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PYRIMIDINE DERIVATIVES OF *N*-ACETYLGUANIDINE: NOVEL INHIBITORS OF SODIUM-HYDROGEN EXCHANGER 1

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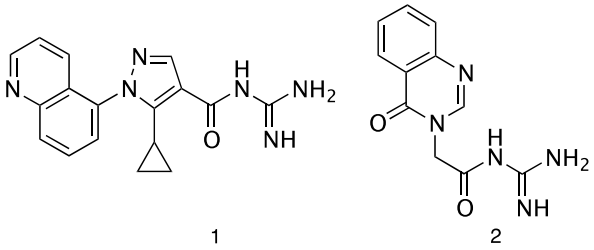
Abstract – Sodium-hydrogen exchanger (Na^+/H^+) type 1 (NHE-1) inhibitors have been shown to protect the heart during ischaemia and early reperfusion. As such, NHE-1 inhibitors are of special interest for clinical development for the attenuation of both acute and chronic post-myocardial infarction responses. New pyrimidine derivatives of *N*-acetylguanidine containing fragments of uracil, thymine, and their 3-benzyl derivatives were synthesised. These compounds showed *in vitro* inhibitory effects on NHE-1 that were significantly higher than those of zoniporide in platelet swelling assays.

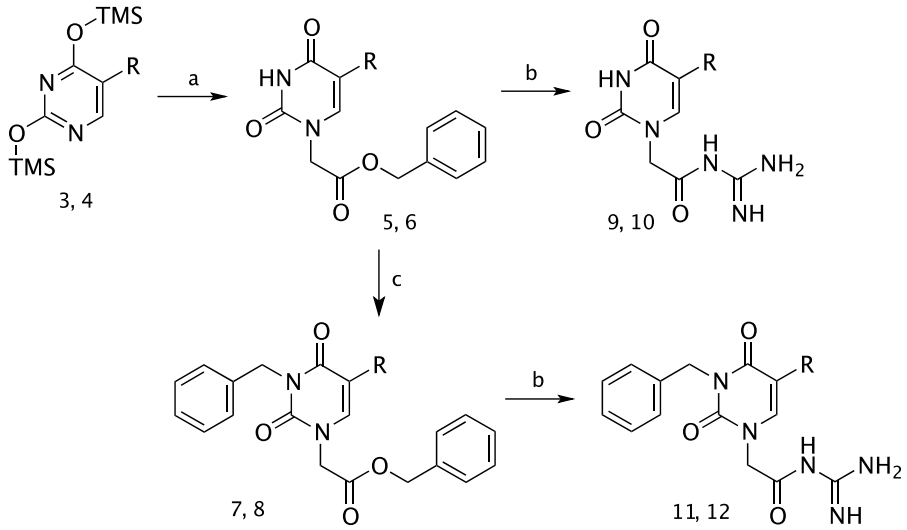
Sodium-hydrogen exchanger (Na^+/H^+) type 1 (NHE-1) inhibitors have been shown to protect the heart during ischaemia and early reperfusion. As such, NHE-1 inhibitors are of special interest for clinical development for the attenuation of both acute and chronic post-myocardial infarction responses.[1] Most of the NHE-1 inhibitors studied here are *N*-acyl derivatives of guanidine containing various heterocyclic systems (pyrazine, indole, quinoline, benzoxazine, and benzimidazole) in the *N*-acyl substituent.[2] The quinoline derivative of zoniporide (**1**) is the most active for selective pharmacological inhibition of NHE-1.[3,4] Previously, we obtained quinazoline derivatives of *N*-acetylguanidine, in particular *N*-[2-[4-oxo-3(4*H*)-quinazolinyl]acetyl]guanidine (**2**), which demonstrated higher *in vitro* inhibitory

activity against NHE-1 than zoniporide.[5] This article describes the synthesis and NHE-1 inhibitory activity of new analogues of compound **2** with various substituents at positions 3 and 5 of the pyrimidine system.



