Three-Carbon Linked Dihydroartemisinin-Isatin Hybrids: Design, Synthesis and Their Antiproliferative Anticancer Activity

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Fifteen dihydroartemisinin-isatin hybrids (5a-e and 6a-j) linked with three-carbon were designed, synthesized. The antiproliferative activity against lung cancer cell lines including drug-sensitive A549, doxorubicin-resistant A549 (A549/DOX) and cisplatin-resistant A549 (A549/DDP) lung cancer cell lines was tested. The cytotoxicity towards normal lung epithelial BEAS-2B cell line was also investigated. From the structure-activity relationship (SAR), it was found that hydrogen bond donors (especially hydroxime and thiosemicarbazide) at C-3 position and electron-withdrawing groups (fluoro and chloro) at C-5 position of isatin moiety were beneficial for the activity. A significant part of them (half maximal inhibitory concentration/IC50: 5.72–55.52 μM) demonstrated considerable antiproliferative activity, and the activity was superior to that of dihydroartemisinin (IC50: 69.42–88.03 μM) and artemisinin (IC50: >100 μM). In particular, two hybrids 6a, e (IC50: 5.72–9.84 μM) were not inferior to doxorubicin (IC50: 4.06 μM) and cisplatin (IC50: 9.38 μM) against drug-sensitive A549 cells and were more potent than doxorubicin (IC50: 54.32 and 15.10 μM) and cisplatin (IC50: 19.74 and 66.89 μM) against multidrug-resistant A549/DOX and A549/DDP lung cancer cell lines. In addition, hybrids 6a, e (IC50: >100 μM) showed no toxicity towards BEAS-2B cells, proving their excellent selectivity profile. Furthermore, hybrid 6a also possessed good stability in mouse and human microsomes, as well as excellent pharmacokinetic properties. Accordingly, hybrid 6a could serve as a promising anti-lung cancer chemotherapeutic candidate for further preclinical evaluations.

Keywords: dihydroartemisinin, hybrid molecules, multidrug resistance, structure-activity relationship, anticancer

INTRODUCTION

Lung cancer, caused by several environmental and genetic variables (Zhang et al., 2021; Yang et al., 2022), is the leading cause of cancer related deaths and is responsible for around 20% of all cancer deaths with an estimated 1.8 million new cases and 1.6 million deaths annually (Hirsch et al., 2017; Kramer and Annema, 2021). According to the histopathological characteristics, lung cancer is mainly divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), and NSCLC accounts for about 80–85% of lung cancers (Slawiński et al., 2020; Xie et al., 2021). Despite the advances in cancer diagnosis and therapy, the advent of chemotherapeutic resistance in lung cancer and serious side effects are the major obstacles for the effective treatment (Bade and Dela...
Therefore, it is imperative to develop novel anti-lung cancer agents.

Dihydroartemisinin (DHA, Figure 1) has a unique sesquiterpene endoperoxide lactone moiety and could form highly reactive free radicals in the presence of ferrous ion (FeII). The concentration of FeII in cancer cells is 1,000 times higher than that in normal cells (Dai et al., 2017; Kiani et al., 2020). DHA could interact with FeII, resulting in an obvious inhibitory effect on lung cancer cells including lung squamous carcinoma, lung adenocarcinoma and large cells lung carcinoma without significant cytotoxicity to normal cells (Gao et al., 2020; Li et al., 2021). Isatin derivatives displayed promising anti-lung cancer activity, and the isatin-based nintedanib plus docetaxel has already been approved for treatment of patients with advanced NSCLC after first-line chemotherapy in Europe (Ding et al., 2020; Hou et al., 2020). Hence, DHA and isatin may provide useful templates for the development of novel anti-lung cancer agents.

Hybrids synthesized by fusing or conjugating different active pharmacophores together are capable to modulate multiple targets, consequently enhancing the efficacy, overcoming drug resistance and reducing side effects (Nepali et al., 2014; Sharma et al., 2014; Mishra and Singh, 2016; Choudhary et al., 2018; Hou et al., 2021). Since both DHA and isatin hold potent anti-lung cancer activity, there is a high possibility that hybridization of DHA with isatin may provide novel anti-lung cancer candidates with high activity and efficacy.

Compared with other linkers like thiazole linker, alkyl linkers are more flexible, probably making the hybrids with alkyl linkers much easier to bind with different targets. To evaluate the influence of the length of the alkyl linker between dihydroartemisinin and isatin, various of dihydroartemisinin-isatin hybrids with two-four-carbon linkers were designed and
synthesized by our group. In this paper, we only designed and synthesized a series of dihydroartemisinin-isatin hybrids with three-carbon linker (Figure 2). Afterwards, the antiproliferative activity against drug-sensitive (A549), and drug-resistant [doxorubicin-resistant A549 (A549/DOX) and cisplatin-resistant A549 (A549/DDP)] lung cancer cell lines was evaluated. The cytotoxicity towards normal lung epithelial cell line (BEAS-2B) was also investigated. Moreover, the liver stability and pharmacokinetic properties of the representative hybrids were also tested to search for the potential anti-lung cancer candidates for further studies. The biological activity and the pharmacokinetic properties of the hybrids with two carbon or four carbon linker are still under investigation, and the anticancer characteristics of the hybrids with different length of linkers will be reported in the near future.

