Synthesis and biological investigation of 1,2,4-triazolo[4,3-*a*]azines as potential HSF1 inductors

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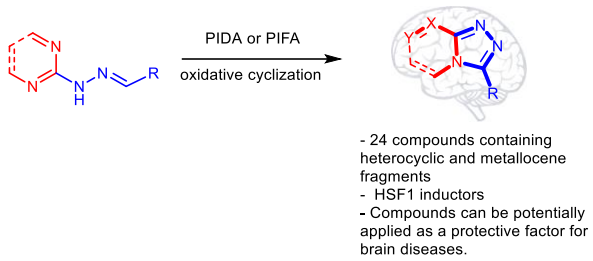

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Abstract Derivatives of fused 1,2,4-triazines containing heterocyclic and metallocene fragments were obtained by one-pot oxidative cyclization of heterocyclic hydrazones in the presence of hypervalent iodine(III) reagents. For 1,2,4-triazolo[4,3-a]azines the ability to HSF1 activation was investigated. The obtained compounds were shown to increase the degree of HSF1 activation. It was shown that the 1,2,4-triazines are able to induce the Hsp70 expression and decrease the mutant HTT aggregate formation.

Key words Azoloazine, hypervalent iodine (III), cyclization, HSF1 inductors, heteroaromatic, metallocene

1,2,4-Triazole and their annulated derivatives were found to to have strong antibacterial,[1] antifungal,[2] anticancer[3] and antiviral[4] activities. Their derivatives have found application in the treatment of neurodegenerative diseases. For example, [1,2,4]triazolo[1,5-a]pyrimidine (**1**) can be used in the therapy for Alzheimer's disease,[5] triazolopyrimidine (**2**) is AChE and BuChE inhibitor[6] (Fig. 1). Compound SCH-58261 is an antagonist of A_{2A} and A_3 adenosine receptors[7] and compound CNDR-51657 is a potential candidate for the treatment of tauopathies,[8] compound (**3**) is an inhibitor of CK1δ,[9] S-enantiomer (**4**) has significant *in vivo* memory-enhancing effects (Fig. 1).[10]

A wide variety of oxidants has been employed for the cyclization of arylhydrazones to their respective fused triazole derivatives, including chemical oxidation with iron(III)[11] and copper(II)[12] chlorides, chloramine T trihydrate,[13] *N*-bromosuccinimide with 1,8-diazabicyclo[5.4.0]-undec-7-ene,[14] $RuCl_3$/oxone,[15] and electrochemical synthesis.[16] Today, hypervalent organoiodine(III) reagents are used as mild oxidizing agents. These reagents have been successfully employed in oxidative transformation of (hetero)arylhydrazones to 1,2,4-triazole systems.[17]

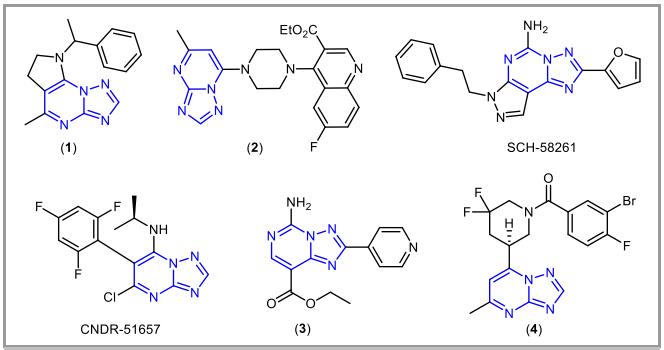
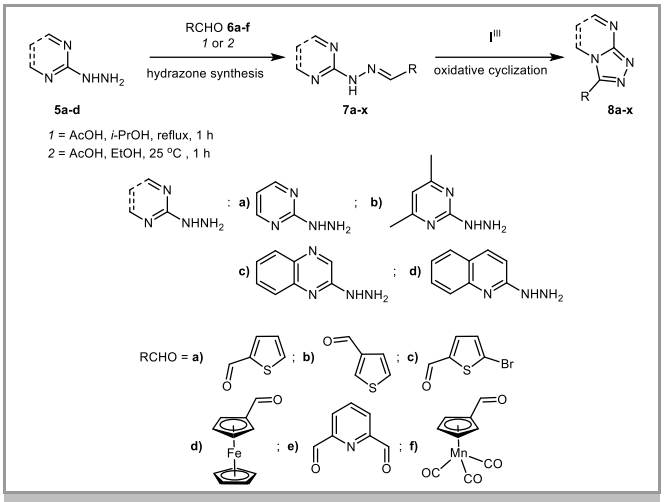


Figure 1 Biologically active *N*-containing heterocycles.

Here we report the synthesis of novel heterocyclic derivatives of 1,2,4-triazolo[4,3-a]azines using hypervalent iodine(III) reagent as mind an oxidant. Notably, the developed method allows the synthesis of heterocyclic metallocene derivatives. Moreover, it was shown that the obtained azoloazines are able to activate of HSF1 as a protective factor for brain diseases.

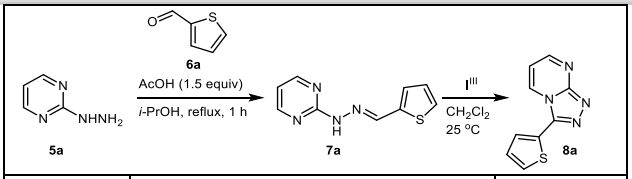
A straightforward method for the preparation of 1,2,4-triazolo[4,3-*a*]azines **8** is an oxidative cyclization of 2-azinylhydrazones **7** (Scheme 1). Hydrazones **7** were obtained by condensation of azinylhydrazines **5**[18] with heteroaromatic or metallocene aldehydes **6** when refluxing in *i*-PrOH (for compounds **8a-d,g-j,p,v**) or at 25 °C in EtOH (for compounds **8e,f,k,l,m-o,q-u,w,x**) in the presence of 20-30 mol% acetic acid (Scheme 1).[3a]



Scheme 1 General procedure for 1,2,4-triazolo[4,3-*a*]azine synthesis.

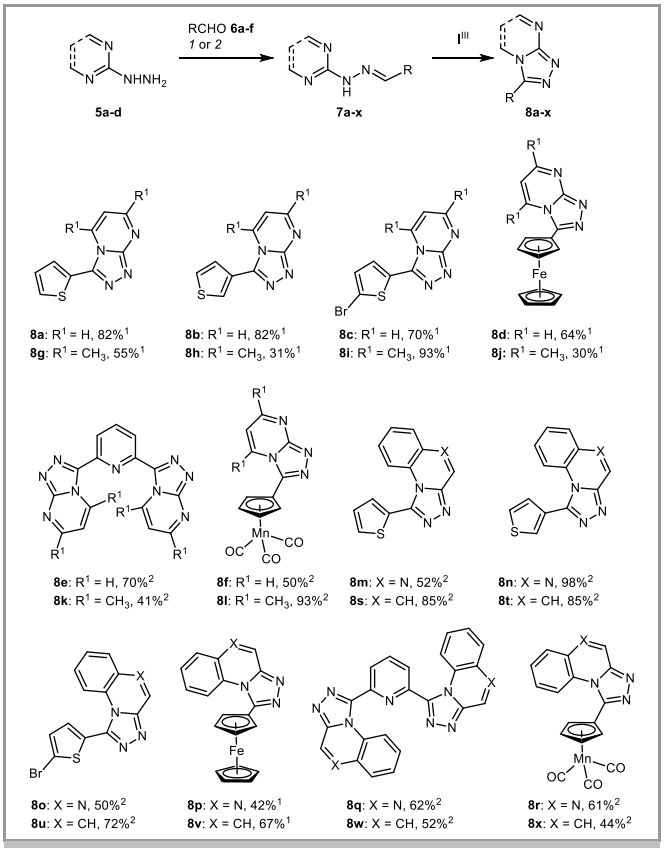
Optimization of the oxidative cyclization step was carried out using the synthesis of 3-(thiophen-2-yl)-1,2,4-triazolo[4,3-*a*]pyrimidine **8a**.[17a,d] We began our investigation by exploring the cyclization of 2-(2-(thiophen-2-ylmethylene)hydrazinyl)pyrimidine **7a** mediated by (diacetoxyiodo)benzene (PIDA). Using 1.1 equiv or 1.5 equiv of PIDA at 25 °C in CH_2Cl_2 (Table 1, entries 1 and 2), we isolated the desired product **8a** in 20% and 60% yield, respectively. Using 1.1 equiv or 1.5 equiv of [bis(trifluoroacetoxy)iodo]benzene (PIFA) at 25 °C in CH_2Cl_2 (Table 1, entries 3 and 4), we isolated the desired **8a** in 82% and 79% yield, respectively. Afterward, various hypervalent iodine(III) reagents such as PhIO, PhI and PhI(OH)OTs (Koser's reagent or [hydroxy(tosyloxy)iodo]benzene) were screened (Table 1, entries 5-7). Subsequently, we discovered that the use of PIFA as an oxidant at 25 °C gives the product **8a** in highest yield.

