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Laboratory note

Studies on the trypanocidal activity of semi-synthetic pyran[*b*-4,3]naphtho[1,2-*d*]imidazoles from β-lapachone

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Abstract

We synthesized new naphthoimidazoles from β-lapachone with an aromatic moiety linked to the imidazole ring, using phenylic and heterocyclic aldehydes. The most active compound against *Trypanosoma cruzi* had a *p*-methyl group linked to the phenyl ring, presenting an EC_{50} value of 15.5 ± 2.9 μM. No reliable correlation could be established with the biological activity and the structure of in the phenylic series. For the heterocyclic series, activity was associated with a three bond-distance from nitrogen to the imidazole ring, in accordance with our previous work.

Keywords: *Trypanosoma cruzi*; Chagas' disease; Naphthoquinones; β-lapachone; Naphthoimidazoles; Chemotherapy

1. Introduction

Naphthoquinones present a broad distribution in the plant kingdom and their structures endow them with redox properties, which could interfere in different biological oxidative processes [1]. In folk medicine, plants containing naphthoquinones have been employed for the treatment of several diseases [2]. Among naturally occurring naphthoquinones we have lapachol and β-lapachone, from the heartwood of trees of Bignoniaceae and Verbanaceae families. Their microbicidal activity had been described, in Brazil, by Gonçalves Lima et al. [3], and subsequent studies described other biological activities, such as anti-tumoural, anti-inflammatory, bactericidal, fungicidal and virucidal [4].

The potential use of β-lapachone and semi-synthetic derivatives as chemotherapeutic agent against Chagas disease has been investigated. This disease, endemic in Latin America, is caused by the protozoa *Trypanosoma cruzi* and affects about 16–18 million people [5]. Efforts have been addressed by several research groups to find more efficient and safe agents for the treatment of this disease [6], since the available nitroheterocyclic (benznidazole) presents severe side effects, and its efficacy depends on the susceptibility of different parasite populations. β-lapachone, among several natural naphthoquinones, showed the highest activity against *T. cruzi*, leading to the generation of free radicals and inhibition of nucleic acid and protein synthesis [7–9].

Due to the variety of microbicidal effects, the easy access to natural sources of quinones from Brazilian flora, and the synthetic alternative routes already developed by our group for the reactivity of quinoidal carbonyl towards nucleophylic agents, we took naphthoquinones, especially β-lapachone, as starting points for medicinal chemistry studies [7,10–17]. We have previously synthesized several derivatives from the reaction of naphthoquinones isolated from *Tabebuia* (*Tecoma*) spp., leading to naphthoimidazoles, naphthoxazoles, and other groups of heterocyclics. The original quinones plus 37 derivatives were assayed against trypomastigotes of *T. cruzi*, the infective form for the mammalian host [4,18,19]. Among the 13 naphthoimidazoles and 14 naphthoxazoles assayed, 50% of the compounds presented activity higher than crystal violet, indicating a trend of trypanocidal activity among these naphthalenic heterocyclics. Two naphthoimida-

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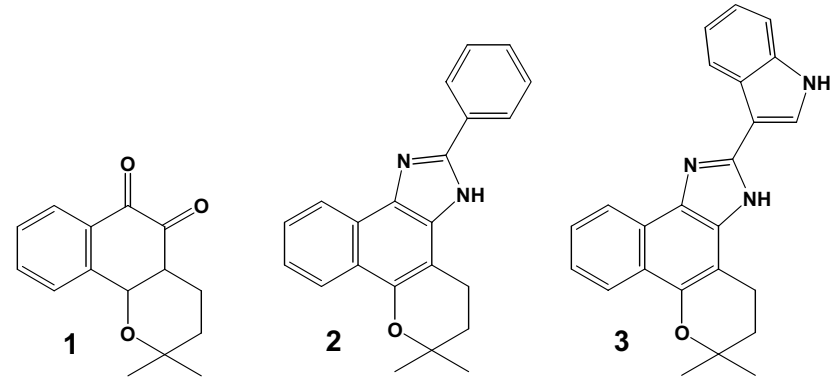
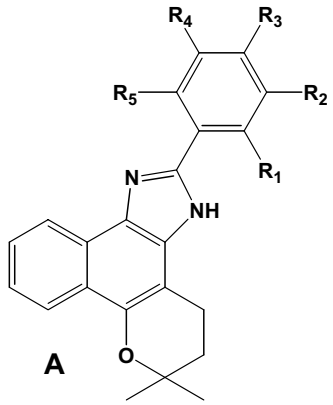
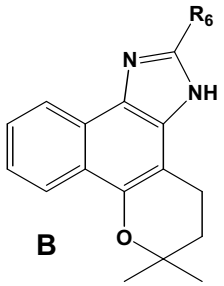






4,**7**,**10**	R_1=*X*	R_2-R_5 =H
5,**8**,**11**	R_2=*X*	R_1,R_3-R_5=H
6,**9**,**12**	R_3=*X*	R_1,R_2,R_4,R_5=H
13	R_1=CH_3	R_2-R_5 =H
14	R_2=CH_3	R_1,R_3-R_5=H
15	R_3=CH_3	R_1,R_2,R_4,R_5=H
16	R_1=CF_3	R_2-R_5 =H
17	R_2=CF_3	R_1,R_3-R_5=H
18	R_3=CF_3	R_1,R_2,R_4,R_5=H
19	R_1=CN	R_2-R_5 =H
20	R_2=CN	R_1,R_3-R_5=H
21	R_3=CN	R_1,R_2,R_4,R_5=H

R_6

22

23

4-6 *X*=F; **7-9** *X*=Cl; **10-12** *X*=Br

Scheme 1. Structures of the naphtoimidazoles obtained from β-lapachone.

zoles derived from β-lapachone (**1**), with the aromatic moieties phenyl (**2**) and 3-indolyl (**3**) linked to the imidazole ring, showed the highest activity against the parasite. These results stimulated us to continuing the screening of the naphthoimidazoles, synthesizing new derivatives with structures related to **2** and **3** (Scheme 1).

