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Curcumin derivatives with 1,5-diaryl-3-oxo-1,4-pentadiene: novel dual-function lead compounds showing anticancer and MDR reversal effects

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1,5-Diaryl-3-oxo-1,4-pentadiene is an excellent pharmacophore derived from Curcumin. Synthesis of six focused 1,5-diaryl-3-oxo-1,4-pentadiene libraries (**1a–1e**, **2a–2e**, **3a–3e**, **4a–4e**, **5a–5e** and intermediates **A–E**) and cytotoxicity screening identified compounds **1d–5d** with N-3-F-PhCH$_2$, which are generally more active than the corresponding molecules possessing the unsubstituted benzylidene moiety. The IC$_{50}$ values of 14 out of 35 compounds are lower than 5 μM, particularly **5d** and **4d**, which have IC$_{50}$ values of 0.17 μM (A549) and 0.68 μM (HeLa), respectively. Most compounds exhibited a preferential and selective toxicity to cancer cells *versus* normal liver cells (LO2), except for **3c**, **4c**, **4e** and **5e**, which exhibited slightly poor activity. In total, 7 human cancer cell lines were analyzed, with all derivatives showing broad-spectrum activity. Simultaneously, multidrug resistance (MDR) reversal activity and cellular uptake were determined. Analysis of data revealed that most compounds with simple 2 F, 4 F or 3-Br at benzylidene possessed potent MDR-reversing properties, reducing the expression level of the drug-resistance gene MDR1 to below 50% *versus* control, in particular, **4a** and **4e**, with reductions to less than 10% and 15%, respectively. Importantly, the intermediates (**C**, **D** and **E**), **3a**, **3b**, **4a**, **4d**, **4e**, **5d** and **5e** displayed both potent MDR-reverting properties and strong cytotoxicity, which may serve as a guideline for future molecular modification strategies to develop dual-function agents in cancer therapy.

Introduction

Cancer is a fatal disease initiated by progressive genetic mutations and alterations, which drive the transformation of normal cells into malignant cells.[1–4] It is regarded as a major global health issue and the second most frequent cause of mortality across the world.[4–7] The treatment strategies for cancer include several therapies, such as surgical treatment, chemotherapy, radiotherapy, immunotherapy, and targeted therapy. Among these, chemotherapy is the standard mainstay treatment, involving single or several anticancer drugs as a standard regimen.[8–11] However, the outcome of chemotherapy is adversely influenced by serious side effects of anticancer agents and MDR.[11–13] The crucial reason for chemotherapy resulting in adverse side effects is owing to its cytotoxic effect on normal cells. Therefore, developing novel anticancer drugs, which display preferential and potent cytotoxicity to cancer cells, or multi-targeting drugs remains an immense challenge and an enormous medical need for cancer chemotherapy.[11,13–18] Moreover, MDR is a critical factor that results in the failure of tumor chemotherapy.[9,13,19–21] Currently, finding novel MDR reversal agents or dual drugs with both anticancer and MDR reversing activities is a promising solution.[22]

Curcumin is studied widely due to its diverse pharmacological activities[23] that include cytotoxic properties against various cancer cells,[24–26] exhibiting anti-angiogenesis,[27] along with anti-metastatic[28] and antioxidant activities.[29] Moreover, curcumin can reverse MDR and enhance the sensitivity of resistant cells to chemotherapy.[8,11] Nevertheless, its clinical application is significantly restrained by poor oral bioavailability, weak pharmacokinetics, and poor solubility in aqueous media such as water and plasma; however, curcumin displays an excellent safety profile and diverse pharmacological activities.[23] As a result, curcumin has become a modification target for the development of potential antitumor candidates and potent chemosensitizers.[11,23] The modification sites of curcumin are mainly concerned with the aromatic rings, the *β*-diketone moiety, and the methylene groups, which are regarded as the main reason for its poor oral absorption, low pharmacokinetics and unstable properties *in vivo*.[23,30] Especially, an excellent pharmacophore of 1,5-diaryl-3-oxo-1,4-pentadiene (Fig. 1) was obtained after the α,β-unsaturated *β*-diketone in curcumin was converted into α,β-unsaturated monoketone, which displays both antiproliferative and MDR-reverting activities.[23,30–35]

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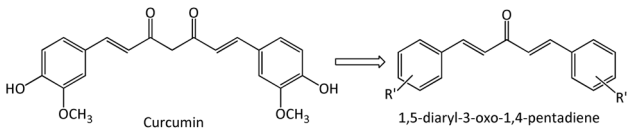


Fig. 1 Modification of curcumin to 1,5-diaryl-3-oxo-1,4-pentadiene.

