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Research Article

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Anticancer and antimicrobial activities of Imine containing compounds

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Abstract

A series of imine derivatives (3a-m) including thio-semicarbazone, semicarbazone, thiazole and oxazole functional moieties were examined for anti-cancer activity in-vitro by MTT assay using glioblastoma cell line (U87). Among all compound 3m was most potent compound with IC50 value of 8.86 ± 0.15 µM. Cytotoxicity of compounds were evaluated using normal human embryonic kidney cell line i.e HEK-293. Moreover, antibacterial activity of synthesized derivatives was also evaluated by disc diffusion method.

Introduction

Cancer, a diversified group of ailments characterized by uncontrolled growth of irregular cells. It is a fatal disease subsequent to the cardiac ailments in terms of morbidity and mortality. The challenges of cancer is significantly rising globally due to the increased life expectancy[1]. However, the investigations linked to cancer have lead towards numerous innovative and useful solutions, the medications used as the therapy of these diseases have clear constraints and regrettably cancer will be anticipated as the prominent reason of fatalities in future [2, 3].

Numerous ways to tumour cell resistance from conventional drugs involve variations in DNA cell cycling incidents. Latest fragments that particularly targets cell cycle events may overcome chemotherapeutic resistance, or possibly promote tumour cell death when consumed alone. A persistent research is needed to discover novel therapeutical agents that can be consumed in combination with immune therapies and biological agents to reduce the systemic diseases non-treatable by surgery or irradiation [4-8].

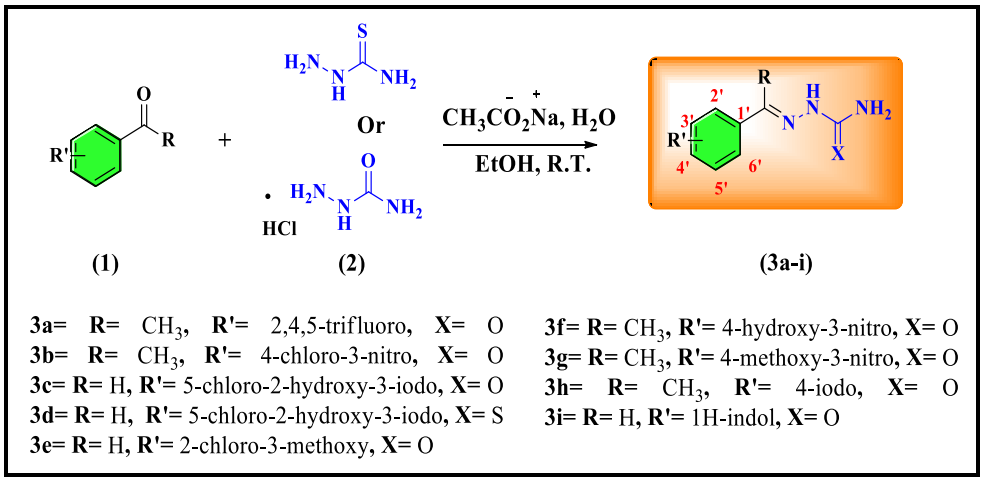
In spite of increased number of anticancer drugs, lack of attainment of multiple-drug resistance and selectivity symbolise considerable impediments to effective cancer treatment and, consequently, variety of efforts have been devoted to find new potent and selective anti-cancer agents [9-13].

The imine (C=N) containing compounds, obtained under certain conditions by the reaction of primary with amines an aldehyde or a ketone [14]. These own numerous biological activities, such as antifungal [15], antibacterial [16], antitumor [17], antimalarial, anti-pyretic [18] and anti-viral [14, 19, 20]. Several imine analogues including semicarbazide and thio-semicarbazide groups possessed anti-convulsant and anti-protozoal activities while few semicarbazones showed significant anticancer activity [21]. Oxazoles and thiazoles are heterocyclic imines having 5-membered unsaturated ring structure containing one nitrogen and an oxygen or a sulphur as hetero-atoms [22, 23] respectively.

