

Supplementary Materials

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A novel therapeutic deep eutectic solvent based on tyrosinase inhibitor neohesperidin dihydrochalcone and pyruvic acid, for enhancing the transdermal delivery of neohesperidin dihydrochalcone

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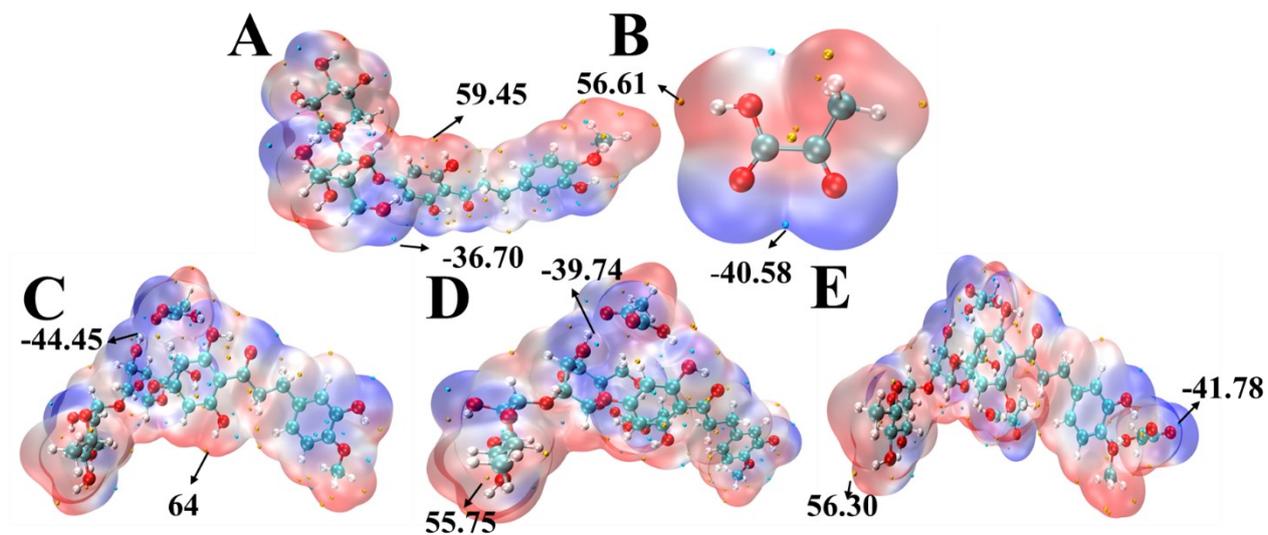