2. Chemistry

β-lapachone was obtained through cyclization in sulfuric acid by nucleophylic attack of oxygen of the isoprenyl side chain of lapachol, which was extracted in large scale from the heartwood of *Tabebuia* sp. and purified by recrystalizations as previously described [13].

The naphtoimidazoles were synthesized by the reaction of β-lapachone and the proper aldehyde, in the presence of ammonium acetate [4,18,19]. The spectral data for the synthesized compounds are in agreement with the structures depicted in Scheme 1.

3. Results and discussion

Although quinoidal substances are important sources of heterocyclics, there are only a few reports about the reactivity

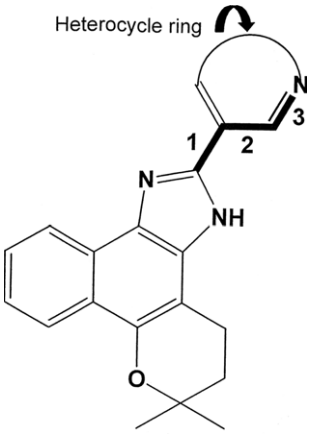
Table 1
Effect of naphthoimidazoles derived from β-lapachone against *T. cruzi*

#	EC_{50}/24 h (μM)	RA [a]
1 [b]	391.5 ± 16.5 [c]	1.37
2 [b]	37.0 ± 0.7	14.49
3 [d]	15.4 ± 0.2	34.81
4 [d]	243.3 ± 24.6	2.20
5 [d]	372.0 ± 38.7	1.44
6	98.0 ± 4.8	5.47
7	39.4 ± 8.1	13.60
8	1064.2 ± 61.6	0.50
9	2286.3 ± 21.1	0.23
10	2004.0 ± 22.9	0.27
11	147.8 ± 12.5	3.63
12	84.9 ± 3.2	6.31
13	90.8 ± 5.8	5.90
14	37.5 ± 12.8	14.29
15	15.5 ± 2.9	34.58
16	448.0 ± 55.7	1.20
17	128.7 ± 29.4	4.16
18	227.5 ± 58.0	2.36
19	>8000	–
20	518.5 ± 78.9	1.03
21	1095.9 ± 92.9	0.49
22	154.9 ± 10.4	3.46
23	190.5 ± 30.3	2.81
CV	536.0 ± 3.0	1.00

[a] RA (relative activity) = ED_{50} **CV**/ED_{50} compound.
[b] Ref. [18].
[c] Mean ± standard deviation of at least four separate experiments.
[d] Ref. [4].

of 1,2-quinoidal carbonyls towards nucleophilic reagents [4,14,15,18]. In this work, through the reaction of β-lapachone with phenylic and heterocyclic aromatic aldehydes in the presence of ammonium acetate were synthesized a series of pyran[*b*-4,3]naphtho[1,2-*d*]imidazoles with substituted aromatic rings at the 2-position. The structural pattern of the synthesized compounds can be divided according to the groups appended at the imidazole ring: (**A**) phenyl with halogens (F, Cl, Br), methyl, trifluoromethyl or cyane, and with disubstituted with hydroxy plus nitro (**4–21**) (Scheme 1A); (**B**) pyridinyl and quinolyl rings (**22 e23**) (Scheme 1B).

The new naphthoimidazoles were assayed against bloodstream forms of *T. cruzi* and only six of them showed lower activity when compared with crystal violet (Table 1). Three derivatives (*o*-fluoro, *m*-fluoro and *m*-chloro) (**4,5,8**) have been previously published [4], but were included in order to compare with the new related phenyl derivatives here described. Among the substituted phenyl derivatives (Scheme 1A), those more active against the parasite were *p*-fluoro (**3**), *o*-chloro (**7**), *p*-bromo (**11**) and the three methyl derivatives (**10–12**), all with EC_{50}/24 below 100 μM. The assays with trifluoromethyl derivatives (**16–18**) showed EC_{50} values in the range of 128–448 μM. As their synthesis was performed aiming to find a correlation between trypanocidal activity and structure, it was possible to verify that only one compound of this series (**15**), with a methyl group at the *para* position in the phenyl ring showed higher activity than the non-substituted imidazole (**2**). Although the compounds **4–21** have substituents with different size and electronic characteristics, no consistent correlation between their structures and biological activity could be established. The only association that could be suggested is that all the substituents are prone to enhance the lipophylic character around the phenyl moiety, condition that would facilitate non-polar–non-polar interactions with vital biomolecules of the parasite.



Scheme 2. Naphthoimidazoles with pyridinyl and quinolyl rings (**22** and **23**), in which the nitrogen atom of the heterocyclic substituent are be located three bonds-distant from its point of attachment to the pyran[*b*-4,3]naphtho[1,2-*d*]imidazolic skeleton.

