Supporting Information

Stabilizing photo-sensitive Colchicine through rebalancing electron distribution of the reactive tropolone ring †

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Experimental Section

Materials

The tropone compounds, acids and all analytical-grade solvents in the experiment were purchased from Sinopharm Chemical Reagent Co., Ltd, with purity higher than 98%, and used without further purification.

Salt design and preparation

High quality salts for single-crystal X-ray diffraction (XRD) were crystallized through solvent evaporation. The bulk of salts were enlarged by solvent evaporation or solvent assisted grinding. According to the handbook of pharmaceutical salts,[1] sulfonic acids are not grouped into the GRAS, but considered as ADI (Acceptable Daily Intake) and grouped into the second class salt-formers, which show low toxicity and good tolerability. Considering the genotoxicity of sulfonic acid esters with alcoholic solvents like methanol, ethanol or propanol,[2] alcoholic solvents were avoided to be used during the preparation of Col salts.

Preparation of Col-PTA Salt (1:1): An equal-molar mixture of Col (0.1 mmol) and PTA (0.1 mmol) were dissolved in 0.5 mL tetrahydrofuran (THF), and bulk yellow powder precipitated immediately. The powder was filtered and supernatant was evaporated at 25 ℃ to obtain needle-like single crystals.

Preparation of Col-NSA Salt (1:1): Salt was synthesized by adding equal-molar (1 mmol) Col and NSA into 5 mL ethyl acetate (EA), and then yellow powder precipitated. High-quality single crystals were obtained by vapor diffusion (40 mg powder was dissolved in 0.5 mL Methyl ethyl ketone (good solvent), and 3mL EA acts as poor solvent) at 25 ℃.

Preparation of Col-BSA Salt (1:1): An equal-molar of Col and BSA (1 mmol) were dissolved in 3 mL acetone at ambient environment near saturation, and the resulting solution was left to evaporate at 50 °C. Bulk yellow block crystals were obtained within several hours. Single crystal for X-ray diffraction was isolated from this mixture.

Preparation of Tr-PTA Salt (1:1): Tr (0.5 mmol) and PTA (0.5 mmol) were dissolved in 5 mL methanol. The

solution was left to evaporate at ambient environment. Crystals suitable for single-crystal X-ray analysis were obtained within one day.

Preparation of Tr-NSA Salt monohydrate (1:1:1): Tr (0.5 mmol) and NSA (0.5 mmol) were dissolved in 5 mL ethyl acetate. The solution was left to evaporate at ambient environment. Crystals suitable for single-crystal X-ray analysis were obtained within three days.

Preparation of Tr-DNSA Salt monohydrate (2:1:2): Tr (0.5 mmol) and DNSA (0.25 mmol) were dissolved in 5 mL methyl ethyl ketone and 4 mL methanol. And then the solution was left to evaporate at ambient environment. Crystals suitable for single-crystal X-ray analysis were obtained from the solution.

Preparation of Tro-PTA Salt (1:1): Tro (0.5 mmol) and PTA (0.5 mmol) were dissolved in 5 mL ethyl acetate with warming. The solution was then placed into 4 ℃ to cool down. Crystals for single-crystal X-ray analysis were obtained within several hours.

Preparation of Tro-NSA Salt trihydrate (1:1:3): Tro (0.5 mmol) and NSA (0.5 mmol) were dissolved in 5 mL methanol. The solution was left to evaporate at ambient environment. Crystals suitable for single-crystal X-ray analysis were obtained within several days.

Preparation of Tro-DNSA Salt (2:1): Tro (0.5 mmol) and DNSA (0.25 mmol) were dissolved in 5 mL methyl ethyl ketone and 4 mL methanol with warming. The solution was then placed into 4 ℃ to cool down. Crystals for single-crystal X-ray analysis were obtained within several hours.

Preparation of Hin-PTA Salt (1:1): Hin (0.5 mmol) and PTA (0.5 mmol) were dissolved in 5 mL ethyl acetate with warming. The solution was then placed into 4 ℃ to cool down. Crystals for single-crystal X-ray analysis were obtained within several hours.

Preparation of Hin-NSA Salt monohydrate (1:1:1): Hin (0.5 mmol) and NSA (0.5 mmol) were dissolved in 5 mL methyl ethyl ketone with warming. The solution was then placed into 4 ℃ to cool down. Crystals for single-crystal X-ray analysis were obtained within several hours.

Preparation of Hin-DNSA Salt (2:1): Hin (0.5 mmol) and DNSA (0.25 mmol) were dissolved in 5 mL methyl ethyl ketone and 4 mL methanol. And then the solution was placed into 4 ℃ to cool down. Crystals suitable for single-crystal X-ray analysis were obtained from the solution.

X-ray Powder Diffraction (XRPD)

XRPD patterns were obtained using a Bruker D8 Advance X-ray diffractometer with Cu Kα radiation. Voltage and current of the generator were set to 40 kV and 40 mA, respectively. Data in the range 2θ = 3–40° were collected with a scan rate of 0.1 s/step at ambient temperature. Data were imaged and integrated with RINT Rapid and peaks

were analyzed with Jade 6.0 software.