### RESULTS AND DISCUSSION

#### Preparation of the Desired Hybrids

Scheme 1 describes the synthetic route of desired dihydroartemisinin-isatin hybrids 5a-e and 6a-j. (5-substituted) isatins 1 alkylating with 3-bromopropanol 2, using potassium carbonate (K₂CO₃) as base yielded intermediates 3. Intermediates 3 then reacted with dihydroartemisinin 4 in presence of boron trifluoride diethyl etherate (BF₃·OEt₂), giving the desired dihydroartemisinin-isatin hybrids 5a-e. Finally, the reaction of hybrids 5a-e with hydroxyamine/semicarbazide/thiosemicarbazide hydrochlorides using sodium bicarbonate (NaHCO₃) as base provided the desired dihydroartemisinin-isatin hybrids 6a-j. The chemical structures and yields of the desired hybrids were listed in Table 1. The structure of the desired dihydroartemisinin-isatin hybrids 5a-e and 6a-j were determined by HRMS, ¹H NMR and ¹³C NMR. All target compounds should be a mixture of α- and β-configuration in dihydroartemisinin skeleton, and for hybrids 6a-j, E- and Z-configuration should be existed at C-3 position of isatin moiety. Taking hybrid 6a for an example, the ¹H NMR (600 Hz, DMSO-d₆) was as follows: δ 0.86–0.99 (m, 7H, H-15, H-16 and H-5a), 1.26–1.57 (m, 7H, H-5a, H-7a, H-6, H-14 and H-8a), 1.64–1.90 (m, 3H, H-7a, and H-8), 1.95–2.06 (m, 3H,

### Table 1: Chemical structures and yields of dihydroartemisinin-isatin hybrids 5a-e and 6a-j.

| Compd | R₁ | R₂ | Yield (%) |
|-------|----|----|-----------|
| 5a    | H  | O  | 61        |
| 5b    | F  | O  | 48        |
| 5c    | Cl | O  | 53        |
| 5d    | Me | O  | 57        |
| 5e    | OMe| O  | 39        |
| 5a    | H  | NOH| 87        |
| 5b    | H  | NNHCONH₂| 41 |
| 5c    | H  | NNHCSNH₂| 33 |
| 5d    | F  | NNHCONH₂| 48 |
| 5e    | F  | NNHCSNH₂| 26 |
| 5f    | Cl | NOH| 72        |
| 5g    | Me | NOH| 91        |
| 5h    | Me | NNHCSNH₂| 30 |
| 5i    | OMe| NOH| 88        |
| 5j    | OMe| NNHCSNH₂| 25 |

### Table 2: In vitro antiproliferative activities, cytotoxicity of dihydroartemisinin-isatin hybrids 5a-e and 6a-j.

| Compd | IC₅₀ (µM) | A549 | A549/DOX | A549/DDP | BEAS-2B | SIA | RI₁ | RI₂* |
|-------|----------|------|----------|----------|---------|-----|-----|------|
| 5a    | >100     | >100 | >100     | >100     | >100    | -   | -   | -    |
| 5b    | >100     | >100 | >100     | >100     | >100    | >1.8| 0.93| 1.49 |
| 5c    | 5.72     | 7.35 | 9.84     | >100     | >100    | >17.4| 1.28| 1.66 |
| 5d    | >100     | >100 | >100     | >100     | >100   | >4.3 | 1.57| 1.24 |
| 5e    | >100     | >100 | >100     | >100     | >100   | -   | -   | -    |
| 5f    | 5.99     | 8.93 | 6.17     | >100     | >100   | >16.6| 1.49| 1.03 |
| 5g    | 24.74    | 26.88| 31.96    | >100     | >100   | >4.0 | 1.08| 1.29 |
| 5h    | 55.52    | 29.90| 49.67    | >100     | >100   | >1.8 | 0.54| 0.89 |
| 5i    | 99.57    | 87.31| 94.23    | >100     | >100   | >1.0 | 0.88| 0.95 |
| 5j    | 51.59    | 73.53| 60.17    | >100     | >100   | >1.9 | 1.46| 1.17 |
| 6a    | 52.62    | 78.46| 40.68    | >100     | >100   | >1.9 | 1.68| 0.77 |
| Artemisinin | >100 | >100 | >100 | >100 | >1.4 | 1.27 | 1.09 |
| DHAfl | 69.42    | 88.03| 75.91    | >100     | >100   | >1.4 | 1.27| 1.09 |
| cisplatin | 9.38 | 19.74| 66.89    | 89.63    | 9.5    | 2.10| 7.13|
| doxorubicin | 4.06 | 54.32| 15.10    | 93.76    | 23.1   | 13.38| 3.72|

