Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2661–2664

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Biological Evaluation of 2,5-Dihydropyrazolo[4,3-*c*]quinolin-3-ones, a Novel Series of PDE 4 Inhibitors with Low Emetic Potential and Antiasthmatic Properties

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Received 18 May 2000; revised 7 September 2000; accepted 18 September 2000

Abstract—A novel series of 2,5-dihydropyrazolo[4,3-*c*]quinolin-3-ones has been prepared. These compounds showed good PDE 4 inhibitory activity and weak affinity for rolipram's binding site. They also exhibited a good anti-inflammatory profile without emetic side effects. © 2000 Published by Elsevier Science Ltd.

Introduction

Selective PDE 4 inhibitors, lacking adverse effects such as emesis, have potential utility in asthma therapy.[1] Following our initial strategy based on the pharmacophore of compounds structurally related to nitraquazone[2] and arofylline,[3] a new series of 2,5-dihydropyrazolo[4,3-*c*]quinolin-3-ones (DHPQ) has been designed and synthesised.

The synthesis, structure–activity relationship (SAR), and the antiasthmatic potential of these new PDE 4 inhibitors are described.

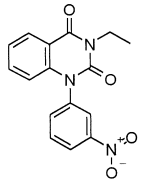
Molecular Modelling Studies

In order to evaluate the 2,5-dihydropyrazolo[4,3-*c*]quinolin-3-ones in the nitraquazone-related pharmacophore, the possible areas of interaction as hydrogen bond acceptors for nitraquazone (represented in purple) and a representative DHPQ (displayed in green, compound **3** in Table 1) have been studied by means of the programme GRID.[4]

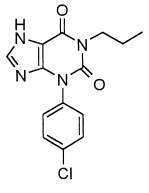
The maps have been obtained using the N1 probe (NH flat amide) and contoured at the level of −4 kcal/mol. Figure 1 shows the interaction pattern of the two molecules derived from the GRID computation. Although the carbonyl group on the right-hand side is situated in different positions, both the maps of interactions and the aromatic rings show good overlap.

Synthesis

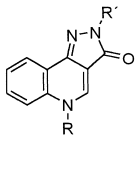
The DHPQs were synthesised according to literature procedures[5] (Scheme 1). Condensation of aniline with ethoxymethylenemalonate diethyl ester yielded the quinolone **26**, which was first treated with phosphorous oxychloride and then with the corresponding alkylated hydrazine, to give DHPQ **14**. Alkylation of **26** afforded the *N*-substituted quinolones **27**, which reacted with phosphorous pentasulfide to yield the thioderivatives **28**. The final DHPQs **1**–**13**, **15**–**25** were obtained by direct reaction of **28** with the corresponding alkylated hydrazine or via initial condensation with hydrazine and subsequent *N*-alkylation.



Nitraquazone



Arofylline



DHPQ

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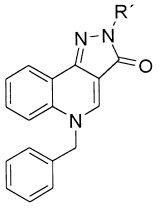
PII: S0960-894X(00)00548-5

Results and Discussion

Structure–activity relationships

Compounds were tested for their inhibitory potencies against isolated guinea pig ventricular PDE 4. Selectivity versus PDE 3, isolated from the same tissue, was also determined. A representative group of compounds was also evaluated for their ability to displace [^{3}H]rolipram from its high-affinity binding site (HARBS) in rat brain membranes. Interaction with this site has been proposed as responsible for the unwanted CNS[6] and emetic side effects[7] of PDE 4 inhibitors. The results are shown in Tables 1 and 2.

Table 1.



Compound	R′	PDE 3[a]	PDE 4[a]	HARBS[a]
1	H	48	37	n.t.[b]
2	Methyl	67	14	n.t.
3	Ethyl	10	3	n.t.
4	Isopropyl	86	1.2	2
5	Butyl	31	2.1	4.7
6	*tert*-Butyl	>100	2.1	5.6
7	Pentyl	120	6.4	n.t.
8	Benzyl	25	7	n.t.
9	Phenethyl	>100	10	n.t.
10	Cyclopentyl	26	0.7	35
11	Norbornyl	>20	1.4	6
12	Cyclobutylmethyl	>200	0.8	5
13	Cyclohexylmethyl	102	24	n.t.
Rolipram		242	0.32	0.006
Nitraquazone		>200	0.05	0.01

[a]IC_{50} (μM).
[b]n.t., not tested.

