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Short communication

# An expeditious access to 5-pyrimidinol derivatives from cyclic methylglyoxal diadducts, formation of argpyrimidines under physiological conditions and discovery of new CFTR inhibitors

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ABSTRACT

In the study of previously reported modulators of CFTR chloride channels that are cyclic methylglyoxal (MG) diadducts (CMGD) to aromatic α-aminoazaheterocycles, we optimized a new expeditious one pot route for preparing in water novel aromatic polycyclic azaheterocycles and described 5-pyrimidinols antioxidants through the formation of 2-oxoaldehyde diadducts to aromatic α-aminoazaheterocycles, amidines, guanidines and thiourea. In regard to the importance as biomarkers of diabetic complications of the 5-pyrimidinols "argpyrimidines" formed in proteins from MG and arginine residues, we demonstrated that argpyrimidines are slowly formed under physiological conditions from CMGD to arginine derivatives according to the synthesis route described. Among the 5-pyrimidinol derivatives prepared, two polycyclic derivatives appeared to inhibit strongly the activity of CFTR channels in wt-CHO cells.


## 1. Introduction

Previously, we have reported the remarkable stereoselective diaddition of methylglyoxal (MG) to aromatic α-aminoazaheterocycles, like, 2-aminopyridine (2AP), adenine, adenine nucleosides and polyadenylic acid [1]. This reaction leads to cyclic methylglyoxal diadducts (CMGD) which are highly functionnalized heterocycles (for example CMGD to 2AP **1a** and **1b**, Fig. 1). More recently, we showed that the CMGD to 2′-deoxyadenosine and 1-aminoisoquinoline (1IQ) **2a** and **3a-b**, respectively, are potent inhibitors of wild-type Cystic Fibrosis Transmembrane conductance Regulator (CFTR) chloride channels and CFTR affected by CF-related mutations [2,3]. The 9-propyladenine CMGD **4a** (Fig. 1) also were able to activate *in vivo* CFTR at micromolar concentrations [2,4].

Searching for potent and specific small molecules able to modulate $Cl^-$ channels is crucial to understand their physiological role in cell functions and also for the development of molecules of therapeutic interest for diseases caused by mutations of these proteins like Cystic Fibrosis which is the most common lethal autosomal recessive genetic disease in Caucasians [5], myotonia, Bartler's and Dent's diseases and osteopetrosis, or due to loss of regulation of CFTR activity (secretory diarrheas, cholera) [4].

In the study of the reactions of the CFTR modulators that we have previously identified, here, we report on the remarkable conversion in one pot in water of such CMGD to polycyclic 5-pyrimidinol derivatives. This conversion was extended to amidines and guanidines derivatives and thiourea and, was related to the *in vivo* formation of the fluorescent 5-pyrimidinols named argpyrimidines (ArgPy) involved in diabete complications and formed from MG and proteins carrying arginine residues. Among the 5-pyrimidinol derivatives prepared, two polycyclic derivatives were found to be strong inhibitors of the CFTR activity.

## 2. Results and discussion

### 2.1. Chemistry

In the aim to identify the common pharmacophore present in the prepared modulators of CFTR channels, it appeared interesting

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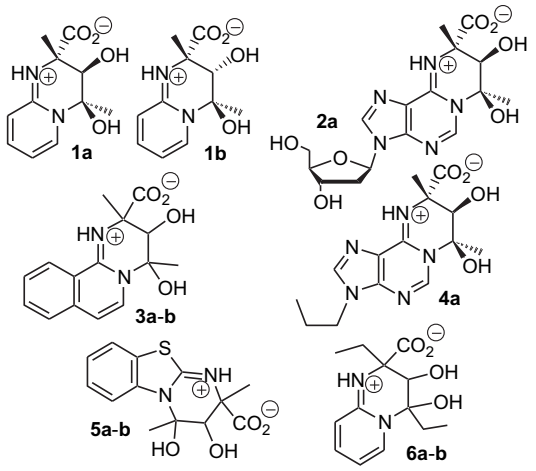


**Fig. 1.** Structures of the prepared cyclic methylglyoxal diadducts to α-amino-azaheterocycles used in this study (one enantiomer drawn).

to remove the carboxylic acid function and/or water for creating a double bond in the heterocyclic ring formed from MG.

The 2AP CMGD **1a-b** were prepared in mixture as model compounds in large scale with 63% yield (30 g of starting amine). When heated under basic conditions, in aqueous NaOH, the mixture of CMGD **1** led to 2AP and to the yellow fluorescent compound **7** which was isolated in low yield (Fig. 2). Interestingly, **7** results from three reactions in a same pot: decarboxylation, dehydration and oxidation. In order to optimize the formation of **7** and avoid the formation of 2AP, the influence of NaOH concentration and temperature on the course of the reaction was studied. At high concentration (>0.5 M), the yield in 2AP decreased strongly. At temperature lower than 70 °C, the produced amount of 2AP increased. Finally, the best result was obtained at 70 °C in 1 M aqueous NaOH. Under these conditions, **7** was obtained in high 81% yield.

We tried to prepare analogues of compound **7** from the CMGD **2a**, **3a-b**, **4a** and **5a-b** (**5a-b** were not described previously). The main products formed under different basic conditions were the aromatic α-aminoazaheterocycle: 2′-deoxyadenosine, 1-aminoisoquinoline (1AI), 9-propyladenine, 2-aminobenzothiazole, respectively. However, the 1AI-CMGD **3** led to the phenolate **8** in low 10% yield (Fig. 2). A high 2 M NaOH concentration in water was also required in order to increase the yield. The ethylglyoxal diadducts (CEGD) to AP **6a-b** (Fig. 1) were prepared (30% yield) and converted under the same conditions to the phenolate **9** in 55% yield (Fig. 2).

