N-Substituted Hydroxyureas as Urease Inhibitors

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In order to seek a urease inhibitor more potent than hydroxyurea (1), its alkyl- or phenyl-substituted derivatives were synthesized and evaluated for their effect on the jack bean urease. Of 16 compounds tested, *m*-methyl- (10) and *m*-methoxy-phenyl substituted hydroxyurea (13) showed the most potent inhibitory activities against the enzyme.

Key words urease inhibitor; *N*-hydroxy-*N*′-(*m*-methylphenyl)urea; *Helicobacter pylori*; jack bean

Persons infected with *Helicobacter pylori* are exposed to a high risk for stomach cancer. As eradication therapy, a combination of antibiotics and a proton inhibitor has been adopted. However, this therapy is unsatisfactory due to the induction of drug-resistance. Thus, the urease activity of *H. pylori* may be an attractive target for drug design of an anti-*H. pylori* agent. Hydroxyurea (**1**), an antineoplastic agent,[1] has been known to inhibit various ureases from microorganisms and vegetables, including *H. pylori*[2,3] and jack bean.[4] In the present work, we have searched for a potent therapeutic drug through the derivatization of **1** to *N*-substituted hydroxyureas.

Phenyl- or low alkyl-substituted hydroxyureas were first synthesized as shown in Table 1. Thus, aniline was converted to the isocyanate with triphosgene in the two-phase system of CH_2Cl_2 and aq. Na_2CO_3, which was treated with *O*-benzylhydroxylamine to give *N*-(benzyloxy)-*N*′-phenylurea (**5a**). This compound was hydrogenated over 10% Pd–C to give the desired *N*-hydroxy-*N*′-phenylurea[5] (**5**). Further, *N*-ethyl-*N*′-hydroxyurea[6] (**2**), *N*-hydroxy-*N*′-propylurea[6] (**3**) and *N*-butyl-*N*′-hydroxyurea[7] (**4**) were prepared by treating the corresponding isocyanates with hydroxylamine hydrochloride in aq. NaOH solution, respectively, according to the procedure by Harmon, *et al.*[6]

The inhibitory activities against jack bean urease were measured as explained in a separate paper[8]: the control solution was prepared by dissolving Phenol Red and blank solvent (H_2O, EtOH, or dimethyl sulfoxide (DMSO)) in 0.1 M phosphate buffer (pH 7.7), whereas the sample solution was made by dissolving Phenol Red and a test sample in a blank solvent in 0.1 M phosphate buffer (pH 6.7). These control and sample solutions, after addition of the urease solution, were successively pre-incubated at 30 °C for 30 min, and diluted 6-fold with the control buffer of pH 7.7 and the buffered urea solution of pH 6.7, respectively, according to the colorimetric timing method.[9—11] The absorbance at 560 nm of the resulting control solution was adjusted to zero on the spectrophotometer, and the time interval for the absorbance of the sample solution to reach zero was measured at 560 nm. The percentage inhibition (%) of each sample was calculated based on the following equation.[8]

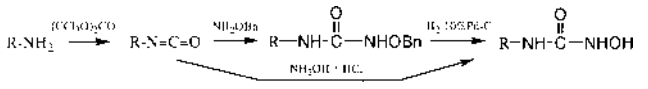
$$\text{percentage inhibition (\%)} = [(t - t_0)/t] \times 100$$

t: time interval (s) measured at each molar sample concentration

t_0: time interval (s) measured at zero molar sample concentration

As a result, substitution with the lower alkyl groups at **1** led to a marked decrease in the inhibitory power [IC_{50} (μM): >800 (**2**); >800 (**3**); 706 (**4**)] against the jack bean urease. However, *N*-phenyl-substituted derivative **5** inhibited the enzyme to the same extent [IC_{50} (μM): 19.3 μM] as did the lead compound, hydroxyurea (**1**) [22.3 μM]. In the next experiment, the *o*-, *m*- and *p*-substituted derivatives of **5** shown below were thus synthesized in order to examine the effect of substituents at the phenyl group: *N*-(fluorophenyl)-*N*′-hydroxyureas (**6**—**8**), *N*-(*o*-methylphenyl)- (**9**),[6] *N*-(*m*-methylphenyl)- (**10**) and *N*-(*p*-methylphenyl)-*N*′-hydroxyurea (**11**),[12] *N*-(*o*-methoxyphenyl)- (**12**),[6] *N*-(*m*-methoxylphenyl)- (**13**)[13] and *N*-(*p*-methoxylphenyl)-*N*′-hydroxyurea (**14**)[12] were prepared *via* the *N*-benzyloxy derivatives **6a**—**14a**, respectively, in the same two-phase system as for **5**. *N*-Hydroxy-*N*′-(*o*-nitrophenyl)- (**15**), *N*-hydroxy-*N*′-(*m*-nitrophenyl)- (**16**) and *N*-hydroxy-*N*′-(*p*-nitrophenyl)-urea (**17**)[12] were synthesized using the procedure of Harmon, *et al.*,[6] but with the addition