Table 1 Optimization of the reaction conditions for the preparation of **8a**.



Entry	I^{III} (equiv)	Yield (%)
1	PIDA (1.1)	20
2	PIDA (1.5)	60
3	PIFA (1.1)	82
4	PIFA (1.5)	79
5	PhIO (1.5)	42
6	PhI (5.0) + AcOOH (25 mol%)	n.d.
7	Koser's reagent (1.5)	n.d.

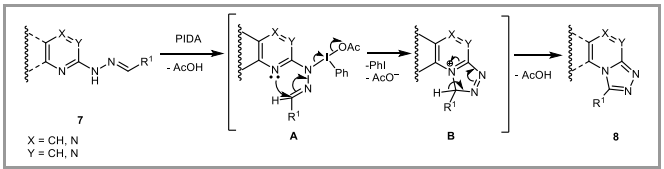
Having in the hands the optimized reaction conditions (1.1 equiv PIFA, CH_2Cl_2, r.t.), we next explored the substrate scope with various 2-azinylhydrazones **7** (Scheme 2). The results showed that various 2-azinylhydrazones **7** were tolerated in the reaction. For example, compounds **7a-c,g-i**, which contain 3-thiophenecarboxaldehyde **6b** and 5-bromo-2-thiophenecarboxaldehyde **6c** in their structure, were cyclized in the presence of PIFA to products **8a-c,g-i** in yields from 31 to 93% (Scheme 2).



Scheme 2 Synthesis of 1,2,4-triazolo[4,3-*a*]azine **8a-x**. Reaction conditions: (1) *i*-PrOH, AcOH, reflux, 1 h, I^{III} = PIFA; (2) EtOH, AcOH, 25 °C, 1 h, I^{III} = PIDA.

The synthesis of ferrocenyl-containing 1,2,4-triazolo[4,3-*a*]azines **8d,j,p,v** could not be carried out by *one-pot* procedure. In this case, by-products of unknown structure were formed. Therefore, the synthesis was carried out in two steps. At the first step, hydrazones **7d,j,p,v** were obtained by the reaction of ferrocene carboxaldehyde **6d** with hydrazines **5a-c**. At the second step, products **8d,j,p,v** were obtained in yields from 30 to 67% by the oxidative cyclization of hydrazones **7** in the presence of PIFA (Scheme 2). Unfortunately, the optimized reaction conditions of the oxidative cyclization were ineffective for the synthesis of quinoline- and quinoxaline-containing 1,2,4-triazoles **8e,f,k,l,m-o,q-u,w,x**. Nevertheless, the replacement of PIFA to PIDA (Scheme 2) afforded products **8e,f,k,l,m-o,q-u,w,x** in yields from 41 to 93%. The synthesis of compounds **8e,f,k,l,m-o,q-u,w,x** was carried out by the *one-pot* procedure, similarly to derivatives **8a-c,g-i**. Unequivocal evidence for the structure of compounds **8** was obtained by X-ray analysis (see Supplementary data).

A plausible mechanism for the PIDA mediated oxidative cyclization was described in Scheme 3.[17a-d] Intermediate **A** was generated through ligand exchange between PIDA and hydrazones **7** with elimination of the molecule of acetic acid. Intermediate **B** was apparently formed via elimination of iodobenzene and an acetate anion. In the next step, the intermediate **B** underwent intramolecular cyclization in the presence of the acetate anion to form the desired products **8**.



Scheme 3 Proposed mechanism for the reaction of cyclization of 2-azinylhydrazones.

Conformational neurodegenerative pathologies such as Alzheimer's disease, Parkinson's disease, and Huntington's disease develop due to defects in structure of certain proteins specific for each pathology. Huntington's disease is a hereditary neurological pathology resulting from a mutation within exon 1 of the huntingtin (HTT) gene, which is located on the short arm of human chromosome 4. Genetic expansion of CAG triplets in the HTT gene (glutamine, polyQ 35) leads to the production of mutated huntingtin protein (mHTT) and causes the formation of cytotoxic oligomers and aggregates[19] associated with numerous violations in neuronal physiology, ultimately resulting in massive cell death in the striatum and cerebral cortex.[20]

Molecular chaperones, mostly related to heat shock protein families (HSPs), guide polypeptides through conformational changes, such as de novo folding, assembly and disassembly, transport, and targeting for degradation.[21] The expression of the molecular chaperones is controlled by specific heat shock transcription factor 1 (HSF1).[22] Since an activation of the HSF1 causes elevated synthesis of molecular chaperones, it appears to be a novel strategy for the treatment of the most common neurodegenerative conditions for which no effective treatment currently exists.[23]

A screening was carried out using a HSE-luciferase reporter system expressed in HeLa cells (HeLa-Luc).[24] In this assay, the HeLa cells expressing a genetic construct consisting of a heat-shock-activated luciferase gene were subjected to a potential HSF1 inductor (Fig. 2). Three compounds (**8m-o**) at a concentration of 0,5 μM were able to increase the degree of HSF1 activation by more than 200% that was comparable to the activity of a reference compound, U133,[23] which increased the HSF1 activity by 3.63 times compared with the untreated cells.

We analyzed the effect of the three compounds on viability of two neuroblastoma cells, human SH-SY5Y and rat PC-12 cells that had a neurological phenotype,[25] and two glioma cells, rat C6 cells and human T98G cells, in comparison with reference substance U133 using CytoTox96 assay. The cells were treated with U-133 and **8m-o** at concentrations in the range of 0.1 to 2.5 μM and **8m** and **8o** were found to possess similar toxicity as U133 at the maximum concentration of 2.5 μM in rat cells; the number of dead cells reached 35.98 ± 2.55% for U133, 36.23 ± 3.52 % for **8m** and 36.83 ±0.77% for **8o** in PC-12 cells, and 42.54 ± 1.53% for U133, 43.28 ± 4.13% for **8m** and 45.71 ± 2.03% for **8o** in C6 glioma cells. The less toxic compound was found to be **8n** that caused death of 21.98 ± 0.37% and 21.88 ± 2.16% of cells at the maximum concentration for PC-12 and C6 cells respectively. In human cells, U133 demonstrated lower toxicity (26.22 ± 0.77%) than **8m** (31.62 ± 0.36%) and **8o** (31.29 ± 0.48%) in SH-SY5Y cells and 25.12 ± 0.11% comparing to 36.66 ± 0.25% for **8m** and 37.25 ± 0.15 % for **8o** in T98G cells. However, the compound **8n** possessed the toxicity even lower than U133, 18.99 ± 0.78% for SH-SY5Y cells and 18.93 ± 1.24% for T98G cells.

