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Design and Synthesis of 6-oxo-1, 6 dihydropyrimidine derivatives as inhibitors of E. histolytica

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1. Introduction

Entamoeba histolytica is prevalent throughout the world and causes amoebic diarrhea, colitis, amoebic liver abscess mostly in developing countries [1- 4] where there is insufficiency of clean water. About 90% of infections include asymptomatic colonization of the intestinal tract. Symptomatic disease symptom comprises dysentery, toxic mega colon, peritonitis and extra intestinal amoebiasis [2, 5]. E. histolytica is substantially found in the colon as inactive, but after certain period it becomes calamitous to human beings by causing rigorous intricacies like Vermiform appendix, [6-12] obstruction of intestine, [13, 14] chronic cholecystisis, [15] ulcerative lesions in perianal mucosa [16,17, 18], entero-enteric fistula [16, 19, 20] are some of the physical symptoms often encountered. The intensely investigated 5- nitroimidazoles like metronidazole, tinidazole, secnidazole are the most effective antiamoebic drugs against invasive intestinal and extra intestinal amoebiasis The toxicology and metabolism of nitroimidazoles, particularly metronidazole, is well defined but the survival of this aggressive invader despite adequate treatment and reappearance of amoebic liver abscess jeopardize its clinical utility, also this drug have several pernicious effects such as genotoxicity, gastric mucus irritation, spermatozoid damage and neurologic toxicity. However, the extended continuances of tinidazole and ornidazole permit for a single dosage with better tolerance, and shorter treatment time than metronidazole which is an optimal pharmaceutical [21, 22]. Thus, it is mandatory to acquaint with this consistently progressing disease to avert more intense outcomes. This scenario has craved the synthesis and functional characterization of new therapeutic drugs enriched with finer activity and low toxicity to enhance global antiamoebic regimens.

Dihydropyrimidines or DHPMs are widely acknowledged as Biginelli's compounds and can be synthesised through condensation reaction of urea/thiourea, β-ketoester, and aryl aldehyde [23]. They display varied biological activities [24] like antitumor [25, 26], antibacterial [27-29], antifungal [30], antioxidant [31], anticancer [32], antimalarial [33], analgesic and anti-inflammatory [34], antiplatelet aggregation [35], and antihypertensive [36]. Biologically active 1, 6-dihydropyrimidine derivatives are the most significant organic compounds exhibiting diverse pharmacological efficacy [37]. The association of two or more

small-molecular-weight fragments in a single procedure helps in constructing variegated heterocyclic scaffolds with structural modifications for biological evaluation [38-43].

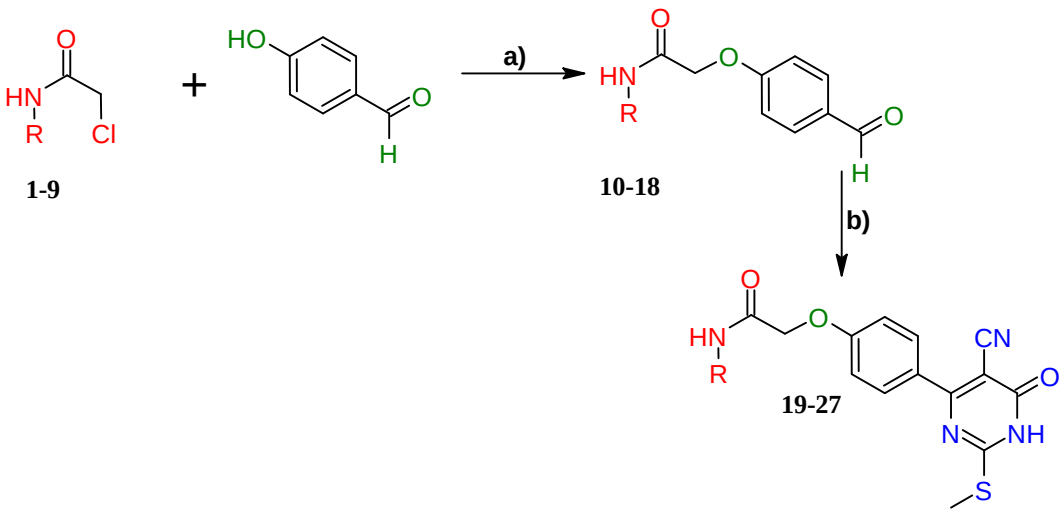
Thus the present work is focused on the synthesis of some novel 6-oxo-1,6 dihydropyrimidine derivatives and their screening against *E. histolytica*.