For the heterocyclic series two derivatives with the nitrogen heteroatom located at a three bonded-distance from the imidazolic ring, *R* = 3′-pyridinyl (**22**) and 3′-quinolyl (**23**) (Scheme 1B), were also active against *T. cruzi*. These results are in accordance with our previous work that showed that the naphthoimidazole (**3**) with a 3′-indolic substituent at the imidazole moiety, was 34.8 times more active than crystal violet [19].

In relation to the mechanism of action, we can exclude damage to the parasite caused by oxidative stress since, unlike the original β-lapachone, **4–21** do not easily undergo redox reactions. However, it is possible that the compounds of in the phenyl series (Scheme 1A), with smaller lipophilic phenyl substituted groups, could act through intercalation within a polar regions of proteins. On the other hand, for the heterocyclic series (Scheme 1B), the planar substituted heterocyclic rings at the 2-position could facilitate a polar–polar interaction of the compounds with macromolecules through the non-bonding electron pair of the nitrogen atom. The present results and those previously reported [19] led us to suggest that for enhancement of the trypanocidal activity, the nitrogen atom of the heterocyclic substituent should be located three bonds-distant from its point of attachment to the pyran[*b*-4,3]naphtho[1,2-*d*]imidazolic skeleton (Scheme 2).

In previous reports [4,18,19], we have already detected that the cyclofunctionalizations of β-lapachone to imidazolic structures led to two of the most active derivatives with phenyl (**2**) and 3-indolyl (**3**) linked to the imidazolic ring. The present work shows another very active naphthoimi-

dazole, compound **15**, with *p*-methyl as substituent (EC_{50}/24 h = 15.5 ± 2.9 μM). In continuity to our work we will investigate in more detail these three naphthoimidazoles, assaying their effect against intracellular amastigotes, toxicity to mammalian cells, possible alterations in ultrastructural organization of the parasite, aiming to detect possible organelle(s) susceptible to these derivatives, and also the direct effect of these compounds upon selected targets in *T. cruzi*.

4. Experimental section

4.1. Chemistry

Melting points were determined in a capillary Thomas Hoover apparatus and are uncorrected. ^{1}H and ^{13}C NMR were recorded using a Varian Gemini 200 and Brucker 200 MHz spectrophotometer, in the solvents indicated, with TMS as internal standard at room temperature. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hertz. Infrared spectra were recorded on a Perkin–Elmer 783 spectrophotometer and Nicolet IRTF in KBr pellets or liquid films. UV spectra were obtained in Shimadzu UV-1601 spectrophotometer in ethanol or hexane. The mass spectra were obtained at 70 eV in a VG Autospec apparatus. The fragments were described as a relation between atomic mass units and the charge (*m/z*) and the relative abundance in percentage. Elemental analysis of the compounds was within ±0.4% of the theoretical values. Column chromatography was performed using silica gel (0.063–0.200 mm/70–230 mesh). Removal of the solvents during isolation and purification of the compounds was done in a Büchi rotatory evaporator under reduced pressure. When necessary, a high vacuum system (0.1–10 mm Hg) was also used. Visualization of the compounds on the chromatographic plates was done under ultraviolet light, exposure to iodine vapor or spraying 2% cerium sulfate in 2 N sulfuric acid followed by gentle heating.

*4.2. General procedure for the preparation of the naphthoimidazoles **4–23***

To a solution of β-lapachone (1.1 mmoles) in acetic acid (6 ml), the proper aldehyde (2.5 mmoles) was added and the mixture gently heated till 70 °C; at this point ammonium acetate (16.5 mmoles) was slowly added and reflux was maintained for a determined time. The course of all the reactions was monitored by thin layer chromatography. At the end of the reaction, after addition into water, the precipitate was purified by column chromatography using as eluent a mixture of hexane/ethyl acetate, with the ratio 9:1, with increasing polarity gradient, as previously described [18]. Other minor products, of oxazole type, were also isolated and are under study.

*4.2.1. 4,5-dihydro-6,6-dimethyl-6H-2-(2′-fluorophenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**4**)*

The aldehyde employed was 2-fluorobenzaldehyde after 30 min of reflux. This compound was obtained in 91% yield, m.p. 195 °C.

UV λ_{max} nm (log ϵ) 348 (4.22), 290 (4.18), 253.0 (4.48), 223 (4.48). IR (KBr) cm^{-1} 3423, 3152, 3107, 3064, 2974, 2948, 2929, 1599, 1583, 1462, 1444, 1252, 1223, 1160, 1120, 1056, 952, 880, 772, 758, 733. MS *m/z* (%) 346 (29), 347 (7), 317 (12), 303 (8), 290 (67), 275 (2): 262 (6), 241 (3). ^{1}H NMR ($CDCl_3$) δ 9.8 (ls, 1H), 8.5 (dd, 1H), 8.3 (d, 1H), 7.4 (m, 2H), 7.2 (m, 4H), 3.0 (sl, 2H), 1.95 (t, 2H), 1.4 (s, 6H).).

*4.2.2. 4,5-dihydro-6,6-dimethyl-6H-2-(3′-fluorophenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**5**)*

The aldehyde employed was 3-fluorobenzaldehyde after 20 min of reflux. This compound was obtained in 90% yield, m.p. 238 °C.

