Res Chem Intermed (2013) 39:177–183
DOI 10.1007/s11164-012-0641-7

Syntheses and electrochemical properties of novel aminopyrimidinone derivatives as a new class of abasic-site binders

Junya Chiba · Yasuhiro Doi · Masahiko Inouye

Received: 3 October 2011 / Accepted: 7 January 2012 / Published online: 22 June 2012

Abstract We describe syntheses and electrochemical properties of a new class of aminopyrimidinone derivatives: 2-amino-4-(3′,4′-dimethoxyphenyl)-6-pyrimidinone, 2-amino-4-(3′,4′-dihydroxyphenyl)-6-pyrimidinone, 2,4-diamino-5-(3′,4′-dimethoxyphenyl)-6-pyrimidinone, 2,4-diamino-5-(3′,4′-dihydroxyphenyl)-6-pyrimidinone, and 4-amino-5-(3′,4′-dihydroxyphenyl)-2,6-pyrimidinione, three of which possess a catechol unit as an oxidation-active moiety for developing electrochemically detectable abasic-site binders. These compounds were synthesized via a pyrimidine-ring forming reaction with guanidine. Cyclic voltammetry measurements revealed that the catechol-bearing derivatives showed oxidation potentials lower than that of DNA, indicating that they satisfied the requirements for the purpose.

Keywords Electrochemistry · Abasic site · Aminopyrimidinone · Catechol

Introduction

Abasic sites, also known as AP (apurinic/apyrimidinic) sites, are locations in DNA duplexes that have neither a purine nor a pyrimidine base. Abasic sites are naturally occurring and one of the well-known forms of DNA damage [1–3]. Various types of small organic molecules have been studied as "abasic-site binders" that are able to bind selectively to nucleobases opposite an abasic site in DNA duplexes, attempting to develop anti-cancer agents, to modulate gene expression and to detect SNPs (single nucleotide polymorphisms) [4–8]. In addition, abasic-site binders with an

J. Chiba (✉) · Y. Doi · M. Inouye (✉)
Graduate School of Pharmaceutical Sciences, University of Toyama, Sugitani 2630, Toyama 930-0194, Japan
e-mail: chiba@pha.u-toyama.ac.jp

M. Inouye
e-mail: inouye@pha.u-toyama.ac.jp

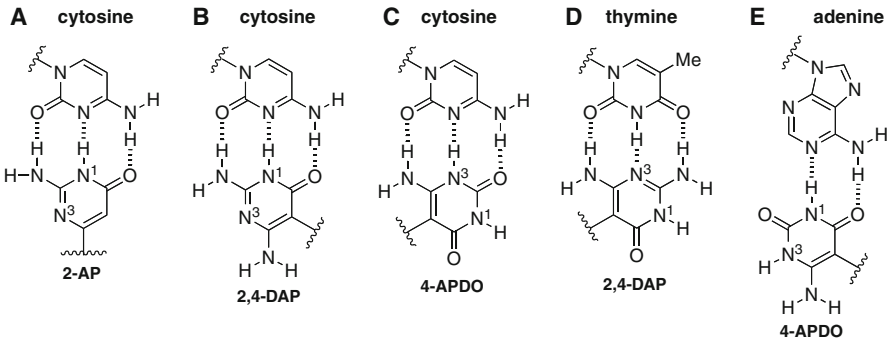


Chart 1 Possible hydrogen-bonding structures of **a** 2-amino-6-pyrimidinone (2-AP) with cytosine, **b** 2,4-diamino-6-pyrimidinone (2,4-DAP) with cytosine, **c** 4-amino-2,6-pyrimidindione (4-APDO) with cytosine, **d** 2,4-DAP with thymine and **e** 4-APDO with adenine

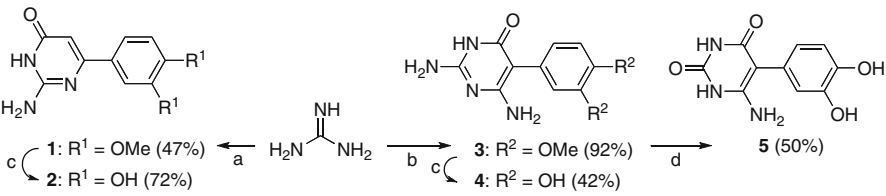
information-signaling ability will be useful reporter molecules for detecting an abasic site in a DNA duplex.