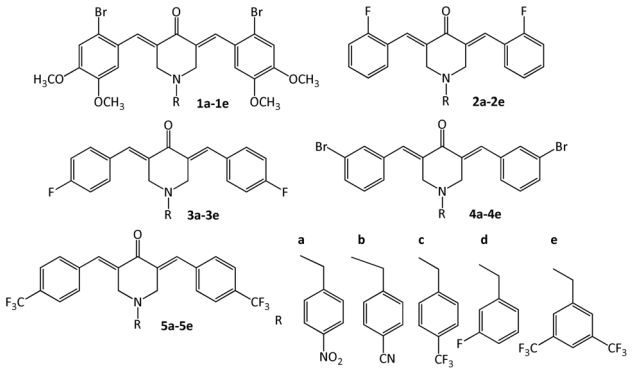
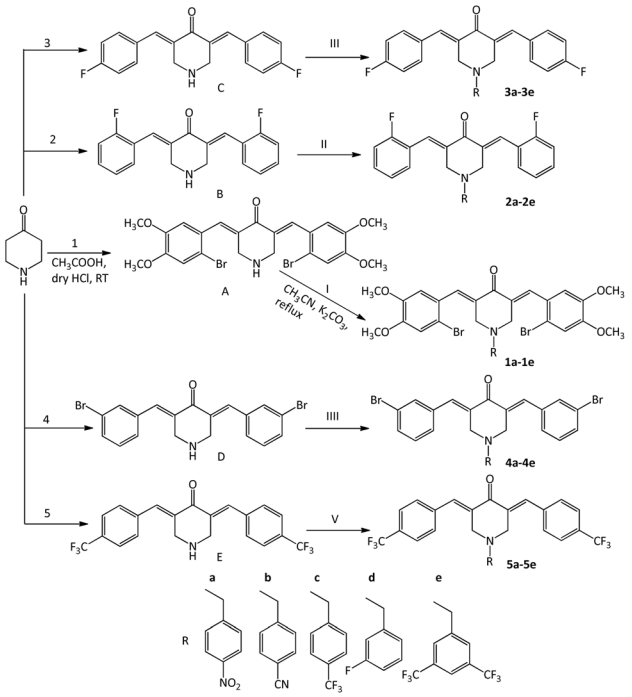


Fig. 2 Chemical structures of 1,5-diaryl-3-oxo-1,4-pentadiene small molecules.



Scheme 1 Synthetic route of small molecules in focused libraries **1–5**.

According to the "sequential cytotoxicity" hypothesis, 1,5-diaryl-3-oxo-1,4-pentadiene can provide continuous chemical attack, which is more harmful for tumor cells compared with that for normal cells; thus, the compounds with this pharmacophore may possess low toxicity and high selectivity.[25,26] Meanwhile, in our previous study, a series of novel piperidine α,β-unsaturated carbonyl compounds with 1,5-diaryl-3-oxo-1,4-pentadiene were found to show good to significant bioactivity;[27–29] in particular, some quaternary ammonium salt derivatives of piperidone N exhibited both potent MDR reversal activity and cytotoxicity.[29] Herein, in order to further investigate the influence of *N*-substituents and different benzylidenes on bioactivity and optimize the structure, the development and cytotoxicity of focused analogue libraries of 1,5-diaryl-3-oxo-1,4-pentadiene with *N*-benzyl are reported (Fig. 2).

Results and discussion

Synthesis and structures

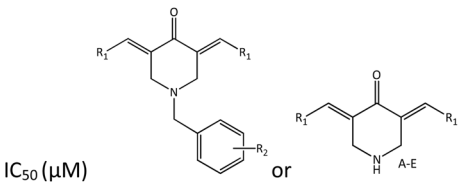
Compounds **1a–1e**, **2a–2e**, **3a–3e**, **4a–4e** and **5a–5e** were synthesized through the synthetic routes shown in Scheme 1. Various aryl aldehydes were subjected to reactions with 4-piperidone *via* a Claisen–Schmidt condensation, using dry hydrogen chloride as the catalyst, resulting in the target intermediates of **A**, **B**, **C**, **D** and **E**. Then, these compounds reacted with 4-nitrobenzyl bromide, 4-cyanobenzyl bromide, 4-trifluoromethylbenzyl bromide, 3-fluorobenzyl bromide or 3,5-ditrifluoromethylbenzyl bromide, respectively, through *N*-acylation, and the corresponding small molecules with 1,5-diaryl-3-oxo-1,4-pentadiene (**1a–1e**, **2a–2e**, **3a–3e**, **4a–4e** and **5a–5e**) were accomplished. The yields of the compounds were between 25% and 82%. Their structures were thoroughly elucidated *via* UV, IR, ^{1}H NMR, ^{13}C NMR and HRMS.

Antiproliferative and antitumor activity *in vitro*

Seven human tumor cell lines, including HepG2 (liver), HT-29 (colon), SGC7901 (stomach), A549 (lung), HeLa (cervix), K562 (leukemia) and K562/DOX (Doxorubicin-resistant human myelogenous leukemia cell line) from the digestive system, respiratory system, reproductive system, circulatory system and a human normal liver cell line (LO2) were utilized to evaluate compounds **1a–1e**, **2a–2e**, **3a–3e**, **4a–4e** and **5a–5e** to validate if their antiproliferative activities against various neoplasms can be demonstrated. The outcomes are summarized in Table 1, tabulating the corresponding IC_{50} (Half maximal inhibitory concentration) values (The concentration of the drug that inhibits cell growth by 50%). Cisplatin (DDP) and Doxorubicin (DOX) were used as the control.

