3’,5’-Dibromo-2’,4’-dihydroxy Substituted Chalcones: Synthesis and in vitro Trypanocidal Evaluation

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Abstract

A new series of 3’, 5’-dibromo-2’, 4’-dihydroxy substituted chalcones was synthesized and evaluated for their inhibitory effect against Trypanosoma cruzi (Chagas disease). Some of these compounds 3c, 3g and 3m showed 85.53, 85.03 and 83.34% in vitro percentage growth of inhibition respectively; while compounds 3b, 3e, 3i and 3l showed 71.23, 71.95, 67.53 and 68.88 percentage growth of inhibition respectively with nifurtimox and benznidazole as reference drugs. 3l was the compound with a good anti-trypanocidal activity, the lower cytotoxicity, higher therapeutic index (14.5), and was the best candidate in comparison with the others. The structures of the newly synthesized compounds (3a-t) were determined by elemental analysis, FTIR, 1HNMR, 13CNMR and mass spectroscopic studies.

Keywords: Chalcones; Trypanosoma cruzi; Chagas disease; Inhibition

Introduction

Currently, an infectious disease crisis of global proportion is threatening hard-won gains in health and life expectancy. Infectious diseases are the world’s largest killer of children and young adults. Chagas disease is one of them which are caused by the protozoan parasite T. cruzi. It is a major cause of illness, morbidity, long-term disability, and death in Latin America. This disease is the third largest parasitic disease burden in the world and an estimated 10 million people are infected with this disease worldwide, mostly in Latin America [1]. In Latin America, infection with T. cruzi is responsible for Chagas disease, which is the leading cause of heart disease [2]. Despite the alarming health, economic and social consequences of this parasite infection and the limited existing drug therapy (nifurtimox and benznidazole) suffer from a combination of drawbacks including poor efficacy and serious side effects. Therefore, there is an urgent need for new chemotherapeutic agents with novel mechanisms of action [3-6]. Chalcones are a diverse group of compounds that can be synthesized or obtained from natural sources. This type compounds is 1, 3-diaryl-2-propan-1-ones and belong to the flavonoid family. These compounds are small molecules that exert various biological activities [7-10]. Moreover, they provide an opportunity for chemist to synthesize a wide variety of bioactive heterocycles [11-14] due to the presence of α, β-unsaturated carbonyl functionality. However, the search for an efficient synthesis for chalcones remains a challenging task.

The most widely used classical method for synthesizing chalcones is the Claisen-Schmidt condensation or through microwave irradiation [15-17] and ultrasonic irradiation because of their rapidity and improvement in yields [18,19]. In a continuation of our earlier endeavour [20-22] to design and synthesize novel bioactive heterocycles, and to consider the biological and medicinal importance of chalcones, herein, we reported a new series of chalcones via conventional as well as non-conventional microwave irradiation method and their trypanocidal evaluation.

Results and Discussion

Chemistry

3’,5’-dibromo-2’,4’-dihydroxyacetophenone (1) was treated with substituted aromatic aldehydes (2) (Note: From the structure, they are not aromatic aldehydes) to give substituted chalcones (3a-t) as shown in Scheme 1, with 74-84% yields. The structures of the newly synthesized compounds (3a-t) were determined on the basis of analytical and spectroscopic data. Thus, FTIR spectrum showed bands at 1688-1722 (C=O), 1642-1654 (-CH=CH), 1’H NMR spectrum revealed the presence of a doublet at δ 7.48-7.98 corresponding to α Hydrogen and doublet at δ 8.11-8.42 corresponding to β Hydrogen and 13C NMR spectrum revealed the presence of Cα group (122.96-131.94), Cβ group (133.36-155.64), C=O group (187.87-192.40) ppm. All these newly synthesized compounds were evaluated for their in vitro trypanocidal evaluation using nifurtimox and benznidazole as reference drugs.

Biological evaluation

The results of percentage growth of inhibition are summarized in (Table 1). The compounds 3a, 3d, 3f, 3h, 3j, 3k, 3n, 3o, 3r, 3s, 3t don’t have trypanocidal activity but there are nine active compounds that do have trypanocidal activity, which were evaluated for their IC50 and their cytotoxicity. Table 2 shows the compounds’ IC50 cytotoxicity, therapeutic index, and the values of the compounds with more anti trypanocidal activity. When the range of therapeutic index is short, testing in vivo is not recommended; we found that compounds 3b, 3c, 3e, 3g, 3i and 3l have a good IC50 and their cytotoxicity is low, which means that these compounds could be used in vivo studies. 3l was the compound with a good anti-trypanocidal activity, the lower cytotoxicity, higher therapeutic index 14.5, and is the best candidate in comparison with the others. This compound has a therapeutic index lower than that of benznidazole and nifurtimox.

