— **Minireview** —

Antichagasic Activities of Natural Products against *Trypanosoma cruzi*

Nahoko Uchiyama*

National Institute of Health Sciences (NIHS), 1–18–1, Kamiyoga, Setagaya-Ku, Tokyo 158–8501, Japan

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Trypanosoma cruzi (*T. cruzi*) is a parasitic protozoan transmitted to mammalian hosts by blood-sucking triatomine bugs. Infections by *T. cruzi*, known as Chagas' disease, pose a major public health problem in endemic countries in Central and South America. New chemotherapeutic agents are desired because of the lack of effective vaccines, undesirable side effects of anti-chagasic drugs in use such as nifurtimox and benznidazole, and the emergence of parasite resistance to these drugs. In the past two decades, novel advances and an improved understanding of the biology and biochemistry of *T. cruzi* have led to the identification of various targets for chemotherapy to treat Chagas' disease. In addition, many efforts have been undertaken to develop antichagasic agents, such as designed and synthesized compounds, natural products, and their derivatives, against a number of targets. Here, I mainly review recent studies on the antichagasic activities of natural products.

Key words —— *Trypanosoma cruzi*, antichagasic activity, natural product

INTRODUCTION

Chagas' disease is a major public health problem endemic in Central and South American countries, with 18–20 million infected people, 25 % of the human population at risk of infection, *ca.* 200000 new cases annually, and 21000 deaths per year.[1)] Its causative agent is *Trypanosoma cruzi* (*T. cruzi*), a parasitic protozoan transmitted to mammalian hosts by blood-sucking triatomine bugs. *T. cruzi* undergoes three main developmental stages during its life cycle: the replicative epimastigote form in insect vectors and the trypomastigote and amastigote forms in mammalian hosts. Non-dividing and infective trypomastigotes circulate in the blood with their free flagellum before invading host cells, preferably muscle cells, where they lose their flagellum to differentiate into replicative amastigotes.[2)] Infections by *T. cruzi* result in a life-threatening, acute, and/or chronic disease with severe cardiac complications. This situation is worsened by the lack of effective vaccines, undesirable side effects of anti-chagasic drugs in use such as nifurtimox and benznidazole, and the emergence of parasite resistance to these drugs. Therefore, new chemotherapeutic agents are urgently needed. Advances in our understanding of the basic biochemistry of *T. cruzi* have allowed the identification of new specific pathways and novel drug targets for chemotherapy, such as sterol biosynthesis, trypanothione reductase, and cystein protease.[1,3–6)] This has led to some rationally designed and synthesized candidates for the treatment of Chagas' disease.[4)]

On the other hand, the use of natural products for the treatment of protozoal infections is well known and long documented.[4)] A number of recent works reported the trypanocidal activity of a wide variety of extracts of natural medicines or its constituents.[7,8)] In our screening work on many medicinal plants used in Asian countries, especially Vietnam and Uzbekistan for trypanocidal activity, several extracts of the plants or its constituents showed trypanocidal activity against *T. cruzi*.[9)] Extensive reviews of chemotherapy against Chagas' disease have been reported.[4,7,8)] Thus, I mainly review recent studies on the antichagasic activities of natural products.

*To whom correspondence should be addressed: National Institute of Health Sciences (NIHS), 1–18–1, Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan. Tel.: +81-3-3700-8764; Fax: +81-3-3707-6950; E-mail: nuchiyama@nihs.go.jp