The structure of compounds **7**–**9** appeared to be related to the structure of the fluorescent 5-pyrimidinol **10a** named argpyrimidine (ArgPy, Fig. 2) isolated in the study of the reaction at 37 and

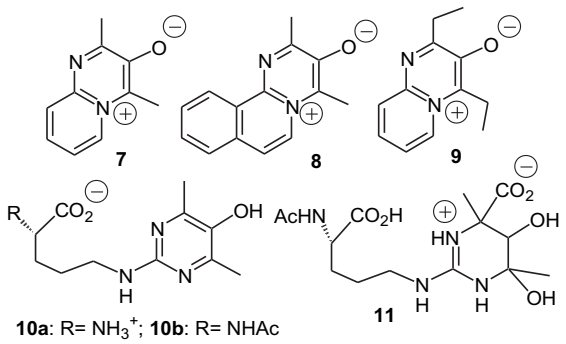


**Fig. 2.** Structures of the prepared phenolates **7**–**9** compared to the structure of the reported argpyrimidines **10a-b** [6,7] and structures of the previously described CMGD to $N_\alpha$-acetylarginine 11 [7].

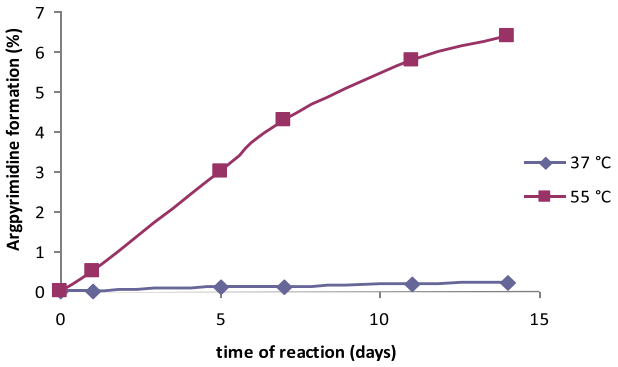


**Fig. 3.** Kinetic profiles at 37 and 55 °C of formation of ArgPy **10b** from $N_\alpha$-acetyl-L-arginine CMGD **11a,b** (36 mg, 0.1 mmol, 1 mL of 100 mM aq. phosphate buffer, pH 7.4) monitored by HPLC analysis.

55 °C of MG in excess with $N_\alpha$-Boc-arginine as a model reaction of proteins (isolation after removal of the Boc group with HCl) [6]. More recently, after reaction of MG with $N_\alpha$-acetyl-L-arginine at pH 7.4 and 37 °C during 14 days, the ArgPy **10b** and also the CMGD **11** were isolated as major products (Fig. 2) [7].

The presence of the argpyrimidines also was detected *in vivo* by immunochemistry in MG-modified proteins in the kidneys from diabetic patients and in the rat brain suggesting that ArgPy may contribute to the progression of not only long term diabetic complications, such as nephropathy and atherosclerosis but also the tissue injury caused by ischemia/reperfusion [7,8]. MG can be formed *in vivo* by slow glucose degradation and is involved in the development of diabetic complications and in mutagenesis [9–15]. It also has appeared to be a signal molecule in apoptosis [16] and was used as an anti-cancer agent [17]. In diabetic complications, MG produced from glucose reacts with nucleic acids and proteins to form advanced glycation end products. The formation of ArgPy has been explained *in vitro* through the condensation of two molecules of MG to lead to the unstable 3-hydroxypentane-2,4-dione and then reaction of this latter with $N_\alpha$-Boc-arginine [6].

In regard to importance of ArgPys as biomarkers of pathologies, their possible formation from CMGD to arginine derivatives was investigated.

First, we studied the conversion of $N_\alpha$-Fmoc-L-arginine-CMGD to ArgPy **10a** under the conditions used for preparing the polycyclic pyrimidinols **7** and **8** without isolation of the intermediate CMGD. Interestingly, **10a** was isolated in 22% after two short steps in one pot: (i) formation of the adducts in water in 40% aqueous MG at 80 °C (8 and 6 equiv. added in twice) and (ii) co-evaporation of MG/water and then aromatisation by heating at 80 °C in 3 M aqueous NaOH.

After this result, we studied the formation of ArgPys from CMGD to arginine derivatives under the conditions previously used to isolate ArgPys that are close to physiological conditions [6,7]. We prepared [18] the $N_\alpha$-acetyl-L-arginine CMGD **11** and studied its behaviour in water at pH 7.4 (phosphate buffer) at 37 and 55 °C [6,7]. Under both conditions, the formation of ArgPy **10b** was detected by HPLC and the yields were measured after 7 and 14

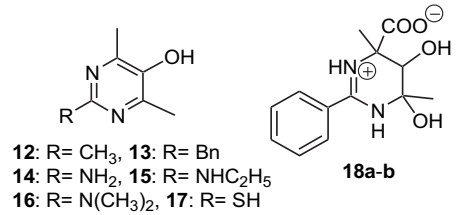


**Fig. 4.** Structure of the prepared 5-pyrimidinols and benzamidine CMGD.

**Table 1**
Yields of preparation from methylglyoxal of the phenolates **7** and **8** and pyrimidinols **10a**, **12**–**17** in water in two steps one pot (heating or microwave irradiation). Previously, some of these compounds have been prepared from 2,4-dimethyl-5-acetyloxazole (*) or 3-acetoxypentan-2,4-dione (†), both prepared from commercially available 3-chloropentan-2,4-dione (30 and 80% yields, respectively). The corresponding references and yields calculated from 3-chloropentan-2,4-dione are mentioned.

| Reagent | 2AP | 1AI | $N_\alpha$-Fmoc-Arg | RC(NH)$NH_2$, R= | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $CH_3$ | Bn | $NH_2$ | NHAc | $NHC_2H_5$ | $N(CH_3)_2$ | SH |
| Product, yields (%, heating/microwave) | **7** 51/41 | **8** 8/14 | **10a** 22/19 | **12** 24/7 | **13** 48/44 | **14** 7/3 | **14** 20/20 | **15** 27/40 | **16** 25/27 | **17** 17/15 |
| Previous synthesis: reference (), yield % | – | _ | [23]† 37 | [19]* 18 | _ | [21]† 62 | [21]† 62 | _ | [19]† 24 | [27]† 38 |

days: at 37 °C, 0.1 and 0.2%, respectively and, at 55 °C, 4.3 and 6.4%, respectively (Fig. 3). Clearly, ArgPys can be formed from arginine CMGD under mild conditions close to physiological conditions.