Table 1. Synthesis of Substituted Hydroxyureas



			Yield (%)
R=H	—	**1**	—
C_2H_5	—	**2**	50.7[a]
C_3H_7	—	**3**	15.6[a]
C_4H_9	—	**4**	54.8[a]
Ph	**5a**	**5**	59.0[b]
o-F-Ph	**6a**	**6**	48.6[b]
m-F-Ph	**7a**	**7**	42.3[b]
p-F-Ph	**8a**	**8**	57.3[b]
o-Me-Ph	**9a**	**9**	42.8[b]
m-Me-Ph	**10a**	**10**	38.6[b]
p-Me-Ph	**11a**	**11**	78.7[b]
o-MeO-Ph	**12a**	**12**	53.5[b]
m-MeO-Ph	**13a**	**13**	21.3[b]
p-MeO-Ph	**14a**	**14**	72.3[b]
o-NO_2-Ph	—	**15**	21.4[a]
m-NO_2-Ph	—	**16**	8.9[a]
p-NO_2-Ph	—	**17**	11.8[a]

a) Based on the corresponding isocyanates. *b*) Based on the corresponding benzyloxyureas.

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Table 2. Effects of Substitution at Phenylhydroxyureas on Jack Bean Urease (**5**: IC_{50}=19.3 μM)

Substituent	IC_{50} (μM)		
	ortho	*meta*	*para*
–F	37.7	35.7	48.0
$-CH_3$	121.3	12.7	152.8
$-OCH_3$	191.7	16.2	340.0
$-NO_2$	N.D.[a)]	N.D.[a)]	533.3

a) Not determined due to insolubility in the assay system.

of MeOH to improve the solubility of the starting isocyanates in the reaction mixture. Table 2 illustrates the substitutional and positional effects of F-, CH_3-, CH_3O- and NO_2-groups at the phenyl group. Introduction of these substituents at the *o*- and *p*-position significantly decreased the anti-urease activity. This can be interpreted on the basis of: i) steric hindrance by the *ortho*-substitution, which disturbs the hydroxamic acid group from accessing the active site. ii) steric compression due to bulky groups at the *para*-position, which hampers the contact between the substituted phenyl group and a hydrophobic cavity around the active site. Substitution with F did not significantly influence their activities, presumably due to its small atomic radius. In contrast, *m*-methyl- and *m*-methoxy-phenyl derivatives (**10**, **13**) had a high potency, inhibiting the enzyme more strongly than **5**, as well as **1**, though to a small degree (IC_{50} 12.7 or 16.2 μM). We plan to examine further the potency of these compounds as therapeutic drugs.

Experimental

All melting points (mp) were determined on a Yanagimoto MP-32 micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrometer, and low resolution (LR)-FAB-MS on a JEOL JMS-HX 100 instrument. ^{1}H-NMR (300 MHz) spectra were recorded on a Bruker AX-300 instrument, using tetramethylsilane (TMS) as a reference. High resolution (HR)- and LR-electron impact (EI)-MS were measured with JEOL The Tandem MStation JMS-700. Chromatography was carried out using silica gel 60 (Kanto Chemical, 70—230 mesh). Analytical TLC and Preparative TLC were performed using Silica gel 60 F_{254} (Merck, 0.25 mm) and Silica gel 60 F_{254} (Merck, 2 mm) glass plates, respectively. Phenol Red solution (1 g/l phenol red in 47 vol% ethanol) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Urease (115 units/mg derived from Jack bean) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Hydroxyurea and isocyanates were purchased from Sigma-Aldrich Japan Co., Inc. (Tokyo).