UV λ_{max} nm (log ϵ) 351 (4.48), 295 (4.38), 255 (3.60): 227 (4.62), 208 (4.65). IR (KBr) cm^{-1} 3447, 3105, 3074, 2975, 2927, 2849, 1615, 1584, 1485, 1473, 1458, 1212, 1159, 1121, 1056, 969, 954, 885, 866, 766, 713. MS *m/z* (%) 346 (45), 347 (11), 331 (1), 317 (2), 303 (8), 290 (100), 275 (2), 261 (5). ^{1}H NMR ($(CD3)_2CO$) δ 8.5 (d, 1H), 8.24 (d, 1H), 8.1 (d, 1H), 8.01 (m, 1H), 7.5 (m, 2H), 7.4 (dd, 1H), 7.15 (m, 1H), 3.1 (t, 2H), 2.0 (t, 2H), 1.4 (s, 6H). ^{13}C NMR (CD_3CO) δ 1416 (s), 1412 (s), 1404 (s), 1304 (s), 1295 (d), 1294 (s), 1293 (s), 1292 (d), 1291 (d), 1286 (d), 1277 (s), 1231 (d), 1229 (d), 1218 (d), 1218 (d), 1217 (d), 1213 (d), 1038 (s), 915 (d), 745 (s), 325 (t), 260 (q), 220 (t).

*4.2.3. 4,5-dihydro-6,6-dimethyl-6H-2-(4′-fluorophenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**6**)*

The aldehyde employed was 4-fluorobenzaldehyde after 25 min of reflux. This compound was obtained in 90% yield, m.p. 295 °C, $C_{22}H_{19}N_2OF$.

UV λ_{max} nm (log ϵ) 346 (4.26), 291 (4.26), 254 (4.45), 212 (4.50). IR (KBr) cm^{-1} 3445, 3172, 3134, 3077, 3048, 2978, 2972, 2955, 2932, 2697, 1631, 1611, 1585, 1548, 1528, 1488, 1435, 1379, 1372, 1333, 1287, 1259, 1232, 1197, 1160, 1197, 1144, 1054, 1015, 970, 953, 881, 835, 816, 769, 726, 688, 647, 634, 580, 518. MS *m/z* (%) 346 (2), 290 (4), 252 1, 140 (0,5), 44 (12), 40 (100). ^{1}H NMR (DMSO) δ 8.40 (d, 1H), 8.30 (m, 2H), 8.10 (d, 1H), 7.50 (t, 1H), 7.40 (m, 3H), 3.1 (t, 2H), 2.00 (t, 2H), 1.40 (s, 6H).

*4.2.4. 4,5-dihydro-6,6-dimethyl-6H-2-(2′-chlorophenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**7**)*

The aldehyde employed was 2-chlorobenzaldehyde after 30 min of reflux. This compound was obtained in 85% yield, m.p. 134 °C.

UV λ_{max} nm (log ϵ) 340.8 (3.62), 288.2 (3.58), 253.2 (4.09), 220.2 (4.12), 202.4 (4.05). IR (KBr) cm^{-1} 3644, 3445, 3220, 3166, 3123, 3068, 2970, 2939, 2926, 2841, 2657, 1609, 1589, 1567, 1475, 1463, 1449, 1439, 1381, 1367, 1341, 1321, 1260, 1242, 1159, 1121, 1061, 1037, 953, 881,

766, 755, 722, 695, 653, 630, 462, 453. MS *m/z* (%) 364(12), 362 (36), 319 (7), 308 (36), 306 (100), 271 (3), 181 (4), 166 (7), 148 (8,5), 140 (37), 130 (14), 115 (22), 102 (14), 89 (11), 77 (5). ^{1}H NMR ($CDCl_3$) δ 8.50 (d, 2H), 8.30 (d, 2H), 7.70–7.30 (m, 4H), 3.10 (t, 2H), 2.00 (t, 2H), 1.10 (s, 6H).

*4.2.5. 4,5-dihydro-6,6-dimethyl-6H-2-(3′-chlorophenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**8**)*

The aldehyde employed was 3-chlorobenzaldehyde after 25 min of reflux. This compound was obtained in 84% yield, m.p. 234 °C.

UV λ_{max} nm (log ϵ) 354 (4.41), 296 (4.31), 253 (4.54), 210 (4.63). IR (KBr) cm^{-1} 3095, 3074, 3051, 2973, 2947, 2928, 1595,1587, 1446, 1367, 1259, 1159, 1121, 1056, 970, 951, 778, 721. MS *m/z* (%) 364 (12), 363 (10), 362 (39), 347 (2.5), 319 (6), 306 (100), 292 (1), 271 (4), 242 (7). ^{1}H NMR ($CDCl_3$) δ 8.5 (d, 1H), 82 (m, 1H), 8.1 (d, 2H), 7.5 (m, 1H), 7.4 (m, 3H), 3.0 (t, 2H), 1.9 (t, 2H), 1.4 (s, 6H).

*4.2.6. 4,5-dihydro-6,6-dimethyl-6H-2-(4′-chlorophenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**9**)*

The aldehyde employed was 4-chlorobenzaldehyde after 20 min of reflux. This compound was obtained in 81% yield, m.p. 288 °C.

