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Short communication

Synthesis, characterization and antiamoebic activity of chalcones bearing *N*-substituted ethanamine tail

Saadia Leeza Zaidi [a], Sonam Mittal [b], Maitreyi S. Rajala [b], Fernando Avecilla [c], Mohammad Husain [d], Amir Azam [a, *]

[a] Department of Chemistry, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India
[b] School of Biotechnology, Jawaharlal Nehru University, New Delhi 110067, India
[c] Departamento de Química Fundamental, Universidade da Coruña, Campus da Zapateira s/n, 15071 A Coruña, Spain
[d] Department of Biotechnology, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India

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ABSTRACT

A series of chalcones (4–21) possessing N-substituted ethanamine were synthesized by the aldol condensation reaction of 1-(4-(2-substituted ethoxy)phenyl)ethanones with different aldehydes preceded by the reaction of 2-chloro N-substituted ethanamine hydrochloride and 4-hydroxy acetophenone. The structure of all the synthesized compounds was elucidated by various spectral and X-ray diffraction studies. The compounds were screened against HM1: IMSS strain of *Entamoeba histolytica* and cytotoxicity was performed on A549 (non-small cell lung cancer cell line) cells by MTT assay. Out of eighteen compounds twelve showed better activity then the standard drug metronidazole. The compound 9, 14 and 19 showed good cell viability, hence were least toxic.

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1. Introduction

Amoebiasis, the devastating disease of the gastrointestinal tract is caused by a parasitic protozoan *Entamoeba histolytica*. It is responsible for 100,000 fatalities worldwide per annum [1]. The spread of the infection is accelerated by poor sanitation. The treatment is usually done by nitroimidazoles such as metronidazole (MNZ) and tinidazole but they have shown several side effects [2]. MNZ is known to be carcinogenic in rodents and some major side effects such as irritation of the gastric mucus lining, spermatozoid damage, convulsions, central nervous system disorders, blood in urine [3–7]. Recent reports have shown clinical resistance towards the conventional drugs [8].

Chalcones are 1, 3-diaryl-2-propen-1-ones possessing a diverse range of biological activities including anticancer [9,10], antimicrobial [11,12], anti-HIV [13,14], anti-oxidant [15,16], anti-inflammatory [17,18] and anti-protozoal activities [19,20]. Modification in their structure leads to an enhancement in their biological activity and therapeutic efficacy [21]. Thus chalcones are a very promising structural motif to be explored in the quest for newer drugs. Literature review revealed that chalcones have been studied for their activity against various protozoal diseases but their potential against the neglected diseases such as amoebiasis remains to be explored. Previously, we have reported antiprotozoal activity of a series of chloroquinoline based chalcones (Fig. 1) which showed some promising results against *E. histolytica* [22]. Compounds containing morpholine, piperadine (Fig. 2) and N, N-dimethylamino scaffolds have shown promising antiprotozoal and antibacterial activities [23–25].

In our continuous effort towards the development of more potent antiamoebic agents and taking into consideration that aliphatic and cyclic amine bearing heterocycles and chalcones are biologically active. We herein, report the synthesis, characterization, antiamoebic activity and toxicity of a series of hybrid molecules containing chalcone and N-substituted ethanamine tail (Fig. 3).

2. Results and discussion

2.1. Chemistry

A series of chalcones (4–21) containing N-substituted ethanamine were synthesized as outlined in Scheme 1. 4-hydroxy

* Corresponding author.
E-mail address: amir_sumbul@yahoo.co.in (A. Azam).

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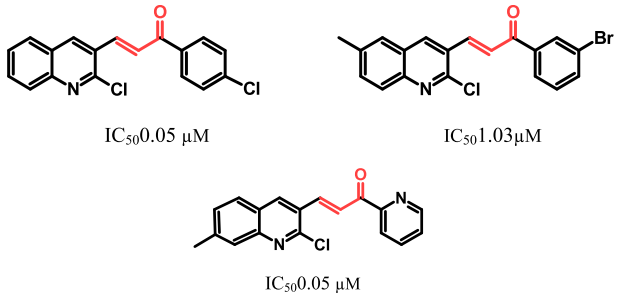


Fig. 1. Structures of antiprotozoal chalcones showing promising antiamoebic activity.

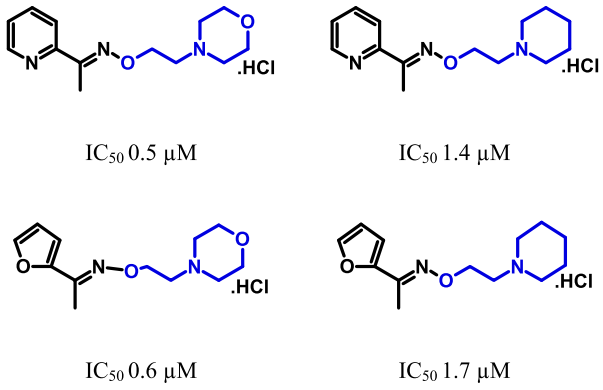


Fig. 2. Structures of compounds containing N-substituted ethanamine side chain showing good antiamoebic activity.

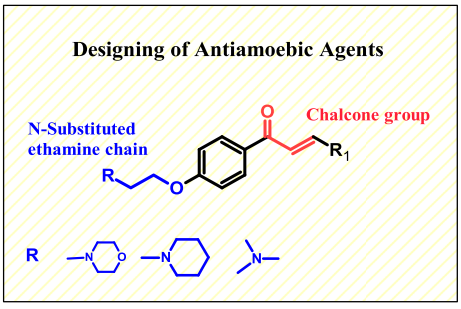
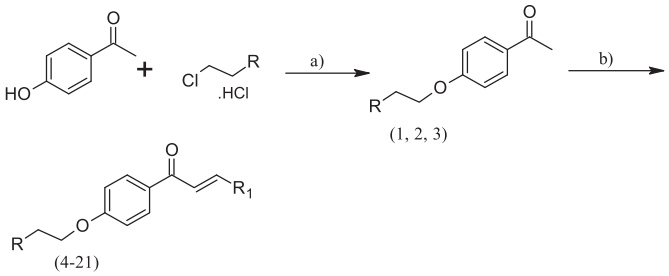


Fig. 3. Designing of hybrid molecule.

acetophenone is converted to 1-[4-(2-dimethylamino/piperidino/morpholinoethoxy)phenyl)ethanone derivative by the reaction with 2-chloro N-substituted ethanamine hydrochloride salts under basic conditions using potassium tert-butoxide (*t*-BuOK) as a base. The aldol condensation reaction of 1-(4-(2-substituted ethoxy)phenyl)ethanones with different aldehydes resulted in the desired chalcones. All the compounds synthesized were characterized by ^{1}H NMR, ^{13}C NMR, IR and mass spectroscopy. The molecular composition of the synthesized compounds was established on the basis of their CHNS elemental analysis data. A good agreement between the calculated and the observed values for various elements supported the formation of expected compounds as given in the experimental section. The structures of 1-(4-(2-substituted ethoxy) phenyl) ketones and their chalcones containing N-substituted ethanamine were also supported by their ^{1}H NMR spectra. The chemical shift values for all the compounds are given in the experimental section. The signals due to characteristic olefinic protons (α and β) were found in all the



Scheme 1. Synthesis of N-substituted ethanamine chalcones. *Reagents and Conditions:* (a) *THF, t-BuOK, Reflux* (b) *NaOH, EtOH, rt.*

compounds with *J* values around 15 Hz. In the IR spectra the carbonyl group appeared in the range 1650–1700 cm^{-1} and at around 1580 cm^{-1}for the alkenylic proton was also observed for the final compounds. The values of the ^{13}C NMR are also in good agreement with the proposed structure. The characteristic peak [M+1] in the mass spectra for chalcones helped to further establish the structure.

