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# Effects of the aryl linker and the aromatic substituent on the anti-HCV activities of aryl diketoacid (ADK) analogues

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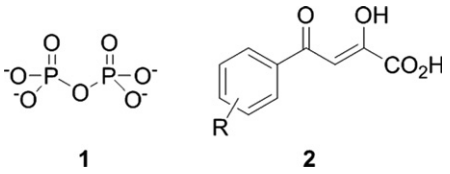
ABSTRACT

Based on our pharmacophore model of the aryl diketoacids (ADKs), we designed and prepared a series of novel ADK analogues, which showed potent inhibitory activities against the NS5B polymerase in the submicromolar range. Pharmacophore-guided docking study revealed that the antiviral activities of the ADKs are highly dependent upon the aryl linker as well as the size and position of the aromatic substituent. It is of another importance that, unlike previously reported ADKs, three ADK analogues synthesized in this study effectively blocked Hepatitis C Virus (HCV) replication in the replicon systems.



From the therapeutic point of view, hepatitis C virus (HCV) offers a number of latent targets for small molecule intervention.[1] Initial effort for the development of HCV-directed antiviral agent has focused on the inhibition of essential virus-encoded enzymes,[1b] and it is likely that agents able to disrupt function of the HCV NS5B polymerase will prove effective in the treatment of HCV infections due to its essential role in viral replication. Advances in high throughput-screening assays have resulted in the identification of numerous anti-NS5B compounds broadly categorized as nucleoside (NIs) and non-nucleoside (NNIs) inhibitors.[2] Particularly, NNIs represent a chemically diverse family of compounds which act by complex and various mechanisms by binding near the active site or discrete allosteric sites of NS5B.[3] Apart from allosteric inhibitors, pyrophosphate (**1**, Fig. 1) mimics like aryl diketoacids (**2**, Fig. 1) have been reported to inhibit the HCV NS5B polymerase through chelation of the divalent cation in the active site.[4–7]

Even though the inherent reactivity of the diketoacid moiety presented a problem for the further development of these compounds as drug candidates,[8] ADKs represent a general scaffold from which an intensive search for chemically and biologically stable diketoacid replacements has been initiated.[9] Thus, information about the binding mode of the ADKs to the target enzyme might provide valuable insights into designing novel NS5B polymerase inhibitors.




1 2

**Figure 1.** Structures of pyrophosphate (**1**) and aryl diketoacid (**2**).

Recently, we proposed a pharmacophore model of ADK analogues which is composed of a metal-binding diketoacid functionality, two hydrogen-bonding acceptors, and/or a hydrogen-bond donor (Fig. 2).[10] The pharmacophore model was successfully applied for the pharmacophore-guided docking followed by 3D-QSAR study to generate a 3D-QSAR (CoMSIA) model which gave a good correlation of the biological activities of ADKs with their three-dimensional structures.[11] However, effects of the linker moiety bridging two aryl groups ($XCH_2$, Fig. 2) as well as the substituent at the terminal aromatic group (R, Fig. 2) on the antiviral activities of the ADKs have yet to be evaluated.

The docking study revealed that the hinge-like aryl linker which connects the diketoacid moiety with the terminal aryl group (Fig. 2) is directly hydrogen-bonded to the protein, and, with the metal-binding diketoacid as well as the hinge-like linker bound to

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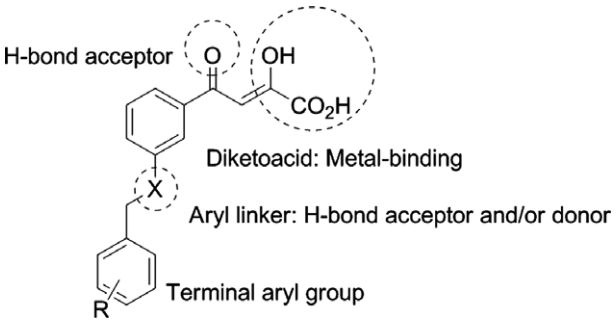



**Figure 2.** Pharmacophore model of the ADKs: metal-binding diketoacid, two hydrogen-bond acceptors, and/or a hydrogen-bond donor.