2-Substituted-3,6-dimethyl-5-pyrimidinols having the aromatic ring of ArgPy have been recently synthesised for the study of their radical-scavenging ability [19–22]. In the main preparation, the commercially available 3-chloropentan-2,4-dione have been converted to the intermediate 3-acetoxypentan-2,4-dione [19]. The reaction of this dione with different amidines and guanidines has led to the corresponding 5-pyrimidinols. 3,6-Dialkyl-5-pyrimidinols have appeared to transfer their phenolic hydrogen atom to peroxyl radicals as quickly as equivalently substituted phenols, while their reactivity toward alkyl radicals far exceeds that of the corresponding phenols. 3,6-Dimethyl-5-pyrimidinol is very efficient to inhibit enzyme-catalysed lipid peroxidation by ovine cyclooxygenase 1 (oCOX-1) and is non cytotoxic [22]. ArgPy also has been prepared from 3-acetoxypentan-2,4-dione in 47% yield (37% from 3-chloropentan-2,4-dione) and also has been reported to be an antioxidant agent [23].

In regard to the observed conversion of CMGD and CEGD to 5-pyrimidinols or azapolycyclic phenols, we investigated a possible direct conversion of amidines, guanidines and thiourea to 5-pyrimidinols from MG without isolation of the intermediate MG-adducts in two steps one pot in water: (i) formation at 80 °C of the CMGD in 40% aqueous MG (addition of 8 and then 6 equiv.) and (ii) after co-evaporation of MG and water, aromatisation by heating at 80 °C in 3 M aqueous NaOH. The conversion was successful with *N,N*-dimethylguanidine, *N*-ethylguanidine, *N*-acetylguanidine, acetamidine, benzamidine and thiourea leading to the corresponding 2-substituted-3,6-dimethyl-5-pyrimidinols **12**–**17** in low to good yields (Fig. 4, Table 1). Like **10a**-**b**, pyrimidinols **14**–**16** appeared to be fluorescent on TLC. The 2-aminopyrimidinol **14** was obtained with a low 3.5% yield from guanidine (7% after partial purification of the diadducts) and in 20% from *N*-acetylguanidine. From benzamidine, the intermediate adducts **18a**-**b** also were isolated (88%) and then treated with 1 M aqueous NaOH to lead to the pyrimidinol **13** (39%).

The procedure was strongly shortened upon microwave irradiation according to the following procedure in one pot: (i) reaction with MG (4 equiv.) in water at 80 °C, 1 h and (ii) addition of aqueous NaOH and reaction at 80 °C, 1 h (Table 1).

Compounds **7** and **8** also were prepared using the developed short procedures in one pot (heating and upon microcrowave) (Table 1).

In comparison to the methods of synthesis of pyrimidinols reported, the method described here appears to be competitive in regard to its simplicity and rapidity (one step of purification) and to the low cost of the commercially available reagents (commercial aqueous MG solution) and solvent (water).

### 2.2. Biological activity

Previously, we have used a simple and robust robotic HTS assay to screen small molecules able to modulate CFTR $Cl^-$ channel activity through measurements of iodide ($^{125}I$) efflux [2–4]. Among the pyrimidinols prepared, compounds **7** and **8** were found able to inhibit strongly the CFTR channel activity in wt-CHO cells. Low $IC_{50}$ values of 5 and 16 nM were measured for compounds **7** (GPinh-12) and **8** (GPinh-3), respectively, whereas the known inhibitor glibenclamide showed a 15 μM $IC_{50}$. The parent CMGD **1a**-**b** have been found inactive and **3a**-**b** have shown a low 15 nM $IC_{50}$ [3]. Interestingly, under physiological conditions, **7** and **8** are in the zwitterionic form like the previously identified active adducts **2a**, **3a**-**b** and **4a** (for **8**, the $pK_a$ value of approximately 4 was determined by UV spectrophotometry, data not shown). This zwitterionic character at physiological pH could be related to the inhibition of CFTR channels observed. Molecular dockings are underway in the search for a common targeted site using the recently reported models of the CFTR 3D structure [24–26].

## 3. Conclusions

In the study of the synthesis of previously reported modulators of CFTR chloride channels that are cyclic methylglyoxal diadducts (CMGD) to aromatic α-aminoazaheterocycles, we optimized a new one pot route for preparing in water novel aromatic polycyclic azaheterocycles and described 5-pyrimidinols antioxidants.

The addition of 2-oxoaldehydes to α-aminoazaheterocycles, amidines, guanidines and thiourea is a general and versatile reaction leading to cyclic diadducts which can be converted to 5-pyrimidinol derivatives. The involved reaction sequence constitutes a new expeditious route for preparing such heterocycles that allowed the preparation and identification of the new polycyclic 5-pyrimidinols **7** and **8** as strong inhibitors of wild-type CFTR channels in wt-CHO cells.

In regard to the importance as biomarkers in diabetic complications of the 5-pyrimidinols "argpyrimidines" formed in proteins from methylglyoxal and arginine residues, we demonstrated here that argpyrimidines are slowly formed under physiological conditions from CMGD to arginine derivatives according to the synthesis route described. The observed great instability of the CMGD to arginine derivatives and the corresponding easy formation of ArgPys under physiological conditions show that the analysis and quantification *in vitro* and *in vivo* of ArgPys and related CMGD have to be performed carefully. The adenine-CMGD such as **2a** which could be formed *in vivo* in DNA during diabetic complications appeared to be stable under physiological conditions.

## 4. Experimental protocols

### 4.1. Chemistry

All starting materials were commercially available research-grade chemicals and used without further purification except ethylglyoxal which was synthesised according to Gi et al. [28]. Reactions were monitored by analytical TLC on silica gel (Alugram Sil G/$UV_{254}$) from Macherey–Nagel with fluorescent indicator $UV_{254}$. Melting points were determined in open glass capillaries using a Büchi 510 apparatus and are reported uncorrected. LRMS were achieved with a ZQ Waters for the ESI. HRMS and elemental

analysis were obtained from the Mass Spectrometry Service, CRMPO, at the University of Rennes I, France, using a Micro-Tof-Q-II Micromass Zabspec-Tof spectrometer for ESI and a Varian Mat311 spectrometer for EI technique, and a Microanalyseur Flash EA1112 CHNS/O Thermo Electron.

