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Original article

Identification, synthesis and pharmacological activity of moxonidine metabolites

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Abstract

The metabolism of moxonidine, 4-chloro-*N*-(4,5-dihydro-1H-imidazol-2-yl)-6-methoxy-2-methyl-5-pyrimidinamine, LY326869, in rats, mice, dogs, and humans has been examined. At least 17 metabolites were identified or tentatively identified in the different species by HPLC, LC/MS and LC/MS/MS. The identities of seven of the major metabolites have been verified by independent synthesis. The metabolites are generally derived from oxidation and conjugation pathways. Oxidation occurred at the imidazolidine ring as well as the methyl at the 2 position of the pyrimidine ring. All seven metabolites were examined in the spontaneously hypertensive rats (3 mg kg^{-1}, i.v.) for pressure and heart rate. Only one, 2-hydroxymethyl-4-chloro-5-(imidazolidin-2-ylidenimino)-6-methoxypyrimidine, exerted a short-lasting decrease in blood pressure, albeit attenuated in magnitude compared to moxonidine.

Keywords: moxonidine; antihypertensive; metabolites

1. Introduction

Moxonidine (4-chloro-*N*-(4,5-dihydro-1H-imidazol-2-yl)-6-methoxy-2-methyl-5-pyrimidinamine, LY326869, **1**) is a new antihypertensive agent that acts within the rostroventral lateral medulla to decrease sympathetic outflow [1,2]. It is marketed throughout Europe under the trade names Cynt™ and Physiotens®. Moxonidine is generally well tolerated by hypertensive patients with fewer adverse reactions such as sedation and rebound hypertension compared to the first generation α_2 agonist, clonidine [3–5]. The basis for the greater therapeutic index of moxonidine compared to clonidine is not clear, but studies suggest that moxonidine has greater selectivity in the central nervous system for specific imidazoline receptive sites over α_2 sites as compared to clonidine [6,7]. Thus, in pre-clinical studies, the antihypertensive effects of moxonidine and related imidazolines appear to correlate with its potency at brainstem imidazoline sites rather than to α_2 receptors [8–10].

In elderly patients, the clearance of moxonidine is reduced and the area under the curve of unchanged drug is greater in comparison to younger patients suggesting the existence of an age-related decrease in metabolism [11]. However, despite widespread clinical use of moxonidine, there is little information available on its metabolic fate or on the activity its metabolites. In part, this reflects the limited analytical technologies available when the compound was originally developed. Data are available demonstrating that the structurally related imidazoline clonidine is degraded by extensive first-pass metabolism after oral administration in rabbits and that levels of intact drug in the brain correlate with blood pressure effects [12]. The pharmacological

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activity of clonidine metabolites was also assessed following peripheral or central administration in rabbits, and only one metabolite, *p*-hydroxy clonidine, induced weak decreases in blood pressure by either route. Five metabolites of clonidine, derived from hydroxylation of the phenyl ring and oxidation of the imidazoline ring followed by splitting of the ring were also identified in dog urine [13].

Moxonidine has a relatively short half-life (about 2–3 h), however; it has long duration of effect and is given only once daily clinically. The differences between the pharmcokinetics and pharmacodynamics of moxonidine were not clear. To determine whether the long duration of moxonidine is attributed to the production of active metabolite(s), we have identified and synthesised the major metabolites of moxonidine and tested their pharmacological activity.

2. Chemistry

The key synthetic approach to moxonidine and its analogs has been the $POCl_3$-mediated coupling of a 5-aminopyrimidine with a protected cyclic urea such as *N*-acetylimidazolidin-2-one, methodology used for the synthesis of moxonidine itself [14]. This tactic was used for the synthesis of metabolites derived from oxidation on the pyrimidine ring while those involving functionalisation of the imidazolidine ring were prepared by closing an oxidised intermediate to form this five-membered ring. The structures and key mass spectral fragmentations of the metabolites are shown in Table 1.

The simplest route to hydroxy compound **5** would be direct oxidation of the methyl group of moxonidine itself. Attempts to use a number of oxidizing agents, including ceric ammonium nitrate [15–17], chromic acid [18–20], peroxydisulfate [21,22], and *N*-bromosuccinimide [23–25] failed. This failure was initially attributed to the sensitivity of the guanidine fragment toward the oxidation. However, application of the same methods to the corresponding nitro-compound (4,6-dichloro-5-nitro-2-methylpyrimidine) was also unsuccessful due to its poor stability under oxidation conditions. Thus, a total synthesis of **5** was required and is summarised in Fig. 1.

The key structural feature of the target molecule is the 2-hydroxymethylpyrimidine fragment, which can be made by condensation of hydroxyacetamidine with malonates [26,27]. Hydroxyacetamidine hydrochloride (**9**), was prepared by known procedures from paraformaldehyde and hydrogen cyanide and subsequent reaction with ethanolic hydrogen chloride, and ammonolysis of the resulting imino-ether hydrochloride [26,27]. Reaction of **9** with diethyl malonate in the presence of sodium ethoxide in refluxing ethanol smoothly gave 4,6-dihydroxy-2-hydroxymethylpyrimidine (**10**). Nitration of **10** with a mixture of nitric and acetic acids led to 4,6-dihydroxy-2-hydroxymethyl-5-nitropyrimidine (**11**). Minimal volume of the nitration solution was used in order to achieve a reasonable yield of the nitro product. Initial attempts to transform **11** into a dichloropyrimidine using phosphorus oxychloride, resulted in formation of 2-chloromethyl-4,6-dichloro-5-nitropyrimidine as a main product. Protection of the primary hydroxyl as an acetate under standard conditions using acetic anhydride in the presence of base in an aprotic solvent was not possible because of the very low solubility of triol **11** in most organic solvents. Its reaction with acetic anhydride in the presence of trimethylsilyl chloride [28,29] also failed, leaving only unreacted starting material. Acetylation was successfully accomplished when **11** was heated in a mixture of acetic acid and acetyl chloride or acetic acid and acetic anhydride, following by simple evaporation of the reaction mixture under vacuum. The resulting 2-acetoxymethyl-4,6-dihydroxy-5-nitropyrimidine (**12**), was treated with phosphorus oxychloride in the presence of *N*,*N*-diethylaniline to form 2-acetoxymethyl-4,6-dichloro-5-nitropyrimidine (**13**). Reduction of the nitro group by hydrogenation over Raney nickel smoothly gave 2-acetoxymethyl-4,6-dichloro-5-aminopyrimidine (**14**), without hydrogenolysis of the aromatic chloride. Coupling of amine **14** with *N*-acetylimidazolidin-2-one [30] in the presence of phosphorus oxychloride afforded **15**. Finally, simultaneous hydrolysis of the acetyl groups and nucleophilic substitution using sodium methoxide in methanol gave the desired product **5**.

For the synthesis of acid **4**, outlined in Fig. 2, the acetate **13** was reduced with diisobutylaluminum hydride under mild conditions to give 2-hydroxymethyl-4,6-dichloro-5-nitropyrimidine (**16**) [31]. Jones oxidation of alcohol **16** followed by the methylation of the resulting 2-carboxy-4,6-dichloro-5-nitropyrimidine yielded 2-methoxycarbonyl-4,6-dichloro-5-nitropyrimidine (**17**). Reduction of its nitro group afforded 2-methoxycarbonyl-4,6-dichloro-5-aminopyrimidine (**18**), which was converted into 2-methoxycarbonyl-4-chloro-5-amino-6-methoxypyrimidine (**19**), by selective methanolysis. A low yield of **20** in the next step of the coupling with *N*-acetylimidazolidin-2-one may be explained by the low nucleophilicity of the heteroaromatic amino group due to the presence of the electron withdrawing methoxycarbonyl group. Finally, simultaneous hydrolysis of the ester and amide fragments of **20** with lithium hydroxide quantitatively gave the target compound **4**.