Analysis of the MTT screening data arising from the examination of the synthesized small molecules displayed a remarkable inhibitory effect *in vitro*. Broad spectrum activity was observed against HepG2, HT-29, SGC7901, A549, HeLa, K562 and K562/DOX cell lines (Table 1), among which K562 is the most sensitive to these compounds. Except for **3a**, **1b**, **1c**, **5b** and C, the IC_{50} values of all other compounds towards K562 are lower than 10 μM, with 12 IC_{50} values out of 30 below 5 μM. While HT-29 is the most refractory to these derivatives, the IC_{50} value of compound A is below 5 μM (4.95 μM). Additionally, all compounds showed moderate to significant potency against K562/DOX from 2.33 to 22.99 μM; particularly, the IC_{50}values of **2d** (4.20 μM), **2e** (4.25 μM), **3d** (2.34 μM), and **E** (2.33 μM) are lower than 5 μM, while the IC_{50} value of the control (DDP) is only 31.94 μM. Importantly, almost all molecules preferentially and selectively act on cancer cells from a good to excellent range *versus* the normal liver cell line (LO2), except **3c**, **4c**, **4e** and **5e**,

Table 1 Determination of IC_{50} (μM) of all compounds against various human cancer cell lines and the normal cell line LO2 (normal liver); Av: the average IC_{50} values