2.2. Single crystal structures of 1, 8 and 9

1-(4-(2-morpholinoethoxy)phenyl)ethanone(1), *3-(2-methylthiophen-2-yl)-1-(4-(2-morpholino ethoxy)phenyl)prop-2-en-1-*one (8) and *3-(3-bromo-4,5 dimethoxyphenyl)-1-(4-(2-morpholino ethoxy)phenyl)ethanone* (9) crystallize from methanol as colorless prism in 1, as yellow prism in 8 and as orange prism in 9. Figs. 4–6 show an ORTEP representation of 1, 8 and 9 respectively. Hydrogen bonds were not found in the structures. In the crystal packing of 8, the compound forms parallel dimers through π–π interactions between the C=C bonds of propene groups and phenyl rings [26]. The distances between the centroids is for 8:d_{c1-c2} = 3.532 Å [c1 (C14A–C15A), c2 (C7B–C8B–C9B–C10B–C11B–C12B)] (Fig. 7). Instead the compound 9 prefers to form antiparallel dimers in supramolecular structure (Fig. 8). π–π stacking interactions between phenyl rings predominate in this case. The distance between centroids are: d_{c3-c4} = 3.427 Å [c3 (C7A–C8A–C9A–C10A–C11A–C12A), c4 (C16L–C17L–C18L–C19L–C20L–C21L)] and d_{c5-c6} = 3.427 Å [c5 (C7L–C8L–C9L–C10L–C11L–C12L), c6 (C16A–C17A–C18A–C19A–C20A–C21A)]. Crystal data and details of the data collection and refinement for the new compounds are collected in Table 1 and Table 2 contains selected bond lengths and angles for compound 1, 8 and 9.

2.3. Pharmacological screening

2.3.1. Antiamoebic activity

Preliminary experiments were carried out to determine the *in vitro* antiamoebic activity of all the compounds (4–21) by microdilution method using HM1: IMSS strain of *E. histolytica* and their IC_{50} values are reported in Table 3. Metronidazole was used as reference drug having IC_{50} = 1.86 μM in our experiment. The results were estimated as the percentage of growth inhibition compared with the untreated controls and plotted as probit values as a function of the drug concentration. IC_{50} and 95% confidence limits were interpolated in the corresponding dose response curve. Out of the 18 compounds synthesized 12 showed better antiamoebic activity than MNZ. Compounds containing electron withdrawing nitro group were found to have IC_{50} value greater than MNZ. Compound 4, 10, 16 had IC_{50} values 4.76 μM, 4.17 μM and 2.33 μM respectively. Compound 12 containing piperidinoethoxy side chain and anisaldehyde as R_1 was found to have the least IC_{50} value of 0.03 ± 0.01 μM. The activity decreased tenfold when the

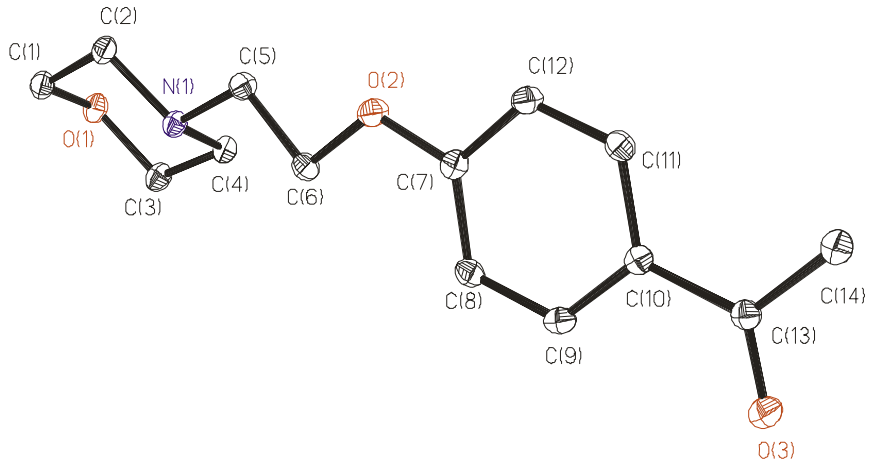


Fig. 4. ORTEP plot of compound *1-(4-(2-morpholinoethoxy)phenyl)ethanone* (1). All the non-hydrogen atoms are presented by their 30% probability ellipsoids. Hydrogen atoms are omitted for clarity.

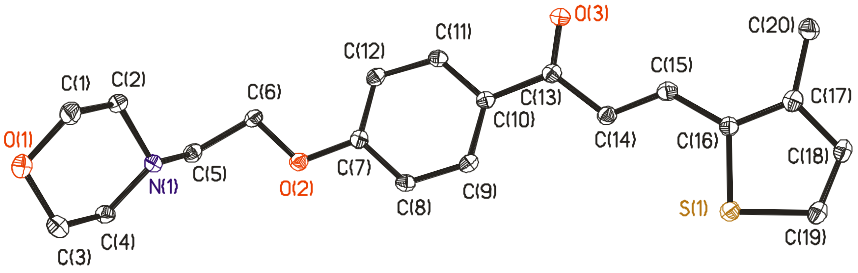


Fig. 5. ORTEP plot of compound *3-(2-methylthiophen-2-yl)-1-(4-(2-morpholinoethoxy)phenyl)prop-2-en-1-one* (8). All the non-hydrogen atoms are presented by their 30% probability ellipsoids. Hydrogen atoms are omitted for clarity.

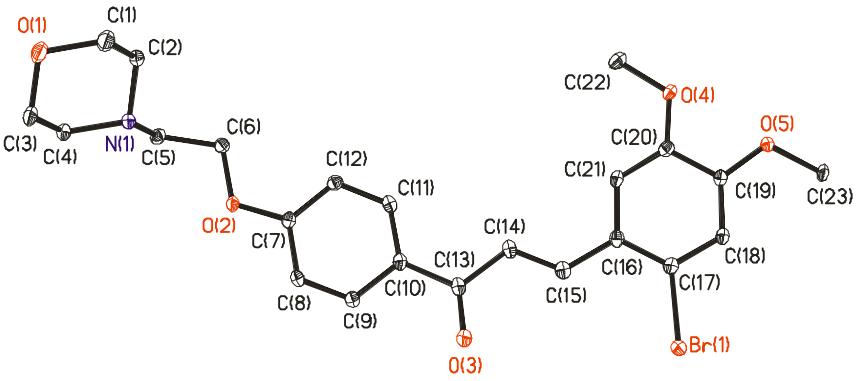


Fig. 6. ORTEP plot of compound *3-(3-bromo-4, 5dimethoxyphenyl)-1-(4-(2-morpholinoethoxy)phenyl)ethanone* (9). All the non-hydrogen atoms are presented by their 30% probability ellipsoids. Hydrogen atoms are omitted for clarity.

piperidinoethoxy side chain was replaced with dimethylaminoethoxy side chain (18; IC_{50} = 0.12 μM) but for 6 with similar R_1 substitution having morpholinoethoxy side chain the activity decreased further (IC_{50} = 2.86 μM) and was comparable to MNZ. A similar trend was also observed for compounds 13, 19 and 7 having R_1 as dimethylamino group with IC_{50} values 0.05 μM, 0.11 μM and 2.38 μM respectively. Compound 11, 17 and 5 were all found to be active (IC_{50} = 0.10 μM, 0.20 μM and 0.85 μM respectively) but the activity showed a trend of gradual decrease of tenfold while moving from piperidinoethoxy side chain to dimethylaminoethoxy side chain and a further fourfold decrease in the morpholinoethoxy side chain. Compound 14 and 20 with R_1 as 2-methylthiophene in piperidinoethoxy and dimethylaminoethoxy side chain respectively showed comparable antiamoebic activity (IC_{50} = 0.20 μM, 0.22 μM) but the activity decreased sharply and compound 8 was inactive (IC_{50} = 9.93 μM) with morpholinoethoxy side chain. Compound 15 and 21 with R_1 2-bromo-4, 5-dimethoxyphenyl showed very good and comparable antiamoebic activity (IC_{50} = 0.07, IC_{50} = 0.08 μM) and compound 9 had (IC_{50} = 0.37 μM).

