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Tricyclic Thiazolopyrazole Derivatives as Metabotropic Glutamate Receptor 4 Positive Allosteric Modulators

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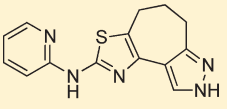
Supporting Information

ABSTRACT: There is an increasing amount of evidence to support that activation of the metabotropic glutamate receptor 4 (mGlu4 receptor), either with an orthosteric agonist or a positive allosteric modulator (PAM), provides impactful interventions in diseases such as Parkinson's disease, anxiety, and pain. mGlu4 PAMs may have several advantages over mGlu4 agonists for a number of reasons. As part of our efforts in identifying therapeutics for central nervous system (CNS) diseases such as Parkinson's disease, we have been focusing on metabotropic glutamate receptors. Herein we report our studies with a series of tricyclic thiazolopyrazoles as mGlu4 PAMs.



EC_{50} = 9 nM
E_{max} = 120%

INTRODUCTION

Activation or inhibition of metabotropic glutamate receptor function by small molecules is a strategy that has been utilized by several research groups to identify novel therapeutic agents.[1,2] Given the localization of the different metabotropic glutamate receptors, many of the target indications for these small molecule ligands are as CNS therapeutics. Recently, an increasing amount of evidence has been accumulating to support the hypothesis that activation of the metabotropic glutamate 4 receptor (mGlu4 receptor), either with an orthosteric agonist or a positive allosteric modulator (PAM), may provide impactful pharmacological interventions in diseases such as Parkinson's disease[3] and anxiety.[4] Among these two types of ligands (orthosteric or allosteric), the mGlu4 agonists reported to date are only subtype-selective, acting with some level of functional efficacy at all Group 3 mGlu receptors, mGlu4, 6, 7, and 8 (based on in vitro studies).[5] Thus, the use of a PAM, targeting a unique site on the mGlu4 receptor away from the native ligand binding site, would have the potential to deliver selective receptor activation. Over the past several years, a number of mGlu4 PAMs including compounds **1**,[6] **2**,[7] **3**,[8] **4**,[9] **5**,[9] **6**,[10] **7**,[11] and **8**[12] have been reported, several of which are characterized by good brain exposure upon peripheral administration (Figure 1). Herein we report our efforts to identify a series of tricyclic pyrazoles as mGlu4 PAMs to enable further testing of the biological hypothesis using in vivo animal models.

Molecular Design. We have taken a multipronged approach in an effort to identify lead compounds for our mGlu4 program. These include high-throughput screening (HTS) of the Lundbeck compound collection and rational design based on existing knowledge of mGlu4 PAMs. Among the known mGlu4 ligands, the thiazolopyrazole derivative **9** (Figure 2)[13] attracted our attention due to the low molecular weight and other favorable physical properties such as cLogP (2.2) and tPSA (61 Å^2). Hence, we synthesized **9** together with several of its analogues (e.g., **10** and **11**, Figure 2) as potential tool compounds. Compound **9** displayed an EC_{50} of 410 nM when tested in a calcium mobilization FLIPR assay of mGlu4 receptor modulation, and it was inactive in the corresponding agonist, antagonist, positive and negative modulation assays for the mGlu1, 2, 3, 5, and 7 receptors. In a broad counterscreen of 70 CNS-relevant GPCR receptors and ion channels, compound **9** showed some level of cross-reactivity with the adenosine A2A and A3 receptors, monoamine oxidase MAO-A, and norepinephrine transporter (54.9, 77.4, 69.6, and 72.1% inhibition at 10 μM, respectively; see Supporting Information). In terms of physicochemical properties, compound **9** is characterized by reasonable lipophilicity for a CNS drug ($LogD_{7.4}$ = 3.1), good kinetic solubility at pH 7.4 (120 μM), and good passive permeability in a PAMPA assay (P_{app} = 32.9 × 10^{-6} cm/s). Human and rat plasma protein binding are moderate (free fractions of 3.5% and 9.2%, respectively), and nonspecific binding in a rat brain homogenate dialysis study indicates a brain free fraction of 1.4%. hERG channel inhibition potential is low (IC_{50} = 33 μM in an electrophysiology screen). In vitro human and rat microsomal stability studies yielded CL_{int} of 11 L/min and 61 mL/min, respectively. In an in vivo study of CNS partition in rat, subcutaneous dosing of compound **9** (10 mg/kg, dosed as a solution in 20% hydroxypropyl-β cyclodextrin) produced at 1 h brain, plasma, and CSF concentrations of 744 ng/g, 766 ng/mL, and 52 ng/mL, respectively (brain/plasma ratio of 1).

In terms of structure−activity relationships, substitution with a methyl group on the thiazole ring increased the mGlu4 PAM potency by over 30-fold (i.e., **10**, EC_{50} = 13 nM), suggesting that substitution at the 5-position of the thiazole ring enabled a favorable interaction with the receptor. Likewise, substitution with a methyl group on the pyrazole ring also significantly

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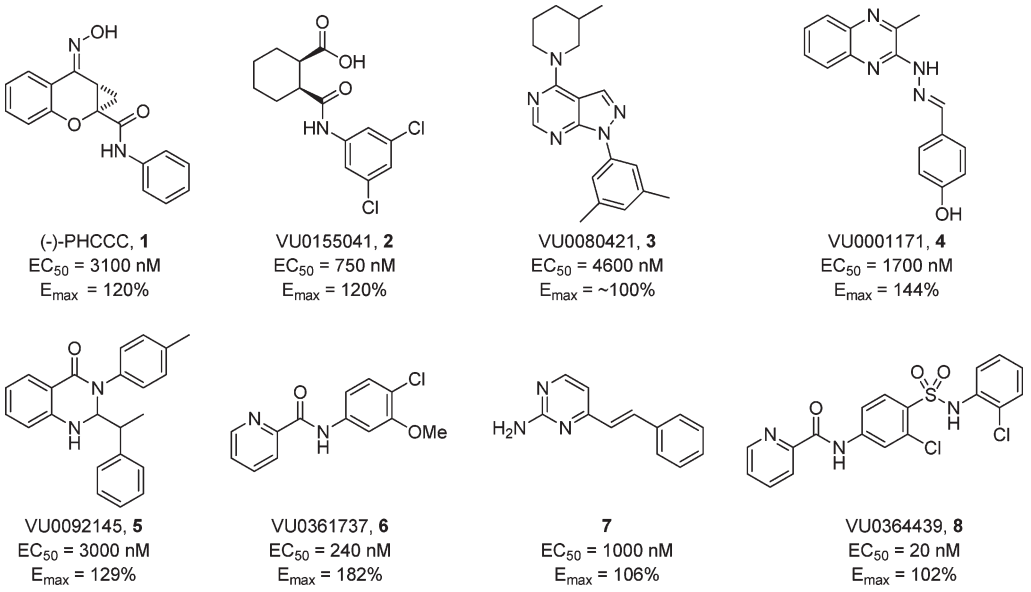

Figure 1. Select known mGlu4 PAMs with reported activity at human receptors.

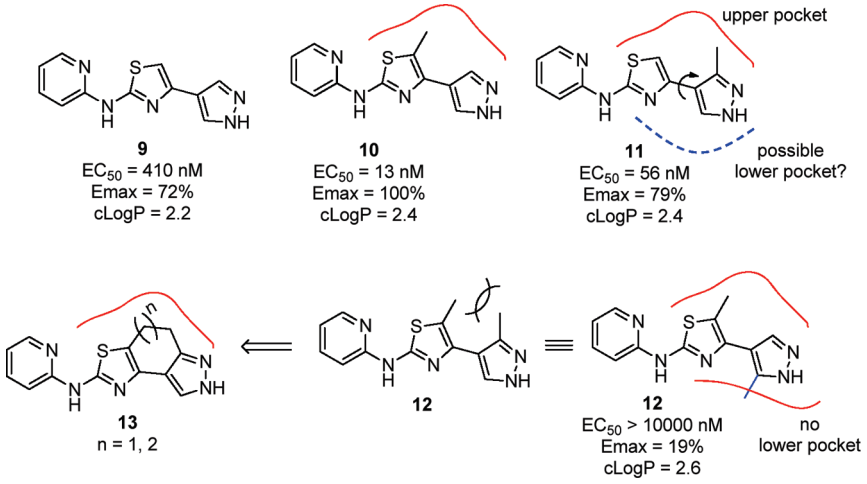

Figure 2. Design of mGlu4 PAMs.

