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Short communication

Synthesis, characterization, antiamoebic activity and cytotoxicity of novel 2-(quinolin-8-yloxy) acetohydrazones and their cyclized products (1,2,3-thiadiazole and 1,2,3-selenadiazole derivatives)

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ABSTRACT

A series of 1,2,3-thiadiazole and 1,2,3-selenadiazole derivatives were synthesized by the cyclization of novel 2-(quinolin-8-yloxy) acetohydrazones. *In vitro* antiamoebic activity was performed against HM1: IMSS strain of *Entamoeba histolytica*. The results showed that all the 2-(quinolin-8-yloxy) acetohydrazones were more active than their cyclized products (1,2,3-thiadiazole and 1,2,3-selenadiazole derivatives). SAR showed that the compounds having quinoline ring and hydrazone linkage with free N–H group are responsible for higher antiamoebic activity. The cytotoxic studies of these compounds on human breast cancer MCF-7 cell line showed that all the compounds were nontoxic at the concentration range of 1.56–50 μM.

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1. Introduction

Amoebiasis is the second leading cause of death from parasitic disease worldwide [1]. *Entamoeba histolytica*, the causative organism of amoebiasis, is a protozoan parasite which affects two main organ systems in human body: the gastrointestinal tract and the liver. Gastrointestinal involvement occurs as a result of ingestion of the cysts of the parasite from food or water contaminated with faeces. The cysts are digested in the intestinal lumen releasing trophozoites [2]. Occasionally these trophozoites penetrate through to the portal circulation, reaching the liver and brain and result in the formation of abscesses which could be life threatening [3]. Metronidazole is known to be a highly effective amoebicide and is considered to be the drug of choice for the treatment of amoebiasis, but recent studies have shown that this drug has several toxic effects such as genotoxicity, gastric mucus irritation, and spermatozoid damage [4,5]. Furthermore, failures in the treatment of several intestinal protozoan parasites may result from drug resistant to parasites [6,7]. Therefore, it is especially necessary to search constantly for new molecules for the treatment of amoebiasis, and to diminish and/or eliminate the use of metronidazole and other toxic drugs.

It is well known that nitrogen, oxygen, sulfur and selenium containing heterocycles possess different pharmacophoric properties. Among these type of molecules, quinolinyl hydrazones, thiadiazoles and selenadiazoles have shown various important biological activities such as antimalarial, antitubercular, antitumor, antibacterial, anticancer, CNS depressant, anticonvulsant, molluscicidal, analgesic, anti inflammatory and anti HIV [8–21]. In our earlier studies, we have reported the synthesis and antiamoebic activity of different heterocyclic compounds such as thiosemicarbazones, pyrazolines and dioxazoles which showed very promising results [22–25].

Considering the fact that nearly all classes of heterocyclic compounds are biologically active and as a part of our continuous efforts towards the development of more potent amoebicidal agents, we herein report the synthesis, characterization, antiamoebic activity and cytotoxicity of novel 2-(quinolin-8-yloxy) acetohydrazones **2**–**7** and their cyclized products, 1,2,3-thiadiazole and 1,2,3-selenadiazole derivatives **8**–**19**.

2. Chemistry

The synthesis of 1,2,3-thiadiazole and 1,2,3-selenadiazole derivatives **8**–**19** was performed by the cyclization of corresponding

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2-(quinolin-8-yloxy) acetohydrazones **2**–**7** on treatment with selenium dioxide/acetic acid and thionyl chloride in a manner as outlinedin Scheme 1. The probable mechanism for cyclization is well known [26]. All the compounds were insoluble in water but soluble in most of the organic solvents. Melting points were recorded on KSW melting point apparatus and are uncorrected. All the compounds **2**–**19** were characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectra. The purity of the compounds was confirmed by elemental analysis and data was found in accordance with ±0.3%.

3. Pharmacology

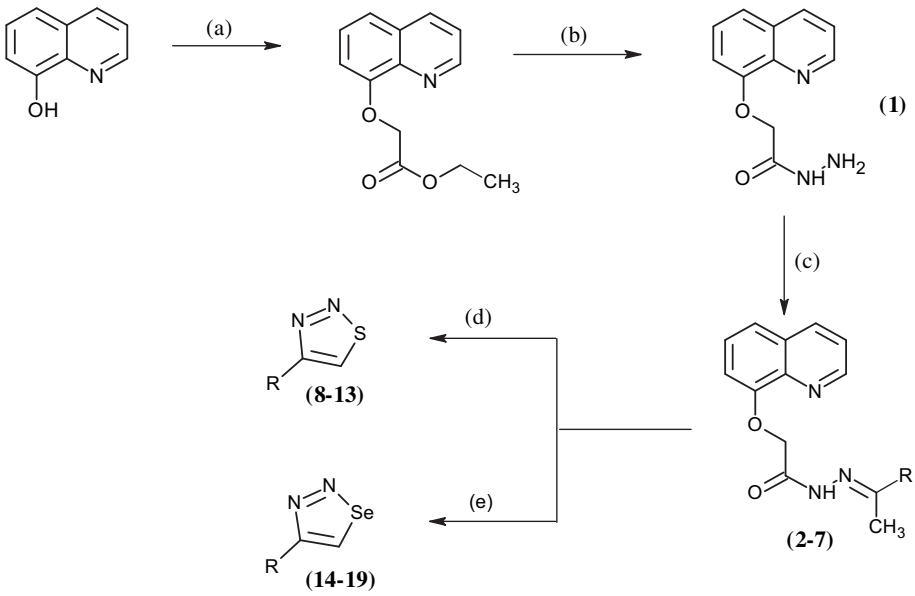
All the synthesized compounds **2**–**19** were screened *in vitro* against HM1: IMSS strain of *E. histolytica* by microdilution method [27]. All the experiments were carried out in triplicate at each concentration level and repeated thrice. Cytotoxicity of compounds **2**–**7**, **9**, **10**, **12**, **16**, **17** and metronidazole were studied by MTT assay on human breast cancer MCF-7 cell line. The results of biological activity and cytotoxicity are summarized in Tables 1 and 2.