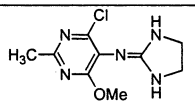
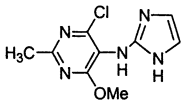
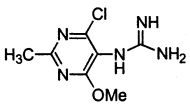
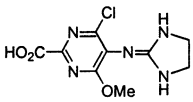
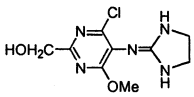
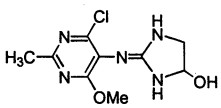
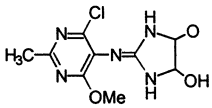
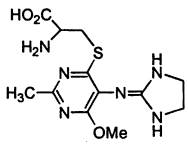
The synthesis of metabolites **2**, **3**, **6**, and **7** is summarised in Fig. 3. Although 5-amino-4-chloro-6-methoxy-2-methylpyrimidine (**22**), was previously known [32,33], its present synthesis represents a significant improvement in its yield from the known

dichloropyrimidine (**21**) [34]. Reaction of **22** with an excess of cyanamide in ethanol provided the metabolite **3**.

The synthesis of **6** and **2** was more challenging, however. Attempted alkylation of **3** with bromoacetaldehyde diethyacetal was unsuccessful. Likewise, the possible condensation of amine **22** with the known cyanamide derived from aminoacetaldehyde diethyl acetal [35] failed to produce the desired coupling. Similarly disappointing was the failure of the amine **22** to react with cyanogen bromide to give the cyanamide, **25** [36].

The successful synthesis of hydroxymoxonidine **6**, is shown in Fig. 3. Condensation of **22** with benzoyl thiocyanate gave the acyl urea **23** in high yield as long as the temperature was kept at ambient or below. The thiourea **24**, produced by methanolysis of **23**, was unstable even at ambient temperature. The 100 °C temperature recommended for the lead-based

Table 1
Moxonidine and its metabolites

Compound	Structure	Observed in species	$[M+H]^+$	Characteristic Product Ions (m/z)
1			242	44, 56, 137, 149, 199, 206
2		rat mouse dog human	240	56, 82, 135, 147, 184, 189, 204
3		rat dog	216	56, 136, 138, 143, 159, 184, 199
4		rat	272	150, 151, 165, 177, 192
5		rat mouse dog human	258	44, 137, 151, 192, 215, 222, 228
6		human rat mouse dog	258	60, 136, 174, 184, 199, 204, 240
7		human	274	56, 136, 172, 184, 199, 228, 256
8		rat mouse human	327	167, 206, 223, 240, 281

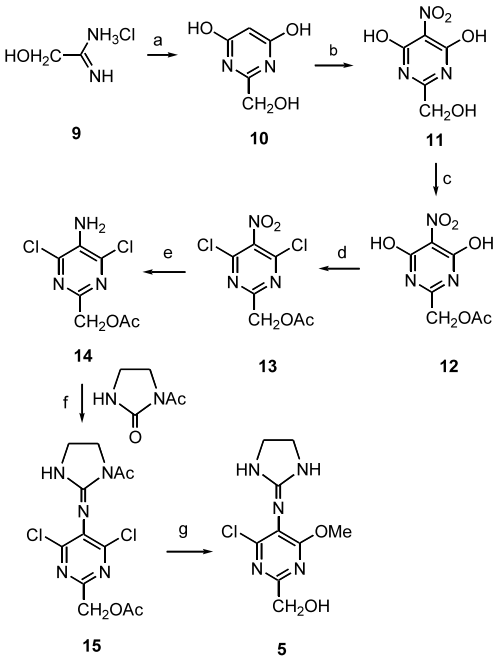


Fig. 1. Synthetic scheme for metabolite **5**. Reagents: (a) (i) $CH_2(CO_2Et)_2$, EtONa, EtOH, (ii) HCl; (b) HNO_3, AcOH; (c) AcOH, Ac_2O; (d) $POCl_3$, $PhNEt_2$; (e) H_2, Ni–Ra; (f) $POCl_3$; (g) MeONa, MeOH.

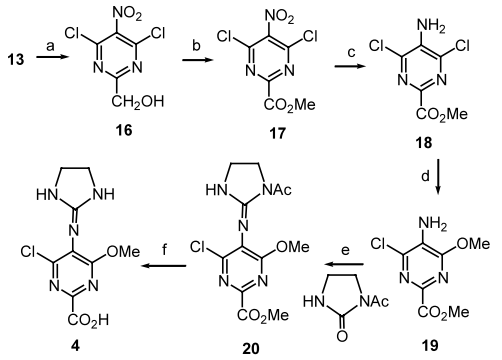


Fig. 2. Synthesis of metabolite **4**. Reagents: (a) DIBAL-H, CH_2Cl_2; (b) (i) H_2CrO_4, (ii) CH_2N_2; (c) H_2, Ra–Ni; (d) MeONa, MeOH; (e) $POCl_3$; (f) LiOH, THF, MeOH.

desulfurisation reaction [37], of thioureas to cyanamides caused extensive degradation. The cyanamide **25** was best prepared at 60 °C and, although it could be isolated and characterised somewhat, it proved beneficial to condense it directly with commercially-available aminoacetaldehyde diethylacetal to produce the acetal **26**. Careful hydrolysis of this acetal and isolation of the resultant hydroxymoxonidine **6**, under acidic conditions resulted in a filterable solid which was quite pure. Czarnik and Leonard developed these conditions for a similar compound [38]. When subjected to neutral or alkaline conditions, the hydroxymoxonidine readily dehydrated to the imidazole metabolite **2**.

The synthesis of the dihydroxylated compound **7**, was effected by the addition of glyoxal to the guanidine **3**. This compound was a mixture of *cis* and *trans* diols and the ratio was somewhat variable and changed as a function of pH, suggesting an equilibrium with open-chain forms.

Due to the ready displacement of the chlorine of moxonidine, the synthesis of metabolite **8** was accomplished by simple heating of a mixture of moxonidine, L-cysteine, and sodium bicarbonate in water. It was purified by chromatography on a polystyrene resin.

3. Metabolism studies

The metabolism of moxonidine was first characterised in Fisher 344 rats after a single oral administration

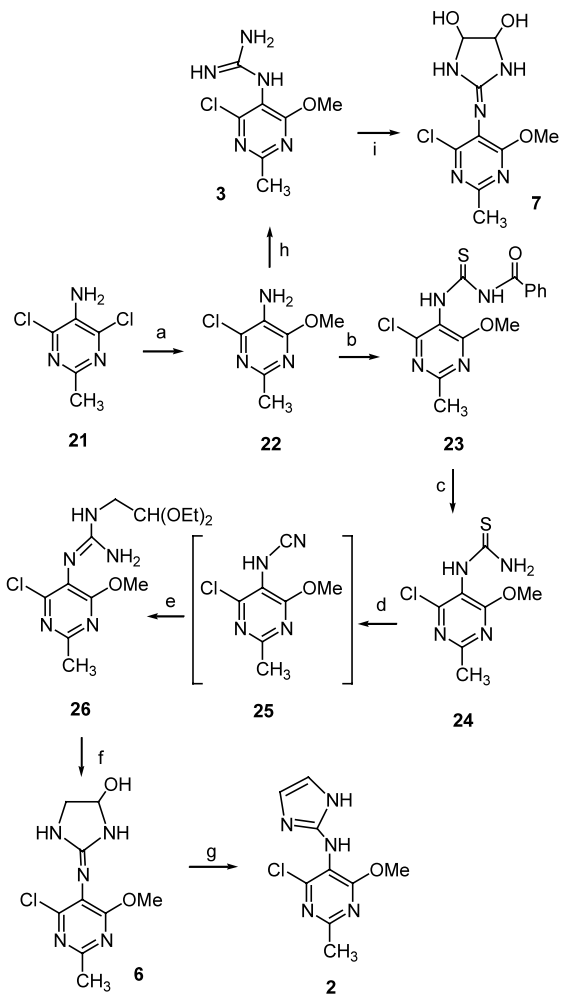


Fig. 3. Synthesis of metabolites **2**, **3**, **6**, and **7**. Reagents: (a) MeONa, MeOH; (b) NH_4SCN, PhCOCl, C_3H_6O; (c) MeONa, MeOH; (d) $Pb(OAc)_2$, H_2O; (e) $H_2NCH_2CH(OEt)_2$, KH_2PO_4; (f) HCl; (g) KOH; (h) H_2NCN; (i) HCOCHO.

