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Journal

Bioorganic & Medicinal Chemistry Letters, 25(22)

ISSN

0960-894X

Authors

Avilés, Edward
Prudhomme, Jacques
Le Roch, Karine G
et al.

Publication Date

2015-11-01

DOI

10.1016/j.bmcl.2015.09.033

Peer reviewed

Published in final edited form as:

Bioorg Med Chem Lett. 2015 November 15; 25(22): 5339–5343. doi:10.1016/j.bmcl.2015.09.033.

Synthesis and preliminary biological evaluation of a small library of hybrid compounds based on Ugi isocyanide multicomponent reactions with a marine natural product scaffold

Edward Avilés[a], **Jacques Prudhomme**[b], **Karine G. Le Roch**[b], **Scott G. Franzblau**[c], **Kevin Chandrasena**[d], **Alejandro M. S. Mayer**[d], and **Abimael D. Rodríguez**[*,a]

[a]Department of Chemistry, University of Puerto Rico, P.O. Box 23346, U.P.R. Station, San Juan, Puerto Rico 00931-3346

[b]Department of Cell Biology and Neuroscience, University of California at Riverside, California 92521

[c]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

[d]Department of Pharmacology, Chicago College of Osteopathic Medicine, Midwestern University, Downers Grove, Illinois 60515

Abstract

A mixture-based combinatorial library of five Ugi adducts (**4**–**8**) incorporating known antitubercular and antimalarial pharmacophores was successfully synthesized, starting from the naturally occurring diisocyanide **3**, via parallel Ugi four-center three-component reactions (U-4C-3CR). The novel α-acylamino amides obtained were evaluated for their antiinfective potential against laboratory strains of *Mycobacterium tuberculosis* H_{37}Rv and chloroquine-susceptible 3D7 *Plasmodium falciparum*. Interestingly, compounds **4**–**8** displayed potent *in vitro* antiparasitic activity with higher cytotoxicity in comparison to their diisocyanide precursor **3**, with the best compound exhibiting an IC_{50} value of 3.6 nM. Additionally, these natural product inspired hybrids potently inhibited *in vitro* thromboxane B_2 (TXB_2) and superoxide anion (O_2^-) generation from *Escherichia coli* lipopolysaccharide (LPS)-activated rat neonatal microglia, with concomitant low short-term toxicity.

Keywords

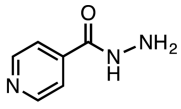
Tuberculosis; Malaria; Anti-neuroinflammatory activity; Ugi multicomponent reaction; Isocyanide; Natural product hybridization

[*]abimael.rodriguez1@upr.edu.

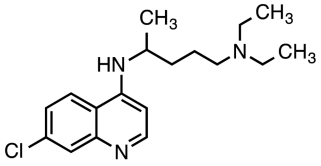
Supplementary data

Supplementary data (experimental details for the synthesis, molecular structure characterization, and biological evaluation of new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/.....

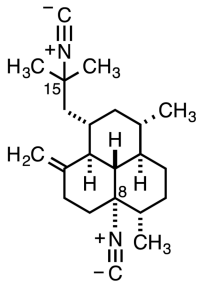
During the course of our investigations of new biologically active compounds from Caribbean marine invertebrates, we have focused our attention to discover new drugs for the treatment of tropical diseases (TDs), which affect billions of people worldwide.[1] Tuberculosis and malaria are two of the major TDs with the highest rates of death.[2] Current first-line drugs for the treatment of these diseases include isoniazid (**1**) and chloroquine (**2**), respectively. Owing to the emergence of microbes that are resistant to currently available antiinfective drugs, there exist an urgent need for new, effective and affordable backup drugs.[3] As part of our strategy to the discovery of novel drugs to combat TDs, we have paid particular attention to determine the antiinfective action of marine isocyanides, since it was previously established that a number of secondary metabolites belonging to this class of compounds possess putative antitubercular and antiplasmodial activity.[4] Moreover, recent investigations appear to support the hypothesis that marine isocyanides and derivatives could become lead compounds for the development of novel agents to modulate excessive release of TXB_2 by activated microglia cells in neuroinflammatory disorders.[5]