UV λ_{max} nm (log ϵ) 353.2 (4.16), 296.2 (4.06), 254.2 (4.28), 227.2 (4.28). IR (KBr) cm^{-1} 3619, 3432, 3177, 2975, 2931, 1633, 1587, 1519, 1432, 1377, 1338, 1258, 1159, 1118, 1054, 1013, 953, 834, 769, 722. MS *m/z* (%) 364 (2.5), 362 (5.7), 319 (0.8), 306 (12), 140 (1,8), 44 (12), 40 (100). ^{1}H NMR ($(CD3)_2CO$) δ 8.50 (d, 1H), 8.30 (d, 2H), 8.10 (d, 1H), 7.60 (m, 3H), 7.40 (m, 1H), 3.10 (t, 2H), 1.90 (t, 2H), 1.40 (s, 6H).

*4.2.7. 4,5-dihydro-6,6-dimethyl-6H-2-(2′-bromophenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**10**)*

The aldehyde employed was 2-bromo benzaldehyde after 30 min of reflux. This compound was obtained in 78% yield, m.p. 135 °C.

UV λ_{max} nm (log ϵ) 354 (4.36), 296.2 (4.25), 252.6 (4.49, 212.6 (4.57). IR (KBr) cm^{-1} 3418, 3071, 3048, 2973, 2928, 2847, 1587, 1567, 1444, 1366, 1259, 1242, 1159, 1144, 1121, 1056, 969, 765, 720, 709, 693. MS *m/z* (%) 409 (12), 408 (49), 407 (17), 406 (54), 365 (7), 363 (7), 353 (32), 352 (94), 351 (36), 350 (100), 242 (8), 195 (4,5), 169 (5.6), 155 (8), 140 (33), 130 (8), 115 (13), 114 (11), 113 (9), 102 (9), 89 (8), 41 (6). ^{1}H NMR ($(CD3)_2CO$) δ 12,42 (sl, 1H), 11,88 (s, 1H), 8,56 (d, 1H), 8,38 à 8,22 (m, 1H), 7,88 (dd, 1H), 7,76 (dd, 1H), 7,62 à 7,32 (m, 4H), 1,44 (s, 6H).

*4.2.8. 4,5-dihydro-6,6-dimethyl-6H-2-(3′-bromophenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**11**)*

The aldehyde employed was 3-bromo benzaldehyde after 40 min of reflux. This compound was obtained in 75% yield, m.p. 251 °C.

UV λ_{max} nm (log ϵ) 354.4 (4.34), 296.2 (4.22), 252.8 (4.48), 212.2 (4.56). IR (KBr) cm^{-1} 3646, 3434, 3068, 2973, 2926, 2849, 1593, 1566, 1446, 1367, 1341, 1259, 1242, 1160, 1145, 1121, 1056, 968, 881, 765, 719, 709, 694, 648. MS *m/z* (%) 409 (11), 408 (47), 407 (17), 406 (51), 365 (7), 363 (8), 353 (33), 352 (100), 351 (36), 350 (98), 271 (12), 242 (10), 195 (5), 169 (5), 155 (10), 148 (15), 140 (26), 130 (10), 115 (13), 114 (11), 113 (8), 102 (8), 89 (6), 44 (29). ^{1}H NMR (DMSO) δ 13.24 (s, 1H), 12.78 (s, 1H), 8.44 (d, 1H), 8.30–8.20 (m, 1H), 8.20–8.10 (m, 1H), 7.70–7.66 (m, 4H), 3.16–2.96 (m, 2H), 2.04–1.86 (m, 2H), 1.40 (s, 6H).

*4.2.9. 4,5-dihydro-6,6-dimethyl-6H-2-(4′-bromophenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**12**)*

The aldehyde employed was 4-bromo benzaldehyde after 30 min of reflux. This compound was obtained in 85% yield, m.p. 280 °C.

UV λ_{max} nm (log ϵ) 354 (4.29), 297 (4.20), 254 (4.41), 227.5 (4.43), 205 (4.52). IR (KBr) cm^{-1} 3435, 3219, 3183, 3136, 3069, 2975, 2927, 1633, 1585, 1519, 1432, 1381, 1333, 1258, 1242, 1160, 1144, 1119, 1055, 1011, 953, 828, 771, 722, 646. MS *m/z* (%) 409 (12), 408 (45), 407 (16), 406 (45), 365 (6), 363 (8), 353 (32), 352 (100), 351 (31), 350 (94), 271 (10), 242 (10), 195 (5), 169 (6), 155 (11), 148 (12), 142 (11), 140 (24), 130 (10), 115 (17), 114 (11), 113 (8), 102 (8), 89 (10), 44 (23). ^{1}H NMR (DMSO) δ 8.44 (d, 1H), 8.20 (m, 3H), 7.76 (d, 2H), 7.60-7.50 (m, 2H), 1.44 (s, 6H).

*4.2.10. 4,5-Dihydro-6,6-dimethyl-6H-2-(2′-methylphenyl)-pyran[*b*-4,3]naphth[1,2-*d*] imidazole (**13**)*

The aldehyde employed was 2-methylbenzaldehyde after 50 min of reflux. This compound was obtained in 70% yield, m.p. 135 °C.

