JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Design, Synthesis, and Structure–Activity Relationship Studies of Tryptanthrins As Antitubercular Agents

Jae-Min Hwang,[†] Taegwon Oh,[‡] Takushi Kaneko,[§] Anna M. Upton,[§] Scott G. Franzblau,[⊥] Zhenkun Ma,[§] Sang-Nae Cho,[‡] and Pilho Kim*[,†,∥]

[†]Cancer and Infectious Diseases Therapeutics Research Group, Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea

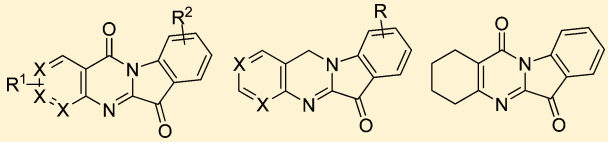
[‡]Department of Microbiology and Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine, Seoul 120-752, Korea

[§]Global Alliance for TB Drug Development, New York, New York 10005, United States

[⊥]Institute for Tuberculosis Research, University of Illinois, Chicago, Illinois 60612, United States

[∥]Department of Medicinal and Pharmaceutical Chemistry, University of Science and Technology, Daejeon 306-350, Korea

ABSTRACT: The natural product tryptanthrin (**1a**) represents a potential lead for new tuberculosis (TB) drugs since tryptanthrin and its synthetic analogues possess potent in vitro activity against *Mycobacterium tuberculosis* (Mtb). However, in spite of their in vitro activity, none of these agents have been shown to be efficacious in vivo against animal models of TB. Described herein are syntheses of new tryptanthrin analogues together with a systematic investigation of their in vitro antitubercular activity and ADME properties followed by pharmacokinetic characterization in rodents for the most promising compounds. Those with the best potency and oral bioavailability were progressed to evaluations of efficacy against acute murine TB. The work aimed to prove the concept that this compound class can limit growth of Mtb during infection as well as to establish the SAR for in vitro activity against Mtb and the range of in vitro ADME parameters for this class of natural products. Novel C-11-deoxy (**5b**) and A-ring-saturated (**6**) tryptanthrin analogues were discovered that maintained activity against Mtb and showed improved solubility compared to tryptanthrin as well as evidence of oral bioavailability in rodents. However, neither **5b** nor **6** demonstrated efficacy against acute murine TB following administration at doses up to 400 mg/kg daily for 4 weeks. Although **5b** and **6** failed to inhibit replication or kill Mtb in vivo, they illuminate a path to new structural variations of the tryptanthrin scaffold that may maximize the potential of this class of compounds against TB.



Tryptanthrin (**1a**) is a natural product containing an indolo[2,1-*b*]quinazoline ring system isolated from the indigo plant *Strobilanthes cusia* Kuntze (Acanthaceae) and its relatives.[1] It is also obtained from *Candida lipolytica* when it is fermented in a tryptophan-rich medium.[2] A number of biological activities have been reported for tryptanthrin or its analogues. These compounds exhibit growth inhibition of *Bacillus subtilis*,[3] permeabilized *Escherichia coli*,[4] methicillin-resistant *Staphylococcus aureus*,[5] dermatophytic fungal pathogens,[1] *Plasmodium falciparum*,[6,7] *Leishmania donovani*,[8] *Trypanosoma brucei*,[9] and *Toxoplasma gondii*.[10] Further, cytostatic or cytotoxic activities have been demonstrated against mammalian tumor cell lines,[11−13] as well as reversal of multidrug resistance in cancer cell lines.[14] Finally, wide-ranging immune-modulatory effects have been observed, including inhibition of Th2 development, IgE-mediated degranulation, and IL-4 production,[15] interferon-γ production,[16] nitric oxide and prostaglandin E2 synthesis,[17] NF-κB,[18] and leukotriene activities,[19] and immune enhancement.[20]

In 1998, Mitscher and Baker reported the antitubercular activity of tryptanthrin (**1a**) and its derivatives.[21] They carried out an extensive analogue development study and identified PA-505 (**2**) and PA-510 (**3**) as the most promising compounds. However, in a subsequent in vivo study little efficacy could be demonstrated in a mouse acute tuberculosis (TB) infection model. The reason for the lack of efficacy was not presented or conjectured on.

TB is a major infectious disease killer, causing an estimated 1.4 million deaths globally in 2010.[22] Although the main burden of TB remains in developing countries, developed countries are not immune to outbreaks of TB.[23] The recent emergence of multidrug resistant (MDR)- and extensively drug resistant (XDR)-TB is of particular concern, posing serious challenges for global TB control and for the treatment of individual patients. Compounding this issue, no novel TB drugs have been developed in the past four decades, leaving healthcare providers with an old and limited TB treatment armamentarium. Therefore, there is a pressing need to develop

Special Issue: Special Issue in Honor of Lester A. Mitscher

Received: October 14, 2012

dx.doi.org/10.1021/np3007167 | *J. Nat. Prod.* XXXX, XXX, XXX−XXX

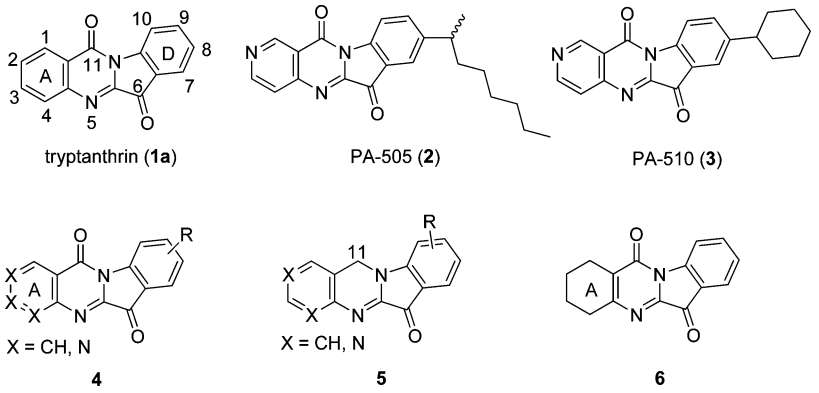


Figure 1. Structures of tryptanthrin (**1a**) and its analogues.

novel TB drugs to address the substantial burden of both drug-sensitive and drug-resistant diseases.

In the field of TB drug development, a key goal is the discovery of compounds that are able to shorten the duration of combination drug therapy required to achieve relapse-free cure of both drug-sensitive and drug-resistant diseases. Current TB treatment comprises 6−9 months of combination therapy for patients with drug-sensitive disease and 18−24 months for patients with drug-resistant TB. There are numerous reports suggesting the opportunity to shorten such regimens through the inclusion of compounds that are active against both replicating and nonreplicating *Mycobacterium tuberculosis* (Mtb).[24] For the past 10 years, the TB drug discovery and development pipeline has been getting stronger.[25] However, tremendous efforts are needed to further strengthen the pipeline. Drugs representative of new chemotypes and with new mechanisms of action are essential to avoid cross-resistance with existing TB drugs and for use in the multidrug combinations that are necessary for the successful treatment of TB.

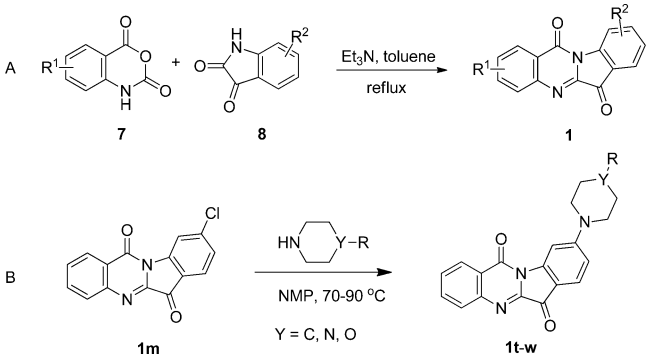
With these circumstances in mind, this work aimed to further investigate the potential of tryptanthrin and its analogues, a novel class with respect to currently used TB drugs, for utility against TB. An exploration of the tryptanthrin scaffold was conducted by repeating some early, published work, in addition to synthesizing and testing new analogues. The goal of this work was demonstration of in vivo efficacy for this class of compounds against murine acute TB. To achieve this, new analogues were designed with the aim of maintaining or improving potency in vitro against Mtb, while also improving physicochemical properties compared to tryptanthin, in particular, solubility. It was anticipated that these improved physicochemical properties would in turn enhance the in vitro ADME profiles and pharmacokinetic (PK) characteristics of these compounds compared to tryptanthrin. In addition, to better understand the bactericidal versus bacteriostatic potential of these compounds, minimum bactericidal activity (MBC) was evaluated for a range of the new analogues.

While the molecular weight (248) and logD (2.29) of tryptanthrin (**1a**) appear reasonable as a starting point, the low solubility of the compound (5.4 μM) and the flatness of the ring system were considered problematic. To potentially address these issues, analogues were designed to incorporate solubilizing groups in ring A or D, A-ring-aza analogues (**4**), C-11-deoxy analogues (**5**), and an A-ring-saturated analogue (**6**) to disrupt planarity of the compounds (Figure 1).

RESULTS AND DISCUSSION

Chemistry. At the outset, several previously reported A-ring- and D-ring-substituted analogues of tryptanthrin (**1a**) were synthesized followed by development of new syntheses of more extensively modified analogues. Tryptanthrins (**1**) were prepared from isatoic anhydride (**7**) and isatin (**8**) by a reference method (Scheme 1A).[11] With this chemistry in hand,

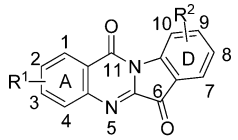
Scheme 1. Synthesis of Tryptanthrins



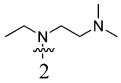
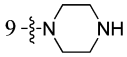
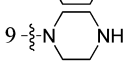
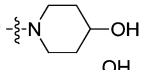
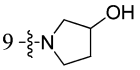
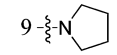
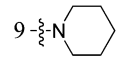
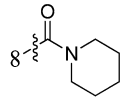
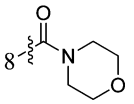
relatively simple A-ring- and/or D-ring-substituted tryptanthrin analogues were prepared from their correspondingly substituted starting materials. Halogen-, nitro-, amido-, ester-, or methoxy-substituted analogues were prepared in this fashion (Table 1). In order to introduce a solubilizing group into tryptanthrin, analogues containing cyclic amines in the D-ring (**1t−w**) were prepared by substituting the chlorine atom of **1m** with appropriate cyclic amines under heating at 70−90 °C in NMP (Scheme 1B).[26] Since Mitscher et al. reported that the A-ring-aza version of tryptanthrins (**4**) was effective in increasing activities as well as demonstrating favorable PK profiles,[26] **4** was also prepared. 4- or 2-Aminonicotinic acid or 3-amino-isonicotinic acid (**9**) was reacted with isatin (**8**) in the presence of HBTU, *N*-methylmorpholine, and DBU in DMF to give the 2- or 4- or 3-aza version of tryptanthrin (**4**), respectively (Scheme 2A).[27] Two approaches were taken to improve solubility compared to tryptanthrin. First, analogues containing a pyridine moiety in place of the A-ring phenyl group were synthesized from **4g** for 4-aza or **4l** for 2-aza analogues and corresponding cyclic amines by a similar method to that described above (Scheme 2B). Second, the phenyl A-ring was replaced with a cyclohexyl ring to alter the flat and aromatic nature of the molecule. This second approach was considered advantageous since the flat aromatic ring system of the

dx.doi.org/10.1021/np3007167 | J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 1. MIC, LORA, and Cytotoxicity of Tryptanthrins (1)



1a-y

compound	substituent		MIC	MBC_{90}	MIC-s[a]	LORA	IC_{50}[b]
	R^1	R^2	(μg/mL)	(μg/mL)	(μg/mL)	(μg/mL)	(μg/mL)
1a	H	H	1	1	1	NA	>8
1b	H	8-NO_2	0.032	0.25	0.5	2.4	28
1c	H	8-CO_2Et	0.5	NA	1	NA[c]	>16
1d	H	8-Cl	0.125	0.125	0.25	>50	>50
1e		8-Cl	16	4	16	NA	32
1f	2-F	8-Cl	0.156	NA	0.3125	>50	23.5
1g	3-F	8-Cl	0.5	0.25	0.5	>50	>50
1h	2-Br	H	0.25	0.5	0.5	>50	>50
1i	H	8-OMe	1	0.5	2	NA	5.5
1j	2-NO_2	H	16	32	32	NA	64
1k	H		0.5	>2	>2	NA	>2
1l	H		8	>16	8	NA	16
1m	H	9-Cl	0.25	0.5	0.5	>50	>50
1n	4-OMe	8-CO_2Et	0.125	1	4	NA	16
1o	H	8-NH_2	8	NA	16	NA	NA
1p	H	8-$NHSO_2$Me	4	2	>8	NA	>8
1q	H	8-Br	0.125	NA	0.5	>50	35
1r	H	8-OCF_3	0.125	NA	0.5	>50	>50
1s	H	8-F	0.125	NA	0.5	>50	>50
1t	H		16	NA	16	NA	31
1u	H		8	NA	8	NA	27
1v	H		IS[d]	NA	IS	IS	IS
1w	H		>128	NA	>128	NA	18
1x	H		0.25	NA	2	>10	>10
1y	H		8	NA	32	>10	>10

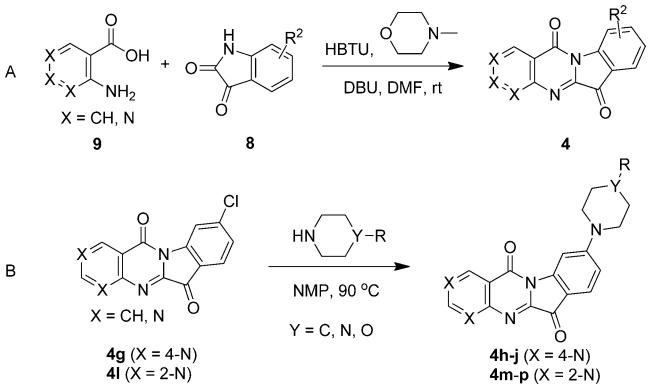
[a]MIC-s: MIC with 40% serum. [b]Cytotoxicity in Vero cells. [c]NA: not available. [d]IS: insoluble.

compound has been postulated to intercalate with DNA, which could result in undesirable cytotoxicity.[27] Thus, C-11-deoxy tryptanthrins (**5**)[28] were synthesized by tandem reduction of tryptanthrins **1** or **4** under $LiAlH_4$ in ether at 40 °C to give an intermediate having both C-6 and C-11 carbonyl groups reduced and oxidation in the presence of MnO_2 in CH_2Cl_2 to reoxidize the C-6-carbinol group (Scheme 3). These C-11-deoxy analogues are of considerable interest because the stereoelectronic properties of the conjugated system has been considerably modified compared with that of the parent compound.[8] In order to construct an A-ring-saturated version of tryptanthrin (**6**), first, A-ring-saturated isatoic anhydride (**11**)[29] was prepared from ethyl 2-oxocyclohexanecarboxylate (**10**) in the presence of ethyl carbamate and $POCl_3$ (Scheme 4). Subsequently, compound **11** was reacted with isatin (**8**) to facilitate **6**. The analogues thus prepared were then examined for in vitro antimycobacterial activity and ADME properties.