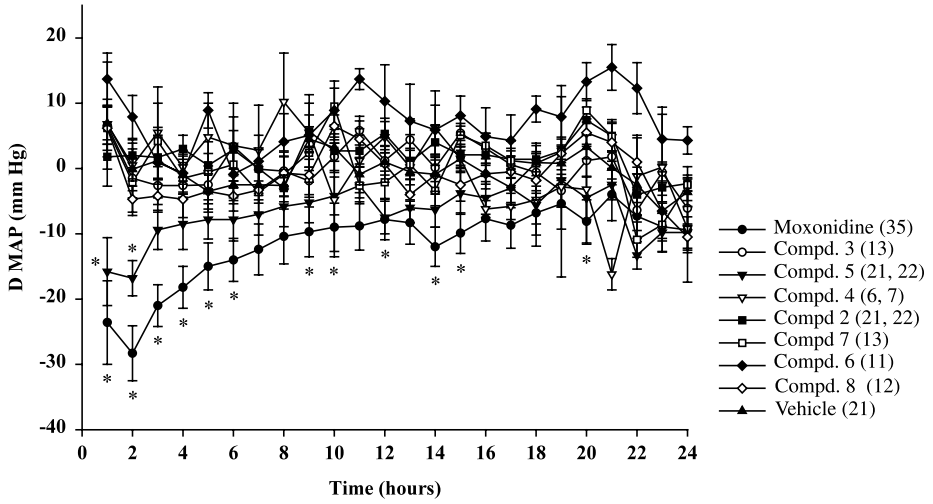


Fig. 4. The effect of moxonidine and its metabolites (3 mg kg^{-1}, i.v.) on the change in mean arterial pressure (MAP) in conscious SHR monitored by telemetry. The numbers in parenthesis indicate replicate animals. Asterisks indicate significantly different from the vehicle group by Dunnett's test.

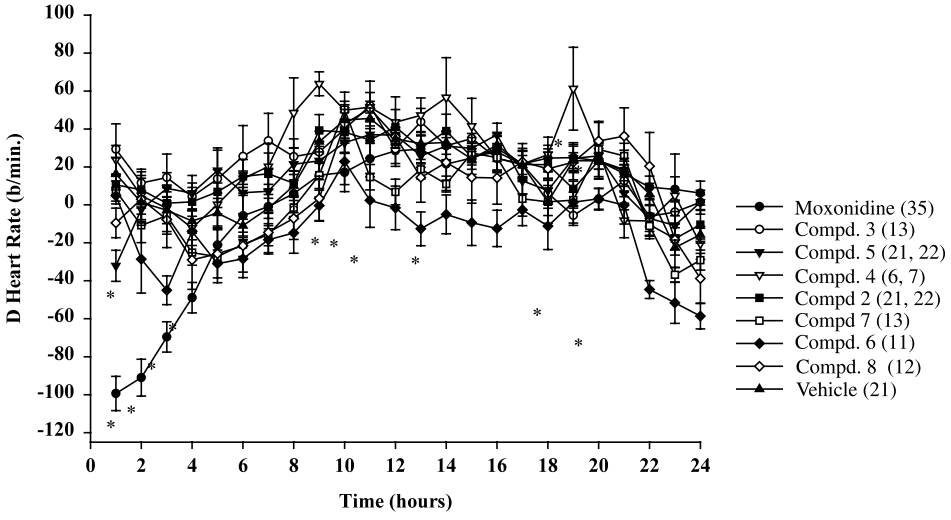


Fig. 5. The effect of moxonidine and its metabolites (3 mg kg^{-1}, i.v.) on the heart rate in conscious SHR monitored by telemetry. The numbers in parenthesis indicate replicate animals. Asterisks indicate significantly different from the vehicle group by Dunnett's test.

of 5 mg kg^{-1} of ^{14}C-moxonidine. Metabolites were identified in rat urine, plasma and bile samples by HPLC, LC/MS and LC/MS/MS [39]. Additional metabolism studies were conducted in CD-1 mice, beagle dogs and healthy volunteers at a single dose of 10, 0.2 mg kg^{-1} and 1 mg of ^{14}C-moxonidine, respectively. At least 17 metabolites have been identified or tentatively identified in biological fluids (i.e. urine, bile and plasma) in different species. The metabolites are generally derived from oxidation and conjugation pathways. Oxidation occurred at the imidazolidine ring as well as the methyl at the two position of the pyrimidine ring. The most abundant and frequently observed moxonidine metabolites were synthesised for determination of pharmacological activities. Their structures and mass spectral information are given in Table 1.

4. Results and discussion

As shown in Fig. 4, of all the compounds studied only moxonidine and its hydroxymethyl metabolite **5**, lowered mean blood pressure compared to vehicle in the spontaneously hypertensive rat (SHR).

The effects of moxonidine on mean pressure were apparent within the first hour and lasted for ca. 12 h. Only compound **5** showed a decrease in pressure that lasted 2 h as compared to vehicle. Accompanying the effect of moxonidine on blood pressure were pronounced falls in heart rate that were most prominent 4 h after dosing as shown in Fig. 5.

Consistent with the shorter effect of **5** on blood pressure, there was also a significant fall compared to vehicle in heart rate apparent during the first hour after

dosing. Compound **6** also demonstrated a significant decrease in heart rate 11–18 h after dosing that was not accompanied by changes in mean blood pressure.

5. Conclusions

Seven metabolites of moxonidine were definitively identified and prepared by independent synthesis. The hydroxymethyl metabolite of moxonidine **5**, has been identified in urine of mice, rats, dogs, and humans and has been shown to possess antihypertensive and bradycardic activity in conscious unrestrained hypertensive rats when administered intravenously at 3 mg kg^{-1}. Compound **5** was less active and shorter acting than moxonidine at this dose; its half-life remains to be determined, however. Although, this metabolite is less active and shorter acting than moxonidine, it may contribute somewhat to the long antihypertensive effect and the overall clinical profile of moxonidine. No other identified metabolites to date show similar antihypertensive activity in this model.

6. Experimental

6.1. Chemistry

The NMR spectra were obtained on a Bruker AC300 or a General Electric QE-300 at 300 (^{1}H) and 75 (^{13}C) MHz. Chemical shifts are reported in parts per million (ppm) downfield from TMS. The Physical Chemistry Research Department of Lilly Research Laboratories provided Microanalytical, IR, UV, and MS data. Flash chromatography was performed on silica gel 60 (230–400 mesh). TLC was done on silica gel 60 F_{254} precoated plates.

