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1,3,5-Triazino-Peptide Derivatives: Synthesis, Characterization and Preliminary Antileishmanial Activity

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Abstract: A library of short di- to tetra-peptides with s-triazine moiety at the *N*-terminal and the C-terminal in the form of either ethyl ester or amide were prepared in solution and in solid-phase. The two remaining positions of the s-triazine moiety were substituted by dimethoxy, dimorpholino, or dipiperidino groups. All the synthesized peptide derivatives were analyzed by HPLC and fully characterized by IR, NMR (^{1}H-NMR and ^{13}C-NMR), elemental analysis, and mass spectra analysis (MALDI TOF/TOF). A preliminary study of the antileishmanial activity of the 1,3,5-triazinyl-peptide derivatives revealed that four dipeptide amide derivatives showed better antipromastigote or antiamastigote activity than that of the reference standard drug miltefosine with no significance acute toxicity.

Introduction

Peptides are characterized by broad chemical diversity, which is translated into a range of biological activities of interest.[1] In this regard, even small peptides have marked physiological effects on living organisms; for example, the tripeptide glutathione is found in most living cells; the pentapeptide enkephalin is a naturally occurring analgesic; and the nonapeptide oxytocin induces the contraction of the uterine muscle in women during labor.[2] Small peptides (3-9 amino acid residues) find applications in diverse therapeutic areas including cancer,[3] asthma, allergy, Ca^{2+} metabolism, and central nervous system disorders and they are also used as antimicrobial, antiviral and analgesic agents, among others.[4] To date, radiolabeled small peptides have mainly been utilized in oncology for diagnostic imaging and targeting radiotherapy.[5] Furthermore, given that peptides can be taken up by cells, these compounds are particularly useful for drug delivery purposes.[6]

Antimicrobial peptides (AMPs) deserve special mention as they are found endogenously in many species of animals and plants. Most AMPs have a low molecular mass and serve as defense and protection against predators and microorganisms.[7] Amino acid and dipeptide esters that contain at least one hydrophobic amino acid show leishmanicidal activities.[8] The intralesional administration of several compounds also restricts the growth of mouse lesions.[8] However, the esters are known to be toxic in vitro for monocytes and certain lymphoid cells.[9] Furthermore, triazines, which act as dihydrofolate reductase inhibitors (DHFRs),[10] also show potential as antileishmanial agents.[11]

The World Health Organisation considers leishmaniasis to be among the seventeen most negelected dangerous tropical protozoal diseases.[12] Indeed, the prevalence of this disease continues to increase, causing morbidity worldwide. Leishmaniasisis is caused by insect vectorborne protozoan parasites and it affects an estimated 12 million people a year, with a further 350 million people at risk of infection.[13] Despite attempts to control this disease over the last few decades, its prevalence has increased in developing countries.[14]

The various clinical forms of leishmaniasis constitute a serious public health concern. Visceral leishmaniasis (VL) is usually fatal when untreated, muco-cutaneous leishmaniasis (MCL) is a mutilating disease, diffuse cutaneous leishmaniasis (DCL) is a disabling disease, and cutaneous leishmaniasis (CL) is also disabling when lesions are multiple.[15] Cutaneous leishmaniasis (CL) is endemic in over 80 countries. In the Americas, CL is widely distributed, from southern Texas to northern Argentina. Other endemic areas include the Middle East, India, Pakistan, Iran, and North and East Africa.[15a, 16] It is worth mentioning that Cutaneous leishmaniasis is caused mainly by the following species *L. major*, *L. tropica*, and *L. aethiopica* in the Old World while by *L. mexicana*, *L. amazonensis*, *L. braziliensis*, *L. panamanesis*, and *L. guyanensis* in the New World.[15a, 16]

Almost all the drugs currently used to treat the disease show resistance and side effects.[14] A vaccine to prevent leishmaniasis is not available despite considerable research efforts in this field.[17] Consequently, the treatment of this condition relies entirely on a considerably limited arsenal of chemotherapeutics. Both VL and CL are treated mostly with pentavalent antimonials, such as sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime),[18] which are highly toxic. However, an increasing non-susceptibility of the parasites to antimonials injection in the area with the world's highest prevalence of disease (North Bihar, India), led to widespread treatment failure and a shift to amphotericin B and Miltefosine.[19] Different formulations of amphotericin B are

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currently available, and amphotericin B deoxycholate (Fungizone) is the most highly effective.[19d, 19f] Amphotericin B deoxycholate has some unfortunate side effects and some infusion-related side effects such as fever, chills, and thrombophlebitis.[20] Miltefosine was the first oral antileishmanial drug that reached the market and has been used for treatment of VL and CL.[19d, 19f] However, side effects include disturbance of the gastrointestinal tract[21] and an elevation of hepatic enzymes.[22] Furthermore, miltefosine has a teratogenic effect and is contra indicated for use during pregnancy.[19f, 21] Similarly, mandatory contraception is also suggested for women of child-bearing age. [21] In addition, Paromomycin,[19f, 23] pentamidine, [19d, 19f, 20] and Sitamaquine[19f, 24] were also investigated.

In this regard, the search for new drugs with strong leishmanicidal activity and a safer profile is critical if we are to tackle this devastating disease. Here we studied the potential antileishmanial activity of a new family of compounds, considered hybrid molecules of 1,3,5-triazine moiety and short hydrophobic peptides, against *L. aethiopica*, the causative agent of CL.

Results and Discussion

Chemistry

Although the solid-phase methodology is the method of choice for the synthesis of most peptides, very short peptides are best synthesized in solution, because this approach involves only one or two reactions.[25] Furthermore, C-terminal esters are difficult to synthesize in solid-phase due to the inherent characteristic of the solid-phase, which is based on anchoring the first amino acid to the solid support through the carboxylic function.

Synthesis of *N*-(4,6-Disubstituted-1,3,5-triazin-2-yl)dipeptide /tripeptide ethyl esters/amides (4-10). 1,3,5-Triazine-based amino acid derivatives [*N*-(4,6-disubstituted-1,3,5-triazin-2-yl)amino acid], which were prepared from 4,6-disubstituted-1,3,5-triazine and the corresponding L-amino acid, were coupled to glycine ethyl ester hydrochloride, L-valine amide hydrochloride, and L-phenylalanyl-L-valine amide p-toluene sulfonic acid salt to give di- and tri-peptide-1,3,5-triazine derivatives, respectively (Scheme 1). Reactions were performed using *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) as a coupling reagent in the presence of DIEA as base, followed by reaction with a solution of amino acid ethyl ester/amide hydrochloride/p-toluensulphonate* and DIEA in DMF at 0°C to afford the desired derivatives in high yields and purities, as confirmed by elemental analysis, IR, and NMR spectroscopy (supplementary data).

The ^{13}C-NMR spectrum for the dimethoxy-s-triazine derivatives **4a, 4b, 4c, 4e, 4f** and **7g** showed three peaks corresponding to the triazine ring. For example, compound **4f** showed peaks at δ 168.13, 170.26, 172.21, 172.38 and 173.28 ppm, corresponding to the two carbonyl carbons (amide CO and ester CO) and the triazine ring carbons. The observation of three peaks for the triazine ring carbons confirms that the two methoxy groups on the triazine ring are non-equivalent, thereby allowing the formation of two conformers. This observation is in agreement with the data reported for methoxy-s-triazine amino acid derivatives by Loeser and Babiak.[26]

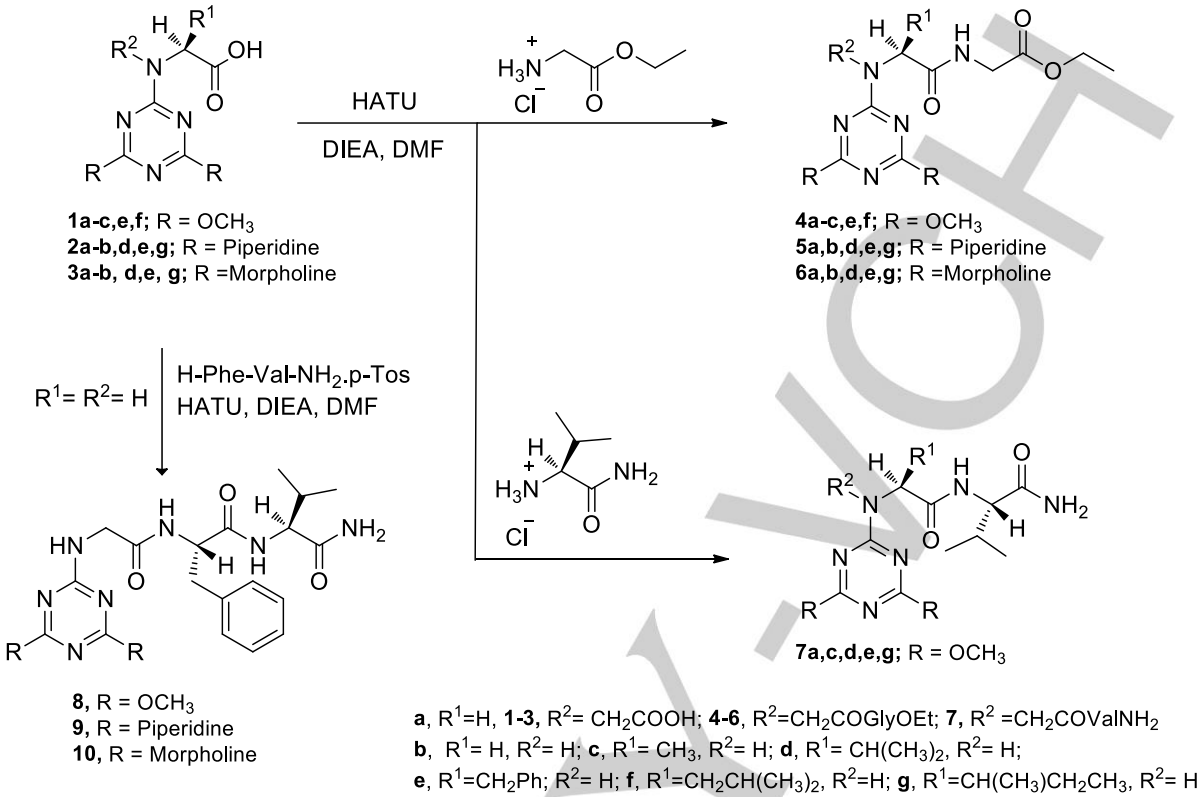
The HPLC chromatogram for *N*-(4,6-dipiperidino-1,3,5-triazin-2-yl)-Ile-Gly-OC_2H_5 **5g** revealed two conformers at retention times t_R = 5.14 and 11.19 min in a ratio of 15:85, respectively. The two conformers were confirmed by the ^{1}H-NMR spectrum. The chemical shift of the two α-CHs and of the two NHs of these conformers differed. Two peaks were observed to be equivalent to one proton at δ 5.55 and 4.25 ppm, in a ratio of 85:15, corresponding to the α-CH. In addition, four peaks were observed to be equivalent to two protons, corresponding to the two NHs. NMR analysis indicated that the conversion between the two conformations was slow enough to be observed by NMR spectrum at room temperature (rt).[26]