Table 1 summarises the influence of substitution at the *N*2 position (R′) for a series of compounds with a benzyl at *N*5. Consistent with the nitraquazone pharmacophore,[2] a lipophilic group is required for activity. The best results are obtained with cyclopentyl or cyclobutylmethyl groups (compounds **10** and **12**).

Table 2 shows the effect on activity of the lipophilic group at the *N*5 position (R). Interesting potencies and selectivities are observed with compound **17**, with a cyclohexylmethyl group, and particularly with compound **25** (R: 2-thienylmethyl, R′: cyclopentyl), which has a PDE 4 activity comparable to that of rolipram.

With regard to the rolipram binding site, all DHPQs evaluated have an improved HARBS/PDE 4 ratio with respect to the reference compounds. For example, compound **25** has an improvement in ratio of >100-fold and >10-fold relative to rolipram and nitraquazone, respectively. Further SAR and molecular modelling studies will be carried out in order to ascertain the cause of this improvement.

Activity in asthma models

Table 3 summarises the in vivo results obtained for a selected group of DHPQs. All compounds tested show anti-inflammatory activity in the guinea pig eosinophilia model.[8] By way of contrast, they are only moderately effective in the histamine-induced bronchoconstriction in guinea pig.[9] In order to evaluate the side-effect profile of this new series, the selected DHPQs were tested in an emesis model.[10] Consistent with their favourable HARBS/PDE 4 ratio, these compounds fail to provoke emesis in dog.

In conclusion, we have identified a new series of 2,5-dihydropyrazolo[4,3-*c*]quinolin-3-ones which show PDE 4 inhibitory activities comparable to rolipram and reduced affinity for its high-affinity binding site. They also present a good anti-inflammatory profile and no emetic effects. Compound **25** has been selected for further evaluation.