4. Results and discussion

4.1. Synthesis

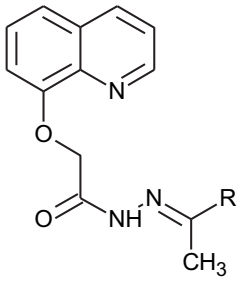
The 1,2,3-thiadiazole and 1,2,3-selenadiazole derivatives **8**–**19** were prepared by the cyclization of novel 2-(quinolin-8-yloxy) acetohydrazones **2**–**7** as shown in Scheme 1. The starting material, 2-(quinolin-8-yloxy) acetohydrazide **1** was synthesized according to a known method [28]. The key intermediates, 2-(quinolin-8-yloxy) acetohydrazone derivatives **2**–**7** were synthesized by the condensation of 8-quinolinoxyacetic acid hydrazide with different aromatic ketones. The cyclization of corresponding 2-(quinolin-8-yloxy) acetohydrazone derivatives **2**–**7** on treatment with thionyl chloride gave 1,2,3-thiadiazoles **8**–**13**, whereas the same 2-(quinolin-8-yloxy) acetohydrazone derivatives **2**–**7** when treated with selenium dioxide/acetic acid gave the compounds 1,2,3-selenadiazoles **14**–**19**. All the compounds **2**–**19** were characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectra. The purity of the compounds was confirmed by elemental analysis and data was found in accordance with ±0.3%.

Characteristic IR spectra showed significant bands for the formation of 2-(quinolin-8-yloxy) acetohydrazone derivatives **2**–**7** where the appearance of two characteristic bands at 3137–3268 cm^{-1} and 1668–1691 cm^{-1} were assigned to the N–H and C=O stretching and the band at 1621–1646 cm^{-1} due to (C=N) suggested the condensation of different aromatic ketones with 2-(quinolin-8-yloxy) acetohydrazide **1**. The symmetric and asymmetric C–H stretching modes of methyl group appeared as a shoulder just below 3000 cm^{-1} in all the 2-(quinolin-8-yloxy) acetohydrazone derivatives **2**–**7**, which was found absent in case of compounds **8**–**19**, revealed their formation. The appearance of bands at 1538–1595 cm^{-1} (C=C) and 1447–1474 cm^{-1} (N=N) also showed the formation of 1,2,3-thiadiazole and 1,2,3-selenadiazole rings in all the compounds **8**–**19**. The structures of the compounds **2**–**7** and **8**–**19** were further confirmed by ^{1}H NMR and ^{13}C NMR. The NMR spectra of all the compounds were recorded in $CDCl_3$ and that also favors the proposed structures. In all the compounds **2**–**7**, (N–H) protons appeared as singlet at δ 9.94–11.8 ppm and the appearance of two doublets in the range of 8.90–8.96 ppm and 8.21–8.32 ppm corresponds to the C-2 and C-4 protons of quinoline moiety. Further, the protons of methyl group of substituted aromatic ketones appeared at δ 2.40–2.48 ppm (Fig. 1). The structures of **2**–**7** were further supported by ^{13}C NMR spectra. The disappearance of ketonic carbon (C=O) signal at 191 ppm confirmed the formation of 2-(quinolin-8-yloxy) acetohydrazone derivatives **2**–**7**. The formation of 1,2,3-thiadiazole and 1,2,3-selenadiazole rings was also supported by the absence of signals at 2.44 and 10.9 ppm due to $-CH_3$ and (–N=NH–) respectively in all the compounds **8**–**19**. The singlets at 8.51–8.67 and 9.27–9.47 ppm, which arised due to (C=C–H) group present at C-5′ of the 1,2,3-thiadiazole and 1,2,3-selenadiazole rings (Fig. 2), confirmed the cyclization of 2-(quinolin-8-yloxy) acetohydrazons **2**–**7** into 1,2,3-thiadiazole **8**–**13** and 1,2,3-selenadiazole rings **14**–**19**. In addition, the signals for aromatic region appeared in the range of 6.67–7.99 ppm for all the compounds.



Scheme 1. General method for the synthesis of 2-(quinolin-8-yloxy) acetohydrazone (**2**–**7**), 1,2,3-thiadiazole (**8**–**13**) and 1,2,3-selenadiazole (**14**–**19**) derivatives. Reagent and conditions: (a) Ethyl chloroacetate, Acetone, K_2CO_3, Reflux (b) $NH_2NH_2 \cdot 2H_2O$, C_2H_5OH, reflux 3 h (c) $RCOCH_3$, C_2H_5OH, reflux 4 h (d) 0–5 °C, $SOCl_2$, Stirring at room temp. 24 h. (e) SeO_2, Glacial acetic acid, (R = Aryl group of different ketones).

Table 1
2-(Quinolin-8-yloxy) acetohydrazones (**2**–**7**), their *in vitro* antiamoebic activity against HM1: IMSS strain of *E. histolytica* and cytotoxicity profile.



(2-7)

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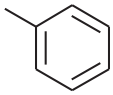
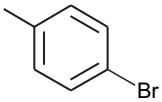
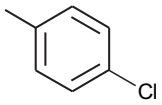
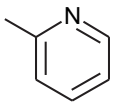
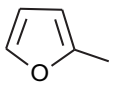
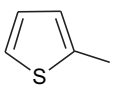
Compound	R	Antiamoebic activity		Cytotoxicity profile	
		IC50 (μM)	SD[a]	IC50 (μM)	SD[a]
2		**0.03**	0.12	>100	0.11
3		**0.04**	0.08	>100	0.24
4		**0.04**	0.05	>100	0.18
5		**0.05**	0.06	>100	0.14
6		**0.05**	0.09	>100	0.11
7		**0.08**	0.16	>100	0.21
Metronidazole		**1.80**	0.13	>100	0.16

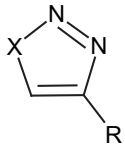
The compound with bold font IC50 values are more active than metronidazole.
[a] Standard deviation, N.D. = Not Done.