1 Isoniazid (INH)



2 Chloroquine (CQ)



3 (–)-DINCA

While a number of amphilectane-based diterpene isocyanides with interesting bioactivity profiles have been reported, effective methods for their synthesis or for generating analogues are limited.[6] This is not only due to the lack of compound supply, but equally important, the limited availability of general methods based on mild reaction conditions required for further functional group manipulations. For instance, the synthesis of complex natural products like 8,15-diisocyano-11(20)-amphilectene [(–)-DINCA] (**3**)[7] and analogues required for structure-activity relationship (SAR) studies, poses a difficult challenge, often involving rather lengthy syntheses with introduction of the isocyanide functionality late in the synthesis.[8] An alternative approach to drug discovery, which we have explored previously, is to use an abundant natural product as a scaffold or building block and to convert it into a small focused library of analogues.[9] Our intended target was **3**, which is accessible in milligram amounts and contains both the amphilectane framework and two isocyanide moieties, one of which could serve as a “handle” with potential for further synthetic elaboration. A mounting body of evidence, which suggest that both the isocyanide functional groups and the diterpene skeleton are responsible for the antituberculosis and antiplasmodial activity of (–)-DINCA (**3**), provides further support for this approach.[10]

An ideal tool for accessing a small library of isocyanide analogues of increased structural diversity, both quickly and in a synthetically inexpensive way, is the use of isocyanide-based multicomponent reactions (IMCRs).[11] Over the past two decades the field of IMCR research has experienced a steadfast growth with the discovery and development of new variations of the classical Passerini and Ugi IMCRs.[12] Multicomponent reactions are defined as reactions in which more than two starting compounds react to form a product that incorporates essentially all of the carbon atoms. In particular, the multicomponent isocyanide condensation discovered by Ivar Ugi is a versatile four-component reaction involving a carboxylic acid, an aldehyde, a primary or secondary amine, and an isocyanide.[12] Thus, the Ugi isocyanide-based multicomponent reactions (Ugi IMCRs) are a powerful tool with which libraries of new compounds that are potentially effective against infectious diseases can be constructed in a single-stage reaction. Furthermore, Ugi IMCRs are typically easy to perform, tolerate a wide range of group functionalities, and provide quick access to structurally complex compounds that otherwise would require lengthy syntheses.[13] An example of this approach to drug discovery using the Ugi IMCR is outlined below (Scheme 1).

In our ongoing investigations to discover novel compounds for screening against *Mycobacterium tuberculosis* and the most common and deadly human malaria parasite, *Plasmodium falciparum* (malaria tropica), we initially sought to design Ugi four-component condensation (U-4CC) reaction adducts (α-acylamino amides) that incorporate known pharmacophores found in two existing antitubercular and antimalarial drugs, namely, isoniazid (**1**) and chloroquine (**2**).[14] Combining these pharmacophores through the U-4CC method has many attractive features, including the opportunity to rationally design novel drugs aimed at multiple targets within the tuberculosis bacterium and malaria parasite. These substructures would be incorporated as part of our amine or carboxylic acid component of the U-4CC reactions (Table 1).[15] Our selection of formaldehyde as the aldehyde component was driven by our desire to synthesize low-molecular-weight adducts while avoiding the formation of epimeric mixtures at C-23, thus simplifying the purification process. Our choice for the isocyanide component was limited to (–)-DINCA (**3**) because of its remarkable potency against *M. tuberculosis* and chloroquine-resistant *P. falciparum* strains and preponderance to react preferentially through its C-15 isocyanide group.[5,10] We expected that our natural product inspired molecular hybridization approach would lead us to the expeditious development of new hybrid molecules with noteworthy antiinfective properties. Table 1 summarizes our selection of carboxylic acid, amine, and aldehyde building blocks for the Ugi multicomponent-based library.