Preparation of *N*-Substituted Hydroxyureas (5—14) These compounds were prepared *via* the corresponding *N*-(benzyloxy)ureas. In a typical experiment, a solution of Na_2CO_3 (14.56 mmol) in water (100 ml) was added to a solution of triphosgene (2.67 mmol) and non-substituted or substituted aniline (7.28 mmol) in CH_2Cl_2 (100 ml), and the mixture was stirred vigorously at room temp. for 1.5 h. Then, a solution of *O*-benzylhydroxylamine (14.56 mmol) in MeOH (50 ml) was added to the mixture, which was stirred for another 15 h. The CH_2Cl_2 layer was separated, dried over $MgSO_4$ and concentrated *in vacuo* to give *N*-(benzyloxy)urea. This compound was dissolved in MeOH (60 ml) and hydrogenated over 10% Pd–C (90 mg) at room temp. for 3 h. After removal of the catalyst, MeOH was evaporated *in vacuo*, affording a desired substituted hydroxyurea.

N-(Benzyloxy)-*N*′-phenylurea (**5a**): Colorless needles (1550 mg, 87.7%). mp 106—107.6 °C (AcOEt). IR (KBr) cm^{-1}: 3323, 3204, 2860, 1659, 1537, 1450, 1335, 1236, 1070, 949, 905, 789, 691. ^{1}H-NMR (CD_3OD) δ: 4.56 (2H, s, –CH_2–), 7.0—7.5 (10H, m, arom. H_{10}). EI-MS *m*/*z*: 242 (M^+).

N-Hydroxy-*N*′-phenylurea[5)] (**5**): Colorless plates (574.2 mg). mp 138—141.8 °C (AcOEt). IR (KBr) cm^{-1}: 3238, 2895, 1636, 1543, 1449, 1230, 1074, 757, 690. ^{1}H-NMR (DMSO-d_6) δ: 6.96 (1H, t, *J*=7.3 Hz), 7.24 (2H, t, *J*=7.4 Hz), 7.60 (2H, d, *J*=8.7 Hz), 8.71 (1H, s), 8.78 (1H, s), 8.91 (1H, d, *J*=0.7 Hz). HR-EI-MS *m*/*z*: 152.0582 (M^+, Calcd for $C_7H_8N_2O_2$: 152.0586).

N-(Benzyloxy)-*N*′-(*o*-fluorophenyl)urea (**6a**): Colorless plates (1010 mg, 53.3%). mp 101.8—102.3 °C (AcOEt). IR (KBr) cm^{-1}: 3166, 3074, 2888, 1688, 1618, 1537, 1491, 1454, 1371, 1329, 1188, 1072, 984, 820, 752, 700. ^{1}H-NMR (CD_3OD) δ: 4.87 (2H, s, –CH_2–), 7.0—7.9 (9H, m, arom. H_9). EI-MS *m*/*z*: 260 (M^+).

N-(*o*-Fluorophenyl)-*N*′-hydroxyurea (**6**): Colorless plates (320.7 mg). mp 140—142.8 °C (AcOEt). IR (KBr) cm^{-1}: 3227, 2846, 1648, 1559, 1458, 1420, 1251, 1084, 752, 638. ^{1}H-NMR (CD_3OD) δ: 7.03—7.17 (3H, m), 7.90—7.98 (1H, m). HR-EI-MS *m*/*z*: 170.0474 (M^+, Calcd for $C_7H_7FN_2O_2$: 170.0492).

N-(Benzyloxy)-*N*′-(*m*-fluorophenyl)urea (**7a**): Colorless needles (762.8 mg, 40.3%). mp 113—114.5 °C (AcOEt). IR (KBr) cm^{-1}: 3321, 3206, 2860, 1661, 1597, 1537, 1493, 1452, 1362, 1312, 1277, 1252, 1148, 1072, 973, 770. ^{1}H-NMR (CD_3OD) δ: 4.56 (2H, s, –CH_2–), 6.7—7.5, m, arom. H_9). EI-MS *m*/*z*: 260 (M^+).

N-(*m*-Fluorophenyl)-*N*′-hydroxyurea (**7**): Colorless columns (210.8 mg). mp 103.4—105.7 °C (AcOEt). IR (KBr) cm^{-1}: 3228, 2852, 1684, 1560, 1450, 1406, 1278, 1144, 763, 638. ^{1}H-NMR (CD_3OD) δ: 6.65—6.78 (1H, m), 7.22—7.26 (2H, m), 7.41—7.47 (1H, m). HR-EI-MS *m*/*z*: 170.0500 (M^+, Calcd for $C_7H_7FN_2O_2$: 170.0492).