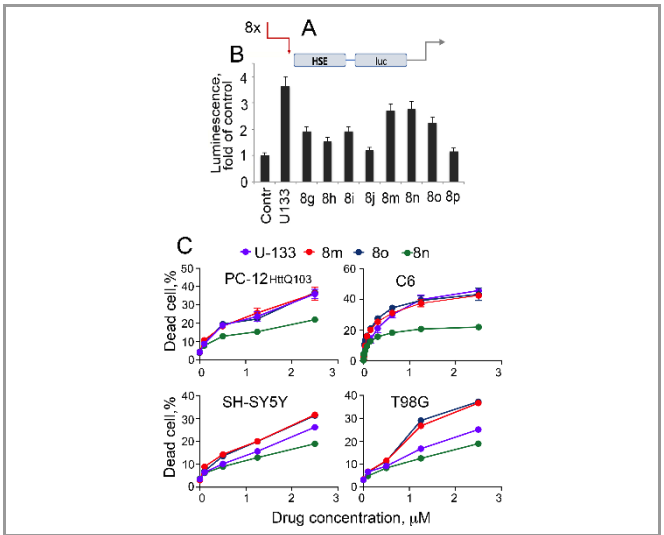


Figure 2 Analysis of biological activity of compounds **8g-j,m-p**. (A) Diagram of HSE-luc, a HSF1 promoter reporter integrated to HeLa cells. (B) Reporter assay of eight compounds **8g-j,m-p**. Reporter HeLa cells (transfected with plasmid bearing the luciferase gene under the control of HSE promoter) were seeded into wells of a 96-well plate. The cells were incubated with the compounds taken at the final concentration of 0.5 μM at 37°C for 20 h before analysis. U-133 was used as the reference compound. (C) The toxicity of the selected compounds compared to toxicity of U133 was measured as LDH activity in the cell medium of rat neuroblastoma PC-12HttQ103 cells, human neuroblastoma SH-SY5Y cells, rat glioblastoma C6 cells, and human glioblastoma T98G cells.

To understand whether the selected compounds are able to prevent formation of mutant HTT aggregates in the cell model of Huntington's disease we used pheochromocytoma PC-12HttQ103 cells bearing the 1st exon of the HTT gene under inducible promoter. Firstly, we performed western blotting of these cells treated with **8m-o** at indicated concentrations and found that **8n** and **8o** are able to induce a Hsp70 accumulation that is approximately 3.5-fold higher than in control cells at maximum concentration. The compound **8m** was less effective and induced approximately 2-fold Hsp70 increase (Fig. 3).

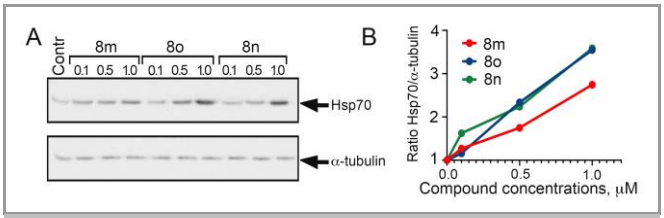


Figure 3 Compounds **8m-o** induce the Hsp70 dose-depend accumulation in PC-12HttQ103 cells. (A) Western blot analysis of PC-12HttQ103 cells treated with the compounds **8m-o** at the indicated concentrations. (B) Intensity of chemiluminescence measured using Image Studio software was calculated as a Hsp70/α-tubilin ratio. Representative data of two independent experiments are presented.

Being convinced that the selected compounds are capable to induce the Hsp70 accumulation in PC-12HttQ103 cells, we induced the expression of mutant HTT with PonA simultaneously adding the compounds **8m-o** to the cell culture (Fig. 4A). Confocal microscopy data demonstrated that the amount and size of the aggregates became lower in the presence of 1 μM of the tested compounds, especially when using **8n** (Fig. 4A). Filter trap assay data confirmed the highest efficiency of **8n** that decreased the amount of aggregated HTT already at concentration of 0.5 μM.

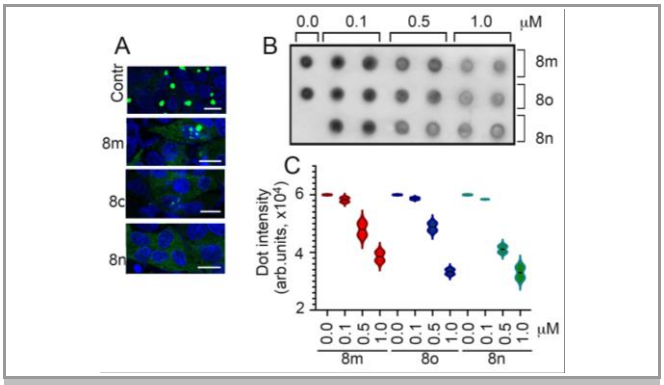


Figure 4 The compounds **8m-o** decrease mutant HTT aggregation in PC-12HttQ103 cells. (A) Data of confocal microscopy. PC-12HttQ103 cells were treated with compounds at a concentration of 1 µM: PC-12HttQ103 fused to EGFP are presented in green, nuclei stained with DAPI are presented in blue. Scale bar: 5 µm (B) Filter trap assay of lysates of PC-12HttQ103 cells treated with **8m-o** at the indicated concentrations. (C) The intensity of the dots from (B) calculated using Image Studio software. Representative data of two independent experiments are presented.

The obtained compounds were investigated as inducers of HSF1.

A wide range of 1,2,4-triazolo[4,3-a]azines was obtained from available aldehydes and heterocyclic hydrazines. The oxidative cyclization of azinylhydrazones in the presence of PIFA or PIDA was found to proceed under mild metal-free conditions. The approach allows to use of the hypervalent iodine (III) compounds to obtain derivatives of triazolopyrimidines, triazoloquinolines, triazoloquinoxalines containing heterocyclic and metallocene fragments. The obtained compounds were investigated as HSF1 inducers. Three compounds (**8m-o**) were shown to be able to increase the degree of HSF1 activation. These results were comparable to the activity of the reference substance, echinochrome triacetyl glucoside U133. The compounds **8m** and **8o** were highly toxic to neuroblastoma SH-SY-5Y cells whereas the compound **8n** possessed low toxicity. All three compounds are able to induce the Hsp70 expression and decrease the mutant HTT aggregate formation. Taking into account the low toxicity and the highest ability to suppress HTT aggregation, we suggest that the compound **8n** can potentially be used as a protective factor for brain diseases.

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The ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance II, using $SiMe_4$ as internal reference in DMSO-d6 and CF_3COOD. Chemical shifts (δ) are reported in parts per millions (ppm) and spin multiplicities are given as singlet (s), doublet (d), triplet (t), or multiplet (m). Coupling constants (*J*) are reported in Hz. Electrospray mass spectra were recorded in positive mode with maXis impact high resolution Q-TOF mass spectrometer (Bruker Daltonics) and a mass spectrometer SHIMADZU GCMS-QP2010 Ultra with sample ionization by electron impact (EI). The elemental analysis was carried out on an automated Perkin Elmer PE 2400 series II CHNS/O analyzer. Melting points were determined using a Boetius heating stage. X-ray diffraction analysis was performed on an X-ray diffractometer equipped with a CDD detector (Xcalibur 3). The course of the reactions was monitored by TLC on 0.25 mm silica gel plates (Merck 60F 254). The column chromatography was performed on silica gel (silica gel 60, 0.035–0.070 mm, 220–440 mesh).