Figure S1 ESP of (A): NHDC, (B): PA, (C): NHDC-PA THEDES-1, (D): NHDC-PA THEDES-2, and (E): NHDC-PA THEDES-3



Figure S2 Appearance of PA used in this experiment

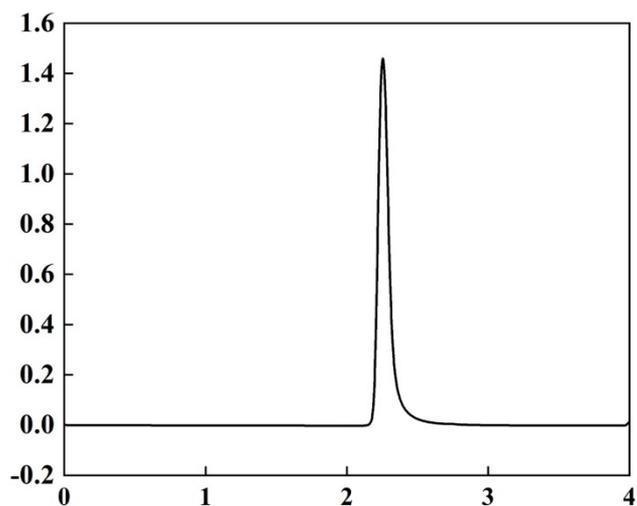


Figure S3 HPLC Chromatogram of THEDES

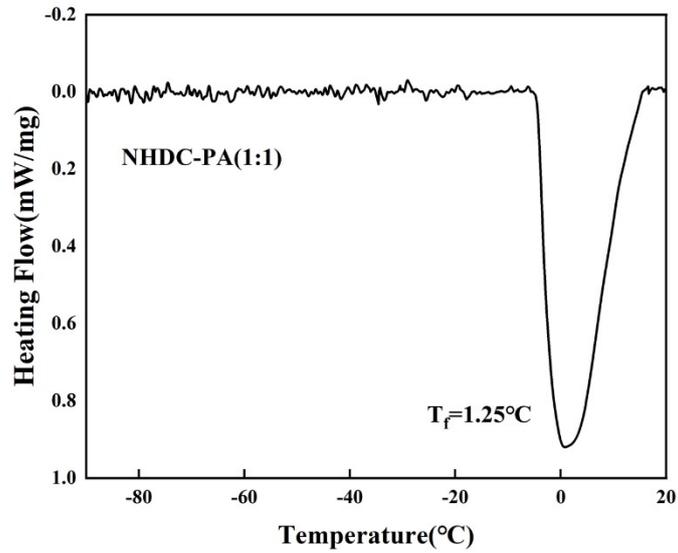


Figure S4 DSC curve of NHDC-PA THEDES at a molar ratio of 1:3

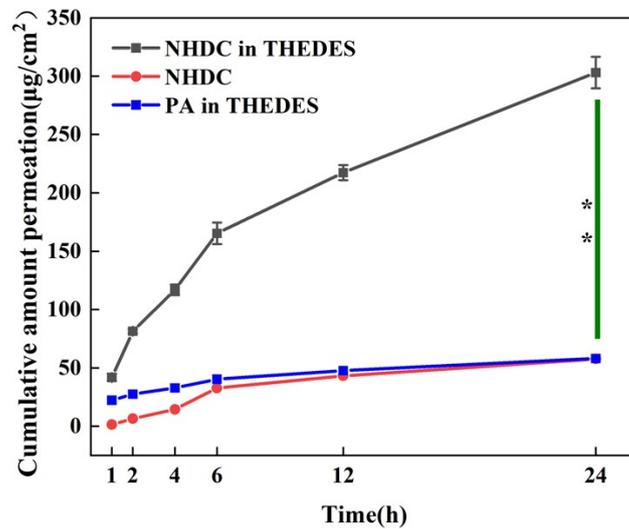


Figure S5 Cumulative permeation of THEDES and NHDC

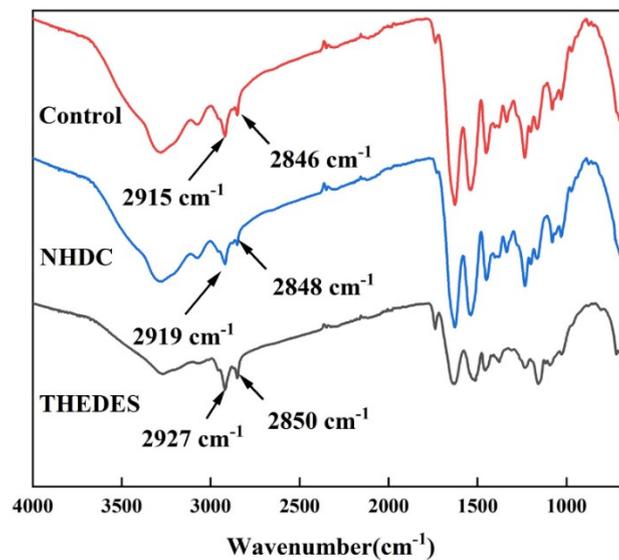


Figure S6 FTIR spectra of skin samples treated with different formulation

Table S1 Topological parameters of BCP and binding energy of hydrogen bonds formed between NHDC and PA