1 2

Regioselective N^1-alkylation of trimethylsilyl derivatives of uracil (**3**) and thymine (**4**) with benzyl bromoacetate in boiling 1,2-dichloroethane for 12 h, as described for 1-bromo-2-aryloxyethanes,[6] furnished the corresponding pyrimidine benzyl acetate derivatives **5** and **6** with a yield of 55% and 65%, respectively. Their subsequent alkylation with benzyl chloride in anhydrous DMF in the presence of potassium carbonate at a temperature of 80-85 °C produces corresponding N^3-benzyl derivatives **7** and **8,** with a high yield (88-92%). The final amination of pyrimidine esters **5-8** was performed with the guanidine-base, obtained *in situ* from guanidine hydrochloride and potassium hydroxide in a boiling MeOH solution, resulting in the desired uracil and thymine derivatives *N*-[2-[2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetyl]guanidines **9** and **10** and their 3-benzyl derivatives **11** and **12**, with a yield of 76-84% (Scheme).



3, **5**, **7**, **9, 11** R = H

4, **6**, **8**, **10, 12** R = Me

Scheme. Reagents and conditions: (**a**) $BrCH_2CO_2Bn$, $ClCH_2CH_2Cl$, reflux, 12 h, 50% EtOH, 1 h, 55-65%; (**b**) $NH_2C(NH)NH_2$•HCl, KOH, MeOH, reflux, 5 min, 25 °C, 10-12 h, 76-84%; (**c**) BnCl, DMF, K_2CO_3, 80-85 °C, 1 h, 88-92%.

The structures of all synthesised substances were confirmed via ^{1}H and ^{13}C NMR spectroscopy. In the mass spectra of target compounds **11** and **12**, peaks corresponding to molecular ions were not observed because of their low stability and rapid decomposition with the cleavage of cyanamide fragments and formation of protonated amides of 2-[3-benzyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetic acids (*m*/*z* 260 and 274, respectively). This indirectly confirms the presence of guanidine moieties in the structure of the original compounds. The presence of monosubstituted guanidine moieties in compounds **11** and **12** was also confirmed by Sakaguchi reaction producing a red stain in the aqueous solution of the substances after oxidation with sodium hypochlorite in the presence of α-naphthol.

A study of the ability of the new pyrimidine derivatives of *N*-acetylguanidine to inhibit sodium-hydrogen exchange revealed their high activities against NHE-1 *in vitro*. A comparison of the inhibitory concentrations (IC_{50}) indicates that, in terms of inhibition of NHE-1 *in vitro*, the activities of new compounds **9** and **10** are respectively 42- and 174-fold higher than that of zoniporide, and these compounds have much simpler chemical structures. In this case, the thymine derivative **10** was 4.2-fold more active than the uracil derivative **9**, indicating the significant effect of the nature of the substitution in the pyrimidine cycle on NHE-1 inhibition. The appending of the benzyl substituent to the nitrogen atom of N^3 in compounds **9** and **10** leads to a significant decrease in their inhibitory effects on NHE-1. Nevertheless, both compounds **11** and **12** outperform zoniporide in their ability to inhibit NHE-1 (Table).

Table. Inhibitory effects of new pyrimidine derivatives of *N*-acetylguanidine on NHE-1 activity *in vitro*

Compound	Inhibition of NHE-1 at a concentration of 10^{-8} M (%)	Inhibitory concentration, IC_{50} (M)
1	48.05 ± 7.09	2.70×10^{-8}
2	52.42 ± 10.76	1.15×10^{-8}
9	70.21 ± 4.33	6.50×10^{-10}
10	79.20 ± 5.71	1.55×10^{-10}
11	61.33 ± 12.28	4.25×10^{-9}
12	70.12 ± 4.61	1.56×10^{-9}

Thus, we synthesised new uracil and thymine derivatives containing fragments of *N*-acetylguanidine in position N^1 of the pyrimidine system. The new compounds significantly outperform the reference compound zoniporide in their abilities to inhibit the first isoform of NHE, which indicates the promising prospects of developing novel neuro- and cardioprotective drugs based on the substances in this series.

