Supporting Information

A novel class of succinimide-derived negative allosteric modulators of metabotropic glutamate receptor subtype 1 provides insight into a disconnect in activity between the rat and human receptors

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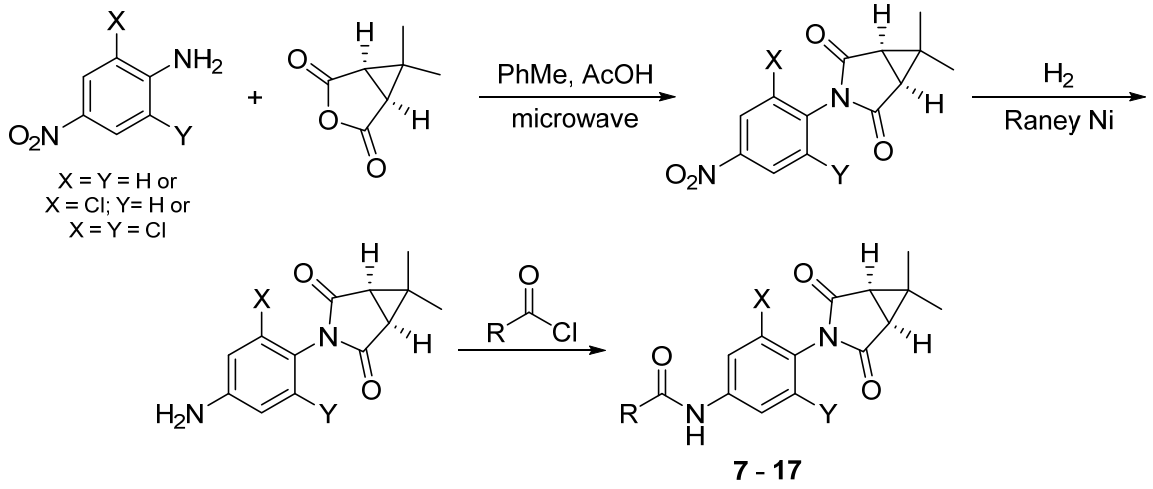
Synthetic Procedures and Characterization Data

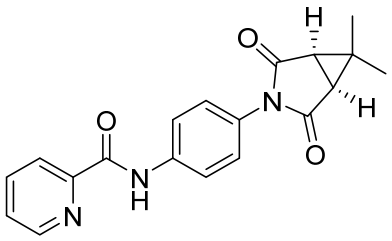
General. All NMR spectra were recorded on a 400 MHz AMX Bruker NMR spectrometer. ^{1}H chemical shifts are reported in δ values in ppm downfield with the deuterated solvent as the internal standard. Data are reported as follows: chemical shift (multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet), coupling constant, integration. Reversed-phase LCMS analysis was performed using an Agilent 1200 system comprising a binary pump with degasser, high-performance autosampler, thermostatted column

compartment, diode-array detector (DAD) and a C18 column. Flow from the column was split to a 6130 SQ mass spectrometer and Polymer Labs ELSD. The MS detector was configured with an electrospray ionization source. Data acquisition was performed with Agilent Chemstation and Analytical Studio Reviewer software. Samples were separated on a ThermoFisher Accucore C18 column (2.6 um, 2.1 x 30 mm) at 1.5 mL/min, with column and solvent temperatures maintained at 45 C. The gradient conditions were 7% to 95% acetonitrile in water (0.1% TFA) over 1.1 minutes. Low-resolution mass spectra were acquired by scanning from 135 to 700 AMU in 0.25 seconds with a step size of 0.1 AMU and peak width of 0.03 minutes. Drying gas flow was 11 liters per minute at a temperature of 350 C and a nebulizer pressure of 40 psi. The capillary needle voltage was 3000 V, and the fragmentor voltage was 100V.

General Experimental Procedure I. Compounds **7-17** were prepared via the route pictured immediately below. The synthesis of compound **7** is described as representative for this set of analogs. All compounds were ≥ 95% pure as measured by UV spectroscopy at 215 and 254 nm.



