WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 12, 1881-1891. Research Article ISSN 2277– 7105

SYNTHESIS AND IN VITRO ANTI MALARIAL ACTIVITY OF ESTRONE BASED CARBOHYDRAZIDE

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Article Received on
09 August 2021,

Revised on 29 August 2021,
Accepted on 19 Sept. 2021

DOI: 10.20959/wjpr202112-21846

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ABSTRACT

A new series of estrone base carbohydrazide has been designed and synthesized. Characterization and in vitro antimalarial evaluation of the newly synthesized compounds are discussed. Estrone and 4'-bromomethyl-2-cyanobiphenyl reacted in presence of potassium carbonate and potassium iodide in anhydrous acetone leads ether 4'-(3-yloxymethyl)-estrone-biphenyl-2-carbonitrile formation, which on sequential condensation with hydrazine hydrate and acid chloride give steroidal carbohydrazide in quantitative yield. Their structures were confirmed by Mass spectra, IR spectra and ^{1}H, ^{13}C NMR spectroscopy. The formation of carbohydrazide linkage with different acid chlorides had an excellent effect on the activity of the compounds. The newly synthesized steroidal carbohydrazide are screened for antimalarial activity against the P. Falciparum.

KEYWORDS: Estrone, 4'-bromomethyl-2-cyanobiphenyl, acid chloride, steroidal carbohydrazide, P. Falciparum, antimalarial activity.

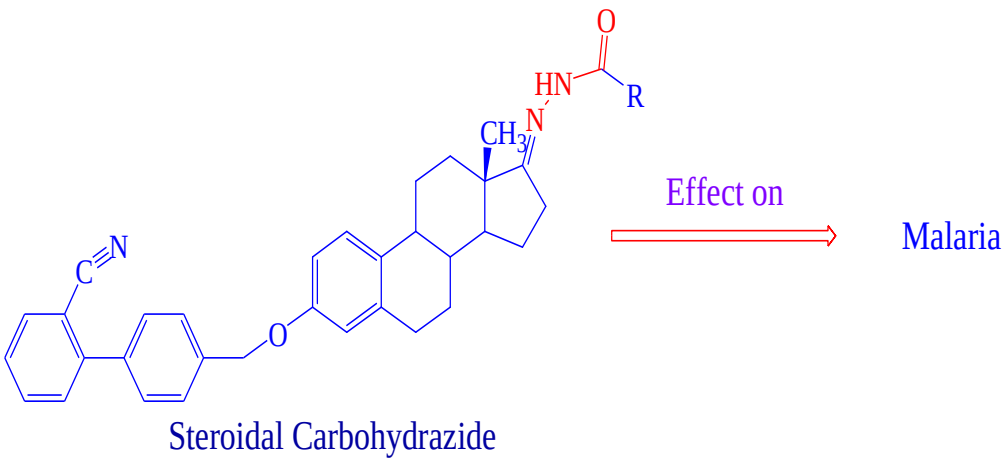


Fig. 1: Steroidal carbohydrazide tested for antimalarial activity.

INTRODUCTION

Parasite causes malaria and is one of the major health diseases in people. Parasites are transmitted to human by infected female mosquitoes. Fever, headache and chills are the symptoms of malaria. Worldwide 229 million cases were estimated in 2019. If patient will not be treated within 24 hours then malaria can progress to severe fever and to death. . Children and young people are mostly affected by malaria in Africa. Children under the age of 5 years and pregnant women are severely affected[1,2] so they are on the high risk. In 2016, it was estimated that there were 216 million cases of malaria globally and 445,000 deaths due to malaria[3] Moreover, exterior Africa, it is the profusely populated Southeast Asia where 30% of the total population is approximated to be at risk of malaria, of which India contributes (80%) most of the cases.[4] People are killing the mosquito larve by spraying the bio-degradable control substance at mosquito breeding sites. This is the time to rethink and eliminate the malaria by discover the new antimalarial agents.

