Bioorganic & Medicinal Chemistry Letters 26 (2016) 1772–1775

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Individual and combined antiparasitic effect of six plant metabolites against *Leishmania amazonensis* and *Trypanosoma cruzi*

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ARTICLE INFO

Article history:
Received 22 December 2015
Revised 15 February 2016
Accepted 16 February 2016
Available online 17 February 2016

Keywords:
Secondary metabolites
Antiparasitic effect
Activity in combination

ABSTRACT

Six plant metabolites including isobavachalcone (**1**), 4-hydroxylonchocarpine (**2**), and (*E*)-1-(2,2-dimethyl-2*H*-chromen-6-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**3**), 6,8-(di-3-methyl-but-2-enyl) eriodictyol (**4**), damnacanthal (**5**), and buesgenine (**6**) were evaluated for their leishmanicidal and trypanocidal activities against intracellular amastigotes of *Leishmania amazonensis* and *Trypanosoma cruzi*. Compounds **2**–**4** and **6** displayed antileishmanial activity while **3** and **5** showed trypanocidal effect. The leishmanicidal activity of **6** was expressed with the lowest IC_{50} (5.70 μg/mL) whereas the most trypanocidal metabolite (**5**) showed its activity with IC_{50} at 11.14 μg/mL. In addition, antiprotozoal effect of mixtures of **1**–**6** prepared at different ratios (3:1, 1:1, and 1:3) was also investigated. Interestingly, **1** and **2** initially inactive against *T. cruzi*, displayed trypanocidal activities when mixed together. This activity increased when **3** (13.63 μg/mL) was combined with **1** in ratios 1:1 (10.01 μg/mL) and 3:1 (7.78 μg/mL). Moreover, the leishmanicidal effect of **4** against *L. amazonensis* increased in the mixture **6**/**4** (1:3).

Plant metabolites have been widely investigated for their antileishmanial and antitrypanosomal activities.[1–5] Independently of the metabolites classes (alkaloids, terpenoids, flavonoids and other polyphenols), significant to no antiprotozoal effects were observed.[1,5] Despite the continuous search for drugs against neglected diseases (NDs) from natural sources, only few of them derive from plants.[6,7] However, natural products remain one of the promising sources of new hits and can also inspire the development of bioactive analogs to combat resistant forms of these protozoan parasites. It is worth mentioning that parasitic diseases such as leishmaniasis and trypanosomiasis have a significant negative impact on developing countries demography by causing death of thousands of people every year. According to WHO statistics, 42.2% of worldwide countries including sub-Saharan, Latin America, South and Middle-East Asian countries were endemic areas in 2013 of cutaneous and/or visceral leishmaniasis.[8] Besides, up to 7 million cases of trypanosomiasis infection have been diagnosed worldwide with prevalence in Latin America.[9] Chagas disease is transmitted mainly by the contact with infected triatomine faeces, but other forms such as congenital, blood transfusion, organ transplantation and ingestion of contaminated food are also involved in the parasite transmission.

Sandflies are known as the vector of *Leishmania* parasites responsible for the cutaneous, mucocutaneous, and visceral forms of the disease. Through decades, these protozoans have developed resistance which combined to the toxicity of available drugs used for the treatment led to a high demand of new therapeutic entities.[10] Therefore, the investigation of the antiprotozoan activity of secondary metabolites remains a crucial contribution for the development of new antiparasitic chemotherapy. Literature reported various alkaloids, anthraquinones, chalcones and flavonoids with exciting inhibitory activity on *Leishmania*[1–3] and African *Trypanosoma* species.[5] This has motivated the assessment of the antiparasitic potential of previously isolated metabolites belonging to these classes. The biological evaluation of mixtures of metabolites was also considered since drug combination has always been a model in drug discovery[11,12] and has recently been used in clinical trials for the development of drugs against leishmaniasis.[12] On this basis, the present study emphasizes the antiprotozoal activity

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http://dx.doi.org/10.1016/j.bmcl.2016.02.044
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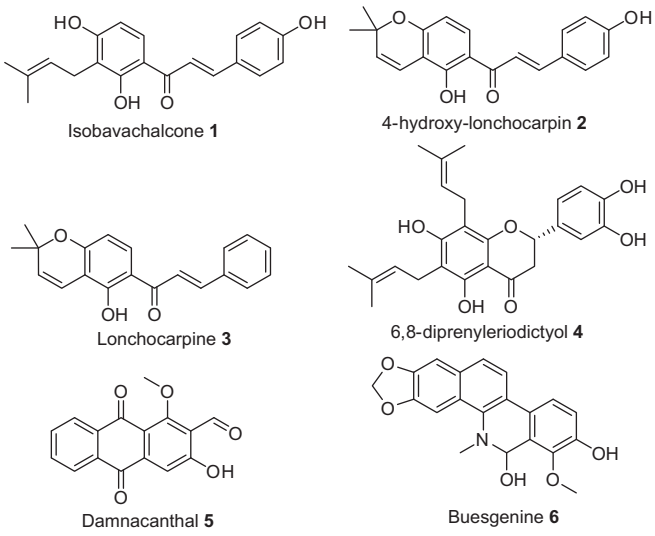

