Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for:

Luciferin methyl ester illuminates the activity of multiple serine hydrolases

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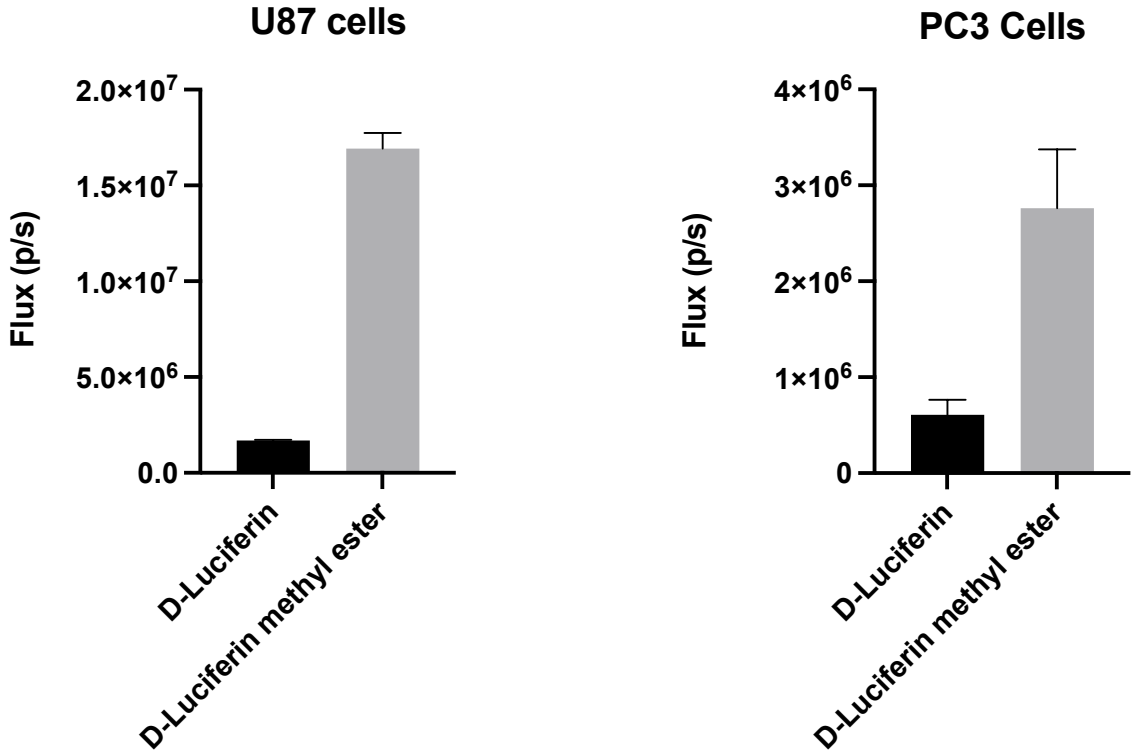

Figure S1. Comparison of bioluminescence from 10 μM D-luciferin and 10 μM D-luciferin methyl ester in luciferase-expressing U87 and PC3 cells.