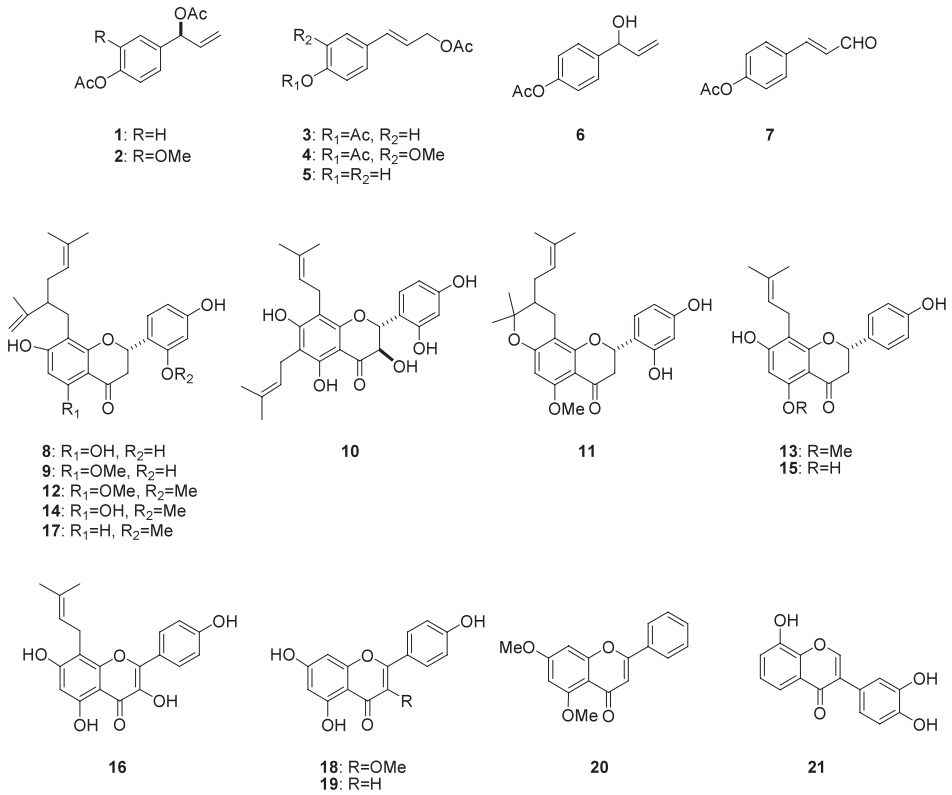

Fig. 1. Structures of Phenylpropanoids and Flavonoids with Trypanocidal Activity

TRYPANOCIDAL COMPOUNDS ISOLATED FROM MEDICINAL PLANTS

Phenylpropanoids

An acetone extract of rhizomes of *Alpinia galanga* (Zingiberaceae) showed strong trypanocidal activity against epimastigotes of *T. cruzi* with a minimum lethal concentration (MLC) of 1.56 μg/ml. Fractionation of the extract resulted in the isolation of seven known phenylpropanoids, 1′-acetoxychavicol acetate (**1**), 1′-acetoxyeugenol acetate (**2**), *p*-coumaryl diacetate (**3**), coniferyl diacetate (**4**), *p*-coumaryl acetate (**5**), 1′-hydroxychavicol acetate (**6**), and *p*-acetoxycinnamaldehyde (**7**), as shown in Fig. 1. The MLCs of these compounds against epimastigotes of *T. cruzi* were 6.7 μM (**1**), 12 μM (**2**), 27 μM (**3**), 24 μM (**4**), 8.1 μM (**5**), > 260 μM (**6**), and 132 μM (**7**), respectively (Table 1).[9)]

Flavonoids

An acetone extract of roots of *Sophora flavescens* (Leguminosae) exhibited trypanocidal activity against epimastigotes of *T. cruzi* with an MLC of 6.25 μg/ml.[9)] Ten prenylated flavonoids, sophoraflavanone G (**8**), (−)-kurarinone (**9**), kushenol L (**10**), sophoraflavone J (**11**), 2′-methoxykurarinone (**12**), 7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**13**), leachianone A (**14**), 8-prenylnaringenin (**15**), noranhydroicaritin (**16**), and alopecurone G (**17**) were isolated as trypanocidal compounds (Fig. 1). The MLCs of these compounds against epimastigotes of *T. cruzi* were 3.7 μM (**8**), 14 μM (**9**), 7.1 μM (**10**), 7.2 μM (**11**), 6.9 μM (**12**), 71 μM (**13**), 5.5 μM (**14**), 18 μM (**15**), 4.4 μM (**16**), and 3.6 μM (**17**), respectively (Table 1).[10)] Although some flavonoids have been reported to have trypanocidal activity,[14−16)] this was the first report of trypanocidal activity for prenylated flavonoids. Among the isolated flavanones, those with a lavandulyl group (**8**, **9**, **11**, **12**, **14**, **17**) showed stronger activity than those with a prenyl group (**13**, **15**), suggesting that prenyl groups may play important roles in the activity. In fact, naringenin, a flavanone with no prenyl group, showed only very weak trypanocidal activity (MLC = 370 μM).[10)] Furthermore, two flavonoids were isolated from the ethyl acetate (EtOAc) extract of *Dracocephalum kotschyi* (Labiatae) and identified as isokaempferide (**18**) and apigenin (**19**), as shown in Fig. 1. These compounds have trypanocidal activity against epimastigotes of *T. cruzi* with MLCs of 30 μM and

Table 1. Trypanocidal Activity of Phenylpropanoids and Flavonoids against Epimastigotes (epi) and Trypomastigotes (trypo) of *T. cruzi*

Compound	MLC[a] or IC_{50} (μM)		
1′-acetoxychavicol acetate (**1**)[9]	MLC	6.7	(epi)
1′-acetoxyeugenol acetate (**2**)[9]	MLC	12.0	(epi)
p-coumaryl diacetate (**3**)[9]	MLC	27.0	(epi)
coniferyl diacetate (**4**)[9]	MLC	24.0	(epi)
p-coumaryl acetate (**5**)[9]	MLC	8.1	(epi)
1′-hydroxychavicol acetate (**6**)[9]	MLC	> 260.0	(epi)
p-acetoxycinnamaldehyde (**7**)[9]	MLC	132.0	(epi)
sophoraflavanone G (**8**)[10]	MLC	3.7	(epi)
(−)-kurarinone (**9**)[10]	MLC	14.0	(epi)
kushenol L (**10**)[10]	MLC	7.1	(epi)
sophoraflavone J (**11**)[10]	MLC	7.2	(epi)
2′-methoxykurarinone (**12**)[10]	MLC	6.9	(epi)
7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**13**)[10]	MLC	71.0	(epi)
leachianone A (**14**)[10]	MLC	5.5	(epi)
8-prenylnaringenin (**15**)[10]	MLC	18.0	(epi)
noranhydroicaritin (**16**)[10]	MLC	4.4	(epi)
alopecurone G (**17**)[10]	MLC	3.6	(epi)
isokaempferide (**18**)[11]	MLC	30.0	(epi)
apigenin (**19**)[11]	MLC	70.0	(epi)
chrysin dimethylether (**20**)[12]	IC_{50}	14.8 (3.9 μg/ml)	(trypo)
3′-hydroxydaidzein (**21**)[12]	IC_{50}	17.4 (4.7 μg/ml)	(trypo)
Gentian violet[b, 13]	MLC	6.3	(epi)

a) Minimum lethal concentration against *T. cruzi*. *b*) Positive control.