Furthermore, the ^{1}H-NMR spectrum of **6e** also revealed two conformers in a ratio of 56:44. The chemical shift of the two α-CH and of the two NH's of these conformers differed.

The purity of *N*-(4,6-dimethoxy-1,3,5-triazin-2-yl)-Gly-Phe-Val-NH_2 **8** was 100%, as detected by reverse- phase HPLC at retention time t_R = 9.05 min. Product **8** was identified from its mass spectral analysis, and it showed an exact mass $[M+Na]^+$ = 482.201 (Supplementary data). Reverse-phase HPLC chromatogram for *N*-(4,6-dipiperidino-1,3,5-triazin-2-yl)-Gly-Phe-Val-NH_2 **9** revealed two conformers at retention times t_R = 5.11 and t_R = 6.49 min in a ratio of 91:9, respectively, while only one mass signal at *m/z* = $[M+H]^+$ = 566.613 was observed. In addition, reverse-phase HPLC chromatogram for *N*-(4,6-dimorpholino-1,3,5-triazin-2-yl)-Gly-Phe-Val-NH_2 **10** showed the presence of two conformers with retention times t_R = 8.54 min and t_R = 9.25 min in a ratio of 85 :15, with an exact mass $[M+H]^+$ = 570.568.

Synthesis of *N*-(4,6-Disubstituted-1,3,5-triazin-2-yl)tetrapeptide amides (11-13). The peptide derivatives **11-13** were manually assembled stepwise on Fmoc-Rinkamide-AM-PS resin, Scheme 2. Preactivation was carried out using Fmoc-L-amino acid (4-fold excess), HATU (4-fold excess), and DIEA (8-fold excess) in DMF, and then the solution was added to the resin, and the resulting mixture was periodically stirred for 2 h. The loaded resin was washed with DMF, and the Fmoc group was removed with 20% piperidine in DMF. Washing of the deblocked resin with DMF, CH_2Cl_2, and DMF was followed by an analogous coupling step with the second Fmoc-L-amino acid.

* For compounds **8-10,** H-Phe-Val-NH_2·*p*-Tos was first prepared starting by the coupling of Boc-L-Phe-OH and HCl·L-Val-NH_2 with HATU in presence of DIEA as a base and DMF solvent at 0°C for 1 h and overnight at room temperature to afford the dipeptide Boc-Phe-Val-NH_2. The crude Boc-Phe-Val-NH_2 was treated with *p*-toluene sulfonic acid in acetonitrile, and stirred for 2 h at room temperature to remove the Boc-group forming the H-Phe-Val-NH_2.*p*-TsOH salt, which was used without further purification.

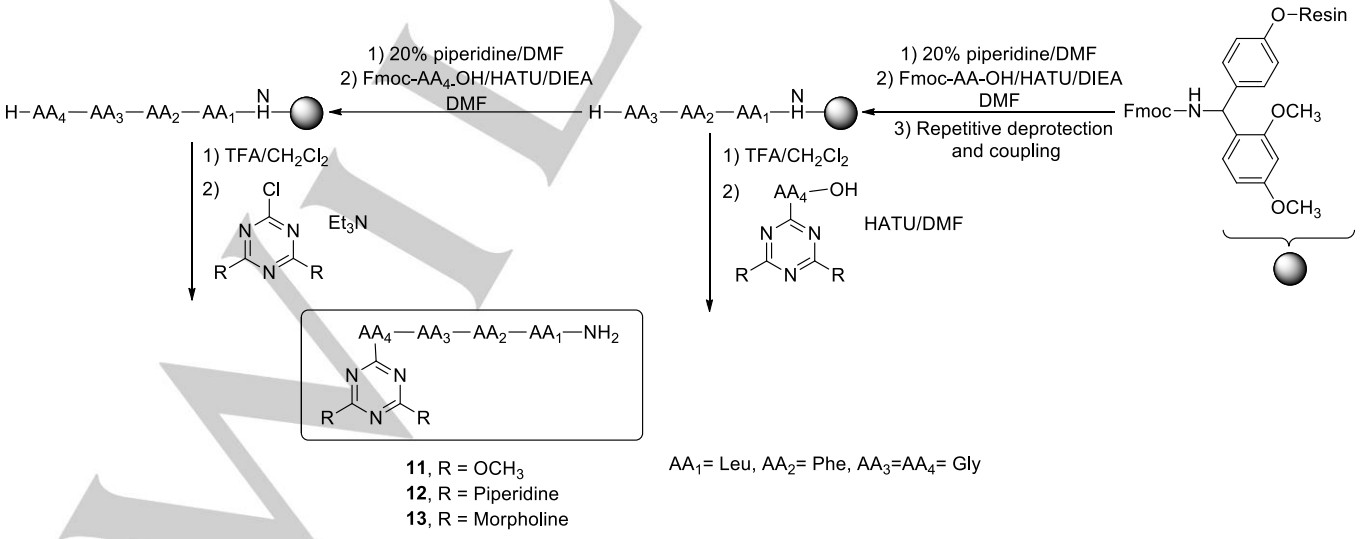


Scheme 1. Synthesis of *N*-(4,6-disubstituted-1,3,5-triazin-2-yl)dipeptide/tripeptide ethyl esters/amides **4-10**.

The assembly of the peptide derivatives was achived via one of the following **two routes** (Scheme 2):

Route (i): The tripeptide H-Gly-Phe-Val-NH_2 was cleaved from the resin with TFA-DCM (9:1) and then coupled to *N*-(4,6-disubstituted-1,3,5-triazin-2yl)-Gly-OH **1b/2b/3b** using HATU/DIEA in DMF solvent at 0°C for 1 h and overnight at rt to give the corresponding *N*-(4,6-disubstituted-1,3,5-triazin-2yl) tetrapeptide **11/12/13,** respectively (Scheme 2).

Route (ii): The tetrapeptide H-Gly-Gly-Phe-Val-NH_2 was allowed to react with 2-chloro-4,6-dimethoxy-1,3,5-triazine in the presence of Et_3N at rt overnight to give *N*-(4,6-dimethoxy-1,3,5-triazin-2yl)-peptide **11** (Scheme 2).



Scheme 2. Synthesis of *N*-(4,6-disubstituted-1,3,5-triazin-2-yl)tetrapeptide amides **11-13.**

Biological Activity

***In vitro* antipromastigote activity.** A quantitative colorimetric assay using the oxidation-reduction indicator Alamar blue® was developed to measure cytotoxicity of the synthesized compounds against the protozoan parasite Leishmania.[27] Alamar blue assay was used to determine the viability of promastigotes and evaluate the antileishmanial activity of the synthesized compounds, using as references the drugs miltefosine and amphotericin B.[7b, 28]

The dimethoxy triazine derivatives **4** showed greater antileishmanial activity than the corresponding dipepridino **5** and dimorpholino **6** derivatives.

Compounds **7a, 7d, 7e,** and **7g** showed greater antileishmanial activity than miltefosine (Table 1). The most active compound, **7a** (IC_{50} = 1.4 µM), showed about 5-fold the activity of miltefosine (IC_{50} = 7.8 µM). On the other hand, all the compounds tested showed lower antipromastigote activity than amphotericin B.

Table 1. Antipromastigote activity (IC_{50}) of the compounds synthesized and reference standards in µM.

Compound number	IC_{50}*	Compound number	IC_{50}*
4a	13.6±0.18	**6g**	28.4±0.22
4b	15.9±0.16	**7a**	**1.4±0.04**
4c	15.0±0.22	**7c**	17.2±0.28
4e	12.7±0.18	**7d**	**4.7±0.11**
4f	16.8±0.26	**7e**	**2.3±0.06**
5a	19.7±0.18	**7g**	**5.0±0.08**
5b	16.8±0.14	**8**	13.1±0.16
5d	17.9±0.36	**9**	23.3±0.22
5e	19.5±0.28	**10**	27.5±0.36
5g	17.0±0.32	**11**	9.4±0.26
6a	28.7±0.24	**12**	24.0±0.32
6b	31.4±0.22	**13**	25.6±0.34
6d	21.7±0.26	Miltefosine	7.8±0.34
6e	29.2±0.38	Amphotericin B deoxycholate	0.04±0.01

* IC_{50}: values indicate the effective concentration of a compound required to achieve 50% growth inhibition in µM. IC_{50} is expressed as mean ± SD of triplicate experiments, P < 0.05.