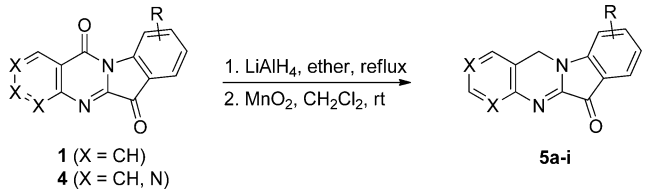
In Vitro Activities (MIC, LORA, Cytotoxicity). The antitubercular activity of each compound against Mtb H37Rv cultured under aerobic conditions was measured using the microplate Alamar Blue assay (MABA).[30] Aerobically grown Mtb culture was exposed to serial dilutions of test compounds for a week in 96-well microplates. The aerobic minimum inhibitory concentration (MIC) was determined by observing the color change of the Alamar Blue dye, which indicates the presence of live bacilli.

dx.doi.org/10.1021/np3007167 | J. Nat. Prod. XXXX, XXX, XXX−XXX

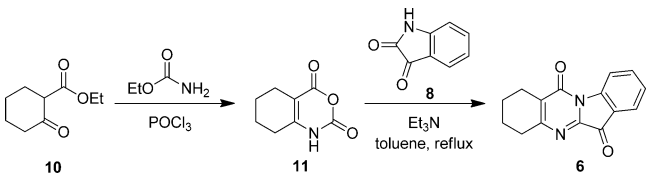
Scheme 2. Synthesis of A-Ring-Aza Tryptanthrins



Scheme 3. Synthesis of C-11-Deoxy Tryptanthrins



Scheme 4. Synthesis of A-Ring-Saturated Tryptanthrin (6)



In addition, the low oxygen recovery assay (LORA)[31] was used to determine the activity of a subset of compounds against low-oxygen-adapted nonreplicating Mtb. In this case, the MIC refers to the concentration of compound that inhibits by ≥90% the ability of low-oxygen-adapted Mtb to recover the ability to express a reporter gene after 10 days exposure to low-oxygen conditions. The LORA is designed to detect compounds that may have the potential for shortening the duration of TB therapy through more efficient killing of the nonreplicating population of bacteria believed to persist in the face of clinical chemotherapy, thus necessitating long durations of treatment. Finally, the cytotoxicity of each compound was measured against the Vero cell line.

The in vitro antitubercular activities of A-ring- and/or D-ring-substituted tryptanthrins are presented along with their cytotoxicities in Table 1. Tryptanthrin (**1a**) has a MABA MIC value of 1 μg/mL. Introduction of a nitro group at the 2-position of tryptanthrin (**1j**) resulted in a less potent compound (MABA MIC: 16 μg/mL), while the corresponding 8-nitro analogue (**1b**) is highly active (MABA MIC: 0.032 μg/mL). On the other hand, 2-bromotryptanthrin (**1h**) exhibits a 4-fold lower MABA MIC than tryptanthrin. Compared with the A-ring-substituted tryptanthrins, D-ring-substituted tryptanthrins are more active. For instance, both 8- and 9-chlorotryptanthrin (**1d** and **1m**) demonstrated 8-fold lower MABA MICs than tryptanthrin. Incorporating an additional substituent on the A-ring while keeping an 8-chloro substituent, such as **1e**–**g**, did not improve potency. Substitution of the C-8 position with bromo (**1q**), OCF_3 (**1r**), and fluoro (**1s**) resulted in MABA MICs as potent as that of 8-Cl tryptanthrin (**1d**).

Introduction of a cyclic amine at the C-9-position to potentially increase solubility (**1t**, **1u**, and **1w**) resulted in MABA MICs far higher than that of tryptanthrin, indicating that such a group was not tolerated for maintenance of in vitro antitubercular activity. The MABA MIC of 9-pyrrolidinetryptanthrin (**1v**) could not be measured due to its low solubility. Introduction of a carboethoxy group at the C-8-position (**1c**) resulted in a compound 2-fold more active than tryptanthrin. Compound **1c** and another carboethoxy-containing analogue (**1n**) are of interest as starting points for the preparation of further derivatives. The potency of **1x**, despite its bulky substituent at the C-8-position, merits special attention. As mentioned above, 8-nitrotryptanthrin (**1b**) has a MABA MIC value of 0.032 μg/mL. This compound also has a LORA MIC value of 2.4 μg/mL, while the majority of analogues lack LORA activity. In general, any electron-withdrawing group at the C-8-position appears to enhance in vitro antitubercular activity. Following consideration of their MABA MIC, LORA MIC, and cytotoxicity IC_{50} values, **1b**–**d**, **1f**–**h**, **1m**, **1q**–**s**, and **1x** (Table 1) were selected for in vitro ADME studies. Tryptanthrin itself (**1a**) was included for the sake of comparison.

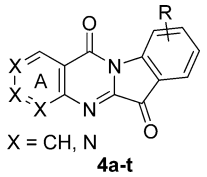
The MABA MIC, LORA MIC, and cytotoxicity IC_{50} values of A-ring-aza tryptanthrins are summarized in Table 2. Introduction of a nitrogen atom at position 2 or 4 as in 2-aza (**4r**) or 4-aza (**4c**) as compared with **1d** indicated that such a substitution is allowed without affecting the MABA MIC (see **1d**). However, their LORA MIC values are far better than that of **1d**. As for the A-ring phenyl analogues, cyclic amines at the C-9-position (**4h**–**j** and **4m**–**p**) produced essentially inactive analogues. Considering their MABA MIC, LORA MIC, and cytotoxicity values (Table 2), **4b** and **4c** were selected for further in vitro ADME studies.

Both C-11-deoxy tryptanthrins (**5**) and A-ring-saturated tryptanthrin (**6**) analogues were designed primarily to reduce the planar characteristics of tryptanthrins to improve solubility and address potential genotoxicity issues related to their planarity. The MABA MIC of C-11-deoxy tryptanthrin (**5c**) is as good as that of tryptanthrin (**1a**), suggesting that the C-11 carbonyl group may not be essential for activity. Moreover, **5c** also has a moderate LORA MIC of 20 μg/mL. However, an A-ring-pyridine in conjunction with a C-11-deoxy modification as in **5d** and **5g** demonstrates reduced potency, with MABA MIC values of 16 and 32 μg/mL, respectively. Compared with 8-chlorotryptanthrin (**1d**), its C-11-deoxy version (**5b**) exhibits a MABA MIC value of 0.13 μg/mL as well as a LORA MIC of 24 μg/mL. However, in the case of 2-aza-8-OCF_3-tryptanthrin (**4a**) and 4-aza-8-chlorotryptanthrin (**4c**), their C-11-deoxy versions (**5f** and **5h**, respectively) are more than 10-fold less active, suggesting that introducing a C-11-deoxy substituent is not universally well-tolerated in terms of activity. Compared with tryptanthrin, A-ring-saturated tryptanthrin has a 2-fold lower MABA MIC value and a moderate LORA MIC value of 10.5 μg/mL. Following consideration of the MABA MIC, LORA MIC, and cytotoxicity IC_{50} values for these compounds, **5b** and **6** (Table 3) were selected for in vitro ADMETox profiling.

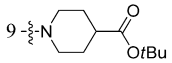
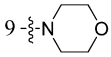
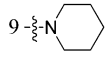
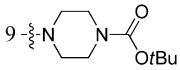
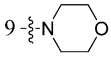
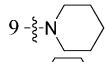
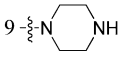
In general, a wide range of in vitro activities against Mtb, as defined by MABA and LORA MIC values, were observed for the compounds synthesized, and these values varied with the structural modifications made to the compounds. As for cytotoxicity, there seems to be no apparent correlation between activity against Mtb and cytotoxicity for compounds included in Tables 1 and 3. However, there may be a closer correlation

dx.doi.org/10.1021/np3007167 | J. Nat. Prod. XXXX, XXX, XXX–XXX

Table 2. MIC, LORA, and Cytotoxicity of A-Ring-Aza Tryptanthrins (4)



4a-t

compound	substituent X	substituent R	MIC (μg/mL)	MBC_{90} (μg/mL)	MIC-s[a] (μg/mL)	LORA (μg/mL)	IC_{50}[b] (μg/mL)
4a	2-N	8-OCF_3	0.063	0.125	0.5	0.64	5.7
4b	2-N	8-Br	1.0	0.25	0.5	>50	>50
4c	4-N	8-Cl	0.125	0.25	0.125	1.4	5.4
4d	3-N	8-OCF_3	0.125	NA	0.25	1.5	5.4
4e	4-N	8-NO_2	0.5	4	4	NA[c]	<0.2
4f	4-N	8-F	1	1	2	NA	0.3
4g	4-N	9-Cl	1	NA	2	NA	7
4h	4-N		IS[d]	NA	IS	IS	IS
4i	4-N		IS	NA	IS	IS	IS
4j	4-N		4	NA	16	NA	81
4k	4-N	8-Br	16	NA	32	NA	>20
4l	2-N	9-Cl	0.125	NA	1	NA	9
4m	2-N		16	NA	>32	NA	24
4n	2-N		32	NA	>32	NA	>20
4o	2-N		16	NA	>32	NA	20
4p	2-N		>4	NA	>4	NA	11
4q	2-N	H	0.5	NA	0.25	1.6	5.3
4r	2-N	8-Cl	0.25	NA	0.25	0.68	8.5
4s	4-N	H	4	NA	8	23	24
4t	4-N	8-OCF_3	0.25	NA	0.5	2.5	7.6

[a]MIC-s: MIC with 40% serum. [b]Cytotoxicity in Vero cells. [c]NA: not available. [d]IS: insoluble.

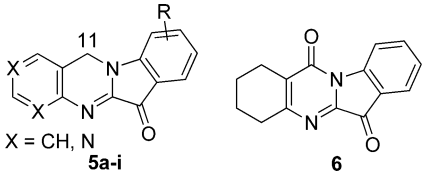
between those two activities among the aza analogues (see Table 2). To further explore the in vitro activities of this group of compounds against Mtb, the ability to kill Mtb, as opposed to inhibit bacterial growth, was determined for a subset of analogues selected based on structural diversity and potency. The ability to kill Mtb is represented by the minimal bactericidal concentration value, which is defined as the minimal concentration of an analogue that reduces the number of colony forming units (CFU) by 90% compared with those growing on analogue-free medium. These MBC values, presented in Tables 1 and 2, indicate that the analogues are in general bactericidal. In addition, to assess the impact of the addition of physiological levels of protein to the MABA MICs of this series, MABA MICs were assessed in the presence of 40% serum for a subset of structurally diverse analogues (Tables 1−3). These data indicate that the addition of supplemental serum results in a modest increase in MABA MIC against Mtb for the compounds tested.

In Vitro ADME. Since a goal of this work was to discover tryptanthrin analogues with improved PK characteristics compared to previously described compounds, in particular by improving their solubility compared to tryptanthrin (**1a**), aqueous solubility was assessed for the 15 more potent and selective analogues selected for progression, along with tryptanthrin (**1a**) for comparison (Table 4). In addition, in vitro permeability, plasma protein binding, metabolic stability, and lipophilicity were evaluated for a subset of these compounds (Table 4).