$^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance 400 at 400 MHz and 100 MHz, respectively, using the residual solvent signal as internal standard. Chemical shifts are reported in ppm (parts per million) relative to the residual signal of the solvent, and the signals are described as singlet (s), broad singlet (bs), doublet (d), triplet (t), doublet of doublet (dd), quartet (q), multiplet (m); coupling constants are reported in Hertz (Hz). Columns chromatography was performed on silica gel (MN Kieselgel 60, 0.063–0.2 mm/70–230 mesh, Macherey–Nagel) or on $C_{18}$ or $C_8$ reversed phase (Macherey–Nagel polygoprep 60-50$C_{18}$).

The synthesis and characterization of compounds **1**, **2** and **3** have been previously described [1]. Only one enantiomer of the racemic mixture of compounds **1**, **6**, **18** is drawn here. When indicated, the ratio of diadducts **a** and **b** are measured by NMR.

Microwave reactions were conducted using a CEM Discover Synthesis Unit (CEM Corp., Matthews, NC) with PC control, equipped with a continuous focused microwave power delivery system which operates at 2.45 GHz with selectable power output from 0 to 300 W. The reaction was performed in glass vessels (capacity 10 mL) sealed with a septum. The temperature of the reaction mixture was monitored using a calibrated infrared temperature control mounted under the reaction vessel. All experiments were conducted under magnetic stirring (rotating magnetic plate located under the microwave cavity and using Teflon-coated magnetic stir bar in the vessel). The solutions were irradiated in a sealed tube at 80 °C for 1 h (ramp time 1 min, $T_{max} = 80$ °C, Power max = 150 W).

The kinetic study was performed with an Agilent 1100 series HPLC machine using a diode array detector and a $C_{18}$ reversed phase column (Nucleodur $C_{18}$ ISIS, Macherey–Nagel, 5 μm particle size, 250 mm × 4.6 mm), with a mobile phase composed of A = water and B = methanol containing 0.5% TFA with a gradient 90:10 to 0:100 A:B over 30 min, 0.7 mL/min, 50 μL injection, detection at 340 nm.

#### 4.1.1. Synthesis of cyclic methylglyoxal diadducts (CMGD)

*4.1.1.1. Synthesis in large scale of the 2-aminopyridine methylglyoxal diadducts (2AP CMGD) **1a,b** (60:40, respectively).* To the commercially available concentrated aqueous solution of MG (40%) (380 mL, 1.9 mol), 2-aminopyridine (30.0 g, 318.8 mmol) was added. Argon was flushed through the solution and the mixture was heated at 60 °C for 20 h. After evaporation under reduced pressure, the residue was dissolved in methanol and precipitated in tetrahydrofuran (300 mL). After filtration, the solid was washed with diethyl ether to give the adducts **1a,b** [1] (47.9 g, 201.1 mmol, 63%) as a white solid.

*4.1.1.2. 2-Aminopyridine ethylglyoxal adducts (2AP CEGD) **6a,b** (75:25).* To a solution of 2-aminopyridine (1.0 g, 10.6 mmol) in water (10 mL), ethylglyoxal (4.1 g, 47.6 mmol) was added. Argon was flushed through the solution and the mixture was heated at 60 °C for 10 h. After evaporation, the residue was chromatographed on Sep-Pak® C18 cartridges (10 g) eluting with $H_2O$ and then $H_2O$-MeOH (95:5) to give **6a,b** (850 mg, 3.2 mmol, 30%).

The adducts **6a** were isolated as a white solid (600 mg, 2.3 mmol, purification yield 94%) after chromatography on Sep-Pak® $C_{18}$ cartridges (10 g) eluting with $H_2O$ and $H_2O$-MeOH (95:5), mp: 152–154 °C. Found C 56.88, H 6.67, 10.46. $C_{13}H_{18}N_2O_4$, 1/2$H_2O$ requires C 56.72, H 6.96, N 10.18; $^1$H (400 MHz, $D_2O$) $\delta$ (ppm): 8.16 (1H, d, *J* 7.2, $CH_{Ar}$), 7.75 (1H, t, *J* 8.4, $CH_{Ar}$), 7.05 (1H, d, *J* 8.4, $CH_{Ar}$), 6.89 (1H, t, *J* 7.2, $CH_{Ar}$), 4.55 (1H, s, CH), 2.02–1.93 (1H, m, $CH_2$), 1.87–1.78 (3H, m, $CH_2$), 0.97 (3H, t, *J* 7.2, $CH_3$), 0.91 (3H, t, *J* 7.2, $CH_3$); $^{13}$C (100 MHz, $D_2O$) $\delta$ (ppm): 176.1 (COOH), 149.5 ($C_{IV}$), 141.8 ($CH_{Ar}$), 133.0 ($CH_{Ar}$), 114.3 ($CH_{Ar}$), 112.9 ($CH_{Ar}$), 91.0 ($C_{IV}$), 68.7 (CH), 65.9 ($C_{IV}$), 30.1 ($CH_2$), 29.1 ($CH_2$), 7.3 ($CH_3$), 6.4 ($CH_3$); HRMS (ESI) calc. for $C_{13}H_{18}N_2O_4$: $[M + H]^+$ 267.1345, found 267.1345.

*4.1.1.3. $N_\alpha$-Acetyl-L-arginine CMGD **11a,b**.* To a commercial aqueous solution of MG (40%) (10 mL, 66.6 mmol), $N_\alpha$-Acetyl-L-arginine (2.5 g, 11.56 mmol) was added under Argon and the mixture was heated at 70 °C for 16 h. After two co-evaporation with water and MeOH, the solid was crystallised in THF then in MeOH to afford **11a,b** [7] (1.30 g, 3.61 mmol, 31%), mp: 175 °C (dec.). LRMS (ESI) calc. for $C_{14}H_{25}N_4O_7$: $[M + H]^+$ 361.5, found 361.1.