From a structure-activity point of view and taking into account that compounds **7a, 7d, 7e, 7g** (Figure 1) showed greater or comparable activity to that of miltefosine, we can conclude that hydrophobic amino acids with an amide at the C-terminal show the best results. The presence of a methoxy group on the triazine moiety enhanced the activity of the s-traizine derivatives, as reported in the literature.[29] Compound **7a** showed the greatest activity. This observation may be explained by the combination of two valine amides and the dimethoxy groups on the triazine moiety. Furthermore, the least active compound within this series was **7c,** which contains alanine adjacent to the s-triazine.

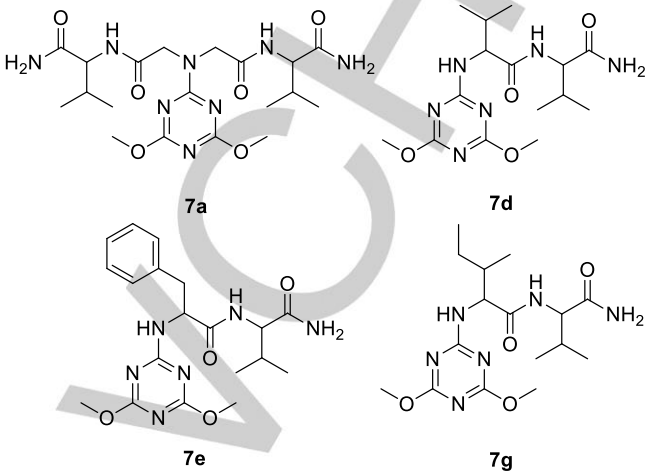


Figure 1. Structure of compounds **7a, 7d, 7e,** and **7g,** which showed greater activity than miltefosine.

***In vitro* antiamastigote activity.** Axenic amastigote were produced appling the method described by Teixeira *et al*.[30] The most active compounds, namely **7a, 7d, 7e, 7g,** were further tested for their antiamastigote activity.[31] They showed 3.4-, 1.6-, 2- and 1.4-fold activity of that of miltefosine, respectively, while they showed lower activity than the standard reference drug amphotericin B (Table 2). The most active compounds were **7a** and **7e,** which have iminodiacetic acid and phenylalanine adjacent to the s-triazine. Compounds **7a, 7d, 7e, 7g** showed slightly lower activity than amphotericin B. However, **7a** showed an activity very close to amphotericin B (Table 2).

Table 2. Antiamastigote activity of the compounds synthesized and the reference standards in µM.

Compound number	IC_{50}*
7a	0.22±0.02
7d	0.47±0.08
7e	0.37±0.06
7g	0.55±0.08
Miltefosine	0.74±0.04
Amphotericin B deoxycholate	0.15±0.02

*IC_{50}: effective concentration required to achieve 50% growth inhibition in µM. IC_{50} is expressed as mean ± SD of triplicate experiments, P < 0.05.

***In vitro* cytotoxicity assay**. The cytotoxicity of the most active compounds, **7a, 7d, 7e** and **7g,** was tested in a VERO cell line using the Mosmann method with modifications, as described in the literature.[32] Briefly, the cells were incubated for 72 h with a range of dilutions of the selected compounds, using MTT as

reagent for the detection of cytotoxicity. 50% cytotoxic concentration (CC_{50}) values represent the concentration of compound required to kill 50% of the cells (Table 3).

Combining the cytotoxic and antiparasitic activities of **7a**, **7d**, **7e** and **7g** by means of the selectivity index (Table 3), these compounds showed greater selectivity toward L. aethiopica than mammalian cells, thereby indicating a safe toxicity profile.

Table 3. CC_{50} and therapeutic values of the most active compounds on VERO cells.

Compound number	CC_{50}[a]	IC_{50}[a]	SI[b]
7a	533.61	0.22±0.02	2457.88
7d	705.42	0.47±0.08	1503.45
7e	310.6	0.37±0.06	841.27
7g	678.55	0.55±0.08	1240.96

[a] CC_{50} is the concentration at which 50% cells survive and IC_{50} is the concentration causing 50% cell death.

[b] SI is the selectivity index regarding antiamastigote activity; SI = CC_{50} / IC_{50}.

***In vivo* acute toxicity testing.** The most active antileishmanial compounds, **7a, 7d, 7e** and **7g** were tested for their toxicity in mice, following the reported method.[33] The mice did not show any sign of toxicity after treatment. There was no significant difference in the weight of the mice and no deaths were recorded during three days of observation after administration of the compounds (data not shown). Oral administration of the compounds up to 250 mg/kg of body weight were well tolerated by the animals. Moreover, the toxicity of the compounds when administered via the parenteral route was tested. All of them were found to be non-toxic up to 100 mg/kg of body weight.

The kidney, spleen and liver of mice receiving 250 mg/kg of body weight orally or 100 mg/kg parenterally showed normal textures. Histopathological studies of samples of these organs did not show any abnormalities.

The results of this study compare favourable with others in the literature for both triazine derivatives and peptides. For instance, Kgokong, *et al.*[34] studied the antileishmanial effect of 1,3,5-triazine derivatives, where the most active compound (supporting info.) had an IC_{50} of 4.01 μM and toxicity (CC_{50} = 227.04 μM) with an SI of 56.57. Furthermore, the antileishmanial effect of much more structurally complex peptides has also been reported. For example, viridamide A (supporting info.) obtained from *Oscillatoria nigrowiridis* showed antileishmanial activity toward *L. mexicana* with an IC_{50} of 1.5 μM;[35] symplocamide A (supporting info.) exhibited antileishmanial activity toward *L. donovani* with an IC_{50} of 9.5 μM;[36] and the most active cecropin A-melittin hybrid peptide KWKLFKKIGAVLKVL-amide had an IC_{50} of 1.8 μM (SI = 26.8) against *L. donovani*.[7d] In comparison to these observations, some of the 1,3,5-triazine-peptide hybrids synthesized herein showed an improvement in both anti-leishmanial activity and SI regarding antiamastigote activity, as exemplified by **7a** (IC_{50} = 1.4±0.04 μM and SI = 2457.88), while other derivatives, such as **7d**, **7e**, and **7g**, showed an improvement in the SI.

Conclusions

New hybrid molecules that hold a 1,3,5-triazine moiety along with di-tri-and tetra-peptides were synthesized and tested for leishmanicidal activity. The piperidino and moroholino derivatives showed low activities while the combination of dimethoxy and valine amide on the s-triazine ring demonstrated the highest antipromastigote and antiamastigote activity. The *in vitro* antipromastigote activity revealed **7a, 7d, 7e,** and **7g** as having a better IC_{50} for promastigotes and amastigotes than that of the standard drug miltefosine. Refering to amphotericin B, these compounds showed an IC_{50} in the same range for amastigotes and less active for promastigotes. It is worth mentioning that amphotericin B uses to show higher activity toward *L. Mexicana* promastigote than amastigote, as occurs with other active compounds.[37]

The compounds presenting greatest activity, namely **7a, 7d, 7e,** and **7g**, showed low toxicity against mammalian cells and therefore a promising SI. In addition, *in vivo* acute toxicity studies for these compounds indicated their safety when administered orally and parenterally up to 250 and 100 mg/kg of body weight, respectively.

Therefore, compounds **7a, 7d, 7e,** and **7g** emerge as promising derivatives for the development of a new class of antileishmanial agents and deserve further attention. In special, **7a** is more active than miltefosin towards both proamastigote and amastigote and of similar activity than amphotericin B for amastigote. Taking into account its great SI makes it an excellent HIT for a medicinal chemistry program.

Experimental Section

Chemistry

Solvents and all reagents were purchased from Sigma-Aldrich. All 1,3,5-triazine L-amino acid derivatives **1a-g**, **2a,b,d,e,g** and **3a,b,d,e,g** were prepared earlier in our lab.[38] Unless otherwise stated, normal workup from organic solvent involved drying over Na_2SO_4 and rotary evaporation. TLC was performed using aluminum-backed Merck Silica Gel 60 F-254 plates using suitable solvent systems, with spots being visualized by a Spectroline UV Lamp (254 or 365 nm) or I_2 vapor. Melting points were obtained in open capillary tubes by using a MEL-Temp II melting point apparatus and they are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 series Fourier transform instrument as KBr pellets. The absorption bands ($\tilde{\nu}$max) are given in wave numbers (cm^{-1}). Nuclear magnetic resonance (NMR) spectra (^{1}H-NMR and ^{13}C-NMR) were recorded on JEOL 400 MHz and JEOL 500 MHz spectrometers at ambient temperature. Chemical shifts are reported in parts per million (ppm) and are referenced relative to residual solvent (e.g. $CHCl_3$ at δ 7.26 ppm for $CDCl_3$, DMSO at δ 2.50 ppm for [D_6]DMSO). Elemental analyses were performed on a Perkin-Elmer 2400 elemental analyzer, and the values found were within ±0.3% of the theoretical ones. HPLC analysis was performed using a reverse-phase Agilent 1200 HPLC separation module, coupled to an Agilent 1200 PDA UV detector. The

chromatograms were processed with Empower software. Separation was accomplished using an Eclipse plus C_{18} column (3.5 µm 4.6x100 mm) or Eclipse plus C_8 column (3.5 µm 4.6x250 mm), and linear gradients of solvent A [0.045% trifluoroacetic acid (TFA) in H_2O] in solvent B (0.036% TFA in CH_3CN) with a flow = 1.0 mL min^{-1}. Exact mass measurements were carried out using a Bruker Daltonics Ultraflex MALDI TOF/TOF Mass Spectrometer in a solvent mixture of acetonitrile: 0.1% aqueous TFA (30/70).

