Supplementary Information for

Combining same-target drugs exhibits additive efficacy with minimal toxicity

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Content of Supplementary Information

1. Supplementary Note

1.1 Supplementary evidence for the additive effects of DNA TOPO II inhibitors

1.1.1 Animal experiments

1.1.1.1 Survival details in the 8-drug ascites tumour study

Mice began to die from day 6 after inoculation, and all mice died in three groups (Model, epirubicin, and pirarubicin) by day 10. The onset of death occurred on day 13 in the combination-drug group (Cdg), whereas the onset of death occurred on days 6–8 in the other groups. No deaths occurred in the Cdg by day 12; however, in the single-drug groups (Sdgs) only one mouse survived in the aclarubicin group by day 12. Between days 13–19, all mice in the Cdg died, whereas all 90 mice died between days 6–14 in the Sdgs and the Model group (Extended Data Table 3).

1.1.1.2 Tumour inhibition rate in 4-drug solid tumour study

Extended Data Fig. 2 shows tumour mass index, tumour inhibition rate (TIR), and tumour volume results. The TIR of the 4-drug-Cdg was 59%, which was higher than the TIRs for the doxorubicin (11.5%), etoposide (19.5%), idarubicin (10.6%), and amsacrine (7.7%) groups (all $P < 0.01$) and tended to be higher than the High-dose doxorubicin [Dox (H)] group (49.2%, $P > 0.05$). Based on the efficacy criteria, which require a TIR greater than 50%[67] or 58%[65], treatments in the single-drug groups were ineffective. The dose of each drug in this experiment was used at 1/10 of its 50% lethal dose (LD_{50}) in both the Sdgs and Cdg, except the Dox (H) group, which had a dose of 1/2 of the LD_{50} (Extended Data Table 4).

1.1.2 Cell experiments

1.1.2.1 Additive effects of four inhibitors on HepG2 cells

I. Four inhibitors in different combinations

i. Four inhibitors with similar IC_{50} values

HepG2 cells were used in these experiments. Four inhibitors, including doxorubicin, daunorubicin, idarubicin, and amsacrine, at concentrations (Supplementary Table 8) corresponding to their IC_{18} were used in the single-drug treatments. The inhibition rates (16.0%, 18.9%, 18.9%, and 17.2%) in the single-drug treatments were close to the expected inhibition rate of 18% (Fig. 4d). The inhibition rate (18.9%) of the four inhibitors combined at 4.5% inhibitor concentration ($IC_{4.5}$) was closed to the individual inhibitors at IC_{18}, indicating the four inhibitors had an additive effect. The inhibition rate of the combination increased (Fig. 4d) as the concentrations (Supplementary Table 8) of the inhibitors in the combination increased to IC_9, IC_{18}, and IC_{36}.

The combination of the four inhibitors at $IC_{4.5}$ achieved an inhibition rate of 18.9%, which is close to the expected inhibition rate of 18% if the inhibitors induced an additive effect, which was the sum of the individual 4.5% rates. Combining the four inhibitors at IC_9 achieved an inhibition rate of 32.9%, which was close to the sum (36%) of the four inhibitors at 9%. These two experiments indicated that the four inhibitors in the combination produced an additive effect, and each of the four inhibitors contributed to the additive effect. However, the combination of the four inhibitors at IC_{18} had an inhibition rate of only 55.6% instead of the expected inhibition rate (72%, 4 × 18%) if the 4 inhibitors elicited an additive effect. The combination of the four inhibitors at IC_{36} had an inhibition rate of 83% instead of the expected inhibition rate of 144% (which is impossible to reach) if the inhibitors elicited an additive effect. These results indicated

that the four inhibitors exhibit additive inhibitory effects on the proliferation of HepG2 cells, but the additive effects became saturated at higher concentrations. The differences between the single-drug treatment at IC_{18} and the combination treatments of the four inhibitors combined at IC_{18} were significant ($P < 0.05$), indicating that combination treatment significantly improved the inhibitory activity compared with the single-drug treatments (Fig. 4d).

ii. Four inhibitors with large differences in IC_{50} values

The effects of doxorubicin, etoposide, daunorubicin, and podophyllotoxin with large differences in IC_{50} values, which represent large differences in their chemical structures (Supplementary Fig. 1), on HepG2 cell proliferation were examined. As shown in Fig. 4e, the inhibition rates (18.3% and 18.4%) of etoposide and daunorubicin were close to the expected inhibition rate (18%). The inhibition rates (20.5%, 33.6%, 60.5%, and 87.4%) of the combination of the four inhibitors increased (Fig. 4e) as the concentrations (Supplementary Table 9) of the individual inhibitors in the combination increased ($IC_{4.5}$, IC_9, IC_{18}, and IC_{36}).

The results indicate that the four inhibitors with large differences in IC_{50} values also elicited additive effects. The inhibition rate was significantly higher for the IC_{18} combination group compared with the inhibition rates for etoposide and daunorubicin used separately at IC_{18} ($P < 0.05$).

II. Proportionally reducing the concentration of the four inhibitors in the combinations

The concentrations of inhibitors (doxorubicin, etoposide, daunorubicin, and podophyllotoxin) in the 4-in-1 combinations were proportionally reduced from the IC_{36} concentrations (Supplementary Table 10). In HepG2 cells, the inhibition rates (45.6% and 43.3%) of the single inhibitor group (doxorubicin and etoposide at IC_{36}) were close to the expected inhibition rates (36%). As the inhibitor combination at IC_{36} was diluted ($IC_{36} \times 1$, $IC_{36} \times 0.75$,

IC_{36} × 0.5, IC_{36} × 0.25, and IC_{36} × 0.125), the inhibition rates (93.7%, 73.6%, 64.2%, 46.1%, and 37.5%; Fig. 4g) decreased proportionally. The difference between the combination group (IC_{36}) and the doxorubicin or etoposide group at IC_{36} was significant ($P < 0.05$).

1.1.2.2 The additive effects of the six-inhibitor combination on HepG2 cells

The additive effects of combining doxorubicin, etoposide, daunorubicin, idarubicin, amsacrine, and ellipticine at IC_{18} concentrations (Supplementary Table 5) on HepG2 cell proliferation were assessed. The inhibition rates (16.3%–23.6%) of single-drug treatments were close to the expected inhibition rates (18%), while the inhibition rate of the combination treatment was 87.4% (Fig. 4b). Theoretically, combining 6 inhibitors at IC_{18} should result in a theoretical inhibition rate of 108% (6 × 18% = 108%). Based on the actual inhibition rates of the six inhibitors in the combination, the calculated inhibition rate should be 123.2% (22.6% + 19.0% + 23.6% + 21.2% + 16.3% + 20.5% = 123.2%). Although both the theoretical (108%) and calculated (123.2%) inhibition rates are impossible to reach, the inhibition rate of the 6-in-1 combination reached 87.4%, demonstrating that combining the six inhibitors has an additive effect but the additive effect is restricted and becomes saturated at higher concentrations. The inhibition rates for the single-drug treatments at IC_{18} and the combination 6-in-1 inhibitor at IC_{18} for each inhibitor were significantly different ($P < 0.05$), indicating the inhibitory activity of the combination treatment was significantly better than the single-drug treatments.

1.1.2.3 Antitumour activity of combinations with different numbers of inhibitors at the same inhibition rates in HepG2 cells

The inhibition rates of the six-drug combinations were significantly higher than the inhibition rates of the four-drug combinations (Fig. 4f and Supplementary Table 11) when combined at the same inhibition rates. Thus, the additive inhibitory effects of the combination treatments are

related to both the number and concentration of inhibitors in the combination. The combination of four inhibitors exhibited an additive inhibitory effect on HepG2 cell proliferation, and the inhibition rates (63.2% at IC_9 or 87.4% at IC_{18}) of the six-drug combinations (doxorubicin, etoposide, daunorubicin, idarubicin, amsacrine, and ellipticine) were higher than the inhibition rates for the corresponding four-drug combinations (33.6% and 32.9% at IC_9 and 60.5% and 55.6% at IC_{18}) (Fig. 4f and Supplementary Table 11). Thus, the inhibition rate increased as the number of inhibitors in the combination or the concentration of the inhibitors increased (Fig. 4f).

1.1.2.4 The additive effects of six inhibitors on U87, HL-60, BxPC-3, and Hela cells

The effects of combinations of doxorubicin, etoposide, daunorubicin, idarubicin, amsacrine, and ellipticine at $IC_{4.5}$, IC_9, IC_{18}, and IC_{36} concentrations (Supplementary Table 6) were assessed in U87, HL-60, BxPC-3, and Hela cells. The methods were similar to the experiments in HepG2 cells. As shown in Fig. 4c, the additive inhibitory effects of combination treatments on U87, HL-60, BxPC-3, and Hela cells were similar to the effects on HepG2 cells. Combining the six inhibitors significantly increased the inhibitory effects on cell proliferation compared with the single-drug treatments at the same concentrations in all cell lines. The combination (Comb $IC_{4.5}$) of six inhibitors at $IC_{4.5}$ achieved inhibition rates of 28.3%, 27.6%, 30.1%, and 25.8% in U87, HL-60, BxPC-3, and Hela cells, respectively (Fig. 4c); the inhibition rates were similar to the expected inhibition rate of 27% (6 × 4.5%). Thus, the six inhibitors in the combination produced an additive effect, with each of the six inhibitors contributing to the additive effect. However, the combination (Comb IC_9) of six inhibitors at IC_9 achieved an inhibition rate of 36.4%–46.5% (Fig. 4c), which was slightly lower than the expected rate of 54% (6 × 9%). The combination (Comb IC_{18}) of six inhibitors at IC_{18} exhibited an inhibition rate of only 47.5%–76.8% (Fig. 4c), which did not reach the expected inhibition rate of 108% (6 × 18%), which is impossible to reach. The

157 combination (Comb IC_{36}) of six inhibitors at IC_{36} had an inhibition rate of 59.2%–94.2% (Fig. 4c)
158 instead of the expected rate of 216% (also impossible to achieve). These results were similar to
159 the four-drug combinations, indicating that the six-drug combinations exhibited additive
160 inhibitory effects on cancer cell proliferation in multiple cancer cell lines. However, the additive
161 effects become saturated at higher concentrations.

162 **1.1.2.5 Dose-response curves and calculation of the combination index (CI)**

163 SRB assays were used to evaluate cell proliferation. The inhibitory effects of doxorubicin,
164 etoposide, daunorubicin, idarubicin, amsacrine, and ellipticine on HepG2, U87, HL-60, BxPC-3,
165 and Hela cell proliferation were determined, and the dose-response curves were plotted
166 (Supplementary Fig. 2). The corresponding IC_{50} values (Supplementary Table 7) were obtained
167 for the subsequent determination of dosing concentrations. For example, the concentrations of
168 each of the six inhibitors corresponding to their 4.5% inhibitory rates against HepG2, U87, HL-
169 60, BxPC-3, and Hela cells were obtained from the dose-response curves and these
170 concentrations were used for the six inhibitors in combination 1 (Comb $IC_{4.5}$; Fig. 4c and
171 Supplementary Table 6). Similarly, the concentrations corresponding to the inhibitory rates at
172 9%, 18%, and 36% were obtained from the dose-response curves and used in combinations 2, 3,
173 and 4 (Comb IC_9, Comb IC_{18}, and Comb IC_{36}; Fig. 4 b,c, and Supplementary Tables 5 and 6). All
174 combination groups 1–4 were also tested in U87 cells; the inhibition rates were 28.3%, 36.4%,
175 47.5%, and 63.2% and the CI values were calculated (Supplementary Table 17).

176 The results from the experiments on the cell lines showed that the combined use of the six
177 TOPO II inhibitors at low concentrations exhibited additive inhibitory effects in tumour cell lines.
178 The CI values of these combinations were all between 0.92 and 1.01 (Supplementary Table 17),
179 suggesting that the combined effects of the 6 TOPO II inhibitors were mainly additive. This

result was consistent with a previous study showing that two-drug combinations of amsacrine with the TOPO II inhibitors doxorubicin, etoposide, or daunorubicin exhibited additive effects while combining amsacrine with the TOPO II inhibitor mitoxantrone exhibited a synergistic effect in human T-cell leukemia cells[68]. These results indicated that the interactions of most drugs targeting TOPO II are additive.