6.1.1. 4,6-Dihydroxy-2-hydroxymethylpyrimidine (**10**)

To a solution of C_2H_5ONa prepared from Na (1.38 g, 60.0 mmol) and EtOH (29 mL), was added hydroxyacetamidine hydrochloride (**9**) [26] (2.21 g, 20.0 mmol) and after 5 min ethyl malonate (3.2 g, 20.0 mmol). The reaction mixture was refluxed for 3 h, cooled to room temperature (r.t.), diluted with H_2O (10 mL) and acidified with concd. HCl (4 mL). The precipitate was filtered off, washed with H_2O (10 mL), EtOH (5 mL) and Et_2O (5 mL), and dried under vacuum to give 2.24 g (79%) of **10** as a light-brown solid. IR (KBr): 527, 1106, 1324, 1571, 1639, 1682, 2910, 3029, 3084 cm^{-1}; UV (EtOH): λ_{max} (ε) 256 (4235), 239 nm (3398); ^{1}H-NMR (DMSO-d_6) δ 4.28 (s, 2H), 5.14 (s, 1H); MS (FD): 142 [M^+, 100%]. Anal. Calc. for $C_5H_6N_2O_3$: C, 42.26; H, 4.26; N, 19.71. Found: C, 41.85; H, 3.96; N, 19.02%.

6.1.2. 4,6-Dihydroxy-2-hydroxymethyl-5-nitropyrimidine (**11**)

To a stirred mixture of HNO_3 (fuming, 4.2 mL) and AcOH (glacial, 2.1 mL) was added **10** (2.3 g, 16.2 mmol) over the period of 40 min at 10–15 °C. The reaction mixture was stirred for 5.5 h at r.t. then cooled to 5 °C and diluted with cold H_2O (3 mL). The precipitate was collected by filtration, washed with EtOH (5 mL) and Et_2O (5 mL), and dried under vacuum to give 2.44 g (80.5%) of **11** as a colorless solid. IR (KBr): 539, 789, 1105, 1297, 1358, 1630, 1674, 2828, 2920, 3402, 3460 cm^{-1}; UV (EtOH): λ_{max} (ε) 322 (3354), 213 nm (17626); ^{1}H-NMR (DMSO-d_6) δ 4.42 (s, 2H); MS (FD): 187 [M^+, 100%]. Anal. Calc. for $C_5H_5N_3O_5$: C, 32.10; H, 2.69; N, 22.46. Found: C, 31.44; H, 2.91; N, 21.83%.

6.1.3. 2-Acetoxymethyl-4,6-dihydroxy-5-nitropyrimidine (**12**)

A mixture of **11** (2.35 g, 12.56 mmol), AcOH (glacial, 15 mL), and Ac_2O (15 mL) was heated at 105 C for 2 h, evaporated under vacuum, and re-evaporated with $C_6H_5CH_3$ (three times). The residue was dried under vacuum to give 2.843 g (99%) of **12** as a yellow solid. IR (KBr): 545, 787, 1212, 1326, 1354, 1367, 1493, 1650, 1673, 1758, 2939, 3082, 3141 cm^{-1}; UV (EtOH): λ_{max} (ε) 327 nm (3365); ^{1}H-NMR (DMSO-d_6): δ 2.14 (s, 3H), 4.98 (s, 2H); MS (FD): 229 [M^+, 100%]. HRMS Calc. for $C_7H_7N_3O_6$: 229.0335. Found: 229.0339.

6.1.4. 2-Acetoxymethyl-4,6-dichloro-5-nitropyrimidine (**13**)

To a mixture of **12** (2.75 g, 12.0 mmol) and phosphorus oxychloride (13 mL) was added *N*,*N*-diethylaniline (2.5 mL, 15.7 mmol) dropwise. The reaction mixture was refluxed for 2 h and evaporated under vacuum. The residue was diluted with Et_2O (15 mL) and poured onto ice. After stirring for 3 min, the organic layer was separated, washed with saturated aq. solutions of $NaHCO_3$ and NaCl, dried over Na_2SO_4 and evaporated under vacuum. Flash chromatography of the residue on silica gel (20% ether in C_6H_{14}) gave 2.446 g (77%) of **13** as a white solid. R_f 0.60 (C_6H_{14}–EtOAc, 3:2); IR (KBr): 836, 853, 1218, 1550, 1755 cm^{-1}; UV (EtOH): λ_{max} (ε) 259 nm (3290); ^{1}H-NMR ($CDCl_3$): δ 2.28 (s, 3H), 5.33 (s, 2H). Anal. Calc. for $C_7H_5Cl_2N_3O_4$: C, 31.60; H, 1.89; N, 15.79. Found: C, 31.84; H, 1.85; N, 15.69%.

6.1.5. 2-Acetoxymethyl-4,6-dichloro-5-aminopyrimidine (**14**)

A mixture of **13** (255 mg, 0.96 mmol) and Raney nickel (30 mg) in EtOH (25 mL) was hydrogenated in a Parr apparatus (50 psi of hydrogen) for 5.5 h. The resulting suspension was filtered through celite and evaporated under vacuum. Flash chromatography of the residue on silica gel (40% EtOAc in C_6H_{14}) gave 202

mg (89%) of **14** as a colorless solid. R_f 0.41 (C_6H_{14}–EtOAc, 3:2); IR (KBr): 793, 907, 1249, 1366, 1459, 1513, 1621, 1731, 3332, 3428 cm^{-1}; UV (EtOH): λ_{max} (ε) 314 (5676), 253 nm (11 629); ^{1}H-NMR ($CDCl_3$): δ 2.21 (s, 3H), 4.55 (s, 2H) 5.16 (s, 2H); MS (FD): 235 [M^+, 100%]. HRMS Calc. for $C_7H_7Cl_2N_3O_2$: 234.9917. Found: 234.9920.

6.1.6. 2-Acetoxymethyl-4,6-dichloro-5-(1-acetyl-imidazolidin-2-ylidenimino)-pyrimidine (**15**)

To a solution of **14** (191 mg, 0.81 mmol) in phosphorus oxychloride (1 mL) was added *N*-acetylimidazolidin-2-one [15] (114 mg, 0.89 mmol) in one portion. The reaction mixture was heated at 105–110 °C (bath) for 3 h and evaporated under vacuum. The residue was diluted with ice H_2O (2 mL), made alkaline with aq. NaOH (5*N*, 2 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The extract was washed with saturated aq. solutions of $NaHCO_3$ and NaCl, dried over Na_2SO_4 and evaporated under vacuum. Flash chromatography of the residue on silica gel (from 50 to 60% EtOAc in C_6H_{14}) gave 166 mg (59%) of **15** as a colorless solid. R_f 0.21 (C_6H_{14}–EtOAc, 1:1); IR (KBr): 815, 1033, 1074, 1219, 1355, 1378, 1414, 1497, 1657, 1675, 1752, 3254, cm^{-1}; UV (EtOH): λ_{max} (ε) 245 nm (12 118); ^{1}H-NMR ($CDCl_3$): δ 2.23 (s, 3H), 2.70 (s, 3H), 3.58 (t, $J = 7.98$ Hz, 2H), 4.11 (t, $J = 7.98$ Hz, 2H), 4.74 (s, 1H), 5.19 (s, 2H); HRMS (FAB) Calc. for $C_{12}H_{13}Cl_2N_5O_3$: 346.0474. Found: 346.0481.

6.1.7. 2-Hydroxymethyl-4-chloro-5-(imidazolidin-2-ylidenimino)-6-methoxypyrimidine (**5**)

A solution of **15** (87 mg, 0.25 mmol) and CH_3ONa (14 mg, 0.26 mmol) in MeOH (1.2 mL) was refluxed for 3 h. The reaction mixture was evaporated under vacuum to one half of the initial volume and cooled to 5 °C. The precipitate was collected by filtration, washed with H_2O (1 mL) and dried under vacuum to give 32 mg (50%) of **5** as a colorless solid. IR (KBr): 720, 1042, 1091, 1283, 1307, 1376, 1440, 1471, 1533, 1659, 3030, 3225, 3340 cm^{-1}; UV (EtOH): λ_{max} (ε) 254 nm (8577); ^{1}H-NMR (DMSO-d_6): δ 3.34 (s, 4H), 3.87 (s, 3H), 4.39 (d, $J = 6.18$ Hz, 2H), 5.19 (t, $J = 6.18$ Hz, 1H), 6.26 (s, 2H); HRMS (FAB) Calc. for $C_9H_{12}ClN_5O_2$: 258.0758. Found: 258.0759.

