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Il Farmaco 58 (2003) 961–970
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2-(Dialkylamino)-4H-1-benzopyran-4-one derivatives modify chloride conductance in CFTR expressing cells

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Received 11 December 2002; accepted 6 March 2003

Abstract

Some 2-(diethylamino)-7-hydroxy-4H-1-benzopyran-4-one derivatives, potentially useful as activators of the cystic fibrosis transmembrane conductance regulator (CFTR), were prepared. The synthesized compounds were tested, together with others 2-(dialkylamino)-7-hydroxybenzopyran-4-one derivatives, by measuring their capacity to modify the kinetics of iodide influx in Fisher rat thyroid cells expressing wild type CFTR and the halide-sensitive yellow fluorescent protein. Among the tested compounds the dinitrile derivatives **8** and **9** are endowed with an activity comparable to the reference compound apigenin.

Keywords: Cystic fibrosis; CFTR; Benzopyran-4-one derivatives

1. Introduction

Cystic fibrosis (CF) is a genetic disease due to an alteration of a protein named cystic fibrosis transmembrane conductance regulator (CFTR) which acts as a cAMP-regulated Cl^- channel [1]. This disorder affects one in 2000–3000 new-borns and is due to severe impairment of electrolyte and fluid secretion in lung, pancreas and other organs. Progressive loss of lung function by bacterial colonization and consequent inflammation is a major cause of death in CF patients. Up to date, more than 800 mutations of the gene have been found. The most common being the deletion of phenylalanine 508 (DF-508).

The search for substances potentially useful in the treatment of the primary defect in CF is still in its infancy. Nevertheless, some classes of organic compounds have been found that activate native and mutant CFTR [2,3]. Among the most interesting modifiers of CFTR channel activity, we must cite purine derivatives (CPX, **1**) and flavone/isoflavone derivatives (genistein, **2**, and apigenin, **3**) (Fig. 1).

A recent screening of a lead-based chemical library has identified 7,8-benzoflavones (UCCF-029, **4**) as potent activators of CFTR Cl^- conductance [4].

Due to our interest in benzopyran-4-one derivatives, and made curious by the intriguing occurrence of benzopyran moiety in molecules active on CFTR, we desired to verify the ability of 2-(diethylamino)-7-hydroxychromone and its derivatives to modulate the CFTR-dependent anion transport.

Other hydroxybenzopyrans and substituted naphthopyrans will be the object of coming investigations in this field.

2. Chemistry

In order to amplify the bulk of 2-(dialkylamino)benzopyran-4-ones to test as CFTR modifiers, we selected the 2-(diethylamino)-7-hydroxychromone **5d** as lead compound to produce and test a reasonable number of derivatives.

7-Hydroxychromone **5d** was treated with *N*,*N*-diethylaminoethyl chloride in anhydrous potassium carbonate

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doi:10.1016/S0014-827X(03)00155-1

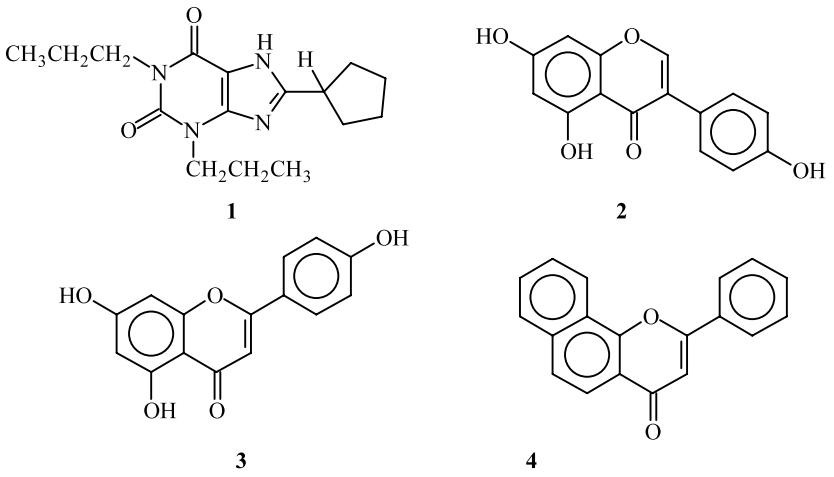
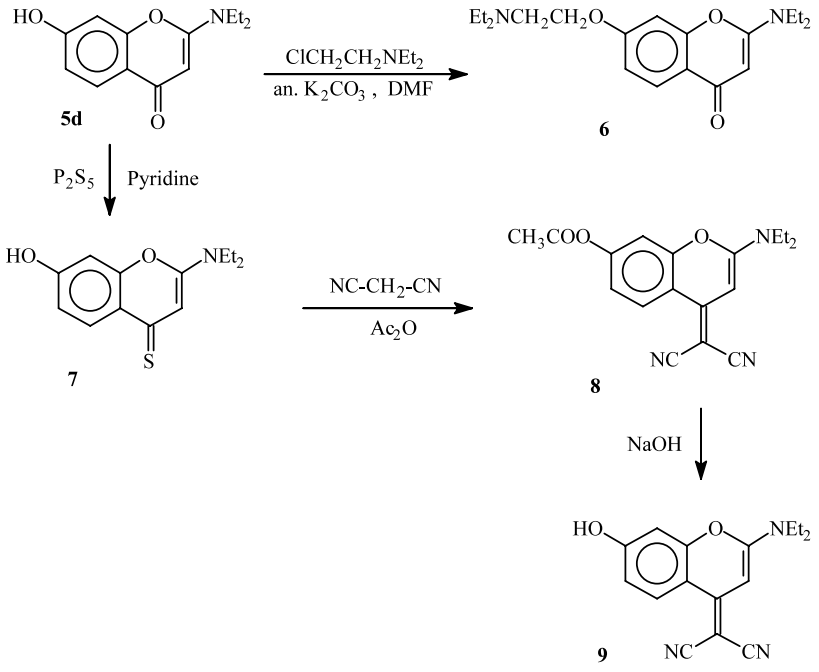


Fig. 1. Structures of CFTR ion channel modifiers: (**1**) CPX, (**2**) Genistein, (**3**) Apigenin, (**4**) UCCF-029.