General procedure for the synthesis of *N*-(4,6-dimethoxy-1,3,5-triazin-2-yl) dipeptide ethyl ester (4a,b,c,e,f)

HATU (0.38 g, 1 mmol) was added to an ice-cold solution of *N*$^{\alpha}$-(4,6-dimethoxy-1,3,5-triazin-2-yl) amino acid (1 mmol) and DIEA (0.34 mL, 2 mmol) in DMF with stirring. A solution of glycine ethyl ester hydrochloride (0.14 g, 1 mmol) and DIEA (0.17 mL, 1 mmol) in DMF was then added to this mixture. The mixture was left overnight with stirring at rt (in the case of IDA derivative, (2 mmol) HATU and amino acid ester were used with equiv. DIEA). The reaction mixture was diluted with 70 mL ethyl acetate and then washed with 5% citric acid (2 × 10 mL), a saturated $NaHCO_3$ solution (2 × 10 mL), and a saturated NaCl solution (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed in vacuo with the aid of a water aspirator. The purity of compounds **4a,b,c,e,f** was detected by reverse-phase HPLC using the following conditions: detection at 220 nm (Agilent 1200 PDA detector); Eclipse plus C_{18} column (3.5 µm 4.6 x 100 mm); linear gradient over 16 min (25 to 50% CH_3CN in H_2O/ 0.1% TFA); and flow rate 1.0 mL/min.

***N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-IDA-(Gly-OEt)$_2$ (4a).**

The product was obtained as a white solid, (0.41 g, 91.7%); mp: 164-166°C; t_R = 4.46 min, purity 100% as detected by HPLC. IR (KBr): 3316 (NH, amide), 1740 (CO, ester) 1657 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D_6]DMSO): δ 1.13 (t, 3H, *J* = 6.9 Hz, $\underline{CH_3}CH_2$), 3.74 (s, 3H, OCH_3), 3.83 (d, 2H, *J* = 4.6 Hz, $\underline{CH_2}NH$), 4.04 (q, 2H, *J* = 6.9 Hz, $O\underline{CH_2}CH_3$), 4.24 (s, 2H, CH_2N), 8.82 (s, 1H, NH); ^{13}C-NMR (125 MHz, [D_6]DMSO): 14.54, 40.35, 52.42, 54.88, 60.99, 167.74, 170.08, 172.20. Anal. calcd for $C_{17}H_{26}N_6O_8$: C, 46.15; H, 5.92; N, 19.00. Found: C, 46.01; H, 6.12; N, 19.17.

***N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-Gly-Gly-OEt (4b).**

The product was obtained as a white solid, 0.29 g (96.1%) yield; mp: 163-165°C; t_R = 10.99 min. (93.60%). IR (KBr): 3459 (NH, amine), 3275 (NH, amide), 1733 (CO, ester) 1632 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D_6]DMSO): δ 1.14 (t, 3H, *J* = 6.9 Hz, $\underline{CH_3}CH_2$), 3.78 (s, 3H, OCH_3), 3.78 (d, 2H, *J* = 6.1 Hz, α-CH_2COO), 3.79 (s, 3H, OCH_3), 3.86 (d, 2H, *J* = 6.1 Hz, α-$\underline{CH_2}$CONH), 4.03 (q, 2H, *J* = 6.9 Hz, $\underline{CH_2}CH_3$), 8.03 (t, 1H, *J* = 6.1 Hz, NH), 8.29 (t, 1H, *J* = 6.1 Hz, N-H amide); ^{13}C-NMR (125 MHz, [D_6]DMSO): 14.57, 41.19, 44.00, 54.66, 60.92, 168.55, 169.94, 170.29, 172.21, 172.39. Anal. calcd for $C_{11}H_{17}N_5O_5$: C, 44.14; H, 5.73; N, 23.40. Found: C, 43.91; H, 5.99; N, 23.47.

***N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-Ala-Gly-OEt (4c).**

The product was obtained as a white solid, 0.26 g (85.8%) yield; mp: 108-110°C; t_R = 2.73 min. (100%). IR (KBr): 3444 (NH, amine), 3293 (NH, amide), 1759 (CO, ester), 1658 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 1.10-1.15 (m, 3H, $\underline{CH_3}CH_2$), 1.26-1.30 (m, 3H, $\underline{CH_3}CH$), 3.75-3.82 (m, 2H, α-CH_2), 3.75-3.82 (m, 6H, 2 × OCH_3), 4.01-4.03 (m, 2H, $\underline{CH_2}CH_3$), 4.37-4.42 (m, 1H, α-CH), 7.97 (s, 1H, NH), 8.26 (s, 1H, NH amide); ^{13}C-NMR (125 MHz, [D6]DMSO): 14.54, 18.26, 41.52, 50.52, 54.67, 60.89, 167.74, 170.28, 172.22, 172.35, 173.54. Anal. calcd for $C_{12}H_{19}N_5O_5$: C, 46.00; H, 6.11; N, 22.35. Found: C, 46.27; H, 6.19; N, 22.06.

N-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-Phe-Gly-OEt (4e).

The product was obtained as a white solid, 0.37 g (95.3%; mp) yield: 166-168°C; t_R = 11.37 min. (100%). IR (KBr): 3446 (NH, amine), 3283 (NH, amide), 1748 (CO, ester), 1652 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 1.13 (t, 3H, *J* = 6.9 Hz, $\underline{CH_3}CH_2$), 2.88 (dd, 1H, ^2J = 13.8 Hz, ^3J = 10.7, CH_2-Ph), 3.07 (dd, 1H, ^2J = 13.8 Hz, ^3J = 3.9 Hz, CH_2-Ph), 3.747 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.77-3.87 (m, 2H, α-CH_2), 4.04 (q, 2H, *J* = 6.9 Hz, $\underline{CH_2}CH_3$), 4.64-4.69 (m, 1H, α-CH), 7.13-7.31 (m, 5H, Ph-H), 8.02 (d, 1H, *J* = 8.4 Hz, NH), 8.53 (t, 1H, *J* = 6.1 Hz, NH amide); ^{13}C-NMR (125 MHz, [D6]DMSO): 14.54, 37.59, 41.35, 54.65, 56.58, 60.95, 126.80, 128.62, 129.69, 138.81, 168.15, 170.27, 172.13, 172.23, 172.58. Elemental Analysis Calcd for $C_{18}H_{23}N_5O_5$: C, 55.52; H, 5.95; N, 17.98. Found: C, 55.31; H, 5.76; N, 17.69.

***N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-Leu-Gly-OEt (4f).**

The product was obtained as a white solid, 0.30 g (85.3%) yield; mp: 126-128°C; t_R = 9.34 min. (100%). IR (KBr): 3441 (NH, amine), 3282 (NH, amide), 1747 (CO, ester), 1656 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 0.82 (d, 3H, *J* = 6.9 Hz, $\underline{CH_3}CH$), 0.86 (d, 3H, *J* = 6.1 Hz, $\underline{CH_3}CH$), 1.13 (t, 3H, *J* = 6.9 Hz, $\underline{CH_3}CH_2$), 1.43-1.49 (m, 1H, CH), 1.56-1.65 (m, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.69-3.83 (m, 2H, α-CH_2), 4.03 (q, 2H, *J* = 6.9 Hz, $\underline{CH_2}CH_3$), 4.39-4.44 (m, 1H, α-CH), 7.95 (d, 1H, *J* = 7.6 Hz, NH), 8.28 (t, 1H, *J* = 6.1 Hz, NH amide); ^{13}C-NMR (125 MHz, [D6]DMSO): 14.52, 21.90, 23.58, 24.75, 40.20, 53.38, 54.66, 60.87, 168.13, 170.26, 172.21, 172.38, 173.28. Elemental Analysis Calcd for $C_{15}H_{25}N_5O_5$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.91; H, 6.93; N, 19.51.

General procedure for the synthesis of *N*-(4,6-dipiperidino-1,3, 5-triazin-2-yl) dipeptide ethyl ester (5a,b,d,e,g)

HATU (0.19 g, 0.5 mmol) was added to an ice-cold solution of Nα-(4,6-dipiperidino-1,3,5-triazin-2-yl) amino acid (0.5 mmol) and DIEA (0.17 mL, 1 mmol) in DMF with stirring. A solution of glycine ethyl ester hydrochloride (0.07 g, 0.5 mmol) and DIEA (0.09 mL, 0.5 mmol) in DMF was added to this mixture. The mixture was left overnight at rt with stirring (in the case of IDA derivative, 2 mmol of HATU and amino acid ester were used with equiv. DIEA). The reaction mixture was diluted with 70 mL ethyl acetate and then washed with 5% citric acid (2 × 10 mL), a saturated NaHCO3 solution (2 × 10 mL), and a saturated NaCl solution (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed in vacuo with the aid of a water aspirator.

***N*-(4,6-Dipiperidino-1,3,5-triazin-2-yl)-IDA-(Gly-OEt)$_2$ (5a).**

The product was obtained as a white solid, 0.18 g (80.6%) yield; mp:138-140°C; IR (KBr): 3306 (NH, amide), 1744 (CO, ester), 1662 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 1.13 (t, 6H, *J* = 7.7 Hz, 2×CH_3), 1.37-1.39 (m, 8H, 4×b-CH_2), 1.54 (quint, 4H, *J* = 6.2 Hz, 2×c-CH_2), 3.57 (t, 8H, *J* = 6.2 Hz, 4×a-CH_2), 3.80 (d, 2H, *J* = 6.1 Hz, 2×α-$\underline{CH_2}$NH), 4.03 (q, 4H, *J* = 7.7 Hz, 2× O$\underline{CH_2}CH_3$), 4.10 (s, 2H, 2×α-$\underline{CH_2}$N), 8.87 (t, 2H, *J* = 6.1 Hz, 2×NH); Anal. calcd for $C_{25}H_{40}N_8O_6$: C, 54.73; H, 7.35; N, 20.42. Found: C, 54.59; H, 7.47; N, 20.33.

