Bioorganic & Medicinal Chemistry Letters 22 (2012) 5177–5181

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Distinct biological effects of golgicide a derivatives on larval and adult mosquitoes

Daniel J. Mack [c], Jun Isoe [b], Roger L. Miesfeld [b,*], Jon T. Njardarson [a,c,*]

[a] Department of Chemistry & Biochemistry, University of Arizona, 1306 E. University Blvd., Tucson, AZ 85721, USA
[b] Department of Chemistry & Biochemistry, BioSciences West Room 518, 1041 E. Lowell St., University of Arizona, Tucson, AZ 85721, USA
[c] Department of Chemistry and Chemical Biology, Cornell University, Baker Laboratory, Ithaca, NY 14850, USA

ARTICLE INFO

Article history:
Received 28 May 2012
Revised 22 June 2012
Accepted 25 June 2012
Available online 5 July 2012

Keywords:
Golgicide A
Malaria
Mosquito
COPI
GBF1

ABSTRACT

A collection of Golgicide A (GCA) analogs has been synthesized and evaluated in larval and adult mosquito assays. Commercially available GCA is a mixture of four compounds. One enantiomer (GCA-2) of the major diastereomer in this mixture was shown to be responsible for the unique activity of GCA. Structure–activity studies (SAR) of the GCA architecture suggested that the pyridine ring was most easily manipulated without loss or gain in new activity. Eighteen GCA analogs were synthesized of which five displayed distinct behavior between larval and adult mosquitos, resulting in complete mortality of both *Aedes aegypti* and *Anopheles stephensi* larvae. Two analogs from the collection were shown to be distinct from the rest in displaying high selectivity and efficiency in killing *An. stephensi* larvae.

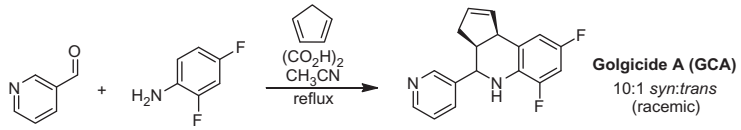
Aedes aegypti mosquitoes, the primary vector of dengue virus, have spread rapidly in the last decade into highly populated urban areas.[1] This has led to a dramatic rise in the number of clinical cases of dengue fever in many parts of the world, with current estimates of 50 million infections/year resulting in 500,000 hospitalizations due to dengue hemorrhagic fever.[2] On an even greater scale, *Anopheline* mosquitoes, primarily the biological vectors *Anopheles gambiae* and *Anopheles stephensi*, account for 250 million cases of malaria/year, leading to ~1 million deaths worldwide.[3] Vector control has proven to be an effective strategy to combat mosquito-borne diseases, and there are many examples in which decreasing mosquito populations reduces disease incidence.[4] However, because of increased mosquito-resistance to pyrethroid-based insecticides,[5] we have been investigating the possibility that proteins required for efficient blood meal metabolism may be novel mosquito-selective targets. Three properties of mosquito blood meal metabolism make it an attractive biological target, (1) it is highly specialized and restricted to blood feeding arthropods, (2) it is a metabolically stressful process in that a ~2.0 mg female mosquito must digest/excrete ~0.5 mg of blood protein within 48 h, and (3) it is both time- and tissue-dependent, which is unlike homeostatic metabolism in non-blood feeding organisms. Taken together, control agents targeting mosquito blood meal metabolism could be more selective and chemically safer than the pyrethroid neurotoxins currently in use.

We have recently shown by RNA interference methods that blood fed mosquitoes are extremely sensitive to defects in COPI vesicle transport, displaying a ~90% mortality by 72 h post blood meal (PBM).[6] The COPI vesicle transport system is known to function in retrograde vesicle transport between the Golgi and ER membrane compartments, as well as receptor targeting to the plasma membrane, and formation of lipid droplets.[7–9] The COPI system consists of three functional components, (1) coatomer proteins (α,β,β′,γ,δ,ε,ζ), which comprise the outer layer of newly formed vesicles, (2) Arf proteins, which are small G proteins that recruit coatomer proteins to the membrane and are activated by GTP–GDP exchange, and (3) the Arf regulatory proteins GBF1 and GAP1/2/3, which are prototypical guanine nucleotide exchange factors (GEFs) and GTPAse activating proteins (GAPs), respectively, that control Arf–GTP and Arf–GDP levels in the cell.[10] A small molecule inhibitor of COPI vesicle transport, golgicide A (GCA, Scheme 1), has recently been shown to disrupt the interaction of GBF1 with Arf proteins, and thereby interfere with COPI-dependent protein targeting.[11]