***N*-(4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)picolinamide (7)**

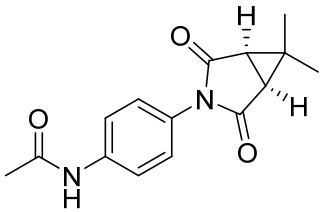


To a solution of 4-nitroaniline (276 mg, 2.0 mmol) in toluene:acetic acid (3:1, 4.5 mL) was added caronic anhydride (308 mg, 2.2 mmol) and the mixture was heated to 150 ºC in the microwave for 60 min. After LCMS confirmed the product, the reaction was added to EtOAc:$NaHCO_3$ (saturated) (1:1, 100 mL) and the organic layer was separated, washed with brine and dried ($MgSO_4$). After the organic layer was filtered and concentrated, the material was carried through to the next step without further purification.

6,6-dimethyl-3-(4-nitrophenyl)-3-azabicyclo[3.1.0]hexane-2,4-dione was dissolved in EtOAc: MeOH (3:1, 100 mL) and subjected to an Argon atmosphere. Raney-Nickel (50 mg) was added. The reaction was evacuated and purged with Argon (3x). The reaction was evacuated and purged with hydrogen (1 atm) (3x). After 3h at rt, no starting material was evident by LCMS. The reaction was filtered through Celite, and the Celite was thoroughly washed with MeOH (2 x 50 mL). The solvent was removed and the material (>80% pure by LCMS) was taken forward to the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.79 (d, J = 8.6 Hz, 2H), 6.58 (d, J = 8.7 Hz, 2H), 5.30 (br s, 2H), 2.59 (s, 2H), 1.29 (s, 3H), 1.24 (s, 3H). HPLC R_T = 0.122 min. ES–MS $[M+H]^+$: 231.2. HRMS, calculated for $C_{13}H_{15}N_2O_2$ ($M+H^+$), 231.1134; found 231.1136.

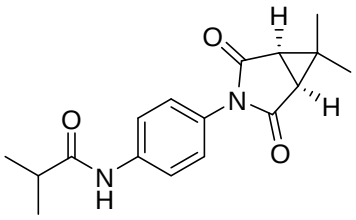
To a solution of 3-(4-aminophenyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione (48 mg, 0.210 mmol) in DMF (3mL) was added triethylamine (0.07 mL, 0.520mmol), followed by picolinoyl chloride hydrochloride (41mg, 0.230mmol) at rt. The reaction was monitored by LCMS. After 3 h at rt, the reaction was purified directly by HPLC reverse phase chromatography (20-75% acetonitrile:water w/0.1% TFA). The fractions were collected and added to EtOAc:$NaHCO_3$ (saturated) (1:1, 20 mL). The organic layer was separated, washed with brine and dried ($MgSO_4$). The organic layer was filtered and concentrated to afford *N*-(4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)picolinamide (27 mg, 39% yield over 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (br s, 1H), 8.77-8.75 (m, 1H), 8.18 (ddd, J=7.8, 1.2, 1.2 Hz, 1H), 8.09 (ddd, J=7.6, 7.6, 1.7 Hz, 1H), 7.99, (d, J=8.9 Hz, 2H), 7.70 (ddd, J=7.5, 4.8, 1.2 Hz, 1H), 7.59 (d, J=9.1 Hz, 2H), 2.69 (s, 2H), 1.34 (s, 3H), 1.28 (s, 3H). HPLC R_T = 0.714 min. ES–MS $[M+H]^+$: 370.0. HRMS, calculated for $C_{19}H_{18}N_3O_3$ ($M+H^+$), 336.1348; found 336.1350.

_N_-(4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)acetamide (8)



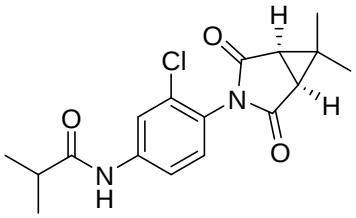
HPLC R_T = 0.488 min. ES–MS $[M+H]^+$: 273.1.