2. Result and discussion

2.1.Chemistry

The synthetic pathway leading to the title compounds is given in **Scheme 1** Different chloroacetamides (**1–9**) were synthesized by substitution of the amine with chloro acetyl chloride [44]. The aldehyde intermediates (**10-18**) were synthesized from aromatic chloroacetamides and 4-hydroxybenzaldehyde using K_2CO_3 in acetone by a reported method [45]. Heating a mixture of ethyl cyanoacetate with intermediate aldehydes and S-methylisothiourea hemisulfate gave the corresponding 4-aryl-5-cyano-2-methylthio-6-oxo-1, 6-dihydropyrimidine derivatives (**19-27**).

Scheme 1. Synthesis of 6-oxo-1, 6 dihydropyrimidine derivatives



Reagents and condition: (a)KI, K_2CO_3, 4-hydroxybenzaldehyde, ethanol, reflux, 24 h; (b) $CNCH_2COOC_2H_5$, S-methylisothiourea hemisulfate, ethanol, reflux.

The compounds are stable in solid states at room temperature. All the synthesized compounds gave satisfactory spectral data for the proposed structures. The ^{1}H NMR of the aromatic chloroacetamides (**1–9**) showed a broad singlet for –NH in the range δ 7.87–9.27 ppm. The intermediate aldehyde proton showed a singlet in the range δ 9.94-9.96 ppm. The disappearance of the peak of aldehyde proton and the appearance of the peak of NH proton of pyrimidine ring as a broad singlet in the range δ 6.53-6.97 ppm along with the three protons of S-CH_3 shows a sharp singlet in range δ 2.34-2.76 ppm confirms the formation of dihydropyrimidine. The signals due to the aromatic protons appeared

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in the expected region. Characteristic IR spectra showed CN absorption band around 2212 -2218 cm^{-1} in dihydropyrimidine derivatives (**19-27**). The CN carbon showed peak around δ 115 ppm and S-CH_3 around 13.7 ppm in the ^{13}C NMR of compounds (**19-27**). The structure of the synthesised compounds was further elucidated by mass spectral studies. The purity of the compounds was confirmed by the elemental analysis.