2.3.2. Cytotoxicity

In vitro toxicity is to analyze the effect of synthetic and natural chemical compounds on cultured bacterial and mammalian cells. This method is primarily used to identify potentially hazardous

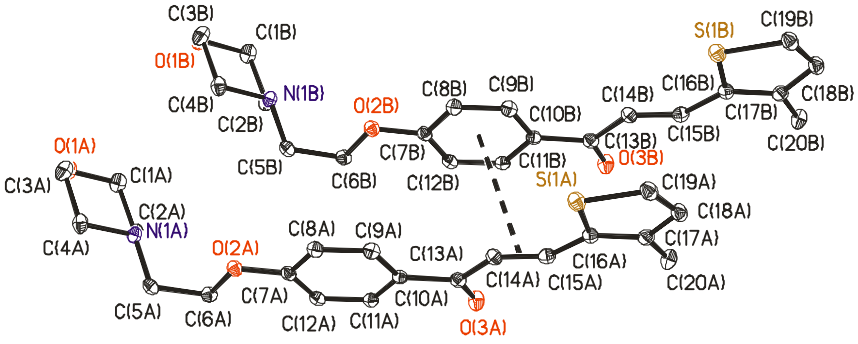


Fig. 7. Intermolecular π–π interactions in 8. Dashed lines link the centres of the π clouds involved in each interaction.

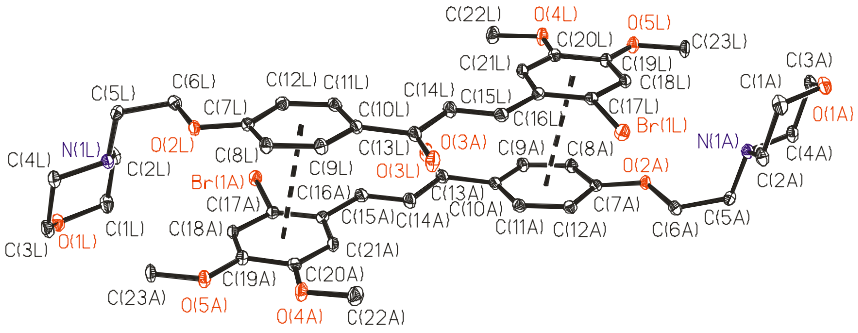


Fig. 8. Intermolecular π–π interactions in 9. Dashed lines link the centres of the π clouds involved in each interaction.

Table 1
Crystal data and structure refinement for 1-(4-(2-morpholinoethoxy)phenyl)ethanone (1), for 3-(2-methylthiophen-2-yl)-1-(4-(2-morpholinoethoxy)phenyl)prop-2-en-1-one (8) and for 3-(3-bromo-4,5 dimethoxyphenyl)-1-(4-(2-morpholinoethoxy)phenyl)ethanone (9).

	1	8	9
Formula	$C_{14}H_{19}NO_3$	$C_{20}H_{23}NO_3S$	$C_{23}H_{26}BrNO_5$
Formula weight	249.30	357.45	476.36
T, *K*	100(2)	100(2)	100(2)
Wavelength, Å	0.71073	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic	Triclinic
Space group	C2/c	C2/c	$P\bar{1}$
a/Å	20.4886(12)	34.479(3)	7.4199(9)
b/Å	6.3550(4)	4.9814(4)	11.8414(15)
c/Å	20.0370(12)	23.268(2)	12.8403(16)
α/°	90	90	74.226(8)
β/°	101.203(4)	117.892(5)	79.908(8)
γ/°	90	90	76.582(8)
V/Å^3	2559.2(3)	3532.1(5)	1048.4(2)
Z	8	8	2
F_{000}	1072	1520	492
D_{calc}/g cm^{-3}	1.294	1.344	1.509
μ/mm^{-1}	0.091	0.202	1.996
θ/(°)	2.03 to 22.24	1.34 to 26.45	1.66 to 26.50
R_{int}	0.0385	0.1540	0.1078
Crystal size/mm^3	0.30 × 0.23 × 0.15	0.32 × 0.17 × 0.05	0.18 × 0.13 × 0.11
Goodness-of-fit on F^2	1.104	1.026	1.069
$R_1[I > 2\sigma(I)]$[a]	0.0270	0.0598	0.0396
wR_2 (all data)[b]	0.0742	0.1951	0.1102
Largest differences peak and hole (eÅ^{-3})	0.133 and −0.133	0.522 and −0.397	0.500 and −0.544

[a] $R_1 = \sum||F_o|-|F_c||/\sum|F_o|$.
[b] $wR_2 = \{\sum[w(|F_o|^2-|F_c|^2|)^2]|/\sum[w(F_o^4)]\}^{1/2}$.

compounds and their toxic effect in the early stage of development of therapeutics drugs. Cytotoxic effects of the compounds (4–21) and standard drug MNZ were tested in A549 (non small cell lung cancer) cells. Few compound of the morpholinoethoxy side tail bearing series, such as 6, 7 and 8 didn't dissolve completely in DMSO. We tried to dissolve in other solvents such as ethanol, methanol and chloroform, but homogeneous drug suspension was not obtained. Thus, we failed to determine the toxic effects of these three compounds. Cytotoxic effects of each compound were measured by calculating percentage of cell viability in dose dependent manner (Figs. 9A, 10A and 11A) subsequently IC_{50} value of each compound was determined. The IC_{50} values of the morpholinoethoxy side chain bearing series compounds 4–9 are in the range of 48–94.5 μM concentration while value of 9 was found to

Table 2
Bond lengths [Å] and angles [°] for *1-(4-(2-morpholinoethoxy)phenyl)ethanone* (*1*), for *3-(2-methylthiophen-2-yl)-1-(4-(2-morpholinoethoxy)phenyl)prop-2-en-1-one*(*8*) and for *3-(3-bromo-4,5 dimethoxyphenyl)-1-(4-(2-morpholinoethoxy)phenyl)ethanone* (*9*).

Bond lengths	1	8	9
O(1)–C(1)	1.4281(18)	1.418(4)	1.426(4)
O(1)–C(3)	1.4297(18)	1.420(4)	1.425(4)
N(1)–C(5)	1.4580(18)	1.464(4)	1.461(4)
N(1)–C(2)	1.4679(18)	1.461(4)	1.467(4)
N(1)–C(4)	1.4697(18)	1.463(4)	1.463(4)
O(2)–C(7)	1.3621(17)	1.363(3)	1.364(3)
O(2)–C(6)	1.4377(17)	1.440(3)	1.436(4)
O(3)–C(13)	1.2242(17)	1.220(4)	1.222(4)
S(1)–C(19)		1.716(3)	
S(1)–C(16)		1.730(3)	
O(4)–C(20)			1.355(4)
O(4)–C(22)			1.427(4)
Br(1)–C(17)			1.901(3)
Angles	**1**	**8**	**9**
C(1)–O(1)–C(3)	108.90(11)	110.2(3)	109.5(2)
C(5)–N(1)–C(2)	109.14(11)	110.3(2)	110.9(2)
C(5)–N(1)–C(4)	111.06(12)	110.0(2)	111.1(2)
C(2)–N(1)–C(4)	108.72(11)	108.7(2)	107.8(2)
C(7)–O(2)–C(6)	119.73(10)	116.6(2)	117.8(2)
C(19)–S(1)–C(16)		91.37(16)	
C(20)–O(4)–C(22)			117.0(2)
C(18)–C(17)–Br(1)			116.0(2)
C(16)–C(17)–Br(1)			121.1(2)

be very high i.e., 94.5 μM. Even at 2.5 μM concentration of 9, A549 (non-small cell lung cancer cell line) cells remained 100% viable at 24 h post drug treatment. IC_{50} values of compounds processing dimethylaminoethoxy side chain (16–21) ranged from 36.4 μM to 69.3 μM. Compound 19 was found to be the least toxic against A549 lung cancer cell line with IC_{50} value 69.3 μM. The IC_{50} values of piperidinoethoxy side tail bearing compounds ranged from 24.5 to 69.0 μM. In this series compounds 14 was found to be the least cytotoxic with IC_{50} value 69.0 μM. Thus out of the three series of compounds 9, 14 and 19 showed the least toxic effect against A549 lung cancer cell line. Corresponding IC_{50} values of each compound was given in Table 3 and graphical representation was also shown besides the graph showing percentage of viability (Figs. 9B, 10B and 11B).