*Selectivity index: IC₅₀(A549/DDP)/IC₅₀(A549).
*Doxorubicin-resistant A549 cells.
*Cisplatin-resistant A549 cells.
*Resistance index: IC₅₀(A549/DDP)/IC₅₀(A549).
*Dihydroartemisinin.
-CH₂- linker and H-5a1), 2.25–2.66 (m, 3H, H-9, H-4a1 and H-4a2), 3.45–4.12 (m, 4H, -OCH₂- linker and -NCH₂- linker), 4.95 (d, J = 2.0 Hz, 1H, H-10), 5.47 (s, 1H, H-12), 6.88 (d, J = 4.0 Hz, 1H, isatin-H), 7.09 (t, J = 4.0 Hz, 1H, isatin-H), 7.36 (t, J = 4.0 Hz, 1H, isatin-H), 8.12 (d, J = 4.0 Hz, 1H), 11.04 (brs, 1H, NOH).

**Antiproliferative Activity and Cytotoxicity Study in vitro**

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determined the antiproliferative activity of dihydroartemisinin-isatin hybrids 5a-e and 6a-j with three-carbon linker against A549, multidrug-resistant A549/DOX and A549/DDP lung cancer cell lines as well as cytotoxicity towards normal lung epithelial cell line (BEAS-2B). The inhibitory effects were expressed in terms of half maximal inhibitory concentration (IC₅₀) values. It can be seen from Table 2 that nine desired dihydroartemisinin-isatin hybrids (5c, 6a, 6c, 6e-j, IC₅₀: 5.72–99.57 μM) showed decent antiproliferative activity against A549, A549/DDO and A549/DDP lung cancer cell lines. These nine hybrids were much more active than artemisinin (IC₅₀: >100 μM), and seven of them (6a, 6c, 6e-g, 6i-j) displayed superior inhibition to the parent drugs DHA (IC₅₀: 69.42–88.03 μM). The SAR revealed that introducing hydroxime and thiosemicarbazide into C-3 position of isatin moiety were beneficial for the activity, while carbonyl and semicarbazide had negative influence on the activity. Electron-withdrawing groups such as fluoro and chloro at C-5 position of isatin moiety could improve the activity to some extent, while electron-donating groups such as methyl and methoxy led to a significant loss of activity.

The cytotoxic results showed that all the desired hybrids (IC₅₀: >100 μM) were non-cytotoxic towards normal lung epithelial cell line (BEAS-2B), and nine of them (5c, 6a, 6c, 6e-j) had the selectivity index (SI: IC₅₀(BEAS-2B)/IC₅₀(A549)) values > 1.0. In addition, the desired hybrids possessed the activity in the same level against both drug-sensitive and multidrug-resistant A549 lung cancer cell lines, and the drug resistance index (RI: IC₅₀(MDR A549)/IC₅₀(A549)) values were 0.54–1.68, demonstrating their potential to overcome drug resistance.

In particular, hybrids 6a, e (IC₅₀: 5.72–9.84 μM) showed pronounced activity against A549, A549/DOX and A549/DDP lung cancer cell lines. The activity of hybrids 6a, e was not inferior to that of cisplatin (IC₅₀: 9.38 μM) and doxorubicin (IC₅₀: 4.06 μM) against A549 cells. These two hybrids were 1.5–10.8 times superior to cisplatin (IC₅₀: 19.74 and 66.89 μM) and doxorubicin (IC₅₀: 54.32 and 15.10 μM) against multidrug-resistant A549/DOX and A549/DDP lung cancer cell lines. Moreover, the SI index values of hybrids 6a, e were >16.6, revealing that both of them possessed high specificity.

**Conclusion**

In this study, we designed and synthesized fifteen novel dihydroartemisinin-isatin hybrids 5a-e and 6a-j with three-carbon linker. Their antiproliferative activity against both drug-sensitive and multidrug-resistant lung cancer cell lines was also evaluated. Among them, hybrids 6a, e (IC₅₀: 5.72–9.84 μM) were highly potent against the three tested lung cancer cell lines, and the activity was comparable or superior to that of cisplatin (IC₅₀: 9.38–66.89 μM) and doxorubicin (IC₅₀: 4.06–54.32 μM). These two hybrids (IC₅₀: >100 μM) also demonstrated non-cytotoxicity towards normal lung epithelial cell line (BEAS-2B), and the SI index values of these two hybrids were >16.6, demonstrating their excellent safety profile. The RI values of hybrids 6a, e were 1.03–1.66, indicating that these two hybrids could overcome drug resistance. In addition, hybrid 6a also exhibited good stability in mouse and human microsomes and excellent pharmacokinetic properties. From these results, hybrid 6a could be considered as a promising anti-lung cancer chemotherapeutic candidate for further evaluations.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding authors.

**ETHICS STATEMENT**

The animal study was reviewed and approved by the Animal Care and Use Committee of Shandong University with the corresponding ethical approval code (LL-201602040, 2016–2022).
AUTHOR CONTRIBUTIONS

FG designed the experiment; MD and GZ performed the experiment and wrote the draft. FG, ML and CZ corrected the manuscript. All authors approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.834317/full#supplementary-material

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