The crystallographic data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Procedures

General procedure for the preparation of 8a-d,g-j,p,v. The glacial acetic acid (20-30 mol%) was added to a solution of 2-azinylhydrazine **5a-d** (1 equiv) and the corresponding carboxaldehyde **6a-d** (1 equiv) in dry *i*-PrOH (5 ml). The reaction mixture was stirred at reflux for 1 h. Further the reaction mixture was evaporated under reduced pressure. Then the PIFA (1.1 equiv) was added to a solution of azinylhydrazone **7a-d,g-j,p,v** in dry CH_2Cl_2. The reaction mixture was stirred at 25 °C for 4 h. Finally, the solution was concentrated under reduced pressure and the product was purified on silica gel (using an appropriate solvent as eluent).

General procedure for the preparation of 4e,f,k,l-o,q-u,w,x. The glacial acetic acid (20-30 mol%) was added to a solution of 2-azinylhydrazine **5a-d** (1 equiv) and the corresponding carboxaldehyde **6a-c,e,f** (1 equiv) in dry EtOH (5 ml). The reaction mixture was stirred at 25 °C for 1 h. Further the reaction mixture was evaporated under reduced pressure. Then the PIDA (1.1-2.2 equiv) was added to a solution of azinylhydrazone **7e,f,k,l-o,q-u,w,x** in dry CH_2Cl_2. The reaction mixture was stirred at 25 °C for 4 h. Finally, the solution was concentrated under reduced pressure and the reaction mixture was purified on silica gel (using an appropriate solvent as eluent) for compounds **8k,l-o,q-u,w,x**. Compounds **8e,f** were recrystallized from CH_2Cl_2.

3-(Thiophen-2-yl)-[1,2,4]triazolo[4,3-*a*]pyrimidine (8a).

The product was purified by column chromatography (EtOAc).

Yield: 165 mg (82%); beige solid; R_f = 0.1 (TLC: EtOAc); mp 192 °C.

FTIR: 3120, 3070, 1614, 1492, 1419, 1353, 762 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.13 (1 H, dd, *J* = 7.0, 1.7 Hz, 7-H), 8.76 (1 H, dd, *J* = 3.9, 1.8 Hz, 5-H), 7.90 (1 H, dd, *J* = 3.7, 0.8 Hz, 3′-H), 7.85 (1 H, dd, *J* = 5.1, 0.8 Hz, 5′-H), 7.33 (1 H, dd, *J* = 5.0, 3.8 Hz, 4′-H), 7.21 (1 H, dd, *J* = 7.0, 3.9 Hz, 6-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 154.9 (C5), 153.5 (C4a), 140.6 (C3), 133.1 (C7), 128.8 (C4′), 128.3 (C5′), 127.1 (C2′), 127.0 (C3′), 110.6 (C6)

MS (ESI): m/z (%) = 202 (100) [M]$^+$.

Anal. Calc. for $C_9H_6N_4S$: C, 53.45; H, 2.99; N, 27.70; S, 15.85. Found: C, 53.42; H, 3.00; N, 27.68; S, 15.87.

3-(Thiophen-3-yl)-[1,2,4]triazolo[4,3-*a*]pyrimidine (8b).

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The product was purified by column chromatography (EtOAc).

Yield: 165 mg (82%); beige solid; R_f = 0.1 (TLC: EtOAc); mp 218 °C.

FTIR: 3123, 3092, 1614, 1520, 1419, 1496 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.10 (1 H, dd, *J* = 7.0, 1.8 Hz, 7-H), 8.75 (1 H, dd, *J* = 3.8, 1.8 Hz, 5-H), 8.36 (1 H, dd, *J* = 2.8, 1.3 Hz, 2′-H), 7.85 (1 H, dd, *J* = 5.0, 2.8 Hz, 5′-H), 7.76 (1 H, dd, *J* = 5.0, 1.2 Hz, 4′-H), 7.19 (1 H, dd, *J* = 7.0, 3.9 Hz, 6-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 154.7 (C7), 153.2 (C3), 141.7 (C8a), 133.2 (C7), 127.8 (C5′), 126.9 (C4′), 126.5 (C3′), 125.5 (C2′), 110.3 (C6).

MS (ESI): m/z (%) = 202 (100) [M]$^+$.

Anal. Calc. for $C_9H_6N_4S$: C, 53.45; H, 2.99; N, 27.70; S, 15.85. Found: C, 53.48; H, 3.00; N, 27.73; S, 15.87.

3-(5-Bromothiophen-2-yl)-[1,2,4]triazolo[4,3-*a*]pyrimidine (8c).

The product was purified by column chromatography (EtOAc/MeOH 9:1).

Yield: 196 mg (70%); beige solid; R_f = 0.5 (TLC: EtOAc/MeOH 9:1); mp 213 °C.

FTIR: 3099, 3077, 1612, 1472, 1416 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.14 (1 H, d, *J* = 6.0 Hz, 7-H), 8.77 (1 H, d, *J* = 2.3 Hz, 5-H), 7.75 (1 H, d, *J* = 3.9 Hz, 3′-H), 7.46 (1 H, d, *J* = 3.9 Hz, 4′-H), 7.23 (1 H, dd, *J* = 6.9, 3.9 Hz, 6-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 155.1 (C5), 153.5 (C8a), 139.7 (C3), 133.4 (C7), 131.7 (C4′), 129.0 (C2′), 127.8 (C3′), 114.3 (C5′), 110.7 (C6).

MS (ESI): m/z (%) = 282 (100) $[M+H]^+$.

Anal. Calc. for $C_9H_5BrN_4S$: C, 38.45; H, 1.79; N, 19.93; Br, 28.42; S, 11.40. Found: C, 38.49; H, 1.77; N, 19.95; Br, 28.45; S, 11.37.

3-Ferrocenyl-[1,2,4]triazolo[4,3-*a*]pyrimidine (8d).

The product was purified by column chromatography (EtOAc/MeOH 9:1).

Yield: 194 mg (64%); dark red solid; R_f = 0.3 (TLC: EtOAc/MeOH 9:1); mp 200 °C.

FTIR: 3109, 2925, 1678, 1128 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.12-9.10 (1 H, m, 7-H), 8.71-8.67 (1 H, m, H-5), 7.15 (1 H, dd, J = 7.0, 3.8 Hz, 6-H), 5.00 (2 H, m, C_5H_4), 4.53 (2 H, m, C_5H_4), 4.21 (5 H, s, CpH).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 154.4 (C5), 153.5 (C3), 144.7 (C8a), 133.1 (C7), 110.0 (C6), 70.0 (C-Cp), 69.8 (C_5H_4), 69.0 (Cp), 67.1 (C_5H_4).

HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{15}H_{12}FeN_4$: 304.0411; found 305.0478.

Anal. Calc. for $C_{15}H_{12}FeN_4$: C, 59.24; H, 3.98; N, 18.42. Found C, 59.01; H, 4.15; N, 18.59.

2,6-Bis([1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)pyridine (8e).

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The product was recrystallized from CH_2Cl_2.

Yield: 220 mg (70%); beige solid; mp 359 °C.