***N*-(4,6-Dipiperidino-1,3,5-triazin-2-yl)-Gly-Gly-OEt (5b).**
The product was obtained as a white solid, 0.14 g (70.8%) yield; mp: 152-154°C; IR (KBr): 3421 (NH, amine), 3287 (NH, amide), 1749 (CO, ester), 1665 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.21-1.26 (m, 3H, CH_3), 1.51-1.66 (m, 12H, 6×CH_2), 3.61-3.74 (m, 8H, 4×CH_2N), 3.93 (d, 1H, *J* = 5.4 Hz, α-CH_2), 4.00 (d, 1H, *J* = 5.4 Hz, α-CH_2), 4.10-4.16 (m, 2H, CH_2), 4.66-4.68 (m, 1H, α-CH_2), 4.83 (d, 2H, *J* = 5.4 Hz, α-CH_2), 6.73 (t, 1H, *J* = 5.4 Hz, NH), 7.10 (t, 1H, *J* = 5.4 Hz, NH); Anal. calcd for $C_{19}H_{31}N_7O_3$: C, 56.28; H, 7.71; N, 24.18. Found: C, 56.07; H, 7.61; N, 24.49.

***N*-(4,6-dipiperidino-1,3,5-triazin-2-yl)-Val-Gly-OEt (5d).**
The product was obtained as a white solid, 0.18 g (76.8%) yield; mp: 88-90°C; IR (KBr): 3433 (NH, amine), 1692 (CO, amide), 1749 (CO, ester) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.94-1.08 (m, 6H, 2×CH_3), 1.22-1.26 (m, 3H, $CH_2\underline{CH_3}$), 1.41-1.67 (m, 12H, 6×CH_2-pip), 2.18-2.24 (m, 1H, CH), 3.63-4.01 (m, 10H, 4×CH_2N, α-CH_2), 4.11-4.4.16 (m, 2H, CH_2), 4.64-4.72 (m, 1H, α-CH), 7.41-7.52 (m, 1H, NH), 8.69 (br. s, 1H, NH). Anal. calcd for $C_{22}H_{37}N_7O_3$: C, 59.04; H, 8.33; N, 21.91. Found: C, 59.31; H, 8.07; N, 22.05.

***N*-(4,6-Dipiperidino-1,3,5-triazin-2-yl)-Phe-Gly-OEt (5e).**
The product was obtained as a white solid, 0.21 g (83.9%) yield; mp: 112-115°C; IR (KBr): 3424 (NH, amine), 3296 (NH, amide), 1749 (CO, ester), 1687 (CO, amide) cm^{-1}; ^{1}H-NMR (125 MHz, $CDCl_3$): δ 1.22-1.26 (m, 3H, CH_3), 1.41-1.76 (m, 12H, 6×CH_2-pip), 2.97-3.36 (m, 2H, CH_2-Ph), 3.51-3.70 (m, 8H, 4×CH_2N), 3.75-4.01 (m, 2H, α-CH_2), 4.09-4.14 (m, 2H, CH_2), 5.39-5.48 (m, 1H, α-CH), 6.98-7.35 (m, 7H, 5Ph-H, 2NH); Anal. calcd for $C_{26}H_{37}N_7O_3$: C, 63.01; H, 7.52; N, 19.78. Found: C, 63.21; H, 7.27; N, 19.91.

***N*-(4,6-Dipiperidino-1,3,5-triazin-2-yl)-Ile-Gly-OEt (5g).**
The product was obtained as a white solid, 0.19 g (83.3%) yield; mp: 127-130°C; the purity of **32** was detected by reverse-phase HPLC with conditions: detection at 240 nm, using Agilent 1200 PDA detector; Eclipse plus C_8 column (3.5 µm 4.6 x 250 mm); linear gradient over 16 min (40 to 90 % CH_3CN in H_2O/ 0.1% TFA); flow rate 1.0 mL/min., t_R = 5.14 min. (14.86 %) and t_R = 11.19 min. (85.14 %). IR (KBr): 3429 (NH, amine), 3381 (NH, amide), 1749 (CO, ester), 1690 (CO, amide) cm^{-1}; ^{1}H-NMR (400 MHz, [D6]DMSO) Isomer A (85.14%): δ 0.79-0.85 (m, 3H, CH_3), 0.88-0.94 (m, 3H, CH_3), 1.15 (t, 3H, *J* = 6.6 Hz, $\underline{CH_3}CH_2O$), 1.35-1.61 (m, 14H, 6 × $\underline{CH_2}$, $\underline{CH_2}CH_3$), 1.89-1.93 (m, 1H, $\underline{CH}CH_3$), 3.54-3.71 (m, 8H, 4 × CH_2N), 3.75-3.90 (m, 2H, α-CH_2), 4.05 (q, 2H, *J* = 6.6 Hz, O$\underline{CH_2}CH_3$), 5.55 (t, 1H, *J* = 8.8 Hz, α-CH), 6.18-6.23 (m, 1H, NH), 7.71-7.82 (m, 1H, NH); Isomer B (14.86 %): δ 0.79-0.85 (m, 3H, CH_3), 0.88-0.94 (m, 3H, CH_3), 1.15 (t, 3H, *J* = 6.6 Hz, $\underline{CH_3}CH_2O$), 1.35-1.61 (m, 14H, 6 × CH_2, $\underline{CH_2}CH_3$), 1.89-1.93 (m, 1H, $\underline{CH}CH_3$), 3.54-3.71 (m, 8H, 4 × CH_2N), 4.05 (q, 2H, *J* = 6.6 Hz, O$\underline{CH_2}CH_3$), 4.25 (t, 1H, *J* = 8.8 Hz, α-CH), 4.70 (d, 2H, *J* = 9.5 Hz, α-CH_2), 6.27 (d, 1H, *J* = 8.1 Hz, NH), 8.21-8.27 (m, 1H, NH). ^{13}C-NMR (100 MHz, [D6]DMSO): 11.41, 11.77, 14.56, 16.10, 18.30, 24.71, 25.02, 25.83, 25.98, 44.45, 60.99, 164.53, 167.90, 170.13, 170.61, 175.00. Anal. calcd for $C_{23}H_{39}N_7O_3$: C, 59.85; H, 8.52; N, 21.24. Found: C, 59.99; H, 8.37; N, 21.04.

General procedure for the synthesis of *N*-(4,6-dimorpholino-1,3,5-triazin-2-yl) dipeptide ethyl ester (6a,b,d,e,g)
HATU (0.19 g, 0.5 mmol) was added to an ice-cold solution of N^{α}-(4,6-dimorpholino-1,3,5-triazin-2-yl) amino acid (0.5 mmol) and DIEA (0.17 mL, 1 mmol) in DMF with stirring. A solution of glycine ethyl ester hydrochloride (0.07 g, 0.5 mmol) and DIEA (0.09 mL, 0.5 mmol) in DMF was added to this mixture. The mixture was left stirring at rt overnight (in the case of IDA derivative, 2 mmol of HATU and amino acid ester were used with equiv. DIEA). The reaction mixture was diluted with ethyl acetate and then washed with 5% citric acid (2 × 10 mL), a saturated $NaHCO_3$ solution (2 × 10 mL), and a saturated NaCl solution (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed in vacuo with the aid of a water aspirator.

***N*-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-IDA-(Gly-OEt)$_2$ (6a).**
The product was obtained as a white solid, 0.24 g (86.9%) yield; mp: 211-214°C; IR (KBr): 3299 (NH, amide), 1748 (CO, ester), 1657 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 1.13 (t, 6H, *J* = 6.9 Hz, 2×CH_3), 3.50-3.53 (m, 8H, 4×CH_2N), 3.55-3.58 (m, 8H, 4×CH_2O), 3.80 (d, 4H, *J* = 5.4 Hz, 2×α-CH_2COO), 4.02 (q, 4H, *J* = 6.9 Hz, 2×O$\underline{CH_2}CH_3$), 4.10 (s, 4H, 2×α-$\underline{CH_2}$CONH), 8.88 (t, 2H, *J* = 5.4 Hz, 2×NH amide); ^{13}C-NMR (125 MHz, [D6]DMSO): 14.54, 43.73, 52.74, 56.57, 60.93, 66.54, 164.97, 165.35, 170.14, 171.41. Anal. calcd for $C_{23}H_{36}N_8O_8$: C, 49.99; H, 6.57; N, 20.28. Found: C, 49.84; H, 6.71; N, 20.35.

***N*-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-Gly-Gly-OEt (6b).**
The product was obtained as a white solid, 0.20 g (94.2%) yield; mp: 204-206°C; IR (KBr): 3419 (NH, amine), 3287 (NH, amide), 1753 (CO, ester), 1663 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 1.13 (t, 3H, *J* = 6.9 Hz, CH_3), 3.53-3.65 (m, 8H, 4×CH_2N), 3.53-3.65 (m, 8H, 4×CH_2O), 3.76-3.79 (m, 2H, α-CH_2), 4.03 (q, 2H, *J* = 6.9 Hz, CH_2), 4.52-4.63 (m, 2H, α-CH_2), 6.93-6.97 (m, 1H, NH), 8.05-8.10 (m, 1H, NH). Anal. calcd for $C_{17}H_{27}N_7O_5$: C, 49.87; H, 6.65; N, 23.95. Found: C, 50.06; H, 6.43; N, 23.87.

***N*-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-Val-Gly-OEt (6d).**
The product was obtained as a white solid, 0.20 g (87.1%) yield; mp: 161-164°C; IR (KBr): 3445 (NH, amine), 3289 (NH, amide), 1729 (CO, ester), 1655 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 0.86 (d, 3H, *J* = 6.9 Hz, $\underline{CH_3}CH$), 0.88 (d, 3H, *J* = 6.9 Hz, $\underline{CH_3}CH$), 1.12 (t, 3H, *J* = 6.9 Hz, $\underline{CH_3}CH_2O$), 1.95 (octet, 1H, *J* = 6.9 Hz, CH), 3.52 (br. s, 8H, 4×CH_2N), 3.58 (br. s, 8H, 4×CH_2O), 3.70 (dd, 1H, *J* = 16.8 Hz, *J* = 4.6 Hz, α-CH_2), 3.81 (dd,

1H, *J* = 16.8 Hz, *J* = 5.4 Hz, α-CH_2), 4.02 (q, 2H, *J* = 6.9 Hz, O$\underline{CH_2}CH_3$), 4.10-4.15 (m, 1H, α-CH), 6.47 (d, 1H, *J* = 8.4 Hz, NH), 8.23 (t, 1H, *J* = 5.4 Hz, NH); ^{13}C-NMR (125 MHz, [D6]DMSO): 14.55, 19.25, 19.82, 30.76, 41.15, 43.71, 60.33, 60.88, 66.59, 165.07, 165.18, 166.21, 170.31, 173.02. Anal. calcd for $C_{20}H_{33}N_7O_5$: C, 53.20; H, 7.37; N, 21.71. Found: C, 53.55; H, 7.18; N, 21.54.