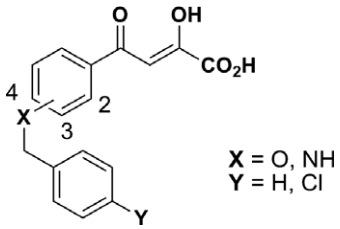



**Figure 4.** ADK analogues studied.

the protein, the terminal aryl group has only limited chance to interact with the enzyme. Moreover, the pharmacophore-guided docking study suggested that, in addition to the pharmacophore model described above (Fig. 2), there are two separate aryl-binding sites (hydrophobic pocket and groove, Fig. 3) which can accommodate the terminal aryl groups of the ADKs. In particular, both aryl-binding domains have additional hydrophobic holes which are in perfect size and shape for accommodating a chlorine substituent (Fig. 3). Taken together, we envisaged that, upon binding of the ADKs to the enzyme active site, the aryl linker determines the location of the terminal aryl group of which hydrophobic interaction with the aryl-binding site governs the antiviral activities of the ADKs.

This paper describes a proof-of-concept effort designed to verify the effects of the aryl linker (X=O or NH, Fig. 4) and the terminal aryl substituents (Y = H, or Cl, Fig. 4) of the ADKs on their antiviral activities.

The general procedure for syntheses of the ADK analogues was adapted from the previous structure–activity relationship study by Summa et al.[4] (Scheme 1). Thus, *O*-benzylacetopheonones and *N*-benzylacetopheonoes (**4**) were prepared by benzylation of the appropriate acetophenones (**3**) with benzyl bromide or *p*-chloro benzyl bromide in DMF in the presence of $K_2CO_3$ at 60 °C for 24 h (Scheme 1). Reaction of the acetophenones (**4**) thus obtained with dimethyl oxalate in the presence of sodium methoxide in THF, followed by hydrolysis with sodium hydroxide in a mixture of methanol and water provided the desired ADKs (**5**–**12**) in 70–88% yields.

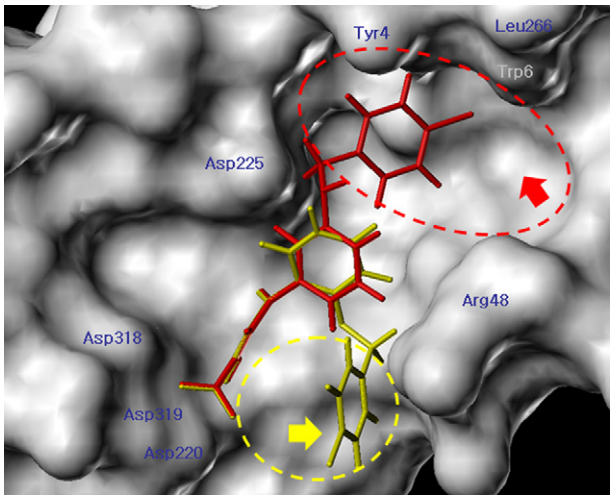
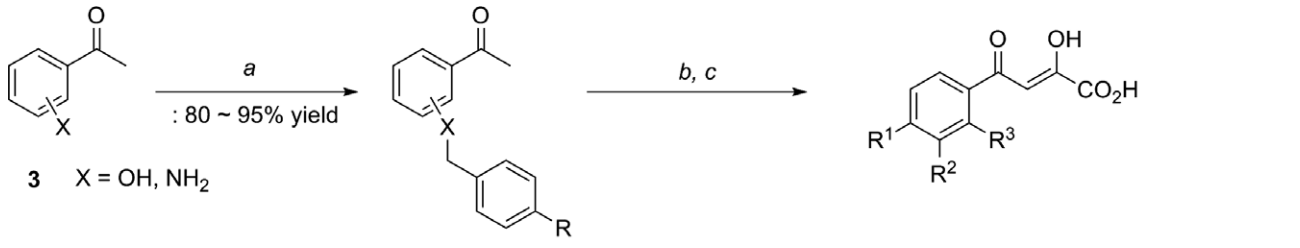



**Figure 3.** Two binding modes of the ADK analogues at the HCV NS5B polymerase: Red and yellow sticks indicate ADK molecules **12** and **10** (Scheme 1) docked at the hydrophobic pocket (red dotted circle) and groove (yellow dotted circle), respectively. Hydrophobic holes inside the aryl-binding domains are denoted as blocked arrows.