6.1.8. 2-Hydroxymethyl-4,6-dichloro-5-nitropyrimidine (**16**)

To a solution of **13** (1.42 g, 5.34 mmol) in CH_2Cl_2 (40 mL) at −78 °C was added di-isobutylaluminum hydride (1*M* in $C_6H_5CH_3$, 15 mL, 15.0 mmol) dropwise. The reaction mixture was stirred at −78 °C for 15 min, treated with isopropyl alcohol (1.5 mL), diluted with CH_2Cl_2 (40 mL), washed with saturated aq. solutions of potassium sodium tartrate and NaCl, dried over Na_2SO_4 and evaporated under vacuum. Flash chromatography of the residue on silica gel (from 55 to 60% ethyl ether in C_6H_{14}) gave 634 mg (53%) of **16** as a white solid. R_f 0.31 (C_6H_{14}–ethyl ether, 1:1); IR (KBr): 833, 841, 1056, 1107, 1264, 1309, 1345, 1437, 1519, 1554, 3335, cm^{-1}; UV (EtOH): λ_{max} (ε) 260 nm (2713); ^{1}H-NMR ($CDCl_3$): δ 3.00 (s, 1H), 5.00 (s, 2H). Anal. Calc. for $C_5H_3Cl_2N_3O_3$: C, 26.81; H, 1.35; N, 18.76. Found: C, 27.11; H, 1.35; N, 18.72%.

6.1.9. 2-Methoxycarbonyl-4,6-dichloro-5-nitropyrimidine (**17**)

To a solution of **16** (625 mg, 2.79 mmol) in C_3H_6O (15 mL) at 0–5 °C was added Jones reagent (8*N*, 2.9 mL) dropwise. The reaction mixture was allowed to reach r.t. over the period of 2 h and treated with isopropyl alcohol (2 mL). The resulting suspension was stirred for 15 min and evaporated under vacuum. The residue was diluted with H_2O (5 mL) and extracted with Et_2O (60 mL). The extract was washed with saturated aq. NaCl, and the combined water layer was re-extracted with Et_2O. The combined organic extract was dried over $MgSO_4$ and evaporated under vacuum to give 2-carboxy-4,6-dichloro-5-nitropyrimidine as a white solid, which was dissolved in Et_2O (35 mL), treated with an excess of ethereal diazomethane, and evaporated under vacuum. Flash chromatography of the residue on silica gel (45% ethyl ether in C_6H_{14}) gave 449 mg (64%) of **17** as a white solid. R_f 0.61 (C_6H_{14}–ethyl ether, 1:1); IR (KBr): 816, 845, 836, 974, 1171, 1211, 1283, 1300, 1330, 1345, 1430, 1521, 1536, 1558, 1746, 3420 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 4.14 (s, 3H). Anal. Calc. for $C_6H_3Cl_2N_3O_4$: C, 28.60; H, 1.20; N, 16.67. Found: C, 28.87; H, 1.31; N, 16.56%.

6.1.10. 2-Methoxycarbonyl-4,6-dichloro-5-aminopyrimidine (**18**)

A mixture of **17** (449 mg, 1.78 mmol) and Raney nickel (80 mg) in EtOH (40 mL) was hydrogenated in a Parr apparatus (50 psi of hydrogen) for 3.5 h. The resulting suspension was filtered through silica gel and evaporated under vacuum. Flash chromatography of the residue on silica gel (from 50 to 60% EtOAc in C_6H_{14}) gave 235 mg (59%) of **18** as a light yellow solid. R_f 0.42 (C_6H_{14}–EtOAc, 1:1); IR (KBr): 742, 983, 1181, 1222, 1341, 1365, 1442, 1511, 1606, 1717, 3301, 3461 cm^{-1}; UV (EtOH): λ_{max} (ε) 301 nm (12932); ^{1}H-NMR ($CDCl_3$): δ 4.06 (s, 3H), 5.03 (s, 2H). Anal. Calc. for $C_6H_5Cl_2N_3O_2$: C, 32.46; H, 2.27; N, 18.93. Found: C, 32.55; H, 2.47; N, 18.90%.

6.1.11. 2-Methoxycarbonyl-4-chloro-5-amino-6-methoxypyrimidine (**19**)

A solution of **18** (390 mg, 1.76 mmol) in MeOH (25 mL) containing CH_3ONa (90 mg, 1.67 mmol), was kept at r.t. for 30 min, diluted with EtOAc, washed with brine, dried over Na_2SO_4, and evaporated under

vacuum to give 320 mg (82%) of **19** as an amorphous solid. ^{1}H-NMR ($CDCl_3$): δ 6.4 (br s, 2H), 4.1 (s, 3H), 3.9 (s, 3H). HRMS Calc. for $C_7H_8ClN_3O_3$: 217.0255. Found: 217.0261.

*6.1.12. 2-Methoxycarbonyl-4-chloro-5-(1-acetyl-imidazolidin-2-ylidenimino)-6-methoxypyrimidine (**20**)*

A mixture of **19** (460 mg, 2.11 mmol), *N*-acetyl-2-imidazolidone (285 mg, 2.22 mmol), and phosphorous oxychloride (15 mL) was heated in an oil bath of 110 °C for 2.25 h, cooled, and evaporated under vacuum. The residue was stirred with CH_2Cl_2–ice water, made strongly basic with NaOH, and extracted well with 3:1 CH_2Cl_2–isopropyl alcohol. The organic solution was washed with brine, dried over Na_2SO_4, and evaporated under vacuum. The residue was chromatographed on silica gel, eluting with a gradient from 50 to 100% C_6H_{14}–EtOAc to give 125 mg (18%) of **20** as an amorphous solid. ^{1}H-NMR ($CDCl_3$) δ 7.4 (br s, 1H), 4.1 (t, 2H), 4.0 (s, 3H), 3.9 (s, 3H), 3.5 (t, 2H), 2.6 (s, 3H); MS (FD): 327.2 [M^+, ^{35}Cl, 100%], 329.2 [M^+, ^{37}Cl, 38%]. HRMS Calc. for $C_{12}H_{14}ClN_5O_4$: 327.0736. Found: 327.0732.

*6.1.13. 2-Carboxy-4-chloro-5-(imidazolidin-2-ylidenimino)-6-methoxypyrimidine (**4**)*

To a solution of **20** (120 mg, 0.366 mmol) in THF (20 mL) and MeOH (5 mL) was added LiOH (15 mg, 0.626 mmol). After stirring for 16.75 h the suspension was treated with aq. HCl (1*N*, 0.63 mL, 0.63 mmol) and evaporated under vacuum. The residue was triturated with THF and filtered to give 110 mg (100%) of **4** as a hygroscopic solid. MS (FD): 271.2 [M^+, ^{35}Cl, 100%], 273.2 [M^+, ^{37}Cl, 37%]. HRMS Calc. for $C_9H_{10}ClN_5O_3$: 271.0474. Found: 271.0475.