_N_-(4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)isobutyramide (9)



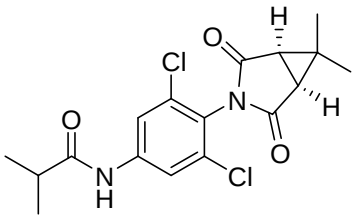
HPLC R_T = 0.594 min. ES–MS $[M+H]^+$: 301.1.

***N*-(3-chloro-4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)isobutyramide (10)**



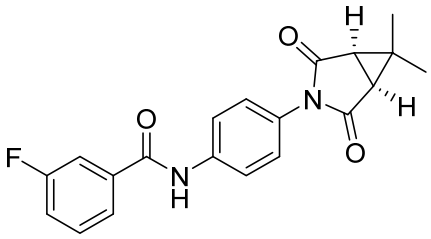
HPLC R_T = 0.653 min. ES–MS $[M+H]^+$: 335.0.

***N*-(3,5-dichloro-4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)isobutyramide (11)**



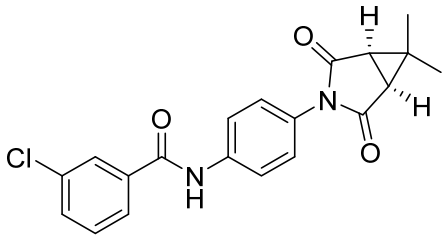
HPLC R_T = 0.725 min. ES–MS $[M+H]^+$: 369.0.

***N*-(4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)-3-fluorobenzamide (12)**



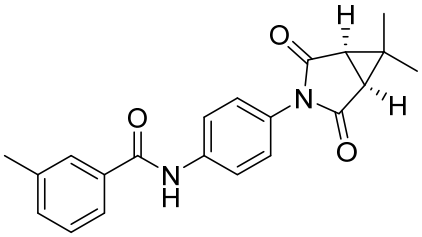
HPLC R_T = 0.679 min. ES–MS $[M+H]^+$: 353.0.

3-chloro-*N*-(4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)benzamide (13)



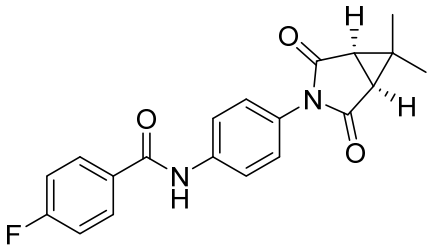
HPLC R_T = 0.724 min. ES–MS $[M+H]^+$: 369.0.

***N*-(4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)-3-methylbenzamide (14)**



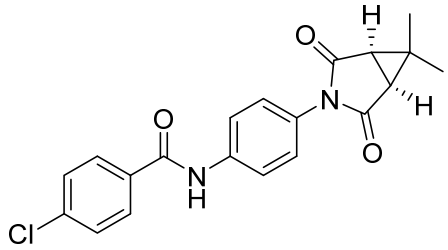
HPLC R_T = 0.702 min. ES–MS $[M+H]^+$: 349.1.

***N*-(4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)-4-fluorobenzamide (15)**



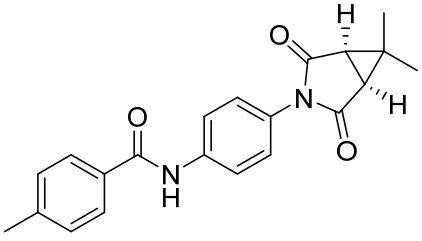
HPLC R_T = 0.671 min. ES–MS $[M+H]^+$: 353.0.

4-chloro-*N*-(4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)benzamide (16)



HPLC R_T = 0.719 min. ES–MS $[M+H]^+$: 369.0.