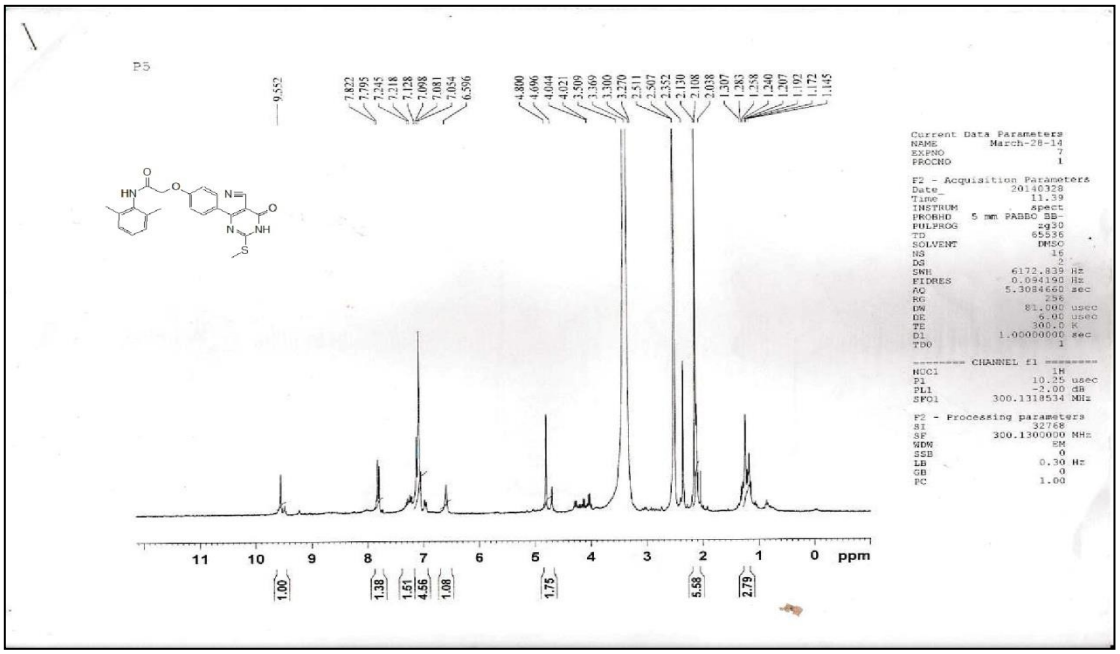

Fig. 1. ^{1}H NMR spectrum of compound 22

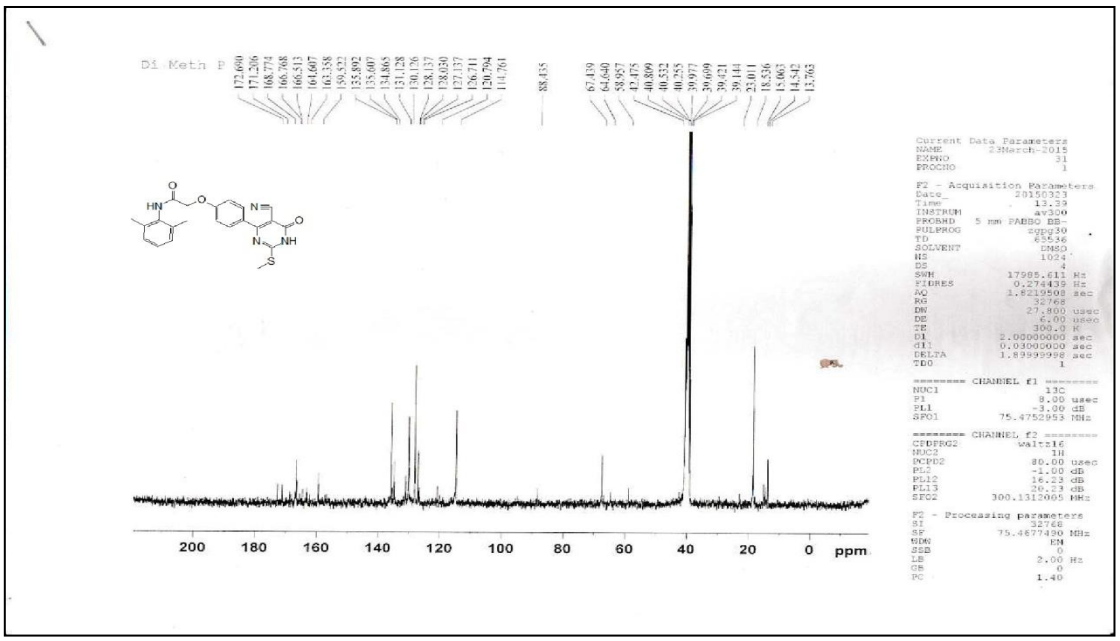

Fig.2. ^{13}C NMR spectrum of compound 22

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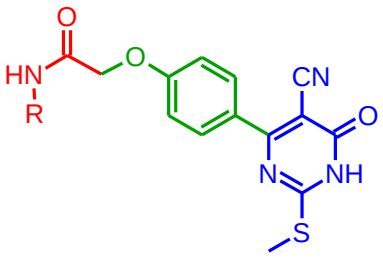
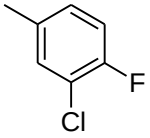
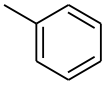
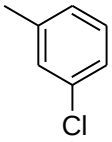
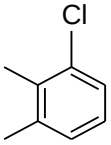
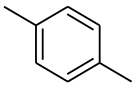
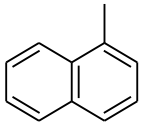
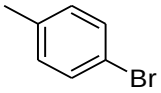
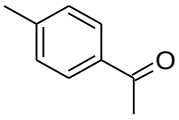
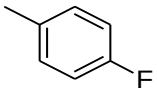
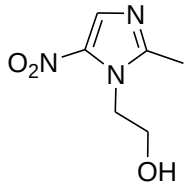
3. Biological activities

3.1. Antiamoebic activity

To scrutinize the antiamoebic potential of the synthesised compounds **(19-27),** they were screened against HM1: IMSS strain of E. histolytica using metronidazole as reference amoebicidal drug which had a 50% inhibitory concentration (IC_{50}) 1.80 μM in our experiments. All the experiments were carried out in triplicate at each concentration level and repeated thrice. The results of antiamoebic activity are summarized in **Table 1**. All the final compounds have IC_{50} values in the range 0.12-7.16μM. Out of the nine synthesised compounds, five compounds: **19** (IC_{50} = 0.12 μM), **21** (IC_{50} = 0.82 μM), **25** (IC_{50} = 1.66 μM), **26** (IC_{50} = 0.51 μM) and **27** (IC_{50} = 0.34 μM) were found to exhibit better antiamoebic activity than the standard drug **MNZ** (IC_{50} =1.80 μM). Compound **19** having weak deactivating chloro group at meta position and fluoro group at para position of phenyl ring, exhibited most promising activity (IC_{50} = 