Comp.	R_1	R_2	HepG2	A549	HT-29	HeLa	K562	K562D	SGC7901	LO2
1a	2-Br-4,5-diOCH_3Ph	4-NO_2	4.81 ± 0.01	14.6 ± 0.09	23.38 ± 0.01	11.04 ± 0.11	8.00 ± 0.06	18.18 ± 0.07	13.06 ± 0.01	98.99 ± 0.08
2a	2-FPh	4-NO_2	9.01 ± 0.01	5.65 ± 0.03	15.44 ± 0.03	12.9 ± 0.01	9.02 ± 0.02	13.05 ± 0.09	11.9 ± 0.03	63.63 ± 0.14
3a	4-FPh	4-NO_2	7.53 ± 0.06	9.10 ± 0.05	15.64 ± 0.01	11.63 ± 0.22	10.57 ± 0.02	6.92 ± 0.08	10.45 ± 0.02	94.58 ± 0.25
4a	4-BrPh	4-NO_2	3.97 ± 0.05	6.60 ± 0.03	8.55 ± 0.03	13.47 ± 0.07	2.83 ± 0.08	10.92 ± 0.09	8.26 ± 0.04	103.85 ± 0.01
5a	4-CF_3Ph	4-NO_2	11.02 ± 0.18	10.76 ± 0.01	7.88 ± 0.11	10.59 ± 0.15	3.91 ± 0.06	13.81 ± 0.04	8.33 ± 0.08	135.04 ± 0.01
1b	2-Br-4,5-diOCH_3Ph	4-CN	15.1 ± 0.24	16.07 ± 0.01	15.76 ± 0.01	19.35 ± 0.02	11.3 ± 0.09	21.85 ± 0.13	6.97 ± 0.05	176.88 ± 0.05
2b	2-FPh	4-CN	3.07 ± 0.09	11.29 ± 0.08	20.72 ± 0.01	1.21 ± 0.07	2.80 ± 0.004	10.76 ± 0.08	11.85 ± 0.15	18.40 ± 0.17
3b	4-FPh	4-CN	2.86 ± 0.03	7.97 ± 0.08	11.62 ± 0.17	18.85 ± 0.03	2.29 ± 0.03	5.15 ± 0.02	7.26 ± 0.08	392.56 ± 0.00
4b	3-BrPh	4-CN	11.02 ± 0.08	10.76 ± 0.01	7.88 ± 0.02	10.59 ± 0.15	3.91 ± 0.06	13.81 ± 0.04	8.33 ± 0.08	135.04 ± 0.11
5b	4-CF_3Ph	4-CN	29.43 ± 0.01	22.09 ± 0.01	24.75 ± 0.09	7.05 ± 0.03	17.9 ± 0.13	29.57 ± 0.17	22.99 ± 0.05	155.43 ± 0.05
1c	2-Br-4,5-diOCH_3Ph	4-CF_3	14.7 ± 0.31	13.93 ± 0.02	13.13 ± 0.01	13.41 ± 0.04	15.83 ± 0.04	17.35 ± 0.19	21.05 ± 0.04	757.01 ± 0.01
2c	2-FPh	4-CF_3	12.67 ± 0.04	7.08 ± 0.04	11.44 ± 0.03	8.94 ± 0.04	5.89 ± 0.03	13.85 ± 0.08	4.94 ± 0.02	39.32 ± 0.01
3c	4-FPh	4-CF_3	7.23 ± 0.05	5.88 ± 0.04	11.95 ± 0.18	11.66 ± 0.01	3.52 ± 0.01	7.60 ± 0.09	5.62 ± 0.05	14.40 ± 0.05
4c	3-BrPh	4-CF_3	5.08 ± 0.05	3.04 ± 0.17	5.79 ± 0.05	12.41 ± 0.01	4.30 ± 0.03	6.02 ± 0.07	3.69 ± 0.04	10.91 ± 0.38
5c	4-CF_3Ph	4-CF_3	7.58 ± 0.05	7.82 ± 0.03	5.05 ± 0.08	15.82 ± 0.01	7.47 ± 0.05	10.7 ± 0.04	5.25 ± 0.03	26.58 ± 0.07
1d	2-Br-4,5-diOCH_3Ph	3 F	19.94 ± 0.14	13 ± 0.011	12.93 ± 0.12	4.51 ± 0.04	9.60 ± 0.04	19.17 ± 0.03	9.86 ± 0.05	274.07 ± 0.42
2d	2-FPh	3 F	7.23 ± 0.02	10.03 ± 0.01	8.69 ± 0.03	6.11 ± 0.02	5.65 ± 0.09	4.20 ± 0.02	4.46 ± 0.05	102.36 ± 0.05
3d	4-FPh	3 F	5.22 ± 0.17	6.03 ± 0.05	8.09 ± 0.06	4.05 ± 0.01	2.00 ± 0.04	2.34 ± 0.01	2.73 ± 0.04	74.28 ± 0.06
4d	3-BrPh	3 F	4.66 ± 0.02	5.48 ± 0.05	9.88 ± 0.09	0.68 ± 0.05	6.38 ± 0.04	14.41 ± 0.01	4.06 ± 0.02	51.51 ± 0.03
5d	4-CF_3Ph	3 F	3.19 ± 0.02	0.17 ± 0.06	10.61 ± 0.06	1.90 ± 0.05	7.81 ± 0.08	11.92 ± 0.05	3.93 ± 0.04	69.19 ± 0.08
1e	2-Br-4,5-diOCH_3Ph	3,5-diCF_3	6.45 ± 0.21	10.14 ± 0.01	10.22 ± 0.03	0.64 ± 0.08	13.02 ± 0.01	14.9 ± 0.02	14.58 ± 0.04	57.76 ± 0.16
2e	2-FPh	3,5-diCF_3	3.41 ± 0.02	2.66 ± 0.09	9.94 ± 0.21	5.76 ± 0.09	3.46 ± 0.03	4.25 ± 0.09	4.20 ± 0.04	65.47 ± 0.24
3e	4-FPh	3,5-diC_3F	3.51 ± 0.08	4.03 ± 0.09	8.68 ± 0.09	3.98 ± 0.01	1.58 ± 0.01	9.75 ± 0.05	5.86 ± 0.02	25.84 ± 0.04
4e	3-BrPh	3,5-diCF_3	7.36 ± 0.01	7.28 ± 0.22	11.42 ± 0.04	11.35 ± 0.02	4.67 ± 0.05	9.40 ± 0.05	3.91 ± 0.07	11.61 ± 0.54
5e	4-CF_3Ph	3,5-diCF_3	14.65 ± 0.04	10.92 ± 0.09	6.29 ± 0.03	11.62 ± 0.02	9.57 ± 0.09	8.98 ± 0.05	11.49 ± 0.02	18.90 ± 0.04
A	2-Br-4,5-diOCH_3Ph	—	5.66 ± 0.07	10.86 ± 0.01	4.95 ± 0.03	3.86 ± 0.08	7.10 ± 0.06	13.17 ± 0.04	3.65 ± 0.09	37.31 ± 0.02
B	2-FPh	—	14.15 ± 0.02	9.63 ± 0.05	10.89 ± 0.1	10.99 ± 0.01	8.95 ± 0.01	22.85 ± 0.01	15.61 ± 0.05	23.02 ± 0.04
C	4-FPh	—	8.30 ± 0.07	5.81 ± 0.07	5.98 ± 0.05	7.43 ± 0.01	13.11 ± 0.03	13.79 ± 0.04	8.96 ± 0.02	16.78 ± 0.01
D	3-BrPh	—	8.06 ± 0.03	7.06 ± 0.01	7.43 ± 0.01	9.16 ± 0.05	3.87 ± 0.07	9.91 ± 0.02	9.12 ± 0.05	74.32 ± 0.05
E	4-CF_3Ph	—	4.04 ± 0.05	3.58 ± 0.02	9.85 ± 0.03	3.75 ± 0.05	9.36 ± 0.08	2.33 ± 0.08	8.60 ± 0.01	39.02 ± 0.01
Av	—	—	8.70	8.64	11.16	8.82	7.19	12.03	8.69	105.47
DDP	—	—	67.71 ± 0.08	78.81 ± 0.01	57.31 ± 0.08	29.21 ± 0.05	42.31 ± 0.03	31.94 ± 0.03	38.01 ± 0.01	41.33 ± 0.03
DOX	—	—	2.01 ± 0.01	1.38 ± 0.02	2.07 ± 0.09	0.95 ± 0.03	0.69 ± 0.07	22.60 ± 0.01	1.20 ± 0.05	0.98 ± 0.02