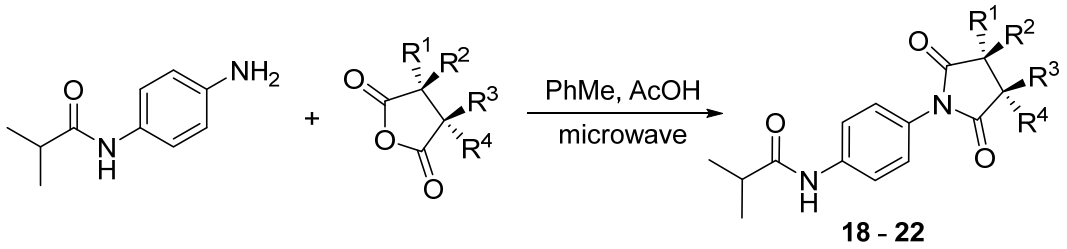
***N*-(4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)-4-methylbenzamide (17)**



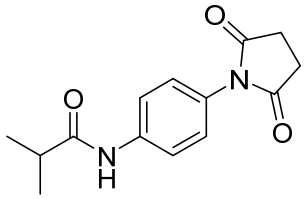
HPLC R_T = 0.697 min. ES–MS $[M+H]^+$: 349.1.

General Experimental Procedure II. Compounds **18-22** were prepared via a single step from commercially available *N*-(4-aminophenyl)isobutyramide as pictured immediately below. The

synthesis of compound **18** is described as representative for this set of analogs. All compounds were ≥ 95% pure as measured by UV spectroscopy at 215 and 254 nm.



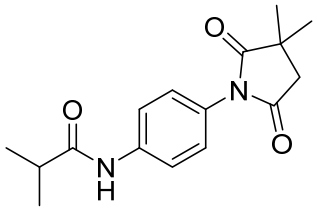
***N*-(4-(2,5-dioxopyrrolidin-1-yl)phenyl)isobutyramide (18)**



To a solution of 1-(4-aminophenyl)pyrrolidine-2,5-dione (50 mg, 0.28 mmol) in toluene:acetic acid (3:2, 2.5 mL) was added succinic anhydride (42 mg, 0.42 mmol) and the mixture was heated to 150 ºC in the microwave for 60 min. After LCMS confirmed conversion to product, the reaction was added to EtOAc:$NaHCO_3$ (saturated) (1:1, 30 mL) and the organic layer was separated, washed with brine and dried ($MgSO_4$). After the organic layer was filtered and concentrated, the crude material was purified by HPLC reverse phase chromatography (20-75% acetonitrile:water w/0.1% TFA). The fractions were collected and added to EtOAc:$NaHCO_3$ (saturated) (1:1, 20 mL). The organic layer was separated, washed with brine and dried ($MgSO_4$). The organic layer was filtered and concentrated to afford *N*-(4-(2,5-dioxopyrrolidin-1-yl)phenyl)isobutyramide (36 mg, 49% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (br s, 1H), 7.69 (d, J=8.8 Hz, 2H), 7.17 (d, J=8.8 Hz, 2H), 2.86 (s, 4H), 2.61 (dq, J=6.8, 6.8, 6.8, 6.8 Hz,

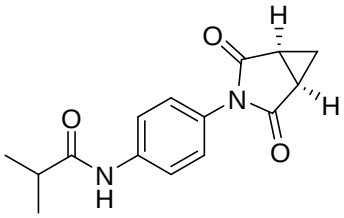
1H), 1.22 (d, J=6.7 Hz, 6H). HPLC R_T = 0.401 min. ES–MS $[M+H]^+$: 261.1. HRMS, calculated for $C_{14}H_{17}N_2O_3$ ($M+H^+$), 261.1239; found 261.1236.

***N*-(4-(3,3-dimethyl-2,5-dioxopyrrolidin-1-yl)phenyl)isobutyramide (19)**



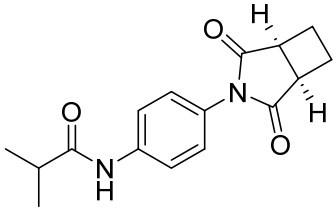
HPLC R_T = 0.563 min. ES–MS $[M+H]^+$: 289.1.

***N*-(4-(2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)phenyl)isobutyramide (20)**



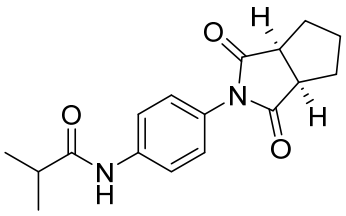
HPLC R_T = 0.479 min. ES–MS $[M+H]^+$: 273.1.

