Synthesis and anti-enterovirus activity of new analogues of MDL-860

Georgi M. Dobrikov a,⇑, Ivaylo Slavchev a, Ivanka Nikolova b, Adelina Stoyanova b, Nadya Nikolova b, Lucia Mukova b, Rosica Nikolova c, Boris Shivachev c, Angel S. Galabov b,⇑

a Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, bl. 9, Acad. G. Bonchev str., Sofia 1113, Bulgaria
b Stephen Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, bl. 26, Acad. G. Bonchev str., Sofia 1113, Bulgaria
c Institute of Mineralogy and Crystallography, Bulgarian Academy of Sciences, bl. 107, Acad. G. Bonchev str., Sofia 1113, Bulgaria

A R T I C L E   I N F O

Keywords:
Entero virus
Coxsackieviruses
Polio
Diarylethers
MDL-860

A B S T R A C T

A series of twelve novel compounds, analogues of antiviral agent MDL-860 were synthesized and their antiviral activity was evaluated in vitro against enteroviruses poliovirus 1 (PV1), Coxsackieviruses B1 (CVB1) and Coxsackieviruses B3 (CVB3). Compounds 14, 24 and 25 manifested strong antiviral effects against CVB1 and PV1 (SI values of 405 and 118 for CVB1 and PV1 respectively). In contrast to the wide anti-enteroviral activity of MDL-860, these three compounds were inactive against CVB3. Compounds 14, 24 and 25 along with MDL-860 were tested in vivo in mice infected with CVB1. Marked protective effects of compounds 14 and 24 were established, PI values of 50% and 33.3%, respectively. In addition, almost all of the tested compounds manifested very low toxicity.

© 2017 Elsevier Ltd. All rights reserved.

Enteroviruses (EV) are non-enveloped, single-stranded (+) RNA viruses belonging to the Picornaviridae family. This large family includes several pathogens that are implicated in a wide range of clinical manifestations, affecting humans as well as animals. Indeed, enteroviruses are responsible for at least 10–15 million symptomatic infections yearly. EV may also be linked to even more serious illnesses, which can subsequently be life-threatening. Such conditions includes meningitis, encephalitis, myocarditis and insulin dependent diabetes etc. Coxsackieviruses, and in particular Coxsackie B group, have often been associated with the development of myocarditis, which may lead to sudden death in young adults or progress to dilated cardiomyopathy if untreated. Over the past decades, several classes of non-peptidic compounds have been reported to be selective inhibitors of enterovirus replication after in vitro testing (cell culture experiments). However, a sharp discrepancy exists between the antiviral activity established in vitro and in vivo (experiments involving laboratory animals). In contrast with hundreds virus replication inhibitors showing in vitro effects less than twenty manifested some in vivo activity. Unfortunately, the efficient anti-enteroviral chemotherapy for clinical use is still not established. The development of drug-resistance is the main reason for the lack of antivirals in clinical use for enteroviral infections. Nevertheless, some anti-enteroviral compounds have entered clinical trials – isoxazoles (“WIN compounds” – disoxaril, pleconaril), pirodavir and its analogues, imidazolidinones, chalcones, flavanes, diarylethers etc. (Fig. 1).

Diarylether derived compound MDL-860 (2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile, also known as DNB) was first reported in 1980’s. Indeed MDL-860 possesses a broad-spectrum of in vitro activity against picornaviruses, by inhibiting an early event in virus replication, after initial uncoating. MDL-860 mechanism of action was elucidated in our recent publication, the identified target being the host phosphatidylinositol-4 kinase III beta (PI4KB). MDL-860 also elicited in vivo efficacy in a model of coxsackievirus B3 (CVB3) induced myocarditis. The promising results inspired the development of many analogues of MDL-860 over the past decades. For example, Markley et al. synthesized and tested over than 70 diarylethers (and their isosteric analogues) against several picornaviruses. The latest work of Pürstinger et al. reports on the synthesis of 60 new diarylethers and their activity against CVB3 replication. All obtained results clearly showed that the 2-cyano-4-nitrophenoxypy group is an essential building block for the existence of antiviral activity of this class of compounds. However, varying the substituents in the other aromatic ring can have a significant impact on both the antiviral activity and cytotoxicity. Identified hit compounds, usually contain two to three halogen atoms in the second aromatic ring.

Despite the reported promising results, recently this class of compounds has been neglected in terms of further investigation with respect to their antiviral activity. Thus, the current study...
similar to those of Markley et al. and Pürstinger et al. Diarydry DMSO by heating the corresponding phenols single step synthesis as shown in Scheme 1, by using procedures groups via O, N and S atoms.

