Open Source Malaria (OSM): Triazolopyrazines as new anti-malarials

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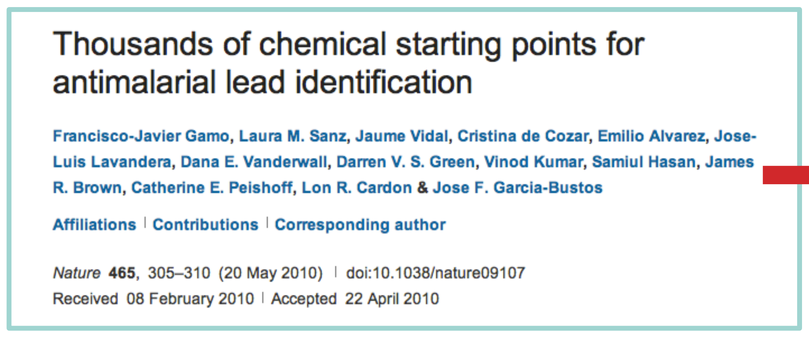
Medicines for Malaria Venture

Introduction

In 2010, GlaxoSmithKline (GSK) released chemical and biological data pertaining to some 13,500 compounds that displayed potent antimalarial activity *in vitro*.[1] Medicinal chemists at the Medicines for Malaria Venture[2] identified several compounds present in this dataset, which they believed to be excellent hits.[3] These compounds had the combination of a drug-like profile, high potency, low cytotoxicity, no known intellectual property issues, and to the best of MMV's knowledge, they were not the subject of antimalarial research by any other group, and hence suitable for an open source project.


Thousands of chemical starting points for antimalarial lead identification

Francisco-Javier Gamo, Laura M. Sanz, Jaume Vidal, Cristina de Cozar, Emilio Alvarez, Jose-Luis Lavandera, Dana E. Vanderwall, Darren V. S. Green, Vinod Kumar, Samiul Hasan, James R. Brown, Catherine E. Peishoff, Lon R. Cardon & Jose F. Garcia-Bustos

Affiliations | Contributions | Corresponding author

Nature **465**, 305–310 (20 May 2010) | doi:10.1038/nature09107
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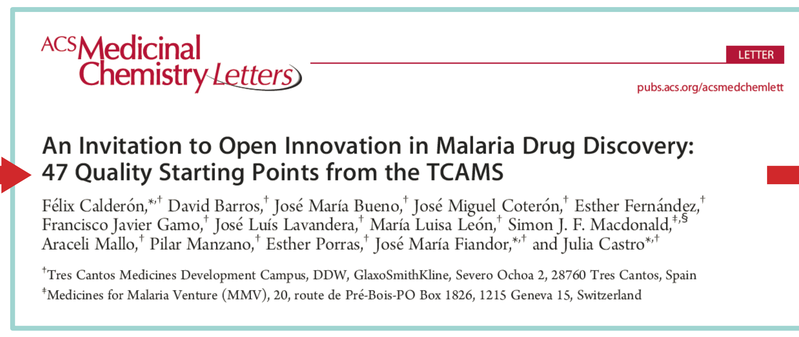
ACS Medicinal Chemistry Letters — LETTER — pubs.acs.org/acsmedchemlett

An Invitation to Open Innovation in Malaria Drug Discovery: 47 Quality Starting Points from the TCAMS

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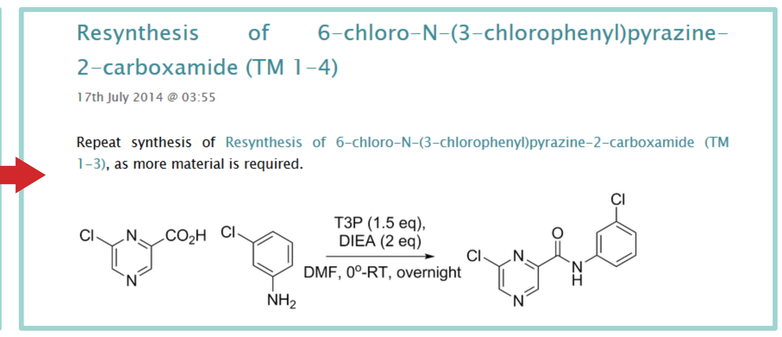
†Tres Cantos Medicines Development Campus, DDW, GlaxoSmithKline, Severo Ochoa 2, 28760 Tres Cantos, Spain
‡Medicines for Malaria Venture (MMV), 20, route de Pré-Bois-PO Box 1826, 1215 Geneva 15, Switzerland


Resynthesis of 6-chloro-N-(3-chlorophenyl)pyrazine-2-carboxamide (TM 1-4)