FTIR: 3119, 3080, 1619, 1593, 1572, 1518, 1503, 1488, 1412, 1390, 1293, 1083, 996, 820, 792, 772 cm^{-1}.

^{1}H NMR (400 MHz, CF_3COOD): δ = 9.87 (2 H, d, J = 6.1 Hz, 5-H), 9.28 (2 H, m, 7-H), 8.59 (2 H, d, J = 7.8 Hz, 3'-H, 5'-H), 8.42 (1 H, t, 4'-H), 7.69 (2 H, m, 6-H).

^{13}C NMR (100 MHz, CF_3COOD): δ = 180.0 (C8a), 164.0 (C7), 148.2 (C3), 143.7 (C2), 142.3 (C6'), 140.9 (C4'), 136.2 (C5), 127.3 (C3',C5'), 115.9 (C6).

MS (ESI): m/z (%) = 315 (100) $[M]^+$.

Anal. Calc for $C_{15}H_9N_9$: C, 57.14; H, 2.88; N, 39.98. Found C, 57.04; H, 2.84; N, 39.95.

3-Cymantrenyl-[1,2,4]triazolo[4,3-*a*]pyrimidine (8f).

The product was recrystallized from CH_2Cl_2.

Yield: 161 mg (50%); yellow solid; mp 269 °C.

FTIR: 3124, 3086, 2016, 1952, 1917, 1620, 1518, 1433 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.97 (1H, dd, J = 7.0, 1.4 Hz, 7-H), 8.78 (1H, dd, J = 3.7, 1.6 Hz, 5-H), 7.25 (1H, dd, J = 7.0, 3.9 Hz, 6-H), 5.99-6.00 (2H, m, C_4H_5), 5.30-5.31 (2H, m, C_5H_4).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 224.1 (CO), 154.8 (C5), 153.6 (C8a), 139.7 (C3), 132.9 (C7), 110.5 (C6), 87.2 (C-Cp), 84.0 (C_5H_4), 83.6 (C_5H_4).

HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{13}H_7MnN_4O_3$: 321.9899; found 322.9975.

Anal. Calc. for $C_{13}H_7MnN_4O_3$: C, 48.47; H, 2.19; N, 17.39. Found C, 48.46; H, 2.20; N, 17.38.

5,7-Dimethyl-3-(thiophen-2-yl)-[1,2,4]triazolo[4,3-*a*]pyrimidine (8g).

The product was purified by column chromatography (acetone/benzene 1:1).

Yield: 126 mg (55%); beige solid; R_f = 0.1 (TLC: acetone/benzene 1:1); mp 179 °C.

FTIR: 3073, 3052, 1623, 1511, 1444, 851, 709 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.88 (1H, d, J = 1.9 Hz, 4′-H), 7.56 (1H, dd, J = 4.8, 3.0 Hz, 5′-H), 7.24 (1H, d, J = 4.9 Hz, 2′-H), 6.89 (1H, s, 6-H), 2.51 (3H, s, CH_3), 2.30 (3H, s, CH_3).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 164.3 (C5), 154.6 (C8a), 144.5 (C7), 139.0 (C3), 132.9 (C5′), 129.8 (C3′), 127.9 (C4′), 126.9 (C2′), 111.5 (C6), 24.2 (CH_3), 18.5 (CH_3).

MS (ESI): m/z (%) = 230 (100) $[M]^+$.

Anal. Calc. for $C_{11}H_{10}N_4S$: C, 57.37; H, 4.38; N, 24.33; S, 13.92. Found C, 57.39; H, 4.40; N, 24.30; S, 13.95.

5,7-Dimethyl-3-(thiophen-3-yl)-[1,2,4]triazolo[4,3-*a*]pyrimidine (8h).

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The product was purified by column chromatography ($CHCl_3$/MeOH 9:1).

Yield: 71.3 mg (31%); beige solid; R_f = 0.2 (TLC: $CHCl_3$/MeOH 9:1); mp 210 °C.

FTIR: 3065, 3040, 1625, 1515, 1444, 1378 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.00 (1H, d, J = 1.9 Hz, 4'-H), 7.73 (1H, dd, J = 4.8, 3.0 Hz, 5'-H), 7.46 (1H, d, J = 3.8 Hz, 2'-H), 6.85 (1H, d, J = 0.6 Hz, 6-H), 2.52 (3H, s, CH_3), 2.21 (3H, s, CH_3).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 164.1 (C5), 154.3 (C8a), 144.5 (C7), 141.1 (C3′), 130.2 (C2′), 129.8 (C4′), 128.7 (C3), 126.1 (C5′), 111.2 (C6), 24.3 (CH_3), 18.7 (CH_3).

MS (ESI): m/z (%) = 230 (100) $[M+H]^+$.

Anal. Calc. for $C_9H_6N_4S$: C, 57.37; H, 4.38; N, 24.33; S, 13.92. Found C, 57.39; H, 4.40; N, 24.30; S, 13.95.

3-(5-Bromothiophen-2-yl)-5,7-dimethyl-[1,2,4]triazolo[4,3-a]pyrimidine (8i).

The product was purified by column chromatography (acetone/benzene 1:1).

Yield: 287 mg (93%); beige solid; R_f = 0.1 (TLC: acetone/benzene 1:1); mp 235 °C.

FTIR: 3081, 3064, 1625, 1515, 1423, 1304, 1195 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.43 (1H, d, J = 3.8 Hz, 3′-H), 7.38 (1H, d, J = 3.8 Hz, 4′-H), 6.93 (1H, d, J = 0.6 Hz, 6-H), 2.55 (3H, s, CH_3), 2.37 (3H, s, CH_3).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 164.6 (C5), 154.6 (C8a), 144.8 (C7), 138.2 (C3), 133.9 (C3′), 130.4 (C4′), 129.9 (C2′), 115.1 (C5′), 111.7 (C6), 24.3 (CH_3), 18.8 (CH_3).

MS (ESI): m/z (%) = 310 (100) $[M+H]^+$.

Anal. Calc. for $C_{17}H_{16}FeN_4$: C, 61.47; H, 4.86; N, 16.87. Found C, 61.45; H, 4.84; N, 16.79.

3-Ferrocenyl-5,7-dimethyl-[1,2,4]triazolo[4,3-*a*]pyrimidine (8j).

The product was purified by column chromatography (EtOAc).

Yield: 99.6 mg (30%); red solid; R_f = 0.1 (TLC: EtOAc); mp 167 °C.

FTIR: 3106, 3091, 2921, 1669, 1516, 1446, 1299, 1103 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.72 (1H, c, 6-H), 4.67 (2H, m, C_5H_3), 4.46 (5H, s, CpH), 4.43 (2H, m, C_5H_3), 2.48 (3H, s, CH_3), 2.13 (3H, s, CH_3).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 163.8 (C5), 154.8 (C8a), 144.5 (C7), 143.0 (C3), 110.8 (C6), 75.4 (C-Cp), 72.7 (C_5H_3), 69.6 (Cp), 68.3 (C_5H_3), 24.2 (CH_3), 18.8 (CH_3).

HRMS (ESI): m/z $[M]^+$ calcd for $C_{17}H_{16}FeN_4$: 332.0724; found 332.0718.

Anal. Calc. for $C_{17}H_{16}FeN_4$: C, 61.47; H, 4.86; N, 16.87. Found C, 61.27; H, 4.80; N, 16.59.

2,6-Bis(5,7-dimethyl-[1,2,4]triazolo[4,3-a]pyrimidin-3-yl)pyridine (8k).

The product was purified by column chromatography (EtOAc/MeOH 1:1).

