Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(2):1016-1021

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis, Characterization, trypanosomal activities on *Trypanosoma brucei brucei* and toxicity against *Artemia salina* Leach of N(4)-aryl semicarbazones and thiosemicarbazones

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ABSTRACT

*N(4)-phenyl substituted semicarbazones and thiosemicarbazones (**1-4**) of propiophenone and 4'-methylacetophenone have been synthesized and characterized by spectrometrical methods analyses (IR, RMN ^{1}H &^{13}C, SM). All compounds were evaluated for their in vitro trypanosomal activity against the bloodstream form of the strain 427 of Trypanosomabruceibrucei and have been tested on larvaeofbrine shrimp, Artemiasalina LEACH, for their toxic activity. The selectivity index (SI) of each molecule was too designed. In the group, propiophenone 4-phenyl-3-thiosemicarbazone **4** has exhibited greater trypanocidal activity with a half-inhibitory concentration (IC_{50}) value equal to 7.63 micromolar (µM). 4'-methylacetophenone 4-phenylsemicarbazone **1** showed moderate anti-trypanosomal activity (IC_{50} = 62.54 µM). Other, **2** and **3**, presented little or no activity against the parasite (IC_{50}> 100 µM). Except propiophenone 4-phenylsemicarbazone **2** which offered a toxic activity on larvae given the half-lethal concentration LC_{50} = 107.49 µM and SI = 0.518 < 1 and has then a good selectivity on cells of larval shrimp Artemiasalina, all compounds showed negligible toxicity (LC_{50}> 281 µM and SI > 1, compounds **1**, **3** and **4**). They turn out quite selective on the parasite. Synthesized compounds could constitute a new class of anti-trypanosomal drug candidates.*

Keywords:Propiophenone, 4'-methylacetophenone, N(4)-phenylsemicarbazone, N(4)-phenyl-3-thiosemicarbazones, Anti-trypanosomal Activity, toxicity, Selectivity Index,.

INTRODUCTION

Livestock plays a vital role in the production systems in Africa south of the Sahara in general. It helps to improve the income of people and contributes to increase the performance of the agricultural sector not only by the supply of organic manure but also through the production of energy for traction and transportation. Livestock production is and remains an asset to the development of the African continent. Protozoan parasites are fearsome pathogens that are responsible for a significant proportion of mortality and morbidity as well as human poverty. [1]. In Africa, the protozoan parasite of the genus *Trypanosoma* causes animal and human African trypanosomiasis[2]. It infects cattle and is a major problem for livestock [3]. In wild animals, these parasites cause relatively mild infections while in domestic animals they cause a severe, often fatal disease. When the illness progresses the animals weaken more and more and eventually become unfit for work [4]. Once the cattle affected, it results in reduced productivity or death [5]. This form of trypanosomiasis lost livelihoods impeding economic development and land settlement in tropical Africa [3, 5]. In addition, these wild and domestic animals may play a major role as parasite reservoirs for human

infections with trypanosomes [6-8]. The AAT is thus one of the greatest constraints to increasing livestock productivity and increased agricultural production resulting in profound effects on the economy, social structure and quality of life in endemic areas.

Indeed, the semicarbazones, thiosemicarbazones and their derivatives in recent years have presented multiple biological activities [9-18] and in particular anti-trypanosomal activities [19-24]. They could therefore help to fight against this disease in Africa.

To contribute to the fight against this scourge, this study focuses on the synthesis of N(4)-phenyl substituted semicarbazones and thiosemicarbazones of 4'-methylacetophenone and propiophenone and on the evaluation of their anti-trypanosomal activities on *Trypanosomabruceibrucei* and larval toxicity test on *Artemiasalina* Leach.

