# Core Modifications

Modifications to the triazolopyrazine core were undertaken to establish the structure-activity relationships surrounding this key motif of the series. Systematic replacement of triazole nitrogens with -CH groups (**2**, **3**) resulted in a loss of antiplasmodial activity compared to the parent compound (**1**). Modification of the pyrazine half of the core were not tolerated; neither aromatic (**4**-**6**) nor aliphatic (**7**, **8**) cores attained submicromolar potency. These analogs establish the importance of the original triazolopyrazine core to potent activity against *P. falciparum*.

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| **Manuscript ID** | **External ID** | **Core** | **Pfal EC50 (μM)**a | **cLogP** | **LLE** |
| 1 | MMV639565 |  | 0.091b | 3.79 | 3.25 |
| 2 | MMV670250 |  | 0.830 | 4.34 | 1.74 |
| 3 | MMV669846 |  | 0.110 | 4.29 | 2.67 |
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| **Manuscript ID** | **External ID** | **Core** | **Pfal EC50 (μM)a** | **cLogP** | **LLE** |
| 4 | MMV672939 |  | 6.78 | 3.31 | 1.85 |
| 5 | MMV669025 |  | 4.13 | 3.27 | 2.12 |
| 6 | MMV668822 |  | 4.01 | 2.88 | 2.52 |
|  | | | | | |
| **Manuscript ID** | **External ID** | **R1** | **Pfal EC50 (μM)a** | **cLogP** | **LLE** |
| 7 | MMV669310 |  | 8.76 | 3.47 | 1.59 |
| 8 | MMV668960 | H | 10 | 1.13 | 3.87 |

**Table X**. Biological activity of core replacements. aData inherited from MMV unless otherwise noted. bAverage of inherited data and Dundee assay. cLogP calculated using DataWarrior.

# Pyrazine - Substitution pattern

# Pyrazine: Substituents with ethylene linker

Both aromatic and aliphatic substituents were installed at the 5-position of the triazolopyrazine scaffold (**Table X**). Of the aromatic groups, the *meta*-substituted methyl (**3**) and benzyl (**5**) ethers were the most potent, although the phenyl (**1**) and 3,4-diflurophenyl (**2**) compounds also achieved sub-micromolar potency. The para-substituted methyl (**4**) and benzyl (**6**) ethers are both at least 5-fold less potent than their meta-substituted counterparts, and the phenyl ether (**7**) loses significant activity as well. Although they have improved clogPs over aromatic substitutents, all of the saturated substituents at this position which contain a heteroatom (**9-15**) are completely inactive (>10 μM). The small, lipophilic cyclopropyl (**8**) does retain some activity, although it is ~10-fold less potent than most aromatic compounds. A variety of phenyl isosteres were synthesized (**16**-**22**), several of which (**16**, **20**) maintained sub-micromolar activity. Interestingly, the caged boranes **20** and **21** show some of the highest LLEs of this series. Finally, a few examples with a propylene linker (**22**, **23**) were synthesized, which were 3 to 5-fold less active than their ethylene-linked matched pairs (**1** and **16**, respectively). In general, nonpolar aromatic substituents linked by a two-carbon spacer to the 5-position of the core are favored for activity.

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| **Manuscript ID** | **External ID** | **R** | **Pfal EC50 (μM)**a | **cLogP** | **LLE** |
| 1 | MMV897698  OSM-S-369 |  | 0.362 | 3.00 | 2.74 |
| 2 | MMV675960  OSM-S-260 |  | 0.281b | 3.35 | 3.19 |
| 3 | MMV897711  OSM-S-383 |  | 0.135 | 3.08 | 3.79 |
| 4 | MMV897712  OSM-S-364 |  | 0.928 | 3.08 | 2.95 |
| 5 | MMV1581304  OSM-S-567 |  | 0.147 | 4.50 | 2.33 |
| 6 | MMV1581303  OSM-S-566 |  | 3.85 | 4.50 | 0.913 |
| 7 | MMV1581346  OSM-S-580 |  | 1.21 | 2.73 | 3.18 |
| 8 | MMV1577581  OSM-S-583 |  | 3.03 | 2.48 | 3.03 |
| 9 | MMV1576788  OSM-S-422 |  | >10 | 1.66 | <3.34 |
| 10 | MMV1576786  OSM-S-421 |  | >10 | 0.939 | <4.06 |
| 11 | MMV1577577  OSM-S-500 |  | >10 | 1.80 | <3.20 |
| 12 | MMV1577576  OSM-S-499 |  | >10 | 2.14 | 2.86 |
| 13 | MMV1577574  OSM-S-497 |  | >10 | 0.83 | <4.17 |
| 14 | MMV1577578  OSM-S-501 |  | >10 | 2.49 | <2.51 |
| 15 | MMV1577575  OSM-S-498 |  | >10 | 0.97 | <4.02 |
| 16 | MMV897700  OSM-S-371 |  | 0.372 | 2.50 | 3.92 |
| 17 | MMV1557939  OSM-S-399 |  | 1.28 | 3.91 | 1.98 |
| 18 | MMV1557935  OSM-S-395 |  | 2.15 | 4.06 | 1.61 |
| 19 | MMV1577573  OSM-S-433 |  | 1.08 | 3.47 | 2.50 |
| 20 | MMV1576784  OSM-S-418 |  | 0.050 | 2.14 | 5.16 |
| 21 | MMV1576790  OSM-S-424 |  | 1.90 | 1.68 | 4.04 |
| 22 | MMV1581344  OSM-S-578 |  | 1.32 | 3.61 | 2.27 |
| 23 | MMV897699  OSM-S-370 |  | 2.00 | 2.96 | 2.74 |