0.12 μM) in the series. Compound **27** (IC_{50} = 0.34 μM) having strong electronegative fluoro group at para position exhibited better activity than compound **21** (IC_{50} = 0.82 μM) having chloro group at meta position and compound **25** (IC_{50} = 1.66 μM) having weak deactivating bromo group at para position. Introduction of strong electron withdrawing acetyl group in compound **26** (IC_{50} = 0.51 μM), at para position of phenyl ring exerts significant inhibitory activity, whereas the presence of weak activating methyl group which increases the electron density through hyperconjugation to the aromatic ring, at ortho positions, compound **22** (IC_{50}= 5.91 μM) and at para position in compound **23** (IC_{50} = 3.25 μM) of pheny ring exhibited less activity. The study of structure activity relationships of the synthesised compounds indicate that the substitution of the phenyl ring of 4-aryl-5-cyano-2-methylthio-1,6-oxopyrimidine derivatives with bulky naphthyl group in compound **24** (IC_{50} = 7.16 μM) resulted in the dramatic decrease in the activity due to the reduced aromaticity or the bulky nature of the group which might be imposing steric hindrance. Thus from the above discussion it can be concluded that compound **19** seems to be the most potent of all the compounds screened.

Table 1. In vitro antiamoebic activity of 4-aryl-5-cyano-2-methylthio-1,6-oxopyrimidine derivatives (**19-27**) and metronidazole against HM1: IMSS strain of E. histolytica



Compound Code	R	Antiamoebic Activity (IC_{50}±S.D.)
19		0.12 ± 0.01
20		3.25 ± 0.01
21		0.82 ± 0.03
22		5.91 ± 0.03
23		4.16 ± 0.01
24		7.16 ± 0.08
25		1.66 ± 0.05
26		0.51±0.01
27		0.34 ± 0.03
MNZ		1.80 ± 0.01

