**SUPPLEMENTARY**

**Table S1.** UV scanning of GA in the 190-500 nm range

|  |  |  |
| --- | --- | --- |
| **No.** | **Wavelength nm (λ)** | **Absorbance** |
| **1** | 450.40 | 0.010 |
| **2** | 258.20 | 0.811 |
| **3** | 195.60 | 2.721 |
| **4** | 467.80 | 0.006 |
| **5** | 219.80 | 0.274 |

**Table S2.** UV scanning of PBS in the 190-500 nm range

|  |  |  |
| --- | --- | --- |
| **No.** | **Wavelength ( λ) (nm)** | **Absorbance** |
| **1** | 476.20 | -0.026 |
| **2** | 195.60 | 2.441 |
| **3** | 468.60 | -0.027 |
| **4** | 338.60 | -0.021 |

**Table S3.** UV scanning of blank PLGA solution in the range of 190-500 nm

|  |  |  |
| --- | --- | --- |
| **No.** | **Wavelength ( λ) (nm)** | **Absorbance** |
| **1** | 196.80 | 3.893 |

**Table S4.** Intraday and interday precision results

|  |  |  |  |
| --- | --- | --- | --- |
|  | **20 µg/mL** | **55 µg/mL** | **60 µg/mL** |
| **Intraday precision** | 0.074±0.001 | 0.841±0.002 | 0.933±0.001 |
| **Interday precision** | 0.074±0.001 | 0.841±0.002 | 0.933±0.001 |
| **%RSD** | 1.209 | 0.208 | 0.361 |

**Table S5.** Recovery study results

|  |  |  |
| --- | --- | --- |
| **Amount added (µg)** | **Recovery (%)** | **% RSD** |
| 30.01 | 100.0 ± 0.29 | 0.26 |
| 34.99 | 100.1 ± 0.27 | 0.24 |
| 40.00 | 100.0 ± 0.12 | 0.11 |

**Table S6.** Recovery study results

|  |  |  |
| --- | --- | --- |
| **Time (Hour)** | **Absorbance** | **Variance %** |
| **0** | 0.940 ± 0.002 | **-** |
| **24** | 0.932 ± 0.001 | 99.1 |
| **48** | 0.935 ± 0.002 | 99.5 |

**Table S7.** Genotoxic effects of GA, and GA-PLGA NPs on *S. typhimurium* TA98 and TA100 mutant strains

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment** | **Concentration**  **(mg/mL)** | **Number of revertant colony/Plate** | |
| **TA98** | **TA100** |
| **Mean±SD** | **Mean±SD** |
| **GA** | **0.219** | 30.33± 2.51 | 114.00 ±28.21 |
| **0.438** | 27.33± 2.45 | 97.00±19.67 |
| **0.875** | 27.66± 2.31 | 105.33±13.31 |
| **1.750** | 25.66 ± 2.08 | 95.66±6.42 |
| **GA-PLGA-NPs** | **0.125** | 30.00± 2.00 | 104.66±13.31 |
| **0.25** | 32.66 ± 1.52 | 110.00 ±21.93 |
| **0.50** | 26.00± 3.00 | 103.33±20.81 |
| **1.00** | 32.00±1.00 | 125.33±4.50 |
| **Positive Control (NPD)** | **0.005** | 897.66± 5.87\* |  |
| **Positive Control (SA)** | **0.0005** |  | 990.66±9.71\* |
| **Negative Control** |  | 27.00±2.64 | 100.66±10.06 |
| **Spontaneous Control** |  | 22.66± 3.05 | 102.66±2.51 |
| **NPD**: 4‐Nitro‐*o*‐phenylenediamine  **SA:** Sodium azide | | | |
|  | | | |

\*The mean revertant colony number difference between negative control and application groups is significant at the level of p <0.05.

