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RESEARCH ARTICLE

LIPOPHILIC AND STRUCTURE ACTIVITY RELATIONSHIPS STUDY OF THIOSEMICARBAZONES AND DERIVATIVES.

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Abstract

Traditionally, small molecules have been a reliable source for discovering novel biologically active compounds because these molecules are easily synthesized and their smooth structural optimization would usually lead to a feasible candidate compound. Here, some thiosemicarbazones, N(4)-methyl and N(4)-phenyl-3-thiosemicarbazones were synthesized in good yield (52-84%), characterized and then their anti-parasitic activity were evaluated. The structure and lipophilic-activity relationships of compounds were particularly studied. Among them, some products exhibited trypanocidal activity with their half inhibitory concentration ($IC_{50} \leq 10$ micromolar "µM") especially compounds **L_{1-3}, D_2, B_3, C_3, D_1** (from 2 to 8.73 µM). Other showed moderate antitrypanosomal activity with their IC_{50} between 12 to 87 µM (**L_4, C_2, C_1, B_2**) while certain showed little activity ($IC_{50} \geq 100\mu M$). Some active products turned out quick selective on the parasite with their selectivity index greater than to unit ($SI \geq 1$).

Several factors including lipophilicity, steric and electronic effects of the substituents have played a vital role in this activity. The elongation of the carbon chain of the carbonyl, the substitution on a phenyl radical, the fixing of a methyl or phenyl on the N(4) nitrogen atom induced significantly the increased trypanocidal activity of compounds. This is the case specifically of N(4)-methyl and especially of N(4)-phenyl-substituted thiosemicarbazones. Such compounds could be able to have applications in the treatment of parasitic diseases.

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Introduction:-

Nowadays, microbial and parasitic diseases are resistant to existing treatments. For these reasons, chemists have been made a great effort to the development of compounds with biological activity that will be used in pharmaceutical chemistry. Currently, most antiparasitic drugs are considered orphan drugs, with the main exception of antimalarials. The pharmaceutical considerations outweigh all others, because the economic return on the development of anti-parasitic drugs is limited. Therefore, it is necessary to find less expensive alternatives for the treatment of parasitic disease (Soates et al., 2011). Recently, due to having wide-spectrum biological activity of thiosemicarbazones derivatives that synthesis studies made, interest on these compounds has been considerably increased in the pharmaceutical sector at the present time (Rogolino et al., 2015 ; Büscher et al., 2017 ; Zani et al., 2017). Thiosemicarbazones and semicarbazones due to be a small molecule widely used in the treatment of antiviral, anticancer and antiparasital disease. More recently, it has been found that they are highly effective antiparasital compounds against *Trypanasoma cruzi* parasites that cause especially Malaria and Chagas diseases. Generally, thiosemicarbazones show this effect by causing the inhibition of cysteine proteases in this type of parasites and derivatives (Du et al., 2002 ; Beraldo and Gambino, 2004 ; Greenbaum et al., 2004 ; Fujii et al., 2005 ; Jeremy et al., 2008).

In the light of this important data which have been achieved with the literature survey considering that the thiosemicarbazones are biologically active compounds, synthesis of the thiosemicarbazone derivatives expected to show positive activity was carried out.

African trypanosomiases are still a serious health and economic problem that requires not only the application of the knowledge and resources currently available, but also their improvement through multidisciplinary research (Simarro et al., 2012 ; Grant et al., 2015). Animal trypanosomiasis is a major constraint for the livestock industry in developing countries (Amer et al., 2011). In East Africa, animal trypanosomiasis is caused by numerous protozoan parasites transmitted by tsetse flies, including *Trypanosoma vivax, T. congolense* and subspecies of *T. brucei* sl (*T. brucei* and *T. b* zoonotic *rhodesiense* infectious for humans) that can co-circulate in domestic and wild animals (Cox et al., 2010).

In this paper, we described the synthesize of thiosemicarbazones, N(4)-methyl and N(4)-phenyl-3-thiosemicarbazones of benzaldehyde, 2'-methylacetophenone, propiophenone, benzophenone with its substituted derivatives. The compounds were tested for their antitrypanosomal activity against *Trypanosoma brucei brucei* and their toxicity on larvae shrimp Artemia salina Leach. And then, some structure-activity and lipophilicity-activity relationships were examined.

Material And Methods:-

Reagents

All reagents were obtained from chemical societies: Sigma-Aldrich, Acros Organic, Janssen Chimica, Prolabo and Riedel-de Haen. Substrates, reagents, catalysts and solvents were used directly for syntheses without any further purification. There are : benzaldehyde, 4'-methylacetophenone, benzophenone and derivate, hydrochloric acid (HCl), glacial acetic acid (AAG), Technical ethanol (EtOH), thiosemicarbazone, 4-methyl-3-thiosemicarbzide, 4-phenyl-3-thiosemicarbazide.

Equipment

All synthesized compounds were characterized by Nuclear Magnetic Resonance spectra using Bruker Avance 400. UltraSheild with dimethylsufoxide (DMSO)-d_6 or chloroform $CDCl_3$ and then Mass Spectrophotometer spectra obtained using the method of Atmospheric-pressure chemical ionization and mass is given in m/z of $[MH^+]$. The frequencies for ^1H and ^{13}C are 400.130 and 100.612 MHz respectively. Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane as internal standard. Multiplicity was designated as singlet (s), doublet (d), doublet dedouble (dd), triplet (t), quintuplet (qi) and multiplet (m). Melting points (m.p.) were determined on a fusionometer of the type electrothermal 1A 9000 and were not corrected.