We previously reported an aminopyrimidinone derivative that serves as a hydrogen-bonding array in an acceptor–donor–donor (ADD, A: acceptor; D: donor) fashion and selectively binds with a cytosine (DAA) derivative (Chart 1a–c) [9]. We also reported the duplex formation of artificial oligonucleotides bearing 2-amino-pyrimidinones with native deoxycytidine homooligomers even in an aqueous buffer solution [10]. Moreover, it is well known that diaminopyrimidine (DAD) and pyrimidindione (ADA) skeletons can selectively interact with thymine (Chart 1d) and adenine (Chart 1e), respectively [11]. With these in mind, we thought that these three frameworks would be useful as hydrogen-bonding sites for designing new abasic-site binders. Thus, we planned to develop some abasic-site binders as an electrochemically detectable reporter molecule. For this purpose, we introduced catechol-derived units into the abasic-site binders described above. In particular, 1,2-dihydroxyphenyl groups will serve as an oxidation active moiety because of the reversible redox combination of catechol/*o*-quinone [12, 13]. In this paper, we report the syntheses and electrochemical properties of a new class of aminopyrimidinone derivatives; 2-amino-4-(3′,4′-dimethoxyphenyl)-6-pyrimidinone (**1**), 2-amino-4-(3′,4′-dihydroxyphenyl)-6-pyrimidinone (**2**), 2,4-diamino-5-(3′,4′-dimethoxyphenyl)-6-pyrimidinone (**3**), 2,4-diamino-5-(3′,4′-dihydroxyphenyl)-6-pyrimidinone (**4**), and 4-amino-5-(3′,4′-dihydroxyphenyl)-2,6-pyrimidindione (**5**) (Scheme 1).

Experimental

General

^{1}H and ^{13}C NMR spectra were obtained at 500 and 125 MHz, respectively, on a Varian Unity 500 spectrometer. IR spectra were measured on a JASCO FT/IR-460



[a]Key: (a) NaOH, ethyl 3-(3',4'-dimethoxyphenyl)-3-oxopropanoate (**6**), EtOH; (b) EtONa, methyl 2-(3',4'-dimethoxyphenyl)-2-cyanoethanoate (**7**), EtOH; (c) Pyridine•HCl; (d) 47% HBr, AcOH.

Scheme 1 Synthetic routes for aminopyrimidinones 1–5

plus spectrometer. ESI-HRMS analyses were carried out on a JEOL JMS-T100LC mass spectrometer. Melting points were determined with Yanako MP-500D and not corrected.

Materials

Ethyl 3-(3′,4′-dimethoxyphenyl)-3-oxopropanoate (**6**) [14, 15] and methyl 2-(3′,4′-dimethoxyphenyl)-2-cyanoethanoate (**7**) [16] were prepared as previously described. All other materials used were commercially available.

*2-Amino-4-(3′,4′-dimethoxyphenyl)-6-pyrimidinone (**1**)*

An EtOH (50 mL) solution of guanidine carbonate (25.0 g, 139 mmol) and NaOH (2.78 g, 69.4 mmol) was added to a toluene (100 mL) solution of **6** (17.5 g, 69.4 mmol), then the mixture was refluxed for 5 h. After removal of the solvent, the residue was resolved in 1 M NaOH and neutralized with AcOH (pH 6–7). The resulting precipitate was then filtered and washed with H_2O, acetone and Et_2O to give crude **1** (8.03 g, 47 %). Recrystallization from MeOH gave pure **1** as a colorless solid. Mp 271–274 °C; IR (KBr) 3,410, 3,170, 3,101, 1,654, 1,264 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.72 (brs, 1 H), 7.55 (dd, $J = 9.0$, 2.0 Hz, 1 H), 7.51 (d, $J = 2.0$ Hz, 1 H), 7.00 (d, $J = 9.0$ Hz, 1 H), 6.56 (brs, 2 H), 6.09 (s, 1 H), 3.81 (s, 3 H), 3.80 (s, 3 H) ppm; ^{13}C NMR (DMSO-d_6) δ 163.4, 162.5, 155.6, 150.6, 148.5, 129.9, 119.8, 111.3, 110.1, 96.6, 55.7, 55.6 ppm; HRMS calculated for MH^+, $C_{12}H_{14}N_3O_3$: 248.1035; found 248.1026.