The structures of the compounds **8**–**19** were further supported by ^{13}C NMR spectra. The appearance of characteristic signals for C-4′ and C-5′ carbon atoms in the range of 161.1–162.8 ppm and 133.2–134.8 ppm clearly favored the formation of 1,2,3-thiadiazole and 1,2,3-selenadiazole rings in all the compounds **8**–**19**. The signals due to the aromatic carbons resonate at their usual positions and the values are given in the Experimental section.

4.2. Antiamoebic activity

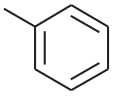
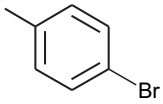
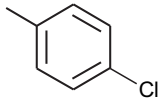
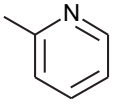
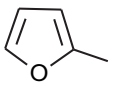
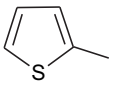
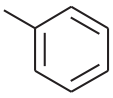
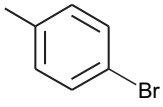
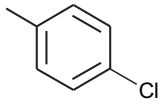
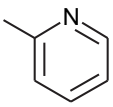
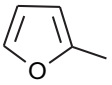
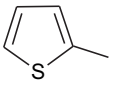
All the compounds **2**–**19** were screened *in vitro* for antiamoebic activity against HM1: IMSS strain of *E. histolytica* by the microdilution method. *E. histolytica* trophozoites were cultured in TYIS-33 growth medium in 96-well microtitre plate. The test compounds (1 mg) were dissolved in DMSO (40 μl). The maximum concentrations of DMSO in the test did not exceed 0.25%, at which level no inhibition of amoebal growth occurred. The stock solutions of the compounds were prepared freshly before use at a concentration of 1 mg/ml. The IC50 values in μM are given in Tables 1 and 2. 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. The IC50 and 95% confidence limits were interpolated in the corresponding dose–response curve. Metronidazole was used as the reference

Table 2
1,2,3-Thiadiazoles (**8**–**13**) and 1,2,3-selenadiazoles (**14**–**19**), their *in vitro* antiamoebic activity against HM1: IMSS strain of *E. histolytica* and cytotoxicity profile of compounds **9**, **10**, **12**, **16**, **17** and metronidazole.



X= S (8-13)

X= Se (14-19)

Compound	R	X	Antiamoebic activity		Cytotoxicity profile	
			IC50 (μM)	SD[a]	IC50 (μM)	SD[a]
8		S	7.46	0.18	N.D	N.D
9		S	**0.24**	0.05	68.11	0.18
10		S	**0.39**	0.08	>100	0.29
11		S	5.46	0.04	N.D	N.D
12		S	**0.23**	0.13	95.07	0.27
13		S	2.25	0.06	66.90	0.25
14		Se	4.56	0.07	N.D	N.D
15		Se	2.95	0.04	N.D	N.D
16		Se	**0.58**	0.11	57.05	0.13
17		Se	**0.46**	0.05	86.62	N.D
18		Se	5.72	0.04	N.D	N.D
19		Se	5.28	0.02	N.D	N.D
Metronidazole			**1.80**	0.13	>100	0.16

The compound with bold font IC50 values are more active than metronidazole.
[a] Standard deviation, N.D. = Not Done.

drug which had a 50% inhibitory concentration of 1.80 μM in our experiments. The 2-(quinolin-8-yloxy) acetohydrazone derivatives **2**–**7** showed an IC50 value in the range 0.03–0.08 μM. However, the cyclized compounds **8**–**19** showed a decreased antiamoebic activity in the range of 0.23–7.46 μM. Among all the 1,2,3-thiadiazole and 1,2,3-selenadiazole derivatives **8**–**19**, the compounds having 4-chloro phenyl (**9**, IC50 = 0.24 μM), 4-bromo phenyl (**10**, IC50 = 0.39 μM; **16**, IC50 = 0.58 μM), furan ring (**12**, IC50 = 0.23 μM) and thiophene ring (**17**, IC50 = 0.46 μM) substituted at **C-4** position of 1,2,3-thia and selenadiazole rings were distinctly more active. Compounds **9** and **12** are the most active among the azole series **8**–**19**. In terms of structural activity relationship it was concluded that among the whole series of compounds **2**–**19**, all the 2-(quinolin-8-yloxy) acetohydrazone derivatives **2**–**7** showed excellent antiamoebic activity compared to the cyclized products **8**–**19**. This may be due to the presence of quinoline nucleus and hydrazone linkage [–NH–N=C(CH_3)–R] which resembles Idoquinol, currently used as an antiamoebic drug [29] and quinoline based hydrazones have shown antimalarial and antitubercular activity [19–21]. Many natural product bearing quinoline nucleus such as quinidine, quinidinone, and quinine from the plant *Cinchona ledgeriana* were also found to be antiamoebic [30].

The considerable loss of antiamoebic activity displayed in the 1,2,3-thiadiazoles and 1,2,3-selenadiazoles is attributed to the loss of active quinoline nucleus in the conversion of hydrazone linkage bearing a free N–H group to a ring constrained structures

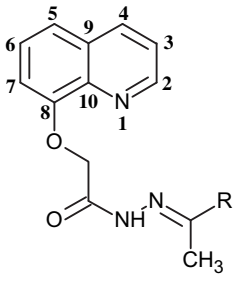
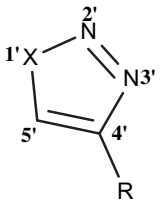


Fig. 1. Structure of 2(quinolin-8-yloxy) acetohydrazones (**2**–**7**).



X = S, Se

Fig. 2. Structure of 1,2,3-thiadiazole and 1,2,3-selenadiazole derivatives (**8**–**19**).

8–**19**. All the biologically active hydrazones possess a free N–H group in their structure [31]. Therefore it can be concluded that a quinoline nucleus with a hydrazone linkage [–NH–N=C(CH_3)–R] bearing a free N–H group is responsible for higher antiamoebic activity.

