Supporting Information

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SI Text

Methods for Correlation of Potencies as Inducers of NQO1 and Suppressors of iNOS Up-Regulation by LPS. Highly quantitative comparisons of potencies of compounds for phase 2 induction and antiinflammation were clearly required for understanding how these processes were mechanistically related. We therefore considered the most informative ways of expressing and comparing potencies of compounds for induction (activation) of the NQO1 specific activity and for suppression (inhibition) of the inflammatory LPS-stimulated up-regulation of iNOS activity. Among the innumerable methods for analyzing dose-response relations, the Median Effect Equation of Chou (1) is one of the most widely useful. It is based on mass action principles, and was originally devised for evaluating the effects of multiple inhibitors to determine whether their behavior was additive, synergistic, or antagonistic. The Median Effect Equation, however, is also useful for quantifying the effects of single inhibitors. The equation: $f_a/f_u = [D/D_m]^m$, where f_a is the fraction of a process that is affected and f_u is the fraction unaffected (i.e., $1 - f_a$), D is the dose of compound required to produce the effect f_a, and D_m is the concentration at which a 50% effect is obtained (i.e., $f_a = f_u$). The analysis involves plotting log (f_a/f_u) with respect to log D, and determining D_m, the slope m of the plots, and the goodness of fit to linearity (r^2). These operations may be performed with the CompuSyn program (2). The advantage of this analysis is that it utilizes a linear transformation of all experimental observations in the determination of D_m, in contrast to conventional methods of expressing inhibitory potencies (IC_{50}, LD_{50}) which often rely on interpolation between adjacent concentration values near the midpoint of effectiveness.

By applying the Median Effect Equation to the relation of fractional inhibition to inhibitor concentration, determination of D_m for inhibition of iNOS is straightforward. To our knowledge, such derived D_m values have been used in the past only for quantifying inhibitory processes. Application of the Median Effect Equation to the induction of NQO1 activity required additional considerations. In the NQO1 assay, values for specific enzyme activity are obtained in the absence of inducers (Control or C values) and in the presence of a series of concentrations (usually serial dilutions) of inducers (Treated or T values). Moreover these values are normalized for protein concentration to compensate for cytotoxicity (3). We reasoned that if the f_a value at any D concentration is represented by $(T - C)/T$, the f_u (i.e., $1 - f_a$) value is therefore C/T. Hence, the ratio of f_a/f_u is $(T - C)/C$, and $\log(T - C)/C$ is plotted against log D to obtain the median effect plot. In this way a D_m value for induction for any compound may be derived. The correlation analysis of concentration-dependence between inhibition of iNOS and induction of NQO1 was performed by plotting the logarithm of D_m values for iNOS inhibition against the logarithm of corresponding D_m values for NQO1 induction by the test compounds, and determining the goodness of fit to linearity (r^2).

As an example, Fig. S1 shows the dose-response curves (upper plots) and Median Effect graphs (lower plots) for both iNOS inhibition and NQO1 induction for three triterpenoids. Over the concentration ranges examined, the inhibitory effects on NO production and induction of NQO1 activity increased with the concentrations of triterpenoids. The median effect plots of the above data (Fig. S1*C* and Fig. S1*D*) are families of linear graphs. The D_m values, the slope (m) of the curves, and the goodness of fit (r^2) to linearity are provided by a computer program, and are recorded in the figure legend.

1. Chou T-C (2006) Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacol Rev* 58:621–681.
2. Chou T-C, Martin N (2005) CompuSyn for Drug Combinations. A Computer Software for Quantitation of Synergism and Antagonism, and the Determination of IC50, ED50 and LD50 Values. [PC software and user's guide.] (ComboSyn, Paramus, NJ).
3. Fahey JW, Dinkova-Kostova AT, Stephenson KK, Talalay P (2004) The "Prochaska" microtiter plate bioassay for inducers of NQO1. *Methods Enzymol* 382:243–258.

