Supplementary Materials

Amide Conjugates of Ketoprofen and Indole as Inhibitors of Gli1-Mediated Transcription in the Hedgehog Pathway

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Author Contributions. NM designed and prepared the compounds, performed cell growth suppression assay and part of microsome stability assay, and initiated the first draft. MCC maintained all cell cultures, performed all Gli-Luc assays, and gave grammatical advice for the manuscript. CP performed real-time RT-PCR, AP1-Luc assay, alkaline phosphatase assay, HDAC inhibition assay, and part of microsome stability assay. LY performed microsome stability assay, PAMPA, and Caco-2 permeability assay. BY is supervisor of LY. NF conducted the overall research and wrote the paper.

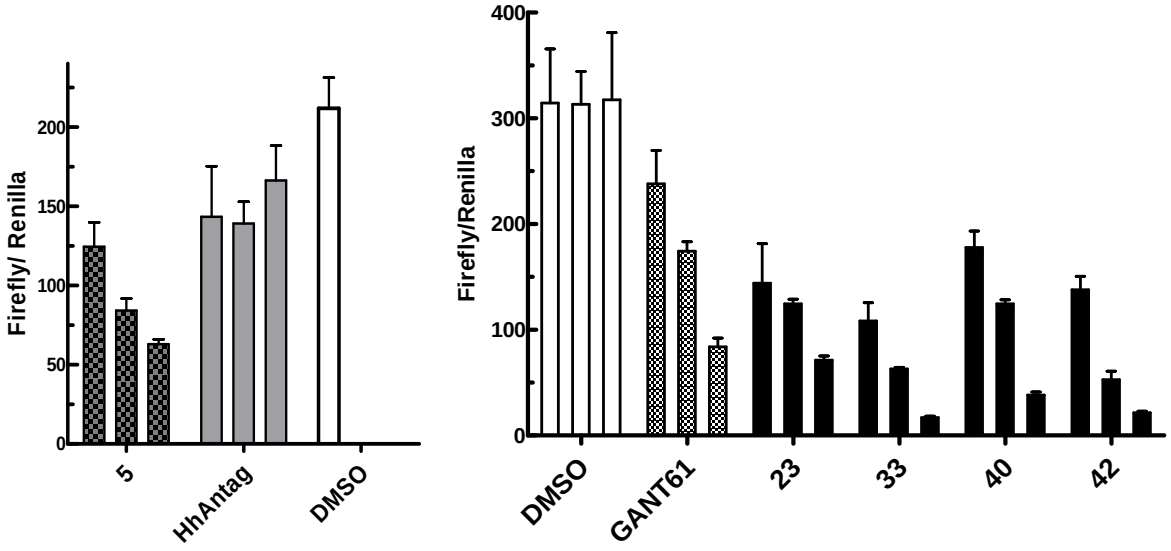


Figure S1 (shown as two panels because of separated experiments)**.** Gli-Luc signal in *Gli1I*-transfected C3H10T1/2 cells treated with 10, 20, and 40 μM (left to right) of each indicated compound.

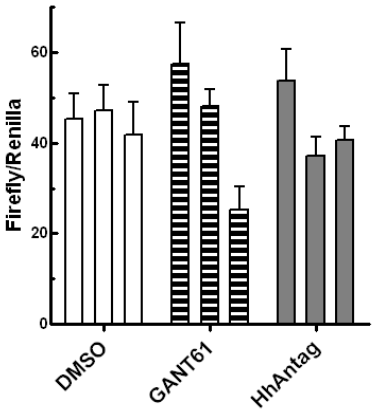
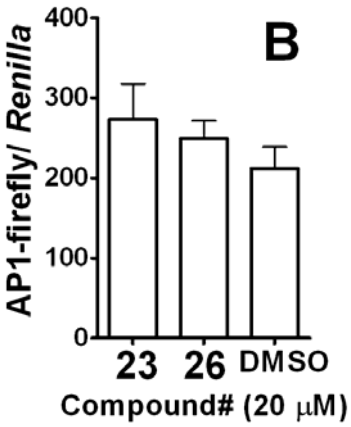


Figure S2 (left)**.** Gli-Luc signal in Rh30 cells cells treated with 10, 20, and 40 μM (left to right) of each indicated compound.

Figure S3 (right, indicated as panel B)**.** Compounds show no inhibition for AP-1 reporter transcription. Assay has been performed by using AP-1 and pRL-TK 24 h after incubation with compounds (20 μM).



Activity againt induction of alkaline phosphatase, an osteoblast differentiation marker

C3H10T1/2 cells were plated in 24-well plates in basal medium Eagle (BME) (Invitrogen Corporation, Carlsbad, CA) supplemented with 2 mM L-glutamine and 10% fetal bovine serum (FBS) (Hyclone Laboratories, Logan, UT). At approximately 80% confluence, the cells were treated with 200 nM Smoothened agonist SAG (N-methyl-N′-(3-pyridinylbenzyl)-N′-(3-chlorobenzo[b]thiophene-2-carbonyl)-1,4-diaminocyclohexane, Santa Cruz Biotechnology Inc, Santa Cruz, CA) and each test compound, in BME containing 10% FBS that has been treated with charcoal/dextran (Hyclone Laboratories, Logan, UT). Cells were incubated for four days before harvest in 50 μL of buffer B of Sensolyte pNPP Alkaline Phosphotase Assay Kit (AnaSpec, Inc, Fremont, CA). The cell suspensions were incubated for 10 min at 4°C under agitation, and centrifuged at 2500g for 10 min. The alkaline phosphotase assay was carried out using the Great EscApe SEAP chemiluminiscence kit (Clontech, Mountain View, CA). Briefly, 5 μL of each lysate was mixed with 15 μL dilution buffer and 20 μL of SEAP substrate solution in a 384-well plate. The luminescence of the sample was measured following incubation of the plate for 45 min at room temperature. Finally, the luminescence data were normalized against protein concentration of each lysate, measured using the Micro BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL).

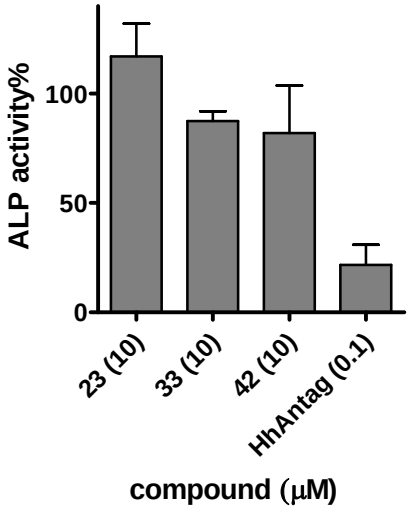
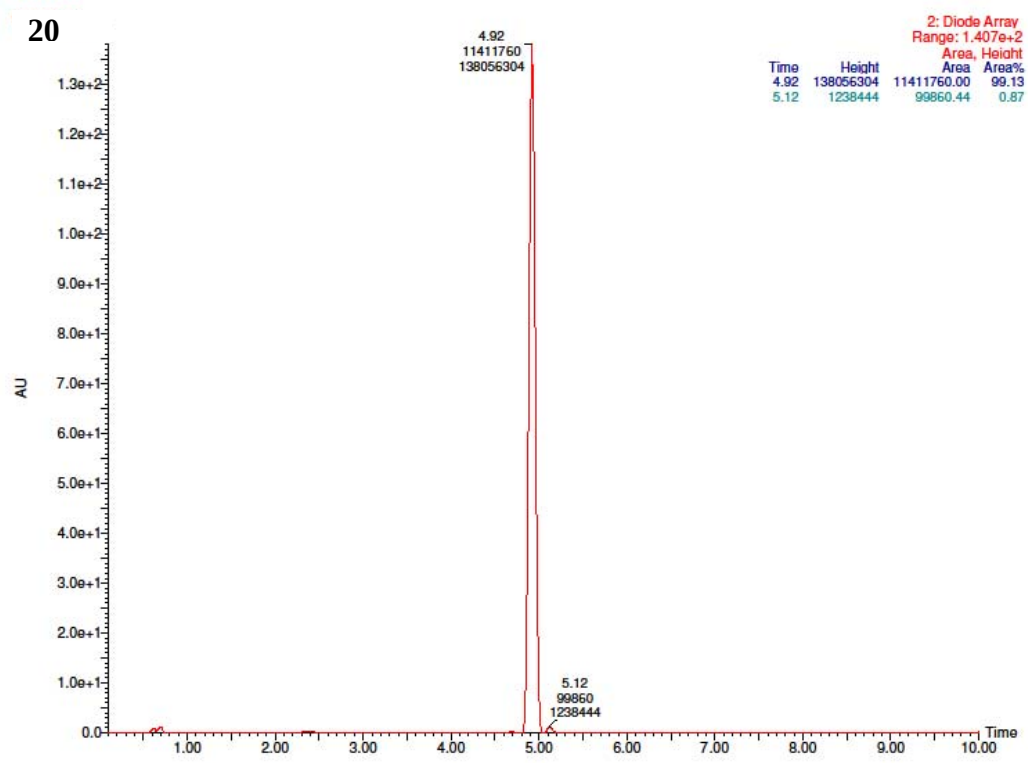


Figure S4 (right)**.** Ketoprofen-indole amide conjugates suppress Hh signal-induced alkaline phosphatase (ALP) activity in C3H10T1/2 cells much more weakly than HhAntag does. Cells were incubated with indicated compound and an Hh agonist (SAG, 200 nM) for activating upstream Hh signal input for 4 days. ALP activity in each cell lysates was normalized by each lysate protein concentration. HhAntag inhibits the Hh signaling with nanomolar IC50 thus was tested in 100 nM. Data are shown as % normalized with the signals of SAG only treatment (=100%) and DMSO (no SAG) treatment (=0%) controls. Error bars represent standard error of duplicate data.