Thermogravimetric analysis (TGA).

Thermogravimetric analysis was carried out on TA TGA55 Discovery equipment. Samples were placed in open aluminum oxide pans and heated at 10 °C min^{-1} to 400 °C. Nitrogen was used as purge gas at 20 mL min^{-1}.

Differential scanning calorimetry (DSC).

DSC experiments were performed on a DSC TA Q2000 instrument under a nitrogen gas flow of 50 mL·min^{-1} purge. Ground samples weighing 1-3 mg were heated in sealed aluminum pans at a heating rate 10 °C·min^{-1}. Two-point calibration using indium and tin was carried out to check the temperature axis and heat flow of the equipment.

Single-Crystal X-ray Diffraction (SCXRD)

X-ray diffractions of all single crystals were carried out on a Bruker Apex II CCD or a Bruker D8 VENTURE diffractometer using Mo-Kα radiation (λ = 0.71073 Å). Integration and scaling of intensity data was performed using the SAINT program. Data were corrected for the effects of absorption using SADABS. The structures were solved by direct method and refined with full-matrix least-squares technique using SHELX-2014 software. Non-hydrogen atoms were refined with anisotropic displacement parameters, and hydrogen atoms were placed in calculated positions and refined with a riding model.

Fourier-transform Infrared (FTIR)

Fourier-transform Infrared (FTIR) spectra were collected by Nicolet-Magna FT-IR 750 spectrometer in the range of 4000 to 350 cm^{-1} with a resolution of 4 cm^{-1} at ambient conditions.

Electrocyclic Reaction Study

The samples about 20 mg were assayed between two glasses conducted in a stability chamber (SHH-GD China), which were carried out at 25 ℃ with an illumination of 5500 lx. Col samples were taken out at an interval of 2 days (2, 4, 6, 8 and 10 days). Other tropone derivatives were taken out at 5 and 10 days.

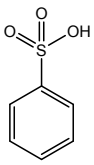
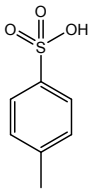
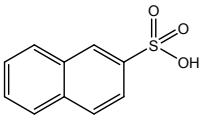
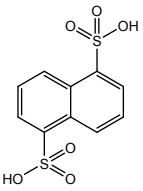
HPLC

The remained assays of Colchicine were analyzed by Agilent 1260 series Infinite HPLC instrument equipped with a ZORBAX ECLIPSE C18 column (4.6 ⌀ 150 mm, 5μm). An injection volume of 5 μL was used with 1 mL/min flow rate. The detection UV-vis wavelength was set at 244 nm. The samples were gradient-eluted with a mobile phase containing a mixture of methanol and water. The gradient started at 40% methanol and 60% water. After 2 min, it was changed to 60% methanol and 40% water in the following 10 min. Then the mobile phase was changed to 40% methanol and 60% water within 0.1 min and maintained for 2 min prepared for the next sample analysis. The observed retention time point for Col is 7 min.

NBO analysis

The Gaussian 09, Gaussian view 5.0[3] and NBO programs[4-9] were performed to study the NBO analysis using DFT-B3LYP method at 6-311G(d,p) basis set.

Table S1. Structures and pKa of acids

acid	Benzenesulfonic acid (BSA)	p-Toluenesulfonic acid (PTA)	2-Naphthalenesulfonic acid (NSA)	1,5-Naphthalenedisulfonic aicd (DNSA)
Structure				
pKa (in water)	**0.7**	**-0.43**	**0.27**	**-0.6**

XRPD analysis

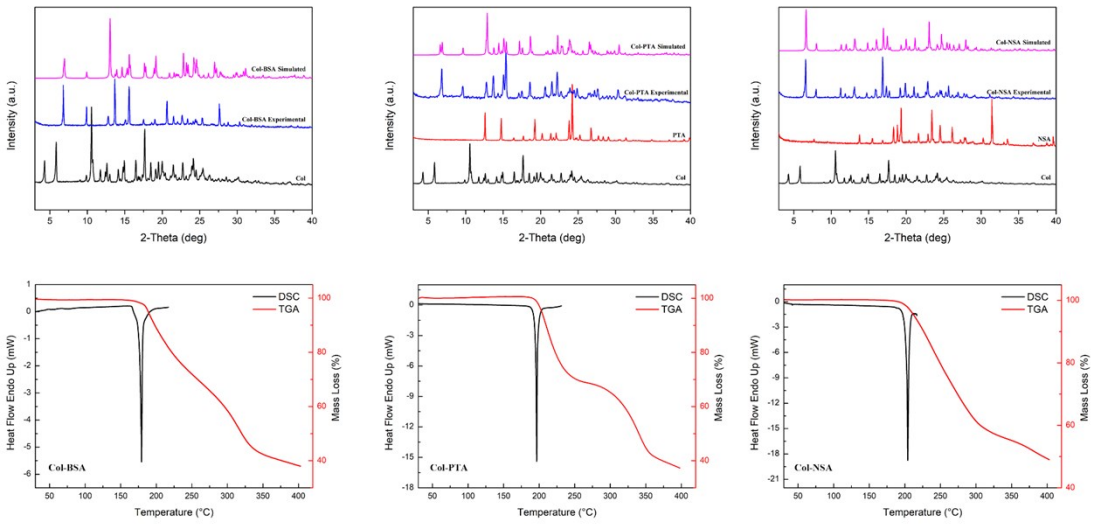


Figure S1. XRPD, DSC and TGA of Col-BSA, Col-PTA, and Col-NSA salts

Single-crystal analysis

The crystallographic data were summarized in Table S2. Although many attempts have been made, the crystalline Col-DNSA salt was not obtained. From the data, we can know that Col-BSA and Col-PTA are isomorphous salts, however, Col-NSA is quite different.