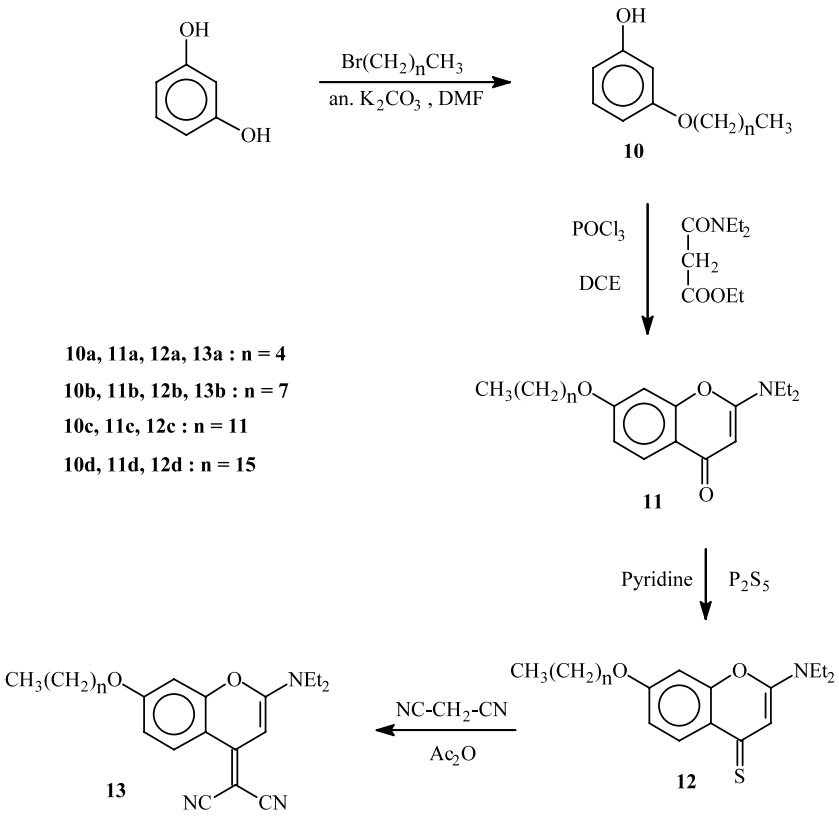
and *N*,*N*-dimethylformamide (DMF) to obtain the *N*,*N*-diethylaminoethyl derivative **6**. Then, compound **5d** was allowed to react with phosphorus pentasulfide in refluxing pyridine to yield the 4-thiochromone derivative **7**; finally, this derivative was condensed with malononitrile in the presence of acetic anhydride at 110 °C to give the 4H-chromene derivative **8**. The hydrolysis of this latter in alkaline medium afforded the corresponding 7-hydroxy derivative **9** (see Scheme 1).

In order to obtain more hydrophobic chains in the position 7 of 1H-benzopyran-4-ones, instead to alkylate the 7-hydroxychromone **5d**, we preferred to synthesize suitable starting phenols **10a**–**d**. This pathway permits to avoid the use of the expensive hydriodic acid as, normally, we obtained **5d** by demethylation of the corresponding 7-methoxyderivative [5]. Therefore, resorcinol was treated with an equimolar amount of bromoalkyl derivative in the presence of anhydrous potassium carbonate and DMF to yield 3-alkyloxyphenol (**10**). In turn, 2-(diethylamino) chromones **11** were prepared by a cyclocondensation reaction between **10** and 3-(diethylamino)-3-oxo-propanoic acid ethyl ester in the presence of phosphorus oxychloride using 1,2-dichloroethane (DCE) as solvent, as previously reported for similar compounds [6,7] (see Scheme 2).

Compounds **11** were allowed to react with phosphorus pentasulfide in refluxing pyridine to yield the 4-thiochromone derivatives **12**; then these derivatives were condensed with malononitrile in the presence of acetic



Scheme 1.



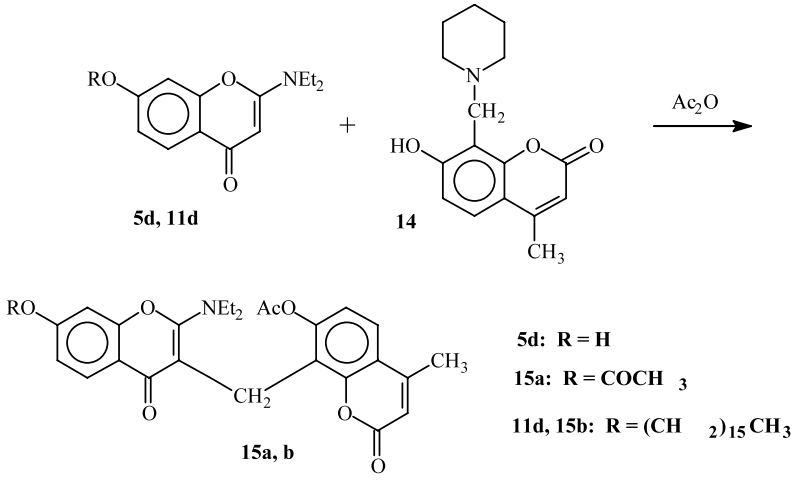
Scheme 2.

anhydride at 110 °C to give the 4H-chromene derivatives **13** (see Scheme 2).