N-(Benzyloxy)-*N*′-(*p*-fluorophenyl)urea (**8a**): Colorless fine crystals (541.8 mg, 28.6%). mp 122.4—124.5 °C (AcOEt). IR (KBr) cm^{-1}: 3312, 3223, 2926, 2860, 1659, 1524, 1412, 1211, 1074, 939, 831, 808, 698. ^{1}H-NMR (CD_3OD) δ: 4.54 (2H, s, –CH_2–), 7.0—7.5 (9H, m, arom. H_9). EI-MS *m*/*z*: 260 (M^+).

N-(*p*-Fluorophenyl)-*N*′-hydroxyurea (**8**): Colorless fine crystals (202.7 mg). mp 135.6—137.1 °C (AcOEt). IR (KBr) cm^{-1}: 3238, 1654, 1559, 1509, 1412, 1213, 1093, 830. ^{1}H-NMR (CD_3OD) δ: 6.93—7.05 (2H, m), 7.29—7.50 (2H, m), 8.81. HR-EI-MS *m*/*z*: 170.0500 (M^+, Calcd for $C_7H_7FN_2O_2$: 170.0492).

N-(Benzyloxy)-*N*′-(*o*-methylphenyl)urea (**9a**): Colorless needles (710 mg, 33.2%). mp 126.7—127.0 °C (AcOEt). IR (KBr) cm^{-1}: 2862, 1686, 1533, 1458, 1252, 1074, 968, 916, 754, 702. ^{1}H-NMR (CD_3OD) δ: 2.15 (3H, s, CH_3), 4.89 (2H, s, –CH_2–), 7.0—7.5 (9H, m, arom. H_9). EI-MS *m*/*z*: 256 (M^+).

N-Hydroxy-*N*′-(*o*-methylphenyl)urea[6)] (**9**): Colorless needles (517.8 mg). mp 233.1—235.3 °C (AcOEt). IR (KBr) cm^{-1}: 3236, 2887, 2359, 2340, 1647, 1612, 1458, 1354, 1288, 1248, 1082, 1042, 746. ^{1}H-NMR (CD_3OD) δ: 2.27 (3H, s, CH_3), 7.0—7.5 (4H, m, arom. H_4). HR-EI-MS *m*/*z*: 166.0727 (M^+, Calcd for $C_8H_{10}N_2O_2$: 166.0742).

N-(Benzyloxy)-*N*′-(*m*-methylphenyl)urea (**10a**): Colorless needles (1012 mg, 47.5%). mp 96.3—97.2 °C (AcOEt). IR (KBr) cm^{-1}: 2912, 2858, 1611, 1541, 1489, 1420, 1362, 1292, 1175, 1088, 951, 905, 878, 824, 775, 694, 627. ^{1}H-NMR (CD_3OD) δ: 2.29 (3H, s, CH_3), 4.86 (2H, s, –CH_2–), 6.8—7.5 (9H, m, arom. H_9). EI-MS *m*/*z*: 256 (M^+).

N-Hydroxy-*N*′-(*m*-methylphenyl)urea (**10**): Colorless needles (467.0 mg). mp 106.8—107.7 °C (AcOEt). IR (KBr) cm^{-1}: 3383, 2883, 1647, 1555, 1489, 1437, 1250, 1173, 1090, 891, 866, 777. HR-EI-MS *m*/*z*: 166.0749 (M^+, Calcd for $C_8H_{10}N_2O_2$: 166.0742).

N-(Benzyloxy)-*N*′-(*p*-methylphenyl)urea (**11a**): Colorless needles (1488 mg, 64.5%). mp 106—106.8 °C (AcOEt). IR (KBr) cm^{-1}: 3331, 3206, 3031, 2916, 2860, 1659, 1591, 1537, 1454, 1412, 1360, 1325, 1236, 1074, 945, 814, 745, 694. ^{1}H-NMR (CD_3OD) δ: 2.19 (3H, s, CH_3), 4.80 (2H, s, –CH_2–), 7.0—7.4 (9H, m, arom. H_9). EI-MS *m*/*z*: 256 (M^+).