Table S2. The crystallographic data of Col salts

	Col hydrate	Col-BSA	Col-PTA	Col-NSA
Formula	$C_{44}H_{56}N_2O_{15}$	$C_{28}H_{31}NO_9S$	$C_{29}H_{33}NO_9S$	$C_{32}H_{33}NO_9S$

Crystal system	Monoclinic	Monoclinic	Monoclinic	Orthorhombic
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_12_12_1$
Temperature (K)	205	100	220	220
a (Å)	13.829 (4)	12.7125 (6)	12.858 (3)	7.825 (2)
b (Å)	10.484 (4)	8.0135 (3)	8.1256 (19)	16.685 (5)
c (Å)	15.998 (5)	12.9098 (6)	13.356 (3)	21.993 (6)
α (°)	90	90	90	90
β (°)	110.885 (6)	91.920 (2)	91.313 (7)	90
γ (°)	90	90	90	90
Volume (Å^3)	2167.1(12)	1314.40 (10)	1395.1 (6)	2871.4 (14)
ρ_{calc}g/cm^3	1.307	1.409	1.361	1.406
Z	2	2	2	4
GooF	0.958	1.080	0.936	1.015
R_{int}	0.924	0.0558	0.0680	0.1153
R_1	0.0760	0.0406	0.0542	0.0524
wR_2	0.2126	0.1000	0.1199	0.1160
CCDC number	2036740	2027384	2027385	2027386

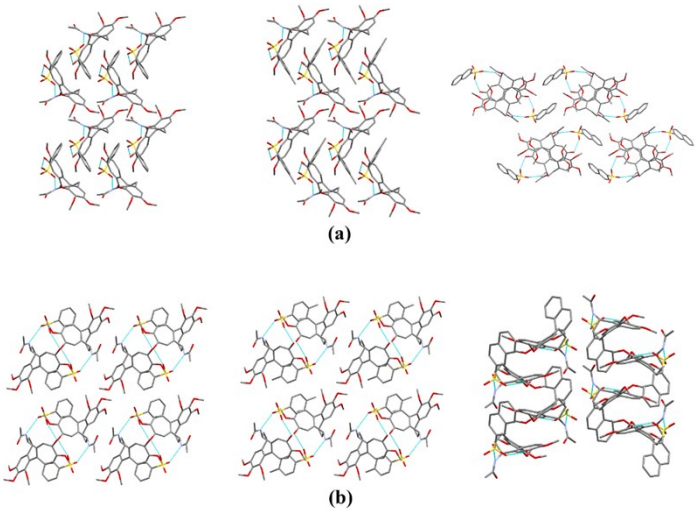


Figure S2 (a) Packing pattern of Col-BSA, Col-PTA, Col-NSA along the a axis (from left to right) (b) Packing pattern of Col-BSA, Col-PTA, Col-NSA along the b axis (from left to right)