EXPERIMENTAL

All reagents were of the highest grade available from Panreac and Acros Organics and were used without further purification unless otherwise noted. Anhydrous DMF was purchased from Sigma-Aldrich Co. Anhydrous 1,2-dichloroethane was obtained by distillation over P_2O_5. NMR spectra were registered on a Bruker Avance 600 spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C) in DMSO-d_6 or D_2O with tetramethylsilane as an internal standard. Electrospray ionization (ESI) mass spectra were recorded with a Finnigan LCQ Advantage spectrometer (USA). Melting points were determined in glass capillaries on a Mel-Temp 3.0 (Laboratory Devices Inc., USA). Elemental analysis was performed on a Vario EL Cube device. Yields refer to spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

Platelet swelling assay. The effects of new compounds on NHE-1 activity were studied *in vitro* using rabbit platelets according to a method previously described.[7] Blood samples were collected from the marginal ear vein of rabbits into a tube containing 3.8% sodium citrate, at a ratio of 1:10. To obtain platelet-rich plasma, the blood was centrifuged at 1000 rpm for 12 min. Increases in light transmission associated with cell swelling were measured with a laser aggregometer ("BIOLA-220 LA", Russia). Briefly, platelet-rich plasma (PRP, 200 μL) was stirred at 1000 rpm in a cuvette and pre-warmed for 5 min at 37 °C. An increase in light transmission of PRP at 550 nm induced by platelet swelling was observed after application of sodium propionate solution (600 μL in mmol/L: sodium propionate 135, HEPES 20, $CaCl_2$ 1, $MgCl_2$ 1, glucose 10, pH 6.7). Application of sodium propionate (pH 6.7) produced an acidic intracellular pH at which platelet NHE-1 was activated, and the increase in Na^+ influx associated with excretion of cytosolic H^+ via NHE-1 resulted in cellular swelling as a result of water accumulation in the cytoplasm. Light transmission through PRP was increased since the density of the cellular component decreases with swelling. Tested compounds and reference drug zoniporide (10 μL) were added to a cuvette containing PRP (200 μL) 3-5 min before the addition of sodium propionate solution, then incubated at 37 °C and continuously stirred with a magnetic stirrer (1000 rpm).

Benzyl 2-[2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetate (5). A suspension of uracil (2.5 g, 22.3 mmol) and ammonium chloride (50 mg) in hexamethyldisilazane (50 mL) was refluxed to a clear solution, the excess hexamethyldisilazane was distilled *in vacuo*, and the residue was dissolved in anhydrous 1,2-dichloroethane (25 mL). Benzyl bromoacetate (5.0 g, 21.8 mmol) was then added and the reaction mixture was refluxed for 12 h, protected from atmospheric moisture. The solution was cooled, and 95% EtOH (10 mL), water (10 mL), and concentrated ammonium hydroxide (1 mL) were added. The reaction mixture was then stirred for 1 h and filtered. The precipitate was washed with water, air-dried, and crystallised from 2-propanol-DMF (2:1, 30 mL) to give 3.25 g (55%) of compound **3** as a white acicular crystalline substance with mp 196.5-198.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6), δ ppm 4.59 (2H, s, NCH_2CO_2), 5.20 (2H, s, OCH_2), 5.60 (1H, d, J = 7.8 Hz, H^5), 7.32-7.40 (5H, m, C_6H_5), 7.61 (1H, d, J =

7.8 Hz, H^6); 11.20 (1H, s, NH). ^{13}C NMR (150 MHz, DMSO-d_6), δ ppm 52.07, 69.85, 104.55, 131.20, 131.52, 131.80, 138.95, 149.04, 154.35, 166.94, 171.34.