which show poor activity. The maximum and minimum selectivity indices (SI, the rate between the IC_{50} of LO2 and IC_{50} of each cancer cell line) of each compound are tabulated in Table 2.

Furthermore, comparing different substituted groups at the *N*-benzene ring, in general, **1d–5d** with 3 F were more potent than the corresponding molecules possessing the same benzylidene; these included 27 IC_{50} values out of 35 under 10 μM towards seven tumor cell lines, with 14 IC_{50} values below 5 μM. Particularly, **4d** and **5d** showed marked potency against A549 (IC_{50}: 0.17 μM) and HeLa cells (IC_{50}: 0.68 μM), respectively. These were followed by **1e–5e** with 3,5-diCF_3, which also showed stronger inhibitory activity, and 12 IC50 values were below 5 μM. While **1a–5a** (4-NO_2), **1b–5b** (4-CN), and **1c–5c** (4-CF_3) were uniformly less active, with only 4–7 IC_{50} values out of 35 under 5 μM. This outcome suggests that 4-substituents at the *N*-benzene ring are responsible for the reduced potency. As for the influence of substituents at benzylidene on the bioactivity, it can be checked from Table 1 that overall 2-Br-4,5-diOCH_3 **1a–1e** with both electron-withdrawing (–Br) and electron-donating (–OCH_3) groups displayed the lowest levels of inhibitory activity, with only seven IC_{50} values out of 35 below 10 μM against seven tumor cell lines. However, compounds bearing only electron-withdrawing groups in **2a–2e** (2 F), **3a–3e** (4 F), **4a–4e** (3-Br) and **5a–5e** (4-CF_3) with the same N-substituents uniformly are more potent than the corresponding analogues in **1a–1e** (2-Br-4,5-diOCH_3), with 23, 27, 25, and 17 IC_{50} values lower than 10 μM, respectively. This analysis implies that electron-donating groups at the benzylidene moiety are probably detrimental to the activity.

Table 2 The maximum and minimum selectivity indices (SI) of synthesized compounds

Comp.	1a	2a	3a	4a	5a	1b	2b	3b	4b	5b
SI (Min–Max)	4–21	4–11	6–14	8–37	10–35	9–25	1–15	21–171	10–35	5–22
Comp.	1c	2c	3c	4c	5c	1d	2d	3d	4d	5d
SI (Min–Max)	44–58	3–8	1–4	1–4	2–5	14–61	10–24	9–37	5–76	6–407
Comp.	1e	2e	3e	4e	5e	A	B	C	D	E
SI (Min–Max)	4–90	7–25	3–16	1–3	1–2	3–10	1–3	1–3	8–19	4–17

A subsequent analysis of the data in Table 1 indicated that some changes in the antiproliferative activity occurred after the intermediates **A**, **B**, **C**, **D** and **E** were *N*-substituted, respectively. First of all, the activity of **1a–1e** bearing the bulky 2-Br-4,5-diOCH_3 at benzylidene apparently decreased in comparison with **A**; in the second place, **5a–5e** containing another bulky 4-CF_3 also showed a certain decrease in activity by contrast with **E**. However, on the whole, most compounds in **2a–2e**, **3a–3e** and **4a–4e** with small 2 F, 4 F or 3-Br displayed greater or similar potency compared to **B**, **C** and **D**, respectively. This result suggests the addition of *N*-substituents to **1a–1e** and **5a–5e** with bulky groups at benzylidene further increased the steric effect, which is detrimental to bioactivity.

In vitro cell uptake detection

Some of the synthesized compounds possess inherent fluorescence, thus allowing direct detection of cellular uptake through confocal microscopy. The represented compounds exhibiting a high level of fluorescent activity include A and **1a–1e**, which contain two aromatic rings linked together by conjugated double bonds, facilitating the generation of fluorescence. In addition, the substituents at the benzylidene moiety can cause shifts in the maximum absorption wavelength and changes in corresponding fluorescent peaks. Additionally, –OCH_3 in A and **1a–1e** can enhance fluorescence since it is an electron-donating group. Moreover, the overall rigid molecular structures of these molecules promote their fluorescent activity as well. As a result, compound **1d** was chosen for cell uptake detection.