HPLC traces of the compounds 20-44

20



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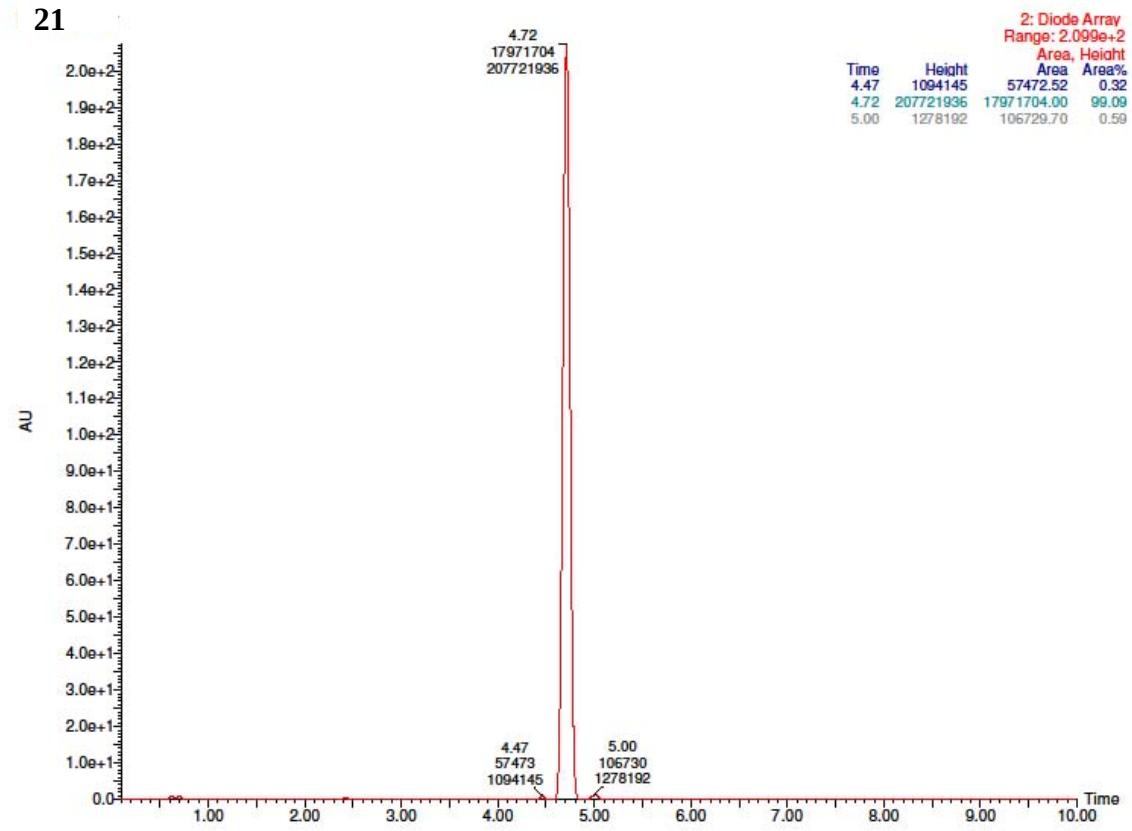

