**Introduction**

Parasitic human diseases, such as malaria, leishmaniasis, trypanosomiasis and toxoplasmosis, represent a severe global health concern. The life cycles of protozoan parasites, including *Plasmodium* spp., *Leishmania* spp., *Trypanosoma* spp. and *Toxoplasma* spp., are rather complex and typically comprise of transitions between hosts and vectors as well as intracellular and extracellular environments. To survive these changes, the protozoan cell must be able to adjust e. g. for fluctuating osmotic pressures. The key mechanism involves membrane-bound pyrophosphatas (mPPases).

Inhibition of membrane-bound pyrophosphatase (mPPase) with small molecules offer a new approach in the fight against pathogenic protozoan parasites. mPPases are absent in humans, but essential for many protists as they couple pyrophosphate hydrolysis to the active transport of protons or sodium ions across acidocalcisomal membranes. So far, only few nonphosphorous inhibitors have been reported. Here, we explore the chemical space around previous hits using a combination of screening and synthetic medicinal chemistry, identifying compounds with low micromolar inhibitory activities in the *Thermotoga maritima* mPPase test system. We furthermore provide early structure-activity relationships around a new scaffold having a pyrazolo[1,5-a]pyrimidine core. The most promising pyrazolo[1,5-a]pyrimidine congener was further investigated and found to inhibit *Plasmadium falciparum* mPPase in membranes as well as the growth of *P. falciparum* in an ex vivo survival assay.

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**Exploration of Pyrazolo[1,5-a]pyrimidines as Membrane-Bound Pyrophosphatase Inhibitors**

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Inhibition of membrane-bound pyrophosphatase (mPPase) with small molecules offer a new approach in the fight against pathogenic protozoan parasites. mPPases are absent in humans, but essential for many protists as they couple pyrophosphate hydrolysis to the active transport of protons or sodium ions across acidocalcisomal membranes. So far, only few nonphosphorous inhibitors have been reported. Here, we explore the chemical space around previous hits using a combination of screening and synthetic medicinal chemistry, identifying compounds with low micromolar inhibitory activities in the *Thermotoga maritima* mPPase test system. We furthermore provide early structure-activity relationships around a new scaffold having a pyrazolo[1,5-a]pyrimidine core. The most promising pyrazolo[1,5-a]pyrimidine congener was further investigated and found to inhibit *Plasmadium falciparum* mPPase in membranes as well as the growth of *P. falciparum* in an ex vivo survival assay.

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cally outside the catalytic side, near the so-called exit channel (PDB code: 6QXA). Here, starting from 2 and its congeners, we aimed to identify novel molecular scaffolds that would lead to gain in binding affinity. These could be translated into therapeutic molecules but could also have a higher probability to be co-crystallized at the mPPase catalytic site, which so far has eluded us. Following a screening of 52 compounds, we set to explore the pyrazolo[1,5-a]pyrimidine core. An advantage of swapping the central core structure is that it would allow further chemical expansion of the 2-position in pyrazolo[1,5-a]pyrimidines via Suzuki coupling or other coupling methods. Furthermore, pyrazolo[1,5-a]pyrimidines have been reported to have antibacterial activity against Mycobacterium tuberculosis, anti-parasitic activity including antileishmanial, antimalarial (P. falciparum dihydroorotate dehydrogenase inhibitors) and antitrypanosomal, as well as antiviral activity against HIV.

Results and Discussion

Exploration of the isoxazole and sulfonamide space through screening

We started from the previously identified 5-arylisoxazole-3-carboxylate core present in 2–5 (Figure 1, highlighted in blue). Using KNIME, we did a substructure search for commercial analogues in the ZINC12 database. From our experience, computational methods (including docking simulations) do not offer a robust and/or reliable mean of prioritization at the mPPase. Therefore, we manually picked compounds from a diverse collection provided by a single vendor (Ambinter, 174 matches for the substructure query). Out of the search results, we selected fifteen isoxazoles for testing in vitro. For all compounds in this manuscript, we tested activity using a 96-well based assay that detects the inhibition of TmPPase.