Aqueous solubility was evaluated in three matrixes: buffer at pH 7.4, and simulated gastric and intestinal fluids (SGF and SIF) with a view to predicting the potential for solubility and absorption in the upper and lower gastrointestinal tract. In buffer at pH 7.4, the solubility of the analogues ranges from <1 μM (below the limit of quantitation) to 64 μM, while solubility in SIF (pH 7.6) is generally higher than this, ranging from 10.3 to 151.9 μM, most likely due to the presence of protein in SIF. Solubility in SGF (pH 1.5) is broadly lower than that in SIF (solubility in SGF: <1 to 79.2 μM). The behavior of individual compounds differs from each other; for example, **1b** is more soluble in neutral buffer and in SIF than in SGF, while the opposite is true for the C-11-deoxy compound, **5b**. In the case of 8-bromo derivatives, the 2-aza analogue (**4b**) is more soluble than the non-aza analogue (**1q**), presumably due to increased hydrogen-bonding feasibility.[32] However, in other cases, the difference between the aza and non-aza analogues was not clear (e.g., **4c** and **1d**). Deoxygenation at C-11 appears to increase solubility under acidic conditions, although solubility under neutral conditions is minimal (see **5b**). An A-ring-saturated

dx.doi.org/10.1021/np3007167 | J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 3. MIC, LORA, and Cytotoxicity of C-11-Deoxy (5) and A-Ring-Reduced (6) Tryptanthrins



	substituent					
compound	X	R	MIC (μg/mL)	MIC-s[a] (μg/mL)	LORA (μg/mL)	IC_{50}[b] (μg/mL)
5a	no aza	8-OCF_3	0.125	0.5	NA[c]	1.3
5b	no aza	8-Cl	0.125	0.125	24	40
5c	no aza	H	1	2	20	42
5d	2-N	H	16	NA	>20	>20
5e	2-N	8-Cl	2	NA	8.3	3.7
5f	2-N	8-OCF_3	2	NA	9.0	5.2
5g	4-N	H	32	NA	NA	4.8
5h	4-N	8-Cl	16	NA	NA	>10
5i	4-N	8-OCF_3	16	NA	NA	4.4
6			0.5	1	10.5	8.7

[a]MIC-s: MIC with 40% serum. [b]Cytotoxicity in Vero cells. [c]NA: not available.

analogue (**6**) exhibited increased solubility compared to other compounds prepared under all three conditions, suggesting that reduced planarity may enhance solubility.[33]

Lipophilicity was determined for several analogues and ranged from 1.6 to 3.37; several compounds demonstrated a lower logD value than tryptanthrin (logD 2.37). The in vitro permeability of these compounds varied greatly. This property was assessed across a monolayer of human intestinal epithelial cells (Caco-2) in both apical to basolateral (A to B) and B to A directions and revealed good permeability in both directions for some compounds, notably tryptanthrin (**1a**), **1g**, **1q**–**s**, **1x**, **4c**, **5b**, and **6**, which possess A → B permeability up to 85.4×10^{-6} cm/s and B → A rates up to 52.4×10^{-6} cm/s, with A/B:B/A ratios all exceeding 1. However, other compounds displayed poor permeability in this assay. The favorable permeability of tryptanthrin (**1a**) itself suggests that this is not a key characteristic for improvement in new analogues, but rather it is important to maintain this behavior when designing new derivatives.

A small subset of compounds exhibited solubility in SIF and SGF higher than 10 μM accompanied by superior permeability, suggesting that reasonable absorption may be expected in vivo. These compounds include tryptanthrin (**1a**), **5b**, **6**, and **1x**, with **1s** also coming close to meeting these criteria. To predict in vivo clearance, plasma protein binding and metabolic stability in human liver microsomes were assessed for several compounds. The analogues tested ranged broadly in their metabolic stability, with **4b**, **4c**, **1g**, and **1x** providing examples of highly stable compounds, while tryptanthrin (**1a**), **1c**, and **6** were less stable under the conditions of the assay. Plasma protein binding was high for all analogues tested, ranging from 94.9% to 99%, and the new analogues did not differ significantly from tryptanthrin with respect to this property. It is interesting to note that, based on the assays used in the present study, tryptanthrin possesses an ADME profile suggestive of reasonable absorption with rapid clearance.

TB is treated with combination therapy, in which multiple anti-TB drugs are administered daily to limit resistance development and maximize efficacy. Additionally, TB drugs are often co-administered with anti-retroviral therapies for patients co-infected with Mtb and HIV. Therefore, it is important to understand the potential for drug–drug interactions for any class of compounds with possible use for TB treatment. Toward understanding this aspect of the profile of tryptanthrin and related compounds, a select set of analogues was screened for their capacity to inhibit the CYP isoenzymes 2C9, 2C19, and 3A4 (Table 5). Moderate inhibition of all three enzymes is exhibited by 4-aza-8-chlorotryptanthrin (**4c**), of CYP2C9 by most analogues tested, and of 2C19 by 8-chlorotryptanthrin (**5b**) in addition to **4c**, suggesting only limited potential for this class to cause drug–drug interactions mediated by inhibition of these enzymes. Tryptanthrin itself

Table 4. Aqueous Solubility, in Vitro Absorption, and Metabolic Stability of Selected Tryptanthrins

compound	solubility, μM (buffer at pH 7.4)	solubility, μM (SIF pH 7.6)[a]	solubility, μM (SGF pH 1.5)[b]	logD	metabolic stability at 1 h in HLM (% parent remaining)[c]	plasma protein binding (%)	Caco-2: A → B permeability (10×10^{-6} cm/s)	Caco-2: B → A permeability (10×10^{-6} cm/s)
1a	5.4	12.9	11.7	2.37	15	95.9	60.1	22.5
1b	64.3	151.9	5.1	NA[d]	33	96.9	ND[e]	ND
1c	3.8	11.1	1.5	2.94	0	98.4	4.2	0.8
1d	3.3	16.7	26.8	2.76	ND	ND	ND	ND
1f	BLQ[f]	29.8	BLQ	NA	63	NA	2.2	2.1
1g	1.1	10.3	2.5	NA	91	NA	28.5	12.7
1h	BLQ	49.4	BLQ	1.69	ND	ND	ND	ND
1m	BLQ	20.3	71.9	2.45	27	99	0.4	0.1
1q	2.6	92.9	2.3	NA	59	NA	23.8	7.6
1r	1	31.3	1.5	NA	59	NA	22	5.8
1s	4.9	18.8	8.5	NA	46	NA	66.6	29.9
1x	16.3	27.5	10.2	NA	105	98.9	59.9	26
4b	35.4	34.4	45.0	1.6	96.0	NA	ND	ND
4c	8.0	18.9	6.0	1.77	96	94.9	73.8	44
5b	BLQ	19.3	79.2	3.37	53	98.7	60.8	20.8
6	54.9	73.4	53	NA	18	95.7	85.4	52.4

[a]SIF: simulated intestinal fluid. [b]SGF: simulated gastric fluid. [c]HLM: human liver microsomes. [d]NA: not available. [e]ND: compound not detectable. [f]BLQ: below limit of quantitation.

dx.doi.org/10.1021/np3007167 | J. Nat. Prod. XXXX, XXX, XXX–XXX

Table 5. Recombinant CYP Inhibition by Selected Tryptanthrins

compound	CYP2C9 % inhibition	CYP2C19 % inhibition	CYP3A4 % inhibition
1a	25	9	12
1c	38	10	5
1d	38	13	10
1h	20	10	10
1m	25	4	7
4b	10	16	14
4c	54	67	62
5b	27	31	1

demonstrated a low percentage of inhibition of each isoenzyme (25% or below, at 10 μM),

PK Studies. Due to their potency and selectivity and their possession of ADME profiles that are consistent with or improved compared with tryptanthrin (**1a**), **1x**, **5b**, and **6** were selected for progression to in vivo studies of their PK characteristics. Following iv dosing at 5 mg/kg to rats, **5b** exhibited limited exposure with an $AUC_{0\text{-inf}}$ of 0.93 μg·h/mL with a high volume of distribution and clearance, resulting in an elimination half-life of 1.42 h. Compound **1x** demonstrates a similar profile following iv dosing, with an $AUC_{0\text{-inf}}$ of 1.07 μg·h/mL, a moderate volume of distribution, and fast clearance, with an elimination half-life of 0.88 h. The particularly short half-life of compound **1x** despite its stability in liver microsomes (Table 4) suggests that this compound is subject to other elimination pathways besides liver metabolism. Oral dosing of each compound to rats at 20 mg/kg resulted in low exposures and oral bioavailabilities of only 8% (**5b**) and 3.2% (**1x**) (Table 6). On the other hand, compound **6** exhibited good exposure following iv dosing at 5 mg/kg to rats with an $AUC_{0\text{-inf}}$ of 16.5 μg·h/mL, a lower volume of distribution than the other compounds, and low clearance, with an elimination half-life of 3.98 h. This compound also demonstrated reasonable exposure following oral dosing at 20 mg/kg to rats, with an $AUC_{0\text{-inf}}$ of 19.89 μg·h/mL, resulting in an oral bioavailability of 30.1%. It is notable that **6** is more soluble in pH 7.4 buffer and in SIF than the other two compounds and shows a higher rate of permeability (across a human epithelial cell layer) and slightly lower human plasma protein binding. Compound **6** is also less metabolically stable in human liver microsomes than **5b** or **1x**. While this may appear contradictory to the longer half-life of **6** in vivo, biphasic elimination, with a fast initial elimination phase, followed by a slower elimination phase, would be consistent with the microsomal data and would give rise to an overall longer half-life and greater AUC for **6** than for the other compounds. The apparent lack of correlation between microsomal stability data and half-life for the tested compounds suggests that elimination pathways other than or in addition to hepatic metabolism are significant for this class. Further investigation of the PK behavior of these compounds may aid in design of new derivatives with enhanced oral bioavailability.

The superior oral exposure of **6** compared to **5b** and especially to **1x** was maintained after oral dosing to mice at 40 mg/kg (Table 6). In an attempt to optimize plasma exposure following oral dosing to mice of **5b** and **6**, two formulations of each compound were used and compared. For **5b**, formulation in 0.1% SLS or in 20% DMSO, 20% PEG400, and 60% (30% HP-beta-CD, 2-hydroxypropyl-β-cyclodextrin, in water) resulted in similar exposures and PK parameters (Table 6). For **6**, formulation in 0.5% methylcellulose resulted in somewhat superior exposure to formulation in 30% HP-beta-CD.

Due to the encouraging potency, ADME, and PK profiles of **5b** and **6**, a micro-Ames assay for mutagenic potential was performed for both of these compounds (Table 7). Owing to their limited solubility, precipitates were observed at ≥25 μg/well for **5b** and at ≥75 μg/well for **6**. In addition, cytotoxicity was observed for **5b** at ≥2.5 μg/well and for **6** at ≥25 μg/well in TA100, both with and without metabolic activation. However, neither compound, at any concentration tested, produced an increase in the number of revertant colonies as compared with the vehicle control in either strain with or without metabolic activation. Therefore, both compounds were classified as nonmutagenic under the conditions of the assay.

In Vivo Efficacy Studies. The in vivo efficacy of **5b** and **6** was evaluated in a murine acute TB infection model. Mice were infected with Mtb Erdman strain via inhalation and were given each drug solution daily by gavage for 4 weeks starting on the 10th day postinfection. The numbers of viable bacteria in the lung and spleen were determined at the end of treatment (Table 8). Assuming that the exposure achieved following administration of a single dose at 40 mg/kg (in the PK study described above) was not the maximum achievable exposure, much higher dose levels were selected for use in the efficacy study, up to 400 mg/kg daily. However, even after 4 weeks of treatment at these high dose levels, both analogues failed to reduce the viable Mtb counts measurable in either the lungs or the spleens of the mice. On the other hand, the positive control compound, rifampicin, dosed at 10 mg/kg daily for 4 weeks was able to control the growth of Mtb in the lungs of mice, resulting in lung CFU 1 log lower than in mice receiving vehicle only.

Table 6. PK Parameters Following Administration of Tryptanthrins to Rats and Mice

		oral administration				IV administration				
species	compound	$AUC_{0\text{-inf}}$ (ug·h/mL)	$-t_{max}$ (h)	$-C_{max}$ (μg/mL)	$-t_{1/2}$ (h)	$AUC_{0\text{-inf}}$ (ug·h/mL)	V_d (L/kg)	Cl (L/h/kg)	$t_{1/2}$ (h)	%F
rat	**1x**	0.14	1	0.03	7.34	1.07	6.21	4.86	0.88	3.16
	5b	0.3	2	0.05	3.33	0.93	11.73	5.57	1.42	8
	6	19.89	4.67	2.03	6.29	16.50	1.62	0.34	3.98	30.14
mouse	**1x**	1.301	2	0.112	ND	NA	NA	NA	NA	NA
	5b[a]	1.4	1	0.197	3.08	NA[e]	NA	NA	NA	NA
	5b[b]	0.968	1.5	0.371	ND[f]	NA	NA	NA	NA	NA
	6[c]	5.281	1.5	1.934	4.99	NA	NA	NA	NA	NA
	6[d]	2.571	0.5	2.258	ND	NA	NA	NA	NA	NA

[a]Formulated in 0.1% SLS. [b]Formulated in 20% DMSO, 20% PEG400, 60% (30% HP-beta-CD in water). [c]Formulated in 0.5% methylcellulose. [d]Formulated in 30% HP-beta-CD. [e]NA: not available. [f]ND: not determined because insufficient data were available.

dx.doi.org/10.1021/np3007167 | J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 7. Mutagenic Potential of 5b and 6

without S9 activation				with S9 activation			
	concentration (μg/well)	TA 98[a]	TA 100[a]		concentration (μg/well)	TA 98[a]	TA 100[a]
DMSO	3.5 μL	1	7	DMSO	3.5 μL	1	7
positive control (−S9) NQNO[e]	5.0×10^{-4}	1	8	positive control (+S9) 2-AA[f]	1.0×10^{-3}	2	9
	5.0×10^{-3}	4	16[g]		1.0×10^{-2}	9[g]	14[g]
	5.0×10^{-2}	42[g]	>60[g]		1.0×10^{-1}	>60[g]	>60[g]
	5.0×10^{-1}	>60[g]	>60[g]		1.0	>60[g]	>60[g]
5b (−S9)	0.25	2	8	**5b** (+S9)	0.25	1	8
	2.5	1	2[b]		2.5	2	0[b]
	12.5	1	1[b]		12.5	1	0[b]
	25[c]	1	0[b]		25[c]	1	0[b]
	75[c]	1	1[b]		75[c]	1	1[b]
	250[c]	1	2[b]		250[c]	1	1[b]
6 (−S9)	0.25	2	7	**6** (+S9)	0.25	3	6
	2.5	2	7		2.5	2	7
	12.5	4	4		12.5	3	7
	25	2	–[d]		25	2	–[d]
	75[c]	1	–[d]		75[c]	1	–[d]
	250[c]	1	–[d]		250[c]	2	–[d]

[a]Mean revertant colonies from duplicate wells (for DMSO controls, means are from 12 wells). [b]Cytotoxicity (reduction in the mean number of revertant colonies). [c]Precipitates present. [d]Wells not able to be counted due to a reduced background lawn. [e]NQNO: 4-nitroquinoline-*N*-oxide. [f]2-AA: 2-aminoanthracene. [g]Greater than 6 revertant colonies and an increase over vehicle mean by 2-fold for TA100 and 3-fold for TA98.