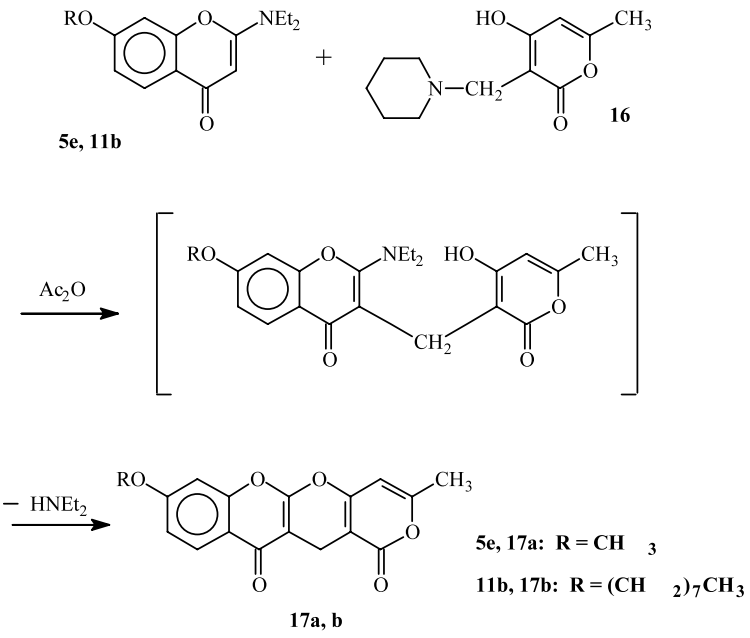
Two 1H-benzopyran-4-ones (**5d**, **11d**) were treated in acetic anhydride with the Mannich base **14**. As previously reported such reaction affords in good yield the unsymmetrical methylene derivatives **15** [8,9] (see Scheme 3).

When two 1H-benzopyran-4-ones (**5e**, **11b**) were treated with the 3-piperidinomethyl-4-hydroxy-6-methylpyran-2-one (**16**) the reaction pattern was modified in respect to the previously reported Scheme 3. In fact, the unsymmetrical methylene derivatives spontaneously loose the diethylamine yielding the polycyclic derivatives **17** (see Scheme 4). This behavior was already observed when, instead of the α-pyrone derivative, 4-hydroxycoumarin derivatives were used. However, in the cited case, the intermediate was easily recovered and the next cyclization occurred under strong conditions (using boiling acetic acid) [10].

Compounds **6**, **11**, **15**, **16** and **17** are white crystals; compounds **7** and **12** are yellow crystals; compounds **8**, **9** and **13** are yellow–orange crystals. The structures of all



Scheme 3.



Scheme 4.

synthesized compounds are in agreement with elemental analyses and spectral data (see Section 3).

3. Experimental protocols

Melting points were determined using a Fisher–Johns apparatus and are uncorrected. Microanalyses were carried out on a Carlo Erba 1106 elemental analyzer. The results of elemental analysis were within $\pm 0.3\%$ of the theoretical value. ^{1}H NMR spectra were performed on a Hitachi Perkin–Elmer R 600 (60 MHz) spectrometer using TMS as internal standard ($\delta = 0$). IR spectra were recorded on a Perkin–Elmer 398 spectrophotometer.

3.1. 2-(Diethylamino)-7-(2-diethylamino)ethyloxy-4H-1-benzopyran-4-one (6)

To 1.0 g (4.3 mmol) of 2-(diethylamino)-7-hydroxy-4H-1-benzopyran-4-one (**5d**) dissolved in 15 ml of DMF, 1.25 g of anhydrous potassium carbonate and 0.78 g (4.5 mmol) of diethylaminoethyl chloride were added and the resulting mixture was heated at 120 °C for 6 h. The final solution was poured onto crushed ice and the solid, which separated out, was extracted three times with chloroform. The pooled organic extracts were treated with anhydrous sodium sulfate and filtered. The solid was then crystallized from acetone/light petroleum ether yielding the chromone **6**. M.p. 75–76 °C; 30.3% yield. IR (KBr) ν (cm^{-1}): 1610, 1625, 2881, 2940, 2976. ^{1}H NMR (δ, $CDCl_3$): 0.83–1.54 (m, 12H, CH_3), 2.40–3.10 (m, 6H, $OCH_2CH_2N(CH_2)_2$) 3.48 (q, 4H, 2-N–CH_2), 4.16 (t, 2H, OCH_2), 5.40 (s, 1H, H-3), 6.66–7.09 (m, 2H, H-6, 8), 8.10 (d, 1H, H-5). *Anal.* $C_{19}H_{28}N_2O_3$: C, H, N.

3.2. 2-(Diethylamino)-7-hydroxy-4H-1-benzopyran-4-thione (7)

To 1.0 g (4.3 mmol) of 2-(diethylamino)-7-hydroxy-4H-1-benzopyran-4-one (**5d**) dissolved in 7 ml of pyridine, 0.8 g of phosphorus pentasulfide were added and the resulting mixture was refluxed for 1 h. The final solution was poured onto crushed ice and the brown solid, which separated out, was stirred for 0.5 h and filtered. The solid was then crystallized from ethanol yielding the thiochromone **7**. M.p. 131–132 °C; 91.3% yield. IR (KBr) ν (cm^{-1}): 1475, 1557, 1593, 1611, 2951, 2974. ^{1}H NMR (δ, $CDCl_3$): 1.39 (t, 6H, CH_3), 3.66 (q, 4H, N–CH_2), 6.79 (s, 1H, H-3), 7.31–7.88 (m, 2H, H-6, 8), 8.80 (d, 1H, H-5). *Anal.* $C_{13}H_{15}NO_2S$: C, H, N.

