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An efficient synthesis of new caffeine-based chalcones, pyrazolines and pyrazolo[3,4-*b*][1,4]diazepines as potential antimalarial, antitrypanosomal and antileishmanial agents

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ABSTRACT

A new series of chalcones **5a**–**f** were synthesized from caffeine-based aldehyde **3** and substituted acetophenones **4a**–**f**. Treatment of compounds **5a**–**f** with hydrazine hydrate led to pyrazolines **6a**–**f**, and their subsequent reaction with acetic anhydride or formic acid afforded the corresponding N-substituted pyrazolines **7a**–**f** and **8a**–**f** respectively. Additionally, the regioselective cyclocondensation reaction of chalcones **5a**–**f** with 4,5-diaminopyrazole **9** afforded the diazepine derivatives **10a**–**f**. Synthesis of the above novel compounds was carried out through a simple procedure involving an easy work-up and mild reaction conditions. In vitro antimalarial activity against Plasmodium falciparum was evaluated for the obtained compounds. Among of them, just pirazoline **6a** showed an outstanding growth inhibition percentage 85.2 ± 5.4%, while diazepines **10a**–**f** showed remarkable growth inhibitions in the range of 80.3 ± 13.5 to 94.2 ± 0.2% when were tested at 20 μg/mL. Compounds **5b**, **5e**, **7c** and **7f** showed remarkable activities against Leishmania panamensis with growth inhibition of 88.3 ± 1.5, 82.6 ± 2.2, 82.8 ± 1.7 and 87.6 ± 0.5% respectively, at 20 μg/mL. In vitro assays against Trypanozoma cruzi showed that pyrazoline **6d** displayed a growth inhibition of 61.9 ± 7.8% at 20 μg/mL while chalcone **5f** was considered especially active with a growth inhibition of 9.7 ± 1.5% for a very low concentration of 1.0 μg/mL.

1. Introduction

Caffeine is an important heterocyclic compound found in nature; its biological activity has been associated with the central nervous system "CNS" and the literature highlight reports in which substitutions on the caffeine ring at C-8 position exhibited important activity against Monoamine Oxidase "MAO" (Type A and B) [1–4]. In fact (*E*)-8-styrylcaffeine derivatives are structures reported with activity against MAO [5]. Strydom and co-workers reported the synthesis of 8-alkyloxy derivatives and their inhibition of MAO with good results [6,7].

On the other hand, chalcones containing several functional groups have been exhibited a wide spectrum of biological activities including antitumor [8,9], antibacterial [10,11], anti-inflammatory [12], antileishmanial [13,14], antimalarial [15–17] and antitrypanosomal [18] activities.

The five membered nitrogen-containing heterocyclic compounds as pyrazolines have shown important antitumor [9,19], antitrypanosomal [20] and antileishmanial activity [21]. We recently reported the synthesis of novel chalcones and pyrazolines containing the 7-chloroquinoline fragment, which showed remarkable antimalarial activity against *Plasmodium falciparum* [22].

The seven membered nitrogen-containing heterocyclic compounds are a scaffold of high synthetic interest because they have shown diverse biological properties including antiviral [23–25], antimicrobial and antioxidant effects [26]. We recently report the synthesis and antitumor activity studies of new pyrimido[4,5-*b*]

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[1,4]diazepines with important values of (GI_{50}) values in the range of 0.46–1.46 μM [27], 0.473–0.638 μM [28] and 0.068–0.35 μM [29] when submitted to *in vitro* assays.

To the best of our knowledge there are not reports in the literature about synthetic caffeine-based compounds showing antimalarial, antileishmanial or antitrypanosomal activity. For that, in this work we report an efficient and straightforward approach for the synthesis of chalcones, pyrazolines and pyrazolo[3,4-*b*][1,4] diazepines, containing the caffeine pharmacophore in their structures in order to evaluate their *in vitro* antimalarial, antileishmanial and antitrypanozomal activities.

2. Results and discussion

2.1. Chemistry

By using a previously reported methodology [30], 8-chlorocaffeine **1** was synthesized and subjected to nucleophilic aromatic substitution reaction (S_NAr) with vanillin **2** in DMF and potassium carbonate under microwave irradiation. This reaction proceeded with the formation of aldehyde **3** in 90% yield. Then a Claisen–Schmidt reaction between aldehyde **3** and substituted acetophenones **4a**–**f** led the novel caffeine-based chalcones **5a**–**f** (Scheme 1), in good yields (51–89%).

Structure elucidation of compounds **5a**–**f** was performed from analysis of their spectroscopic data (FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry). The IR spectrum of compound **5d** as representative of this series, showed an absorption band at 1713 cm^{-1} associated to the carbonyl group from the α,β-unsaturated fragment and absorption bands at 1703 and 1662 cm^{-1} associated to the carbonyl groups of the caffeine fragment. In the ^{1}H NMR spectrum of compound **5d**, three singlets at 3.21, 3.22 and 3.79 ppm indicated the presence of the three methyl groups from caffeine unit, two doublets at 7.73 and 7.94 ppm with coupling constant $^3J = 15.6$ Hz assigned to H_α and H_β respectively, indicating the *trans* configuration for the vinylic system. The ^{13}C NMR spectrum of compound **5d** showed a signal at 189.1 ppm corresponding to the carbonyl group of the α,β-unsaturated fragment. The signals corresponding to caffeine carbonyl groups appear at 150.5 and 153.6 ppm assigned to $C_{2'}$ and $C_{6'}$ respectively.