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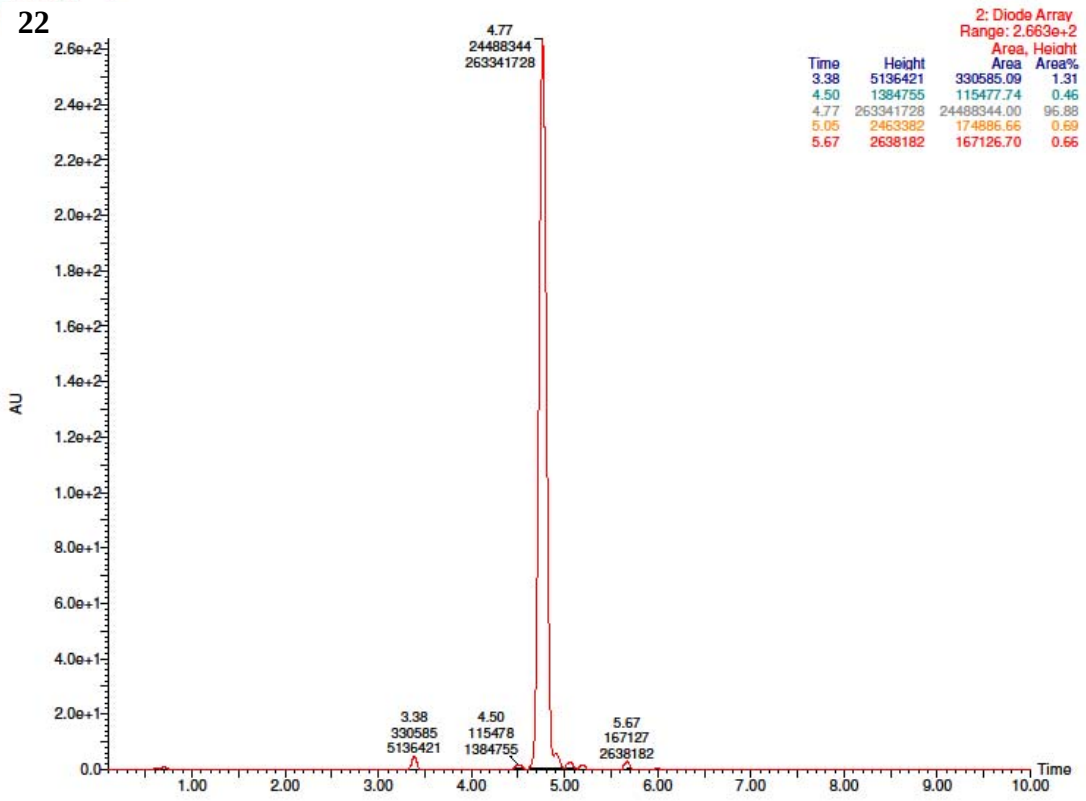
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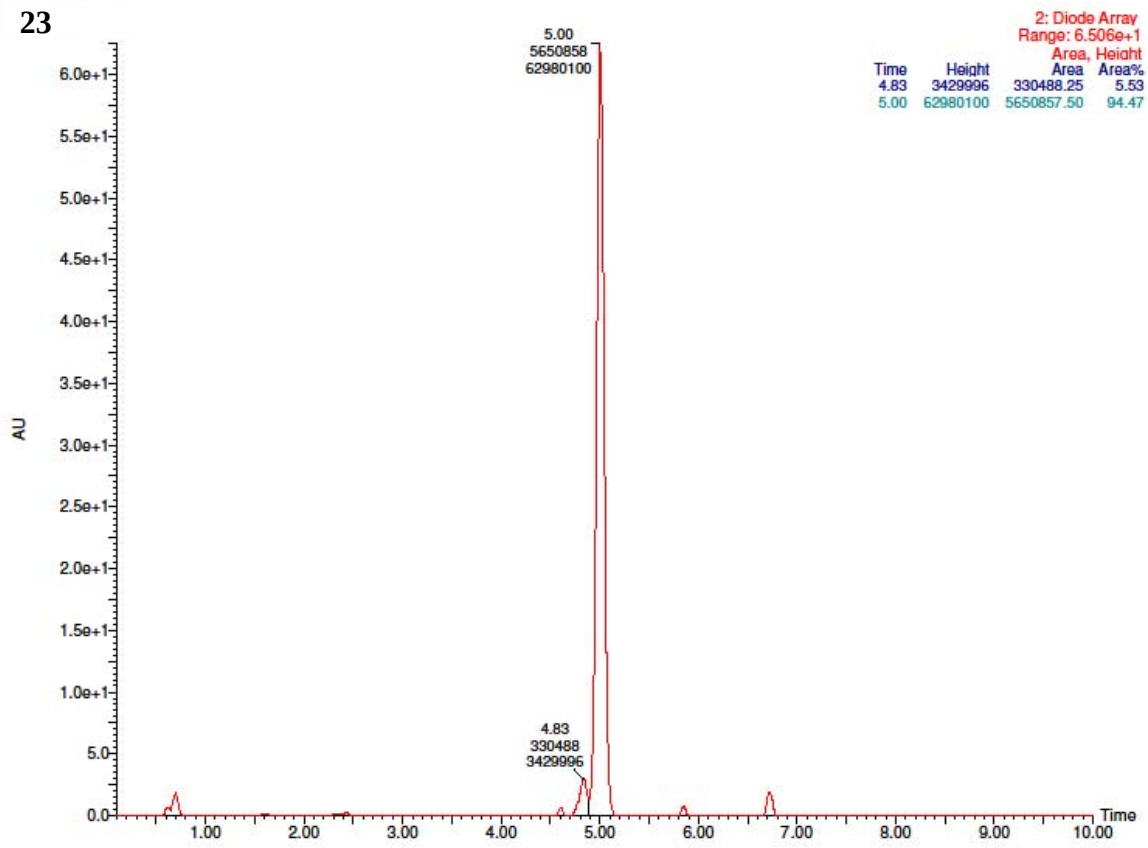
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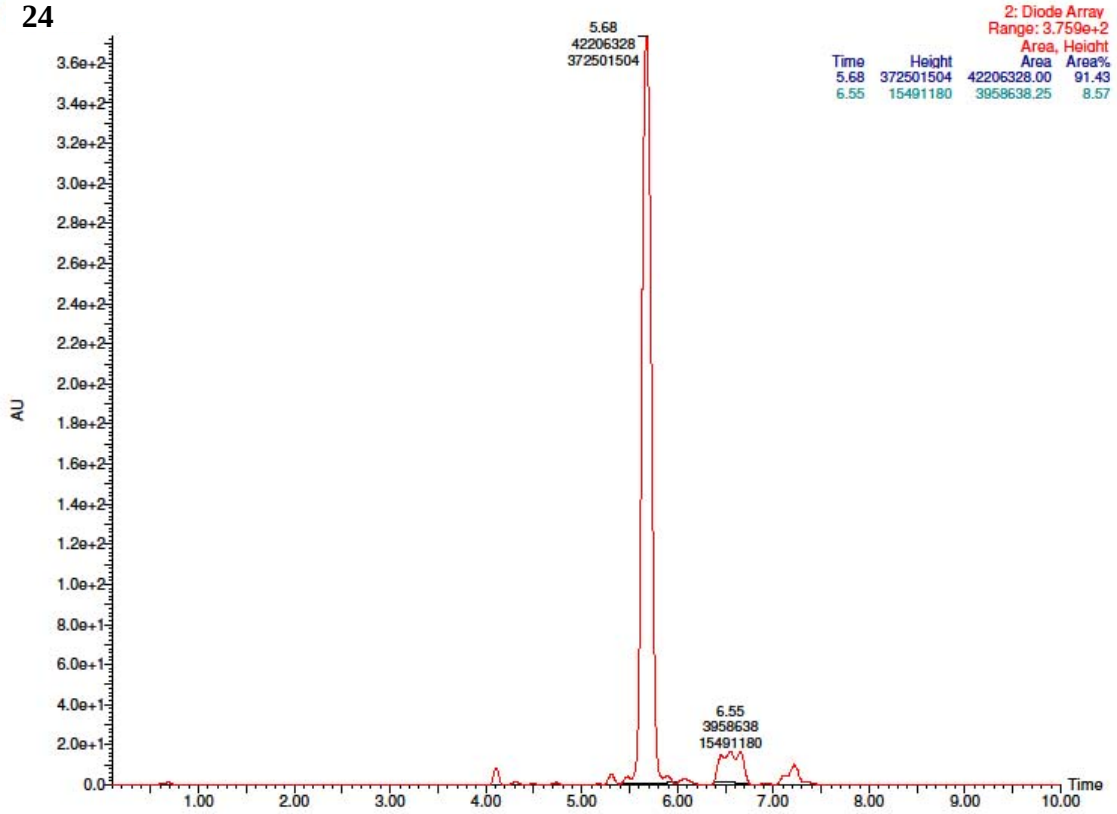
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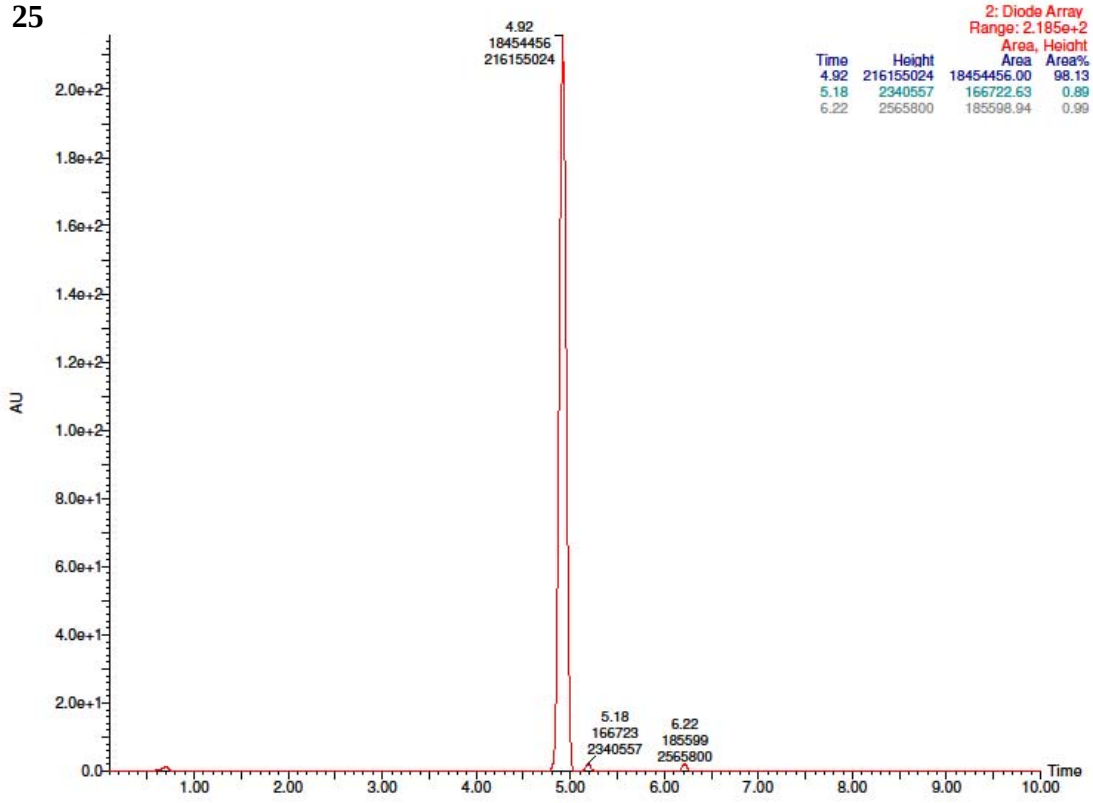