Steroids play a very important role in human. Steroids, an important family of polycyclic molecules with various structure, have drawn extensive attention due to their diverse bio-activities and highly bioavailable.[5] Steroids are naturally occurring compounds with broad spectrum of biological activities such as anti-microbial,[6] anti-inflammatory,[7] anti-cancer,[8,9,10] Anti-inflammatory,[11] Anti tuberculosis[12] and antimalarial.[13,14,15] Steroidal ring modification and incorporation of heteroatom, heterocycle, amides or replacing one or more carbon atoms in steroidal molecule may improves its biological activities have been researched and reported.[16,17,18,19] Many steroid-based drugs have been applied in clinical treatments and become one of the highest marketed classes of pharmaceuticals.[20] Estrone based steroidal drugs are widely used in the treatment of breast cancer (Fulvestrant) and in birth control (Ethinyl estradiol). So the modification of estrone is important for developing a new therapeutic agent. Rational modification of steroid molecule with improved biological activities have been reported. Steroidal nucleus having alkaloids possess antimalarial activity could be found in literature. Dua et al. investigated the *in vitro* antimalarial and cytotoxic effects of the known compound, conessine.[21]

Carbohydrazide is considered to be a versatile scaffold for application in coordination chemistry, agrochemicals, pharmacology and asymmetric catalysis.[22,23,24] The Carbohydrazide derivative have been reported such as antifungal, anti-inflammatory, antiplatelets, antimalarial and anticancer activities.[25,26,27,28]

Asurvey of the literature revealsthat many researchers have synthesized carbohydrazide by condensation ofaldehyde and hydrazide. The synthesis of carbohydrazide based on estrone isexpected to yield new antimalarial substances. Carbohydrazide derivative of3-O-ether-estrone-17-hydrazone with acid chloride is still not reported. Thisencourages to synthesize steroidal carbohydrazide by the condensation of acidchloride with hydrazide and evaluated their antimalarial activity against P.falciparum strain. In the search of new antimalarial agents, a series ofsteroidal carbohydrazide containing -NHCO- group attached to steroidal17-hydrazone were synthesized from estrone as starting material. The structuralmodification of estrone building block is carried out on C-3 and C-17 in ring-Aand D respectively. As per our knowledge, Estrone based carbohydrazide withantimalarial activity have not been reported.

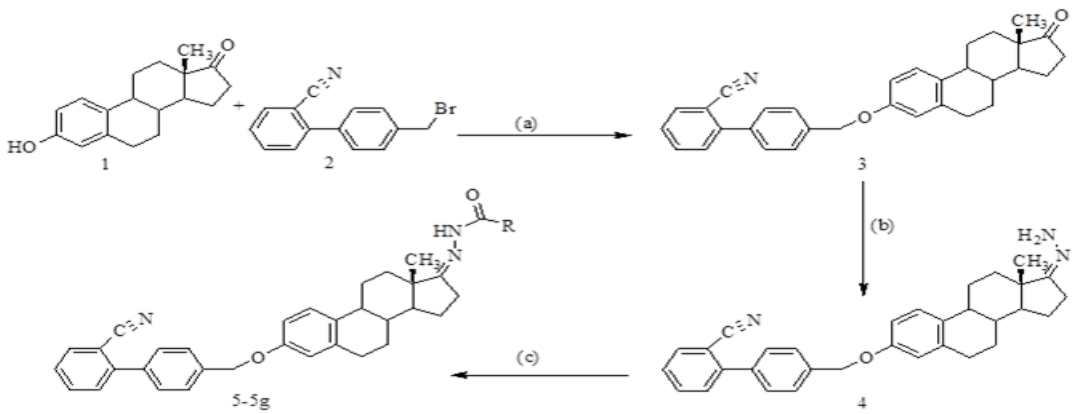
MATERIALS AND METHODS

All the chemicals were used as received from commercial sources. All reaction progress were monitored by thin-layer chromatography (TLC) analysis using silica gel 60 F_{254} TLC plates.

