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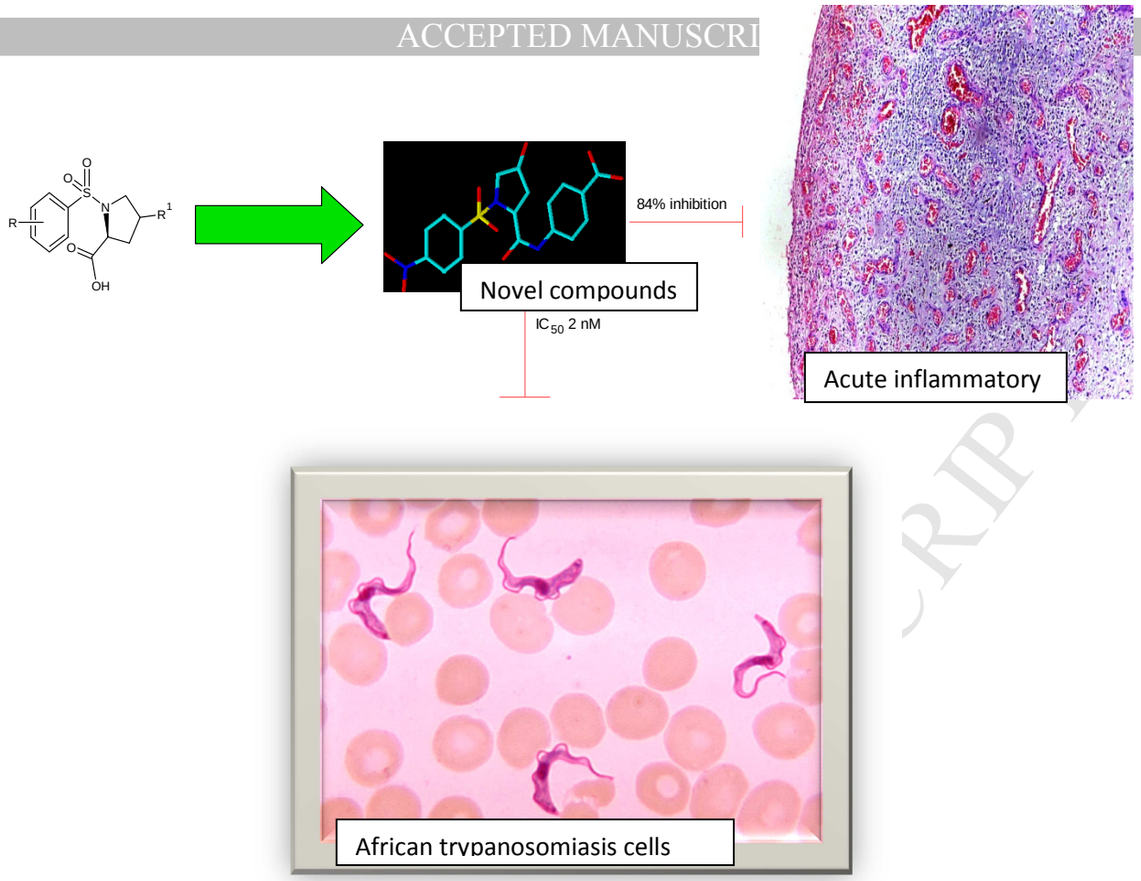
Graphical Abstract



Synthesis of Proline Derived Benzenesulfonamides: a Potent anti-*Trypanosoma brucei gambiense* agent

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Abstract

Thousands of death in Africa and other developing nations are still attributed to trypanosomiasis. Excessive sleep has been associated with increased inflammation. We report herein, the synthesis, antitrypanosomal and anti-inflammatory activities of eight new carboxamide derivatives bearing substituted benzenesulfonamides. The base promoted reactions of L-proline and L-4-hydroxyproline with substituted benzenesulfonyl chlorides gave the benzenesulfonamides (**11a-h**) in excellent yields. Boric acid mediated amidation of the benzenesulfonamides (**11a-h**) and *p*-aminobenzoic acid (**12**) gave the new carboxamides (**13a-h**) in excellent yields. The new carboxamides were tested for their antitrypanosomal and anti-inflammatory activities against *Trypanosome brucei gambiense* and inhibition of carrageenan-induced rat paw edema. Compound **13f** was the most potent antitrypanosomal agent with an IC_{50} value of 2 nM as against 5 nM for melarsoprol; whereas compound **13a** was the most potent anti-inflammatory agent with percentage inhibition of carrageenan-induced rat paw edema of 58, 60, 67 and 84% after 0.5 h, 1 h, 2 h and 3 h administration respectively. The structure-activity relationship study revealed that substitution at the *para* position in the benzenesulfonamide ring increased both the antitrypanosomal and anti-inflammatory activities. The 4-hydroxyprolines (**13a-d**) showed higher anti-inflammatory activity than the prolines (**13e-h**). In contrast, the prolines (**13e-h**) had higher antitrypanosomal activities than the 4-hydroxyprolines. The link between excessive sleep and inflammation makes the report of this class of compounds possessing both antitrypanosomal and anti-inflammatory activity worthwhile. The pharmacokinetic studies showed that the compounds would not pose oral bioavailability, transport and permeability problems.

Keywords: African trypanosomiasis; anti-inflammatory; carboxamides; catalysis; *Trypanosome brucei;* sulfonamides.

1. Introduction

African trypanosomiasis also known as sleeping sickness is an insect-borne parasitic disease of humans caused by the protozoa specie *Trypanosoma brucei.* Of the two species of *Trypanosoma brucei*, *Trypanosoma brucei gambiense* causes over 98% of reported cases [1]. *Trypanosoma brucei* has over 800 genes that make proteins of the parasite mixes and matches to evade immune system detection [2]. This has tampered with the development of vaccine for the diseases. There is emerging increase in African trypanosoma due to growing poverty of the countries affected, the need to take immediate action against human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) and above all, the absence of non-toxic treatment that is easily administered, reasonably priced and effective at every stage of the disease [3]. Suramin (**1**) and pentamidine (**2**) are effective drugs used during the initial phase of the disease but are almost totally ineffective in the neurological phase, since they cannot cross the blood-brain barrier [3]. Melarsoprol (**3**) was found to be effective in all stages but it has associated drawback of being expensive (US$100 per patient in 2000) and toxic. Eflornithine (**4**) has an advantage of being less toxic and as well active against all stages of the disease but is overwhelmingly expensive (US$ 700 per patient in 2000) and the recipient has to be hospitalized and given the drug by perfusion for at least two weeks. Robays *et al* reported that the cost per life saved for melarsoprol was 600.4 Euros against 627.6 Euros for eflornithine [4]. Nifurtimox (**5**) when taken orally for 1-2 months and alpha-difluoromethylornithine (**6,** alpha-DFMO) with an administration scheme spread over 5 weeks including 14 days of intravenous injections provides alternatives for all cases [5]. Although, eflornithine was better tolerated than melarsoprol, it produces several side effects, including gastrointestinal problems, fever, hypertension and convulsion [6].