Methods

Synthesis of the compounds

An equimolar mixture (0.01 mol) of thiosemicarbazide and analogues dissolved in 10 mL ethanol (EtOH 96°) was added slowly to a solution (0.01 mol) of arylketone dissolved in 20-30 mL of EtOH in presence of acid (HCl, 1 N or

GAA). The mixture was heated at reflux for 4 h with stirring. After cooling, the precipitate was filtered, washed with cold distilled water until neutrality, dried and then recrystallized in ethanol.

All compounds after synthesis have been submitted to the *in vitro* anti-trypanosomal test on the bloodstream form of the strain 427 of *Trypanosoma brucei brucei* and were evaluated for their *in vitro* cytotoxicity on *Artemia salina* Leach following standard biological methods.

Pharmacology

Anti-trypanosomal test

The assessment is performed on the bloodstream form of the strain 427 of *Trypanosoma brucei brucei* by the «LILIT Alamar Blue™» method (Baltz et al., 1985 ; Räz et al., 1997). The stock solutions of each thiosemicarbozone have been prepared from an initial concentration of 10 mg/mL in dimethylsufoxide (DMSO). The trypanosomes are grown in a medium containing 10% of heat inactivated fetal calf serum and bloodstream form supporting factor. The trypanosome suspensions were adjusted to 5×10^4 tryp/mL. In each well, 50 µL of different dilutions of the stock solution were added to 50 µL of suspension of trypanosomes. The plates were then incubated at 37°C for 72 hours in an atmosphere with 5% CO_2. 10 µL of dye "Alamar BlueTM" is added to each well and then incubated for 4 hours. The dye "Alamar BlueTM" 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 half-inhibitory concentration is the concentration of unstained wells in which there is the lowest amount of thiosemicarbazones. 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. We carried out the test in triplicate for each compound. All data were expressed as means ± standard deviation of triplicate measurements.

Cytotoxicity screen

The cytotoxicity test was performed on larvae of brine shrimp (*Artemia salina* Leach) by the method of Sleet and Brendel (1983). *Artemia salina* eggs were incubated in seawater until hatching of young larvae (48 hours). Then, series of solutions of test compound at varying concentrations were prepared in DMSO/seawater. A defined number of larvae were introduced into each solution and incubated under rocking condition for 24 h. To evaluate the toxicity of the solution, counting of larvae viability was performed under microscope by determining the number of dead larvae in each solution. In the case where there was death in the control medium, the data was corrected by Abbott's formula:

% death = [(nd test - nd control)/ nd control)] x 100 (Abott, 1925) with nd = number of dead larvae.

Data (dose-response) were transformed by logarithm and the halflethal concentration LC_{50} was determined by linear regression (Hafner et al., 1977). Tests were carried out in triplicates. All data were expressed as mean ± standard deviation of triplicate measurements.

Results And Discussion:-

Results

Chemistry

Before synthesizing the compounds, we carried out a theoretical study based on the pharmacokinetic properties rules (Lipinski et al., 1997 ; 2001) and results are summarized in the table 1. Fifteen compounds were synthesized and their physico-chemical properties are described in the table 2.

Compounds were synthesized following the condensation reaction (figure 1).

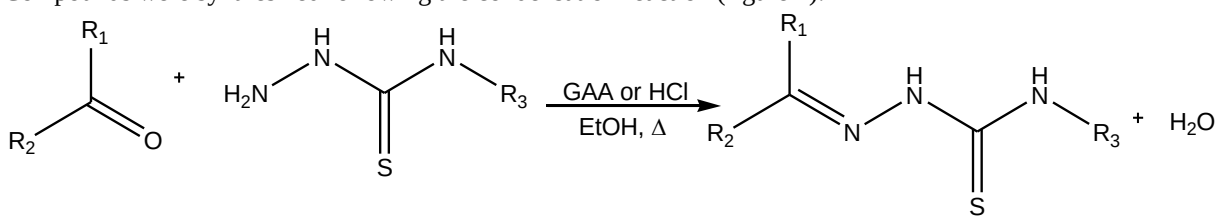


Figure 1:-Synthetic routes of thiosemicarbazones (scaffold).

Compounds : series Bi : R_1 = Et, R_2 = Ph ; **B_1** R_3 = H ; **B_2** R_3 = Me, **B_3** R_3 = Ph

Compounds : series Ci : R_1 = R_2 = Ph ; **C_1** R_3 = H ; **C_2** R_3 = Me, **C_3** R_3 = Ph

Compounds : series Di : R_1 = Me, R_2 = p-Me-Ph ; **D_1** R_3 = H ; **D_2** R_3 = Me, **D_3** R_3 = Ph

Compounds : series Ki : R_1 = H, R_2 = Ph ; **K_1** R_3 = H ; **K_2** R_3 = Ph

Compounds : series Li : R_3 = Ph ; **L_1** R_1 = Ph ; R_2 = o-H_2N-Ph ; **L_2** R_1 = Ph ; R_2 = o-HO-Ph ; **L_3** R_1 = Ph ; R_2 = p-HO-Ph ; **L_4** R_1 = Ph ; R_2 = 2-H_2N-5-Cl-Ph.