To synthesize the quinoline-containing amine required for the U-4CC, we reacted commercially available 4,7-dichloroquinoline (**9**) with excess ethylenediamine in the absence of solvents at 80 °C for 1 h and at 135–140 °C for 3 h to afford **10** in 90% yield (Scheme 2).[13a] The condensation of stoichiometric amounts of the amine, aldehyde, carboxylic acid and diisocyanide **3** in anhydrous EtOH at 20 °C furnished the desired Ugi adducts. A summary of the synthesized target compounds **4**–**8** is provided in Figure 1. Following solvent removal under reduced pressure, purification of the crude reaction

mixtures was easy achieved by flash silica-gel chromatography to afford the products in modest to good isolated yields (Table 1).

Compounds **4**–**8** were structurally analyzed on the basis of conventional spectroscopic data (IR, UV, HRESI-MS, and 1D and 2D NMR). For adducts **4** and **8**, molecular characterization was swift and straightforward because each compound was obtained as a homogeneous stable entity. In the case of compounds **6** and **7**, the initial characterization by ^{1}H and ^{13}C NMR was complicated because of the duplication of many of the proton and carbon signals. Rotation around the tertiary amide bond in these Ugi adducts gave rise to two quickly interchanging rotational isomers with notably different chemical shift values in a ratio of approximately 1:1. We confirmed this phenomenon by running the experiments in DMSO-d_6 and briefly observed the coalescence of the duplicating peaks. The two sets of signals reappeared shortly thereafter (~2 h), indicating that the two rotamers had reached equilibrium. Rotational isomerization was observed only in the two latter adducts, which is reasonable given the inherent conformational flexibility introduced by the ethylenediamine bridge of **6** and **7** relative to that of **4**, **5**, and **8**. As we predicted, the Ugi adduct **5** was obtained as a diastereomeric mixture (dr 67:33) that could not be easily separated by flash silica-gel chromatography and was therefore characterized as such without further optimization (the isomer ratio was determined via the integration of selected signals in the ^{1}H NMR spectra of the reaction products).

Compounds **3**–**8** were tested against *M. tuberculosis* $H_{37}Rv$ with the results as shown in Table 2. From the modest library, compound **3** with an MIC of 3.2 μg/mL exhibited the best activity, being nearly as potent in this strain as the powerful mycobactericide isoniazid (**1**) (MIC = 0.44 μg/mL). On the other hand, the MIC values for the Ugi adducts obtained from **3**, compounds **4**–**8**, were between 14.9 and 101.8 μg/mL. Based on a comparison of the MIC results obtained for these compounds, it appears that manipulation of the isocyanide group at the C-15 position in **3** to an α-acylamino amide function, as in **4**–**8**, results in a marked decrease in activity. In general, it can be seen from the Table 3 that the antitubercular activity decreases for all of the Ugi adducts whether based on isonicotinic acid (e.g. **4**, **5**, **7**, and **8**) or aminoquinoline (e.g. **6**) pharmacophores.

As derivatives based on an antiparasitic natural product scaffold, compounds **4**–**8** were also screened against a chloroquine (CQ) non-resistant *Plasmodium falciparum* 3D7 strain to ascertain their potentials as effective antimalarial agents. The antiplasmodial activities were determined as the inhibitory concentrations at 50% parasite survival (IC_{50}) in the strain; the results are tabulated in Table 2. The antiplasmodial activity and selectivity index (SI) of CQ (**2**) are also shown for comparative purposes. Interestingly, all of the α-acylamino amides obtained from the U-4CC reactions displayed potent antiplasmodial activity (IC_{50} values ≤ 13.0 nM) against this strain. Compound **7**, which was based on isonicotinic acid and aminoquinoline pharmacophores, was observed to be the most active (IC_{50} = 3.6 nM) of the investigated compounds, although **8**, which was based only on isonicotinic acid, was nearly as active (IC_{50} = 6.3 nM) as **7**. Compound **5**, the only adduct with a bulky group at C-23 that was analyzed to be a mixture of diastereomers, was the least active against the 3D7 strain (IC_{50} = 13.0 nM). However, we speculate that the separation and testing of each diastereomer may result in a marked increase in activity of one of the compounds. Inasmuch