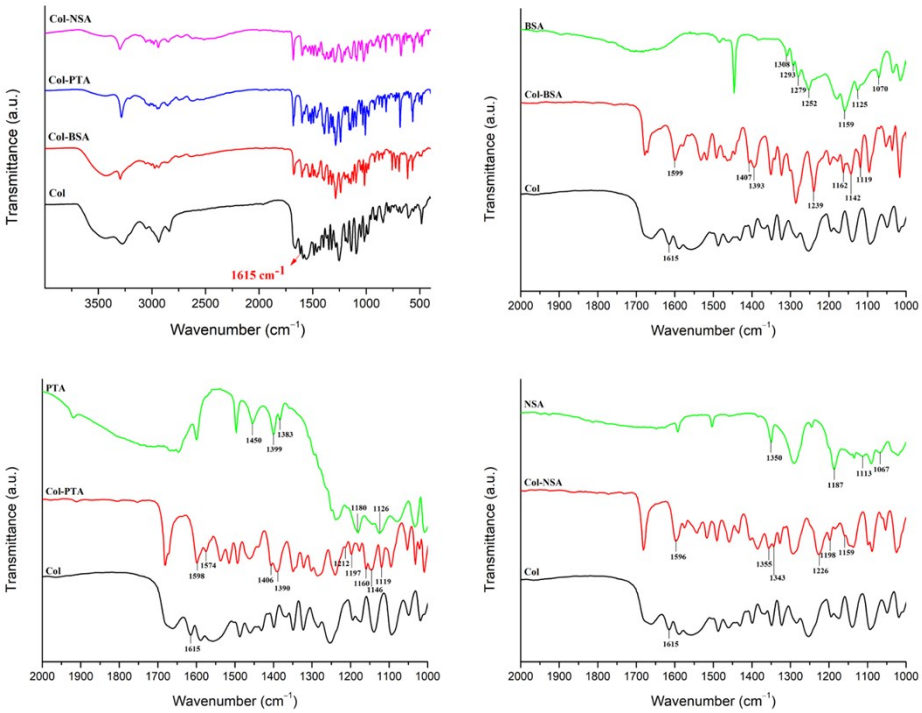


Figure S3. IR of Col salts

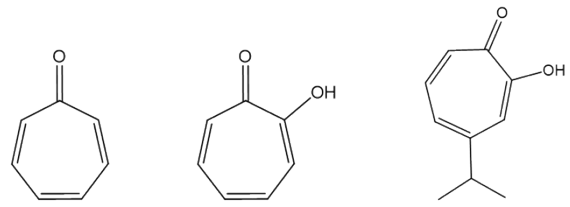


Figure S4. Chemical structures of tropones

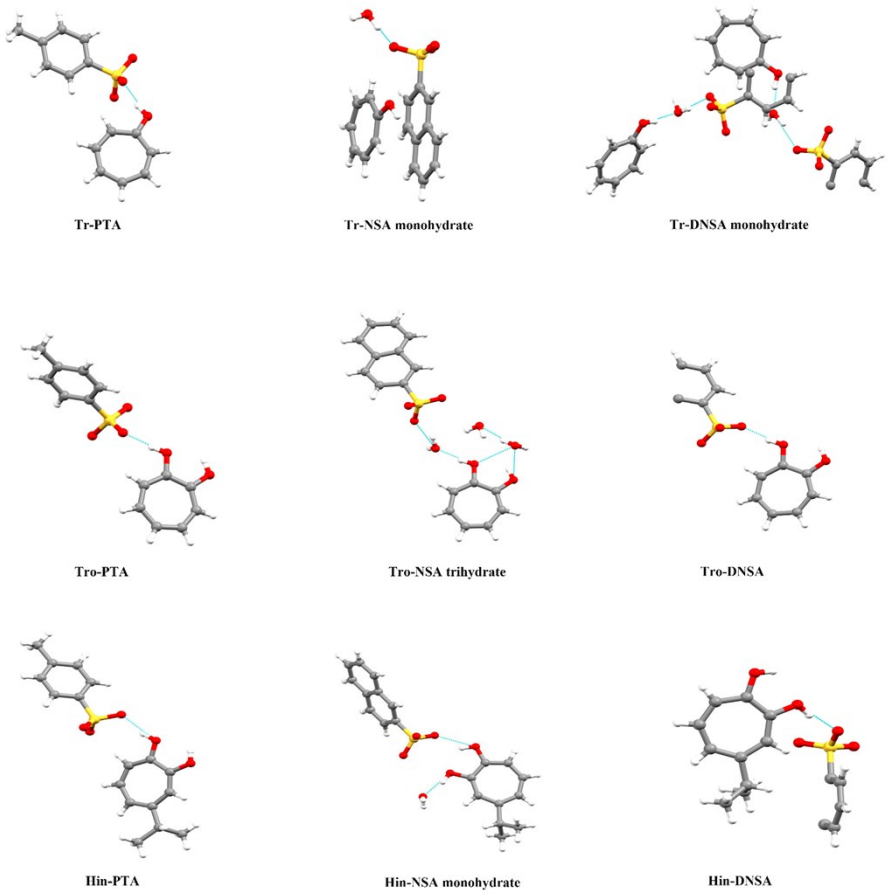


Figure S5. The asymmetric unit of tropone salts

SCXRD of Tr, Tro and Hin salts

Table S3. The crystallographic data of Tr salts

	Tr-PTA	Tr-NSA monohydrate	Tr-DNSA monohydrate
Formula	$C_{14}H_{14}O_4S$	$C_{17}H_{16}O_5S$	$C_{24}H_{24}O_{10}S_2$
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/n$	$P2_1/c$
Temperature (K)	170	170	170
a (Å)	9.5417 (4)	6.7177 (11)	15.4803 (5)
b (Å)	10.8327 (3)	16.233 (3)	12.7831 (4)
c (Å)	12.7884 (5)	14.530 (3)	12.1312 (5)
α (°)	90	90	90
β (°)	101.427 (1)	98.666 (5)	91.739 (1)
γ (°)	90	90	90

Volume (Å^3)	1295.64 (8)	1566.4 (5)	2399.49 (15)
ρ_{calc}g/cm^3	1.427	1.409	1.485
Z	4	4	4
GooF	1.047	1.046	1.027
R_{int}	0.0338	0.0805	0.0460
R_1	0.0371	0.0688	0.0448
wR_2	0.0982	0.1489	0.1096
CCDC number	2027398	2027392	2027394