4. Experimental Protocols

4.1. Materials and measurements

All the required chemicals were purchased from Merck and Aldrich Chemical Company (USA). Precoated aluminium sheets (Silica gel 60 F254, Merck Germany) were used for thin-layer chromatography (TLC) and spots were visualized under UV light. The melting points were recorded on Veego instrument with model specifications REC-22038 A2 and are uncorrected. Elemental

analyses were performed on Elementar Vario analyzer and the results are within ±0.4% of the theoretical values. IR spectra (KBr) were acquired at Bruker FT-IR spectrophotometer. ^{1}H and ^{13}C NMR were recorded on a Bruker Spectrospin DPX 400 MHz and Bruker Spectrospin DPX 75 MHz spectrometer respectively, using DMSO-d_6 as a solvent and trimethylsilane (TMS) as the internal standard. Splitting patterns are designated as follows; s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shift values are given in ppm. Mass spectra were recorded by ESI-MS (AB-Sciex 2000, Applied Biosystem).

4.2. General procedure for the synthesis of chloroacetamide derivatives (1-9)

Chloroacetamide derivatives (1-9) were synthesised by a method reported in the literature [44].

4.3.Synthesis of aldehyde intermediates (10-18)

The aldehyde intermediates which contained 2-(4-formyl-phenoxy)-N-substituted-Phenylacetamides were synthesised by a reported method [45].

4.4. Synthesis of 4-aryl-5-cyano-2-methylthio-6-oxo-1, 6-dihydropyrimidine derivatives (19-27)

4-aryl-5-cyano-2-methylthio-6-oxo-1, 6-dihydropyrimidine derivatives were synthesised by a reported method [46].

4.4.1. N-(3-chloro-4-fluorophenyl)-2-(4-(5-cyano-2-(methylthio)-6-oxo-1, 6-dihydropyrimidin-4-yl)phenoxy)acetamide (19)

Yield 82.5 %; mp: 287°C; Anal. Calc. (%) for $C_{20}H_{14}ClFN_4O_3S$: C, 54.00; H, 3.17; Cl, 7.97; F, 4.27; N, 12.59; O, 10.79; S, 7.21; found: C, 53.91; H, 3.16; Cl, 7.98; F, 4.25; N, 12.58; O, 10.77; S, 7.22; FT-IR ν_{max} (cm^{-1}): 3310 (NH), 2215(C=N), 1655(C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 10.37(s, 1H, NH), 7.77 (d, 1H, J=8.7 Hz, Ar-H), 7.47 (d, 1H, J=8.1 Hz, Ar-H), 7.35(t 1H, J=7.8 Hz, Ar-H), 7.30-7.22(m, 2H, Ar-H), 7.07(d, 2H, J=7.8 Hz, Ar-H), 6.97(s, 1H, NH), 4.81 (s,2H,CH_2), 2.34(s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 172.61, 169.05, 167.18, 161.59, 159.40, 136.08, 132.28, 130.17, 127.72, 122.72, 121.76, 120.80, 117.18, 115.61, 114.67, 88.60, 67.54, 13.74 ; ESI-MS: m/z = 446 (M^{+}+1).

4.4.2. 2-(4-(5-cyano-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-4-yl)phenoxy)-N-phenylacetamide (20)

Yield 81.3 %; mp: 290°C; Anal. Calc. (%) for $C_{20}H_{16}N_4O_3S$: C, 61.21; H, 4.11; N, 14.28; O, 12.23; S, 8.17; found: C, 61.20; H, 4.10; N, 14.29; O, 12.24; S, 8.16; FT-IR ν_{max} (cm^{-1}): 3330 (NH), 2212 (C=N), 1666(C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 10.30(s, 1H, NH), 7.84 (d, 1H, J=7.8 Hz, Ar-H), 7.80(d,2H, J=8.4 Hz, Ar-H), 7.65(d, 2H, J=7.8 Hz, Ar-H), 7.39-7.23(m, 2H, Ar-H), 7.08(d, 2H, J=7.5 Hz, Ar-H), 6.97(s, 1H, NH), 4.86 (s,2H,CH_2), 2.34(s,3H,CH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 172.65, 170.43, 168.25, 167.51, 159.83, 138.26, 133.69, 129.27, 127.47, 123.23, 121.21, 115.87, 114.32, 88.47, 67.09, 13.21 ; ESI-MS: m/z = 393 (M^{+}+1).

