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ORIGINAL ARTICLE

Artemisinin-isatin hybrids with potential antiproliferative activity against breast cancer

Yanhua Wang [a,1], Ruiqi Ding [a,1], Zijian Tai [a,1], Haodong Hou [b], Feng Gao [b], Xiangyang Sun [c,*]

[a] *Department of Critical Care Medicine, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong 250012, China*
[b] *Key Laboratory for Experimental Teratology of the Ministry of Education and Center for Experimental Nuclear Medicine, School of Basic Medical Sciences, Cheeloo College of Medicine, Shandong University, Jinan, Shandong 250012, China*
[c] *Department of Interventional Radiology, Qilu Hospital of Shandong University, Jinan, Shandong 250012, China*

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KEYWORDS

Artemisinin;
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Structure–activity relationship

Abstract Three series of artemisinin-isatin hybrids **8a-i**, **10a-c** and **11a-e** were designed, synthesized and evaluated for their antiproliferative activity against breast cancer cells (MCF-7, MDA-MB-231 and doxorubicin-resistant MCF-7 (MCF-7/DOX)), as well as the cytotoxicity towards normal MCF-10A breast cells. The preliminary results showed that a significant part of the synthesized hybrids (IC_{50}: 20.7–99.9 μM) were active against both drug-sensitive and doxorubicin-resistant breast cancer cell lines. The structure–activity relationship illustrated that the linker between artemisinin and isatin moieties as well as the substituents on C-3 and C-5 position of isatin motif had great influence on the activity. In particular, hybrids **11c,d** were found to be most active against all tested breast cancer cell lines, and their activity was not inferior to that of doxorubicin. Therefore, hybrids **11c,d** could serve as useful templates for the development of novel anti-breast cancer agents.

1. Introduction

Breast cancer, a highly heterogeneous disease, is one of the most prevalent and reoccurring cancers, and it is also considered as the second most common cause of death in women (Houssein et al., 2021; Ansari et al., 2021). It is estimated by International Agency for Research on Cancer that 2.3 million new cases of breast cancer were diagnosed and 684,996 deaths occurred in 2020, and the worldwide incidence of female breast cancer will be projected to approximately 3.2 million new cases per year by 2050 (International Agency for Research on Cancer, 2021; Tao et al., 2015). Many synthetic drugs and medications are provided with their beneficial actions, but the advent of chemotherapeutic resistance in breast cancer and serious side

* Corresponding author.
E-mail address: rgsuper@163.com (X. Sun).
[1] Yanhua Wang, Ruiqi Ding and Zijian Tai contributed equally.
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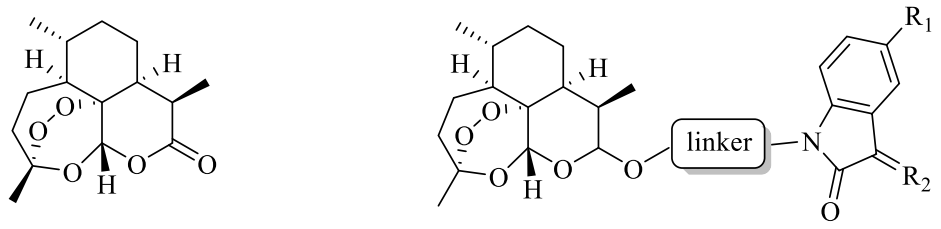
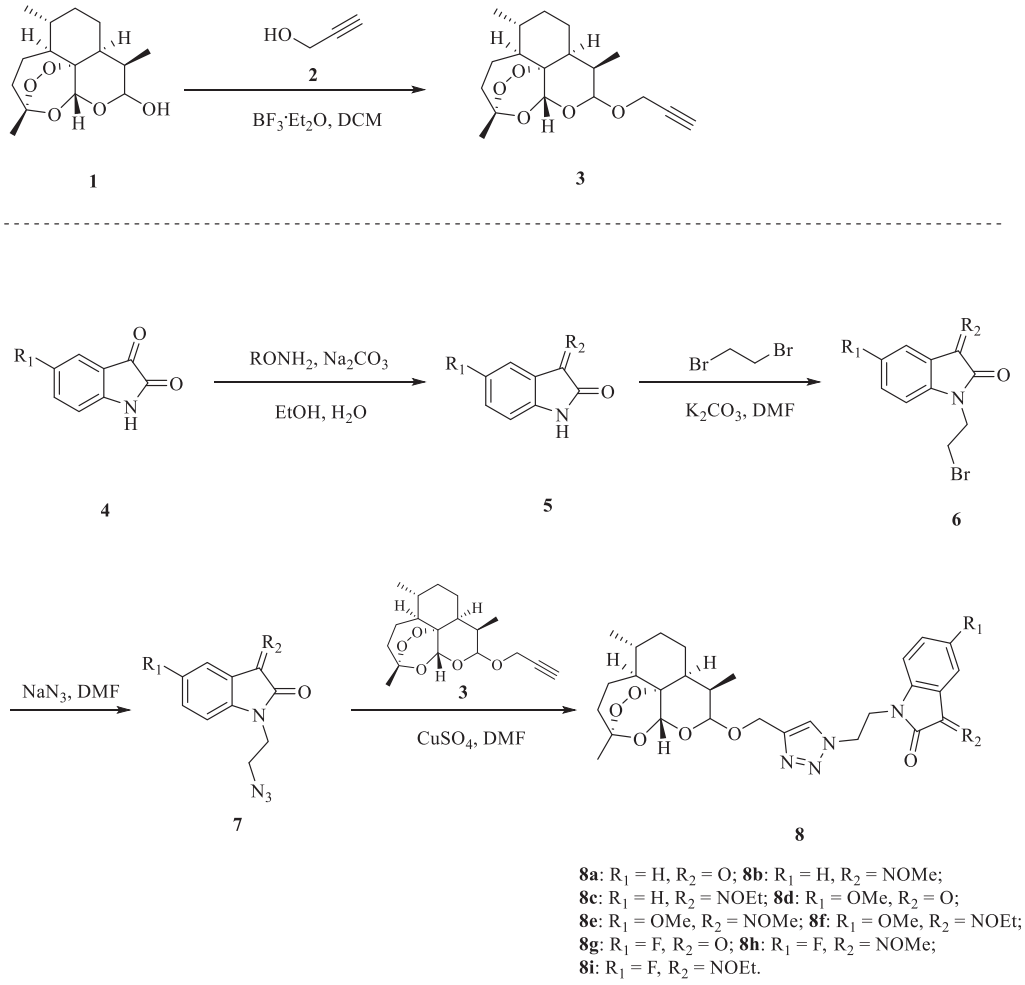