Benzyl 2-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetate (6) was synthesised similarly from thymine, yield 65%, mp 200.5-202.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6), δ ppm 1.78 (3H, s, CH_3), 4.55 (2H, s, NCH_2CO_2), 5.20 (2H, s, OCH_2), 7.32-7.40 (5H, m, C_6H_5), 7.48 (1H, s, H^6), 11.19 (1H, s, NH). ^{13}C NMR (150 MHz, DMSO-d_6), δ ppm 15.06, 51.89, 69.81, 112.11, 131.20, 131.50, 131.79, 138.97, 144.79, 154.35, 167.57, 151.44.

Benzyl 2-[3-benzyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetate (7). A mixture of compound **5** (2.5 g, 9.6 mmol) and finely ground anhydrous potassium carbonate (2.0 g, 14.5 mmol) was stirred in anhydrous DMF (25 mL) at 80-85 °C for 30 min. Benzyl chloride (1.3 mL, 11.3 mmol) was then added and the reaction mixture was stirred at the same temperature for 1 h, cooled, and filtered. The filtrate was then evaporated *in vacuo*. The residue was washed with water (25 mL), and the resulting pale yellow oil was dried *in vacuo* at 80-85 °C, cooled, and crystallised from Et_2O (25 mL) to give 2.97 g (88%) of a white crystalline solid with mp 83.5-85 °C. ^{1}H NMR (600 MHz, DMSO-d_6), δ ppm 4.67 (2H, s, NCH_2CO_2), 5.01 (2H, s, NCH_2), 5.20 (2H, s, OCH_2), 5.79 (1H, d, J = 7.8 Hz, H^5), 7.22-7.43 (10H, m, C_6H_5), 7.72 (1H, d, J = 7.8 Hz, H^6). ^{13}C NMR (150 MHz, DMSO-d_6), δ ppm 46.87, 53.23, 69.95, 103.88, 130.48, 130.84, 131.23, 131.54, 131.62, 131.80, 138.87, 140.35, 147.89, 154.73, 165.64, 171.19.

Benzyl 2-[3-benzyl-5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetate (8) was synthesised similarly from compound **6**, yield 92%, mp 98-99.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6), δ ppm 1.85 (3H, s, CH_3), 4.63 (2H, s, NCH_2CO_2), 5.02 (2H, s, NCH_2), 5.20 (2H, s, OCH_2), 7.21-7.41 (10H, m, C_6H_5), 7.60 (1H, s, H^6). ^{13}C NMR (150 MHz, DMSO-d_6), δ ppm 15.72, 47.15, 53.04, 69.89, 111.44, 130.44, 130.91, 131.21, 131.51, 131.59, 131.78, 138.90, 140.46, 143.78, 154.61, 166.39, 171.27.

***N*-[2-[2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetyl]guanidine (9).** Granular potassium hydroxide (0.60 g, 10.18 mmol) was added to a boiling solution of compound **5** (2.00 g, 7.69 mmol) and guanidine hydrochloride (0.90 g, 9.42 mmol) in anhydrous MeOH (100 mL). The reaction mixture was boiled for 5 min, filtered, maintained overnight (10-12 h) at room temperature and evaporated *in vacuo*. The residue was extracted with hot MeOH (20 mL), filtered, and the filtrate was cooled, diluted with Et_2O (20 mL), and maintained at a temperature of -15 °C for 24 h. The precipitate was filtered off, washed with Et_2O (10 mL), air-dried, and re-crystallised from MeOH (10 mL) to give 1.36 g (84%) of a white crystalline solid with mp of 227-229 °C (decomp). ^{1}H NMR (600 MHz, D_2O), δ ppm 4.10 (2H, s, NCH_2CO_2), 4.98 (2H, s, NCH_2), 5.66 (1H, d, J = 7.8 Hz, H^5), 7.20-7.31 (5H, m, C_6H_5), 7.59 (1H, d, J = 7.8 Hz, H^6). ^{13}C NMR (150 MHz, D_2O), δ ppm 43.57, 52.19, 99.30, 127.34, 127.86, 128.57, 137.62, 146.21, 151.74, 159.06, 162.99, 171.58. Anal. Calcd for $C_7H_9N_5O_3$: C 39.81, H 4.30, N 33.16. Found: C 39.97, H 4.38, N 32.91.