EXPERIMENTAL SECTION

Equipment

Melting points of the products were taken on a fusionometer of the type electrothermal 1A 9000 and are uncorrected. The IR spectra were recorded on a Perkin-Elmer FTIR 286. The frequencies of absorption bands are expressed in cm^{-1}. The NMR spectra were registered on a spectrophotometer type Brucker500 in DMSO-d_6 or $CDCl_3$ and the frequencies for ^1H and ^{13}C are 400 MHz and 100 MHz respectively. Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane as a benchmark. Multiplicity is designated as singlet (s), singlet dedoubled (s_d), triplet (t), triplet dedoubled (t_d), doublet (d), quartet dedoubled (q_d) and multiplet (m). Mass spectrometrical data of compounds were reported in APCI mode.

Reagents

4-phenylsemicarbazide and 4-phenyl-3-thiosemicarbazide obtained from ^{A}ALDRICH^R (SIGMA-ALDRICH Chemie GmbH, Germany) were used on 4'-methylacetophenone obtained from Fluka AG-Buchs SG and propiophenone purchased from MERCK-Schuchardt. Sodium acetate and the glacial acetic acid using in the reactions are obtained from PROLABO (EMB de 45-Briare, France).

Compounds were synthesized using the following synthesis route (scheme 1)

Synthesis of 4-phenylsemicarbazones

A solution of 4-phenylsemicarbazide (1 mmol), sodium acetate (1 mmol) in 1 mL of hydrochloric acid (1N) and 10 mL of water was added slowly to a stirring solution of appropriate ketone (1 mmol) in 2.5 mL of ethanol. If the reaction mixture becomes turbid, we added ethanol to remove the turbidity. The reaction mixture was stirred at room temperature until precipitation. The precipitate obtained were frozen, filtered and recrystallized from aqueous ethanol (95°) to give desired product (**1** and **2**).

Synthesis of 4-phenyl-3-thiosemicarbazones[10]

A solution of 4-phenyl-3-thiosemicarbazide (1 mmol) in ethanol (1 mL) was added slowly to a stirring solution of appropriate carbonyl compound (1 mmol) in 0.5 mL of ethanol (EtOH) containing (0.2 mL) of glacial acetic acid (AAG). The solution was heated on a water bath for 10 minutes and cooled on an ice bath. The precipitate obtained on cooling were filtered and recrystallized from ethanol (95°) to give desired product (**3** and **4**).

All compounds synthesized were submitted to the *invitro* antiparasitical activity against the bloodstream form of the strain 427 of *Trypanosomabruceibrucei* and have been evaluated for their *invitro* cytotoxicity on *Artemiasalina* Leach followed biological methods.

Pharmacology

Anti-trypanosomal activity (LILIT, AlamarBlue^{TM})

The test is performed on the bloodstream form of the strain 427 of *Trypanosoma bruceibrucei* by the «LILIT Alamar Blue^{TM}» method [25-28]. The stock solutions of thiosemicarbazones have been prepared from an initial concentration of 10 mg/mL^{-1} in DMSO. The trypanosomes are grown in a medium containing 10% of heat-inactivated fetal calf serum and bloodstream form supporting factor. The trypanosome suspensionswere adjusted to $5x10^{-4}$ $tryp \cdot mL^{-1}$. In each well, 50 µL of different dilutions of the stock solution were added to 50 µL of suspension of trypanosomes. The plates were thenincubated at 37°C for 72 hours in an atmosphere with 5% CO_2. 10 µL of dye "Alamar Blue^{TM}" is added to each well and then incubated for 4 hours. The dye "Alamar Blue^{TM}"is a reagent for detecting enzymatic activity. The wells in which the concentration of compound is insufficient to inhibit the proliferation of trypanosomes are stained. The MIC is the concentration of unstained wells in which there is the

lowest amount of thiosemicarbazone. The plate reading is made in comparison with control wells on a fluorescence plate reader using an excitation wavelength of 530 nm and an emission wavelength 590 nm.