***N*-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-Phe-Gly-OEt (6e).**
The product was obtained as a white solid, 0.22 g (89.7%) yield; mp: 135-138ºC; IR (KBr): 3428 (NH, amine), 1745 (CO, ester), 1681 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ isomer A (55.6 %): δ 1.12-1.16 (m, 3H, CH_3), 2.94-3.12 (m, 2H, CH_2-Ph), 3.45-3.56 (m, 8H, 4×CH_2N), 3.45-3.56 (m, 8H, 4×CH_2O), 3.78-3.83 (m, 2H, α-CH_2), 4.01-4.06 (m, 2H, CH_2), 4.54-4.58 (m, 1H, α-CH), 6.76 (d, 1H, *J* = 7.7 Hz, NH), 7.02-7.18 (m, 5H, Ph-H), 7.22 (t, 1H, *J* = 6.9 Hz, NH); isomer B (44.4 %): δ 1.12-1.16 (m, 3H, CH_3), 2.94-3.12 (m, 2H, CH_2-Ph), 3.45-3.56 (m, 8H, 4×CH_2N), 3.45-3.56 (m, 8H, 4×CH_2O), 4.01-4.06 (m, 2H, CH_2), 5.44-5.42 (m, 2H, α-CH_2), 5.93-5.96 (m, 1H, α-CH), 7.02-7.18 (m, 5H, Ph-H), 7.29 (d, 1H, *J* = 7.7 Hz, NH), 7.43 (t, 1H, *J* = 6.9 Hz, NH); Anal. calcd for $C_{24}H_{33}N_7O_5$: C, 57.70; H, 6.66; N, 19.63. Found: C, 57.89; H, 6.51; N, 19.49.

***N*-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-Ile-Gly-OEt (6g).**
The product was obtained as a white solid, 0.21 g (86.8%) yield; mp: 170-172ºC; IR (KBr): 3438 (NH, amine), 3301 (NH, amide), 1727 (CO, ester), 1651 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 0.79 (t, 3H, *J* = 6.9 Hz, $\underline{CH_3}CH_2$), 0.85 (d, 3H, *J* = 6.1 Hz, $\underline{CH_3}$CH), 1.12 (t, 3H, *J* = 6.9 Hz, $\underline{CH_3}CH_2$O), 1.10-1.14 (m, 1H, $\underline{CH_2}CH_3$), 1.43-1.50 (m, 1H, $\underline{CH_2}$CH3), 1.70-1.75 (m, 1H, $\underline{CH}CH_3$), 3.52 (br. s, 8H, 4×CH_2N), 3.58 (br. s, 8H, 4×CH_2O), 3.68-3.84 (m, 2H, α-CH_2), 4.02 (q, 2H, *J* = 6.9 Hz, O$\underline{CH_2}CH_3$), 4.19 (t, 1H, *J* = 7.7 Hz, α-CH), 6.48 (d, 1H, *J* = 7.7 Hz, NH), 8.23 (t, 1H, *J* = 5.4 Hz, NH); ^{13}C-NMR (125 MHz, [D6]DMSO): 11.47, 14.54, 15.88, 25.27, 36.93, 41.16, 43.70, 59.05, 60.87, 66.59, 165.09, 165.19, 166.09, 170.29, 173.00. Anal. calcd for $C_{21}H_{35}N_7O_5$: C, 54.18; H, 7.58; N, 21.06. Found: C, 54.41; H, 7.43; N, 21.11.

General procedure for the synthesis of *N*-(4,6-dimethoxy-1,3,5-triazin-2-yl) dipeptide amide derivatives (7a,c,d,e,g)
HATU (0.38 g, 1 mmol) was added to an ice-cold solution of N^{α}-(4,6-dimethoxy-1,3,5-triazin-2-yl) amino acid (1 mmol) and DIEA (0.34 mL, 2 mmol) in DMF with stirring. A solution of L-valine amide hydrochloride (0.15 g, 1 mmol) and DIEA (0.17 mL, 1 mmol) in DMF was added to this mixture. The mixture was left overnight at rt with stirring (in the case of IDA derivative, 2 mmol of HATU and amino acid ester were used with equiv. DIEA). The reaction mixture was diluted with 70 mL ethyl acetate and then washed with 5% citric acid (2 × 10 mL), a saturated $NaHCO_3$ solution (2 × 10 mL), and a saturated NaCl solution (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed in vacuo with the aid of a water aspirator.

***N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-IDA-(Val-NH_2)$_2$ (7a).**
The product was obtained as a white solid, 0.40 g (85.0%) yield; mp: 260-262ºC; IR (KBr): 3397 (NH, amide), 1654 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 0.74 (d, 6H, *J* = 6.9 Hz, 2×CH_3), 0.77 (d, 6H, *J* = 6.1 Hz, 2×CH_3), 1.96 (m, 2H, 2×CH), 3.75 (s, 6H, 2×OCH_3), 4.05-4.09 (m, 2H, 2×α-CH), 4.17-4.30 (m, 4H, 2×α-CH_2), 6.99 (s, 2H, 2×NH), 7.23 (s, 2H, 2×NH), 8.53 (d, 2H, *J* = 9.2 Hz, 2×NH); ^{13}C-NMR (125 MHz, [D6]DMSO): 18.12, 19.74, 30.62, 53.31, 54.81, 58.18, 167.61, 169.72, 172.12, 173.39. Anal. calcd for $C_{19}H_{32}N_8O_6$: C, 48.71; H, 6.88; N, 23.92. Found: C, 48.55; H, 7.17; N, 23.69.

***N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-Ala-Val-NH_2 (7c).**
The product was obtained as a white solid, 0.29 g (88.2%) yield; mp: 244-246ºC; IR (KBr): 3463 (NH, amide), 1648 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 0.73-0.82 (m, 6H, 2×a-CH_3), 1.22-1.26 (m, 3H, b-CH_3), 1.60-1.94 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.03-4.09 (m, 1H, α-$\underline{CH}$CH), 4.40-4.49 (m, 1H, α-$\underline{CH}CH_3$), 7.00-7.02 (m, 1H, NH_2), 7.37-7.46 (m, 1H, NH_2), 7.62-7.76 (m, 1H, NH), 7.88-8.01 (m, 1H, N-H); ^{13}C-NMR (125 MHz, [D6]DMSO): 11.34, 14.42, 18.41, 19.77, 31.25, 50.61, 54.70, 57.90, 167.67, 172.20, 172.40, 172.79, 173.41. Anal. calcd for $C_{13}H_{22}N_6O_4$: C, 47.84; H, 6.79; N, 25.75. Found: C, 47.61; H, 6.99; N, 25.86.

***N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-Val-Val-NH_2 (7d).**
The product was obtained as a white solid, 0.29 g (83.1%) yield; mp: 203-206ºC; IR (KBr): 3441 (NH, amide), 1662 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 0.77 (d, 3H, *J* = 7.7 Hz, a-CH_3), 0.78 (d, 3H, *J* = 7.7 Hz, b-CH_3), 0.86 (d, 6H, *J* = 6.9 Hz, 2×c-CH_3), 1.85-1.89 (m, 1H, CH), 2.03 (octet, 1H, *J* = 6.9 Hz, CH), 3.80 (s, 6H, 2 × OCH_3), 4.07-4.12 (m, 1H, α-CH), 4.29 (t, 1H, *J* = 7.7 Hz, α-CH), 7.00 (s, 1H, NH_2), 7.38 (s, 1H, NH_2), 7.69 (d, 1H, *J* = 8.4 Hz, NH), 7.89 (d, 1H, *J* = 9.1 Hz, NH); ^{13}C-NMR (125 MHz, [D6]DMSO): 18.24, 18.52, 19.17, 19.75, 19.80, 30.63, 31.23, 54.72, 57.79, 60.85, 168.34, 171.31, 172.23, 172.38, 173.24. Anal. calcd for $C_{15}H_{26}N_6O_4$: C, 50.83; H, 7.39; N, 23.71. Found: C, 51.07; H, 7.11; N, 23.61.

***N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-Phe-Val-NH_2 (7e).**
The product was obtained as a white solid, 0.34 g (83.5%) yield; mp: 224-226ºC; IR (KBr): 3282 (NH, amide), 1664 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 0.75-0.86 (m, 6H, 2×$\underline{CH_3}$), 1.92 (octet, 1H, *J* = 6.9 Hz, CH), 2.84-2.94 (m, 1H, CH_2-Ph), 2.99-3.05 (m, 1H, CH_2-Ph), 3.75 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 4.08-4.15 (m, 1H, α-$\underline{CH}$CH), 4.70-4.75 (m, 1H, α-$\underline{CH}CH_2$), 7.03 (s, 1H, NH), 7.10-7.31 (m, 5H, Ph-H), 7.41 (s, 1H, NH), 7.90 (d, 1H, *J* = 9.2 Hz, NH), 8.02 (d, 1H, *J* = 8.4 Hz, NH amide); ^{13}C-NMR (125 MHz, [D6]DMSO): 18.38, 18.61, 19.74, 31.28, 37.51, 54.66, 56.49, 57.85, 58.18, 126.77, 128.58, 129.71, 138.70, 168.07, 171.73, 172.12, 172.26, 173.26. Anal. calcd for $C_{19}H_{26}N_6O_4$: C, 56.70; H, 6.51; N, 20.88. Found: C, 56.96; H, 6.23; N, 20.76.