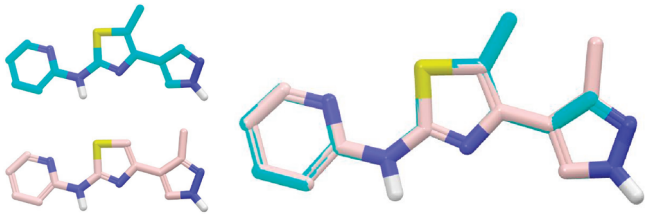

Figure 3. Overlay of **10** (with cyan carbon atoms) and **11** (with light-pink carbon atoms) with each methyl group in the "top pocket".

improved the potency as compared to that of **9** (i.e., **11**, EC_{50} = 56 nM), which suggested to us that the methyl group on the pyrazole **11** ring may occupy the same pocket ("upper pocket", Figure 2) as that of the methyl group from thiazole ring in compound **10**. Computational models[14] of low energy conformations of methyl derivatives **10** and **11** are superimposed in Figure 3, portraying this "same-pocket" hypothesis. Methylation in compound **10** resulted in a dihedral angle of only 13° between the thiazole and pyrazole rings. These rings are essentially coplanar in compound **11**. Alternatively, in another low energy conformation of **11**, the pyrazole methyl group may point away from the "upper pocket" and occupy another possible pocket ("lower pocket", highlighted by a blue dotted line for **11**). To further investigate this hypothesis, we synthesized dimethyl derivative **12**, only to find it to be inactive (EC_{50} > 10000 nM). We surmised that due to the steric hindrance, the two methyl groups in **12** cannot reside on the same side of the molecule but will likely adopt a more favorable conformation with both methyl groups pointing away from each other, as shown computationally (Figure 4). The poor functional activity of **12** may further imply that there is no room in the lower pocket to accommodate the pyrazole methyl group when **12** adopts such conformation. To further explore the "upper pocket" hypothesis and to identify

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mGlu4 positive allosteric modulators with potentially improved properties, we constrained the two methyl groups by forming an ethylene or a propylene bridge, therefore tethering the molecule between the thiazole and pyrazole rings. This work yielded a class of tricyclic thiazolopyrazole derivatives **13** with potent and selective mGlu4 PAM affinity. It should be noted that a more recent patent[15] from Addex was published disclosing similar compounds to those described herein after most of this work at Lundbeck had been completed.[16,17]

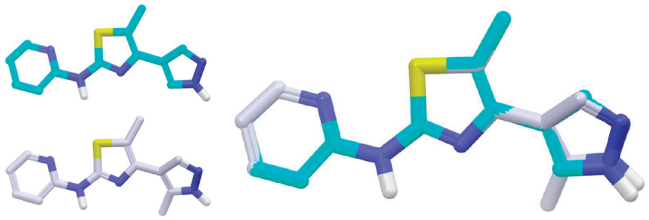
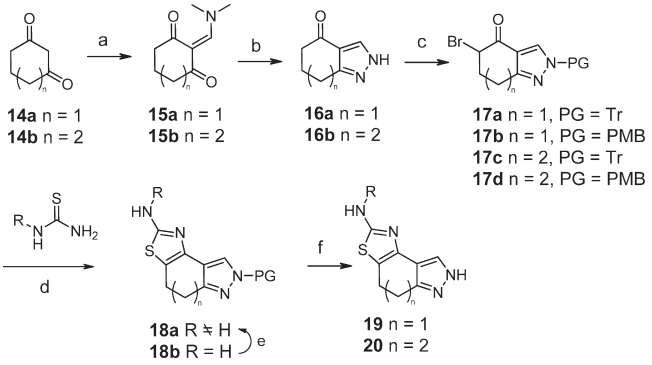


Figure 4. Preferred conformation of **12** (with light-gray carbon atoms) to avoid steric hindrance of its two methyl groups, relative to the conformation of **10** (with cyan carbon atoms).

Scheme 1[a]



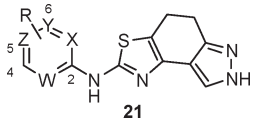
[a] Reagents and conditions: (a) $Me_2NCH(OMe)_2$, reflux (**15a**, 80−100%; **15b**, 69%); (b) hydrazine, MeOH, reflux (**16a**, 74−97%; **16b**, 60%; (c) for PG = Tr, (i) Ph_3CCl, Et_3N, DCM, (ii) $CuBr_2$, EtOAc, reflux, for PG = PMB, (i) PMBCl, K_2CO_3, CH_3CN, 60 °C, (ii) $CuBr_2$, EtOAc, reflux (**17b**, 21%; **17d**, 36%, 2-step) (d) EtOH, reflux, overnight; (e) aryl halide, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, DMF/THF, microwave, 120 °C, 1 h; (f) TFA, microwave, 150 °C, 30 min (PG = PMB) (5−50% overall from **17**).

Chemistry. The synthesis of the tricyclic thiazolopyrazole derivatives is outlined in Scheme 1. Heating 1,3-cyclohexanedione **14** to reflux in *N*,*N*-dimethylformamide dimethoxyacetal (DMF-DMA) generated 2-dimethylamino-cyclohexane-1,3-dione **15**, and subsequent treatment of **15** with hydrazine in methanol afforded the pyrazolo-cyclohexananone **16**. The free nitrogen of the pyrazole was then protected by either a trityl (Tr) or a 4-methoxybenzyl (PMB) group as regioisomeric mixtures (only the 2-substituted regioisomer is shown), which were carried on without further separation. Bromination of the isomeric mixtures with $CuBr_2$ in ethyl acetate afforded the common intermediate **17**, which was then reacted with a variety of thioureas to provide derivatives **18**. The Tr or PMB protecting groups were then removed, under standard conditions, to afford the final tricyclic pyrazoles **20** and **21**. An alternative synthesis was developed when the aryl thioureas were not synthetically or commercially available. In this case, unsubstituted thiourea and the corresponding **17** were heated at reflux in EtOH overnight to afford corresponding amino-thiazolo tricycles **18b**. A typical Buchwald coupling was then carried out between **18b** and a variety of aryl halides to afford derivatives **18a**, which upon deprotection, as before, provided final compounds **19** and **20**.

RESULTS AND DISCUSSION

To aid in the exploration of the structure−activity relationship (SAR), a number of 6-membered ring derivatives were prepared, and the mGlu4 EC_{50} and E_{max} data, as well as some physicochemical and in vitro dmpk parameters, are summarized in Table 1. The 2-pyridyl derivative **21a**, which displayed an EC_{50}

Table 1. SAR of 6-Membered Tricyclic Pyrazoles



21

compd	X	Y	Z	W	R	EC_{50}[a] (nM)	E_{max}[a] (%)	cLogP	PSA (Å^2)	solubility[b] (μM)	permeability P_{app}[c] (10^{-6} cm/s)	rCL_{int}[d] (mL/min)	hCL_{int}[d] (L/min)	rPPB[e] (%)	hPPB[e](%)
21a	N	CH	CH	CH	H	220	150	2.6	66.5	0.1	3.0	37	5.6	97.7	99.2
21b	CH	N	CH	CH	H	>10000	11	2.6	66.5	17	18.7				
21c	CH	CH	N	CH	H	>10000	10	2.6	66.5	180	2.5				
21d	CH	CH	CH	CH	H	2200	160	3.9	53.6	<0.1	<0.1				
21e	N	CH	CH	N	H	65	180	1.8	79.4	20	3.4	42	4.4	89.5	96.4
21f	N	CH	CH	CH	4-Me	>10000	11	3.1	66.5	9	12.4				
21g	N	CH	CH	CH	5-Me	>10000	12	3.1	66.5	<0.1	1.9				
21h	N	CH	CH	CH	6-Me	910	120	3.1	66.5	2.9	2.0	400	48		
21i	N	CH	CH	CH	4,6-di-Me	>10000	11	3.6	66.5	0.1	1.0				

[a] EC_{50} was for the potentiation of an EC_{20} glutamate concentration; E_{max} (%) was the percent response compared with the maximum response of glutamate alone. [b] Kinetic solubility from DMSO stock solutions at pH 7.4 and room temperature. [c] Passive permeability in a PAMPA assay. [d] Rat and human microsomal intrinsic clearance values. Rat and human hepatic blood flows are 20 mL/min and 1.5 L/min, respectively. [e] Rat and human plasma protein binding percentage.

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of 220 nM, is twice as potent as its nontethered analogue **9** but is ~15-fold less potent than the monomethylated analogue **10**. When computational models (Figure 5) of **21a** and **10** were superimposed, we noticed a change in the orientation of the pyrazole N−H unit, a potential hydrogen-bond donor, in **21a** and **10** and that the thiazole and pyrazole rings in **21a** are rotated by only 11° from coplanarity. Thus, the lower potency of **21a** relative to that of **10** may be due to the change in the orientation of the pyrazole NH moiety. The importance of the N−H group of the pyrazole ring was further supported by the lack of activity

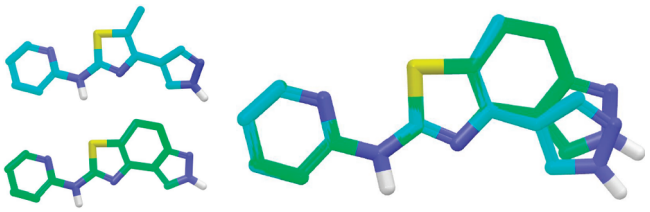
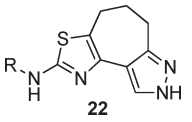


Figure 5. An overlay of **21a** (with green carbon atoms) and **10** (with cyan carbon atoms) to show different orientations of the pyrazole ring.

of the corresponding 1-methyl pyrazole analogue as well as its 2-methyl regioisomer (data not shown). Alternatively, it may implicate that the relatively bigger cyclohexyl group cannot be accommodated by the "top pocket". The latter hypothesis was quickly dismissed by the great potency of the 7-membered ring analogues (vide infra). Compounds **21b** and **21c** were then synthesized to investigate the effect of the pyridine nitrogen position on the activity. As it can be seen from the Table 1, both compounds were inactive, suggesting that the 2-pyridyl group either participates in a very specific interaction with the receptor or may be needed to stabilize a particular conformation. The somewhat narrow SAR observed in this investigation is consistent with what has been seen with other reported mGlu4 PAMs. Phenyl derivative **21d** was active although with a much reduced potency as compared to that of **21a**, confirming the favorable interaction of the optimized pyridyl amine moiety. Introduction of an additional nitrogen atom at the 3-position of the pyridine ring (**21e**) increased the potency by 3-fold. One may hypothesize that the improved potency of pyrimidine over pyridine derivatives may be because the second nitrogen atom is involved in additional interactions with the receptor or due to the reduced