***N*-(4-(2,4-dioxo-3-azabicyclo[3.2.0]heptan-3-yl)phenyl)isobutyramide (21)**



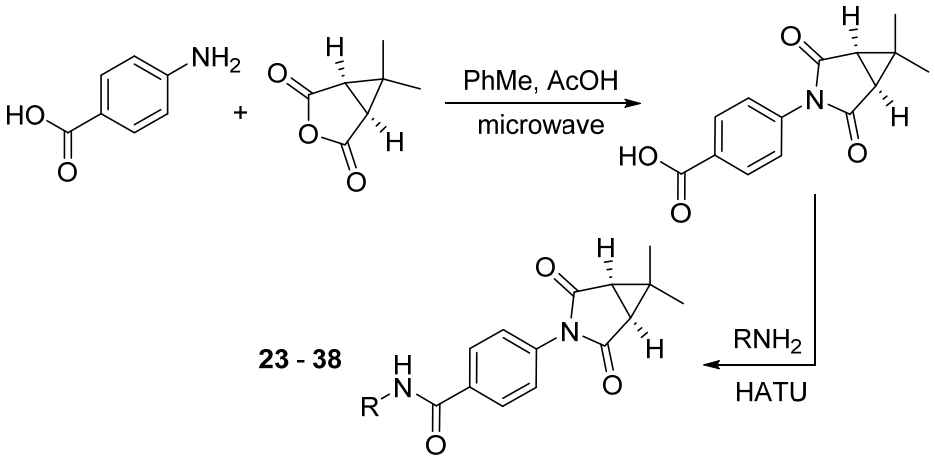
HPLC R_T = 0.514 min. ES–MS $[M+H]^+$: 287.1.

***N*-(4-(1,3-dioxohexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)phenyl)isobutyramide (22)**

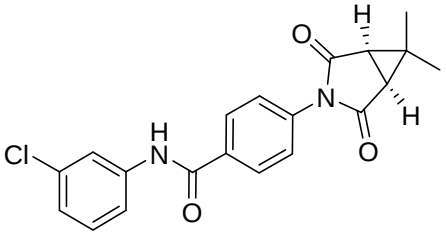


HPLC R_T = 0.575 min. ES–MS $[M+H]^+$: 301.1.

General Experimental Procedure III. Compounds **23-38** were prepared via a two-step sequence as pictured immediately below. The synthesis of compound **29** is described as representative for this set of analogs. All compounds were ≥ 95% pure as measured by UV spectroscopy at 215 and 254 nm.



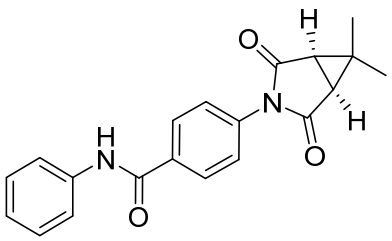
***N*-(3-chlorophenyl)-4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)benzamide (29)**



To a solution of 4-aminobenzoic acid (274 mg, 2.0 mmol) in toluene:acetic acid (3:1, 4.5 mL) was added caronic anhydride (308 mg, 2.2 mmol) and the mixture was heated to 150 ºC in the microwave for 60 min. After LCMS confirmed the product, the reaction was added to EtOAc:$NaHCO_3$ (saturated) (1:1, 100 mL) and the organic layer was separated, washed with brine and dried ($MgSO_4$). After the organic layer was filtered and concentrated, the material was carried through to the next step without further purification. HPLC R_T = 0.522 min. ES–MS $[M+H]^+$: 260.0.