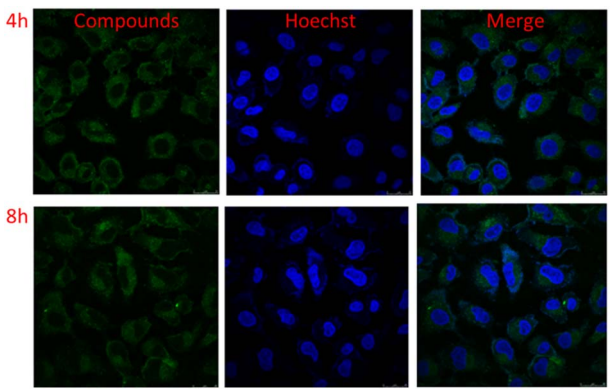


Fig. 3 Cellular uptake of **1d** in HeLa cells observed by CLSM.

The uptake of **1d**, with an IC_{50} value of 4.51 μM, in HeLa cells is shown in Fig. 3. A small number of molecules were observed aggregating outside the nucleus when treated for 4 h, while most compounds entered the cells when the treatment duration reached 8 h. Analysis of Fig. 3 revealed that compound **1d** was primarily distributed in the cytoplasm, with increasing fluorescence intensity over time, which indicates the inhibitory effect mainly occurred in the cytoplasm.

Evaluation of MDR reversal activity

MDR is primarily driven by the aberrant overexpression of specific transmembrane proteins in the ATP-binding cassette (ABC) transporter superfamily. Among these membrane transporters, P-glycoprotein (P-gp) emerges as the most critical mediator of MDR, and its excessive expression represents a prominent factor in the failure of cancer therapy, which is a 170 kDa phosphorylated glycoprotein encoded by the MDR1 gene.[22]

Due to pharmacokinetic conflicts between MDR reversal agents and anti-tumor drugs when used in combination, it is very difficult to accomplish the desired clinical therapeutic outcome. Therefore, developing small molecule drugs possessing anti-tumor and MDR reversal dual effects has become a new direction in the research of MDR reversal agents.

In our previous work, by investigating the expression levels of both the MDR1 gene and P-gp, we proved that 4-piperidone quaternary ammonium salt derivatives with 1,5-diaryl-3-oxo-1,4-pentadiene showed an MDR reversal effect.[35] Consequently, in this work, only the expression level of the MDR1 gene was determined in order to tentatively make sure the MDR reversal effect of compounds prepared and establish compound libraries with dual effects. As for the deep mechanism research, including apoptosis pathway and cell cycle effects, some related reports have been checked; for example, Mohammad reported one of the possible mechanisms is influencing percentage of cells in the G2/M phase and promoting expressions of caspase-3 and -9 to enhance the cell apoptosis,[36] which is the focus of our subsequent work.

The MDR reversal activity was determined using RT-PCR, and the control is the K562/DOX cells without being treated with the synthesized compounds. The results are illustrated as

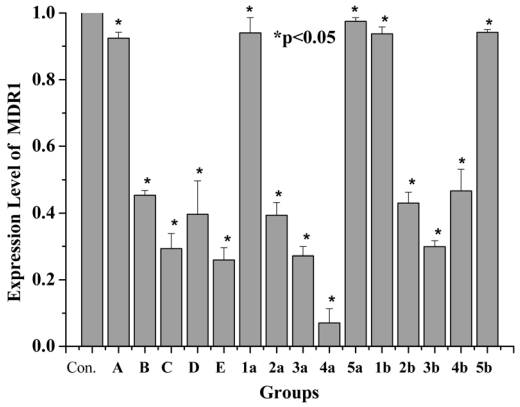


Fig. 4 Effect of A–E, 1a–5a and 1b–5b on the expression level of the resistance gene MDR1 using RT-PCR.

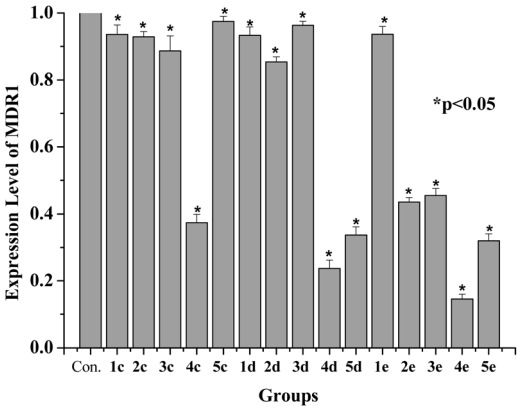


Fig. 5 Effect of 1c–5c, 1d–5d and 1e–5e on the expression level of the resistance gene MDR1 using RT-PCR.

a column diagram in Fig. 4 and 5. Examination of both figures revealed that all 30 molecules displayed certain MDR reversal activity. Of which, the activity of 17 molecules was remarkable, reducing the expression level of the drug-resistant gene MDR1 to lower than 50% *versus* control. Most compounds with small 2 F, 4 F or 3-Br at benzylidene, such as B, C, D, **2a**, **3a**, **4a**, **2b**, **3b**, **4b**, **2e**, **3e**, **4e**, **4c** and **4d** had excellent MDR reversal effect. Uniformly, **4a–4e** with 3-Br at benzylidene all showed strong MDR reversing potency; particularly, 4-NO_2-PhCH_2 **4a** and 3,5-bisCF_3-PhCH_2 **4e** decreased the MDR1 expression level to lower than 10% and 15%, respectively, *versus* control.