as hybrids **4**–**8** showed excellent *in vitro* inhibitory activity (IC_{50} values in the range of 3.6–13.0 nM) when compared to the standard drug CQ (IC_{50} = 6.6 nM), they showed more toxicity (SI values in the range 19788–49173) than the CQ (SI = 35526). Interestingly, our starting scaffold **3** proved to be the most promising compound of the series (IC_{50} = 1.2 nM, SI = 83112), which was 5.5 times more active and significantly less toxic than the standard drug. When compared to **3**, the observation that the antiplasmodial activity of the new compounds is comparable to that of the parent compound raises the question as to whether the isocyanide group at C-15 in the natural product is responsible for the observed activity.

Derivatives **4**–**8** along with scaffold **3** were screened further in order to determine the effect of these compounds on *E. coli* LPS-activated microglia TXB_2 and O_2^- generation *in vitro*. O_2^- and TXB_2 release, as well as short term cell viability, were assessed as described in the Supplementary data. As shown in Table 3, α-acylamino amides **4, 5** and **8** (based on the isonicotinic acid pharmacophore only) inhibited TXB_2 generation with IC_{50}'s = 3.0, 0.1 and 1.1 μM, respectively, demonstrating minimal effect on O_2^- release (IC_{50} > 10 μM) and minimal short-term toxicity (LDH_{50} > 10 μM). In contrast, **6** and **7**, the only derivatives prepared based on the aminoquinoline pharmacophore, potently inhibited TXB_2 generation (IC_{50} = 0.8 and 0.9 μM, respectively) and O_2^- release (IC_{50} = 1.7 and 5.0 μM, respectively), but with evidence of short-term toxicity (LDH_{50} = 5.0 μM). Thus, in our *in vitro* experimental conditions, it appeared that inhibition of microglia TXB_2 generation by Ugi adducts **4, 5** and **8** resulted from a pharmacologic effect, in contrast with that of analogs **6** and **7** that were toxic to the microglia cells. To summarize, comparison of the IC_{50}'s of starting diisocyanide **3** (IC_{50} ~ 0.23 μM) and α-acylamino amides **4**–**8** supports the observation that the observed bioactivity is mainly associated with the presence of an amphilectane diterpenoid core bearing a C-8 isocyanide functionality. Within the series of semi-synthetic products **4**–**8**, analog **5** displayed the highest antineuroinflammatory activity (IC_{50} = 0.1 μM), and when compared to **4**, the least active congener bearing the isonicotinic acid pharmacophore (IC_{50} = 3.0 μM), the presence in **5** of a phenyl residue at C-23 seems to further potentiate its biological activity. On the other hand, substitution of R^2 (Scheme 1) with bulkier functional groups, as observed in compound **7**, appears to lower the activity. Moreover, comparison of the IC_{50}'s of **4** and **8** suggests that the presence of an isopentyl group (R^2) also seems to play a role in lowering the observed pharmacological activity. Lack of O_2^- inhibition in compounds **3**–**5** and **8** would appear to suggest that they inhibit TXB_2 synthesis through a cyclooxygenase-dependent mechanism.