**Table S8.** The docking score energies and probable interactions between GA and MMP Targets

|  |  |  |  |
| --- | --- | --- | --- |
|  | **MMP-1** | **MMP-3** | **MMP-9** |
|  | **PDB ID:966c** | **PDB ID:2JT6** | **PDB ID:5CUH** |
| **Docking Score(Kcal/mol)** | -8.26 | -4.57 | -8.64 |
| **H.Bonding interaction (Angstrom)** | TYR240(2.41)  ASN180(2.04)  LEU181(2.08)  GLU219(1.62) | HIS201(2.33) | TYR248(1.68)  HIS236(1.74)  HIS236(2.72)  ASP235(1.79)  ALA191(1.75) |
| **Salt Bridge interaction(Angstrom)** | Zn265(2.06) | - | Zn301(4.19) |
| **Metal coordination interaction**  **(Angstrom)** | Zn265(2.06)  Zn265(2.27) | Zn256(2.26) | Zn301(1.98) |

**Table S9.** Virtual screening of GA showing drug-likeliness by Molinspiration and SwissADME servers.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Molinspiration** | | | | |
| Molinspiration property engine | | Molinspiration bioactivity score | | |
| miLogP | 1.97 | GPCR ligand | | -1.78 |
| TPSA | 267.04 | Ion channel modulator | | -3.09 |
| natoms | 58 | Kinase inhibitor | | -3.09 |
| MW | 822.94 | Nuclear receptor ligand | | -2.36 |
| nON | 16 | Protease inhibitor | | -1.26 |
| nOHNH | 8 | Enzyme inhibitor | | -1.93 |
| nviolations | 3 |  | |  |
| nrotb | 7 |  | |  |
| volume | 741.93 |  | |  |
| **SwissADME** | | | | |
| Physicochemical Properties | | | Lipophilicity | |
| Formula | C42H62O16 | | LogPO/W(iLOGP) | 2.15 |
| MW | 822.93 g/mol | | LogPO/W(XLOGP3) | 2.80 |
| TPSA | 267.04 Å² | | LogPO/W(WLOGP) | 2.25 |
| No. of H-bond acceptors | 16 | | LogPO/W(MLOGP) | 0.02 |
| No. of H-bond donors | 8 | | LogPO/W(SILICOS-IT) | 0.52 |
| Molar refractivity | 202.84 | | Consensus LogPO/W | 1.55 |
| Rotatable bond count | 7 | |  |  |
| Heavy atom count | 58 | | Water Solubility | |
| Aromatic heavy atom count | 0 | | Log S (ESOL) | -6.24 |
|  |  | | Log S (Ali) | -8.06 |
|  |  | | Log S (SILICOS-IT) | -1.39 |
| Pharmacokinetics | | | Druglikeness | |
| GI absoption | Low | | Lipinski | No; 3 violations: MW>500,  N or O >10,  NH or OH >5 |
| BBB permeant | No | | Ghose | No; 3 violations: MW>480,  MR>130 |
| P-gp substrate | Yes | | Veber | No; 1 violation: TPSA>140 |
| Log KP (Skin Permeation) | -9.33 cm/s | | Muegge | No; 4 violations: MW>600, TPSA>150, H-acc>10, H-don>5 |
|  |  | | Bioavailability Score | 0.11 |