4.4.3.N-(3-chlorophenyl)-2-(4-(5-cyano-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-4-yl)phenoxy)acetamide (21)

Yield 79.3 %; mp: 275°C; Anal. Calc. (%) for $C_{20}H_{15}ClN_4O_3S$: C, 56.27; H, 3.54; Cl, 8.31; N, 13.12; O, 11.24; S, 7.51; found: C, 56.26; H, 3.53; Cl, 8.32; N, 13.11; O, 11.25; S, 7.52; FT-IR ν_{max} (cm^{-1}): 3335 (NH), 2214 (C=N), 1660(C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 10.33(s, 1H, NH), 7.85 (d, 3H, J=9.0 Hz, Ar-H), 7.58(d,1H, J=7.8 Hz, Ar-H), 7.38-7.31(m, 2H, Ar-H), 7.24(d, 2H, J=8.4 Hz, Ar-H), 6.95(s, 1H, NH), 4.78 (s,2H,CH_2), 2.35(s,3H,CH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 172.45, 170.77, 167.25, 166.53, 159.55, 140.32, 133.50, 130.9, 130.24, 123.88, 120.65, 119.63, 118.56, 114.92, 114.66, 88.56, 67.47, 13.77; ESI-MS: m/z = 428 (M^++1).

4.4.4.2-(4-(5-cyano-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-4-yl)phenoxy)-N-(2,6-dimethylphenyl)acetamide (22)

Yield 79%; mp: 278°C; Anal. Calc. (%) for $C_{22}H_{20}N_4O_3S$: C, 62.84; H, 4.79; N, 13.32; O, 11.41; S, 7.63; found: C, 62.85; H, 4.78; N, 13.31; O, 11.42; S, 7.64; FT-IR ν_{max} (cm^{-1}): 3330 (NH), 2212 (C=N), 1668(C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 10.14(s, 1H, NH), 7.84 (d, 1H, J=9.0 Hz, Ar-H), 7.64(d, 1H, J=7.8 Hz, Ar-H), 7.41-7.28(m, 1H, Ar-H), 7.20(d, 2H, J=7.8 Hz, Ar-H), 7.08(d,2H, J=8.1 Hz, Ar-H), 6.95(s, 1H, NH), 4.80(s,2H,CH_2), 2.35(s,3H,CH_3), 2.06 (s,6H,CH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 172.69, 171.20, 168.77, 166.51, 163.35, 159.52, 135.89, 134.86, 131.12, 130.12, 128.13, 128.03, 127.13, 126.71, 120.79, 114.76, 88.43, 67.43, 14.54 ,13.76; ESI-MS: m/z = 421 (M^++1).

4.4.5.2-(4-(5-cyano-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-4-yl)phenoxy)-N-p-tolylacetamide (23)

Yield 88.1 %; mp: 292°C; Anal. Calc. (%) for $C_{21}H_{18}N_4O_3S$: C, 62.05; H, 4.46; N, 13.78; O, 11.81; S, 7.89 ; found: C, 62.04; H, 4.45; N, 13.79; O, 11.82; S, 7.88; FT-IR ν_{max} (cm^{-1}): 3350 (NH), 2218 (C=N), 1655(C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 10.05(s, 1H, NH), 7.80(d, 2H, J=8.4 Hz, Ar-H), 7.48-7.41(m,2H,Ar-H), 7.20(d, 2H, J=7.8 Hz, Ar-H), 7.08(d, 2H, J=8.7 Hz, Ar-H), 6.96(s, 1H, NH),4.74(s, 2H, CH_2) , 2.76 (s,3H,CH_3), 2.28(s,3H,CH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 171.35, 167.73, 167.04, 166.71, 159.56, 138.40, 131.10, 129.01, 127.78, 121.40, 120.74, 114.89, 114.63, 88.36, 67.57, 27.19, 13.76 ; ESI-MS: m/z = 407 (M^++1).