To summarize, Ugi IMCRs were used to accomplish four-component couplings of amines, aldehydes, acids, and diisocyanide **3**, allowing for the design and construction of a small library of α-acetylamino amides (**4**–**8**) that, while less active than isoniazid (**1**), are as potent as the antimalarial drug chloroquine (**2**) against the 3D7 strain of *P. falciparum*. All of the compounds in our modest library exhibited IC_{50} values in the low nanomolar range, with adduct **7** exhibiting the highest antiplasmodial activity (IC_{50} = 3.6 nM, SI = 7154). To our delight, this hybrid was twice as potent as chloroquine against the 3D7 strain (Table 2), however, it showed the most toxicity. Although some general trends related to the activities of the compounds are evident, further exploration, including the synthesis and testing of additional compounds against multiple parasite strains with various degrees of sensitivity

and resistance, will be needed before definite conclusions can be drawn. Notwithstanding, the results presented herein clearly underscore the importance of isocyanide-based multicomponent reactions in antimalarial drug discovery, particularly when combined with a rational choice of inputs based on known antimalarial pharmacophores. Thus, hybrid approaches inspired by natural products can lead to the rapid generation of novel chemotypes for the development of diverse, biologically rich SAR libraries.[16] The development of new treatment strategies is of great importance because parasitic resistance to existing antimalarial agents is spreading rapidly. In time, this seemingly inevitable predicament will diminish the effectiveness of the artemisinins, the current mainstay of treatment against drug-resistant parasites.[3] Finally, in addition to their pharmacologic effects on enhanced TXB_2 generation and their reduced cytotoxic effects, it is perhaps of interest to note that derivatives **4**–**8** are more potent inhibitors of rat microglia TXB_2 generation than acetylsalicylic acid (aspirin) (IC_{50} = 3.12–10.0 μM)[17,18] and flurbiprofen (apparent IC_{50} = 100 nM),[19] which are two clinically used NSAIDS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We are grateful to Dr. J. Marrero for the initial characterization of (–)-DINCA (**3**) and L. Gómez for technical assistance during the Ugi reactions. Mass spectral determinations were provided by the Mass Spectrometry Laboratory of the University of Illinois at Urbana–Champaign. Financial support to E. Avilés was provided by the PRLSAMP-BDP, UPR-RISE, GK-12, and Eli Lilly del Caribe Foundation fellowships. A.M.S.M. thanks the Office of Research and Sponsored Programs at Midwestern University for a CCOM Student Summer Research Award to support K.C. and Mary L. Hall for expert technical assistance with the O_2^{-}, TXB_2 and LDH assays. This research was supported by the NIH Grant 1SC1GM086271-01A1 awarded to A. D. Rodríguez.

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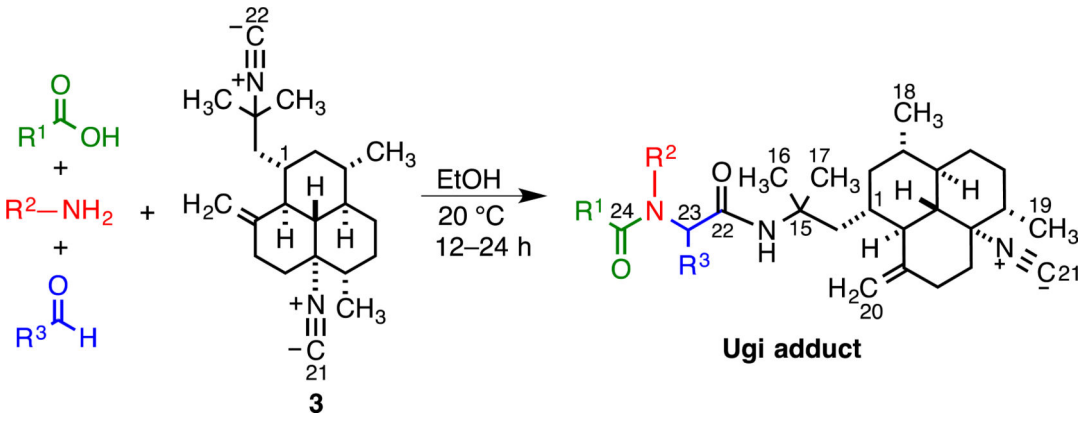
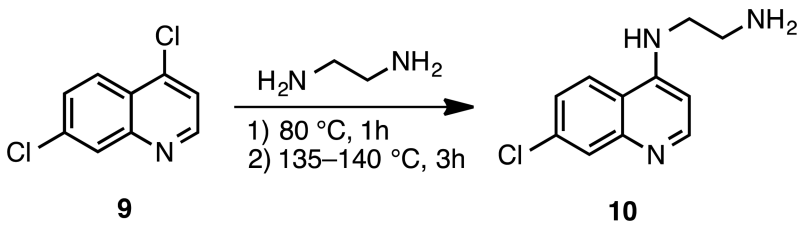


Scheme 1.
Coupling of the four components utilized in the U-4CC reactions.