In this first set (Supporting Information, Table S1 and S2), eleven of the fifteen isoxazoles had a sulfonamide-linked 2-methoxyphenyl moiety. The sulfonamides 6 and 7 (Figure 2) were the two best compounds with 6 (IC$_{50}$ = 5.4 μM) being one of the most potent TmPPase inhibitors found this far. Interestingly, in comparison with 7–10, the sulfonamide-linked 2,3,4,5-tetrahydrobenzo[b][1,4]thiazepine moiety found in 6 is critical for mPPase activity. Even the simple addition of a methyl group (as in 8) was not tolerated. Also, minor alterations of the seven-membered ring (as in 9 and 10) caused the loss of all TmPPase inhibitory activity. Other ring modifications also proved unsuccessful (Table S2). Because of the sharp, difficult to rationalize, structure-activity relationships of the isoxazole-sulfonamide core, we in this manuscript decided to pursue other scaffolds (see below).

We also tested another set of 18 sulfonamides (Table S3) and 19 amides (Table S4) that were readily available to us and thus were candidates for repurposing. These compounds share some chemical features with 2, with many having a central polar core bearing N and/or O atoms surrounded by more

Figure 1. Selected examples of nonphosphorus mPPase inhibitors previously reported. Blue highlight, common substructure used for similarity searches in this manuscript; IC$_{50}$, half maximal inhibitory concentration; CI$_{95}$%, half maximal inhibitory concentration expressed as a 95% confidence interval (given in square brackets).

Figure 2. Top two compounds and some of their inactive analogues from the substructure-based search.
hydrophobic/aromatic functional groups. Of this set, compounds 11 and 12 (Figure 3) were the most potient with IC_{50} values below 25 μM. Sulfonamide 11 (IC_{50} = 14 μM; ligand efficiency (LE) = 0.23) share the 2-methoxyphenyl-sulfonamide moiety of 6 but lacks its isoxazole core, and therefore shows potential for further exploration. Compound 12 (IC_{50} = 25 μM) is structurally different from the hitherto most potent nonphosphorus TmPPase inhibitors and thus was picked as a template for further design. Compound 12 was the only compound from this subset with a 4,5-dihydropyrazolo[3,4-c]pyrazol-6(1H)-one core.

Chemical exploration of the pyrazolo[1,5-a]pyrimidine core

Next, we decided to further explore the chemical space around the recently discovered 4,5-dihydropyrazolo[3,4-c]pyrazol-6(1H)-one scaffold, using combinations of substituents already found to be favorable for activity. We nonetheless decided to swap the original bicyclic scaffold with a related nitrogen-rich, pyrazolo[1,5-a]pyrimidine core. This approach was synthetically more achievable, allowing easy modifications at three positions. In addition, the synthetic exploration could take advantage of readily available starting materials, such as various aromatic or aliphatic ketones. The scaffold change can be rationalized by comparing the pharmacophores of 17a with 2 and 12 (Supporting Information, Figure S1 and S2).

We relied on a method reported by Childress et al. as we could adapt the first two steps of their synthesis route to access the key intermediate 15 (Scheme 1). Crossed condensation at room temperature of commercially available 3,5-dimethylacetophenone or acetylcyclopropane with diethyl oxalate and potassium tert-butoxide in THF gave 14. Subsequent ring condensation of 14 with 3-bromo-1H-pyrazol-5-amine in refluxing ethanol resulted in the formation of pyrazolo[1,5-a]pyrimidine 15. Hydrolysis of the formed ethyl ester 15 using lithium hydroxide in a mixture of ethanol and water gave the corresponding carboxylic acid 16, which was reacted with 2-bromophenol and 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) and 2-bromophenol to give the desired 2-bromophenyl carboxylate 17.

The bromine substituent in the 2-position of 15 could serve as a coupling handle for further exploration of the chemical space by various Pd-mediated cross-coupling reactions. Suzuki coupling of ethyl ester 15 in n-propanol with commercially available potassium vinyltrifluoroborate was done under microwave conditions. The product obtained, 18, could be transformed into the corresponding 2-bromophenyl ester 20 following the same hydrolysis and esterification procedures used for 16 and 17, respectively. However, changing trimethylamine used in the Suzuki coupling to ethylenediamine serendipitously led to both Suzuki coupling and amidation occurring in the same reaction mixture, yielding 21 in a single step.

