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Supplementary Materials for

Design and tuning of ionic liquid–based HNO donor through intramolecular hydrogen bond for efficient inhibition of tumor growth

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NMR characterization

[Ch][Tau]: ^{1}H NMR (400 MHz, DMSO): 3.83 (ddd, 2H), 3.44 – 3.38 (m, 2H), 3.12 (s, 9H), 2.77 (t, 2H), 2.51 (dd, 2H).^{13}C NMR (101 MHz, DMSO): 67.09 – 66.76, 55.15, 54.56, 53.32 – 52.95, 38.40 ppm.

[EMIm][APA]: ^{1}H NMR (400 MHz, DMSO): 9.83 (s, 1H), 7.84 (t, 1H), 7.75 (t, 1H), 4.21 (q, 2H), 3.87 (s, 3H), 2.56 (t, 2H), 1.90 (dd, 2H), 1.40 (t, 3H). ^{13}C NMR (101 MHz, DMSO): 174.55, 137.18, 123.46, 121.88, 43.93, 42.14, 35.50, 15.16 ppm.

[Ch][APA]: ^{1}H NMR (400 MHz, DMSO): 3.81 (d, 2H), 3.49 – 3.36 (m, 2H), 3.13 (s, 9H), 2.58 (t, 2H), 1.97 (t, 2H).^{13}C NMR (101 MHz, DMSO): 175.99, 72.35, 67.31, 64.51, 64.28, 59.89, 55.05, 53.42 – 52.93, 41.74, 40.04, 39.81, 39.49, 39.14, 39.00 ppm.

[APMim][BF_4]: ^{1}H NMR (400 MHz, DMSO): 8.94 (d, 1H), 7.66 (t, 1H), 7.60 (t, 1H), 4.27 – 4.15 (m, 2H), 3.83 (s, 3H), 3.38 (s, 2H), 2.59 – 2.51 (m, 2H), 1.94 – 1.81 (m, 2H).^{13}C NMR (101 MHz, DMSO): 136.77, 123.75, 122.45, 46.77, 37.75, 35.86, 32.30.

[EMIm][Tau]: ^{1}H NMR (400 MHz, DMSO): 9.20 (s, 1H), 7.81 (d, 1H), 7.72 (d, 1H), 4.20 (q, 2H), 3.86 (s, 3H), 2.76 (t, 2H), 2.50 (t, 2H), 1.41 (t, 3H). ^{13}C NMR (101 MHz, DMSO): 136.32, 123.54, 121.95, 54.92, 44.06, 38.52, 35.63, 15.10 ppm.

[HEMN][Tau]: ^{1}H NMR (400 MHz, DMSO): 3.84 (t, 4H), 3.54 –3.45 (m, 4H), 3.13 (s, 6H), 2.76 (t, 2H), 2.51 (t, 2H). ^{13}C NMR (101 MHz, DMSO): 65.74, 54.93, 54.73, 51.56, 51.52, 51.48, 38.40 ppm.

[P_{66614}][Tau]: ^{1}H NMR (400 MHz, DMSO): 2.75 (t, 2H), 2.48 (t, 2H), 2.19 (m, 12H), 1.70 –1.08 (m, 48H), 1.02 –0.62 (m, 8H). ^{13}C NMR (101 MHz, DMSO): 54.93, 38.58,

31.28, 30.82, 30.39, 30.21, 30.02, 29.81, 29.66, 29.03, 29.00, 28.95, 28.70, 28.63, 28.08, 22.07, 21.90, 21.79, 21.19, 20.54, 20.49, 17.68, 17.58, 17.21, 17.11, 13.89, 13.81 ppm.

[EMIm][AMS]: ^{1}H NMR (400 MHz, DMSO): 9.20 (s, 1H), 7.79 (t, 1H), 7.71 (t, 1H), 4.19 (q, 2H), 3.85 (s, 3H), 3.12 (s, 1H), 2.54 (t, 1H), 2.45 – 2.39 (m, 1H), 1.61 (ddt, 1H), 1.41 (t, 3H).^{13}C NMR (101 MHz, DMSO): 136.41, 123.59, 121.99, 60.54, 49.39, 44.12, 41.11, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 35.69, 29.40, 15.16 ppm.

[EMIm][APS]: ^{1}H NMR (400 MHz, DMSO): 9.22 (s, 1H), 7.81 (t, 1H), 7.72 (t, 1H), 4.20 (q, 2H), 3.86 (s, 3H), 2.57 –2.48 (m, 2H), 2.41 (dt, 2H), 1.69 –1.55 (m, 2H), 1.41 (t, 3H). ^{13}C NMR (101 MHz, DMSO): 136.36, 123.54, 121.94, 49.36, 44.05, 41.13, 35.63, 29.53, 15.10 ppm.