Golgicide A represented an excellent small molecule starting point for evaluating our COPI vehicle transport inhibitory strategy for mosquitoes. Golgicide A is made in a single step using the Povarov reaction (Scheme 1),[12] which involves imine formation followed by an acid catalyzed addition/cyclization cascade. This one step approach is not only an ideal way of providing rapid access to golgicide A, which is particularly attractive for long term practical applications, but it also lends itself well to being readily manipulated to allow access a vast number of golgicide A analogs. Since no follow up studies have been reported for golgicide A, it is

* Corresponding author.
E-mail address: njardars@email.arizona.edu (J.T. Njardarson).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.06.076



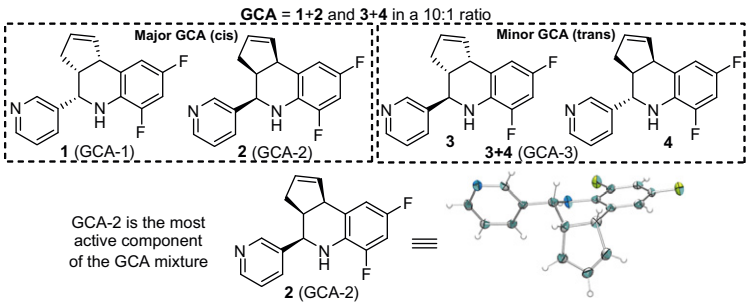
Scheme 1. Synthesis of golgicide A (GCA).

not known how significant each of its five functional groups (two fluorines, piperidine and pyridine nitrogens, as well as the cyclopentene olefin) are in contributing to its biological activity. Moreover, no studies have been reported regarding the potential for GCA to inhibit mosquito metabolism. Furthermore, since golgicide A is now sold commercially,[13] it is important to know which of the four compounds in the commercial preparation are the most active (Scheme 2). This racemic diastereomeric mixture, which results from the acid catalyzed Povarov reaction, is primarily composed of the *cis*-diastereomer (**1** and **2**) in favor of the *trans*-diastereomer (**3** and **4**). The first question we set out to answer was, are all four components of Golgicide A (GCA **1**–**4**) equally potent with respect to their COPI vesicle inhibitory activity? It seemed unlikely to us that this would be the case as the fused rigid tricyclic architecture of GCA would present the pyridine substituent in a dramatically different non-flexible fashion to the target. Furthermore, if we were to assume that the major *cis*-components (**1** and **2**) of GCA were responsible for its biological response, it was still quite likely that the two enantiomeric rigid semi-bowl shaped structures would interact with different efficiency to their chiral target. We addressed this challenge by synthesizing GCA, separating the diastereomers (**1** + **2** from **3** + **4**) using a combination of chromatography and crystallization. The enantiomers were separated from each other using chiral HPLC-chromatography.[14] These pure compounds (GCA-**1**, GCA-**2** and GCA-**3**) were tested against GCA (mixture) using DMSO as a control in a mosquito ovarian development assay (Table 1). This mosquito biological assay was performed by microinjecting each of the compounds into blood fed *Ae. aegypti* mosquitoes and measuring follicle lengths in the developing ovaries 48 h post blood meal. As we predicted, the individual components of the GCA mixture responded in a dramatically different fashion. The minor racemic *trans*-diastereomer (GCA-**3**) was shown to be close to inactive compared to GCA. When the two enantiomers (GCA-**1** vs GCA-**2**) of the major *cis*-diastereomer were compared to each other the significance of chirality was shown to be strongly on display, with one enantiomer (GCA-**1**) performing poorly, while the other enantiomer (GCA-**2**) was superior to all four components as well as the commercial GCA mixture. With this new insight into golgicide-A (GCA), we set out to unambiguously determine the absolute configuration of the active component (GCA-**2**). We were fortunate to be able to grow X-ray quality crystals of GCA-**2**, which in turn allowed us to determine its absolute configuration shown in Scheme 2.[15]