The record of severe reactive encephalopathies in 5-10% of cases, death in 3-5% of the patient against melarsoprol and side effects against eflornithine underscore the need for new anti-trypanosomal agents [7]. The potential for resistance when used in monotherapy ushered in

combinatorial therapy in which nifurtimox-eflornithine combination was the most successful. The work of Alirol *et al* however, observed that nifurtimox-eflornithine (NECT) cannot be considered as an ideal treatment for African trypanosomiasis because it requires 7 days of intravenous administration which is a huge challenge for primary healthcare systems in developing countries [8]. Additionally, some adverse effects like frequent vomiting which may cause insufficient bioavailability of nifurtimox, requiring frequent use of antiemetics and occasional readministration of nifurtimox are worrisome. Although, psychotic behaviour and convulsions are less frequent, they can be a source of distress for patients and relatives. NECT treatment regimen is usually accompanied with painful lumbar punctures and difficult examination of the cerebrospinal fluid [9].

There is an urgent need for more effective treatments for Human African Trypanosomiasis (HAT), as the current therapy has many shortcomings viz. high toxicity, prohibitive costs, undesirable route of administration and poor efficacy. The above problems associated with the current antitrypanosomal agents highlights the need for research aimed at developing new drugs for the treatment of human African trypanosomiasis.

To direct the research, some proline derivatives have been reported to possess activity against human African trypanosomiasis in micromolar concentration (IC_{50} 30- 50 µM) [10,11].

Papadopoulou *et al.* reported a nitro based sulfonamide derivative possessing activity as low as 0.218 µM against HAT [12]. Junqueira *et al.* reported novel galactosyl-triazolobenzenesulfonamides possessing IC_{50} in micromolar concentration [13]. Brand *et al.* reported the synthesis of pyrazole sulfonamides (**7**) that inhibited *N*-myristoyltransferase [14]. The IC_{50} of the reported lead compound was 0.003 mM with blood brain barrier of 1.5. Sykes *et al.* reported a lead agent against *T. brucei gambiense* having IC_{50} of 1.3 micromolar bearing benzenesulfonamide moiety (**8**) [15]. Kulman *et al.* reported some series of sulfonamide derivatives (**9**) having micromolar inhibition of tubulin of *T. brucei* with good selectivity [16]. This class of compound presents a huge breakthrough in the chemotherapy of HAT because the toxicity of current HAT drugs has been associated with poor selectivity between mammalian and parasitic

tubulin [16]. Bhattacharya *et al.* reported *N*-phenyl-3,5-dinitro-N^4,N^4-di-n-butylsulfanilamide with an IC_{50} value of 0.12 μM against *T. brucei* [17]

Some carboxamides have been reported to possess interesting antitrypanosomal activity against *T. brucei gambiense* [18-20]. Report has shown that each additional hour of self-reported sleep duration was associated with an eight percent increase in C-reactive protein (CRP) levels and a seven percent increase in interleukin-6 (Il-6) which are two inflammatory mediators. Chronic elevations in cytokines such as CRP and Il-6 have been associated with an increased risk of problems such as diabetes and heart diseases [21]. It can therefore be adduced from these findings that sleeping sickness can induce inflammatory diseases by raising the levels of CRP and Il-6.

In search for suitable coupling reagents for direct amidation reaction, Bala *et al* [22], Mylavarapu *et al* [23], Tang *et al* [24] and Lanigan *et al* [25] have reported boric acid and its derivatives as a good catalyst for the direct amidation of un-activated carboxylic acids. The work of these groups was unable to check the feasibility of the catalyst system in the presence of other functional groups.