1.1.3 Enzyme experiments

1.1.3.1 TOPO II-mediated kDNA decatenation assay

In a 1% agarose gel, kDNA mostly remains in the loading well. TOPO II disrupts the interlinks in kDNA and liberates small DNA minicircles, which can migrate into the agarose gel (Fig. 4h and Supplementary Tables 12 and 13). Lane D of Fig. 4h contained the kDNA control, which did not migrate. Lane T contained a mixture of kDNA and TOPO II; only a small amount of kDNA remained in the loading well, indicating that the majority of the kDNA was enzymatically digested by TOPO II to generate DNA minicircles after decatenation. Thus, TOPO II can enzymatically degrade kDNA in the absence of any inhibitor. Lanes 1–3 were loaded with mixtures of kDNA, TOPO II, and doxorubicin at low, medium, and high doses (Supplementary Table 12). As the inhibitor concentration increased, the amount of migrating DNA minicircles decreased. Analysis of the electrophoretic pattern showed that the low, medium, and high doses of doxorubicin inhibited TOPO II activity by 9.1%, 15.6%, and 88.7%, respectively (Supplementary Table 13). Lanes 6–8 and 9–11 contained kDNA treated with TOPO II and low, medium, and high doses of daunorubicin and idarubicin, respectively. The inhibitory effects of the two inhibitors on TOPO II activity were also dose-dependent, with inhibition rates of 0.8%, 11.9%, 87.1%, and 7.1%, 11.5%, 86.1% for daunorubicin and idarubicin, respectively. The high

dose of each inhibitor was considered the positive control because they almost completely inhibited TOPO II. Lanes 4–5, 12–13, and 14–15 correspond to 2 doses (low and medium doses) of etoposide, amsacrine, and ellipticine, respectively. The inhibition rates for etoposide, amsacrine, and ellipticine were 8.0% and 16.6%, 8.1% and 19.5%, and 11.6% and 22.2%, respectively. Lanes 16–17 (Fig. 4h) showed the effects of the 6-in-1 combinations of doxorubicin, etoposide, daunorubicin, idarubicin, amsacrine, and ellipticine at low and medium doses, respectively. The low-dose combination included the individual inhibitors at their low doses, and the medium-dose combination included the individual inhibitors at their medium doses (Supplementary Table 12). The corresponding inhibition rates were 17.8% and 84.2%, respectively (Supplementary Table 13). Thus, the inhibitory effects of the combinations on TOPO II activity were dose-dependent. The medium dose was more effective than the single inhibitor treatments. The inhibitory effects of the six individual TOPO II inhibitors were much lower than the inhibitory effects of the combined inhibitors at the corresponding dose. These results confirm the additive effect of combining multiple drugs targeting TOPO II.

1.1.3.2 TOPO II-mediated DNA uncoiling assay

In the TOPO II-mediated DNA uncoiling assay, supercoiled DNA (**SC**) and relaxed DNA (**RLX**) are distinguished by their different gel migration rates (Supplementary Tables 12 and 14, and Fig. 4i). In Fig. 4i, Lane D shows the pBR322 control, which exhibits the SC band properties of the negatively supercoiled structure. Lane T shows pBR322 treated with TOPO II; the SC band is not detectable, indicating that the majority of the DNA was uncoiled and transformed into RLX by TOPO II. Lane 1 was loaded with a mixture of pBR322, TOPO II, and medium-dose etoposide. The absence of the SC signal and the presence of the RLX signal in the lane indicates that a majority of the DNA was uncoiled by TOPO II. Thus, the medium-dose

etoposide did not completely inhibit TOPO II (inhibition rate 11.6%; Supplementary Table 14). Lane 2 shows a mixture of pBR322, TOPO II, and the combination consisting of the six TOPO II inhibitors (doxorubicin, etoposide, daunorubicin, idarubicin, amsacrine, and ellipticine) at medium doses (Supplementary Table 12). Only the SC band appeared in this lane, and no RLX band was visible (Fig. 4i). This result indicates that the combination drug significantly inhibited TOPO II-mediated uncoiling of the DNA, with an inhibition rate of 89.2% (Supplementary Table 14). Lane 3 was loaded with a mixture of pBR322, TOPO II, and high-dose etoposide. Only the SC band was detected (Fig. 4i), indicating that high-dose etoposide inhibited the ability of TOPO II to uncoil the supercoiled structure; the corresponding inhibition rate was 81.7% (Supplementary Table 14). These results indicate that etoposide did not exhibit a significant inhibitory effect at the medium dose, but the combination of six TOPO II inhibitors at medium doses significantly inhibited TOPO II. Thus, the combination suppressed TOPO II better than the single-drug treatments, confirming the additive effect on the same target.

1.2 Supplementary evidence for the reduced toxicity of the 8-drug combination in the 8-drug solid tumour study

1.2.1 Hepatotoxicity

The liver index of the Model group significantly increased by 28.6% compared with the Blank group ($P < 0.01$), indicating that the liver was slightly swollen after tumour inoculation (Fig. 3g). The liver index decreased by 12.3% ($P < 0.05$) in the aclarubicin group and 13.2% ($P < 0.05$) in the teniposide group compared with the Model group, indicating that the 2 single-drug groups (Sdgs) reversed the liver swelling to some extent. The liver indices in the other groups were not different from the Model group.

Serum albumin (ALB) levels in the Model group tended to be lower than ALB levels in the Blank group. None of the drug groups induced abnormalities in ALB compared with the Model group. The combination-drug group (8-drug-Cdg) tended to reverse the reduction in ALB. Compared with the Cdg (Fig. 3f), ALB was significantly lower in the doxorubicin, amsacrine, teniposide, and epirubicin groups, indicating that the ability of the liver to synthesize proteins was impaired in these 4 Sdgs compared with the Cdg, whereas the Cdg did not impair protein synthesis.

1.2.2 Cardiotoxicity

Cardiac indices (Fig. 3j) were not significantly different when any two of the following groups were compared: the combination-drug, Blank, Model, and single-drug groups. Compared with the Cdg, Serum lactate dehydrogenase (LDH) and creatine kinase (CK) levels were significantly increased in the doxorubicin and idarubicin groups (both $P < 0.01$ and both $P < 0.05$, respectively) (Fig. 3h,i). Differences in LDH (5.3; Table 1) and CK (1.9; Table 1) levels in the Cdg compared with the Model group were lower than differences (48.5 and 25.5; Table 1) between the doxorubicin group and the Model group (89.1% and 92.5%, respectively) [Note: $(48.5 - 5.3)/48.5 = 89.1\%$; $(25.5 - 1.9)/25.5 = 92.5\%$]. Differences in LDH and CK levels in the Cdg compared with the Model group were lower than the differences (115.5 and 29.7; Table 1) between the idarubicin group and the Model group (95.4% and 93.6%, respectively). Thus, doxorubicin and idarubicin caused severe myocardial injury and the Cdg reduced myocardial damage by about 90%.

1.2.3 Physical condition

Mice developed visible tumours in their right axilla after tumour cell inoculation. The physical status of mice in the Blank and Model groups was relatively good. Mice in the

combination-drug, Model, and Blank groups were all relatively active and healthy and had sleek shiny coats, with no significant differences between the three groups. However, mice in the Sdgs had dull and ruffled coats and gradually worsening vitality. The mice became increasingly less aggressive when handled. These mice had increased piloerection and consumed less food, and 10%–50% of mice in these groups died during the treatment period (Fig. 3a and Extended Data Table 5). These results indicate that the toxic effects in the Sdgs were more severe than the toxic effects in the Cdg.

1.2.4 Growth status and adverse gastrointestinal effects

Growth status and adverse gastrointestinal effects, which are common toxic effects of anticancer drugs, were assessed using body weight, daily food intake, and daily water intake. On the last day of the experiment, the Cdg exhibited overall good physical condition but body weight (Fig. 3n) declined by 22.2% ($P < 0.01$) compared with the Model group (Table 1). Body weights (Fig. 3n) in the Sdgs declined, especially in the idarubicin group, compared with the Model group (7.8%–20.3%, $P < 0.01$), except the amsacrine group (Table 1). A dose was considered toxic if either the recipient died or their body weight dropped by more than 15% from their initial weight[64,110]. All eight drugs in the Sdgs are toxic, based on the mortality criterion, and the Cdg and four Sdgs (body weight loss: 17.3%, 20.3%, 17.0%, and 17.0% for the Eto, Ida, Acl, and Epi groups, respectively; Table 1) in the present study are toxic, based on the body weight criterion.The average daily food intake per mouse in the Cdg declined by 30.0% compared with the Model group ($P < 0.05$), suggesting that the body weight loss in the Cdg was due to reduced food intake (Fig. 3o). Mice in the epirubicin and idarubicin groups had the lowest average daily food intake per mouse on the last day of the experiment (35.0%, $P < 0.01$ and

294 32.5%, $P < 0.05$, respectively) compared with the Model group (Table 1). Thus, the epirubicin
295 and idarubicin groups suffered more adverse gastrointestinal effects than the Cdg.

296 No significant differences in average daily water intake per mouse were detected between
297 the groups (Fig. 3p). The epirubicin and idarubicin groups and the Cdg had the lowest water
298 intake on Day 9 (Fig. 3p and Table 1).

299 Overall, the data for body weight, daily food intake, and daily water intake suggest that the
300 idarubicin and epirubicin groups and the Cdg experienced moderate adverse effects. As the Cdg
301 demonstrated similar adverse effects to idarubicin and epirubicin groups, the adverse effects of
302 the Cdg may have been predominantly caused by idarubicin and epirubicin in the drug
303 combination. The decrease in body weights in the Cdg was slightly worse than the weight
304 changes in the Sdgs. Daily food intake decreased less in the Cdg compared with the epirubicin
305 and idarubicin groups but more than the other Sdgs.

306 **1.2.5 Myelosuppression**

307 Myelosuppression is a common toxic effect of anticancer drugs. All of the drugs in this
308 study cause varying degrees of myelosuppression (Supplementary Tables 1 and 2). Therefore, a
309 routine blood test was used to evaluate myelosuppression; declines in white cell and platelet
310 counts indicated myelosuppression. No significant differences in white cell and platelet counts
311 were detected between the Model and Blank groups (Fig. 3q,r), indicating that no
312 myelosuppression occurred after tumour inoculation. Mice in the Cdg suffered slight
313 myelosuppression, and more severe myelosuppression was detected in the idarubicin and
314 amsacrine groups compared with the Cdg (Fig. 3q,r, and Table 1). The doxorubicin, pirarubicin,
315 etoposide, and epirubicin groups tended to develop myelosuppression and suffered from drug

toxicity comparable to the Cdg. No significant changes in white cell or platelet counts were detected in the aclarubicin and teniposide groups, indicating less toxicity than the Cdg.

1.2.6 Immune system damage

Immune system damage may occur in the mice in response to the drug intervention (Supplementary Table 2). Thus, damage to the immune system was evaluated using thymus and spleen indices. The thymus (Fig. 3s) and spleen (Fig. 3t) indices increased by 17.6% ($P > 0.05$) and 93.6% ($P < 0.01$), respectively, in the Model group compared with the Blank group, indicating that immunity was activated by tumour inoculation. Immune system damage was detected in both the Cdg and Sdgs, but less damage was detected in the Cdg (Fig. 3s,t, and Table 1). Larger changes in the thymus index were detected in seven Sdgs and larger changes in the spleen index were detected in six Sdgs compared with changes in the thymus and spleen indices in the Cdg (Table 1).

1.3 Toxicities of four drugs alone or in combination in solid tumour mouse model

1.3.1 Survival rate (Extended Data Figs. 1c and 3a)

The dose of each drug (doxorubicin, etoposide, idarubicin, and amsacrine; Extended Data Table 4) in both the single-drug groups (Sdgs) and the combination-drug group (4-drug-Cdg) was set at 1/10 of the LD_{50} for each drug, and each drug was administered in six doses in the 4-drug solid tumour study. The high-dose doxorubicin [Dox (H)] group had a dose (Extended Data Table 4) of 1/2 of the LD_{50} and was administered in only four doses. Administration was halted after the fourth dose due to severe toxicity, resulting in the death of six mice in the Dox (H) group. Over the 12-day administration period (Extended Data Fig. 1c), no mice died in the Blank, Model, doxorubicin, etoposide, or idarubicin groups, and no differences in survival rates were observed. However, in the other drug groups, 1 to 6 deaths per group occurred starting at day 8

after tumour inoculation. The survival rates were 90% (mortality rate 10%) in the 4-drug-Cdg, 80% (mortality rate 20%) in the amsacrine group, and 40% (mortality rate 60%) in the Dox (H) group (Extended Data Fig. 3a).