Table 2. SAR of 7-Membered Tricyclic Pyrazoles (Part 1)



22

Compound	R	EC_{50}[a] (nM)	Emax[a] (%)	cLogP	PSA (Å^2)	Solubility[b] (μM)	Permeability *Papp*[c] (10^{-6} cm/s)	rCLint[d] (mL/min)	hCLint[d] (L/min)	rPPB[e] (%)	hPPB[e] (%)
22a		9	120	3.2	66.5	1.2	9.4	36	10	98.9	99.4
22b		>10,000	26	3.2	66.5	3.8	35	---	---	---	---
22c		1,300	150	2.3	79.4	1.8	41	---	---	---	---
22d		>10,000	18	2.3	79.4	78	3.8	---	---	---	---
22e		7	160	2.3	79.4	3.0	1.5	92	9	99.4	96.9
22f		3,200	140	2.8	82.3	190	25	---	---	---	---
22g		2,400	39	3.3	82.3	10	15	---	---	---	---
22h		3,000	130	2.6	71.4	160	19	---	---	---	---
22i		>10,000	35	3.0	66.5	---	---	---	---	---	---
22j		>10,000	6.3	2.7	79.4	21	31	---	---	---	---
22k		1,300	150	4.5	53.6	<0.1	2.8	---	---	---	---

[a] EC_{50} was for the potentiation of an EC_{20} glutamate concentration; E_{max} (%) was the percent response compared with the maximum response of glutamate alone. [b] Kinetic solubility from DMSO stock solutions at pH 7.4 and room temperature. [c] Passive permeability in a PAMPA assay. [d] Rat and human microsomal intrinsic clearance values. Rat and human hepatic blood flows are 20 mL/min and 1.5 L/min, respectively. [e] Rat and human plasma protein binding percentage.

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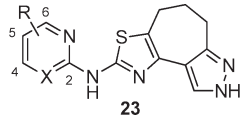
Table 3. SAR of 7-Membered Tricyclic Pyrazoles (Part 2)



23

compd	R	X	EC_{50}[a] (nM)	E_{max}[a] (%)	cLogP	PSA (Å^2)	solubility[b] (μM)	permeability P_{app}[c] (10^{-6} cm/s)
23a	3-Me	CH	3400	84	3.7	66.5	0.2	8.0
23b	5-Me	CH	>10000	28	3.7	66.5	<0.1	2.7
23c	6-Me	CH	140	200	3.7	66.5	110	4.9
23d	4,6-di-Me	CH	>10000	4	4.2	66.5	0.4	1.2
23e	5-F	CH	750	170	3.4	66.5	<0.1	0.3
23f	6-F	CH	105	170	3.4	66.5	0.2	0.1
23g	6-Cl	CH	1100	220	3.9	66.5	38	15.4
23h	6-OMe	CH	650	100	4.0	75.7	0.1	0.1
23i	6-OEt	CH	290	78	4.5	71.7	<0.1	<0.1
23j	6-CN	CH	4900	50	2.9	90.3	<0.1	<0.1
23k	6-CO_2Me	CH	>10000	5	2.9	93.0	1.9	0.1
23l	6-(CH_2OH)	CH	2700	76	2.6	82.7	30	38.8
23m	6-($OCH_2CH_2OCH_3$)	CH	390	90	3.9	85.0	0.1	0.1
23n	6-N Me_2	CH	340	150	3.9	69.7	<0.1	<0.1
23o	6-NHEt	CH	600	63	4.2	78.5	0.9	3.1
23p	6-(pyrrolidin-1-yl)	CH	>10000	14	4.0	69.7	<0.1	0.1
23q	6-Me	N	51	210	2.8	79.4	0.6	0.5
23r	6-MeO	N	100	230	3.3	88.6	0.6	0.3

[a] EC_{50} was for the potentiation of an EC_{20} glutamate concentration; E_{max} (%) was the percent response compared with the maximum response of glutamate alone. [b] Kinetic solubility from DMSO stock solutions at pH 7.4 and room temperature. [c] Passive permeability in a PAMPA assay.

basicity of the pyrimidine ring affording an optimized interaction with the receptor. While the 4-methyl and 5-methyl derivatives, **21f** and **20g**, respectively, were totally inactive, the 6-methyl derivative **21h** had an EC_{50} of 910 nM, which represents less than a 5-fold reduction from the potency of **21a**. The 4,6-dimethyl derivative **21i** was totally inactive, which further underscores the low tolerance for substitution on the pyridine ring of this series of mGlu4 PAMs. Additionally, several heterocycles were explored to replace the pyrazole ring, but all of them resulted in compounds with significantly reduced potency and the respective series were not followed up (data not shown). In terms of physicochemical properties, cLogP values for these analogues have increased over that for compound **9**, as had been expected from the ethylene tether. While pyrimidine **21e** has reasonable aqueous solubility and plasma free fraction, the values of these important parameter for other compounds showing some level of mGlu4 PAM activity (**21a** and **21h**) are suboptimal. Passive permeabilities for these three analogues are similar. The addition of the methyl group in the 6-position in **21h** impacts negatively the microsomal stability, increasing human and rat intrinsic clearances by 10-fold compared with unsubstituted **21a** and **21e**.

To further explore the SAR, a number of 7-membered derivatives (**22a**−**22l**) were then synthesized, with the data summarized in Tables 2 and 3. The 7-membered ring 2-pyridyl derivative **22a** has an EC_{50} of 9 nM and is 24-fold more potent than its 6-membered ring counterpart **21a**, and equipotent to the monomethylated analogue **10**. In **22a**, a dihedral angle of only 4° is found between the pyrazole and thiazole rings (Figure 6). Unlike its 6-membered ring derivative **21a**, in which the pyrazole

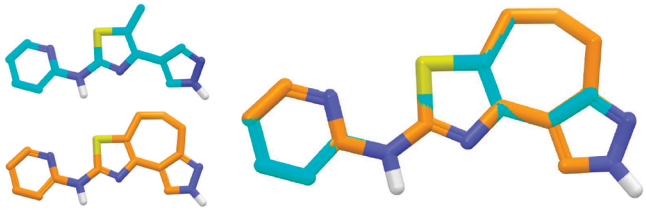


Figure 6. An overlay of **22a** (with orange carbon atoms) and **9** (with cyan carbon atoms).

NH moiety shifts from that of noncyclic **9**, low energy conformations of compounds **22a** and **10** are almost completely superimposed. As expected, the corresponding 3-pyridyl derivative **22b** was inactive, consistent with the SAR from the 6-membered ring compounds. While the 4-pyrimidyl and the 2-pyrazinyl compounds **22c** and **22d**, respectively, had significantly reduced or no activity, the 2-pyrimidyl (**22e**) and 2-pyridyl were shown to be the optimal groups, again in concert with the SAR of the 6-membered ring system. Other groups including some 5-membered heterocycles, substituted phenyls and cyclohexyl derivatives (**22f**−**22k**, Table 2) have also been explored, and all were significantly less active. Some of these compounds displayed good levels of aqueous solubility and passive permeability (**22f**, **22h**), however those for active analogues **22a** and **22e** were suboptimal, as it was their rat and human plasma protein binding.

Once the 2-pyridyl and 2-pyrimidyl derivatives indicated these were the ring systems enabling mGlu4 PAM activity, the

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Table 4. Profile of Compounds 9, 21a, 22a, and 22e

compd	9	21a	22a	22e
EC_{50}[a]	410	220	9	7
E_{max}[a]	72	150	120	160
selectivity @ mGlu 1,2,3,5,7	>10 μM	>10 μM	>10 μM	>10 μM
MW	243	269	283	284
cLogP	2.2	2.6	3.2	2.3
$LogD_{7.4}$	3.1	4.0	5.0	3.8
solubility pH 1.5 (μM) [b]	220	220	240	100
hERG IC_{50} (μM)	33	17	28	30
permeability P_{app} [c] (10^{-6} cm/s)	32.9	3.0	9.4	1.5
MDCK/mdr1[d] (10^{-6} cm/s, A→B; B→A; ratio)	n/a	22; 7.7; 0.36	24; 13; 0.54	33; 21; 0.64
hCL_{int} (L/min)[e]	11	5.6	10	9
rCL_{int} (mL/min)[e]	61	37	36	92
rBrain free fraction, f_u (%)	9.2	0.43	0.43	1.6
rPlasma free fraction, f_u (%)	1.4	2.3	1.1	0.6
	exposure (10 mg/kg, PO, rats)			
plasma (ng/mL), 1 h	766	987	259	0.7
brain (ng/g), 1 h	744	517	200	3.1
brain/plasma ratio	1	0.5	0.8	[f]