Biological activity of the pyrazolo[1,5-a]pyrimidine core

We started our exploration by taking inspiration from 12 (IC_{50} = 25 μM) and changing the isoxazole core of 2 (IC_{50} = 6.9 μM) to the corresponding pyrazolo[1,5-a]pyrimidine analogue 17a (Table 1). This scaffold change was relatively well tolerated, with just a 2-fold loss in activity compared to 2. As previously

![Figure 3. Best hits from the readily available compound set.](image)

![Scheme 1. Synthesis of compounds 14–21. Reagents and conditions: (a) t-BuOK, diethyl oxalate, THF, rt, 1 h, 87–88%; (b) 5-Bromo-1H-pyrazol-3-amine; HCl (aq), ETOH, mw, 78 °C, 30 min, 64–81%; (c) LiOH, ETOH/H_{2}O, rt, overnight, 89–96%; (d) 2-Bromophenol, HATU, DIPEA, DMF, rt, overnight, 36–61%; (e) Potassium vinyltrifluoroborate, Et,N, Pd(dpdpf)Cl_{2}, ETOH, mw, 125 °C, 15 min, 53–56%; (f) Potassium vinyltrifluoroborate, ethylenediamine, Pd(dpdpf)Cl_{2}, n-PrOH, mw, 125 °C, 15–30 min, 49–87%.)](image)
noticed for the isoxazole series,[24] the corresponding carboxylic acid 16a was inactive, but the ethyl ester 15a retained weak activity.

Since the central pyrazolo[1,5-a]pyrimidine core is bulkier than the original isoxazole moiety (the molecular weight of 17a is approximately 500 Da), we tried to introduce lighter substitutions. The exchange of the R-group at the 7-position, from a 3,5-dimethylphenyl group to a cyclopropyl ring, was only slightly effective for the ethyl carboxylate 15b. Furthermore, it proved completely unsuccessful for the carboxylic acid 16b and the 2-bromophenyl carboxylate 17b. Similarly, replacing the R-substituent with other phenyl moieties than the original 3,5-dimethylphenyl substituent (unsubstituted or bearing electron withdrawing/donating groups), were generally not well tolerated for the isoxazole derivatives.[24]

We next studied further functionalization of the 2-position in pyrazolo[1,5-a]pyrimidines. Bromine atoms are very useful in X-ray crystallography (due to their anomalous scattering, which can aid in identifying the presence of the compound as well as its orientation in low-resolution, 3.5 Å or worse). Additionally, aryl bromides are highly useful e.g. in Suzuki coupling with various organoboron substrates. We introduced a well-accepted vinyl group at the 5-position, showing nearly no loss of activity for 20a in comparison to the hitherto best pyrazolo[1,5-a]pyrimidine 17a (IC_{90} = 14 μM). Moreover, there was a 2.2-fold improvement in the inhibition comparing the ethyl ester 18a to the 2-bromo analogues 15a. Interestingly, the 2-vinyl-substituted carboxylic acid 19a (IC_{90} = 14 μM) was as active as the best pyrazolo[1,5-a]pyrimidines and superior to its inactive 2-bromo analogue 16a. As presented above, the corresponding 2-vinyl-substituted cyclopropyl analogues 18b-20b were all inactive. In the same way, the 3,5-dimethylphenyl-substituted amide 21a showed 3.6-fold higher inhibition than the corresponding cyclopropyl-substituted analogue 21b.

**Follow-up studies of 12, 17a, 19a and 20a**

In order to rule out a cause of false positives, colloidal aggregation in the TmPPase model assay was evaluated for compounds 17a, 19a and 20a at six concentrations (100 μM, 50 μM, 20 μM, 10 μM, 1 μM, and 0.1 μM) using the assay conditions (Figure S5). Compounds 17a and 20a showed aggregate formation at concentrations above 20 μM, which is above their IC_{90} values. Compound 19a showed no detectable aggregation.

Further hit validation was done against the purified mPPase from *P. falciparum* (PPPase-VP1) expressed in baculovirus-infected insect cells. Compound 17a was able to inhibit the PPPase-VP1 activity with an IC_{50} of 58 μM (Figure 4A). Compounds 19a and 20a had lower inhibition activities with IC_{50} values of 130 μM and 74 μM, respectively (Figure 5S). Overall, these compounds have higher IC_{50} values for PPPase-VP1 than for TmPPase. In a survival assay in erythrocytes culture 17a was able to inhibit the growth of *P. falciparum* with an IC_{50} of 31 μM (Figure 4B), better than its inhibition on the PPPase-VP1. This
could mean that the compound inhibits other proteins in the parasite, e.g. through soluble pyrophosphatase, or via some other mechanism, which could be linked to colloidal aggregation. Interestingly, compound 12 was able to inhibit the growth of *P. falciparum* with the IC\textsubscript{50} of 3.6 μM (Figure S6) even though the activity on PfPPase-VP1 (Figure S4) was comparably weak. No hemolysis of human erythrocytes was observed, suggesting no significant cytotoxicity.