**Table X**. Biological activity of 5-position substituents. aDundee*P. fal* assay unless otherwise noted. bSyngene *P. fal* assay. cLogP calculated using DataWarrior.

# Pyrazine: Substituted linkers (Benzylic position)

The SAR of the linker between the core and the distal substituent at the 5-position was explored (**Table X**). In general, substitution of the ethylene linker was tolerated, with most of these compounds maintaining sub-micromolar potency. Compounds containing primary alcohols (**2**, **4**, and **7**) were all more potent than the unsubstituted ethylene linker (**1**) with LLE >4. The ketone **3** was notably less potent than its alcohol counterpart **2**. Fluorine substitution (**5**, **6**) also resulted in compounds that were more active than the unsubstituted ethylene. The SAR around N-containing linkers is somewhat unclear; however, the dimethylamine **11** is one of the most potent compounds of this series and has a high LLE. Several matched-pair analogs with an unsubstituted phenyl group in place of the 3,4-difluorophenyl substituent were also synthesized (**SI Table X**) and were consistently less potent than the difluorinated compound.

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| **Manuscript ID** | **External ID** | **R** | **Pfal EC50 (μM)a** | **cLogP** | **LLE** |
| 1 | MMV675960  OSM-S-260 |  | 0.281b | 3.35 | 3.19 |
| 2 | MMV672687  OSM-S-390 |  | 0.124c | 2.28 | 4.72 |
| 3 | MMV1557932  OSM-S-392 |  | 0.832d | 2.47 | 3.61 |
| 4 | MMV670947  OSM-S-381 |  | 0.024 | 2.86 | 4.75 |
| 5 | MMV672936  OSM-X-003 |  | 0.065 | 3.00 | 4.20 |
| 6 | MMV672727  OSM-X-006 |  | 0.123 | 3.57 | 3.34 |
| 7 | MMV672723  OSM-X-004 |  | 0.101 | 2.87 | 4.12 |
| 8 | MMV670438  OSM-X-022 |  | 0.483 | 2.66 | 3.66 |
| 9 | MMV671651  OSM-X-010 |  | 0.279 | 1.89 | 4.67 |
| 10 | MMV670763  OSM-X-067 |  | 10 | 2.24 | 2.76 |
| 11 | MMV670437  OSM-X-002 |  | 0.044 | 2.50 | 4.85 |
| 12 | MMV1576792  OSM-S-431 |  | 8.85d | 2.91 | 2.14 |
| 13 | MMV1576791  OSM-S-430 |  | >10d | 3.32 | <1.68 |
| 14 | MMV671647  OSM-X-030 |  | 0.613 | 2.47 | 3.74 |

**Table X**. Biological activity of substituted ethylene linker analogs. aPotency data inherited from MMV unless otherwise noted. bSyngene *P. fal* assay. cAverage of inherited data and Dundee assay. dDundee *P. fal* assay. cLogP calculated using DataWarrior.

# Pyrazine: Benzyl ethers

A series of benzyl ethers was explored at the 5-position of the core. Although a few compounds (**3**, **4**, and **9**) achieved sub-micromolar potency, none of these analogs had activity <100 nM or LLE >4. Further exploration of direct comparison between a pyridyl benzyl ether and a phenethyl ether also showed the benzyl ethers to be without activity against *P. fal* (**SI Table X**). Although changing from an ether to a sulfoxide (**11**) or sulfone (**12**) did result in a lower clogP, these compounds were also inactive.