[a] EC_{50} was for the potentiation of an EC_{20} glutamate concentration; E_{max} (%) was the percent response compared with the maximum response of glutamate alone. [b] Kinetic solubility from DMSO stock solutions at pH 7.4 and room temperature. [c] Passive permeability in a PAMPA assay. [d] Permeability in Madin−Darby canine kidney cells transfected with the human MDR1 gene; P-gp substrate assay. [e] Rat and human microsomal intrinsic clearance values. Rat and human hepatic blood flows are 20 mL/min and 1.5 L/min, respectively. [f] Not calculated as absolute brain and plasma values are low.

tolerance of substitution on these skeletons was extensively investigated aiming to extract the most benefit from this chemotype. The data for select examples are summarized in Table 3. Although EC_{50} values were used as the primary parameter to drive SAR for this chemical series, E_{max} may also play a significant role in determining the in vivo effects of mGlu4 PAMs, the exact nature of which deserves further investigations. It became immediately obvious that the 6-position is the only one that allows substitution, even with a small group such as fluorine (**23a**−**23f**, Table 3). A variety of other moieties (**23g**−**23r**) were then explored, however, all resulted in compounds with significantly reduced potency further confirming the limited SAR for this chemotype around this allosteric site. Most of these analogues are within the cLogP range for CNS drug candidates and are characterized by borderline solubility and passive permeability. Notably, in spite of being substituted with a lipophilic group on position 6, compounds **23c** and **23g** are more soluble than their unsubstituted counterpart **22a**. This effect may arise from disturbances to crystal packing caused by lack of coplanarity by methyl or chloro C-6 substitution.[18] Among polar C-6 substituents, only the hydroxymethyl analogue **23l** shows solubility in an appropriate range.

Compounds **21a**, **22a**, and **22e** were selected for further characterization, and selected properties are summarized in Table 4. All three compounds were found to be inactive [EC_{50} >10 μM in agonist and PAM mode, IC_{50} >10 μM in negative allosteric modulator (NAM) mode] at mGlu 1, 2, 3, 5, and 7 receptors and showed low potential for hERG channel inhibition. These compounds have excellent drug-like properties with low molecular weight (<300) and favorable cLogP values for CNS drugs (2.5−3.5). While kinetic solubilities at pH 7.4 are in the low μM range, those at pH 1.5 are higher. Experimental $LogD_{7.4}$ values are significantly larger than cLogP, indicating the lipophilic nature of these compounds and in agreement with the relatively low free fractions observed both in plasma and brain homogenate. High in vitro intrinsic clearance (CL_{int}) in both human and rat liver microsomes and high protein binding in a brain homogenate and in plasma were observed for all these. The pyridine derivatives **21a** and **22a** displayed good plasma (987 and 259 ng/mL, respectively) and brain exposure levels (517 and 200 ng/g, respectively) as well as good brain penetration (brain/plasma ratios of 0.5 and 0.8, respectively) after 1 h following a 10 mg/kg oral administration in Sprague−Dawley rats (compounds dosed as suspensions in 20% aqueous hydroxypropyl-β cyclodextrin). Asymmetry ratios in the MDCK-mdr1 assay (B→A/A→B) were less than 1, indicating no evidence of these compounds being a P-glycoprotein substrate. On the other hand, the pyrimidine derivative **22e** was characterized by very poor in vivo exposure levels and was not profiled further. In a broad counterscreen of 70 CNS-relevant GPCR receptors and ion channels, compound **22a** (10 μM concentration) maintained the level of cross-reactivity previously seen with compound **9** for the case of the adenosine A3 receptor (65% inhibition) and monoamine oxidase MAO-A (71% inhibition). Notably, interactions with the A2A receptor (13% inhibition) and the norepinephrine transporter (28.9% inhibition) were significantly reduced compared with those liabilities for compound **9** (see Supporting Information). The improved selectivity against these two receptors will eliminate caveats interpreting results while testing the mGlu4 PAM biological hypothesis in animal models of Parkinson's disease.

CONCLUSION

In summary, we identified a series of tricyclic thiazolopyrazole derivatives as mGlu4 PAMs through medicinal chemistry design

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aided by molecular modeling. The SAR and SPR of this series were explored in detail. While several mGlu4 PAM chemotypes reported in the literature show very shallow and narrow SAR, in this chemotype some characteristics of mGlu4 PAM SAR and SPR translated between the open chain (e.g., **9**), the 5,7-dihydro-4*H*-thiazolo[4,5-*e*]indazol-2-amine analogues (e.g., **21e**), and the 4,5,6,8-tetra-hydropyrazolo-[3′,4′:6,7]cyclohepta[1,2-*d*]thiazol-2-amine (e.g., **22a**) analogues. Potent and orally bioavailable compounds were identified (**21a** and **22a**) with excellent brain penetration and good physicochemical properties. Among these, compound **22a** showed an improved selectivity profile over lead compound **9**, warranting further studies to elucidate the value of mGlu4 PAMs as potential CNS therapeutics.

EXPERIMENTAL SECTION

General. Unless specifically stated otherwise, the experimental procedures were performed under the following conditions. All operations were carried out at room temperature (about 18 °C to about 25 °C) under nitrogen atmosphere. Evaporation of solvent was carried out using a rotary evaporator under reduced pressure or in the high performance solvent evaporation system HT-4X (Genevac Inc., Valley Cottage, NY, USA). The microwave oven used was a Biotage Initiator synthesizer (Charlottesville, VA, USA). The course of the reaction was followed by thin layer chromatography (TLC) or liquid chromatography−mass spectrometry (LC-MS), and reaction times are given for illustration only. Silica gel chromatography was carried out on a Combi*Flash* system (Teledyne Isco, Inc., Lincoln, NE, USA) with prepacked silica gel cartridges or performed on Merck Silica Gel 60 (230−400 mesh). The final compound structures were confirmed using both nuclear magnetic resonance (NMR) and low and high resolution mass spectrometry. Purity of all final products was determined to be >95% based on LC-MS trace using UV detection in the range of 240−400 nm as well as at a single 254 nm wavelength. Purifications were carried out on a reversed phase liquid chromatography/mass spectrometry (RP-HPLC/MS) purification system. Flow rate: 100 mL/min. Mobile phase additive: 48 mM of ammonium formate. Column: Inertsil C18, 30 mm × 50 mm, 5 μm particle size. Gradient conditions were as follows:

time (min)	0	0.50	0.65	3.00	3.50	4.95	5.00
acetonitrile %	18	18	22	45	95	95	18

High resolution mass spectra were recorded using an LTQ Orbitrap XL, Thermo Electron Corp., Waltham, MA, USA. NMR spectra were recorded on a Bruker Avance 300 spectrometer (Bruker BioSpin Corp., Billerica, MA, USA) or a Varian UNITY INOVA 400 (Varian, Inc., Palo Alto, CA, USA) using the indicated solvent. Chemical shift (δ) is given in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. Coupling constants (J) are expressed in hertz (Hz), and conventional abbreviations used for signal shape are: s = singlet; d = doublet; t = triplet; m = multiplet; br = broad, etc. Unless stated otherwise, mass spectra were obtained using electrospray ionization (ESMS) via either a Micromass Platform II system or a Quattro Micro system (both from Waters Corp., Milford, MA, USA) and $(M + H)^+$ is reported.

Calcium Mobilization Assay. The hmGlu4 and murine Gα15 (G-protein) cDNAs were stably expressed in a BHK cell line and grown in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, CA) with supplements (10% dialyzed fetal bovine serum, 1% glutamax, 1% sodium pyruvate, 1% Pen/strep, 1 mg/mL Geneticin, and 0.2 mg/mL hygro B) at 37 °C, 5% CO_2 . Twenty-four hours prior to assay, cells were seeded into 384-well black-wall microtiter plates coated with poly-D-lysine. Just prior to assay, media was aspirated and cells dye-loaded (30 μL/well) with Calcium 3 no wash dye (Molecular Devices, Sunnyvale, CA) made in assay buffer (Hank's Balanced Saline Solution (HBSS)): 150 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, plus 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.4, 0.1% bovine serum albumin (BSA), and 2.5 mM probenecid) for 1 h in 5% CO_2 at 37 °C. Basal fluorescence is monitored in a fluorometric imaging plate reader (FLIPR) (Molecular Devices, Sunnyvale, CA). Cells were stimulated with an EC_{20} concentration of glutamate in the presence of a compound to be tested, and relative fluorescent units were measured at defined intervals. Concentration−response curves derived from the maximum change in fluorescence were analyzed by nonlinear regression (Hill equation). A positive modulator can be identified from these concentration−response curves if a compound produces a concentration dependent increase in the EC_{20} glutamate response. EC_{50} and E_{max} values were measured at least in two independent experiments, each one in duplicate, and the mean values are reported.

4-(1*H*-Pyrazol-4-yl)-*N*-(pyridin-2-yl)thiazol-2-amine (9). The title compound was prepared according the procedure reported in the literature.[13] ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (br, 1H), 8.30 (d, J = 5.0 Hz, 1H), 7.94 (s, 2H), 7.70 (t, J = 7.8 Hz, 1H), 7.08 (d, J = 7.87 Hz, 1H), 7.01 (s, 1H), 6.92 (dd, J = 6.3, 5.2 Hz, 1H). ESMS m/e: 244.0 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{11}H_9N_5S^+$ 244.06514; found 244.06450.