### Conclusion

Altogether, this manuscript presents novel scaffolds with potential for further exploration in the drug discovery against parasitic diseases. Using a screening approach (52 compounds) together with a medicinal chemistry exploration (14 compounds) and the TmPPase test system, we discovered new TmPPase inhibitors: the 4,5-dihydropyrrolo[3,4-c]pyrazol-6(1H)-one core (compound 12) and pyrazolo[1,5-a]pyrimidines (17a, 19a and 20a). We explored the SARs around this latter core and maintained low micromolar activity (IC\textsubscript{50} = 14–18 μM) for three of the synthesized pyrazolo[1,5-a]pyrimidines. Molecular modelling suggests that the substrate binding sites are highly conserved in many protozoan pathogens, which should allow transferability of the findings.\textsuperscript{11,34} Indeed, compound 17a binds to the PFPase-VP1 with an IC\textsubscript{50} of 58 μM and inhibits parasite growth.

### Experimental Section

#### Computational methods

Pharmacophore modelling was conducted using the Schrödinger Maestro suite.\textsuperscript{35} Substructure searches were conducted from the ZINC12 database\textsuperscript{31} (clean drug-like subset; 13,195,609 compounds; downloaded on 2018.11.11). Ligand efficiencies were computed using the pIC\textsubscript{50} and the "Heavy Atom Count" normalization method with Accelrys’s Discovery Studio.\textsuperscript{36}

#### Chemistry

**General experimental methods:** All chemicals were available from commercial vendors and used without any further purification. Anhydrous reactions were conducted in oven-dried (130 °C, > 24 h) glassware that were purged with argon prior to use. Microwave reactions were done in sealed reaction vials using a Biotage\textsuperscript{®} Initiator\textsuperscript{+} instrument (Uppsala, Sweden). The progress of the reactions was monitored using thin-layer chromatography on silica gel 60-F\textsubscript{254} aluminum plates and visualized by a dual short/long wave (254/366 nm) UV lamp. Combined organic solutions from extractions were dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, filtered and concentrated with a rotary evaporator at reduced pressure. Flash SiO\textsubscript{2} column chromatography was performed with automated high performance flash chromatography, Biotage\textsuperscript{®} Isolera\textsuperscript{™} Systems with ACI\textsuperscript{™} and Assist (ISO-15W Isolera One) equipped with a variable UV-VIS (200–800 nm) photodiode array (Uppsala, Sweden) using SNAP KP-Sil/Ultra 10, 25, 50 or 100 g cartridges and the indicated mobile phase gradient. The reactions were not optimized and all the yields are given for purified products.

The synthesized products were characterized by NMR and MS analysis. \textsuperscript{1}H and \textsuperscript{13}C NMR spectra were acquired at 298 K on a Bruker Ascend 400 MHz-Avance III HD NMR spectrometer (Bruker Corporation, Billerica, MA, USA). Chemical shifts (δ) are reported in parts per million (ppm) relative to the residual solvent signals: CDCl\textsubscript{3} 7.26 and 77.16 ppm, CD\textsubscript{3}OD 3.31 and 49.00 ppm for \textsuperscript{1}H and \textsuperscript{13}C NMR, respectively. Multiplicities are indicated as bs (broad singlet), s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), tt (triplet of triplets) and m (multiplet). Purity of the final compounds (> 95 %) was confirmed by LC–MS using a Waters Acquity\textsuperscript{®} UPLC system (Waters, Milford, MA, USA) equipped with an Acquity PDA detector, a Waters Synapt G2 HDMS mass spectrometer (Waters, Milford, MA, USA) and an Acquity UPLC\textsuperscript{®} BEH C18 column (1.7 μm, 50 mm × 2.1 mm, Waters, Ireland). Mass range was set from 100 to 600 Da. High resolution mass (HRMS-ESI) data was...
reported for the calculated and experimentally found molecular ions [M + H]+ or [M – H].

**General procedure for synthesis of compound 14**: The synthesis of compound 14 was adapted from a previously described method.21 In brief, t-BuOK (2 equiv) was dissolved in anhydrous THF (1.2 mL/mmol) under argon, followed by addition of diethyl oxalate (2 equiv) and the mixture was stirred for 30 min. Then, ketone 13 (1 equiv) dissolved in anhydrous THF (2.2 mL/mmol) was added dropwise and stirred for 1 h. The reaction was quenched by addition of a 1 M solution of HCl in H2O (3.0 mL/mmol).

