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Efficient synthesis and biological evaluation of 1,3-benzenedicarbonyl dithioureas

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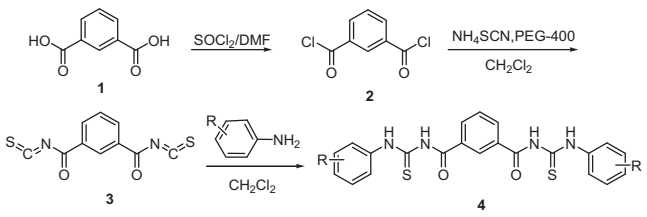
ABSTRACT

The synthesis and biological activity of 1,3-benzenedicarbonyl dithioureas are described. Bioassay results indicated that these compounds exhibited cytotoxicity against various cancer cells. For example, compounds **4a** showed the best inhibition activities against KB and CNE2 with IC_{50} 10.72 and 9.91 μM, respectively.

Acylthioureas (ATUs) possess various biological activities, such as antiviral,[1,2] antibacterial,[3] fungicidal,[4,5] herbicidal,[6,7] plant growth regulating,[8] antiaggregating,[9] antiarrythmic,[10] analgesic,[10] antihyperlipidemic,[10] local anesthetic.[10] Especially some thieno[3,2-*b*]pyridine acylthioureas have been recently described as potent antitumor agents inhibiting c-Met/VEGFR2 tyrosine kinase[11] and some quinoline and quinazoline–acylthiourea derivatives have been reported as potent and selective inhibitors of the platelet-derived growth factor (PDGF) receptor autophosphorylation.[12]

In the past, the pharmacological potential of this chemical class had attracted our attention and a number of ATUs endowed with various biological activities have been synthesized in our group.[13] Recently, we have become interested in the synthesis of symmetric acylthioureas aiming to finding novel antitumor agents. Herein, we would like to describe an efficient synthesis and antitumor activities of 1,3-benzenedicarbonyl dithiourea **4** from easily accessible 1,3-benzenedicarbonyl diisothiocyanate **3**.

All of the compounds studied in this work were synthesized according to the similar methods described in the literature,[14,15] in which compounds **4b**, **4g**, **4j**, **4m** and **4n** have been reported. The isophthaloyl dichloride **2**, obtained by reaction of isophthalic acid **1** with sulfuryl dichloride and *N*,*N*-dimethylformamide, was converted easily to 1,3-benzenedicarbonyl diisothiocyanate **3** via reaction with ammonium thiocyanate by solid–liquid phase transfer catalysis of polyethylene glycol-400 (PEG-400). 1,3-Benzenedicarbonyl diisothiocyanate **3** reacted with substituted benzeneamine to give 1,3-benzenedicarbonyl dithiourea **4** in satisfactory yields at room temperature.[16] The results are listed in Table 1.



All the compounds of the **4** series were obtained as white or yellow solids after recrystallization from DMF/H_2O. Their structures were fully characterized by IR, ^{1}H NMR, EI-MS and elemental analysis. For example, the IR spectrum of **4a** revealed absorption bands at 1671 (C═O), 3045 (C_6H_5), and 3217 (NH) cm^{-1}. The corresponding ^{1}H NMR spectrum showed the OH group at d(H) 10.27 (s), and the NH signals of the thiourea appeared at d(H) 11.47 and 12.90. The other signals resonated at d(H) 6.80–8.53 (m, 12 Ar-H). The structures of **4a** and the other analogs were further confirmed on the basis of elemental analysis. In the case of **5n**, single-crystal X-ray diffraction was employed to confirm the structure.[17]

The biological activity of the series of compounds **4** was investigated, and the results showed that most of them exhibited good to moderate cytotoxicity against KB and CNE2 cancer cell lines.[18] Compounds **4a** (R = 2-OH) showed the best inhibitory activity

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Table 1
Yields and in vitro cytotoxicity (IC_{50}[a], μM) of 1,3-benzenedicarbonyl dithiourea **4**

Compd	R	Yield[b] (%)	Cytotoxicity against KB[c]	Cytotoxicity against CNE2[c]
4a	2-OH	83	10.72	9.91
4b	2-Cl	72	38.75	>50
4c	2-Br	74	47.1	>50
4d	2-F	69	44.95	38.56
4e	2-CF_3	76	26.05	18.50
4f	2-CH_3	60	>50	>50
4g	3-Cl	82	12.02	16.27
4h	3-Br	81	15.16	18.56
4i	4-OH	89	20	16.61
4j	4-Cl	69	13.70	15.72
4k	4-Br	81	23.17	32.66
4l	4-F	71	43.2	32.97
4m	4-CH_3	78	15.32	32.98
4n	4-NO_2	73	35.7	>50
4o	4-OCH_3	78	>50	30.34
4p	H	88	>50	>50
Fluorouracil			15.9	

