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Preliminary communication

Novel bronchodilatory quinazolines and quinoxalines: Synthesis and biological evaluation

Marcel Špulák [a], Jana Pourová [b,**], Marie Vopršálová [b], Jiří Mikušek [a], Jiří Kuneš [a], Jan Vacek [c], Mukund Ghavre [a], Nicholas Gathergood [d], Milan Pour [a,*]

[a] Department of Inorganic and Organic Chemistry, Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Heyrovského 1203, CZ-500 03 Hradec Králové, Czech Republic
[b] Department of Pharmacology and Toxicology, Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Heyrovského 1203, CZ-500 03 Hradec Králové, Czech Republic
[c] Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 3, Olomouc 77515, Czech Republic
[d] School of Chemical Sciences and National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland

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ABSTRACT

A series of heterocyclic derivatives analogous to (−)vasicinone, in which the vasicinone C-ring was replaced with alkyl chain terminated by tertiary amine was prepared. N3, C4–O, C4–S or C4–N were used as the sites of attachment. The 4-[3-(1-piperidyl)propylsulfanyl]derivatives displayed bronchodilatory effect at low micromolar concentrations on isolated rat trachea, and low toxicity both on Balb/c 3T3 mouse fibroblast cells and in mice.

1. Introduction

Asthma bronchiale is a chronic inflammatory disease [1]. Recent trends indicate that it is becoming increasingly prevalent in most developed countries, and in many developing countries (China, India). Somewhat more alarming is the fact that morbidity and mortality associated with asthma bronchiale is also increasing. Even though there is no cure for asthma, most cases can be managed with appropriate treatment based on: (1) long term suppression of airway inflammation; and (2) bronchodilation in case of acute need [2]. Inhaled corticosteroids (e.g. beclomethasone), mast cell stabilizers (sodium cromoglycate), leukotriene inhibitors (montelukast), and xanthines (theophyline, aminophylline) serve to control inflammation of the trachea and bronchi. Bronchodilators are used in cases of acute attack or as a prophylactic. Inhaled β_2 sympatomimetics (albuterol), and parasympatolytics (ipratropium) are prescribed for this purpose; xanthines can also be used to prevent night dyspnea (theophylline, aminophylline). A more recent (alternative) therapy based on the identification of possible targets in the immune cascade has underpinned the development of IgG1 monoclonal antibody, omalizumab [3].

Even though the above-mentioned small molecule drugs have been in clinical use for some time, it is well-known that their efficacy is not always optimal. For example [2], β_2 sympatomimetics are not 100% selective, while xanthines have a narrow therapeutic window and need to be administered in very high doses (vide infra). Despite an obvious need for the development of novel and safer therapeutics, there are, to the best of our knowledge, no serious candidates currently under development.

Natural products have historically been a fertile source of new drugs for the pharmaceutical industry [4], and considerable efforts have recently been reported that align natural products with the modern drug discovery paradigm [5,6]. (−)Vasicine (**1**) and (−) vasicinone (**2**) (Fig. 1) are major alkaloids isolated from *Justicia adhatoda* L. known to have a moderate bronchodilatory effect [7,8]. To date, several literature reports have described the modification

* Corresponding author. Tel.: +420 495067277.
** Corresponding author. (pharmacology). Tel.: +420 495067295.
E-mail addresses: pourova@faf.cuni.cz (J. Pourová), pour@faf.cuni.cz (M. Pour).

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of the vasicinone structure, especially the C-ring containing the stereogenic C atom, e.g. removal of the hydroxyl group [9], expanding the ring [10–13] or converting it to a heterocycle [14–17]. Of these analogs, compounds with a seven-membered C ring and alkoxy substituents in the A ring were found to be more active compared with theophylline as a standard. By way of example, the EC_{50} of the 7,9-diethoxy derivative **3a** ranged between 12 and 70 μmol/L [13], while the 8,9-dimethoxy analog **3b** caused 82% relaxation at 20 μg/mL (ie. 73 μmol/L) [12], and the 8,9-dioxymethylene compound **3c** as well as its 8,9-deoxy congener, **3d**, effected 100% relaxation at 30 μg/mL (i.e., 116 and 140 μmol/L, respectively) against a guinea pig tracheal chain contracted by acetylcholine, histamine or ovalbumine [18]. Furthermore, SAR studies have shown that compounds without the C-ring also displayed a bronchodilatory effect, however, their activity was limited to histamine-contracted trachea. Importantly, the presence of the A-ring was claimed to be crucial for activity [18].