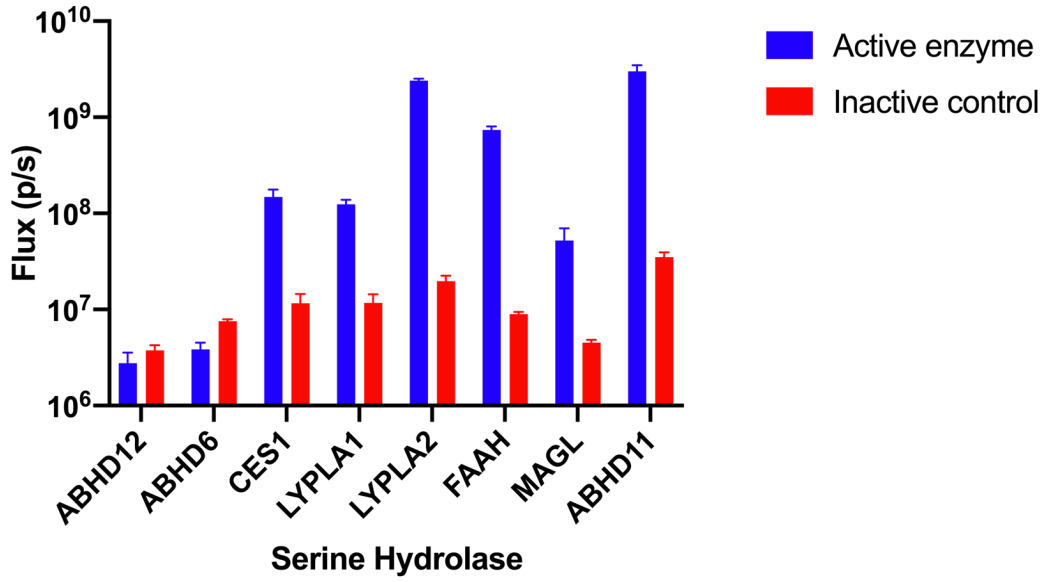

Figure S2. Effect of serine hydrolase expression on bioluminescence with 10 μM D-luciferin methyl ester in luciferase-expressing HEK293 cells. The active serine hydrolase is compared to its control inactive serine-to-alanine mutant. The ratio is reported in Figure 3.

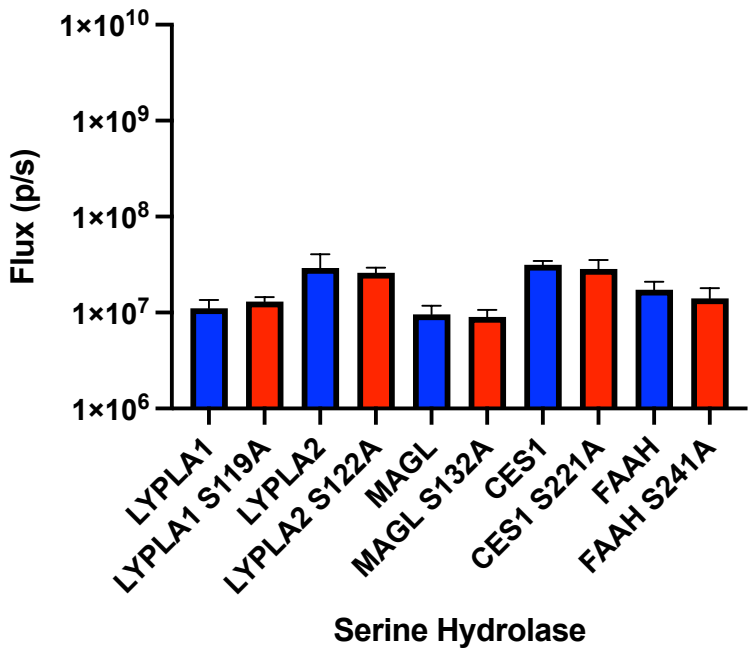

Figure S3. Effect of active vs inactive serine hydrolase expression on bioluminescence with 10 μM D-luciferin in luciferase-expressing HEK293 cells.

Experimental Procedures

Materials and Methods

General

Chemicals for synthesis were obtained from AstaTech, ChemImpex, or Sigma-Aldrich unless otherwise noted. D-luciferin was obtained from Gold Bio. Serine hydrolase inhibitors were purchased from Cayman. Cell lines were purchased from ATCC. Serine hydrolases cloned into pcDNA3.1 vectors were purchased from GenScript. Protein concentrations were determined using Coomassie Plus (Thermo Scientific). Bioluminescence assays were performed on a Xenogen IVIS-100 in the Small Animal Imaging facility. Data acquisition and analysis were performed with Living Image® software. Data were plotted and analyzed with GraphPad Prism 6.0. Data are reported as total flux (p/s) for each region of interest (ROI). For *in vitro* and cellular assays, the ROIs correspond to each well of a 96-well plate. NMR spectra were acquired on a Bruker Avance III HD 500 MHz NMR. High resolution mass spectral data were recorded on a Waters QTOF Premier spectrometer (University of Massachusetts Medical School Proteomics and Mass Spectrometry Facility).