All compounds synthesized were assayed against HCV genotype 1b NS5B Δc21 enzyme to assess their inhibitory activity ($IC_{50}$, Table 1).[12] Also, all analogues were evaluated in a cell-based assay, in which the HCV subgenomic replicon RNA-harboring NS5B gene was transfected and expressed in the Huh-7 hepatoma cell lines, and $EC_{50}$ and $CC_{50}$ values are reported (Table 1).[13]

The inhibitory activities of two known compounds (**7** and **11**) against the target enzyme ($IC_{50}$'s: 4.6 and 28 μM, respectively) were comparable to the previously reported data (8.0 and 17 μM, respectively).[7,14] In general, the unsubstituted ADKs (**5**, **9** and **11**) showed only marginal anti-HCV activities ($IC_{50}$'s: 8.3, 30, and 28 μM), but the corresponding *para*-chloro analogues (**6**, **10** and **12**) were clearly more potent inhibitors of the HCV NS5B polymerase ($IC_{50}$'s: 1.5, 2.0, and 0.96 μM, respectively). Unlike other *para*-chloro analogues, compound **8** showed decreased antiviral activity compared with its unsubstituted counterpart **7** (Table 1) whereas the *N*-benzyl analogue of **8** (**12**) showed very potent enzyme inhibitory activity ($IC_{50}$: 0.96 μM, Table 1). It is of another interest that, even though the regioisomers **6** and **10** have different configurations and thereby different spatial orientations of the terminal aryl groups with respect to the diketoacid moieties, they show almost equipotent antiviral activities.

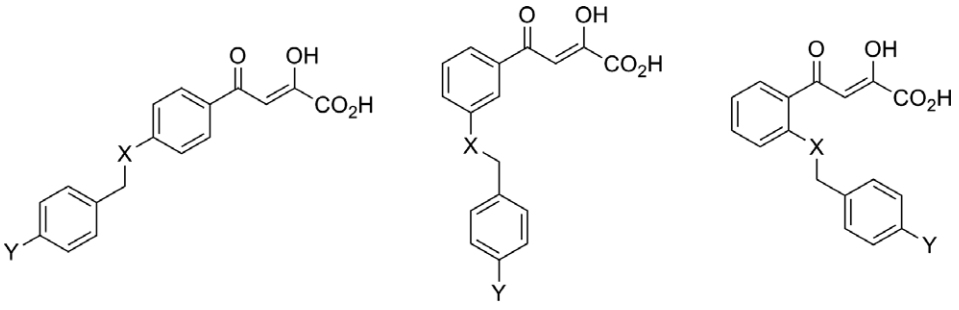
Binding modes of the ADK analogues provided by the pharmacophore-guided docking were in fairly good agreement with the structure–activity discussion described above (Fig. 5). First, compounds with $IC_{50}$ values less than 10 μM (**5**, **6**, **10**, **11**, and **12**) gave docking poses at the active site but others (**7**, **8**, and **9**) failed to dock. In particular, the *meta* configurations of **7** and **8** proved unable to locate the terminal aryl groups at either of the terminal aryl-binding sites, and, as a result, the *para*-chloro substitution has no effect on the anti-HCV effect of **8**. Second, due to the different locations of the aryl linkers as well as the terminal aryl groups between **6** and **10** with respect to the diketoacid moieties, the regioisomers **6** and **10** gave completely different docking poses (Fig. 5a and b) of which terminal aromatic groups were bound to the two different aryl-binding sites: the hydrophobic pocket and the hydrophobic groove, respectively. Most importantly, even though **7** and **8** failed to produce binding poses due to the lack of the hydrophobic interactions with the aryl-binding sites, their *N*-benzyl analogue **12** successfully located its terminal aryl group at the hydrophobic pocket (Fig. 5c). The *N*-benzyl linker of **12** is hydrogen-bonded to the carbonyl oxygen of Leu159 (Fig. 5c) as a hydrogen-bond donor whereas the oxygen atom in the *O*-benzyl linker is hydrogen-bonded to the amide NH of Leu159 (Fig. 5a) as a hydrogen-bond acceptor. Thus, the subtle change in the hydrogen bonding pattern of **12** induced by the presence of an amino group as a hydrogen-bond donor instead of an acceptor enabled **12** to locate its terminal aromatic group at the hydrophobic pocket (Fig. 5c) leading to the remarkably improved $IC_{50}$ value. Analysis of the binding modes of **6** (Fig. 5a) and **12** (Fig. 5c) also reveals that the interaction of the chlorine substituent with the hydrophobic