[a] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control.
[b] Isolated yields based on 1,3-benzenedicarbonyl diisothiocyanate **3**.
[c] KB cells were the drug sensitive human oral carcinoma cells and CNE2 cells were the nasopharyngeal carcinoma cells.

against KB and CNE2 with IC_{50} 10.72 and 9.91 μM, respectively, the inhibitory activity against KB even higher than that of fluorouracil (Table 1), while its unsubstituted analog **4p** showed no effect in inhibitory activity. Replacement of hydroxyl group in compound **4a** with chloro (**4b**, IC_{50} = 38.75 μM) reduced the cytotoxicity against KB by three-fold. The analogs **4c** (IC_{50} = 47.10 μM) and **4d** (IC_{50} = 44.95 μM), with halo substitution in the benzene ring, show reduction in the inhibitory activity by 4–5-fold as compared to **4a**. In the case of halo substituted derivatives, the compounds **4g** and **4h**, with halo substitution at *meta* position in benzene ring showed better activity compared to that substituted at *ortho* (**4b** and **4c**) and *para* (**4j** and **4k**) position. Replacement of R hydroxyl group with methyl group showed marginal reduction in the inhibitory activity as can be seen by comparing the IC_{50} values of compound **4a** with **4f** (IC_{50} >50 μM). Compound **4i** with R hydroxyl group at *para* position in benzene ring showed two-fold decreases in potency, compared to **4a** with hydroxyl group at *ortho* position. And introduction bulk groups like nitro and methoxy at *para* position in benzene ring, resulted in the compounds **4n** and **4o** with marginal reduction or no inhibitory activity. Furthermore, some 1,4-benzenedicarbonyl dithioureas were also synthesized, but their cytotoxicity showed almost inactive, for example, the corresponding 1,4-benzenedicarbonyl dithiourea of compound **4a** (R = 2-OH) showed no inhibitory activity against KB and CNE2 with IC_{50} more than 50 μM.

In conclusion, we have synthesized a series of 1,3-benzenedicarbonyl dithiourea via reaction of functionalized diisothiocyanate with various benzeneamine. The preliminary investigation on the biological activities of **4** showed that some of them exhibited cytotoxicity against various cancer cells.