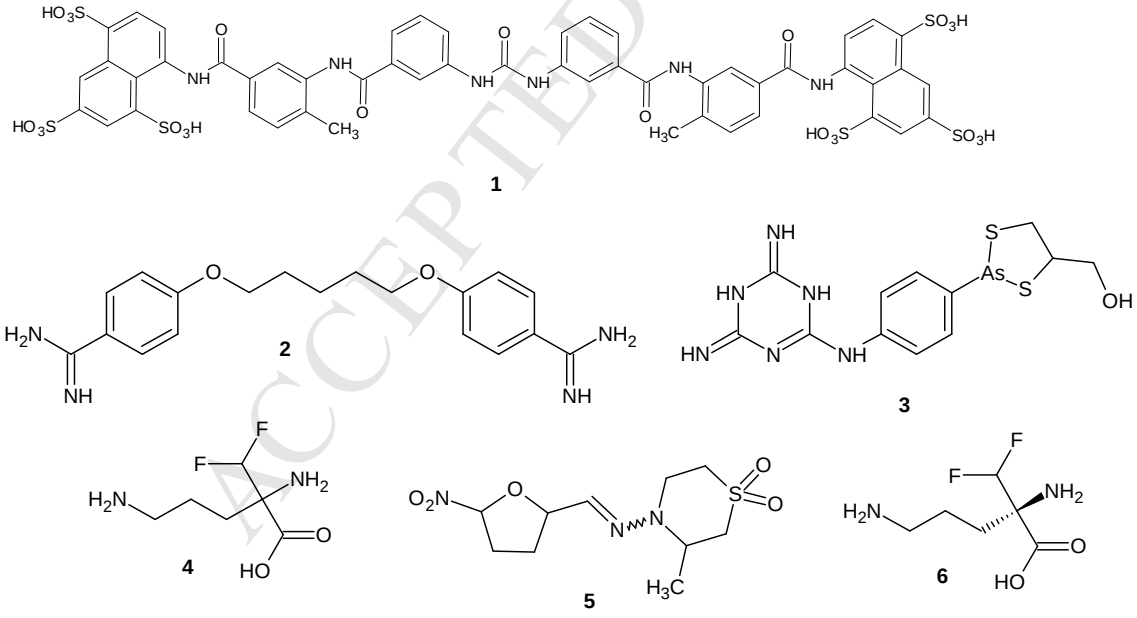


Fig 1: examples of drugs in use for the treatment of HAT

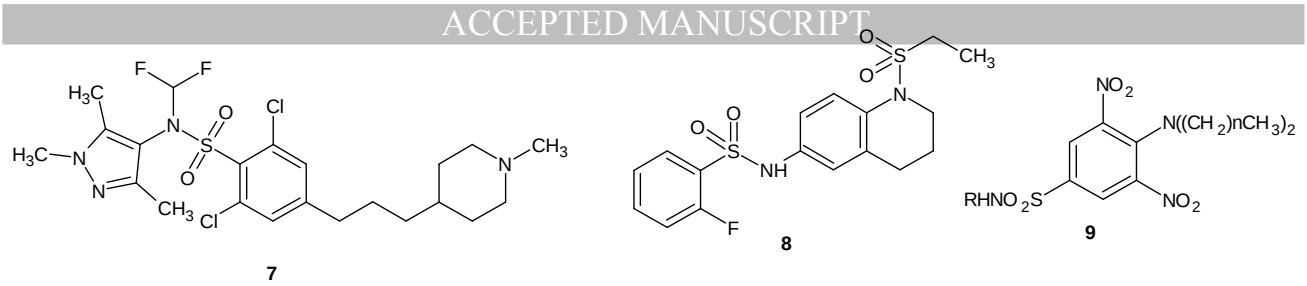
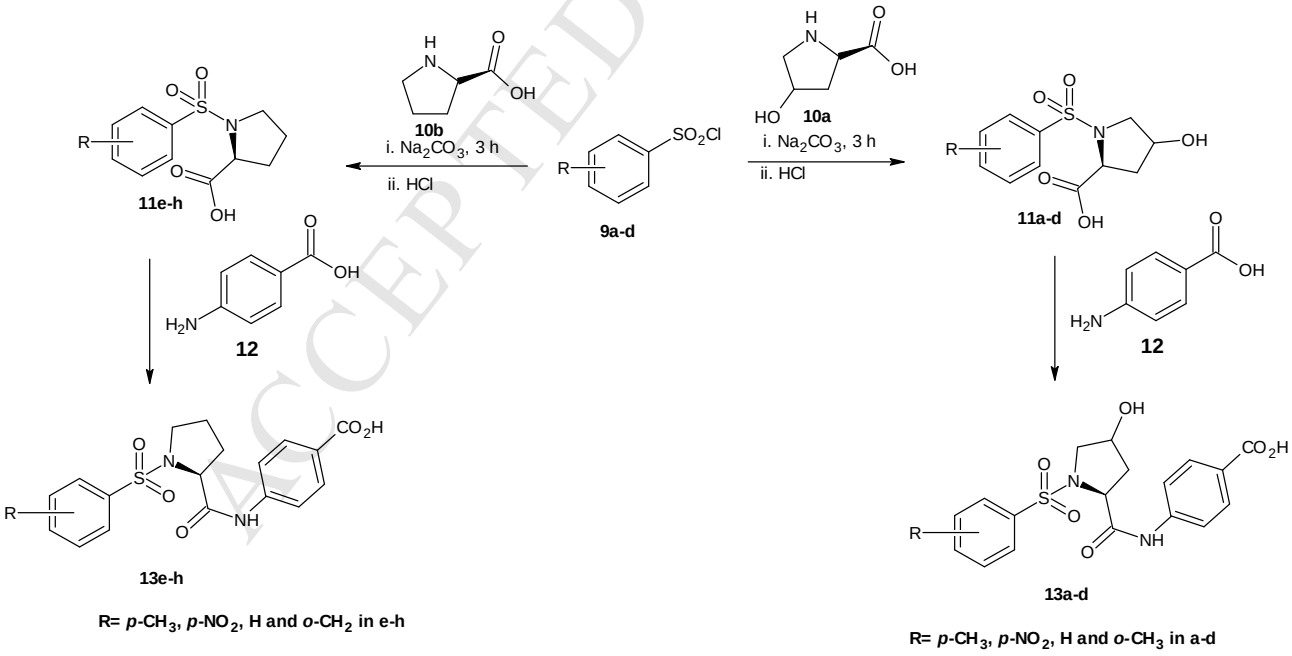


Fig 2: examples of recently reported antitrypanosomal agent

The presence of sulfonic acid group, sulfone and carboxamide functionalities in conventional drugs used for the treatment of HAT informs the desire to make new compounds containing sulfonamide and carboxamide functionalities. Proline was considered in this study because of the earlier report on some proline derivatives that showed activities against *T. brucei gambiense.* We report herein the boric acid catalysed amidation of un-activated carboxylic acids bearing sulfonamide, carboxylic acids and alcoholic group. Additionally, the antitrypanosomal and anti-inflammatory activities of the derivatives were also tested.

2. Results and Discussion

2.1 Chemistry



Scheme 1: Protocol for the synthesis of new carboxamide derivatives bearing sulfonamide functionalities.

Table 1: Description of substituents

S/N	a	b	c	d	e	f	g	h
R	*p*-CH_3	*p*-NO_2	H	*o*-CH_3	*p*-CH_3	*p*-NO_2	H	*o*-CH_3

The reactions of substituted benzenesulfonyl chloride (**9a-d**) and L-4-hydroxyproline (**10a**) or L-proline (**10b**) in the presence of sodium carbonate at 0 ^{o}C after 3 h gave the substituted benzenesulfonamides (**11a-h**) in excellent yields. Further reactions of the benzenesulfonamides (**11a-h**) with *p*-aminobenzoic acid (PABA, **12**) in the presence of catalytic amount of boric acid under reflux gave the new carboxamides (**13a-h**) in excellent yields (scheme 1, Table 1). The compounds were characterised using FTIR, ^{1}H NMR, ^{13}C NMR and high resolution mass spectroscopy (HRMS). In the FTIR spectra, the hydroxyl band of compounds **13a-d** appeared between 3473-3460 cm^{-1}. These bands were not observed in compounds **13e-h.** The NH band appeared between 3462-3363 cm^{-1} indicating the successful coupling of PABA with the benzenesulfonamide to form the carboxamide. In addition to the NH band, the appearance of two carbonyl bands between 1740-1688 cm^{-1} for the carboxylic acid and 1704-1660 cm^{-1} for the carboxamide is diagnostic of successful coupling. In the ^{1}H NMR spectra, the proton count accounted for all the expected protons and additionally, the appearance of the NH peak between 5.93-5.03 ppm is supportive of successful synthesis of target molecules. In the carbon-13 NMR spectra, the peaks between 174.6-172.9 ppm were assigned to the carbonyl of carboxylic acid. In addition, the C-CO_2H peaks appeared between 153.7-152.6 ppm.

2.2 Biological assay

2.2.1 Anti-trypanosoma activity

Table 2: *In vitro* antitrypanosomal activity potential of the newly synthesized derivatives (µM)

Compd. No.	IC_{50} (µM)	LD_{50} (µM/kg)
13a	0.155±0.12	12.01±2.05
13b	0.144±0.11	10.28±1.08

13c	0.160±0.15	12.07±2.68
13d	0.157±0.14	9.98±1.10
13e	0.101±0.11	11.92±2.76
13f	0.002±0.01	8.65±0.98
13g	0.159±0.32	10.06±1.43
13h	0.134±0.21	13.09±3.12
melarsoprol	0.005±0.02	8.75±1.04

The *in vitro* antitrypanosomal activity (Table 2) showed that except compound **13f** that had twofold better activity than melarsoprol, other derivatives had good anti-*Trypanosoma brucei* activities in micromolar concentration but not comparable with melarsoprol. The drug-likeness studies revealed that five of the new carboxamides are drug-like inclusive of compound **13f**. This findings, therefore, calls for more experimental work aimed at developing any of the drug-like derivatives into a potential drug. The structure-activity relationship studies showed that the L-proline derivatives (**13e-h**) had better anti-*Trypanosome brucei* activities than L-hydroxyproline derivatives (**13a-d**). The nitro-substituted benzene derivative **13f** with an IC_{50} of 0.002 µM was the most active derivative among the proline derivatives. The order of activity is **13f>13e>13h>13g.** Among the 4-hydroxyproline derivatives (**13a-d**), **13b** was the most active with an IC_{50} of 0.144 µM. The order of activity is **13b>13a>13d>13c**. Generally, substitution on the benzene ring increased the anti-*Trypanosome brucei* activities. The presence of electron withdrawing NO_2 improved the antitrypanosomal activity. The LD_{50} suggests that the compounds would not pose toxicity problems in clinical trials.