Yield: 152 mg (41%); yellow solid; R_f = 0.2 (TLC: EtOAc/MeOH 1:1); mp 279 °C.

FTIR: 2988, 1629, 1520, 1110 cm^{-1}.

^{1}H NMR (400 MHz, CF_3COOD): δ = 8.35-8.39 (1H, m, H-4'), 8.28 (2H, d, *J* = 7.7 Hz, H-3', H-5'), 7.29 (2H, s, H-6), 2.75 (6H, s, CH_3), 2.44 (6H, s, CH_3).

^{13}C NMR (100 MHz, CF_3COOD): δ = 179.9 (C8a), 177.2 (C7), 149.2 (C5), 148.7 (C3), 144.3 (C2, C6), 143.2 (C3, C5), 129.6 (C4), 117.3, 117.2 (C6), 23.5 (CH_3), 19.2 (CH_3).

MS (ESI): m/z (%) = 371 (100) $[M]^+$.

Anal. Calc. for $C_{19}H_{17}N_9$: C, 61.44; H, 4.61; N, 33.94. Found C, 61.48; H, 4.65; N, 34.00.

3-Cymantrenyl-5,7-dimethyl-[1,2,4]triazolo[4,3-*a*]pyrimidine (8l).

The product was purified by column chromatography (EtOAc/MeOH 9:1).

Yield: 325 mg (93%); beige solid; R_f = 0.2 (TLC: EtOAc/MeOH 9:1); mp 230 °C.

FTIR: 2923, 2014, 1922, 1627, 1510 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.88 (1H, s, 6-H), 5.77 (2H, m, C_4H_5), 5.14 (2H, m, C_5H_4), 2.54 (3H, s, CH_3), 2.43 (3H, s, CH_3).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 224.7 (CO), 164.3 (C5), 155.1 (C8a), 144.5 (C7), 138.9 (C3), 111.1 (C6), 91.8 (C_5H_4), 89.2 (C-Cp), 82.0 (C_5H_4), 24.3 (CH_3), 19.1 (CH_3).

HRMS (ESI): m/z $[M]^+$ calcd for $C_{15}H_{11}MnN_4O_3$: 350.0212; found 350.0284.

Anal. Calc. for $C_{15}H_{11}MnN_4O_3$: C, 51.44; H, 3.17; N, 16.00. Found C, 51.45; H, 3.19; N, 15.56.

1-(Thiophen-2-yl)-[1,2,4]triazolo[4,3-*a*]quinoxaline (8m).

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The product was purified by column chromatography (EtOAc).

Yield: 131 mg (52%); brown solid; R_f = 0.3 (TLC: EtOAc); mp 199 °C.

FTIR: 3021, 1610, 1449, 1397, 1044 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.41 (1H, s, 10-H), 8.09 (2H, m, 3'-H, 8-H), 7.74 (1H, m, 7-H), 7.67 (1H, m, 5-H), 7.61 (2H, m, 5′-H, 6-H), 7.33-7.43 (1H, m, 4'-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 144.6 (C10a), 143.8 (C10), 143.2 (C3), 136.1 (C4a), 132.1 (C3'), 131.2 (C8), 130.3 (C4'), 129.5 (C5'), 128.2 (C9), 127.7 (C7), 126.9 (C2′),125.8 (C8a), 115.4 (C6).

MS (ESI): m/z (%) = 252 (95%) $[M]^+$.

Anal. Calc. for $C_{13}H_8N_4S$: C, 61.89; H, 3.20; N, 22.21; S, 12.71. Found C, 61.90; H, 3.23; N, 22.19; S, 12.69.

1-(Thiophen-3-yl)-[1,2,4]triazolo[4,3-*a*]quinoxaline (8n).

The product was purified by column chromatography (EtOAc).

Yield: 246 mg (98%); beige solid; R_f = 0.3 (TLC: EtOAc); mp 249 °C.

FTIR: 3097, 2920, 1553, 1453, 1406 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.39 (1H, s, 10-H), 8.19-8.20 (1H, m, 8-H), 8.11-8.13 (1H, d, *J* = 7.8 Hz, 5'-H), 7.93-7.95 (1H, m, 4'-H), 7.65-7.69 (1H, m, 7-H), 7.57-7.61 (1H, m, 2'-H), 7.52-7.54 (2H, m, 5-H, 6-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 145.0 (C10a), 144.2 (C10), 143.8 (C3), 136.0 (C4a), 130.3 (C5'), 129.9 (C8), 129.5 (C2'), 128.5 (C6), 128.3 (C4'), 127.7 (C7), 127.6 (C8a), 125.9 (C3'), 115.6 (C5).

MS (ESI): m/z (%) = 252 (100) $[M]^+$.

Anal. Calc. for $C_{13}H_8N_4S$: C, 61.89; H, 3.20; N, 22.21; S, 12.71. Found C, 61.89; H, 3.23; N, 22.24; S, 12.70.

1-(5-Bromothiophen-2-yl)-[1,2,4]triazolo[4,3-a]quinoxaline (8o).

The product was purified by column chromatography (EtOAc).

Yield: 165 mg (50%); beige solid; R_f = 0.4 (TLC: EtOAc); mp 208 °C.

FTIR: 3107, 2922,1555, 1451, 1393 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.43 (1H, s, 10-H), 8.14 (1H, d, *J* = 6.8 Hz, 8-H), 7.69-7.76 (2H, m, 6-H, 3'-H, 5-H), 7.55-7.59 (2H, m, 4'-H, 7-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 144.5 (C10a), 143.7 (C10), 142.3 (C2'), 136.1 (C4a), 133.2 (C7), 131.4 (C4'), 130.3 (C8), 129.7 (C6), 128.8 (C3), 127.8 (C3'), 125.8 (C8a), 116.45 (C5), 115.7 (C5').

MS (ESI): m/z (%) = 332 (80) $[M+H]^+$.

Anal. Calc. for $C_{13}H_7BrN_4S$: C, 47.15; H, 2.13; Br, 24.13 N, 16.92; S, 9.68. Found C, 47.20; H, 2.16; Br, 24.03; N, 16.89; S, 9.65.

1-Ferrocenyl-[1,2,4]triazolo[4,3-*a*]quinoxaline (8p).

The product was purified by column chromatography (EtOAc).

Yield: 148 mg (42%); red solid; R_f = 0.3 (TLC: EtOAc); mp 200 °C.

FTIR: 3073, 2920, 1540, 1459, 1403, 769 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.36 (1H, s, 10-H), 8.08 (1H, d, *J* = 7.5 Hz, 8-H), 7.54-7.64 (3H, m, 6-H, 5-H, 7-H), 4.76 (2H, m, C_5H_3), 4.66 (2H, m, C_5H_3), 4.51 (5H, s, CpH).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 147.5 (C10a), 144.7 (C10), 143.8 (C3), 136.0 (C4a), 130.2 (C8), 129.0 (C6), 127.4 (C7), 126.0 (C8a), 115.9 (C5), 74.3 (C-Cp), 71.2 (C_5H_4), 70.0 (Cp), 69.6 (C_5H_4).

HRMS (ESI): m/z $[M]^+$ calcd for $C_{19}H_{14}FeN_4$: 354.0568; found 354.0561.

Anal. Calc. for $C_{19}H_{14}FeN_4$: C, 64.43; H, 3.98; N, 15.82. Found C, 64.48; H, 4.01; N, 15.89.

2,6-Bis([1,2,4]triazolo[4,3-a]quinoxalin-1-yl)pyridine (8q).

The product was purified by column chromatography (EtOAc/MeOH 9:1).