*6.1.14. 5-Amino-4-chloro-6-methoxy-2-methyl-pyrimidine (**22**)*

Sodium metal (5.43 g, 0.236 mol) was added in portions to 265 mL of anhydrous MeOH in a 500 mL round-bottomed flask fitted with a mechanical agitator, a nitrogen purge, and a thermocouple. After a homogeneous solution was obtained, it was cooled to 20 °C and 40.0 g (0.225 mol) of 5-amino-4,6-dichloro-2-methylpyrimidine (**21**), [34] was added along with a small MeOH rinse. The mixture was stirred overnight at ambient temperature, filtered, and concentrated in vacuo to a residue. Ethyl acetate (200 mL) and water (65 mL) were added and the phases were separated. The organic layer was washed with 30 mL of dilute aq. NaCl and the combined aq. layers were extracted with 30 mL of EtOAc. The combined organic layers were distilled at atmosphere pressure until the still temperature reached 93 °C. Hexane, 133 mL, was added and the mixture was distilled again until the internal temperature was 80 °C. Another 335 mL of C_6H_{14} was added and the mixture was cooled to about 30 °C whereupon it crystallised. After stirring for 1 h at ambient temperature, the product was collected by filtration and was rinsed with about 35 mL of C_6H_{14}. The filtrate was distilled at atmospheric pressure until about 275 mL had been removed. When cooled to about 40 °C, an oil separated but formed crystals upon continued stirring. After a stir of one-half hour at ambient temperature, the slurry was stirred for 1 h in an ice bath. The second crop was isolated by filtration and was rinsed with cold C_6H_{14}. Both crops were dried in vacuo overnight at 30 °C. The yield was 26.66 g in the first crop and 10.42 g in the second crop for a combined yield of 95%. The purity of the first crop by isocratic HPLC was 99% (m.p. 58–59 °C) [40,41] and the second about 95% (m.p. 56–58 °C); they were combined and used in the next step. ^{1}H-NMR: 5.1 (s, 2H), 3.9 (s, 3H), 2.3 (s, 3H). ^{13}C-NMR: 157.55, 153.13, 138.27, 124.52, 54.18, 24.12. MS EI+: 173 (50, M+1), 175 (18), 144 (22), 120 (15), 89 (30), 42 (100); CI+: 174 (100), 176 (30), 202 (12), 138 (22); HRMS found 174.0437, theory for $C_6H_9N_3OCl$ (M+H) is 174.0434.

*6.1.15. N-[4-Chloro-6-methoxy-2-methylpyrimid-5-yl]-N′-benzoylthiourea (**23**)*

Ammonium thiocyanate (16.3 g, 0.21 mol) was added to a 1 L round-bottomed flask fitted with a mechanical agitator, a nitrogen purge, and a thermocouple. Acetone (222 mL) was added and the mixture stirred until homogeneous. Benzoyl chloride (24.4 mL, 0.21 mol) was added and the mixture was stirred for 2 h at ambient temperature. Aminopyrimidine **22** (37.0 g, 0.21 mol) was added along with 148 mL of C_3H_6O. After stirring for 2 h at ambient temperature, 370 mL of water was added. The mixture was stirred for 1.5 h at ambient temperature, filtered, and the product was washed with 150 mL of a 1:1 mixture of C_3H_6O and water. The product was dried in vacuo at 50 °C overnight to give 67.7 g (94%) of an off-white solid. ^{1}H-NMR: 11.9 (s, 1H), 11.8 (s, 1H), 7.9 (d, 2H), 7.6 (m, 1H), 7.5 (t, 2H), 3.9 (s, 3H), 2.6 (s, 3H). ^{13}C-NMR: 181.28, 168.00, 165.49, 165.38, 156.82, 133.15, 133.67, 128.63, 128.35, 116.36, 54.75, 25.08. MS, CI+: 301 (100), 337 (40), 339 (18); ES+: 301 (100), 337 (90), 339 (35); due to its instability, HRMS analysis showed only the ion from $C_7H_9N_4OS$ corresponding to the loss of benzamide and HCl, found 197.0492, theory is 197.0497.

*6.1.16. N-(4-Chloro-6-methoxy-2-methylpyrimid-5-yl)-thiourea (**24**)*

Sodium metal (4.09 g, 0.18 mol) was added in portions to 600 mL of anhydrous MeOH in a 1 L round-bottomed flask fitted with a mechanical agitator, a nitrogen purge, and a thermocouple. After a homogeneous solution was obtained, it was cooled to 17 °C

and 60.0 g (0.18 mol) of compound **23** was added, along with a rinse of 20 mL of MeOH. The mixture was stirred for 5 h at this temperature, then at ambient temperature for one-half hour. It was cooled in an ice bath and 5% aq. HCl was added until a pH of 7 was obtained. The resultant slurry was stirred for an hour in the ice bath then an hour in an ice–C_3H_6O bath. The product was collected by filtration, rinsed with 60 mL of cold 4:1 MeOH–water, and dried in vacuo overnight at ambient temperature to give 38.5 g (93%) of a white solid which was 96.4% pure by gradient HPLC. The sample was stored in the freezer to minimise decomposition. ^{1}H-NMR: 9.02 (br s, 1H), 7.8 and 7.2 (very br singlets, 1H ea.), 3.93 (s, 3H), 2.47 (s, 3H). ^{13}C-NMR: 182 (br), 166.46, 165.25 (br), 157.95, 128.5 (3 or 4 lines). MS ES+: 233 (9, M+1), 235 (3), 197 (100); EI+: 196 (30), 167 (25), 125 (20), 60 (75), 29 (100); due to its instability, HRMS analysis showed only the ion from $C_7H_9N_4OS$ corresponding to the loss of HCl, found 197.0497, theory is 197.0497.

*6.1.17. N-(2,2-Diethoxyethyl)-N′-(4-chloro-6-methoxy-2-methylpyrimid-5-yl)-guanidine (**26**)*

Potassium hydroxide (10.6 g, 85% assay, 0.16 mol) was added to a 250 mL round-bottomed flask fitted with a mechanical agitator and a thermocouple. Water (75 mL) was added and the solution was heated to 55 °C. Lead(II) acetate trihydrate (9.0 g, 0.024 mol) was added followed by thiourea **24** (5.0 g, 0.022 mol) and a rinse of 10 mL of water. The slurry was stirred rapidly at 60 °C for 45 min and cooled to ambient temperature. Glacial AcOH was added until the pH of the black slurry was 7. About 3 g of filter aid was added. The mixture was filtered on a pad of filter aid and the cake was rinsed with about 10 mL of water [42]. The aq. solution was washed twice with 100 mL portions of EtOAc and evaporated in vacuo (bath temperature of 50 °C) to a weight of 46 g. The purity of this solution of cyanamide **25** was 98% by gradient HPLC. The aq. solution of the cyanamide and 40 mL of MeCN were combined in a clean flask and aminoacetaldehyde diethyl acetal (6.2 mL, 0.043 mol) was added followed by sufficient solid KH_2PO_4 to achieve a pH of 8.5. The mixture was heated at 60 °C for 3 h, cooled to ambient temperature, and treated with solid KOH until the pH was 10. Filter aid was added and the mixture was filtered to remove polymeric materials. The phases were separated and the upper layer was evaporated to a weight of 15 g in vacuo. Water (50 mL) and EtOAc (100 mL) were added and the mixture was agitated, then separated. The organic layer was concentrated in vacuo to a weight of about 14 g. Upon standing overnight in the freezer, the product crystallised. This slurry was combined with similar slurries from one and one-half other such reactions (total of 12.5 g of **24** starting material). Hexane (150 mL) was added to the mixture and the resultant slurry was stirred at ambient temperature for 15 min. The crude material was isolated by filtration, rinsed with C_6H_{14}, and dried in vacuo at 30 °C for 30 min. Its purity was 85% by the gradient HPLC method. The acetal was purified by adding the partially dried solid to a clean flask with 80 mL C_6H_{14} and heating to 60 °C. Ethyl acetate (40 mL) was added at this temperature and the heating was removed. After stirring for one-half hour at ambient temperature, the product was isolated by filtration, was rinsed with 2:1 C_6H_{14}–EtOAc, and was dried in vacuo at 30 °C. The yield was 6.0 g (34%) of an off-white solid, m.p. 81–91 °C. The purity by gradient HPLC was 96.8%. ^{1}H-NMR: 5.58 (t, $J=7$, 1H), 5.2 (s, 2H), 4.63 (t, $J=7$, 1H), 3.82 (s, 3H), 3.6 (m, 2H), 3.5 (m, 2H), 3.15 (t, $J=7$, 2H), 2.38 (s, 3H), 1.1 (t, $J=7$, 6H). ^{13}C-NMR: 163.25, 156.77, 152.46, 150.29, 126.86, 100.55, 61.67, 53.79, 43.53, 24.55, 15.29. MS ES+: 332 (58, M+1), 334 (20), 286 (100), 288 (60); HRMS found 332.1493, theory for $C_{13}H_{23}N_5O_3Cl$ (M+H) is 332.1489.