The structures of synthesized compounds were characterized with the TLC, spectrometrical analysis MS, IR, and especially with NMR ^{13}C & ^{1}H.

Table 1:-Theoretical Pharmacokinetic and drug availability study

Compounds	Molecular weight (M in g.mol^{-1})	C logP	Number of H-bond donors	Number of H-bond acceptors	Number of criteria met
Rules	**M < 500**	**< 5**	**≤ 5**	**< 10**	**at least 3**
B_1	208.08	2.930	3	3	all
B_2	221.09	2.816	2	3	all
B_3	283.11	4.600	2	3	all
C_1	255.09	4.102	3	3	all
C_2	269.11	4.071	2	3	all
C_3	331.11	5.400	2	3	3
D_1	207.08	2.900	3	3	all
D_2	221.09	2.786	2	3	all
D_3	283.11	4.570	2	3	all
K_1	179.24	1.865	3	3	all
K_2	255.34	3.960	2	3	all
L_1	346.43	4.673	4	4	all
L_2	347.43	5.263	3	4	3
L_3	347.43	5.263	3	4	3
L_4	380.89	5.509	4	4	3

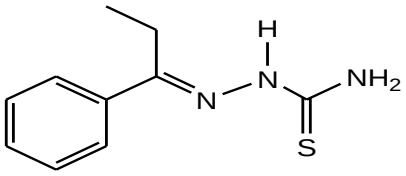
Table 2:-Physico-chemical properties of synthesized compounds

Compounds	**Formula brute**	**[MH^+]^a in g.mol^{-1}**	**Melting point in °C**	**Yields in %**
B_1	$C_{10}H_{13}N_3S$	208.11	116-117	74
B_2	$C_{11}H_{15}N_3S$	222.06	98-99	67
B_3	$C_{16}H_{17}N_3S$	284.16	113-114	80
C_1	$C_{14}H_{13}N_3S$	256.07	167-168	84
C_2	$C_{15}H_{15}N_3S$	270.10	164-165	52
C_3	$C_{20}H_{17}N_3S$	332.02	153-154	82
D_1	$C_{10}H_{13}N_3S$	208.14	148-149	81
D_2	$C_{11}H_{15}N_3S$	222.13	144-145	71
D_3	$C_{16}H_{17}N_3S$	284.07	175-176	77
K_1	$C_8H_9N_3S$	180.39	162-163	65
K_2	$C_{14}H_{13}N_3S$	256.07	199-200	75
L_1	$C_{20}H_{18}N_4S$	347.39	136-137	73
L_2	$C_{20}H_{17}N_3OS$	348.37	186-187	57
L_3	$C_{20}H_{17}N_3OS$	348.41	172-173	65
L_4	$C_{20}H_{17}ClN_4S$	381.87	143-144	61

a : mass obtained of MS

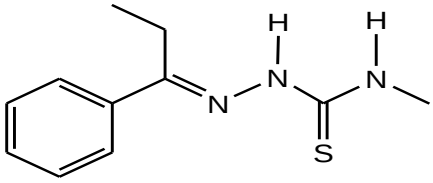
Compounds in series B_i

Propiophenone thiosemicarbazone (B_1)



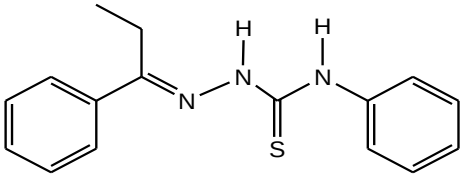
^{13}C NMR δ (DMSO-d_6, ppm): 179.13 & 178.76 (C=S); 155.81 & 152.97 (C=N); 136.14; 132.92; 130.03; 129.96; 129.81; 128.69; 126.76; 126.46 (C-Ar); 31.54 & 20.30 (-CH_2-); 10.73 & 10.65 (CH_3). **^{1}H NMR** δ ($CDCl_3$, ppm): 8.85 & 8.65 (sd, 1H, C=NNH-); 7.70-7.20 (m, 10H, H-Ar); 6.70 & 6.55 (sd, 2H, CS-NH_2); 2.83 & 2.61 (qd, 2H, -CH_2-); 1.23 & 1.11 (td, 3H, CH_3). **IR** ν(KBr, cm^{-1}): 3406, 3301, 3203 (NH); shoulder at 3200 (NH_2); 1600 (C=N); 1074, 1053, 840 (N-CS-N). **MS** m/z [MH^+]: 208.11.

Propiophenone 4-methyl-3-thiosemicarbazone (B_2)



^{13}C NMR δ ($CDCl_3$, ppm): 178.99 & 178.62 (C=S); 154.50 & 151.50 (C=N); 136.39; 133.17; 129.85; 129.72; 129.69; 128.85; 126.79; 126.35 (C-Ar); 31.44 & 31.30 (N-CH_3); 31.09 & 20.18 (-CH2-); 10.85 & 10.55 (CH_3). **^{1}H NMR** δ ($CDCl_3$, ppm): 8.80 & 8.50 (sd, 1H, C=NNH-); 7.17 (q, 1H, CSNH-CH_3); 7.80-7.30 (m, 5H, H-Ar); 3.30 & 3.20 (sd, 3H, N-CH_3); 2.70 & 2.50 (qd, 2H, -CH_2-); 1.20 & 1.10 (td, 3H, CH_3). **IR** ν(KBr, cm^{-1}): 3414, 3290 (NH); 1544 (C=N); 1062, 1033, 845 (N-CS-N). **MS** m/z [MH^+]: 222.06.