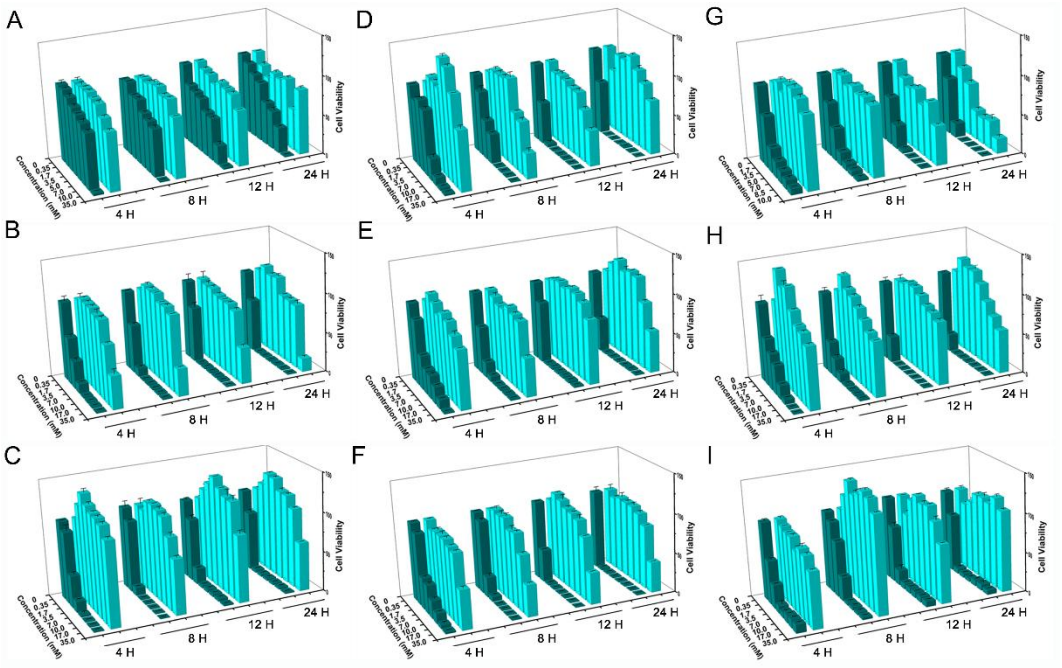

Fig. S1. *In vitro* cytotoxicity of ILs and IL-NONOates. ILs (Dark cyan) and IL-NONOates (cyan). (**A**, **D** and **G)** [Ch][Tau] and [Ch][Tau]-NONOate. (**B**, **E** and **H)** [EMIm][APA] and [EMIm][APA]-NONOate. (**C**, **F** and **I)** [Ch][APA] and [Ch][APA]-NONOate. (**A - C**) CT-26. (**D - F**) MCF-7 and (**G - I**) HEK-293 cells. The cytotoxicity was evaluated after 4, 8, 12 or 24 hour of incubation with the sample by MTT assay (n = 5).

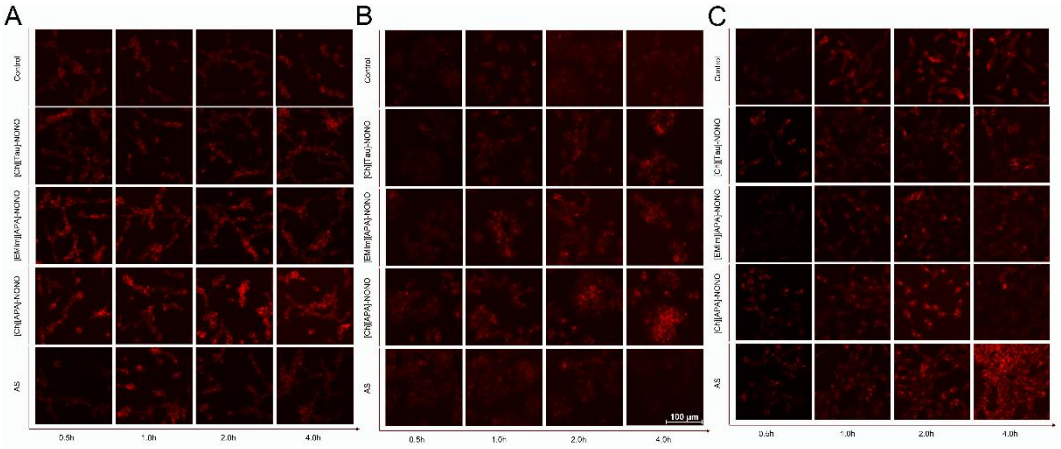

Fig. S2. Temporal changes in RNS production in cells treated with IL-NONOates or Angeli's salt. Angeli's salt (AS). (**A)** CT-26 (**B)** MCF-7 and (**C)** HEK-293 cells.