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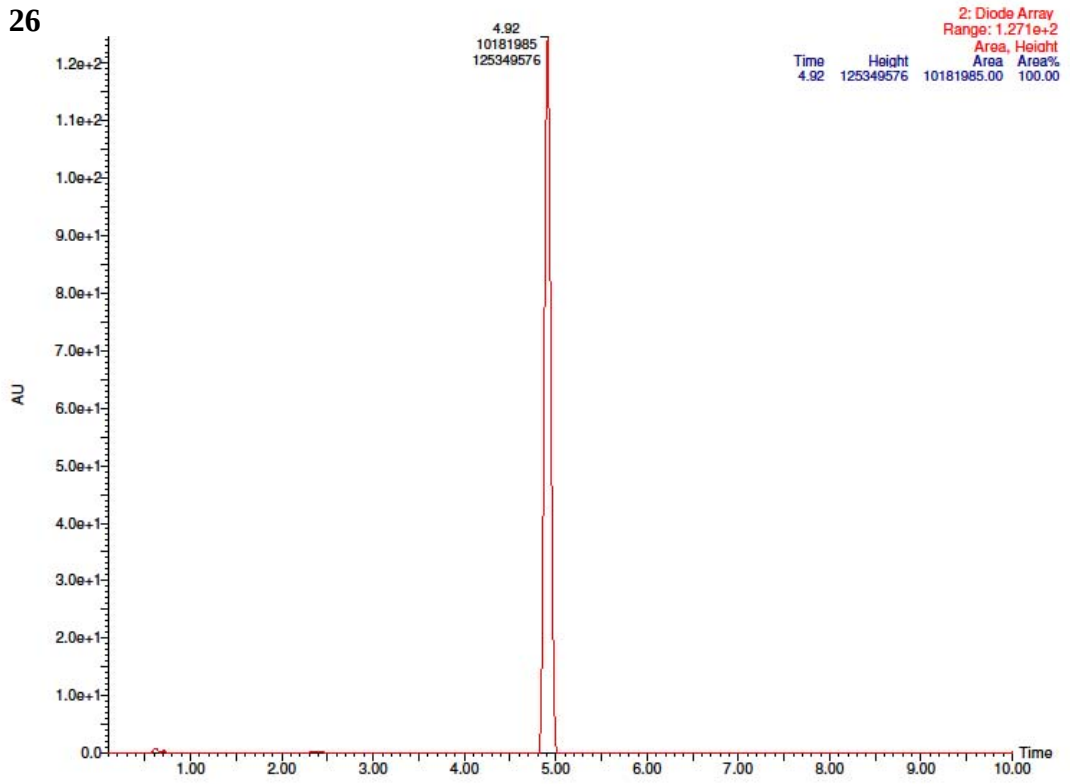
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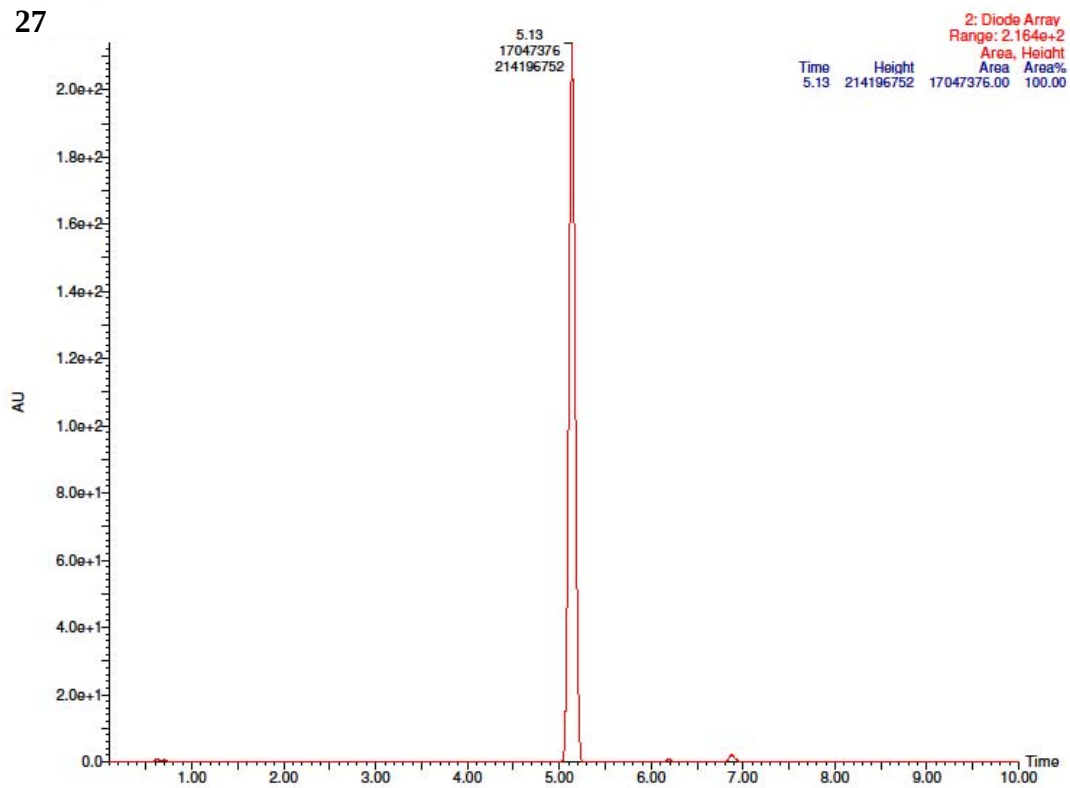
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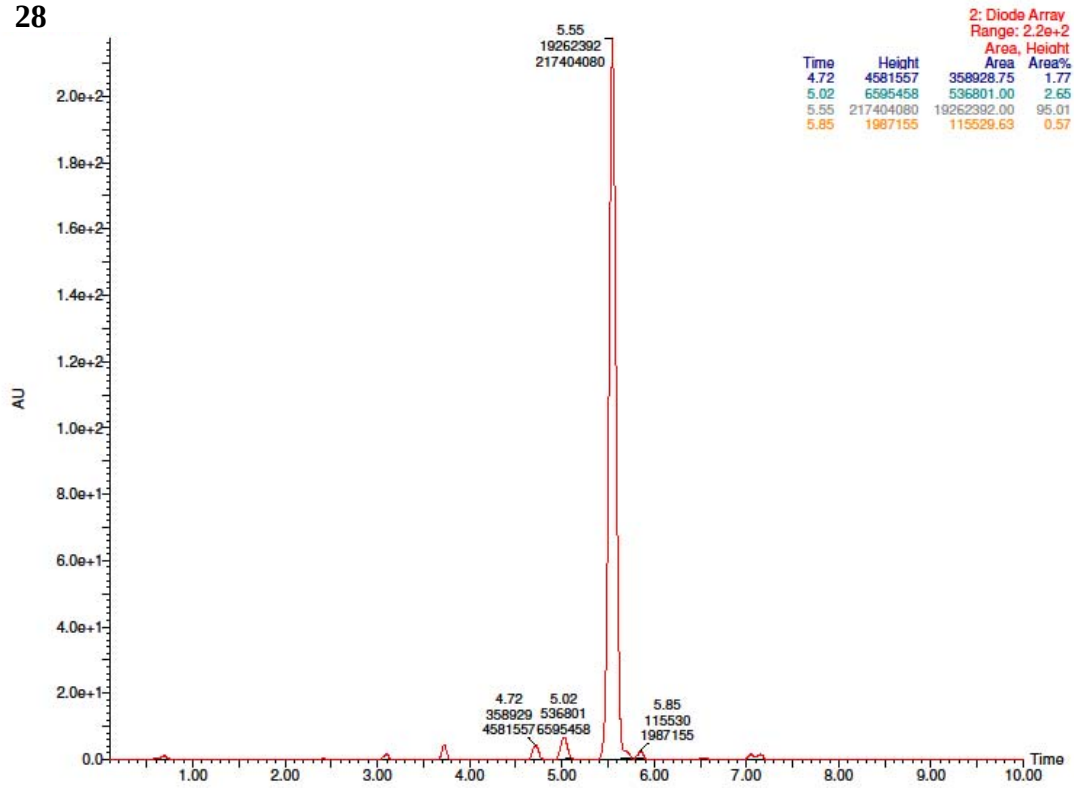
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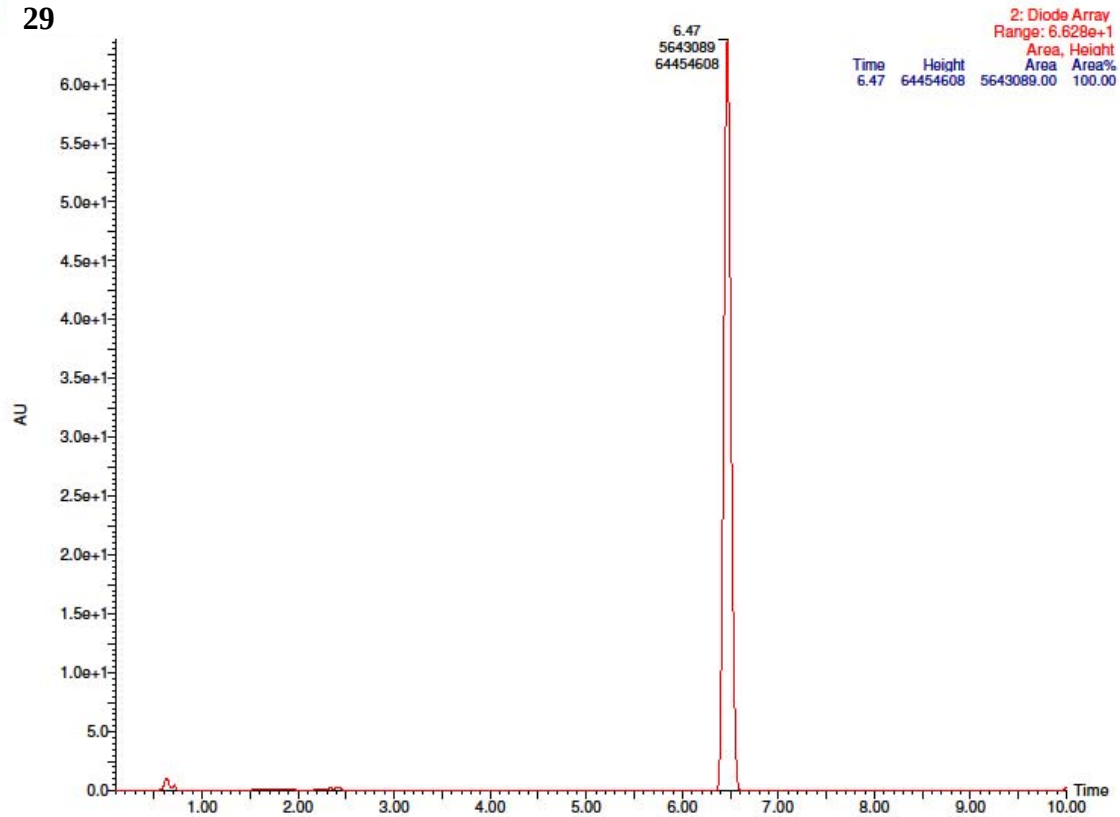