Acknowledgments

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16. *Preparation of 1,3-benzenedicarbonyl dithiourea* **4**: A suspension of 1,3-benzenedicarbonyl chloride (1.02 g, 5 mmol), ammonium thiocyanate (1.14 g, 15 mmol) and PEG-400 (3% based on ammonium thiocyanate) in methylene chloride was stirred for 2 h at room temperature. The reaction mixture was filtered to give the soln of **3**, which was used directly without further purification. To a soln of **3** in CH_2Cl_2 (10 ml), the appropriate benzeneamine was added and the mixture was stirred for another 1 h, the yellow precipitate was formed. The reaction mixture was filtered and washed with a small quantity of ethanol, Et_2O and water successively, then recrystallized from DMF-water to give the target compounds.
Compound **4a**. Yellow solid. mp 201–203 °C. IR(KBr) υ: 3408, 3217, 3045, 1671(C=O), 1607, 1539, 1459, 1356 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 6.80–8.53 (m, 12H, Ar-H), 10.27 (s, 2H, OH), 11.47 (s, 2H, NH), 12.90 (s, 2H, NH). MS (70 eV) *m*/*z* (%): 466 (M^+). Anal. Calcd for $C_{22}H_{18}N_4O_4S_2$: C, 56.64; H, 3.89; N, 12.01. Found: C, 56.91; H, 3.41; N, 12.47.
Compound **4b**. Yellow solid. mp 185–187 °C. IR(KBr) υ: 3370, 3232, 2960, 1673(C=O), 1591, 1547, 1524, 1463, 1442, 1341 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 7.34–8.66 (m, 12H, Ar-H), 11.86 (s, 2H, NH), 12.61 (s, 2H, NH). MS (70 eV) *m*/*z* (%): 502 (M^+). Anal. Calcd for $C_{22}H_{16}Cl_2N_4O_2S_2$: C, 52.49; H, 3.20; N, 11.13. Found: C, 52.11; H, 2.83; N, 11.56.
Compound **4c**. Yellow solid. mp 191–193 °C. IR(KBr) υ: 3433, 3200, 3010, 1695(C=O), 1590, 1520, 1516, 1472, 1426, 1367 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 7.26–8.64 (m, 12H, Ar-H), 11.86 (s, 2H, NH), 12.50 (s, 2H, NH). MS (70 eV) *m*/*z* (%): 590 (M^+). Anal. Calcd for $C_{22}H_{16}Br_2N_4O_2S_2$: C, 44.61; H, 2.72; N, 9.46. Found: C, 44.94; H, 2.25; N, 9.02.
Compound **4d**. Yellow solid. mp 192–194 °C. IR(KBr) υ: 3386, 3023, 1683(C=O), 1618, 1598, 1557, 1527, 1483, 1459, 1354 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 7.26–8.61 (m, 12H, Ar-H), 11.80 (s, 2H, NH), 12.47 (s, 2H, NH). MS (70 eV) *m*/*z* (%): 470 (M^+). Anal. Calcd for $C_{22}H_{16}F_2N_4O_2S_2$: C, 56.16; H, 3.43; N, 11.91. Found: C, 55.70; H, 3.51; N, 11.54.
Compound **4e**. Yellow solid. mp 194–196 °C. IR(KBr) υ: 3417, 3246, 3040, 1681(C=O), 1603, 1559, 1527, 1414, 1350, 1323 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 7.71–8.57 (m, 12H, Ar-H), 11.70 (s, 2H, NH), 12.65 (s, 2H, NH). MS (70 eV) *m*/*z* (%): 570 (M^+). Anal. Calcd for $C_{24}H_{16}F_6N_4O_2S_2$: C, 50.52; H, 2.83; N, 9.82. Found: C, 50.26; H, 2.62; N, 9.52.
Compound **4f**. Yellow solid. mp 183–185 °C. IR(KBr) υ: 3315, 3171, 3027, 1675(C=O), 1611, 1588, 1521,1458, 1352 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 2.27 (s, 6H, CH_3), 7.22–8.62 (m, 12H, Ar-H), 11.64 (s, 2H, NH), 12.23 (s, 2H, NH). MS (70 eV) *m*/*z* (%): 462 (M^+). Anal. Calcd for $C_{24}H_{22}N_4O_2S_2$: C, 62.31; H, 4.79; N, 12.11. Found: C, 61.85; H, 4.70; N, 11.75.
Compound **4g**. Yellow solid. mp 189–191 °C. IR(KBr) υ: 3417, 3227, 3156, 3046, 1678(C=O), 1589, 1538, 1432, 1333, 1303 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 7.37–8.58 (m, 12H, Ar-H), 11.68 (s, 2H, NH), 12.54 (s, 2H, NH). MS (70 eV) *m*/*z* (%): 502 (M^+). Anal. Calcd for $C_{22}H_{16}Cl_2N_4O_2S_2$: C, 52.49; H, 3.20; N,11.13. Found: C, 52.79; H, 3.32; N, 11.17.
Compound **4h**. White solid. mp 190–192 °C. IR(KBr) υ: 3433, 3221, 3032, 1680(C=O), 1588, 1534, 1516, 1472, 1426, 1349 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 7.39–8.57 (m, 12H, Ar-H), 11.67 (s, 2H, NH), 12.53 (s, 2H, NH). MS (70 eV) *m*/*z* (%): 590 (M^+). Anal. Calcd for $C_{22}H_{16}Br_2N_4O_2S_2$: C, 44.61; H, 2.72; N, 9.46. Found: C, 44.71; H,2.69; N,9.75.
Compound **4i**. Yellow solid. mp 190–192 °C. IR(KBr) υ: 3321, 1670(C=O), 1613, 1515, 1438, 1345 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 6.78–8.52 (m, 12H, Ar-H), 9.58–9.59 (d, 2H, OH), 11.46 (s, 2H, NH), 12.33 (s, 2H, NH). MS (70 eV) *m*/*z* (%): 466 (M^+). Anal. Calcd for $C_{22}H_{18}N_4O_4S_2$: C, 56.64; H, 3.89; N, 12.01. Found: C, 56.77; H, 3.41; N, 12.47.
Compound **4j**. Yellow solid. mp 212–214 °C. IR(KBr) υ: 3437, 3204, 3021, 1671(C=O), 1643, 1587, 1548, 1522, 1491, 1433, 1400, 1382, 1344 cm^{-1}. ^1H

NMR (600 MHz DMSO-d_6) δ(ppm): 7.48–8.55 (m, 12H, Ar-H), 11.62 (s, 2H, NH), 12.47 (s, 2H, NH). MS (70 eV) m/z (%): 502 (M^+). Anal. Calcd for $C_{22}H_{16}Cl_2N_4O_2S_2$: C, 52.49; H, 3.20; N, 11.13. Found: C, 52.55; H, 3.26; N, 11.17.