Figure 1. Structures of tested compounds.

of the individual compounds and of samples obtained from various proportions of the combination of two metabolites.

The tested compounds were composed of three chalcones: isobavachalcone (**1**), 4-hydroxylonchocarpine (**2**) and lonchocarpine (**3**), one flavanone: 6,8-diprenyleriodictyol (**4**), one anthraquinone: damnacanthal (**5**) and one benzophenanthridine alkaloid: buesgenine (**6**) (Fig. 1). Compounds **1**, **2** and **4** were provided by Professor Ngadjui and he previously described their isolation as well as their structure elucidation. Thus, **1** was isolated from *Dorstenia kameruniana* and was identified as isobavachalcone[13] while **2** and **4** were obtained from *Dorstenia mannii* and identified, respectively, as 4-hydroxylonchocarpine and 6,8-diprenyleriodictyol.[14] Compound **3** was recently isolated from *D. mannii* and was identified as lonchocarpine[15] by the group of Professor Kuete as described in the experimental part (Supporting information). He also provided damnacanthal (**5**), an anthraquinone identified from *Pentas schimperi* and previously reported in the literature.[16] As a part of our chemical studies, we recently identified buesgenine (**6**), a benzophenanthridine alkaloid from *Zanthoxylum buesgenii*.[17] The structures of provided metabolites were checked and confirmed by NMR spectroscopy data in comparison to those reported in the literature.

The leishmanicidal and trypanocidal potential of the six secondary metabolites (**1**–**6**) were evaluated against intracellular amastigotes of *Leishmania amazonensis* and *Trypanosoma cruzi*. In addition, pairs of metabolites **1**–**6** prepared in various ratios were also assessed for their antiprotozoal activity. Amphotericin B and benznidazole were used as reference drugs, respectively, for *L. amazonensis* and *T. cruzi*. Except for **1** and **5**, other metabolites including 4-hydroxylonchocarpine (**2**) and lonchocarpine (**3**), 6,8-diprenyleriodictyol (**4**) and buesgenine (**6**) showed activities against the amastigotes of *L. amazonensis*. Alkaloid **6** showed the most promising antileishmanial effect with IC_{50} at 5.70 μg/mL (Table 1). Moreover, it had the highest selectivity index (SI >7.69) suggesting that it is more antileishmanial than toxic on THP-1 cells. Besides, interesting leishmanicidal effects were also obtained with compounds **2** and **3** with IC_{50} values at 7.41 μg/mL and 6.66 μg/mL, respectively. Both compounds revealed SI value around 3. While a moderate activity was observed for compound **4** against *L. amazonensis* (IC_{50} 12.38 μg/mL), compounds **1** and **5** showed no activity. As emerged in Figure 2, among the diaryl propanoids only those containing a chromene core such as 4-hydroxylonchocarpine (**2**) and lonchocarpine (**3**), showed leishmanicidal activities while their analog, isobavachalcone (**1**) without this heterocycle did not demonstrate any activity. Interestingly, 6,8-diprenyleriodictyol (**4**), a flavanone with a chromenone moiety as part of its structure demonstrated the same bioactivity. Literature supporting this observation reported synthetic chromene-containing chalcones with interesting antileishmanial activity against the amastigote form of *Leishmania donovani*.[18] In addition, synthetic chromenes based on chalcone scaffold were further described as strong inhibitors of *Leishmania major* promastigote.[19] Benzophenantridine alkaloids and their quaternary ammonium salts were formerly reported as significant inhibitor of *L. amazonensis*[1,20] supporting the activity observed for **6**.