Yield: 257 mg (62%); yellow solid; R_f = 0.4 (TLC: EtOAc/MeOH 9:1); mp 279 °C.

FTIR: 3075, 2930, 1704, 1572, 1454, 1377, 1232 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.47 (1H, s, 10-H), 8.57 (1H, m, 4'-H), 8.47 (2H, d, *J* = 7.7 Hz, 3'-H, 5'-H), 8.08 (2H, d, *J* = 7.9 Hz, 8-H), 7.89 (2H, d, *J* = 8.5 Hz, 5-H), 7.60 (2H, t, *J* = 7.6 Hz, 6-H), 7.25 (2H, t, *J* = 7.9 Hz, 7-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 147.7 (C3), 147.6 (C2', C6'), 144.6 (C10a), 143.8 (C10), 140.0 (C4'), 136.1 (C4a), 130.2 (C8), 129.1 (C7), 127.9 (C3', C5'), 127.9 (C6), 125.4 (C8a), 117.4 (C5).

MS (ESI): m/z (%) = 414 (100) $[M+H]^+$.

Anal. Calc. for $C_{23}H_{13}N_9$: C, 66.50; H, 3.15; N, 30.35. Found C, 66.45; H, 3.20; N, 30.40.

1-Cymantrenyl-[1,2,4]triazolo[4,3-*a*]quinoxaline (8r).

The product was purified by column chromatography (EtOAc).

Yield: 219 mg (61%); brown solid; R_f = 0.4 (TLC: EtOAc); mp 198 °C.

FTIR: 3075, 2019, 1934, 1456 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.46 (1H, s, 10-H), 8.19 (1H, m, 8-H), 7.77 (3H, m, 5-H, 6-H, 7-H), 5.92 (2H, m, C_4H_5), 5.39 (2H, m, C_5H_4).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 224.4 (CO), 145.1 (C10a), 143.7 (C10), 143.1 (C3), 135.9 (C4a), 130.5 (C8), 129.7 (C6), 127.7 (C7), 125.8 (C8a), 115.7 (C5), 90.7 (C_5H_4), 88.0 (C-Cp), 83.3 (C_5H_4).

HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{17}H_9MnN_4O_3$: 372.0055; found 373.0122.

Anal. Calc. for $C_{17}H_9MnN_4O_3$: C, 54.86; H, 2.44; N, 15.05. Found C, 54.82; H, 2.42; N, 15.06.

1-(Thiophen-2-yl)-[1,2,4]triazolo[4,3-a]quinoline (8s).

The product was purified by column chromatography (EtOAc).

Yield: 213 mg (85%); beige solid; R_f = 0.2 (TLC: EtOAc); mp 148 °C.

FTIR: 3458, 2921, 1558, 1444, 1396 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.00-8.04 (2H, m, 8-H, 5'-H), 7.85 (1H, d, *J* = 9.5 Hz, 9-H), 7.76 (1H, d, *J* = 9.5 Hz, 10-H), 7.65 (1H, m, 3'-H), 7.53 (3H, m, 5-H, 6-H, 7-H), 7.40 (1H, m, 4'-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 149.4 (C10a), 142.2 (C3), 131.7 (C7), 131.3 (C9), 130.5-130.7 (C5'), 130.2-130.3 (C2'), 129.5-129.6 (C3'), 129.27 (C4a), 128.4 (C8), 128.1-128.2 (C4'), 126.3 (C6), 124.2 (C8a), 115.6 (C10), 114.4 (C5).

MS (ESI): m/z (%) = 251 (100) $[M]^+$.

Anal. Calc. for $C_{14}H_9N_3S$: C, 66.91; H, 3.61; N, 16.72; S, 12.76. Found C, 66.90; H, 3.60; N, 16.69; S, 12.74.

1-(Thiophen-3-yl)-[1,2,4]triazolo[4,3-a]quinoline (8t).

The product was purified by column chromatography (EtOAc).

Yield: 213 mg (85%); white solid; R_f = 0.2 (TLC: EtOAc); mp 179 °C.

FTIR: 3100, 2921, 1614, 1533, 1443, 1356, 1164 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 8.17 (1H, d, *J* = 1.8 Hz, 2'-H), 8.03-8.07 (1H, m, 8-H), 7.96 (1H, dd, *J* = 4.8, 3.0 Hz, 5'-H), 7.87 (1H, d, *J* = 9.5 Hz, 9-H), 7.78 (1H, d, *J* = 9.5 Hz, 10-H), 7.54-7.58 (2H, m, 6-H, 7-H), 7.52 (1H, d, *J* = 4.7 Hz, 4'-H),7.48 (1H, d, *J* = 7.8 Hz, 5-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 149.0 (C10a), 144.3 (C3), 131.4 (C9), 129.88 (C4a), 129.83 (C3'), 129.4-129.5 (C5'), 129.2 (C8), 129.0 (C7), 128.61-128.65 (C2'), 128.0-128.1 (C4'), 126.1 (C6), 124.1 (C8a), 115.7 (C5), 114.5 (C10).

MS (ESI): m/z (%) = 251 (100) $[M]^+$.

Anal. Calc. for $C_{14}H_9N_3S$: C, 66.91; H, 3.61; N, 16.72; S, 12.76. Found C, 66.90; H, 3.62; N, 16.70; S, 12.74.

1-(5-Bromothiophen-2-yl)-[1,2,4]triazolo[4,3-a]quinoline (8u).

The product was purified by column chromatography (EtOAc).

Yield: 237 mg (72%); white solid; R_f = 0.2 (TLC: EtOAc); mp 141 °C.

FTIR: 2921, 1657, 1396, 970 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 8.07 (1H, d, *J* = 7.1 Hz, 5-H), 7.91 (1H, d, *J* = 9.5 Hz, 8-H), 7.80 (1H, d, *J* = 9.5 Hz, 10-H), 7.74 (1H, d, *J* = 7.9 Hz, 6-H), 7.63-7.65 (2H, m, 9-H, 3'-H), 7.56 (2H, m, 4'-H, 7-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 149.5 (C10a), 141.4 (C5'), 132.7 (C4'), 131.4 (C8), 131.2 (C7), 130.3 (C3'), 129.5 (C5), 126.4 (C9), 124.2 (C2', C3), 115.8 (C6), 115.6 (C10), 114.3 (C4a, C8a).

MS (ESI): m/z (%) = 329 (95) $[M+H]^+$.

Anal. Calc. for $C_{14}H_8BrN_3S$: C, 50.92; H, 2.44; Br, 24.20; N, 12.73; S, 9.71. Found C, 50.94; H, 2.42; Br, 24.19; N, 12.71; S, 9.70.

1-Ferrocenyl-[1,2,4]triazolo[4,3-a]quinoline (8v).

The product was purified by column chromatography (EtOAc).

Yield: 236 mg (67%); brown solid; R_f = 0.2 (TLC: EtOAc); mp 201 °C.

FTIR: 3090, 3066, 2921, 1541, 1506, 1414, 820 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 7.96 (1H, d, *J* = 7.4 Hz, 8-H), 7.77 (1H, d, *J* = 9.4 Hz, 9-H), 7.70 (1H, d, *J* = 9.4 Hz, 10-H), 7.39-7.53 (3H, m, 5-H, 6-H, 7-H), 4.69 (2H, m, C_5H_4), 4.61 (2H, m, C_5H_4), 4.51 (5H, s, CpH).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 149.5 (C10a), 146.4 (C3), 131.5 (C4a), 129.5 (C9), 129.3 (C8), 128.7 (C5), 125.9 (C7), 124.1 (C8a), 116.0 (C6), 114.5 (C10), 76.2 (C-Cp), 71.2 (C_5H_4), 69.9 (Cp), 69.3 (C_5H_4).