3.3. [2-(Diethylamino)-7-acetoxy-4H-chromen-4-ylidene]malononitrile (8)

A mixture of 1.0 g (4.0 mmol) of thiochromone **7**, 0.55 g (8.0 mmol) of malononitrile and 8 ml of freshly distilled acetic anhydride was heated at 110–115 °C for 1 h cooled and poured onto crushed ice. After stirring for a 30 min the brown solid which separated out was collected, washed with water and recrystallized from ethanol yielding the 4H-chromene **8**. M.p. 136–137 °C; 44.8% yield. IR (KBr) ν (cm^{-1}): 1606, 1625, 1767, 2177, 2198, 2937, 2984, 3073. ^{1}H NMR ($CDCl_3$): 1.33 (t, 6H, N–CH_2CH_3), 2.34 (s, 3H, CH_3CO), 3.60 (q, 4H, N–

CH_2CH_3), 5.91 (s, 1H, H-3), 6.93–7.40 (m, 2H, H-6, 8), 8.92 (d, 1H, H-5). *Anal.* $C_{18}H_{17}N_3O_3$: C, H, N.

3.4. [2-(Diethylamino)-7-hydroxy-4H-chromen-4-ylidene]malononitrile (**9**)

A mixture of 0.56 g (2.0 mmol) of 4H-chromene **8** and 2.0 mmol of NaOH in 10 ml of water was heated at 60 °C for 4 h. At the end, the solution was acidified with 6M HCl. The resulting precipitate was filtered off and crystallized from ethyl acetate yielding the 7-hydroxy derivative **9**. M.p. 289–290 °C; 32.6% yield. IR (KBr) ν (cm^{-1}): 1606, 2183, 2201, 2924, 2950, 3188. ^1H NMR (δ, DMSO): 1.21 (t, 6H, CH_3), 3.68 (q, 4H, N–CH_2), 5.68 (s, 1H, H-3), 6.84–7.04 (m, 2H, H-6, 8), 8.54 (d, 1H, H-5). *Anal.* $C_{15}H_{15}N_3O_2$: C, H, N.

3.5. 3-Alkyloxyphenol (**10**)

To 4.0 g (0.036 mol) of resorcinol dissolved in 10 ml of DMF, 8.0 g of anhydrous potassium carbonate and 0.036 mol of suitable bromoalkyl derivative were added. The mixture was heated at 110–115 °C for 5 h under a stream of nitrogen. The final mixture was cooled, poured onto crushed ice (adjusting the pH to 7) and stirred for 30 min. The aqueous solution was extracted several times with chloroform. The pooled organic extracts were counter extracted with 2N NaOH. The alkaline solution was acidified with 6M HCl and the oil obtained was distilled in vacuo. The following products were obtained:

3-*n*-Pentyloxyphenol (**10a**) (b.p. 140 °C/5 mm Hg) [11]
3-*n* Octyloxyphenol (**10b**) (b.p. 170 °C/5 mm Hg) [11]
3-*n*-Dodecyloxyphenol (**10c**) (b.p. 180 °C/3 mm Hg) [12]
3-*n*-Hexadecyloxyphenol (**10d**) (b.p. 210 °C/3 mm Hg) [12]

3.6. 2-(Diethylamino)-7-alkyloxy-4H-1-benzopyran-4-one (**11**)

In an ice cooled flask, protected from moisture with a calcium chloride drying tube, 7.0 ml (78.0 mmol) of phosphorus oxychloride were added drop wise with stirring to 10.30 g (55.0 mmol) of 3-(diethylamino)-3-oxo-propanoic acid ethyl ester [5]. After the addition, the mixture was removed from the ice bath and maintained at room temperature for 0.5 h. To the resulting yellow mixture, a solution of 9.56 g (50.0 mmol) of 3-alkyloxyphenol (**10**) in 40 ml of DCE was slowly added with stirring. The reaction mixture was then heated for 5 h at reflux. After cooling, a solution of 68 g of sodium acetate trihydrate in 200 ml of water was added and the mixture was then heated for 1.5 h at 70 °C. After cooling, the organic phase was discarded and the aqueous one was extracted three times with chloroform. The pooled organic extracts were washed with water, dried and evaporated under reduced pressure giving dark red oil. The oil was stirred at room temperature for 2 h with 200 ml of 2 N NaOH and 50 ml of light petroleum ether. The obtained solid was filtered off and washed with water. The crude product was crystallized from ethyl acetate obtaining **11**.

The following products were obtained:

3.6.1. 2-(Diethylamino)-7-n-pentyloxy-4H-1-benzopyran-4-one (**11a**)

M.p. 92–93 °C; 16% yield. IR (KBr) ν (cm^{-1}): 1598, 1617, 2870, 2936, 2954, 2966. ^1H NMR (δ, $CDCl_3$): 0.95–1.61 (m, 15H, N–CH_2CH_3, $(CH_2)_3CH_3$), 3.47 (q, 4H, N–CH_2), 4.04 (t, 2H, OCH_2), 5.37 (s, 1H, H-3) 6.63–7.10 (m, 2H, H arom), 8.10 (d, 1H, H arom). *Anal.* $C_{18}H_{25}NO_3$: C, H, N.

3.6.2. 2-(Diethylamino)-7-n-octyloxy-4H-1-benzopyran-4-one (**11b**)

M.p. 87–88 °C; 18% yield. IR (KBr) ν (cm^{-1}):1601, 1620, 2863, 2942, 2955, 2972. ^1H NMR ($CDCl_3$): 0.80–1.92 (m, 21H, N–CH_2CH_3, $(CH_2)_6CH_3$), 3.48 (q, 4H, N–CH_2), 4.04 (t, 2H, OCH_2), 5.39 (s, 1H, H-3), 6.72–7.04 (m, 2H, H-6, 8), 8.10 (d, 1H, H-5). *Anal.* $C_{21}H_{31}NO_3$: C, H, N.