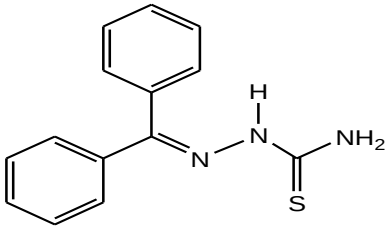
Propiophenone 4-phenyl-3-thiosemicarbazone (B_3)



^{13}C NMR δ (DMSO-d_6, ppm): 176.35 & 176.01 (C=S); 154.79 & 152.02 (C=N); 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 (C-Ar); 31.54 & 20.45 (-CH_2-); 10.83 & 10.67 (CH_3). **^{1}H NMR** δ ($CDCl_3$, ppm): 9.40 (s, 1H, C=NNH-); 8.90 & 8.60 (nd, 1H, CSNH-Ph); 7.85-7.15 (m, 10H, H-Ar); 2.80 & 2.65 (qd, 2H, -CH_2-); 1.30 & 1.15 (td, 3H, CH_3). **IR** ν(NaCl, cm^{-1}): band 3450, 3294 (NH); 1598, 1588 (C=N); 1114, 1055, 920 (N-CS-N). **MS** m/z [MH^+]: 284.16.

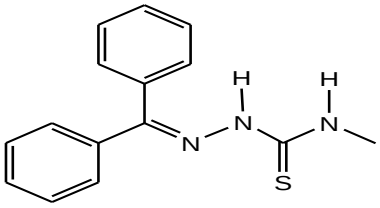
Compounds in series C_i

Benzophenone thiosemicarbazone (C_1)



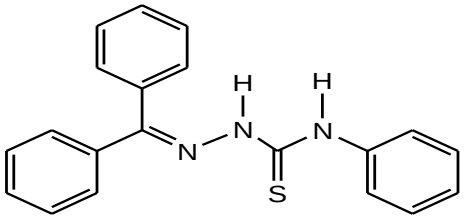
^{13}C NMR δ (DMSO-d_6, ppm): 179.05 (C=S); 150.21 (C=N); 136.50; 131.16; 128.54; 127.88 (C-Ar). **^{1}H NMR** δ ($CDCl_3$, ppm): 8.60 (s, 1H, C=NNH-); 7.60-7.20 (m, 10H, H-Ar); 6.42 (s, 2H, CS-NH_2). **IR** ν(NaCl, cm^{-1}): 3410, 3346, 3248 (NH_2); 3151 (NH); 1608 (C=N); 1069, 1026, 846 (N-CS-N). **MS** m/z [MH^+]: 256.07.

Benzophenone 4-methyl-3-thiosemicarbazone (C_2)



^{13}C NMR δ ($CDCl_3$, ppm): 178.67 (C=S); 149.57 (C=N); 136.79; 131.46; 128.56; 127.72 (C-Ar); 31.27 (N-CH_3). **^{1}H NMR** δ ($CDCl_3$, ppm): 8.65 (s, 1H, C=NNH-); 7.70 (q, 1H, CSNH-CH_3); 7.55-7.25 (m, 10H, H-Ar); 3.25 (s, 3H, N-CH_3). **IR** ν(KBr, cm^{-1}): 3439, 3315 (NH); 1531 (C=N); 1073, 1024, 823 (N-CS-N). **MS** m/z [MH^+]: 270.10.

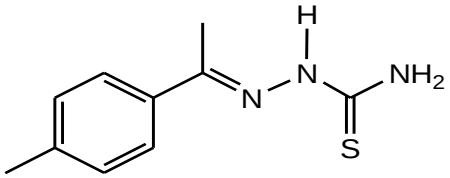
Benzophenone 4-phenyl-3-thiosemicarbazone (C_3)



^{13}C NMR δ (DMSO-d_6, ppm): 175.47 (C=S); 149.22 (C=N); 137,11; 135.73; 130.49; 129.65; 129.15; 128.02, 127.75; 125.38 (C-Ar). **^{1}H NMR** δ ($CDCl_3$, ppm): 9.45 (s, 1H, C=NNH-); 8.75 (s, 1H, CSNH-Ph); 7.60-7.25 (m, 15H, H-Ar). **IR** ν(NaCl, cm-1): 3338, 3304 (NH); 1594 (C=N); 1071, 1031, 855 (N-CS-N). **MS** m/z [MH^+]: 332.02.