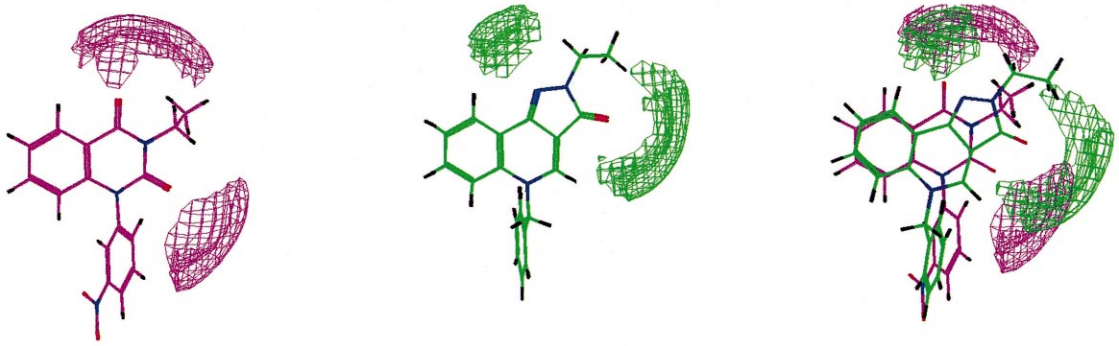
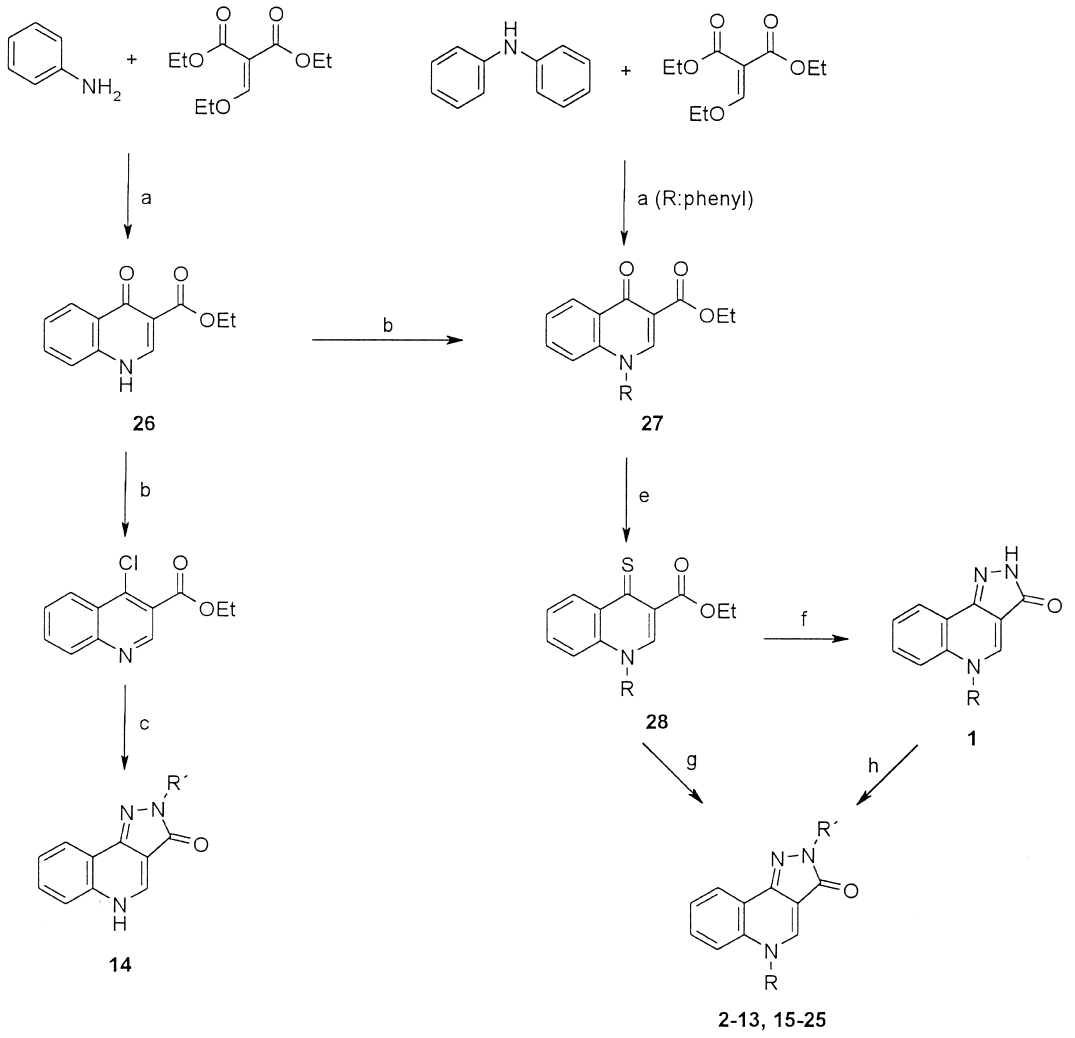
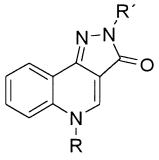


Figure 1. Interaction maps obtained with the N1 probe (NH flat amide) of GRID software for nitraquazone (purple) and DHPQ **3** (green).



Scheme 1. Reagents: (a) 100 °C then PPA; (b) $POCl_3$, reflux; (c) R′-$NHNH_2$, dioxane, reflux; (d) R-X, KI, K_2CO_3, DMF; (e) P_4S_{10}, pyridine, reflux; (f) NH_2NH_2, ethanol, reflux; (g) R′-$NHNH_2$. $C_2O_4H_2$, 2 N NaOH, pyridine, reflux; (h) R′-X, 2 N NaOH, DMF, 50 °C.

Table 2.



