Bioorganic & Medicinal Chemistry 19 (2011) 6309–6315

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Discovery of pyridine-2-ones as novel class of multidrug resistance (MDR) modulators: First structure–activity relationships

Sören Krawczyk [a], Monika Otto [a], Alexander Otto [a], Claudius Coburger [a], Martin Krug [a], Marianne Seifert [a], Volkmar Tell [a], Joséf Molnár [b], Andreas Hilgeroth [a,*]

[a] Institute of Pharmacy, Martin Luther University, 06120 Halle, Germany
[b] Department of Medical Microbiology, University of Szeged, 6720 Szeged, Hungary

ARTICLE INFO

Article history:
Received 10 May 2011
Revised 1 September 2011
Accepted 5 September 2011
Available online 10 September 2011

Keywords:
Structure–activity relationships (SAR)
Multidrug resistance (MDR)
P-glycoprotein (P-gp)
Lead structure
Efflux pump inhibition

ABSTRACT

A novel facile synthesis led to pyridine-2-one target structures of which first series with varying substituents have been yielded and biologically characterized as novel multidrug resistance (MDR) modulators inhibiting P-glycoprotein (P-gp). Structure–activity relationships prove a dependency of the MDR-modulating properties from the kind and positioning of hydrogen bond acceptor functions within the molecular skeleton. Cyano functions turned out as biologically effective substituents for a potential hydrogen bonding to the protein target structure.

1. Introduction

Multidrug resistance (MDR) is an ongoing main problem in anticancer therapies.[1–3] Main causative agents for this problem have been multidrug efflux pumps which are found overexpressed in tumor cells.[4,5] Such efflux pumps transport anticancer drugs out of the tumor cells so that insufficient intracellular concentrations result and reason a tumor cell resistance.[3] Various efflux pump proteins have been identified meanwhile.[3–5] P-glycoprotein (P-gp) is the most spread efflux pump in MDR-resistant tumor cells.[6] Numerous cytostatic agents have been found as substrates of P-gp.[6–8] Also novel cytostatics like tyrosine kinase inhibitors or monoclonal antibodies turned out as P-gp substrates.[9–11]

So there have been ongoing efforts to find strategies to circumvent the MDR problem. Although novel methods like gene silencing have been perspective in vitro techniques the in vivo realization remains doubtful due to various reasons.[2,12,13]

The main perspective strategy has been the development of MDR modulators which effectively inhibit the efflux pump activity.[6,14,15] However, most of the identified MDR modulators are unfortunately substrates of the efflux pumps so that their effectiveness is given only at higher concentrations which easily reach toxic ranges.[7,8,16]

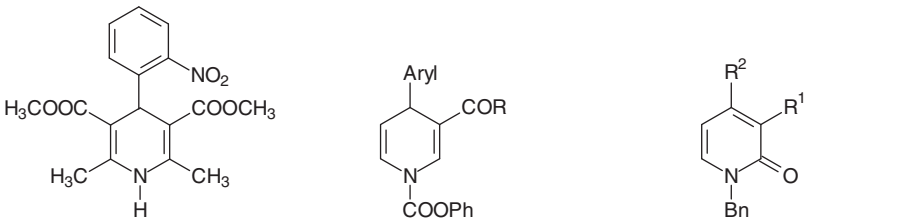
Such toxic problems concern also representatives of MDR modulators of the third generation which presently undergo clinical trials.[6,17] With many problems of the presently developed MDR modulators there is still a great challenge to discover novel MDR lead structures. Many of the recently reported MDR modulators have been derived from natural sources like plants.[6,18,19] They have complex structures and their high molecular weights are unfavorable molecular properties because molecules with high molecular weights often have bioavailability-problems. Moreover, natural products are often difficult to synthesize because of many necessary reaction steps. Thus, such resynthesized compounds will finally be expensive for a therapeutical use.

Pharmacologically used 1,4-dihydropyridines like nifedipin have been identified to act as MDR modulators beside their blood pressure-influencing properties (Fig. 1).[13,20,21] The introduction of more lipophilic phenyl substituents into first lead structures led to increased MDR-modulating properties.[22,23] However, the water solubility of such lipophilic derivatives with various phenyl substituents was limited. Such a lowered water solubility was unfavorable for in vivo applications. Moreover, essential structural changes within the molecular skeleton were necessary to reduce or avoid the originally pharmacological properties of the early 1,4-dihydropyridines. First attempts to design novel MDR-modulating 1,4-dihydropyridines led to *N*-phenoxycarbonyl substituted compounds (Fig. 1).[6]

However, the *N*-carbamide ester function in these structures was sensitive to hydrolysis. This structural sensitivity made a

* Corresponding author. Tel.: +49 345 55 25168; fax: +49 345 55 27207.
E-mail address: andreas.hilgeroth@pharmazie.uni-halle.de (A. Hilgeroth).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.09.005



Nifedipin | ***N*-Phenoxycarbonyl-1,4-dihydropyridines** | **Pyridine-2-one Target Structure**

Figure 1.

further in vivo application to a critical procedure because of the early inactivation of the bioactive molecules by such an easy carbamide ester hydrolysis.