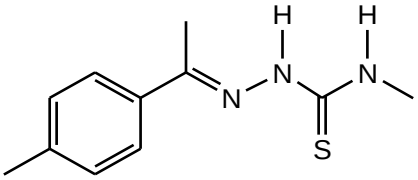
Compounds in series D_i

4'-methylacetophenone thiosemicarbazone (D_1)



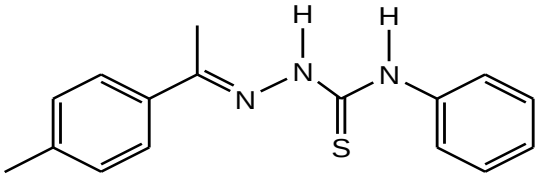
^{13}C NMR δ (DMSO-d_6, ppm): 179.12 (C=S); 148.30 (C=N); 140.29; 134.38; 130.45; 129.32; 126.44; 126.33 (C-Ar); 21.33 (p-CH_3-Ar); 13.62 (CH_3). **^{1}H NMR** δ ($CDCl_3$, ppm): 8.85 (s, 1H C=NNH-); 7.60-7.15 (m, 4H, H-Ar); 6.62 (s, 2H, CS-NH_2); 2.41 (s, 3H, p-CH_3); 2.25 (s, 3H, CH_3). **IR** ν(NaCl, cm^{-1}): 3411, 3379, 3227 (NH_2); 3145 (NH); 1598 (C=N); 1093, 1016, 848 (N-CS-N); 817, 717 (p-CH_3-Ar). **MS** m/z [MH^+]: 208.14.

4'-methylacetophenone 4-methyl-3-thiosemicarbazone (D_2)



^{13}C NMR δ ($CDCl_3$, ppm): 178.85 (C=S); 146.89 (C=N); 139.95; 134.64; 130.39; 129.29; 126.47; 126.26 (C-Ar); 31.27 (N-CH_3); 24.78 & 21.32 (p-CH_3); 13.50 (CH_3). **^{1}H NMR** δ ($CDCl_3$, ppm): 8.70 (s, 1H, C=NNH-); 7.20 (q, 1H, CSNH-CH_3); 7, 65-7.30 (m, 4H, H-Ar); 3.37 (s, 3H, N-CH_3) 2.85 (s, 3H, p-CH_3) 2.39 (s, 3H, CH_3). **MS** m/z [MH^+]: 222.13.

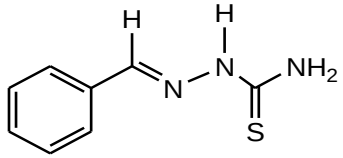
4'-methylacetophenone 4-phenyl-3-thiosemicarbazone (D_3)



^{13}C NMR δ (DMSO-d_6, ppm): 176.25 (C=S); 147.32 (C=N); 140.33; 137.94; 134.44; 129.43; 128.89; 126.33; 126.13; 124.22 (C-Ar); 21.36 (p-CH_3); 13.71 (CH_3). **^{1}H NMR** δ ($CDCl_3$, ppm): 9.45 (s, 1H, C=NNH-); 8.75 (s, 1H, CSNH-Ph); 7.70-7.25 (m, 9H, H-Ar); 2.42 (s, 3H, p-CH_3); 2.35 (s, 3H, CH_3). **IR** ν(NaCl, cm^{-1}): band 3398-3299 (NH); 1633 (C=N); 1100, 1027, 928 (N-CSN); 815, 756 (p-CH_3-Ar). **MS** m/z [MH +]: 284.07.

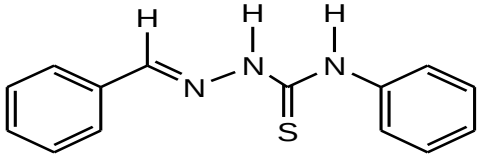
Compounds in series K_i

Benzaldehyde-thiosemicarbazone (K_1):



^{13}C NMR δ (DMSO-d_6 ppm): 177.97 (C=S); 142.24 (C=N); 127.26-134.15 (aromatic C). **^{1}H NMR** δ (DMSO-d_6 ppm): 7.39-7.79 (5H, several signals, Ar-H); 8.00 (1H, s, NH_2); 8.06 (1H, s, CH=N); 8.21 (1H, s, NH_2); 11.44 (1H, s, NH). **IR** ν(KBr cm^{-1}): 3401, 3145 (NH); 1600, 1584 (C=N); 1217 (C=S). **MS** m/z [MH^+]: 180.39.

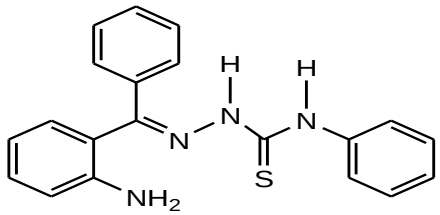
Benzaldehyde 4-phenyl-3-thiosemicarbazone (K_2)



^{13}C NMR δ (DMSO-d_6, ppm): 176.00 (C=S); 142.83 (C=N); 139.04; 133.99; 130.01; 128.62; 127.61; 125.91; 125.31 (C-Ar). **^{1}H NMR** δ ($CDCl_3$, ppm): 10.25 (s, 1H, C=NNH-); 9.20 (s, 1H, CSNH-Ph); 7.97 (s, 1H, HC=N); 7.65-7.25 (m, 10H, H-Ar). **IR** ν(KBr, cm-1): band 2989 (NH); 1590 (C=N); 1227 (C=S). **MS** m/z [MH^+]: 256.32.