Apart from **3** and **5** that inhibited moderately *T. cruzi*, no further effect was observed with other metabolites. **3** and **5** had no relevant features on their chemical structures except for the fact that both contain α,β-unsaturated carbonyls which are Michael acceptors. Based on previous studies, chalcones barely demonstrated antitrypanosome activity while quinones-derived metabolites displayed remarkable trypanocidal effect.[21]

Various investigations showed that in vitro and in vivo biological activities of plant extract are significantly greater than those of its isolated constituents. Synergism of the phytoconstituents has always been highlighted as the explanation.[22] Thus, the potency of crude extracts versus isolated compounds presumably inspired therapies based on drugs combinations. The antimalarial cure ASAQ containing artesunate/amodiaquine (10:27, w/w) represents one of the drug combinations in the market.[12] Based on this concept, several mixtures were prepared in proportions 1:1, 1:3 and 3:1 of two metabolites chosen among **1**–**6** and were tested against the aforementioned parasites. Depending on the proportion of the metabolites in the pair, the results compiled in Table 2 revealed an improvement, no change, a decrease, and a loss of the activity in different cases. So, the leishmanicidal activity of **3** did not change despite its combination with **2** in proportions 1:1 (6.88 μg/mL) and 3:1 (6.64 μg/mL) otherwise, it decreased when both compounds were in a proportion 1:3 (9.29 μg/mL). In general, the comparison of the IC_{50} values in Figure 3 revealed no change for the mixtures **3**/**2** (1:1) and (3:1) tested against *L. amazonensis* (Fig. 3). In contrast, the trypanocidal action of **3** was abolished in

Table 1
IC_{50} ± SD, CC_{50} ± SD and SI values of compounds **1**–**6** as well as the reference drugs against intracellular amastigotes of *Trypanosoma cruzi* and *Leishmania amazonensis* and THP-1 cell line

Compounds	*T. cruzi*		*L. amazonensis*		THP-1		*L. amazonensis* SI	*T. cruzi* SI
	(μM)	(μg/mL)	(μM)	(μg/mL)	(μM)	(μg/mL)		
Isobavachalcone (**1**)	–	–	–	–	35.95 ± 1.33	11.65 ± 0.43	–	–
4-Hydroxylonchocarpine (**2**)	–	–	23.02 ± 0.86	7.41 ± 0.28	76.25 ± 1.44	24.55 ± 0.46	3.31	–
Lonchocarpine (**3**)	44.53 ± 0.49	13.63 ± 0.15	21.75 ± 5.43	6.66 ± 1.66	66.04 ± 2.13	20.21 ± 0.65	3.03	1.48
6,8-Diprenyleriodictyol (**4**)	–		29.20 ± 8.01	12.38 ± 3.39	46.27 ± 18.45	19.62 ± 7.82	1.58	
Damnacanthal (**5**)	39.51 ± 0.39	11.14 ± 0.11	–		31.47 ± 2.90	8.87 ± 0.82	–	0.79
Buesgenine (**6**)	–	–	16.25 ± 1.17	5.70 ± 0.41	>125.4	>43.83	>7.69	–
Amphotericin B			0.07 ± 0.01		10		142	
Benznidazole	10.18 ± 0.3				>125.4			>12

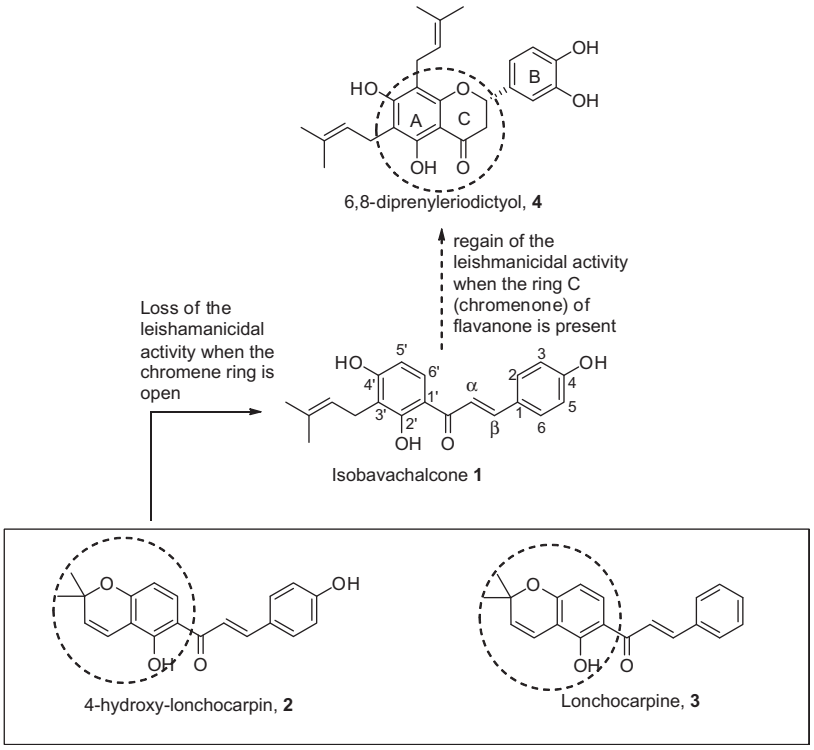

Figure 2. SAR between compounds **1**–**4**.