70 μM, respectively (Table 1).[11] On the other hand, Tasdemir *et al.* reported that several flavonoid glycosides from a Turkish plant were tested in mouse models showing moderate activity against trypomastigotes of *T. cruzi*, and only chrysin dimethylether (**20**) and 3′-hydroxydaidzein (**21**) had IC_{50} values of 3.9 μgml (14.8 μM) and 4.7 μgml (17.4 μM), respectively (Fig. 1, Table 1).[12]

Terpenoids

Monoterpenes

Four monoterpene hydroperoxides, (−)-(2*S*,4*S*)- and (−)-(2*R*,4*S*)-*p*-mentha-1(7),8-dien-2-hydroperoxide (**23** and **24**) and (−)-(1*R*,4*S*)- and (−)-(1*S*,4*S*)-*p*-mentha-2,8-dien-1-hydroperoxide (**25** and **26**), were isolated from aerial parts of *Chenopodium ambrosioides* (Chenopodiaceae) along with monoterpene endoperoxide ascaridole (**22**) as trypanocidal compounds (Fig. 2). The MLCs of these compounds against epimastigotes of *T. cruzi* were 23 μM (**22**), 1.2 μM (**23**), 1.6 μM (**24**), 3.1 μM (**25**), and 0.8 μM (**26**), respectively (Table 2).[17] Meanwhile, the alcohols, corresponding to the hydroperoxides **23**–**26**, did not show trypanocidal activity even at 400 μM, indicating that the hydroperoxy group is essential for the activity of this type of compound. On the other hand, in the HeLa cell infection assay, **26** almost completely inhibited the infection of trypomastigotes to HeLa cells at 1 μg/ml (6.5 μM), and **24** and **25** inhibited the infection by 63 % and 88 % at 1 μg/ml (6.0 and 6.5 μM), respectively. However, they did not inhibit the proliferation of amastigotes in infected cells.[17] Another monoterpene hydroperoxide (1*R*,4*S*)-1-hydroperoxy-*p*-menth-2-en-8-ol acetate (**27**), isolated from *Laurus nobilis* (*L. nobilis*) L. (Lauraceae), also showed trypanocidal activity against epimastigotes of *T. cruzi* with MLC of 1.4 μM (Fig. 2, Table 2). In a HeLa cell infection assay at 1 μg/ml (4.4 μM), 27 inhibited the infection of trypomastigotes to HeLa cells and the proliferation of amastigotes in HeLa cells by 98 % and 83 %, respectively.[18]

Sesquiterpenes

Sesquiterpene hydroperoxides, (1*S*,4*R*,7*R*,8a*S*)-1,2,4,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-4-azulenyl hydroperoxide (**28**), (1*S*,3a*S*,7*R*,8a*S*)-1,2,3,5,6,7,8,8a-octahydro-1-methyl-4-methylene-7-(1-methylethenyl)-3a(1*H*)-azulenyl hydroperoxide (**29**) and [(1*S*,7*R*,8a*S*)-1,2,3,5,6,7,8,8a-octahydro-1-methyl-7-(1-methylethenyl)-4-azulenyl]methyl

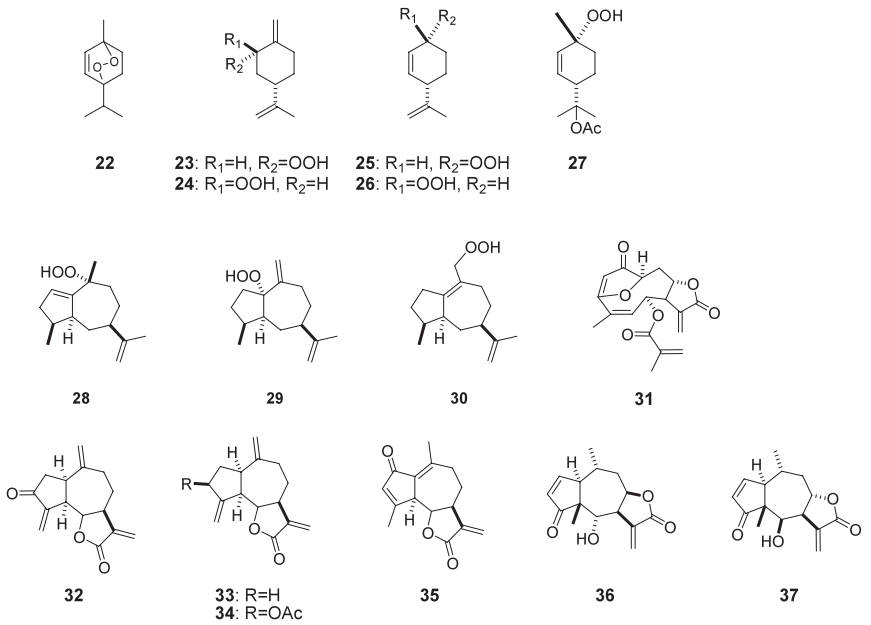

Fig. 2. Structures of Mono- and Sesquiterpenes with Trypanocidal Activity

Table 2. Trypanocidal Activity of Mono- and Sesquiterpenes against Epimastigotes (epi) and Trypomastigotes (trypo) of *T. cruzi*