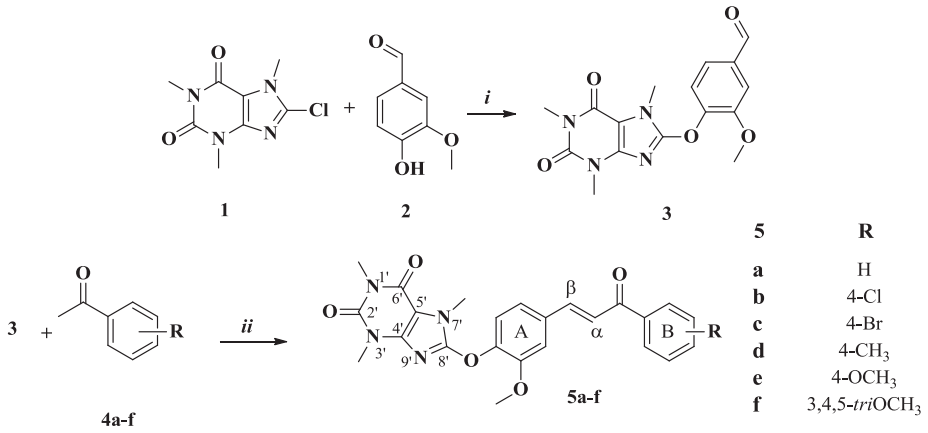
Treatment of chalcones **5a**–**f** with hydrazine hydrate in ethanol at room temperature for 1 h, led to the formation of the pyrazolo-derivatives **6a**–**f** (Scheme 2), in good yields (90–96%).

In order to functionalize the –NH group of the dihydro-1*H*-pyrazole ring, the treatment of compounds **6a**–**f** with acetic anhydride or formic acid, both acting as reagents and as solvents, at room temperature for 1 h (Scheme 3), afforded the acylated and formylated products **7a**–**f** and **8a**–**f** respectively, in good yields (86–97%).

Structure elucidation of pyrazolines **6a**–**f**, **7a**–**f** and **8a**–**f** was performed from their spectroscopic data (FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry). The IR spectrum of compound **6b** as representative of the series, showed an absorption band at 3322 cm^{-1} associated to the –NH group from the pyrazole moiety, which disappeared in the IR spectra of acylated and formylated compounds **7b** and **8b**, respectively. Regarding to the ^{1}H NMR, the spectrum of compound **6b** showed a singlet at 7.79 ppm corresponding to –NH group, while for compound **7b** this signal is absent and contrary, a singlet at 2.33 ppm, assigned to the methyl of the acyl-inserted group, was observed. Spectral data of compound **8b**, showed a singlet at 8.93 ppm associated to the proton of the formyl-inserted group. In all cases the two methylene protons of the carbon atom C-4 and the stereogenic proton in C-5 generates an ABX spin system. As discussed for compound **8b**; the signal associated to proton H-4_A appears as a double-doublet at 3.28 ppm with coupling constant values $^2J_{AB} = 18.2$ Hz and $^3J_{AX} = 5.1$ Hz, for proton H–4_B the double-doublet appears at 3.98 ppm, with coupling values $^2J_{AB} = 18.2$ Hz and $^3J_{BX} = 11.8$ Hz while the corresponding signal of the H-5_X proton appeared as a double-doublet at 5.58 ppm with coupling values $^3J_{BX} = 11.8$ Hz and $^3J_{AX} = 5.2$ Hz. Furthermore, the ^{13}C NMR spectra of compound **7b** and **8b** showed signals at 168.2 and 159.9 ppm corresponding to the carbonyls of the acyl and formyl groups respectively.

In accordance with our current program on the synthetic utility of chalcones in the obtention of aromatic and heteroaromatic annelated 1,4-diazepines, the treatment of equimolar amounts of chalcones **5a**–**f** with 4,5-diamino-3-methyl-1-phenylpyrazole **9** in *N*,*N*-dimethylformamide and $BF_3.OEt_2$ as catalyst under microwave irradiation resulted in the regioselective formation of the desired products **10a**–**f** regioselectively (Scheme 4). In all cases reactions proceeded with the same behavior and yields in the range of 60–75%.

Due to the presence of non-equivalent amino groups in the pyrazole ring of **9**, two possible regioisomeric cyclization products could be expected. However, selective formation of the single product type **10** was observed, which could be associated with the electronic effect of the pyrazole ring which enhances selectively the nucleophilicity of the amino group on C-4. Therefore, condensation of such amino group with the α,β-unsaturated carbonyl group of **5a**–**f** followed by a Michael-type addition of the less nucleophilic amino group to the C=C double bond should afford the isolated products **10a**–**f**.



Scheme 1. General methodology for the synthesis of aldehyde **3** and chalcones **5a**–**f**: *i* = DMF, K_2CO_3 (2 equiv), MW, *ii* = NaOH 20%, EtOH, rt, 8 h.