Table 8. In Vivo Short-Term Efficacy of Tryptanthrin Analogues[a]

group (mg/kg/day)	$\log_{10}$ CFU/lung	$\log_{10}$ CFU/spleen
vehicle	6.49 ± 0.10	4.79 ± 0.21
rifampin (10)	5.75 ± 0.16	4.53 ± 0.07
5b (200)	6.58 ± 0.27	4.85 ± 0.15
5b (400)	6.64 ± 0.13	4.89 ± 0.19
6 (200)	6.50 ± 0.27	4.88 ± 0.29
6 (400)	6.60 ± 0.23	4.89 ± 0.14

[a]The resulting values are based on the experimental group of five mice and are presented as means of $\log_{10}$ CFU ± standard deviation per group.

It is challenging to explain the discrepancy between the in vitro antitubercular activity of compounds **5b** and **6** and their in vivo efficacy data. The laboratory strain of Mtb used for in vitro MIC determinations (H37Rv) differed from that used for the in vivo efficacy study (Erdman), and this may provide a partial explanation.

More probably, the limit of absorption of these analogues may be much lower than was anticipated; it is possible that most of the high dose administered during the efficacy study was not in fact absorbed, resulting in subefficacious exposure. Since the pharmacodynamics driver of efficacy for this class is unknown, a critical length of time or magnitude of AUC or C_{max} above MIC may not have been achieved. It is also possible that the PK of these compounds is affected by TB infection (in the mice used in the efficacy study) or that the compounds distributed poorly to the lungs and spleens of the infected mice; further efficacy studies that include evaluation of plasma and tissue concentrations of each compound over the course of treatment may allow identification of one or more of these limitations that later analogue development work may seek to improve upon.

The PK properties of this class are not yet well understood. Rat PK data for tryptanthrin itself (**1a**), following oral dosing, were published very recently and indicated not unexpected PK behavior.[34] Specifically, after an oral dose of 56 mg/kg, C_{max} values of of 2.62 g/mL (in male rats) and 1.89 g/mL (in female rats) were achieved with $t_{1/2}$ values of 5.60 h (in male rats) and 4.35 h (female rats). The corresponding values in Table 6 indicate that the C_{max} values achieved following oral dosing of 20 mg/kg **5b** and **1x** to rats were low compared with that of tryptanthrin (**1a**), even taking the lower dose administered during the present studies into account. Although the MABA MIC values for **5b** and **1x** are similar and significantly lower than the MABA MIC of tryptanthrin, respectively (Tables 1 and 3), it appears unlikely, based on the data in hand, that **5b** and **1x** possess superior PK properties in conjunction with superior potency against Mtb and therefore greater potential for efficacy against murine TB than tryptanthrin itself.

On the other hand, oral dosing of 20 mg/kg of **6** to rats resulted in a C_{max} comparable to that achieved in the recently published study following oral administration of a tryptanthrin dose almost 3-fold higher; these limited data are suggestive of improved bioavailability of **6** compared to tryptanthrin. Given that the MABA MICs for tryptanthrin and **6** are similar (Tables 1 and 3), **6** appears more likely to be efficacious against murine TB than tryptanthrin. However, proof of the concept and understanding of the basal requirements for efficacy against murine TB remain to be demonstrated for this class of compounds, and the data in hand are insufficient to explain the lack of efficacy demonstrated by **6**, in particular.

Conclusion. More than 50 analogues of tryptanthrin (**1a**) were synthesized and tested for their activity against Mtb. Electron-withdrawing substituents in ring A or D enhanced antitubercular activity, while introduction of a cyclic amine moiety in ring D resulted in generally poor antitubercular activity. A-Ring-aza-modifications appear to produce slightly more cytotoxic analogues than their non-aza counterparts. In handling these analogues, however, it was discovered that many of them have extremely limited aqueous solubility, with some possessing lower aqueous solubility than tryptanthrin (Table 4). For selected analogues, in vitro ADME properties were examined and their PK properties were evaluated in mice and

dx.doi.org/10.1021/np3007167 | J. Nat. Prod. XXXX, XXX, XXX−XXX

rats. Compound **6** exhibited MICs of 0.5 μg/mL (MABA) and 11.5 μg/mL (LORA) and an oral bioavailability of 30% in rats, the highest among the analogues evaluated. Compound **5b** demonstrated MICs of 0.14 μg/mL (MABA) and 24.0 μg/mL (LORA) and an oral bioavailability of 8% in the rat. Both compounds failed to demonstrate efficacy against acute murine TB at doses up to 400 mg/kg daily, given orally for 4 weeks. The exact cause of this gap between the in vitro and in vivo activities is not yet known but may be due to insufficient plasma and/or target tissue concentrations. Despite the lack of efficacy of the compounds tested, we have shown that it is possible to synthesize compounds in this series with superior potency against Mtb than tryptanthrin (**1a**) and with a range of ADME and PK profiles compared with tryptanthrin. Further, it has been shown for the first time that ring A can be saturated or the C-11 carbonyl can be converted to a methylene group while retaining antitubercular activity. This may shed light on the possible mode of action of this class of compounds against Mtb since the stereoelectronic properties of the molecules were altered considerably. Against permeabilized *E. coli*, the tryptanthrin analogues were mainly bacteriostatic at 6−40 μM, although cell viability decreased with prolonged exposure.[4] In spite of the numerous biological activities reported as mentioned earlier, a careful review of the published data indicates there are only a handful of cases where in vivo efficacy was observed when a tryptanthrin analogue was given orally.[11,15]

EXPERIMENTAL SECTION

MIC Determinations. The minimal inhibitory concentration of each compound against Mtb H37Rv was determined by the microplate Alamar Blue assay.[35] Mtb was grown in 10 mL of Middlebrook 7H9 broth (Difco) supplemented with 0.2% (v/v) glycerol (Sigma), 1.0 g of Casitone (Difco) per liter, 10% (v/v) OADC (oleic acid, albumin, dextrose, catalase; Difco), and 0.05% (v/v) Tween 80 (Sigma), until its optical density at 600 nm reached 0.4. Initial compound solutions were prepared in DMSO (Sigma), and 2-fold serial dilutions were made in 7H9 broth in microplates. The culture was diluted 1:50 in 7H9 and was inoculated to yield 2×10^5 CFU/mL in plate wells. The plates containing diluted compounds and Mtb inoculum were incubated at 37 °C for 7 days, and then 20 μL of Alamar Blue solution (Serotec) was added to each well. A color change from blue to pink was observed after 24 h incubation, and the MIC was defined as the lowest concentration of compounds that inhibited a color change.

Serum Shift MIC. The human serum (PAA Laboratories) was added to Middlebrook 7H9 medium to make 40% serum 7H9 broth. The MIC at 40% serum was determined by the same method as above except that the incubation period was 14 days. The minimal bactericidal concentration was determined by the agar dilution susceptibility test. Twofold serial dilutions of test compounds were made in Middlebrook 7H10 agar medium (Difco) in plastic quadrant Petri dishes, and separate 10^{-2} and 10^{-4} dilutions of the frozen Mtb H37Rv stock of known CFU were prepared. The diluted stocks were inoculated onto the control quadrants and onto each of the compound-containing quadrants. The plates were incubated at 37 °C for 3 weeks; then the colonies were counted and the MBCs were determined.

In Vitro Activity against Nonreplicating Mtb. MIC values against nonreplicating Mtb, under low oxygen conditions, were measured using the low oxygen recovery assay.[31] Briefly, Mtb H37Rv transfected with the plasmid pFCA-luxAB was adapted to low oxygen through gradual, monitored, self-depletion of oxygen during culture in a sealed flask with slow stirring, then exposed for 10 days to test compounds in 96-well microplates that were maintained under an anaerobic environment using an Anoxomat system, thus precluding growth. Mtb viability was then evaluated via determination of the ability to produce a luminescent signal after a 28 h "recovery" in ambient oxygen and exposure to the *n*-decanal (luciferase) substrate. The LORA MIC is defined as the lowest concentration of compound that reduces luminescence by 90% compared to untreated controls.

In Vitro ADMETox Assays. *a. Aqueous Solubility.* Aqueous solubility was measured for each compound,[36] in phosphate buffered saline (PBS, pH 7.4), simulated intestinal fluid (35 mM NaCl, 85 mM HCl, 2000 units/mL pepsin), and simulated gastric fluid (50 mM KH_2PO_4, 38 mM NaOH, 10 mg/mL pancreatin, pH 7.5). Briefly, each analogue was incubated at a final concentration of 200 μM in 2% DMSO plus the appropriate aqueous medium, with shaking, for 24 h at room temperature. Compound detection was performed via HPLC with photodiode array detection where the detection wavelength was 230 nm. All tests were performed in duplicate, and mean values are presented.

b. In Vitro Absorption. In vitro absorption or transport across a human intestinal epithelial cell line (TC7, a subclone of Caco-2, CEREP) monolayer was determined as previously described.[37,38] In brief, to determine permeability in the apical (A) to basolateral (B) direction, each analogue was provided to the donor (A) side of the monolayer at 10 μM in 1% DMSO in Hank's balanced salt solution (Invitrogen) plus 5 mM 2-(*N*-morpholino)ethanesulfonic acid (Sigma) (HBSS-MES) at pH 6.5; the receiver (B) compartment was maintained at pH 7.4. Samples were taken from the A and B compartments at the start of the assay and from the B compartment following incubation with shaking at 37 °C for 1 h. Sample analysis was via HPLC-MS/MS. For measurements of permeability in the B to A direction each analogue was provided to the B compartment at 10 μM in 1% DMSO in HBSS plus 2-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES, Sigma) pH 7.4; the A compartment was maintained at pH 6.5. Samples were taken from the A and B compartments at the start of the assay and from the A compartment following incubation with shaking at 37 °C for 40 min. Sample analysis was via HPLC-MS/MS. Measurements were made in duplicate, and mean values are presented.

c. LogD. The partition coefficient between *n*-octanol and phosphate-buffered saline pH 7.4 was measured for each analogue[39] following 1 h incubation with shaking at room temperature. Analysis was by HPLC with photodiode assay detection. LogD was calculated as the $\log_{10}$ of the amount of compound in the organic phase divided by the amount of compound in the aqueous phase.

d. Metabolic Stability in Human Liver Microsomes. Metabolic stability in human liver microsomes was determined as described previously.[40] Each analogue was incubated at 1 μM with pooled, mixed gender human liver microsomes that contained 0.3 mg/mL protein (Xenotech), 1 mM NADP (Sigma), 5 mM D-glucose-6-phosphate (Sigma), 1 U/mL glucose-6-phosphate dehydrogenase (Sigma), 0.6% methanol, and 0.6% acetonitrile in phosphate buffer pH 7.4 for 1 h at 37 °C. The parent compound concentration was determined at the start and end of the incubation period by HPLC-MS/MS. Measurements were performed in duplicate, and mean values are presented.

e. Inhibition of CYP Isoenzymes 2C9, 2C19, and 3A4. Inhibition of human recombinant cytochrome P450 (CYP) isoenzymes 2C19, 2C19, and 3A4 by each analogue was determined.[41−43] Specifically, each analogue, at 10 μM, was incubated at 37 °C with either CYP2C9 for 80 min (15 pmol/mL, BD Biosciences), CYP 2C19 for 60 min (10 pmol/mL, BD Biosciences), or CYP3A4 for 30 min (2.5 pmol/mL, Invitrogen) in 1.3 mM NADP (Sigma), 3.3 mM D-glucose-6-phosphate (Sigma), 0.4 U/mL glucose-6-phosphate dehydrogenase (Sigma), and 0.4 mg/mL bovine serum albumin. Probe substrates included in the assays were 50 μM 7-methoxy-4-trifluoromethylcoumarin (Sigma) for CYP2C9; 25 μM 3-cyano-7-ethoxycoumarin (Molecular Probes) for CYP2C19, and 50 μM 7-benzyloxy-4-(trifluoromethyl)coumarin (Discovery Labware) for CYP3A4. Following the incubation period, fluorimetric quantitation of the following metabolites was performed and % inhibition of each CYP by each test analogue calculated: 7-hydroxy-4-trifluoromethylcoumarin (Sigma) for CYP2C9 and CYP3A4; 3-cyano-7-hydroxycoumarin (Molecular Probes) for CYP2C19.

dx.doi.org/10.1021/np3007167 | J. Nat. Prod. XXXX, XXX, XXX−XXX

f. MicroAmes Reverse Mutation Assay. A MicroAmes assay was performed for each compound to determine their potential to induce a positive response in a full Ames test. Two Ames *Salmonella* tester strains were used: TA98, which detects frameshift mutations, and TA100, which detects base pair substitutions. Briefly, each tester strain was preincubated both with and without an Aroclor-induced rat liver S9 fraction in duplicate in multiwell plates with six concentrations (0.25−250 μg equivalents/well) of each compound. Four concentrations of each positive control mutagen (in duplicate) and six replicates of vehicle controls (DMSO) were also used. The positive control without S9 was 4-nitroquinoline-*N*-oxide. The positive control with S9 was 2-aminoanthracene. Following preincubation, treated bacteria were added to the agar wells and incubated. Cultures were examined for signs of cytotoxicity (decreased spontaneous revertant colonies and/or background lawn), compound precipitation, and number of mutant colonies. A positive response was defined as the appearance of at least 2-fold revertant colonies for TA100 and at least 3-fold for TA98 over the control revertant colonies, where there were at least six colonies per well. Concentration−response relationships were also taken into account.

g. Plasma Protein Binding. The proportion of each analogue bound to human plasma proteins was determined via equilibrium dialysis as described previously.[44] In brief, each analogue was incubated at 10 μM in 1% DMSO for at least 8 h at 37 °C in human plasma (Bioreclamation) on the sample side of a 12−14 K M_w cutoff dialysis membrane, with 0.05 M phosphate buffer pH 7.5 at the dialysate side. Following incubation, sample analysis was via HPLC with photodiode assay detection at 230 nm. Measurements were made in duplicate, and means are presented.