9 **10**

Scheme 2.
Synthesis of quinoline-containing amine **10**.

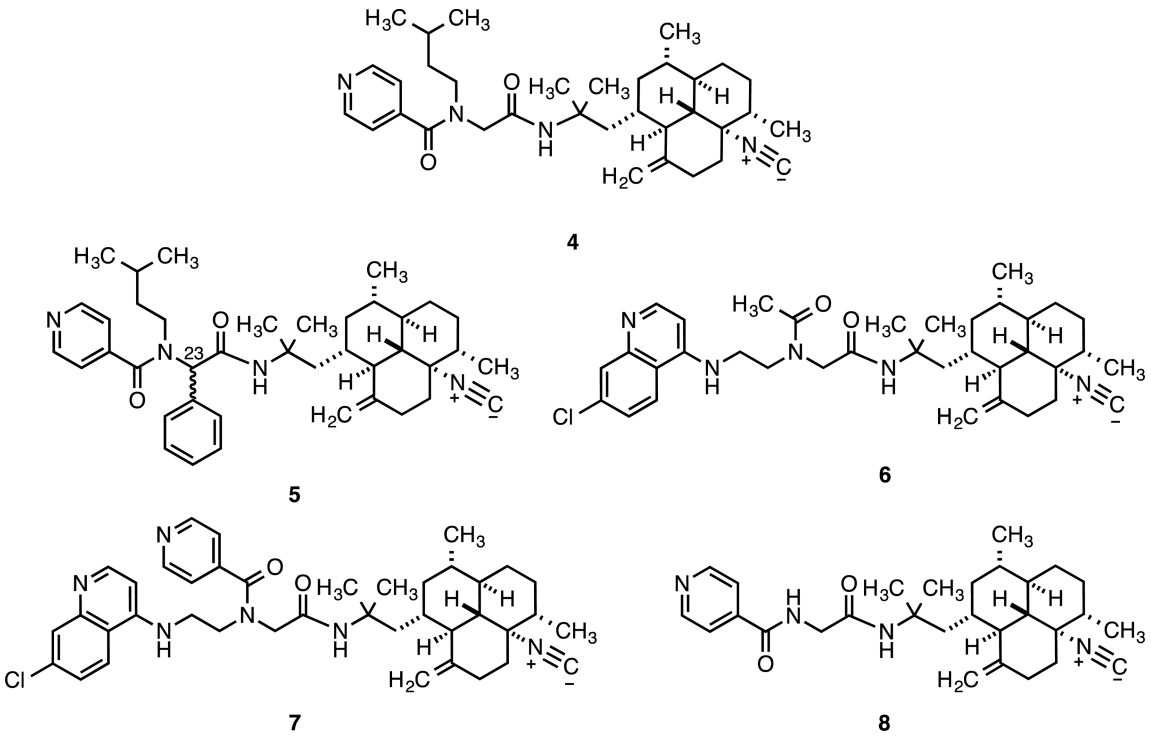


Fig. 1.

Structural formulas of congeners **4**–**8** synthesized by U-4CC reactions with (–)-DINCA (**3**).