3. Conclusion

In summary chalcones bearing N-substituted ethanamine side chain were synthesized. The *in vitro* antiamoebic activity was examined using HM1:1MSS strain of *E. histolytica* and results showed that out of 18 compounds synthesized 12 exhibited better antiamoebic activities than the reference drug metronidazole. Cytotoxicity of the compounds on A549 (non small cell lung cancer cell line) cells was measured by MTT assay. Based on percentage of cell viability IC_{50} values of the compounds was determined. Compound 9, 14 and 19 were found to be the least toxic on lung cancer cell line. It is hoped that these studies will fuel further efforts towards the development of effective antiamoebic agents.

4. Experimental protocol

All the chemicals were purchased from Aldrich Chemical Company (USA). Precoated aluminum sheets (silica gel 60 F254, Merck Germany) were used for thin-layer chromatography (TLC) and spots were visualized under UV light. Elemental analysis (C, H and N) was carried out on CHNS Elementar analyzer (Vario EL-III). Electrospray (ES) mass spectra were carried out on Microtof-Q II 10262. Melting points were recorded on a Veego melting point apparatus (model REC-2203882) and were uncorrected. IR spectra were recorded on a Perkin Elmer model 1600 FT-IR RX1 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were obtained at ambient temperature using a Bruker spectrospin DPX-300 MHz instrument, in $CDCl_3$ using tetramethylsilane as an internal standard. Splitting patterns are designated as follows: s, singlet; d; doublet; t, triplet; m, multiplet. Chemical shift values are given in ppm.

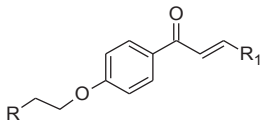
4.1. X-ray crystal structure determination

Three-dimensional X-ray data were collected on a Bruker Kappa Apex CCD diffractometer at low temperature for 1, 8 and 9 by the $\phi-\omega$scan method. Reflections were measured from a hemisphere of data collected from frames, each of them covering 0.5° inω. Of the 19,611 for 1, 62,019 for 8 and 54641 for 9 reflections measured, all were corrected for Lorentz and polarization effects and for absorption by multi-scan methods based on symmetry-equivalent and repeated reflections, 1445, 2300 and 3476 respectively, independent reflections exceeded the significance level $(|F|/\sigma|F|) > 4.0$. Complex scattering factors were taken from the program package SHELXTL [27]. The structures were solved by direct methods and refined by full matrix least-squares on F^2. In 1, hydrogen atoms were located in difference Fourier map and left to refine freely, except for C (11), which was included in calculation position and refined in the riding mode. In 8, hydrogen atoms were located in difference Fourier map and left to refine freely, except for C(6) and C(20), which were included in calculation positions and refined in the riding mode. In 9, hydrogen atoms were located in difference Fourier map and left to refine freely, except for C(8), C(22), and C(23), which were included in calculation positions and refined in the riding mode. Refinements were done with allowance for thermal anisotropy of all non-hydrogen atoms. A final difference Fourier map showed no residual density outside: 0.133 and −0.133 e $Å^{-3}$ for 1, 0.522 and −0.397 e $Å^{-3}$ for 8 and 0.500 and −0.544e $Å^{-3}$ for 9. A weighting scheme $w = 1/[\sigma^2(F_o^2) + (0.039400\ P)^2 + 1.210700\ P]$ for 1, $w = 1/[\sigma^2(F_o^2) + (0.116400\ P)^2 + 0.618300P]$ for 8 and $w = 1/[\sigma^2(F_o^2) + (0.063800P)^2 + 0.000000\ P]$ for 9, where $P = (|F_o|^2 + 2|F_c|^2)/3$, were used in the latter stages of refinement. CCDC 1035422 for 1, 1035420 for 8 and 1035421 for 9 contain the supplementary crystallographic data for the structures reported in this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk.

4.2. General procedure for the synthesis of ketone (1, 2, 3)

To a stirred solution of *t*-BuOK (2.5 eq) in tetrahydrofuran (250 mL) and 4-hydroxyacetophenone (1 eq) was added in portions which resulted in a white precipitate. Different N-substituted 2-chloroethanamine hydrochloride (1 eq) were subsequently added. The reaction mixture was refluxed for 15 h and the reaction was monitored using TLC. The reaction mixture was then concentrated *in vacuo*. The crude solid thus obtained was treated with water, extracted with dichloromethane and dried over anhydrous sodium sulphate. 1, 2 were recrystallized with ethanol and 3 was purified by column chromatography using ethyl acetate:hexane (1:9) as eluent. The compounds were obtained in approximately 70–80 % yield.

Table 3
In vitro antiamoebic activity of chalcones (4–21) against HM1: IMSS strain of *E. histolytica* and measurement of cell viability by MTT assay.



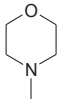
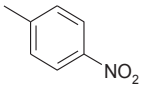
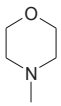
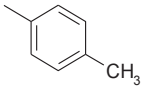
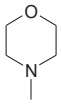
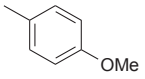
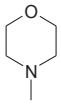
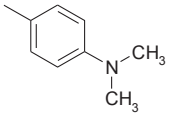
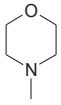
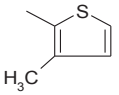
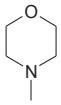
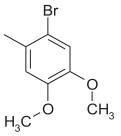
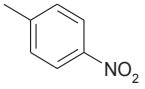
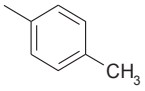
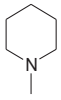
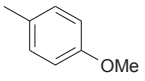
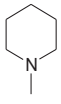
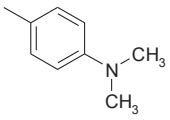
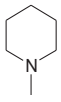
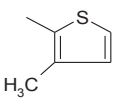
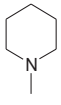
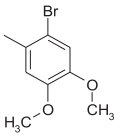
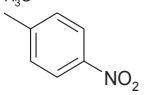
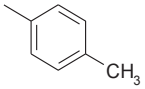
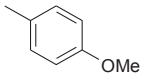
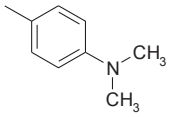
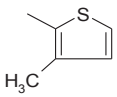
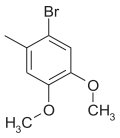
Compound code	R	R1	Antiamoebic activity (IC_{50}±S.D.)	Cytotoxicity (IC_{50}±S.D.)
4			4.76 ± 0.02	48.86 ± 1.23
5			0.85 ± 0.01	49.76 ± 0.03
6			2.86 ± 0.02	N.D
7			2.38 ± 0.01	N.D
8			9.93 ± 0.01	N.D
9			0.37 ± 0.01	94.53 ± 0.80
10			4.17 ± 0.01	24.54 ± 1.20
11			0.10 ± 0.01	30.96 ± 3.03
12			0.03 ± 0.01	28.57 ± 0.02
13			0.05 ± 0.01	39.45 ± 1.70
14			0.20 ± 0.01	69.00 ± 0.03
15			0.07 ± 0.01	47.84 ± 0.40
16			2.33 ± 0.01	N.D
17			0.20 ± 0.01	40.95 ± 0.20
18			0.12 ± 0.02	36.45 ± 1.82

Table 3 (*continued*)

Compound code	R	R1	Antiamoebic activity (IC_{50}±S.D.)	Cytotoxicity (IC_{50}±S.D.)
				
19			0.11 ± 0.02	69.32 ± 0.50
20			0.22 ± 0.01	44.54 ± 2.30
21			0.08 ± 0.01	36.82 ± 1.20
MNZ			1.86 ± 0.02	>160

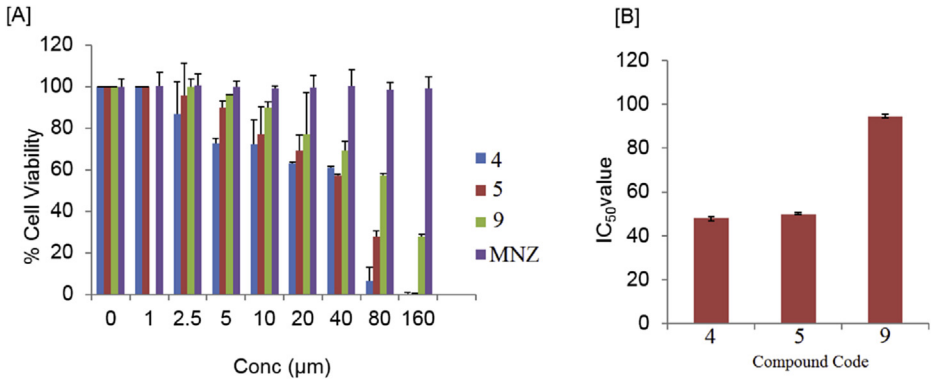


Fig. 9. (A) Measurement of cell viability following treatment with morpholinoethoxy side chain bearing compounds by MTT assay (B) IC_{50} values of corresponding compounds.