Cell Culture

PC3, U87, and HEK293 cells were grown in a CO_2 incubator at 37°C with 5% CO_2 and were cultured in F-12K Nutrient Mixture (GIBCO) and Dulbecco's Modified Eagle's Medium (DMEM) (GIBCO) respectively. Both media were supplemented with 10% fetal bovine serum and 100 U/mL penicillin/streptomycin.

Transfections

Cells were transfected with codon-optimized firefly luciferase (luc2) as previously described.[1] Transient transfections were performed at RT using Lipofectamine 2000 on cells plated at 60%–80% confluency in 96-well black tissue culture-treated plates (Costar 3916). For PC3 and U87

cells, 0.075 µg DNA/well of pcDNA3.1-luc2 was transfected; for HEK293 cells, 0.0375 µg DNA/well each of pcDNA3.1-luc2 and either pcDNA3.1-serine hydrolase or control pcDNA3.1 serine hydrolase S>A mutant was transfected. Assays were performed in triplicate 48 hrs after transfection.

Live Cell Serine Hydrolase Activity Assays

Transfected cells were washed with HBSS. For inhibitor assays, the cells in 96-well plates were incubated with 50 µL of the indicated concentration of serine hydrolase inhibitor in HBSS at ambient temperature for 30 minutes. Then, 50 µL of 20 µM luciferin or luciferin ester substrate was added to each well to a final luciferin or luciferin ester concentration of 10 µM. Bioluminescence imaging was performed one minute after the addition of substrate.

Serine hydrolases used in this study. For each serine hydrolase, the indicated inactive serine-to-alanine mutant was expressed as a control:

MAGL (NP_009214.1)

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  1 metgpedpss mpeessprrt pqsipyqdlp hlvnadgqyl fcrywkptgt pkalifvshg
 61 agehsgryee larmlmgldl lvfahdhvgh gqsegermvv sdfhvfvrdv lqhvdsmqkd
121 ypglpvfllg hsmggaiail taaerpghfa gmvlisplvl anpesattfk vlaakvlnlv
181 lpnlslgpid ssvlsrnkte vdiynsdpli craglkvcfg iqllnavsrv eralpkltvp
241 flllqgsadr lcdskgayll melaksqdkt lkiyegayhv lhkelpevtn svfheinmwv
301 sqrtatagta spp
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MAGL S132A

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121 ypglpvfllg h[A]mggaiail taaerpghfa gmvlisplvl anpesattfk vlaakvlnlv
181 lpnlslgpid ssvlsrnkte vdiynsdpli craglkvcfg iqllnavsrv eralpkltvp
241 flllqgsadr lcdskgayll melaksqdkt lkiyegayhv lhkelpevtn svfheinmwv
301 sqrtatagta spp
```

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181 pfvhtnvpqs mfsydcsnpl fgqtvnpwks skspggssgg egaligsggs plglgtdigg
241 sirfpssfcg icglkptgnr lsksglkgcv ygqeavrlsv gpmardvesl alclrallce
301 dmfrldptvp plpfreevyt ssqplrvgyy etdnytmpsp amrravletk qsleaaghtl
361 vpflpsniph aletlstggl fsdgghtflq nfkgdfvdpc lgdlvsilkl pqwlkgllaf
421 lvkpllprls aflsnmksrs agklwelqhe ievyrktvia qwraldldvv ltpmlapald
481 lnapgratga vsytmlyncl dfpagvvpvt tvtaedeaqm ehyrgyfgdi wdkmlqkgmk
541 ksvglpvavq cvalpwqeel clrfmrever lmtpekqss
```

FAAH1 S241A

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241 [A]irfpssfcg icglkptgnr lsksglkgcv ygqeavrlsv gpmardvesl alclrallce
301 dmfrldptvp plpfreevyt ssqplrvgyy etdnytmpsp amrravletk qsleaaghtl
361 vpflpsniph aletlstggl fsdgghtflq nfkgdfvdpc lgdlvsilkl pqwlkgllaf
421 lvkpllprls aflsnmksrs agklwelqhe ievyrktvia qwraldldvv ltpmlapald
481 lnapgratga vsytmlyncl dfpagvvpvt tvtaedeaqm ehyrgyfgdi wdkmlqkgmk
541 ksvglpvavq cvalpwqeel clrfmrever lmtpekqss
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ABHD6 (NP_001307055.1)