GCA, which is not a rationally designed inhibitor, surfaced as a candidate from an activity screen of a known compound library.[11] Having demonstrated the significance of substitution (*trans* vs *cis*) and chirality on inhibitory activity, we next set out to decipher the importance of each one of its substituents. The carbon skeleton of most drug architectures sets the conformational and three dimensional boundaries by which its substituents can interact with the biological target. With the functional groups being the most likely candidates responsible for target binding, we decided to evaluate how important the contribution of each one of GCA's five functional groups (two aryl fluorides, piperidine and pyridine nitrogen atoms and the cyclopentene olefin) was to the overall biological activity using the same in vivo biological assay (Table 1). The analogs we first synthesized are shown in Scheme 3. Each analog has one of the key GCA functional groups either replaced or substituted (piperidine nitrogen). These analogs were readily accessed by one of two approaches. GCA analogs **4**–**6** were made by reducing or substituting the major GCA-*syn* isomer (**1** + **2**), while analogs **7**–**9** took advantage of the flexibility of the Povarov reaction.[16] When these new analogs were subjected to the same ovarian development assay as GCA's **1**–**3**, it was revealed that all but one functional group contribute substantially to inhibitory activity. Removing the pyridine nitrogen (GCA-**8**) proved to be beneficial. This most active GCA derivative provided us with an opportunity for pursuing further analogs inspired by this new hit structure.

Inspired by GCA-**8** we synthesized GCA analogs **10**–**17** (GCA-**13**, not shown, has the 4-fluoro group replaced with a trifluoromethyl group), wherein the 3-pyridine group has been replaced with heterocycles (**10** and **12**), alkyl- (**11**) or aryl (**14**–**17**) groups. Two analogs from this new set surfaced as noteworthy based on the in vivo mosquito reproduction assay. GCA-**12**, wherein the pyridine nitrogen is in the 2-position compared to the 3-position for GCA proved to be equipotent to GCA, but inferior to lead candidate GCA-**8**. Substituting the phenyl group of GCA-**8** with a 4-trifluoromethyl group (GCA-**17**) proved to be a favorable substitution that resulted in a slight improvement in inhibitory activity.

Our analysis of this new data suggested that less polar aromatic replacements for the 3-pyridine group of GCA were performing better than other substitutions. With these hit structures, we decided to explore the potential of increasing the size of this substituent further in a flexible chainlike fashion with the hope of gaining useful secondary target interactions. Towards that end we decided to functionalize the amine group of GCA-**16** with a di-

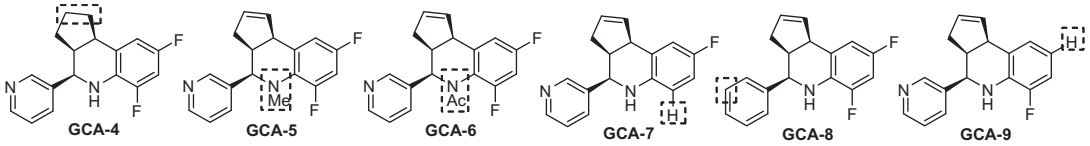


Scheme 2. Four components of golgicide A – Crystal structure and absolute configuration of most active component (GCA-**2**).