2.2.2 *In vivo* anti-inflammatory activity

Table 3: *In vivo* anti-inflammatory activities of the newly synthesized carboxamide derivatives (%)

Compd. No.	30 min (%)	1 h (%)	2 h (%)	3 h (%)
13a	58.01	59.95	67.23	84.01

13b	44.10	48.48	52.25	62.81
13c	33.85	24.71	27.34	1.85
13d	38.58	21.18	23.44	25.31
13e	28.75	31.49	36.27	35.28
13f	42.12	45.88	50.08	52.33
13g	23.56	26.34	29.44	31.15
13h	35.78	39.38	36.33	40.05
Indomethacin	56.92	63.53	64.84	63.58

The *in vivo* anti-inflammatory activity of the compounds in percentages (%) are presented in Table 3. All the reported derivatives had anti-inflammatory activities reported as the percentage decrease in edema thickness induced by carrageenan. The structure-activity relationship studies showed that the hydroxyl group in the proline ring (**13a-d**) increased the anti-inflammatory activities. This could be as a result of utilization of the lone pair of the hydroxyl group in the formation of hydrogen bonds with the amino acids of the possible target. The substitution at the *para* position was shown to contribute significantly to the anti-inflammatory activities of the derivatives as evident in the decreased activity in compounds **13c, 13d, 13g** and **13h** when compared with compounds **13a, 13b, 13e** and **13f**. The effect of electron availability is made evident from the results of compounds **13a** and **13b** and **13e** and **13f**, the methyl substituent had better activity than the nitro-substituted derivatives. The substitution in ortho position (**13d** and **13h**) favoured anti-inflammatory activity than the unsubstituted derivatives (**13c** and **13g**). Compound **13a** and **13b** had comparable anti-inflammatory activity with indomethacin. The drug-likeness showed that compound **13b** is non-drug-like thereby making compound **13a** the only likely anti-inflammatory drug candidate.

2.2.3 Physicochemical Properties Study

Table 4: Physicochemical Properties calculation of the new analogs

Compd. No.	MW	clogP	HBD	HBA	TPSA (Å^2)	NRB	ABS (%)	NV
13a	404	0.418	3	6	79.90	6	81.43	0
13b	435	-1.132	3	6	123.04	7	66.55	0
13c	390	-0.253	2	6	79.90	6	81.43	0
13d	404	0.418	3	6	79.90	7	81.43	0
13e	388	-0.190	1	5	79.90	6	81.43	0
13f	419	1.305	2	5	123.04	6	66.55	0
13g	374	-0.103	3	5	79.90	6	81.43	0
13h	388	1.305	2	5	79.90	6	81.43	0

MW= molecular weight, HBA= hydrogen bond acceptor, HBD= hydrogen bond donor, NRB= number of rotatable bond, ABS= solubility, TPSA= topological polar surface area, NV= ro5 number of violations.

Lipophilicity has long been recognized as an important factor for successful passage of drugs through clinical development [26]. Generally, calculated logP (clogP) is being used for assessment of lipophilicity and the key events of molecular desolvation, transfer from aqueous phases to cell membranes and protein binding sites [27]. With the evidenced role as a predictor of eventual compound success, computation of logP (clogP) for lipophilicity is essential for the development of a successful therapeutic compound. All the tested compounds complied with ro5, where logP values ranged between -1.132 to 1.305 (<5); MW range 374-435 (<500); HBA range 5-6 (<10) and HBD range 1-3 (<5), suggesting that these compounds would not pose oral bioavailability problems. All the compounds showed NRB values range 6-7 (<10), indicating their acceptable molecular flexibility with consequent expected good permeability and oral bioavailability. In addition, all the compounds evaluated showed TPSA values range 79.90-123.04 Å^2 (<140 Å^2), showing good permeability and transport of the compounds in the cellular plasma membrane. Furthermore, the

evaluated compounds showed good % ABS range 66.55-81.43% calculated from %ABS= 109-0.345×TPSA [28], which indicates their good bioavailability upon oral administration.

3. Conclusions

The synthesis of eight new carboxamide derivatives has been reported. The compounds were characterised using FTIR, NMR and HRMS. The anti-inflammatory and antitrypanosomal (*T. brucei gambiense*) activities results showed that the compounds were active. Particularly, compound **13f** had twofold anti-*Trypanosoma brucei gambiense* activity when compared with melarsoprol. Other derivatives had antitrypanosomal activities in micromolar concentration but were not comparable to melarsoprol. Like the antitrypanosomal activities, the anti-inflammatory activities showed that all the new derivatives were active and of particular attention were compounds **13a** and **13b** which had comparable activity with indomethacin. The pharmacokinetic study showed that the reported derivatives would not have oral bioavailability, transport or permeability problems. The connectivity between excess sleep and inflammation-related diseases makes it resounding to have a compound that could act as both antitrypanosomal agent and anti-inflammatory agent.

4.0 Experimental

All reactions requiring inert atmosphere were carried out under nitrogen atmosphere. Drying of solvents was achieved using molecular sieve for 48 h. All reagents were purchased from commercial suppliers, Aldrich, Merck, Fluka, Avra, SD fine and Alfa Aesar. Thin layer chromatography was carried out using silica plates purchased from Avra. The plates were visualized under UV light (popular India). FT-IR spectroscopy of the compounds were run in PerkinElmer Spectrum version 10.03.06 and the bands presented in wavenumber. Proton and carbon-13 NMR spectroscopy were run in $DMSOd_6$ and CD_3OD, on either Jeol 500 MHz or 400 MHz. The chemical shifts were reported in part per million with reference to tetramethylsilane. High Resolution Mass

spectroscopy were carried out using micro TOF electrospray time of flight (ESI-TOF) mass spectrometer, sodium formate was used as the calibrant. All experiments were carried out at Prof. Sandeep Verma's Laboratory, Department of Chemistry, Indian Institute of Technology, Kanpur. Melting points were determined using digital melting point apparatus and were uncorrected.