3.6.3. 2-(Diethylamino)-7-n-dodecyloxy-4H-1-benzopyran-4-one (**11c**)

M.p. 63–64 °C; 15% yield. IR (KBr) ν (cm^{-1}): 1472, 1540, 1603, 1625, 2851, 2916. ^1H NMR (δ, $CDCl_3$): 0.74–1.60 (m, 29H, N–CH_2CH_3, $(CH_2)_{10}CH_3$), 3.46 (q, 4H, N–CH_2), 3.98 (t, 2H, OCH_2), 5.40 (s, 1H, H-3), 6.44–7.03 (m, 2H, H-6, 8), 8.12 (d, 1H, H-5). *Anal.* $C_{25}H_{39}NO_3$: C, H, N.

3.6.4. 2-(Diethylamino)-7-n-hexadecyloxy-4H-1-benzopyran-4-one (**11d**)

M.p. 82–83 °C; 15% yield. IR (KBr) ν (cm^{-1}): 1473, 1540, 1605, 1630, 2851, 2916. ^1H NMR (δ, $CDCl_3$): 0.80–1.75 (m, 37H, N–CH_2CH_3, $(CH_2)_{14}CH_3$), 3.45 (q, 4H, N–CH_2), 4.07 (t, 2H, OCH_2), 5.40 (s, 1H, H-3), 6.35–6.90 (m, 2H, H-6, 8), 8.05 (d, 1H, H-5). *Anal.* $C_{29}H_{47}NO_3$: C, H, N.

3.7. 2-(Diethylamino)-7-n-alkyloxy-4H-1-benzopyran-4-thiones (**12**)

To 3.3 mmol of 2-(diethylamino)-7-alkyloxy-4H-1-benzopyran-4-one **11** dissolved in 7 ml of pyridine, 0.8 g of phosphorus pentasulfide were added and the resulting mixture was refluxed for 1 h. The final solution was poured onto crushed ice and the brown solid, which separated out, was stirred for 0.5 h and filtered. The

solid was then crystallized from ethanol yielding yellow crystals.

The following products were obtained:

*3.7.1. 2-(Diethylamino)-7-n-pentyloxy-4H-1-benzopyran-4-thione (**12a**)*

M.p. 135–136 °C; 73.5% yield. IR (KBr) ν (cm^{-1}): 1622, 2857, 2938, 2955, 2969 ^{1}H NMR (δ, $CDCl_3$): 0.95–1.61 (m, 15H, N–CH_2CH_3, $(CH_2)_3CH_3$), 3.54 (q, 4H, N–CH_2), 4.04 (t, 2H, OCH_2), 6.63–7.10 (m, 3H, H-3, 6, 8), 8.72 (d, 1H, H-5). *Anal.* $C_{18}H_{25}NO_2S$: C, H, N.

*3.7.2. 2-(Diethylamino)-7-n-octyloxy-4H-1-benzopyran-4-thione (**12b**)*

M.p. 144–145 °C; 71.2% yield. IR (KBr) ν (cm^{-1}): 1479,1557,1594,1620, 2851, 2921, 2939, 2980. ^{1}H NMR (δ, $CDCl_3$): 0.92–1.87 (m, 21H, N–CH_2CH_3, $(CH_2)_6CH_3$), 3.54 (q, 4H, N–CH_2), 4.06 (t, 2H, OCH_2), 6.70–7.12 (m, 3H, H-3, 6, 8), 8.65 (d, 1H, H-5). *Anal.* $C_{21}H_{31}NO_2S$: C, H, N.

*3.7.3. 2-(Diethylamino)-7-n-dodecyloxy-4H-1-benzopyran-4-thione (**12c**)*

M.p. 142–143 °C; 67.9% yield. IR (KBr) ν (cm^{-1}): 1620, 2848, 2921, 2980. ^{1}H NMR (δ, CF_3COOD): 0.93–1.68 (m, 29H, N–CH_2CH_3, $(CH_2)_{10}CH_3$), 3.79 (q, 4H, N–CH_2), 4.24 (t, 2H, OCH_2), 6.82–7.48 (m, 3H, H-3, 6, 8), 7.83 (d, 1H, H-5). *Anal.* $C_{25}H_{39}NO_2S$: C, H, N.

*3.7.4. 2-(Diethylamino)-7-n-hexadecyloxy-4H-1-benzopyran-4-thione (**12d**)*

M.p. 134–135 °C; 46.3% yield. IR (KBr) ν (cm^{-1}): 1479, 1555, 1593, 1620, 2848, 2922. ^{1}H NMR (δ, CF_3COOD): 0.97–1.76 (m, 37H, N–CH_2CH_3, $(CH_2)_{14}CH_3$), 3.75–4.50 (m, 6H, N–CH_2, OCH_2), 6.80–7.40 (m, 3H, H-3, 6, 8), 8.00 (d, 1H, H-5). *Anal.* $C_{29}H_{47}NO_2S$: C, H, N.

*3.8. [2-(Diethylamino)-7-alkyloxy-4H-chromen-4-ylidene]malononitriles (**13**)*

A mixture of 4.0 mmol of thiochromones **12**, 0.55 g (8.0 mmol) of malononitrile and 8 ml of freshly distilled acetic anhydride was heated at 110–115 °C for 1 h, cooled and poured onto crushed ice. After stirring for a 30 min the brown solid which separated out was collected, washed with water and recrystallized from ethanol yielding orange crystals.

The following products were obtained:

*3.8.1. [2-(Diethylamino)-7-n-pentyloxy-4H-chromen-4-ylidene]malononitrile (**13a**)*

M.p. 141–142 °C; 33.8% yield. IR (KBr) ν (cm^{-1}): 1597, 1628, 2179, 2196, 2875, 2944, 2962. ^{1}H NMR (δ, $CDCl_3$): 0.88–1.65 (m, 15H, N–CH_2CH_3, $(CH_2)_3CH_3$), 3.56 (q, 4H, N–CH_2), 4.07 (t, 2H, OCH_2), 5.80 (s, 1H, H-3), 6.67–6.90 (m, 2H, H-6, 8), 8.78 (d, 1H, H-5). *Anal.* $C_{21}H_{25}N_3O_2$: C, H, N.