Table 1
Effect of GCA derivatives on mosquito reproduction

Short name	Chemical description	Follicle size ± SEM (μm)	Statistics[a]	Statistics[b]
DMSO	Control	482.6 ± 4.8	–	–
GCA	Commercial	291.8 ± 5.6	***	ns
GCA-1	Ent 1	408.2 ± 8.8	***	D***
GCA-2	**Ent 2**	**270.0 ± 7.4**	***	–
GCA-3	Diasteromer	433.6 ± 7.3	ns	D***
GCA-4	Hydrogenated	407.7 ± 7.6	***	D***
GCA-5	*N*-Methyl	416.3 ± 5.5	**	D***
GCA-6	*N*-Acyl	442.7 ± 4.9	ns	D***
GCA-7	de-2-F-GCA	343.8 ± 12.5	***	D***
GCA-8	***de-Pyridyl-GCA***	***235.1 ± 6.8***	***	***I****
GCA-9	de-4-F-GCA	312.1 ± 10.3	***	D**
GCA-10	thiazole-GCA	330.8 ± 8.4	***	D***
GCA-11	*t*Bu-GCA	326.2 ± 9.3	***	D***
GCA-12	2-Pyridyl-GCA	287.5 ± 5.4	***	ns
GCA-13	4-CF3-GCA	315.0 ± 10.2	***	D***
GCA-14	GCA-ArCN	309.5 ± 13.5	***	D**
GCA-15	GCA-ArBnNHAc	375.4 ± 11.6	***	D***
GCA-16	GCA-ArBnAm	301.5 ± 12.5	***	ns
GCA-17	***GCA-ArCF3***	***230.4 ± 5.2***	***	***I****
GCA-18	GCA-ArAmPip	275.3 ± 5.3	***	ns
GCA-19	GCA-ArAmHept	243.4 ± 8.5	***	ns
GCA-20	GCA-ArAmNapth	255.9 ± 5.2	***	ns
GCA-21	GCA-ArAmArCF3	297.5 ± 16.6	***	ns

[a] Statistical comparison between DMSO and GCA derivatives.
[b] Statistical comparisons between GCA-2 and other GCA derivatives. Symbol 'I' and 'D' indicate the increased and decreased activity of GCA derivatives compared to GCA-2. Italics indicates compounds significantly more inhibitory than GCA-2 in this assay. Significance levels are shown as ns: not significant, *: $P<0.05$, **: $P<0.01$, and ***: $P<0.001$.



Scheme 3. Molecular editing of GCA's reactive functional groups.

verse set of groups to increase our chance of identifying a new hit. For this purpose, a heterocycle (GCA-**18**), alkyl- (GCA-**19**) and aryl- (GCA-**20** and **21**) groups were chosen.

We were encouraged to find that all four analogs were superior to the starting structure (GCA-**16**). Three of these four analogs (GCA-**18**, **19** and **20**) performed better than GCA (Table 1). This new data seems to support our assessment that more greasy compounds perform better. For example, substituting the polar benzylic nitrogen atom of GCA-**16** with a greasy heptyl- (GCA-**19**) or napthyl- (GCA-**20**) groups affords analogs that are similar in potency to GCA-**8** and GCA-**17**.

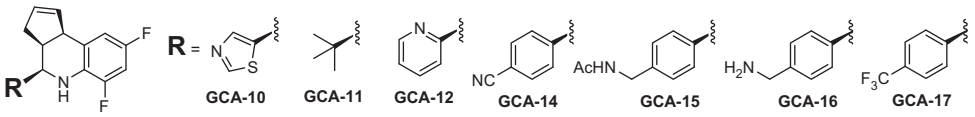
Of the eighteen GCA inspired analogs we synthesized, four performed better than the active enantiomer GCA-**2** (GCA-**8**, GCA-**18**, GCA-**19**, GCA-**20**). However, only GCA-**8** and GCA-**17** displayed statistically significant improvement in inhibitory activity over GCA-**2** (*$P<0.05$*). Equipped with these new insights about GCA and the collection of new analogs from our ovarian development assay in blood fed mosquitoes (Table 1), we next tested the performance of the compounds in a more sensitive cytotoxic assay in second instar *Ae. aegypti* and *An. stephensi* mosquito larvae (Table 2). In this larval assay, GCA derivatives were added directly to the culture media and larval mortality was measured 24 h later. The results from these studies are quite intriguing. GCA and its individual components (GCA **1**–**3**) displayed minimal to no cytotoxicity in the *Ae. aegypti* assay, with the most interesting exception being GCA-**2**, which demonstrated strong cytotoxicity against *An. stephensi* mosquito larvae. Of the 21 compounds evaluated, four (GCA-**5**, GCA-**9**, GCA-**12** and GCA-**18**) proved very effective in killing larvae from both mosquito species. GCA-**16** followed closely behind in terms of general cytotoxicity. These five hits are quite diverse and represent all phases of our derivatization studies. Of these active analogs, two are from our initial functional group SAR editing campaign (Scheme 3), wherein the piperidine nitrogen has been methylated (GCA-**5**) and the 4-fluoro group of GCA removed (GCA-**9**). One (GCA-**12**) is from the second phase of our SAR studies in which we focused on replacing the GCA 3-pyridine group (Scheme 4) and the last two (GCA-**16** and GCA-**18**) are members of our last compound collection (Scheme 5) whose goal was to achieve a second molecular target interaction. An equally interesting observation from the data presented in Table 2 is the high mosquito species selectivity for four of the GCA analogs (GCA-**2**, GCA-**4**, GCA-**10** and GCA-**11**), which in all cases are about five times more selective in killing *An. stephensi* larvae compared to *Ae. aegypti* larvae. Despite being similar in terms of mosquito selectivity, their cytotoxicity varies significantly, ranging from 86.4% (GCA-**11**) to 37.6% (GCA-**10**). It is worth mentioning, that the most active component of the initial GCA mixture (GCA-**2**) again emerges as being unique.