4.3. Cytotoxicity profile

To examine the effect of antiamoebic compounds **2**–**7**, **9**, **10**, **12**, **16**, **17** and metronidazole on cell proliferation, we studied their cytotoxicity on human breast cancer MCF-7 cell line. A subconfluent population of MCF-7 cells was treated with increasing concentrations of compounds and the number of viable cells was measured after 48 h by MTT cell viability assay based on mitochondrial reduction of the yellow MTT tetrazolium dye to a highly coloured blue formazone product. This assay usually shows high correlation with number of living cells and cell proliferation. The concentration range for all the compounds was 1.56–100 μM. Fig. 3 depicts the compounds **2**–**6** and metronidazole exhibited 100% viability at the concentration range of 1.56–100 μM whereas the compound **7** showed 88% viability at 100 μM. These results mean that all the compounds **2**–**7** and metronidazole were nontoxic against the human breast cancer MCF-7 cell line. Compounds **9**, **10**, **12**, **17** and metronidazole exhibited >80% viability (Fig. 4); compound **16** showed 66% viability at the concentration range of 1.56–50 μM (Fig. 4). On increasing the concentration range up to 100 μM only compounds **9** and **16** (Viability 68% and 57%) showed moderate cytotoxicity against the breast cancer MCF-7 cell line. The cytotoxicity IC50 values along with the standard deviation values of compounds **2**–**7**, **9**, **10**, **12**, **16**, **17** and metronidazole are given in Tables 1 and 2.

5. Conclusion

This research involves the synthesis of 2-(quinolin-8-yloxy) acetohydrazone, 1, 2, 3-thiadiazole and 1,2,3-selenadiazole derivatives **2**–**19**. The antiamoebic activity of all these compounds was examined using HM1: IMSS strain of *E. histolytica* and the results showed that the compounds **2**–**7** having quinoline nucleus with a hydrazone linkage [–NH–N=C(CH_3)–R] bearing a free N–H group exhibited higher antiamoebic activity as compared with derived cyclized products **8**–**19**. SAR studies showed that the considerable loss of antiamoebic activity displayed in the 1,2,3-thiadiazole and 1,2,3-selenadiazole derivatives **8**–**19** is attributed to the loss of 2-(quinolin-8-yloxy) acetohydrazones moiety. The MTT assay results showed that all the compounds were nontoxic. Only compounds **9** and **16** showed moderate cytotoxicity against the human breast cancer MCF-7 cell line at a concentration of 100 μM.

6. Experimental protocol

All the chemicals were purchased from 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. Elemental analyses were performed on Heraeus Vario EL III analyzer the results were within ±0.3% of the theoretical values. IR spectra on KBr disks were recorded on a Perkin Elmer model 1620 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Spectrospin DPX 300 MHz and Bruker Spectrospin DPX 75 MHz spectrometer respectively using $CDCl_3$ as a solvent and trimethylsilane (TMS) 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. The FAB mass spectra of the compounds were recorded on JEOL SX 102/DA-6000 mass spectrometer using Argon/Xenon 6 KV, 10 mA as the FAB gas and m-nitrobenzyl alcohol (NBA) was used as the matrix.

6.1. Preparation of 2-(quinolin-8-yloxy) acetohydrazide (*1*)

2-(Quinolin-8-yloxy) acetohydrazide was prepared by a reported method [27].

6.2. Synthesis of 2-(quinolin-8-yloxy) acetohydrazones (*2*–*7*): general procedure

A mixture of 2-(quinolin-8-yloxy) acetohydrazide **2** (10 mmol) and appropriate aromatic ketone (10 mmol) in absolute ethanol

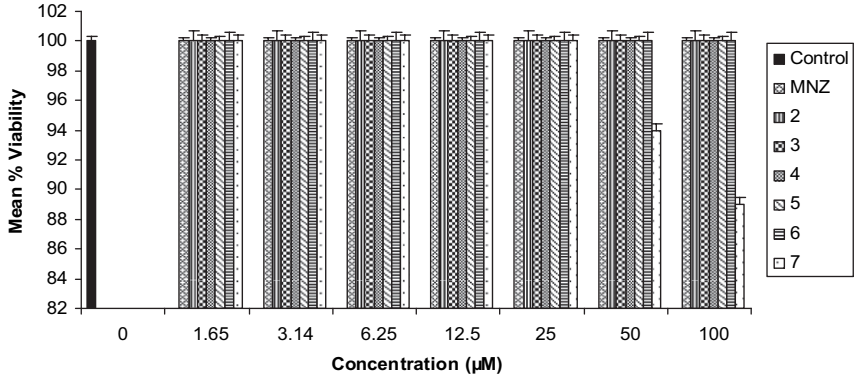


Fig. 3. Percentage of viable cells after 48 h pre-treatment of human breast cancer MCF-7 cells with compounds **2**, **3**, **4**, **5**, **6**, **7** and metronidazole (MNZ), evaluated by MTT assay.

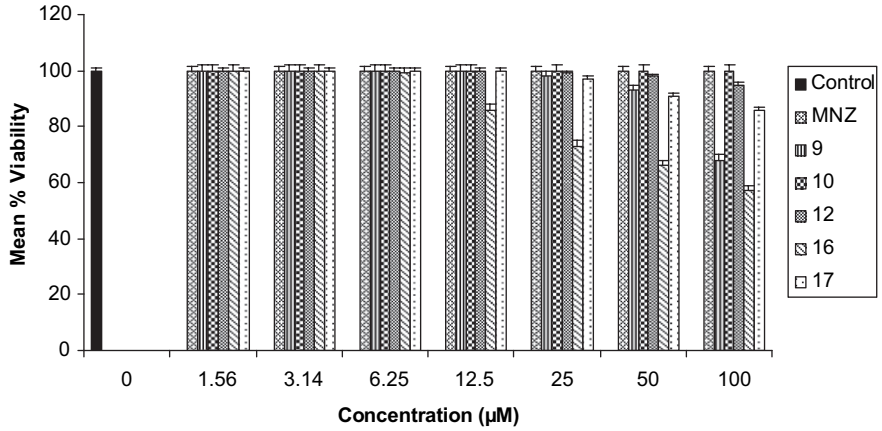


Fig. 4. Percentage of viable cells after 48 h pre-treatment of human breast cancer MCF-7 cells with compounds **9**, **10**, **12**, **16**, **17** and metronidazole (MNZ), evaluated by MTT assay.