IR spectra were recorded using potassium bromide discs on a Bruker Optics with software Opus (4.2). ^{1}H and ^{13}C NMR spectra were recorded on Varian spectrometer 200 MHz and 50 MHz respectively; the chemical shifts δ were measured in ppm with respect to the solvent. High resolution mass spectra were recorded on Waters make Acquity model and UPLC connected with SQ detector (Single Quadra pole) with Software Mass Lynx (401). Measurements were performed in positive (MS+) ion mode.

Synthesis

Our prime focused on the preparation and evaluation of steroidal carbohydrazide, The title compounds (5-5g) were synthesized as depicted in Scheme-1.



Scheme 1: Synthesis Carbohydrazide (a) K_2CO_3 (previously dry at 105°C), KI, TBAB, Acetone, 50-55°C, 16 h; (b) Hydrazine hydrate (80%), glacial acetic acid, methanol, reflux; (c) Methylene chloride, Triethylamine, Acid chloride.

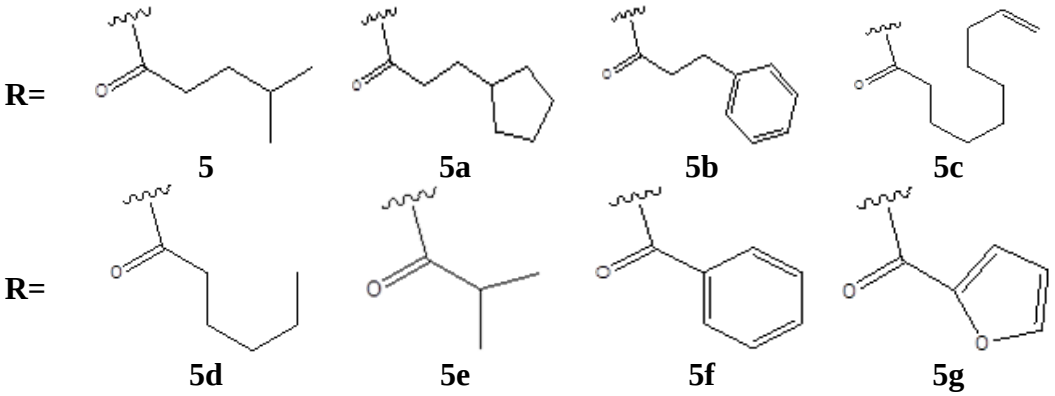


Fig 2: R= Acid chloride.

General procedure for the synthesis 5-5i (Steroidal carbohydrazide).

Synthesis of 4'-(3yloxymethyl)-1,3,5(10)-estratriene-biphenyl-2-carbonitrile 3

A suspension of Estrone 1 (15g, 55 mmol), potassium carbonate (K_2CO_3) (11.4g, 82 mmol) (previously dried at 105°C), potassium iodide (KI) (10% mol), tetra butyl ammonium bromide (TBAB) (10% mol) in a mixture of acetone (150ml) and DMF (Dimethyl formamide) (15ml) was stirred for 10-15 minutes at 25-30°C. Charged 4'-bromomethyl-2-cyanobiphenyl 2 (16.5g, 60 mmol) in to reaction mass at 25-30°C. The reaction went to completion within 16h at 50-55°C. Progress of reaction was monitored by TLC [mobile phase: Chloroform/Acetone (7/3) (v/v)]. Distilled out solvent under vacuum at about 60°C and replaced with water (250ml). The mass was agitated for 30 minutes at 25-30°C. The solid was removed by filtration, washed with water till neutral pH, sequentially washed with chilled (5-10°C) methanol (25ml x 2). Dried under vacuum at 45-50°C resulted in a white solid of **3** (20g, 80%).

Synthesis of 4'-[17-(hydrazone)-3yloxymethyl-1,3,5(10)-estratriene]-biphenyl-2-carbonitrile 4

The intermediate 3 (10g, 21 mmol) was reflux with hydrazine hydrate (80%) (20ml) in methanol (100ml) using 2-3 drops of glacial acetic acid as catalyst. Progress of reaction was monitored by TLC [mobile phase: Chloroform/Acetone (7/3) (v/v)]. TLC indicated that reaction was complete (about 3h). The mixture was cooled to 25-30°C and resulting white solid was filtered and washed with Methanol (20ml). The wet solid dried under vacuum at about 50°C to provide 7.5g (75%) of 4.