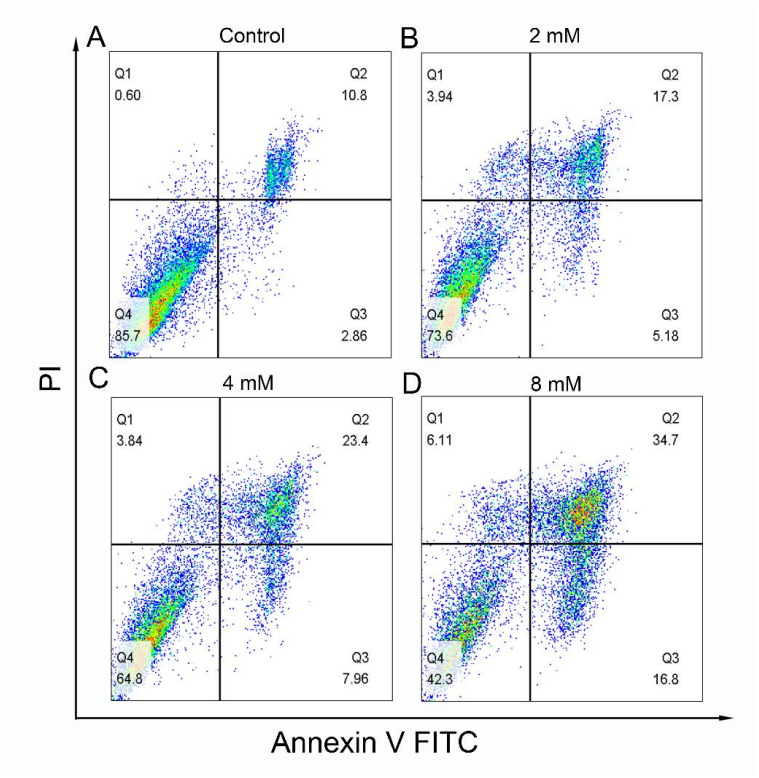

Fig. S3. Representative flow-cytometric analysis of cell apoptosis. Flow-cytometric analysis of Annexin-V/PI staining of CT-26 cells after different concentrations of [Ch][Tau]-NONOate.

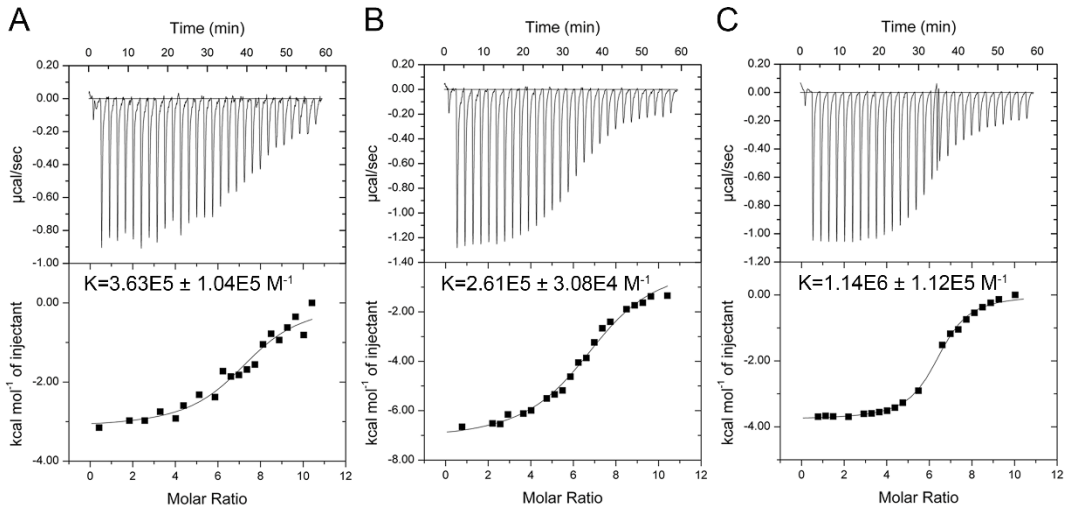

Fig. S4. ITC curves obtained by titrating ILs into cardiolipin. (**A**) [Ch][Tau], (**B**) [EMIm][APA] and (**C**) [Ch][APA].

Fig. S5. Representative images of *in vivo* tumor growth. The yellow dash line denotes the tumor position. Photo Credit: Xiaoyu Lv, Zhejiang University.