4.1 Synthesis of substituted proline-based benzenesulfonamides

Sodium carbonate (Na_2CO_3, 1.590 g, 15 mmol) was added to a solution of amino acids (**10a-b,** 12.5 mmol) in water (15 mL) with continuous stirring until all the solutes dissolved. The solution was cooled to -5 ^oC and the appropriate benzenesulfonyl chloride (**9a-d,** 15 mmol) was added in four portions over a period of 1 h. The slurry was further stirred at room temperature for about 3 h. The progress of the reaction was monitored using TLC (MeOH/DCM, 1:9). Upon completion, the mixture was acidified using 20% aqueous hydrochloric acid to pH 2. The crystals were filtered via suction and washed with pH 2.2 buffer. The pure products (**11a-h**) were dried over self-indicating fused silica gel in a desiccator [29].

4.2 Boric Acid Catalysed Amidation of Un-Activated Carboxylic Acid and *p*-aminobenzoic acid.

To a suspension of substituted benzenesulfonamides (**11a-h**) (1.0 mmol) in dry toluene (40 mL) equipped with Dean-Stark apparatus for azeotropic removal of water, was added 4-aminobenzoic acid (1.0 mmol) and boric acid (0.1 mmol) at room temperature and then refluxed for 4 h. On completion (as monitored by TLC), reaction mixture was precipitated to amides by adding about 40 mL *n*-hexane. The new proline derivatives were obtained via suction filtration, washed with *n*-hexane and dried over fused silica gel or concentrated using rotary evaporator and dried over vacuum in the case of oily products.

4.2.1 4-[4-Hydroxy-1-(4-methylbenzenesulfonyl)pyrrolidine-2-amido]benzoic acid (13a):

Yield (0.40 g, 100%), mp 155.30-155.60 ^oC; FTIR (KBr) ν/cm^{-1} 3460, 3364, 2550, 1740, 1666, 1601, 1574, 1442, 1327, 1313, 1174, 1157, 1072, 1000; ^1H NMR (400 MHz, $DMSOd_6$) δ_H 7.66-7.64 (d, 1H, *J* 8.24 Hz, ArH), 7.59-7.56 (d, 3H, *J* 8.68 Hz, ArH), 7.37-7.35 (d, 1H, *J* 8.24 Hz,

ArH), 6.51-6.49 (d, 3H, *J* 8.24 Hz, ArH), 5.81 (s, 1H, NH), 4.17 (s, 1H, OH), 4.02-3.98 (t, 1H, *J* 7.80 Hz, CH-C=O), 3.43-3.40 (m, 1H, CH-OH), 3.06-3.04 (d, 2H, *J* 10.56 Hz, CH_2N), 2.34 (s, 3H, CH_3), 1.92-1.89 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $DMSOd_6$) δ_C 173.8, 168.0, 153.7, 143.7, 134.9, 131.8, 130.1, 127.9, 117.4, 113.1, 68.9, 60.2, 56.8, 21.5; HRMS (FTMS+ESI) *m/z*, observed: 405.1122; $C_{19}H_{20}N_2O_6S$ (M+H), requires: 405.1121.

4.2.2 4-[4-Hydroxy-1-(4-nitrobenzenesulfonyl)pyrrolidine-2-amido]benzoic acid (13b):

Yield (0.44 g, 99.98%), mp 164.70-164.90 ^oC; FTIR FTIR (KBr) v/cm^{-1} 3460, 3365, 2968, 2555, 1713, 1667, 1602, 1442, 1423, 1575, 1528, 1327, 1312, 1174, 1130, 1093, 1071, 1011; ^1H NMR (400 MHz, DMSO-d_6) δ_H 12.07 (s, 1H, OH of COOH), 8.38-8.36 (d, 2H, *J* 7.36 Hz, ArH), 8.04-8.02 (d, 2H, *J* 6.88 Hz, ArH), 7.58-7.56 (d, 2H, *J* 6.88 Hz, ArH), 6.51-6.49 (d, 2H, *J* 6.88 Hz, ArH), 5.83 (s, 1H, NH), 4.17 (s, 1H, OH of alcohol), 4.13-4.09 (t, 1H, *J* 6.42 Hz, CH-C=O), 3.47-3.44 (m, 1H, CH-OH), 3.21-3.19 (d, 2H, *J* 8.68 Hz, CH_2N), 2.03-2.00 (m, 1H, CH of CH_2), 1.93-1.87 (m, 1H, CH of CH_2); ^{13}C NMR (100 MHz, $DMSOd_6$) δ_C 173.5, 168.0, 153.7, 150.3, 146.9, 131.7, 129.5, 124.8, 117.4, 113.1, 69.0, 60.4, 57.1; HRMS (FTMS+ESI) *m/z*, observed: 454.0912; $C_{18}H_{20}N_3O_9S$ ($M+H_3O$), requires: 454.0913.

4.2.3 4-[1-(benzenesulfonyl)-4-hydroxypyrrolidine-2-amido]benzoic acid (13c):

Yield (0.39 g, 100%); mp 142.50-142.70 ^oC; FTIR (KBr) v/cm^{-1} 3461, 3365, 2956, 2672, 1700, 1666, 1601, 1574, 1443, 1422, 1385, 1313, 1174, 1128, 1099, 1070, 1010; ^1H NMR (400 MHz, DMSO-d_6) δ_H 7.78-7.76 (d, 2H, *J* 6.88 Hz, ArH), 7.65-7.54 (m, 5H, ArH), 6.52-6.50 (d, 2H, *J* 8.68 Hz, ArH), 5.83 (s, 1H, NH), 4.18 (s, 1H, OH), 4.06-4.02 (t, 1H, *J* 8.00 Hz, CH-C=O), 3.45-3.41 (m, 1H, CH-OH), 3.11-3.08 (d, 2H, *J* 5.26 Hz, CH_2N), 1.95-1.89 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $DMSOd_6$) δ_C 173.8, 168.1, 153.7, 137.8, 133.5, 131.8, 129.6, 128.7, 127.9, 125.8, 117.4, 113.1, 68.9, 60.2, 56.8, 21.6; HRMS (FTMS) *m/z*, observed: 413.0788; $C_{18}H_{18}N_2O_6SNa$ (M+Na), requires: 413.0786.

4.2.4 4-[4-Hydroxy-1-(2-methylbenzenesulfonyl)pyrrolidine-2-amido]benzoic acid (13d):

Yield (0.40 g, 100%); mp 134.50-134.60 ^{o}C; FTIR (KBr) v/cm^{-1} 3473, 3415, 3029, 2988, 1692, 1669, 1601, 1442, 1422, 1313, 1290, 1174, 1154, 1093, 1072, 1015; ^{1}H NMR (400 MHz, CD_3OD) δ_H 7.77-7.69 (m, 2H, ArH), 7.50-7.43 (m, 1H, ArH), 7.33-7.28 (m, 2H, ArH), 7.19-7.16 (t, 1H, *J* 7.32 Hz, ArH), 7.12-7.07 (t, 1H, *J* 9.38 Hz, ArH), 6.62-6.60 (d, 2H, *J* 8.68 Hz, ArH), 4.45 (s, 1H, OH), 4.30-4.28 (t, 1H, *J* 2.32 Hz, CH-C=O), 3.56-3.51 (m, 1H, CH-OH), 3.38-3.25 (m, 2H, CH_2N), 2.60 (s, 3H, CH_3-Ar), 2.16-2.10 (m, 2H, CH_2); ^{13}C NMR (100 MHz, CD_3OD) δ_C 172.9, 169.4, 153.2, 138.2, 131.5, 129.3, 128.6, 127.6, 125.8, 124.9, 117.7, 113.0, 69.3, 59.4, 38.8, 20.2, 19.5; HRMS (FTMS+ESI) *m/z*, observed: 403.0968; $C_{19}H_{19}N_2O_6S$ (M-H); requires: 403.0963.