Cytotoxicity screen

The test is performed on larvae fbrine shrimp (Artemia *salina* Leach) by the method of Michael *et al*. [27] resumed by Vanhaecke *et al*. [28] and by Sleet and Brendel [29]. Thus, *Artemiasalina* eggs are incubated in sea water until hatching of young larvae(48h). Then, series of solution softest substance sat varying concentrations and progressive were prepared in DMSO (dimethylsufoxide)/seawater. A defined number of larvae introduced into each solution. All solutions and control solutions containing no active substance were left stirring for 24hours.Counting under a microscope the number of death larvae in each solution used to evaluate the toxicity of the solution. In the case where there was death in the control medium, the data was corrected by Abbott's formula:

%death=[(test - control) /control)] x100[30]

Data(dose-response) are transformed by logarithm and the half-lethal concentration LC_{50}isdeterminedbylinear regression [31]. Tests were carried out in triplicate. All data were expressed as means±standard deviation of triplicate measurements.

RESULTS

Chemistry

Four semicarbazones and thiosemicarbazones N(4)-phenylsubstituted (**1**-**4**) were synthesized with good yield such as 4'-methylacetophenone 4-phenylsemicarbazone (**1**), propiophenone 4-phenylsemicarbazone (**2**), 4'-methylacetophenone 4-phenyl-3-thiosemicarbazone (**3**), propiophenone 4-phenyl-3-thiosemicarbazone (**4**) The structures of synthesized compounds were characterized with spectrometrical analysis IR, NMR ^{1}H &^{13}C and MS.

Characterization of synthesized compounds

4'-methylacetophenone4-phenylsemicarbazone (**1**)

Yield:73%m.p:194-195°C; **IR** (NaCl) ν(cm^{-1}): broad 3212-3185 ν(NH); 1681 ν(C=O); 1591 ν(C=N); 815, 748 ν(p-CH_3-Ar) ; **^{13}C NMR** (DMSO-d_6, 100MHz) δ(ppm): 154.36, 146.76, 138.42, 129.34, 129.07, 126.16, 123.26, 119.56, 21.30, 13.86 ; **^{1}H NMR** ($CDCl_3$, 400MHz) δ(ppm): 2.42 (s, 3H, CH_3); 2.45 (s, 3H, CH_3); 7.75-7.40-6.79 (m, 9H, H-Ar);8.40 (s, 1H, CONH-Ph); 9.40 (s, 1H, =NNH−). **MS** (m/z): [MH^+]268.13 ; [MH^+]found268.14

Propiophenone4-phenylsemicarbazone (**2**)

Yield:71%m.p: 112-113°C; **IR** (NaCl) ν(cm^{-1}): broad 3434-3410, 3330 ν(NH); 1658ν(C=O); 1617 ν(C=N); **^{13}C NMR** (DMSO-d_6, 100MHz) δ(ppm): 154.01, 150.94, 138.15, 137.08, 133.58, 129.65, 129.26, 128.97, 128.64, 126.88, 126.15 123.32, 119.49, 31.43, 20.44, 10.87, 10.48 ; **^{1}H NMR** ($CDCl_3$, 400MHz) δ (ppm): 1.75, 1.20 (t_d, 3H, CH_3); 2.80, 2.69 (q_d, 2H, CH_2); from 7.75-7.10 (m, 10H, H-Ar);8.79, 8.67 (s_d, 1H, CONH-Ph); 9.12 (s_d, 1H, =NNH−). **MS** (m/z): [MH^+] 268.12 ; [MH^+]found 268.14

4'-methylacetophenone4-phenyl-3-thiosemicarbazone (**3**)