Compound	MLC,[a] IC_{50} or IC_{90} (μM)		
ascaridole (**22**)[17]	MLC	23.0	(epi)
(−)-(2*S*,4*S*)-*p*-mentha-1(7),8-dien-2-hydroperoxide (**23**)[17]	MLC	1.2	(epi)
(−)-(2*R*,4*S*)-*p*-mentha-1(7),8-dien-2-hydroperoxide (**24**)[17]	MLC	1.6	(epi)
(−)-(1*R*,4*S*)-*p*-mentha-2,8-dien-1-hydroperoxide (**25**)[17]	MLC	3.1	(epi)
(−)-(1*S*,4*S*)-*p*-mentha-2,8-dien-1-hydroperoxide (**26**)[17]	MLC	0.8	(epi)
(1*R*,4*S*)-1-hydroperoxy-*p*-menth-2-en-8-ol acetate (**27**)[18]	MLC	1.4	(epi)
(1*S*,4*R*,7*R*,8a*S*)-1,2,4,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-4-azulenyl hydroperoxide (**28**)[19]	MLC	0.8	(epi)
(1*S*,3a*S*,7*R*,8a*S*)-1,2,3,5,6,7,8,8a-octahydro-1-methyl-4-methylene-7-(1-methylethenyl)-3a(1*H*)-azulenyl hydroperoxide (**29**)[19]	MLC	1.7	(epi)
[(1*S*,7*R*,8a*S*)-1,2,3,5,6,7,8,8a-octahydro-1-methyl-7-(1-methylethenyl)-4-azulenyl]methyl hydroperoxide (**30**)[19]	MLC	1.7	(epi)
15-deoxygoyazensolide (**31**)[20]	lysis	100.0	(trypo)
dehydrozaluzanin C (**32**)[21]	IC_{90}	10.0–200.0	(trypo)
dehydrocostuslactone (**33**)[18]	MLC	6.3	(epi)
zaluzanin D (**34**)[18]	MLC	2.5	(epi)
helenalin (**36**)[22]	IC_{50}	0.7	(trypo)
mexicanin I (**37**)[22]	IC_{50}	1.9	(trypo)

a) Minimum lethal concentration against *T. cruzi*.

hydroperoxide (**30**), were isolated as the trypanocidal constituents of *Pogostemon cablin* (Labiatae), as shown in Fig. 2. The MLCs of 28–30 against epimastigotes of *T. cruzi* were 0.84 μM (**28**), 1.7 μM (**29**), and 1.7 μM (**30**) (Table 2). However, the activity levels of the corresponding alcohols of **28**–**30** were very weak (> 200 μM). Thus the trypanocidal activity of the sesquiterpene hydroperoxides is ascribable to the hydroperoxy function.[19]

On the other hand, several sesquiterpene lactones having an α,β-unsaturated γ-lactone moiety have shown trypanocidal activity. The structures and trypanocidal activity of these compounds are exhibited in Fig. 2 and Table 2. 15-Deoxygoyazensolide (**31**) lyses trypomastigotes of *T. cruzi* at 100 μM,[20] dehydrozaluzanin C (**32**) showed an IC_{50} value in the range of 10–200 μM against 15 strains of epimastigotes of *T. cruzi*.[21] De-

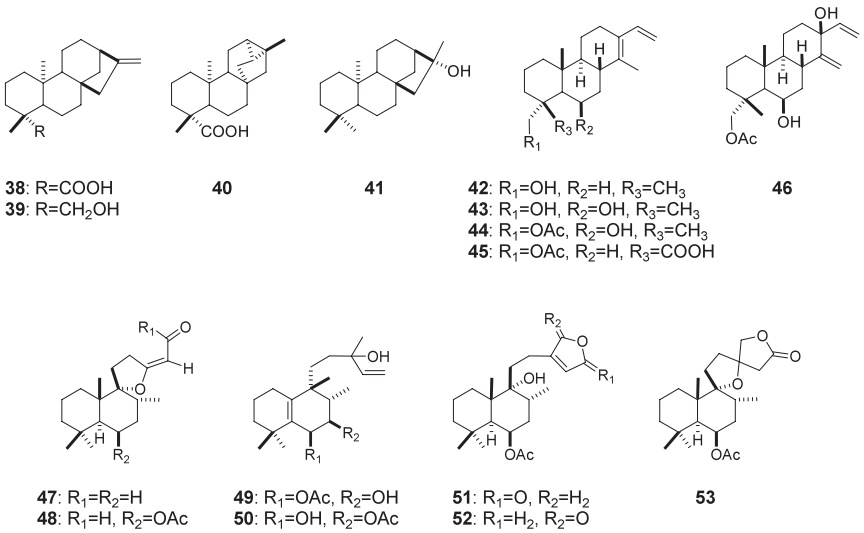

Fig. 3. Structures of Diterpenes with Trypanocidal Activity

hydrocostus lactone (**33**) and zaluzanin D (**34**), isolated with **27** from *L. nobilis*, exhibited trypanocidal activity against epimastigotes of *T. cruzi* with MLCs of 6.3 and 2.5 μM. Compound **33** also inhibited the infection of HeLa cells by trypomastigotes and inhibited the proliferation of amastigotes in HeLa cells by 74 % and 75 %, respectively, at 1 μg/ml (4.3 μM), whereas **34** weakly inhibited proliferation and did not inhibit infection at 1 μg/ml (3.4 μM).[18] Brengio *et al.* reported that the trypanocidal effect of dehydrocouledine (**35**) was irreversible but could be blocked by the presence of reducing substances such as thiol compound.[23] This suggested that the trypanocidal activity of this group of compounds depends on covalent bond formation of the α,β-unsaturated γ-lactone moiety with nucleophiles that are essential for the life of the parasite. Trypanosomes are known to be sensitive to oxidative stresses. As a defense against these stresses, they use trypanothione (1,8-bis-glutathionyl spermidine), whose counterpart in mammalian cells is glutathione. α,β-Unsaturated γ-lactones will react with -SH groups of trypanothione and of enzymes involved in the trypanothione cycle,[24] thus inactivating the defense system and exposing the parasite to oxidative damage.[18] Helenalin (**36**) and mexicanin I (**37**), which also have an α,β-unsaturated γ-lactone moiety, exhibited IC_{50} values of 0.7 and 1.9 μM, respectively, against trypomastigotes of *T. cruzi*.[22] However, Jimenez-Ortiz *et al.* reported that **36** and **37** are deleterious for epimastigotes of *T. cruzi* and that their mechanism of action is different from that of the related lactone (**35**).[25]