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| **Manuscript ID** | **External ID** | **X** | **R** | **Pfal EC50 (μM)a** | **cLogP** | **LLE** |  |
| 1 | MMV897697  OSM-S-368 | O |  | 2.24 | 2.72 | 2.93 |  |
| 2 | MMV693150  OSM-S-348 | O |  | 1.64b | 3.33 | 2.46 |  |
| 3 | MMV693149  OSM-S-347 | O |  | 0.53b | 3.93 | 2.34 |  |
| 4 | MMV693148  OSM-S-346 | O |  | 0.70b | 4.22 | 1.93 |  |
| 5 | MMV672688  OSM-X-040 | O |  | 1.67c | 3.92 | 1.86 |  |
| 6 | MMV689977  OSM-S-301 | O |  | >5b | 1.78 | <3.52 |  |
| 7 | MMV1576787  OSM-S-420 | O |  | >10 | 2.13 | <2.87 |  |
| 8 | MMV1557937  OSM-S-397 | O |  | 4.80 | 3.61 | 1.71 |  |
| 9 | MMV1557938  OSM-S-398 | O |  | 0.486 | 2.68 | 3.63 |  |
| 10 | MMV693161  OSM-S-359 | S |  | >10b | 3.08 | 1.92 |  |
| 11 | MMV693162  OSM-S-360 | SO |  | >2.5b | 2.11 | 3.49 |  |
| 12 | MMV693163  OSM-S-361 | SO2 |  | >10b | 1.60 | 3.39 |  |

**Table X**. Biological activity of benzylic ethers. aDundee *P. fal* assay unless otherwise noted. bSyngene *P. fal* assay. cData inherited from MMV. cLogP calculated using DataWarrior.

# Pyrazine: Ethers

As the unsubstituted ethylene linker was seen as a potential metabolic liability, direct ether attachment of substituents to the 5-position of the core was explored. A naphthalene (**9**) was the most potent compound discovered through this strategy, although its activity was still >100 nM. Fluoro-substituted naphthyl derivatives (**10**, **11**) also had some activity, although fused ring systems containing heteroatoms (**13**-**22**) showed potencies in the 1-10 μM range. An unsubstituted phenyl ring (**1**) was inactive; however, the addition of lipophilic substituents such as halogens, CF3, or methyl groups, (**4**-**8**) resulted in compounds with sub-micromolar activity. Lipophilicity therefore seems to be a requirement for potency in this instance, resulting in few analogs with LLE >3: even when compounds are active, this is offset by a high clogP.

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| **Manuscript ID** | **External ID** | **R** | **Pfal EC50 (μM)a** | **cLogP** | **LLE** |
| 1 | MMV1581345  OSM-S-579 |  | >25b | 2.77 | 1.83 |
| 2 | MMV669784  OSM-S-516 |  | 4.08c | 3.38 | 2.01 |
| 3 | MMV670935  OSM-X-050 |  | 3.07 | 3.38 | 2.14 |
| 4 | MMV670764  OSM-X-018 |  | 0.383 | 3.61 | 2.80 |
| 5 | MMV671680  OSM-X-009 |  | 0.263 | 7.85 | -1.27 |
| 6 | MMV671648  OSM-X-033 |  | 0.738 | 4.35 | 1.78 |
| 7 | MMV670765  OSM-X-020 |  | 0.455 | 3.98 | 2.36 |
| 8 | MMV671650  OSM-X-028 |  | 0.597 | 4.22 | 2.00 |
| 9 | MMV670659  OSM-X-005 |  | 0.115 | 3.69 | 3.00 |
| 10 | MMV672941  OSM-X-008 |  | 0.173 | 4.17 | 2.59 |
| 11 | MMV672940  OSM-X-034 |  | 0.839 | 4.17 | 1.91 |
| 12 | MMV671649  OSM-X-048 |  | 2.58 | 3.93 | 1.66 |
| 13 | MMV672989  OSM-X-041 |  | 1.67 | 3.37 | 2.41 |
| 14 | MMV672620  OSM-X-039 |  | 1.63 | 3.99 | 1.80 |
| 15 | MMV672730  OSM-X-046 |  | 2.03 | 3.26 | 2.43 |
| 16 | MMV672622  OSM-X-042 |  | 1.82 | 3.88 | 1.86 |
| 17 | MMV672725  OSM-X-058 |  | 6.04 | 3.51 | 1.70 |
| 18 | MMV672937  OSM-X-027 |  | 0.576 | 3.61 | 2.63 |
| 19 | MMV672621  OSM-X-043 |  | 1.93 | 2.90 | 2.82 |
| 20 | MMV672686  OSM-X-059 |  | 6.20 | 2.15 | 3.06 |
| 21 | MMV672623  OSM-X-087 |  | 10 | 2.15 | 2.85 |
| 22 | MMV672618  OSM-X-044 |  | 1.98 | 2.94 | 2.76 |

**Table X**. Biological activity of directly attached 5-position substituents. aData inherited from MMV unless otherwise noted. bDundee *P. fal* assay. cAverage of inherited data and Dundee assay.