1.3.2 Hepatotoxicity (Extended Data Fig. 3b–g)

The liver index increased by 37.7% in the Model group ($P < 0.01$) compared with the Blank group, indicating mild liver swelling after tumour inoculation. The liver index decreased by 22.3% in the 4-drug-Cdg and increased by 13.2% in the amsacrine group (both $P < 0.01$) compared with the Model group, indicating abnormal liver function in both groups. No differences in liver index were observed between the other Sdgs and the Model group.

ALT and AST levels increased by 42.4% ($P < 0.05$) and 187.5% ($P < 0.01$) and ALB levels decreased by 18.4% ($P < 0.05$) in the Model group compared with the Blank group, indicating that tumour inoculation induced liver inflammation leading to liver swelling. ALT, AST, and ALP increased by 61.6%, 116.9%, and 49.2% (all $P < 0.01$), respectively, and TP and ALB decreased by 14.3% and 22.9% (both $P < 0.05$), respectively, in the Dox (H) group compared with the Model group. No significant differences in the five liver indicators were detected between the doxorubicin, etoposide, idarubicin, and amsacrine groups compared with the Model group. TP decreased by 12.3% in the 4-drug-Cdg compared with the Model group ($P < 0.05$); no significant differences in the other four indicators were detected between the 4-drug-Cdg and the Model group. ALT, AST, and ALP levels decreased by 34.7%, 49.1%, and 37.2% (all $P < 0.01$), respectively, while ALB levels increased by 19.1% ($P < 0.05$) in the 4-drug-Cdg compared with the Dox (H) group. These results suggest much lower liver damage in the 4-drug-Cdg compared with the Dox (H) group. In summary, the Dox (H) group exhibited the most severe damage, the 4-drug-Cdg showed mild liver injury, and none of the other Sdgs exhibited liver damage.

1.3.3 Cardiotoxicity (Extended Data Fig. 3h, i)

No significant differences in the cardiac indices were detected in pairwise comparisons between the 4-drug-Cdg and the Model group and other Sdgs (data not shown). CK increased by 24.2% ($P < 0.01$) and LDH increased by 15.0% ($P > 0.05$) in the 4-drug-Cdg compared with the Model group. LDH increased by 83.1% and CK increased by 79.1% in the Dox (H) group compared with the Model group (both $P < 0.01$), and the increases were significantly higher than the 4-drug-Cdg (both $P < 0.01$). CK levels were significantly lower in the doxorubicin, etoposide, idarubicin, and amsacrine groups compared with the 4-drug-Cdg (all $P < 0.05$). In summary, the Dox (H) group exhibited severe myocardial damage, and the 4-drug-Cdg also showed myocardial damage. However, none of the other Sdgs caused myocardial damage.

1.3.4 Nephrotoxicity (Extended Data Fig. 3j–l)

The kidney index, indicating renal impairment, increased by 11.7% in the Dox (H) group compared with the Model group ($P < 0.05$). No significant differences in kidney index were detected between the other drug groups and the Model group. No significant differences in urea levels were detected between the drug groups and the Model group. The SCr levels, indicating kidney damage, increased by 25.8% in the Dox (H) group compared with the Model group ($P < 0.01$). No significant differences in SCr levels were detected between the other drug groups and the Model group. In summary, the Dox (H) group exhibited some kidney damage, while none of the other drug groups showed evidence of kidney damage.

1.3.5 Immune system damage (Extended Data Fig. 3m,n)

The thymus index increased by 32.1% ($P > 0.05$) and the spleen index increased by 88.9% ($P < 0.01$) in the Model group compared with the Blank group, indicating immune system stimulation and enhanced immunity in response to tumour inoculation. The thymus index

decreased significantly in all drug groups compared with the Model group ($P < 0.05$ for the amsacrine and Dox (H) groups; $P < 0.01$ for all others). The thymus index decreased by 48.6%, 24.3%, 51.4%, 29.7%, 24.3%, and 54.1% in the 4-drug-Cdg, doxorubicin, etoposide, idarubicin, amsacrine, and Dox (H) groups, respectively. Overall, the Dox (H) and etoposide groups exhibited greater thymus indices compared with the 4-drug-Cdg.

Compared with the Model group, the spleen index decreased by 49.0% and 44.1% in the 4-drug-Cdg and Dox (H) group, respectively (both $P < 0.01$). The spleen index in the amsacrine, doxorubicin, etoposide, and idarubicin groups increased by 22.5% ($P < 0.05$), 33.3%, 14.7%, and 6.9%, respectively (all $P > 0.05$), compared with the Model group. In summary, all drug groups experienced some level of immune system damage.

1.3.6 Growth status and adverse gastrointestinal effects (Extended Data Fig. 3o,p)

The 4-drug-Cdg exhibited a 25.6% decrease in body weight on day 14 compared with the Model group ($P < 0.01$). Dox (H) caused an 11.8% decrease in body weight compared with the Model group ($P < 0.05$). The other Sdgs (excluding the amsacrine group) caused a slight decrease in body weight, but the weight losses were not significantly different from the Model group.

The daily food intake between the 6th and 11th days decreased by 25.0% and 32.5% in the 4-drug-Cdg (3.0 g) and idarubicin group (2.7 g), respectively, compared with the Model group (4.0 g). No significant differences in food intake were detected between the other drug groups and the Model group.

1.3.7 Comparison of toxicities in the 4-drug-Cdg and the single-drug groups in the 8-drug solid tumour study

The total dose (1/10 × 4 drugs = 4/10 LD_{50}; 4/10 LD_{50} × 6 injections = 24/10 LD_{50}) in the 4-

drug-Cdg (doxorubicin, etoposide, idarubicin, and amsacrine combined at 1/10 LD_{50} each; Extended Data Table 4) was larger than the doses (1/4=2.5/10 LD_{50}; 2.5/10 LD_{50}×5 injections=12.5/10 LD_{50}; Extended Data Table 1) of each drug in the 4 single-drug groups (Sdgs) in the 8-drug solid tumour study. However, the mortality was 10% in the 4-drug-Cdg (Extended Data Fig.3a), which was lower than the mortality (20%–50%; Table 1) in the 4 Sdgs (doxorubicin, etoposide, idarubicin, and amsacrine;). Other indicators of toxicity in the 4-drug-Cdg were also lower than the toxicities of the Sdgs, as shown by the ALT, AST, ALP, LDH, and Scr levels in Fig. 3 for the Sdgs and Extended Data Fig. 3 for the 4-drug-Cdg (The values for the Model groups in the two figures can be used as a baseline for comparison). These data suggest that the 4-drug-Cdg did not elicit an additive effect on toxicity. The tumour inhibition rate (TIR) of the 4-drug-Cdg (59%) was significantly higher than the TIR (24.6%–38.1%) of the Sdgs in the 8-drug solid tumour study. Thus, if the doses of each Sdg were increased to achieve a TIR of 59%, the toxicity of each Sdgs (doxorubicin, etoposide, idarubicin, and amsacrine) would be much greater than the toxicity of the 4-drug-Cdg. The toxicities of these anticancer drugs are dose-limiting or dose-dependent[1,9,10,12,13]. Overall, these results indicate that the 4-drug-Cdg elicited a toxicity scattering effect.

2. Supplementary Discussion

2.1 Does the observed toxicity reduction of the 8-drug-Cdg in this study hold significant clinical implications？-----Comparison of the toxicities of the 8-drug-Cdg with the clinical toxicities of the eight anticancer drugs

Based on the reported clinical toxicities (Supplementary Table 1) of the eight drugs used in the 8-drug solid tumour study, the toxicity reduction in the 8-drug-Cdg is highly significant. For

example, long-term use of doxorubicin causes congestive heart failure with a high mortality rate (Supplementary Table 1), and the incidence of cardiotoxicity positively correlates with the total cumulative dose. When the cumulative dose of doxorubicin reaches 550 mg/m^2 (being equivalent to 14.9 mg/kg, according to the equation mg/m^2 = K_m × mg/kg, where K_m = 60 kg/1.62 m^2, APPENDIX B of the FDA document[69] and other literature[70–72]) or above, the incidence of congestive heart failure is 7%[73], > 18%[74], or 26%[11,18,75]. When the cumulative dose reaches 700 mg/m^2 (equivalent to 18.9 mg/kg), the incidence of congestive heart failure is up to 18%[73], > 36%[74], or 48%[18,75]. A survey by the Paediatric Cardiomyopathy Registry of more than 100 paediatric cardiology centres in North America showed that more than 15% of all patients with cardiomyopathy were previously treated for cancer with anthracyclines (doxorubicin, idarubicin, epirubicin etc.) during childhood or adolescence[76]. In addition, mortality directly related to anthracycline-induced cardiac failure is substantial, with some large series reporting rates of over 20% mortality[76]. Furthermore, the use of doxorubicin at a dose of 25 mg/kg can cause renal, myocardial, and liver damage in rats after 2 days of treatment[77]. If the cumulative dose (2.5 mg/kg × 5 doses = 12.5 mg/kg) of intraperitoneal injections for the doxorubicin group in the present study, which can be considered as the dose by intravenous injection due to the similar bioavailability[78,79], is converted to the cumulative dose of intravenous injection for adults, the dose is 37.5 (37.7) mg/m^2 [being equivalent to 1.02 mg/kg; according to the equations of HED (mg/m^2) = HED (mg/kg) × Human K_m = Animal dose (mg/kg) × (Animal K_m/Human K_m) × Human K_m = Animal dose (mg/kg) × Animal K_m, (where Human K_m = 60 kg/1.62 m^2; Animal (mouse) K_m = 0.02 kg/0.007 m^2; HED, human equivalent dose), in APPENDIX B of a FDA document[69] and other literature[70–72]]. At this dose, the toxicity including a 20% mortality rate (Table 1) of doxorubicin in mice in our experiments was similar to the toxicity observed in the

clinical settings in which death, cardiotoxicity, myelosuppression, liver toxicity, and renal damage occurred (Supplementary Table 1). These toxicity responses occurred at the drug dose at which the efficacy in the doxorubicin group was only about 1/2 the efficacy of the 8-drug-Cdg (TIR of the doxorubicin group to the Cdg was 27.0%/54.3% = 49.7%; Fig. 2b). If the doxorubicin group dose was increased to exhibit the same efficacy as the Cdg, the toxicity caused by doxorubicin would be much more severe. We used doxorubicin alone by increasing the dose to 1/2 of its LD_{50} (the cumulative dose = 5.0 mg/kg × 4 doses = 20.0 mg/kg), resulting in a TIR of 49.2%, but with a 60% mortality rate (see Section 1.3 for the detail). In contrast, the Cdg at this dose (the total dose of the Cdg was 3.02 times the dose used in the doxorubicin group) did not exhibit liver toxicity, cardiotoxicity, or renal toxicity, and no deaths occurred (Table 1). Thus, our drug combination exhibited good anticancer efficacy (TIR being 54.3%) with greatly reduced toxicity.

In this study, the dose of the doxorubicin group (2.5 mg/kg × 5 doses = 12.5 mg/kg in mice, equivalent to 1.02 mg/kg in humans) was comparable to the clinical dose for doxorubicin (0.68–3.24 mg/kg; Supplementary Table 15). However, this dose did not exhibit significant tumour-inhibitory efficacy (TIR 27.0%; Fig. 2b). In contrast, the TIR (54.3%; Fig. 2b) of the 8-drug-Cdg group was twice TIR of the doxorubicin group, demonstrating effective tumour suppression with almost no detectable toxicity. Therefore, applying this combination of 8 drugs in the clinical setting, which shows a greater therapeutic window, should achieve more desirable tumour treatment effects and significantly reduce the incidence of toxic reactions. Thus, the observed toxicity reduction of the 8-drug-Cdg in this study is clinically significant.

2.2 Why and how is the 'toxicity scattering effect' elicited? Do drugs with different structures that produce distinct toxicity profiles underlie the 'toxicity scattering effect,'

resulting in significantly reduced toxicity when combining multiple drugs with the same target? -----Relationship between the chemical structures and toxicities of drugs and the 'toxicity scattering effect'

Based on our experimental data and data reported in the literature[11,64,80–82], we found that drugs acting on the same target exhibit variations in their chemical structures (Supplementary Fig. 1), resulting in diverse toxicity profiles, including the types and intensities of toxicities (Table 1, Fig. 3, and Supplementary Tables 1 and 2). These differences suggest varying 'off-target toxicities'. We believe this phenomenon is the basis for the elicitation of the 'toxicity scattering effect' and the significant reduction in toxicity when combining multiple drugs with the same target. The following section presents specific differences in toxicities due to differences in the chemical structure of the related drugs based on our experimental data analysis.