its mixtures (1:1, 1:3, and 3:1) with **2**. Figure 4 depicted a potentiating effect of **3** by **1** against *T. cruzi* as well as the induction of the trypanocidal activity of **1** in its combination with **3** in the proportion **3**/**1** (1:3). Interestingly, IC_{50} at 10.01 and 7.78 μg/mL obtained, respectively, for the proportions **3**/**1** (1:1) and (3:1) were better than that of compound **3** alone (13.63 μg/mL). The IC_{50} value of 10.93 μg/mL obtained with **3**/**1** (1:3) suggested an induction of the trypanocidal effect of **1** when combined with **3** (Fig. 4). This latter turned inactive against *L. amazonensis* in its mixtures with **1**. A slight decrease of the trypanocidal activity of **5** when mixed with **1**, **2**, and **3** was noted (Table 2). Subsequently, its SI value in the pair **5**/**2** (1:1) remained low and almost similar to that of **5**. Moreover, its trypanocidal effect was even lost in some cases. In the other hand, **2** and **3** lost their potency against *L. amazonensis* when administered with **5**. The pair of metabolites resulting from the mixture **6**/**2** and **6**/**4** in same ratios did not show any trypanocidal activity whereas an unchanged effect was observed for the leishmanicidal activity of **6** in the mixture **6**/**4** (3:1). Leishmanicidal action of **4** was improved by 1.2-fold in the mixture **6**/**4** (1:3). Other couples of **6**/**2** and **6**/**4** did not show a promising

Table 2
IC_{50} ± SD (μg/mL), CC_{50} ± SD (μg/mL), and SI values of the binary mixtures

Mixture of compounds	*T. cruzi*	*L. amazonensis*	THP-1	*L. amazonensis* SI	*T. cruzi* SI
3/**2** (1:1)	–	6.88 ± 1.58	16.37 ± 6.12	2.38	
3/**2** (3:1)	–	6.64 ± 1.47	–	–	–
3/**2** (1:3)	–	9.29 ± 0.12	–	–	–
3/**1** (1:1)	10.01 ± 1.44	–	17.24 ± 0.79	–	1.72
3/**1** (3:1)	7.78 ± 1.06	–	–	–	–
3/**1** (1:3)	10.93 ± 0.11	–	–	–	–
5/**3** (1:1)	–	–	6.11 ± 1.43	–	–
5/**3** (3:1)	11.11 ± 0.04	–	–	–	–
5/**3** (1:3)	14.19 ± 0.12	–	–	–	–
5/**1** (1:1)	–	–	<4.77	–	–
5/**1** (3:1)	12.30 ± 0.12	–	–	–	–
5/**2** (1:1)	13.90 ± 0.19	–	10.73 ± 1.71	–	0.77
5/**2** (3:1)	11.94 ± 0.14	–	–	–	–
5/**2** (1:3)	12.99 ± 0.06	–	–	–	–
6/**2** (1:1)	–	12.14 ± 0.79	101.45 ± 10.73	13.89	–
6/**2** (3:1)	–	6.69 ± 1.55	–		–
6/**2** (1:3)	–	–	–	–	–
6/**4** (1:1)	–	16.21 ± 3.38	12.39 ± 0.82	0.76	–
6/**4** (3:1)	–	5.80 ± 2.42	–	–	–
6/**4** (1:3)	–	9.94 ± 2.70	–	–	–
2/**4** (1:1)	–	16.07 ± 1.01	45.80 ± 5.33	2.85	
2/**4** (3:1)	–	–	–	–	–
2/**4** (1:3)	–	15.2 ± 1.84	–	–	–
2/**1** (1:1)	12.47 ± 0.11	–	23.54 ± 1.58		1.88
2/**1** (3:1)	14.24 ± 0.50	–	–	–	–
2/**1** (1:3)	15.26 ± 1.19	–	–	–	–