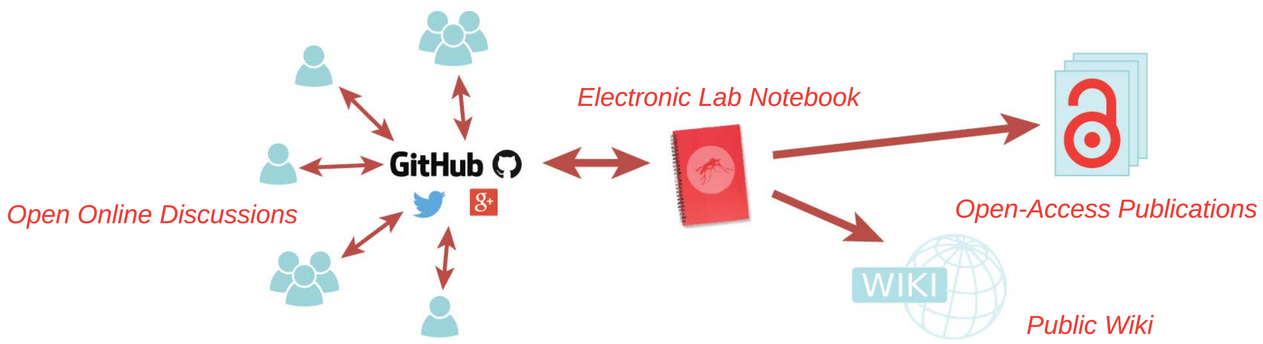
17th July 2014 @ 03:55

Repeat synthesis of Resynthesis of 6-chloro-N-(3-chlorophenyl)pyrazine-2-carboxamide (TM 1-3), as more material is required.

T3P (1.5 eq), DIEA (2 eq), DMF, 0°-RT, overnight

In 2011, the Todd lab started a pilot project in open source drug discovery.[4] The aim of the project is to find a small molecule that is effective for the treatment of malaria, the difference being that everything is open, meaning that all the experiments are published on the web in real-time (including the ones that did not turn out well).[5] Additionally, all of the data are available and anyone can do anything they wish with the compounds, with the proviso that the project is cited.[6]

An Open Source Drug Discovery Project



OSM operates under a completely open philosophy, following the '**Six Laws**' (see right)[7]. Participation is open to all interested parties at any level of engagement, with local and international collaborators ranging from research groups to high school students. All experimental data obtained are posted to publicly-accessible electronic lab notebooks, with resulting discussions and decision-making occurring over open media such as Twitter, GitHub, and G+. Collected and organised results are presented on a wiki, and eventually published in open access journals.

The Six Laws

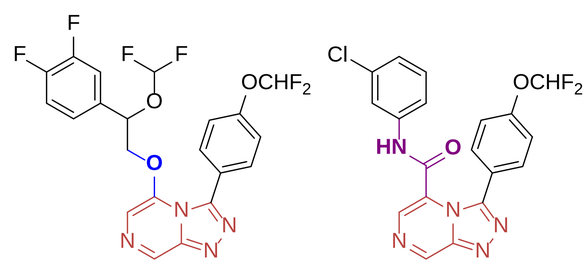
First law:	All data are open and all ideas are shared
Second Law:	Anyone can take part at any level of the project
Third Law:	There will be no patents
Fourth Law:	Suggestions are the best form of criticism
Fifth Law:	Public discussion is much more valuable than private email
Sixth Law:	The project is bigger than, and is not owned by, any given lab.

The aim is to find a good drug for malaria, by whatever means, as quickly as possible

OSM Series Four: The Triazolopyrazines

In 2014 the OSM team have largely focused on the triazolopyrazine series, which has already been assessed by Pfizer and subsequently a CRO funded by MMV. Much of the data suggests that this series could be very promising as many of the compounds are potent antimalarials with good pharmacokinetic properties. Additionally, some information on the mode of action of this series also exists as some compounds were found to be positive in an ion regulation assay, suggesting that they could be hitting PfATP4, a new and interesting target in antimalarial drug discovery.[8]