(50 ml) was refluxed for 4 h with continuous stirring. After cooling, the solid was filtered and recrystallized from appropriate solvent.

*6.2.1. N′-[(1E)-1-Phenylethylidene]-2-(quinolin-8-yloxy) acetohydrazone (**2**)*

Yield 82%; m.p. 189 °C; Anal. calc. for $C_{19}H_{17}N_3O_2$: C 71.46, H 5.37, N 13.16%; found: C 71.38, H 5.29, N 13.11%; IR ν_{max} (cm^{-1}): 3249 (NH), 3023 (Ar-H), 2912, 2827 (C—H), 1668 (C═O), 1626 (C═N), 1545 (C═C); ^{1}H NMR ($CDCl_3$) δ (ppm): 9.94 (s, 1H, NH), 8.95 (d, 1H, $J = 4.1$ Hz, quinoline ring C-2), 8.24 (d, 1H, $J = 8.3$ Hz, quinoline ring C-4), 7.64–6.96 (m, 9H, quinoline and phenyl ring), 4.95 (s, 2H, CH2), 2.44 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 165.66 (C═O), (149.51, 147.32, 136.35, 135.12, 127.23, 122.54, 121.55 quinoline and phenyl ring), 70.56 (CH_2), 11.87 (methyl); FAB-MS (*m*/*z*): 319 (M^+, 100%), 320 ($M^+ + 1$, 65%), 319 ($M^+ - CH_3$, 28%).

*6.2.2. N′-[(1E)-1-(4-bromophenyl) ethylidene]-2-(quinolin-8-yloxy) acetohydrazone (**3**)*

Yield 76%; m.p: 189 °C; Anal. calc. for $C_{19}H_{16}N_3O_2Br$: C 56.27, H 3.67, N 10.94%; found: C 56.19, H 3.62, N 10.88%; IR ν_{max} (cm^{-1}): 3165 (NH), 3065 (Ar-H), 2987, 2834 (C—H), 1672 (C═O), 1628 (C═N), 1546 (C═C); ^{1}H NMR ($CDCl_3$) δ (ppm): 10.5 (s, 1H, NH), 8.94 (d, 1H, $J = 4.2$ Hz, quinoline ring C-2), 8.22 (d, 1H, $J = 8.3$ Hz, quinoline ring C-4), 7.66–6.97 (m, 8H, quinoline and phenyl ring), 4.94(s, 2H, CH_2), 2.42 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 165.23 (C═O), (149.21, 147.32, 136.86, 135.23, 126.83, 121.93 quinoline and phenyl ring), 70.12 (CH_2), 11.93 (methyl); FAB-MS (*m*/*z*): 398 (M^+, 100%), 399 ($M^+ + 1$, 74%).

*6.2.3. N′-[(1E)-1-(4-chlorophenyl) ethylidene]-2-(quinolin-8-yloxy) acetohydrazone (**4**)*

Yield 78%; m.p: 171 °C; Anal. calc. for $C_{19}H_{16}N_3O_2Cl$: C 63.63, H 4.15, N 12.37%; found: C 63.57, H 4.09, N 12.31%; IR ν_{max} (cm^{-1}): 3246 (NH), 3035 (Ar-H), 2954, 2842 (C—H), 1682 (C═O), 1621 (C═N), 1535 (C═C); ^{1}H NMR ($CDCl_3$) δ (ppm): 10.3 (s,1H, NH), 8.92 (d, 1H, $J = 4.3$ Hz, quinoline ring C-2), 8.21(d, 1H, $J = 8.4$ Hz, quinoline ring C-4), 7.63–6.97 (m, 8H, quinoline and phenyl ring), 4.93 (s, 2H, CH_2), 2.45 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 164.98 (C═O), (149.86, 148.68, 135.43, 128.68, 125.12, 122.13 quinoline and phenyl ring), 70.58 (CH_2), 12.16 (methyl); FAB-MS (*m*/*z*): 353 (M^+, 100%), 354 ($M^+ + 1$, 5%), 338 ($M^+ - CH_3$, 43%).

*6.2.4. N′-[(1E)-1-(Pyridin-2-yl) ethylidene]-2-(quinolin-8-yloxy) acetohydrazone (**5**)*

Yield 87%; m.p: 188 °C; Anal. calc. for $C_{18}H_{16}N_4O_2$: C 67.49, H 5.03, N 17.49%; found: C 67.36, H 5.07, N 17.38%; IR ν_{max} (cm^{-1}): 3137 (NH), 3097 (Ar-H), 2921, 2844 (C—H), 1686 (C═O), 1624 (C═N), 1579 (C═C); ^{1}H NMR ($CDCl_3$) δ (ppm): 11.08 (s,1H, NH), 8.96 (d, 1H, $J = 4.1$ Hz, quinoline ring C-2), 8.32 (d, 1H, $J = 8.1$ Hz, quinoline ring C-4), 8.24–7.25 (m, 8H, quinoline and pyridine ring), 4.92 (s,2H, CH_2), 2.40(s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 165.32 (C═O), (149.79, 148.44, 136.31, 129.64, 126.84, 122.14, 121.55 quinoline and pyridine ring), 70.46 (CH_2), 11.85 (methyl); FAB-MS (*m*/*z*): 320 (M^+, 100%), 321 ($M^+ + 1$, 82%).