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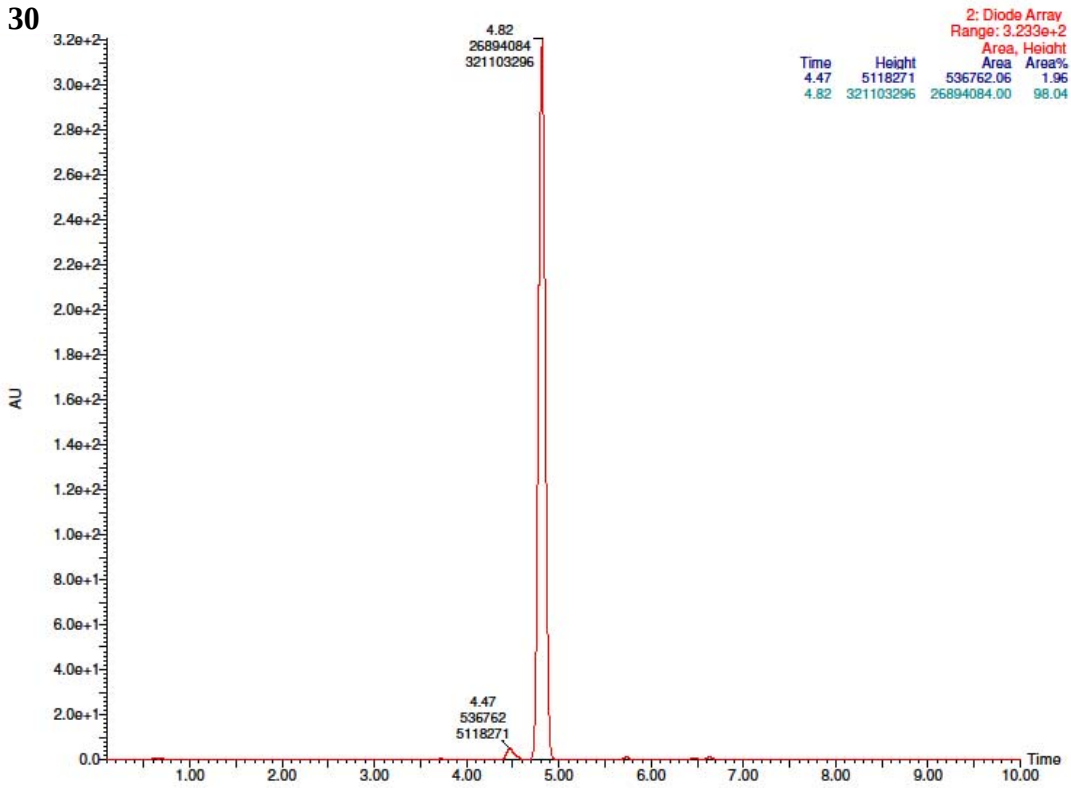
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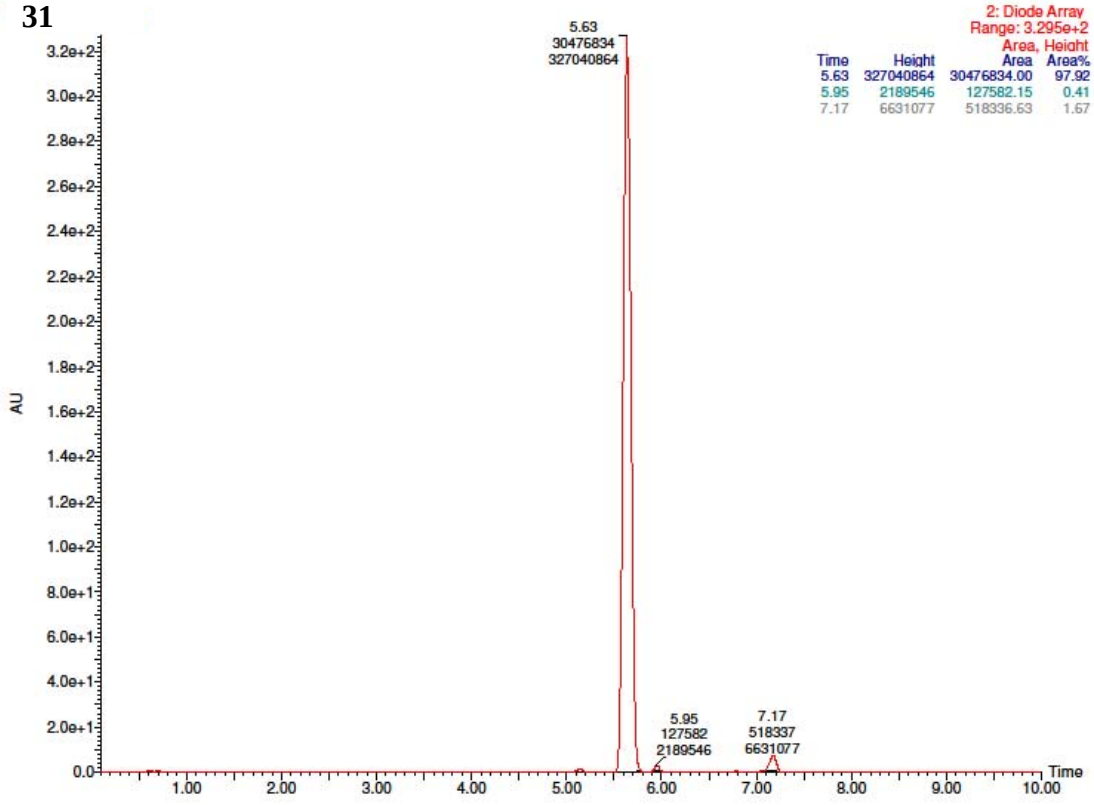
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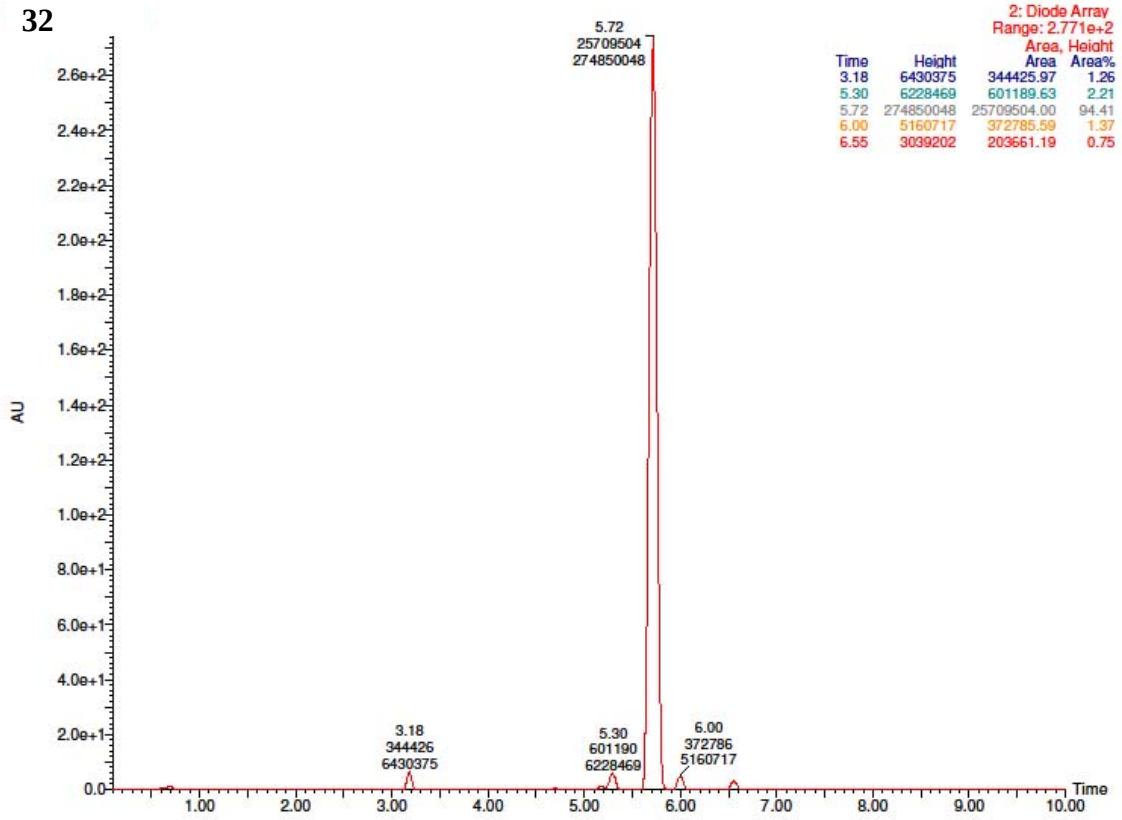