| | $R^1$ | $R^2$ | $R^3$ | Yield (%) |
|---|---|---|---|---|
| **5** | $OCH_2Ph$ | H | H | 88 |
| **6** | $OCH_2$(4-ClPh) | H | H | 82 |
| **7** | H | $OCH_2Ph$ | H | 83 |
| **8** | H | $OCH_2$(4-ClPh) | H | 78 |
| **9** | H | H | $OCH_2Ph$ | 87 |
| **10** | H | H | $OCH_2$(4-ClPh) | 81 |
| **11** | H | $NHCH_2Ph$ | H | 75 |
| **12** | H | $NHCH_2$(4-ClPh) | H | 70 |

**Scheme 1.** Syntheses of the ADK analogues. Reagents and conditions: $K_2CO_3$, $PhCH_2Br$ or 4-$ClPhCH_2Br$, acetone, rt; (b) dimethyl oxalate, NaOMe, THF, rt; (c) NaOH, MeOH/$H_2$0, rt.

**Table 1**
Biological activity of the ADK analogues




| Compound | $IC_{50}$ (μM) | $EC_{50}$ (μM) | $CC_{50}$ (μM) |
|---|---|---|---|
| **5** | 8.3 | 0.66 | > 100 |
| **6** | 1.5 | 3.1 | > 100 |
| **7** | 4.6 (8.0[a]) | 30 | > 100 |
| **8** | 28 | > 50 | > 100 |
| **9** | 30 | 40 | > 100 |
| **10** | 2.0 | 0.54 | > 100 |
| **11** | 28 (17[a]) | 28 | > 100 |
| **12** | 0.96 | 0.82 | > 100 |

[a] $IC_{50}$ values from the literature Refs. 7 and 14.

hole (Fig. 3) inside the pocket controls the inhibitory activities of the ADKs: the *para*-chloro substituent of **12** is well oriented to snuggle into the hole deep inside the hydrophobic pocket, which explains lower $IC_{50}$ value of **12** (Fig. 5c) than that of **6** (Fig. 5a).

To our knowledge, no data of antiviral activity in the cell-based assay for the pyrophosphate mimics including diketoacids are available presumably due to the high ionic nature and instability of these compounds. Thus, it is of particular interest that the inhibitory activities of **10** and **12** against the NS5B enzyme were maintained in the HCV replicon assay. It is also noteworthy that **5**, **10**, and **12** showed more potent antiviral activities in the cell-based assay ($EC_{50}$ values of 0.66, 0.54 and 0.82 μM, respectively) than in the enzyme inhibition assay ($IC_{50}$ values of 8.3, 2.02 and 0.96 μM, respectively), which warrants further investigation of these ADKs regarding their mode of actions in the cell-based assay. No ADK analogues synthesized showed cytotoxicity up to 100 μM.

In summary, due to the chemical and enzymatic instability of ADK analogues, many ADK derivatives including 5,6-dihydroxypyrimidine-4-carboxylic acids[9] are under development. However, as these derivatives are only different variations of the diketoacid functionality, understanding the binding mode of the ADK would provide valuable insights into designing novel inhibitors of the