PK Studies in Rats. Plasma exposure to each analogue was evaluated following both intravenous (iv) and oral (po) administration of single doses of each formulated compound to male Sprague−Dawley rats (n = 3). For iv administration, compounds were prepared as solutions in polyethyleneglycol 300/DMSO (50:50, v/v) and administered to achieve a single 5 mg/kg body weight dose. For po administration, compounds were prepared as suspensions or solutions in 0.5% carboxymethylcellulose/Tween 80, and a single 20 mg/kg body weight dose was delivered via oral gavage. Blood was collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, and 24 h (iv) and at 0.25, 0.5, 1, 1.5, 2, 4, 8, and 24 h (po) and processed to plasma. Bioanalysis was performed via LC-MS, and PK analyses of the plasma concentration−time relationships for the compounds were performed using PK Solutions (Summit PK). A noncompartmental model was used to calculate PK parameters, which are reported in Table 5 as means.

PK Studies in Mice. Plasma levels of each analogue were measured following po administration of single doses of each formulated compound to female C57BL/6 mice, with three mice used per compound per time point. Compounds were prepared in 0.1% sodium lauryl sulfate and in 20% DMSO, 20% polyethylene glycol 400 (PEG400), and 60% (30% HP-beta-CD) (for **5b**); in 0.5% methyl cellulose and in 30% HP-beta-CD (for **6**); or in 0.5% methyl cellulose only (for **1x**) and administered via oral gavage to achieve a single dose of 40 mg/kg body weight. Blood was collected at 30 min and 1, 1.5, 2, 4, 8, and 24 h and processed to plasma. Bioanalysis was performed via LC-MS, and PK analyses of the plasma concentration−time relationships for the compounds were performed using PK Solutions (Summit PK). A noncompartmental model was used to calculate PK parameters, which are reported in Table 5 as means.

In Vivo Efficacy Determination. *a. Mice.* Specific pathogen-free female C57BL/6 mice at 5−6 weeks of age were purchased from Japan SLC, Inc. (Shijuoka, Japan), maintained under barrier conditions in a BL-3 biohazard animal room at Yonsei University Medical Research Center, and fed a sterile commercial mouse diet and water ad libitum.

b. Bacteria. Mtb Erdman (ATCC 35801) was used and grown for about 10 days at 37 °C as a surface pellicle on Sauton medium enriched with 0.4% sodium glutamate (Sigma) and 3.0% glycerin (Sigma). The surface pellicles were collected and disrupted with 6 mm glass beads by gentle vortexing. After the clumps settled, the upper suspension was collected and aliquots were stored at −70 °C until used. After thawing, viable organisms were then counted by plating serial dilutions on the Middlebrook 7H11 agar (Difco). For inoculation of Mtb into mice, bacterial suspensions were sonicated briefly in a sonic bath and diluted with PBS to reach the desired numbers.

c. Mtb Infection and Bacterial Counts. Mice were challenged by aerosol exposure with Mtb H37Rv using an inhalation device (Glas-Col, Terre Haute, IN, USA) calibrated to deliver about 300 bacteria into the lungs. Five mice were sacrificed for bacterial count the next day to count initial infection dose per organ. Drug treatment started on the 10th day postinfection. Compound **6** was suspended in 30% HP-beta-CD in sterile water, and **5b** was suspended in DMSO (Sigma)/PEG400 (polyethylene glycol 400, Sigma)/HP-beta-CD (30% in sterile water) (2:2:6) solution. Rifampin (Sigma) was dissolved in sterile water. The drug was given to mice daily by gavage 5 days a week for 4 weeks. The mice of 400 mg/kg/day dose groups were given a 200 mg/kg dose twice daily. Mice were sacrificed for bacterial count at the end of a 4-week drug treatment. The numbers of viable bacteria in the lung and spleen were determined by plating serial dilution of whole organ homogenates on Middlebrook 7H11 agar. Colonies were counted after 3−4 weeks of incubation at 37 °C.

Chemistry. General Procedure for the Synthesis of Tryptanthrins (1). A solution of isatoic anhydride (1 mmol), isatin (1 mmol), and Et_3N (5 mmol) in toluene (5 mL) was refluxed for 5−6 h. The reaction mixture was quenched with H_2O and then extracted with EtOAc (2×). The organic layers were dried with $MgSO_4$, filtered, and concentrated. The reaction mixture was purified via column chromatography (eluted with EtOAc/hexanes) to give tryptanthrins.

Indolo[2,1-*b*]quinazoline-6,12-dione (1a): yellow solid (83%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.47 (1H, t, J = 7.4 Hz), 7.70−7.76 (1H, m), 7.83−7.89 (2H, m), 7.94 (2H, d, J = 3.6 Hz), 8.31 (1H, d, J = 7.8 Hz), 8.47 (1H, d, J = 7.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 117.9, 121.9, 123.7, 125.3, 127.1, 127.5, 130.2, 130.7, 135.1, 138.2, 144.3, 146.3, 146.6, 158.0, 182.5; HRESIMS m/z 248.0583 [M$^+$] (calcd for $C_{15}H_8N_2O_2$, 248.0586).

8-Nitroindolo[2,1-*b*]quinazoline-6,12-dione (1b): yellow solid (79%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76−7.81 (1H, m), 8.01 (2H, d, J = 3.7 Hz), 8.36 (1H, d, J = 7.7 Hz), 8.56 (1H, s), 8.66−8.75 (2H, m); HRESIMS m/z 293.0419 [M$^+$] (calcd for $C_{15}H_7N_3O_4$, 293.0437).

Ethyl 6,12-dioxo-6,12-dihydroindolo[2,1-*b*]quinazoline-8-carboxylate (1c): yellow solid (34%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (3H, t, J = 7.1 Hz), 4.43 (2H, q, J = 7.1 Hz), 7.67−7.73 (1H, m), 7.85−7.90 (1H, m), 8.04 (1H, dd, J = 8.0, 0.6 Hz), 8.44−8.50 (2H, m), 8.58−8.58 (1H, m), 8.71 (1H, dd, J = 8.4, 0.5 Hz); HRESIMS m/z 320.0794 [M$^+$] (calcd for $C_{18}H_{12}N_2O_4$, 320.0797).

8-Chloroindolo[2,1-*b*]quinazoline-6,12-dione (1d): yellow solid (87%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72−7.78 (1H, m), 7.92 (1H, dd, J = 8.5, 2.2 Hz), 7.95−7.97 (3H, m), 8.33 (1H, dt, J = 7.8, 0.9 Hz), 8.47 (1H, d, J = 8.4 Hz); HRESIMS m/z 282.0208 [M$^+$] (calcd for $C_{15}H_7ClN_2O_2$, 282.0196).

8-Chloro-2-((2-(dimethylamino)ethyl)(ethyl)amino)indolo-[2,1-*b*]quinazoline-6,12-dione (1e). To a solution of **1f** (200 mg, 0.670 mmol) and K_2CO_3 (180 mg, 1.33 mmol) in DMF (10 mL) was added *N*,*N*-dimethyl-*N*′-ethylethylenediamine (0.13 mL, 0.80 mmol), and the reaction mixture was refluxed for 14 h. The reaction mixture was quenched with H_2O and then extracted with EtOAc (3×). The organic layers were dried with $MgSO_4$, filtered, and concentrated. The reaction mixture was purified via column chromatography (eluted with 5% MeOH in CH_2Cl_2) to give the title compound as a yellow solid (140 mg, 53%). ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.17 (3H, t, J = 6.9 Hz), 2.23 (6H, s), 2.2−2.47 (2H, m), 3.54−3.59 (4H, m), 7.29 (1H, dd, J = 9.5, 3.9 Hz), 7.38 (1H, d, J = 3.0 Hz), 7.74 (1H, d, J = 9.0 Hz), 7.86−7.90 (2H, m), 8.47 (1H, d, J = 9.2 Hz); LC-MS m/z 397.11 [M + H$^+$].

8-Chloro-2-fluoroindolo[2,1-*b*]quinazoline-6,12-dione (1f): yellow solid (76%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.85 (1H, td, J = 8.6, 2.8 Hz), 7.91−7.97 (2H, m), 8.02−8.08 (2H, m), 8.46 (1H, d, J = 8.6 Hz); HRESIMS m/z 300.0075 [M$^+$] (calcd for $C_{15}H_6ClFN_2O_2$, 300.0102).

dx.doi.org/10.1021/np3007167 | *J. Nat. Prod.* XXXX, XXX, XXX−XXX

8-Chloro-3-fluoroindolo[2,1-*b*]quinazoline-6,12-dione (1g): yellow solid (77%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.59−7.65 (1H, m), 7.83 (1H, dd, J = 7.7, 2.2 Hz), 7.90−8.01 (2H, m), 8.38 (1H, dd, J = 8.9, 6.1 Hz), 8.45 (1H, d, J = 8.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 116.52 (d, J_{C-F} = 22.5 Hz), 118.86 (d, J_{C-F} = 23.0 Hz), 119.18, 120.25, 122.87, 130.12 (d, J_{C-F} = 10.25 Hz), 133.49, 137.87, 144.40, 144.90, 148.78 (d, J_{C-F} = 12.62 Hz), 157.15, 166.71 (d, J_{C-F} = 255.8 Hz), 181.17; HRESIMS m/z 300.0092 [M$^+$] (calcd for $C_{15}H_6ClFN_2O_2$, 300.0102).

2-Bromoindolo[2,1-*b*]quinazoline-6,12-dione (1h): yellow solid (90%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.47−7.52 (1H, m), 7.88 (1H, s), 7.91 (2H, d, J = 2.1 Hz), 8.12 (1H, dd, J = 8.5, 2.3 Hz), 8.40 (1H, d, J = 2.2 Hz), 8.46 (1H, d, J = 7.8 Hz); HRESIMS m/z 325.9694 [M$^+$] (calcd for $C_{15}H_7BrN_2O_2$, 325.9691).

8-Methoxyindolo[2,1-*b*]quinazoline-6,12-dione (1i): red solid (73%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.89 (3H, s), 7.28−7.33 (1H, m), 7.38 (1H, d, J = 2.5 Hz), 7.64−7.69 (1H, m), 7.81−7.86 (1H, m), 8.02 (1H, d, J = 8.2 Hz), 8.41−8.44 (1H, m), 8.52 (1H, d, J = 8.8 Hz); HRESIMS m/z 278.0694 [M$^+$] (calcd for $C_{16}H_{10}N_2O_3$, 278.0691).

2-Nitroindolo[2,1-*b*]quinazoline-6,12-dione (1j): yellow solid (82%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (1H, t, J = 7.5 Hz), 7.88−7.96 (2H, m), 8.18 (1H, d, J = 8.8 Hz), 8.49 (1H, d, J = 7.7 Hz), 8.67 (1H, dd, J = 8.8, 2.7 Hz), 8.96 (1H, d, J = 2.7 Hz); HRESIMS m/z 293.0431 [M$^+$] (calcd for $C_{15}H_7N_3O_4$, 293.0437).

9-(Piperazin-1-yl)indolo[2,1-*b*]quinazoline-6,12-dione (1k). Step 1: To a solution of **1m** (260 mg, 0.920 mmol) in 1-methyl-2-pyrrolidinone (NMP; 5 mL) was added *tert*-butyl-1-piperazine carboxylate (210 mg, 1.11 mmol), and the reaction mixture was heated at 70 °C for 2 h. The reaction mixture was quenched with H_2O and then extracted with EtOAc (3×). The organic layers were dried with $MgSO_4$, filtered, and concentrated. The reaction mixture was purified via column chromatography (eluted with 5% MeOH in CH_2Cl_2) to give a Boc-protected version of the title compound as a yellow solid (320 mg, 80%). Step 2: To a solution of the Boc-protected title compound (300 mg, 0.790 mmol) in CH_2Cl_2 (10 mL) was added TFA (5 mL), and the reaction mixture was stirred at rt for 30 min. The reaction mixture was quenched with $NaHCO_3$ and then extracted with $CHCl_3$ (3×). The organic layers were dried with $MgSO_4$, filtered, and concentrated. The reaction mixture was purified via column chromatography (eluted with 5% MeOH in CH_2Cl_2) to give the title compound as a red solid (160 mg, 69%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.35−3.63 (8H, m), 6.88 (1H, dd, J = 8.9, 2.1 Hz), 7.67 (1H, d, J = 8.8 Hz), 7.91−7.97 (4H, m), 8.28 (1H, d, J = 7.5 Hz); HRESIMS m/z 332.1250 [M$^+$] (calcd for $C_{19}H_{16}N_4O_2$, 332.1273).

9-(Piperazin-1-yl)indolo[2,1-*b*]quinazoline-6,12-dione hydrochloride (1l). To a solution of **1k** (100 mg, 0.300 mmol) in MeOH/CH_2Cl_2 (1:1; 40 mL) was added a 4 M solution of HCl in dioxane (0.11 mL, 0.44 mmol), the reaction mixture was stirred at rt for 3 days, and a precipitate was formed. The solid precipitate was filtered and washed with ether. The title compound was obtained as a red solid (93 mg, 84%): ^{1}H NMR (300 MHz, D_2O) δ 3.20−3.34 (8H, m), 5.99−6.03 (1H, m), 6.71 (1H, s), 6.83 (1H, d, J = 8.9 Hz), 7.37 (3H, m), 7.69 (1H, t, J = 6.9 Hz); LC-MS m/z 333.0 [M + H$^+$].