Yield:77% m.p: 175-176°C; **IR** (NaCl) ν(cm^{-1}): broad 3398-3299 ν(NH); 1633 ν(C=N); 1100, 1027, 928 ν(N-CS-N); 815, 756 ν(p-CH_3-Ar) ; **^{13}C NMR** (DMSO-d_6, 100MHz) δ(ppm): 176.25, 147.32, 140.33, 137.94, 134.44, 129.43, 128.89, 126.33, 126.13, 124.22, 21.36, 13.71 ; **^{1}H NMR** ($CDCl_3$, 400MHz) δ(ppm): 2.35 (s, 3H, CH_3); 2.42 (s, 3H, CH_3); 7.7-7.25 (m, 9H, H-Ar); 8.75 (s, 1H, CSNH-Ph); 9.45 (s, 1H, =NNH−). **MS** (m/z): [MH^+] 284.13 ; [MH^+]found 284.11

Propiophenone4-phenyl-3-thiosemicarbazone (**4**)

Yield: 80% m.p: 113-114°C; **IR** (NaCl) ν(cm^{-1}): broad 3450, 3294 ν(NH); 1598, 1588 ν(C=N); 1114, 1055, 920 ν(N-CS-N); **^{13}C NMR** (DMSO-d_6, 100MHz) δ(ppm): 176.35, 176.01, 154.79, 152.02, 138.05, 137.96, 136.21, 133.06, 130.08, 129.99, 129.85, 128.81, 126.83, 126.46, 126.13, 125.05, 124.21, 31.54, 20.45, 10.83, 10.67 ; **^{1}H NMR** ($CDCl_3$, 400MHz) δ (ppm): 1.30, 1.15 (t_d, 3H, CH_3), 2.80, 2.65 (q_d, 2H, CH_2), 7.85-7.15 (m, 10H, H-Ar); 8.90, 8.60 (s_d, 1H, CSNH-Ph); 9.40 (s, 1H, =NNH−);. **MS** (m/z): [MH^+] 284.16; [M]found 284.11

Pharmacology

Anti-trypanosomal activities

All compounds synthesized were evaluated for their trypanosomal activities against *Trypanosomabruceibrucei*. The half-concentrations inhibitions IC_{50} of products are summarized in table 2. Analyzes of the data mean that compounds **4** and **1** show greater IC_{50} value below 100 µM. The molecule **2**with IC_{50} = 207 µM presents little or no activity on the parasite. Product **3**, during the test, precipitates in the cell culture medium.

Cytotoxicity screen

The cytotoxicity of each compound has been tested on *Atemiasalina* and lethality assays were evaluated by Excel computer statistical program to determine the LC_{50}. Results are registered in table 2. Dataindicate that only compound **2**(LC_{50} = 107.49 µM) shows low value on the group. Other products give their LC_{50} values at higher than 700 µM.

Selectivity index

The index of selectivity of each product is calculated by the ratio of the half-lethal concentration and the half-inhibitory concentration (LC_{50} larvae / IC_{50} parasite). Table 2 contains the values found. Except molecule **2** giving its value less than unity, the other three compounds **1**, **3** and **4** give their values significantly greater than unity.