Diterpenes

Previously, the trypanocidal activity of several types of diterpenes has been reported. Da Costa *et al.* reported that kaurane diterpenes, (−)-*ent*-kaur-16-en-19-oic acid (**38**), (−)-kaur-16-en-19-ol (**39**), (−)-trachyloban-19-oic acid (**40**), and (−)-kauran-16-α-ol (**41**), were effective against trypomastigotes of *T. cruzi* with IC_{50} values of 1.7, 0.7, 1.7, and 1.7 mM, respectively (Fig. 3, Table 3).[26, 27] Cassane diterpenes, 18-hydroxycassan-13,15-diene (**42**), 6β,18-dihydroxycassan-13,15-diene (**43**), 6β-hydroxy-18-acetoxycassan-13,15-diene (**44**), 18-acetoxy-13,15-diene-19-cassanoic acid (**45**), and 6β,13β-dihydroxy-18-acetoxycassan-14(**17**),15-diene (**46**), were also reported to show trypanocidal activity against trypomastigotes and amastigotes of *T. cruzi* with IC_{50} values in the range of 11.5–104 μM and 16.6–35.8 μM, respectively (Fig. 3, Table 3).[28]

Two new norditerpene aldehydes, (2*E*)-*rel*-(−)-2-[(1′*R*,2′*R*,4′a*S*,8′a*S*)-decahydro-2′,5′,5′,8′a-tetramethylspiro[furan-2(3*H*),1′(2′*H*)-naphthalen]-5-ylidene]-acetaldehyde (**47**), and (2*E*)-*rel*-(−)-2-[(1′*R*,2′*R*,4′*R*,4′a*S*,8′a*S*)-4′-(acetyloxy)decahydro-2′,5′,5′,8′a-tetramethylspiro[furan-2(3*H*),1′(2′*H*)-naphthalen]-5-ylidene]-acetaldehyde (**48**), and five known diterpenes, vitexifolin E (**49**), vitexifolin F (**50**), vitexilactone (**51**), 6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide (**52**), and previtexilactone (**53**), which were isolated from *Vitex trifolia* (Verbenaceae), exhibited trypanocidal activity with MLCs against epimastigotes of *T. cruzi* (Fig. 3). The MLCs of **47**–**53** against epimastigotes of *T.*

Table 3. Trypanocidal Activity of Diterpenes against Epimastigotes (epi), Trypomastigotes (trypo), and Amastigotes (ama) of *T. cruzi*

Compound	MLC[a] or IC_{50} (μM)		
(−)-*ent*-kaur-16-en-19-oic acid (**38**)[26,27]	MLC	1.7 mM	(epi)
(−)-kaur-16-en-19-ol (**39**)[26]	MLC	0.7 mM	(epi)
(−)-trachyloban-19-oic acid (**40**)[26]	MLC	1.7 mM	(epi)
(−)-kauran-16-a-ol (**41**)[26]	MLC	1.7 mM	(epi)
18-hydroxycassan-13,15-diene (**42**)[28]	IC_{50}	48.6/17.4	(trypo/ama)
6*β*,18-dihydroxycassan-13,15-diene (**43**)[28]	IC_{50}	56.0/16.6	(trypo/ama)
6*β*-hydroxy-18-acetoxycassan-13,15-diene (**44**)[28]	IC_{50}	11.5/25.9	(trypo/ama)
18-acetoxy-13,15-diene-19-cassanoic acid (**45**)[28]	IC_{50}	104.0/ND[b]	(trypo/ama)
6*β*,13*β*-dihydroxy-18-acetoxycassan-14(17),15-diene (**46**)[28]	IC_{50}	16.5/35.8	(trypo/ama)
(2*E*)-*rel*-(-)-2-[(1′*R*,2′*R*,4′a*S*,8′a*S*)-decahydro-2′,5′,5′,8′a-tetramethylspiro[furan-2(3*H*),1′(2′*H*)-naphthalen]-5-ylidene]-acetaldehyde (**47**)[29]	MLC	11.0	(epi)
(2*E*)-*rel*-(-)-2-[(1′*R*,2′*R*,4′*R*,4′a*S*,8′a*S*)-4′-(acetyloxy)deca-hydro-2′,5′,5′,8′a-tetramethylspiro[furan-2(3*H*),1′(2′*H*)-naphthalen]-5-ylidene]-acetaldehyde (**48**)[29]	MLC	36.0	(epi)
vitexifolin E (**49**)[29]	MLC	34.0	(epi)
vitexifolin F (**50**)[29]	MLC	34.0	(epi)
vitexilactone (**51**)[29]	MLC	66.0	(epi)
6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide (**52**)[29]	MLC	66.0	(epi)
previtexilactone (**53**)[29]	MLC	> 265.0	(epi)
komaroviquinone (**54**)[13]	MLC	0.4	(epi)
cyclocoulterone (**55**)[13]	MLC	20.0	(epi)
dracocequinone A (**56**)[13]	MLC	12.5	(epi)
dracocequinone B (**57**)[13]	MLC	25.0	(epi)
dracocephalone A (**58**)[13]	MLC	200.0	(epi)
komarovinone A (**59**)[13]	MLC	> 200.0	(epi)
komarovispirone (**60**)[13]	MLC	23.0	(epi)
5-*epi*-icetexane (**61**)[30]	IC_{50}	6.5	(epi)

a) Minimum lethal concentration against *T. cruzi*. *b*) Not determined.