*4.1.1.4. Benzamidine CMGD **18a,b** (70:30).* To the aqueous solution of MG (40%) (11.8 mL, 76.8 mmol), benzamidine chlorhydrate (1.5 g, 9.6 mmol), water (2 mL) and ethanol (2 mL) were added. Argon was flushed through the solution and the mixture was heated at 50 °C for 24 h. After evaporation, the residue was chromatographed on Sep-Pak® $C_{18}$ cartridges (10 g) eluting with $H_2O$ and $H_2O$-MeOH (9:1). Crystallization from propan-2-ol afforded **18a**,**b** (2.23 g, 8.45 mmol, 88%) as a white solid. The adducts **18a** were isolated as a white solid (651 mg, 2.23 mmol) after chromatography on silica gel in DCM-MeOH (9:1 then 8:2), mp: 177 °C (dec.). Found C 52.42, H 6.37, N 8.39. $C_{13}H_{16}N_2O_4$, HCl, 1/2$C_3H_7OH$ requires C 52.65, H 6.40, N 8.47; $^1$H (400 MHz, $D_2O$) $\delta$ (ppm): 7.70–7.66 (3H, m, $CH_{Ar}$), 7.57–7.53 (2H, m, $CH_{Ar}$), 4.21 (1H, s, CH), 1.65 (3H, s, $CH_3$), 1.64 (3H, s, $CH_3$); $^{13}$C (100 MHz, $D_2O$) $\delta$ (ppm): 176.6 (COOH), 161.9 ($C_{IV}$), 135.6 ($C_{IV}$), 130.9 ($CH_{Ar}$), 129.4 ($CH_{Ar}$), 128.8 ($CH_{Ar}$), 82.0 ($C_{IV}$), 70.8 (CH), 62.9 ($C_{IV}$), 24.8 ($CH_3$), 22.3 ($CH_3$); HRMS (ESI) calc. for $C_{13}H_{16}N_2O_4$: $[M + H]^+$ 265.1188, found 265.1172.

#### 4.1.2. Preparation of phenolates **7**, **8**, **9** and pyrimidinols **10a**, **13** after isolation of the diadducts

*4.1.2.1. Phenolate **7**.* A solution of **1a**,**b** (200 mg, 0.8 mmol) in 1 M aqueous NaOH (25 mL) was heated at 70 °C for 5 h. After cooling at rt, the solution was neutralized by addition of 1 M aqueous HCl. After evaporation, the residue was chromatographed on Sep-Pak® $C_{18}$ cartridges (10 g) eluting with $H_2O$ and $H_2O$-MeOH (95:5) to give **7** (119 mg, 0.68 mmol, 81%) as a yellow solid, mp: 140 °C (dec.). $^1$H (400 MHz, $D_2O$) $\delta$ (ppm): 8.92 (1H, m, $CH_{Ar}$), 8.36–8.32 (1H, m, $CH_{Ar}$), 8.26–8.23 (1H, m, $CH_{Ar}$), 8.00–7.96 (1H, m, $CH_{Ar}$), 2.89 (3H, s, $CH_3$), 2.81 (3H, s, $CH_3$); $^{13}$C (100 MHz, $D_2O$) $\delta$ (ppm): 164.6 ($C_{IV}$), 145.8 ($C_{IV}$), 143.9 ($C_{IV}$), 137.5 ($CH_{Ar}$), 136.8 ($C_{IV}$), 131.0 ($CH_{Ar}$), 126.7 ($CH_{Ar}$), 123.3 ($CH_{Ar}$), 20.7 ($CH_3$), 12.0 ($CH_3$); HRMS (EI) calc. for $C_{10}H_{10}N_2O$: $[M]^+$ 174.0793, found 174.0780.

*4.1.2.2. Phenolate **8**.* A solution of **3a**,**b** [3] (200 mg, 0.63 mmol) in 2 M aqueous NaOH (3 mL) was heated at 70 °C for 3 h. After cooling at rt, the solution was neutralized by addition of aqueous $H_3PO_4$ (30%) and then, extracted with DCM. After evaporation, the residue was chromatographed on silica gel in DCM-MeOH (95:5) to give **8** (14 mg, 0.06 mmol, 10%) as a yellow solid, mp: 187 °C (dec.). Found C 69.84, H 5.81, N 11.37. $C_{14}H_{12}N_2O$, 1/2$H_2O$ requires C 69.86, H 6.06, N 11.24; $^1$H (400 MHz, $CD_3OD$) $\delta$ (ppm): 8.74–8.71 (1H, m, $CH_{Ar}$), 8.14 (1H, d, *J* 7.6, $CH_{Ar}$), 7.80–7.78 (1H, m, $CH_{Ar}$), 7.73–7.66 (3H, m, $CH_{Ar}$), 2.67 (3H, s, $CH_3$), 2.63 (3H, s, $CH_3$), $^{13}$C (100 MHz, $CD_3OD$) $\delta$ (ppm): 166.3 ($C_{IV}$), 160.0 ($C_{IV}$), 136.6 ($C_{IV}$), 136.4 ($C_{IV}$), 132.1 ($CH_{Ar}$), 131.1 ($CH_{Ar}$), 130.8 ($C_{IV}$), 128.0 ($CH_{Ar}$), 128.0 ($C_{IV}$), 126.4 ($CH_{Ar}$), 125.4 ($CH_{Ar}$), 122.6 ($CH_{Ar}$), 22.0 ($CH_3$), 12.3 ($CH_3$); HRMS (EI) calc. for $C_{14}H_{12}N_2O$: $[M]^+$ 224.0950, found 224.0947.

*4.1.2.3. Phenolate **9**.* A solution of **6a**,**b** (50 mg, 0.19 mmol) in 1 M aqueous NaOH (20 mL) was heated at 60 °C for 6 h. After cooling at rt, the solution was neutralized by addition of 1 M aqueous HCl and, then, extracted with DCM. After evaporation, the residue was

chromatographed on a $C_{18}$ reversed phase eluting with $H_2O$ and $H_2O$-MeOH (9:1) to afford **9** (21 mg, 0.10 mmol, 55%) as a yellow hygroscopic solid. $^1H$ (400 MHz, $CD_3OD$) $\delta$ (ppm): 8.68 (1H, d, *J* 6.8, $CH_{Ar}$), 8.05 (1H, d, *J* 8.4, $CH_{Ar}$), 7.82–7.78 (1H, m, $CH_{Ar}$), 7.68–7.65 (1H, m, $CH_{Ar}$), 3.39–3.34 (2H, m, $CH_2$), 3.11–3.06 (2H, m, $CH_2$), 1.40–1.33 (3H, m, $CH_3$), 1.31–1.27 (3H, m, $CH_3$); $^{13}C$ (100 MHz, $CD_3OD$) $\delta$ (ppm): 170.8 ($C_{IV}$), 156.9 ($C_{IV}$), 138.6 ($C_{IV}$), 136.2 ($C_{IV}$), 128.9 ($CH_{Ar}$), 127.4 ($CH_{Ar}$), 127.1 ($CH_{Ar}$), 121.4 ($CH_{Ar}$), 26.3 ($CH_2$), 17.2 ($CH_2$), 9.3 ($CH_3$), 8.0 ($CH_3$); HRMS (ESI) calc. for $C_{12}H_{15}N_2O$: $[M + H]^+$ 203.1184, found 203.1182.