9-Chloroindolo[2,1-*b*]quinazoline-6,12-dione (1m): brown solid (77%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (1H, dd, J = 8.1, 1.6 Hz), 7.67−7.72 (1H, m), 7.83−7.90 (2H, m), 8.03 (1H, d, J = 8.1 Hz), 8.44 (1H, dd, J = 7.8, 0.9 Hz), 8.69 (1H, d, J = 1.2 Hz); HRESIMS m/z 282.0179 [M$^+$] (calcd for $C_{15}H_7ClN_2O_2$, 282.0196); anal. C 63.73, H 2.50, N 9.91%, calcd for $C_{15}H_7ClN_2O_2$, C 63.67, H 2.48, N 9.77%.

Ethyl 4-methoxy-6,12-dioxo-6,12-dihydroindolo[2,1-*b*]quinazoline-8-carboxylate (1n): yellow solid (33%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (3H, t, J = 7.1 Hz), 4.07 (3H, s), 4.42 (2H, q, J = 7.1 Hz), 7.32 (1H, d, J = 9.2 Hz), 7.63 (1H, t, J = 8.0 Hz), 8.01 (1H, dd, J = 7.9, 1.1 Hz), 8.47 (1H, dd, J = 8.4, 1.7 Hz), 8.57 (1H, d, J = 1.5 Hz), 8.70 (1H, d, J = 8.4 Hz); HRESIMS m/z 350.0903 [M$^+$] (calcd for $C_{19}H_{14}N_2O_5$, 350.0903).

8-Aminoindolo[2,1-*b*]quinazoline-6,12-dione (1o). To a solution of **1b** (1.00 g, 3.41 mmol) in MeOH/THF (4:1; 25 mL) was added ammonium formate (430 mg, 6.82 mmol), and the reaction mixture was degassed under an argon atmosphere. To the reaction mixture was added $Pd(OH)_2$ on carbon (200 mg, 1.70 mmol), and the reaction mixture was stirred at rt for 2 h. The reaction mixture was filtered and then extracted with EtOAc (3×). The organic layers were dried with $MgSO_4$, filtered, and concentrated. The reaction mixture was purified via column chromatography (eluted with 5% MeOH in CH_2Cl_2) to give the title compound as a black solid (540 mg, 60%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.66 (2H, s), 6.96−7.00 (2H, m), 7.67−7.72 (1H, m), 7.88−7.90 (1H, m), 7.90 (1H, d, J = 1.0 Hz), 8.13 (1H, d, J = 9.1 Hz), 8.25−8.28 (1H, m); HRESIMS m/z 263.0693 [M$^+$] (calcd for $C_{15}H_9N_3O_2$, 263.0695).

***N*-(6,12-Dioxo-6,12-dihydroindolo[2,1-*b*]quinazolin-8-yl)-methanesulfonamide (1p).** To a solution of **1o** (540 mg, 2.05 mmol) in MeOH (20 mL) was added Et_3N (430 mg, 6.82 mmol) and MsCl (0.20 mL, 2.5 mmol) at 0 °C. The reaction mixture was stirred at rt for 2 h, quenched with H_2O, and then extracted with EtOAc (3×). The organic layers were dried with $MgSO_4$, filtered, and concentrated. The reaction mixture was purified via column chromatography (eluted with 3% MeOH in CH_2Cl_2) to give the title compound as a gray solid (850 mg, 72%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.58 (3H, s), 7.73−7.78 (1H, m), 7.95−7.97 (2H, m), 8.02 (1H, dd, J = 8.5, 2.3 Hz), 8.13 (1H, d, J = 2.1 Hz), 8.34 (1H, d, J = 7.8 Hz), 8.55 (1H, d, J = 8.6 Hz); HRESIMS m/z 341.0457 [M$^+$] (calcd for $C_{16}H_{11}N_3O_4S$, 341.0470).

8-Bromoindolo[2,1-*b*]quinazoline-6,12-dione (1q): yellow solid (67%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66−7.71 (1H, m), 7.83−7.90 (2H, m), 8.01−8.04 (2H, m), 8.42 (1H, dd, J = 7.8, 1.0 Hz), 8.52 (1H, d, J = 8.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 119.5, 120.7, 123.3, 123.6, 127.6, 128.2, 130.5, 130.9, 135.3, 140.6, 143.7, 144.9, 146.5, 157.9, 181.2; HRESIMS m/z 325.9689 [M$^+$] (calcd for $C_{15}H_7BrN_2O_2$, 325.9691).

8-(Trifluoromethoxy)indolo[2,1-*b*]quinazoline-6,12-dione (1r): yellow solid (67%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60−7.63 (1H, m), 7.66−7.71 (1H, m), 7.75 (1H, s), 7.83−7.89 (1H, m), 8.02 (1H, d, J = 8.0 Hz), 8.42 (1H, dd, J = 7.9, 0.9 Hz), 8.68 (1H, d, J = 8.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 117.6, 119.4, 123.0, 123.5, 127.6, 130.5, 130.6, 130.9, 135.3, 144.0, 144.3, 146.4, 147.7, 157.8, 181.4; HRESIMS m/z 332.0408 [M$^+$] (calcd for $C_{16}H_7F_3N_2O_3$, 332.0409).

8-Fluoroindolo[2,1-*b*]quinazoline-6,12-dione (1s): yellow solid (74%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (1H, dd, J = 8.6, 2.7 Hz), 7.55−7.58 (1H, m), 7.65−7.71 (1H, m), 7.85 (1H, dt, J = 7.6, 1.4 Hz), 8.02 (1H, dd, J = 8.0, 0.5 Hz), 8.42 (1H, dd, J = 7.9, 0.9 Hz), 8.63 (1H, dd, J = 8.8, 4.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 112.0 (d, J_{C-F} = 24.3 Hz), 119.0 (d, J_{C-F} = 7.5 Hz), 123.4 (d, J_{C-F} = 7.6 Hz), 124.8 (d, J_{C-F} = 23.8 Hz), 127.5, 130.5, 130.8, 135.2, 142.4, 144.2, 146.4, 157.8, 161.1 (d, J_{C-F} = 248.8 Hz), 181.7; HRESIMS m/z 266.0493 [M$^+$] (calcd for $C_{15}H_7FN_2O_2$, 266.0492).

9-(4-Hydroxypiperidin-1-yl)indolo[2,1-*b*]quinazoline-6,12-dione (1t). To a solution of **1m** (340 mg, 1.21 mmol) in NMP (5 mL) was added 4-hydroxypiperidine (150 mg, 1.45 mmol) at rt. The reaction mixture was stirred at 90 °C for 2 h. The reaction mixture was concentrated and dissolved in MeOH. To the reaction mixture was added ether to precipitate solids, which were filtered and dried to give the title compound as a red solid (320 mg, 76%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.99−2.02 (2H, m), 2.00−2.08 (2H, m), 3.41−3.50 (2H, m), 3.90−3.98 (2H, m), 4.05−4.10 (1H, m), 6.72 (1H, dd, J = 8.8, 2.4 Hz), 7.63 (1H, t, J = 7.1 Hz), 7.75 (1H, d, J = 8.9 Hz), 7.80−7.85 (1H, m), 8.02 (1H, d, J = 9.4 Hz), 8.12 (1H, d, J = 2.0 Hz), 8.38−8.40 (1H, m); HRESIMS m/z 347.1269 [M$^+$] (calcd for $C_{20}H_{17}N_3O_3$, 347.1270).

9-(3-Hydroxypyrrolidin-1-yl)indolo[2,1-*b*]quinazoline-6,12-dione (1u). Using 3-pyrrolidinol instead of 4-hydroxypiperidine, the procedure for **1t** was adopted to give the title compound as a red solid (72%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.99−2.04 (1H, m), 2.19−2.24 (1H, m), 3.54−3.75 (4H, m), 4.71 (1H, s), 6.38−6.42 (1H, m), 7.58−7.64 (1H, m), 7.72 (2H, d, J = 8.8 Hz), 7.79−7.84 (1H, m), 8.01 (1H, d, J = 7.6 Hz), 8.36 (1H, d, J = 8.5 Hz); HRESIMS m/z 333.1110 [M$^+$] (calcd for $C_{19}H_{15}N_3O_3$, 333.1113).

dx.doi.org/10.1021/np3007167 | J. Nat. Prod. XXXX, XXX, XXX−XXX

9-(Pyrrolidin-1-yl)indolo[2,1-*b*]quinazoline-6,12-dione (1v). Using pyrrolidine instead of 4-hydroxypiperidine, the procedure for **1t** was adopted to give the title compound as a red solid (73%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.10 (4H, s), 3.53 (4H, s), 6.39 (1H, d, J = 8.8 Hz), 7.60 (1H, t, J = 7.1 Hz), 7.70 (2H, d, J = 8.7 Hz), 7.80 (1H, t, J = 7.0 Hz), 7.99 (1H, d, J = 7.7 Hz), 8.36 (1H, d, J = 7.3 Hz); HRESIMS m/z 317.1162 [M^+] (calcd for $C_{19}H_{15}N_3O_2$, 317.1164).

9-(Piperidin-1-yl)indolo[2,1-*b*]quinazoline-6,12-dione (1w). Using piperidine instead of 4-hydroxypiperidine, the procedure for **1t** was adopted to give the title compound as a red solid (71%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.71 (6H, s), 3.57 (4H, s), 6.62 (1H, d, J = 8.6 Hz), 7.59 (1H, t, J = 7.4 Hz), 7.67 (1H, d, J = 8.7 Hz), 7.79 (1H, d, J = 7.4 Hz), 7.99 (2H, d, J = 7.8 Hz), 8.34 (1H, d, J = 7.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.2, 25.5, 48.6, 100.5, 110.2, 110.8, 123.3, 127.1, 127.7, 129.2, 130.1, 134.8, 146.9, 149.1, 156.8, 158.6, 177.7; HRESIMS m/z 331.1304 [M^+] (calcd for $C_{20}H_{17}N_3O_2$, 331.1321).

8-(Piperidine-1-carbonyl)indolo[2,1-*b*]quinazoline-6,12-dione (1x) (ref 45): yellow solid (50%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46−1.70 (6H, m), 3.32−3.75 (4H, m), 7.65−7.71 (1H, m), 7.84−7.71 (3H, m), 8.04 (1H, d, J = 8.0 Hz), 8.44 (1H, d, J = 7.6 Hz), 8.67 (1H, d, J = 8.1 Hz); LC-MS m/z 360.0 [M + H^+].

8-(Morpholine-4-carbonyl)indolo[2,1-*b*]quinazoline-6,12-dione (1y) (ref 45): yellow solid (60%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.63 (8H, s), 7.73−7.78 (1H, m), 7.90−7.94 (2H, m), 7.97 (2H, d, J = 3.6 Hz), 8.34 (1H, d, J = 7.7 Hz), 8.53 (1H, dd, J = 7.9, 0.7 Hz); LC-MS m/z 362.1 [M + H^+].

General Procedure for the Synthesis of A-Ring-Aza Tryptanthrins (4). To a solution of 2-[1*H*-benzotriazole-1-yl]-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU; 1 mmol), *N*-methylmorpholine (1.8 mmol), and 4- or 2-aminonicotinic acid or 3-aminoisonicotinic acid (1 mmol) in DMF (3 mL) was added a solution of isatin (0.9 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 2 mmol) in DMF (3 mL) over 10 min at rt. After 20 h, the reaction mixture was quenched with 1 N citric acid aqueous solution (10 mL). The reaction mixture was filtered, and the filtrate was extracted with $CHCl_3$ (3×). The organic layers were dried with $MgSO_4$, filtered, and concentrated. The reaction mixture was purified via column chromatography (eluted with 5% MeOH in CH_2Cl_2) to give 4- or 2- or 3-aza tryptanthrin, respectively.

8-(Trifluoromethoxy)indolo[2,1-*b*]-2-azaquinazoline-6,12-dione (4a): yellow solid (30%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.92 (2H, d, J = 5.1 Hz), 7.96 (1H, d, J = 8.2 Hz), 8.56 (1H, d, J = 8.6 Hz), 9.03 (1H, d, J = 5.4 Hz), 9.50 (1H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 117.9, 118.0, 118.3, 118.3, 118.7, 121.7, 122.6, 123.6, 130.3, 144.2, 146.5, 148.5, 149.8, 151.9, 154.9, 157.1, 180.7; LC-MS m/z 334.1 [M + H^+].

8-Bromoindolo[2,1-*b*]-2-azaquinazoline-6,12-dione (4b): red solid (55%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.92 (1H, d, J = 5.4 Hz), 8.07−8.13 (2H, m), 8.39 (1H, d, J = 8.8 Hz), 9.02 (1H, dd, J = 5.4, 0.9 Hz), 9.49 (1H, s); HRESIMS m/z 326.9643 [M^+] (calcd for $C_{14}H_6BrN_3O_2$, 326.9629).

8-Chloroindolo[2,1-*b*]-4-azaquinazoline-6,12-dione (4c): yellow solid (59%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (1H, dd, J = 7.9, 4.5 Hz), 7.76 (1H, dd, J = 8.5, 2.2 Hz), 7.89 (1H, d, J = 2.1 Hz), 8.57 (1H, d, J = 8.5 Hz), 8.76 (1H, dd, J = 7.9, 1.9 Hz), 9.11 (1H, dd, J = 4.5, 1.9 Hz); HRESIMS m/z 283.0149 [M^+] (calcd for $C_{14}H_6ClN_3O_2$, 283.0149); anal. C 59.28, H 2.13, N 14.81%, calcd for $C_{14}H_6ClN_3O_2$, C 59.18, H 2.31, N 14.80%.