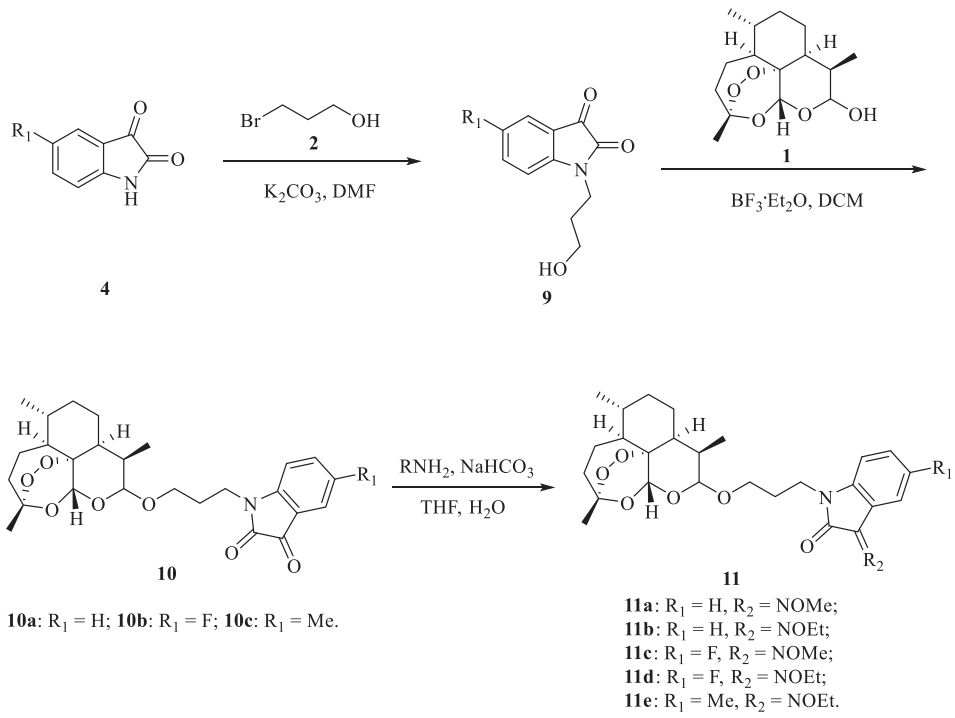
Fig. 1 Chemical structures of artemisinin and artemisinin-isatin hybrids.



Scheme 1 Synthesis of artemisinin-1,2,3-triazole-isatin hybrids **8a-i.**

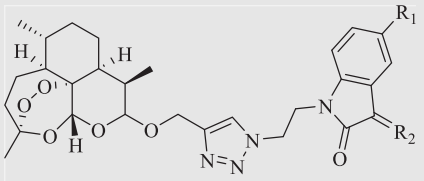
effects are the major obstacles to effective cancer chemotherapy (Li et al., 2021; Guo and Jiang, 2021). Therefore, there is an urgent need to develop novel anti-breast cancer agents.