Synthesis of carbohydrazide 5-5g

A solution of compound 4 (1.0 mmol), in dry methylene dichloride (25ml) with triethylamine (3ml) was stirred and cooled to 5 to 10°C. Then solution of acid chloride (1.5 mmol) in methylene dichloride (10ml) were added and the reaction mass was warmed to 25-30°C. Progress of reaction was monitored by TLC. [mobile phase: Toluene/Ethyl acetate (8/2) (v/v)]. TLC indicated that reaction was completed (about 30 minutes). The reaction mass was diluted with water (5ml), settled and separated the lower organic layer which was washed with sodium carbonate solution and then wash with dil. HCl solution, finally adjusted neutral pH by water washing. Solid isolated by filtration from methanol. The wet solid dried under vacuum at about 50°C. The yield of desired compound 5-5g is 70±5%.

4'-{17-[-(Isocaproic)-hydrazide]-3-yloxymethyl-1, 3, 5(10)-estratriene}-biphenyl-2-carbonitrile 5

White solid, 0.6g, Yield 66%; m.p. 190-192°C; IR (KBr, ν_{max}, cm^{-1}): 1251, 1496, 1605(C=N), 1662(C=O), 2223(C≡N), 2930, 3060, 3165; ^{1}H NMR (200 MHz $CDCl_3$, δ, ppm) : 0.98(s,3H,-CH_3), 1.1(d,6H J=6.9,-CH_3), 5.18(s,2H,-OCH_2-), 6.44-7.67(m,11H,Ar-H); ^{13}C NMR (50 MHz $CDCl_3$): 16.9(-CH_3), 22.1, 26.4, 28.4, 30.2, 31.5, 33.5, 34.7, 35.4, 36.2, 39.2, 42.4, 44.2, 53.4(-OCH_2), 69.9, 111.5, 112.3, 114.8, 118.7(C≡N), 126.4, 127.6, 127.7, 129.0, 130.0, 132.7, 132.8, 133.8, 137.6, 137.9, 145.1, 157.3(C3), 161.0(C=N), 177.0(C=O); M/S m/z: 574.32$[M+K]^+$; Anal. Calcd. for $C_{39}H_{37}N_3O$.

4'-{17-[-(3-Cyclopentylpropionic)-hydrazide]-3-yloxymethyl-1,3,5(10)-estratriene}-biphenyl-2-carbonitrile (5a).

White solid, 0.61g,Yield 71%; m.p. 170-172°C; IR (KBr, ν_{max}, cm^{-1}): 1253, 1498, 1610(C=N), 1665(C=O), 2224(C≡N), 2934, 3063, 3176; ^{1}H NMR (200 MHz $CDCl_3$, δ, ppm) : 0.94(s,3H,-CH_3), 5.13 (s,2H,-OCH_2-), 6.77-7.91(m,11H,Ar-H); ^{13}C NMR (50 MHz $CDCl_3$): 17.1(-CH_3), 23.2, 25.1, 25.2, 26.2, 27.1, 29.7, 31.1, 31.9, 32.5, 34.1, 38.1, 39.9, 44.1, 44.8, 52.4 (-OCH_2), 69.5, 111.2, 112.3, 114.8, 118.7(C≡N), 126.4, 127.6, 127.7, 129.0, 130.0, 132.7, 132.8, 133.8, 137.6, 137.9, 145.1, 156.7(C3), 165.0(C=N), 176.0(C=O); M/S m/z: 601.31$[M+H]^{+\cdot}$; Anal. Calcd. for $C_{40}H_{90}N_3O_2$.

4'-{17-[-(3-Phenyl propionic)-hydrazide]-3-yloxymethyl-1,3,5(10)-estratriene}-biphenyl-2-carbonitrile (5b).