4.4.6.2-(4-(5-cyano-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-4-yl)phenoxy)-N-(naphthalen-1-yl)acetamide (24)

Yield 86.3 %; mp: 295°C; Anal. Calc. (%) for $C_{24}H_{18}N_4O_3S$: C, 65.14; H, 4.10; N, 12.66; O, 10.85; S, 7.25; found: C, 65.15; H, 4.11; N, 12.65; O, 10.84; S, 7.26; FT-IR ν_{max} (cm^{-1}): 3320 (NH), 2212 (C=N), 1668(C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 10.19(s, 1H, NH), 7.94 (d, 2H, J=7.8 Hz, Ar-H), 7.48 (t, 2H, J=8.1 Hz, Ar-H), 7.74(d, 2H, J=7.2 Hz, Ar-H), 7.64 (d, 2H, J=7.2 Hz, Ar-H), 7.54-7.49 (m,1H,Ar-H), 7.49(d, 2H, J=8.4 Hz, Ar-H), 6.53(s, 1H, NH),4.91(s, 2H, CH_2) , 2.50 (s,3H,CH_3); ^{13}C

NMR (DMSO-d_6) δ (ppm): 170.80, 167.72, 166.59, 159.74, 134.16, 133.32, 131.44, 130.74, 129.13, 128.70, 127.18, 126.56, 126.35, 123.29, 122.94, 120.59, 115.03, 114.78, 88.65, 67.63, 14.31 ; ESI-MS: m/z = 443 (M^++1)

4.4.7.N-(4-bromophenyl)-2-(4-(5-cyano-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-4-yl)phenoxy)acetamide (25)

Yield 82.6 %; mp: 274°C; Anal. Calc. (%) for $C_{20}H_{15}BrN_4O_3S$: C, 50.97; H, 3.21; Br, 16.95; N, 11.89; O, 10.18; S, 6.80 ; found: C, 50.98; H, 3.20; Br, 16.96; N, 11.88; O, 10.17; S, 6.81; FT-IR ν_{max} (cm^{-1}): 3310 (NH), 2212 (C=N), 1658(C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 10.40(s, 1H, NH), 7.79(d, 2H, J=8.7 Hz, Ar-H), 7.65(d, 2H, J=8.7 Hz, Ar-H), 7.52(d, 2H, J=9.0 Hz, Ar-H), 7.07(d, 2H, J=8.7 Hz, Ar-H), 6.88(s, 1H, NH),4.77(s, 2H, CH_2) , 2.50 (s,3H,CH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 170.65, 169.30, 167.01, 166.50, 159.45, 138.24, 132.02, 130.16, 127.64, 126.27, 121.04, 115.81, 114.63, 88.40, 67.70, 13.75 ; ESI-MS: m/z = 472 (M^++1).

4.4.8.N-(4-acetylphenyl)-2-(4-(5-cyano-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-4-yl)phenoxy)acetamide (26)

Yield 84.5 %; mp: 298°C; Anal. Calc. (%) for $C_{22}H_{18}N_4O_4S$: C, 60.82; H, 4.18; N, 12.90; O, 14.73; S, 7.38; found: C, 60.81; H, 4.19; N, 12.91; O, 14.72; S, 7.39; FT-IR ν_{max} (cm^{-1}): 3343 (NH), 2212 (C=N), 1660(C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 10.36(s, 1H, NH), 8.28(m, 1H, Ar-H), 7.93-7.83(m, 2H, Ar-H), 7.71(d, 1H, J=6.9 Hz, Ar-H), 7.25(d, 2H, J=7.5 Hz, Ar-H), 7.16-7.09 (m,2H,Ar-H), 6.55(s, 1H, NH),4.80(s, 2H, CH_2) , 2.56(s,3H,CH_3), 2.50 (s,3H,CH_3);^{13}C NMR (DMSO-d_6) δ (ppm): 198.07, 172.78, 170.35, 167.14, 166.53, 159.81, 139.23, 137.80, 130.68, 129.64, 124.70, 120.10, 115.57, 114.76, 88.59, 67.57, 27.19, 13.76 ; ESI-MS: m/z = 435 (M^++1).