*4.1.2.4. ArgPy* ***10b****.* 2 mL of 3 M aqueous NaOH was added to 50 mg of **11a,b** (0.14 mmol) and the mixture was stirred 24 h at 70 °C. After cooling, the pH was adjusted to 7.5 by addition of 3 M aqueous HCl. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt-MeOH (1:2) and on a $C_{18}$ reversed phase eluting with $H_2O$ and $H_2O$-MeOH (9:1) to afford **10b** (25 mg, 0.08 mmol, 61%) as a white solid, mp: 187 °C (dec.). $^1H$ (400 MHz, $D_2O$) $\delta$ (ppm): 4.10 (1 H, q, CH), 3.23 (2 H, t, *J* 6.4, N-$CH_2$), 2.24 (6 H, s, $CH_3$), 1.95 (3 H, s, $CH_3$), 1.81 (1 H, m, $CH_2$), 1.62 (3 H, m, CH+$CH_2$); $^{13}C$ (100 MHz, $D_2O$) $\delta$ (ppm): 180.8 ($COO^-$), 175.1 (CO), 159.5 (2×$C_{IV}$-$CH_3$) 157.9 ($C_{IV}$-OH), 139.9 ($C_{IV}$-NH), 56.7 (CH), 42.7 (HN–$CH_2$), 30.6 ($CH_2$), 26.7 ($CH_2$), 23.4 ($CH_3$), 19.2 (2×$CH_3$); LRMS (ESI) calc. for $C_{13}H_{20}N_4O_4$: $[M - H]^-$ 295.3, found 294.9.

*4.1.2.5. 2-Phenyl-5-pyrimidinol* ***13****.* A solution of **18a**,**b** (100 mg, 0.30 mmol) in 1 M aqueous NaOH (20 mL) was heated to 50 °C for 3 h. After cooling at rt, the solution was neutralized by addition of 1 M aqueous HCl and then, extracted with DCM. After evaporation under reduced pressure, the residue was chromatographed on silica gel eluting with DCM and DCM-MeOH (95:5) to give **13** (24 mg, 0.12 mmol, 39%) as a white solid, mp: 142 °C (dec.). Found: C, 71.77; H, 5.93; N, 13.86. Calc. for $C_{12}H_{12}N_2O$: C, 71.98; H, 6.04; N, 13.99; $^1H$ (400 MHz, $CDCl_3$) $\delta$ (ppm): 8.24 (2H, m, $CH_{Ar}$), 7.42–7.38 (3H, m, $CH_{Ar}$), 2.47 (6H, s, $CH_3$); $^{13}C$ (100 MHz, $CDCl_3$) $\delta$ (ppm): 156.7 ($C_{IV}$), 153.0 ($C_{IV}$), 146.0 ($C_{IV}$), 138.0 ($C_{IV}$), 129.7 ($CH_{Ar}$), 128.6 ($CH_{Ar}$), 127.8 ($CH_{Ar}$), 18.8 ($CH_3$); HRMS (EI) calc. for $C_{12}H_{12}N_2O$: $[M]^+$ 200.0950, found 200.0947.

### *4.1.3. Expeditious synthesis of 5-pyrimidinols and phenolates without isolation of the intermediate diadducts*

General procedure for preparation of phenolates and pyrimidinols upon conventional heating: To a commercial aqueous solution of MG (40%) (4.41 mL, 29.4 mmol), 3.67 mmol of reactant were added and the mixture was heated at 80 °C for 6 h, Then 6 equiv of MG (40%) (3.30 mL, 22.02 mmol) was added and the mixture stirred at 80 °C for 6 h. After two co-evaporation with water, the residue was dissolved in 3 M aqueous NaOH (15 mL) and the solution was heated at 80 °C for 8 h.

General procedure for preparation of phenolates and pyrimidinols upon microwave irradiation: To a commercial aqueous solution of MG (40%) (2.21 mL, 14.7 mmol) in a sealed tube, 3.67 mmol of reactant were added and the mixture was heated at 80 °C for 1 h upon microwave irradiation. Then 3 M aqueous NaOH (10 mL) was added and the solution was heated at 80 °C for 1 h upon microwave irradiation.

*4.1.3.1. Phenolate* ***7****.* Microwave procedure: After reaction and cooling at rt, the pH was adjusted to 7 by addition of 3 M aqueous HCl. After evaporation, the residue was chromatographed on a $C_{18}$ reversed phase eluting with $H_2O$ and $H_2O$-MeOH (9:1) to give **7** (260 mg, 1.49 mmol, 41%) as a yellow solid (characteristics described in Section 4.1.2.1.).

*4.1.3.2. Phenolate* ***8****.* Microwave procedure: After reaction and cooling at rt, the pH was adjusted to 7 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt and AcOEt-MeOH (9:1) to give **8** (114 mg, 0.51 mmol, 14%) as a yellow solid (characteristics described in Section 4.1.2.2.).

*4.1.3.3. ArgPy* ***10a****.* Thermic procedure: Fmoc-Arg-OH (1.455 g, 3.67 mmol). After cooling at rt, the pH was adjusted to 7.5 by addition of 3 M aqueous HCl. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt-MeOH (1:2) and on a $C_{18}$ reversed phase eluting with $H_2O$ and $H_2O$-MeOH (9:1) to afford **10a** (205 mg, 0.81 mmol, 22%) as a white solid.