5-Methyl-4-(1*H*-pyrazol-4-yl)-*N*-(pyridin-2-yl)thiazol-2-amine (10). The title compound was prepared according the procedure reported in the literature.[13] ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (br, 1H), 11.15 (br, 1H), 8.26 (d, J = 4.8 Hz, 1H), 7.88 (br, 2H), 7.67 (t, J = 7.8 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.88 (t, J = 5.8 Hz, 1H), 2.41 (s, 3H). ESMS m/e: 258.0 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{12}H_{11}N_5S^+$ 258.08079; found 258.08017.

4-(3-Methyl-1*H*-pyrazol-4-yl)-*N*-(pyridin-2-yl)thiazol-2-amine (11). The title compound was prepared according the procedure reported in the literature.[13] ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (br, 1H), 11.28 (s, 1H), 8.30 (d, J = 4.9 Hz, 1H), 7.77 (br, 1H), 7.69 (t, J = 7.8 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 6.91 (t, J = 6.3 Hz, 1H), 6.83 (s, 1H), 2.45 (s, 3H). ESMS m/e: 258.0 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{12}H_{11}N_5S^+$ 258.08079; found 258.08029.

2-Dimethylaminomethylene-cyclohexane-1,3-dione (15a). Into a round-bottom flask, 1,3-cyclohexanedione (2.00 g, 17.8 mmol) and 1,1-dimethoxy-*N*,*N*-dimethylmethanamine (30 mL, 200 mmol) were added. The reaction was heated to reflux for 3 h and was concentrated in vacuo to remove 1,1-dimethoxy-*N*,*N*-dimethylmethanamine. The desired title compound was obtained as a brown solid (2.89 g, 100% crude yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 3.40 (s, 3H), 3.20 (s, 3H), 2.50 (m, 4H), 2.00 (m, 2H).

2-Dimethylaminomethylene-cycloheptane-1,3-dione (15b). A mixture of cycloheptane-1,3-dione (10 g, 79 mmol) and DMF-DMA (29 g, 244 mmol) was stirred at refluxing for 1 h. The reaction mixture was concentrated in vacuo. The resulting residue was triturated with toluene and filtered to give the title compound (10 g, 69%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.84 (s, 1H), 3.37 (s, 3H), 2.82 (s, 3H), 2.61−2.50 (m, 4H), 1.90−1.72 (m, 4H).

2,5,6,7-Tetrahydro-indazol-4-one (16a). The crude material of step 1 (2-dimethylaminomethylene-cyclohexane-1,3-dione, 2.89) was dissolved in methanol (50 mL), followed by addition of hydrazine (0.629 g, 19.6 mmol). The reaction was heated to reflux for 5 h. The reaction was cooled to room temperature and filtered. A light-brown solid was generated. The solid was washed with MeOH/hexanes and dried to give the title compound (2.30 g, 95% crude yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.4−7.7 (m, 1H), 2.8−2.3 (s, 4 h), 2.1−1.8 (m, 2H).

5,6,7,8-Tetrahydro-2*H*-cycloheptapyrazol-4-one (16b). To a cold solution of 2-dimethylaminomethylene-cycloheptane-1,3-dione

(20 g, 110 mmol) in MeOH (500 mL) was added dropwise a solution of hydrazine in THF (112 mL, 112 mmol, 1 M THF) over 5 min. The reaction was stirred for 1 h and concentrated in vacuo. The resulting residue was recrystallized from EtOAc to afford the title compound (10 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (s, 1H), 3.04−2.95 (m, 2H), 2.78−2.65 (m, 2H), 2.04−1.85 (m, 4H).

5-Bromo-2-trityl-2,5,6,7-tetrahydro-indazol-4-one (17a). Into a round-bottom flask, the 2,5,6,7-tetrahydro-indazol-4-one (1.00 g, 7.34 mmol), triethylamine (2.05 mL, 14.7 mmol), triphenylmethyl chloride (2.25 g, 8.08 mmol), and methylene chloride (30 mL) were added. The reaction was stirred for 3 h. The resulting mixture was quenched with saturated $NaHCO_3$ and extracted with DCM (3×). The combined organic phase was washed with brine, dried with $MgSO_4$, and concentrated in vacuo. The crude material was purified over silica gel by eluting with 0−60% EtOAc:hexanes to afford the intermediate as an isomeric mixture (1.78, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.5−7.1 (m, 16H), 2.9−2.5 (m, 4H), 2.2−2.0 (m, 2H).

The 2-trityl-2,5,6,7-tetrahydro-indazol-4-one (0.5 g, 1.32 mmol) was dissolved in ethyl acetate (20 mL), followed by addition of copper(II) bromide (0.5 g, 2.24 mmol). The reaction was heated at 50 °C overnight, filtered through Celite, and concentrated in vacuo. The crude title compound was used without further purification.

5-Bromo-2-(4-methoxy-benzyl)-2,5,6,7-tetrahydro-indazol-4-one (17b). A suspension of 2,5,6,7-tetrahydro-indazol-4-one (6 g, 0.044 mol), PMBCl (10 g, 0.064 mol), and K_2CO_3 (9.1 g, 0.66 mol) in acetonitrile (300 mL) was stirred at 60 °C overnight. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated to afford the crude product, which was purified by column chromatography on silica gel (4:1 petroleum ether:ethyl acetate) to give the intermediate as a mixture of regioisomers (8 g, 71%).

To a solution of 2-(4-methoxy-benzyl)-2,5,6,7-tetrahydro-indazol-4-one (4 g, 16 mmol) in EtOAc (400 mL) was added $CuBr_2$ (7 g, 31 mmol). The reaction mixture was stirred at refluxing for 4 h. The resulting mixture was cooled to room temperature and filtered. The filtrate was concentrated to give the crude compound, which was purified by column chromatography on silica gel (3:1 petroleum ether:ethyl acetate) to give the title compound as a mixture of regioisomers in a ratio of 1:2. (1.5 g, 29%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.49 (s, 2H),7.93 (s, 1H), 7.31 (d, J = 8.4 Hz, 4H), 7.22 (d, J = 8.4 Hz, 2H), 6.99−6.87 (m, 6H), 5.31 (s, 2H), 5.26 (s, 4H), 4.85−4.75 (m, 3H), 3.80−3.70 (m, 9H), 3.07−3.02 (m, 1H), 2.89−2.75 (m, 5H), 2.48−2.29 (m, 6H). ESMS m/e: 337 $(M + H)^+$.

5-Bromo-2-(4-methoxy-benzyl)-5,6,7,8-tetrahydro-2*H*-cycloheptapyrazol-4-one (17d). To a solution of 5,6,7,8-tetrahydro-2*H*-cycloheptapyrazol-4-one (10 g, 67 mmol) in CH_3CN (200 mL) was added PMBCl (12.5 g, 80 mmol) and K_2CO_3 (13.8 g, 100 mmol). The reaction mixture was stirred at 60 °C for 2 h. The resulting mixture was filtered, and the filtrate was concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (10:1−1:1 petroleum ether:EtOAc) to afford the intermediate (15 g, 83%). ^{1}H NMR ($CDCl_3$ 400 MHz): δ 8.01 (s, 0.5H), 7.78 (s, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.09 (d, J = 8.8 Hz, 1H), 6.92−6.85 (m, 3H), 5.25 (s, 1H), 5.17 (s, 2H), 3.83 (s, 3H), 3.81 (s,1.5H), 2.97 (t, J = 6.0 Hz, 2H), 2.85 (t, J = 6.0 Hz, 1H), 2.77−2.65 (m, 3H), 2.01−1.85 (m, 6H).

A solution of compound 2-(4-methoxy-benzyl)-5,6,7,8-tetrahydro-2*H*-cycloheptapyrazol-4-one (8 g, 30 mmol) and $CuBr_2$ (11.2 g, 50.2 mmol) in EtOAc (150 mL) was stirred at reflux for 1 h. The reaction mixture was filtered, and the filter cake was washed with EtOH several times. The combined filtrate was concentrated in vacuo. The resulting residue was purified by reverse-phase HPLC to afford the title compound (5.4 g, 52%). ^{1}H NMR ($CDCl_3$ 400 MHz): δ 8.02 (s, 0.5H), 7.81 (s, 1H), 7.22 (dd, J = 6.4 Hz, 2.0 Hz, 2H), 7.07 (d, J = 8.4 Hz, 1H), 6.91−6.83 (m, 3H), 5.31−5.17 (m, 1H), 5.14 (s, 2H), 4.88−4.76 (m, 1.5H), 3.80 (s,3H), 3.78 (s, 1.5H), 3.17−3.10 (m, 1H), 3.03−2.96 (m, 0.5H), 2.91−2.83 (m, 1H), 2.78−2.70 (m, 0.5H), 2.38−2.22 (m, 4.5H), 2.05−1.90 (m,1.5H). MS (ES^+) m/e 351 $(M + H)^+$.

8-(4-Methoxy-benzyl)-4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[e]azulen-2-ylamine (18b, *n* = 2). The reaction mixture of **17d** (2.00 g, 5.73 mmol) and thiourea (436 mg, 5.73 mmol) in EtOH (120 mL) was refluxed overnight, cooled to room temperature, and concentrated under the reduced pressure to afford the title compound (1.82 g, 97%), which was used without further purification. MS (ES^+) m/e 327 $(M + H)^+$.