Compound **4k**. Yellow solid. mp 202–204 °C. IR(KBr) υ: 3324, 3027, 2923, 1680(C=O), 1591, 1552, 1515, 1485, 1346 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 7.64–8.57 (m, 12H, Ar-H), 11.64 (s, 2H, NH), 12.49 (s, 2H, NH). MS (70 eV) m/z (%): 590 (M^+). Anal. Calcd for $C_{22}H_{16}Br_2N_4O_2S_2$: C, 44.61; H, 2.72; N, 9.46. Found: C, 44.94; H, 2.35; N, 9.92.

Compound **4l**. Yellow solid. mp 196–197 °C. IR(KBr) υ: 3151, 3042, 1680(C=O), 1654, 1612, 1551, 1507, 1438, 1355, 1319 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 7.25–8.55 (m, 12H, Ar-H), 11.59 (s, 2H, NH), 12.41 (s, 2H, NH). MS (70 eV) m/z (%): 470 (M^+). Anal. Calcd for $C_{22}H_{16}F_2N_4O_2S_2$: C, 56.16; H, 3.43; N, 11.91. Found: C, 55.78; H, 3.02; N, 11.90.

Compound **4m**. Yellow solid. mp 210–212 °C. IR(KBr) υ: 3410, 3164, 3030, 1675(C=O), 1596, 1531, 1361, 1343 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 2.31 (s, 6H, CH_3), 7.20–8.54 (m, 12H, Ar-H), 11.52 (s, 2H, NH), 12.47 (s, 2H, NH). MS (70 eV) m/z (%): 462 (M^+). Anal. Calcd for $C_{24}H_{22}N_4O_2S_2$: C, 62.31; H, 4.79; N, 12.11. Found: C, 62.19; H, 4.52; N, 11.86.

Compound **4n**. Yellow solid. mp 210–212 °C. IR(KBr) υ: 3247, 3007, 1679(C=O), 1614, 1565, 1514, 1320 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 7.76–8.59 (m, 12H, Ar-H), 11.80 (s, 2H, NH), 12.78 (s, 2H, NH). MS (70 eV) m/z (%): 525 (M^++1). Anal. Calcd for $C_{22}H_{16}N_6O_6S_2$: C, 50.38; H, 3.07; N, 16.02. Found: C, 50.40; H, 3.15; N, 16.33.

Compound **4o**. Yellow solid. mp 186–188 °C. IR(KBr) υ: 3377, 3189, 2963, 1669(C=O), 1608, 1515, 1330 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 3.77 (s, 6H, OCH_3), 6.97–8.54 (m, 12H, Ar-H), 11.50 (s, 2H, NH), 12.38 (s, 2H, NH). MS (70 eV) m/z (%): 494 (M^+). Anal. Calcd for $C_{24}H_{22}N_4O_4S_2$: C, 58.28; H, 4.48; N, 11.33. Found: C, 58.64; H, 4.68; N, 11.47.

Compound **4p**. Yellow solid. mp 190–192 °C. IR(KBr) υ: 3443, 3332, 3169, 3033, 1671(C=O), 1598, 1555, 1516, 1447, 1349 cm^{-1}. ^1H NMR (600 MHz DMSO-d_6) δ(ppm): 7.27–8.56 (m, 12H, Ar-H), 11.57 (s, 2H, NH), 12.53 (s, 2H, NH). MS (70 eV) m/z (%): 434 (M^+). Anal. Calcd for $C_{22}H_{18}N_4O_2S_2$: C, 60.81; H, 4.18; N, 12.89. Found: C, 60.83; H, 3.95; N, 12.99.

17. Crystallographic data for the structural analysis have been deposited at the Cambridge Crystallographic Data Centre, CCDC No 802615. (E-mail: deposit@ccdc.cam.ac.uk).
18. Cytotoxic activities were evaluated by using standard MTT assay after exposure of cells to the tested compounds for 72 h. Results are presented as mean values of three independent experiments done in quadruplicates. Coefficients of variation were <10%.