*6.1.18. 4-Chloro-5-[4-hydroxy-imidazolidin-2-ylidenimino]-6-methoxy-2-methyl-pyrimidine (**6**)*

Acetal **26** (2.0 g, 0.006 mol) and 21 mL of water were combined in a flask with a magnetic stirrer and concd. HCl (5.0 mL, 0.066 mol) was added dropwise at ambient temperature. After 85 min, the solution was concentrated over a period of 6 min in vacuo with a 50 °C bath, removing 23 g. Acetone (200 mL) was added to the residue and the resulting mixture stirred rapidly at ambient temperature for one-half hour and then in an ice bath for 1 h. The mixture was filtered, and the product was rinsed with C_3H_6O and dried in vacuo at 27 °C for 1 h to give 1.21 g (68%) of a white solid. The purity based on the isocratic HPLC method (but 70:30 aqueous–MeCN) was 98.1%. The NMR spectra show hindered rotation, i.e. two structures of equal amounts: ^{1}H-NMR: 8.8 (d, $J=8$, 1H), 8.3 (br s), 5,55 (m, 1H), 3.9 and 3.85 (s, s, 3H), 3.9 (m, 1H), 3.35 (m, 1H), 2.5 (s, 3H). ^{13}C-NMR: 168.56 and 168.43, 168.36 and 167.44, 161.13 and 159.31, 156.76 and 156.61, 111.15, 84.21 and 84.04, 56.26 and 56.08, 50.15 and 49.88, 26.26. MS ES+: 258 (100, M+1), 260 (38), 242 (95), 240 (92); HRMS found 258.0748, theory for $C_9H_{13}N_5O_2Cl$ (M+H) is 258.0758.

*6.1.19. N-(4-Chloro-6-methoxy-2-methylpyrimid-5-yl)-1H-imidazol-2-amine (**2**)*

Acetal **26** (1.0 g, 0.003 mol) and 10 mL of water were combined in a flask with a magnetic stirrer and concd. HCl (2.5 mL, 0.033 mol) was added dropwise at ambient temperature. After 4 h solid KOH was added until the pH rose to 12. The mixture was stirred one-half hour at ambient temperature and filtered. The solid was rinsed with water and dried in vacuo, at 35 °C for

2 days. The yield was 0.43 g (60%) of a pink solid, m.p. 175–178 °C. The purity by HPLC was 98.2%. ^{1}H-NMR: 6.55 (s, 2H), 3.88 (s, 3H), 2.49 (s, 3H). ^{13}C-NMR: 164.35, 160.66, 152.65, 145.85, 119.26, 118 (br), 54.48, 24.77. MS EI+: 239 (50, M+1), 241 (18), 204 (100), 189 (19), 174 (29), 56 (65), 42 (90); CI+: 240 (100), 242 (32), 268 (20), 204 (85); ES−: 238 (100), 240 (33), 202 (32), 206 (35); HRMS found 240.0651, theory for $C_9H_{11}N_5OCl$ (M+H) is 240.0652.

*6.1.20. N-(4-Chloro-6-methoxy-2-methylpyrimid-5-yl)-guanidine (**3**)*

Compound **22** (4.0 g, 0.023 mmol) and 2.0 g cyanamide (2.0 equiv. Aldrich Chem. Co.) were combined in a 50 mL round-bottomed flask. Ethanol (20 mL) and concd. HCl (2.2 mL, 3 equiv.) were added and the mixture was heated at 50 °C. An additional 2 equiv. of cyanamide and 2 equiv. concd. HCl were added at this temperature and at 1.5–2 h intervals until 8 equiv. of cyanamide had been added. The mixture was stirred overnight and the EtOH was evaporated. The residue was dissolved in 30 mL water and washed twice with 20 mL of methylene chloride. The pH of the aq. layer was raised to 9.5 with solid NaOH. The resultant slurry was stirred 1 h at ambient temperature and one-half hour in an ice bath. The product was isolated by filtration, washed with water, and dried for 2 days at 40 °C to yield 4.22 g (85%). Its purity was 98.9% by the isocratic HPLC method. ^{1}H-NMR: 5.25 (s, 4H), 3.82 (s, 3H), 2.36 (s, 3H). ^{13}C-NMR: 163.56, 156.64, 153.53, 150.36, 126.96, 53.80, 24.58. MS EI+: 215 (20, M+1), 217 (8), 173 (18), 150 (12), 143 (15), 43 (100); CI+: 216 (100, M+1), 218 (30); HRMS found 216.0658, theory for $C_7H_{11}N_5OCl$ (M+H) is 216.0652.

*6.1.21. 4-Chloro-5-(3-4-dihydroxyimidazolidin-2-ylidenimino)-6-methoxy-2-methyl-pyrimidine (**7**)*

Compound **3** (1.0 g, 4.7 mmol) and 10 mL of water were combined in a 50 mL round-bottomed flask and 1.0 equiv. of concd. HCl (0.38 mL) was added. Aqueous glyoxal (40%, 0.96 mL, Aldrich Chem. Co.) was added and the mixture was heated at 50 °C for 4 h. After standing for 3 days at ambient temperature, it was heated for 4 h at 60 °C. This produced a mixture of the two isomers of the product and **3** in a ratio of 19:74:1.5 by the isocratic HPLC method (retention times 5.1, 5.4, and 6.6 min, respectively). The water was removed on the rotary evaporator with a bath temperature of 50 °C. Acetone, 15 mL, was added and evaporated. Ethyl acetate, 25 mL, was added and the mixture stirred at ambient temperature for 1 h. The solid was filtered, rinsed with EtOAc, and dried at 40 °C for a day to give 1.68 g of a white powder. It was purified by HPLC on a 25 cm × 23 mm Zorbax RX-C8 column eluted with 92% water (containing 0.08% TFA) and 8% MeCN at 22 mL min^{-1}. The main fractions were collected, combined, and concentrated in vacuo to produce 0.43 g of colorless oil with a purity of 99.3% by the gradient HPLC method. ^{1}H-NMR (C_3H_6O-d_6): 11.3, 11.2, 10.2, 7.6 (broad d, d, s, s, total 2H), 5.7 and 5.4 (m, m, total 2H), 4.10, 4.02, 4.00 (s, s, s, total 3H), 2.61, 2.60, 2.55 (s, s, s, total 3H). MS ES+: 274 (100, M+1), 276 (29); HRMS found 274.0709, theory for $C_9H_{13}N_5O_3Cl$ (M+H) is 274.0707.