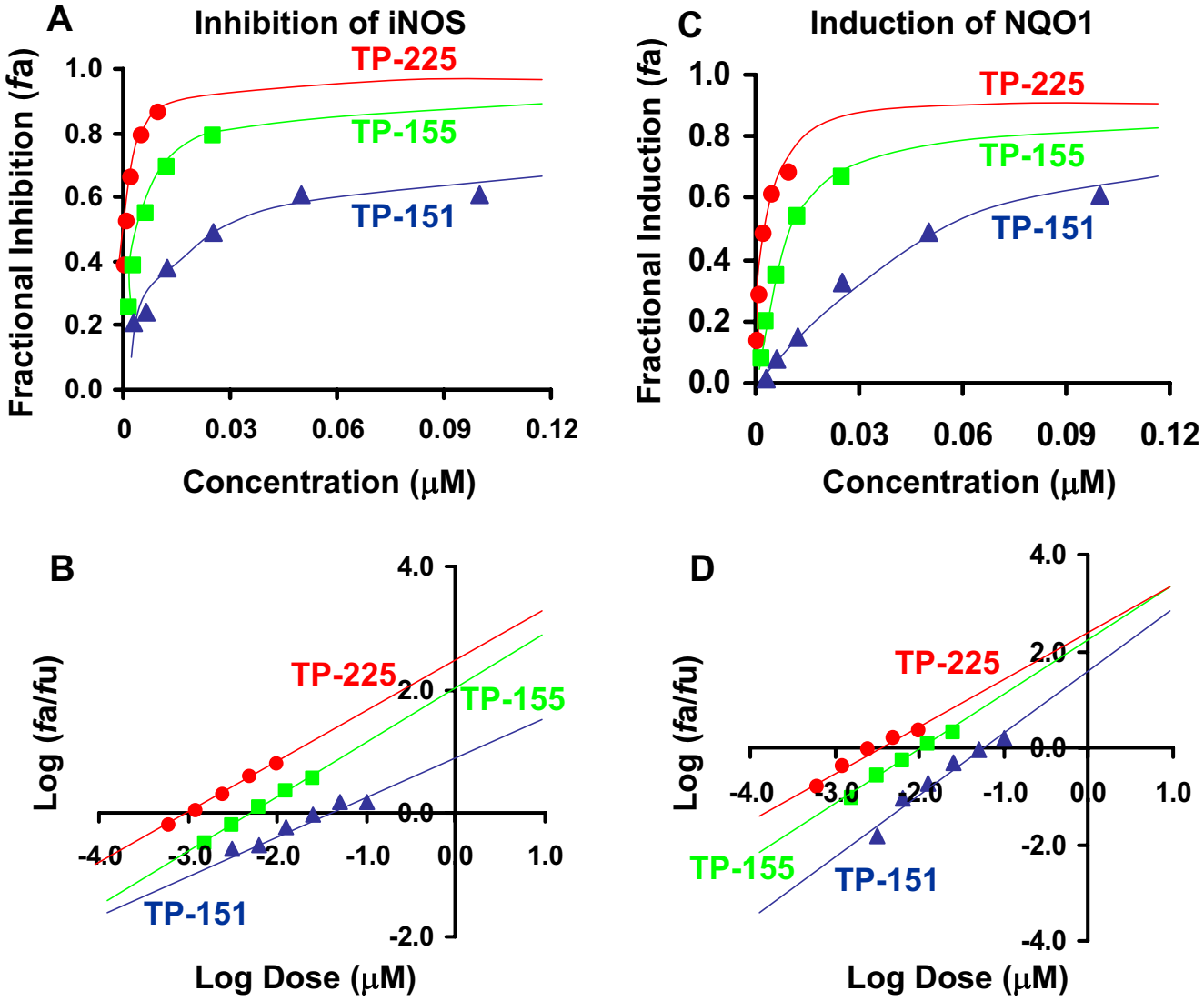

Fig. S1. Data analysis by Median Effect principle of iNOS inhibition and NQO1 induction. Plots for three triterpenoids (TP-225, TP-155, and TP-151. Structures are shown in Fig. 4. (*A*) Fractional inhibition of iNOS up-regulation (f_a) by LPS in RAW264.7 cells as a function of triterpenoid concentration. (*B*) Median Effect plot of iNOS inhibition by triterpenoids (TP-225: D_m = 0.0011 μM, m = 0.84, r^2 = 1.0: TP-155: D_m = 0.0,052 μM, m = 0.88, r^2 = 1.0; TP-151: D_m 0.033 μM, m = 0.58, r^2 = 0.98). (*C*) Fractional induction of NQO1 (effect, f_a) as a function of triterpenoid concentration. (*D*) Median Effect plot of NQO1 induction by triterpenoids (TP-225: D_m = 0.0035 μM, m = 0.95, r^2 = 0.98; TP-155: D_m = 0.012 μM, m = 1.1, r^2 = 0.99; TP-151: D_m = 0.054 μM, m = 1.3, r^2 = 0.98).