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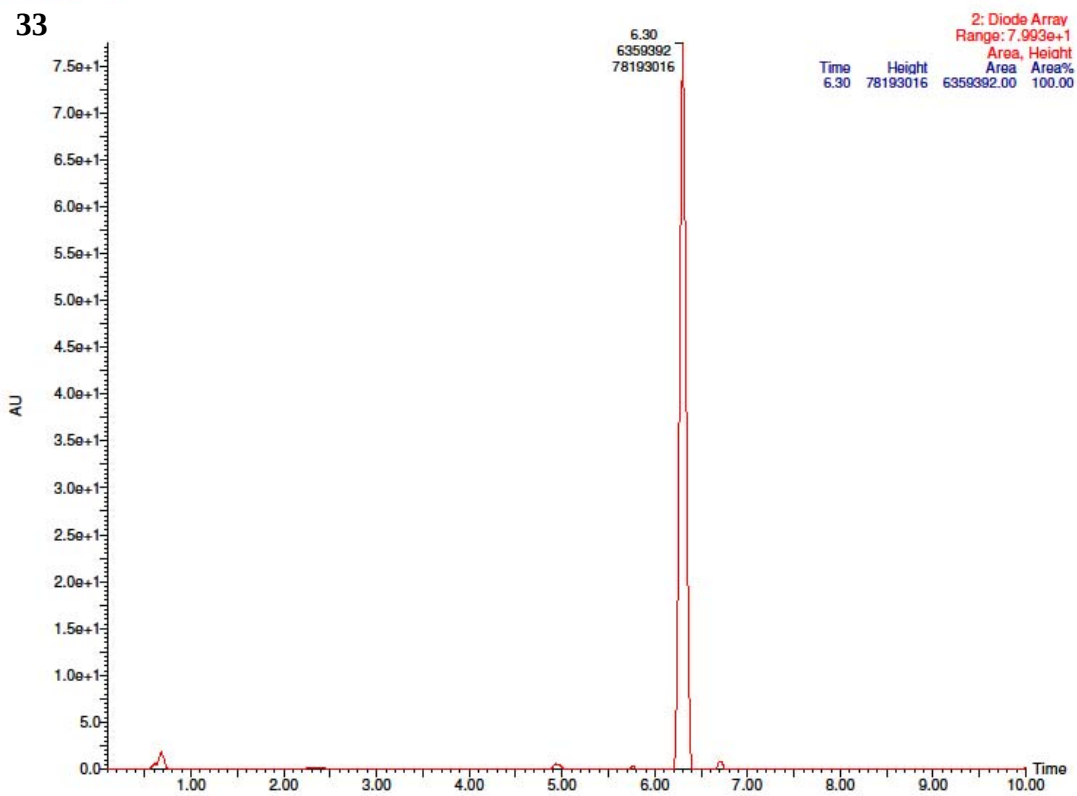
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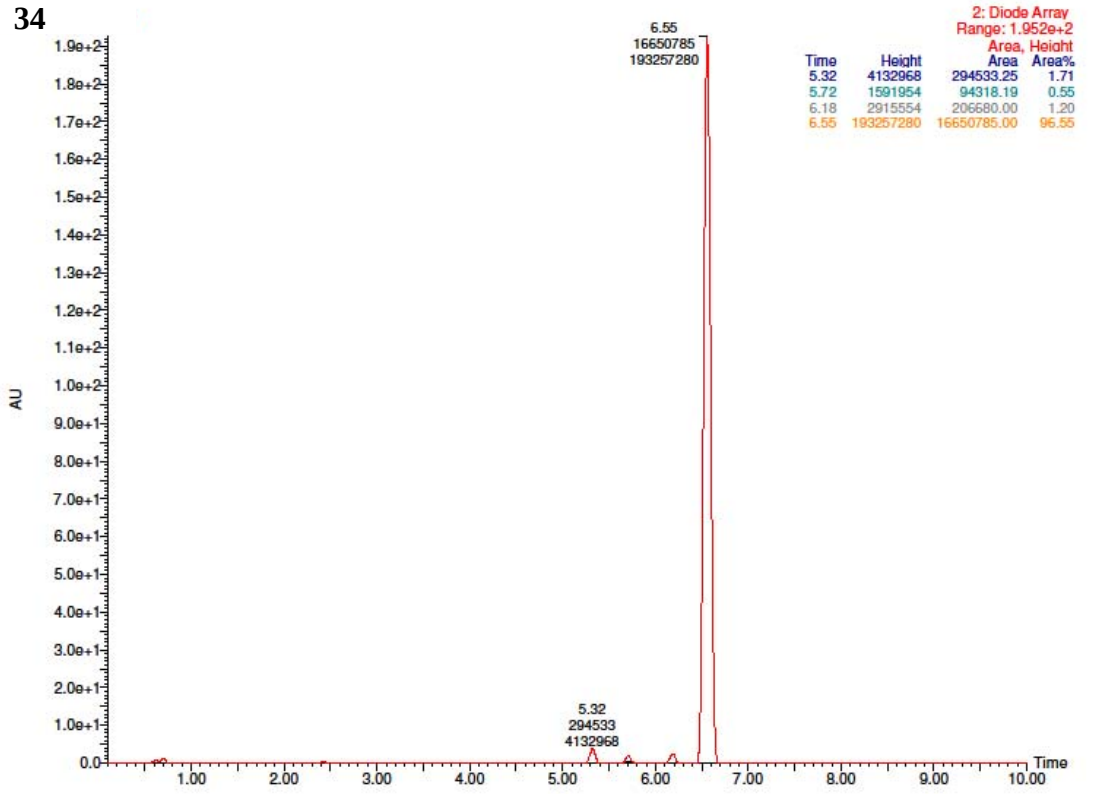
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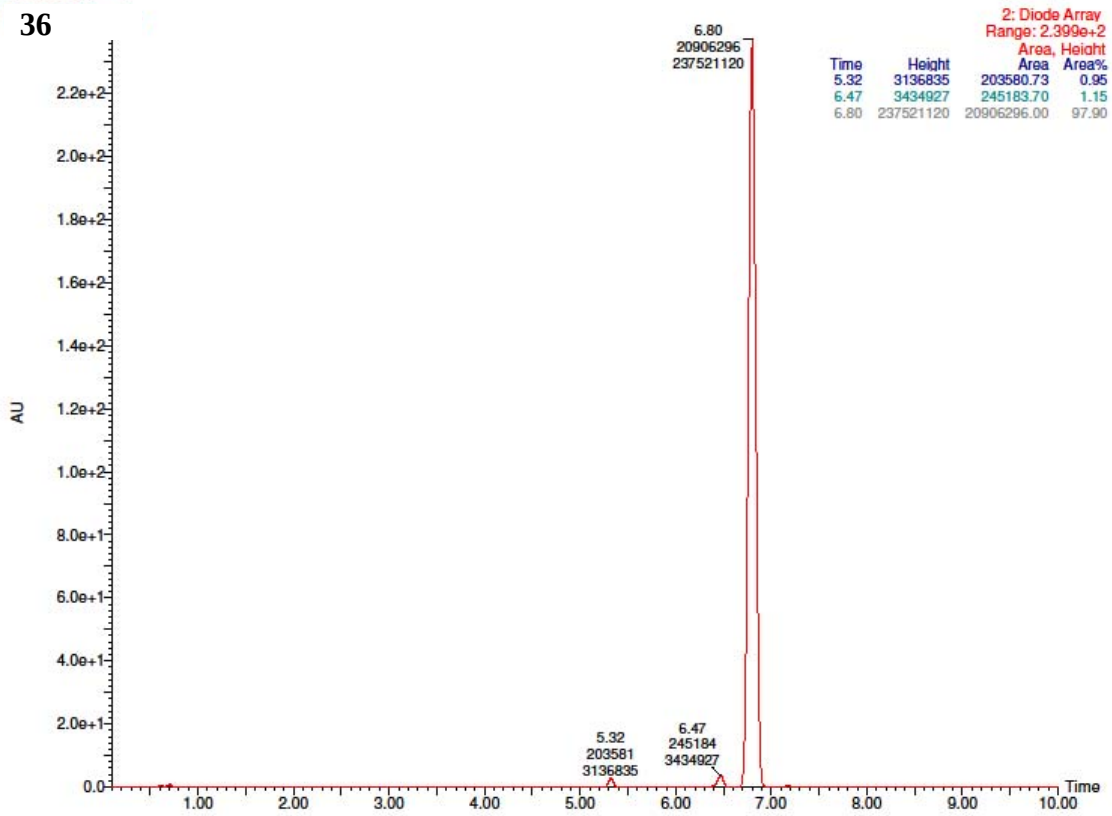
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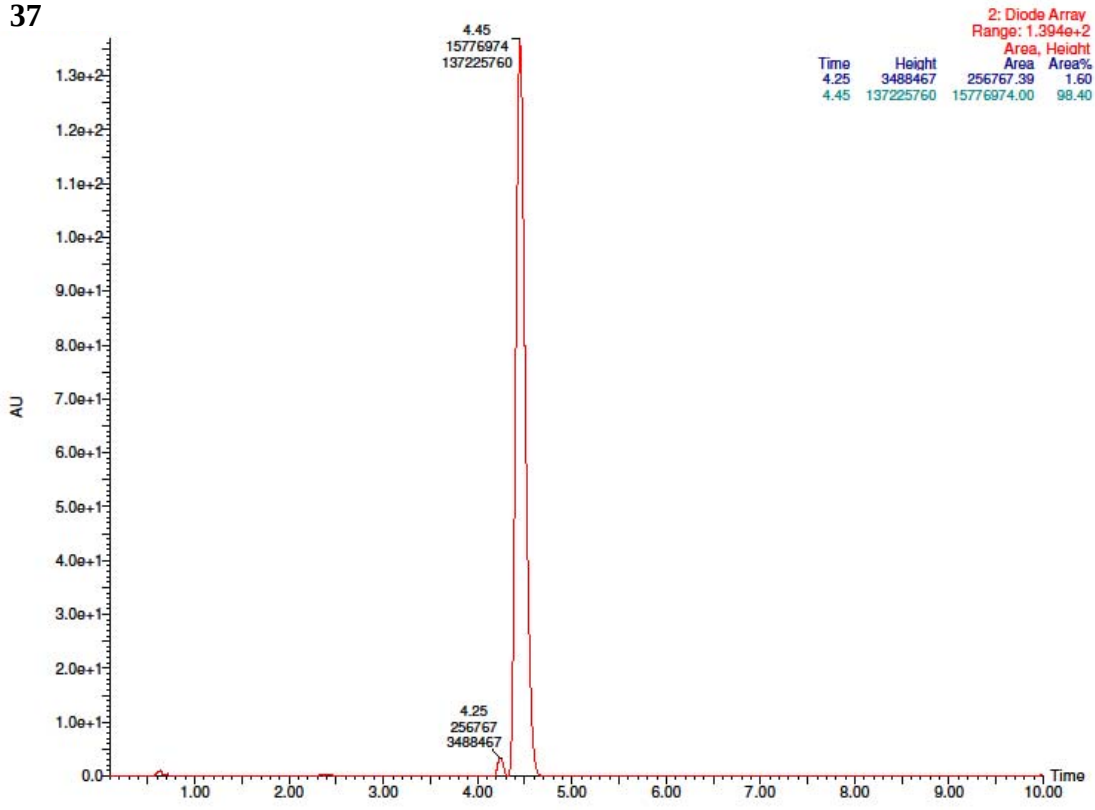