# Pyrazine: Amides

A library of amides at the 5-position was synthesized. Amides derived from substituted anilines (**1**-**10**) generally showed some activity; in particular, those containing a *meta*-chloro substitution (**2**-**6**) were sub-micromolar hits (the exception being the 3-chloro-2-methylaniline derivative **4**). A 4-fluoro substitution (**7**) also resulted in potent compounds, particularly when combined with a meta halogen (**6**, **9**). However, these halogenated analogs were fairly lipophilic, resulting in LLEs <4 even for the more potent compounds. A 3,5-ditrifluoromethylaniline derivative (**10**) showed reasonable activity, while the related trifluoromethyl pyridyl derivative (**11**) was almost equally potent and much less lipophilic, resulting in a desirable LLE value of 4.40. Unsubstituted pyridyl analogs (**12**, **13**), however, were totally inactive.

Other amides were derived from benzyl- or aliphatic amines. Benzylamine-derived amides were also synthesized. Again, halogenated derivatives tend to be active at ~1 μM or less. *Meta*- and *ortho*-chloro groups (**14** and **17**, respectively) were favored over the *para* analog (**16**), and methylation of the amide *N* (**15**) caused a ~4-fold loss in potency. The difluorinated compound (**20**) was also fairly potent; in addition, its lower clogP in comparison to the chlorinated derivatives resulted in a desirable LLE of 4.42. Methylation of the benzyl position (**21**, **22**) consistently resulted in an EC50 >1 μM. Constraining the benzyamine using an aminoindane (**23**) ring did improve potency over its unconstrained matched pair (**16**), while indolines **24** and **25** were inactive. A variety of other aliphatic amides were assessed (**26**-**33**) but none were discovered to have sub-micromolar potency against *P. falciparum*.

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| **Manuscript ID** | **External ID** | **R** | **Pfal EC50 (μM)** | **cLogP** | **LLE** |
| 1 | MMV670246  OSM-S-367 |  | 7.40b | 2.83 | 2.30 |
| 2 | MMV669542  OSM-S-202 |  | 0.24c | 2.83 | 3.84 |
| 3 | MMV675947  OSM-S-254 |  | 0.679d | 3.17 | 2.99 |
| 4 | MMV675718  OSM-S-201 |  | 5 | 3.17 | 2.20 |
| 5 | MMV675946  OSM-S-204 |  | 0.85d | 2.93 | 3.13 |
| 6 | MMV670767  OSM-S-379 |  | 0.40b | 2.93 | 3.60 |
| 7 | MMV669849  OSM-X-053 |  | 3.74 | 2.33 | 3.10 |
| 8 | MMV671654  OSM-X-057 |  | 5.58 | 2.93 | 2.32 |
| 9 | MMV669850  OSM-X-031 |  | 0.652 | 2.43 | 3.76 |
| 10 | MMV675719  OSM-S-206 |  | 0.31e | 3.92 | 2.59 |
| 11 | MMV670944  OSM-S-175 |  | 0.45b | 2.12 | 4.40 |
| 12 | MMV1557948  OSM-S-407 |  | >10f | 1.05 | <3.95 |
| 13 | MMV669026  OSM-X-082 |  | >10 | 1.58 | 3.42 |
| 14 | MMV668958  OSM-S-176 |  | 0.25 | 2.54 | 4.06 |
| 15 | MMV669105  OSM-X-036 |  | 1.06 | 2.81 | 3.17 |
| 16 | MMV669001  OSM-S-178 |  | 1.34 | 2.54 | 3.33 |
| 17 | MMV669002  OSM-X-017 |  | 0.379 | 2.54 | 3.88 |
| 18 | MMV671676  OSM-X-015 |  | 0.332 | 3.15 | 3.33 |
| 19 | MMV671677  OSM-X-013 |  | 0.309 | 3.39 | 3.12 |
| 20 | MMV669543  OSM-X-012 |  | 0.276 | 2.14 | 4.42 |
| 21 | MMV669024  OSM-S-186 |  | 1.35 | 2.22 | 3.65 |
| 22 | MMV669021  OSM-X-094 |  | 1.59 | 2.22 | 3.57 |
| 23 | MMV672624  OSM-X-025 |  | 0.547 | 3.23 | 3.04 |
| 24 | MMV669000  OSM-S-177 |  | >10 | 1.57 | <3.43 |
| 25 | MMV670768  OSM-X-080 |  | >10 | 2.71 | <2.29 |
| 26 | MMV669022  OSM-S-182 |  | >10 | 1.57 | <3.43 |
| 27 | MMV669104  OSM-S-183 |  | 3.56 | 3.09 | 2.36 |
| 28 | MMV669003  OSM-S-179 |  | >10 | 1.10 | <3.90 |
| 29 | MMV669011  OSM-S-181 |  | >10 | 0.185 | <4.81 |
| 30 | MMV669027  OSM-X-051 |  | 3.17 | 2.36 | 3.13 |
| 31 | MMV669010  OSM-S-180 |  | >10 | 1.71 | <3.29 |
| 32 | MMV669023  OSM-S-184 |  | >10 | 0.480 | <4.52 |
| 33 | MMV669020  OSM-S-185 |  | >10 | 0.752 | <4.25 |