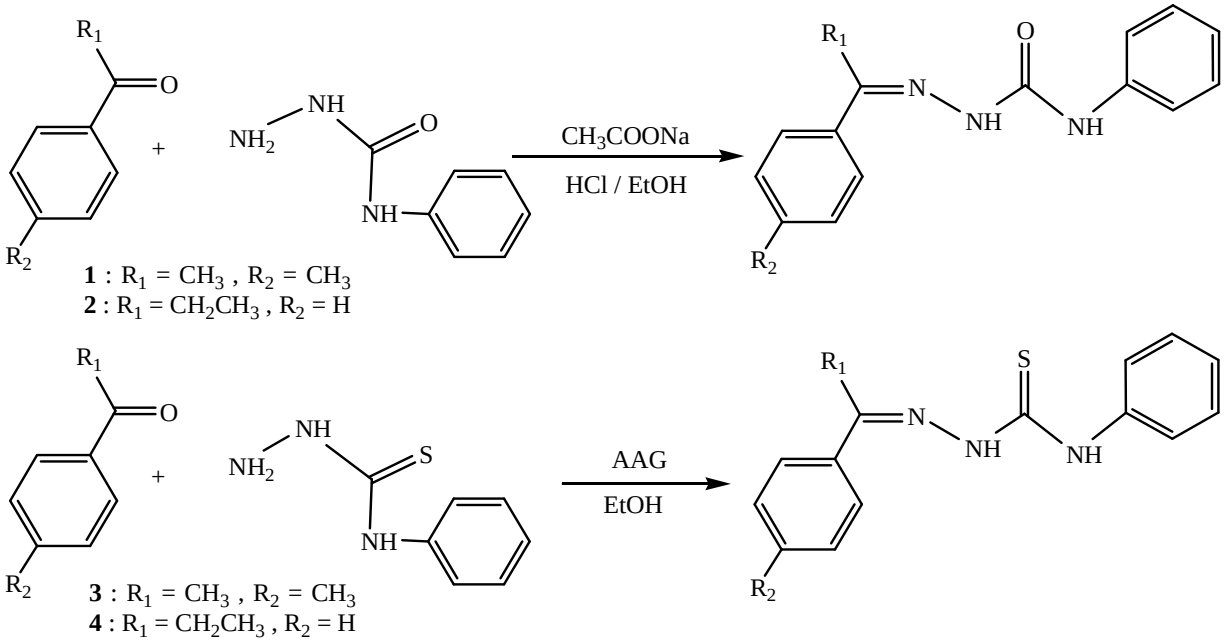
DISCUSSION

Four compounds have beensynthesized andcharacterized. There are 4'-methylacetophenone 4-phenylsemicarbazone **1**, propiophenone 4-phenylsemicarbazone **2**, 4'-methylacetophenone 4-phenyl-3-thiosemicarbazone **3** and propiophenone 4-phenyl-3-thiosemicarbazone **4**. The scaffold (scheme 1) has advantageous properties: low molecular weight, reasonable *C*log *P*, good hydrogen bond donating and accepting capabilities (table 1), easy, and economical synthetic routes [32]. Their IR spectra show the frequencies of the typical bands of −NH− between 3430-3185; C=O bands are 1681 and 1658 and C=N bands from 1617 to 1591 cm^{-1} in the structures of compounds **1**-**2** and −NH− bands in the range from 3455 to 3294; C=N bands 1633-1588 $cm^{-1}$of compounds **3**-**4** and we note the disappearance of C=O bands in these structures.Fundamentally functions have been confirmed in the analysis of the ^{13}C NMR spectra. The C=S peaks appear in the range from 176.35 to 175.25 ppm, peaks of C=N between 154.79 and 147.32 ppm in the structure of compounds **3** and **4**while in **1** and **2** we note C=O peaks appearing at 154.36 and151.04, C=N 146.76 and 150.94 ppm respectively. All compounds aromatic carbons are shown from 140.33 to 119.49 ppm.Methyl carbons peaks are observed at 10.87, 10.48 and 10.83, 10.67 ppm, the methylene carbonsat 31.54, 20.45 and 31.43, 20.44 ppm in propiophenone. It should be noted that at the level of propiophenone, all peaks are almost dedoubled. Peaks of methyl and arylmethyl (CH_3-Ar)in 4'-methylacetophenone appeared respectively at 13.71, 13.86 and 21.36, 21.30.^1H NMR spectra analysis gives the characteristic protons existing in each structure. The protons signals in =NN**H**− are shown between9.45-9.12 ppm (**1**-**4**). Protons (CSN**H**-Ph) appear at 8.90, 8.60 and 8.75 ppm for **4** and **3** respectively and in CON**H**-Ph, proton signalsof **2** and **1** are observed at 8.79 8.67 and 8.40ppm correspondingly. We note that the protons associated with N(2) are more deshielded than those associated with N(4), an effect that is due to its environment electro attractor.The molar mass of each synthesized molecule given by mass spectrometry is consistent with theoretical mass found. Various spectrometrical analyses done on each compound have really confirmed the presence of functional groups and different types of protons and carbons in each structure.