N-Hydroxy-*N*′-(*p*-methylphenyl)urea[12)] (**11**): Colorless columns (760.7 mg). mp 149—153.5 °C (AcOEt). IR (KBr) cm^{-1}: 3240, 2918, 2540, 1896, 1641, 1597, 1555, 1416, 1331, 1232, 1082, 1003, 870, 820, 704. HR-EI-MS *m*/*z*: 166.0727 (M^+, Calcd for $C_8H_{10}N_2O_2$: 166.0742).

N-(Benzyloxy)-*N*′-(*o*-methoxyphenyl)urea (**12a**): Colorless columns (1136 mg, 57.3%). mp 89.0—90.2 °C (*n*-hexane–AcOEt). IR (KBr) cm^{-1}: 3078, 2839, 1680, 1601, 1537, 1489, 1462, 1366, 1250, 1215, 1175, 1119, 986, 918, 810, 702, 665, 631. ^{1}H-NMR (CD_3OD) δ: 3.87 (3H, s, OCH_3), 4.86 (2H, s, –CH_2–), 6.8—8.0 (9H, arom. H_9). EI-MS *m*/*z*: 272 (M^+).

N-Hydroxy-*N*′-(*o*-methoxyphenyl)urea[6)] (**12**): Powdery crystals (709.6 mg). mp 163.2—165.3 °C (AcOEt). IR (KBr) cm^{-1}: 3215, 2841, 2340, 1643, 1595, 1547, 1462, 1427, 1259, 1215, 1177, 1124, 1082, 1047, 1003, 939, 876, 802, 764, 646. ^{1}H-NMR (CD_3OD) δ: 3.93 (3H, s, OCH_3), 6.8—8.1 (4H, m, arom. H_4). HR-EI-MS *m*/*z*: 182.0676 (M^+, Calcd for $C_8H_{10}N_2O_3$: 182.0691).

N-(Benzyloxy)-*N*′-(*m*-methoxyphenyl)urea (**13a**): Colorless needles (1205 mg, 60.8%). mp 118.1—119.2 °C (*n*-hexane–AcOEt). IR (KBr) cm^{-1}: 2837, 1655, 1612, 1572, 1501, 1418, 1333, 1311, 1213, 1178, 1159, 1080, 1032, 941, 891, 833, 789, 741, 702. ^{1}H-NMR (CD_3OD) δ: 3.59 (3H, s, OCH_3), 4.86 (2H, s, –CH_2–), 6.5—7.5 (9H, m, arom. H_9). EI-MS *m*/*z*: 272 (M^+).

N-Hydroxy-*N*′-(*m*-methoxyphenyl)urea[13] (**13**): Powdery crystals (282.5 mg). mp 113.3—114.6 °C (AcOEt). IR (KBr) cm^{-1}: 3371, 3214, 2839, 1456, 1435, 1296, 1202, 1163, 1092, 1053, 991, 887, 766, 689. ^{1}H-NMR (CD_3OD) δ: 3.76 (3H, s, OCH_3), 6.6—7.2 (4H, m, arom. H_4). HR-EI-MS *m*/*z*: 182.0703 (M^+, Calcd for $C_8H_{10}N_2O_3$: 182.0691).

N-(Benzyloxy)-*N*′-(*p*-methoxyphenyl)urea (**14a**): Colorless needles (1292.6 mg, 86.5%). mp 105.5—107 °C (AcOEt). IR (KBr) cm^{-1}: 3344, 3211, 3067, 2862, 1649, 1518, 1420, 1323, 1269, 1232, 1178, 1069, 1030, 947, 827, 804, 750, 654. ^{1}H-NMR (CD_3OD) δ: 3.75 (3H, s, OCH_3), 6.8—7.5 (9H, m, arom. H_9). EI-MS *m*/*z*: 272 (M^+).

N-Hydroxy-*N*′-(*p*-methoxyphenyl)urea[12] (**14**): Colorless plates (625.3 mg). mp 137.8—141.5 °C (AcOEt). IR (KBr) cm^{-1}: 3441, 3320, 1654, 1597, 1551, 1511, 1240, 1025, 825. ^{1}H-NMR (CD_3OD) δ: 3.70 (3H, s, OCH_3), 6.7—7.4 (4H, m, arom. H_4). HR-EI-MS *m*/*z*: 182.0692 (M^+, Calcd for $C_8H_{10}N_2O_3$: 182.0691).