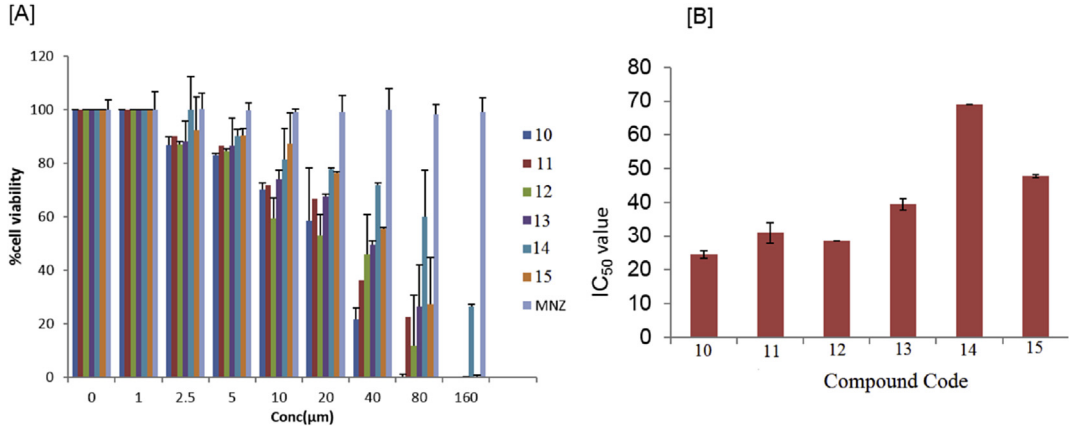


Fig. 10. (A) Measurement of cell viability following treatment with piperidinoethoxy side chain bearing compounds by MTT assay (B) IC_{50} values of corresponding compounds.

*4.2.1. 1-(4-(2-morpholinoethoxy)phenyl)ethanone (**1**)*

Yield: 82%; m.p. 80 °C; Anal. Calc. (%) for $C_{14}H_{19}NO_3$: C 67.45, H 7.68, N 5.62; found: C 67.67, H 7.66, N 5.75; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.94 (d, 2H, J = 9.0 Hz, Ar–H), 6.95 (d, 2H, J = 9.0 Hz, Ar–H), 2.84 (t, 2H, J = 5.7 Hz), 4.19 (t, 2H, J = 4.8 Hz, CH_2O), 3.75 (t, 4H, J = 5.7 Hz, CH_2O), 2.60–2.53 (7H, CH_2N, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 196.71, 162.58, 130.40, 129.67, 114.83, 66.88, 66.45, 54.54, 53.67, 27.70. ES-MS: m/z: 250 [M+1].

*4.2.2. 1-(4-(2-(piperidin-1-yl)ethoxy)phenylethanone (**2**)*

Yield: 87%; m.p.76 °C; Anal. Calc. (%) for: $C_{14}H_{21}NO_2$: C 72.84, H 8.56, N 5.66; found: C 72.76, H 8.45, N 5.76; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.94 (d, 2H, J = 8.7 Hz, Ar–H), 6.95 (d, 2H, J = 8.7 Hz, Ar–H), 4.18 (t, 2H, J = 6.0 Hz, CH_2O), 2.81 (t, 2H, J = 6.0 Hz, CH_2N), 2.56 -2.51 (m, 7H), 1.63–1.57 (m, 6H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 196.72, 162.65, 131.71, 130.35, 129.69, 66.11, 57.62, 54.98, 26.90, 25.02; ES-MS: m/z: 248 [M+1].

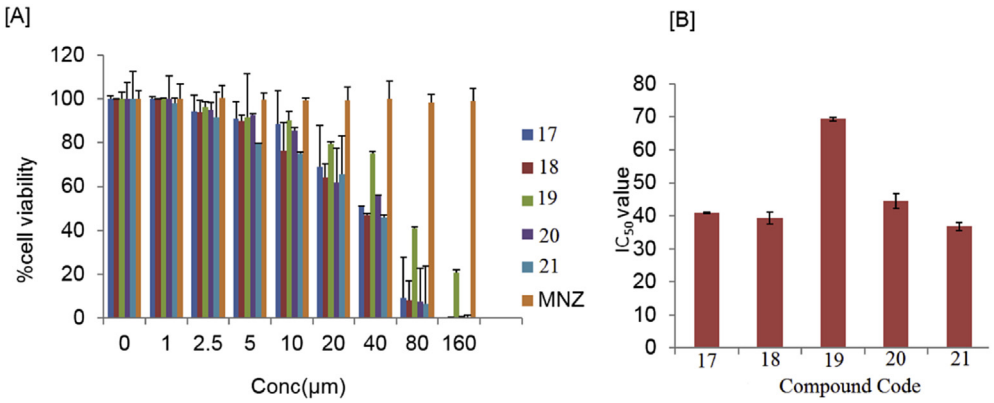


Fig. 11. (A) Measurement of cell viability following treatment with dimethylaminoethoxy side chain bearing compounds by MTT assay (B) IC_{50} values of corresponding compounds.

*4.2.3. 1-(4-(2-(Dimethylamino)ethoxy)phenyl)ethanone (**3**)*

Yield: 75%; Anal. Calc. (%) for $C_{12}H_{17}NO_2$: C 69.54, H 8.27,N 6.76; found: C 69.91, H 8.32, N 6.75; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.94 (d, 2H, J = 7.4 Hz, Ar–H), 7.27 (d, 2H, J = 7.4 Hz, Ar–H), 4.15 (t, 2H, J = 5.4 Hz, CH_2), 2.78 (t, 2H, J = 5.4 Hz, CH_2), 2.56 (s, 3H, CH_3), 2.35 (s, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 196.80, 162.64, 130.52, 115.55, 114.187, 65.97, 57.91, 45.67, 26.24; ES-MS: *m/z*: 208 [M+1].

*4.3. General procedure for the synthesis of chalcones (**4**–**21**)*

A mixture of 4-substituted acetophenone derivatives (1 eq), aromatic ketones (1 eq), and sodium hydroxide (2 mL, 40% aqueous) in 10 mL ethanol was stirred at room temperature for 24 h. The reaction was monitored by TLC. After the completion of the reaction the solvent was evaporated *in vacuo*. The concentrate was extracted with dichloromethane and dried over anhydrous sodium sulphate. The product was recrystallized from methanol. Compound 19 has been reported earlier [25].

*4.3.1. 1-(4-(2-morpholinoethoxy)phenyl)-3-(4-nitrophenyl)prop-2-en-1-one (**4**)*

Yield: 78%; m.p.: 94 °C; Anal. Calc. (%) for $C_{21}H_{22}N_2O_5$: C 65.96, H 5.80, N 7.33; found: C 65.76, H 5.65, N 7.45; FT-IR ν_{max} (cm^{-1}): 1655 (C=O), 1583 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.28 (d, 2H, J = 8.7 Hz, Ar–H), 8.06 (d, 2H, J = 8.7 Hz, Ar–H), 7.83–7.78 (m, 3H), 7.67 (d, 1H, J = 15.6 Hz, 1H$_\beta$), 7.02 (d, 2H, J = 8.7 Hz), 4.22 (t, 2H, J = 5.7 Hz, CH_2O), 3.76 (t, 4H, J = 4.2 Hz, CH_2O), 2.88 (t, 2H, J = 5.7 Hz, CH_2N), 2.61 (t, 4H, J = 4.5 Hz, CH_2N); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 187.69, 162.97, 148.44, 141.27, 140.71, 130.61, 128.83, 125.61, 124.16, 114.59, 66.84, 66.13, 57.41, 54.09; ES-MS: *m/z*: 383 [M+1].