4.4.9.2-(4-(5-cyano-2-(methylthio)-6-oxo-1,6-dihydropyrimidin-4-yl)phenoxy)-N-4-fluorophenyl)acetamide (27)

Yield 86.1 %; mp: 265°C; Anal. Calc. (%) for $C_{20}H_{15}FN_4O_3S$: C, 58.53; H, 3.68; F, 4.63; N, 13.65; O, 11.69; S, 7.81; found: C, 58.52; H, 3.69; F, 4.62; N, 13.64; O, 11.68; S, 7.82; FT-IR ν_{max} (cm^{-1}): 3347 (NH), 2216 (C=N), 1664(C=O); ^{1}H NMR (DMSO-d_6) δ (ppm): 10.39(s, 1H, NH), 7.88(d, 2H, J=8.7 Hz, Ar-H), 7.74(d, 2H, J=8.7 Hz, Ar-H), 7.52(d, 2H, J=9.0 Hz, Ar-H), 7.07(d, 2H, J=8.7 Hz, Ar-H), 6.88(s, 1H, NH),4.77(s, 2H, CH_2) , 2.50 (s,3H,CH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 170.93, 166.73, 166.04, 162.92, 159.03, 131.70, 129.37, 127.47, 125.93, 124.79, 120.28, 115.41, 114.67, 88.39, 66.85, 13.27 ; ESI-MS: m/z = 411 (M^++1).

5. Biological assays

5.1.In vitro antiamoebic assay

All the synthesized compounds were screened in vitro for antiamoebic activity against HM1: IMSS strain of E. histolytica by microdilution method. [47]. All the experiments were carried out in

triplicate at each concentration level and repeated thrice. E. histolytica trophozoites were cultured in the TYI-S-33 growth medium in wells of 96-well microtiter plates [48]. DMSO (40 lL) was added to all the samples (1 mg) followed by enough culture medium to obtain concentration of 1 mg/mL. The maximum concentration of DMSO in the test did not exceed 0.1%, and at this level no inhibition of amoebal growth had occurred. Compounds were further diluted with medium to a concentration of 0.1 mg/mL. Two fold serial dilutions were made in the wells of 96-well microtiter plate. Each test included metronidazole (MNZ) as the standard amoebicidal drug, control (culture medium plus parasite) and a blank (culture medium only). The cell suspension was then diluted to 105 organism/mL by adding fresh medium and 170 lL of this suspension was added to the test and control well in the plate. Plate was sealed and gassed for 10 min with nitrogen before incubation at 37ºC for 72 h. After incubation, the growth of amoebae in the plate was checked with a low power microscope and the optical density of the solution in each well was determined at 490 nm with a microplate reader. The % inhibition of amoebal growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the

best-fitted straight line from which the IC_{50} value was found

6. Conclusions

To conclude, this paper describes the synthesis and biological evaluation of 6-oxo-1, 6 dihydropyrimidine derivatives as inhibitors of E. histolytica. The in vitro study suggested that some derivatives exhibited potent amoebicidal activity than the standard drug. Out of the nine synthesised compounds, five compounds: **19** (IC_{50} = 0.12 μM), **21** (IC_{50} = 0.82 μM), **25** (IC_{50} = 1.66 μM), **26** (IC_{50} = 0.51 μM) and **27** (IC_{50} = 0.34 μM) were found to exhibit better antiamoebic activity than the standard drug **MNZ** (IC_{50} =1.80 μM). Compound **19** having weak deactivating chloro group at meta position and fluoro group at para position of phenyl ring, exhibited potential outcome (IC_{50} = 0.12 μM) in the series

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