4-nitrophenyl moiety bridged to various aromatic or aliphatic

tivity and cytotoxicity of a series of new compounds bearing 2-cyano-
was focused on the synthesis and evaluation of the antiviral activ-

The target compounds were obtained in high yields via single step synthesis as shown in Scheme 1, by using of procedures similar to those of Markley et al. and Pürstinger et al. Diarylethers and the thioether were synthesized in dry DMSO by heating the corresponding phenols and 2-chlorothiophenol (9) with 2-chloro-5-nitrobenzonitrile (1) in the presence of NaOH. Arylamines and were obtained through reaction of 1 with 2.2 equivalents of the amines and, respectively, in excess of N-methylmorpholine utilized as a basic agent. All starting compounds (except phenols and) are commercially available products. The synthesis of intermediates and was previously described. All target compounds were isolated and purified by column chromatography. Chemical structure elucidation was achieved by NMR and MS spectroscopy. Additionally, the structure of compound was confirmed by X-ray crystallography. The obtained information could be helpful for further molecular docking studies of this class of compounds.

Details on the synthesis, purification, melting temperatures, spectroscopy, X-ray and elemental analyses data of the target compounds are provided in the Supplementary data.

Compounds were evaluated for their in vitro cytotoxicity and antiviral activities in cell culture experiments against three enteroviruses, namely PV1, CVB1 and CVB3. MDL-860 was used as a reference compound (Table 1). Compound manifested a strong activity against PV1 and CVB1 (SI values 118 and 405, respectively), being inactive against CVB3. Compounds and showed a moderate effect (SI 20.5 and 19.6, respectively) against CVB3. A borderline activity toward CVB1 was established for (SI 10.9). Compounds could be considered as inactive against the three viruses included in the screening (except for a low activity against CVB3). A remarkable activity of was demonstrated against PV1 and CVB1 viruses. None of the tested compounds replicated MDL-860 antiviral spectrum and effects embracing all the three enteroviruses (MDL-860 SI values of 72.5, 586.9 and 182 vs PV1, CVB1 and CVB3, respectively). The remarkable insusceptibility of CVB3 to the active compounds has to be emphasized. Moreover, compound (with closest structural resemblance to MDL-860) demonstrated one of the highest activities to two other viruses.

Regarding the cytotoxicity (against HEp-2 cells) of the compounds studied, all were non-toxic in general (except ). Some of them and demonstrated even lower toxicity than MDL-860.

Unfortunately, the number of compounds in this study is not enough to provide adequate QSAR analysis, but some structure-activity relations can be commented. An initial structure – activity analysis showed that a marked efficiency is usually demonstrated by two- or three halogen-substituted diarylether analogues of MDL-860. The results of compounds and confirmed this deduction. Other substituents (aromatic heterocycles in and replacements of bridged oxygen atom with other heteroatom) led to lack of activity. Similar effect was observed when halogen substituents were replaced with other (–). It is interesting to note, that compound (structurally nearest difluoro analogue of MDL-860) lack the activity only against CVB3. On the other hand, analogues of MDL-860, containing more than three halogen atoms in the second ring were not known. In this study we provided only one pentachloro substituted compound, which demonstrated moderate antiviral effect. Of course, this single example is not representative, so this could be one of the directions for further investigations.

It can be concluded that the future synthesis of new active diarylethers could be promising and to be directed to other halogen-substituted analogues of MDL-860. In addition, QSAR and some other issues (for example insolubility of the MDL-860 and its analogues in water) could be targeted in future investigations.

The selected in the in vitro screening compounds and were tested in experimental CVB1 neuroinfection in newborn mice of ICR random bred line, infected with a massive virus inoculum (20 LD50). They were administered in 12-days treatment course starting on the day of virus inoculation. The subcutaneous daily dose of the compounds was as follow: compounds and demonstrated moderate antiviral effect. Of course, this single example is not representative, so this could be one of the directions for further investigations.

As seen, compound demonstrated the highest activity, attaining a protection effect of 50% and a very pronounced lengthening of the MST by 5 days. A marked activity was established at the course with compound (PI = 33.3% and MST lengthened by 5.4 days). Compound showed a weak protective effect (PI = 11.6% and A days of 2.6). In contrast, MDL-860 activity was marked by a lengthening of MST only, by 3 days. Markley et al. tested in vivo MDL-860 and five other analogues (against Coxsack-
ievirus A21 in mice), however it is not correct to compare results obtained in this study (by using of subcutaneous administration) with those of Markley et al. (by using of oral administration against different virus). Evidently, compounds 14 and 24 could be characterized as a perspective anti-CVB agents which need further study.