***N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-Ile-Val-NH_2 (7g).**
The product was obtained as a white solid, 0.35 g (94.0%) yield; mp: 205-208ºC; IR (KBr): 3439 (NH, amide), 1656 (CO, amide) cm^{-1}; ^{1}H-NMR (500 MHz, [D6]DMSO): δ 0.76-0.83 (m, 12H, 4×CH_3), 1.10-1.24 (m, 1H, CH), 1.41-1.44 (m, 1H, CH), 1.81-1.90 (m, 2H, CH_2), 3.79 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.10

(t, 1H, *J* = 8.4 Hz, α-CH), 4.33 (t, 1H, *J* = 8.4 Hz, α-CH), 7.01 (s, 1H, NH), 7.41 (s, 1H, NH), 7.77 (d, 1H, *J* = 8.4 Hz, NH), 7.93 (d, 1H, *J* = 8.4 Hz, NH); ^{13}C-NMR (125 MHz, [D6]DMSO): 11.27, 15.91, 18.54, 19.75, 25.25, 31.20, 36.57, 54.69, 54.76, 57.85, 59.61, 168.19, 171.36, 172.22, 172.39, 173.28. Anal. calcd for $C_{16}H_{28}N_6O_4$: C, 52.16; H, 7.66; N, 22.81. Found: C, 51.00; H, 7.81; N, 22.67.

***N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-Gly-Phe-Val-NH$_2$ (8).**

HATU (0.76 g, 2 mmol) was added to an ice-cold solution of Boc-L-phenyl alanine (0.53 g, 2 mmol) and DIEA (0.7 mL, 4 mmol) in DMF with stirring. A solution of L-valine amide hydrochloride (0.30 g, 2 mmol) and DIEA (0.35 mL, 2 mmol) in DMF was added to this mixture. The reaction mixture was stirred at 0°C for 1 h and overnight at rt. The mixture was diluted with 70 ml of ethyl acetate and then washed with 5% citric acid (2 × 10 mL), saturated $NaHCO_3$ (2 × 10 mL), and saturated NaCl solution (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed in vacuo with the aid of a water aspirator. The crude product was dried under reduced pressure, to afford a white solid of Boc-Phe-Val-NH_2 in an overall yield 0.65 g (89.5%), m.p. 193-194°C. The crude product (0.64 g, 1.76 mmol) and p-toluene sulfonic acid (1.01 g, 5.29 mmol) were dissolved in acetonitrile (10 mL), and stirred for 2 h to remove the Boc-group, thereby forming the H-Phe-Val-NH_2.*p*-TsOH. The product was filtered and washed with acetonitrile to give an overall yield of 0.651 g (85%), m.p. 234-235°C. The crude H-Phe-Val-NH_2.*p*-TsOH was allowed to undergo a further coupling as follows: HATU (0.062 g, 0.069 mmol) was added to a stirred solution of *N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-Gly-OH **1b** (0.015 g, 0.069 mmol), the crude H-Phe-Val-NH_2.*p*-TsOH (0.03 g, 0.069 mmol) and DIEA (0.015 mL, 0.207 mmol) in DMF (3 mL) at 0°C. The reaction mixture was stirred at 0°C for 1 h and overnight at rt. The mixture was diluted with 70 ml of ethyl acetate and then washed with 5 % citric acid (2 x 10 ml), saturated $NaHCO_3$ (2 x 10 ml), and saturated NaCl (2 x 10 ml). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed in vacuo with the aid of a water aspirator. The crude *N*-(4,6-dimethoxy-1,3,5-triazin-2-yl)-Gly-Phe-Val-NH_2 was dried under reduced pressure. The purity of **8** was measured by reverse-phase HPLC with the following conditions: detection at 220 nm, using an Agilent 1200 PDA detector; Eclipse plus C_{18} column (3.5 µm 4.6 x 100 mm); linear gradient over 14 min (0 to 50 % CH_3CN in H_2O/ 0.1% TFA); and flow rate 1.0 mL/min., t_R = 9.05 min. (100 %). Yield: 0.02 g (66.7 %), mp: 264-266ºC. For exact mass determination, a sample of **8** was prepared by dissolution in H_2O/CH_3CN and dilution in H_2O/CH_3CN/1%TFA: *m*/*z* = 482.201 $[M+Na]^+$.

***N*-(4,6-dipiperidino-1,3,5-triazin-2-yl)-Gly-Phe-Val-NH$_2$ (9)**

HATU (0.087 g, 0.23 mmol) was added to an ice-cold solution of *N*-(4,6-dipiperidino-1,3,5-triazin-2-yl)-Gly-OH **2b** (0.074 g, 0.23 mmol) and DIEA (0.081 mL, 0.46 mmol) in DMF with stirring. A solution of H-Phe-Val-NH_2.*p*-TsOH (0.1 g, 0.23 mmol) and DIEA (0.041 mL, 0.23 mmol) in DMF was added to this mixture. The reaction mixture was stirred at 0°C for 1 h and overnight at rt. The mixture was diluted with 50 ml of ethyl acetate and then washed with 5% citric acid (2 × 10 mL), saturated $NaHCO_3$ (2 × 10 mL), and saturated NaCl solution (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed in vacuo with the aid of a water aspirator. The crude product was dried under reduced pressure. Yield: 0.073 g (56.2 %), mp: 242-246°C. The purity of **9** was measured by reverse-phase HPLC with the following conditions: detection at 220 nm using an Agilent 1200 PDA detector; Eclipse plus C_{18} column (3.5 µm 4.6 x 100 mm); linear gradient over 18 min (0 to 50 % CH_3CN in H_2O/ 0.1% TFA); and flow rate 1.0 mL/min., t_R = 5.11 min. (90.84 %), t_R = 6.49 min. (9.16%). For exact mass determination, a sample of **9** was prepared, by dissolution in H_2O/CH_3CN and dilution in H_2O/CH_3CN/1%TFA: *m*/*z* = 566.613 $[M+H]^+$.

***N*-(4,6-dimorpholino-1,3,5-triazin-2-yl)-Gly-Phe-Val-NH$_2$ (10).**

HATU (0.087 g, 0.23 mmol) was added to an ice-cold solution of *N*-(4,6-dimorpholino-1,3,5-triazin-2-yl)-Gly-OH **3b** (0.075 g, 0.23 mmol) and DIEA (0.081 mL, 0.46 mmol) in DMF with stirring. A solution of H-Phe-Val-NH_2.*p*-TsOH (0.1 g, 0.23 mmol) and DIEA (0.041 mL, 0.23 mmol) in DMF was added to this mixture. The reaction mixture was stirred at 0°C for 1 h and overnight at rt. It was then diluted with 50 ml of ethyl acetate, and then washed with 5% citric acid (2 × 10 mL), saturated $NaHCO_3$ (2 × 10 mL), and then saturated NaCl (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed in vacuo with the aid of a rotary evaporator. The crude product was dried under reduced pressure. The purity of **10** was measured by reverse-phase HPLC with the following conditions: detection at 220 nm (Agilent 1200 PDA detector); Eclipse plus C_8 column (3.5 µm 4.6 x 250 mm); linear gradient over 15 min (30 to 55 % CH_3CN in H_2O/ 0.1% TFA); and flow rate 1.0 mL/min., t_R = 8.54 min. (84.50 %), t_R = 9.25 min. (15.50 %). Yield: 0.05 g (38.5 %), mp: 259-262°C. For exact mass determination, a sample of **10** was prepared by dissolution in H_2O/CH_3CN and dilution in H_2O/CH_3CN/1%TFA: *m*/*z* = 570.568 $[M+H]^+$.

General Procedure for Solid-Phase Assembly of Model Peptides (11-13)

The general synthesis was carried out manually using a disposable plastic syringe attached to a water aspirator as a reaction vessel. The synthesis was carried out as follows: 100 mg of Fmoc-Rinkamide-AM-PS resin (0.059 mmol/g) in a 10-mL disposable syringe fitted with a teflon filter was washed with CH_2Cl_2 (3 x 10 mL) and DMF (3 x 10 mL) and deprotected with 10 mL of 20% piperidine in DMF for 7 min. The deprotected resin was washed with DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL) and again with DMF (3 x 10 mL). Preactivation was carried out for 5 min using Fmoc-L-Val-OH or Fmoc-L-Leu-OH (0.236 mmol, 4 equiv.), HATU (0.09 g, 0.236 mmol, 4 equiv.), and DIEA (0.09 mL, 0.472 mmol, 8 equiv.) in 0.2 ml of DMF. The solution of the activated amino acid was added to the resin, and the resulting mixture was periodically stirred with a teflon stick every 5 min over a period of 2 h. The loaded resin was washed with DMF (3

x 10 mL), and the Fmoc group was removed with 10 mL of 20 % piperidine in DMF for 7 min. Washing of the deblocked resin with DMF, CH_2Cl_2, and DMF was followed by an analogous coupling step with the second Fmoc-L-amino acid. Subsequent L-amino acids were added in the same manner. To complete the synthesis of the desired peptides, the previously synthesized peptide-resin underwent one of the following three routes (route i or ii).

Route i: The peptide-resin was treated with 10 mL of 50% TFA in CH_2Cl_2 with shaking for 2.5 h. The peptide formed was filtered and concentrated in vacuo and then precipitated by the addition of cold ether (40 mL). The crude peptide was filtered. The free peptide underwent an additional coupling step with *N*-(4,6-disubstituted-1,3,5-triazin-2yl)-Gly-OH (**1b**, **2b** or **3b**) to afford the desired *N*-(4,6-disubstituted-1,3,5-triazin-2yl)-peptides.