In addition, **E**, **5d** and **5e** with bulky 4-CF_3 at benzylidene demonstrated moderate MDR reversing activity as well, while the other compounds with bulky groups (4-CF_3 **5a–5c** or 2-Br-4,5-diOCH_3 **1a–1e**) demonstrated the least MDR reversal activity. This suggests that bulky groups at benzylidene may not contribute to MDR reversal activity, which is similar to cytotoxicity. Furthermore, subsequent analysis of the data in Table 1 and Fig. 4 and 5 disclosed that some compounds displayed dual effects of strong anti-cancer and potent MDR reversal activities, such as intermediates (**C**, **D** and **E**), **3a**, **3b**, **4a**, **4d**, **4e**, **5d** and **5e**, which reduced the MDR1 expression level to below 30% of control.

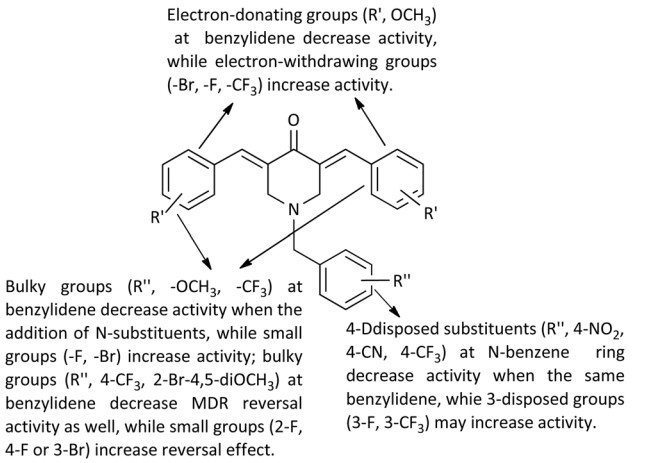


Fig. 6 Summary of SAR developed for the synthesized compounds.

Summary of SAR for the synthesized compounds

Based on the above discussion of biological data, a structure–activity relationship (SAR) for compounds prepared in this work is summarized in Fig. 6. In future work, this qualitative SAR can provide more explicit guidance for the modification, optimization and establishment of the corresponding compound libraries, which will promote the descriptive SAR to a quantitative model and computational research.

Experimental

General methods

All reactions were performed using standard laboratory equipment and glassware. Solvents and reagents were purchased from Sigma-Aldrich or Innochem and used as received. Organic solvent extracts were dried over sodium sulfate (Na_2SO_4) and dried under reduced pressure with rotary evaporators. Melting points were recorded on SGW X-4 Microscopic melting point apparatus. Samples for ultraviolet (UV) spectroscopy were dissolved at 10^{-5} M in methanol, and spectra were detected using a Beckman Du-70 ultraviolet spectrophotometer (USA). The IR Spectra were recorded with a NicoletIS50 Fourier transform infrared spectrometer. Electrospray mass spectra were recorded using an Agilent Technologies UHPLC system with a 6545XT LC/Q-TOF LC/MS. TLC was performed on Merck silica gel 60 F254 precoated aluminium plates with a thickness of 0.2 mm. Nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker Avance III 400 MHz (^{1}H and ^{13}C NMR) at 400 and 101 MHz, respectively. All spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d_6) from Cambridge Isotope Laboratories Inc. Chemical shifts (δ) were measured in parts per million (ppm) relative to TMS.

Biology

Cell culture and stock solutions

The cell lines used in this study were from Shandong Provincial Key Laboratory of Medicine and Health Sciences, Shandong

Medical and Pharmaceutical University, China; Fetal calf serum was purchased from Gibco Company, America; RPMI 1640 medium, high-glucose DMEM culture medium and trypsin were purchased from HyClone Company, America; The total RNA Extraction Kit and RT-PCR kit were purchased from Roche Company, America.

Stock solutions were prepared as described below and stored at −20 °C: the cell lines were cultured under a humidified atmosphere containing 5% CO_2 at 37 °C. The cell lines were cultured in RPMI-1640 medium (HyClone Laboratories, Inc, America) supplemented with 10% foetal bovine serum (FBS), 10 mmol L^{-1} sodium bicarbonate, penicillin (100 U per mL), streptomycin (100 U per mL), and glutamine (4 mmol L^{-1}).