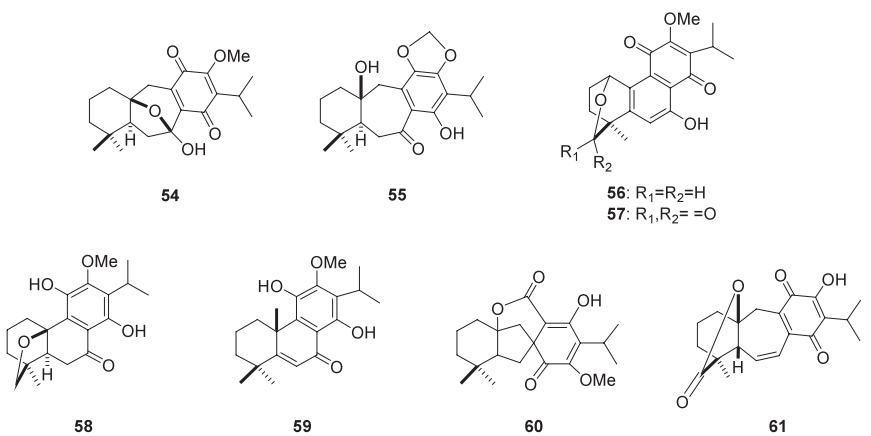

Fig. 4. Structures of Diterpenes with Trypanocidal Activity

cruzi were 11 μM (**47**), 36 μM (**48**), 34 μM (**49**), 34 μM (**50**), 66 μM (**51**), 66 μM (**52**), and > 265 μM (**53**) (Table 3).[29]

On the other hand, two novel icetexanes, komaroviquinone (**54**) and cyclocoulterone (**55**), four new 20-norabietane diterpenes, dracocequinones A (**56**), B (**57**), dracocephalone A (**58**) and komarovinone A (**59**), and a new diterpene with a spiro-octahydroindene skelton (komarovispirone, **60**) were isolated from *Dracocephalum komarovi* (Labiatae, Fig. 4).[13,31,32] The trypanocidal activities of the isolated compounds are summarized in

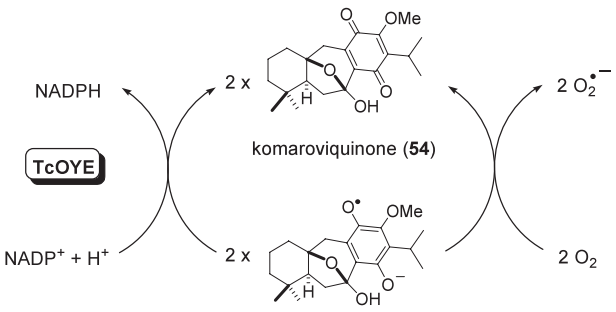

Fig. 5. Redox-cycling Scheme Showing the One-electron Reduction of 54 Catalyzed by TcOYE

Table 3. The MLCs of **55** (20 μM), **56** (12.5 μM), **57** (25 μM), and **60** (23 μM) are similar values, but higher than that of **54** (0.4 μM) under the same conditions. On the other hand, **59**, which lacks the quinone moiety as does **58** (200 μM), did not show trypanocidal activity even at 200 μM. Several types of natural quinones have been reported to show trypanocidal activity, which has been ascribed in part to the production of a reactive oxygen species in the parasite.[33] In fact, we found that **54** was reduced by *T. cruzi* old yellow enzyme (TcOYE) to its semiquinone radical, which subsequently generates superoxide anion radicals (Fig. 5). The komaroviquinone (**54**) reductase activity in trypanosoma lysates was completely immunoabsorbed by anti-TcOYE antibody. These results indicate that TcOYE is the main source of **54** reductase activity in *T. cruzi*, and this in turn implies that the trypanocidal activity of **54** is specifically due to its reduction by the parasite enzyme TcOYE.[34] Therefore, the trypanocidal activity of **54**, **56**, and **57** may be due to the generation of a reactive oxygen species.

Furthermore, a novel icetexane diterpene, 5-*epi*-icetexane (**61**, Fig. 4) obtained from *Salvia gilliessi*, was effective against epimastigotes of *T. cruzi* with IC_{50} value of 6.5 ± 0.75 μM (Table 3).[30] Thus, the trypanocidal activity of diterpenes (**42**–**52**, **55**–**57**, **60**, and **61**) were in a similar range except for kaurane diterpenes (**38**–**41**). However, **54** showed more potent activity than the other diterpenes. Compound **54** at its high concentration (3 μM) did not inhibit the intracellular growth of amastigotes within HeLa cells. However, **54** inhibited the survival of trypomastigotes more potently than that of epimastigotes with an IC_{50} value of 9 nM and inhibited infection with trypomastigotes to HeLa cells with the same IC_{50} value (9 nM). Furthermore, **54** showed selective toxicity between the parasite and HeLa cells, calculated to be about 2200-fold.[34] These results clearly indicate that **54** is a good candidate for developing new antichagasic drugs.

OTHER COMPOUNDS

In our current study, fungal metabolites, isolated from Aspergillaceae, were identified as a new type of trypanocidal compounds. These compounds showed potent trypanocidal activity against amastigotes, epimastigotes, and trypomastigotes of *T. cruzi* in the μM range (data not shown).[35] Thus, further study is necessary to clarify the mechanisms of action of these compounds.

CONCLUSION

Despite many research efforts, currently there are no drugs in clinical trial for the treatment of Chagas' disease. However, the wide range of therapeutic targets under investigation should hasten the discovery of novel trypanocidal compounds that can be made into effective, widely available drugs. Although various natural products have been identified as trypanocidal compounds, there is little information that enables the identification of the mechanisms of action with most of these products. Further investigation is required, not only to identify trypanocidal compounds from natural medicines but also to reveal those compounds' mechanisms of action.