**Ethyl 4-(3,5-dimethylphenyl)-2-hydroxy-4-oxobut-2-enate (14a)**: 1-(3,5-Dimethylphenyl)ethan-1-one (2.0 g, 13 mmol) was used to give 14a (2.9 g, 88%) as a yellow solid. 1H NMR (400 MHz, CDCl3): δ = 7.59 (s, 2H), 7.23 (s, 1H), 7.04 (s, 1H), 4.40 (q, J = 7.1 Hz, 2H), 2.38 (s, 6H), 1.41 (t, J = 7.1 Hz, 3H), 1H not observed (exchangeable); 13C NMR (101 MHz, CDCl3): δ = 191.3, 169.6, 162.4, 138.7, 135.7, 135.0, 125.8, 98.2, 62.7, 21.3, 14.2.

**Ethyl 4-cyclopropyl-2-hydroxy-4-oxobut-2-enate (14b)**: 1-Cyclopropylethan-1-one (1.4 g, 17 mmol) was used to give 14b (2.8 g, 87%) as a pale yellow liquid. 1H NMR (400 MHz, CDCl3): δ = 6.46 (s, 1H), 4.33 (q, J = 7.1 Hz, 2H), 1.87 (t, J = 7.8, 4.5 Hz, 1H), 1.35 (t, J = 7.1 Hz, 3H), 1.22–1.17 (m, 2H), 108.102 (m, 2H), 1H not observed (exchangeable); 13C NMR (101 MHz, CDCl3): δ = 204.7, 163.2, 162.4, 102.2, 62.5, 20.8, 14.1, 12.3.

**General procedure for synthesis of compound 15**: The synthesis of compound 15 was adapted from a previously reported method.21 Briefly, to a solution of 14 (1 equiv) in EtOH (5.0 mL/mmol) were added a catalytic amount of a 1 M solution of HCl in H2O (5 drops) and 5-bromo-1H-pyrazol-3-amine (1.25 equiv). The reaction mixture heated by microwave irradiation at 78°C for 30 min, followed by filtration once the product had precipitated.

**Ethyl 2-bromo-7-(3,5-dimethylphenyl)pyrazolo[1,5-a]pyrimidine-5-carboxylate (15a)**: Compound 14a (1.0 g, 4.1 mmol) was used to give 15a (1.2 g, 81%) as a pale yellow solid. 1H NMR (400 MHz, CDCl3): δ = 7.65 (s, 2H), 7.64 (s, 1H), 7.22 (s, 1H), 7.02 (s, 1H), 4.54 (q, J = 7.1 Hz, 2H), 2.42 (s, 6H), 1.48 (t, J = 7.1 Hz, 3H); 13C NMR (101 MHz, CDCl3): δ = 164.0, 149.8, 147.6, 134.7, 138.7, 136.1, 133.7, 129.9, 127.1, 107.4, 102.1, 62.9, 21.5, 14.4; HRMS (ESI) m/z: [M + H]+ calcld for C13H14BrN2O, 274.0504; found 274.0503.

**Ethyl 2-bromo-7-cyclopropylpyrazolo[1,5-a]pyrimidine-5-carboxylate (15b)**: Compound 14b (0.77 g, 4.2 mmol) was used to give 15b (0.82 g, 64%) as a white solid. 1H NMR (400 MHz, CDCl3): δ = 7.05 (s, 1H), 6.93 (s, 1H), 4.49 (q, J = 7.1 Hz, 2H), 2.91 (t, J = 8.5, 5.3 Hz, 1H), 1.47–1.38 (m, 5H), 1.19–1.13 (m, 2H); 13C NMR (101 MHz, CDCl3): δ = 164.1, 153.3, 148.7, 147.2, 135.8, 101.8, 101.1, 62.9, 14.9, 10.8, 10.7; HRMS (ESI) m/z: [M + H]+ calcld for C13H14BrN2O2, 310.0191, found 310.0190.

**General procedure for synthesis of compounds 16 and 19**: The synthesis of compounds 16 and 19 were done according to a method described elsewhere.24 In brief, a mixture of the ethyl ester (1 equiv) and LiOEt (2 equiv) in a 4:1 ratio of EtOH/H2O (75 mL/mmol) was stirred for 16 h (3 d for 16b), followed by back-extraction.