4.2.5 4-[1-(4-Methylbenzenesulfonyl)pyrrolidine-2-amido]benzoic acid (13e):

Yield (0.36 g, 93.25%); mp 161.40-161.60 ^{o}C; FTIR (KBr) v/cm^{-1} 3462, 3060, 2984, 2879, 2559, 1738, 1669, 1603, 1422, 1556, 1524, 1345, 1291, 1174, 1134, 1092, 1072, 1025; ^{1}H NMR (400 MHz, $DMSOd_6$) δ_H 7.75-7.69 (m, 4H, ArH), 6.65-6.63 (m, 4H, ArH), 5.02 (s, 1H, NH), 4.16-4.12 (t, 1H, *J* 6.40 Hz, CH-C=O), 3.45-3.39 (m, 1H, CH_a of CH_2N), 3.22-3.16 (m, 1H, CH_b of CH_2N), 2.40-2.30 (m, 3H, CH_3-Ar), 1.92-1.82 (m, 3H), 1.60-1.56 (m, 1H, CH); ^{13}C NMR (100 MHz, $DMSOd_6$) δ_C 174.6, 169.3, 152.6, 144.1, 134.6, 131.5, 128.6, 127.9, 125.6, 118.2, 60.7, 30.7, 24.3, 20.2, 20.0; HRMS (FTMS+ESI) *m/z*, observed: 389.1172; $C_{19}H_{20}N_2O_5S$ (M+H), requires: 389.1178.

4.2.6 4-[1-(4-Nitrobenzenesulfonyl)pyrrolidine-2-amido]benzoic acid (13f):

Yield (0.42 g, 99.90%); mp 132.40-132.50 ^{o}C; FTIR (KBr) v/cm^{-1} 3378, 3082, 2980, 2874, 2540, 1745, 1704, 1604, 1446, 1417, 1529, 1349, 1315, 1177, 1162, 1105, 1076, 1023, 1011; ^{1}H NMR (400 MHz, $DMSOd_6$) δ_H 12.35 (OH of COOH), 8.38-8.36 (d, 2H, *J* 9.16 Hz, ArH), 8.07-8.05 (d, 2H, *J* 8.68 Hz, ArH), 7.58-7.56 (d, 2H, *J* 8.24 Hz, ArH), 6.51-6.48 (d, 2H, *J* 8.64 Hz, ArH), 5.93 (s, 1H, NH), 4.19-4.16 (dd, 1H, *J* 3.64, 3.04 Hz, CH-C=O), 3.40-3.34 (m, 1H, CH_a of CH_2N), 3.23-3.17 (m, 1H, CH_b of CH_2), 2.00-1.91 (m, 1H, CH of CH_2), 1.86-1.75 (m, 2H, CH_2), 1.65-1.59 (m, 1H, CH); ^{13}C NMR (100 MHz, $DMSOd_6$) δ_C 173.4, 168.0, 153.7, 150.4, 143.7, 131.7, 129.2, 125.2,

117.4, 113.1, 61.1, 48.9, 30.9, 24.7; HRMS (FTMS+ESI) *m/z*, observed: 438.0953; $C_{18}H_{20}N_3O_8S$ (M+H_3O), requires: 438.0957.

4.2.7 4-[1-(Benzenesulfonyl)pyrrolidine-2-amido]benzoic acid (13g):

Yield (0.37 g, 99.98%); mp 171.50-171.60 ^oC; FTIR (KBr) v/cm^{-1} 3363, 2958, 2688, 1688, 1677, 1602, 1573, 1446, 1422, 1354, 1313, 1201, 1163, 1095, 1073, 1016; ^1H NMR (400 MHz, $DMSOd_6$) δ_H 7.80-7.78 (d, 2H, *J* 7.32 Hz, ArH), 7.67-7.63 (t, 1H, *J* 7.32 Hz, ArH), 7.59-7.55 (t, 2H, *J* 7.24 Hz, ArH), 7.21-7.07 (m, 2H, ArH), 6.52-6.50 (d, 2H, *J* 8.68 Hz, ArH), 5.83 (s, 1H, NH), 4.08-4.05 (dd, 1H, *J* 4.60, 5.04 Hz, CH-C=O), 3.34-3.28 (m, 1H, CH of CH_2N), 3.13-3.08 (m, 1H, CH of CH_2N), 1.85-1.71 (m, 3H), 1.51-1.47 (m, 1H, CH); ^{13}C NMR (100 MHz, $DMSOd_6$) δ_C 173.7, 168.1, 153.7, 133.6, 131.8, 129.9, 129.4, 128.7, 127.6, 125.8, 117.4, 113.1, 60.9, 48.9, 30.9, 24.7466; HRMS (FTMS+ESI) *m/z*, observed: 393.1121; $C_{18}H_{21}N_2O_6S$ (M+H_3O), requires: 393.1122.

4.2.8 4-[1-(2-Methylbenzenesulfonyl)pyrrolidine-2-amido]benzoic acid (13h):

Yield (0.39 g, 100%); mp 119.90-120.10 ^oC; FTIR (KBr) v/cm^{-1} 3382, 3020, 2981, 2632, 1692, 1688, 1604, 1574, 1443, 1422, 1313, 1290, 1158, 1131, 1091, 1016; ^1H NMR (400 MHz, CD_3OD) δ_H 7.75-7.72 (m, 2H, ArH), 7.44-7.40 (t, 1H, *J* 6.64 Hz, ArH), 7.34-7.28 (m, 1H, ArH), 7.19-7.13 (m, 2H, ArH), 7.11-7.05 (m, 2H, ArH), 4.15-4.13 (t, 1H, *J* 4.30 Hz, ArH), 3.45-3.43 (m, 2H, CH_2N), 2.59 (m, 3H, CH_3-Ar), 1.85-1.66 (m, 2H, CH_2), 1.59-1.42 (m, 2H, CH_2); ^{13}C NMR (100 MHz, CD_3OD) δ_C 174.3, 169.4, 153.2 (C-COOH), 137.6, 132.9, 131.5, 129.6, 127.9, 126.0, 125.0, 117.8, 113.1, 60.1, 30.9, 24.3, 20.2, 19.5; HRMS (FTMS+ESI) *m/z*, observed: 389.1163; $C_{19}H_{21}N_2O_5S$ (M+H), requires: 389.1164.