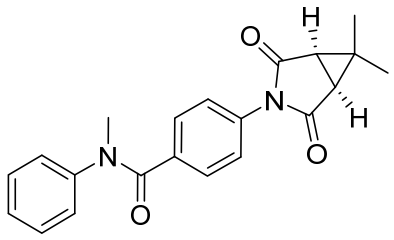
To a solution of 4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)benzoic acid (25 mg, 0.1 mmol), triethylamine (21 µl, 0.15 mmol), 3-chloroaniline (14mg, 0.11 mmol) in DMF (1mL) was added HATU (42mg, 0.11 mmol) at rt. After 16 h, the reaction was purified directly by HPLC reverse phase chromatography (25-90% acetonitrile:water w/0.1% TFA). The fractions were dried down with an air concentrator to provide *N*-(3-chlorophenyl)-4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)benzamide (21 mg, 60% yield). ^1H NMR (400 MHz, DMSO-d_6) δ 10.48 (br s), 8.03 (d, J=8.4 Hz, 2H), 7.97 (s, 1H), 7.71 (d, J=8.0 Hz, 1H), 7.43 (d, J=8.4 Hz, 2H), 7.40 (t, J=8.0 Hz, 1H), 7.18 (d, J=7.8 Hz, 1H), 2.74 (s, 2H), 1.35 (s, 3H), 1.29 (s, 3H). HPLC R_T = 0.750 min. ES–MS $[M+H]^+$: 369.0. HRMS, calculated for $C_{20}H_{18}N_2O_3Cl$ ($M+H^+$), 369.1006; found 369.1007.

4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)-*N*-phenylbenzamide (23)



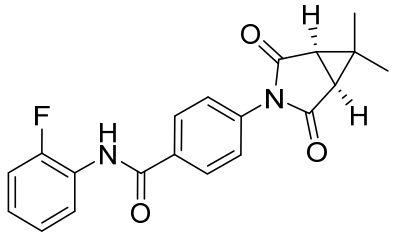
HPLC R_T = 0.676 min. ES–MS $[M+H]^+$: 335.0.

4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)-*N*-methyl-*N*-phenylbenzamide (24)



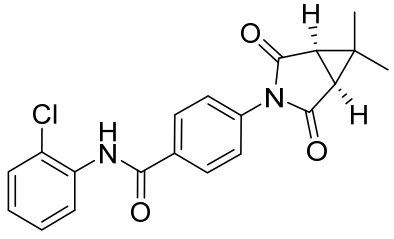
HPLC R_T = 0.668 min. ES–MS $[M+H]^+$: 349.1.

4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)-*N*-(2-fluorophenyl)benzamide (25)



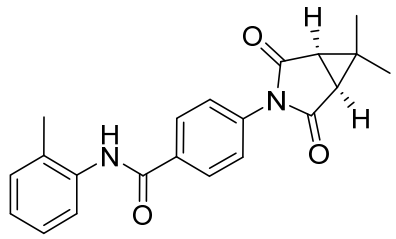
HPLC R_T = 0.672 min. ES–MS $[M+H]^+$: 353.0.

***N*-(2-chlorophenyl)-4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)benzamide (26)**



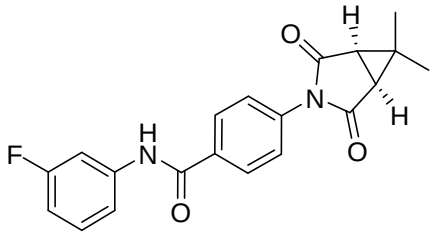
HPLC R_T = 0.716 min. ES–MS $[M+H]^+$: 369.0.

4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)-*N*-(o-tolyl)benzamide (27)



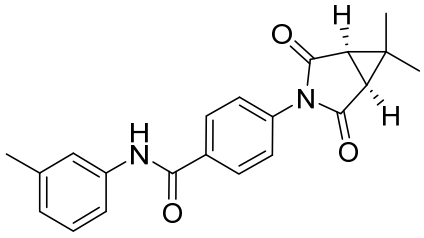
HPLC R_T = 0.673 min. ES–MS $[M+H]^+$: 349.1.

4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)-*N*-(3-fluorophenyl)benzamide (28)



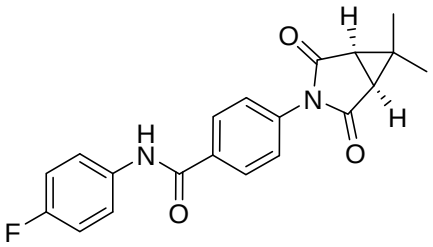
HPLC R_T = 0.710 min. ES–MS $[M+H]^+$: 353.0.