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 61 cysfrgrpgh kpsilmlhgf sahkdmwlsv vkflpknlhl vcvdmpgheg ttrssldlls
121 idgqvkrihq fveclklnkk pfhlvgtsmg gqvagvyaay ypsdvsslcl vcpaglqyst
181 dnqfvqrlke lqgsaaveki plipstpeem semlqlcsyv rfkvpqqilq glvdvriphn
241 nfyrklflei vseksryslh qnmdkikvpt qiiwgkqdqv ldvsgadmla ksiancqvel
301 lencghsvvm erprktakli idflasvhnt dnnkkld
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ABHD6 S148A

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 61 cysfrgrpgh kpsilmlhgf sahkdmwlsv vkflpknlhl vcvdmpgheg ttrssldlls
121 idgqvkrihq fveclklnkk pfhlvgt[A]mg gqvagvyaay ypsdvsslcl vcpaglqyst
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181 dnqfvqrlke lqgsaaveki plipstpeem semlqlcsyv rfkvpqqilq glvdvriphn
241 nfyrklflei vseksryslh qnmdkikvpt qiiwgkqdqv ldvsgadmla ksiancqvel
301 lencghsvvm erprktakli idflasvhnt dnnkkld
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 61 algrrkgvwl rlrkilfcvl glyiaipfli klcpgiqakl iflnfvrvpy fidlkkpqdq
121 glnhtcnyyl qpeedvtigv whtvpavwwk naqgkdqmwy edalasshpi ilylhgnagt
181 rggdhrvely kvlsslgyhv vtfdyrgwgd svgtpsergm tydalhvfdw ikarsgdnpv
241 yiwghslgtg vatnlvrrlc eretppdali lespftnire eakshpfsvi yryfpgfdwf
301 fldpitssgi kfandenvkh iscpllilha eddpvvpfql grklysiaap arsfrdfkvq
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ABHD12 S246A

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121 glnhtcnyyl qpeedvtigv whtvpavwwk naqgkdqmwy edalasshpi ilylhgnagt
181 rggdhrvely kvlsslgyhv vtfdyrgwgd svgtpsergm tydalhvfdw ikarsgdnpv
241 yiwgh[A]lgtg vatnlvrrlc eretppdali lespftnire eakshpfsvi yryfpgfdwf
301 fldpitssgi kfandenvkh iscpllilha eddpvvpfql grklysiaap arsfrdfkvq
361 fvpfhsdlgy rhkyiykspe lprilreflg ksepehqh
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CES1 (NP_001020365.1, isoform b)