(5,7-Dihydro-4*H*-3-thia-1,6,7-triaza-as-indacen-2-yl)-pyridin-2-yl-amine (21a). Crude 5-bromo-2-trityl-2,5,6,7-tetrahydro-indazol-4-one (**17a**) (0.300 g, 0.657 mmol) and 2-pyridylthiourea (0.0486 g, 0.317 mmol) were dissolved in ethanol (5 mL), followed by refluxing overnight. Using a rotary evaporator, the excess EtOH was removed from the reaction mixture and the crude material was purified by reverse-phase HPLC to afford the title compound (3 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.4 (m, 2H), 7.7 (s, 1H), 7.6 (m, 1H), 6.9 (m, 2H), 3.1 (m, 4H). MS (ES^+) m/e 270 $(M + H)^+$; HRMS calcd for $(M + H)^+$: $C_{13}H_{12}N_5S^+$ 270.08079; found 270.08066.

(5,7-Dihydro-4*H*-3-thia-1,6,7-triaza-as-indacen-2-yl)-pyridin-3-yl-amine (21b). Regio-mixture of 5-bromo-2-(4-methoxy-benzyl)-2,5,6,7-tetrahydro-indazol-4-one (**17b**) (0.050 g, 0.179 mmol) and (6-methyl-pyridin-2-yl)-thiourea (0.030 g, 0.18 mmol) were dissolved in ethanol (10 mL), followed by refluxing overnight. Using a rotary evaporator, the excess EtOH was removed from the reaction mixture and the crude was dissolved in TFA (3−5 mL), followed by microwave irradiation at 150 °C for 30 min. The reaction mixture was concentrated in vacuo, and the material was purified by reverse-phase HPLC to afford the title compound (22 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (s, 1H), 10.3 (s, 1H), 8.8 (d, J = 2.54 Hz, 1H), 8.2 (m, 1H), 8.1 (m, 1H), 7.7 (bs, 1H), 7.3 (dd, J = 4.6, 8.3 Hz, 1H) 2.9 (m, 4H). MS (ES^+) m/e 270 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{13}H_{12}N_5S^+$ 270.08079; found 270.08108.

(5,7-Dihydro-4*H*-3-thia-1,6,7-triaza-as-indacen-2-yl)-pyridin-4-yl-amine. (21c). The title compound was prepared by the same method as **21b** (14 mg, 37%). ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.3 (m, 2H), 7.7 (bs, 1H), 7.6 (m, 2H), 3.0 (m, 4H). MS (ES^+) m/e 270 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{13}H_{12}N_5S^+$ 270.08079; found 270.08051.

(5,7-Dihydro-4*H*-3-thia-1,6,7-triaza-as-indacen-2-yl)-phenyl-amine (21d). The title compound was prepared by the same method as **21b** (6 mg, 10%). ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.7 (s, 1H), 7.5 (d, J = 8.3 Hz, 2H), 7.3 (t, J = 8.3 Hz, 2H), 7.0 (t, J = 7.6 Hz, 1H), 3.0 (bs, 4H). MS (ES^+) m/e 269 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{14}H_{13}N_4S^+$ 269.08554; found 269.08550.

(5,7-Dihydro-4*H*-3-thia-1,6,7-triaza-as-indacen-2-yl)-pyrimidin-2-yl-amine (21e). The title compound was prepared by the same method as **21b** (15 mg, 37%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (s, 1H), 11.6 (s, 1H), 8.6 (d, J = 4.9 Hz, 2H), 7.6 (s, 1H), 7.0 (t, J = 4.9 Hz, 1H), 3.0−2.8 (m, 4H). MS (ES^+) m/e 271 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{12}H_{11}N_6S^+$ 271.07604; found 271.07602.

(5,7-Dihydro-4*H*-3-thia-1,6,7-triaza-as-indacen-2-yl)-(4-methyl-pyridin-2-yl)-amine (21f). The title compound was prepared according to the method described for compound **21b** (5 mg, 8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.3 (br, 1H), 11.1 (s, 1H), 8.00 (d, J = 5.2 Hz, 2H), 7.46 (s, 1H), 6.67 (s, 1H), 6.62 (d, J = 5.3 Hz, 1H), 2.87−2.74 (m, 4H), 2.15 (s, 3H). MS (ES^+) m/e 284 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{14}H_{14}N_5S^+$ 284.09644; found 282.08084.

(5,7-Dihydro-4*H*-3-thia-1,6,7-triaza-as-indacen-2-yl)-(5-methyl-pyridin-2-yl)-amine (21g). The title compound was prepared according to the method described for compound **21b** (3 mg, 5%). ^{1}H NMR (400 MHz, MeOD) δ 8.13 (s, 1H), 7.69 (s, 1H), 7.56−7.50

(m, 1H), 6.97 (d, J = 8.4 Hz, 1H), 3.07−2.97 (m, 4H), 2.29 (s, 3H). MS (ES^+) m/e 284 $(M + H)^+$.

(5,7-Dihydro-4*H*-3-thia-1,6,7-triaza-as-indacen-2-yl)-(6-methyl-pyridin-2-yl)-amine (21h). The title compound was prepared according to the method described for compound **21a** (3 mg, 6%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (s, 1H), 11.2 (s, 1H), 7.6 (s, 1H), 7.5 (t, J = 8.0 Hz, 1H), 6.8 (dd, J = 8.0, 16.8 Hz, 2H), 3.0−2.9 (m, 4H), 2.4 (s, 3H). MS (ES^+) m/e 284 $(M + H)^+$.

(5,7-Dihydro-4*H*-3-thia-1,6,7-triaza-as-indacen-2-yl)-(4,6-dimethyl-pyridin-2-yl)-amine (21i). The title compound was prepared according to the method described for compound **21b** (3 mg, 4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.3 (br, 1H), 11.1 (s, 1H), 7.38 (s, 1H), 6.60 (s, 1H), 6.54 (s, 1H), 2.99−2.86 (m, 4H), 2.40 (s, 3H), 2.22 (s, 3H). MS (ES^+) m/e 298 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{15}H_{16}N_5S^+$ 298.11209; found 298.11240.

Pyridin-2-yl-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (22a). The title compound was prepared according to the method described for compound **23c** (0.16 g, 39%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (s, 1H), 11.1 (s, 1H), 8.2 (d, J = 8.2 Hz, 1H), 7.7 (m, 2H), 7.0 (d, J = 8.2 Hz, 1H), 6.8 (dd, J = 5.7, 6.8 Hz, 1H), 3.0 (m, 4H), 2.0 (m, 2H). MS (ES^+) m/e 284 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{14}H_{14}N_5S^+$ 284.09644; found 284.09650.

Pyridin-3-yl-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (22b). The title compound was prepared according to the method described for compound **23c** (9 mg, 10%). ^{1}H NMR (400 MHz, MeOD) δ 8.73 (s, 1H), 8.45 (s, 1H), 8.21−8.14 (m, 1H), 8.05−7.95 (m, 1H), 7.77 (s, 1H), 7.28 (dd, J = 8.4, 4.8 Hz, 1H), 2.97−2.86 (m, 4H), 2.05−1.96 (m, 2H). MS (ES^+) m/e 284 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{14}H_{14}N_5S^+$ 284.09644; found 284.09646.

Pyrimidin-4-yl-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (22c). The title compound was prepared according to the method described for compound **23q** (16 mg, 23%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (br, 1H), 11.6 (s, 1H), 8.70 (s, 1H), 8.34 (d, J = 5.9 Hz, 1H), 7.63 (s, 1H), 6.97 (d, J = 5.7 Hz, 1H), 2.97−2.87 (m, 4H), 1.96−1.88 (m, 2H). MS (ES^+) m/e 285 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{13}H_{13}N_6S^+$ 285.09169; found 285.09175.

Pyrazin-2-yl-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (22d). The title compound was prepared according to the method described for compound **23q** (2 mg, 4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70−7.66 (m, 4H), 3.07−2.93 (m, 4H), 2.04−1.95 (m, 2H). MS (ES^+) m/e 285 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{13}H_{13}N_6S^+$ 285.09190; found 285.09208.

Pyrimidin-2-yl-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (22e). The title compound was prepared according to the method described for compound **23c** (60 mg, 10%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (s, 1H), 11.5 (s, 1H), 8.6 (d, J = 4.9 Hz, 2H), 7.7 (s, 1H), 7.0 (d, J = 4.9 Hz, 1H), 3.0 (m, 4H), 2.0 (m, 2H). MS (ES^+) m/e 285 $(M + H)^+$; HRMS Calcd for $(M + H)^+$: $C_{13}H_{13}N_6S^+$ 285.09169; found 285.09177.

(1*H*-Pyrazol-3-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (22f). The title compound was prepared according to the method described for compound **23c** (45 mg, 38%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (br s, 1H), 12.1 (s, 1H), 10.3 (s, 1H), 7.66 (s, 1H), 7.59 (s, 1H), 5.98 (s, 1H), 3.00−2.88 (m, 4H), 2.04−1.91 (m, 2H). MS (ES^+) m/e 273 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{12}H_{13}N_6S^+$ 273.09169; found 273.09164.

(5-Methyl-1*H*-pyrazol-3-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (22g). The title compound was prepared according to the method described for compound **23c** (50 mg, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (br s, 1H), 11.8 (s, 1H), 10.2 (s, 1H), 7.65 (s, 1H), 5.73 (s, 1H), 2.98−2.87 (m, 4H), 2.19 (s, 3H), 2.01−1.92 (m, 2H). MS (ES^+) m/e 287 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{13}H_{15}N_6S^+$ 287.10734; found 287.10762.