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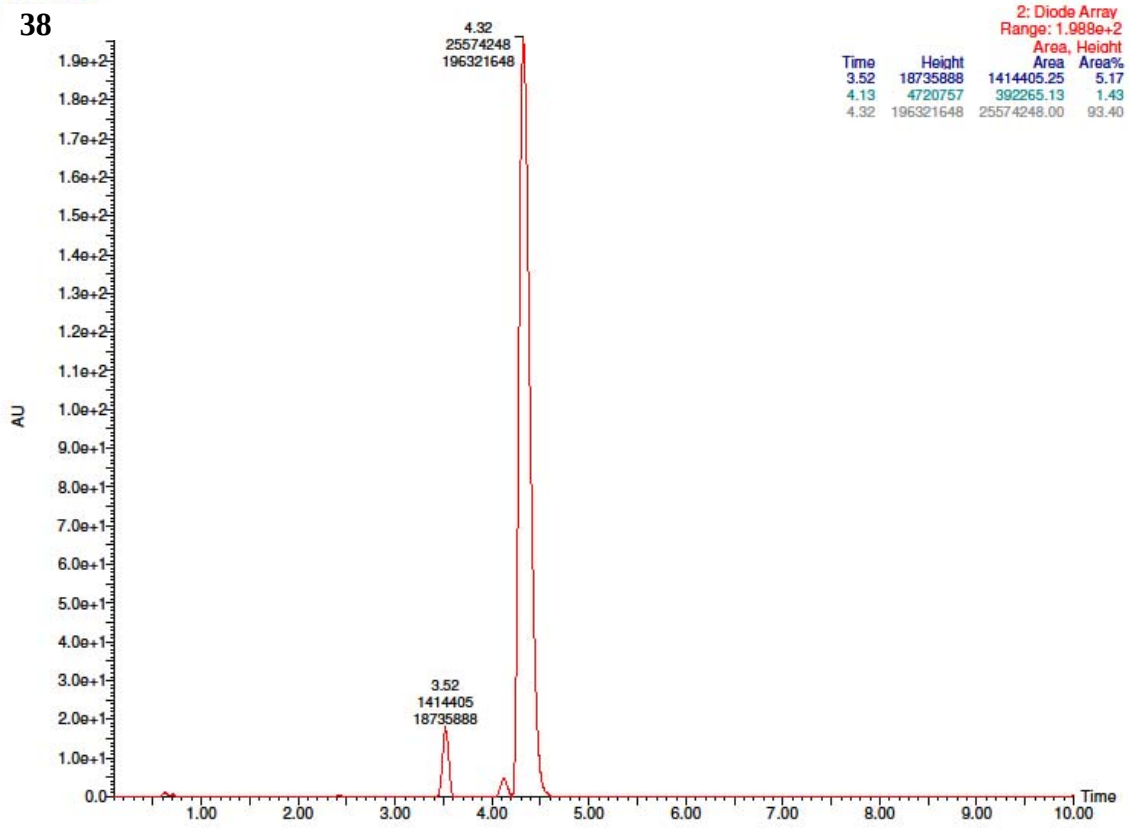
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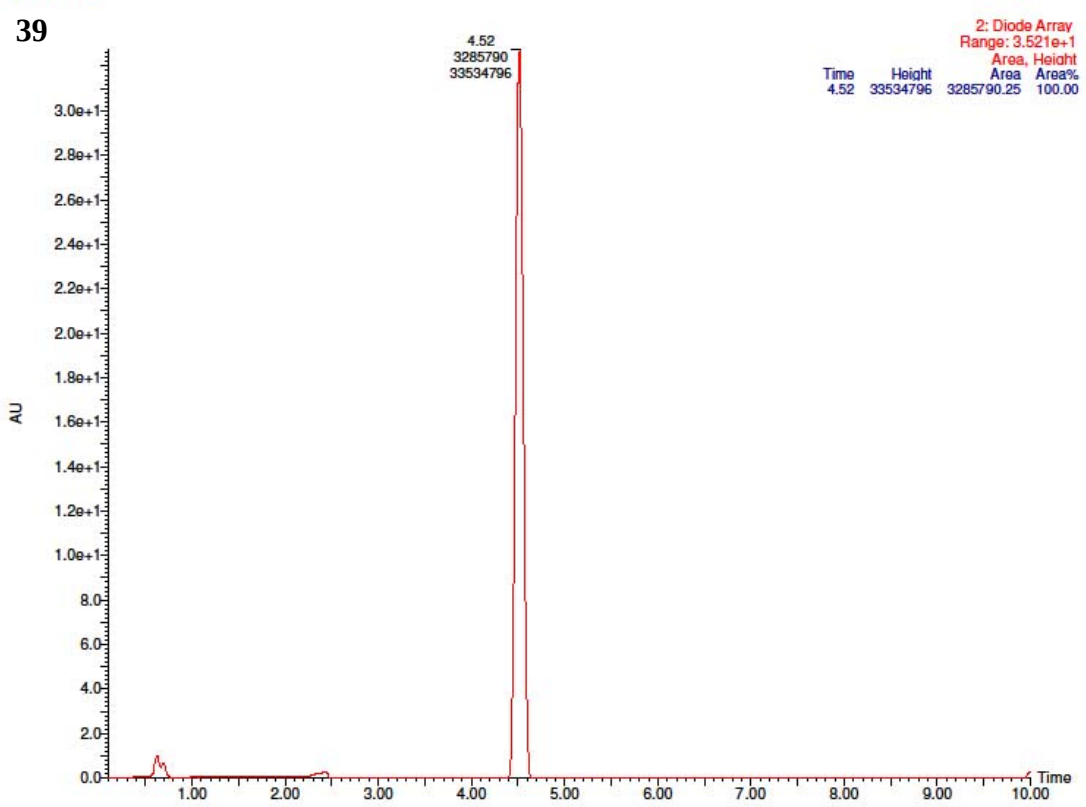
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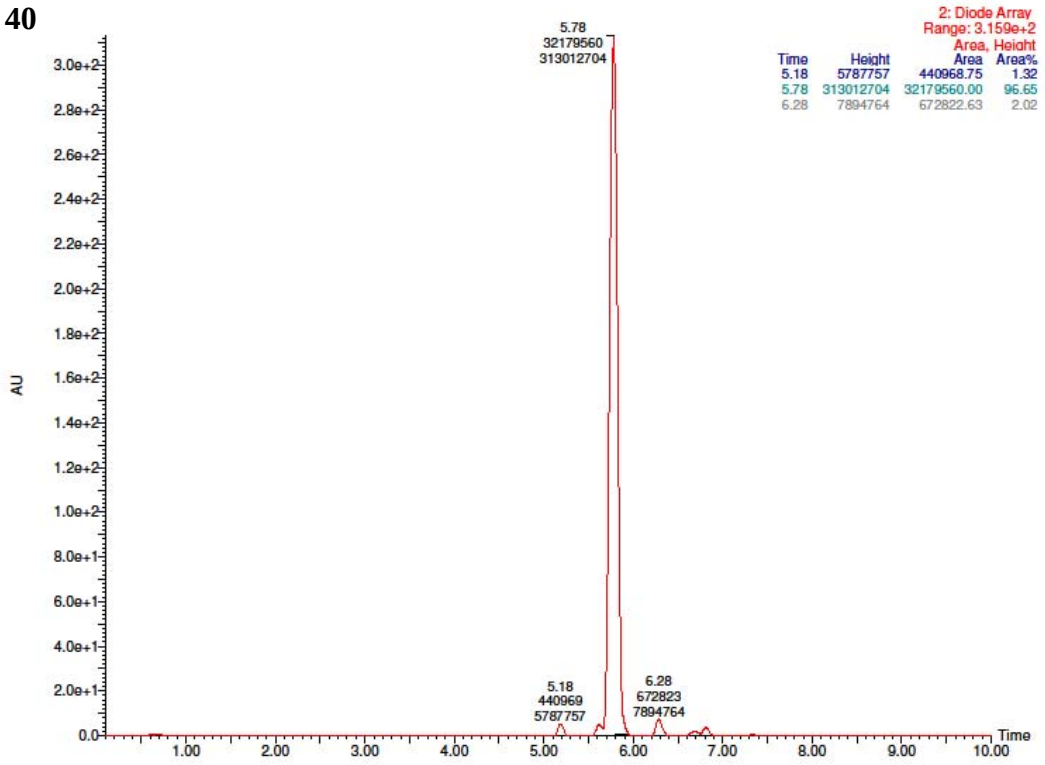
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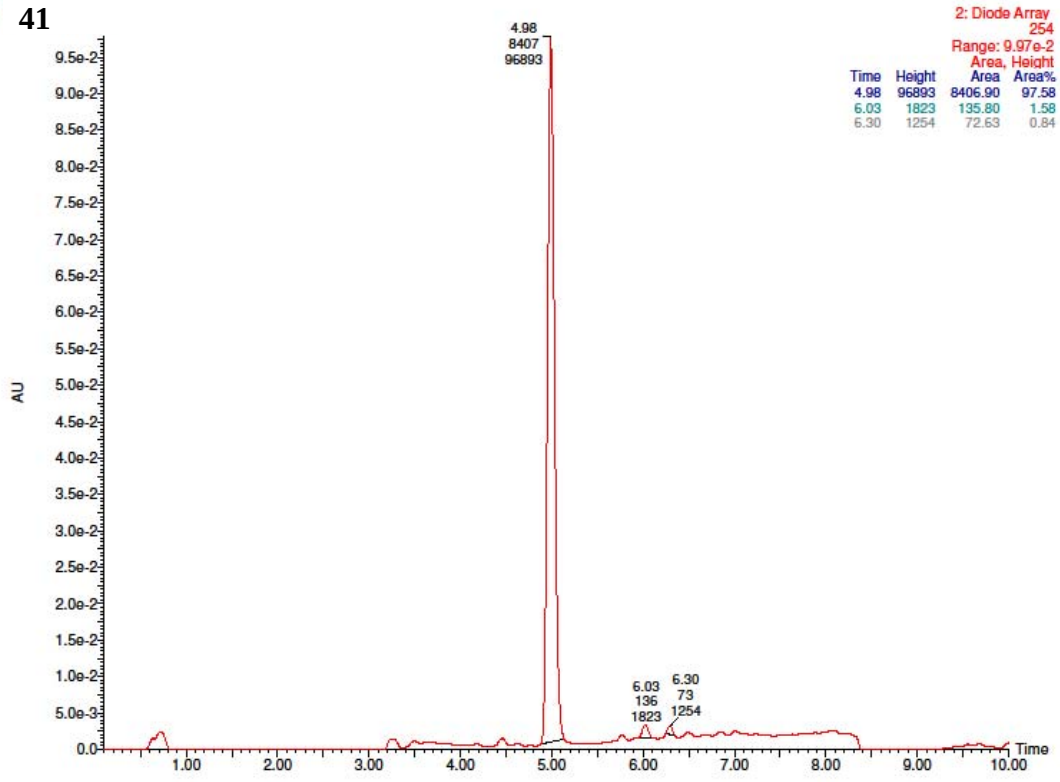