UV λ_{max} nm (log ϵ) 340 (3.61), 252.2 (4.10), 221.2 (4.10). IR (KBr) cm^{-1} 3654, 3099, 3057, 3012, 2971, 2945, 2923, 2848, 2795, 2707, 2653, 2597, 1633, 1587, 1548, 1519, 1487, 1430, 1402, 1382, 1366, 1322, 1259, 1240, 1205, 1158, 1121, 1091, 1055, 1027, 966, 952, 882, 838, 768, 760, 728, 719, 703, 674, 644, 629, 598, 465, 448. MS *m/z* (%) 343 (9), 342 (44), 327 (3), 300 (2), 299 (7), 287 (34), 286 (100), 258 (1), 257 (5), 156 (5), 154 (7), 143 (6), 142 (7), 130 (8), 129 (7), 115 (8), 114 (4), 102 (4), 41 (4). ^{1}H NMR (DMSO) δ 8.40 (d, 1H), 8.20 (d, 1H), 7.80 (m, 1H), 7.50–7.30 (m, 4H), 3.10 (t, 2H), 2.70 (s, 3H), 1.90 (t, 2H), 1.40 (s, 6H).

*4.2.11. 4,5-Dihydro-6,6-dimethyl-6H-2-(3′-methylphenyl)-pyran[*b*-4,3]naphth[1,2-*d*] imidazole (**14**)*

The aldehyde employed was 3-methylbenzaldehyde after 45 min of reflux. This compound was obtained in 73% yield, m.p. 230 °C.

UV λ_{max} nm (log ϵ) 347.4 (4.29), 292.8 (4.28), 254.4 (4.45), 213 (4.49). IR (KBr) cm^{-1} 3153, 3093, 3050, 3011, 2972, 2944, 2926, 2848, 2809, 27858, 2702, 2651, 1631, 1604, 1584, 1542, 1444, 1435, 1366, 1322, 1260, 1242, 1208, 1159, 1142, 1121, 1099, 1056, 1039, 1018, 969, 950, 906, 881, 848, 833, 784, 767, 722, 711, 687, 660, 647, 634, 597, 543. MS *m/z* (%) 343 (11), 342 (43), 341 (9), 327 (3), 300 (3), 299 (7), 287 (36), 286 (100), 258 (2), 257 (4), 162

(8), 156 (6), 154 (7), 143 (4), 141 (5), 140 (8), 130 (7), 129 (3), 115 (8), 114 (4), 102 (4), 441 (11). ^{1}H NMR (DMSO) δ 13.1 (s, 1H), 12.6 (s, 1H), 8.40 (d, 1H), 8.10 (m, 3H), 7.50 (t, 1H), 7.40 (t, 2H), 7.10 (d, 1H), 3.10 (sl, 2H), 2.20 (s, 3H), 1.90 (t, 2H), 1.40 (s, 6H).

*4.2.12. 4,5-dihydro-6,6-dimethyl-6H-2-(4′-methylphenyl)-pyran[*b*-4,3]naphth[1,2-*d*] imidazole (**15**)*

The aldehyde employed was 4-methylbenzaldehyde after 45 min of reflux. This compound was obtained in 78% yield, m.p. 250 °C.

UV λ_{max} nm (log ϵ) 347 (4.21), 293 (4.22), 255 (4.37), 209.5 (4.63). IR (KBr) cm^{-1} 3201, 3166, 3132, 3075, 3044, 2971, 2952, 2930, 2851, 2755, 2693, 1631, 1599, 1585, 1574, 1527, 1488, 1459, 1442, 1433, 1379, 1370, 1332, 1319, 1283, 1259, 1242, 1159, 1144, 1119, 1054, 1018, 970, 953, 880, 840, 820, 769, 724, 690, 646, 638, 508. MS *m/z* (%) 342 (4), 287 (4), 286 (13(, 149 (4), 59 (4), 58 (26), 44 (37), 43 (100), 42 (9), 41 (5). ^{1}H NMR (DMSO) δ 13.2 (sl, 1H), 12.7 (sl, 1H), 8.40 (d, 1H), 8.20 (d, 3H), 7.50 (t, 1H), 7.30 (t 3H), 3.10 (t, 2H), 2.40 (s, 3H), 2.00 (sl, 2H), 1.40 (s, 6H).

*4.2.13. 4,5-dihydro-6,6-dimethyl-6H-2-(2′-trifluoromethylphenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**16**)*

The aldehyde employed was 2-trifluoromethylbenzaldehyde after 15 min of reflux. This compound was obtained in 86% yield, m.p. 146 °C.

UV λ_{max} nm (log ϵ) 338.6 (3.81), 289.4 (3.71), 253.4 (4.39), 221.8 (4.38). IR (KBr) cm^{-1} 3688, 3645, 3446, 3160, 3108, 3070, 3023, 2971, 2925, 2867, 2823, 1633, 1602, 1587, 1548, 1526, 1445, 1434, 1383, 1369, 1316, 1289, 1261, 1242, 1161, 1122, 1058, 1054, 1035, 967, 952, 882, 839, 766, 739, 719, 687, 647, 629, 596, 551, 530, 446. MS *m/z* (%) 397 (23), 396 (89), 353 (7), 342 (33), 340 (90), 321 (37), 320 (100), 292 (13), 291 (17), 273 (14), 198 (5), 180 (11), 173 (25), 153 (8), 152 (13), 140 (11), 113 (6), 102 (7), 44 (23). ^{1}H NMR ($CDCl_3$) δ 8.30 (d, 2H), 7.90–7.70 (ml, 2H), 7.40 (m, 4H), 3.00 (sl, 2H), 1.90 (t, 2H), 1.40 (s, 6H).

*4.2.14. 4,5-dihydro-6,6-dimethyl-6H-2-(3′-trifluoromethylphenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**17**)*

The aldehyde employed was 3-trifluoromethylbenzaldehyde after 15 min of reflux. This compound was obtained in 88% yield, m.p. 193 °C.