*6.2.5. N′-[(1E)-1-(Furan-2-yl) ethylidene]-2-(quinolin-8-yloxy) acetohydrazone (**6**)*

Yield 78%; m.p: 176 °C; Anal. calc. for $C_{17}H_{15}N_3O_3$: C 66.01, H 4.89, N 13.58%; found: C 66.04, H 4.72, N 13.46%; IR ν_{max}(cm^{-1}): 3268 (NH), 3107 (Ar-H), 2972, 2851 (C—H), 1675 (C═O), 1646 (C═N), 1542 (C═C); ^{1}H NMR ($CDCl_3$) δ (ppm): 10.8 (s,1H, NH), 8.90 (d, 1H, $J = 4.2$ Hz, quinoline ring C-2), 8.22 (d, 1H, $J = 8.4$ Hz, quinoline ring C-4), 7.53–6.42 (m, 7H, quinoline and furan ring), 4.94 (s, 2H, CH_2), 2.48 (s, 3H, CH3); ^{13}C NMR ($CDCl_3$) δ (ppm): 164.77 (C═O), (149.64, 145.88, 136.36, 135.12, 129.61, 122.17 quinoline and furan ring), 70.40 (CH_2), 12.86 (methyl); FAB-MS (*m*/*z*): 309 (M^+, 100%), 310 ($M^+ + 1$, 68%), 294 ($M^+ - CH_3$, 28%).

*6.2.6. 2-(Quinolin-8-yloxy) N′-[(1E)-1-(thiophen-2-yl) ethylidene]-acetohydrazone (**7**)*

Yield 81%; m.p: 164 °C; Anal. calc. for $C_{17}H_{15}N_3O_2S$: C 61.72, H 4.21, N 13.50%; found: C 61.64, H 4.17, N 13.41%; IR ν_{max}(cm^{-1}): 3248 (NH), 3062 (Ar-H), 2972, 2863 (C-H), 1691 (C═O), 1626 (C═N), 1552 (C═C); ^{1}H NMR ($CDCl_3$) δ (ppm): 10.8 (s, 1H, NH), 8.90 (d, 1H, $J = 4.2$ Hz, quinoline ring C-2), 8.22 (d, 1H, $J = 8.1$ Hz, quinoline ring C-4), 7.54–7.01 (m, 7H, quinoline and thiophene ring), 4.98 (s, 2H, CH2), 2.44 (s, 3H, CH3); ^{13}C NMR ($CDCl_3$) δ (ppm): 165.93 (C═O), (149.28, 148.72, 134.86, 132.23, 127.83, 121.93 quinoline and thiophene ring), 70.42 (CH2), 12.03 (methyl); FAB-MS (*m*/*z*): 325 (M^+, 100%), 326 ($M^+ + 1$, 42%), 319 ($M^+ - CH_3$, 21%).

*6.3. Synthesis of 1,2,3-thiadiazole derivatives (**8**–**13**): general procedure*

All the 1,2,3-thiadiazole derivatives were synthesized by the cyclization of 2-(quinolin-8-yloxy) acetohydrazones **2**–**7** (5 mmol) in an excess amount of thionyl chloride (10 ml) at 0–5 °C for 30 min and then stirred at room temperature overnight until no more hydrogen chloride was produced. The remaining thionyl chloride was evaporated under vacuum to afford the crude title compounds **8**–**13**, which were further purified by column chromatography using 7:3 hexane: ethylacetate as eluent [32].

*6.3.1. 4-Phenyl-[1,2,3]-thiadiazole (**8**)*

Yield: 69%; mp: 162 °C; Anal. calc. for $C_8H_6N_2S$: C 59.23, H 3.73, N 17.27%; found: C 59.12, H 3.67, N 17.19%; IR ν_{max} (cm^{-1}): 3018 (Ar–H), 1550 (C=C), 1447(N=N); ^1H NMR ($CDCl_3$) δ (ppm): 8.67 (s, 1H, thiadiazole), 7.68–7.65 (m, 2H, Ar-H), 7.45–7.28 (m, 3H, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 161.5 (C-4′, thiadiazole), 134.7 (C-5′, thiadiazole), 130.5, 129.2 128.6, 126.6 (Ar-C); FAB-MS (*m*/*z*): 162 (M^+, 100%), 163 ($M^+ + 1$, 37%).

*6.3.2. 4-(4-Bromophenyl)-[1,2,3]-thiadiazole (**9**)*

Yield: 55%; mp: 123 °C; Anal. calc. for $C_8H_5N_2SBr$: C 39.85, H 2.09, N 11.62%; found: C 39.74, H 2.16, N 11.57%; IR ν_{max} (cm^{-1}): 3087 (Ar–H), 1542 (C=C), 1454 (N=N); ^1H NMR ($CDCl_3$) δ (ppm): 8.54 (s, 1H, thiadiazole), 7.66–7.63 (m, 2H, Ar-H), 7.41–7.39 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 162.5 (C-4′, thiadiazole), 133.2 (C-5′, thiadiazole), 130.4, 129.7, 128.2, 126.5 (Ar-C); FAB-MS (*m*/*z*): 241(M^+, 100%), 242($M^+ + 1$, 22%), 161 ($M^+ - Br$, 5%).

*6.3.3. 4-(4-Chlorophenyl)-[1,2,3]-thiadiazole (**10**)*

Yield: 62%; mp: 113 °C; Anal. calc. for $C_8H_5N_2SCl$: C 48.86, H 2.56, N14.24%; found: C 48.77, H 2.49, N 14.18%; IR ν_{max} (cm^{-1}): 3086 (Ar–H), 1551 (C=C), 1457 (N=N); ^1H NMR ($CDCl_3$) δ (ppm): 8.56 (s,1H, thiadiazole), 7.79–7.81 (m, 2H, Ar-H), 7.61–7.48 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 162.6 (C-4′, thiadiazole), 134.2 (C-5′, thiadiazole), 130.2, 129.6 128.7, 127.1126.6 (Ar-C); FAB-MS (*m*/*z*): 196 (M^+, 100%), 197 ($M^+ + 1$, 24%), 198 ($M^+ + 2$, 32%), 192 ($M^+ - 4$, 98%).

*6.3.4. 2-(1,2,3-Thiadiazole-4-yl)pyridine (**11**)*

Yield: 38%; mp: 163 °C; Anal. calc. for $C_7H_5N_3S$: C 51.52, H 3.09, N 25.75%; found: C 51.41, H 3.12, N 25.67%; IR ν_{max} (cm^{-1}): 3011 (Ar–H), 1544 (C=C), 1471 (N=N); ^1H NMR ($CDCl_3$) δ (ppm): 8.52 (s, 1H, thiadiazole), 8.03 (d, 1H, $J = 7.1$ Hz, Ar-H), 7.95 (d, 1H, $J = 7.1$ Hz, Ar-H), 7.59–6.31 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 162.6 (C-4′, thiadiazole), 134.3 (C-5′, thiadiazole), 128.6, 124.7, 123.7, 126.8 (Ar-C); FAB-MS (*m*/*z*): 163 (M^+, 100%), 164 ($M^+ + 1$, 28%).