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Experimental section

General: All melting points (m.p.) were determined in open capillaries on Veego (VMP – PM) melting point apparatus and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) with Silica Gel-G (Merck). The instruments used for spectroscopic data are the IR spectrophotometer Brucker Alpha-Zn-Se, 1H NMR and 13C NMR (CDCl3) on 500 MHz FT-NMR spectrometer Bruker AV III, GC-MS (EI-MS fragment) performed on JEOL GC Mate spectrometer and elemental analysis was carried out on a Carlo Erba 1108 analyzer within ± 0.5% of the theoretical values. Column chromatography was performed on silica.

Scheme 1: Synthesis of the title compounds (3a-t).

| Entry | Concentration Used | % Growth of Inhibition | Entry | Concentration Used | % Growth of Inhibition |
|-------|---------------------|------------------------|-------|---------------------|------------------------|
| 3a    | 10 ug/mL            | 9.84                   | 3l    | 10 ug/mL            | 68.88                  |
| 3b    | 10 ug/mL            | 71.23                  | 3m    | 10 ug/mL            | 83.34                  |
| 3c    | 10 ug/mL            | 85.53                  | 3n    | 10 ug/mL            | 33.14                  |
| 3d    | 10 ug/mL            | 29.51                  | 3o    | 10 ug/mL            | 9.58                   |
| 3e    | 10 ug/mL            | 71.95                  | 3p    | 10 ug/mL            | 54.22                  |
| 3f    | 10 ug/mL            | 25.57                  | 3q    | 10 ug/mL            | 51.41                  |
| 3g    | 10 ug/mL            | 85.03                  | 3r    | 10 ug/mL            | 15.41                  |
| 3h    | 10 ug/mL            | 13.06                  | 3s    | 10 ug/mL            | 10.40                  |
| 3i    | 10 ug/mL            | 67.53                  | 3t    | 10 ug/mL            | 5.80                   |
| 3j    | 10 ug/mL            | 19.34                  | Nifurtimox | 10 ug/mL            | 68.50                  |
| 3k    | 10 ug/mL            | 23.32                  | Benznidazole | 10 ug/mL            | 86.77                  |

*Each value is the mean of three experiments

Table 1: Biological evaluation of synthesized chalcones against Trypanosoma cruzi; % Growth inhibition.

| Entry | Concentration used | % Growth of Inhibition | IC50* (ug/mL) | Cytotoxicity* (ug/mL) | T.I.* |
|-------|---------------------|------------------------|---------------|-----------------------|-------|
| 3b    | 10 ug/mL            | 71.23                  | 6.04          | 32                    | 5.3   |
| 3c    | 10 ug/mL            | 85.53                  | 4.55          | 10                    | 2.2   |
| 3e    | 10 ug/mL            | 71.95                  | 5.53          | 32                    | 5.8   |
| 3g    | 10 ug/mL            | 85.03                  | 5.24          | 25                    | 4.8   |
| 3i    | 10 ug/mL            | 67.53                  | 5.48          | 30                    | 5.5   |
| 3l    | 10 ug/mL            | 68.88                  | 2.07          | 14                    | 14.5  |
| 3m    | 10 ug/mL            | 83.34                  | 5.42          | 30                    | 2.6   |
| 3p    | 10 ug/mL            | 54.22                  | 4.54          | 28                    | 6.2   |
| 3q    | 10 ug/mL            | 51.41                  | 9.23          | 25                    | 2.7   |
| Nifurtimox | 10 ug/mL            | 68.50                  | 0.47          | 27                    | 57.44 |
| Benznidazole | 10 ug/mL            | 86.77                  | 0.81          | >50                   | >62   |

*Each value is the mean of three experiments

Table 2: Biological evaluation of active samples against Trypanosoma cruzi; IC50, Cytotoxicity & therapeutic index.
gel (Merck, 60-120 mesh). Microwave assisted reaction was carried out on a commercially modified MW synthesis system model CATA-R, operating 700W, generating 2450 MHz frequency.