4.3 Biological Assay

4.3.1 *In vitro* antitrypanosomal activities

In vitro antitrypanosomal activities against *T. b. gambiense* strain was described by Otoguro *et al* [30]. In brief, *T. b. gambiense* strain was cultured in IMDM with various supplements and 10% heat-inactivated FBS at 37 °C, under 5.0% CO_2/95% air according to the method of Baltz *et al* [31].

Ninety five mL of *T. brucei gambiense* suspension (2.0-2.5 trypanosomes/mL for strain GUTat 3.1) was seeded in a 96-well microplate, and 5.0 mL of a test compound solution (dissolved in 5.0% dimethylsulfoxide) was added followed by incubation for 72 h. Ten mL of the fluorescent dye Alamar Blue was added to each well. After incubation for 3-6 h, the resulting solution was read at 528/20 nm excitation wavelength and 590/35 nm emission wavelength by a FLx800 fluorescent plate reader (Bio-Tek Instrument, Inc. Vermont, USA). Data were transferred into a spreadsheet program (Excel). The IC_{50} values were determined using fluorescent plate reader software (KC-4, Bio-Tek). Successive subcultures were done in 24-well tissue culture plates under the same conditions.

4.3.2 *In vivo* anti-inflammatory activities determination

Edema was produced by using 1% carrageenan solution. Foot volumes were measured in plethysmometer by mercury displacement. The instrument was calibrated before performing the experiment using standard calibrated probe number and standard drug used was indomethacin. Anti-inflammatory activity was determined by carrageenan induced rat hind paw edema method. The tested compounds and reference drug (indomethacin) were administered orally at a dose level of 100 mg/kg and 25 mg/kg respectively. After an hour of oral medication, all rats were injected with 1% carrageenan suspension (0.05 mL/animal) into the sub-planter surface of the right hind paw. The thickness of both paws were measured at different time intervals of zero, 1 h, 2 h and 3 h, after carrageenan injection. The anti-inflammatory activity of the tested compounds and indomethacin were calculated as the percentage decrease in carrageenan-induced edema thickness and was determined with the following formula [32]. *In vivo* efficacy studies in rat were conducted according to the rules and regulations for the protection of animal rights of Nigeria. They were approved by the veterinary office of University of Nigeria, Nsukka, Nigeria.

Percentage inhibition= $\frac{Vc-Vt}{Vc} * 100$ where V_c = volume of control, V_t = volume of test sample.

4.3.3 Determination of LD_{50} for the active anti-inflammatory compounds

Male mice were divided into various groups and test compounds were administered in various doses intraperitoneally. Following treatments, the animals were observed for up to 6 h continuously and were then kept under observation for 72 h. All behavioral changes and deaths during the observation periods were recorded. The percentage of death at each dose level was then calculated, converted to probits and the LD_{50} (µM/kg) values were calculated [33]. To observe the health status of the mice, they were monitored 4 times a day. Humane endpoints were used when the animal shows sign of weight loss, weakness accompanied by the inability to get food, complete anorexia and convulsion for 24 h. For the purpose of ameliorating the suffering of the dying mice, CO_2 euthanasia was applied. All the dead mice were disposed in bio-safety containers in accordance with local standard protocols. The mortality rate in each group was calculated according to the formula:

Mortality rate (%)= (the number of dead mice/the number of mice in the group)×100.

4.3.4 Physicochemical Properties

The prediction was based on molecular weight (MW), calculated logP, topological polar surface area (TPSA), hydrogen bond donors (HBD), hydrogen bond acceptor (HBA) and number of rotatable bond (NRB) and number of violation (NV) which is a record of the number of violations. The Physicochemical Properties Study was carried out using molinspiration, MedChem designer 3.0 and DrugLiTo software.

5.0 Supplementary Information

The ^{1}H NMR, ^{13}C NMR and HRMS spectra are available

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References

[1] R. Brun, J. Blum, F. Chappuis, C. Burri, Human African Trypanosomiasis, Lancet, 375 (2010) 159.

[2] G. Berriman, E. Ghedin, C. Hertz-Fowler, G. Blandin, H. Renauld, D.C. Barthol, The genome of the African trypanosome Trypanosoma brucei, Science, 309(5733) (2005) 422.

[3] M. Dumas, B. Bouteille, Treatment of Human African Trypanosomiasis, Bulletin of the World Health Organisation, 78(12) (2000) 1474.

[4] J. Robays, M.E. Raguenaud, T. Josenando, M. Boelaert, Eflornitine is a cost-effective alternative to melarsoprol for the treatment of second-stage human west African trypanosomiasis in Caxito, Angola, Trop. Med. Int. Health, 13(2*)* (2008) 271.

[5] M. Wery, Drugs used in the treatment of sleeping sickness, International Journal of Antimicrobial Agents, 4(3) (1994) 238.

[6] G. Priotto, L. Pinoges, I.B. Fursa, B. Burke, N. Nicolay, G. Grillet, C. Hawison, M. Balasegaram, Safety and effectiveness of first line eflornithine for Trypanosoma brucei gambiense sleeping sickness in Sudan: cohort study, British Medical Journal, 336 (2010) 708.

[7] J. Blum, S. Nkunku, C. Burri, Clinical description of encephalopathic syndromes and risk factors for their occurrence and outcome during melarsoprol treatment of human African trypanosomiasis, Trop. Med. Int. Health, 6 (2001) 400.

[8] E. Alirol, D. Schrumpf, J.A. Heradi, A. Riedel, C. de Patoul, M. Quere, F. Chappuis, Nifurtimox-Eflornithine Combination Therapy for Second-Stage Gambiense Human African Trypanosomiasis: Médecins Sans Frontières Experience in the Democratic Republic of the Congo, Clinical Infectious Diseases, 56(2) (2013) 203.

[9] O. Yun, G. Priotto, J. Tong, L. Flevaud, F. Chappuis, NECT is Next: Implementing the New Drug Combination Therapy for *Trypanosoma brucei gambiense* Sleeping Sickness, PloS Neglected Tropical Diseases, 4(5) (2010) e720.

[10] A.C.L. Leite, D.R. Moreira, M.Z. Hemandes, A.C. Kiperstock, R.S. de Lima, M.B.P. Soares, Synthsis, docking and in vitro activity of thisemicarbazones and acyl-thiazolidones against trypanosoma cruzi, Sociedade Brasileira de Quimica, 2 (2014) 344.

[11] G.C. Muscia, S.I. Carzola, F.M. Frank, G.L. Borosky, G.Y. Buldain, S.E. Asis, E.L. Malchiodi, Synthesis, trypanocidal activity and. molecular modeling studies of 2-alkylaminomethylquinoline derivatives, European Journal of Medicinal Chemistry, 46(9) (2011) 3703.