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241 hraisesgva ltsvlvkkgd vkplaeqiai tagcktttsa vmvhclrqkt eeellettlk
301 mkflsldlqg dpresqpllg tvidgmlllk tpeelqaern fhtvpymvgi nkqefgwlip
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421 iadvmfgvps vivarnhrda gaptymyefq yrpsfssdmk pktvigdhgd elfsvfgapf
481 lkegaseeei rlskmvmkfw anfarngnpn geglphwpey nqkegylqig antqaaqklk
541 dkevafwtnl fakkavekpp qtehiel
```

CES1 S221A

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  1 mwlrafilat lsasaawghp ssppvvdtvh gkvlgkfvsl egfaqpvaif lgipfakppl
 61 gplrftppqp aepwsfvkna tsyppmctqd pkagqllsel ftnrkenipl klsedclyln
121 iytpadltkk nrlpvmvwih ggglmvgaas tydglalaah envvvvtiqy rlgiwgffst
181 gdehsrgnwg hldqvaalrw vqdniasfgg npgsvtifge [A]aggesvsvl vlsplaknlf
241 hraisesgva ltsvlvkkgd vkplaeqiai tagcktttsa vmvhclrqkt eeellettlk
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481 lkegaseeei rlskmvmkfw anfarngnpn geglphwpey nqkegylqig antqaaqklk
541 dkevafwtnl fakkavekpp qtehiel
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LYPLA1 (CAG33384.1)

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 61 vtlnmnvamp swfdiiglsp dsqedesgik qaaenikali dqevkngips nriilggfsq
121 ggalslytal ttqqklagvt alscwlplra sfpqgpigga nrdisilqch gdcdplvplm
181 fgsltveklk tlvnpanvtf ktyegmmhss cqqemmdvkq fidkllppid
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LYPLA1 S119A

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  1 mcgnnmstpl paivpaarka taaviflhgl gdtghgwaea fagirsshik yicphapvrp
 61 vtlnmnvamp swfdiiglsp dsqedesgik qaaenikali dqevkngips nriilggf[A]q
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121 ggalslytal ttqqklagvt alscwlplra sfpqgpigga nrdisilqch gdcdplvplm
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LYPLA2 (NP_009191.1)

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121 fsqggalsly taltcphpla givalscwlp lhrafpqaan gsakdlailq chgeldpmvp
181 vrfgaltaek lrsvvtparv qfktypgvmh sscpqemaav keflekllpp v
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LYPLA2 S122A

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121 f**A**qggalsly taltcphpla givalscwlp lhrafpqaan gsakdlailq chgeldpmvp
181 vrfgaltaek lrsvvtparv qfktypgvmh sscpqemaav keflekllpp v
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ABHD11