(1-Methyl-1*H*-pyrazol-3-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (22h). The title compound was prepared according to the method described for compound **23c** (30 mg, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (br, 1H), 10.3 (s, 1H), 7.66 (s, 1H), 7.53 (d, J = 2.2 Hz, 1H), 5.96 (d, J = 2.0 Hz, 1H), 3.74 (s, 3H), 2.98−2.89 (m, 4H), 2.01−1.93 (m, 2H). MS (ES^+) m/e 287 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{13}H_{15}N_6S^+$ 287.10734; found 287.10779.

(4,5,6,8-Tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-thiazol-2-yl-amine (22i). To a solution of **18a** (60 mg, 0.18 mmol) in THF (3.0 mL) was added NaH (60% in mineral oil, 15 mg, 0.37 mmol) at 0 °C, and the mixture was stirred at room temperature for 10 min, followed by the addition of 2-bromothiazole (30 mg, 8.18 mmol). The reaction mixture was refluxed overnight, cooled to room temperature, and quenched with ice. The aqueous layer was extracted with DCM (2 × 10 mL). The combined organic layers were concentrated, and the residue was purified by Combi*Flash* system (gradient: 10−90% ethyl acetate in hexanes) to afford PMB-protected intermediate, which was dissolved in TFA (2.5 mL). The resulting mixture was microwaved at 150 °C for 30 min and concentrated. The residue was quenched with saturated aqueous $NaHCO_3$, and the aqueous layer was extracted with *i*-PrOH/$CHCl_3$ (1:3, 3 × 15 mL). The combined organic layers were concentrated and the resulting residue was purified by reverse-phase HPLC to afford the title compound (2 mg, 4% over 2 steps). MS (ES^+) m/e 290 $(M + H)^+$.

(6-Methyl-pyridazin-3-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (22j). The title compound was prepared according to the method described for compound **23q** (16 mg,c 29%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (br s, 1H), 11.2 (s, 1H), 7.70 (s, 1H), 7.44 (d, J = 9.0 Hz, 1H), 7.26 (d, J = 9.0 Hz, 1H), 3.03−2.93 (m, 4H), 2.51 (s, 3H), 2.05−1.95 (m, 2H). MS (ES^+) m/e 299 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{14}H_{15}N_6S^+$ 299.10734; found 299.10769.

Cyclohexyl-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (22k). The title compound was prepared according to the method described for compound **23c** (14 mg, 17%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (s, 1H), 7.58 (s, 1H), 7.19 (d, J = 7.7 Hz, 1H), 2.99−2.79 (m, 4H), 2.53−2.51 (m, 1H), 1.98−1.89 (m, 4H), 1.75−1.53 (m, 3H), 1.37−1.11 (m, 5H). MS (ES^+) m/e 289 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{15}H_{21}N_4S^+$ 289.14814; found 288.28970.

3-Methyl-pyridin-2-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (23a). The title compound was prepared according to the method described for compound **23q** (25 mg, 46%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (br s, 1H), 10.1 (s, 1H), 8.13 (dd, J = 4.9, 1.2 Hz, 1H), 7.76 (s, 1H), 7.52 (dd, J = 7.2, 0.8 Hz, 1H), (dd, J = 7.2, 5.0 Hz, 1H), 3.02−2.94 (m, 4H), 2.33 (s, 3H), 2.04−1.95 (m, 2H). MS (ES^+) m/e 298 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{15}H_{16}N_5S^+$ 299.10734; found 299.10769.

(5-Methyl-pyridin-2-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (23b). The title compound was prepared according to the method described for compound **23c** (3 mg, 5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (br, 1H), 11.0 (s, 1H), 8.10−8.06 (m, 1H), 7.68 (s, 1H), 7.50 (dd, J = 8.6, 2.2 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 3.02−2.93 (m, 4H), 2.21 (s, 3H), 2.03−1.93 (m, 2H). MS (ES^+) m/e 298 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{15}H_{16}N_5S^+$ 298.11209; found 298.11209.

(6-Methyl-pyridin-2-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (23c). Into a vial was added 5-bromo-2-(4-methoxy-benzyl)-5,6,7,8-tetrahydro-2*H*-cyclohepta-pyrazol-4-one (**17d**) (0.100 g, 0.286 mmol), (6-methyl-pyridin-2-yl)-thiourea (0.0479 g, 0.286 mmol), and ethanol (5 mL, 80 mmol). The

reaction mixture was heated to reflux overnight, and then, using a rotary evaporator, the excess EtOH was removed. The crude material was dissolved in trifluoroacetic acid (3 mL, 40 mmol) and irradiated with microwave at 150 °C for 30 min. The excess TFA was removed in vacuo. The crude material was purified over silica gel eluting with 0−10% MeOH (2 M NH_3) in DCM to afford the title compound **23c** (25 mg, 29%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (s, 1H), 11.1 (s, 1H), 7.7 (s, 1H), 7.5 (t, J = 7.5 Hz, 1H), 6.8 (d, J = 8.3 Hz, 1H), 6.7 (d, J = 7.5 Hz, 1H), 3.0 (m, 4H), 2.4 (s, 3H), 2.0 (m, 2H). MS (ES$^+$) m/e 298 (M + H)$^+$. HRMS calcd for (M + H)$^+$: $C_{15}H_{16}N_5S^+$ 298.11209; found 278.10360.

(4,6-Dimethyl-pyridin-2-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (23d). The title compound was prepared according to the method described for compound **23c** (3 mg, 4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.0 (s, 1H), 7.68 (s, 1H), 6.63 (s, 1H), 6.60 (s, 1H), 3.01−2.92 (m, 4H), 2.39 (s, 3H), 2.22 (s, 3H), 2.03−1.94 (m, 2H). MS (ES$^+$) m/e 312 (M + H)$^+$. HRMS calcd for (M + H)$^+$: $C_{15}H_{16}N_5S^+$ 298.11209; found 278.10360.

(6-Fluoro-pyridin-2-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (23f). The title compound was prepared according to the method described for compound **23c** (5 mg, 6%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (s, 1H), 11.4 (s, 1H), 7.8 (q, J = 8.0 Hz, 1H), 7.7 (s, 1H), 7.0 (d, J = 8.0 Hz, 1H), 6.6 (dd, J = 8.0, 2.1 Hz, 1H), 3.0 (m, 4H), 2.0 (m, 2H). MS (ES$^+$) m/e 302 (M + H)$^+$. HRMS calcd for (M + H)$^+$: $C_{16}H_{18}N_5S^+$ 312.12774; found 312.12770.

(6-Chloro-pyridin-2-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (23g). The title compound was prepared according to the method described for compound **23c** (5 mg, 5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.6 (s, 1H), 11.4 (s, 1H), 7.7 (m, 2H), 7.7 (s, 1H), 7.0 (d, J = 7.8 Hz, 1H), 6.9 (d, J = 7.4 Hz, 1H), 3.0 (m, 4H), 2.0 (m, 2H). MS (ES$^+$) m/e 302 (M + H)$^+$. HRMS calcd for (M + H)$^+$: $C_{14}H_{13}FN_5S^+$ 302.08702; found 302.08736.

(5-Fluoro-pyridin-2-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (23e). The title compound was prepared according to the method described for compound **23q** (6 mg, 10%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (br, 1H), 11.2 (s, 1H), 8.24 (d, J = 3.0 Hz, 1H), 7.72−7.64 (m, 2H), 7.13−7.07 (m, 1H), 3.02−2.91 (m, 4H), 2.04−1.93 (m, 2H). MS (ES$^+$) m/e 302 (M + H)$^+$. HRMS calcd for (M + H)$^+$: $C_{14}H_{13}FN_5S^+$ 302.08702; found 302.08736.

(6-Methoxy-pyridin-2-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (23h). The title compound was prepared according to the method described for compound **23q** (5 mg, 9%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.1 (s, 1H), 7.74−7.52 (m, 2H), 6.57 (d, J = 7.8 Hz, 1H), 6.27 (d, J = 7.8 Hz, 1H), 4.00 (s, 3H), 3.01−2.94 (m, 4H), 2.03−1.95 (m, 2H). MS (ES$^+$) m/e 314 (M + H)$^+$. HRMS calcd for (M + H)$^+$: $C_{15}H_{16}N_5OS^+$ 314.10701; found 314.10631.

(6-Ethoxy-pyridin-2-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (23i). The title compound was prepared according to the method described for compound **23q** (8 mg, 10%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.6 (br, 1H), 11.1 (s, 1H), 7.76 (s, 1H), 7.63 (t, J = 7.9 Hz, 1H), 6.64 (d, J = 7.8 Hz, 1H), 6.32 (d, J = 7.8 Hz, 1H), 4.55 (q, J = 7.0 Hz, 2H), 3.10−3.01 (m, 4H), 2.12−2.02 (m, 2H), 1.45 (t, J = 7.0 Hz, 3H). MS (ES$^+$) m/e 328 (M + H)$^+$. HRMS calcd for (M + H)$^+$: $C_{16}H_{18}N_5OS^+$ 328.12266; found 328.12323.