UV λ_{max} nm (log ϵ) 357.8 (4.22), 355.4 (4.23), 295.4 (4.11), 253.8 (4.34), 228.2 (4.34), 212.2 (4.33). IR (KBr) cm^{-1} 3647, 3071, 3053, 2974, 2924, 2870, 2848, 2781, 2733, 2696, 1584, 1520, 1485, 1456, 1447, 1353, 1341, 1324, 1318, 1305, 1281, 1258, 1239, 1169, 1160, 1122, 1071, 1057, 970, 953, 907, 883, 841, 806, 765, 730, 720, 711, 695, 648, 621, 596, 531, 450, 418. MS *m/z* (%) 397 (10), 396 (45), 353 (7), 341 (35), 340 (100), 292 (1), 291 (1.7), 273 (1), 198 (3), 179 (2), 173 (3), 153 (3), 152 (3), 140 (12), 115 (10), 114 (6), 102 (3), 41 (5). ^{1}H NMR ($CDCl_3$) δ 8.50–8.20 (ml, 4H), 7.60–7.40 (ml, 4H), 3.00 (t, 2H), 1.90 (t, 2H), 1.40 (s, 6H).

*4.2.15. 4,5-dihydro-6,6-dimethyl-6H-2-(4′-trifluoromethylphenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**18**)*

The aldehyde employed was 4-trifluoromethylbenzaldehyde after 15 min of reflux. This compound was obtained in 92% yield, m.p. 200 °C.

UV λ_{max} nm (log ϵ) 359.8 (4.32), 299.2 (4.13), 250.4 (4.39), 228.8 (4.41). IR (KBr) cm^{-1} 3624, 3208, 3160, 3128, 3075, 3030, 2976, 2947, 2923, 2851, 2708, 1632, 1617, 1604, 1587, 1576, 1449, 1443, 1370, 1325, 1287, 1260, 1245, 1165, 1122, 1066, 1058, 1014, 958, 883, 850, 814, 764, 748, 716, 697, 686, 648, 594, 501. MS *m/z* (%) 397 (9), 396 (38), 353 (6), 341 (32), 340 (100), 242 (1), 198 (3), 180 (1), 173 (2), 153 (2), 152 (3), 140 (11), 130 (5), 115 (9), 114 (6), 113 (4), 102 (3), 44 (17). ^{1}H NMR ($(CD3)_2CO$) δ 8.50 (sl, 1H), 8.40 (d, 2H), 8.30 (d, 1H), 7.80 (t, 1H), 7.40 (t, 1H), 3.10 (tl, 2H), 2.00 (t, 2H), 1.40 (s, 6H).

*4.2.16. 4,5-dihydro-6,6-dimethyl-6H-2-(2′-cyanephenyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**19**)*

The aldehyde employed was 2-cyanebenzaldehyde after 15 min of reflux. This compound was obtained in 68% yield, m.p. 192 °C.

UV λ_{max} nm (log ϵ) 363.5 (4.00), 283 (4.00), 253 (4.35), 222 (4.44). IR (KBr) cm^{-1} 3578, 3413, 3222, 3169, 3122, 3067, 2973, 2942, 2923, 2224, 1721, 1712, 1665, 1610, 1590, 1523, 1485, 1441, 1383, 1368, 1341, 1323, 1296, 1260, 1242, 1192, 1161, 1120, 968, 962, 884, 768, 760, 747, 738, 714, 695, 657, 646, 633, 555, 531, 499, 461. MS *m/z* (%) 354 (3), 353 (11), 336 (4), 298 (8), 297 (33), 140 (5), 115 (5), 102 (3), 44 (100). ^{1}H NMR (DMSO) δ 8,40 (d, 1H), 8,20 (t, 2H), 8,00 (d, 1H), 7,80 (m, 1H), 7,60 (m, 2H), 7,50 (d, 1H), 3,00 (t, 2H), 2,00 (t, 2H), 1,40 (s, 6H).

*4.2.17. 4,5-dihydro-6,6-dimethyl-6H-2-(3′-cyanephenyl)-pyran[*b*-4,3]naphth[1,2-*d*] imidazole (**20**)*

The aldehyde employed was 3-cyanebenzaldehyde after 20 min of reflux. This compound was obtained in 72% yield, m.p. 265 °C.

UV λ_{max} nm (log ϵ) 361 (4.35), 296 (4.22), 255 (4.51), 222.5 (4.63). IR (KBr) cm^{-1} 3433, 3287, 3065, 2967, 2936, 2919, 2849, 2242, 1665, 1594, 1584, 1515, 1486, 1446, 1433. 1385, 1370, 1334, 1286, 1268, 1259, 1244, 1201, 1161, 1146, 1123, 1059, 1016, 975, 967, 952, 886, 800, 761, 714, 680, 661, 648, 596, 587, 475. MS *m/z* (%) 354 (9), 353 (43), 336 (4), 310 (4), 298 (33), 297 (100), 161 (6), 140 (11), 115 (11), 102 (5), 44 (22). ^{1}H NMR (DMSO) δ 8.60 (m, 2H), 8.40 (d, 1H), 8.20 (d, 1H), 7.80 (d, 1H), 7.70 (t, 1H), 7.60 (t, 1H), 7.40 (t, 1H), 3.00 (t, 2H), 1.90 (t, 2H), 1.30 (s, 6H).