In previous work we have established the very high activity in experimental Coxsackievirus B1 neuroinfection in mice of the consecutive alternating administration (CAA) treatment course of a triple combination of enterovirus inhibitors including MDL-860 as a component. It would be of great interest the testing the effect

![Scheme 1. Synthesis of compounds 14-25.](image)

**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cytotoxicity, CC_{50} (µM)</th>
<th>PV1 IC_{50} (µM)</th>
<th>SI</th>
<th>CVB1 IC_{50} (µM)</th>
<th>SI</th>
<th>CVB3 IC_{50} (µM)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>320.0</td>
<td>2.7</td>
<td></td>
<td>0.8</td>
<td></td>
<td>405.0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>119.1</td>
<td>NA</td>
<td></td>
<td>10.9</td>
<td>10.9</td>
<td>5.8</td>
<td>20.5</td>
</tr>
<tr>
<td>16</td>
<td>570.3</td>
<td>NA</td>
<td></td>
<td>256.1</td>
<td>2.2</td>
<td>29.6</td>
<td>19.6</td>
</tr>
<tr>
<td>17</td>
<td>22.0</td>
<td>NA</td>
<td></td>
<td>NA</td>
<td></td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>94.3</td>
<td>NA</td>
<td></td>
<td>16.2</td>
<td>5.8</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>570.8</td>
<td>NA</td>
<td></td>
<td>190.1</td>
<td>3.0</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>718.0</td>
<td>NA</td>
<td></td>
<td>NA</td>
<td></td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>53.8</td>
<td>NA</td>
<td></td>
<td>NA</td>
<td>9.9</td>
<td>NA</td>
<td>5.4</td>
</tr>
<tr>
<td>22</td>
<td>675.0</td>
<td>NA</td>
<td></td>
<td>NA</td>
<td>426.0</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>492.2</td>
<td>234.0</td>
<td>2.1</td>
<td>152.2</td>
<td>3.2</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>355.0</td>
<td>6.8</td>
<td>52.2</td>
<td>0.7</td>
<td>507.1</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>517.5</td>
<td>2.7</td>
<td>191.6</td>
<td>0.75</td>
<td>690</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>MDL-860</td>
<td>493.0</td>
<td>6.8</td>
<td>72.5</td>
<td>0.8</td>
<td>586.9</td>
<td>2.7</td>
<td>182.0</td>
</tr>
</tbody>
</table>

NA-not active.
of CAA course by a triple combination in which MDL-860 is replaced by compound 14.

Acknowledgments

This study was supported by the Bulgarian Science Fund - project B02/11 12.12.2014 “Synthesis and anti-enterovirus activity of novel diaryl ethers and their complexes with cyclodextrins”. The financial support of the Bulgarian Science Fund for the purchase of Bruker Avance II+ 600 NMR spectrometer in the framework of the Program ‘Promotion of the Research Potential through Unique Scientific Equipment’ – project UNA-17/2005 is gratefully acknowledged.

A. Supplementary data

Supplementary data (CCDC 1530748 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures. Supplementary data (experimental procedures, characterization of final compounds, NMR spectra and biological assays protocols) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.08.056.

Table 2
Study of the activity of compounds 14, 24 and 25 at CVB1 experimental neuro infection in newborn mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Survivors/Total</th>
<th>MST ± SD days a</th>
<th>Δ days</th>
<th>Mortality, %</th>
<th>PI, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>22/24</td>
<td>8.1 ± 1.0 b</td>
<td>+5.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>24</td>
<td>9/27</td>
<td>8.5 ± 1.0 b</td>
<td>+5.4</td>
<td>66.7</td>
<td>33.3</td>
</tr>
<tr>
<td>25</td>
<td>3/26</td>
<td>5.7 ± 1.2 c</td>
<td>+2.6</td>
<td>88.4</td>
<td>11.6</td>
</tr>
<tr>
<td>MDL-860</td>
<td>0/27</td>
<td>6.1 ± 1.6 c</td>
<td>+3.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Placebo</td>
<td>0/17</td>
<td>3.1 ± 0.3</td>
<td></td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are from three independent experiments (average).

a One-way ANOVA (Bonferroni’s multiple comparison post-test); MST – mean survival time; PI - protection index; SD – standard deviation; ** p < 0.0001 vs. placebo group; 
*** p < 0.01 vs. placebo group; 
* p < 0.05 vs. placebo group.

References


Fig. 2. Individual effects of compounds 14, 24, 25 and MDL-860 in experimental neurotropic infection with Coxsackievirus B1 in newborn mice.