6-(4,5,6,8-Tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]-azulen-2-ylamino)-pyridine-2-carboxylic Acid Methyl Ester (23k). The title compound was prepared according to the method described for compound **23q** (5 mg, 8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (br, 1H), 11.3 (s, 1H), 7.77 (dd, J = 8.3, 7.4 Hz, 1H), 7.63 (s, 1H), 7.50 (dd, J = 7.3, 0.6 Hz, 1H), 7.21(d, J = 8.3 Hz, 1H), 3.84 (s, 3H), 2.95−2.88 (m, 4H), 1.97−1.89 (m, 2H). MS (ES$^+$) m/e 342 (M + H)$^+$. HRMS calcd for (M + H)$^+$: $C_{16}H_{16}N_5O_2S^+$ 342.10192; found 342.10217.

[6-(4,5,6,8-Tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]-azulen-2-ylamino)-pyridin-2-yl]-methanol (23l). The title compound was prepared according to the method described for compound **23q** (8 mg, 10%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (br, 1H), 11.1 (s, 1H), 7.71−7.63 (m, 1H), 6.97 (d, J = 7.0 Hz, 1H), 6.88 (d, J = 8.1 Hz, 1H), 5.34 (s, 1H), 4.55 (s, 1H), 3.02−2.92 (m, 4H), 2.04−1.94 (m, 2H). MS (ES$^+$) m/e 314 (M + H)$^+$. HRMS calcd for (M + H)$^+$: $C_{15}H_{16}N_5OS^+$ 342.10192; found 342.10217.

[6-(2-Methoxy-ethoxy)-pyridin-2-yl]-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (23m). The mixture of (6-fluoro-pyridin-2-yl)-[8-(4-methoxy-benzyl)-4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl]-amine (50 mg, 0.052 mmol) and NaOH (14 mg, 0.36 mmol) in 2-methoxyethanol (0.5 mL) was microwaved at 150 °C for 30 min. The mixture was diluted with DCM (20 mL). The organic layer was washed with brine and concentrated to give a crude intermediate, which was dissolved in TFA (2 mL). The resulting reaction mixture was microwaved at 130 °C for 30 min and concentrated. The residue was dissolved in *i*-PrOH/$CHCl_3$ (1:3. 30 mL) and quenched with saturated aqueous $NaHCO_3$. The combined organic layers were concentrated, and the resulting residue was purified on a reversed-phase liquid chromatography/mass spectrometry (RP-HPLC/MS) purification system (Gradient: acetonitrile in water, 20−95% in 3.3 min with a cycle time of 5 min. A shallow gradient between 25 and 50% of acetonitrile was used between 0.6 andd 3.0 min to separate close-eluting impurities. Flow rate: 100 mL/min. Mobile phase additive: 39 mM of ammonium acetate. Column: Inertsil C8, 30 mm × 50 mm, 5 μm particle size) to afford the title compound (5 mg, 9% over 2 steps). The ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (br, 1H), 11.0 (s, 1H), 7.61 (S, 1H), 7.49 (T, J = 7.9 Hz, 1H), 6.50 (d, J = 7.8 Hz, 1H), 6.20 (d, J = 7.9 Hz, 1H), 4.49−4.44 (m, 2H), 3.67−3.62 (m, 2H), 3.25 (s, 3H), 2.96−2.86 (m, 4H), 1.96−1.87 (m, 2H). MS (ES$^+$) m/e 358 (M + H)$^+$. HRMS calcd for (M + H)$^+$: $C_{17}H_{20}N_5O_2S^+$ 358.13322; found 358.13348.

***N*,*N*-Dimethyl-*N*′-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-pyridine-2,6-diamine (23n).** The title compound was prepared according to the method described for compound **23q** (23 mg, 29%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (br, 1H), 10.7 (s, 1H), 7.59 (s, 1H), 7.29 (t, J = 8.0 Hz, 1H), 6.13 (d, J = 7.7 Hz, 1H), 5.97 (d, J = 8.1 Hz, 1H), 3.03 (s, 6H), 2.93−2.85 (m, 4H), 1.95−1.86 (m, 2H). MS (ES$^+$) m/e 327 (M + H)$^+$. HRMS calcd for (M + H)$^+$: $C_{16}H_{19}N_6S^+$ 327.13864; found 327.13912.

***N*-Ethyl-*N*′-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta-[*e*]azulen-2-yl)-pyridine-2,6-diamine (23o).** The title compound was prepared according to the method described for compound **23q** (21 mg, 26%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (br, 1H), 10.7 (s, 1H), 7.66 (s, 1H), 7.22 (t, J = 7.8 Hz, 1H), 6.43 (d, J = 5.5 Hz, 1H), 6.08 (d, J = 7.6 Hz, 1H), 5.93 (d, J = 7.9 Hz, 1H), 3.48−3.38 (m, 2H), 3.02−2.89 (m, 4H), 2.03−1.94 (m, 2H), 1.18 (t, J = 7.1 Hz, 3H). MS (ES$^+$) m/e 326 (M + H)$^+$; HRMS calcd for (M + H)$^+$: $C_{16}H_{19}N_6S^+$ 327.13864; found 327.13873.

(6-Pyrrolidin-1-yl-pyridin-2-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triazacyclopenta[*e*]azulen-2-yl)-amine (23p). The title compound was prepared according to the method described for compound **23q** (5 mg, 8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (br, 1H), 10.7 (s, 1H), 7.59 (s, 1H), 7.30−7.23 (m, 1H), 6.07 (d, J = 7.7 Hz, 1H), 5.79 (d, J = 8.1 Hz, 1H), 3.46−3.37 (m, 4H), 2.93−2.84 (m, 4H), 1.96−1.84 (m, 6H). MS (ES$^+$) m/e 353 (M + H)$^+$. HRMS calcd for (M + H)$^+$: $C_{18}H_{21}N_6S^+$ 353.15429; found 353.15444.

(4-Methyl-pyrimidin-2-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (23q). The mixture of **18b** (60 mg, 18 mmol), 2-chloro-4-methylpyrimidine (23 mg, 18 mmol), Cs_2CO_3 (120 mg, 0.363 mmol), $Pd_2(dba)_3$ (2 mg, 0.002 mmol), and Xantphos (2 mg, 0.004 mmol) in DMF/THF (1.5 mL/1.5 mL) was microwaved at 125 °C for 1 h, filtered through the Celite pad, and concentrated to afford the crude intermediate, [8-(4-methoxy-benzyl)-

4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl]-(4-methyl-pyrimidin-2-yl)-amine, which was dissolved in TFA (3.0 mL). The resulting reaction mixture was microwaved at 150 °C for 30 min and concentrated. The residue was dissolved in *i*-PrOH/$CHCl_3$ (1:3. 30 mL) and quenched with saturated aqueous $NaHCO_3$. The combined organic layers were concentrated, and the resulting residue was purified on a reverse-phase HPLC to afford the title compound (5 mg, 9% over 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (br, 1H), 11.4 (s, 1H), 8.43 (d, J = 5.0 Hz, 1H), 7.70 (s, 1H), 6.88 (d, J = 5.0 Hz, 1H), 3.01−2.95 (m, 4H), 2.42 (s, 3H), 2.02−1.95 (m, 2H). MS (ES^+) m/e 299 $(M + H)^+$. HRMS calcd for $(M + H)^+$: $C_{14}H_{15}N_6S^+$ 299.10734; found 299.10760.

(4-Methoxy-pyrimidin-2-yl)-(4,5,6,8-tetrahydro-3-thia-1,7,8-triaza-cyclopenta[*e*]azulen-2-yl)-amine (23r). The title compound was prepared according to the method described for compound **23q** (5 mg, 9%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.5 (br, 1H), 11.4 (s, 1H), 8.29 (d, J = 5.7 Hz, 1H), 7.70 (s, 1H), 6.42 (d, J = 5.7 Hz, 1H), 4.01 (s, 3H), 3.03−2.94 (m, 4H), 2.03−1.95 (m, 2H). MS (ES^+) m/e 315 $(M + H)^+$; HRMS calcd for $(M + H)^+$: $C_{14}H_{15}N_6OS^+$ 315.10226; found 315.10278.

■ ASSOCIATED CONTENT

Supporting Information. Cross reactivity panel for compounds **9** and **22a**, reference compounds used in cross reactivity panel and controls used in the MDCK-mdr1 in vitro assay for P-gp substrate activity. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

DMPK, drug metabolism and pharmacokinetics; Tr, trityl; PMB, 4-methoxybenzyl; PAM, positive allosteric modulator; mGlu4, metabotropic glutamate 4 receptor; HTS, high-throughput screening; MTS, medium-throughput screening; DMF-DMA, *N*, *N*-dimethylformamide dimethoxyacetal; CNS, central nervous system; TLC, thin layer chromatography; LC-MS, liquid chromatography−mass spectrometry; NMR, nuclear magnetic resonance; FLIPR, fluorescent imaging plate reader; tPSA, topological polar surface area; CL_{int}, intrinsic clearance; CSF, cerebrospinal fluid; TMS, tetramethylsilane; BHK, baby hamster kidney; DMEM, Dulbecco's Modified Eagle Medium; HBSS, Hank's Balanced Saline Solution; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; BSA, bovine serum albumin; NAM, negative allosteric modulator; SAR, structure−activity relationship; SPR, structure−property relationship; P-gp, P-glycoprotein; GPCR, G protein coupled receptor; hERG, human ether-à-go-go related gene

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