*4.2.18. 4,5-dihydro-6,6-dimethyl-6H-2-(4′-cyanephenyl)-pyran[*b*-4,3]naphth[1,2-*d*] imidazole (**21**)*

The aldehyde employed was 4-cyanebenzaldehyde after 25 min of reflux. This compound was obtained in 76% yield, m.p. 160 °C.

UV λ_{max} nm (log ϵ) 381 (4.95), 305 (4.01), 293 (4.01), 251 (4.37), 234 (4.42), 207.5 (4.37). IR (KBr) cm^{-1} 3627, 3430,

3405, 3211, 3166, 3130, 3070, 2979, 2925, 2225, 2089, 1706, 1601, 1586, 1562, 1525, 1483, 1452, 1440, 1384, 1369, 1354, 1344, 1323, 1288, 1280, 1260, 1244, 1200, 1182, 1164, 1122, 1060, 1039, 970, 958, 949, 880, 853, 808, 738, 716. MS *m/z* (%) 354 (10), 353 (42), 336 (4), 298 (31), 297 (100), 167 (8), 141 (6), 140 (11)), 130 (5), 115 (10), 114 (7), 102 (6), 41 (4). ^{1}H NMR (DMSO) δ 13.50 (sl, 1H), 13.00 (s, 1H), 8.40 (d, 3H), 8.20 (d, 1H), 8.00 (d, 2H), 7.50 (m, 1H), 3.00 (t, 2H), 2.00 (t, 2H), 1.40 (s, 6H).

*4.2.19. 4,5-dihydro-6,6-dimethyl-6H-2-(3′-pyridinyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**22**)*

The aldehyde employed was 3-pyridine carboxyaldehyde after 40 min of reflux. This compound was obtained in 77% yield, m.p. 146 °C.

UV λ_{max} nm (log ϵ) 356 (4.29), 293.5 (4.13), 254 (4.45), 228.5 (4.44). IR (KBr) cm^{-1} 3219, 3161, 3104, 3051, 2976, 2924, 2843, 2727, 2661, 1588, 1575, 1448, 1422, 1382, 1369, 1342, 1322, 1271, 1262, 1245, 1202, 1160, 1147, 1120, 1060, 1028, 958, 883., 813, 767, 718, 705, 662, 649, 630, 598. MS *m/z* (%) 331 (1), 330 (4), 329 (16), 314 (1), 313 (1), 301 (2), 289 (3), 274 (8), 273 (32), 150 (1), 130 (2), 115 (2), 114 (1), 113 (1), 105 (2), 102 (1), 88 (1), 79 (1), 78 (2), 77 (2), 55 (1), 51 (1), 44 (16), 41 (4), 40 (100). ^{1}H NMR (DMSO) δ 9.80 (d, 1H), 9.10 (d, 1H), 8.45 (d, 1H), 8.17 (m, 3H), 7.88–7.70 (m, 1H), 7.75–7.56 (m, 2H), 7.54–7.42 (m, 1H), 3.10 (t, 2H), 2.00 (t, 2H), 1.42 (s, 6H).

*4.2.20. 4,5-dihydro-6,6-dimethyl-6H-2-(3′-quinolinyl)-pyran[*b*-4,3]naphth[1,2-*d*]imidazole (**22**)*

The aldehyde employed was 3-quinoline carboxyaldehyde after 50 min of reflux. This compound was obtained in 75% yield, m.p. 166 °C.

UV λ_{max} nm (log ϵ) 384.5 (4.35), 285.5 (4.43), 255 (4.59), 232 (4.72). IR (KBr) cm^{-1} 3102, 3059, 2973, 2940, 2926, 2849, 1740, 1636, 1587, 1576, 1499, 1484, 1147, 1402, 1382, 1366, 1352, 1319, 1273, 1260, 1241, 1203, 1161, 1145, 1122, 1097, 1040, 969, 952, 943, 913, 885, 862, 835, 781, 762, 745, 718, 705, 667, 645, 579, 475. MS *m/z* (%) 379 (1), 323 (3), 191 (2), 115 (1), 109(3), 77 92), 58 (9), 46 (5), 45 (10), 44 (100), 43 (12), 42 (6). ^{1}H NMR (DMSO) δ 9.44 (s, 1H), 9.43 (s, 1H), 9.40 (d, 1H), 8.68–8.62 (m, 1H), 8.59 (t, 1H), 8.54 (t, 1H), 8.45 (d, 1H), 8.18 (d, 1H), 7.64–7.52 (m, 3H), 3.15 (t, 2H), 2.00 (t, 2H), 1.44 (s, 6H).

4.3. Trypanocidal assay

Stock solutions of the compounds (**4–23**) were prepared in dimethylsulfoxide, with the final concentration of the solvent in the experiments never exceeding 0.1%. Bloodstream forms were obtained at the peak of parasitaemia from *T. cruzi*-infected Swiss mice, isolated by differential centrifugation and resuspended in Dulbecco's modified Eagle medium (DME) containing 10% mice blood to a concentration of 5×10^6 trypomastigotes/ml. This suspension (100 μl) was added to the same volume of the compounds prepared in DME at twice the desired final concentrations in 96-well microplates. After incubation for 24 h at 4 °C, the parasites were counted in a Neubauer chamber. The activity of the compounds was quantified by the EC_{50} that corresponds to the concentration that lyses 50% of the parasite. Untreated and crystal violet-treated parasites were used as controls [20].

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