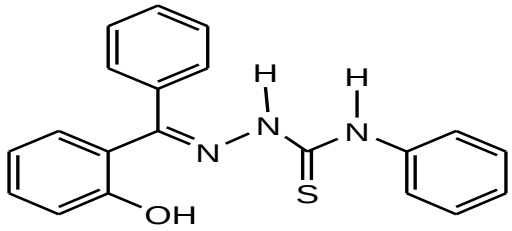
Compounds in series L_i

2-aminobenzophénone 4-phenyl-3-thiosemicarbazone (L_1)



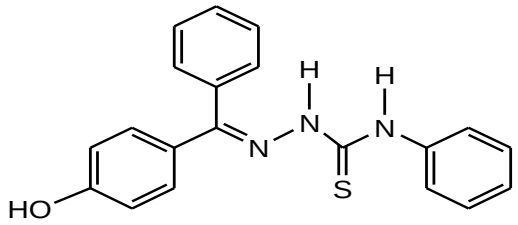
^{13}C NMR δ ($CDCl_3$, ppm): 176.07 (C=S); 143.74 (C=N); 148.30; 137.91; 135.63; 131.76; 130.51; 129.96; 129.57; 128.86; 127.58; 126.21; 124.33; 119.37; 116.80; 115.49 (C-Ar). **^{1}H NMR** δ ($CDCl_3$. ppm): 10.30 (s, 1H, C=NNH−); 8.87 (s, 1H, CSNH-Ph); 7.75-7.17 (m, 14H, H-Ar); 6.87 & 6.70 (s, 2H, Ar-NH_2). **MS** m/z [MH^+] : 347.39.

2-hydroxybenzophenone 4-phenyl-3-thiosemicarbazone (L_2)



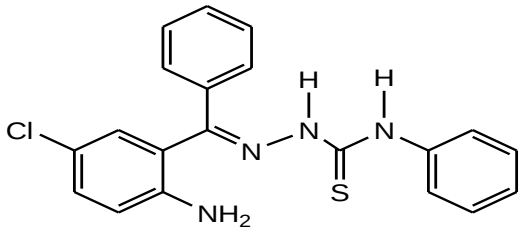
^{13}C NMR δ ($CDCl_3$. ppm): 176.03 (C=S); 148.26 (C=N); 154.56; 138.92; 133.12; 131.67; 129.77; 129.66; 128.53; 128.23; 127.66; 126.10; 125.75; 124.05; 117.69; 116.53 (C-Ar). **^{1}H NMR** δ ($CDCl_3$. ppm): 10.50 (s, 1H, OH); 10.21 (s, 1H, C=NNH−); 8.70 (s, 1H, CSNH-Ph); 7.80-7.00 (m. 14H. H-Ar). **SM** m/z [MH^+] : 348.41.

4-hydroxybenzophenone 4-phenyl-3-thiosemicarbazone (L_3)



^{13}C NMR δ ($CDCl_3$. ppm): 175.75 (C=S); 150.33 (C=N); 159.04; 136.72; 131.70; 130.00; 129.89; 129.79; 128.30; 128.25; 127.07; 125.95; 125;60; 121.34; 116.48 (C-Ar). **^{1}H NMR** δ ($CDCl_3$. ppm): 10.30 (s. 1H. OH); 9.90 (s. 1H. C=NNH−); 8.80 (s. 1H. CSNH-Ph) ; 7.70-6.70 (m. 14H. H-Ar). **MS** m/z [MH^+] : 348.41.

2-amino-5-chlorobenzophenone 4-phenyl-3-thiosemicarbazone (L_4)



^{13}C NMR δ (DMSO-d_6. ppm): 176.79 (C=S); 150.47 (C=N); 144.20; 136.95; 132.08; 131.51; 131.29; 129.86; 129.56; 128.75; 126.18; 124.38; 118.90; 116.98 (C-Ar). **^{1}H NMR** δ ($CDCl_3$. ppm): 10.30 (s, 1H, C=NNH−); 9.00 (s, 1H, CSNH-Ph); 7.70-6.90 (m, 13H, H-Ar); 6.78 & 6.35 (s, 2H, Ar-NH_2). **SM** m/z [MH^+] : 381.87.

Pharmacology

The antiparasitic activity of products was evaluated on the strain 427 of Trypanosoma brucei brucei using lapachol as witness. The toxicity activity of antitrypanosomal compounds was screened on Artemia salina Leach. The results of biological activities of products were obtained and expressed in IC_{50} and LC_{50} respectively. Selectivity of actives products are determined (table 3).

Table 3:-Trypanocidal and toxicity activity of synthesized compounds

Compounds	IC_{50} (µM)	**Trypanocidal activity**	LC_{50} (µM)	**Toxicity**	SI = LC_{50}/IC_{50}	**Selectivity**
B_1	> 100	low or no	-	-	-	-
B_2	87.15 ± 1.13	moderate	149.27 ± 2.05	toxic	1.71	selective
B_3	7.63 ± 1.27	trypanocidal	909.18 ± 0.17	no toxic	119.15	selective
C_1	67.17 ± 3.15	moderate	33.72 ± 0.04	toxic	0.50	no selective
C_2	23.27 ± 1.24	moderate	425.65 ± 0.43	no toxic	18.29	selective
C_3	8.48 ± 0.89	trypanocidal	366.76 ± 0.02	no toxic	43.25	selective
D_1	8.73 ± 0.63	trypanocidal	317.52 ± 0.13	no toxic	36.37	selective
D_2	5.42 ± 1.03	trypanocidal	185.31 ± 1.09	toxic	34.19	selective
D_3	> 100	low or no	-	-	-	-
K_1	> 100	low or no	-	-	-	-
K_2	> 100	low or no	-	-	-	-
L_1	2.83 ± 0.17	trypanocidal	42.50 ± 1.19	toxic	15.01	selective
L_2	3.86 ± 0.86	trypanocidal	14.55 ± 1.32	toxic	3.76	selective
L_3	2.76 ± 1.00	trypanocidal	5.56 ± 0.63	toxic	2.01	selective
L_4	12.16 ± 0.44	moderate	13.62 ± 2.17	toxic	1.12	selective