2.2.1 The relationship between the chemical structures and toxicities of the eight drugs in the present study

The relationship between the chemical structures and toxicity of the eight drugs used in animal experiments in the present study is summarized here based on Fig. 3, Table 1, and Supplementary Fig. 1. The probable basis for the significant reduction in toxicity due to the 'toxicity scattering effect'[38,39] is described.

The eight drugs used in our animal experiments can be classified into the following three categories based on their chemical structures:

2.2.1.1 Amsacrine is an acridine with a markedly different chemical structure compared with the other seven drugs (Supplementary Fig. 1). Amsacrine did not exhibit heart, liver, or kidney toxicity in our study. However, the other seven drugs caused damage to at least one of these

499 organs. Amsacrine did not affect body weight, whereas the other seven drugs caused weight loss
500 of 7.8%–20.3% (Table 1).

501 **2.2.1.2** Etoposide and teniposide are podophyllotoxin analogues. Etoposide showed no toxicity
502 to either the heart or liver in our study, whereas the other six drugs (excluding amsacrine) all
503 exhibited cardiac and/or hepatic toxicity. Etoposide exhibited the highest thymus (65.0%) and
504 spleen (40.7%) indices among the eight drugs (Table 1) and were significantly higher than the
505 indices for amsacrine (50.0% and 29.7%, respectively). Etoposide had no significant effect on
506 WBC, but amsacrine increased the WBC by 26.6%. Teniposide increased the liver index by
507 13.2% but did not affect the spleen index. Thus, teniposide exhibited a different toxicity profile
508 than etoposide, which has no significant effect on liver index (Table 1).

509 **2.2.1.3** Doxorubicin, idarubicin, aclarubicin, epirubicin, and pirarubicin are anthracyclines.
510 Idarubicin had the highest mortality rate (50%), the highest increases in LDH (115.5%), CK
511 (29.7%), ALP (25.2%), and SCr (31.1%) levels, and the greatest decreases in WBC (40.4%),
512 platelet count (35.4%), and body weight (20.3%) among the eight drugs (Table 1). Thus,
513 idarubicin was the most toxic among the eight drugs. The mortality rate in the doxorubicin group
514 was 20%. Doxorubicin did not significantly affect ALP, platelet count, or food intake, but
515 significantly increased urea levels by 31.0%, whereas idarubicin decreased food intake by 32.5%
516 but did not significantly affect urea. Among the eight drugs, only doxorubicin significantly
517 increased ALT (42.9%). Aclarubicin increased AST (44.5%) and urea (42.0%) more than the
518 other seven drugs but did not significantly affect LDH, CK, or WBC. Thus, the toxicities of
519 aclarubicin were different from the toxicities of idarubicin and doxorubicin; doxorubicin
520 increased LDH and CK by 48.5% and 25.5%, respectively, and decreased WBC by 21.3%.
521 Epirubicin decreased food intake the most (35.0%) and was the only drug that decreased TP

levels (14.6%). The mortality rate (10%) and the thymus index (25.0%) for pirarubicin were the lowest among the eight drugs. Pirarubicin induced the lowest body weight loss (7.8%) among the five anthracyclines. Overall, these results indicate significant differences in toxicities among the anthracyclines and the other drugs (Table 1).

2.2.2 Relationship between chemical structures and toxicities of drugs reported in the literature

2.2.2.1 Different toxicities of doxorubicin and pirarubicin

Treatment (i.v.) with 15 mg/kg pirarubicin and doxorubicin produced 85.07% and 74.74% tumour regression, respectively. Treatment with 15 mg/kg doxorubicin caused 10.6% weight loss in mice, whereas no weight loss was observed after treatment with 15 mg/kg pirarubicin. Treatment with 25 mg/kg doxorubicin caused approximately 67% mortality and the remaining mice lost 26% of their initial body weight, whereas no deaths and less than 5% weight loss were observed in mice treated with 25 mg/kg pirarubicin. All mice died after treatment with 37.5 mg/kg doxorubicin. Mice receiving 37.5 mg/kg pirarubicin showed a > 17% decrease in body weight and no deaths[64].

2.2.2.2 Different toxicities of doxorubicin and idarubicin

Administration of 15 mg/kg (i.v.) doxorubicin induced left ventricular dysfunction in rats. Idarubicin displayed a much weaker cardiotoxicity compared with doxorubicin; left ventricular functional parameters were altered at 4.5 mg/kg of idarubicin to a significantly lower degree than 15 mg/kg doxorubicin. Treatment with 4.5 mg/kg of idarubicin caused a 26.0% decrease in body weight and treatment with 15 mg/kg doxorubicin caused a 27.8% decrease in body weight. The maximum tolerated doses were 3 mg/kg per injection for doxorubicin and 0.75 mg/kg per injection for idarubicin. The equivalent dose ratio for general toxicity was around 1:4

(idarubicin:doxorubicin), and the equivalent dose ratio for cardiotoxicity was estimated to be about 1:3 (idarubicin:doxorubicin)[82].

2.2.2.3 Different toxicities of doxorubicin and epirubicin

The risk of congestive heart failure for a cumulative doxorubicin dose of 400 mg/m² is 5%–6%, and the risk increases to over 10% for a cumulative dose of 500 mg/m². For doxorubicin doses over 550 mg/m², approximately 25% of patients develop congestive cardiac failure. A 900 mg/m² cumulative dose of epirubicin is associated with a cumulative congestive heart failure risk of 4%, and the risk increases to 15% for a 1000 mg/m² dose. Thus, doxorubicin incurs a higher risk of congestive heart failure than epirubicin at the same dose[11].

2.2.2.4 Different toxicities of etoposide and teniposide

The LD_{10} values for etoposide (VP-16) and teniposide (VM-26) were 9.4 (7.4–11.8) mg/kg daily and 3.4 (2.5–4.5) mg/kg daily, respectively, in mice. The difference in LD_{10} values indicates that the toxicity of these two drugs differs by approximately threefold[81].

2.2.2.5 Different toxicities of the eight drugs in animal experiments in the present study

In the present study, the LD_{50} values for intraperitoneal administration of the eight drugs in mice ranged from 3 to 29.57 mg/kg (Supplementary Table 3). This indicates a significant variation in acute toxicity among these drugs, with the largest difference being 10-fold[80].

2.2.3 Conclusion for the relationship between the chemical structures and toxicities of drugs and the 'toxicity scattering effect'

Drugs with different chemical structures acting on the same target exhibit diverse toxicity profiles, including the types and intensities of toxicities. These differences suggest varying 'off-target toxicities'. We believe this phenomenon is the basis for the 'toxicity scattering effect', which significantly reduces toxicity when combining multiple drugs with the same target.

2.3 Why is the anticancer efficacy additive, but the toxicity is not additive? Why do low doses of the 8 drugs have additive effects on cancer cells but not on normal cells?

The simplest explanation is that the off-target effects are non-overlapping, while the on-target effects overlap. Our enzyme experiments demonstrate that the TOPO II inhibitors exhibit additive effects on the inhibition of TOPO II at low concentrations. We posit that the fundamental principle underlying the reduction in toxicity is the significant decrease in the dose of each drug in the combination. A toxic drug is less toxic if the dose is reduced to 1/8 of the usual dose.

We attribute the additive effects of the 8 drugs to their on-target effects (action on the same efficacy target) (Extended Data Fig.4a). Their binding sites on the protein (TOPO II) should overlap, otherwise, the drugs would not produce an additive effect. The reduced toxicity of the 8-drug combination is likely due to their off-target effects. These off-target binding sites should be located at different locations on the same protein (TOPO II) or on different proteins. Otherwise, the toxicity of the combination drug would not be significantly reduced. In other words, tumour cells are sensitive to all eight drugs; therefore, they exhibit additive on-target effects, as confirmed by our additive effect experiments (Extended Data Fig.4a). However, the sensitivity of the eight drugs varies in normal cells, resulting in only partially additive off-target effects (leading to a toxicity scattering effect) when the drugs are combined (Extended Data Fig.4a).

For example, we consider a scenario where one drug affects normal heart cells, another drug affects normal liver cells, a third affects normal kidney cells, and the remaining five drugs affect normal bone marrow cells. The first three drugs affect specific cells at low concentrations (for each drug: 1/8 of the whole potency of the combination drug; Extended Data Fig.4a), these drugs

591 do not exert significant toxicity on these cells when combined. Thus, no observable toxicities are
592 observed when these drugs are used together. This is a non-additive effect, resulting in a toxicity
593 scattering effect. On the other hand, the latter five drugs all affect normal bone marrow cells.
594 When combined, the concentration of these drugs adds up (for the five drugs: 5/8 of the whole
595 potency; Extended Data Fig.4a), leading to additive effects on bone marrow suppression. This
596 can be described as a partial additive effect on the same target for the toxicity, resulting in a
597 partial toxicity scattering effect.

598 We do not have direct evidence for off-target effects. However, as indicated in
599 Supplementary Tables 1 and 2, and Section 2.2.2 above-mentioned, the literature shows that each
600 of the eight drugs has individual toxicities resulting in different toxicity profiles in both humans
601 and animals (for our animal data see Table 1, Fig.3, Extended Data Fig. 3 and Section 2.2.1),
602 although some toxicities may overlap among these drugs. Even when the toxicities are the same,
603 the degree of toxicity varies among these drugs. The toxicity targets must also differ because the
604 toxic effects of each drug differ. In other words, the toxicities of these drugs are not additive
605 (what we call the 'toxicity scattering effect'); thus, these drugs act on different toxicity targets.
606 We believe this is evidence for off-target effects.

607 **2.4 Does combining more drugs with the same target lower the required dose of each drug**
608 **and increase the likelihood of the 'toxicity scattering effect'?**

609 **2.4.1 Impact of the number of the same-target drugs in combination on toxicity in the**
610 **present study**

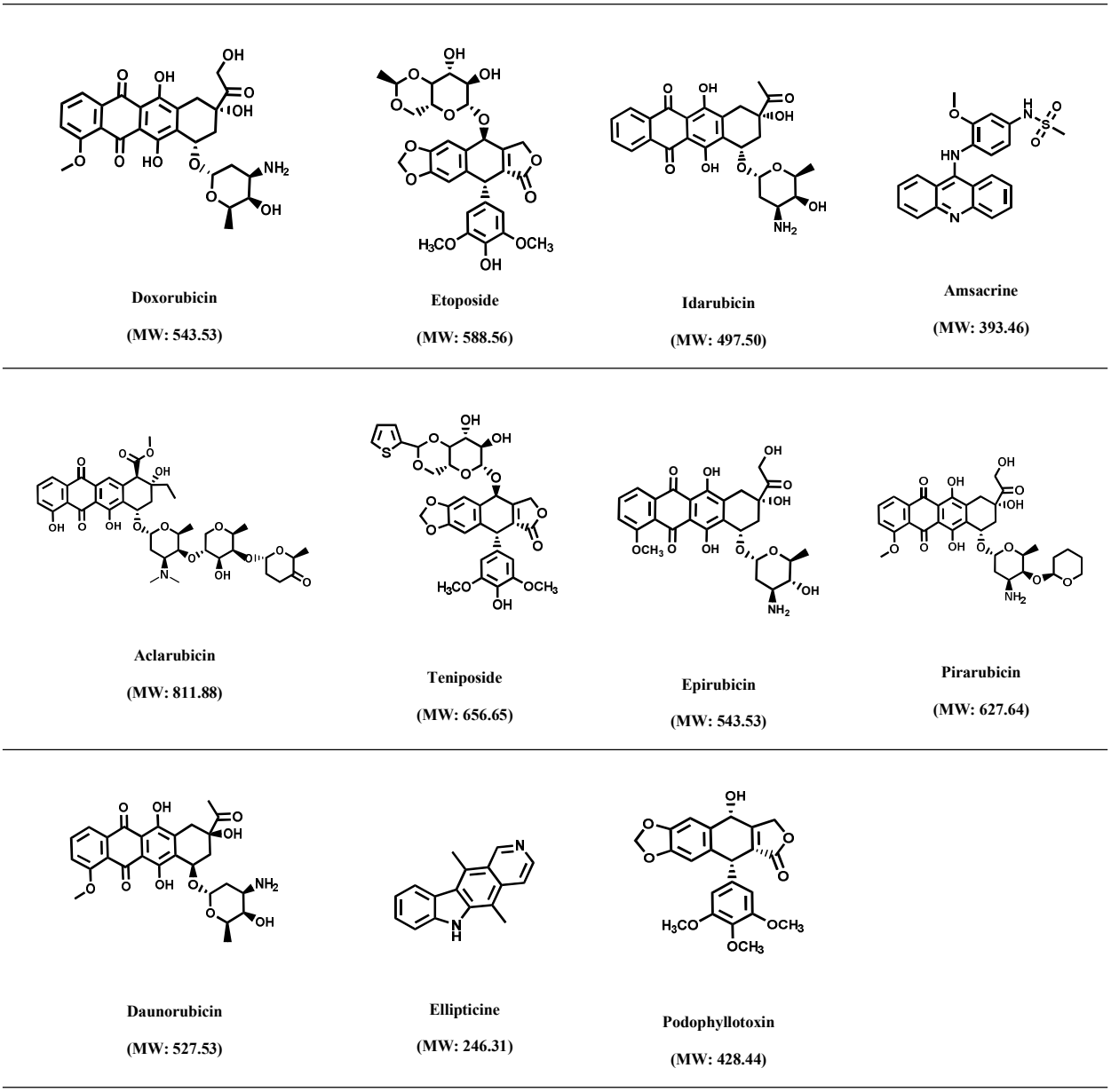
611 The 4-drug-Cdg (doxorubicin, etoposide, idarubicin, and amsacrine combined at 1/10 of their
612 LD_{50}) induced a similar tumour inhibition rate (TIR) (59%; Extended Data Fig. 2) in the 4-drug
613 solid tumour study as the 8-drug- Cdg (TIR 54.3%; Fig. 2) in the 8-drug solid tumour study.