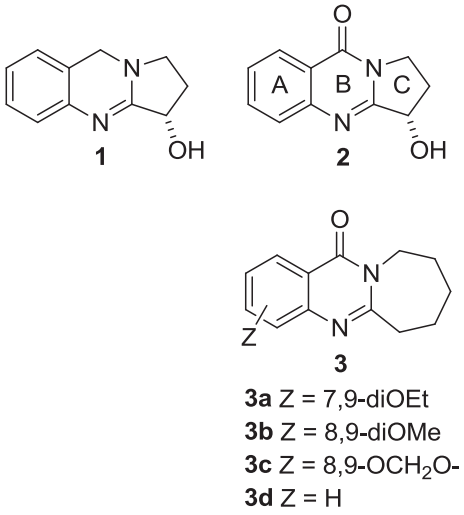


Fig. 1. Major alkaloids of *Justicia adhatoda* and their most active analogs.

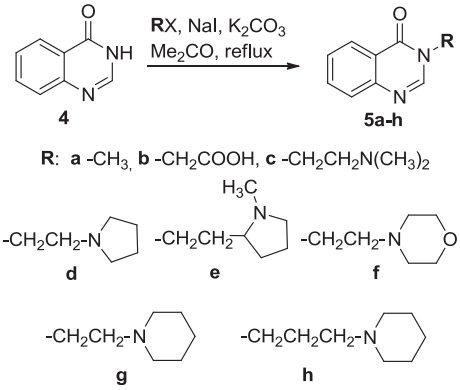
While direct comparison of results obtained in different laboratories can be misleading, given the data were obtained under more or less varying conditions and expressed in different ways (% of relaxation vs. EC_{50}) a partial conclusion can nevertheless be made that the most active vasicinone analogs reported so far have displayed bronchodilatory effect at concentrations of tens of μmol/L at best. Herein we wish to report the design, synthesis and biological evaluation of a new series of readily prepared analogs without the C-ring, in which the most active compounds exhibited improved activity (units of μmol/L) and concomitant low toxicity.

2. Results and discussion

Previous results indicated that the C-ring could probably be excluded or extensively modified. Thus, the C-ring was replaced with a simple alkyl chain capable of mimicking a cycle with N_3, C_4–O, C_4–S or C_4–N as the sites of attachment. The synthesis was thus facilitated using straightforward, high-yielding chemistry starting from the commercially available 3,4-dihydroquinazoline-4-one (**4**). Additionally, testing on the model of isolated trachea indicated that potential drugs should be water soluble, so an acidic or basic function was also incorporated into the chain.

A Williamson ether synthesis using the procedure described in a recent report [19] afforded our initial series of 3-alkyl-3,4-dihydroquinazoline-4-ones, that were subsequently screened for in vitro bronchodilatory activity on isolated rat trachea (Table 1).

Table 1
Synthesis and bronchodilatory activity of *N*-alkyl-3,4-dihydroquinazoline-4-ones.



Compound	Yield (%)	ED_{50} (mmol/L)[a]
4	–	0.810
5a[b]	95	0.747
5b	90	NA[c]
5c	90	0.440
5d	96	0.562
5e	89	0.104
5f	80	1.900
5g	72	0.178
5h	83	**0.0283**
Theophylline	–	2.090
(−)Vasicinone (**2**)	–	2.864

[a] 50% of the maximum relaxation caused by theophylline.
[b] Administered as hydrochloride.
[c] Not active.

Theophylline, and (−)vasicinone prepared according to a reported procedure [20] were used as standards. All substances were sufficiently soluble in aqueous media, with the exception of compound **5a** that had to be converted into the corresponding hydrochloride prior to evaluation.

Screening results indicated that the ED_{50} of theophylline was in the millimolar range, which is in agreement with the high doses being typically prescribed (12–20 mg/kg/day) [21]. While ED_{50} of (−)vasicinone was comparable, unsubstituted quinazolinone **4** and methyl derivative **5a** were somewhat more effective, even though their ED_{50} values were approximately 1 mmol/L. Compound **5b** bearing an acidic moiety did not relax the trachea at all, but the presence of a tertiary amine in substance **5c** gave rise to yet another slight improvement of activity, which shifted the response to hundreds of micromoles/L. This finding suggests that further efforts can be directed towards preparing compounds in which the length of the side chain bearing a terminal tertiary amino group is altered. The ED_{50} values show further improvement following the introduction of a nitrogen-containing saturated heterocyclic ring. Of this subseries, the ED_{50} of the 3-[3-(1-piperidyl)propyl] derivative **5h** decreased by two orders of magnitude compared to the standards (28 μmol/L).

For the C_4–O, C_4–N and C_4–S series, the starting quinazolinone **4** was first converted into the 4-chloro derivative **6** or sulfur analog **7** via established procedures [22,23]. The reaction of the former with various alkoxides or amines gave rise to 4-alkoxy **8** or alkylamino derivatives **9**. Alkylation of the latter yielded 4-alkylsulfanyl compounds **10**.

Screening results are summarized in Table 2. It is noteworthy that the biological effect of almost all derivatives was higher than that of theophylline. The 4-alkylsulfanyl derivatives displayed the most pronounced effect and were more potent than their alkoxy and alkylamino analogs. Similar to derivative **5h**, compound **10h**