*3.8.2. [2-(Diethylamino)-7-n-octyloxy-4H-chromen-4-ylidene]malononitrile (**13b**)*

M.p. 92–93 °C; 45.2% yield. IR (KBr) ν (cm^{-1}): 1593, 1627, 2182, 2197, 2852, 2923. ^{1}H NMR (δ, $CDCl_3$): 0.85–1.70 (m, 21H, N–CH_2CH_3, $(CH_2)_6CH_3$), 3.58 (q, 4H, N–CH_2), 4.09 (t, 2H, OCH_2), 5.80 (s, 1H, H-3), 6.73–7.00 (m, 2H, H-6, 8), 8.78 (d, 1H, H-5). *Anal.* $C_{24}H_{31}N_3O_2$: C, H, N.

*3.9. 8-[2′-(Diethylamino)-7′-alkyloxychromon-3′-yl]methyl-7-acetoxy-4-methylcoumarin (**15**)*

In a 100-ml flask, protected from moisture with a calcium chloride tube, 1.0 mmol of 2-(dialkylamino)-benzopyran-4-one (**5d** or **11d**) was dissolved in 5 ml of freshly distilled acetic anhydride. Then 1.0 mmol of Mannich base **14** [8] was added, and the mixture was heated at 95 °C for 1.5 h, with stirring. At the end, the cooled solution was poured onto crushed ice and water, and stirred for 2 h obtaining a solid residue. The solid was collected by filtration, washed with water and crystallized from ethyl acetate.

The following products were obtained:

*3.9.1. 8-[2′-(Diethylamino)-7′-acetoxychromon-3′-yl]methyl-7-acetoxy-4-methylcoumarin (**15a**)*

M.p. 209–210 °C; 74.5% yield. IR (KBr) ν (cm^{-1}): 1596, 1613, 1725, 1760, 2933, 2972. ^{1}H NMR (δ, $CDCl_3$): 0.94 (t, 6H, N–CH_2CH_3), 2.10 (s, 3H, 7-$OCOCH_3$), 2.26 (s, 3H, 7′-$OCOCH_3$), 2.40 (s, 3H, 4-CH_3), 3.30 (q, 4H, N–CH_2CH_3), 4.15 (s, 2H, CH_2 bridge), 6.27 (s, 1H, H-3), 6.86–7.56 (m, 4H, H-5, 6, 6′, 8′), 8.21 (d, 1H, H-5′). *Anal.* $C_{28}H_{27}NO_8$: C, H, N.

*3.9.2. 8-[2′-(Diethylamino)-7′-n-hexadecylchromon-3′-yl]methyl-7-acetoxy-4-methylcoumarin (**15b**)*

M.p. 120–121 °C; 23.6% yield. IR (KBr) ν (cm^{-1}): 1537, 1582, 1603, 1733, 1770, 2849, 2920, 2956. ^{1}H NMR (δ, $CDCl_3$): 0.94–1.62 (m, 37H, N–CH_2CH_3, $(CH_2)_{14}CH_3$), 2.08 (s, 3H, CH_3CO), 2.41 (s, 3H, 4-CH_3), 3.23 (q, 4H, N–CH_2), 4.05 (t, 2H, OCH_2), 4.20 (s, 2H, CH_2 bridge), 6.29 (s, 1H, H-3), 6.70–7.50 (m, 4H, H-5, 6, 6′, 8′), 8.12 (d, 1H, H-5′). *Anal.* $C_{42}H_{57}NO_7$: C, H, N.

*3.10. 4-Hydroxy-6-methyl-3-piperidinomethyl-2-pyrone (**16**)*

To 20.0 mmol of 4-hydroxy-6-methyl-2-pyrone dissolved in 50 ml of ethanol, 20.0 mmol of piperidine and 2.0 ml of 40% formaldehyde were added. The resulting mixture was refluxed for 6 h. After cooling, the solvent was evaporated under reduced pressure. The pale yellow oil obtained was treated with cool acetone, leaving a

white solid which was crystallized from ethanol obtaining the Mannich base **16**, m.p. 165–166 °C, 74.8% yield. IR (KBr) ν (cm^{-1}): 1525, 1677, 1705, 2940, 2992. ^1H NMR (δ, CF_3COOD): 1.60–1.21 (m, 6H, β+γ-piperidine CH_2), 2.39 (s, 3H, CH_3), 3.42–3.88 (m, 4H, α-piperidine CH_2), 4.27 (s, 2H, CH_2 bridge), 6.46 (s, 1H, H-5). *Anal.* $C_{12}H_{17}NO_3$: C, H, N.

*3.11. 1,11,12 H-3-methyl-8-alkyloxy-2,5,6-trioxanaphthacen-1,11-dione (**17**)*

With the same procedure used for the synthesis of compounds **15**, starting from **5e** and the Mannich base **16** and after crystallization from acetone, the following product was obtained:

*3.11.1. 1,11,12H-3-methyl-8-methoxy-2,5,6-trioxanaphthacen-1,11-dione (**17a**)*

M.p. 180–181 °C; 32.5% yield. IR (KBr) ν (cm^{-1}): 1617, 1637, 1670, 1725, 2845, 2943, 3076. ^1H NMR (δ, CF_3COOD): 2.48 (s, 3H, CH_3), 3.52 (s, 2H, CH_2 bridge), 4.03 (s, 3H, OCH_3), 6.50 (s, 1H, H-4), 7.00–7.35 (m, 2H, H-7, 9) 7.96 (d, 1H, H-10). *Anal.* $C_{17}H_{12}O_6$: C, H, N.