*2-Amino-4-(3′,4′-dihydroxyphenyl)-6-pyrimidinone (**2**)*

A mixture of **1** (1.00 g, 4.05 mmol) and pyridine hydrochloride (10.0 g, 86.5 mmol) was heated at 200 °C for 1 h. After being cooled to room temperature, the mixture was suspended in H_2O (100 mL) and neutralized with 1 M NaOH (pH 6–7). After removal of the solvent, H_2O (100 mL) was added to the residue. The resulting precipitate was filtered and then washed with H_2O, EtOH, and Et_2O to give **2** (638 mg, 72 %). Recrystallization from 1 M HCl gave pure **2** (hydrochloride salt) as a pale yellow powder. Mp 262–264 °C; IR (KBr) 3,282, 3,114, 1,700, 1,637, 1,523 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.62 (brs, 1 H), 9.28 (brs, 1 H), 9.01 (brs, 1

H), 7.36 (d, $J = 2.0$ Hz, 1 H), 7.25 (dd, $J = 8.0$, 2.0 Hz, 1 H), 6.75 (d, $J = 8.0$ Hz, 1 H), 6.45 (brs, 2 H), 5.86 (s, 1 H) ppm; ^{13}C NMR (DMSO-d_6) δ 162.5, 156.9, 154.6, 148.8, 145.6, 124.5, 118.9, 115.8, 114.3, 97.1 ppm; HRMS calculated for MH^+, $C_{10}H_{10}N_3O_3$: 220.0722; found 220.0730.

*2,4-Diamino-5-(3′,4′-dimethoxyphenyl)-6-pyrimidinone (**3**)*

Guanidine hydrochloride (16.3 g, 170 mmol) was added to an EtOH (200 mL) solution of NaOEt (11.6 g, 170 mmol), then the mixture was stirred for 5 min at room temperature. After filtration, **7** (10.0 g, 42.5 mmol) was added to the filtrate. The resulting mixture was stirred for 16 h at ambient temperature and evaporated under atmospheric pressure to remove the EtOH. The residue was solidified with water (300 mL), and the resulting precipitate was filtered, washed with EtOH and Et_2O, and air-dried to give crude **3** (9.80 g, 92 %). Recrystallization from H_2O gave pure **3** as a colorless solid. Mp > 313 °C (decomp.); IR (KBr) 3,437, 3,175, 1,639, 1,600, 1,239 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.93 (brs, 1 H), 6.90 (d, $J = 8.5$ Hz, 1 H), 6.80 (d, $J = 2.0$ Hz, 1 H), 6.76 (dd, $J = 8.5$, 2.0 Hz, 1 H), 6.15 (brs, 2 H), 5.46 (brs, 2 H), 3.74 (s, 3 H), 3.71 (s, 3 H) ppm; ^{13}C NMR (DMSO-d_6) δ 161.4, 153.7, 148.4, 146.9, 127.8, 123.1, 114.9, 111.9, 90.3, 55.6, 55.4 ppm; HRMS calculated for MH^+, $C_{12}H_{15}N_4O_3$: 263.1144; found 263.1141.

*2,4-Diamino-5-(3′,4′-dihydroxyphenyl)-6-pyrimidinone (**4**)*

A mixture of **3** (1.10 g, 4.21 mmol) and pyridine hydrochloride (10.0 g, 86.5 mmol) was heated at 170 °C for 30 min. The mixture was cooled to room temperature, dissolved in H_2O (70 mL) and neutralized with 1 M NaOH (pH 6–7). After removal of the solvent, H_2O (50 mL) was added to the residue. The resulting precipitate was filtered and then washed with H_2O, EtOH and Et_2O to give **4** (412 mg, 42 %). Recrystallization from 1 M HCl gave pure **4** (hydrochloride salt) as a pale yellow powder. Mp 253–255 °C; IR (KBr) 3,185, 2,733, 1,694, 1,657, 1,595, 1,535, 1,431 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.85 (brs, 1 H), 8.69 (brs, 1 H), 8.60 (brs, 1 H), 6.67 (d, $J = 7.5$ Hz, 1 H), 6.64 (d, $J = 2.0$ Hz, 1 H), 6.47 (dd, $J = 7.5$, 2.0 Hz, 1 H), 6.09 (brs, 2 H), 5.29 (brs, 2 H) ppm; ^{13}C NMR (DMSO-d_6) δ 152.1, 151.2, 145.3, 144.7, 121.9, 121.6, 118.5, 115.9, 90.4 ppm; HRMS calculated for MH^+, $C_{10}H_{11}N_4O_3$: 235.0831; found 235.0839.