**Table S10.** Pharmacokinetic Properties of GA showing ADME and Toxicity by pkCSM server

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***ABSOPTION*** | **DESCRIPTOR** | | |  | **UNIT** |
| **Water Solubility** | | | **-2.892** | Numeric  (log mol/L) |
| **Caco2 permeability** | | | **-0.812** | Numeric  (log Papp in 10-6 cm/s) |
| **Intestinal absorption (human)** | | | **0** | Numeric (% Absorbed) |
| **Skin Permeability** | | | **-2.735** | Numeric (log Kp) |
| **P-glycoprotein substrate** | | | **Yes** | Categorical (Yes/No) |
| **P-glycoprotein I inhibitor** | | | **No** | Categorical (Yes/No) |
| **P-glycoprotein II inhibitor** | | | **No** | Categorical (Yes/No) |
| ***DISTRIBUTION*** | **VDss (human)** | | | **-0.615** | Numeric (log L/kg) |
| **Fraction unbound (human)** | | | **0.421** | Numeric (Fu) |
| **BBB permeability** | | | **-1.494** | Numeric (log BB) |
| **CNS permeability** | | | **-4.206** | Numeric (log PS) |
| ***METABOLISM*** | **CYP2D6 substrate** | | | **No** | Categorical (Yes/No) |
| **CYP3A4 substrate** | | | **Yes** | Categorical (Yes/No) |
| **CYP1A2 inhibitior** | | | **No** | Categorical (Yes/No) |
| **CYP2C19 inhibitior** | | | **No** | Categorical (Yes/No) |
| **CYP2C9 inhibitior** | | | **No** | Categorical (Yes/No) |
| **CYP2D6 inhibitior** | | | **No** | Categorical (Yes/No) |
| **CYP3A4 inhibitior** | | | **No** | Categorical (Yes/No) |
| ***EXCRETION*** | **Total Clearance** | | | **-0.304** | Numeric (log ml/min/kg) |
| **Renal OCT2 substrate** | | | **No** | Categorical (Yes/No) |
| ***TOXICITY*** | **AMES toxicity** | | | **No** | Categorical (Yes/No) |
| **Max. tolerated dose (human)** | | | **0.389** | Numeric (log mg/kg/day) |
| **hERG I inhibitor** | | | **No** | Categorical (Yes/No) |
| **hERG II inhibitor** | | | **No** | Categorical (Yes/No) |
| **Oral Rat Acute Toxicity (LD50)** | | | **2.48** | Numeric (mol/kg) |
| **Oral Rat Chronic Toxicity (LOAEL)** | | | **5.889** | Numeric (log mg/kg\_bw/day) |
| **Hepatotoxicity** | | | **No** | Categorical (Yes/No) |
| **Skin Sensitisation** | | | **No** | Categorical (Yes/No) |
| ***T.Pyriformis* toxicity** | | | **0.285** | Numeric (log ug/L) |
| **Minnow toxicity** | | | **5.591** | Numeric (log mM) |
| **MOLECULE PROPERTIES** | | | | | |
| **Descriptor** | | **Value** |  | | **Value** |
| **Molecular Weight** | | 822.942 | **Rotatable Bonds** | | 7 |
| **LogP** | | 2.2456 | **Acceptors** | | 13 |
| **Surface Area** | | 337.426 | **Donors** | | 8 |

**Table S11.** Oral toxicity prediction results for GA by proTOX-II server

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **TOXICITY MODEL REPORT** | | | | |
| Classification | Target | Shorthand | Prediction | Probability |
| Organ toxicity | Hepatotoxicity | dili | Inactive | 0.88 |
| Toxicity end points | Carcinogenicity | carcino | Inactive | 0.61 |
| Toxicity end points | Immunotoxicity | immuno | Active | 0.99 |
| Toxicity end points | Mutagenicity | mutagen | Inactive | 0.96 |
| Toxicity end points | Cytotoxicity | cyto | Inactive | 0.73 |
| Tox21-Nuclear receptor signalling pathways | Aryl hydrocarbon Receptor (AhR) | nr\_ahr | Inactive | 0.99 |
| Tox21-Nuclear receptor signalling pathways | Androgen Receptor (AR) | nr\_ar | Inactive | 0.89 |
| Tox21-Nuclear receptor signalling pathways | Androgen Receptor Ligand Binding Domain (AR-LBD) | nr\_ar\_lbd | Inactive | 0.96 |
| Tox21-Nuclear receptor signalling pathways | Aromatase | nr\_aromatase | Inactive | 0.87 |
| Tox21-Nuclear receptor signalling pathways | Estrogen Receptor Alpha (ER) | nr\_er | Inactive | 0.74 |
| Tox21-Nuclear receptor signalling pathways | Estrogen Receptor Ligand Binding Domain (ER-LBD) | nr\_er\_lbd | Inactive | 0.98 |
| Tox21-Nuclear receptor signalling pathways | Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma) | nr\_ppar\_gamma | Inactive | 0.99 |
| Tox21-Stress response pathways | Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE) | sr\_are | Inactive | 0.95 |
| Tox21-Stress response pathways | Heat shock factor response element (HSE) | sr\_hse | Inactive | 0.95 |
| Tox21-Stress response pathways | Mitochondrial Membrane Potential (MMP) | sr\_mmp | Inactive | 0.83 |
| Tox21-Stress response pathways | Phosphoprotein (Tumor Supressor) p53 | sr\_p53 | Inactive | 0.98 |
| Tox21-Stress response pathways | ATPase family AAA domain-containing protein 5 (ATAD5) | sr\_atad5 | Inactive | 0.97 |