Anti-trypanosomalactivities study showed that compound **4** exhibits trypanocial activity and product **1**a moderate anti-trypanosomal activity (IC_{50} = 7.63 and 62.54 µM respectively). Compound **2** presents little or no activity against the trypanosome whit its IC_{50} equal to 207.34 µM. Molecule **3** which precipitates in the cell culture middle show none activity until 353.35 µM (table 2). Note that these results are consistent with the scale of anti-trypanosomal activity established in the works of Du *et al*. [33] and Fujii*et al*. [19]. According them, thiosemicarbazones are trypanocidal when their IC_{50} values are lower than 10 µM, and are regarded as moderate anti-trypanosomal agents if these values are between 10 and 100 µM, and have little or no activity when their IC_{50} are higher than 100 µM.

In the toxicity study, every product was tested on *Atemiasalina* L. To assess the toxicity with the LC_{50} values of compounds (table 3), we have referred to the LC_{50} value of lapachol (281 µM) which is known as reference compound [34, 35]. In descending order, we have values of LC_{50} = 107.49, 731.46, 897.17 and 909.18 µM respectively for compounds **2**, **1**, **3** and **4**. By comparing these values with that of lapachol (LC_{50} = 281 µM), we contact that only the product **2** has exerted a toxic activity on the larvae of *Artemia*. Othercompounds show any toxic activity on larvae. These tests that are a summary assessment of the toxicity of products reflects the sensitivity of shrimp larvae to the synthesized compounds and by extension that of the human species. Indeed, there is a correlation between toxicity on shrimp larvae and cytotoxicity on cells 9KB and 9PS (human carcinoma nasopharygien) a part [36], cells A-549 lung carcinoma and HT-29 cells of carcinoma of the colon on the other [37]. Consequently, compounds **4** and **1** which exhibit anti-trypanosomal activity can be used at higher doses for trypanosomal treatment. After analyze selectivity index data, we note that the compounds **3**, **1** and **4** (with their SI > 1) turn out quite selective on the parasite *Trypanosomabruceibrucei* and product **2** (SI > 1) have good selectivity on cells of larval shrimp *Artemiasalina* and then is more cytotoxic than anti-parasite. These results are in perfect agreement with the work of Tiuman*et al*., [38] in which if the SI value obtained is greater than unity, the test

compound is considered to be selective on the parasites and if SI value is less than unity, the test compound is more cytotoxic than anti-parasitic.



Scheme 1 Synthetic routes of semicarbazones and thiosemicarbazones (scaffold)

Table 1 :Physical Properties[§]of synthesized compounds

	Molecular weight	Clog *P*	No. of H bond donors	No. of H bond acceptors	No. of criteria met
Rule	< 500	< 5	< 5	< 10	at least 3
1	267	4.365	2	4	All
2	267	4.395	2	4	All
3	283	4.570	2	3	All
4	283	4.600	2	3	All

[§]*Properties Compatible with Reasonable Pharmacokinetics and Drug Availability*

Table 2 :Anti-trypanosomal activities, toxicity and selectivity index of synthesized compounds

Compounds	Half-inhibition concentration IC_{50} (µM)	Anti-trypanosomal activities[#]	Half-lethal concentration LC_{50} (µM)	Toxic Activities[&]	Selectivity index (SI = LC_{50}/IC_{50})
1	62.54±5.88	Moderate	731.46±0.02	No toxic	43.80
2	207.34±0.86	Little or no	107.46±0.07	Toxic	0.51
3	>353.35*****	None	897.71±0.03	No toxic	<2.53
4	7.63±1.27	Trypanocidal	909.18±0.17	No toxic	119.15

