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QUINAZOLINES: COMBINED TYPE 3 AND 4 PHOSPHODIESTERASE INHIBITORS.

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Abstract:

A series of quinazolines has been prepared and evaluated for its ability to inhibit cyclic AMP phosphodiesterase type 3, type 4A, 4B and 4D. The most potent inhibitors showed IC_{50} values in the nanomolar range for type 3 and type 4 isoforms and bind with high affinity to the [^{3}H]rolipram binding site. These quinazolines represent a new family of potent mixed PDE 3 / 4 inhibitors and are expected to have a therapeutic potential. © 1998 Elsevier Science Ltd. All rights reserved.

Isozyme selective phosphodiesterase (PDE) inhibitors may represent a new class of drugs for the treatment of obstructive pulmonary disease (e.g. asthma). PDE 3 inhibitors, and possibly PDE 4 inhibitors possess bronchodilator activity. On the other hand, synthesis and release of inflammatory mediators, chemotaxis and proliferation of inflammatory cells in response to antigen challenge are inhibited by agents that produce an increase in the intracellular concentration of cAMP.[1-3] Increase of cAMP level can be achieved by inhibition of cAMP PDE [3':5'-nucleotidohydrolase, EC 3.1.4.17]. Since the different isoenzymes, that preferentially hydrolyse cAMP, are not uniformly distributed in different cell types,[4,5] they are sensitive to selective inhibitors and are interesting drug targets for a variety of different diseases. The quest of novel antiinflammatory agents has led chemists and pharmacologists to focus on selective inhibitors of PDE 4 because it is the predominant form in human leukocytes.[1-3,5,6] The recent discovery of different cDNA isoforms and splice variants of PDE 4 suggests that the regulation of cAMP metabolism in leukocytes may be more complex than originally thought. Four distinct human isoforms, provisionally designated PDE 4A, 4B, 4C, 4D,[5] have been cloned and expressed.[7-11] An increase in intracellular cAMP induces the synthesis of the A, B, and D isoforms,[6,12] suggesting a role for these enzymes in the long term regulation of cAMP metabolism. Mixed PDE 3 / 4 inhibitors may combine, therefore, both bronchodilator and antiinflammatory properties. Zardaverine [6-

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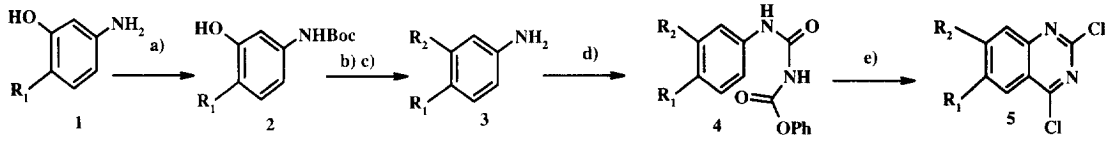
(4-Difluoromethoxy-3-methoxyphenyl)-3[2H]pyridazinone] and AH 21-132, both PDE 3 / 4 inhibitors, exhibit bronchodilator and antiinflammatory properties in animals [13,14] as well as bronchodilator effects in humans.[15,16] Our objective was to develop a series of compounds with a mixed PDE 3 / 4 inhibitor profile based on the quinazoline structure. Quinazolines substituted in position 2 and 4 are well known for their antiinflammatory properties but to date an inhibitory effect on PDE 4 has not been described.

Synthesis: The compounds in this study were synthesized from the key intermediates, 2,4-dichloro-quinazolines (**5a-d**). 2,4-Dichloro-6,7-dimethoxy-quinazoline **5e** was commercially obtained.[17] The synthesis was performed according to previously reported procedures[18,19] and outlined in Scheme 1. 4-Nitrocatechol, starting material for **5d** ($R_1 = R_2 = OCH_2CO_2Et$), was first dialkylated and the nitro function reduced to the amine (H_2, Pd/C, 0.2 bar). The yield of the cyclisation with $POCl_3$ is strongly dependent on the nature of the 6, 7 substitutents (80% range for **5a-c** and 10% for **5d**).

