.67 (48.69)	3.55 (3.77)	17.23 (17.47)	3275, 1597, 1556, 1518, 1332, 1282, 1191, 1110, 851, 709	9.66 (s, 1H), 8.76 (s, 1H), 8.28 (d, 2H), 8.07 (d, 2H), 7.43–7.08 (m, 3H), 2.38 (s, 3H)
III-23	50.41 (50.39)	3.86 (3.90)	13.59 (13.56)	3288, 1519, 1291, 1200, 1087, 1044, 1016, 819, 788, 717	9.27 (s, 1H), 8.73 (s, 1H), 7.67 (d, 2H), 7.37 (d, 2H), 7.39–7.06 (m, 3H), 2.34 (s, 3H)
III-24	50.05 (50.39)	3.90 (3.90)	13.87 (13.56)	3298, 3231, 1578, 1516, 1416, 1255, 1200, 1045, 708, 528	9.31 (s, 1H), 8.70 (s, 1H), 7.82–7.07 (m, 7H), 2.35 (s, 3H)
III-25	45.13 (45.38)	3.10 (3.22)	12.31 (12.20)	3164, 1557, 1478, 1411, 1336, 1280, 1058, 1033, 711, 640	9.37 (s, 1H), 8.67 (s, 1H), 7.56 (s, 2H), 7.21 (s, 1H), 7.41–7.07 (m, 3H), 2.36 (s, 3H)
III-26	62.44 (62.73)	5.01 (4.64)	13.27 (12.91)	3304, 3204, 1597, 1530, 1514, 1488, 1360, 1291, 1249, 1208	9.58 (s, 1H), 8.88 (s, 1H), 8.03–7.06 (m, 10H), 2.40 (s, 3H)

Table 3. The inhibition rates for P-388 and A-549 in vitro (III-1–III-16)

Compound	Inhibition rate of P-388 (%) concentration (mol/L)			Inhibition rate of A-549 (%) concentration (mol/L)		
	10^{-5}	10^{-6}	10^{-7}	10^{-5}	10^{-6}	10^{-7}
III-1	6.4	4.6	4.6	16.4	0.0	0.0
III-2	0.0	0.0	0.0	0.0	0.0	0.8
III-3	4.6	0.0	0.0	0.8	0.0	0.0
III-4	25.8	21.2	28.8	0.0	0.0	0.0
III-5	7.3	3.7	2.8	0.8	0.0	3.3
III-6	53.0	25.8	28.8	0.0	0.0	0.0
III-7	30.3	4.6	12.1	46.8	0.0	0.0
III-8	7.3	4.6	3.7	0.0	16.4	0.8
III-9	68.8	4.6	0.0	4.1	0.8	0.0
III-10	100.0	24.8	0.0	63.9	0.8	0.0
III-11	26.6	4.6	0.0	7.4	16.4	0.0
III-12	100.0	12.8	13.8	100.0	19.7	24.6
III-13	96.5	41.6	39.8	40.0	17.8	0.0
III-14	77.3	50.0	37.9	72.3	0.0	0.0
III-15	94.5	74.3	73.5	13.6	21.5	15.6
III-16	96.5	89.4	28.3	79.3	18.5	12.6

Table 4. The inhibition rates for P-388 and SGC-7901 in vitro (III-15–III-26)

Compound	Inhibition rate of P-388 (%) concentration (mol/L)					Inhibition rate of SGC-7901(%) concentration (mol/L)				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
III-15	—	94.5	74.3	73.5	—	—	92.5	60.8	14.3	—
III-16	—	96.5	89.4	28.3	—	—	90.0	90.0	81.7	—
III-17	100.0	0.0	15.0	0.0	0.0	96.4	94.9	7.2	1.6	12.8
III-18	48.0	1.2	1.6	6.2	8.5	39.2	0.0	0.0	0.0	0.0
III-19	25.9	5.2	6.9	3.6	0.0	65.1	29.2	0.0	0.0	0.0
III-20	91.9	1.3	0.0	5.9	0.0	73.5	41.6	5.5	0.0	7.1
III-21	8.4	9.2	5.7	10.8	0.0	67.8	33.9	15.0	9.6	8.8
III-22	8.6	0.0	2.1	3.6	10.3	51.7	35.7	8.3	14.5	9.1
III-23	21.6	9.9	0.0	0.0	0.0	56.7	12.7	0.0	0.0	0.0
III-24	51.0	6.5	3.7	8.3	0.0	86.8	39.7	0.0	0.0	0.0
III-25	90.1	20.9	7.0	17.6	0.0	72.9	27.9	0.0	0.0	0.0
III-26	30.3	4.3	7.2	0.9	0.0	55.9	24.3	13.5	16.5	20.0

Table 5. IC_{50} of III-16 for SGC-7901

Concentration (mol/L)	10^{-7}	5×10^{-8}	2.5×10^{-8}	1.25×10^{-8}	0.625×10^{-8}	IC_{50} (μM)
III-16-a	65.1	60.1	48.4	31.7	18.4	0.032
III-16-b	83.9	68.9	51.8	49.8	38.8	0.012

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17. Representative procedure for compound III-16: (a) To a mixture of 60 mL distilled water, 50 mL isopropyl alcohol, and 49.5 g (0.75 mol) potassium hydroxide was added 39.0 g (0.75 mol) hydrazine hydrate at 20 °C. The resulted mixture was cooled down at 10 °C with an ice-water bath, then 57.5 g (0.75 mol) carbon disulfide was dropped slowly over the course of 100 min. After the addition, the reaction was continued for 2 h. Then 94.5 g (0.75 mol) dimethyl sulfate was added to the mixture at 15 °C and stirred for 1 h. The resulted precipitate was filtered, washed with water, and dried in vacuum. The crude product was recrystallized with methylene dichloride to give 55.0 g compound (I), yield 60.1%, mp 81–82 °C [81–83 °C lit[18]], IR (KBr, cm^{-1}): 3443, 3200, 2978, 1602, 1510, 1430, 1378, 1293, 1155, 1009, 948, 715, 666, ^1H NMR ($CDCl_3$-DMSO, δ): 5.51 (br, 2H), 4.49 (s, 1H), 2.61 (s, 3H). (b) 2.5g (0.02 mol) compound (I) and 2.5g (0.02 mol) 2-acetylpyrimidine were dissolved in 20 mL isopropyl alcohol. The mixture was stirred for 24 h at room temperature. Then the resulted yellow precipitate was filtered, washed with isopropyl alcohol, and recrystallized with 95% ethanol to give 3.7 g compound (II), yield 81.8%, mp 132–134 °C, IR (KBr, cm^{-1}): 3146, 2916, 1657, 1603, 1457, 1412, 1304, 1270, 1148, 1115, 1082, 1068, 986, 815, ^1H NMR ($CDCl_3$, δ): 8.94 (d, 2H), 7.38 (t, 1H), 2.66 (s, 3H), 2.53 (s, 3H). (c) To a mixture of 2.3 g (0.01 mol) compound (II) in 50 mL ethanol was added 1.6 g (0.01 mol) 1-(2-pyridyl) piperazine. The mixture was refluxed for 24 h and the TLC test showed the reaction is complete. The mixture was cooled down to room temperature and concentrated under vacuum. The residue was recrystallized with the mixture of ethanol and chloroform (1/2) to give a 2.1 g brown compound III-16, yield 66.0%, mp 195–196 °C, the full spectral analysis is given in Table 2.
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