4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)-*N*-(*m*-tolyl)benzamide (30)



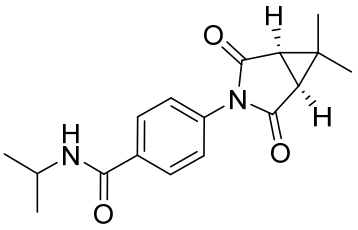
HPLC R_T = 0.718 min. ES–MS $[M+H]^+$: 349.0.

4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)-*N*-(4-fluorophenyl)benzamide (31)



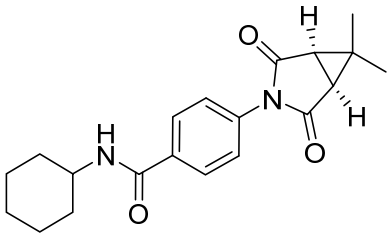
HPLC R_T = 0.694 min. ES–MS $[M+H]^+$: 353.0.

4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)-*N*-isopropylbenzamide (32)



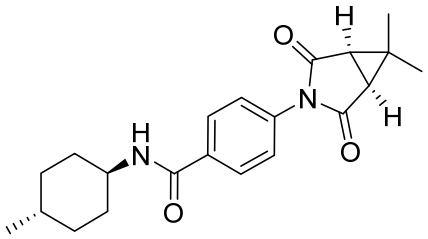
HPLC R_T = 0.576 min. ES–MS $[M+H]^+$: 301.1.

***N*-cyclohexyl-4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)benzamide (33)**



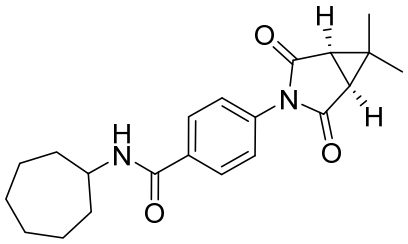
HPLC R_T = 0.692 min. ES–MS $[M+H]^+$: 341.0.

4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)-*N*-(*trans*-4-methylcyclohexyl)benzamide (34)



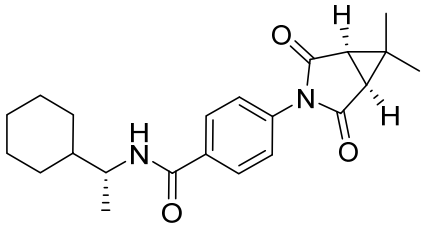
HPLC R_T = 0.737 min. ES–MS $[M+H]^+$: 355.0.

_N_-cycloheptyl-4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)benzamide (35)



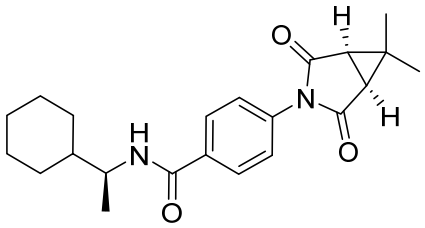
HPLC R_T = 0.729 min. ES–MS $[M+H]^+$: 355.1.

_N_-((_R_)-1-cyclohexylethyl)-4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)benzamide (36)



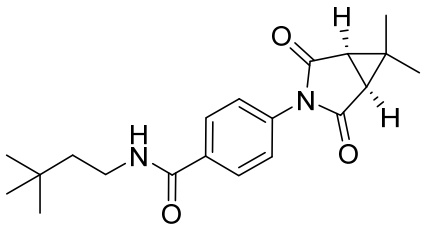
HPLC R_T = 0.767 min. ES–MS $[M+H]^+$: 369.1.

_N_-((_S_)-1-cyclohexylethyl)-4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)benzamide (37)



HPLC R_T = 0.769 min. ES–MS $[M+H]^+$: 369.0.

4-(6,6-dimethyl-2,4-dioxo-3-azabicyclo[3.1.0]hexan-3-yl)-*N*-(3,3-dimethylbutyl)benzamide (38)



HPLC R_T = 0.723 min. ES–MS $[M+H]^+$: 343.1.