However, the 4-drug-Cdg exhibited a 10% mortality rate, increased CK levels, and decreased TP levels and thymus, spleen, and liver indices (Extended Data Fig. 3 and Extended Data Table 6) compared with the 8-drug-Cdg. No mortality or hepatic, cardiac, or renal toxicities were observed in the 8-drug-Cdg (Fig.3 and Table 1). Thus, the antitumour effects were nearly equivalent, but the toxicity of the 8-drug-Cdg was significantly lower than the toxicity of the 4-drug-Cdg. Furthermore, the 4-drug-Cdg exhibited much lower toxicity than doxorubicin at 1/2 of its LD_{50}. The high-dose doxorubicin [Dox (H) group] exhibited a TIR of 49.2%, but a 60% mortality rate, increased levels of ALT, AST, ALP, LDH, CK, SCr, and kidney index, and decreased ALB levels compared with the 4-drug-Cdg (Extended Data Figs. 2 and 3, and Extended Data Table 6). Notably, the Dox (H) group essentially functioned as a 'combination of only one drug'. Therefore, our results indicate that as the number of drugs in the combination increased from 1 to 4 and then to 8, the dose of each drug progressively decreased (from 1/2 LD_{50} to 1/10 LD_{50} and then to 1/20 LD_{50}), leading to a corresponding reduction in toxicity. Thus, the more drugs that act on the same target in a combination, the lower the dose required of each drug and the higher the likelihood of a 'toxicity scattering effect'[38,39]. Increasing the number of drugs with the same target in a combination may progressively lower the toxicity due to the 'toxicity scattering effect'[38,39]. The 8-drug combination dramatically reduced the mortality rate and achieved a TIR of around 50%, demonstrating a much larger therapeutic window than the single drugs and the 4-drug combination, making this combination the most appropriate anticancer therapy (Extended Data Fig. 4 b,c).

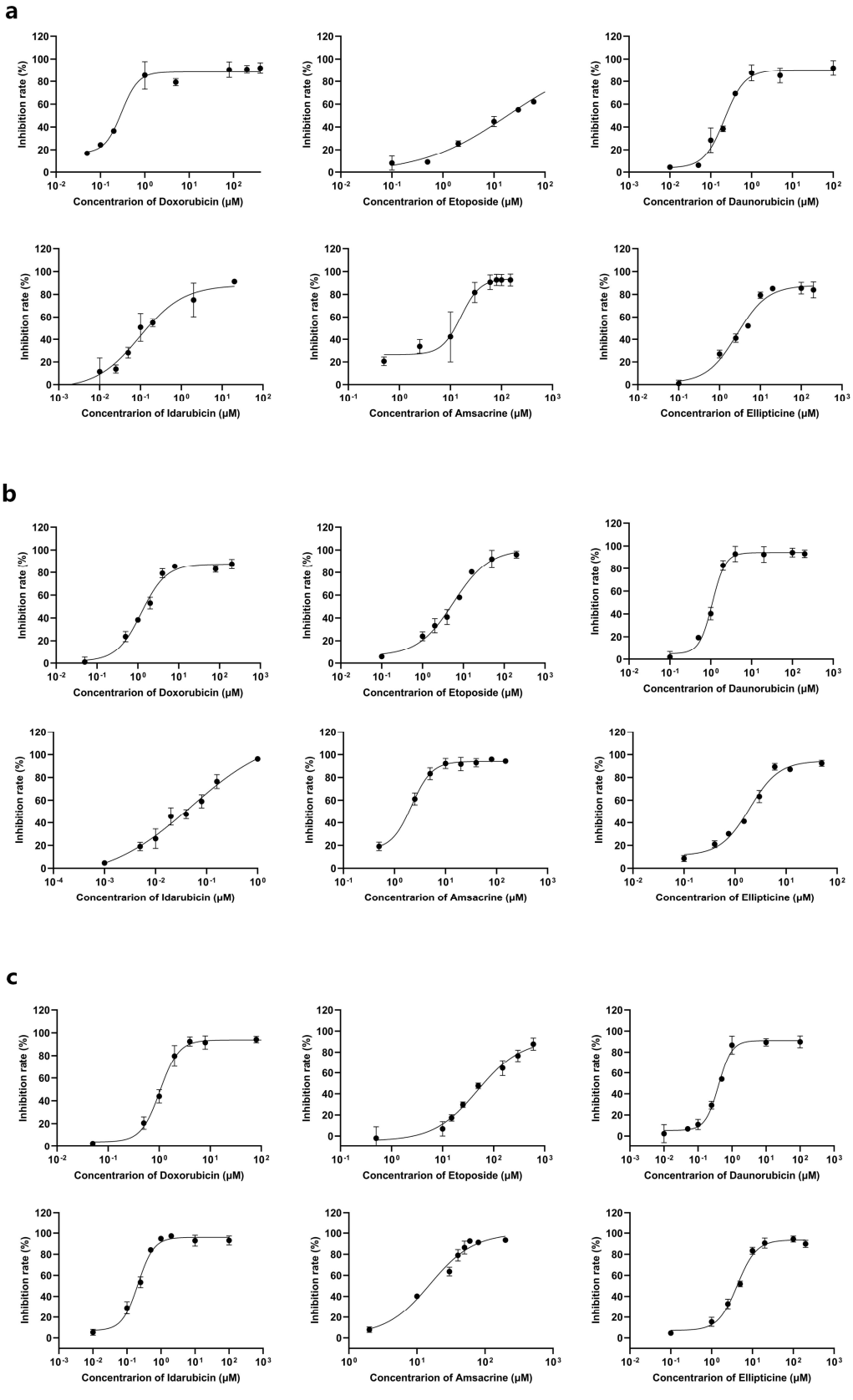
2.4.2 The reason most traditional Chinese medicines (TCMs) have very low toxicity or are non-toxic is that a certain group of active substances of a single TCMs is equivalent to a combination of numerous constituents that collectively act on the same target.

637 Our proposed hypotheses [38,39] regarding TCMs ‘additive effects’ and ‘toxicity scattering
638 effects’ suggest that a certain group of active substances of a single TCMs include chemical
639 constituents and metabolites that can act on the same target. These compounds can produce an
640 ‘additive effect’ and a ‘toxicity scattering effect.’ Each TCMs contains numerous (at least
641 more than ten) compounds with similar chemical structures (including constituents and
642 metabolites) that can produce a same-target ‘additive effect’, leading to the ‘toxicity
643 scattering effect’. Therefore, most TCMs exhibit very low toxicity or are non-toxic. Essentially,
644 a single TCMs is equivalent to a combination of numerous constituents that act on the same
645 target. Thus, “the more drugs in the combination, the lower the dose required for each drug,
646 thereby increasing the likelihood of a ‘toxicity scattering effect’.”
647

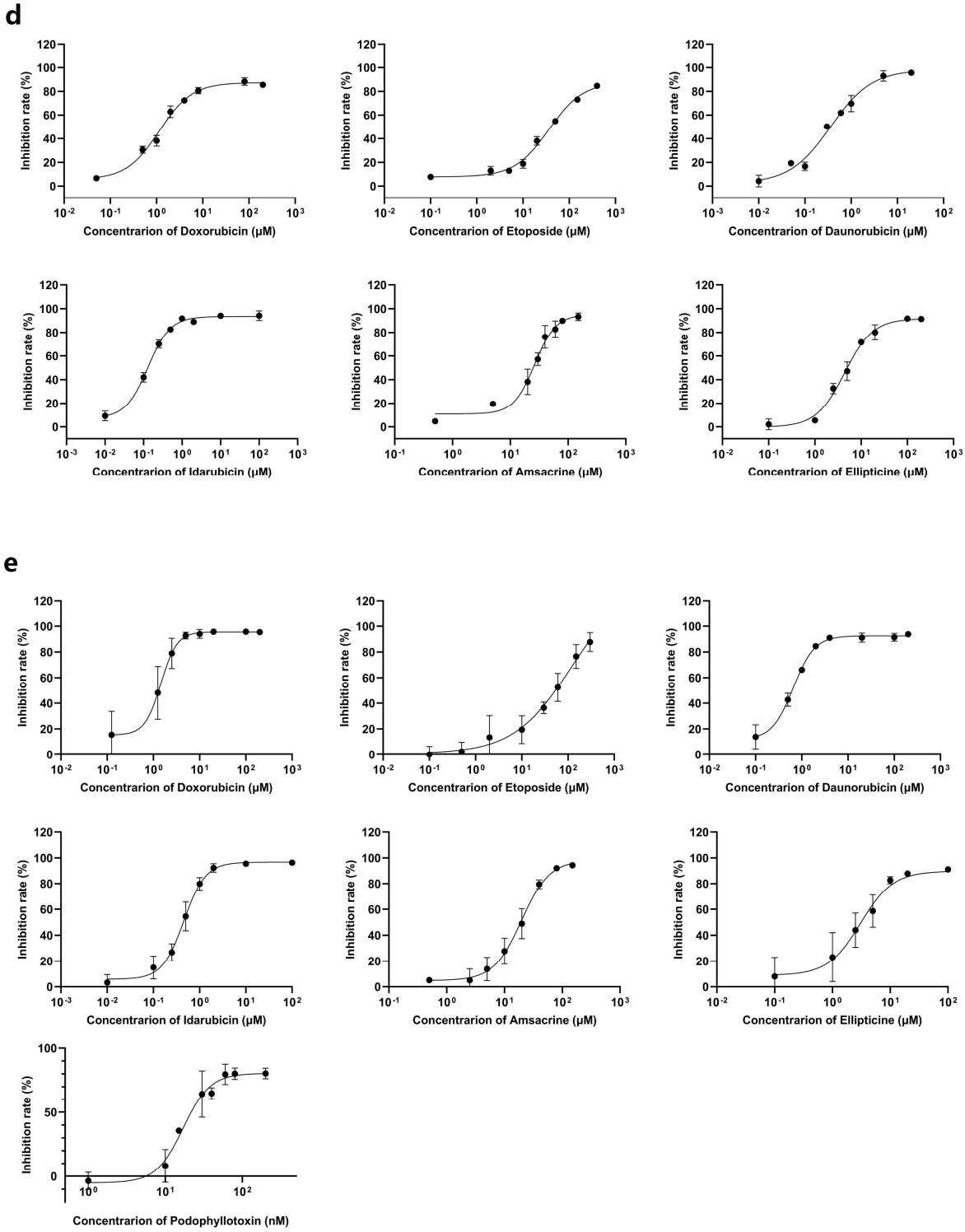
3. Supplementary Figs. 1 and 2



Supplementary Fig. 1. The chemical structures of the 11 anticancer drugs used in this study. All drugs are DNA topoisomerase II inhibitors and marketed anticancer drugs that are used in clinical practice (except ellipticine, which was not used in the animal experiments)**.** The top eight drugs were used in animal experiments, and the top four drugs were also used in cell and enzyme experiments. The bottom three compounds were only used in enzyme and/or cell experiments.


a
Inhibition rate (%)
Concentrarion of Doxorubicin (μM)
Concentrarion of Etoposide (μM)
Concentrarion of Daunorubicin (μM)
Concentrarion of Idarubicin (μM)
Concentrarion of Amsacrine (μM)
Concentrarion of Ellipticine (μM)
b
Inhibition rate (%)
Concentrarion of Doxorubicin (μM)
Concentrarion of Etoposide (μM)
Concentrarion of Daunorubicin (μM)
Concentrarion of Idarubicin (μM)
Concentrarion of Amsacrine (μM)
Concentrarion of Ellipticine (μM)
c
Inhibition rate (%)
Concentrarion of Doxorubicin (μM)
Concentrarion of Etoposide (μM)
Concentrarion of Daunorubicin (μM)
Concentrarion of Idarubicin (μM)
Concentrarion of Amsacrine (μM)
Concentrarion of Ellipticine (μM)

654



Supplementary Fig. 2. Dose-response curves of seven TOPO II inhibitors in U87 (a), HL-60 (b), BxPC-3 (c), Hela (d), and HepG2 (e) cells. All five cell lines (n = 6) were treated with

doxorubicin, etoposide, daunorubicin, idarubicin, amsacrine, and ellipticine. Only HepG2 cells were treated with podophyllotoxin (n = 6).