**2-Bromo-7-(3,5-dimethylphenyl)pyrazolo[1,5-a]pyrimidine-5-carboxylic acid (16a)**: Compound 15a (0.30 g, 0.80 mmol) was used to give 16a (0.25 g, 89%) as a pale yellow solid. 1H NMR (400 MHz, CDOD): δ = 7.69 (s, 2H), 7.67 (s, 1H), 7.28 (s, 1H), 7.01 (s, 1H), 2.43 (s, 6H); 13C NMR (101 MHz, CDOD): δ = 166.5, 151.0, 149.7, 149.0, 139.8, 136.7, 134.3, 131.3, 128.2, 108.4, 102.0, 21.4; HRMS (ESI) m/z: [M + H]+ calcld for C13H14BrN2O, 346.0191, found 346.0196.
General procedure for synthesis of compound 18: The synthesis of compound 18 was adapted from a previously reported method. Briefly, the 2-bromo pyrazolo[1,5-a]pyrimidine 1 (equiv), potassium vinyltrifluoroborate (1.2 equiv), Et(N₂) (2 equiv), and Pd(dppf)Cl₂ (0.05 equiv) were dissolved in EtOH (7.5 mM/mL) in a microwave vial and purged with argon for 10 min. The reaction mixture was heated by microwave irradiation at 125°C for 15 min. Then, the reaction mixture was filtrated through Celite® and purified with automated flash chromatography with n-hexane/EtOAc (1:0) as eluent.

Ethyl 7-(3,5-dimethylphenyl)-2-vinylpyrazolo[1,5-a]pyrimidine-5-carboxylate (18a): Compound 17a (0.2 g, 1.3 mmol) was used to give 18a (0.23 g, 53%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃): δ = 7.70 (s, 2H), 7.60 (s, 1H), 7.21 (s, 1H), 7.05 (s, 1H), 6.99 (dd, J = 17.7, 11.0 Hz, 1H), 6.15 (dd, J = 17.7, 12.1 Hz, 1H), 5.66 (dd, J = 11.0, 1.2 Hz, 1H), 4.54 (q, J = 7.1 Hz, 2H), 2.43 (s, 6H), 1.48 (t, J = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃): δ = 164.4, 156.3, 153.0, 148.7, 146.3, 129.2, 119.7, 106.9, 96.0, 62.7, 21.5, 14.5; HRMS (ESI) m/z: [M + H]⁺ calcd for C₂₁H₂₃NO₂ 322.1555, found 322.1554.

General procedure for synthesis of compound 21: 2-Bromo pyrazolo[1,5-a]pyrimidine 1 (equiv), potassium vinyltrifluoroborate (1.2 equiv), ethylenediamine (4 equiv), and Pd(dppf)Cl₂ (0.05 equiv) were dissolved in n-PrOH (5.5 mL/mmol) in a microwave vial and purged with argon for 10 min. The reaction mixture was heated by microwave irradiation at 125°C for 15 min for (21a) and 2×15 min for (21b). Then, the reaction mixture was filtrated through Celite® and purified with automated flash chromatography with n-hexane/EtOAc (1:0—1) as eluent.

N-(2-Aminoethyl)-7-(3,5-dimethylphenyl)-2-vinylpyrazolo[1,5-a]pyrimidine-5-carboxamide (21a): Compound 15a (0.051 g, 0.14 mmol) was used to give 21a (0.023 g, 49%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃): δ = 7.69 (s, 2H), 7.61 (s, 1H), 7.25 (s, 1H), 6.99 (s, 1H), 6.87 (dd, J = 17.8, 11.1 Hz, 1H), 6.12 (dd, J = 17.8, 1.3 Hz, 1H), 5.57 (dd, J = 11.1, 1.3 Hz, 3.68 (t, J = 5.9 Hz, 2H), 3.11 (t, J = 5.9 Hz, 2H), 2.41 (s, 6H). ¹³C NMR (101 MHz, CDCl₃): 166.4, 157.8, 150.6, 149.8, 148.9, 139.7, 134.0, 132.0, 130.3, 128.1, 120.2, 105.8, 95.5, 41.3, 39.9, 21.4; HRMS (ESI) m/z: [M + H]⁺ calcd for C₁₈H₁₄N₃O 336.1824, found 336.1828.
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Conflict of Interest
The authors declare no conflict of interest.

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