With the same procedure used for the synthesis of compounds **15**, starting from **11b** and the Mannich base **16** and after crystallization from ethanol, the following product was obtained:

*3.11.2. 1,11,12H-3-methyl- 7-n-octyloxy-2,5,6-trioxanaphthacen-1,11-dione (**17b**)*

M.p. 254–255 °C; 24.2% yield. IR (KBr) ν (cm^{-1}): 1570, 1640, 1670, 1705, 2853, 2920, 3430. ^1H NMR (δ, $CDCl_3$): 0.89 (t, 3H, CH_3), 1.10–1.95 (m, 15H, CH_2), 3.82–4.20 (m, 4H, OCH_2 and CH_2 bridge), 6.42–6.68 (m, 3H, H arom), 7.13 (d, 1H, H arom). *Anal.* $C_{24}H_{26}O_6$: C, H, N.

4. CFTR assays

CFTR activity was assessed by using the fluorescent method described previously [4,13]. Briefly, cells coexpressing wild type CFTR and the halide-sensitive yellow fluorescent protein YFP-H148Q were plated in 96-well microplates. After 48 h, cells were washed and incubated in 50 µl PBS with or without test compounds or apigenin at 10 µM. After 15 min, cell fluorescence was measured in a Galaxy plate reader (BMG) equipped with high quality filters for the yellow fluorescent protein (500±10 nm for excitation; 535±15 nm for emission) and two syringe pumps for liquid addition. The assay for each well consisted in: (1) 22 s before fluorescence reading: addition of 10 µl of PBS containing forskolin to give a final submaximal concentration of 0.5 µM; (2) continuous measurement of cell fluorescence for 14 s: 2 s in resting conditions, 12 s after addition of 165 µl of a modified PBS solution in which NaCl was replaced with NaI. Data were sampled at 5 Hz and stored on computer disk. CFTR activity was estimated by subtracting the background, normalizing the data for the initial fluorescence in PBS, and fitting the fluorescence curve after I^- addition with a single exponential function. Maximal slope, which is proportional to $d[I^-]/dt$, was then calculated using the Igor software

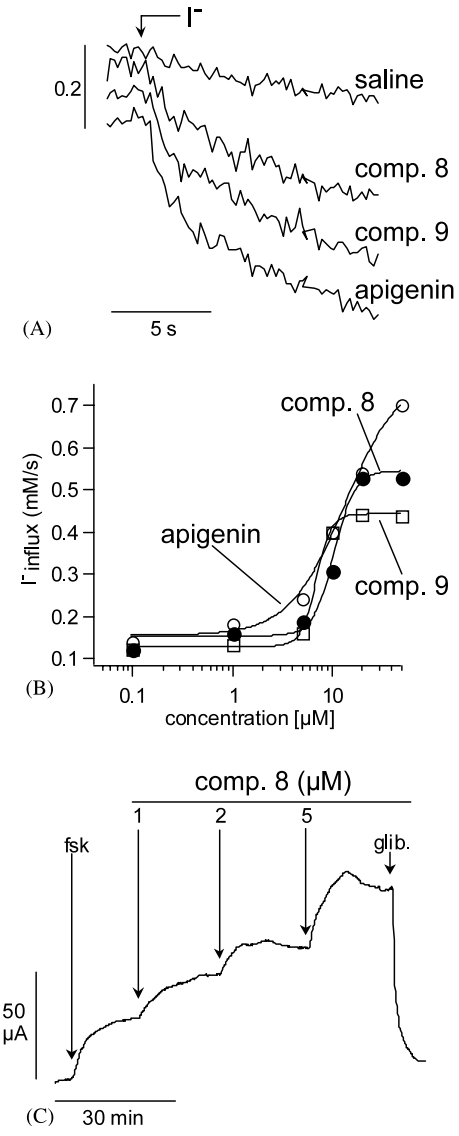
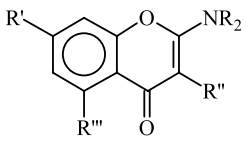


Fig. 2. CFTR activation by 2-(dialkylamino)-7-hydroxy-4H-1-benzopyran-4-one derivatives. (A) Representative traces showing the time course of cell fluorescence measured before and after addition of I^- solution in plate reader measurements. Cells were incubated with physiological saline solution alone or with 10 µM of apigenin or dinitrile derivatives **8** and **9**. (B) Dose–response relationships for active compounds. Each point (mean of three experiments) reports the I^- influx as a function of drug concentration. Data are fitted with Hill equation. (C) Representative short-circuit current experiment showing CFTR activation by compound **8** added at the indicated concentrations in the apical chamber. Forskolin (fsk) and glibenclamide (glib) were added at 0.5 and 400 µM, respectively.

Table 1

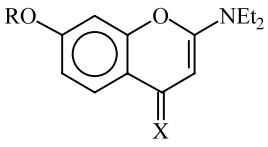


5

Compound	NR_2	R′	R″	R‴	Activity [a]	Reference
5a	NMe_2	OH	H	H	T	[5]
5b	NHEt	OCH_3	H	H	I	[14]
5c	NHEt	OH	H	H	I	[15]
5d	NEt_2	OH	H	H	I	[5]
5e	NEt_2	OCH_3	H	H	I	[5]
5f	NEt_2	CH_3	H	OH	I	[15]
5g	NEt_2	OCH_2CH_3	CHO	H	T	[16]
5h	NEt_2	$O(CH_2)_2CH_3$	H	H	I	[17]
5I	Pyrrolidinyl	OCH_3	H	H	I	[5]
5j	Pyrrolidinyl	H	H	OH	T	[18]
5k	Piperidinyl	OCH_3	H	H	I	[5]
5l	Piperidinyl	OH	H	H	I	[5]
5m	Piperidinyl	H	H	OH	I	[18]
5n	$N(CH_2CH_2OCH_3)_2$	OCH_3	H	H	I	[17]
5o	NEt_2	OCH_2CH_3	[b]	H	T	[16]
5p	NEt_2	$O(CH_2)_4CN$	H	H	T	[19]

T, toxic; I, inactive.
[a] In respect to apigenin (100%).
[b] Morpholinomethyl.