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REFERENCES

1) Urbina, J. A. and Docampo, R. (2003) Specific chemotherapy of Chagas disease: controversies and advances. *Trends Parasitol.*, **19**, 495–501.
2) Nakajima-Shimada, J., Hirota, Y. and Aoki, T. (1996) Inhibition of *Trypanosoma cruzi* growth in mammalian cells by purine and pyrimidine analogs. *Antimicrob. Agents Chemother.*, **40**, 2455–2458.
3) Urbina, J. A. (2002) Chemotherapy of Chagas disease. *Curr. Pharm. Des.*, **8**, 287–295.
4) Duschak, V. G. and Couto, A. S. (2007) An insight

on targets and patented drugs for chemotherapy of Chagas disease. *Recent Patents on Anti-Infective Drug Discovery*, **2**, 19–51.

5) El-Sayed, N. M., Myler, P. J., Bartholomeu, D. C., Nilsson D., Aggarwal, G., Tran, A. N., Ghedin, E., Worthey, E. A., Delcher, A. L., Blandin, G., Westenberger, S. J., Caler, E., Cerqueira, G. C., Branche, C., Haas, B., Anupama, A., Arner, E., Aslund, L., Attipoe, P., Bontempi, E., Bringaud, F., Burton, P., Cadag, E., Campbell, D. A., Carrington, M., Crabtree, J., Darban, H., da Silveira, J. F., de Jong, P., Edwards, K., Englund, P. T., Fazelina, G., Feldblyum, T., Ferella, M., Frasch, A. C., Gull, K., Horn, D., Hou, L., Huang, Y., Kindlund, E., Klingbeil, M., Kluge, S., Koo, H., Lacerda, D., Levin, M. J., Lorenzi, H., Louie, T., Machado, C. R., McCulloch, R., McKenna, A., Mizuno, Y., Mottram, J. C., Nelson, S., Ochaya, S., Osoegawa, K., Pai, G., Parsons, M., Pentony, M., Pettersson, U., Pop, M., Ramirez, J. L., Rinta, J., Robertson, L., Salzberg, S. L., Sanchez, D. O., Seyler, A., Sharma, R., Shetty, J., Simpson, A. J., Sisk, E., Tammi, M. T., Tarleton, R., Teixeira, S., Van Aken, S., Vogt, C., Ward, P. N., Wickstead, B., Wortman, J., White, O., Fraser, C. M., Stuart, K. D. and Andersson, B. (2005) The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. *Science*, **309**, 409–415.
6) Rodriques Coura, J. and de Castro, S. L. (2002) A critical review on Chagas disease chemotherapy. *Mem. Inst. Oswaldo Cruz*, **97**, 3–24.
7) Maya, J. D., Cassels, B. K., Iturriaga-Vásquez, P., Ferreira, J., Faúndez, M., Galanti, N., Ferreira, A. and Morello, A. (2007) Mode of action of natural and synthetic drugs against *Trypanosoma cruzi* and their interaction with the mammalian host. *Comp. Biochem. Physiol. A*, **146**, 601–620.
8) Lockman, J. W. and Hamilton, A. D. (2005) Recent developments in the identification of chemotherapeutics for Chagas disease. *Curr. Med. Chem.*, **12**, 945–959.
9) Kiuchi, F., Matsuo, K., Itano, Y., Ito, M., Honda, G., Qui, T. K., Nakajima-Shimada, J. and Aoki, T. (2002) Screening of natural medicines used in Vietnam for trypanocidal activity against epimastigotes of *Trypanosoma cruzi. J. Nat. Med.*, **56**, 64–68.
10) Matsuo, K., Ito, M., Honda, G., Qui, T. K. and Kiuchi, F. (2003) Trypanocidal flavonoids from *Sophora flavescens. J. Nat. Med.*, **57**, 253–255.
11) Gohari, A. R., Saeidnia, S., Matsuo, K., Uchiyama, N., Yagura, T., Ito, M., Kiuchi, F. and Honda, G. (2003) Flavonoid constituents of *Dracocephalum kotschyi* growing in Iran and their trypanocidal activity. *J. Nat. Med.*, **57**, 250–252.
12) Tasdemir, D., Kaiser, M., Brun, R., Yardley, V., Schmidt, T. J., Tosun, F. and Rüedi, P. (2006) Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: *in vitro*, *in vivo*, structure-activity relationship, and quantitative structure-activity relationship studies. *Antimicrob. Agents Chemother.*, **50**, 1352–1364.
13) Uchiyama, N., Kiuchi, F., Ito, M., Honda, G., Takeda, Y., Khodzhimatov, O. K. and Ashurmetov, O. A. (2006) Trypanocidal constituents of *Dracocephalum komarovi. Tetrahedron*, **62**, 4355–4359.
14) Grael, C. F., Vichnewski, W., Souza, G. E., Lopes, J. L., Albuquerque, S. and Cunha, W. R. (2000) A study of the trypanocidal and analgesic properties from *Lychnophora granmongolense* (Duarte) Semir & Leitão Filho. *Phytother. Res.*, **14**, 203–206.
15) Ribeiro, A., Piló-Veloso, D., Romanha, A. J. and Zani, C. L. (1997) Trypanocidal flavonoids from *Trixis vauthieri. J. Nat. Prod.*, **60**, 836–841.
16) Lopes, N. P., Chicaro, P., Kato, M. J., Albuquerque, S. and Yoshida, M. (1998) Flavonoids and lignans from *Virola surinamensis* twigs and their in vitro activity against *Trypanosoma cruzi. Planta Med.*, **64**, 667–668.
17) Kiuchi, F., Itano, Y., Uchiyama, N., Honda, G., Tsubouchi, A., Nakajima-Shimada, J. and Aoki, T. (2002) Monoterpene hydroperoxides with trypanocidal activity from *Chenopodium ambrosioides. J. Nat. Prod.*, **65**, 509–512.
18) Uchiyama, N., Matsunaga, K., Kiuchi, F., Honda, G., Tsubouchi, A., Nakajima-Shimada, J. and Aoki, T. (2002) Trypanocidal terpenoids from *Laurus nobilis* L. *Chem. Pharm. Bull.* (Tokyo), **50**, 1514–1516.
19) Kiuchi, F., Matsuo, K., Ito, M., Qui, T. K. and Honda, G. (2004). New sesquiterpene hydroperoxides with trypanocidal activity from *Pogostemon cablin. Chem. Pharm. Bull.* (Tokyo), **52**, 1495–1496.
20) Chiari, E., de Oliveira, A. B., Raslan, D. S., Mesquita, A. A. L. and Tavares, K. G. (1991) Screening in vitro of natural products against blood forms of *Trypanosoma cruzi. Trans. R. Soc. Trop. Med. Hyg.*, **85**, 372–374.
21) Fournet, A., Muñoz, V., Roblot, F., Hocquemiller, R., Cavé, A. and Gantier, J. C. (1993) Antiprotozoal activity of dehydrozaluzanin C, a sesquiterpene lactone isolated from *Munnozia maronii* (Asteraceae). *Phytother. Res.*, **7**, 111–115.
22) Schmidt, T. J., Brun, R., Willuhn, G. and Khalid, S. A. (2002) Anti-trypanosomal activity of helenalin and some structurally related sesquiter-