8-(Trifluoromethoxy)indolo[2,1-*b*]-3-azaquinazoline-6,12-dione (4d): yellow solid (15%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (1H, dd, J = 8.7, 2.5 Hz), 7.79 (1H, d, J = 1.3 Hz), 8.22 (1H, d, J = 5.1 Hz), 8.69 (1H, d, J = 8.7 Hz), 8.92 (1H, d, J = 5.1 Hz), 9.41 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 117.9, 119.5, 119.6, 123.1, 128.9, 130.7, 140.9, 143.7, 145.4, 148.2, 150.4, 153.0, 156.6, 180.5; HRESIMS m/z 333.0361 [M^+] (calcd for $C_{15}H_6F_3N_3O_3$, 333.0361).

8-Nitroindolo[2,1-*b*]-4-azaquinazoline-6,12-dione (4e): yellow solid (29%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.80 (1H, dd, J = 7.9, 4.6 Hz), 8.60 (1H, d, J = 2.3 Hz), 8.67 (1H, s), 8.75 (1H, dd, J = 3.5, 2.1 Hz), 8.77 (1H, d, J = 8.7 Hz), 9.12 (1H, dd, J = 4.6, 1.9 Hz); HRESIMS m/z 294.0385 [M^+] (calcd for $C_{14}H_6N_4O_4$, 294.0389).

8-Fluoroindolo[2,1-*b*]-4-azaquinazoline-6,12-dione (4f): yellow solid (33%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.69−7.76 (2H, m), 7.82 (1H, dd, J = 7.1, 2.7 Hz), 8.30 (1H, dd, J = 8.8, 4.1 Hz), 8.69 (1H, dd, J = 7.9, 1.9 Hz), 9.06 (1H, dd, J = 4.5, 1.9 Hz); HRESIMS m/z 267.0442 [M^+] (calcd for $C_{14}H_6FN_3O_2$, 267.0444).

9-Chloroindolo[2,1-*b*]-4-azaquinazoline-6,12-dione (4g): yellow solid (54%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42−7.46 (1H, m), 7.60−7.65 (1H, m), 7.86−7.89 (1H, m), 8.65 (1H, s), 8.73−8.78 (1H, m), 9.09−9.12 (1H, m); HRESIMS m/z 283.0145 [M^+] (calcd for $C_{14}H_6ClN_3O_2$, 283.0149).

***tert*-Butyl 4-(6,12-dioxo-6,12-dihydroindolo[2,1-*b*]-4-azaquinazolin-9-yl)piperazine-1-carboxylate (4h).** To a solution of **4g** (260 mg, 0.92 mmol) in NMP (5 mL) was added *tert*-butyl piperazine-1-carboxylate (210 mg, 1.11 mmol) at rt. The red reaction mixture was stirred at 90 °C for 2 h and was concentrated and dissolved in MeOH. To the reaction mixture was added ether to precipitate solids, which were filtered and dried to give the title compound as a red solid (320 mg, 80%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (9H, s), 3.63 (8H, d, J = 7.8 Hz), 6.61 (1H, dd, J = 8.7, 1.3 Hz), 7.57 (1H, dd, J = 7.7, 4.6 Hz), 7.69 (1H, d, J = 8.8 Hz), 7.91 (1H, d, J = 1.2 Hz), 8.66 (1H, dd, J = 7.6, 1.4 Hz), 9.06−9.07 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.3, 46.6, 80.5, 100.7, 110.6, 111.7, 119.1, 124.1, 127.8, 136.3, 148.4, 149.1, 154.5, 156.3, 156.7, 157.6, 158.5, 177.3; HRESIMS m/z 433.1746 [M^+] (calcd for $C_{23}H_{23}N_5O_4$, 433.1750); anal. C 63.73, H 5.35, N 16.16%, calcd for $C_{23}H_{23}N_5O_4$, C 63.93, H 5.29, N 16.02%.

9-Morpholinoindolo[2,1-*b*]-4-azaquinazoline-6,12-dione (4i). Using morpholine instead of *tert*-butyl piperazine-1-carboxylate, the procedure for **4h** was adopted to give the title compound as a red solid (73%): ^{1}H NMR (300 MHz, TFA-*d*) δ 4.45−4.74 (8H, m), 7.49−7.62 (1H, m), 8.40−8.53 (1H, m), 8.64−8.86 (2H, m), 9.67−9.78 (1H, m), 10.00−10.11 (1H, m); HRESIMS m/z 334.1066 [M^+] (calcd for $C_{18}H_{14}N_4O_3$, 334.1068); anal. C 64.66, H 4.22, N 16.76%, calcd for $C_{18}H_{14}N_4O_3$, C 64.66, H 4.17, N 16.48%.

9-(Piperidin-1-yl)indolo[2,1-*b*]-4-azaquinazoline-6,12-dione (4j). Using piperidine instead of *tert*-butyl piperazine-1-carboxylate, the procedure for **4h** was adopted to give the title compound as a yellow solid (71%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.75 (6H, s), 3.62 (4H, s), 6.68 (1H, dd, J = 9.0, 2.2 Hz), 7.55 (1H, dd, J = 7.8, 4.5 Hz), 7.71 (1H, d, J = 8.9 Hz), 8.00 (1H, d, J = 2.2 Hz), 8.69 (1H, dd, J = 7.8, 1.9 Hz), 9.06 (1H, dd, J = 4.9, 1.9 Hz); HRESIMS m/z 332.1251 [M^+] (calcd for $C_{19}H_{16}N_4O_2$, 332.1273); anal. C 68.34, H 5.06, N 16.39%, calcd for $C_{19}H_{16}N_4O_2$, C 68.66, H 4.85, N 16.86%.

8-Bromoindolo[2,1-*b*]-4-azaquinazoline-6,12-dione (4k): yellow solid (65%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.91 (1H, d, J = 5.4 Hz), 8.06−8.12 (2H, m), 8.39 (1H, d, J = 8.4 Hz), 9.02 (1H, d, J = 5.4 Hz), 9.48 (1H, s); HRESIMS m/z 326.9645 [M^+] (calcd for $C_{14}H_6BrN_3O_2$, 326.9643).

9-Chloroindolo[2,1-*b*]-2-azaquinazoline-6,12-dione (4l): yellow solid (34%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.92 (1H, d, J = 7.6 Hz), 8.07−8.13 (2H, m), 8.39 (1H, d, J = 8.3 Hz), 9.01−9.03 (1H, m), 9.49 (1H, s); HRESIMS m/z 283.0142 [M^+] (calcd for $C_{14}H_6ClN_3O_2$, 283.0149).

***tert*-Butyl 4-(6,12-dioxo-6,12-dihydroindolo[2,1-*b*]-2-azaquinazolin-9-yl)piperazine-1-carboxylate (4m).** Using **4l** instead of **4g**, the procedure for **4h** was adopted to give the title compound as a red solid (70%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (9H, s), 3.65 (8H, s), 6.70 (1H, d, J = 7.2 Hz), 7.77 (1H, d, J = 8.8 Hz), 7.84 (1H, d, J = 5.5 Hz), 8.04 (1H, d, J = 1.7 Hz), 8.97 (1H, d, J = 5.5 Hz), 9.61 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.3, 46.7, 80.6, 101.0, 110.7, 111.4, 118.2, 122.7, 128.1, 148.8, 150.7, 152.4, 154.4, 154.8, 157.0, 157.7, 177.3; HRESIMS m/z 433.1750 [M^+] (calcd for $C_{23}H_{23}N_5O_4$, 433.1750).

9-Morpholinoindolo[2,1-*b*]-2-azaquinazoline-6,12-dione (4n). Using **4l** instead of **4g**, the procedure for **4i** was adopted to give the title compound as a red solid (71%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.60 (4H, t, J = 4.3 Hz), 3.89 (4H, t, J = 4.3 Hz), 6.73 (1H, d, J = 8.7 Hz), 7.79 (1H, d, J = 8.2 Hz), 7.84 (1H, d, J = 5.0 Hz), 8.08 (1H, s), 8.97 (1H, d, J = 5.5 Hz), 9.61 (1H, s); HRESIMS m/z 334.1067 [M^+] (calcd for $C_{18}H_{14}N_4O_3$, 334.1066).

dx.doi.org/10.1021/np3007167 | J. Nat. Prod. XXXX, XXX, XXX−XXX

9-(Piperidin-1-yl)indolo[2,1-*b*]-2-azaquinazoline-6,12-dione (4o). Using **4l** instead of **4g**, the procedure for **4j** was adopted to give the title compound as a red solid (64%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.76 (6H, s), 3.65 (4H, s), 6.70 (1H, dd, J = 9.0, 2.3 Hz), 7.74 (1H, d, J = 9.0 Hz), 7.83 (1H, d, J = 5.3 Hz), 8.04 (1H, d, J = 2.2 Hz), 8.95 (1H, d, J = 5.5 Hz), 9.61 (1H, s); HRESIMS m/z 332.1273 [M$^+$] (calcd for $C_{19}H_{16}N_4O_2$, 332.1273).

9-(Piperazin-1-yl)indolo[2,1-*b*]-2-azaquinazoline-6,12-dione hydrochloride (4p). To a solution of **4m** (100 mg, 0.23 mmol) in NMP (5 mL) was added a 4 M solution of HCl in dioxane (0.20 mL, 0.80 mmol) at rt. The reaction mixture was stirred at rt for 24 h and was concentrated and dissolved in MeOH. To the reaction mixture was added ether to precipitate solids, which were filtered and dried to give the title compound as a red solid (71%): ^{1}H NMR (300 MHz, MeOH-d_4) δ 3.40 (4H, t, J = 5.1 Hz), 3.83 (4H, t, J = 5.2 Hz), 7.02 (1H, dd, J = 8.9, 2.1 Hz), 7.42 (1H, d, J = 6.9 Hz), 7.72 (1H, d, J = 8.8 Hz), 7.89 (1H, d, J = 2.0 Hz), 8.46 (1H, dd, J = 6.9, 1.1 Hz), 9.06 (1H, d, J = 0.7 Hz); HRESIMS m/z 333.1224 [M$^+$] (calcd for $C_{18}H_{16}ClN_5O_2$, 333.1226).

Indolo[2,1-*b*]-2-azaquinazoline-6,12-dione (4q): yellow solid (45%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47−7.49 (1H, m), 7.82−7.86 (2H, m), 7.93 (1H, d, J = 7.5 Hz), 8.60 (1H, d, J = 8.1 Hz), 9.00 (1H, d, J = 5.4 Hz), 9.65 (1H, s); LC-MS m/z 250.1 [M + H$^+$].

8-Chloroindolo[2,1-*b*]-2-azaquinazoline-6,12-dione (4r): yellow solid (45%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (1H, dd, J = 8.6, 2.1 Hz), 7.86 (1H, d, J = 5.2 Hz), 7.89 (1H, d, J = 2.0 Hz), 8.58 (1H, d, J = 8.5 Hz), 9.02 (1H, d, J = 5.4 Hz), 9.66 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.6, 118.2, 119.3, 122.5, 123.1, 125.5, 133.9, 138.2, 144.2, 147.0, 150.9, 151.9, 155.2, 156.9; LC-MS m/z 284.16 [M + H$^+$].

Indolo[2,1-*b*]-4-azaquinazoline-6,12-dione (4s): yellow solid (45%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (1H, t, J = 7.5 Hz), 7.62 (1H, dd, J = 7.9, 4.6 Hz), 7.81 (1H, dt, J = 7.8, 1.0 Hz), 7.94 (1H, d, J = 7.7 Hz), 8.60 (1H, d, J = 8.1 Hz), 8.76 (1H, dd, J = 7.9, 1.8 Hz), 9.10 (1H, dd, J = 4.5, 1.8 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 116.9, 119.1, 122.1, 124.7, 124.9, 127.2, 136.3, 137.9, 145.7, 147.6, 156.1, 157.2, 158.1, 182.3; LC-MS m/z 250.15 [M + H$^+$].

8-(Trifluoromethoxy)indolo[2,1-*b*]-4-azaquinazoline-6,12-dione (4t): yellow solid (40%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61−7.66 (2H, m), 7.77 (1H, s), 8.66 (1H, d, J = 8.7 Hz), 8.76 (1H, dd, J = 7.9, 1.8 Hz), 9.10 (1H, dd, J = 4.5, 1.9 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 117.9, 118.3, 118.6, 118.9, 121.7, 123.9, 124.9, 130.2, 136.4, 144.1, 146.5, 147.7, 156.3, 157.0, 158.0, 181.0; LC-MS m/z 334.0 [M + H$^+$].

General Procedure for the Synthesis of C-11-Deoxy Tryptanthrins (5). Step 1: To a solution of tryptanthrin (1 mmol) in ether (3 mL) was added $LiAlH_4$ (3 mmol) at 0 °C. The reaction mixture was refluxed overnight. To the reaction mixture was added H_2O (10 mL), and the solution extracted with EtOAc (2×). The organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The organic layers were dried with $MgSO_4$, filtered, and concentrated. The reaction mixture was purified via column chromatography (eluted with 5% MeOH in CH_2Cl_2) to give a reduced tryptanthrin. Step 2: To a solution of the reduced tryptanthrin (1 mmol) in CH_2Cl_2 (3 mL) was added MnO_2 (2 mmol) at rt. The reaction mixture was stirred at rt for 48 h. The reaction mixture was filtered and washed with CH_2Cl_2. The organic layers were dried with $MgSO_4$, filtered, and concentrated. The reaction mixture was purified via column chromatography (eluted with 5% MeOH in CH_2Cl_2) to give the title compound.

8-(Trifluoromethoxy)indolo[2,1-*b*]quinazolin-6(12*H*)-one (5a): red solid (79%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.01 (2H, s), 6.90 (1H, d, J = 8.5 Hz), 7.13 (1H, dd, J = 6.8, 1.0 Hz), 7.28−7.35 (2H, m), 7.49 (1H, dd, J = 8.5, 2.4 Hz), 7.55 (1H, dd, J = 7.4, 1.3 Hz), 7.62 (1H, d, J = 1.3 Hz); HRESIMS m/z 318.0603 [M$^+$] (calcd for $C_{16}H_9F_3N_2O_2$, 318.0616).