Table S4. The crystallographic data of Tro salts

	Tro-PTA	Tro-NSA trihydrate	Tro-DNSA
Formula	$C_{14}H_{14}O_5S$	$C_{17}H_{20}O_8S$	$C_{24}H_{20}O_{10}S_2$
Crystal system	Orthorhombic	Monoclinic	Triclinic
Space group	*P*na2$_1$	*P*2$_1$/c	*P*-1
Temperature (K)	173	170	150
a (Å)	6.9880 (2)	6.7888 (4)	7.0583 (2)
b (Å)	30.2686 (8)	20.0960 (12)	8.1581 (3)
c (Å)	6.2510 (2)	13.5491 (8)	10.0680 (3)
α (°)	90	90	102.650 (1)
β (°)	90	102.749 (2)	104.172 (1)
γ (°)	90	90	90.598 (1)
Volume (Å^3)	1322.19 (7)	1802.90 (19)	547.19 (3)
ρ_{calc}g/cm^3	1.479	1.416	1.616
Z	4	4	1
GooF	1.041	1.159	1.081
R_{int}	0.0718	0.0638	0.0194
R_1	0.0413	0.0720	0.0330
wR_2	0.0975	0.1493	0.0815
CCDC number	2027396	2027397	2027393

Table S5. The crystallographic data of Hin salts

	Hin-PTA	Hin-NSA monohydrate	Hin-DNSA
Formula	$C_{17}H_{20}O_5S$	$C_{20}H_{22}O_6S$	$C_{30}H_{32}O_{10}S_2$
Crystal system	Monoclinic	Monoclinic	Triclinic
Space group	$P2_1/c$	Cc	P-1
Temperature (K)	150	180	150
a (Å)	6.6868 (2)	9.2402 (4)	6.8451 (3)
b (Å)	31.6978 (11)	31.6946 (17)	9.0199 (4)
c (Å)	7.7242 (3)	6.4645 (3)	12.2623 (6)
α (°)	90	90	81.489 (2)
β (°)	93.655 (1)	101.052 (2)	78.810 (2)
γ (°)	90	90	70.464 (1)
Volume (Å^3)	1633.87 (10)	1858.11 (15)	697.12 (6)
ρ_{calc}g/cm^3	1.367	1.396	1.469
Z	4	4	1
GooF	1.059	1.066	1.034
R_{int}	0.0585	0.0340	0.0324
R_1	0.0446	0.0345	0.0507
wR_2	0.1141	0.0713	0.1358
CCDC number	2027395	2027391	2027390

Bond Length of salts

The bond length of all salts shows the averageness and charge delocalization in varying degrees.

Table S6. The bond length of Col and salts

	C_9-C_{10}	**C_{10}-C_{11}**	**C_{11}-C_{12}**	**C_{12}-C_{12a}**	**C_{12a}-C_{7a}**	**C_{7a}-C_8**	**C_8-C_9**
Col	1.486	1.358	1.407	1.368	1.459	1.358	1.438
Col-BSA	1.427	1.382	1.388	1.404	1.398	1.390	1.387
Col-PTA	1.419	1.392	1.383	1.409	1.409	1.387	1.386
Col-NSA	1.417	1.382	1.380	1.386	1.411	1.390	1.388

Table S7. The bond length of Tr /Tro/Hin and salts

	C_1-C_2	C_2-C_3	C_3-C_4	C_4-C_5	C_5-C_6	C_6-C_7	C_7-C_1
Tr	1.484	1.342	1.443	1.347	1.443	1.342	1.484
Tr-DNSA MH-1	1.402	1.363	1.388	1.350	1.402	1.364	1.408
Tr-DNSA MH-2	1.404	1.364	1.400	1.364	1.397	1.369	1.411
Tr-PTA	1.412	1.369	1.397	1.362	1.401	1.366	1.410
Tr-NSA MH	1.411	1.371	1.392	1.354	1.412	1.366	1.423
Tro	1.454	1.379	1.393	1.341	1.411	1.372	1.411
Tro-DNSA	1.436	1.395	1.386	1.382	1.388	1.390	1.392
Tro-PTA	1.426	1.394	1.384	1.379	1.382	1.385	1.388
Tro-NSA TH	1.429	1.383	1.385	1.381	1.360	1.385	1.377
Hin	1.450	1.389	1.437	1.381	1.444	1.364	1.453
Hin-DNSA	1.426	1.389	1.398	1.393	1.387	1.391	1.379
Hin-PTA	1.431	1.383	1.400	1.396	1.380	1.381	1.387
Hin-NSA MH	1.431	1.397	1.395	1.390	1.382	1.389	1.380

Note: TH-trihydrate; MH-monohydrate;