** precipitate in the diluted solution, # against Trypanosoma bruceibrucei, [&] on Artemiasalina L*

CONCLUSION

In this study, four N(4)-aryl semicarbazones and thiosemicarbazones (**1**-**4**) of arylketones were synthesized and characterized by spectrometrical methods. Submitted to anti-trypanosomal and toxicity testing, some molecules (**1** and **4**) showed trypanocidal activities with negligible toxicity and have good selectivity on *Trypanosomabruceibrucei,*the studied parasite. Note that compound **2** less anti-trypanosomal has a selectivity more toxic than anti-parasites and could be used in the treatment of cancers. These synthesized compounds may contribute to the treatment of trypanosomiasis and could open a promising avenue in eradication of this scourge.

REFERENCES

[1]. MC Field; CL Allen; V Dhir; D Goulding; BS Hall; GW Morgan; P Veazey; M Engstler. *Microscopy and Microanalysis*.,**2004**, 10(5), 621-636.
[2]. D Courtin; D Berthier; S Thevenon; GK Dayo; A Garcia; B Bucheton. *Infection, Genetics and Evolution*.,**2008**, 8(3), 229-238.

[3]. BM Swallow. Impacts of trypanosomosis on African agriculture.PAAT Technical and ScientificSeries 2. FAO (Organisation Mondiale pour l'Alimentation et l'Agriculture), Rome, **2000**; 52.
[4]. S Winkle. *Geißeln der Menschheit. Kulturgeschichte der Seuchen*. Düsseldorf: Artemis & Winkler; **2005**.
[5].LT Budd. DFID-funded Tsetse and Trypanosomiasis Research and Development since 1980.Vol 2 Economic Analysis.**1999**.
[6]. WHO (World Health Organization). African trypanosomiasis (sleeping sickness). [http://www.who.int/mediacentre/factsheets/fs259/en/] World Health Organ Fact Sheet., **2006**.
[7]. F Njiokou; C Laveissière; G Simo; S Nkinin; P Grébaut; G Cuny; S Herder. *Infect Genet Evol*.,**2006**, 6, 147-153.
[8]. G Simo; T Asonganyi; SW Nkinin; F Njiokou; S Herder. *Vet. Parasitol*.,**2006**, 139, 57-66.
[9]. H Beraldo; D Gambino. *Mini. Rev. Med. Chem*., **2004**, 4, 31-39.
[10]. N Aggarwal; R Aggarwal; P Mishra; JS Jain; SK Bansal; KK Jha. *Cent.Nerv.Syst. Agents Med. Chem*., **2008**, 8, 26-28.
[11]. KSO Ferraz; L Ferandes; D Carrilho; MCX Pinto; MF Leite; EM Souza–Fagundes; NL Speziali; IC Mendes; H Beraldo. *Bioorg. Med. Chemy*.,**2009**, 17, 7138-7144.
[12]. SSKarki; S Bahaduria; VSRana; VKumar; S Subbaro; PG Das; U Balzarini; J De Clercq; EJRDimmock. *J. enzyme inhibition and Med. Chem*., **2009**, 24(2), 537-544.
[13]. A Kshirsagar; MP Toraskar; VM Kulkarn; S Dhanashire; V Kadam. *Int. J. Chem. Tech. Res*., **2009**, 1(3), 696-701.
[14]. AK Parekh; KK Desai. *Indian J. Chem*., **2006**, 45B, 1072-1075.
[15]. K. Shashikala Devi, M. Ramaiah, G.K. Vanita, Veena.K and V.P. Vaidya, *J. Chem. Pharm. Res.*, **2011**, 3(1):445-451