**Table X**. Biological activity of 5-position amides. aData inherited from MMV unless otherwise noted. bAverage of inherited data and Dundee assay. cAverage of inherited data and GSK assay. dAverage of Syngene and Dundee assays. eGSK *P. fal* assay. fDundee *P. fal* assay.

# Pyrazine: Amines and Reverse Amides

The Nitrogen substituted analogs at R1 (entries **1**-**12**) showed only marginal to no potency against *P. falciparum* and overall were inferior to the ether linked analogs. Of the two reversed amide analogs synthesized (**13** & **14**), the shorter chain acetamide analog showed weak activity, which was lost following extension by one additional carbon atom. The reversed sulfonamide (**15**) also was inactive.

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| **Manuscript ID** | **External ID** | **R1** | **R2** | **Pfal EC50 (μM)** | **cLogP** | **LLE** |
| 1 | MMV670934 |  | -OCHF2 | 9.635 | 4.12 | 0.89 |
| 2b | MMV1634424  OSM-S-638 |  | -OCHF2 | >25 | 2.44 | <2.16 |
| 3b | MMV1634422  OSM-S-589 |  | -OCHF2 | >25 | 1.80 | <2.80 |
| 4b | MMV1634423  OSM-S-588 |  | -OCHF2 | >25 | 1.80 | <2.80 |
| 5b | MMV1634421  OSM-S-591 |  | -OCHF2 | >25 | 2.73 | <1.87 |
| 6 | MMV671652 |  | -OCHF2 | 0.569 | 2.00 | 4.24 |
| 7 | MMV669353 |  | -OCHF2 | >10 | 3.27 | <1.73 |
| 8 | MMV669008 |  | -OCHF2 | 4.96 | 2.82 | 2.49 |
| 9 | MMV668957 |  | -OCHF2 | 2.00 | 3.61 | 2.09 |
| 10 | MMV671653 |  | -OCHF2 | 6.72 | 3.49 | 1.68 |
| 11 | MMV693165 |  | -OCHF2 | >10 | 3.03 | <1.97 |
| 12 | MMV671678 |  | -OCHF2 | >10 | 3.99 | <1.01 |
| 13c | OSM-W-6 |  | -CN | 4.17 | 2.39 | 2.99 |
| 14c | OSM-W-7 |  | -CN | >10 | 2.84 | <2.16 |
| 15 | MMV669103 |  | -OCHF2 | >10 | 2.76 | <2.24 |

**Table X**. Biological activity of 5-position amine linked and reversed amide analogs. aData inherited from MMV unless otherwise noted. bAverage of inherited data and Dundee assay. cAverage of Potency data from Broad Institute *P. fal* assay. cLogP calculated using DataWarrior.

# Triazole: Substituted phenyls

The biological activity of analogs containing substituted phenyl groups at the 3-position of the triazolopyrazine core is shown in **Table X**. Generally, small substituents at the *para* position (R1, entries **2**-**4**) resulted in analogs which retained sub-micromolar potency; however, carboxylic acids, amides, and sulfonamides at R1 or R2 (entries **5**-**11**, **25**-**27**) rendered the compounds inactive. A primary amine at R2 (**12**) was moderately active, and its activity was improved by the addition of a halogen at R1 (**13**, **14**): the 3-amino-4-chlorophenyl analog (**13**) is the most potent compound of this series and also has a desirable LLE of 4.23. Acetylating (**17**) or methylating (**18**) this amine caused a loss of activity, and amines at the para position, including cyclic tertiary amines (**19**-**24**), were also inactive against *P. falciparum*. The R3 difluoro substitution (**16**) on the tethered aromatic ring only marginally increased potency over the monofluoro substitution (**15**).