White solid, 0.64g, Yield 70%**;** m.p. 173-175°C; IR (KBr, ν_{max}, cm^{-1}): 1257, 1496, 1609(C=N), 1667(C=O), 2223(C≡N), 2931, 3065, 3159; ^{1}H NMR (200 MHz $CDCl_3$, δ, ppm): 1.1(s,3H,-CH_3), 2.35(s,3H,-CH_3), 5.14(s,2H,-OCH_2-), 6.44-7.66(m,15H,Ar-H),

8.42(s,1H,-CH=N); ^{13}C NMR (0 M5Hz $CDCl_3$): 16.1, 25.4, 26.2, 27.4, 29.7, 31.0, 33.2, 38.2, 42.6, 45.1, 52.9(-OCH_2), 70.1, 110.9, 112.1, 114.2, 118.6, 125.7, 127.1, 127.3, 127.9, 128.4, 129.1, 130.2, 132.8, 137.6, 141.2, 145.1, 154.5(C3), 160.4(C=N), 175(C=O); M/S m/z : 608.118.6$[M+H]^+$; Anal. Calcd. for $C_{40}H_{39}N_3O$.

4'-{17-[-(10-Undecanoic)-hydrazide]-3-yloxymethyl-1,3,5(10)-estratriene}-biphenyl-2-carbonitrile (5c).

White solid, 0.66g, Yield 70%; m.p. 148-150°C; IR (KBr, ν_{max}, cm^{-1}): 1253, 1495, 1608(C=N), 1660 (C=O), 2222(C≡N), 2925, 3060, 3164; ^{1}H NMR (200 MHz $CDCl_3$, δ, ppm): 1.04(s,3H,-CH_3), 4.80(dd, J=17, 10, 2H,=CH_2), 5.16(s,2H,-OCH_2-), 5.61(m,1H,-CH), 6.64-7.75(m,11H,Ar-H); ^{13}C NMR (50 MHz $CDCl_3$): 24.2(-$\underline{C}H_3$), 23.2, 25.1, 25.2, 26.2, 27.1, 29.7, 31.1, 31.9, 32.5, 34.1, 38.1, 39.9, 44.1, 44.8, 52.4(-OCH_2), 69.8, 111.2, 112.3, 114.8, 115.4(=CH_2), 118.6(C≡N), 126.4, 127.6, 127.7, 129.0, 130.0, 132.7, 132.8, 133.8, 137.6, 137.9, 140.1, 145.1, 154.7(C3), 157.2(C=N), 168.1(C=O); M/S m/z: 628.21$[M+H]^+$; Anal. Calcd. For $C_{42}H_{49}N_3O_2$.

4'-{17-[-(Hexanoic)-hydrazide]-3-yloxymethyl-1,3,5(10)-estratriene}-biphenyl-2-carbonitrile (5d).

White solid, 0.8g, Yield 66.6%; m.p. 178-180°C; IR (KBr, ν_{max}, cm^{-1}): 1252, 1497, 1606(C=N), 1666(C=O), 2222(C≡N), 2928, 3061, 3168; ^{1}H NMR (200 MHz $CDCl_3$, δ, ppm): 0.98(s,6H,-CH_3(estrone) & -CH_3 (Hexanoyl chloride), 5.13(s,2H,-OCH_2-), 6.77-7.92(m,11H,Ar-H); ^{13}C NMR (50 MHz $CDCl_3$): 14.01[-$\underline{C}H_3$(terminal methyl group of Hexanoyl chloride)], 17.1(-CH_3), 22.4, 23.2, 24.4, 25.2, 26.1, 27.1, 29.7, 31.6, 32.5, 34.1, 38.1, 44.1, 44.7, 52.4, 69.5(-OCH_2), 111.2, 112.3, 114.8, 118.7(C≡N), 126.4, 127.6, 127.7, 129.9, 130.0, 132.7, 132.8, 133.8, 137.6, 137.9, 145.1, 156.7(C3), 165.0(C=N), 175.8(C=O); M/S m/z: 574.3$[M+H]^+$; Anal. Calcd. For $C_{40}H_{39}N_3O_2$.