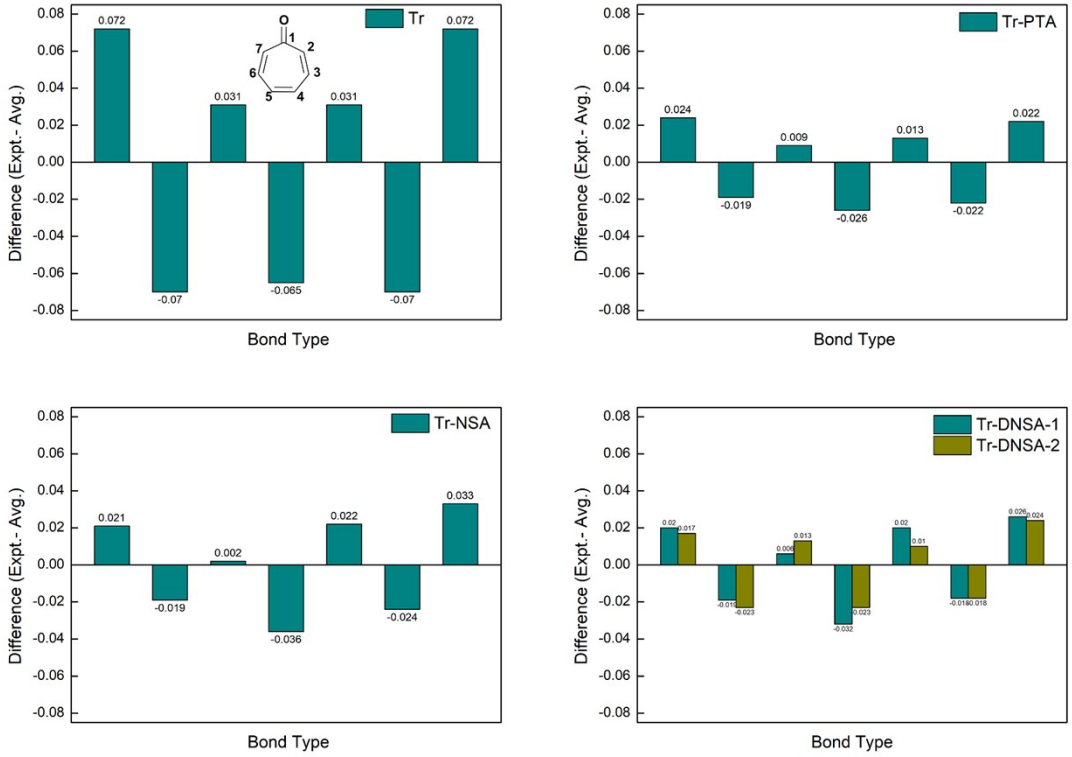


Figure S6. The bond length distribution of Tr and salts (bond type from left to right is C_1-C_2, C_2-C_3, C_3-C_4, C_4-C_5, C_5-C_6, C_6-C_7, C_7-C_1)

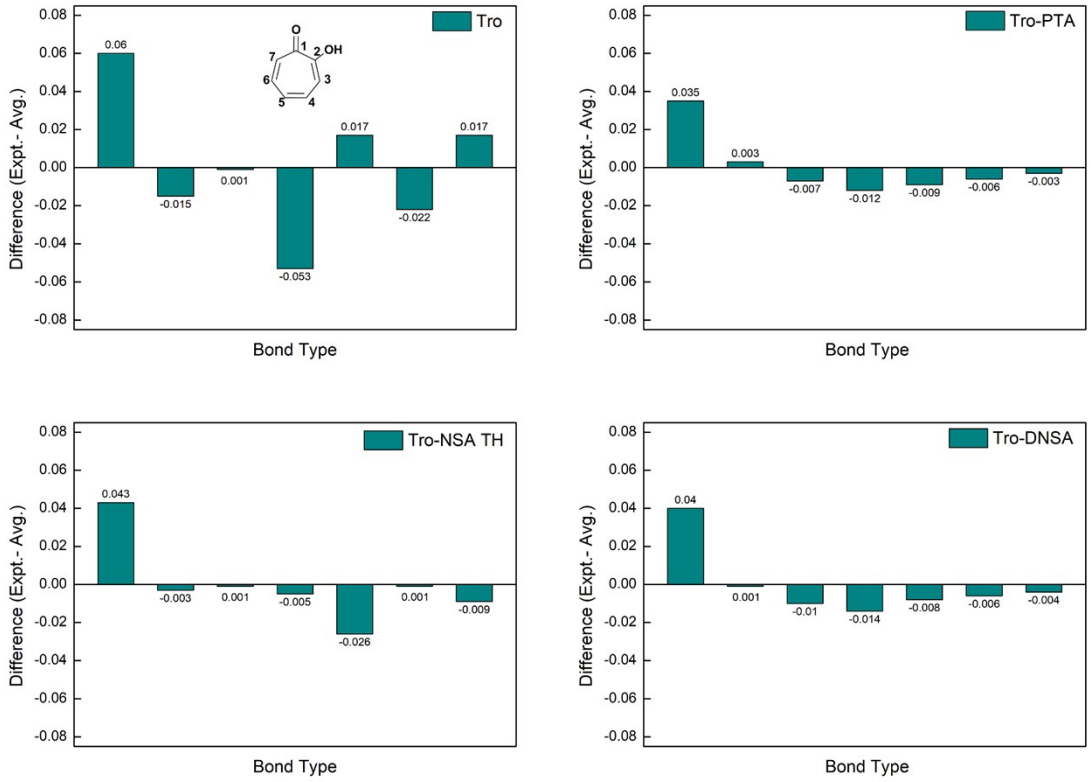


Figure S7. The bond length distribution of Tro and salts (bond type from left to right is C_1-C_2, C_2-C_3, C_3-C_4, C_4-C_5, C_5-C_6, C_6-C_7, C_7-C_1)