*4.3.2. 1-(4-(2-morpholinoethoxy)phenyl)-3-p-tolylprop-2-en-1-one (**5**)*

Yield 73%; m.p.: 81 °C; Anal. Calc. (%) for $C_{22}H_{25}NO_3$: C 75.19, H 7.17, N 3.99; found: C 75.21, H 7.23, N 3.89; FT-IR ν_{max} (cm^{-1}): 1648 (C=O), 1583 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.04 (d, 2H, J = 8.7 Hz, Ar–H), 7.81 (d, 1H$_\beta$, J = 15.6 Hz), 7.55–7.47 (m, 3H), 7.23 (d, 2H, J = 8.1 Hz), 7.00 (d, 2H, J = 9.0 Hz), 4.217 (t, 2H, J = 5.7 Hz, CH_2O), 3.76 (t, 4H, J = 4.2 Hz, CH_2O), 2.85 (t, 2H, J = 5.7 Hz, CH_2N), 2.61 (t, 4H, J = 4.5 Hz, CH_2N), 2.39 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.68, 162.47, 144.05, 140.80, 132.33, 131.36, 130.74, 129.66, 128.38, 120.85, 114.37, 66.90, 66.08, 57.46, 54.11, 21.50; ES-MS: *m/z*: 352 [M+1].

*4.3.3. 3-(4-methoxyphenyl)-1-(4-(2-morpholinoethoxy)phenyl) prop-2-en-1-one (**6**)*

Yield: 85%; m.p.: 83 °C; Anal. Calc. (%) for $C_{22}H_{25}NO_4$: C 71.91, H 6.86, N 3.81; found: C 71.87, H 6.78, N 3.59; FT-IR ν_{max} (cm^{-1}): 1740 (C=O), 1595 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.04 (d, 2H, J = 8.4 Hz, Ar–H), 7.81 (d, 1H$_\alpha$, J = 15.6 Hz), 7.62 (d, 2H, J = 9.9 Hz, Ar–H), 7.45 (d, 1H$_\beta$, J = 15.6 Hz), 7.00–6.92 (m, 4H, Ar–H), 4.21 (t, 2H, J = 5.4 Hz, CH_2O), 3.86 (s, 3H, OCH_3), 3.76–3.73 (m, 4H, CH_2O), 2.86 (t, 2H, J = 5.7 Hz, CH_2), 2.61–2.59 (m, 4H, CH_2N); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm):188.66, 162.38, 161.53, 143.83, 131.49, 130.67, 130.10, 127.79, 119.52, 114.39, 114.34, 66.88, 66.05, 57.35, 55.38, 54.10; ES-MS: *m/z*: 368 [M+1].

*4.3.4. 3-(4-(dimethylamino) -1-(4-(2-morpholinoethoxy)phenyl) prop-2-en-1-one (**7**)*

Yield: 82%; m.p.: 78□C; Anal. Calc. (%) for $C_{23}H_{28}N_2O_3$: C 72.61, H 7.42, N 7.36; found: C 72.45, H 7.44, N 7.62; FT-IR ν_{max} (cm^{-1}): 1646 (C=O), 1596 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.03 (d, 2H, J = 8.7 Hz, Ar–H), 7.80 (d, 1H$_\alpha$, J = 15.6 Hz), 7.56 (d, 2H, J = 8.7 Hz, Ar–H), 7.37 (d, 1H, J = 15.3 Hz), 6.98 (d, 2H, J = 9.0 Hz, Ar–H), 6.71 (d, 2H, J = 8.7 Hz, Ar–H), 4.20 (t, 2H, J = 5.7 Hz, CH_2O), 3.75 (t, 4H, J = 4.8 Hz, CH_2O), 3.04 (s,6H, $(CH_3)_2N$), 2.85 (t, 2H, J = 5.7 Hz, CH_2N), 2.60 (t, 4H, J = 4.5 Hz, CH_2N); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm):188.86, 162.07, 151.94, 144.98, 132.06, 130.52, 130.25, 122.89, 116.72, 114.24, 111.86, 66.91, 66.03, 57.50, 54.12, 40.13; ES-MS: *m/z*: 381[M+1].

*4.3.5. 3-(3-Methylthiophen-2-yl)-1-(4-(2-morpholinoethoxy) phenyl)prop-2-en-1-one (**8**)*

Yield: 77%; m.p.: 110 °C; Anal. Calc. (%) for $C_{20}H_{23}NO_3S$: C 67.20, H 6.49, N 3.92, S 8.97; found: C 67.34, H 6.66, N 4.01, S 9.12; FT-IR ν_{max} (cm^{-1}): 1643 (C=O), 1547 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.08–8.00 (m, 2H, Ar–H), 7.61 (d, 1H$_\beta$, J = 15.9 Hz), 7.17 (s, 1H, Ar–H), 6.99 (d, 2H, J = 8.7 Hz, Ar–H), 6.99 (d, 2H, J = 8.7 Hz, ArH), 6.88 (d, 2H, J = 9.0 Hz), 4.21 (t, 1H, J = 5.7 Hz, CH_2O), 3.87–3.82 (m, 4H, CH_3), 3.75 (t, 3H, J = 4.5 Hz, CH_2O), 2.85 (t, 2H, J = 5.7 Hz, CH_2N), 2.60 (t, 3H, J = 4.5 Hz, CH_2N); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.02, 162.43, 142.41, 134.87, 134.67, 131.42, 131.34, 130.65, 126.97, 119.64, 114.37, 66.83, 66.00, 57.44, 54.08, 14.28; ES-MS: *m/z*: 358 [M+1].

*4.3.6. 3-(2-Bromo-4, 5-dimethoxyphenyl)-1-(4-(2-morpholinoethoxy)phenyl)prop-2-en-1-one (**9**)*

Yield: 76%; m.p.: 93□C; Anal. Calc. (%) for $C_{23}H_{26}BrNO_5$: C 57.99, H 5.50, N 2.94; found: C 57.69, H 5.80, N 3.14; FT-IR ν_{max} (cm^{-1}): 1750 (C=O), 1620 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.07-8.00 (m, 2H, J = 15 Hz, 1H$_\beta$, Ar–H), 7.27–7.26 (m, 1H, Ar–H), 7.19 (s,

1H, Ar–H), 7.08 (s, 1H, Ar–H), 7.00 (d, 2H, J = 8.7 Hz, Ar–H), 4.21–4.18 (m, 2H), 3.95 (s, 3H, CH_3O), 3.92 (s, 3H, CH_3O), 3.76–3.73 (m, 4H), 2.86 (t, 2H, J = 6.0 Hz), 2.61–2.58 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.77, 162.73, 148.99, 142.84, 130.92, 127.65, 122.93, 115.83, 114.39, 66.72, 57.43, 56.24, 54.05; ES-MS: m/z: 477 [M+1].

*4.3.7. 3-(4-nitrophenyl)-1-(4-(2-(piperidin-1-yl)ethoxy)phenyl) prop-2-en-1-one (**10**)*

Yield: 88%; m.p.: 76 °C; Anal. Calc. (%) for $C_{22}H_{24}N_2O_4$: C 69.46, H 6.36, N 7.36; found: C 69.56, H 6.54, N 7.56; FT-IR ν_{max} (cm^{-1}): 1676(C=O), 1589(C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.28 (d, 2H, J = 8.7 Hz, Ar–H), 7.96 (d, 2H, J = 8.4 Hz, Ar–H), 7.88–7.76 (m, 3H, Ar–H), 7.67 (d, 1H$_\beta$, J = 15.6 Hz), 6.99 (d, 2H, J = 6.9 Hz, Ar–H), 4.22–4.13 (m, 2H), 2.84 (t, 2H), 2.55–2.53 (m, 2H), 1.64–1.61 (m, 6H), 1.47–1.46 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 187.72, 163.15, 148.42, 141.31, 140.63, 130.95, 130.44, 128.83, 125.68, 124.16, 114.61, 66.33, 57.66, 55.07, 25.84, 24.08; ES-MS: m/z: 381[M+1].