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| **Manuscript ID** | **External ID** | **R1** | **R2** | **R3** | **Pfal EC50 (μM)** | **cLogP** | **LLE** |
| 1 | MMV1557949  OSM-S-415 | H | H | F | 2.15 | 3.18 | 2.48 |
| 2 | MMV1557931  OSM-S-391 | I | H | F | 0.342 | 3.62 | 2.83 |
| 3 | MMV1580438  OSM-S-553 | NO2 | H | F | 0.980 | 2.26 | 3.75 |
| 4 | MMV1580435  OSM-S-550 | Et | H | F | 0.144 | 3.94 | 2.90 |
| 5 | MMV1580437  OSM-S-552 | H | -CO2H | F | >25 | 2.67 | <1.93 |
| 6 | MMV1580436  OSM-S-551 | -CO2H | H | F | >25 | 2.67 | <1.93 |
| 7 | MMV1577569  OSM-S-506 | -SO2NH2 | H | F | >10 | 1.94 | <3.05 |
| 8 | MMV1576794  OSM-S-495 | H | -CONH2 | F | >10 | 2.27 | <2.73 |
| 9 | MMV1579338  OSM-S-522 | H | -CON(CH3)2 | F | >25 | 2.89 | <1.71 |
| 10 | MMV1576793  OSM-S-494 | -CONH2 | H | F | >10 | 2.27 | <2.73 |
| 11 | MMV1576795  OSM-S-496 | -CON(CH3)2 | H | F | 3.69 | 2.89 | 2.54 |
| 12 | MMV1580434  OSM-S-549 | H | -NH2 | F | 2.8 | 2.51 | 3.05 |
| 13 | MMV1580433  OSM-S-548 | Cl | -NH2 | F | 0.045 | 3.11 | 4.23 |
| 14 | MMV1581334  OSM-S-585 | F | -NH2 | F | 0.813 | 2.61 | 3.48 |
| 15 | MMV639565  OSM-S-272 | Cl | H | F | 0.091 | 3.79 | 3.54 |
| 16 | OSM-W-10\* | Cl | H | H | 0.141 | 3.69 | 3.16 |
| 17 | MMV1579346  OSM-S-530 | H |  | F | >25 | 2.89 | <1.71 |
| 18 | MMV1580430  OSM-S-545 | H | -N(CH3)2 | F | 7.0 | 3.08 | 2.07 |
| 19 | MMV1579342  OSM-S-526 | -NH2 | H | F | 13.0 | 2.50 | 2.38 |
| 20 | MMV1579345  OSM-S-529 |  | H | F | >25 | 2.89 | <1.71 |
| 21 | MMV1579351  OSM-S-531 | -N(CH3)2 | H | F | 5.53 | 3.08 | 2.18 |
| 22 | MMV1579344  OSM-S-528 |  | H | F | >22 | 3.68 | <0.981 |
| 23 | MMV1579343  OSM-S-527 |  | H | F | >20 | 4.02 | <0.681 |
| 24 | MMV1579340  OSM-S-524 |  | H | F | >17 | 2.85 | <1.92 |
| 25 | MMV1577571  OSM-S-508 | H |  | F | >10 | 2.97 | <2.03 |
| 26 | MMV1577568  OSM-S-510 |  | H | F | >10 | 2.97 | <2.03 |
| 27 | MMV1577570  OSM-S-507 |  | H | F | >10 | 2.86 | <2.14 |
| 28\* | OSM-W-8 | -CN | H | H | 0.818 | 2.92 | 3.17 |
| 29\* | OSM-W-9 | -CN | H | F | 0.279 | 3.02 | 3.53 |
| 30\* | OSM-W-3 | -S(O)2Me | H | H | 2.795 | 2.09 | 3.47 |
| 31\* | OSM-W-5 | -S(O)2Me | H | F | 1.092 | 2.19 | 3.78 |