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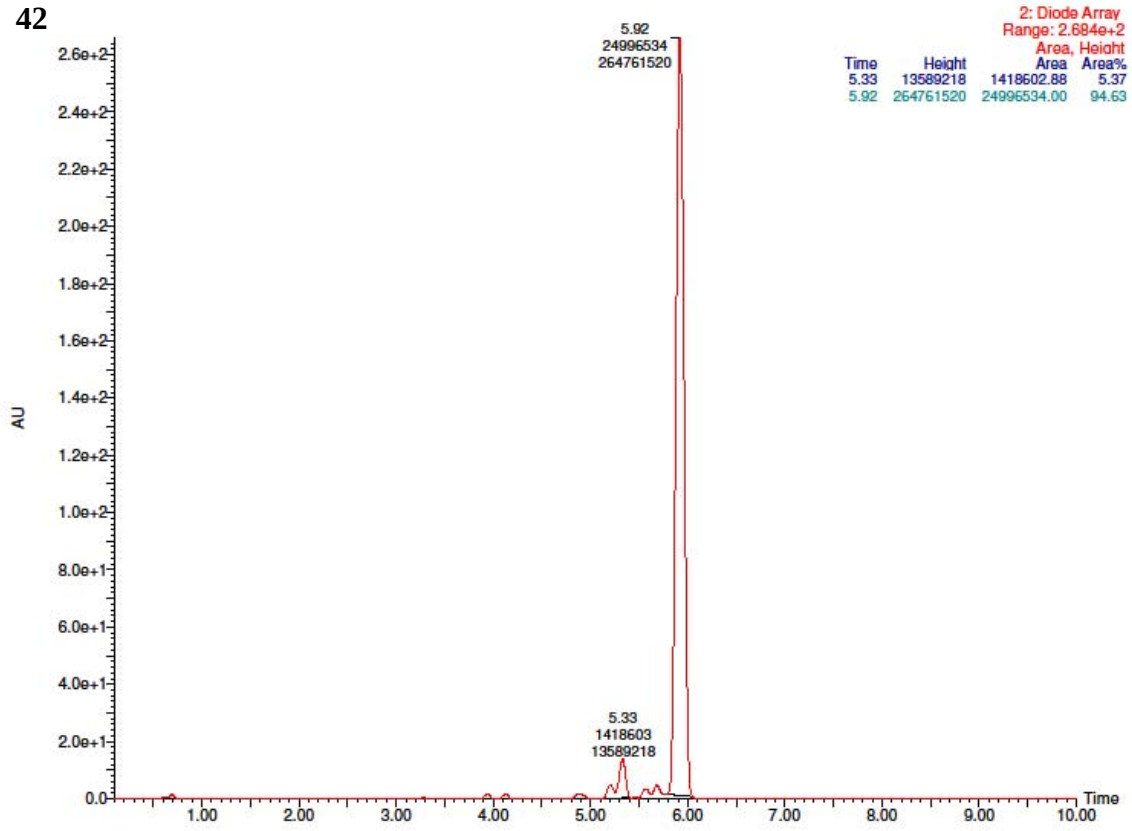
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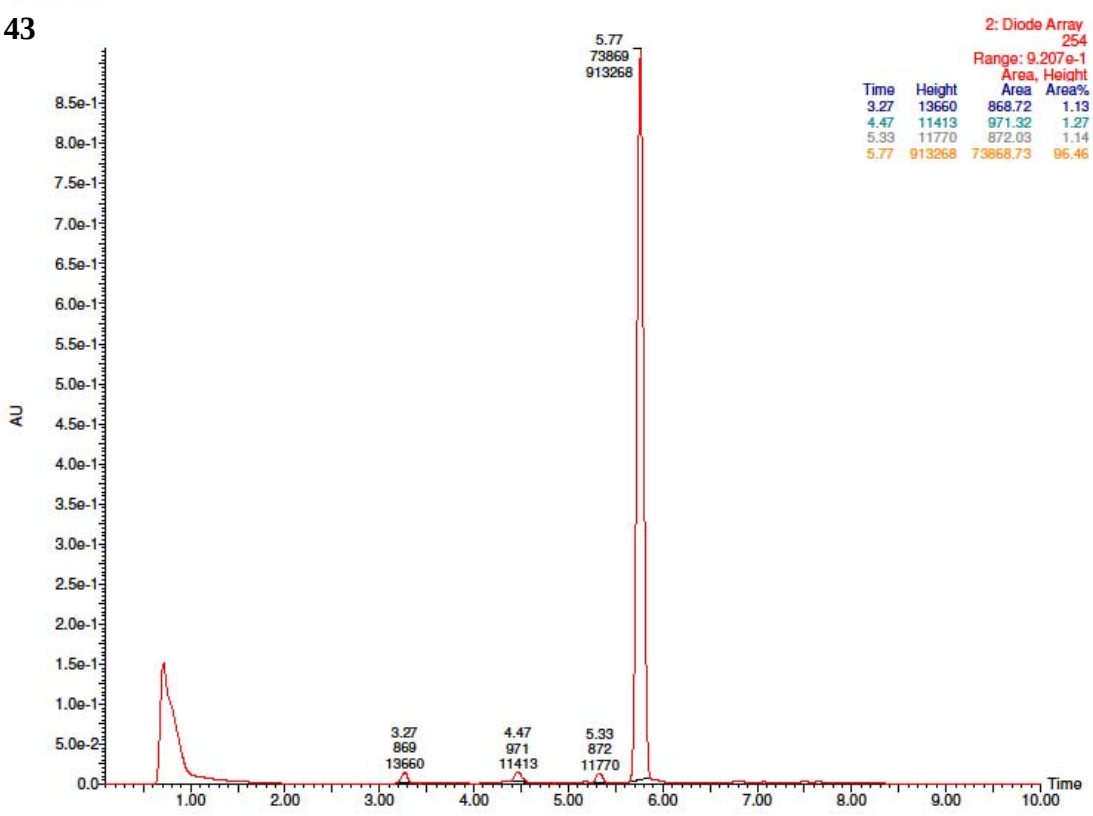
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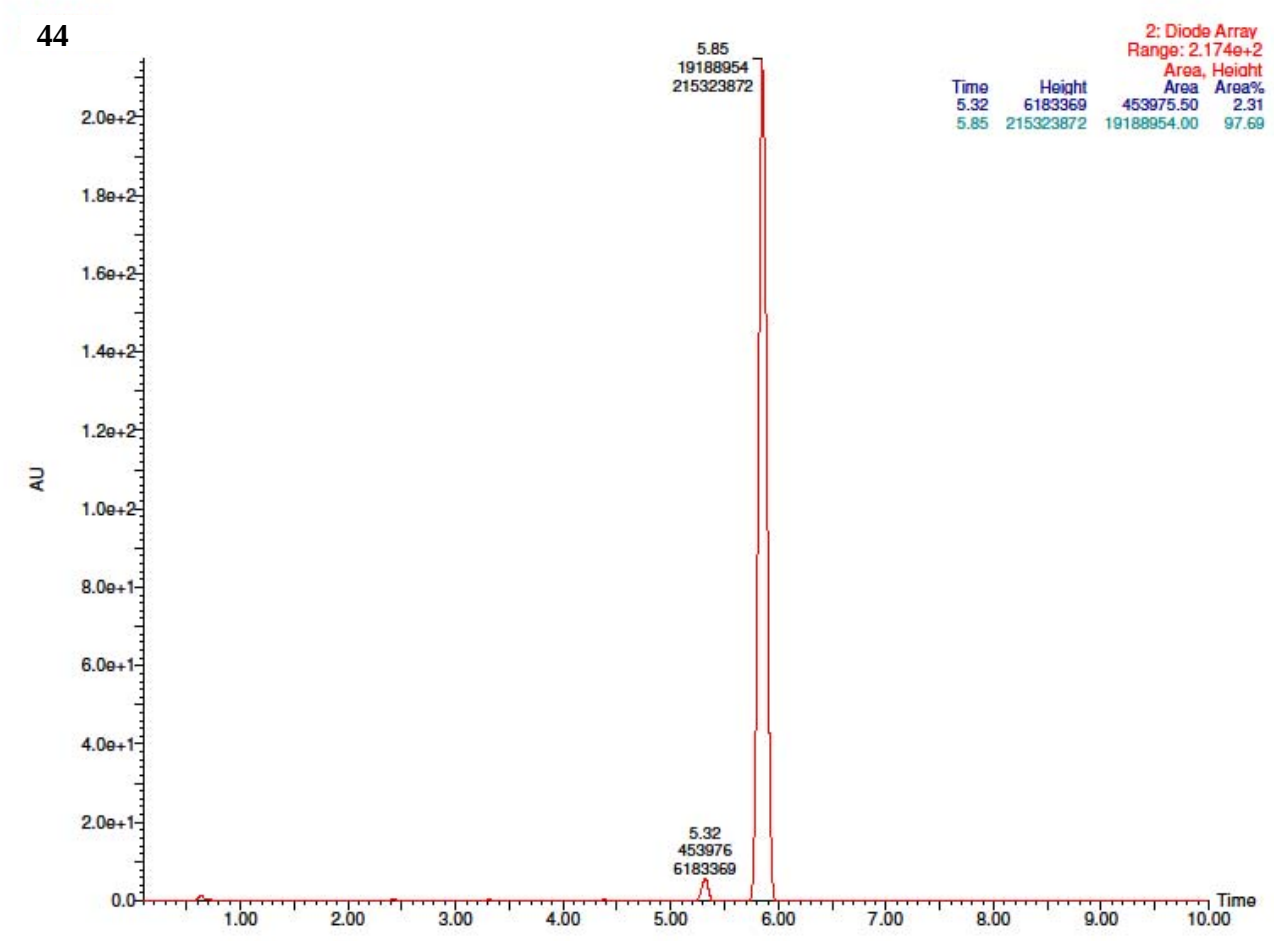
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