4'-{17-[-(Isobutyric)-hydrazide]-3-yloxymethyl-1,3,5(10)-estratriene}-biphenyl-2-carbonitrile (5e).

Off white solid, 0.67g, Yield 75%; m.p. >200°C; IR (KBr, ν_{max}, cm^{-1}): 1244, 1491,1609(C=N), 1664(C=O), 2222(C≡N), 2939, 3061, 3170; ^{1}H NMR (200 MHz $CDCl_3$, δ, ppm): 0.99(s,3H,-CH_3), 1.19(d,6H J=6.9,-CH_3), 5.13(s,2H,-OCH_2-), 6.55-7.95(m,11H,Ar-H); ^{13}C NMR (50 MHz $CDCl_3$): 18.9, 25.4, 26.4, 27.4, 28.3, 29.5, 33.6, 38.7, 42.9, 43.6, 45.1, 52.4(-OCH_2), 69.7, 111.2, 112.3, 114.8, 118.6(C≡N), 126.6, 127.3, 127.5, 129.5, 130.1,

132.7, 132.8, 133.8, 135.6, 137.6, 138.1, 145.1, 157.7(C3), 163.0(C=N), 170.0(C=O); M/S m/z : 546.34$[M+H]^+$; Anal. Calcd. for $C_{36}H_{39}N_3O_2$.

4'-{17-[-(Benzoic)-hydrazide]-3-yloxymethyl-1,3,5(10)-estratriene}-biphenyl-2-carbonitrile (5f).

Off white solid, 0.67g, Yield 75%; m.p. 227-229°C; IR (KBr, ν_{max}, cm^{-1}): 1249, 1498, 1607(C=N), 1663(C=O), 2224(C≡N), 2933, 3064, 3173; ^{1}H NMR (200 MHz $CDCl_3$, δ, ppm): 1.04(s,3H,-CH_3), 5.14(s,2H,-OCH_2-), 6.55-7.95(m,16H,Ar-H); ^{13}C NMR (50 MHz $CDCl_3$): 23.1(-CH_3), 25.5, 26.4, 27.2, 28.3, 29.4, 33.6, 38.7, 42.4, 44.3, 53.3(-OCH_2), 69.6, 111.2, 112.3, 114.8, 118.6(C≡N), 126.6, 127.4, 127.6, 128.3, 128.6, 132.5, 133.3, 135.6, 138.6, 145.1, 155.7(C3), 159.9(C=N), 169.0(C=O) M/S m/z : 580.51$[M+H]^+$; Anal. Calcd. for $C_{39}H_{37}N_3O_2$.

4'-{17-[-(Furan-2-carboxylic)-hydrazide]-3-yloxymethyl-1,3,5(10)-estratriene}-biphenyl-2-carbonitrile (5g).

Off white solid, 0.55g, Yield 65%; m.p. 178-180°C; IR (KBr, ν_{max}, cm^{-1}):1246, 1496, 1608(C=N), 1666 (C=O), 2222(C≡N) , 2931, 3066, 3174; ^{1}H NMR (200 MHz $CDCl_3$, δ, ppm): 1.0(s, 3H, -CH_3), 5.12(s,2H,-OCH_2-), 6.55-7.80(m,14H,Ar-H & Furan-H); ^{13}C NMR (50 MHz $CDCl_3$): 23.2(-$\underline{C}H_3$), 25.7, 25.9, 26.0, 26.5, 27.0, 27.3, 29.2, 29.6, 29.8, 30.2, 31.5, 35.9, 38.3, 38.6, 44.0, 48.0, 50.4, 51.3(-OCH_2), 69.4, 111.2, 111.6, 111.7, 111.9, 112.2, 112.3, 112.4, 113.0, 113.8, 114.4, 114.6, 114.7, 114.8, 116.4, 118.7(C≡N), 119.4, 119.5, 126.3, 126.4, 127.6, 127.7, 128.9, 130.0, 132.8, 133.8, 137.6, 137.9, 138.0, 144.9, 145.1, 145.3, 145.4, 145.5, 145.7, 146.3(C3), 156.6(C=N), 156.7(C=O); M/S m/z: 571.22$[M+H]^+$; Anal. Calcd. for $C_{37}H_{35}N_3O_3$.