Discussion:-

Synthetic molecules have physical properties compatible with reasonable pharmacokinetics and drug availability. The scaffold (Figure 1) has advantageous properties: low molecular weight, reasonable C.logP, good hydrogen bond donating and accepting capabilities (Table 1), easy and economical synthetic routes (Lipinski et al., 1997 , 2001). The analysis of spectrometrical data gave especially in ^{13}C NMR spectra, peaks of C=S from 179.13 to 175.47 ppm and of **C=N** between 150.47 and 143.74 ppm in all molecules. All aromatics carbons of the compounds ranged from 159.04 to 115.49 ppm. ^{1}H NMR spectra gave the characteristic protons in each structure: signals of protons (**H**C=N) were observed between 8.06 and 7.97 ppm (products $\mathbf{K_1}$ & $\mathbf{K_2}$), we generally noted the disappearance of the peaks for the proton NH2 of thiosemicarbazide, except the protons in the amino group H_2N in molecules $\mathbf{L_1}$ and $\mathbf{L_4}$ respectively were identified at 6.87 and 6.70 and 6.78 and 6.35 ppm. In the products $\mathbf{L_2}$ and $\mathbf{L_3}$, the signal of the typical proton HO was observed respectively at 8.70 and 8.80 ppm. Aromatics protons in the compounds were obtained between 7.80 and 6.70 ppm. It is worth mentioning that substituents used here, OH and NH_2, are both electron donating in the ortho and para positions. The analysis of these spectral data further confirms the structure of each molecule synthesized. In mass spectrometry, mass of each molecular ion peak (parent peak) obtained is very consistent and comparable to the theoretically estimated mass.

Pharmacological tests (table 3) showed that compounds presented interesting activity. On the parasites, some molecules $\mathbf{B_3}$, $\mathbf{C_3}$, $\mathbf{D_1}$, $\mathbf{D_2}$ and $\mathbf{L_{1\text{-}3}}$ revealed a high trypanocidal effect with their IC_{50} between 2.76 and 8.73 µM (IC_{50} < 10 µM). Product $\mathbf{B_2}$, $\mathbf{C_1}$, $\mathbf{C_2}$ and $\mathbf{L_4}$ (IC_{50} = 12 < IC_{50} < 90 µM) inhibited a moderate trypanocidal activity. The other compounds showed little or no activity because presented a high value of IC_{50} (> 100 µM). These results are consistent with the scale of trypanocidal activity established in the previous works (Du et al., 2002 ; Greenbaum et al., 2004 ; Fujii et al., 2005). According their previous studies, 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.

Lipophilic and structure-activity relationship of compounds

It has been reported in the literature that N(4)-alkyl or N(4)-arylthiosemicarbazones exhibit greater anti-trypanosomal activity than their unsubstituted analogs, probably due to their increased lipophilicity (Pandeya et al., 2000 ; Beraldo and Gambino 2004 ; Perez-Robellodo et al., 2008). In our work, this is valid for the same series of compounds (table 4).

The data in the table show that for these three series of compounds, the most active are N(4)-methyl- and especially N(4)-phenyl-3-thiosemicarbazones. Indeed, the N(4)-phenyl-3-thiosemicarbazones of propiophenone $\mathbf{B_3}$ (7.63 µM) and benzophenone $\mathbf{C_3}$ (8.48 µM), all trypanocides, are more active than their analogs N(4)-methyl-3-thiosemicarbazones $\mathbf{B_2}$ (87.15 µM) and $\mathbf{C_2}$ (23.27 µM) which presented moderate activity; they are also more active than unsubstituted thiosemicarbazones $\mathbf{B_1}$ (> 100 µM) and $\mathbf{C_1}$ (67.17 µM). The same remark is made at the level of compounds $\mathbf{D_2}$ and $\mathbf{D_1}$, all trypanocides, where 4'-methylacetophenone N(4)-methyl-3-thiosemicarbazone $\mathbf{D_2}$ (5.42 µM) inhibited more trypanocidal activity than the unsubstituted thiosemicarbazone $\mathbf{D_1}$ (8.73 µM).

Lipophilicity is a main physico-chemical determinant influencing the bioavailability, permeability and frequently the toxicity of drugs (Lipinski et al., 2001). A substance is all the more lipophilic as log P is positive. The calculation of the log P (C log P) involves the additivity rules of the hydrophobic constants of Rekker (Rekker, 1977). The higher the logP (C logP) the lower IC_{50} and the more active substance (Du et al., 2002 ; Fujii et al., 2005).