4. Supplementary Tables 1–17

Supplementary Table 1. Common toxicities of the TOPO II inhibitors according to the human organs affected

	Dox	Eto	Ida	Ams	Acl	Ten	Epi	Pir
Death	Yes[76,83,84]		Yes[85]		Yes[86]	Yes[83]	Yes[83]	
Liver	Hepatotoxicity[16,46]; Changes in transaminase levels[87]	Hepatotoxicity[87]	ALP↑/AST↑[87]	Hepatotoxicity[87]; ALP↑/AST↑[87]	Hepatotoxicity[86,88]	Hepatotoxicity ion[83,87]	Transaminases↑[87]	
Heart	Cardiotoxicity[1,16,19,46,73,89]; Cardiomyopathy and congestive[11,74,90]	Congestive heart failure[87]	Cardiotoxicity[85,87,90]; Cardiomyopathy and congestive[74]; LDH↑[87]	Cardiac arrhythmias and congestive heart failure[87]	Cardiotoxicity[74,86,88]; LDH↑[88]	Arrhythmia[83,87]	Cardiotoxicity[74,83,89,90]; Congestive heart failure[11,87]	Cardiotoxicity[33,74]
Kidney	Nephrotoxicity[5,16,46]		SCr↑[87]	Nephrotoxicity[87]; SCr↑/BUN↑[87]		Nephrotoxicity[83,87]		
Myelosuppression	Yes[87,90]; WBC↓/PC↓[83]; WBC↓[87]	Yes[90]	Yes[90]; WBC↓[85]; WBC↓/PC↓[87]	Yes[87]; WBC↓/PC↓[87]	Yes[88]; WBC↓/PC↓[86,88]	Yes[83,90]; WBC↓/PC↓[83,87]	Yes[83,90]; WBC↓/PC↓[83,87]	WBC↓[33]
Gastrointestinal reactions	Nausea/vomiting[16,83,84,87,90]; Diarrhea[83,84,90]	Nausea/vomiting[87,90]; Diarrhea[90]	Nausea/vomiting[87,90]; Diarrhea[87]	Nausea/vomiting[87]; Diarrhea[87]	Yes[86]; Nausea/vomiting[86,88]; Diarrhea[86]	Nausea/vomiting[83,87,90]; Diarrhea[87]	Nausea/vomiting[83,87]; Diarrhea[83,87]; Bleeding[83,87]	Yes[33]; Nausea/vomiting[84]; Diarrhea[84]

An upward arrow (↑) indicates an increase in value; a down arrow (↓) indicates a decrease in value. Acl, aclarubicin; ALP, alkaline phosphatase; Ams, amsacrine; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Dox, doxorubicin; Epi, epirubicin; Eto, etoposide; Ida, idarubicin; LDH, lactate dehydrogenase; PC, platelet count; Pir, pirarubicin; Ten, teniposide; SCr, creatinine; WBC, white blood cell; Yes, meaning that this toxicity is present.

Supplementary Table 2. Drug toxicities in animal models

	Dox	Eto	Ida	Ams	Acla	Ten	Epi	Pir
Death	Yes[22,64,82]	Yes[67,81,91–93]	Yes[82,94]	Yes[95]	Yes[88,96,97]	Yes[81,98]	Yes[99]	Yes[41,100–102]
Liver	Hepatotoxicity[15,43]; ALT↑/AST↑[15,43,77]; AST↑[22]; TP↓/ALB↓[15]	Focal necrosis[91]; TP↓/ALP↓[91]			Hepatotoxicity[88]	Lesions[45,98]; AST↑[45]	Lesions [83,99]; ALT↑/AST↑[103]	Lesions[33,101]; ALT↑[41,101,104]; AST↑[41,101,102,104]; Liver/body weight↑[101]
Heart	Cardiotoxicity [15,43,46,64,82,105–107]; CK ↑[22]; CK-MB↑[15,46,77,105]; LDH↑[15,22,46,77,105]; CK-NAC↑[15,46]; Heart/body weight↑[107]		Cardiotoxicity[82]		Cardiotoxicity[96,108]	Lesions[45,98]; LDH↑[45]	Cardiotoxicity[83,99]; CK-MB↑[103]; Heart weight↓/ CKP↑[99]	Cardiotoxicity[33,41,64,100]; CK↑/LDH↑[41,102]; CK-MB ↑[41,100]; LDH↑[100]
Kidney	Nephrotoxicity[15,43]; BUN↑/SCr↑[15,77]				Congestion[108]		Lesions[83]	Nephrotoxicity[41]; BUN↑ /SCr↑[41]
Thymus		Atrophy[91,92]			Atrophy[88,108]			
Spleen		Atrophy[91]			Atrophy[88,108]; Spleen weight↓[97]		Lesions[83]	Lesions[33]
Body weight	Weight loss[43,64,78,82]	Weight loss[91,92]	Weight loss[82,94]	Weight loss[95]	Weight loss[88,96,97,108]	Weight loss[98]	Weight loss[83,99,111]	Weight loss[33,64,100,101,104,112]
Myelosuppression	Yes[43]; WBC↓[22,43]; RBC↓[43]; PC↓[22]	Yes[92,93]; WBC↓[91,93]; RBC↓[91]; Hypoplasia[91]	WBC↓/PC↓ [94]; LC↓/BMC↓[113]		Yes[88,96,108]	WBC↓[45]	Yes[83]	WBC↓[33,41,102]; PC↓[102]
Gastrointestinal toxicity	Intestinal mucositis [78,106]; Diarrhea[82]; Gastric gland dilation [106]; Vomiting[43]	Feed intake↓[91]; Diarrhea[91]; Bloody stools[92]; Ulcerations[93]	Intestinal ucositis[109]; Diarrhea[82,109]		Bleeding[88,108]; Nausea/vomiting[88]; Degeneration[108]		Yes[83]; Diarrhea, vomiting, and lesions[83]	Yes[41]; Feed intake↓[100,101,104]; Gastrointestinal tract injured[41]

An upward arrow (↑) indicates an increase in value; a down arrow (↓) indicates a decrease in value. Acl, aclarubicin; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; Ams, amsacrine; AST, aspartate aminotransferase; BMC, bone marrow cell count; BUN, blood urea nitrogen; CK, creatine kinase; CK-MB, creatine kinase isoenzyme; CK-NAC, N-acetylcysteine activated creatine kinase; CPK, creatine phosphokinase; Dox, doxorubicin; Epi, epirubicin; Eto, etoposide; Ida, idarubicin; LC, leukocyte count; LDH, lactate dehydrogenase; PC, platelet count; Pir, pirarubicin; RBC, red blood cell count; SCr, creatinine; Ten, teniposide; TP, total protein; WBC, white blood cells; Yes, meaning that this toxicity is present.

Supplementary Table 3. LD_{50} in mice for intraperitoneal injections of the 8 drugs*

Drug	LD_{50} (mg/kg)
Doxorubicin	10.70†
Etoposide	64
Idarubicin	3
Amsacrine	15.47†
Aclarubicin	16.10
Teniposide	29.57
Epirubicin (hydrochloride)	10.80‡
Pirarubicin (hydrochloride)	13.90‡

*see reference[80]; †The doses of doxorubicin and amsacrine were calculated using LD_{50} = 10 and LD_{50} = 15, respectively; ‡ The LD_{50} values of epirubicin hydrochloride and pirarubicin hydrochloride were used as the LD_{50} values of epirubicin and pirarubicin, respectively; no LD_{50} for epirubicin or epirubicin hydrochloride was found for mice, so the LD_{50} for rats was used.

677 **Supplementary Table 4. Literature report of ineffective, effective, or usual doses of 8 anticancer drugs in different mice***

	Dose for i.p. dosing		Dose for i.v. dosing
Dox	**Doses* reported:** **7.02** (T/C§ 126%), **14.04** (T/C 138%), **28.17** (T/C 152%), **56.25** (T/C 167%), **112.5** (T/C 229%), **225** (T/C 214%), and **450** (T/C 143%) mg/kg [65]; **7.5**, **15**, **20**, and **25** mg/kg[15]†††; **7.8** (ILS§ 25%) and **11.7** (ILS 79%) mg/kg[114]; **8** mg/kg (TIR§ ca.23%¶)[115]; **9** (TIR by volume ca.50%) mg/kg[95]; **10** mg/kg[116]; **15** mg/kg[43,105]; **15** and **30** mg/kg[107]	**Doses* in Sdg and Cdg in the present study†:** 12.5 mg/kg (TIR 27.0%§) and 2.50 mg/kg **Estimated minimum effective dose‡:** 14 mg/kg **Percentage of dose in Cdg versus the minimum effective dose:** 2.5/14=17.9%	**Doses* reported:** **5** (TIR§ ca.37%¶) ††† and **10** (TIR ca.54%) mg/kg[106]; **10** mg/kg (TIR 29%)[117]; **10** (none died) and **20** (all mice died) mg/kg[22]; **20** (all mice died) mg/kg[102]; **15** (MTD; Tumour regression 74.74%), **25** (2/3rd mice died), and **37.5** (all mice died) mg/kg[64]
Eto	**Reported:** **10** (cures§ 13%; ILS 242%); **20** (cures 15%, 0%, and 18%; ILS 200%, 188%, and 131%)**; and **40** (cures 81%, 55%, and 27%; ILS -††, -, and 156%)** mg/kg[81]; **11** (ILS 8%), **22** (ILS 66%), **33** (ILS 92%), **70** (ILS 105%), **80** (ILS 82%), and **90** (ILS 47%) mg/kg[93]; **20** (Sd60§ 15%; T/C 183%) and **80** (Sd60 60%; T/C -††) mg/kg[92]; **52**, **73**, **102**, **143** (none died), and **200** (all mice died) mg/kg[92]; **100**–**400** (T/C 134%–300%), **150** (TIR 92%), and **180** (TIR 91%) mg/kg[67]	**Doses in Sdg and Cdg in the present study†:** 80.0 mg/kg (TIR 38.1%) and 16.0 mg/kg **Estimated minimum effective dose‡:** 82 mg/kg **Percentage of dose in Cdg versus the minimum effective dose:** 16/82=19.5%	
Ida	**Reported:** **1** (TIR ca. 7%¶) and **4** mg/kg (TIR ca. 65%¶)[113]; **3**, **6**, and **12** mg/kg[118]	**Doses in Sdg and Cdg in the present study†:** 3.75 mg/kg (TIR 34.5%) and 0.75 mg/kg **Estimated minimum effective dose‡:** 4.0 mg/kg **Percentage of dose in Cdg versus the minimum effective dose:** 0.75/4.0=18.8%	**Reported:** **0.5** (ILS 13%), **1** (ILS 50%), **2** (ILS 113%), **3** (ILS 144%), and **4** (MTD) mg/kg[110]; **2** mg/kg[119]
Ams	**Reported:** **8**, **10**, **15**, **20**, and **24** (TIR by volume ca.27%) mg/kg‡‡[95]; **26.7** (ILS 38%, 42%, and 78%)§§ and **39.9** (ILS 38%, 42%, and 78%)§§ mg/kg[114]; **14.4**, **24**, and **39.9** mg/kg[120]	**Doses in Sdg and Cdg in the present study†:** 18.75 mg/kg (TIR 31.1%) and 3.75 mg/kg **Estimated minimum effective dose‡:** 28 mg/kg **Percentage of dose in Cdg versus the minimum effective dose:** 3.75/28=13.4%	**Reported:** **90** (ILS 58% and 72%)§§ mg/kg[114]
Acl	**Reported:** **2.5** (TIR 18%), **5** (TIR 60%), **12.5** (TIR 100%), and **25** (TIR 100%) mg/kg[97]; **2.5**–**40** mg/kg (T/C 100%)[121];	**Doses in Sdg and Cdg in the present study†:** 20.15 mg/kg (TIR 24.6%) and 4.03 mg/kg **Estimated minimum effective dose‡:**	**Reported:** **20** (ILS 125%, 200%) mg/kg[88]; **20**, **200**, and **400** (all mice died) mg/kg[96]