[16]. S. Shivhare and Mangla Dave Gautam, *J. Chem. Pharm. Res.*,**2011**, 3(5):682-688.
[17]. Ramesh Yamgar, Prasad Kamat, DileepKhandekarandSudhirSawant, *J. Chem. Pharm.Res*., **2011**, 3(1):188-198.
[18]. Sanjay Gaikwad and CharushilaGaikwad, *J. Chem. Pharm. Res.*, **2010**, *2(4): 106-111*.
[19]. N Fujii; JP Mallari; EJ Hansell; Z Mackey; P Doyle; YM Zhou; J Gut; PJ Rosenthal; JH McKerrow; RK Guy. *Bioorg. Med. Chem*., **2005**, 15(1), 121-123.
[20]. B Glinma; AF Gbaguidi; SDS Kpoviessi; HR Fatondji; J Poupaert; CG Accrombessi. *St. Cerc. St. CICBIA*., **2011a**, 12(1), 33-40.
[21]. B Glinma; SDS Kpoviessi; HR Fatondji; AF Gbaguidi; CN Kapanda; J Bero; DM Lambert; V Hannaert; J Quetin-Leclercq; M Moudachirou; J Poupaert; CG Accrombessi. *J. App. Pharm. Sci*.,**2011**b, 01(08), 65-70.
[22]. A Pérez-Rebolledo; LR Teixeira; AA Batista; AS Mangrich; G Aguirre; H Cerecetto; M Gonzalez; P Hernandez; AM Ferreira; NL Speziali; H Beraldo. *Eur. J. Med. Chem*., **2008**, 43(5), 939-948.
[23]. DC Greenbaum; Z Mache; E Hansell; P Doyle; J Gut; CR. Caffrrey; J Lehrman; PJ Rosenthal; JH McKerrow; K Chibale. *J. Med. Chem*., **2004**, 47(12), 3212-3219.
[24]. T Baltz; D Baltz; C Giroud; J Crockett. 1985. *The EMBO Journal*.,**1985**, 4(5), 1273-1277.
[25]. H Hirumi; K Hirumi. *Parasitology today*.,**1994**, 10(2), 80-84.
[26]. B Räz; M Iten; Y Grether-Bühler; R Kaminsky; R Brun. *ActaTropica*.,**1997**, 68, 139-147.
[27]. AS Michael; CG Thompson; M Abramovitz. *Science*.,**1956**, 123, 464.
[28]. P Vanhaecke; G Persoone; C Claus; P Sorgeloos. *Ecotoxicol. Environ. Safety*.,**1981**, 5, 382-387.
[29]. RB Sleet; K Brendel. *Ecotoxicol.Env.Safety*.,**1983**, 7, 435-446.
[30]. WS Abbott. *J. Econ. Entomol*.,**1925**, 18, 265.
[31]. E Hafne,r; E Heiner; E Noack. *ArzneimittelForschung Drug Research*., **1977**, 27, 1871-1873.
[32]. CA Lipinski; F Lombardo; BW Dominy; PJ Feeney. *Drug Delivery Rev*., **1997**, 23, 3-25.
[33]. X Du; C Guo; E Hansell; SP Doyle; CR Caffrey; TP Holler; JH McKerrow; FE Cohen. *J. Med.Chem*., **2002**, 45, 2695-2707.
[34]. PLP Santos; GB Pinto; JA Takahashi; LGF Silva; MAD Boaventura. *Phytomedicine*.,**2003**, 10(2-3), 209-212.
[35]. AE Graminha; AA Batista; SRW Louro; RL Ziolli; LR Teixeira; H Beraldo. *Polyhedron*.,**2008**, 27, 547-551.
[36]. M Pelka; C Danzl; W Distler; A Petschelt. J. Dent., 2000, 28(5), 341-345. doi:10.1016/S0300-5712(00)00007-5.
[37]. JLCarballo;ZL Hernández-Inda; P Pérez;DCGarcía-Grávalos.*BMC Biotechnol*.,**2002, 2,** 17. doi:10.1186/1472-6750-2-17.
[38]. TS Tiuman; T Ueda-Nakamura; DA Garcia Cortez; BP Dias Filho; JA Morgado-Diaz; W de Souza; CV Nakamura. *Antimicrob. Agents chemother*.,**2005**, 492, 176-182.