Preparation of *N*-substituted Hydroxyureas (2—4, 15—17) In a typical experiment,[14] a solution of $NH_2OH \cdot HCl$ (7.61 mmol) in H_2O (1.5 ml) was added to a solution of NaOH (7.61 mmol) in H_2O (1 ml). To this stirred mixture was added isocyanate (7.61 mmol) over a period of 20 min under ice-cooling. After stirring for another 45 min, the reaction mixture was filtered to yield a precipitate.

N-Ethyl-*N*′-hydroxyurea[6] (**2**): Colorless fine crystals (401.2 mg). mp 113—115 °C (AcOEt). IR (KBr) cm^{-1}: 3396, 3238, 2981, 2879, 1684, 1654, 1458, 1153, 1070, 778, 724. ^{1}H-NMR (CD_3OD) δ: 1.13 (3H, t, J=7.2 Hz), 3.22 (2H, q, J=7.2 Hz).

N-Hydroxy-*N*′-propylurea[6] (**3**): Colorless fine crystals (140 mg). mp 116—118.3 °C (AcOEt). IR (KBr) cm^{-1}: 3323, 2964, 2875, 1636, 1560, 1151, 1125, 1082, 824, 749. ^{1}H-NMR (CD_3OD) δ: 0.92 (3H, t, J=7.4 Hz), 1.47—1.59 (2H, m), 3.15 (2H, t, J=7.0 Hz).

N-Butyl-*N*′-hydroxyurea[7] (**4**): Colorless fine crystals (550 mg). mp 122—124 °C (AcOEt). IR (KBr) cm^{-1}: 3328, 3159, 2959, 1560, 1430, 1317, 1145, 1082, 832. ^{1}H-NMR (DMSO-d_6) δ: 0.87 (3H, t, J=7.3 Hz), 1.20—1.32 (2H, m), 1.34—1.44 (2H, m), 3.03 (2H, q, J=6.7 Hz), 6.60 (1H, br t, J=5.8 Hz), 8.18 (1H, s), 8.49 (1H, d, J=1.0 Hz).

N-Hydroxy-*N*′-(*o*-nitrophenyl)urea (**15**): Pale yellow needles (320.9 mg). mp 225—226 °C ($CHCl_3$–MeOH). IR (KBr) cm^{-1}: 3292, 1654, 1584, 1508, 1340, 1276, 1148, 743. ^{1}H-NMR (DMSO-d_6) δ: 7.29 (1H, br t, J=7.8 Hz), 7.71 (1H, br t, J=7.9 Hz), 7.96 (1H, dd, J=1.3, 8.4 Hz), 8.04 (1H, dd, J=1.5, 8.3 Hz), 10.04 (1H, s). HR-EI-MS *m*/*z*: 197.0427 (M^+, Calcd for $C_7H_7N_3O_4$: 197.0437).

N-Hydroxy-*N*′-(*m*-nitrophenyl)urea (**16**): Pale yellow fine crystals (133.5 mg). mp 156—158.5 °C ($CHCl_3$–MeOH). IR (KBr) cm^{-1}: 3199, 2892, 1647, 1597, 1560, 1523, 1420, 1347, 1108, 892, 842. ^{1}H-NMR (DMSO-d_6) δ: 7.53 (1H, t, J=8.2 Hz), 7.81 (1H, ddd, J=0.9, 2.3, 8.2 Hz), 8.04 (1H, ddd, J=0.9, 2.2, 8.2 Hz), 8.70 (1H, t, J=2.2 Hz). HR-EI-MS *m*/*z*: 197.0427 (M^+, Calcd for $C_7H_7N_3O_4$: 197.0437).

N-Hydroxy-*N*′-(*p*-nitrophenyl)urea[12] (**17**): Pale yellow needles (176.9 mg). mp 162.5—165 °C ($CHCl_3$–MeOH). IR (KBr) cm^{-1}: 3323, 2964, 2875, 1610, 1560, 1151, 1125, 1082, 824. ^{1}H-NMR (DMSO-d_6) δ: 7.92 (2H, d, J=9.4 Hz), 8.16 (2H, d, J=9.4 Hz), 9.13 (1H, s), 9.26 (1H, s), 9.50 (1H, s). HR-EI-MS *m*/*z*: 197.0427 (M^+, Calcd for $C_7H_7N_3O_4$: 197.0437).

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