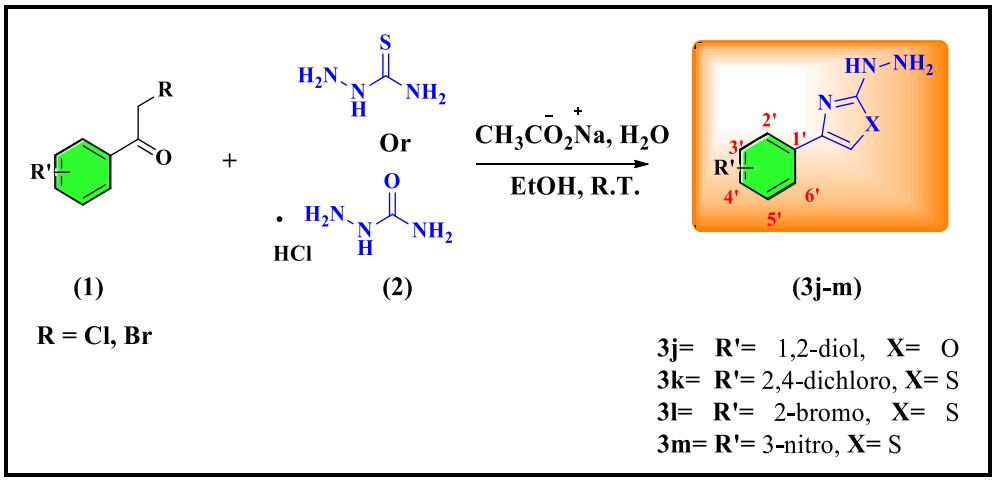
In current study, a series of synthesized imines containing thio-semicarbazone, semicarbazone, thiazole and oxazole moieties (3a-m) were assessed for anti-bacterial and anti-cancer activities.

Results and Discussion

A novel series of imine analogues (**3a-m**) comprising semicarbazone, thio-semicarbazone, thiazole and oxazole moieties were synthesized as displayed in Scheme 1 and 2 reported previously [24]. Thio-semicarbazone and semicarbazone analogues (**3a-i**) were synthesized by the reaction of substituted benzaldehydes or acetophenones (1) with thio-semicarbazide or semicarbazide hydrochloride (2) (Scheme 1) respectively. While oxazole and thiazoles (**3j-m**), were produced by the reaction of ethanolic solution of substituted 2-halo-1-phenylethanone (1) with aqueous solution of semicarbazide hydrochloride or thio-semicarbazide solution (2) resulting in corresponding hydrazinyl-oxazole and hydrazinyl-thiazoles (**3j-m**) (Scheme 2). The final products were obtained after evaporation of solvent and re-crystallization with a suitable solvent (ethanol). Structural elucidation of synthesized compounds was done using various spectroscopic methods, involving IR, mass, H^1NMR and C^{13}NMR spectroscopy as reported in detail earlier [24].



Scheme-1: Synthetic scheme for semicarbazones and thio-semicarbazone [24]



Scheme-2: Synthetic scheme for oxazole and thiazoles [24]

Anticancer activity

Anti-cancer activity of imine derivatives (**3a-m**) were evaluated by MTT assay (**Table 1-3**) using glioblastoma cell line (U87). Doxorubicin was used as positive control. This cell line was selected as compounds showed promising activity against different causative enzymes of neurodegenerative diseases [25]. Among all compound **3g** showed potent anti-cancer activity against U87 cell line with IC_{50} value of 8.83 ± 0.23 µM. Moreover, compounds **3e, 3f, 3h** and **3i** also showed good to moderate anticancer activities having IC_{50} values of 17.73 ± 0.05 µM, 21.38 ± 0.02 µM, 12.37 ± 0.09 µM and 19.68 ± 0.06 µM respectively. Compound **3m** among thiazole was the most potent compound with IC_{50} value of 8.86 ± 0.15 µM. Other analogues **3k** and **3l** also showed good to moderate anticancer activity with IC_{50} values of 13.68 ± 0.11 µM and 20 ± 0.22 µM respectively. Moreover, oxazole containing compound **3j** also showed good activity with IC_{50} value of 13.17 ± 0.06 µM.

The SAR of semicarbazones and thiosemicarbazone indicated that compound **3g** containing meta nitro and para methoxy groups against semicarbazide moiety showed potent inhibitory potential with IC50 value of 8.83 ± 0.23 µM changing methoxy group with hydroxyl in **3f** drastically decrease the activity with IC_{50} value of 21.38 ± 0.02 µM. Changing nitro or methoxy group with halogens in **3e** and **3h** also decreases the activity with IC_{50} values of 17.73 ± 0.05 µM and 12.37 ± 0.09 µM correspondingly.

