Tetrahedron xxx (2017) 1–7

Contents lists available at ScienceDirect

Tetrahedron

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

New chiral Mannich adducts of di-*tert*-butylphenols and a bicyclic imine – Synthesis and antiproliferative activity

Jakub Iwanejko [a], Elżbieta Wojaczyńska [a, *], Justyna Trynda [b], Magdalena Maciejewska [b], Joanna Wietrzyk [b], Andrzej Kochel [c], Jacek Wojaczyński [c]

[a] *Department of Organic Chemistry, Faculty of Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370, Wrocław, Poland*
[b] *Department of Experimental Oncology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, 12 R. Weigla St., 53 114, Wrocław, Poland*
[c] *Department of Chemistry, University of Wrocław, 14 F. Joliot-Curie St., 50-383, Wrocław, Poland*

ARTICLE INFO

Article history:
Received 19 December 2016
Received in revised form
17 February 2017
Accepted 6 March 2017
Available online xxx

Keywords:
Antiproliferative activity
Imines
Mannich bases
Mannich reaction
Stereoselectivity

ABSTRACT

Tests of antiproliferative activity of Mannich adducts of aromatic nucleophiles and a chiral bicyclic imine revealed a di-*tert*-butylphenol derivative as a promising compound for further exploration. To study the influence of substitution pattern and a configuration of stereogenic centers on the inhibition of cancer cell growth, a series of Mannich bases were obtained with a good to high diastereoselectivity from the reaction of the imine and three isomeric di-*tert*-butylphenols. Six new enantiopure adducts were isolated and fully characterized. Selected derivatives were shown to exhibit an interesting antiproliferative activity comparable to cisplatin.

1. Introduction

The Mannich reaction is widely used as a useful tool for carbon-carbon bond formation in organic synthesis.[1–3] Numerous Mannich bases and their derivatives have already found applications as drugs,[4,5] including the antidepressant fluoxetine, painkiller tramadol, and trihexyphenidyl used in treatment of Parkinson's disease. The antibacterial, antiviral, antiparasitic, anti-inflammatory and other activity is connected with the presence of amine and carbonyl groups which can participate in hydrogen bond formation. Certain Mannich bases have been found to act as cytostatics, and the mechanism of their action is based on inhibition of the biosynthesis of RNA and proteins.[6]

The Mannich reaction can be performed in an indirect variant, utilizing a preformed, stable imine which is reacted with nucleophiles. Relatively few cyclic imines have been tried for this purpose.[7] Reaction of bicyclic imines with acetone catalyzed by L-proline was described leading to Mannich adducts in 48–92% yields and enantiomeric excess up to >99%.[8] Minakawa and coworkers converted *cis*- or (±)-*trans*-1,2-diaminocyclohexane into bicyclic imines and investigated their reactions with phenols in aqueous solutions, yielding products as diastereomeric mixtures.[9–11] In our contribution in the field, we reported the use of the enantiopure imine (1*R*,6*R*)-**1** in Mannich-type reactions with aromatic nucleophiles (indole and substituted phenols), leading to adducts **3** and **4** with reasonable yields and good to high diastereoselectivity (Fig. 1).[12]

The obtained adducts **3** and **4** show a structural resemblance to the known diketopiperazine derivatives (such as compound A, Fig. 2) described in a publication and a patent from Kozikowski's group.[13,14] These compounds exhibited neuroprotective activity preventing neuronal death in an *in vitro* model of traumatic injury and increased survival in a model of free radical induced cell death. Diketopiperazine and our compound **3d** (Fig. 2) share not only the 2,6-di-*tert*-butylphenol substituent responsible for the neutralizing of free radicals, but also show the similar location of hydrophobic fragments and of groups responsible for the hydrogen bond formation (Fig. 2). In addition, various phenolic Mannich bases were shown to exhibit an interesting antiproliferative activity, although the mechanism of their action was not explained.[15–17]

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[*] Corresponding author.
E-mail address: elzbieta.wojaczynska@pwr.edu.pl (E. Wojaczyńska).

http://dx.doi.org/10.1016/j.tet.2017.03.017
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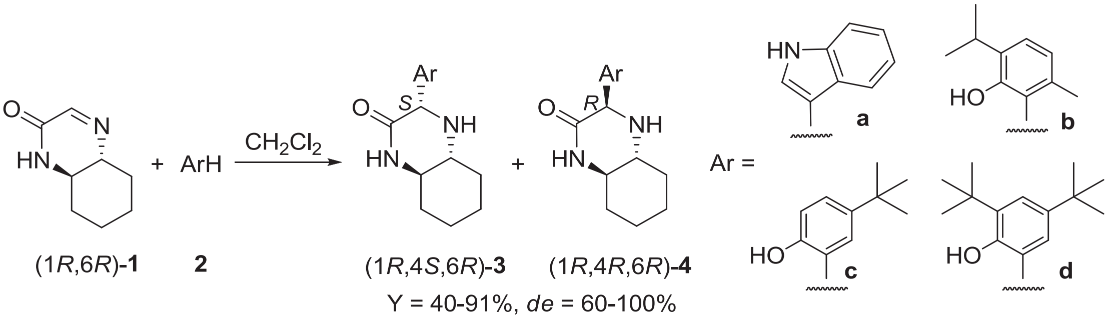

Fig. 1. Synthesis of Mannich adducts **3** and **4**.