# Triazole: Aliphatic

In an effort to lower the lipophilicity of the compound, aliphatic groups were installed at the 3-position (**Table X**). Although the average clogP of these analogs was slightly lower than those presented in **Table X** (2.59 vs 2.90, respectively), these compounds were in general inactive. Excising the 3-position substituent entirely (**1**) lowered the clogP significantly, but did not produce a potent compound. Piperidine derivatives (**2**-**6**) showed moderate activity when lipophilic groups such as a CF3 were appended distal to the core; morpholine (**7**), piperazine (**8**-**9**), and tetrahydropyran (**10**) derivatives were inactive. Difluoroaniline (**11**) and indoline (**12**) substituents did show sub-micromolar activity, and while the incorporation of fluorine resulted in a high clogP, the indoline derivative was only moderately lipophilic, which resulted in an LLE of 3.93.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | | | | |
| **Manuscript ID** | **External ID** | **R** | **Pfal EC50 (μM)a** | **cLogP** | **LLE** |
| 1 | MMV897706  OSM-S-387 | H | >10 | 1.54 | <3.46 |
| 2 | MMV1580442  OSM-S-555 |  | >25 | 2.26 | <2.34 |
| 3 | MMV669009 |  | 2.63b | 2.74 | 2.84 |
| 4 | MMV1579348  OSM-S-519 |  | 8.46 | 2.59 | 2.48 |
| 5 | MMV1579347  OSM-S-518 |  | 1.51 | 2.88 | 2.94 |
| 6 | MMV1579349  OSM-S-520 |  | 2.75 | 3.50 | 2.06 |
| 7 | MMV1579337  OSM-S-517 |  | >19 | 1.58 | <3.14 |
| 8 | MMV1579350  OSM-S-521 |  | >25 | 1.69 | <2.91 |
| 9 | MMV668961 |  | 10b | 2.57 | 2.43 |
| 10 | MMV668959 |  | 10b | 2.40 | 2.60 |
| 11 | MMV669006 |  | 0.85b | 3.64 | 2.43 |
| 12 | MMV669028 |  | 0.47b | 2.4 | 3.93 |
| 13 | MMV670249 |  | 2.05b | 3.86 | 1.83 |

**Table X**. Biological activity of aliphatic substituents. aDundee *P. fal* assay unless otherwise noted. bData inherited from MMV.

# Triazole: Heterocycles

A series of heterocycles at the 3-position was synthesized in an effort to lower the lipophilicity while maintaining potency. Small heterocycles such as a pyrazole (**1**) and imidazole (**2**) were inactive; however, activity was observed with some of the 5,6 fused ring systems that were installed. Notably, the 6-substituted indole (**4**) showed an EC50 ~100 nM and an LLE of 3.75, while the indazole (**8**), although ~4-fold less active, showed a significant reduction in clogP and an LLE of 3.97. Ethers (**9**, **10**) were also installed to the detriment of antiplasmodial activity. Finally, a substituted dimethyl pyrazole (**11**) was installed which was designed to lower clogP and introduce conformational strain. However, these compounds failed to show activity against *P. falciparum* (**SI Table X)**.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | | | | |
| **Manuscript ID** | **External ID** | **R** | **Pfal EC50 (μM)a** | **cLogP** | **LLE** |
| 1 | MMV1580427  OSM-S-542 |  | >25 | 1.35 | <3.25 |
| 2 | MMV1581330  OSM-S-581 |  | >25 | 1.80 | <2.80 |
| 3 | MMV1580432  OSM-S-547 |  | 4.9 | 3.22 | 2.09 |
| 4 | MMV1579341  OSM-S-525 |  | 0.107 | 3.22 | 3.75 |
| 5 | MMV1581331  OSM-S-582 |  | 0.375 | 3.36 | 3.07 |
| 6 | MMV1581333  OSM-S-584 |  | 1.01 | 2.86 | 3.14 |
| 7 | MMV1581332  OSM-S-583 |  | >25 | 2.29 | <2.31 |
| 8 | MMV1580431  OSM-S-546 |  | 0.434 | 2.40 | 3.97 |
| 9 | MMV1580429  OSM-S-544 |  | >25 | 3.91 | <0.689 |
| 10 | MMV1579339  OSM-S-523 |  | >25 | 3.89 | <0.711 |
|  | | | | | |
| 11 | MMV672625 |  | 10b | 2.02 | 2.98 |
| 12 | MMV670944 |  | 0.300c | 2.12 | 4.40 |

**Table X**. Biological activity of heterocyclic substituents. aDundee *P. fal* assay unless otherwise noted. bData inherited from MMV. cAverage of inherited data and Dundee assay. cLogP calculated using DataWarrior.

# Triazole: Phenethyl ether

A related set of analogs was synthesized with an unsubstituted phenyl ring at the 5-position (**Table X**) which showed similar patterns of activity. The unsubstituted phenyl (**1**) showed moderate activity, while compounds including a *para*-halo group (**2**, **5**-**8**) were consistently active in the sub-micromolar range. On the other hand, *ortho*- or *meta*- halides (**3**, **4**, **9**) were detrimental to antiplasmodial activity. Para-cyano (**10**) and -pentafluorosulfanyl (**12**) substituents, as well as a naphthyl (**13**) group, also produced active compounds, while the 4-pyridyl analog (**11**) was not tolerated. None of the aliphatic groups (**15**-**20**) installed at the 3-position resulted in compounds with any activity against *P. falciparum*. In addition, none of these compounds resulted in an LLE >3.5.