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 61 dgeaalpavv flhglfgskt nfnsiakila qqtgrrvltv darnhgdsph spdmsyeims
121 qdlqdllpql glvpcvvvgh smggktamll alqrpelver liavdispve stgvshfaty
181 vaamrainia delprsrark ladeqlssvi qdmavrqhll tnlvevdgrf vwrvnldalt
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301 drpqdfiaai rgflv
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ABHD11 S141A

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121 qdlqdllpql glvpcvvvgh **A**mggktamll alqrpelver liavdispve stgvshfaty
181 vaamrainia delprsrark ladeqlssvi qdmavrqhll tnlvevdgrf vwrvnldalt
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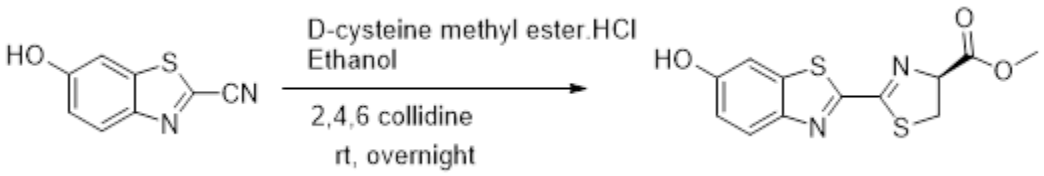
Synthesis of D-luciferin methyl ester

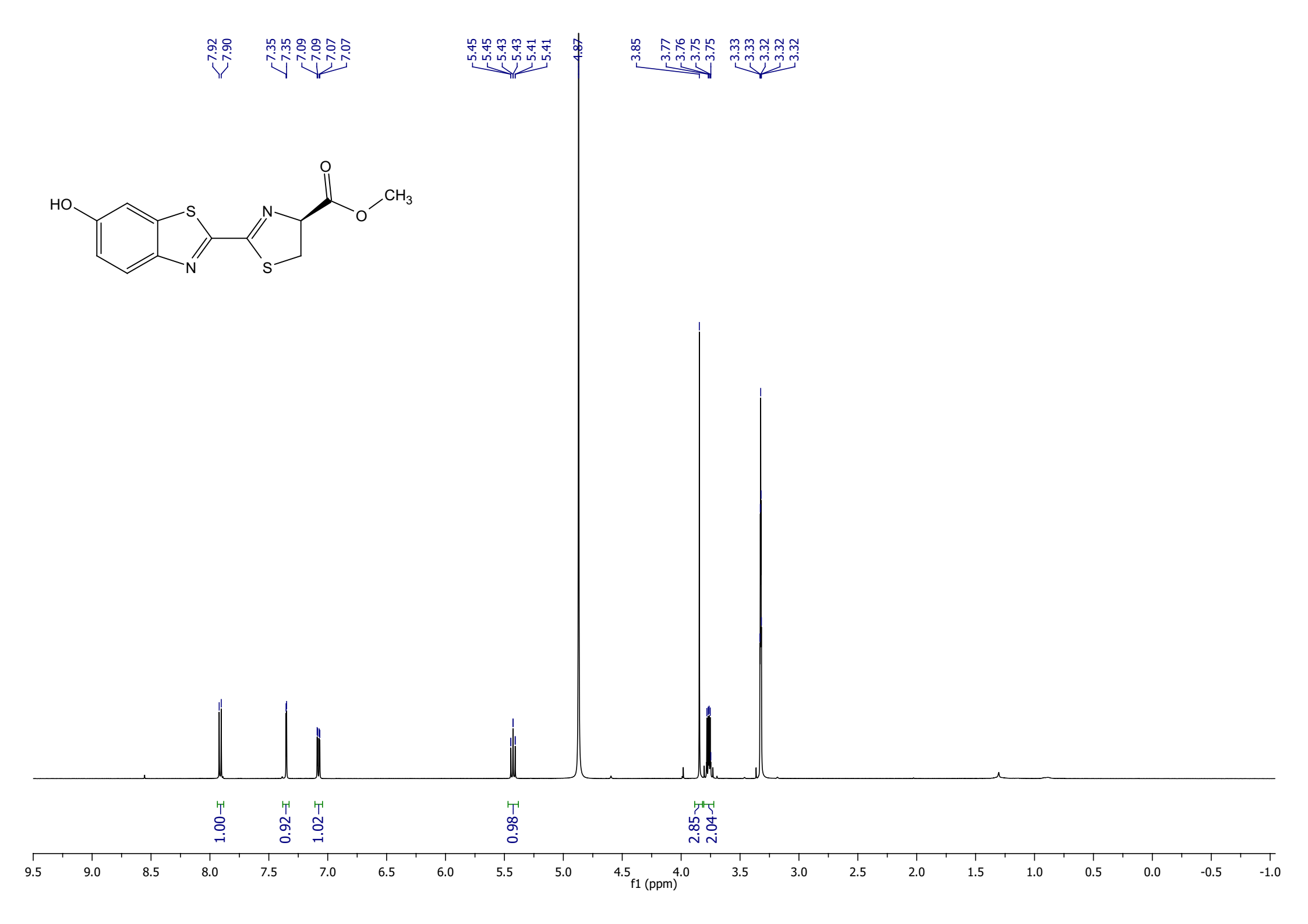


Methyl (S)-2-(6-hydroxybenzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylate

Methyl D-cysteinate hydrochloride salt (55 mg, 0.32 mmol) and 2,4,6-collidine (50 µL, 0.38 mmol) were dissolved in degassed ethanol (500 µL). This solution was added to a flask containing 6-hydroxybenzothiazole-2-carbonitrile (50 mg, 0.28 mmol) dissolved in 500 µL of degassed ethanol under argon gas. The reaction mixture was stirred at room temperature overnight. After the reaction progress was checked on TLC, ethanol was evaporated, and the crude reaction mixture was purified by flash column chromatography (0-30% acetone: hexanes). Pure fractions were combined and evaporated to yield D-luciferin methyl ester as an off-white solid (21.1 mg, 25%). ^{1}H NMR (500 MHz, CD_3OD): δ 7.91 (d, J = 8.9 Hz, 1H), 7.35 (d, J = 2.4 Hz, 1H), 7.08 (dd, J = 8.9, 2.4 Hz, 1H), 5.47 – 5.38 (m, 1H), 3.85 (s, 3H), 3.81 – 3.72 (m, 2H). Consistent with literature values.[2] Collidine has a low pKa of 7.4, so little if any racemization is expected, but we did not measure optical purity. HRMS [M + H]+ calcd for $C_{12}H_{11}N_2O_3S_2^+$: 295.0206 and found: 295.0205.

References

1. Mofford, D. M. *et al.* Luciferase Activity of Insect Fatty Acyl-CoA Synthetases with Synthetic Luciferins. *ACS Chem. Biol.* **12**, 2946–2951 (2017).
2. Rothweiler, U. *et al.* Luciferin and derivatives as a DYRK selective scaffold for the design of protein kinase inhibitors. *European Journal of Medicinal Chemistry* **94**, 140–148 (2015).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