| Structure | Hydrogen bonds | $\rho(r)$ (a.u.) | $\nabla^2\rho(r)$ (a.u.) | $V(r)$ (a.u.) | EHB (kcal/mol) |
|------------------|-----------------|------------------|--------------------------|---------------|----------------|
| NHDC-PA THEDES-1 | O82-H83...O17 | 0.03633 | 0.10269 | -0.02666 | -7.36 |
| NHDC-PA THEDES-1 | O14-H51...O89 | 0.02460 | 0.06896 | -0.01858 | -4.75 |
| NHDC-PA THEDES-2 | O82-H83...O17 | 0.03528 | 0.09894 | -0.02584 | -7.13 |
| NHDC-PA THEDES-2 | O14-H51...O89 | 0.02462 | 0.06911 | -0.01861 | -4.75 |
| NHDC-PA THEDES-2 | O92-H93...O5 | 0.03368 | 0.09547 | -0.02499 | -6.77 |
| NHDC-PA THEDES-3 | O82-H83...O17 | 0.03508 | 0.09834 | -0.02569 | -7.08 |
| NHDC-PA THEDES-3 | O14-H51...O89 | 0.02469 | 0.06930 | -0.01866 | -4.77 |
| NHDC-PA THEDES-3 | O92-H93...O5 | 0.03387 | 0.09609 | -0.02513 | -6.81 |
| NHDC-PA THEDES-3 | O40-H76...O101 | 0.02919 | 0.08574 | -0.02182 | -5.77 |
| NHDC-PA THEDES-3 | O102-H103...O42 | 0.04476 | 0.13077 | -0.03415 | -9.24 |

Table S2 DPPH radical scavenging data

| Concentration | THEDES | standard error | NHDC | standard error | PA | standard error |
|----------------------|-----------|----------------|-----------|----------------|-----------|----------------|
| 120 $\mu\text{g/mL}$ | 99.20917% | 0.20576% | 95.50472% | 0.11448% | 78.69165% | 0.82702% |
| 100 $\mu\text{g/mL}$ | 97.82473% | 0.36098% | 90.66241% | 0.48278% | 77.12042% | 0.46563% |
| 80 $\mu\text{g/mL}$ | 96.52091% | 0.48783% | 86.74444% | 0.72465% | 68.41919% | 2.80497% |
| 60 $\mu\text{g/mL}$ | 94.95296% | 0.17195% | 72.32787% | 1.84573% | 58.88106% | 3.67604% |
| 40 $\mu\text{g/mL}$ | 73.51476% | 1.6309% | 62.3159% | 1.16042% | 46.89087% | 1.18423% |
| 20 $\mu\text{g/mL}$ | 62.00928% | 3.75748% | 44.23283% | 3.518% | 33.44116% | 1.08505% |
| 10 $\mu\text{g/mL}$ | 48.36014% | 2.06813% | 35.39488% | 1.14584% | 22.72176% | 3.83129% |

Table S3 ABTS radical scavenging data

| Concentration | THEDES | standard error | NHDC | standard error | PA | standard error |
|----------------------|-----------|----------------|-----------|----------------|-----------|----------------|
| 120 $\mu\text{g/mL}$ | 93.23181% | 0.23715% | 89.50314% | 0.33155% | 32.91937% | 0.74654% |
| 100 $\mu\text{g/mL}$ | 91.53968% | 0.11502% | 88.81435% | 0.58167% | 33.00739% | 1.14641% |
| 80 $\mu\text{g/mL}$ | 90.53413% | 0.50099% | 86.92101% | 0.67334% | 30.04879% | 2.45944% |
| 60 $\mu\text{g/mL}$ | 89.30224% | 0.35551% | 78.89267% | 1.13718% | 24.89295% | 0.55879% |
| 40 $\mu\text{g/mL}$ | 86.4344% | 0.33182% | 73.727% | 1.10347% | 18.28974% | 1.61167% |
| 20 $\mu\text{g/mL}$ | 61.65809% | 0.98723% | 37.56053% | 3.40755% | 11.21042% | 1.37093% |
| 10 $\mu\text{g/mL}$ | 42.97615% | 3.04296% | 22.7931% | 2.2225% | 9.23173% | 1.09859% |

Table S4 Mushroom tyrosinase inhibition rate data

| Concentration | THEDES | standard error | NHDC | standard error | PA | standard error |
|---------------|-----------|----------------|-----------|----------------|-----------|----------------|
| 20 mg/mL | 93.67662% | 3.5648% | 85.50741% | 0.85252% | 59.7742% | 2.26494% |
| 15 mg/mL | 90.51024% | 2.16147% | 75.65482% | 1.11255% | 44.80146% | 1.04185% |
| 10 mg/mL | 83.00225% | 0.95082% | 68.28928% | 2.32249% | 32.50498% | 0.57384% |
| 5 mg/mL | 70.15709% | 2.4084% | 41.48144% | 1.39724% | 23.0573% | 1.60689% |
| 2.5 mg/mL | 48.61698% | 0.16675% | 25.40153% | 3.70641% | 14.55871% | 3.53976% |
| 1.25 mg/mL | 31.22476% | 0.637% | 13.45523% | 2.74342% | 4.22727% | 0.23257% |