Among thiazoles **3m** possessing nitro at meta position against thiazole moiety was most potent thiazole with IC_{50} value of 8.86 ± 0.15 µM. Changing nitro with halogens in 3k and 3l decreases the activity with IC_{50} values of 13.68 ± 0.11 µM and 20 ± 0.22 µM respectively. Compound **3j** containing oxazole moiety with hydroxy groups at meta and para positions showed good activity with IC_{50} value of 13.17 ± 0.06 µM.

To evaluate the cytotoxic effect of synthesized derivatives on normal human cells, MTT assay was carried out in contrast to HEK-293 (Normal human embryonic kidney Cell Line). Semicarbazones and thiosemicarbazones were found non-cytotoxic with moderate anti-carcinoma activity except **3g** and **3h**. Though thiazole derivatives **3k** and **3m** were found cytotoxic, other derivatives did not show cytotoxicity against HEK-293.

Table 1: Cytotoxicity and anti-cancer assay results of synthesized semicarbazones and thiosemicarbazone

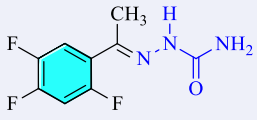
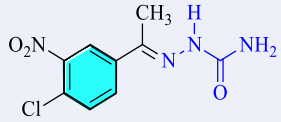
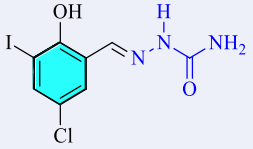
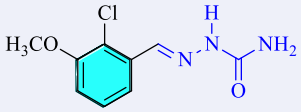
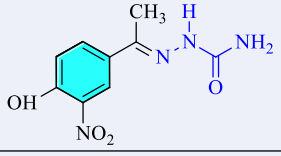
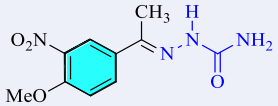
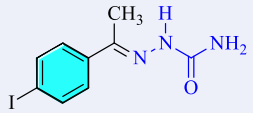
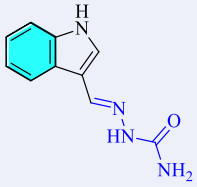
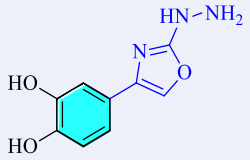
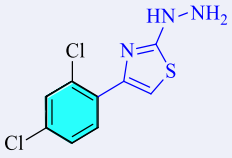
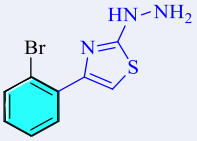
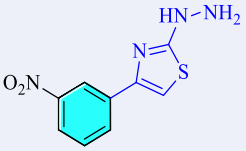
Entry	Comp. No.	Structure	%age or IC_{50} ± SD (µM)	
			HEK	U87
1.	**3a**		43	46
2.	**3b**		42	49
3.	**3c**		37	41
4.	**3e**		38	17.73 ± 0.05
5.	**3f**		37	21.38 ± 0.02
6.	**3g**		12.56 ±0.11	8.83 ± 0.23
7.	**3h**		13.83 ± 0.10	12.37 ± 0.09
8.	**3i**		48	19.68 ± 0.06

Table 2: Cytotoxicity and anti-cancer assay results of synthesized oxazole and thiazole derivatives

Entry	Comp. No.	Structure	%age or $IC_{50} \pm SD$ (µM)a	
			HEK	U87
9	**3j**		37	13.17 ± 0.06
10	**3k**		18.21 ± 0.20	13.68 ± 0.11
11	**3l**		44	20 ± 0.22
12	**3m**		11.60 ± 0.07	8.86 ± 0.15