*4.3.8. 1-(4-(2-(piperidin-1-yl)ethoxyphenyl-3-p-tolylprop-2-en-1-one (**11**)*

Yield: 87%; m.p.: 84 °C; Anal. Calc. (%) for $C_{23}H_{27}NO_2$: C 79.05, H 7.79, N 4.01; found: C 79.32, H 7.56, N 3.98; FT-IR ν_{max} (cm^{-1}): 1633 (C=O), 1540 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.02 (d, 2H, J = 8.8 Hz, Ar–H), 7.78 (d, 1H$_\alpha$, J = 15.2 Hz), 7.54–7.51 (m, 2H, 1H$_\beta$, Ar–H), 7.47 (s, 1H, Ar–H), 6.97 (d, 2H, J = 8.8 Hz, Ar–H), 4.19 (t, 2H, J = 6.0 Hz, CH_2O), 2.81 (t, 2H, J = 5.6 Hz, CH_2N), 2.51 (bs, 4H, CH_2), 2.38 (s, 3H, CH_3), 1.62–1.59 (m, 6H, CH_2), 1.45–1.44 (m, 2H, CH_2);^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.78, 162.65, 144.01, 140.79, 132.37, 131.23, 130.74, 129.66, 128.38, 120.91, 114.40, 66.29, 57.76, 55.10, 53.32, 50.28 25.96, 24.16, 21.51; ES-MS: m/z: 350 [M+1].

*4.3.9. 3-(4-methoxyphenyl)-1-(4-(2-(pipeidin-1-yl)ethoxy)phenyl) prop-2-en-1-one (**12**)*

Yield: 70%; m.p.: 43 °C; Anal. Calc. (%) for $C_{23}H_{27}NO_3$: C 75.59, H 7.45, N 3.83; found: C 75.65, H 7.43, N 3.82; FT-IR ν_{max} (cm^{-1}):1649 (C=O), 1592 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 8.03 (d, 2H, J = 9.0 Hz, Ar–H), 7.80 (d, 1H$_\alpha$, J = 14.4 Hz), 7.61 (d, 2H, J = 9.0 Hz, ArH), 7.45 (d, 1H$_\beta$, J = 16.2 Hz), 6.99–6.92 (m, 4H, Ar–H), 4.20 (t, 2H, J = 6.0 Hz, CH_2O), 3.85 (s, 3H, OCH_3), 2.82 (t, 2H, J = 6.3 Hz, CH_2N), 2.53 (t, 2H, J = 4.5 Hz, CH_2N), 1.65–1.58 (m, 6H, CH_2), 1.49 (t, 2H, J = 5.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.76, 162.65, 143.98, 140.76, 132.38, 131.23, 130.72, 129.65, 128.37, 120.93, 114.39, 66.31, 57.75, 55.09, 25.95, 24.16, 21.49; ES-MS: m/z: 366[M+1].

*4.3.10. 3-(4-(dimethylamino)phenyl)-1-(4-(2-(piperidin-1-yl) ethoxy)phenyl)prop-2-en-1-one (**13**)*

Yield: 86%; m.p.: 65 °C; Anal. Calc. (%) for $C_{24}H_{30}N_2O_2$: C 76.16, H 7.99, N 7.40; found: C 76.24, H 7.87, N 7.34; FT-IR ν_{max} (cm^{-1}): 1664 (C=O), 1593 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.02 (d, 2H, J = 7.7 Hz, Ar–H), 7.80 (d, 1H$_\alpha$, J = 16.0 Hz), 7.55 (d, 2H, J = 9.0 Hz, 2Ar–H), 7.37 (d, 1H$_\beta$, J = 16.0 Hz), 6.98 (d, 2H, J = 9.0 Hz, Ar–H), 6.70 (d, 2H, J = 9.0 Hz, Ar–H), 4.20 (t, 2H, J = 5.7 Hz, CH_2O), 3.07 (s, 6H, NCH_3), 2.82 (t, 2H, J = 6.0 Hz, CH_2N), 2.54–2.52 (m, 4H, CH_2), 1.63–1.61 (m, 4H, CH_2), 1.46–1.45 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.85, 162.25, 151.90,144.88, 130.87, 130.49, 130.24, 122.89, 116.72, 114.26, 111.84, 66.20, 57.76, 55.07, 40.13, 26.32, 25.91, 24.15; ES-MS: m/z: 378 [M+1].

*4.3.11. 3-(3-Methylthiophen-2-yl)-1-(4-(2-(piperidin-1-yl)ethoxy) phenyl)prop-2-en-1-one (**14**)*

Yield: 85%; m.p.: 45 °C; Anal. Calc. (%) for $C_{21}H_{25}NO_2S$: C 70.95, H 7.09, N 3.94, S 9.02 found: C 71.12, H 7.23, N 4.12, S 9.32; FT-IR ν_{max} (cm^{-1}): 1643 (C=O), 1597 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.04–7.99 (m, 3H, Ar–H), 7.31–7.26 (m, 2H, J = 12.3 Hz, 1 H$_\beta$, Ar–H), 6.99 (d, 2H, J = 9.0 Hz, Ar–H), 6.90 (d, 1H, J = 8.0 Hz, Ar–H), 4.20 (t, 2H, J = 6.0 Hz), 2.82 (t, 2H, J = 6.0 Hz), 2.53 (t, 2H, J = 4.8 Hz), 2.39 (s, 3H), 1.66–1.58 (m, 6H), 1.49–1.45 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 187.99, 162.63, 142.30, 134.75, 134.69, 131.38, 131.57, 130.67, 126.92, 119.69, 114.39, 66.25, 57.73, 55.07, 25.91, 24.14, 14.26; ES-MS: m/z: 356 [M+1].

*4.3.12. 3-(2-Bromo-4,5-dimethoxyphenyl)-1-(4-(2-(piperidin-1-yl) ethoxy)phenyl)prop-2-en-1-one (**15**)*

Yield: 76%; m.p.: 78 °C; Anal. Calc. (%) for $C_{24}H_{28}BrNO_4$: C 60.76, H 5.95, N 2.95; found: C 60.88, H 5.76, N 3.01; FT-IR ν_{max} (cm^{-1}): 1771 (C=O), 1600 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.00–7.84 (m, 2H, J = 15.3 Hz, 1H$_\alpha$, ArH), 7.25–7.19 (m, 2H, Ar–H), 7.01 (s, 1H, Ar–H), 6.93 (s, 1H, Ar–H), 6.87 (d, 2H, J = 8.40 Hz, Ar–H), 4.22–4.12 (m, 2H), 3.85 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 2.83–2.81 (m, 2H), 2.58–2.31 (m, 4H), 1.62–1.59 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.66, 162.63, 151.42, 148.68,142.45, 141.61, 131.03, 130.86, 125.79,122.90, 115.78, 114.40, 109.62, 66.17, 57.67, 56.16, 55.04, 25.79, 24.06; ES-MS: m/z: 474 [M+1].

*4.3.13. 1-(4-(2-(diethymlamino)ethoxy)phenyl)-3-(4-nitrophenyl) prop-2-en-1-one (**16**)*

Yield: 75%; m.p: 117 °C; Anal. Calc. (%) for $C_{19}H_{20}N_2O_4$: C 67.05, H 5.92, N 8.23; found: C 67.07, H 5.90, N 8.22; FT-IR ν_{max} (cm^{-1}): 1660 (C=O), 1567 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.31 (d, 2H, J = 8.7 Hz, Ar–H), 8.03 (d, 2H, J = 8.6 Hz, Ar–H), 7.90–7.80 (m, 3H, Ar–H), 7.63 (d, 1H$_\beta$), 7.02 (d, 2H), 4.15 (t, 2H, J = 5.7 Hz, CH_2O), 2.511 (t, 2H, J = 5.0 Hz, CH_2O), 2.21 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.87, 162.73, 161.40, 144.34, 131.60, 130.06, 129.99, 127.76, 119.87, 115.06, 66.34, 58.43, 55.32, 45.86; ES-MS: m/z: 340 [M+1].