Route ii: The peptide-resin was treated with 10 mL of 50 % TFA in CH_2Cl_2 with shaking for 2.5 h. The peptide formed was filtered and concentrated in vacuo and then precipitated by the addition of cold ether (40 mL). The crude peptide was filtered. The free peptide reacts with 2-chloro-4,6-dimethoxy-1,3,5-triazine in the presence of triethyl amine at rt overnight to give *N*-(4,6-dimethoxy-1,3,5-triazin-2yl)-peptide after neutralization with 1N HCl.

***N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-Gly-Gly-Phe-Val-NH_2 (11).**
Route i or Route ii**,** the sticky product was obtained, yield 0.03 g (58.8%). Purity was detected by reverse-phase HPLC with the following conditions: detection at 220 nm (Agilent 1200 PDA detector); Eclipse plus C_{18} column (3.5 µm 4.6 x 100 mm); linear gradient over 14 min (0 to 50 % CH_3CN in H_2O/ 0.1% TFA); and flow rate 1.0 mL/min., t_R = 3.42 min. (100%). For exact mass determination, a sample of **11** was prepared by dissolution in H_2O/CH_3CN and dilution in H_2O/CH_3CN/1%TFA: m/z (MALDI-TOF/TOF) = $[M+H]^+$ = 517.644.

***N*-(4,6-Dipiperidino-1,3,5-triazin-2-yl)-Gly-Gly-Phe-Val-NH_2 (12).**
Route i, the product was obtained as a white solid, 0.042 g (68.0 %) yield; mp: 234-237ºC. Purity was detected by reverse-phase HPLC with the following conditions: detection at 220 nm (Agilent 1200 PDA detector); Eclipse plus C_{18} column (3.5 µm 4.6 x 100 mm); linear gradient over 20 min (0 to 50 % CH_3CN in H_2O/ 0.1% TFA); and flow rate 1.0 mL/min., t_R = 10.71 min. (98.28 %). For exact mass determination, a sample of **12** was prepared by dissolution in H_2O/CH_3CN and dilution in H_2O/CH_3CN/1%TFA: m/z (MALDI-TOF/TOF)= $[M+H]^+$ = 623.548.

***N*-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-Gly-Gly-Phe-Val-NH_2 (13).**
Route i, the product was obtained as a white solid, 0.045 g (71%) yield; mp: 242-245°C. Purity was determined by reverse-phase HPLC with the following conditions: detection at 220 nm (Agilent 1200 PDA detector); Eclipse plus C_{18} column (3.5 µm 4.6 x 100 mm); linear gradient over 20 min (0 to 50 % CH_3CN in H_2O/ 0.1% TFA); and flow rate 1.0 mL/min., t_R = 16.54 min. (70.79 %), t_R = 17.41 min. (29.21 %). For exact mass determination, a sample of **13** was prepared by dissolution in H_2O/CH_3CN and dilution in H_2O/CH_3CN/1%TFA: m/z (MALDI-TOF/TOF) = $[M+H]^+$ = 627.350.

Biology
The protozoan used in this study was *L. aethiopica*, which is the main causal agent of cutaneous leishmaniasis in Ethiopia.

Culture medium for anti-leishmanial activity
RPMI-1640 (Gibco, Invitrogen Co., UK), 10% heat-inactivated fetal calf serum (HIFCS), penicillin-streptomycin and 1% L-glutamine, all from Sigma Chem. Co., St. Louis, USA, were used to make complete culture media.

In vitro antipromastigote activity
All the compounds, dissolved in DMSO to a final concentration of 1 mg/mL, were evaluated for antileishmanial activity. The final concentration of DMSO did not exceed 0.1% and thus had no effect on the parasite. Both test and standard solutions were serially diluted to appropriate concentrations using fresh complete media.[7b, 28a,b] The compounds were prepared by serial dilutions (starting from 10 to 0.04 µg/mL). Amphotericin B deoxycholate and miltefosine were used as positive controls for comparison of the antileishmanial activity of the selected compounds and were used in serial dilutions. Promastigote forms of *L. aethiopica* were used for the assay. 100 µL of culture media containing 3 x 10^6 promastigotes of *L. aethiopica* was seeded in each well of a 96-well flat-bottomed plate. Various dilutions of the test compounds (10, 3.33, 1.11, 0.37, 0.12, 0.04 µg/mL) were added to the parasites. The assay was done in triplicate. Wells containing only the parasites, media and DMSO were used as negative controls. The plates were then kept at rt (21 ± 1°C). After 24 h, 10 µL of Alamar blue (12.5 mg resazurin dissolved in 100 ml of distilled water)[28c] was added to each well. Absorbance of the resulting mixture was measured after 48 h at 540 and 630 nm using a plate reader. Alamar blue works through the conversion of resazurin (7-hydroxy-3H-phenoxazine-3-one-10-oxide), the active ingredient of Alamar blue® (blue and non-fluorescent), to resorufin (pink and highly fluorescent) through reduction reactions of metabolically active cells. The amount of fluorescence produced is proportional to the number of living cells.[28d,e] It is worth mentioning that the assay method used in this article was validated according to the reported procedures.[27] It was found that, there is a direct and linear correlation between the fluorescence intensity and the cell concentration (promastigote or amastigote).

In vitro anti-amastigote activity
Axenic amastigote were produced appling the method described by Teixeira *et al*.[30] The compounds were serially diluted in a 96-well microtitre plate to a final test concentration of 0.04-10 µg/mL in 50 µL culture medium, and 50-µL suspensions containing 2 x 10^7 cells/ml axenic amastigotes were added to each well. The plate contents were then incubated in a humidified atmosphere at 31ºC under a 5% CO_2 for 72 h. After 68 h of incubation, a 10 µL of fluorochrome resazurin solution (12.5 µg dissolved in 100 mL of PBS (pH = 7.2)) was added to each well and the fluorescence intensity was measured after a total incubation time of 72 h using 37 Victor3 Multilabel Counter at excitation

wavelength of 530 nm and emission wavelength of 590 nm. The IC_{50} value for each compound was evaluated from sigmoidal dose-response curves using Graph pad prism 3.0 software. The results were expressed as mean ± SD of triplicate experiments with each test concentration measured in duplicate. Assays with standard anti-leishmanial drugs and negative controls (medium alone and 1% DMSO) were also performed to have reference values. The background fluorescence intensity of each compound and reference drug was measured.[31]

In vitro cytotoxicity assay

The cytotoxicity of the compounds was tested in the Vero cell line using the Mosmann method, with certain modifications, as described in the literature.[32] Briefly, the cells were incubated for 72 h with a range of dilutions of the selected compounds, using MTT as reagent for the detection of cytotoxicity. 50% cytotoxic concentration (CC_{50}) values represent the concentration of compound required to kill 50% of the cells. The SI (Table 3) was calculated using the formula, $SI = CC_{50}/IC_{50}$

In vivo acute toxicity testing

The most active compounds, namely **7a, 7e, 7d** and **7g**, were tested for acute oral toxicity in mice. Six groups of mice, each group consisting of six males (25-30 g), were used for this purpose.[33a] The mice in each group were fasted overnight and weighed prior to the test. The compounds were prepared in suspension form in aqueous vehicle containing 1% gum acacia. Mice in group 1 to 5 were given dose in ascending order by oral gavage. Group I received 25 mg/kg/day, group II 50 mg/kg/day, group III 100 mg/kg/day, group IV 200 mg/kg/day and group V 250 mg/kg/day of body weight of the compounds as single dose on only one day, while group 6 was treated orally with the vehicle gum acacia (control group) at a maximum dose of 1 mL/100 g of body weight. The percentage of mortality in each group was recorded after 24 h and followed up to seven days.[33b, c] Additionally, the acute toxicity of the compounds when administered through the parenteral route was examined in groups comprising six mice, as reported earlier.[33d] The compounds, or their vehicle, namely propylene glycol (control), were administered by intraperitoneal injection in doses of 10, 25, 50, 75, 100 mg/kg of body weight. The percentage of animals surviving was monitored up to seven days.[33e]

The kidney, spleen and liver of the mice that received 250 mg/kg of the test compounds orally or 100 mg/kg parenterally showed normal textures. Histopathological studies of kidney, spleen and liver specimens did not show any abnormalities.

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Ethical conduct of research

The protocols used in this study followed the guidelines set in 'The Guide for the Care and Use of Laboratory Animals,' and got approval by ACUC, Faculty of Pharmacy, Alexandria University, Project No. ACUC17/18, at 29/4/2017 ACUC17/18.

Conflicts of Interest

The authors declare no conflict of interest.

Keywords: 1,3,5-Triazine derivatives • Peptide • Morpholine • Piperidine • Antileishmanial

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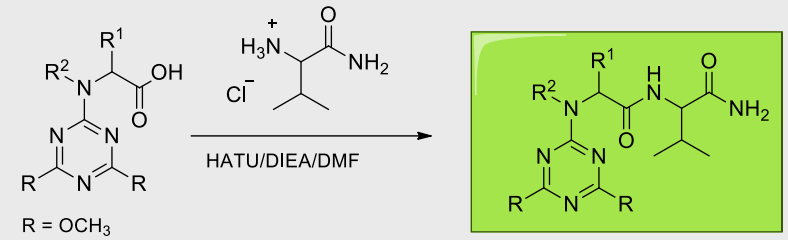
Entry for the Table of Contents

Antipromastigote and Antiamastigote Activity



	Antipromastigote	*Antiamastigote*
R = OCH_3, R^1 =H R^2 =CH_2COVal-NH_2	IC_{50} = 1.4 μM	IC_{50} = 0.22 μM
Miltefosine	IC_{50} = 7.8 μM	IC_{50} = 0.74 μM

The antileishmanial activity of the 1,3,5-triazino-peptide derivatives revealed that four dipeptide amide derivatives showed better antipromastigote or antiamastigote activity than that of the reference standard drug miltefosine with no significance acute toxicity.