Artemisinin (Fig. 1) derivatives are potential anticancer agents for the treatment of highly aggressive cancers by inhibiting cancer proliferation, metastasis, and angiogenesis *via* the induction of ferroptosis by iron-mediated cleavage of the endoperoxide bridge (Zhu et al., 2021; Zhang, 2020). Artemisinin derivatives possess profound *in vitro* and *in vivo* efficacy against both drug-sensitive and drug-resistant breast cancers, and no significant cytotoxicity from artemisinin to normal cells is observed, demonstrating the different mechanism from conventional chemotherapy (Dong et al., 2020; Gao et al., 2020; Kiani et al., 2020). Isatin is an endogenous substance which distributed widely in nature, and its derivatives are potential inhibitors of histone deacetylase, β-carbonic anhydrase, tyrosine kinase and tubulin (Nath et al., 2021; Hou et al., 2020). In addition, almost all positions of isatin moiety can be modified, and the substituents on C-3 (R_1 position) and C-5 (R_1 position) positions of isatin moiety influence the biological activity significantly. Moreover, some isatin-based agents such as semaxanib and sunitinib exhibited excellent *in vivo* anti-breast cancer activity and have already been under clinical evaluations for the treatment of breast cancer (Ding et al., 2020; Ferraz de Paiva et al., 2020).



Scheme 2 Synthesis of dihydroartemisinin-isatin hybrids **10a-c** and **11a-e.**

Table 1 The antiproliferative activity and cytotoxicity of artemisinin-1,2,3-triazole-isatin hybrids **8a-i.**



8a-i

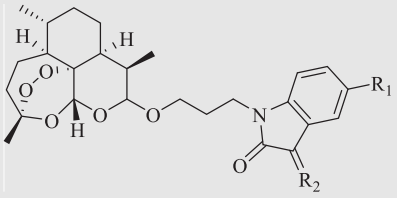
Hybrids	IC_{50} (μM)				SI[a]	RI[b]
	MCF-7	MDA-MB-231	MCF-7/DOX	MCF-10A		
8a	> 100	> 100	> 100	> 100	–	–
8b	> 100	> 100	> 100	> 100	–	–
8c	99.8	94.6	91.3	> 100	> 1.0	0.92
8d	> 100	> 100	> 100	> 100	–	–
8e	> 100	> 100	> 100	> 100	–	–
8f	> 100	> 100	> 100	> 100	–	–
8g	99.8	80.7	93.7	> 100	> 1.0	0.94
8h	98.1	85.4	76.9	> 100	> 1.0	0.78
8i	64.7	59.8	66.6	> 100	> 1.5	1.03
artemisinin	87.6	72.4	96.3	> 100	> 1.1	1.10
doxorubicin	18.9	17.2	83.4	68.8	3.6	4.41

[a] selectivity index: $IC_{50(MCF\text{-}10A)}/IC_{50(MCF\text{-}7)}$.
[b] resistance index: $IC_{50(MCF\text{-}7/DOX)}/IC_{50(MCF\text{-}7)}$.

Hence, it is conceivable that hybridization of the artemisinin with C-3 and or C-5 substituted isatin could provide valuable therapeutic agents for the treatment of breast cancer.

Based on the above considerations, three series of artemisinin-isatin hybrids tethered through different linkers were designed and synthesized in this study. The *in vitro* antiproliferative activity of the syn-

Table 2 The antiproliferative activity and cytotoxicity of artemisinin-isatin hybrids **10a-c** and **11a-e.**



Hybrids	IC_{50} (μM)				SI[a]	RI[b]
	MCF-7	MDA-MB-231	MCF-7/DOX	MCF-10A		
10a	> 100	> 100	> 100	> 100	–	–
10b	76.4	88.3	77.2	> 100	> 1.3	1.01
10c	> 100	> 100	> 100	> 100	–	–
11a	69.9	72.7	58.4	> 100	> 1.4	0.84
11b	57.6	49.5	37.0	> 100	> 1.7	0.64
11c	31.0	34.7	32.8	> 100	–	–
11d	20.7	23.9	21.3	> 100	> 1.3	0.96
11e	99.9	86.8	97.1	> 100	> 4.8	1.03
artemisinin	87.6	72.4	96.3	> 100	> 1.1	1.10
doxorubicin	18.9	17.2	83.4	68.8	3.6	4.41

[a] selectivity index: $IC_{50(MCF\text{-}10A)}/IC_{50(MCF\text{-}7)}$;
[b] resistance index: $IC_{50(MCF\text{-}7/DOX)}/IC_{50(MCF\text{-}7)}$.

thesized hybrids against breast cancer cell lines (MCF-7, MDA-MB-231 and doxorubicin-resistant MCF-7 (MCF-7/DOX)), and the cytotoxicity towards normal MCF-10A breast cells were evaluated. Moreover, the structure–activity relationship (SAR) was also discussed for further rational design of more efficient candidates against breast cancer.