Scheme 1:



1a R_1 = OMe
1b R_1 = OH
1c R_1 = H

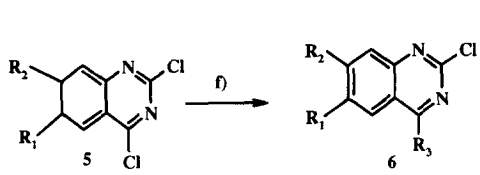
2a R_1 = OMe
2b R_1 = OH
2c R_1 = H

a R_1 = OMe $R_2 = OCH_2Ph$
b R_1 = OMe $R_2 = OCH_2CO_2Et$
c R_1 = H $R_2 = OCH_2CO_2Et$
d $R_1 = R_2 = OCH_2CO_2Et$
e $R_1 = R_2$ = OMe

Reagents and conditions: a) $(Boc)_2O$, NEt_3, r.t., THF. b) Alkyl halide, K_2CO_3, NaI, 2-butanone, reflux. c) CF_3CO_2H, 0°C, pH=7. d) PhOCONCO, THF, r.t. e) $POCl_3$, N,N-dimethylaniline, 110°C, 4h.

Since the position 4 is far more reactive than the position 2, substituents were first introduced in position 4 using the Pd (0) catalysed coupling reaction of the corresponding tin reagent (Scheme 2). An already described four step sequence has been used to prepare the tin reagents.[20] Compounds **6** were obtained with good to excellent yields. Substitution of the position 2 by organotin reagents using the same methodology (compounds **9a-f**) or by alcohols, thiols or amines in the presence of a strong base like NaOH (compounds **9g-m**) was easily performed with yields varying from 50 to 90% (Scheme 3). Substitution in position 2 can be also performed by organozinc reagents (compounds **7a-d** and **10**). Reduction of the protecting group in position 7, compound **11**, followed by appropriate alkylation or a Mitsunobu reaction leads to **12**.

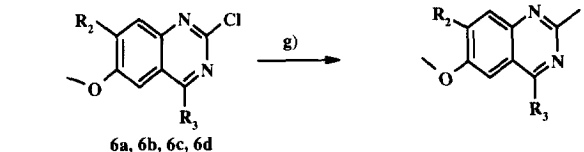
Scheme 2:



	R_1	R_2	R_3
6a	$R_1 = R_2$ = OMe		3,5-dibenzyloxy-phenyl
6b	$R_1 = R_2$ = OMe		3,5-diisopropyloxy-phenyl
6c	$R_1 = R_2$ = OMe		3,5-dicyclopropylmethyloxy-phenyl
6d	OMe	OCH_2CO_2Et	3,5-dicyclopropylmethyloxy-phenyl
6e	OMe	OCH_2Ph	3,5-dicyclopropylmethyloxy-phenyl
6f	OH	OCH_2CO_2Et	3,5-dicyclopropylmethyloxy-phenyl
6g	$R_1 = R_2$ =	OCH_2CO_2Et	3,5-dicyclopropylmethyloxy-phenyl

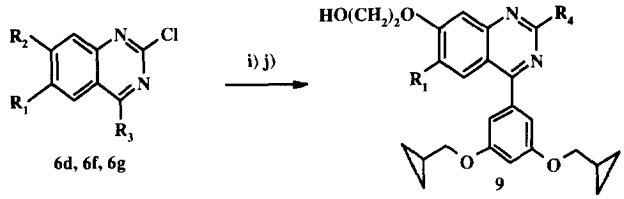
Reagents and conditions: f) R_3SnBu_3, $(PPh_3)_2PdCl_2$, xylene, 110°C, 3h.

Scheme 3:

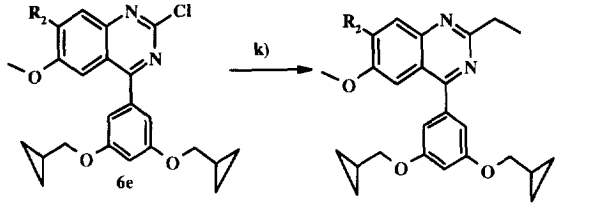


	R_2	R_3
7a	OMe	3,5-dibenzyloxy-phenyl
7b	OMe	3,5-diisopropyloxy-phenyl
7c	OMe	3,5-dicyclopropylmethyloxy-phenyl
7d	OCH_2CO_2Et	3,5-dicyclopropylmethyloxy-phenyl

h) → 8 R_3 = 3,5-dihydroxy-phenyl



	R_1	R_4
9a	OMe	Et
9b	OMe	2-thiophen
9c	OMe	R_3
9d	OMe	n-But
9e	H	Et
9f	R_2	Et
9g	OMe	$O(CH_2)_3OH$
9h	OMe	$NH(CH_2)_3OH$
9i	OMe	$O(CH_2)_2OH$
9j	OMe	N-morpholine
9k	OMe	OMe
9l	OMe	OEt
9m	OMe	SEt



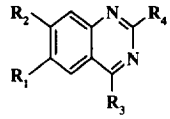
	R_2
10	OCH_2Ph
11	OH
12	$OCH_2CH(CH_2)_2$

h), l)

Reagents and conditions: g) MeLi, $ZnCl_2$, $Pd(PPh_3)_4$, dioxan. h) H_2, Pd/C, ethanol. i) $LiBH_4$, THF, 0°C to r.t. j) R_3SnBu_3, $(PPh_3)_2PdCl_2$ in xylene or ROH (RSH, R_2NH) , NaOH 2N, DMF (40-110°C). k) $Zn(Et)_2$, $Pd(PPh_3)_4$. l) $(CH_2)_2CHCH_2OH$, PPh_3, DEAD.