*6.3.5. 4-(Furan-2-yl)-[1,2,3]-thiadiazole (**12**)*

Yield: 35%; mp: 178 °C; Anal. calc. for $C_6H_4N_2OS$: C 47.36, H 2.65, N 18.41%; found: C 47.28, H 2.57, N 18.29%; IR ν_{max} (cm^{-1}): 3075 (Ar–H), 1546 (C=C), 1456 (N=N); ^1H NMR ($CDCl_3$) δ (ppm): 8.58 (s, 1H, thiadiazole), 7.26–6.13 (m, 2H, Ar-H), 6.37 (d, 1H, $J = 3.3$ Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 162.2 (C-4′, thiadiazole), 133.6 (C-5′, thiadiazole), 121.6, 117.6, 109.4 (Ar-C); FAB-MS (*m*/*z*): 152 (M^+, 100%), 153 ($M^+ + 1$, 46%).

*6.3.6. 4-(Thiophen-2-yl)-[1,2,3]-thiadiazole (**13**)*

Yield: 43%; mp: 189 °C; Anal. calc. for $C_6H_4N_2S_2$: C 42.83, H 2.40, N 16.65%; found: C 42.74, H 2.29, N 16.52%; IR ν_{max} (cm^{-1}): 3063 (Ar–H), 1548 (C=C), 1471 (N=N); ^1H NMR ($CDCl_3$) δ (ppm): 8.51 (s, 1H, thiadiazole), 7.66 (d, 1H, $J = 4.2$ Hz, Ar-H), 7.42 (d, 1H, $J = 4.8$ Hz, Ar-H), 7.12 (t, 1H, $J = 3.6$ Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 161.8 (C-4′, thiadiazole), 134.8 (C-5′, thiadiazole), 131.4, 129.3, 128.9, 126.4, 117.4 (Ar-C); FAB-MS (*m*/*z*): 168 (M^+, 100%), 169 ($M^+ + 1$, 22%).

*6.4. Synthesis of 1,2,3-selenadiazole derivatives (**14**–**19**): general procedure*

All the 1,2,3-selenadiazole derivatives were synthesized by dissolving of respective 2-(quinolin-8-yloxy) acetohydrazones **2**–**7** (5 mmol) in glacial acetic acid (15 ml) at room temperature and the resulting mixture was treated with SeO2 powder (6.5 mmol). The mixture was kept under vigorous stirring. After 2–3 min the color of the mixture becomes red. Monitoring of the reaction by TLC (eluent: 7:3 hexane:ethyl acetate) showed that the reaction was complete in 10–24 h. The mixture was filtered and the filtrates poured into ice water and extracted with chloroform. The combined organic layers were washed with water and dried over anhydrous sodium sulphate. The solvent was removed under vacuum to afford the crude title compounds **14**–**19**, which were further purified by column chromatography using 7:3 hexane:-ethylacetate as eluent [32].

*6.4.1. 4-Phenyl-[1,2,3]-selenadiazole (**14**)*

Yield: 66%; mp: 198 °C; Anal. calc. for $C_8H_6N_2Se$: C 45.95, H 2.89, N 13.40%; found: C 45.88, H 2.82, N 13.29%; IR ν_{max} (cm^{-1}): 3090 (Ar–H), 1542 (C=C), 1474 (N=N); ^1H NMR ($CDCl_3$) δ (ppm): 9.27 (s, 1H, selenadiazole), 7.57–7.54 (m, 2H, Ar-H), 7.38–7.34 (m, 3H, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 163.6 (C-4′, selenadiazole), 134.8 (C-5′, selenadiazole), 130.6, 129.2, 129.1, 128.3, 126.6 (Ar-C); FAB-MS (*m*/*z*): 209 (M^+, 100%), 210 ($M^+ + 1$, 18%).

*6.4.2. 4-(4-Bromophenyl)-[1,2,3]-selenadiazole (**15**)*

Yield: 56%; mp: 177 °C; Anal. calc. for $C_8H_5N_2BrSe$: C 33.36, H 1.75, N 9.73%; found: C 33.24, H 1.66, N 9.66%; IR ν_{max} (cm^{-1}): 3068 (Ar–H), 1558 (C=C), 1474 (N=N); ^1H NMR ($CDCl_3$) δ (ppm): 9.36 (s, 1H, selenadiazole), 7.78–7.69 (m, 2H, Ar-H), 7.45–7.24 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 162.8 (C-4′, selenadiazole), 134.3 (C-5′, selenadiazole), 130.5, 129.7, 128.3, 126.3 (Ar-C); FAB-MS (*m*/*z*): 288 (M^+, 100%), 289 (M^++1, 31%).

*6.4.3. 4-(4-Chlorophenyl)-[1,2,3]-selenadiazole (**16**)*

Yield: 62%; mp: 154 °C; Anal. calc. for $C_8H_5N_2ClSe$: C 39.45, H 2.07, N 11.50%; found: C 39.38, H 2.09, N 11.43%; IR ν_{max} (cm^{-1}): 3079 (Ar–H), 1595 (C=C), 1452 (N=N); ^1H NMR ($CDCl_3$) δ (ppm): 9.47 (s, 1H, selenadiazole), 8.02 (d, 2H, $J = 8.4$ Hz, Ar-H), 7.51–7.48 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 162.4 (C-4′, selenadiazole), 133.7 (C-5′, selenadiazole), 130.9, 129.5, 129.4, 128.6, 126.3 (Ar-C); FAB-MS (*m*/*z*): 243 (M^+, 100%), 244 ($M^+ + 1$, 48%).