**Synthetic procedures for (3a-t):**

- **Conventional solution phase method**

A mixture of 3′,5′-dibromo-2′,4′-dihydroxyacetophenone 1 (0.01 mol) and substituted aromatic aldehydes 2 (Note: From the structure, they are not aromatic aldehydes ) (0.01 mol) in 1 mL DMF placed in 100 mL borosil flask, was added 4 g basic alumina. The mixture was kept overnight at room temperature and then it was poured into crushed ice and acidified with HCl. The solid was obtained by filtering and then it was crystallized from ethanol and offered the analytical samples of (3a-t).

- **Non-conventional solid phase method**

To a solution of 3′,5′-dibromo-2′,4′-dihydroxyacetophenone 1 (0.01 mol) and substituted aromatic aldehyde 2 (Note: From the structure, they are not aromatic aldehydes ) in 30 mL ethanol and then 15mL 40% KOH solution was added to it. The mixture was cooled at room temperature and the product was extracted with dichloromethane (2×20 mL). Removal of the solvent and subsequent adsorbed material was irradiated inside a gel (Merck, 60-120 mesh). Microwave assisted reaction was carried out on a commercially modified MW synthesis system model CATA-R, operating 700W, generating 2450 MHz frequency.

**Spectroscopic data of the synthesized compounds are shown below:**

**Table 3:** Comparison of reaction time and yield of synthesized chalcones under MW and classical method.

| Entry | MW (hrs or min?) | Reaction time | Yield (%) |
|-------|-----------------|---------------|-----------|
| 3a    | 7               | Classical (hrs) | MW Classical |
| 3b    | 8               | 17            | 76         |
| 3c    | 6               | 18            | 79         |
| 3d    | 7               | 18            | 80         |
| 3e    | 8               | 81            | 65         |
| 3f    | 9               | 83            | 66         |
| 3g    | 8               | 80            | 70         |
| 3h    | 8               | 77            | 64         |
| 3i    | 8               | 21            | 81         |
| 3j    | 7               | 19            | 80         |
| 3k    | 7               | 20            | 80         |
| 3l    | 7               | 20            | 84         |
| 3m    | 8               | 19            | 80         |
| 3n    | 9               | 21            | 82         |
| 3o    | 9               | 20            | 79         |
| 3p    | 7               | 19            | 84         |
| 3q    | 8               | 21            | 81         |
| 3r    | 7               | 22            | 77         |
| 3s    | 6.9             | 22            | 78         |
| 3t    | 7               | 20            | 74         |

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Ar-H), 8.25 (d, J=15.6Hz). \(^{13}C\)-NMR (125 MHz, CDCl\(_3\)) δ: 79.73, 98.98, 103.44, 115.23, 127.82 (2a), 129.86, 130.92, 133.67, 145.26 (Cβ), 149.29, 155.66, 163.88, 188.65 (C=O). MS: m/z 415.80 (M\(^+\)). Calcd. for C\(_{15}\)H\(_9\)Br\(_3\)O\(_3\): C, 43.50, H, 2.42%. Found: C, 43.55, H, 2.37%.

(2E)-1-(3', 5'-dibromo-2', 4'-dihydroxyphenyl)-3-(4-fluorophenyl) prop-2-en-1-one prop-2-en-1-one

(3g). Brown solid; Yield (?), m.p. 159-160°C. IR (KBr, cm\(^{-1}\)): 3458 (Ar-OH), 3089, 3007 (Ar-H), 2832 (CH\(_2\)), 1665 (C=CH), 862 (C-Br). \(^{1}H\)-NMR (500 MHz, CDCl\(_3\)) δ: 7.76-7.61 (m, 4H, Ar-H), 8.09 (s, 2H, 2 x Ar-Br), 8.14 (d, J=15.6Hz). MS: m/z 443.80 (M\(^+\)). Calcd. for C\(_{16}\)H\(_{12}\)Br\(_2\): C, 43.26, H, 2.70%. Found: C, 43.32, H, 2.65%.