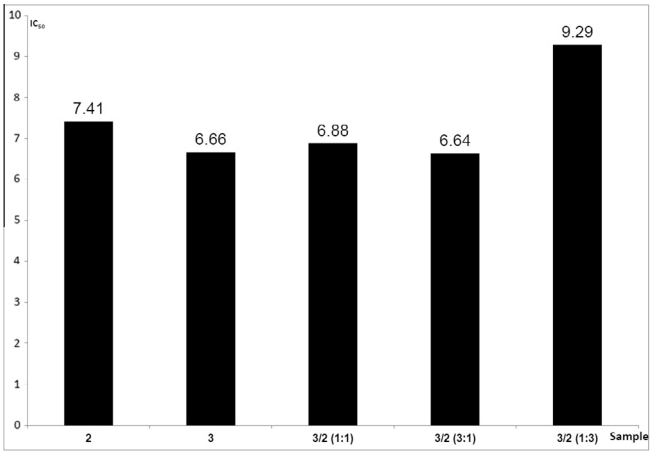

Figure 3. Comparison of leishmanicidal activity of compounds **2** and **3** adiministered alone and as combined samples.

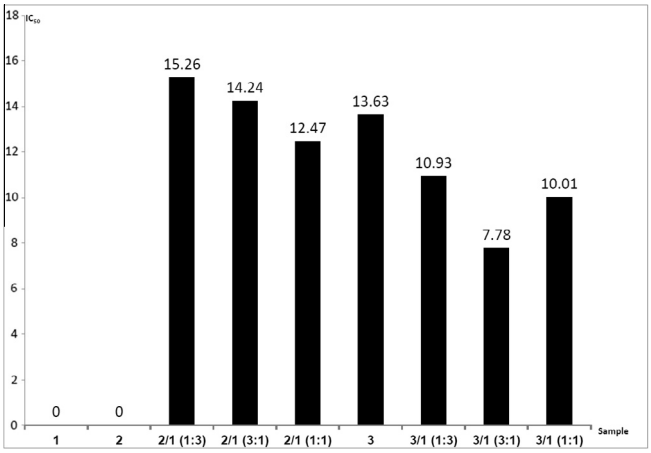

Figure 4. Comparison of trypanocidal activity of compounds **1**–**3** adiministered alone and as combined samples.

improvement of the leishmanicidal activity since their potency decreased or were lost otherwise. Though **6**/**2** (1:1) showed a lower leishmanicidal activity than compounds **6** and **2**, the mixture led to an interesting SI value of 13.89. This finding clearly demonstrates that combination of two components, one less toxic than another decreases the toxicity meanwhile the antiparasitic activity is still effective. Furthermore, this result can also be used as a strategy in drug development to get the toxicity low and still have the expected bioactitivty. Polyphenols **1** and **2** tested individually, had no effect on *T. cruzi* while their combination in proportions 1:1, 3:1, and 1:3 led to three trypanocidal samples with IC_{50} at 12.47, 14.24, and 15.26 μg/mL, respectively. Among the six samples of **2**/**1** and **2**/**4**, only **2**/**4** (1:1) and (1:3) were less active on *L. amazonensis* than the single compounds. The remaining couples did not show any leishmanicidal effect.

In general, combination of chalcones improved or induced the trypanocidal activity in many cases. Besides, the action against *L. amazonensis* of the flavanone was improved while combined with the alkaloid (Table 2).

Except for the alkaloid, the other secondary metabolites (**1**–**5**) showed cytotoxic activity against the human macrophage cell line THP-1 cells with the strongest antiproliferation associated to damnacanthal (Table 1). Further, the combinations **5**/**3** (1:1) and **5**/**1** (1:1) displayed neither the leishmanicidal nor the trypanocidal activities but were significantly cytotoxic with CC_{50} values at 6.11 μg/mL and <4.77 μg/mL, respectively. Develop a good drug mixture becomes a challenging task when besides the expected bioactivity the samples express a strong cytotoxicity which was the case of all the tested samples. Their SI values were weaker than those of the reference drugs.

Six compounds were evaluated for their antiprotozoal effect against *L. amazonensis* and *T. cruzi*. Based on the obtained results, chromene-derived metabolites and isoquinoline alkaloids still have interesting outcomes against *Leishmania* parasites. Therefore, chemical derivatization of these cores might lead to leishmanicidal hit compounds. Furthermore, testing mixtures of metabolites showed activities in some cases suggesting that drug combination remains an alternative to overcome neglected diseases with one of the difficulties being the choice of the right proportion for a better activity. Another limitation that might occur is the increase of the toxicity leading to at least two factors to be considered in drug combination therapy and development.

Acknowledgements

Authors would to thank Nicole Biondo for her technical assistance. Part of this work was performed within the Research Network Natural Products against Neglected Diseases (ResNetNPND).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.044.

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