Results and discussion: Compounds thus prepared were tested for their ability to inhibit PDE 3,[21] 4A, 4B, and 4D.[22] Our results with this series are summarized in Table 1.

Table 1. Inhibitory activity of quinazolines against phosphodiesterases PDE 3, PDE 4A, PDE 4B, PDE 4D.



					PDE 3	PDE 4A	PDE 4B	PDE 4D
No.	**R_1**	**R_2**	**R_3**	**R_4**	**IC_{50}[a]**	**IC_{50}[a]**	**IC_{50}[a]**	**IC_{50}[a]**
8	OMe	OMe	3,5-dihydroxy-phenyl	Me	n.d.	23.5	14	2
7b	OMe	OMe	3,5-diisopropyloxy-phenyl	Me	n.d.	2.3	1.3	1.2
7c	OMe	OMe	3,5-dicyclopropylmethyloxy-phenyl	Me	n.d.	0.9	0.65	0.8
7d	OMe	OCH_2CO_2Et	3,5-dicyclopropylmethyloxy-phenyl	Me	0.7	>10	61	>100
9a	OMe	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	Et	0.016	0.03	0.025	0.022
9b	OMe	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	2-thiophen	n.d.	5.4	1.3	3
9c	OMe	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	$= R_3$	7.8	14	22	8.5
9d	OMe	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	n-But	0.72	1.3	0.3	0.3
9g	OMe	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	$O(CH_2)_3OH$	0.31	0.25	0.2	0.13
9h	OMe	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	$NH(CH_2)_3OH$	0.43	0.5	0.34	0.23
9i	OMe	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	$O(CH_2)_2OH$	0.43	0.07	0.08	0.09
9j	OMe	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	N-morpholine	n.d.	3.3	2.5	1.2
9k	OMe	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	OMe	0.39	0.13	0.11	0.14
9l	OMe	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	OEt	0.34	0.085	0.07	0.06
9m	OMe	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	SEt	n.d.	0.54	0.44	0.7
10	OMe	OCH_2Ph	3,5-dicyclopropylmethyloxy-phenyl	Et	n.d.	>100	>100	>100
11	OMe	OH	3,5-dicyclopropylmethyloxy-phenyl	Et	n.d.	3.3	2.4	0.65
12	OMe	$OCH_2CH(CH_2)_2$	3,5-dicyclopropylmethyloxy-phenyl	Et	n.d.	2.9	0.6	0.54
9e	H	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	Et	n.d.	0.63	0.66	0.85
9f	$O(CH_2)_2OH$	$O(CH_2)_2OH$	3,5-dicyclopropylmethyloxy-phenyl	Et	n.d.	>100	>100	>100

[a] Concentration of compound in μM which inhibited 50% of phosphodiesterase activity.

Modification in position 4. Aryl substitutions were preferred since alkyl groups led usually to inactive compounds (data not shown). Compounds **8**, **7b**, **7c** showed that the 3,5-alkyloxy phenyl substitutions (precisely 3,5-dicyclopropylmethyloxy-phenyl) was favourable. Quinazoline **7c** showed inhibitory activity against PDE 4 isoforms in the micromolar range. Improvement of potency was attempted by maintaining the 3,5-dicyclopropyl methyloxy-phenyl substitution at position 4 and modifiying positions 7, 6 and 2.

Modification in position 7. Bulky substitutions (benzyl, **10**, ester, **7d**) gave inactive compounds. However the introduction of a linear alkyl chain, specially hydroxy ethylene (derivative **9a**), resulted in a dramatic improvement of activity. Compound **9a** showed a reproducible IC_{50} value in the range of 30 nanomolar against PDE 4A, B, D and PDE 3. Probably the presence of a polar "head "(hydroxyl function) at the end of the C_2-alkyl chain is beneficial, since compound **12** showed a similar potency to the free phenol **11** but was significantly less potent than **9a.**

Modification in position 6. Replacement of the 6-methoxy group by a hydrogen, **9e**, resulted in a weak inhibitory activity. Furthermore a hydroxy ethylene group , **9f**, optimal in position 7, was also not well tolerated, even in the presence of previously effective substituents in positions 4 and 2.