*4.3.14. 1-(4-(2-(dimethylamino)ethoxy)phenyl)-3-p-tolylprop-2-en-1-one (**17**)*

Yield: 80%; m.p: 87 °C; Anal. Calc. (%) for $C_{20}H_{23}NO_3$: C 77.64, H 7.49, N 4.53; found: C 77.66, H 7.45, N 4.20; FT-IR ν_{max} (cm^{-1}): 1653 (C=O), 1586 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.02 (d, 2H, J = 8.8 Hz, Ar–H), 7.79 (d, 1H$_\alpha$, J = 16.0 Hz), 7.53–7.51 (m, 2H, 1H$_\beta$, Ar–H), 7.47 (s, 1H, Ar–H), 7.21 (d, 2H, J = 8.0 Hz, Ar–H), 6.99 (d, 2H, J = 8.8 Hz, Ar–H), 4.15 (t, 2H, J = 5.6 Hz, CH_2O), 2.77 (t, 2H, J = 5.6 Hz, CH_2N), 2.37 (s, 3H, CH_3), 2.34 (s, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.66, 162.54, 161.50, 143.73, 131.40, 130.64, 130.07, 127.82, 119.58, 114.37, 66.27, 58.12, 55.36, 45.89; ES-MS: m/z: 310 [M+1].

*4.3.15. 1-(4-(2-(dimethylamino)ethoxy)phenyl)-3-(4-methoxyphenyl)propan-1-one (**18**)*

Yield: 75%; m.p: 89 °C; Anal. Calc. (%) for $C_{20}H_{23}NO_3$: C 73.82, H 7.12, N 4.30; found: C 73.67, H 7.21, N 4.33; FT-IR ν_{max} (cm^{-1}): 1652 (C=O), 1594 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.04 (d, 2H, J = 8.7 Hz, Ar–H), 7.81 (d, 1H$_\alpha$, J = 15.6 Hz), 7.62 (d, 2H, J = 8.7 Hz, Ar–H), 7.46 (d, 1H$_\beta$, J = 15.6 Hz), 7.01 (d, 2H, J = 9.0 Hz, Ar–H), 6.95 (d, 2H, J = 8.7 Hz, Ar–H), 4.16 (t, 2H, J = 5.4 Hz, Ar–H), 3.86 (s, 3H, OCH_3), 2.78 (t, 2H, J = 5.7 Hz, Ar–H), 2.35 (s, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.75, 162.63, 143.97, 140.74, 132.40, 131.32, 130.70, 129.64, 128.35, 120.96, 114.38, 66.32, 58.13, 45.90, 21.46; ES-MS: m/z: 325 [M+1].

*4.3.16. 1-(4-(2-(dimethylamino)ethoxy)phenyl)-3-(4(dimethylamino)phenyl)prop-2-en-1-one (**19**)*

Yield: 77%; m.p: 162 °C; Anal. Calc. (%) for $C_{21}H_{26}N_2O_2$: C 74.53, H 7.74, N 8.28; found: C 74.36, H 7.76, N 8.34; FT-IR ν_{max} (cm^{-1}): 1652 (C=O), 1597 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.02 (d, 2H, J = 8.4 Hz, Ar–H), 7.80 (d, 1Hα, J = 15.3 Hz), 7.55 (d, 2H, J = 8.7 Hz, Ar–H), 7.36 (d, 1H$_\beta$, J = 15.6 Hz), 7.00 (d, 2H, J = 8.7 Hz, Ar–H), 6.70 (d, 2H, J = 8.7 Hz, Ar–H), 4.20 (t, 2H, J = 5.7 Hz, CH_2O),

3.08 (s, 6H, $N(CH_3)_2$), 2.84 (t, 2H, J = 5.4 Hz, CH_2N), 2.40 (s, 6H, $N(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.85, 162.08, 151.94, 144.94, 132.02, 130.48, 130.24, 122.85, 116.71, 114.25, 111.85, 65.95, 57.98, 45.69, 40.08; ES-MS: m/z: 339 [M+1].

4.3.17. 1-(4-(2-(dimethylamino)ethoxy)phenyl)-3-(3-methylthiophen-2-yl)prop-2-en-1-one (**20**)

Yield: 81%; m.p.: 83 °C; Anal. Calc. (%) for $C_{18}H_{21}NO_2S$: C 68.54, H 6.71, N 4.44, S 10.16; found: C 68.57, H 6.67, N 4.34, S 10.23; FT-IR ν_{max} (cm^{-1}): 1655 (C═O), 1594 (C═C); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.06 (d, 2H, J = 8.7 Hz, Ar–H), 7.90 (d, $1H_\alpha$, J = 15.3 Hz, Ar–H), 7.65–7.62 (m, 1H, Ar–H), 7.42 (d, $1H_\beta$, J = 15.0 Hz), 7.07–7.00 (m, 3H, Ar–H), 4.15 (t, 2H, J = 5.7 Hz, CH_2O), 2.62 (t, 2H, J = 4.5 Hz, CH_2N), 2.34 (s, 3H, CH_3), 2.19 (s, 6H, CH_3N); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.06, 162.32, 142.34, 134.85, 134.65, 131.39, 130.62, 126.96, 119.70, 114.39, 65.76, 57.81, 54.67, 45.51, 14.24; ES-MS: m/z: 316 [M+1].

4.3.18. 3-(2-Bromo-4,5-dimethoxyphenyl)-1-(4-(2-(dimethylamino)ethoxy)phenyl)prop-2-en-1-one (**21**)

Yield: 66%; m.p.: 142 °C; Anal. Calc. (%) for $C_{21}H_{24}BrNO_4$: C 58.07, H 5.57, N 3.22; found: C 58.12, H 5.55, N 3.21; FT-IR ν_{max} (cm^{-1}): 1645 (C═O), 1582 (C═C); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.07–7.99 (m, 3H, J = 14.1 Hz, 1 H_α, Ar–H), 7.27–7.26 (m, 1H, Ar–H), 7.19 (s, 1H, Ar–H), 7.08 (s, 1H, Ar–H), 7.00 (d, 2H, J = 8.7 Hz, Ar–H), 4.17 (t, 2H, J = 5.7 Hz, CH_2O), 3.94 (s, 3H, CH_3O), 3.91 (s, 3H, CH_3O), 2.78 (t, 2H, CH_2N), 2.35 (s, 6H, CH_3N); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 188.86, 162.59, 151.40, 148.30, 142.47, 131.11, 130.86, 127.13, 122.88, 117.83, 115.82, 114.39, 109.65, 66.14, 58.04, 56.23, 45.80; ES-MS: m/z: 435 [M+1].

4.4. Pharmacological procedures

4.4.1. Antiamoebic activity

The chalcones (4–21) were screened against the HM1: IMSS strain of *E. histolytica* by using the microdilution method [28]. All the experiments were carried out in triplicates at each concentration level and repeated thrice. *E. histolytica* trophozoites were cultured in TYI-S-33 growth medium in wells of 96-well microtiter plates [29]. DMSO (40 μL) 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 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 μL 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 37 °C 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.

4.4.2. Cytotoxicity assay

Cytotoxic effects of the synthesized compounds were determined in A549 cells (lung epithelial cells). Each compound was tested from 1 μM to 160 μM concentrations. However, for few drugs higher concentrations were used when 100% cell death was not observed at 160 μM concentrations. Compounds were reconstituted initially in DMSO, but further dilutions were made in culture medium. A549 cells were cultured in 96 well plates at a density of 4000cells/well in Ham's F-12 medium (Hi Media) supplemented with 10% FBS. Once the cells attached to 60–70% confluence, medium was aspirated off and replaced with medium containing desired concentration of each drug and 2% FBS. Each drug concentration was taken in triplicates. Cells treated only with medium were used as negative controls. Plates were incubated at 37 °C with 5% CO_2. At 24 h post drug treatment, the cell viability was measured by MTT assay [30]. In brief, cells in each well were incubated with 20 μl of MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide) reagent at a concentration of 5 mg/ml in methanol for 4hr. Then, MTT reagent was removed and the resulting formazan crystals were solubilized in 200 μl of DMSO. The quantity of formazan which is directly proportional to the number of cells viable was measured by recording absorbance at wavelength of 560 nm. The percentage of viability was calculated taking negative controls into account and plotted against the concentration of the drug. IC_{50} value for each compound was determined by linear interpolation of the drug concentration and the corresponding percentage of viability [31] using the given formula: IC_{50} = (50% − Low inhibition %)/(High inhibition% − Low inhibition %) X (HighConc−Low Conc) + Low Conc..

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.05.013.

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