

1 **5.3.9. Animal**

2 A total of twenty 5-week-old, male BALB/c nude mice (weight: 15-17g) were purchased from Henan
3 Skobes Biotechnology Co., Ltd. (Anyang, China; License No.: SCXK (Yu) 2020-0005). Animal use was
4 scientifically justified as the tumor-bearing mouse model is irreplaceable for evaluating systemic efficacy
5 and safety, which cannot be assessed *in vitro*. The sample size of $n=5$ per group was determined as the
6 statistically minimal requirement, in strict compliance with the 3R principles. Mice were housed under
7 specific pathogen-free (SPF) conditions in the animal center of the Henan University of Science and
8 Technology, with a 12 h light/dark cycle, controlled temperature (22 ± 2 °C) and humidity ($50 \pm 10\%$).
9 Standard irradiated rodent diet and autoclaved drinking water were provided. All mice were acclimatized
10 for one week prior to any experimental procedures. The study protocol was reviewed and approved by
11 the IRB of Henan University of Science and Technology Approval Letter (HAUST-024-M081001). All
12 procedures were conducted in strict accordance with the institution's ethical guidelines and the 3R
13 principles (Replacement, Reduction, and Refinement). At the time of the end-of-treatment the mice were
14 9 weeks old and had body weights of 18.5 ± 1.3 g, 17.6 ± 1.2 g, 17.2 ± 1.2 g and 17.6 ± 0.6 g,
15 respectively (mean \pm SEM). All mice were sacrificed by cervical dislocation under deep anesthesia
16 induced by an overdose of pentobarbital sodium.

17 **5.3.10. *In vivo* tumour killing experiment**

18 Mice were housed at the SPF animal center of the School of Basic Medicine and Forensic Science,
19 Henan University of Science and Technology, with solid food and drinking water provided, and a 12 h
20 light–dark cycle each day. After one week of acclimatization, all mice were subcutaneously injected with
21 5×10^6 HepG2 cells at the lower flank. When the tumour size reached about 100 mm³, mice were
22 randomly divided into the blank group, 3c treatment group, 3d treatment group and erlotinib treatment
23 group ($n = 5$). Mice in the 3c, 3d or erlotinib treatment group received intraperitoneal injection of
24 compounds solution (diluted in normal saline solution containing 2% DMSO/40% PEG 300/5% tween
25 80) at 30 mg/kg. The control group was injected with 10 μ L/g of solvent. The day of treatment was set
26 as day 0, and tumour size was recorded at day 4, 6, 8, 10. On day 10, all mice were sacrificed and
27 tumours were dissected. Tumours were sliced and subjected to HE staining. The major organs (heart,
28 liver, spleen, lung and kidney) were sectioned and subjected to HE staining.

29 **5.3.11. Statistical Analyses**

30 Data were presented as mean \pm SD and analysed using Graph Prim 10.0. Data were analysed using
31 one-way analysis of variance (ANOVA). Data were obtained from no fewer than three independent
32 experiments. $p < 0.05$ was considered statistically significant.

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