pene lactones. *Planta Med.*, **68**, 750–751.
23) Brengio, S. D., Belmonte, S. A., Guerreiro, E., Giordano, O. S., Pietrobon, E. O. and Sosa, M. A. (2000) The sesquiterpene lactone dehydroleucodine (DhL) affects the growth of cultured epimastigotes of *Trypanosoma cruzi. J. Parasitol.*, **86**, 407–412.
24) D'Silva, C. and Daunes, S. (2002) The therapeutic potential of inhibitors of the trypanothione cycle. *Expert Opin. Investig. Drugs*, **11**, 217–231.
25) Jimenez-Ortiz, V., Brengio, S. D., Giordano, O., Tonn, C., Sánchez, M., Burgos, M. H. and Sosa, M. A. (2005) The trypanocidal effect of sesquiterpene lactones helenalin and mexicanin on cultured epimastigotes. *J. Parasitol.*, **91**, 170–174.
26) Da Costa, F. B., Albuquerque, S. and Vichnewski, W. (1996) Diterpenes and synthetic derivatives from *Viguiera aspillioides* with trypanomicidal activity. *Planta Med.*, **62**, 557–559.
27) Alves, T. M., Chaves, P. P., Santos, L. M., Nagem, T. J., Murta, S. M., Ceravolo, I. P., Romanha, A. J. and Zani, C. L. (1995) A diterpene from *Mikania obtusata* active on *Trypanosoma cruzi. Planta Med.*, **61**, 85–87.
28) Torres-Mendoza, D., Urena-Gonzalez, L. D., Ortega-Barria, E., Capson, T. L. and Cubilla-Rios, L. (2003) Five new cassane diterpenes from *Myrospermum frutescens* with activity against *Trypanosoma cruzi. J. Nat. Prod.*, **66**, 928–932.
29) Kiuchi, F., Matsuo, K., Ito, M., Qui, T. K. and Honda, G. (2004) New norditerpenoids with trypanocidal activity from *Vitex trifolia. Chem. Pharm. Bull.*, **52**, 1492–1494.
30) Sanchez, A. M., Jimenez-Ortiz, V., Sartor, T., Tonn, C. E., García, E. E., Nieto, M., Burgos, M. H. and Sosa, M. A. (2006) A novel icetexane diterpene, 5-*epi*-icetexone from *Salvia gilliessi* is active against *Trypanosoma cruzi. Acta Trop.*, **98**, 118–124.
31) Uchiyama, N., Ito, M., Kiuchi, F., Honda, G., Takeda, Y., Khodzhimatov, O. K. and Ashurmetov, O. A. (2004) A trypanocidal diterpene with novel skeleton from *Dracocephalum komarovi. Tetrahedron Lett.*, **45**, 531–533.
32) Uchiyama, N., Kiuchi, F., Ito, M., Honda, G., Takeda, Y., Khodzhimatov, O. K. and Ashurmetov, O. A. (2003) New icetexane and 20 norabietane diterpenes with trypanocidal activity from *Dracocephalum komarovi. J. Nat. Prod.*, **66**, 128–131.
33) Sepúlveda-Boza, S. and Cassels, B. K. (1996) Plant metabolites active against *Trypanosoma cruzi. Planta Med.*, **62**, 98–105.
34) Uchiyama, N., Kabututu Z., Kubata, B. K., Kiuchi, F., Ito, M., Nakajima-Shimada, J., Aoki, T., Ohkubo, K., Fukuzumi, S., Martin, S. K., Honda, G. and Urade, Y. (2005) Anti-chagasic activity of komaroviquinone is due to generation of reactive oxygen species catalyzed by *Trypanosoma cruzi* old yellow enzyme. *Antimicrob. Agents Chemother.*, **49**, 5123–5126.
35) Uchiyama, N., Nakamura, N., Konishi, T., Kawahara, N., Itabashi, T., Kawai, K. and Nakajima-Shimada, J. (2008) Trypanocidal activities of fungal products part 3. *Proc. 128th Annu. Meet. Pharm. Soc. Jpn.*, Yokohama, Japan, **2**, p.125.