For each of these series, it is noted that in general the introduction of the alkyl or aryl group on the nitrogen N(4) induces an increase in lipophilicity and also activity : $\mathbf{B_3}$ and $\mathbf{C_3}$ with **ClogP** = 4.600 and 5.400 respectively are all trypanocidal. In the Di series, the two trypanocidal compounds have almost the same degree of lipophilicity.

In the $\mathbf{L_{1\text{-}4}}$ series of 4-phenyl-3-thiosemicarbazones of the benzophenone derivatives, a significant increase in activity is also seen. Among these molecules, 4-hydroxybenzophenone 4-phenyl-3-thiosemicarbazone ($\mathbf{L_3}$) is the most active displaying an IC_{50} of 2.76 µM. A comparative study between 4-phenyl-3-thiosemicarbazones of benzophenone $\mathbf{C_3}$ and its $\mathbf{L_{1\text{-}3}}$ derivatives shows that the substitution of an ortho or para proton of a phenyl radical of benzophenone by an amino or a hydroxy group significantly enhanced trypanocidal activity (Table 3). The IC_{50} values found for the substituted $\mathbf{L_{1\text{-}3}}$ derivatives are still low compared to that of the unsubstituted $\mathbf{C_3}$ compound (IC_{50} = 8.48 µM). It appears that it is the substitution of the hydroxyl group in para position $\mathbf{L_2}$ which gives the best activity (IC_{50} = 2.76 µM). This group enriches its nucleus by its mesomeric donor effect. The ortho amino and

hydroxy benzophenone derivatives are also more active than **C_3**. The difference in activity between these **L_1** and **L_2** molecules could be explained by the effect of the amino group or the substituted hydroxy group on the phenyl radical. Between ortho and para hydroxy-substituted products, it is para the most active **L_2**. This could be explained by the steric OH effect in the ortho position. There is a slight decrease in activity (IC_{50} p-OH = 2.76 and o-OH = 3.86 µM) (Du et al., 2002).

In our study, we have remarked that the substitution of the imine's hydrogen C**H**=N of benzaldehyde 4-phenyl-3-thiosemicarbazone (**K_2**) by the radical **ethyl** (propiophenone **B_3**) and **phenyl** (benzophenone **C_3**) induced a trypanocidal activity. This observation confirms the work of Du et al. (2002). The IC_{50} value goes from more than 300 µM (for **K_2**) to 8.48 µM (for **C_3**) and 7.63 µM (for **B_3**). The last two compounds are also the most lipophilic (table 1).

Table 4:-Evolution of the trypanocidal activity with the thiosemicarbazone N(4)-alkyl or aryl substituted.

Compounds	C log P	IC_{50} en µM	Activity
Propiophenone 4-phenyl-3-thiosemicarbazone **B_3**	**4.600**	7.63	trypanocidal
Propiophenone 4-methyl-3-thiosemicarbazone **B_2**	2.816	87.15	moderate
Propiophenone thiosemicarbazone **B_1**	2.930	210.00	low
Benzophenone 4-phenyl-3-thiosemicarbazone **C_3**	**5.400**	8.48	trypanocidal
Benzophenone 4-methyl-3-thiosemicarbazone **C_2**	4.071	23.27	moderate
Benzophenone thiosemicarbazone **C_1**	4.102	67.17	moderate
4'-methylacetophenone 4-methyl-3-thiosemicarbazone **D_2**	2.786	5.42	trypanocidal
4'-methylacetophenone thiosemicarbazone **D_1**	2.900	8.73	trypanocidal

We then studied the larval toxicity of the active compounds on the trypanosome using the cytotoxicity of the lapachol (LC_{50} = 281 µM) as referred (Santos et al., 2003 ; Graminha et al., 2008). Shrimp larvae were selected in this study as biological model. Our different LC_{50} values obtained using the synthesized products presented toxic or no activity than lapachol. Among compounds, it is **B_2**, **C_1**, **D_2** and the series **$L_{1\text{-}4}$** which showed toxic activity, particularly the 4-phenyl-3-thiosemicarbazones of benzophenone derivatives **$L_{1\text{-}4}$** (Table 3). Indeed, there is a correlation between the toxicity of the compounds on shrimp larvae and their cytotoxicity on 9KB and 9PS cells (human carcinoma nasopharygien) (Pelka et al., 2000), and on A-549 cells of lung carcinoma and HT-29 cells of colon carcinoma (Carballo et al., 2002). In addition, we noticed that the most strongly trypanocidal compounds were also the most cytotoxic. With their LC_{50} and IC_{50} values, we have determined the selectivity indexe of each trypanocidal compound. Except **C_1** all products displayed greater selectivity, especially **B_3** with SI = 119 and compounds **C_2**, **C_3**, **D_2**, **D_1** and **L_1** (SI > 10). These results are in perfect agreement with the work of Tiuman et al., (2005) in which if the SI value obtained is greater than unity, the test compound is considered to be selective on the parasite and if SI value is less than unity, the test compound is more cytotoxic than anti-parasitic.

Conclusion:-

In this work, fifteen thiosemicarbazones and derivatives were synthesized and studied. Their biological activities were evaluated and products showed interesting trypanocidal activity on the parasite study and were selective. Some factors including lipophilicity, steric and electronic effects of the substituents have played a vital role in this activity. This study could open an interesting opportunity to the treatment of the trypanomiasis sickness.

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