When the data from Tables 1 and 2 are compared, several interesting observations emerge (Scheme 5). The most active compounds in the ovarian development assay (Table 1, GCA-**8** and GCA-**17**), were not significantly better than DMSO in the larval mortality assay (Table 2), suggesting that the biological target may be different in larvae than in adults. Alternatively, it could be that the pharmacokinetics of these compounds are very different when injected into the mosquito hemolymph (ovarian development assay), compared to water (larvae cytotoxicity assay). It is remarkable that by simply methylating the piperidine nitrogen of GCA we are able to convert one of the most inactive compounds (GCA-**5**) presented in Table 1 into one of the most active one in the

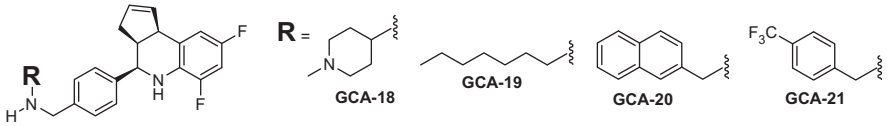
Table 2
Larvicidal effect of GCA derivatives of 2nd instar mosquito larvae

Name		Ae. aegypti mortality				An. stephensi mortality				Statistics[c]
		Mean	SEM	Statistics[a]	LC_{50}[d]	Mean	SEM	Statistics[b]	LC_{50}[d]	
DMSO	control	0.0	0.0	—		0.0	0.0	—		ns
GCA-1	Ent 1	4.7	2.6	ns		0.0	0.0	ns		ns
GCA-2	Ent 2	18.1	4.0	**		83.4	2.8	***		***
GCA-3	Diasteromer	1.5	1.5	ns		0.0	0.0	ns		ns
GCA-4	Hydrogenated	12.3	2.6	ns		57.5	8.1	***		***
GCA-5	*N*-Methyl	96.2	1.9	***	0.1	100.0	0.0	***	0.1	ns
GCA-6	*N*-Acyl	1.7	1.7	ns		0.0	0.0	ns		ns
GCA-7	de-2-F-GCA	0.0	0.0	ns		0.0	0.0	ns		ns
GCA-8	de-Pyridyl-GCA	0.0	0.0	ns		0.0	0.0	ns		ns
GCA-9	de-4-F-GCA	91.4	7.0	***	0.2	100.0	0.0	***	0.2	ns
GCA-10	thiazole-GCA	0.0	0.0	ns		37.6	2.1	***		**
GCA-11	*t*Bu-GCA	15.4	4.4	*		86.4	9.7	***		***
GCA-12	2-Pyridyl-GCA	100.0	0.0	***	0.1	100.0	0.0	***	0.1	ns
GCA-13	4-CF3-GCA	5.3	5.3	ns		0.0	0.0	ns		ns
GCA-14	GCA-ArCN	0.0	0.0	ns		0.0	0.0	ns		ns
GCA-15	GCA-ArBnNHAc	0.0	0.0	ns		0.0	0.0	ns		ns
GCA-16	GCA-ArBnAm	91.7	1.7	***	0.2	81.7	4.6	***	0.1	ns
GCA-17	GCA-ArCF3	5.9	5.9	ns		0.0	0.0	ns		ns
GCA-18	GCA-ArAmPip	100.0	0.0	***	0.05	100.0	0.0	***	0.05	ns
GCA-19	GCA-ArAmHept	0.0	0.0	ns		0.0	0.0	ns		ns
GCA-20	GCA-ArAmNapth	0.0	0.0	ns		0.0	0.0	ns		ns
GCA-21	GCA-ArAmArCF3	0.0	0.0	ns		0.0	0.0	ns		ns