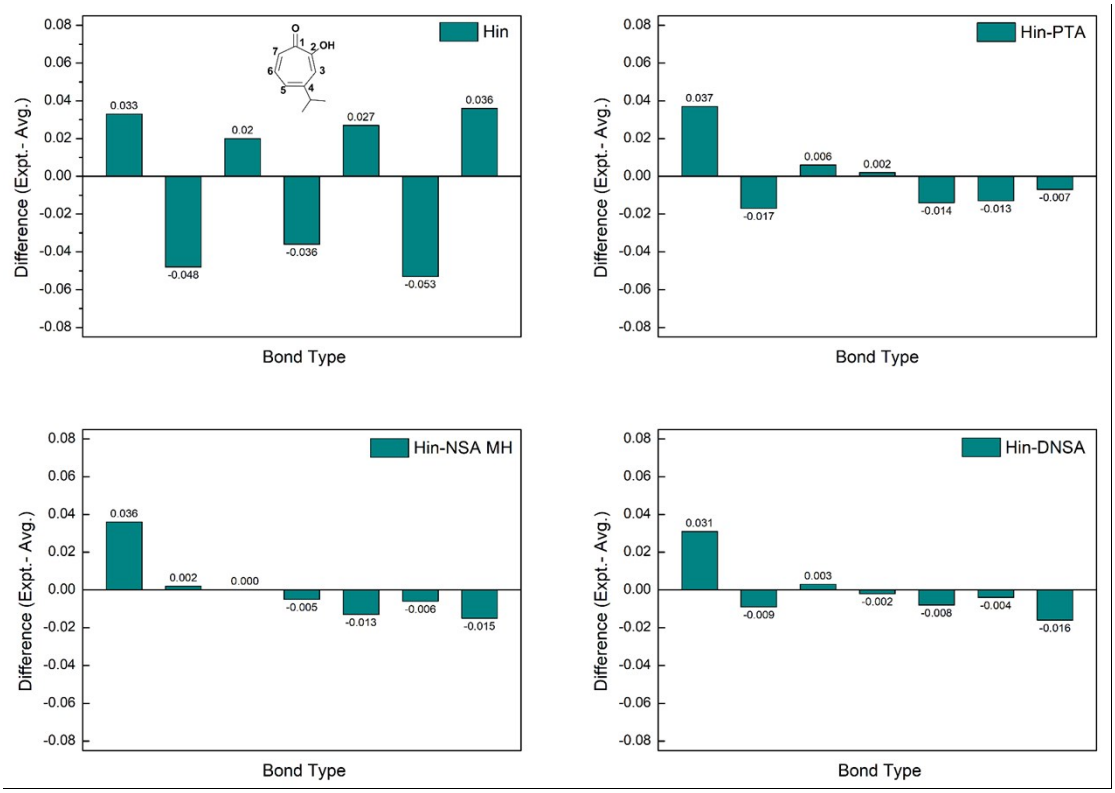


Figure S8. The bond length distribution of Hin and salts (bond type from left to right is C_1-C_2, C_2-C_3, C_3-C_4, C_4-

C_5, C_5-C_6, C_6-C_7, C_7-C_1)

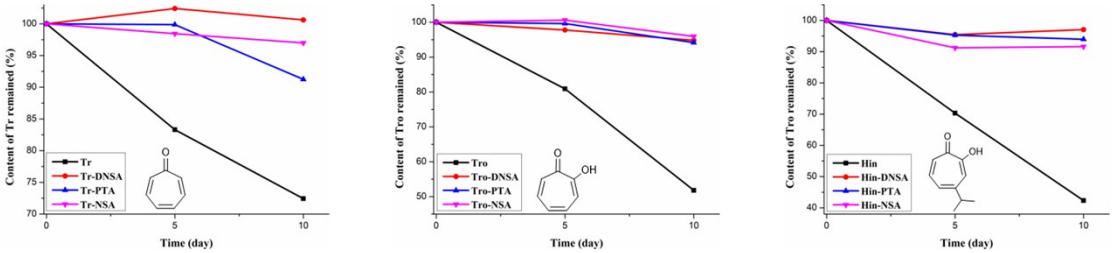


Figure S9. Comparison of relative percentage of pure tropones and salts after an illumination of 5500 lx for 10 days

NBO analysis

The calculated stabilized energy (E(2)) are summarized in following tables. There exist bigger E(2) on carbon-carbon double bond on tropones of Col, Tr, Tro and Hin salts. So the salts are much more stable than the raw materials.