Cell cytotoxicity determination by the MTT assay

Cytotoxicity was determined using the MTT (3-[4,5-dimethyl thiazol-2-yl]-2,5- diphenyl-tetrazolium bromide, Gibco, America) assay. Cytotoxicity for every single compound was detected after 24 h of co-cultivation with each cell line. The different cells in logarithmic growth were transferred to 96-well plates at a concentration of 1000–10000 or 5×10^4 cells per 100 μL per well. The cells were permitted to culture overnight at 37 °C in a humidified atmosphere with 5% CO_2. The detected molecules were initially prepared in DMSO (Sigma), and the working solutions were added to a FBS free culture medium. The final DMSO concentration in each well was 1%. The compounds were added to each well with increasing drug concentrations. After a 24 h incubation period, 20 μL of the MTT (5 mg mL^{-1}) reagent was added, and the plate was incubated for 4 h at 37 °C. The optical density (OD) was detected by an enzyme-linked immunosorbent assay (ELISA) detector (Spectra Max Mz, Molecular Devices, America) at 490 or 570 nm. The outcomes are displayed as a decrease in cell viability (%) compared with the untreated controls. The concentration of each compound was measured in triplicate, and the IC_{50} values are illustrated graphically. The concentrations of each compound used were 100, 80, 60, 40, 20, 10, 5, 1 and 0.1 μg mL^{-1}. Cisplatin (DDP, Gibco, America) was used as a positive control. The concentrations of DDP used were 100, 80, 60, 40, 20, 10, 5, 1 and 0.1 μg mL^{-1}.

Cell uptake detection

The HeLa cell line was used for cellular uptake studies. A concentration of 2–10 × 10^5 cells in a culture medium (2.0 mL) was seeded in 6-well plates, and after culturing for 24 h, different concentrations of the tested compounds were added. After culturing for another 1 h, the final concentration of the compounds was 10 mg mL^{-1}. Then, the culture medium was removed, the cells were washed three times with PBS, fixed for 1 h using 4% (w/v) paraformaldehyde at RT, and stained for 15 min with 100 μL (10 mg mL^{-1}) Hoechst 33 342. Finally, the cells were observed using CLSM (Confocal laser scanning microscopy, TCS SPE, Leica, Germany).

MDR reversal activity inspection by RT-PCR

The MDR reversal activity was inspected by reverse transcription-polymerase chain reaction (RT-PCR). In brief, total RNA was extracted from cells, and then reverse transcription was performed. Finally, the PCR was conducted under the conditions of 94 °C, 40 s; 56 °C, 1 min; and 72 °C, 1 min, with 30 cycles.

Conclusions

Five focused libraries of curcumin analogues comprising 25 compounds in total and five intermediates with 1,5-diaryl-3-oxo-1,4-pentadiene were designed and synthesized. Subsequent MTT analysis revealed that **1d–5d** with *N*-3-F-PhCH_2 are more active than the corresponding molecules containing benzylidene, and 27 IC_{50} values out of 35 are lower than 10 μM. Most compounds exhibited activity against cancer cells preferentially and selectively *versus* the normal liver cells (LO2); an electron-donating (–OCH_3) group at benzylidene and 4-disposed substituents at the *N*-benzene ring probably are detrimental to the activity; the *N*-substituents in **1a–1e** and **5a–5e** with bulky groups at benzylidene further increased the steric effect, resulting in decreased activity as well. The examination of the MDR reversal activity disclosed that most compounds with small 2 F, 4 F or 3-Br groups at benzylidene had good to excellent MDR reversal effect, particularly 3-Br **4a–4e**, while most compounds with bulky groups (4-CF_3 **5a–5c** or 2-Br-4,5-diOCH_3 **1a–1e**) demonstrated the least MDR reversal activity. Importantly, intermediates (C, D and E), **3a**, **3b**, **4a**, **4d**, **4e**, **5d** and **5e** exhibited dual effects of strong anticancer and significant MDR reversal activities. Finally, during investigation, it was observed that the solubility for most of the synthesized compounds remained relatively poor compared with some 4-piperidone quaternary ammonium salt derivatives containing 1,5-diaryl-3-oxo-1,4-pentadiene, as we previously reported,[35] requiring further optimization to increase the solubility. The findings in this study could offer insights into molecular modification strategies for investigating dual-function agents, integrating MDR reversal and anticancer effects.

Author contributions

Qinglong Wang: synthesis and biological evaluation of focused compounds (Scheme 1); Xiaofan Zhang: support in the preparation of experimental apparatus and reagents required for this work; Jiacheng Yuan: data collection and analysis of UV and IR data; Yan ren: determination and analysis of HRMS; Huanhuan Yan: methodology guidance, manuscript reviewing & editing; Jufeng Sun: proposal of research (Scheme 1), methodology guidance, further inspection & analysis of focused compounds, manuscript – writing, reviewing, submission & editing.

Conflicts of interest

The authors report no conflicts of interest.

Data availability

The authors declare that all data analysed in this work can be found in the supplementary information (SI). Supplementary

information: all Uv, IR, ^{1}H NMR, and ^{13}H NMR data. See DOI: https://doi.org/10.1039/d6ra02187e.

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