(2E)-1-(3', 5'-dibromo-2', 4'-dihydroxyphenyl)-3-(4-methoxyphenyl) prop-2-en-1-one

(3i). Red solid; Yield (?). m.p. 3402 (Ar-OH), 3089, 3007 (Ar-H), 2832 (OCH\(_3\)), 1715 (C=O), 1651 (C=CH), 862 (C-Br). \(^{13}C\)-NMR (500 MHz, CDCl\(_3\)) δ: 3.86 (s, 3H, OCH\(_3\)), 7.00-7.48 (m, 4H, Ar-H), 7.84 (d, J=16.0Hz), 10.82 (s, 2H, 2 x Ar-OH), 8.08 (s, 1H, Ar-H), 8.17 (d, J=16.0Hz). \(^{1}C\)-NMR (125 MHz, CDCl\(_3\)) δ: 56.29 (OCH\(_3\)), 80.25, 99.84, 115.05, 116.04, 123.87 (Ca), 129.31, 131.71, 133.40, 138.26 (Cβ), 156.20, 160.35, 161.77, 190.77 (C=O). MS: m/z 427.80 (M\(^+\)). Calcd. for C\(_{16}\)H\(_{12}\)Br\(_2\)O\(_3\), C, 44.88, H, 2.80%. Found: C, 44.83, H, 2.74%.

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The parasite: T. cruzi (Tulahuen C4) transfected with β-galactosidase was measured at 570 nm using a readore boards VersaMax Micro™ microplate reader. The IC50 of the compound was calculated by logartihmic regression analysis of the percentages of OD obtained, compared with the untreated control. Those samples showing IC50 values <50 μg/mL have been further tested for cell viability and cytotoxicity evaluation. Nifurtimox (Bayer) was used as a control at concentrations of 0.1, 1 and 10 μg/mL. Negative control is comprised of 50 μL of a solution containing DMSO, equivalent to the DMSO contained in samples (working dilution).

Cytotoxicity assay: Active Compounds were screened for cytotoxicity against VERO cells line, at a maximum concentration of 50 μg/mL. Briefly, Vero cell line were seeded into 96-well plate at a total concentration of 1x104 cells/well in 100 μl of RPMI-1640 media without phenol red with 10% FBS. Cells were allowed to attach for 24 hrs. The wells were incubated with five decreasing concentrations, diluted in RPMI 1640 modified media or RPMI 1640 modified media alone, used as control. After 72 hrs, a colorimetric MTT assay was performed. Wells were incubated for 4 hrs with 5 μg/mL of the tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide/Aldrich company, St. Louis MO). The IC50 of the compound was determined by the colorimetric method based on reducing the absorbance as compared with untreated controls that were incubated for 24 hrs. The absorbance was measured at 570 nm using an ELISA microplate reader (VersaMax Micro™ microplate reader) at 562 nm. Tetrazolium salts are cleaved to formazan by mitochondrial cells lysed with 100% isopropanol. The absorbance was measured using an ELISA microplate reader (VersaMax Micro™ microplate reader) at 562 nm. Tetrazolium salts are cleaved to formazan by mitochondrial enzymes in viable cells. Therefore, an increase in the OD reading, as a result of production of formazan, indirectly measures cell viability. The IC50 value was defined as the concentration of test sample resulting in a 50% reduction of absorbance as compared with untreated controls that received a serial dilution of the solvent in which the test samples were dissolved, and was determined by linear regression analysis.

Conclusions

Two noteworthy features are apparent from our study project on the synthesis of small molecules of medicinal interest. Firstly, a novel series of substituted chalcones has been synthesized and it is concluded from Table 2 that classical procedure is tedious, time consuming, low yield and requiring an appreciable amount of solvent as compared to the environmentally benign synthetic procedure utilizing microwave irradiation (MWI) under solvent free conditions, over inorganic solid support. Secondly, it was observed from the results obtained by the trypanocidal evaluation that compounds: 3b, 3c, 3e, 3g, 3i, 3l, 3m, 3p, and 3q have a good IC50 and their cytotoxicity is low, this means that these compounds could be used in future in vivo studies. 3l was the compound with a good anti-trypanocidal activity, the lower
cytotoxicity, higher therapeutic index 14.5, and is the best candidate in comparison with the others. This compound has a therapeutic index lower than that of benznidazole and nifurtimox. Our results demonstrate the potential of these compounds as a new class of small molecule inhibitors of T. cruzi. The further biologically assay of the tested compounds gives an idea about the possible development for a new encouraging framework in this field that may lead to the discovery of potent trypanocidal drug.

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