Statistical significane levels are shown as ns: not significant, *: $P<0.05$, **: $P<0.01$, and ***: $P<0.001$.
[a] Statistical comparison between DMSO and GCA derivatives in *Ae. aegypti*.
[b] Statistical comparison between DMSO and GCA derivatives in *An. stephensi*.
[c] Statistical comparison between *Ae. aegypti* and *An. stephensi* for each GCA derivative.
[d] LC50 (50% the lethal concentration in mM) was determined in *Ae. aegypti* and *An. stephensi*.



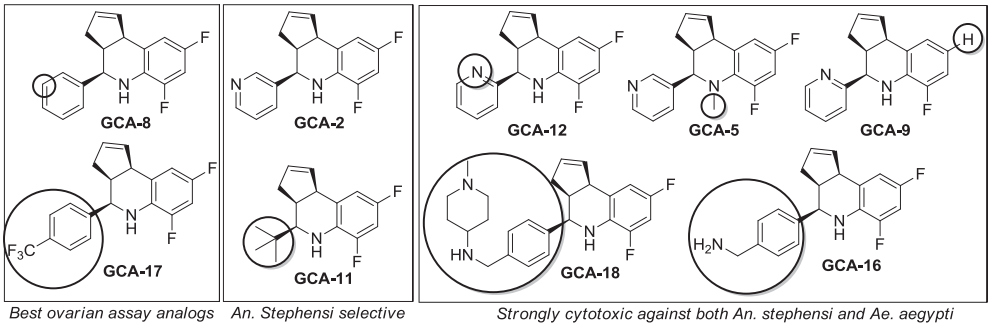
Scheme 4. GCA-**8** Inspired analogs.



Scheme 5. GCA-**17** Inspired analogs – in search of a secondary interaction.

cytotoxic assay (Table 2). The 4-desfluoro GCA analog (GCA-**9**), displayed similarly drastic behavior. Closer inspection of the data from Tables 1 and 2 reveals that GCA-**12** and GCA-**18** are the only analogs that display strong effects in all three assays. In the *Ae. aegypti* ovarian development assay, GCA-**12** and GCA-**18** were shown to be equipotent with GCA (Table 1), while in the larvae cytotoxicity assay (Table 2), GCA-**12** and GCA-**18** were strongly cytotoxic against both *An. stephensi* and *Ae. aegypti* larvae and GCA is not. When comparing the structures of these two analogs to GCA, GCA-**12** represents a miniscule structural change. In GCA-**12** the pyridine nitrogen has simply been moved from the 3-position with respect to the piperidine ring in GCA to the 2-position. Although unknown at this time, this subtle structural change now provides both metal chelating and hydrogen bonding opportunities for the adjacent nitrogen atoms of GCA-**12** which are not possible for GCA or any of the other analogs. GCA-**18** on the other hand represents a significant structural change from GCA having the pyridine ring been replaced with a phenyl group containing a *para*-substituted benzylic secondary amine group attached to an additional piperidine functionality. It is noteworthy that GCA-18 had the lowest LC50 (0.05 mM) in both *An. stephensi* and *Ae. aegypti* larvae (Table 2). Four of the analogs shown in Scheme 6 represent minor structural changes compared to GCA-**2**, with three of these four having one of GCA-**2** two nitrogen atoms either capped (GCA-**5**), deleted (GCA-**8**) or migrated (GCA-**12**). For the other four analogs, the 3-pyridine ring has been replaced by a hindered alkyl group (GCA-**11**) or *para*-substituted aryl group with a diverse group of substituents (GCA-**16**–**18**).