We had the idea to change the compound structure by maintaining two molecular features: A lipophilic *N*-substituent was maintained by changing the phenoxy substituent by a benzyl substituent and, furthermore, the ester amide function should be kept. Such ester functions are potential hydrogen bond acceptor functions within MDR modulators and discussed to be important for the biological activities although the contribution of such functions to the extent of the biological activity is unknown.[24,25] The knowledge of such hydrogen bond acceptor functions is limited to few investigated structures and their effects to reverse MDR.[26,27]

If the *N*-phenoxycarbonyl function was involved in the mode of the biological activity to reverse MDR we thought to place it next to the nitrogen atom of the 1,4-dihydropyridine scaffold and so the novel pyridine-2-one structure with a *N*-benzyl substituent was favored with all the suggested molecular properties: the maintained lipophilic *N*-substituent contributing to the lipophilicity of the molecule, the amide function replacing the ester carbamide function near the nitrogen atom and the two double bonds in the 1,2-dihydropyridine-2-one structure instead of the non-conjugated double bonds within the reported 1,4-dihydropyridines.

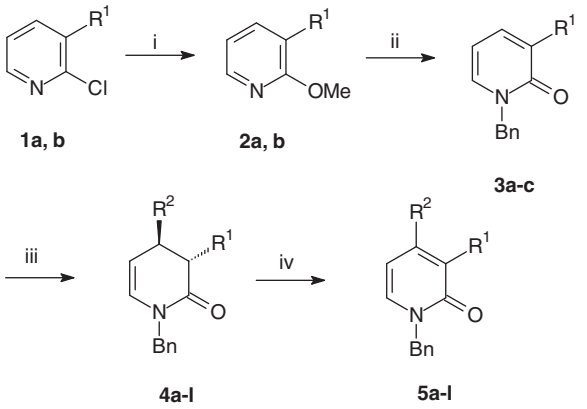
Two other important structural features have also been maintained within the novel MDR-modulating lead structure: the 4-aryl substituent and, finally, the 3-carbonyl function which is found in almost all biologically active 1,4-dihydropyridines.

2. Results and discussion

2.1. Chemistry

The 2-methoxy substituted pyridines **2a** and **2b** with R^1 = COOMe (**2a**) and R^1 = CN (**2b**) have been given by the reaction of the 2-chloro substituted starting compounds **1a** (R^1 = COOMe) and **1b** (R^1 = CN) with freshly prepared sodium methoxide under reflux conditions in methanolic solutions via a nucleophilic substitution reaction (Scheme 1).

The *N*-benzylation of the pyridine nucleus in compounds **2a** and **2b** to **3a** and **3b** succeeded in toluene under reflux conditions with benzyl bromide as alkylating agent. The 2-methoxy function in the primary yielded *N*-benzyl pyridinium cation underwent a bond cleavage by the attack of the bromide anion so that the alkylation reaction product finally owned a pyridine-2-one structure after the rearrangement of the pyridine nucleus bonds. The 3-benzyloxy ester substituted product **3c** (R^1 = COOBn) was given as a side product of the *N*-alkylation reaction of derivative **2a** by a transesterification of the 3-methoxy ester **3a** which reacted with benzyl alcohol as product of a partial benzyl bromide hydrolysis during the reaction course.



	R^1	R^2
1a, **2a**, **3a**	COOMe	-
1b, **2b**, **3b**	CN	-
3c	COOBn	-
4a, **5a**	COOMe	Ph
4b, **5b**	COOMe	Bn
4c, **5c**	COOMe	2-tolyl
4d, **5d**	COOMe	4-tolyl
4e, **5e**	COOMe	4-MeOPh
4f, **5f**	CN	Ph
4g, **5g**	CN	Bn
4h, **5h**	CN	2-tolyl
4i, **5i**	CN	4-tolyl
4j, **5j**	CN	2-MeOPh
4k, **5k**	CN	4-MeOPh
4l, **5l**	COOBn	Ph

Scheme 1. Reagents and conditions: (i) MeOH, NaOMe, reflux, 6 h; (ii) BnBr, 120 °C, 20 h; (iii) THF, Cu(I)I, LiCl, ArylMgBr, −40 °C to rt, 4 h; (iv) toluene, MnO_2, 130 °C, 6–8 h.

The varying 3-substituted pyridine-2-ones **3a**–**c** were then treated with the various grignards reagents at low temperatures using copper(I) iodide as catalyst in dried THF as solvent. The solubility of the used copper(I) iodide was improved by the use of lithium chloride. The pyridine-2-one nucleus may formally be attacked at two favored positions by the grignard reagents, namely the 4- and the 6-position. We exclusively found the 4-grignard addition products. The preferred attack at the 4-position of the pyridine nucleus has been observed to proceed under similar reaction conditions as in the reported reaction of *N*-acylated pyridinium cations with corresponding grignard reagents yielding 4-substituted 1,4-dihydropyridines from pyridines with 3-substituents similar to our pyridine-2-ones.[28–30] The only formation of 4-addition products by the use of copper(I) iodide is much more favorable than the formation of both 4- and 6-addition products described in