HRMS (ESI): m/z $[M]^+$ calcd for $C_{20}H_{15}FeN_3$: 353.0615; found 353.0610.

Anal. Calc. for $C_{20}H_{15}FeN_3$: C, 68.01; H, 4.28; N, 11.90. Found C, 67.98; H, 4.25; N, 11.88.

2,6-Bis([1,2,4]triazolo[4,3-a]quinolin-1-yl)pyridine (8w).

The product was purified by column chromatography (EtOAc/MeOH 9:1).

Yield: 214 mg (52%); white solid; R_f = 0.1 (TLC: EtOAc/MeOH 9:1); mp 279 °C.

FTIR: 3050, 2923, 1727, 1619, 1589, 1564, 1404, 814 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 8.53 (1H, t, *J* = 7.8 Hz, 4'-H), 8.38 (2H, d, *J* = 7.8 Hz, 3'-H, 5'-H), 7.99 (2H, d, *J* = 7.7 Hz, 5-H), 7.86 (2H, d, *J* = 9.5 Hz, 8-H), 7.78 (2H, d, *J* = 9.5 Hz, 10-H), 7.63 (2H, d, *J* = 8.5 Hz, 9-H), 7.49 (2H, t, *J* = 7.5 Hz, 6-H), 7.20 (2H, t, *J* = 7.8 Hz, 7-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 149.3 (C3), 148.8 (C2', C6'), 147.2 (C10a), 139.9 (C4a), 130.8 (C8), 130.51-130.59 (C5'), 129.4-129.5 (C5), 128.8-128.9 (C7), 127.3-127.4 (C3'), 126.4 (C6), 124.1 (C8a), 117.2 (C9), 114.3 (C10).

MS (ESI): m/z (%) = 412 (100) $[M+H]^+$.

Anal. Calc. for $C_{25}H_{15}N_7$: C, 72.63; H, 3.66; N, 23.72. Found: C, 72.60; H, 3.64; N, 23.71.

1-Cymantrenyl-[1,2,4]triazolo[4,3-a]quinoline (8x).

The product was purified by column chromatography (EtOAc/hexane 1:1).

Yield: 163 mg (44%); brown solid; R_f = 0.1 (TLC: EtOAc/hexane 1:1); mp 204 °C.

FTIR: 2923, 2018, 1924, 1658, 1446 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 8.04 (1H, s, 8-H), 7.83 (1H, s, 9-H), 7.74 (2H, m, 7-H, 10-H), 7.61 (2H, m, 5-H, 6-H), 5.85 (2H, m, C_4H_5), 5.32 (2H, m, C_5H_4).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 224.6 (CO), 142.2 (C3), 131.4 (C10a), 130.0 (C9), 129.7 (C8), 129.6 (C6), 129.3 (C8a), 126.2 (C5), 124.1 (C4a), 115.8 (C7), 114.4 (C10), 90.8 (C_5H_4), 89.9 (C-Cp), 83.1 (C_5H_4).

HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{18}H_{10}MnN_3O_3$: 371.0103, found 372.0168.

Anal. Calc. for $C_{18}H_{10}MnN_3O_3$: C, 58.24; H, 2.72; N, 11.32. Found: C, 58.23; H, 2.70; N, 11.31.

Biological evaluations

Cells. Human neuroblastoma SH-SY5Y cells, human glioma T98G and rat glioma C6 cells were obtained from the Russian Collection of Cell Cultures (Institute of Cytology of Russian Academy of Sciences, St. Petersburg, Russia). Rat neuroblastoma PC-12HttQ103 cells containing complete HTT exon 1 fused to enhanced green fluorescent protein (EGFP) under control of the ecdysone-inducible promoter[26] provided by Prof. Michael Sherman (Boston School of Medicine, MA, USA). HeLa-luc cells were kindly provided by Prof. Richard Morimoto (Northwestern University, USA). Cells were grown in Dulbecco's modified Eagle's medium supplemented with L-glutamine, 10% horse serum with 5% fetal calf serum (Gibco Life Technologies, UK) and 50 μg/mL gentamicine sulfate in 5% CO_2 at 37°C. The PC-12HttQ103 cell culture medium also contained 100 μg/mL G-418. To induce the expression of HttQ103, ponasterone A (PonA; Sigma, St Louis, MO, USA) in a concentration of 2 μM was added. Cell viability was measured with aid of Cytotox96 Non Radio Cytotoxicity Assay Kit (Promega, USA) according the manufacturer's recommendation.

Luceferase reporter assay. The screening was carried out with the use of the HSE-reporter system expressed in HeLa cells (HeLa-luc). In this assay, HeLa cells expressing a genetic construct consisting of luciferase gene under the control of heat shock element (HSE) promoter[24] were subjected to compounds **8g-j,m-p** at concentrations of 0.1, 0.5 and 1.0 μM for 24 h and then luciferase activity was measured with Bright Glo Luciferase Kit (Promega, USA) using multichannel Fluorophot Charity (OOO "Probnauchpribor", Russia). The measured time was 5000 ms.

Western blotting. PC12wt cells were treated with **8m-o** in concentration 0.1, 0.5 and 1.0 μM for 24 h, after that cells were collected, washed three times in cold PBS and lysed in buffer containing 20 mM Tris-HCl, pH7.5, 150 mM NaCl, 0.5% Triton X-100, 2 mM EDTA. Electrophoresis was performed in 11% polyacrilamide gel according to standard protocol. Protein bands were transferred onto PVDF membrane, 0.45 μm (Amersham Hybond, Germany) using Power Blotter System (Thermo fisher scientific, USA). The membranes were stained with monoclonal mouse anti Hsp70 antibody (Clone 3C5[27]). Antibody to *α*-tubulin (Abkam, UK) were used as a loading control.

Confocal microscopy. PC-12HttQ103 cells were seeded on coverslips in 24-well plate (Sarstedt, Germany) in concentration 200 000 cell/mL overnight. The next morning 2 μM of PonA was added together with **8m-o** in concentration 1.0 μM for the next 24 h. Then the cells were washed three times with phosphate-buffered saline (PBS), nuclei were stained with 4',6-diamidino-2-phenylindole. Slides were examined on a Leica TCS SL (Wetzlar, Germany) confocal microscope at a 488 nm excitation wavelength for EGFP and 450 nm for 4',6-diamidino-2-phenylindole. The analysis of the images was carried out using the program Leica Application Suite X (Leica, Germany).

Filter Trap Assay. PC-12HttQ103 cells were seeded to wells of 12-well plate (TTP, Switzerland) and when cells reached 70% monolayer 2 μM PonA and compounds **8 m-o** were added in concentration 0.1, 0.5 and 1.0 μM for 24 h. Cell extracts prepared as described above were mixed with 2% sodium dodecyl sulfate (SDS) and subjected to ultrafiltration with the use of 96-well dot-blotting manifold (Bio-Rad Laboratories, USA). The resulring membrane was incubated with polyQ antibodiy (Millipore, Germany) and subsequently with HRP-conjugated secondary anti-mouse antibody. Visualization of resulting dots was peformed by chemiluminescence and dot intensity was measured with aid of Image Studio software (Li-Cor, США). The graphs were prepared with the aid of Prism GraphPad 9.0.2 software.

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Supporting Information

YES (this text will be updated with links prior to publication)

Primary Data

NO.

Conflict of Interest

The authors declare no conflict of interest.

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