[12] M.V. Papadopoulou, W.D. Bloomber, H.S. Rosenzweig, S.R. Wilkinson, J. Szular, M. Kaiser, Novel 3-Nitro-1*H*-1,2,4-triazole-Based Amides and Sulfonamides as Potential Antitrypanosomal Agents, Journal of Medicinal Chemistry, 55(11) (2012) 5565.

[13] G.G. Junqueira, M.R. Carvalho, P. de Andrade, C.D. Lopes, Z.A. Carneiro, R. Sesti-Costa, J.S. Silva, I. Carvalho, Synthesis and in vitro Evaluation of Novel Glactosyl-triazolo-benzenesulfonamides Against Trypanossoma cruzi, Journal of the Brazilian Chemical Society, 25(10) (2014) 1884.

[14] S. Brand, N.R. Norcross, S. Thompson, J.R. Harrison, V.C. Smith, D.A. Robinson, L.S. Torrie, S.P. McElroy, I. Hallyburton, S. Norval, P. Seullion, L. Stojanovski, F.R.C. Simeons, D. Aalten, J.A. Frearson, R. Brenk, A.H. Fairlamb, M.A.J. Ferguson, P.G. Wyatt, I.H. Gilbert, K.D. Read, Lead optimization of a pyrazole sulfonamide series of Trypanosoma brucei N-myristoyltransferase inhibitors: identification and evaluation of CNS penetrant compounds as potential treatments for stage 2 human African trypanosomiasis, Journal of Medicinal Chemistry, 57(23) (2014) 9869.

[15] M.L. Sykes, J.B. Baell, M. Kaiser, E. Chatelain, S.R. Moawad, D. Ganame, J.R. Ioset, V.M. Avery, Identification of Compounds with Anti-Proliferative Activity against *Trypanosoma brucei brucei* Strain 427 by a Whole Cell Viability Based HTS Campaign, PloS Neglected Tropical Diseases, 6 (2012) e1896.

[16] D.G. Kulman, R. Lama, B. Li, B. Su, *Cleveland State University* *www.csuohio.edu/sites/default/files/29-%202015.pdf* retrieved on 22nd February 22, 2017.

[17] G. Bhattacharya, J. Herman, D. Delfin, M.M. Salem, T. Barszcz, M. Mollet, G. Riccio, R. Brun, K.A. Werbovetz, Synthesis and antitubulin activity of N1- and N4-substituted 3,5-dinitro sulfanilamides against African trypanosomes and Leishmania, Journal of Medicinal Chemistry, 47(7) (2004) 1832.

[18] F.T. Silva, C.H. Franco, D.C. Favaro, L.H. Freitas-Junior, C.B. Moraes, E.T. Ferreira, Design, synthesis and antitrypanosomal activity of some nitrofurazone 1,2,4-triazolic bioisosteric analogues, European Journal of Medicinal Chemistry, 121 (2016) 553-560

[19] M.V. Papadopoulou, W.D. Bloomber, H.S. Rosenzweig, S.R. Wilkinson, J. Szular, M. Kaiser, Antitrypanosomal activity of 5-nitro-2-aminothiazole-based compounds, European Journal of Medicinal Chemistry, 117 (2016) 179-186.

[20] J. Spencer, R.P. Rathman, A.L. Harvey, C.J. Clements, R.L. Clark, M.P. Barrett, P.E. Wong, L. Male, S.J. Coles, S.P. Mackey, Synthesis and biological evaluation of 1,4-benzodiazepin-2-ones with antitrypanosomal activity, Bioorganic and Medicinal Chemistry, 19 (2011) 1815

[21] American Academy of Sleep Medicine, Inflammation may be link between extreme sleep duration & poor health, ScienceDaily (2009) www.sciencedaily.com/releases/2009/02/090201094119

[22] M. Bala, P.K. Verma, N. Kumar, B. Singh, Direct waste-free synthesis of amides from nonactivated carboxylic acids and amines: Application to the synthesis of tetrahydroisoquinolines, Synthetic Communications, 45(7) (2015) 847-856

[23] R.K. Mylavarapu, G.C.M. Kondaiah, K. Naveenkumar, V. Raju, K. Purandhar, B. Apurba, B. Rakeshwar, Boric acid catalysed amidation in the synthesis of active pharmaceutical ingredients, Organic Process Research and development, 11 (2007) 1068.

[24] P.W. Tang, Boric Acid Catalyzed Amide Formation from Carboxylic Acids and Amines, Organic Synthesis, 81 (2005) 262

[25] R.M. Lanigan, P. Starkov, T.D.Sheppard, Direct Synthesis of Amides from Carboxylic Acids and Amines Using B(OCH2CF3)3, Journal of Organic Chemistry, 78 (2013) 4523.

[26] J.A. Arnott, R. Kumar, S.L. Planey, Lipophilicity Indices for Drug Development, J. Appl. Biopharm. Pharmacokinet, 1 (2013) 31.

[27] X. Liu, B. Testa, A. Fahr, Lipophilicity and its relationship with passive drug permeation, Pharmaceutical Research, 28 (2011) 962.

[28] Y. Zhao, M.H. Abraham, J. Lee, A. Hersey, N.C. Luscombe, G. Beck, B. Sherborne, I. Cooper, Rate-limited steps of human oral absorption and QSAR studies, Pharmaceutical Research, 19 (2002) 1457.

[29] D.I. Ugwu, U.C. Okoro, P.O. Ukoha, A. Ibezim, S. Okafor, N.M. Kumar, Synthesis, characterization, molecular docking and in vitro antimalarial properties of new carboxamides bearing sulphonamide, European Journal of Medicinal Chemistry, 135 (2017) 349-369.

[30] K. Otoguro, A. Ishiyama, M. Namatame, A. Nishihara, F. Furusawa, R. Masuma, K. Shiomi, Y. Takahashi, H. Yamada, S. Omura, Selective and potent in vitro antitrypanosomal activities of ten. microbial metabolites, The Journal of Antibiotics, 61 (2008) 378.

[31] T. Baltz, D. Baltz, C.H. Giroud, J. Crockett, Cultivation in a semi-defined medium of animal infective forms of Trypanosoma brucei, T. equiperdum, T. evansi, T. rhodesiense and T. Gambiense, EMBO J, 4 (1985) 1277.

[32] P. Selvam, N. Ramlakshmi, G. Uma, S.A. Kumar, A. Umamaheswari, Synthesis, characterisation and biological evaluation of novel coumarin derivatives, Rasayan Journal of Chemistry, 3(2) (2010) 280.

[33] M. Ghosh, Fundamentals of Experimental Pharmacology Scientific Book Agency, Calcutta, (1984) 153-158.

Highlights

1. Structure-based design and synthesis of novel proline derived sulphonamides is reported
2. The link between excessive sleep and inflammation calls for the synthesis of antitrypanosomal agents with additional anti-inflammatory properties.
3. *In vitro* and *in vivo* studies reveal that the reported compounds possessed good antitrypanosomal and anti-inflammatory activities.
4. Physicochemical properties prediction was carried out to assess the oral bioavailability, transport and permeability properties of the compounds.