*6.1.22. L-S-[5-(Imidazolidin-2-ylidenimino)-6-methoxy-2-methyl-pyrimidine-4-yl]-cysteine (**8**)*

Moxonidine (**1**, 2.0 g, 8.3 mmol), $NaHCO_3$ (0.7 g, 1.0 equiv.) and L-cysteine (2.0 g, 2.0 equiv.) were mixed with 20 mL of water and 10 mL of MeOH in a 50 mL round-bottomed flask and heated to 55 °C. After 3 h at this temperature, the mixture was cooled and concentrated on the rotary evaporator. It was purified along with a similar batch from a second reaction at the same scale on an 800 mL Biotage HP20 column, eluted with water. Fractions rich in the desired adduct (retention time 4 min on the isocratic HPLC method) were combined and the water was removed on a rotary evaporator with a bath temperature of 40 °C. The resulting solid was further dried on the vacuum pump to give 1.5 g of a white foam. Its purity by HPLC was 97.2% with 1.2% of a late-eluting (11 min) degradation product. ^{1}H-NMR (D_2O): 4.1 (m, 1H), 3.9 (s, 3H), 3.8 (dd, $J=3$, 15 Hz, 1H), 3.6 (s, 4H), 3.5 (dd, $J=6$, 15 Hz, 1H), 2.4 (s, 3H). ^{13}C-NMR (D_2O): 171.71, 167.28, 166.16, 164.09, 158.21, 110.08, 55.20, 54.27, 42.64, 37.15, 24.37. MS direct infusion ES+: 327 (100), 313 (30), 281 (35), 240 (60); HRMS found 327.1244, theory for $C_{12}H_{19}N_6O_3S$ (M+H) is 327.1239.

6.2. Analytical methods

6.2.1. Isocratic HPLC

A Zorbax RX-C8 column, 25 cm × 4.6 mm with 5 μ particles, was eluted at 1.0 mL min^{-1} at ambient temperature with 75% aq. eluent (20 mmol sodium pentanesulfonate, H_2SO_4 to pH 3) and 25% MeCN. Detection was by UV at 230 nm.

6.2.2. Gradient HPLC

The same column and eluent solutions were used. Detection was at 255 nm and the program was as follows, all at 1.00 mL min^{-1}: 75:25 (aq.: CH_3CN) for 6 min, ramp linearly to 45:55 at 10 min, hold at 45:55 until 20 min, return to 75:25 at 25 min.

6.2.3. Metabolite identification

6.2.3.1. Sample preparation. Urine samples collected from rats, mice and dogs were diluted 1:1 with 25 mM CH_3COONH_4 buffer (pH 5.0) before analysis. Urine samples collected from healthy volunteers were

concentrated by adding an equal volume of MeCN followed by evaporation to dryness at ca. 37 °C under N_2. Samples were reconstituted with 25 mM CH_3COONH_4 buffer (pH 5.0) before analysis. The bile samples collected from bile-duct cannulated rats were diluted 1:1 with 25 mM CH_3COONH_4 (pH 5.0) before analysis. Plasma samples were mixed with 20% of trichloroacetic acid (TCA) and centrifuged at ca. 3800 rpm for ca. 5 min to sediment precipitated proteins. The entire volume of the supernatant fraction of the sample was transferred to a Varian C-18, 200 mg, 3 mL solid phase extraction cartridge, which had been conditioned with 2 mL of MeOH and equilibrated with 2 mL of 1% TCA. The cartridge was then rinsed with 2 mL of 1% TCA and moxonidine-related substances were eluted with 2 mL of 25 mM CH_3COONH_4 (pH 5.0)–MeCN (50:50). The effluent was evaporated to dryness at ca. 37 °C under N_2. Samples were reconstituted with 25 mM CH_3COONH_4 buffer (pH 5.0) before analysis.

6.2.3.2. LC/MS/MS analysis. Separation was performed using a C-18 Supelcosil LC-ABZ column (25 cm × 4.6 mm, 5 μ) at a flow rate of 1 mL min^{-1} (Waters 600MS pump). The flow was split to allow 250 μL to flow into the electrospray source and 750 μL to a Ramona 5-LS radiochemical detector with a solid flow cell, which provided simultaneously detection of radioactivity and total ion chromatogram. A mobile phase gradient of (A) 25 mM CH_3COONH_4 (pH 5) and (B) MeCN was programmed as follows: started with 100% A, changed to A–B at 95:5 from 0 to 20 min, held at 95:5 from 20 to 25 min, changed to 100% A from 25 to 26 min. The gradient was changed slightly to optimise the separation of metabolites in each matrix. The characterisation of the metabolites was performed on a Finnigan TSQ 700 Mass Spectrometer. The samples were introduced using atmospheric pressure ionisation (API) with electrospray. Samples were analyzed in the positive ion mode using a spray voltage of +4500 V, capillary heater temperature of 270 °C, sheath gas of 80 psi (N_2) and an auxiliary gas flow (N_2) of 30 mL min^{-1}. For full scan analysis, the mass spectrometer was scanned from 150 to 600 amu in 1 s. The MS/MS analysis was performed utilizing collision energy of −30 eV and collision gas pressure of 1.5 mTorr of argon.

6.3. Pharmacology

6.3.1. Telemetry implantation

Spontaneously hypertensive rats (Tac:N(SHR)fBR) were obtained from Taconic Farms (Germantown, NY) at 14–16 weeks of age (295–325 gm) and housed under a 12-h light–dark cycle (lights on from 06:00 to 18:00 h). Following a 1-week acclimation period, rats were anesthetised with 2% isoflurane (Aerrane, Anaquest, Madison, WI) for implantation of blood pressure transmitters (model TA11PA-C40, Data Sciences Int. (DSI), St. Paul, MN). The abdomen was shaved, scrubbed with Betadine®, and a 4.5-cm abdominal incision was made beginning just caudal to the approximate location of the kidneys. The abdominal aorta was isolated and gently cleaned of connective tissue with a sterile cotton swab. A small spatula was used to raise a portion of the aorta away from the vena cava in an area just rostral to the iliac artery bifurcation. A bulldog clamp was placed caudal to the left renal artery, and the aorta was punctured rostral to the common iliac using a 21-gauge needle (bent at a 45° angle with the bevel down). A fluid-filled catheter (0.7 mm OD, 8 cm in length) attached to the hermetically sealed transmitter was inserted and advanced to the bulldog clamp using the bent needle as a guide. The area was dried with a cotton swab and tissue adhesive applied at the entry point while the clamp was removed. The entry point was further sealed using tissue adhesive and a cellulose fiber patch (Vetland, 3M Co). The body of the transmitter was sutured to the muscles of the inner abdominal wall using non-absorbable 4-0 silk, the muscle layers were approximated with sterile 3-0 silk, and the final incision closed with sterile metal wound clips. All animals were administered 10,000 units of penicillin intramuscularly (Ambi-Pen®, Butler), housed individually in shoe box cages with food and water ad libitum, and permitted to recover for at least 1 week before study. Digitised pressure signals were acquired for 30 s every 10 min using DSI Dataquest IV 2.0 software. Mean arterial pressure was calculated as the arithmetic mean of the waveform sampled at a frequency of 500 Hz. Heart rate was obtained from the pressure readings. The 6 hourly values were averaged to obtain a mean blood pressure over a 1 h interval. Pressure and heart rate values in the drug groups were compared to the vehicle group using Dunnett's test for multiple comparisons. Statistical comparisons were performed with JMP software (SAS, Cary, NC) with values of $P < 0.05$ considered significant.

6.3.2. Animal studies

Animals were briefly sedated with 2% isoflurane. Test compounds, moxonidine, or vehicle (water) were administered via the tail vein in a total volume of 0.3–0.4 mL. Test solutions were prepared by adding ca. 50 μL of 1 N HCL to the test compound and slowly diluting with water to the appropriate concentration. Rats were returned to telemetry cages and their blood pressure was monitored for the subsequent 24 h. Monitoring was initiated immediately except that the values for 15 min prior and subsequent to dosing were omitted.

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