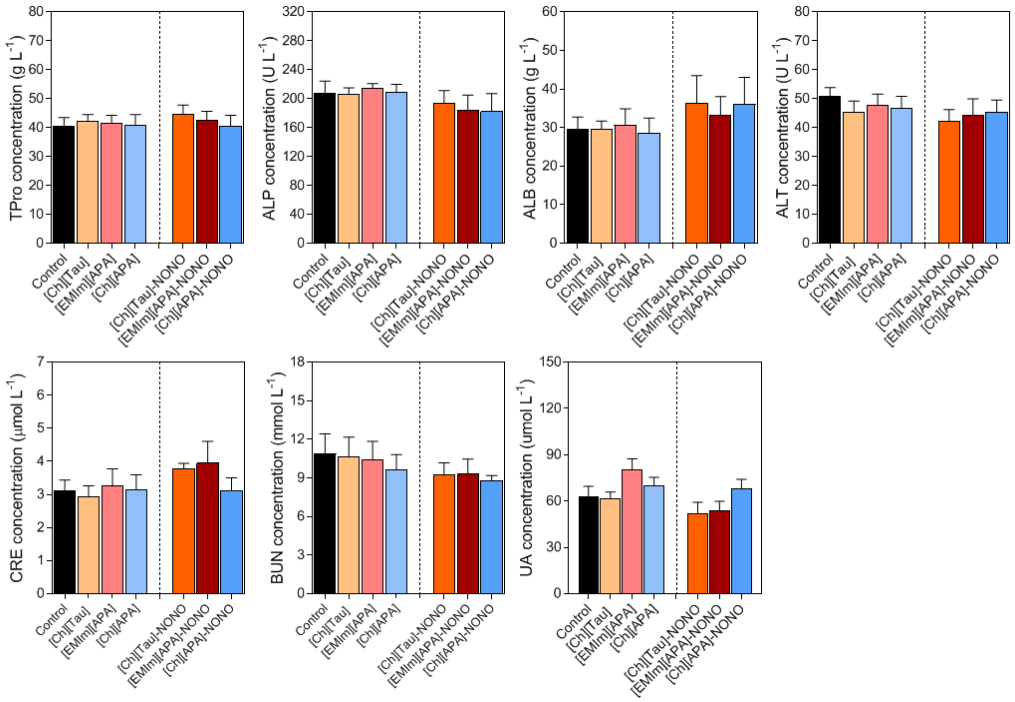

Fig. S6. The biosafety evaluation of ILs and IL-NONOates. Blood biochemical analyses including liver functions and kidney functions. TPro: total protein, ALP: alkaline phosphatase, ALB: albumin, ALT: alanine aminotransferase, CRE: creatinine, BUN: blood urea nitrogen, and UA: uric acid (n = 4). The data were expressed as mean ± s.d.

Table S1. HNO release half-lives of IL-NONOates under phosphate buffer (PBS), fetal bovine serum (FBS) and mouse serum (Serum) conditions (37 ^{o}C, pH = 7.4).

	[Ch][Tau]-NONO	[EMIm][APA]-NONO	[Ch][APA]-NONO
PBS Solutions		$t_{1/2}$ (min)	
PBS	289	996	1061
10% FBS	239	868	892
10% Serum	187	765	990
20% Serum	117	510	990

Table S2. The thermodynamic parameters of ITC curves. [Ch][Tau], [Ch][APA] or [EMIm][APA] was titrated into cardiolipin at 25 ^{o}C.

Sample	Ka ($\times 10^5$ M^{-1})	ΔH (kcal mol^{-1})	TΔS (kcal mol^{-1})
[Ch][Tau]	3.63 ± 1.04	-3.16 ± 0.13	0.37
[EMIm][APA]	2.61 ± 30.8	-7.24 ± 0.14	0.01
[Ch][APA]	11.4 ± 1.12	-3.78 ± 0.03	0.375

Table S3. Summary and comparison of HNO release properties at physiological conditions (37 ^{o}C, pH = 7.4).

Materials	$t_{1/2}$ (min) [a]	Ref.
[APMim][BF_4]	4.2	-
[P_{66614}][Tau]	325	-
[DBU][Tau]	243	-
[EMIm][Tau]	211	-
[EMIm][AMS]	324	-
[EMIm][APS]	959	-
[EMIm][APA]	996	-
[Ch][APA]	1061	-
Angeli's salt	2.5	(25, 27)
Piloty's acid	HNO only generated at > physiological pH	(39)
IsopropylamineNONOate (IPA/NO)	2.3	(26)
Cyanamide (H_2NCN)	-	(25)
Acyloxynitroso	-	(40)

[a] HNO release half-life.

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