	6 (ILS 8%) and **9** (ILS -) mg/kg[93]; **4.8** (ILS 50%), **9** (ILS 43%), **12** (ILS 133%), **20** (ILS 168%, >170%, or 112%), **36** (ILS 113%), **45** (ILS 36%, 81%, 108%, and 122%), and **48** (ILS 80%), **10** (TIR 91%), **11** (TIR 56%), **20** (TIR 24%, 64%, and 80%), **28.2** (TIR 47%), **30** (TIR 64%, 67%, and 56%), **36** (TIR 84%), **54** (TIR 100%), **60** (TIR 94%), and **60** (TIR 74% and 94%) mg/kg¶¶[88]	23 mg/kg **Percentage of dose in Cdg versus the minimum effective dose:** 4.03/23=17.5%	
Ten	**Reported:** **3.75** (cures 13%; ILS 167%); **7.5** (cures 46%, 23%, and 0%; ILS 344%, 175%, and 125%)**; and **15** (cures 72%, 70%, and 27%; ILS -††, -, and 181%)** mg/kg[81]; **30** (TIR 48%) and **100** (all mice died) mg/kg[98]; ED_{50} = 40.46 mg/kg[98]	**Doses in Sdg and Cdg in the present study†:** 37.0 mg/kg (TIR 29.0%) and 7.40 mg/kg **Estimated minimum effective dose‡:** 42 mg/kg **Percentage of dose in Cdg versus the minimum effective dose:** 7.4/42=17.6%	**Reported:** **20**, **40**, and **60** mg/kg[45]
Epi	**Reported:** **10** and **20** mg/kg[99]; **12.5** mg/kg (TIR ca. 25%¶)[103]; **60** mg/kg[111]	**Doses in Sdg and Cdg in the present study†:** 13.5 mg/kg (TIR 28.0%) and 2.70 mg/kg **Estimated minimum effective dose‡:** 35 mg/kg **Percentage of dose in Cdg versus the minimum effective dose:** 2.7/35=7.7%	**Reported:** **20.5** mg/kg (ca. 0.5 times human dose)[83]; **30** mg/kg (TIR by volume ca.25%¶)[122]
Pir	**Reported:** **8** mg/kg (TIR 40%)[115]	**Doses in Sdg and Cdg in the present study†:** 17.4 mg/kg (TIR 28.4%) and 3.48 mg/kg **Estimated minimum effective dose‡:** 19 mg/kg **Percentage of dose in Cdg versus the minimum effective dose:** 3.48/19=18.3%	**Reported:** **5** (TIR by volume ca. 80%¶) mg/kg[112]; **9** (TIR by size ca. 35%¶) mg/kg[123]; **10** (TIR by volume ca. 54%¶) mg/kg[33]; **10** (all mice died) and **20** (all mice died) mg/kg[102]; **10** (TIR 81.4%), **20** (TIR 90.8%), and **30** (all mice died) mg/kg[41]; **15** (Tumour regression 85.07%), **25** (MTD) and **37.5** (toxic) mg/kg[64]
A range for the percentage of the dose of each drug in Cdg equivalent to its minimum effective dose:		7.7%–19.5%, average 16.3%	

* Since the drugs (8 TOPO II inhibitors) used in this study were developed and applied in clinical practice during the early years, the effective anticancer dose of these drugs in animals, especially the effective dose for i.p. in mice with clear and complete information were difficult to find. For example, there was no record of the route of administration and/or the number of doses in some publications. Therefore, the dose information for each drug listed in this table is limited. The minimum effective dose for mouse i.p. was also difficult to determine from the reported doses listed in the table. The doses in the table indicate an accumulated dose in a cycle for each drug in the free-drug form.

† The data for each single-drug group (Sdg) and each drug in the combination-drug group (Cdg) in the 8-drug solid tumour study.

‡ We used the estimated dose to achieve 50% TIR as the minimum effective dose for i.p. in mice, which should be higher than the individual dose of each Sdg because none of the Sdgs had a TIR over 50% in the present study, and because different mouse tumour models[81,88] or different treatment schedule[88] may respond differently to the same drug at the same dose.

§ A drug was considered to have anticancer activity if it produced T/C of ≥ 125% [67,124], ≥ ILS 30% [65], ≥ T/C 130%[65], ≥ TIR 50%[67], or ≥ TIR 58%[65]. For ascites tumours, ILS (%) = (T/C −1) ×100, where T and C are the survival

periods (days) for treated and control mice, respectively[65]. For solid tumours: TIR (%) = (1 − Wt/Wc) ×100, where Wt and Wc are the tumour weights in treated and control mice, respectively[65]. Longer survivals than 40 days in L1210 and P388 and 60 days in other ascites tumours with no retention of ascites were considered to be cured; mice without any trace of tumour at the termination of the experiments were considered to be cured[65].

¶ These values are estimated by us according to the graphs in the literature[33,103,106,112,113,115,122,123].

** These data were obtained from three different mouse models for both etoposide and teniposide[81].

†† The '-' means that no value was shown for groups in which > 50% of the animals were cured[81].

‡‡ At this dose (24 mg/kg), amsacrine was ineffective against human malignant schwannoma, malignant lymphoma, malignant melanoma, liposarcoma, and neuroblastoma, but was active against testicular cancer[95].

§§ These data were obtained from two different mouse models (or dosing schedules) for amsacrine[114].

¶¶ These data were obtained from twelve different mouse ascites models (or dosing schedules) or thirteen different mouse solid (or human xenograft) models for aclarubicin[88].

††† The underlined dose is the ineffective dose for each drug.

Acl, aclarubicin; Ams, amsacrine; ca., circa; Cdg, combination-drug group; Dox, doxorubicin; ED_{50}, 50% effective dose; Epi, epirubicin; Eto, etoposide; Ida, idarubicin; ILS, increase in life span (life-prolonging rate, LPR); i.p., intraperitoneal injection; i.v., intravenous injection; mg/kg, mg/kg of body weight; Mod, model; MTD, maximum tolerated dose; Pir, pirarubicin; Sd60 (%), the number of mice surviving to day 60/number of mice in a group; Sdg, single-drug group; T/C, median survival time of dying mice in a drug-treated (T) group divided by the median survival time of untreated mice in a tumour control (C) group; Ten, teniposide; TIR, tumour inhibition rate (by weight).

Supplementary Table 5. Concentrations of the six inhibitors in the additive experiment in Hep G2 cells

Group	Concentration (μM)
Doxorubicin IC_{18}*	0.74
Etoposide IC_{18}	11.69
Daunorubicin IC_{18}	0.30
Idarubicin IC_{18}	0.10
Amsacrine IC_{18}	4.89
Ellipticine IC_{18}	0.66
Combination IC_{18}	18.38

*Individual inhibitors were used as controls. The combination IC_{18} represented that each inhibitor in the combination was combined at its IC_{18} [a corresponding concentration of a 18% inhibition rate in its dose-response curve (Supplementary Fig.2) obtained in the previous experiment (i.e., an inhibitor concentration that inhibits cell viability by 18%—henceforth referred as IC_{18})].

Supplementary Table 6. Concentrations of the six inhibitors in the additive experiment in U87, HL-60, BxPC-3, and Hela cell lines

		Concentration (μM)						
Cell	Group	Dox	Eto	Dau	Ida	Ams	Ell	Combination
U87	Comb $IC_{4.5}$	0.02	0.08	0.04	0.01	0.12	0.19	0.45
	Comb IC_{9}	0.04	0.23	0.06	0.01	0.27	0.35	0.96
	Comb IC_{18}	0.07	0.71	0.10	0.03	0.66	0.67	2.25
	Comb IC_{36}	0.15	2.79	0.17	0.07	1.90	1.48	6.56
Hela	Comb $IC_{4.5}$	0.04	2.04	0.02	0.01	2.79	0.49	5.39
	Comb IC_{9}	0.09	3.79	0.03	0.01	4.29	0.80	9.01
	Comb IC_{18}	0.19	7.40	0.07	0.02	6.82	1.37	15.87
	Comb IC_{36}	0.50	16.27	0.17	0.06	11.79	2.57	31.37
HL-60	Comb $IC_{4.5}$	0.12	0.22	0.27	0.00	0.15	0.10	0.86
	Comb IC_{9}	0.20	0.47	0.37	0.00	0.25	0.19	1.49
	Comb IC_{18}	0.35	1.05	0.52	0.01	0.45	0.39	2.76
	Comb IC_{36}	0.68	2.71	0.77	0.02	0.87	0.91	5.97
BxPC-3	Comb $IC_{4.5}$	0.23	2.35	0.06	0.02	1.97	0.58	5.21
	Comb IC_{9}	0.32	4.69	0.09	0.04	3.05	0.91	9.10
	Comb IC_{18}	0.46	9.84	0.15	0.06	4.89	1.46	16.86
	Comb IC_{36}	0.70	23.60	0.25	0.12	8.53	2.58	35.77

Ams, amsacrine; Comb, combination; Dau, daunorubicin; Dox, doxorubicin; Ell, ellipticine; Eto, etoposide; Ida, idarubicin.

Supplementary Table 7. IC_{50} values for the seven TOPO II inhibitors in the five cell lines

Cell	Dox (μM)	Eto (μM)	Dau (μM)	Ida (μM)	Ams (μM)	Ell (μM)	Pod (nM)
HepG2	1.59	39.08	0.76	0.40	13.96	2.22	16.52
Hela	0.90	26.39	0.31	0.11	16.49	3.79	
HL-60	1.03	4.84	0.98	0.04	1.32	1.52	
U87	0.23	6.43	0.24	0.12	3.61	2.40	
BxPC-3	0.91	40.23	0.35	0.17	11.98	3.64	

Ams, amsacrine; Dau, daunorubicin; Dox, doxorubicin; Ell, ellipticine; Eto, etoposide; Ida, idarubicin; Pod, Podophyllotoxin

Supplementary Table 8. Concentrations of the four inhibitors with similar IC_{50} values in the additive experiment in HepG2 cells

	Concentration (μM)				
Group	Doxorubicin	Daunorubicin	Idarubicin	Amsacrine	Combination
Doxorubicin IC_{18}*	0.74				
Daunorubicin IC_{18}		0.30			
Idarubicin IC_{18}			0.10		
Amsacrine IC_{18}				4.89	
Comb $IC_{4.5}$	0.34	0.12	0.02	1.68	2.16
Comb IC_{9}	0.5	0.19	0.05	2.81	3.55
Comb IC_{18}	0.74	0.30	0.10	4.89	6.03
Comb IC_{36}	1.19	0.54	0.23	9.38	11.34

*Individual inhibitors were used as controls. Comb, combination.

Supplementary Table 9. Concentrations of the four inhibitors with large IC_{50} differences in the additive experiment in HepG2 cells

	Concentration (μM)				
Group	Doxorubicin	Etoposide	Daunorubicin	Podophyllotoxin	Combination
Etoposide IC_{18}*		11.69			
Daunorubicin IC_{18}			0.30		
Comb $IC_{4.5}$	0.34	3.43	0.12	0.006	3.90
Comb IC_{9}	0.50	6.20	0.19	0.007	6.90
Comb IC_{18}	0.74	11.69	0.30	0.010	12.74
Comb IC_{36}	1.19	24.72	0.54	0.013	26.46

*Etoposide with IC_{18} and daunorubicin with IC_{18} were used as controls. Comb, combination.