8-Chloroindolo[2,1-*b*]quinazolin-6(12*H*)-one (5b): red solid (78%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.98 (2H, s), 6.83 (1H, d, J = 8.3 Hz), 7.12 (1H, d, J = 6.9 Hz), 7.24−7.35 (2H, m), 7.52−7.59 (2H, m), 7.69 (1H, d, J = 1.8 Hz); HRESIMS m/z 268.0428 [M$^+$] (calcd for $C_{15}H_9ClN_2O$, 268.0403); anal. C 67.29, H 3.25, N 10.18%, calcd for $C_{15}H_9ClN_2O$, C 67.05, H 3.38, N 10.43%.

Indolo[2,1-*b*]quinazolin-6(12*H*)-one (5c): red solid (81%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.93 (2H, s), 6.83 (1H, d, J = 7.9 Hz), 7.04−7.09 (2H, m), 7.18−7.28 (2H, m), 7.48 (1H, dd, J = 7.5, 1.2 Hz), 7.56−7.61 (1H, m), 7.69 (1H, d, J = 7.7 Hz); LC-MS m/z 235.2 [M + H$^+$].

Indolo[2,1-*b*]-2-azaquinazolin-6(12*H*)-one (5d): red solid (2%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.08 (2H, s), 6.88−6.92 (1H, m), 7.17−7.23 (2H, m), 7.46−7.53 (2H, m), 7.62−7.65 (1H, m), 7.53−7.57 (1H, m); LC-MS m/z 236.1 [M + H$^+$].

8-Chloroindolo[2,1-*b*]-2-azaquinazolin-6(12*H*)-one (5e): red solid (12%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.06 (2H, s), 6.84 (1H, d, J = 8.4 Hz), 7.16−7.21 (1H, m), 7.45−7.48 (1H, m), 7.58−7.61 (1H, m), 7.71 (1H, d, J = 2.1 Hz), 8.52−8.55 (1H, m); LC-MS m/z 270.0 [M + H$^+$].

8-(Trichloromethoxy)indolo[2,1-*b*]-2-azaquinazolin-6(12*H*)-one (5f): red solid (10%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.04 (2H, s), 6.95 (1H, d, J = 8.5 Hz), 7.38 (1H, d, J = 5.1 Hz), 7.53 (1H, dd, J = 8.5, 2.1 Hz), 7.63 (1H, s), 8.43 (1H, s), 8.56 (1H, d, J = 5.1 Hz); LC-MS m/z 320.0 [M + H$^+$].

Indolo[2,1-*b*]-4-azaquinazolin-6(12*H*)-one (5g): red solid (6%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.06 (2H, s), 6.87 (1H, d, J = 8.0 Hz), 7.12−7.18 (2H, m), 7.46 (1H, d, J = 7.3 Hz), 7.63 (1H, t, J = 7.7 Hz), 7.75 (1H, d, J = 7.5 Hz), 8.52 (1H, d, J = 5.0 Hz); LC-MS m/z 235.9 [M + H$^+$].

8-Chloroindolo[2,1-*b*]-4-azaquinazolin-6(12*H*)-one (5h): red solid (9%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.06 (2H, s), 6.84 (1H, d, J = 8.4 Hz), 7.16−7.21 (1H, m), 7.45−7.48 (1H, m), 7.58−7.61 (1H, m), 7.71−7.73 (1H, m), 8.52−8.55 (1H, m); LC-MS m/z 269.9 [M + H$^+$].

8-(Trifluoromethoxy)indolo[2,1-*b*]-4-azaquinazolin-6(12*H*)-one (5i): red solid (10%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.07 (2H, s), 6.89−6.93 (1H, m), 7.17−7.22 (1H, m), 7.46−7.52 (2H, m), 7.62−7.64 (1H, m), 8.54−8.56 (1H, m); LC-MS m/z 320.0 [M + H$^+$].

1,2,3,4-Ttetrahydroindolo[2,1-*b*]quinazoline-6,12-dione (6). A solution of A-ring-saturated isatoic anhydride[28] (403 mg, 2.41 mmol), isatin (425 mg, 2.89 mmol), and Et_3N (930 mg, 9.19 mmol) in toluene (20 mL) was refluxed for 16 h. The reaction mixture was quenched with H_2O and then extracted with EtOAc (2×). The organic layers were dried with $MgSO_4$, filtered, and concentrated. The reaction mixture was purified via column chromatography (eluted with EtOAc/hexanes) to give the title compound as a yellow solid (85.1 mg, 14%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.76−1.88 (4H, m), 2.63−2.68 (2H, m), 2.76−2.80 (2H, m), 7.39 (1H, dt, J = 7.5, 0.7 Hz), 7.72 (1H, dt, J = 7.8, 1.3 Hz), 7.83 (1H, dd, J = 7.5, 0.8 Hz), 8.51 (1H, d, J = 8.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 21.9, 22.9, 31.5, 117.9, 121.3, 125.4, 127.4, 128.5, 137.9, 144.7, 146.0, 158.4, 159.4, 182.7; ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.9, 21.5, 22.5, 31.0, 116.9, 121.7, 124.8, 126.5, 127.1, 137.4, 145.2, 145.6, 157.9, 158.4, 182.4; LC-MS m/z 253.1 [M + H$^+$].

AUTHOR INFORMATION

Corresponding Author

*Tel: +82-42-860-7541. Fax: +82-42-860-7348. E-mail: pkim@krict.re.kr.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are very grateful to Prof. Lester A. Mitscher for sharing background information on tryptanthrins. Without his encouragement, this work would never have begun. The authors also would like to thank Dr. S.-H. Lee at KRICT for synthetic help and Dr. C. B. Cooper at TB Alliance for helpful discussions. This research was supported in part by KRICT and Brain Korea 21.

DEDICATION

Dedicated to Dr. Lester A. Mitscher, of the University of Kansas, for his pioneering work in the discovery of bioactive natural products and their derivatives.

REFERENCES

(1) Honda, G.; Tabata, M. *Planta Med.* **1979**, *36*, 85−90.
(2) Schindler, F.; Zahner, H. *Arch. Mikrobiol.* **1971**, *79*, 187−203.
(3) Honda, G.; Tabata, M.; Tsuda, M. *Planta Med.* **1979**, *37*, 172−174.
(4) Bandekar, P. P.; Roopnarine, K. A.; Parekh, V. J.; Mitchell, T. R.; Novak, M. J.; Sinden, R. P. *J. Med. Chem.* **2010**, *53*, 3558−3565.
(5) Kawakami, J.; Matsushima, N.; Ogawa, Y.; Kakinami, H.; Nakane, A.; Kitahara, H.; Nagaki, M.; Ito, S. *Trans. Mater. Res. Soc. Jpn.* **2011**, *36*, 603−606.
(6) Pitzer, K. K.; Scovill, J. P.; Kyle, D. E.; Gerena, L. PCT Int. WO 2000018769 A2, 2000.
(7) Bhattacharjee, A. K.; Hartell, M. G.; Nichols, D. A.; Hicks, R. P.; Stanton, B.; van Hamont, J. E.; Milhous, W. K. *Eur. J. Med. Chem.* **2004**, *39*, 59−67.
(8) Bhattacharjee, A. K.; Skanchy, D. J.; Jennings, B.; Hudson, T. H.; Brendle, J. J.; Werbovetz, K. A. *Bioorg. Med. Chem.* **2002**, *106*, 1979−1989.
(9) Scovill, J. B. E.; Konnick, M.; Nenorats, E.; Shapiro, T. *Antimicrob. Agents Chemother.* **2002**, *46*, 882−883.
(10) Krivogorsky, B.; Grundt, P.; Yolken, R.; Jones-Brando, L. *Antimicrob. Agents Chemother.* **2008**, *52*, 4466−4469.
(11) Sharma, V. M.; Prasanna, P.; Seshu, K. V.; Renuka, B.; Rao, C. V. L; Kumar, G. S.; Narasimhulu, C. P.; Babu, P. A.; Puranik, R. C.; Subramanyam, D.; Venkateswarlu, A.; Rajagopal, S.; Kumar, K. B. S.; Rao, C. S.; Mamidi, N. V. S. R.; Deevi, D. S.; Ajaykumar, R.; Rajagopalan, R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2303−2307.
(12) Chan, H. L.; Yip, H. Y.; Mak, N. K.; Leung, K. N. *Cell. Mol. Immunol.* **2009**, *6*, 335−342.
(13) Miao, S.; Shi, X.; Zhang, H.; Wang, S.; Sun, J.; Hua, W.; Miao, Q.; Zhao, Y.; Zhang, C. *Int. J. Mol. Sci.* **2011**, *12*, 3831−3845.
(14) Yu, S. T.; Chern, J. W.; Chen, T. M.; Chiu, Y. F.; Chen, H. T.; Chen, Y. H. *Acta Pharmacol. Sin.* **2010**, *31*, 259−264.
(15) Iwaki, K.; Ohashi, E.; Arai, N.; Kohno, K.; Ushio, S.; Taniguchi, M.; Fukuda, S. *J Ethnopharmacol.* **2011**, *134*, 450−459.
(16) Takei, Y.; Kunikata, T.; Aga, M.; Inoue, S.; Ushio, S.; Iwaki, K.; Ikeda, M.; Kurimoto, M. *Biol. Pharm. Bull.* **2003**, *26*, 365−367.
(17) Ishihara, T.; Kohno, K.; Ushio, S.; Iwaki, K.; Ikeda, M.; Kurimoto, M. *Eur. J. Pharmacol.* **2000**, *407*, 197−204.
(18) Hamburger, M.; Danz, H. PCT Int. WO 2000-CH214 20000412, 2000.
(19) Pergola, C.; Jazzar, B.; Rossi, A.; Northoff, H.; Hamburger, M.; Sautebin, L.; Werz, O. *Br. J. Pharmacol.* **2012**, *165*, 765−776.
(20) Valiante, N. PCT Int. WO 2004064759 A2, 2004.
(21) Mitscher, L. A.; Baker, W. *Med. Res. Rev.* **1998**, *18*, 363−374.
(22) TB Facts.org: http://www.tbfacts.org/tb-treatment.html.
(23) Zumla, A.; Squire, S.; Chintu, C.; Grange, J. *Trans. R. Soc. Trop. Med. Hyg.* **1999**, *93*, 113−l17.
(24) Kaneko, T.; Cooper, C.; Mdluli, K. *Future Med. Chem.* **2011**, *3*, 1373−1400.
(25) For a list of the pipeline of TB drug discovery by TB Alliance, see: http://www.tballiance.org/downloads/mediakit/TBA_Portfolio_%202.22.2011.pdf.
(26) Mitscher, L.; Baker, W. *Pure Appl. Chem.* **1998**, *70*, 365−371.
(27) Baker, W. R.; Shaopei, C.; Keeler, E. L. U.S. Pat. 5,668,127, 1997.
(28) Bergman, J.; Tilstam, U.; Toernroos, K. W. *J. Chem. Soc., Perkin Trans. 1* **1987**, 519−527.
(29) Beccalli, E. M.; Marchesini, A. *J. Org. Chem.* **1987**, *52*, 3426−3434.
(30) Collins, L. A.; Torrero, M. N.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1998**, *42*, 344−347.
(31) Cho, S. H.; Warit, S.; Wan, B.; Hwang, C. H.; Pauli, G. F.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **2007**, *51*, 1380−1385.
(32) Lipinski, C. A. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235−249.
(33) Ishikawa, M.; Hashimoto, Y. *J. Med. Chem.* **2011**, *54*, 1539−1554.
(34) Li, J.; Miao, S.; Xie, Y.; Wang, J.; Cao. W.; Bi. L.; Wang, S. *Chromatographia* **2012**, October 2, 1−6.
(35) Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004−1009.
(36) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **2001**, *46*, 3−26.
(37) Hunter, J.; Jepson, M. A.; Tsuruo, T.; Simmons, N. L.; Hirst, B. H. *J. Biol. Chem.* **1993**, *268*, 14991−14997.
(38) Grès, M. C.; Julian, B.; Bourrié, M.; Meunier, V.; Roques, C.; Berger, M.; Boulenc, X.; Berger, Y.; Fabre, G. *Pharm. Res.* **1998**, *15*, 726−733.
(39) Sangster, J. *Octanol-Water Partition Coefficients: Fundamentals and Physical Chemistry*; Wiley Series in Solution Chemistry, Vol. *2*; John Wiley & Sons: Chichester, U.K., 1997; pp 57−74.
(40) Kuhnz, W.; Gieschen, H. *Drug Metab. Dispos.* **1998**, *26*, 1120−1127.
(41) Crespi, C. L.; Miller, V. P.; Penman, B. W. *Anal. Biochem.* **1997**, *248*, 188−190.
(42) Ono, S.; Hatanaka, T.; Hotta, H.; Satoh, T.; Gonzalez, F. J.; Tsutsui, M. *Xenobiotica* **1996**, *26*, 681−693.
(43) Stresser, D. M.; Blanchard, A. P.; Turner, S. D.; Erve, J. C.; Dandeneau, A. A.; Miller, V. P.; Crespi, C. L. *Drug Metab. Dispos.* **2000**, *28*, 1440−1448.
(44) Banker, M. J.; Clark, T. H.; Williams, J. A. *J. Pharm. Sci.* **2003**, *92*, 967−974.
(45) Kaila, N.; Janz, K.; DeBernardo, S.; Bedard, P. A. W.; Camphausen, R. T.; Tam, S.; Tsao, D. H. H.; Keith, J. C.; Nickerson-Nutter, C.; Shilling, A.; Young-Sciame, R.; Wang, Q. *J. Med. Chem.* **2007**, *50*, 21−39.

dx.doi.org/10.1021/np3007167 | *J. Nat. Prod.* XXXX, XXX, XXX−XXX