Table S8 Selected E(2) of C=C bonds on tropone ring of Col and salts

	Donor NBO(*i*)	Acceptor NBO(*j*)	E(2) (kcal/mol)
Col	BD(2) C7a - C8	BD*(2) O4 - C9	21.95
	BD(2) C10 - C11	BD*(2) C12 - C12a	21.30
	BD(2) C12 - C12a	BD*(2) C7a – C8	20.26
Col-PTA	BD(2) C7a - C8	LP*(1) C9	77.20
	BD(2) C10 - C11	LP*(1) C9	44.65
	BD(2) C10 - C11	BD*(2) C12 - C12a	22.07
	BD(2) C12 - C12a	BD*(2) C10 - C11	21.72
	BD(2) C12 - C12a	BD*(2) C7a - C8	20.88
Col-NSA	BD(2) C7a - C8	LP*(1) C9	75.90
	BD(2) C7a - C8	LP*(1) C12a	50.52
	BD(2) C11 - C12	LP*(1) C10	68.61
	BD(2) C11 - C12	LP*(1) C12a	42.02
Col-BSA	BD(2) C7a – C8	LP*(1) C9	78.16
	BD(2) C10 - C11	LP*(1) C9	42.33
	BD(2) C10 - C11	BD*(2) C12 - C12a	21.48
	BD(2) C12 - C12a	BD* (2) C7a - C8	21.85
	BD(2) C12 - C12a	BD* (2) C10 – C11	20.95

Table S9. E(2) of C=C bonds of tropone in Tr/Tro/Hin and salt

	Donor NBO(i)	Acceptor NBO(j)	E(2) (kcal/mol)
Tr-raw	BD(2) C2 - C3	BD*(2) C4 - C5	12.78
	BD(2) C4 - C5	BD*(2) C6 – C7	13.21
	BD(2) C6 - C7	BD*(2) C4 - C5	12.78
Tr-DNSA MH	BD(2) C2 - C3	LP*(1) C1	59.71
	BD(2) C4 - C5	BD*(2) C2 - C3	20.03
	BD(2) C6 - C7	LP*(1) C1	55.96
Tr-PTA	BD(2) C2 - C3	LP*(1) C1	54.65

	BD(2) C4 - C5	BD*(2) C2 - C3	18.46
	BD(2) C6 - C7	LP*(1) C1	55.70
Tr-NSA MH	BD(2) C2 - C3	LP*(1) C1	69.79
	BD(2) C4 - C5	BD*(2) C2 - C3	20.76
	BD(2) C6 - C7	LP*(1) C1	62.49
Tro-raw	BD(2) C2 - C3	BD*(2) C4 - C5	21.96
	BD(2) C4 - C5	BD*(2) C6 – C7	19.46
	BD(2) C6 - C7	BD*(2) C1 - O1	26.87
Tro-DNSA	BD(2) C2 - C3	BD*(2) C4 - C5	21.21
	BD(2) C4 - C5	LP*(1) C6	57.69
	BD(2) C6 - C7	LP*(1) C6	56.82
Tro-PTA	BD(2) C2 - C3	LP*(1) C4	58.04
	BD(2) C5 - C6	LP*(1) C4	64.35
	BD(2) C1 - C7	BD*(2) C5 - C6	21.13
Tro-NSA TH	BD(2) C3 - C4	LP*(1) C2	68.89
	BD(2) C5 - C6	LP*(1) C7	48.72
Hin-raw	BD(2) C2 - C3	BD*(2) C1 – O1	19.31
	BD(2) C4 - C5	BD*(2) C6 – C7	17.13
	BD(2) C6 - C7	BD*(2) C1 - O1	21.48
Hin-DNSA	BD(2) C2 - C3	LP*(1) C4	52.09
	BD(2) C5 - C6	LP*(1) C4	51.97
	BD(2) C1 - C7	BD*(2) C5 - C6	19.11
Hin-PTA	BD(2) C2 - C3	BD*(2) C4 - C5	21.39
	BD(2) C5 - C6	LP*(1) C6	65.27
	BD(2) C1 - C7	LP*(1) C6	58.47
Hin-NSA MH	BD(2) C2 - C3	LP*(1) C4	56.28
	BD(2) C5 - C6	LP*(1) C4	58.16
	BD(2) C1 - C7	BD*(2) C5 - C6	22.69

Notes and references

1. Stahl and P. Heinrich, *Handbook of pharmaceutical salts*, VHCA ;, 2002.
2. S. Glowienke, W. Frieauff, T. Allmendinger, H. J. Martus, W. Suter and L. Mueller, *Mutat Res*, 2005, **581**, 23-34.
3. M. J. F. Gaussian 09, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N.

Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.

4. J. P. Foster and F. Weinhold, *J Am Chem Soc*, 1980, **102**, 7211-7218.
5. A. E. Reed and F. Weinhold, *The Journal of Chemical Physics*, 1983, **78**, 4066-4073.
6. A. E. Reed, R. B. Weinstock and F. Weinhold, *The Journal of Chemical Physics*, 1985, **83**, 735-746.
7. A. E. Reed and F. Weinhold, *The Journal of Chemical Physics*, 1985, **83**, 1736-1740.
8. J. E. Carpenter and F. Weinhold, *Journal of Molecular Structure: THEOCHEM*, 1988, **169**, 41-62.
9. A. E. Reed, L. A. Curtiss and F. Weinhold, *Chemical Reviews*, 1988, **88**, 899-926.