Supplementary Table 10. Proportionally reduced concentrations of combined inhibitors in HepG2 cell proliferation experiments

	Concentration (μM)				
Group	Doxorubicin	Etoposide	Daunorubicin	Podophyllotoxin	Combination
Doxorubicin IC_{36} *	1.19				
Etoposide IC_{36}		24.72			
Comb IC_{36} × 1	1.19	24.72	0.54	0.013	26.46
Comb IC_{36} × 0.75	0.89	18.54	0.40	0.01	19.84
Comb IC_{36} × 0.5	0.59	12.36	0.27	0.007	13.23
Comb IC_{36} × 0.25	0.30	6.18	0.13	0.003	6.61
Comb IC_{36} × 0.125	0.15	3.09	0.07	0.002	3.31

* Doxorubicin IC_{36} and etoposide IC_{36} were used as controls. Comb, combination.

Supplementary Table 11. Comparison of antitumour activity (inhibition rates) among combination groups composed of different inhibitors or different numbers of inhibitors in HepG2 cells

Group	Combination of 4 inhibitors †	Combination of 4 inhibitors ‡	Combination of 6 inhibitors§
Comb $IC_{4.5}$	20.5 ± 11.1	18.9 ± 6.5	
Comb IC_{9}	33.6 ± 5.1	32.9 ± 7.5	63.2 ± 1. 2*
Comb IC_{18}	60.5 ± 1.6	55.6 ± 4.4	87.4 ± 6.3*
Comb IC_{36}	87.4 ± 2.1	83.6 ± 1.3	

† Four inhibitors (doxorubicin, etoposide, daunorubicin, and podophyllotoxin) with large IC_{50} value differences; ‡ Four inhibitors (doxorubicin, daunorubicin, idarubicin, and amsacrine) with similar IC_{50} values; § Six inhibitors, including doxorubicin, etoposide, daunorubicin, idarubicin, amsacrine, and ellipticine. Comb, combination. * $P < 0.05$ compared with the combination of four inhibitors.

Supplementary Table 12. TOPO II assay treatment doses

Group	Low dose (μM)	Medium dose (μM)	High dose (μM)
Doxorubicin	1	10	50
Etoposide	50	100	200
Daunorubicin	1	10	50
Idarubicin	1	10	50
Amsacrine	10	50	
Ellipticine	1	10	
Drug combination	64	190	

TOPO, topoisomerase.

Supplementary Table 13. Gray values (GV) for TOPO II-mediated kDNA decatenation

Lane	Drug	Dose (μM)	GV	Inhibition rate
D	kDNA		971	
T	kDNA + TOPO II		9713	
1	kDNA + TOPO II + doxorubicin (low)	1	8832	9.1%
2	kDNA + TOPO II + doxorubicin (medium)	10	8198	15.6%
3	kDNA + TOPO II + doxorubicin (high)	50	1093	88.7%
4	kDNA + TOPO II + etoposide (low)	50	8940	8.0%
5	kDNA + TOPO II + etoposide (medium)	100	8105	16.6%
6	kDNA + TOPO II + daunorubicin (low)	1	9633	0.8%
7	kDNA + TOPO II + daunorubicin (medium)	10	8560	11.9%
8	kDNA + TOPO II + daunorubicin (high)	50	1249	87.1%
9	kDNA + TOPO II + idarubicin (low)	1	9023	7.1%
10	kDNA + TOPO II + idarubicin (medium)	10	8597	11.5%
11	kDNA + TOPO II + idarubicin (high)	50	1347	86.1%
12	kDNA + TOPO II + amsacrine (low)	10	8931	8.1%
13	kDNA + TOPO II + amsacrine (medium)	50	7819	19.5%
14	kDNA + TOPO II + ellipticine (low)	1	8589	11.6%
15	kDNA + TOPO II + ellipticine (medium)	10	7561	22.2%
16	kDNA + TOPO II + Comb (low)	64	7981	17.8%
17	kDNA + TOPO II + Comb (medium)	190	1531	84.2%

Comb, combination; TOPO, topoisomerase.

Supplementary Table 14. Gray values (GV) for TOPO II-mediated DNA uncoiling assay

Lane	Drug	Dose (μM)	GV	Inhibition rate
D	pBR322		651	
T	pBR322 + TOPO II		7510	
1	pBR322 + TOPO II + etoposide (medium)	100	6637	11.6%
2	pBR322 + TOPO II + Comb (medium)	190	814	89.2%
3	pBR322 + TOPO II + etoposide (high)	200	1373	81.7%

Comb, combination; TOPO, topoisomerase.

Supplementary Table 15. Comparison of experimental doses for each drug in 8-drug-Cdg used in the solid tumour mouse model with clinical doses*

Drug	Total dose† of each drug in 8-drug-Cdg (mg/kg, i.p.)	Total dose† of each drug in 8-drug-Cdg (mg/kg, i.v.)	Human equivalent dose (mg/kg, i.v.) §	Reported human dose per cycle (mg/kg, i.v.) ¶	Percentage of the dose in 8-drug-Cdg equivalent to the minimum human dose	Percentage of the dose in 8-drug-Cdg equivalent to the maximum human dose
Doxorubicin	2.50	2.50	0.193	0.68–2.03[87]; 0.81–1.08[61]; 0.81–3.24[84]; 1.08[17]; 1.08–2.03[83]; 1.35[18]; 1.62–2.43[90] **Dosage range#: 0.68—3.24**	28.4%	6.0%
Etoposide	16.0	13.6‡	1.049	2.70—13.51[87]; 4.32[17]; 4.73–13.51[90]; 8.11[125]; 10.14[86]; 12.97[126]; 13.51[127] **Dosage range#: 2.70–13.51**	38.9%	7.8%
Idarubicin	0.75	0.64‡	0.049	0.27–0.41[118]; 0.97[90,127]; 0.97–1.08[87,128]; 1.08[118,129] **Dosage range#: 0.27–1.08**	18.1%	4.5%
Amsacrine	3.75	3.19‡	0.246	4.05[130]; 8.11–17.03[131]; 10.14–16.89[87]; 13.51[132] **Dosage range#: 4.05–17.03**	6.1%	1.4%
Aclarubicin	4.05	3.44‡	0.265	1.08–2.65[133]; 1.62–8.11[86]; 2.16[134] **Dosage range#: 1.08–8.11**	24.5%	3.3%
Teniposide	7.40	6.29‡	0.485	1.35–17.84[87]; 2.70–12.16[90] **Dosage range#: 1.35–17.48**	35.9%	2.8%
Epirubicin	2.70	2.30‡	0.177	0.95–4.05[87]; 2.70–3.24[83,90] **Dosage range#: 0.95–4.05**	18.6%	4.4%
Pirarubicin	3.50	2.98‡	0.230	0.81–2.16[84]; 1.62[125] **Dosage range#: 0.81–2.16**	28.4%	10.6%
Average value**					24.9%	5.1%

* The clinical doses for each drug refer to commonly used clinical doses published in literature or on websites.

† The total dose represents the cumulative doses of five injections of the combination.

‡ According to the literature, to convert the intravenous injection (i.v.) dose to intraperitoneal injection (i.p.) dose in mice, the bioavailability of drugs for intraperitoneal injection is usually 80%–85% (the ratio to intravenous injection is 1.18–1.25)[135]; the bioavailability of 5 compounds, including docetaxel, for intraperitoneal injection was 69%, 18%, 94%, 102%, and 106%, with an average of 78% for the 5 compounds[136]; no differences in plasma AUC_{0-6h} between intraperitoneal and intravenous administration routes for doxorubicin[78,79]; therefore, we assumed that the remaining seven drugs (except doxorubicin) in the table had an i.p. bioavailability of 85% (a ratio of 1.18 to i.v.), and used the equation of the dose for i.v. (mg/kg) = the dose for i.p (mg/kg) ÷ 1.18, to convert the i.p. doses of the seven drugs to the i.v. doses of the seven drugs.

§ For estimating the human equivalent dose (HED, mg/kg, i.v.) from the total dose of each drug in the 8-drug-Cdg (mg/kg, i.v.), we used the following equation: HED (mg/kg) = Animal dose (mg/kg) × (Animal Km / Human K_m), (where Human K_m = 60 kg/1.62 m^2 = 37.04 kg/m^2; Animal (mouse) K_m = 0.02 kg/0.007 m^2 = 2.857 kg/m^2, in APPENDIX B of a FDA document[69] and other literature[70–72].

¶ We converted published clinical doses from surface area doses (mg/m^2, i.v.) to body weight doses (mg/kg, i.v.) based on the equation[69]: Human dose (mg/kg) = Human dose (mg/m^2)/ Human K_m, where Human K_m = 37.04 kg/m^2.

The dosage range is derived from the combined results of the doses reported in the literature above.

**The values of 24.9% and 5.1% were averaged to 15.0%.

Supplementary Table 16. Comparison of experimental doses for 8 Sdgs in the solid tumour mouse model with clinical doses*

Drug	Total dose† of each drug in 8 Sdgs (mg/kg, i.p.)	Total dose† of each drug in 8 Sdgs (mg/kg, i.v.)	Human equivalent dose (mg/kg, i.v.) §	Reported human dose per cycle (mg/kg, i.v.) ¶	Percentage of the dose in Sdg equivalent to the minimum human dose	Percentage of the dose in Sdg equivalent to the maximum human dose
Doxorubicin	12.50	12.50	0.964	0.68–2.03[87]; 0.81–1.08[61]; 0.81–3.24[84]; 1.08[17]; 1.08–2.03[83]; 1.35[18]; 1.62–2.43[90] **Dosage range#: 0.68—3.24**	141.8%20	29.8%
Etoposide	80.0	68.0‡	5.243	2.70—13.51[87]; 4.32[17]; 4.73–13.51[90]; 8.11[125]; 10.14[86]; 12.97[126]; 13.51[127] **Dosage range#: 2.70–13.51**	194.2%30	38.8%
Idarubicin	3.75	3.19‡	0.246	0.27–0.41[118]; 0.97[90,127]; 0.97–1.08[87,128]; 1.08[118,129] **Dosage range#: 0.27–1.08**	91.1%50	22.8%
Amsacrine	18.75	15.94‡	1.229	4.05[130]; 8.11–17.03[131]; 10.14–16.89[87]; 13.51[132] **Dosage range#: 4.05–17.03**	30.3%20	7.2%
Aclarubicin	20.25	17.21‡	1.327	1.08–2.65[133]; 1.62–8.11[86]; 2.16[134] **Dosage range#: 1.08–8.11**	122.9%30	16.4%
Teniposide	37.0	31.45‡	2.425	1.35–17.84[87]; 2.70–12.16[90] **Dosage range#: 1.35–17.48**	179.6%20	13.9%
Epirubicin	13.5	11.48‡	0.885	0.95–4.05[87]; 2.70–3.24[83,90] **Dosage range#: 0.95–4.05**	93.2%40	21.9%
Pirarubicin	17.5	14.88‡	1.147	0.81–2.16[84]; 1.62[125] **Dosage range#: 0.81–2.16**	141.7%10	53.1%
Average value**					124.4%	25.5%

Notes for *, ‡, ¶, and #, see Supplementary Table 15.

† The total dose represents the cumulative doses of five injections for each drug in the Sdg.

§ For estimating the human equivalent dose (HED, mg/kg, i.v.) from the total dose of each drug in the Sdg, see § in Supplementary Table 15.

**The values of 124.4% and 25.5% were averaged to 75.0%.

Supplementary Table 17. Combination index (CI) for U87 cell experiment for combinations of six TOPO II inhibitors

Group	Concentration (μM)						Measured Inhibition (%)	CI value
	Dox	Eto	Dau	Ida	Ams	Ell		
Comb 1 ($IC_{4.5}$)	0.02	0.08	0.04	0.01	0.12	0.19	28.3	0.92
Comb 2 (IC_{9})	0.04	0.23	0.06	0.01	0.27	0.35	36.4	0.97
Comb 3 (IC_{18})	0.07	0.71	0.10	0.03	0.66	0.67	47.5	1.01
Comb 4 (IC_{36})	0.15	2.79	0.17	0.07	1.9	1.48	63.2	1.01

Ams, amsacrine; Comb, combination; CI, combination index; Dau, daunorubicin; Dox, doxorubicin; Ell, ellipticine; Eto, etoposide; Ida, idarubicin; TOPO, topoisomerase.

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