***N*-[2-[5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetyl]guanidine (10)** was synthesised

similarly from compound **6**, yield 77%, mp 219-222 °C (decomp). ^{1}H NMR (600 MHz, D_2O), δ ppm 1.82 (3H, s, CH_3), 4.05 (2H, s, NCH_2CO_2), 5.01 (2H, s, NCH_2), 7.20-7.31 (5H, m, C_6H_5), 7.41 (1H, s, H^6). ^{13}C NMR (150 MHz, D_2O), δ ppm 15.75, 46.96, 55.14, 109.67, 130.27, 131.01, 131.51, 140.92, 145.28, 154.68, 162.34, 166.68, 174.22. Anal. Calcd for $C_8H_{11}N_5O_3$: C 42.67, H 4.92, N 31.10. Found: C 42.84, H 5.01, N 30.73.

***N*-[2-[3-Benzyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetyl]guanidine (11).** Granular potassium hydroxide (0.7 g, 11.9 mmol) was added to a boiling solution of compound **7** (2.75 g, 7.9 mmol) and guanidine hydrochloride (1.1 g, 11.5 mmol) in anhydrous MeOH (25 mL). The reaction mixture was boiled for 5 min, filtered, maintained overnight (10-12 h) at room temperature and evaporated *in vacuo*. The residue was extracted with hot isopropyl alcohol (25 mL), filtered, and the filtrate was cooled, diluted with Et_2O (25 mL), and maintained at a temperature of -15 °C for 24 h. The precipitate was filtered off, washed with Et_2O (10 mL), air-dried, and re-crystallised from isopropyl alcohol (20 mL) to give 1.80 g (76%) of a white crystalline solid with mp of 144.5-146 °C. ^{1}H NMR (600 MHz, DMSO-d_6), δ ppm 4.10 (2H, s, NCH_2CO_2), 4.98 (2H, s, NCH_2), 5.66 (1H, d, J = 7.8 Hz, H^5), 7.20-7.31 (5H, m, C_6H_5), 7.59 (1H, d, J = 7.8 Hz, H^6). ^{13}C NMR (150 MHz, DMSO-d_6), δ ppm 43.57, 52.19, 99.30, 127.34, 127.86, 128.57, 137.62, 146.21, 151.74, 159.06, 162.99, 171.58. MS (ESI, 4.5 kV), *m/z* 260 $[M+H-CH_2N_2]^+$. Anal. Calcd for $C_{14}H_{15}N_5O_3$: C 55.81, H 5.02, N 23.24. Found: C, 55.68; H 4.93, N 23.41.

***N*-[2-[3-Benzyl-5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetyl]guanidine (12)** was synthesised similarly from compound **8**, yield 84%, mp 163-166 °C. ^{1}H NMR (600 MHz, DMSO-d_6), δ ppm 1.82 (3H, s, CH_3), 4.05 (2H, s, NCH_2CO_2), 5.01 (2H, s, NCH_2), 7.20-7.31 (5H, m, C_6H_5), 7.41 (1H, s, H^6). ^{13}C NMR (150 MHz, DMSO-d_6), δ ppm 15.75, 46.96, 55.14, 109.67, 130.27, 131.01, 131.51, 140.92, 145.28, 154.68, 162.34, 166.68, 174.22. MS (ESI, 4.5 kV), *m/z* 274 $[M+H-CH_2N_2]^+$. Anal. Calcd for $C_{15}H_{17}N_5O_3$: C 57.13, H 5.43, N 22.21. Found: C 56.90, H, 5.34; N 22.61.

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