*4-Amino-5-(3′,4′-dihydroxyphenyl)-2,6-pyrimidindione (**5**)*

A mixture of 47 % HBr (5 mL), AcOH (2.5 mL) and **3** (540 mg, 2.06 mmol) was heated at 140 °C for 10 h. After being cooled to room temperature, the mixture was treated with H_2O (15 mL). The resulting precipitate was filtered and then washed with H_2O, EtOH and Et_2O to give **5** (243 mg, 50 %). Recrystallization from H_2O gave pure **5** as a colorless solid. Mp > 307 °C (decomp.); IR (KBr) 3,384, 3,039, 2,711, 1,693, 1,609, 1,534, 1,423, 1,291 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.38 (brs, 2 H), 8.48 (brs, 1 H), 8.41 (brs, 1 H), 6.93 (d, $J = 1.5$ Hz, 1 H), 6.74 (dd, $J = 8.0$, 1.5 Hz, 1 H), 6.56 (d, $J = 8.0$ Hz, 1 H), 6.50 (brs, 2 H) ppm; ^{13}C NMR (DMSO-d_6)

δ 163.3, 152.0, 143.8, 142.5, 125.8, 121.9, 118.4, 118.3, 114.5, 91.7 ppm; HRMS calculated for MNa^+, $C_{10}H_9N_3O_4Na$: 258.0491; found 258.0479.

Electrochemical measurements [17]

Cyclic voltammetry (CV) was performed using a Bioanalytical Systems (BAS) Model 620A electrochemical analyzer with a normal three-electrode configuration consisting of a gold working electrode ($\phi = 1.6$ mm, BASi), saturated Ag/AgCl reference electrode (BAS) and platinum wire auxiliary electrode. Measurements for **2**, **4**, and **5** were performed with a scanning rate (v) of 0.1 V/s at 25.0 ± 0.1 °C in 65 mM phosphate buffer (pH 7.0) that had been thoroughly degassed with N_2. Redox potentials of dimethoxy derivatives **1** and **3** (0.5 mM) were measured in N_2 bubbled acetonitrile containing 100 mM tetrabutylammonium perchlorate (conditions: $v = 0.1$ V/s, 25.0 ± 0.1 °C).

Results and discussion

Syntheses of the new aminopyrimidinones

The new aminopyrimidinones were synthesized via a pyrimidine-ring forming reaction with commercially available guanidine carbonate and guanidine hydrochloride (Scheme 1). Base-catalyzed cyclocondensation of guanidine carbonate and

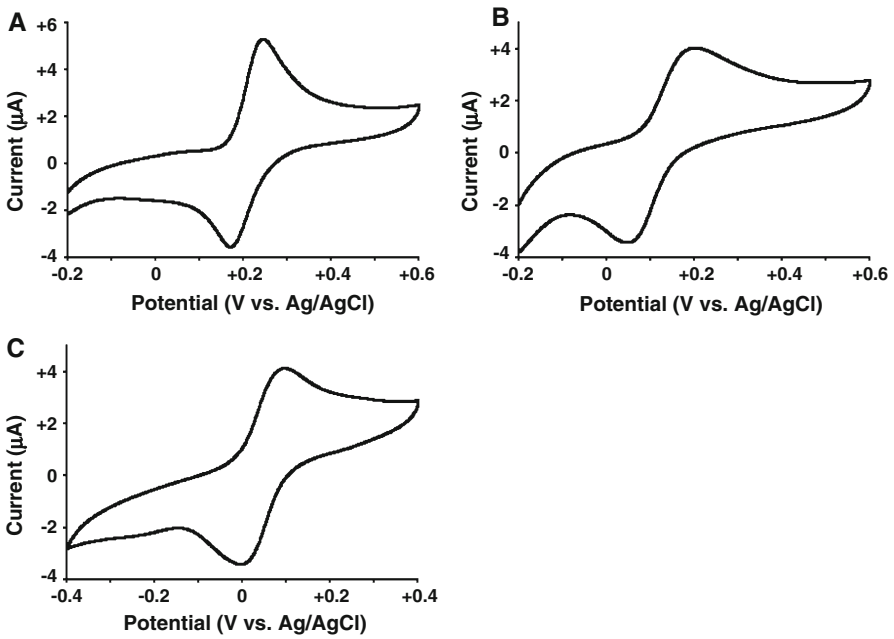


Fig. 1 Cyclic voltamograms of **a 2**, **b 4**, and **c 5** (0.5 mM)