Table S5 Raw cumulative permeation data($\mu\text{g}/\text{cm}^2$)

| Concentration | THEDES | standard error | NHDC | standard error |
|---------------|-----------|----------------|----------|----------------|
| 1h | 41.84837 | 3.17025 | 1.52908 | 0.82497 |
| 2h | 81.4043 | 1.80791 | 6.5942 | 1.14823 |
| 4h | 116.87328 | 4.61429 | 14.52554 | 0.99584 |
| 6h | 165.2749 | 9.25215 | 32.77748 | 1.29041 |
| 12h | 217.21254 | 6.46219 | 43.2706 | 1.25166 |
| 24h | 303.00566 | 13.50479 | 57.64336 | 1.68724 |

Table S6 MTT data of B16F10 cells

| Concentration | THEDES | standard error | NHDC | standard error | PA | standard error | KA | standard error |
|---------------|-----------|----------------|-----------|----------------|-----------|----------------|-----------|----------------|
| 5 µg/mL | 99.86025% | 6.5986% | 96.36946% | 3.77114% | 89.92627% | 3.0829% | 96.0238% | 3.91832% |
| 10 µg/mL | 99.08261% | 4.74291% | 95.38463% | 4.22244% | 86.41102% | 3.11678% | 92.97247% | 3.94972% |
| 50 µg/mL | 95.49959% | 4.94942% | 92.11913% | 3.24367% | 82.18167% | 2.77606% | 89.29563% | 4.54203% |
| 100 µg/mL | 94.29303% | 5.07397% | 89.28996% | 3.55973% | 79.00095% | 2.92242% | 83.54737% | 5.02477% |
| 200 µg/mL | 85.87997% | 3.12641% | 68.91945% | 4.34406% | 48.38639% | 4.27597% | 72.52725% | 4.01819% |

Table S7 MTT data of HaCaT cells

| Concentration | THEDES | standard error | NHDC | standard error | PA | standard error | KA | standard error |
|---------------|------------|----------------|-----------|----------------|-----------|----------------|-----------|----------------|
| 5 µg/mL | 101.51491% | 2.39302% | 98.59353% | 2.29848% | 87.88654% | 2.72682% | 95.14135% | 2.03615% |
| 10 µg/mL | 100.37997% | 2.32998% | 97.93155% | 0.86737% | 80.34895% | 2.03935% | 92.64898% | 1.78677% |
| 50 µg/mL | 99.56242% | 2.46986% | 96.20535% | 1.61244% | 76.93124% | 2.24543% | 91.06468% | 3.24315% |
| 100 µg/mL | 95.73456% | 2.59663% | 94.12182% | 1.83418% | 75.66667% | 2.79084% | 88.03308% | 3.07803% |
| 200 µg/mL | 83.49553% | 3.27872% | 75.60753% | 2.18179% | 46.96895% | 3.86899% | 83.6989% | 3.11071% |

Table S8 Melanin content data

| Concentration | inhibition rate | standard error |
|---------------|-----------------|----------------|
| control | 100% | 0.22361% |
| THEDES | 37.2929% | 3.98902% |
| NHDC | 61.91547% | 3.91778% |
| PA | 89.98851% | 3.41254% |
| KA | 46.78684% | 3.22188% |

Table S9 Tyrosinase activity data

| Concentration | inhibition rate | standard error |
|---------------|-----------------|----------------|
| control | 100% | 0.40373% |
| THEDES | 32.88315% | 2.25977% |
| NHDC | 45.73824% | 3.72475% |
| PA | 87.87889% | 8.31457% |
| KA | 42.18133% | 1.63949% |

Table S10 Stability determination

| Time | Content |
|-------|---------|
| DAY0 | 100% |
| DAY7 | 93.48% |
| DAY14 | 90.91% |
| DAY21 | 78.61% |
| DAY30 | 70.01% |