Table 2



6, 7, 8, 9, 11-13

Compound	X	R	Activity [a]
6	O	$CH_2CH_2NEt_2$	I
7	S	H	I
8	$C(CN)_2$	CH_3CO	A (79%)
9	$C(CN)_2$	H	A (102%)
11a	O	$(CH_2)_4CH_3$	I
11b	O	$(CH_2)_7CH_3$	T
11c	O	$(CH_2)_{11}CH_3$	NS
11d	O	$(CH_2)_{15}CH_3$	NS
12a	S	$(CH_2)_4CH_3$	NS
12b	S	$(CH_2)_7CH_3$	NS
12c	S	$(CH_2)_{11}CH_3$	NS
12d	S	$(CH_2)_{15}CH_3$	NS
13a	$C(CN)_2$	$(CH_2)_4CH_3$	I
13b	$C(CN)_2$	$(CH_2)_7CH_3$	I

A, active; T, toxic; I, inactive; NS, insoluble.
[a] In respect to apigenin (100%).

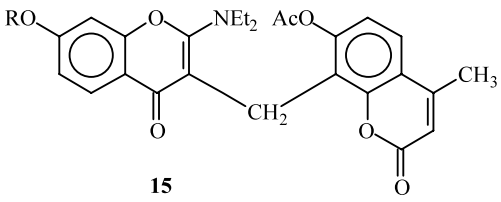
(WaveMetrics, Inc.). Dose–responses were assessed for active compounds and apigenin using different concentrations in the 1–50 μM range. Active compounds were confirmed by measuring the short-circuit current across confluent monolayers of FRT cells expressing CFTR plated on Snapwell permeable supports (Corning Costar) as previously described [13].

5. Results and discussion

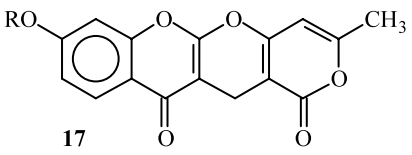
The benzopyrans utilized in this pharmacological screening are shown in Tables 1–3. Table 1 shows compounds previously synthesized (**5a**–**q**). Newly synthesized benzopyran derivatives are instead depicted in Tables 2 and 3 (**6**–**9**, **11**–**13**, **15**, **17**).

Activity of compounds was determined in the plate reader after prestimulation of CFTR with a low concentration of forskolin. Under these conditions, the reference compound apigenin (10 μM) caused a significant increase in CFTR activity as expected (Fig. 2A). Most of compounds were inactive (the slope was indistinguishable from that of cells incubated with PBS alone) or toxic (as evident from the detachment of cells from the well bottom during the assay upon addition of solutions). However, compounds **8** and **9** elicited a CFTR activity which was 79 and 102%, respectively, of that shown by apigenin at the same concentration

Table 3



15



17

Compound	R	Activity[a]	Comp	R	Activity
15a	CH_3CO	NS	**17a**	CH_3	NS
15b	$CH_3(CH_2)_{15}$	NS	**17b**	$(CH_2)_7CH_3$	I

[a] In respect to apigenin (100%). I, inactive; NS, insoluble.

(Fig. 2A). Dose–responses were performed in triplicate and resulting data were fitted with a Hill equation to obtain information on maximal effect and affinity for each active compound. Apigenin caused an estimated maximal I^- influx of 0.75 mM/s with a half effective concentration of 12.6 μM. Compounds **8** and **9** elicited a maximal effect smaller than apigenin (around 0.5 mM/s) whereas the half effective concentration was comparable or lower (10.2 and 7.9 μM, respectively; Fig. 2B).

The ability of new compounds to induce electrogenic activity of CFTR was confirmed in using chamber experiments [13]. CFTR was partially stimulated with forskolin 0.5 μM, as in the plate reader measurements, and subsequently test compounds or apigenin were added at increasing concentrations. Compounds **8** and **9**, as well as apigenin, elicited a dose-dependent increase of short-circuit current which was blocked by the CFTR inhibitor glibenclamide (Fig. 2C).

Though previous studies demonstrated the biological relevance of 2-(dialkylamino)chromones [5–8,14–18], compounds **5** are, in general, devoid of activity on CFTR. The chromones and thiochromones **11** and **12** bearing a long alkyloxy chain are insoluble and, therefore, we cannot express a clear conclusion on their value in this context. The same negative circumstance occurs for more complex derivatives as **15** and **17**. On the other hand, dinitrile derivatives **8** and **9** seem interesting compounds in activating CFTR conductance. Their activity is comparable to that of apigenin and our interest in these new compounds lies in the presence of the dinitrile group. In fact, as long as we can see, this group is not present in substances endowed with this type of activity. It is significant to note that the presence of a long alkyloxy group in position 7 of the benzopyran moiety, as in compounds **13**, causes loss of activity even though the dinitrile substituent is present. As it is likely that the acetyl derivative **8** could be easily hydrolyzed in vivo, the presence of the hydroxy group in position 7, when in the molecule the dinitrile substituent is present, seems to have great importance. This consideration is confirmed from the fact that the 7-hydroxychromones **5a**, **5c**, **5d**, **5l** and 7-hydroxythiochromone **7** are inactive.

Next research will be focused to prove the action of dinitrile group on dihydroxychromones and naphthopyrans.

In conclusion, we have identified a new class of CFTR openers. The enlargement of spectrum of CFTR active compounds is of extreme importance to understand the molecular mechanisms underlying channel regulation and gating. Comparison between various CFTR activators could lead to the identification of consensus structures that could in turn provide information about binding site(s). This will be helpful for synthesis of new effective drugs for the pharmacological correction of CF basic defect.

Acknowledgements

This work was supported by grants from Italian M.I.U.R. and Telethon (Project GP0296Y01).

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