oxopropanoate **6** afforded 2-aminopyrimidinone **1** in moderate yield. Next, a deprotection reaction of the methyl group with pyridine hydrochloride at 200 °C proceeded for 1 h to give the water-soluble dihydroxy compound **2** in good yield. The condensation of cyanoethanoate **7** and guanidine hydrochloride under basic conditions gave 2,4-diaminopyrimidinone **3** in 92 % yield. Subsequent treatment of **3** with pyridine hydrochloride at 180 °C for 30 min provided the corresponding dihydroxy derivative **4**. It is important to regulate the reaction time for increasing the yield of the deprotection reaction. A longer reaction time gave a mixture of **4** and 2,6-pyrimidindione **5**, the hydrolyzed compound of **4**. The by-product **5** could be mainly obtained from **3** by treatment with 47 % HBr and AcOH at 140 °C for 10 h.

Electrochemical properties

Redox potentials of aminopyrimidinones **1**–**5** were measured by CV. Dimethoxy compounds **1** and **3** exhibited no electrochemical response within a scanning range of –0.4 to +0.6 V versus Ag/AgCl. On the other hand, the dihydroxy derivatives **2**, **4** and **5** showed reversible cyclic voltammograms, in which the oxidation potentials were +0.25, +0.21, and +0.10 V versus Ag/AgCl, respectively (Fig. 1). These results suggested that the catechol derivatives **2**, **4**, and **5** showed clear electrochemical responses owing to the catechol/*o*-quinone redox activity. On the other hand, the methyl-protected **1** and **3** were hardly oxidized within the potential range. The oxidation potentials of **2**, **4**, and **5** to give the corresponding *o*-quinone derivatives were similar to that of catechol previously reported (ca. +0.2 V vs. Ag/AgCl in aqueous media [12]). In addition, these values are favorable for a reporter molecule in abasic sites because DNA is electrochemically silent in the potential range lower than ca. +0.8 V [18, 19].

Conclusion

In conclusion, we synthesized novel aminopyrimidinones possessing a catechol moiety for providing oxidation activity. Because all derivatives with the dihydroxy form of the catechol unit showed low oxidation potentials to give the corresponding *o*-quinone derivatives, they will be appropriate for using as abasic-site binders with a function of electrochemically detectable reporter. Development of a simple electrochemical detection system for nucleobases at abasic sites using these compounds is now underway.

References

1. J. Lhomme, J.-F. Constant, M. Demeunynck, Biopolymers **52**, 65 (1999)
2. E.C. Friedberg, Nature **421**, 436 (2003)
3. P.J. Berti, J.A.B. McCann, Chem. Rev. **106**, 506 (2006)

4. J.H.J. Hoeijmakers, Nature **411**, 366 (2001)
5. Y. Shao, K. Morita, Q. Dai, S. Nishizawa, N. Teramae, Electrochem. Commun. **10**, 438 (2008)
6. M. Li, Y. Sato, S. Nishizawa, T. Seino, K. Nakamura, N. Teramae, J. Am. Chem. Soc. **131**, 2448 (2009)
7. Z. Xu, Y. Sato, S. Nishizawa, N. Teramae, Biosens. Bioelectron. **26**, 4733 (2011)
8. Y. Sato, S. Nishizawa, N. Teramae, Chem. Eur. J. **17**, 11650 (2011)
9. H. Abe, M. Takase, Y. Doi, S. Matsumoto, M. Furusyo, M. Inouye, Eur. J. Org. Chem. **2005**, 2931 (2005)
10. Y. Doi, J. Chiba, T. Morikawa, M. Inouye, J. Am. Chem. Soc. **130**, 8762 (2008)
11. S.A. Benner, Acc. Chem. Res. **37**, 784 (2004)
12. R.P. Singh, Analyst **136**, 1216 (2011)
13. B.P. López, A. Merkoçi, Analyst **134**, 60 (2009)
14. V. Bardot, P. Besse, Y. Gelas-Miahle, R. Remuson, H. Veschamber, Tetrahedron **7**, 1077 (1996)
15. M.W. Rathke, J. Deitch, Tetrahedron Lett. **31**, 2953 (1971)
16. M.C. Honan, A. Balasuryia, T.M. Cresp, J. Org. Chem. **50**, 4326 (1985)
17. J. Chiba, Y. Doi, M. Inouye, Heterocycles **79**, 411 (2009)
18. E.E. Ferapontova, E. Dominguez, Electroanalysis **15**, 629 (2003)
19. I. Saito, T. Nakamura, K. Nakatani, Y. Yoshioka, K. Yamaguchi, H. Sugiyama, J. Am. Chem. Soc. **120**, 12686 (1998)