*6.4.4. 2-(1,2,3-Selenadiazole-4-yl)pyridine (**17**)*

Yield: 43%; mp: 186 °C; Anal. calc. for $C_7H_5N_3Se$: C 40.02, H 2.40, N 20.00%; found: C 40.05, H 2.34, N 20.09%; IR ν_{max} (cm^{-1}): 3088 (Ar–H), 1565 (C=C), 1466 (N=N); ^1H NMR ($CDCl_3$) δ (ppm): 9.46 (s, 1H, selenadiazole), 8.04 (d, 1H, $J = 7.1$ Hz, Ar-H), 7.95 (d, 1H, $J = 7.1$ Hz, Ar-H), 7.54–7.33 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 161.1 (C-4′, selenadiazole), 134.2 (C-5′, selenadiazole), 129.4, 128.6, 127.7, 126.1 (Ar-C); FAB-MS (*m*/*z*): 210 (M^+, 100%), 211 ($M^+ + 1$, 42%).

*6.4.5. 4-(Furan-2-yl)-[1,2,3]-selenadiazole (**18**)*

Yield: 28%; mp: 177 °C; Anal. calc. for $C_6H_4N_2OSe$: C 36.20, H 2.03, N 14.07%; found: C 36.13, H 2.11, N 14.05%; IR ν_{max} (cm^{-1}): 3078 (Ar–H), 1538 (C=C), 1462(N=N); ^1H NMR ($CDCl_3$) δ (ppm): 9.32 (s, 1H, selenadiazole), 7.54–6.58 (m, 3H, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 162.8 (C-4′, selenadiazole), 133.8 (C-5′, selenadiazole), 129.8, 128.4, 126.1 (Ar-C); FAB-MS (*m*/*z*): 199 (M^+, 100%), 200 ($M^+ + 1$, 37%).

*6.4.6. 4-(Thiophen-2-yl)-[1,2,3]-selenadiazole (**19**)*

Yield: 28%; mp: 164 °C; Anal. calc. for $C_6H_4N_2SSe$: C 33.50, H 1.87, N 13.02%; found: C 33.48, H 1.76, N 13.07%; IR ν_{max} (cm^{-1}): 3090 (Ar–H), 1542 (C=C), 1474 (N=N); ^1H NMR ($CDCl_3$) δ (ppm): 9.30 (s, 1H, selenadiazole), 7.41 (d, 1H, $J = 4.8$ Hz, Ar-H), 7.16 (t, 2H, $J = 5.1$ Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ (ppm): 162.5 (C-4′, selenadiazole), 133.3 (C-5′, selenadiazole), 128.3, 127.5, 124.9, 104.5 (Ar-C). FAB-MS (*m*/*z*): 215 (M^+, 100%), 216 ($M^+ + 1$, 29%).

6.5. In vitro antiamoebic assay

All the compounds (**2**–**19**) were screened *in vitro* for antiamoebic activity against HM1: IMSS strain of *E. histolytica* by microdilution

method [27]. *E. histolytica* trophozoites were cultured in culture tubes by using Diamond TYIS-33 growth medium [33]. The test compounds (1 mg) were dissolved in DMSO (40 μl, level at which no inhibition of amoeba occurs) [30,34]. The stock solutions of the compounds were prepared freshly before use at a concentration of 1 mg/ml. Two-fold serial dilutions were made in the wells of 96-well microtiter plate (costar). Each test includes metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae) and a blank (culture medium only). All the experiments were carried out in triplicate at each concentration level and repeated thrice. The amoeba suspension was prepared from a confluent culture by pouring off the medium at 37 °C and adding 5 ml of fresh medium, chilling the culture tube on ice to detach the organisms from the side of the flask. The number of amoeba/ml was estimated with the help of a haemocytometer, using trypan blue exclusion to confirm the viability. The suspension was diluted to 10^5 organism/ml by adding fresh medium and 170 μl of this suspension was added to the test and control wells in the plate so that the wells were completely filled (total volume, 340 μl). An inoculum of 1.7×10^4 organisms/well was chosen so that confluent, but not excessive growth, took place in control wells. Plates were sealed and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h. After incubation, the growth of amoeba in the plate was checked with a low power microscope. The culture medium was removed by inverting the plate and shaking gently. Plate was then immediately washed with sodium chloride solution (0.9%) at 37 °C. This procedure was completed quickly and the plate was not allowed to cool in order to prevent the detachment of amoebae. The plate was allowed to dry at room temperature and the amoebae were fixed with methanol and when dried, stained with (0.5%) aqueous eosin for 15 min. The stained plate was washed once with tap water, then twice with distilled water and then allowed to dry. A 200 μl portion of 0.1 N sodium hydroxide solution was added to each well to dissolve the protein and release the dye. The optical density of the resulting 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 fitting line from which the IC50 value was found. The IC50 values in μM are reported in Tables 1 and 2.

6.6. MTT assay

MCF-7 cells were cultured and maintained as monolayer in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% of fetal calf serum (FCS), antibiotics: 100 IU/ml of penicillin and 100 μg/ml of streptomycin. All cells were cultured in flasks at 37 °C in the 100% humidity atmosphere and 5% of CO_2 [35]. Only viable cells were used in the assay. Exponentially growing cells were plated at 1.2×10^4 cells per well into 96-well plates and incubated for 48 h before the addition of drugs. Stock solutions of compounds were initially dissolved in 20% (v/v) DMSO and further diluted with fresh complete medium. The growth-inhibitory effects of the compounds were measured using standard tetrazolium MTT assay. After 48 h of incubation at 37 °C, the medium was removed and 5 μl of MTT reagent (5 mg/ml) in serum free medium was added to each well. The plates were incubated at 37 °C for 4 h. At the end of the incubation period, the medium was removed and pure DMSO (100 μl) was added to each well. The metabolized MTT product dissolved in DMSO was quantified by reading the absorbance at 570 nm. All assays were performed in triplicate. Percent viability was defined as the relative absorbance of treated versus untreated control cells. Plates were analyzed in an ELISA plate reader (Labsystems Multiskan RC, Helsinki, Finland) at 570 nm with a reference wavelength of 655 nm.

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