In conclusion, we have demonstrated that GCA-**2** is the most active component of the commercially available GCA mixture. GCA-**2** was superior to its three other components as shown by an ovarian development assay in blood fed *Ae. aegypti* mosquitoes and a more sensitive larvae cytotoxicity assay. In order to improve upon the performance of GCA-**2**, we synthesized 18 analogs, which were tested in both assays. The results from these studies showed that these analogs behaved distinctly differently in these assays, with GCA-**12** and GCA-**18** being the only two analogs that performed well in both assays. Five of the GCA analogs were shown to be effective in killing both *Ae. aegypti* and *An. stephensi* larvae, while two analogs (GCA-**2** and GCA-**11**), displayed distinct and strongly selective cytotoxicity (>80%) towards *Ae. stephensi* larvae. Future studies are aimed at exploiting the unique biological insights gained from our GCA inspired collection toward making more active and selective compounds.



Scheme 6. Structures of the biologically most active GCA analogs (circle denotes change from starting structure).

Acknowledgments

We would like to thank the University of Arizona for financial support, Dr. Michael Riehle for help in establishing the *An. stephensi* colony in the Miesfeld lab, and NIH grant support to RLM for mosquito metabolic studies (R01AI046451, R01AI031951). Dr. Sue Roberts is gratefully acknowledged for her X-ray structure analysis, which helped determine the structure and absolute configuration of GCA-**2**.

Supplementary data

Supplementary data (supplementray data)associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.076.

References and notes

1. Jansen, C. C.; Beebe, N. W *Microbes Infect.* **2010**, *12*, 272.
2. Guzman, M. G.; Halstead, S. B.; Artsob, H.; Buchy, P.; Farrar, J.; Gubler, D. J.; Husperfer, E.; Kroeger, A.; Margolis, H.; Martinez, E.; Nathan, M. B.; Pelegrino, J. L.; Simmons, C.; Yoksan, S.; Peeling, R. W. *Rev. Microbiol.* **2010**, *8*, S7.
3. Enayati, A.; Hemingway, J. *Ann. Rev. Entomol.* **2010**, *55*, 569.
4. Raghavendra, K.; Barik, T. K.; Reddy, B. P.; Sharma, P.; Dash, A. P. *Parasitol. Res.* **2011**, *108*, 757.
5. Ranson, H.; N'Guessan, R.; Lines, J.; Moiroux, N.; Nkuni, Z.; Corbel, V. *Trends Parasitol.* **2011**, *27*, 91.
6. Isoe, J.; Collins, J.; Badgandi, H.; Day, W. A.; Miesfeld, R. L. *Proc. Nat. Acad, Sci. U.S.A.* **2011**, *108*, E211.
7. Styers, M. L.; O'Connor, A. K.; Grabski, R.; Cormet-Boyaka, E.; Sztul, E. *Am. J. Physiol. Cell. Physiol.* **2008**, *294*, C1485.
8. Rennolds, J.; Tower, C.; Musgrove, L.; Fan, L.; Maloney, K.; Clancy, J. P.; Kirk, K. L.; Sztul, E.; Cormet-Boyaka, E. *J. Biol. Chem.* **2008**, *283*, 833.
9. Beller, M.; Sztalryd, C.; Southall, N.; Bell, M.; Jackle, H.; Auld, D. S.; Oliver, B. *PLoS Biol.* **2008**, *6*, e292.
10. Beck, R.; Rawet, M.; Wieland, F. T.; Cassel, D. *FEBS Lett.* **2009**, *583*, 2701.
11. Saenz, J. B.; Sun, W. J.; Chang, J. W.; Li, J.; Bursulaya, B.; Gray, N. S.; Haslam, D. B. *Nat. Chem. Biol.* **2009**, *5*, 157.
12. For a recent review, consult: Kouznetsov, V. V. Tetrahedron 2009, 65, 2721.
13. (a) Tocris Biosciences at www.tocris.com; (b) Sigma–Aldrich at www.sigmaaldrich.com.; (c) Santa Cruz Biotechnology Inc. at www.scbt.com.; (d) EMD Millipore Chemicals at www.emdmillipore.com.
14. See Supplementary data for details.
15. For X-ray crystal structure details, see Supplementary data. The CCDC deposition number for **GCA-2** is 886176.
16. For these analogs, the major (*syn*) diastereomer was separated from the minor (*trans*) diastereomer using either chromatography or recrystallization approaches. All analogs in this adn following series were tested as racemates.