Microwave procedure: After reaction and cooling at rt, the pH was adjusted to 7 by addition of 3 M aqueous NaOH, and then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt-MeOH (1:2) and followed by a purification on a $C_{18}$ reversed phase eluting with $H_2O$ and $H_2O$-MeOH (9:1) to afford **10a** (182 mg, 0.70 mmol, 19%) as a white solid, mp: 213 °C (dec.) (207 °C, dec., [23]). $^1H$ (400 MHz, $D_2O$) $\delta$ (ppm): 3.72 (2 H, t, *J* 6, CH), 3.26 (2 H, t, *J* 9.6, N–$CH_2$), 2.23 (6 H, s, $CH_3$), 1.86 (2 H, m, $CH_2$), 1.62 (2 H, m, $CH_2$); $^{13}C$ (100 MHz, $D_2O$) $\delta$ (ppm): 176.2 ($COO^-$), 159.6 (2 × $C_{IV}$-$CH_3$) 158.1 ($C_{IV}$-OH), 140.1 ($C_{IV}$-NH), 56.1 (CH), 42.4 (HN–$CH_2$), 29.4 ($CH_2$), 26.0 ($CH_2$), 19.3 (2×$CH_3$); HRMS (ESI) calc. for $C_{11}H_{17}N_4O_3$: $[M - H]^-$ 253.1301, found 253.1306.

*4.1.3.4. 2,4,6-Trimethyl-5-pyrimidinol* ***12****.* Thermic procedure: Acetamidine hydrochloride (347 mg, 3.67 mmol). After cooling at rt, the pH was adjusted to 6.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt and AcOEt-MeOH (9:1) to give **12** (120 mg, 0.87 mmol, 24%) as a white solid.

Microwave procedure: After reaction and cooling at rt, the pH was adjusted to 6.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt and AcOEt-MeOH (9:1) to give **12** (38 mg, 0.28 mmol, 7.5%) as a white solid, mp: 150–152 °C (152–154 °C, [29]). $^1H$ (400 MHz, $CD_3OD$) $\delta$ (ppm): 2.39 (3 H, s, $CH_3$), 2.29 (6 H, s, 2 × $CH_3$); $^{13}C$ (100 MHz, $CD_3OD$) $\delta$ (ppm): 158.5 ($C_{IV}$-OH), 155.2 (2×$C_{IV}$-$CH_3$), 147.4 ($C_{IV}$-$CH_3$), 24.1 ($CH_3$), 18.5 (2 × $CH_3$); HRMS (ESI) calc. for $C_7H_{11}N_2O$: $[M + H]^+$ 139.0871, found 139.0873.

*4.1.3.5. 2-Phenyl-5-pyrimidinol* ***13****.* Thermic procedure: To the concentrated aqueous solution of MG (40%) (4.1 mL, 25.6 mmol), water (2 mL) and benzamidine chlorhydrate (500 mg, 3.2 mmol) were added. Argon was flushed through the solution and the mixture was heated at 50 °C for 24 h. After two co-evaporation with water, the residue was chromatographed on $C_8$ reversed phase eluting with water. The fractions containing **18a**,**b** were evaporated to dryness. A solution of **18a**,**b** in 1 M aqueous NaOH (20 mL) was heated at 50 °C for 5 h. After cooling at rt, the solution was neutralized by addition of 1 M aqueous HCl. After evaporation, the residue was chromatographed on silica gel eluting with ethyl acetate to lead to **12** (300 mg, 1.5 mmol, 48%) as a white solid.

Microwave procedure: After reaction and cooling at rt, the pH was adjusted to 7 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt-cHx (7:3) and AcOEt to give **13** (322 mg, 1.62 mmol, 44%) as a white solid (characteristics described in Section 4.1.2.5.).

*4.1.3.6. 2-Amino-4,6-dimethyl-5-pyrimidinol* ***14*** *from N-acetylguanidine.* Thermic procedure: *N*-acetylguanidine (371 mg, 3.67 mmol). After cooling at rt, the pH was adjusted to 7.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the

residue was chromatographed on silica gel eluting with AcOEt and AcOEt-MeOH (9:1) to give **14** (105 mg, 0.75 mmol, 20%) as a beige solid.

Microwave procedure: After reaction and cooling at rt, the pH was adjusted to 7.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt and AcOEt-MeOH (9:1) to give **14** (100 mg, 0.72 mmol, 20%) as a beige solid, mp: 208 °C (dec.). $^1$H (400 MHz, $CD_3OD$): 2.17 (6 H, s, $CH_3$); $^{13}$C (100 MHz, $CD_3OD$): 157.9 (2 × $C_{IV}$-$CH_3$), 156.3 ($C_{IV}$-OH), 139.4 ($C_{IV}$-$NH_2$), 17.6 (2 × $CH_3$); HRMS (ESI) calc. for $C_6H_9N_3ONa$: $[M + Na]^+$ 162.0643, found 162.0644.

*4.1.3.7. 2-Amino-4,6-dimethyl-5-pyrimidinol **14** from guanidine hydrochloride.* Thermic procedure: Guanidine hydrochloride (350 mg, 3.67 mmol). After cooling at rt, the pH was adjusted to 7.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt and AcOEt-MeOH (9:1) to give **14** (17 mg, 0.12 mmol, 3.5%) as a beige solid.

Thermic procedure with a short partial purification by chromatography on $C_{18}$: After two co-evaporation with water, the residue was chromatographed on a $C_{18}$ reversed phase eluting with $H_2O$ and $H_2O$-MeOH (9:1). The fractions containing the adducts were evapored and then dissolve in 3 M aqueous NaOH (17 mL) and the solution was heated at 70 °C for 14 h. After cooling at rt, the pH was adjusted to 7.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt and AcOEt-MeOH (9:1) to give **14** (36 mg, 0.26 mmol, 7%) as a beige solid.

Microwave procedure: After reaction and cooling at rt, the pH was adjusted to 7.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt and AcOEt-MeOH (9:1) to give **14** (15 mg, 0.10 mmol, 3%) as a beige solid.

*4.1.3.8. 2-Ethylamino-4,6-dimethyl-5-pyrimidinol **15**.* Thermic procedure: *N*-ethylguanidine hydrosulfate (500 mg, 3.67 mmol). After cooling at rt, the pH was adjusted to 7.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt-cHx (7:3) and AcOEt to give **15** (165 mg, 0.99 mmol, 27%) as a white solid.