Modification in position 2. Substitutions of the 2-chloro group with bulky substituents like 2-thiophen, **9b**, 3,5-dicyclopropylmethyloxy-phenyl, **9c**, N-morpholine, **9j**, decreased the inhibitory activity. On the other hand, introduction of a "linear" substituent, C_4-alkyl chain, alcohols, diol, thiol, amine (**9d, 9g-i, 9k-m**) produced compounds with a good to excellent inhibitory activity against PDE 3 and PDE 4 isoforms, comparable to compound **9a**.

On the basis of the above results, we selected the most potent inhibitors to be tested for their affinity for the rolipram binding site,[26] since there is evidence of excellent linear and rank order correlations between inhibition of cAMP hydrolysis, displacement of [^{3}H]R-(-)-rolipram and cAMP accumulation, if membrane-bound PDE 4 was used as the enzyme preparation.[29] Compounds **9(a, d-l)** displaced [^{3}H] rolipram at nanomolar concentrations (Table 2).

Table 2. Quinazolines as inhibitors of [^{3}H]rolipram binding.

No.	R_1	R_2	R_3	R_4	IC_{50}[a]
9a	OMe	$HO(CH_2)_2$	3,5-dicyclopropylmethyloxy-phenyl	Et	0.015
9d	OMe	$HO(CH_2)_2$	3,5-dicyclopropylmethyloxy-phenyl	n-But	0.05
9g	OMe	$HO(CH_2)_2$	3,5-dicyclopropylmethyloxy-phenyl	$O(CH_2)_3OH$	0.145
9h	OMe	$HO(CH_2)_2$	3,5-dicyclopropylmethyloxy-phenyl	$NH(CH_2)_3OH$	0.2
9k	OMe	$HO(CH_2)_2$	3,5-dicyclopropylmethyloxy-phenyl	OMe	0.21
9l	OMe	$HO(CH_2)_2$	3,5-dicyclopropylmethyloxy-phenyl	OEt	0.043

[a] Concentration of compound in μM which inhibited the binding of [^{3}H]rolipram by 50%

In summary we have shown that 6,7-dialkyloxy quinazolines are capable of inhibiting PDE 3, 4A, B, D and compete also for the binding site of [^{3}H]rolipram. Preliminary results have shown that compound **9a** is also a good inhibitor of the release of TNF-α, induced by LPS and interferon γ, from human peripheral blood mononuclear cells (IC_{50} = 79nM). These data suggest strongly that quinazolines could be of therapeutic interest in inflammatory diseases. Further work will focus on evaluating the potential of these compounds *in vivo*.

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21. PDE 3 is prepared from human platelets by ultrasonic homogenisation. Platelets were washed once with PBS, suspended in 10 ml of buffer H containing dithiothreitol (1 mM) and the following protease inhibitor solutions: 5 ml/ml of phenylmethyl-sulphonylfluoride (7 mg/ml in 2-propanol), 1 ml/ml leupeptin and pepstatin A (1 mg/ml each, in ethanol). After sonication (15 s at 4°C, Branson probe sonicator), homogenates were centrifuged at 2200 x g. The pellet was resuspended in the same volume of buffer H and the sonication repeated. Pooled supernates were stored at -20°C. Activity was assayed as described.[24]
22. PDE 4 cDNA coding for the three isoenzymes (human PDE 4A,[23] rat PDE 4B,[7] human PDE4D[25]) was cloned either into an extrachromosomal yeast expression vector (PDE 4D)[24] or integrated (PDE 4A, PDE 4B; single copy) at the pep4 locus of a saccharomyces cerevisiae strain lacking both of the wild-type yeast PDE genes[23]. Expression of human PDE 4B was attempted at several occasions and proved consistently unsuccessful. Since human PDE 4B is 97% homologous to rat PDE 4B, the rat enzyme was substituted for the human PDE 4B. The assay protocol was used as described.[24]
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26. Rolipram receptor-binding assay method: rat brains were suspended in 10 ml/g of ice-cold buffer (tris 20 mM, $MgCl_2$ 1 mM, dithiothreitol 0.1 mM, pH 7.5), homogenised using 2 bursts (30 sec each) of a Polytron homogeniser (Kinematica, Switzerland), centrifuged (600 g, 10 min, 4 C). The supernatants were pooled, and centrifuged (240'000 g, 10 min, 4 C). The pellets were resuspended in 5 ml/g of buffer. Protein content was determined using the method of Bradford,[27] according to the instructions of the manufacturer (BioRad). The assay was carried out as described by Schneider et al.[28]
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