Table 1

Building blocks for the Ugi multicomponent based library and isolated yields

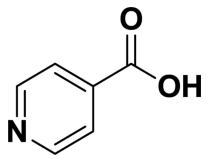
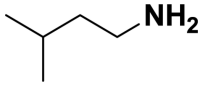
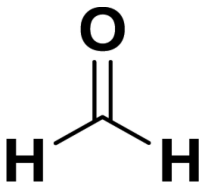
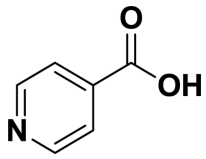
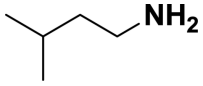
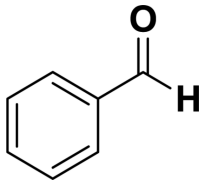
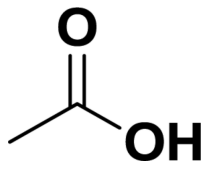
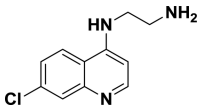
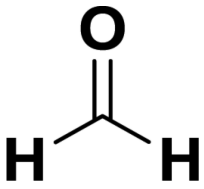
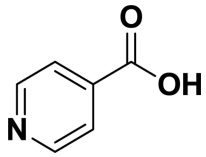
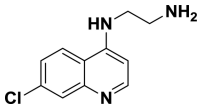
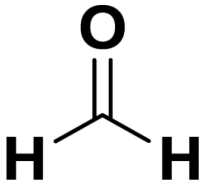
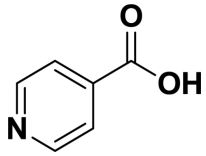
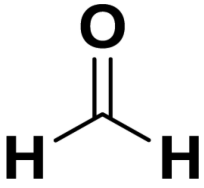
Product	Carboxylic acid (r^1)	Amine (r^2)	Aldehyde (r^3)	Yield (%)
4				61
5				53
6				71
7				67
8		NH_3		42

Table 2

In vitro antimycobacterial and antiplasmodial activities of compounds **3–8**

Compound	MABA MIC (μg/mL)[a]	3D7 IC_{50} ± SE (nM)[b]	Cytotoxicity IC_{50} (nM)	Selectivity Index[c] (SI)
3	3.2	1.2 ± 0.00004	99735	83112
4	14.9	11.5 ± 0.0027	19788	1721
5[d]	56.4	13.0 ± 0.0034	49173	3782
6	17.9	11.1 ± 0.0006	28560	2573
7	52.0	3.6 ± 0.0003	25753	7154
8	101.8	6.3 ± 0.0005	NT	–
CQ	–	6.6 ± 0.0008	234470[e]	35526
INH	0.44	–	–	–

[a]Values are means of three experiments. Minimum inhibitory concentrations (MIC) in μg/mL.

[b]The IC_{50} values are reported as means ± standard errors.

[c]Selectivity index (SI) defined by the ratio: IC_{50} (in mammalian Vero cell lines)/IC_{50} values of antiparasitic activity against 3D7 cell line.

[d]Tested as a mixture of diastereomers.

[e]Value obtained from reference 16. NT indicates that the compound was not tested due to insufficient material. CQ = chloroquine and INH = isoniazid (+Ctrls).

Table 3

Antineuroinflammatory activity of compounds **3–8**[a]

Compound	O_2^- IC_{50} [μM]	TXB_2 IC_{50} [μM]	LDH_{50}[b] [μM]
3	> 10	0.23	> 10
4	> 10	3.0	> 10
5[c]	> 10	0.1	> 10
6	1.7	0.8	5.0
7	5.0	0.9	5.0
8	> 10	1.1	> 10

[a]Effect of compounds **3–8** on LPS-primed rat microglia PMA [1 μM]-stimulated release of TBX_2 [control release: 2,555 ± 718 pg/mL/70 min] and O_2^- [control release: 12.6 ± 2.4 nanomoles/70 min] and LDH. The antineuroinflammatory assay is described in the Supplementary data. Data correspond to 1–4 independent experiments.

[b]LDH_{50} represents the concentration of the compound that caused 50% release of the total LDH content of microglia cells. LDH was measured as described in the Supplementary data.

[c]Tested as a mixture of diastereomers.