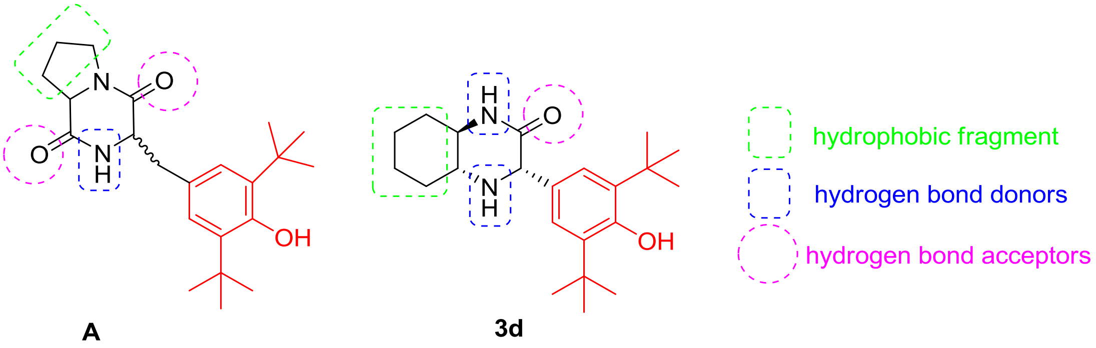

Fig. 2. Structural similarity of diketopiperazines (**A**)[13] and Mannich adduct **3d**.

These observations prompted us to enlarge the family of diastereomerically and enantiomerically pure derivatives of imine (1*R*,6*R*)-**1** and its enantiomer, (1*S*,6*S*)-**1**, and study their biological activity. In this publication, we describe the synthesis of new chiral phenol Mannich adducts and their preliminary biological evaluation as antiproliferative agents.

2. Results and discussion

Encouraged by the reported activities of Mannich bases, and having in hand a series of easily available compounds of this kind, we decided to preliminarily test them for their antiproliferative activity.

A series of 4-substituted derivatives of imine (1*R*,6*R*)-**1** were evaluated for their ability to inhibit the growth of four cancer cell lines. Each compound was tested on two human cancer cell lines displaying various levels of drug resistance, namely human colon adenocarcinoma cell line (LoVo), its doxorubicin resistant subline (LoVo/DX) and human acute promyelocytic leukemia (HL-60). To illustrate the agents' activity against the cells with MDR (multidrug resistance) phenotype the *Resistance Index* (*RI*) was calculated (Table 1). The *RI* value indicates how many times more resistant the subline is in comparison to its parental cell line.

The antiproliferative effect was also studied on normal murine embryonic fibroblast cell line (BALB/3T3) to evaluate the potential selectivity of the compounds studied. The mean values of the IC_{50} ± SD of the tested compounds are collected in Table 1.

Results presented in Table 1 indicate that almost all of the compounds studied were inactive or showed very low activity against HL-60 leukemia and normal mice fibroblasts BALB/3T3. However, all compounds inhibit proliferation of colon cancer cells. The high differences between antiproliferative activity against colon cancer and normal cells as well as leukemia cells suggest that cancer cells of solid tumors will be killed preferentially over normal or leukemic. Among the eight compounds tested, di-*tert*-butylphenol derivative **3d** with IC_{50}s in the micromolar range, emerged as the most potent. This compound is also able to break multidrug resistance mechanisms of LoVo/DX colon cancer cells revealing similar proliferation inhibition towards both colon cancer sublines. The calculated value of *RI* clearly confirmed overcoming of drug resistance by **3d** ($RI = 0.76$, whereas for doxorubicin $RI = 68.2$). Comparing the activity of **3d** with the other derivatives, few SAR indications emerged: the presence of a second *tert*-butyl group in the 3-phenyl ring was very productive, indeed compound **3c** and its epimer **4c** are definitely less active than **3d** (with slightly better results shown by 4*S* isomer **3c**). This observation can be attributed to a better penetration of cell membranes by **3d** derivative. The replacement of the *tert*-butyl with other substituents is detrimental (see thymol derivative **3b**). The phenol fragment cannot be replaced by indole moiety as in compounds **3a** and **4a** which are active only against the sensitive LoVo cell line. Thus, from the first series of derivatives, compound (1*R*,4*S*,6*R*)-**3d** endowed with selective antitumor activity that also encompassed resistant strain, emerged as a valid hit worthy to be further investigated.

Taking into account the results of structure-activity relationship (SAR) analysis, we decided to prepare a series of Mannich adducts reacting bicyclic enantiomeric imines, (1*R*,6*R*)-**1** and (1*S*,6*S*)-**1**, with readily commercially available di-*tert*-butylphenols (2,4-, 3,5- and 2,6-di-*tert*-butylphenol, **2d-2f**). This choice of reactants allowed establishing the effect of substitution pattern on the course of Mannich reaction and on the antiproliferative activity of the isomeric products.

The results of the performed syntheses are shown in Fig. 3 and Table 2. The reactions were performed in dichloromethane solution. In all cases, a complete regioselectivity was observed caused by the directing effect of hydroxyl group combined with the steric hindrance provided by the bulky alkyl substituents. As a result, products of substitution in *ortho* position with respect to —OH

Please cite this article in press as: Iwanejko J, et al., New chiral Mannich adducts of di-*tert*-butylphenols and a bicyclic imine – Synthesis and antiproliferative activity, Tetrahedron (2017), http://dx.doi.org/10.1016/j.tet.2017.03.017