2. Results and discussion

Three series of artemisinin-isatin hybrids **8a-i**, **10a-c** and **11a-e** were prepared by the synthetic routes depicted in Schemes 1 and 2. Etherification of dihydroartemisinin (**1**) with propargyl alcohol (**2**) in the presence of boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$) provided intermediate **3**. Isatins **4** reacting with methoxylamine/ethoxylamine hydrochloride with sodium carbonate (Na_2CO_3) as base yielded intermediates **5**. Alkylation of isatins **5** with 1,2-dibromoethane using potassium carbonate (K_2CO_3) as base generated intermediates **6**, which were then converted to azido precursors **7** by treating with sodium azide. The desired artemisinin-1,2,3-triazole-isatin hybrids **8a-i** were obtained through Cu-promoted azide-alkyne cycloaddition reaction between intermediate **3** and azido precursors **7**.

Alkylation of (5-substituted) isatins **4** with 3-bromopropanol generated intermediates **9**, which were then reacted with dihydroartemisinin **1** in the presence of boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$), yileding desired dihydroartemisinin-isatin hybrids **10a-c**. Finally, introduction of methoxime or ethoxime into C-3 position of isatin moiety in dihydroartemisinin-isatin hybrids **10a-c** provided desired dihydroartemisinin-isatin hybrids **11a-e**.

All of the hybrids **8a-i**, **10a-c** and **11a-e** were characterized by MS, ^1H NMR and ^{13}C NMR, and the corresponding analytical spectra were in the supplementary information section.

The antiproliferative activity of artemisinin-isatin hybrids **8a-i**, **10a-c** and **11a-e** against MCF-7, MDA-MB-231 and doxorubicin-resistant MCF-7 (MCF-7/DOX) breast cancer cell lines was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphe nyltetrazolium bromide (MTT) assay, and half maximal inhibitory concentration (IC_{50}) values were listed in Tables 1 and 2.

From Tables 1 and 2, it can be seen that ten of the synthesized hybrids (IC_{50}: 20.7–99.9 μM) showed considerable activity against MCF-7, MDA-MB-231 and MCF-7/DOX breast cancer cell lines, and five of them were more potent than artemisinin (IC_{50}: 72.4–96.3 μM). The SAR indicated that the linker between artemisinin and isatin moieties influenced the activity remarkably, and alkyl linker (**10a-c** and **11a-e**) was better than 1,2,3-triazole (**8a-i**). Methoxime and ethoxime were more favorable than ketone at C-3 position of isatin moiety, and the relative contribution order was ethoxime (**11b,d**) > methoxime (**11a,c**) > ketone (**10a-c**). Additionally, electron-drawing fluoro (**11c,d**) at C-5 position of isatin motif was beneficial for the activity, while electron-donating methoxy group (**11e**) decreased the activity. In particular, hybrids **11c,d** (IC_{50}: 31.0–34.7 μM and 20.7–23.9 μM, respectively) were comparable to doxorubicin (IC_{50}: 18.9 and 17.2 μM) against drug-sensitive MCF-7 and MDA-MB-231 breast cancer cell lines and ~ 3 folds more active than doxorubicin (IC_{50}: 18.9–83.4 μM) against MCF-7/DOX cells, demonstrating their potential to fight against both drug-sensitive and drug-resistant breast cancers.

All hybrids (IC_{50}: > 100 μM) were non-toxic towards normal MCF-10A breast cells, and the selectivity index (SI) values for the most active hybrids **11c,d** were > 3.2, indicating their excellent selectivity. Moreover, the resistance index (RI) values for hybrids **11c,d** were 1.06 and 1.03 respectively, implying that these hybrids had no cross resistance with doxorubicin.

3. Conclusion

In summary, seventeen artemisinin-isatin hybrids were designed, synthesized and evaluated for their antiproliferative activity against MCF-7, MDA-MB-231 and MCF-7/DOX breast cancer cell lines and cytotoxicity towards normal MCF-10A breast cells. Among them, hybrids **11c,d** not only showed non-toxicity towards normal MCF-10A breast cells, but also demonstrated potent activity against the three tested breast cancer cell lines. The activity of hybrids **11c,d** was not inferior to that of doxorubicin, so they both were considered as useful matrices for the development of novel anti-breast cancer drugs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103639.

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