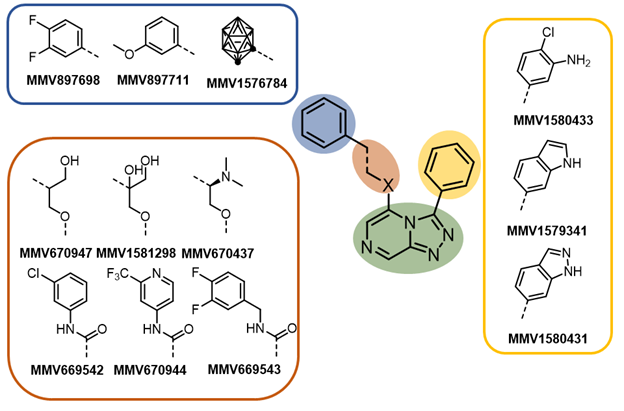
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | | | | |
| **Manuscript ID** | **External ID** | **R** | **Pfal EC50 (μM)a** | **cLogP** | **LLE** |
| 1 | MMV689970  OSM-S-294 |  | 1.51b | 2.98 | 2.84 |
| 2 | MMV663915  OSM-S-293 |  | 0.112b | 3.59 | 3.36 |
| 3 | MMV689968  OSM-S-291 |  | >5 | 3.56 | <1.71 |
| 4 | MMV689969  OSM-S-292 |  | >2.5 | 3.56 | <2.01 |
| 5 | MMV693153  OSM-S-351 |  | 0.259 | 4.20 | 2.39 |
| 6 | MMV693152  OSM-S-350 |  | 0.709 | 4.20 | 1.95 |
| 7 | MMV693154  OSM-S-352 |  | 0.366 | 3.08 | 3.35 |
| 8 | MMV693151  OSM-S-349 |  | 0.582 | 3.19 | 3.05 |
| 9 | MMV689971  OSM-S-295 |  | >5 | 3.19 | <2.12 |
| 10 | OSM-S-187 |  | 0.53c | 2.82 | 3.45 |
| 11 | MMV1557947  OSM-S-408 |  | >10d | 1.98 | <3.01 |
| 12 | MMV1557934  OSM-S-394 |  | 0.485d | 8.07 | -1.75 |
| 13 | MMV689973  OSM-S-297 |  | 0.42 | 4.18 | 2.20 |
| 14 | MMV689972  OSM-S-296 |  | 1.01 | 3.09 | 2.90 |
| 15 | MMV1581339  OSM-S-574 |  | >25d | 3.38 | <1.23 |
| 16 | MMV1581342  OSM-S-577 |  | >25d | 1.72 | <2.88 |
| 17 | MMV1581340  OSM-S-575 |  | >25d | 1.86 | <2.74 |
| 18 | MMV1581337  OSM-S-572 |  | 15d | 3.72 | 1.12 |
| 19 | MMV1581341  OSM-S-576 |  | >25d | 2.06 | 2.54 |
| 20 | MMV1581338  OSM-S-573 |  | >25d | 2.20 | 2.40 |

**Table X**. Biological activity of phenethyl ether substituents. aSyngene *P. fal* assay. bAverage of Syngene and Dundee *P. fal* assays. cGSK *P. fal* assay. dDundee *P. fal* assay.

# Summary

A summary of the structure-activity relationships of this series as a whole is shown in Figure X. The triazolopyrazine core, highlighted in green, is essential for activity; any modification resulted in a loss of P. falciparum activity. The substituent at the 5-position of the core, highlighted in blue, must be aromatic, as none of the analogs containing aliphatic groups at this position were active. A variety of substitution patterns off the aromatic ring were tolerated, but MMV897698 and MMV897711 showed the best combination of potency and lipophilicity. Interestingly, MMV1576784 was also highly active while maintaining a low lipophilicity. Aromaticity was also required for the 3-position substituent, highlighted in yellow. In general, compounds containing para-halogenated rings at this position, such as MMV1580433, displayed sub-micromolar potency. Nitrogen-containing heterocycles, such as MMV1579341 and MMV1580431, were also tolerated at this position and in general resulted in a lower clogP as well.

The linker, highlighted in orange, proved the region most tolerant of modification and some of the best compounds of the series are highlighted in the orange box. Substitution of the ethylene linker with small, polar groups such as the ones of MMV670947, MMV1581298, and MMV670437 resulted in sub-micromolar potency and very low lipophilicity. Extending to a propylene linker or shortening to benzyl or phenyl ethers typically resulted in lower activity, which was generally only seen with highly lipophilic substituents. Changing from X = O to X = S, SO, or SO2 resulted in a loss in activity. A variety of aniline-derived amides were tolerated at this position, with some, such as MMV669542, MMV670944, and MMV669543, exhibiting sub-micomolar potency and tolerable clogP, while amine-derived amides were inactive.



**Figure X**. SAR summary.

# “Crossover” analogs