Microwave procedure: After cooling at rt, the pH was adjusted to 7.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt-cHx (7:3) and AcOEt to give **15** (240 mg, 1.42 mmol, 40%) as a white solid, mp: 122–123 °C. Found: C, 57.55; H, 7.73; N, 23.73. Calc. for $C_8H_{13}N_3O$: C, 57.47; H, 7.84; N, 25.13%; $^1$H (400 MHz, $CD_3OD$) $\delta$ (ppm): 3.35 (2 H, q, *J* 7.2, $CH_2$), 2.30 (6 H, s, 2 × $CH_3$), 1.19 (3 H, t, *J* 7.2, $CH_3$); $^{13}$C (100 MHz, $CD_3OD$) $\delta$ (ppm): 158.1 ($C_{IV}$-OH), 157.7 (2×$C_{IV}$-$CH_3$), 140.6 ($C_{IV}$-NH), 37.6 (2×$CH_3$), 18.9 ($CH_2$), 15.5 ($CH_3$); HRMS (ESI) calc. for $C_8H_{14}N_3O$: $[M + H]^+$ 168.1137, found 168.1137.

Comparison thermic procedure/microwave irradiation under the same other conditions: To a commercial aqueous solution of MG (40%) (2.21 mL, 14.7 mmol), *N*-ethylguanidine hydrosulfate (500 mg, 3.67 mmol) was added and the mixture was heated at 80 °C for 1 h. Then 3 M aqueous NaOH (17 mL) was added and the solution was heated at 80 °C for 1 h. After cooling at rt, the pH was adjusted to 7.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt-cHx (7:3) and AcOEt to give **15** (90 mg, 0.54 mmol, 15%) as a white solid.

*4.1.3.9. 2-Dimethylamino-4,6-dimethyl-5-pyrimidinol **16**.* Thermic procedure: *N*-dimethylguanidine hydrosulfate (503 mg, 3.67 mmol) with pH kept around 6. After cooling at rt, the pH was adjusted to 7.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt-cHx (7:3) and AcOEt to give **16** (155 mg, 0.93 mmol, 25%) as a beige solid.

Microwave procedure: After reaction and cooling at rt, the pH was adjusted to 7.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt-cHx (7:3) and AcOEt to give **16** (166 mg, 1 mmol, 27%) as a beige solid, mp: 146–148 °C (149–151 °C, [19]). $^1$H (400 MHz, $CD_3OD$) $\delta$ (ppm): 3.20 (6 H, s, 2 × $CH_3$), 2.41 (6 H, s, 2 × $CH_3$); $^{13}$C (100 MHz, $CD_3OD$) $\delta$ (ppm): 158.7 ($C_{IV}$-OH), 157.2 (2×$C_{IV}$-$CH_3$), 140.0 ($C_{IV}$-$N(CH_3)_2$), 38.2 (2 × $CH_3$), 19.1 (2 × $CH_3$); HRMS (ESI) calc. for $C_8H_{14}N_3O$: $[M + H]^+$ 168.1137, found 168.1137.

*4.1.3.10. 2-Thio-4,6-dimethyl-5-pyrimidinol **17**.* Thermic procedure: After cooling at rt, the pH was adjusted to 6.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt-cHx (7:3) and AcOEt to give **17** (95 mg, 0.61 mmol, 17%) as a yellow solid.

Microwave procedure: After reaction and cooling at rt, the pH was adjusted to 6.5 by addition of 3 M aqueous HCl and, then, extracted with AcOEt. After evaporation, the residue was chromatographed on silica gel eluting with AcOEt-cHx (7:3) and AcOEt to give **17** (84 mg, 0.54 mmol, 15%) as a yellow solid, mp: 186 °C (dec.). $^1$H (400 MHz, DMSO d6) $\delta$ (ppm): 2.30 (6 H, s, $CH_3$); $^{13}$C (100 MHz, DMSO d6) $\delta$ (ppm): 155.8 ($C_{IV}$-OH), 155.0 (2 × $C_{IV}$-$CH_3$), 146.0 ($C_{IV}$-SH), 19.0 (2 × $CH_3$); HRMS (ESI) calc. for $C_6H_9N_2OS$: $[M + H]^+$ 157.0436, found 157.0437; LRMS (ESI) calc. for $C_{12}H_{15}N_4O_2S_2$: $[2M + H]^+$ 311.5, found 311.1.

### 4.2. Biological activity: inhibition of CFTR channels in wt-CHO cells, iodide efflux assay

Screening of small molecules and concentration response curves were determined by measuring the rate of iodide ($^{125}$I) efflux with a high-capacity robotic system (MultiProbe II EXT; PerkinElmer Life and Analytical Sciences, Courtaboeuf, France) adapted to the determination of iodide efflux as described previously [30–32]. Our protocol of screening is as follows. CHO cells overexpressing wild-type CFTR (wt-CFTR) were cultured in multiwell plates and incubated at 37 °C in Krebs' solution containing 1 μM KI and 1 μCi of $Na^{125}I$/mL (PerkinElmer Life and Analytical Sciences, Boston, MA) during 30 min to permit $^{125}$I to reach equilibrium. The first three aliquots were used to establish a stable baseline in cold Krebs' buffer (from $t_0$ to $t_2$). A medium containing the appropriate drug was then used for the remaining aliquots from $t_3$ to $t_8$. Residual radioactivity was extracted at the end of the experiment with a mixture of 0.1 N NaOH and 0.1% SDS and determined using a gamma counter (Cobra II; PerkinElmer Life and Analytical Sciences). The fraction of initial intracellular $^{125}$I lost during each time point was determined, and time-dependent rates (*k* peak rate, per minute) of $^{125}$I efflux were calculated from the following equation: $k = \ln(^{125}It_1/^{125}It_2)/(t_1 - t_2)$, where $^{125}It$ is the intracellular $^{125}$I at time *t*, and $t_1$ and $t_2$ are successive time points [31]. Relative rates were calculated as $k_{peak} - k_{basal}$ (per minute), i.e., the maximal value for the time-dependent rate ($k_{peak}$, per minute) excluding the third point used to establish the baseline ($k_{basal}$, per minute). Concentration-dependent activation curves were constructed as a percentage of maximal activation (set at 100%) transformed from the calculated relative rates. CFTR-dependent iodide efflux was stimulated by forskolin. Other details have been described elsewhere [30,32].

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