In vitro antimalarial screening

The in vitro antimalarial assay was carried out in 96 well microtitre plates according to the micro assay protocol of Rieckmann and co-workers with minor modifications. The cultures of P. falciparum strain were maintained in medium RPMI 1640 supplemented with 25mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of P. falciparum were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µ l of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitemia (rings) and uniformly maintained with 50% RBCs (O+).

A stock solution of 5mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20 µl volume were added to the test wells so as to obtain final concentrations (at fivefold dilutions) ranging between 0.4 µg/ml to 100 µg/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37oC in a candle jar. After 36 to 40 hours incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC).

Observation of the in vitro Anti-malarial Screening

The mean number of rings, trophozoites and schizonts recorded per 100 parasites from duplicate wells after incubation for 38 hours, and percent maturation inhibition with respect to control group.

Table 1: Anti-malarial activity.

MIC (Minimal Inhibition Concentration)		
Sr. no.	**Compound ID**	**Mean IC 50 Values a**
1	5	0.55 µg/ml
2	5a	1.12 µg/ml
3	5b	0.36 µg/ml
4	5d	0.48 µg/ml
5	5f	0.72 µg/ml
6	5g	0.63 µg/ml
STD	Chloroquine	0.020 µg/ml
	Quinine	0.268 µg/ml

a: mean values in representative assay. All experiments were performed in duplicate.

RESULT AND DISCUSSION

The hydrazide group present in the compound 4 is capable of carbohydrazide formation. Compound 4 is reacted with different acid chloride in presence of base such as triethyl amine in dichloromethane as solvent to give carbohydrazide derivatives (scheme-1). Eight compounds were successfully synthesized as per general procedure. The structures of the carbohydrazide were assigned on the basis of spectral data. The compound 5a was isolated as white solid, showed a $[M+H]^+$ ion peak at m/z 601.31 ESI-MS match with the molecular formula $C_{40}H_{45}N_3O_2$. On other hand compound 5g, obtained as white solid, showed a $[M+H]^+$ion peak at m/z 571.22 ESI-MS match with the molecular formula $C_{37}H_{35}N_3O_3$. The

IR spectrum of steroidal carbohydrazide recorded using KBr pellet, showed strong band at around 1666 cm^{-1} and 3470-3380 cm^{-1} region are due to carbonyl and NH of CONH group respectively. ^{13}C NMR spectra of compound 5a and 5d revealed the presence of $–OCH_2$-(estrone-3-O-ether) at around 52, and two signals at 175-177 and 161-165 corresponding to CO and C=N groups.

The Steroidal carbohydrazide displayed activities in the range of 0.36-1.12µg/mL. Chloroquine and Quinine were used as the reference drug with as much as 0.002 and 0.268 µg/mL IC_{50} (Table-1). The carbohydrazide with alkyl chain 5, 5b and 5d, having Isocaproic, Phenyl Propionic and Hexanoic group, exhibited better anti-malarial activities compared to the ones with no alkyl chain i.e. 5f (Benzoic), 5g (Furoic); exception being 5a which exhibited lowest anti-malarial activity among the series due to inclusion of cyclopentyl ring with alkyl chain.

CONCLUSION

The anti-malarial activities of the steroidal carbohydrazide on the P. falciparum indicated good activity. Best of our knowledge Estronic carbohydrazide with biphenyl moiety has not yet been synthesized and its antimalarial, cytotoxic, and antitumor activities not studied. This result provides a new way for modification of Estronic steroidal derivatives for the development of new antimalarial agents.

ACKNOWLEDGEMENT

We wish to thanks Dr. Mahesh Davadra for providing laboratory set up and chemicals.

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