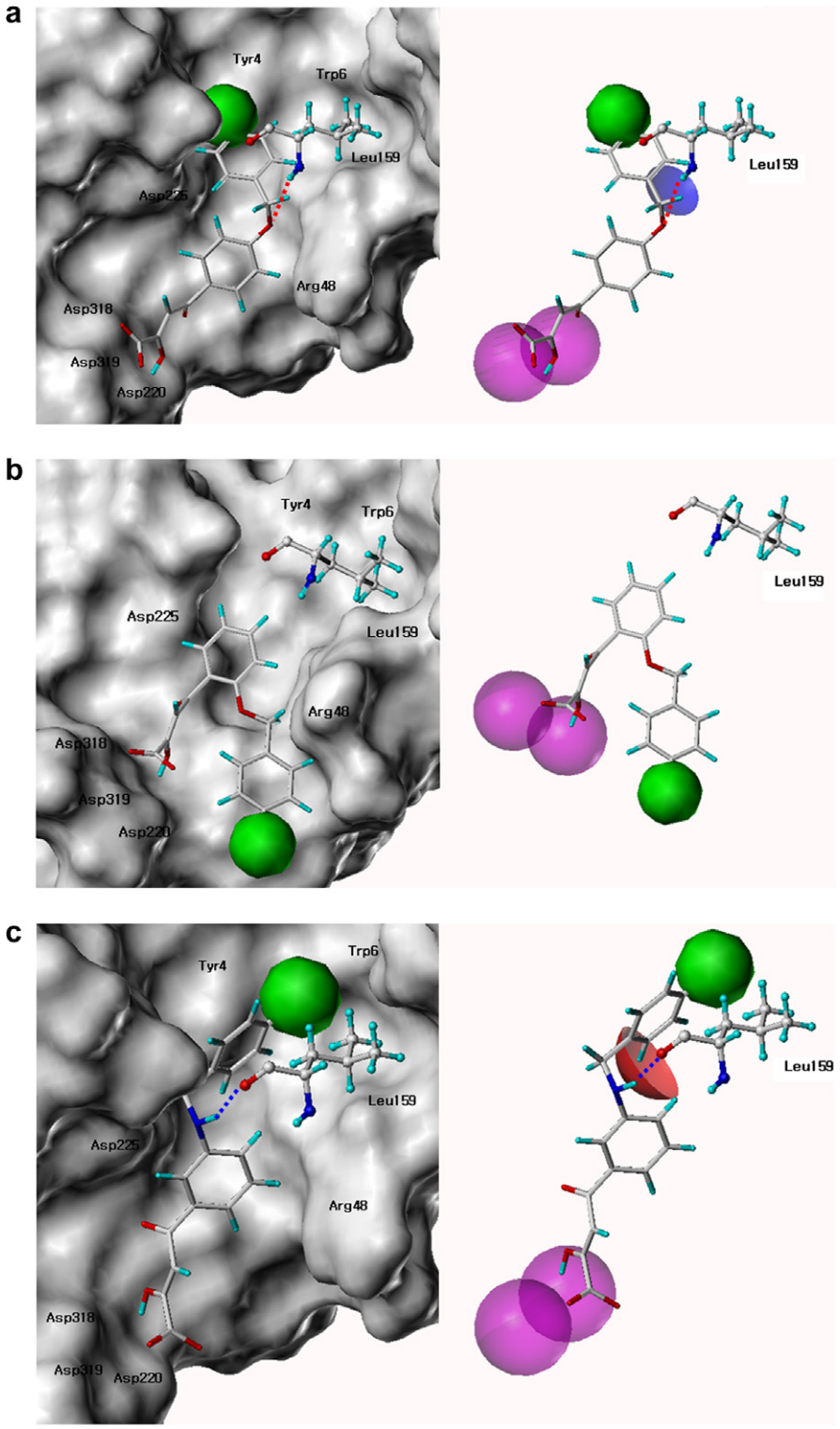



**Figure 5.** Pharmacophore-guided docking poses of (a) **6**, (b) **10**, and (c) **12**. Connolly surface of the enzyme, ADK analogues, and chlorine atoms are represented in gray surface, capped sticks, and space fill model, respectively. Colored dishes indicate specific interactions around the pharmacophore with the enzyme residues: electrostatic interaction with divalent metal ions (purple), H-bond acceptors (blue), and H-bond donors (red).

NS5B polymerase. Thus, based on our pharmacophore model of the ADK analogues, we designed and prepared a series of novel ADK analogues and evaluated their antiviral activities. We showed that ADKs with potent antiviral activities are highly dependent upon the aryl linkers as well as the size and position of the aromatic substituents. More interestingly, the hereto unreported potent antiviral activities of the pyrophosphate mimics in the subgenomic replicon assay were produced by the newly synthesized ADK analogues.

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