MMV670652
P.fal IC_{50}: 16.7 nM
LogP: 4.59

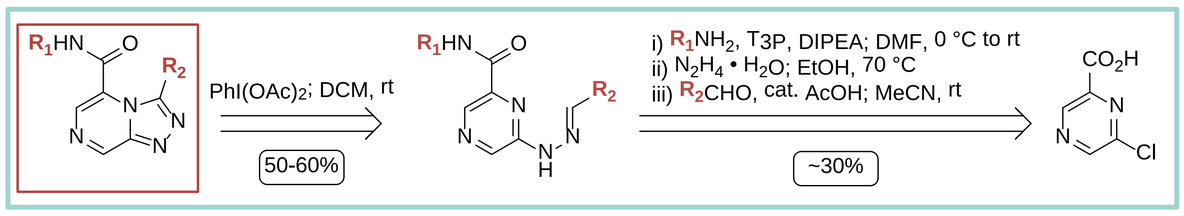


MMV669542
P.fal IC_{50}: 242 nM
LogP: 3.37

Representative ether- and amide-series triazolopyrazines

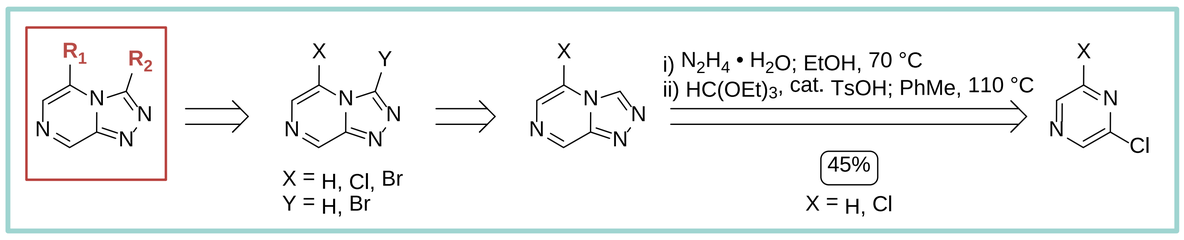
Series four compounds consist of a triazolopyrazine core with two pendant functional groups, one joined by a linking heteroatom. An ether linkage seems to give compounds with the highest potencies, but some early results suggest that an amide linker

Accessing triazolopyrazine amides



Amides were obtained in four steps from a chloropyrazine carboxylic acid starting material, itself accessible from pyrazinoic acid. Bifunctionalised hydrazones are obtained from 6-chloro-2-pyrazine carboxylic acid starting material in three steps by amide bond formation, nucleophilic substitution with hydrazine, and acid-catalysed condensation. The functionalised hydrazones are oxidatively cyclised with phenyliodine diacetate to give the targeted triazolopyrazines.

Synthetic access to [1,2,4]triazolo[4,3-a]pyrazine building blocks



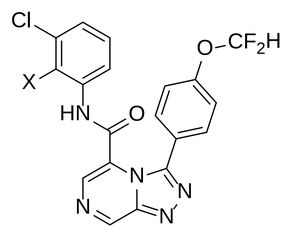
The linear route to access Series 4 analogues would be improved by a divergent scheme starting from simple, functionalisable triazolopyrazine compounds. These building blocks are commercially unavailable, and literature routes are few and low-yielding. A new route was developed to simple [1,2,4]triazolo[4,3-*a*]pyrazine compounds from hydrazinylpyrazines by acid-catalysed condensation with triethyl orthoformate, and performed at the multi-gram scale. Regioselective halogenations were used to selectively brominate at the targeted 3- and 5- positions, but unfortunately attempts to access the desired amides through carbonylative cross-coupling reactions were unsuccessful.

Biological Evaluations of Series 4 triazolopyrazines

Triazolopyrazine amides were synthesised with *Plasmodium falciparum* IC_{50} values measured to 234 nM. Of interest was the extreme sensitivity to substituents on the amidic aryl ring (see right).

The triazolopyrazines have also been identified as potential inhibitors of hERG, a potassium ion channel found in the heart. Of the currently active research strands, the amides appear to show higher hERG binding: further investigations into this undesired off-target effect are underway.

A selection of these compounds were evaluated sent to Pfizer for evaluation of clearance by aldehyde oxidase (AO), an enzyme responsible for metabolising polyaza heterocycles. Both amides sent were found to be substrates for AO: future work will include strategies to block AO metabolism, such as substitution of N-adjacent protons.



X	*P.Fal* IC_{50} (μM)
H	0.242
F	0.702
CH_3	>5

High sensitivity of potencies to amide substituents

Join The Team:

The Open Source Malaria project is experimenting with a new way to do science and hopefully discover a new medicine for malaria. Chemists, biologists, informaticians and many other interested people have all influenced the project in ways that have directly altered its direction.[9]

If you would like to join the team contact us at **@O_S_M** or on the **G+** page. We try to avoid email where possible, but feel free to drop us a line at **opensourcemalaria@gmail.com** if you would like to know how you can become involved in the OSM project.

References

[1] J. F. Garcia-Bustos *et al.*, *Nature*, **2010**, *465*, 305–310; [2] http://www.mmv.org/; [3] *Med. Chem. Lett.*, **2011**, *2*, 741–746; [4] http://openwetware.org/wikiOSDDMalaria:GSK_Arylpyrrole_Series:Story_so_far; [5] http://malaria.ourexperiment.org/; [6] The OSM project's licence unless otherwise stated is CC-BY-3.0 meaning you can use whatever you want for whatever purpose, provided you cite the project; [7] http://www.thesynapticleap.org/node/343 [8] *Science*, **2010**; *329*, 1175–1180; [9] *Parasitology* **2013** *in press* DOI: 10.1017/S0031182013001121

Acknowledgements

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