Compound	R	R′	PDE 3[a]	PDE 4[a]	HARBS[a]
14	H	Butyl	136	90	n.t.[b]
15	Phenyl	Butyl	68	18	n.t.
5	Benzyl	Butyl	31	2.1	4.7
16	Phenethyl	Butyl	>200	160	n.t.
17	Cyclohexylmethyl	Butyl	111	0.7	1.5
18	Cyclohexylmethyl	*tert*-Butyl	>20	0.5	1.2
19	Cyclohexylmethyl	Cyclopentyl	>20	0.4	0.9
20	*m*-Nitrobenzyl	Butyl	>20	11	n.t.
21	*m*-Aminobenzyl	Butyl	>200	12	n.t.
22	*m*-Chlorobenzyl	Butyl	>20	2.3	14
23	2-Thienylmethyl	Butyl	>20	2.7	50
24	2-Thienylmethyl	*tert*-Butyl	>20	0.8	2
25	2-Thienylmethyl	Cyclopentyl	>20	0.4	1.0
Rolipram			242	0.32	0.006
Nitraquazone			>200	0.05	0.01

[a]IC_{50} (μM).
[b]n.t., not tested.

Table 3.

Compound	Eosinophilia[a]	Bronchodilation[b]	Emetic effect[c]
5	52	>100	>3 (iv)
6	51	n.t.	>10 (po)
17	60	>100	>1 (iv)
22	53	62	>10 (po)
23	56	37	>10 (po)
25	62	83	>3 (iv)
Rolipram	58	4	0.03 (iv)

[a]Guinea pig eosinophilia percentage inhibition at 10 mg/kg po.
[b]Inhibition of histamine induced bronchoconstriction in guinea pig, ED_{50} (μg/kg) iv.
[c]Minimum emetic dose in dogs (mg/kg).

References and Notes

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7. Barnette, M. S.; Torphy, T. J.; Christensen, S. B. *Compounds* WO 00139, 1995.
8. *Guinea pig airway eosinophil infiltration*: Male Dunkin–Hartley guinea-pigs (400±50 g) were sensitised with an injection of ovalbumin (OA, 3 mg/kg, 1 mL/kg ip) plus 5% Freund incomplete adjuvant on days 0 and 3. On day 21 the animals were fasted and the compounds were dosed by oral route (1 mL/kg), 2 h prior to and 6 h after aerosolised antigen exposure (1.5 mg/mL OA, 75 seg in 5 min) to pyrilamine protected animals. Under urethane (1.5 g/kg ip) anaesthesia, 72 h later, a bronchoalveolar lavage was done with saline (4 mL, twice) through an intratracheal polyethylene cannula. Cellular suspensions were centrifuged and extended for microscopic observation after staining (Sangodiff®, Merck). Different leucocyte subtypes and proportions were determined under 1000× magnification. Results are expressed as percentage inhibition of OA-induced eosinophil infiltration.
9. *Bronchodilation in anaesthetised guinea pigs:* Male Dunkin–Hartley guinea pigs (450±50 g) were anaesthetised with sodium pentobarbitone (60 mg/kg ip) and externally ventilated (10 mL/kg, 60 strokes/min) through a tracheal tube with a side branch for measuring airway resistance. After stabilisation, the left jugular vein was infused with histamine (10–20 μg/kg/min) in order to achieve a sustained increase of about 150% in inflation pressure. Compounds were dosed cumulatively through a catheter in the right jugular vein. Results are expressed as percentage of inhibition of histamine-induced bronchospasm.
10. *Emetic effects in conscious dogs:* Trained Beagle dogs (10–15 kg) of either sex were fasted overnight. On the day of the experiment the animals were placed in slings and a Teflon catheter was inserted in the right cephalic vein and connected to a perfusion pump. The compounds were dissolved and administered through a 0.22 μM Millipore filter. Cumulative doses of compounds were infused for 3 min and subsequent observation periods of 30 min were allowed before increasing the dose. This procedure was repeated until any emetic episode occurred. Results are expressed as the minimum emetic dose.