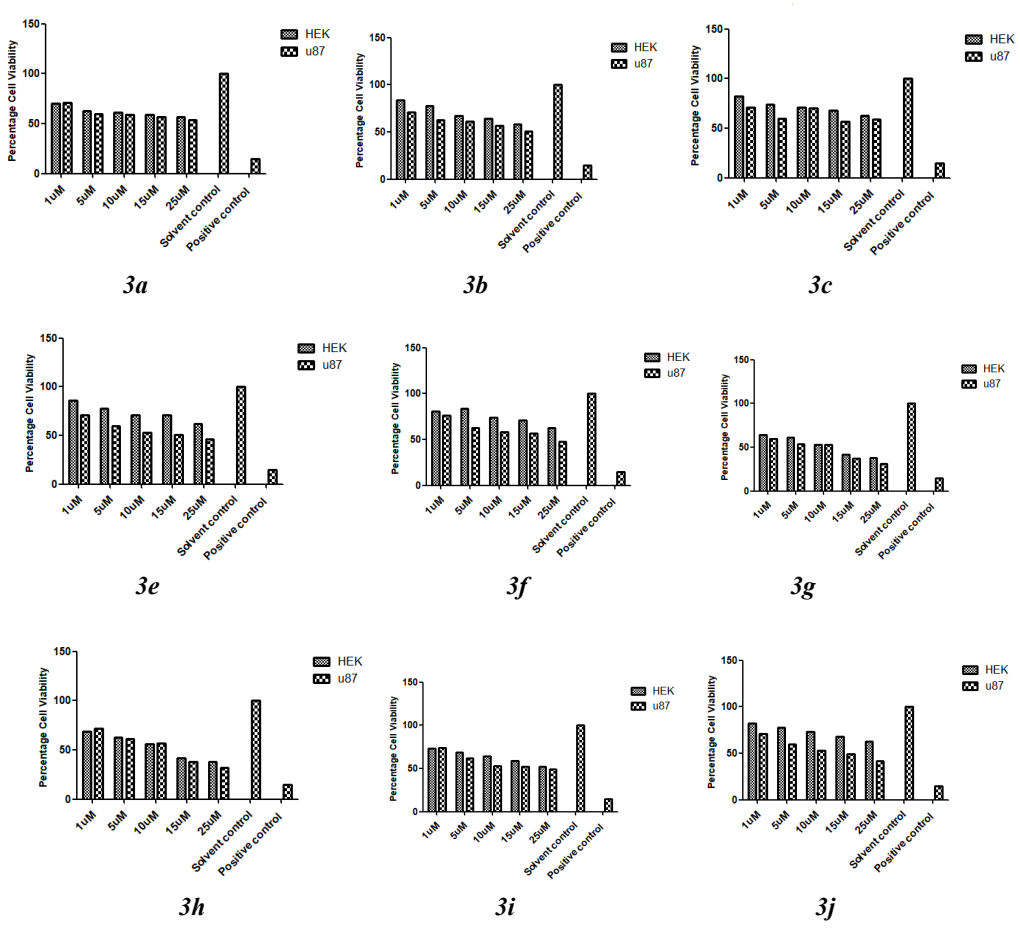


Figure 1: Graphs between percentage cell viability and concentration for synthesized derivatives.

Anti-bacterial Activity

Anti-bacterial agents are of ultimate developments in modern medicine responsible to inhibit the growth of bacteria ultimately results in damaging them. Ideally these focus on bacteria devoid of affecting mammalian cells [26]. Due to increasing bacterial resistance now a days the development of new anti-bacterial agents is of great significance.

The anti-bacterial potential of semicarbazone, thiosemicarbazone, oxazole and thiazole derivatives (**3a-m**) was evaluated using Agar well diffusion method against five Gram-positive bacterial strains including *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Corynebacterium diphtheriae, Klebsiella pneumoniae* and five Gram-negative bacterial strains including *Salmonella Typhi, Eschericia coli, Enterobacter aerogenes, Pseudomonas aeruginosa* and *Proteus mirabilis*. Moxifloxacin was used as reference standard. Most of the synthesized derivatives showed almost negligible anti-bacterial activity. Results are illustrated in table 3.

Among semicarbazones only **3c** showed slight anti-bacterial action only against gram negative strain *i.e., Salmonella Typhi* and gram-positive strain *Cornybacterium diphthereae* with zone of inhibition of 8mm for both the strains at the concentration of 200 µg/ml. Compound **3d** having thio-semicarbazide moiety showed mild anti-bacterial potential against two gram positive strains including *Staphylococcus aureus, Corynebacterium diphtheriae* and a gram negative strain i.e., *Proteus mirabilis* with zone of inhibition of 10mm, 18mm and 10 mm respectively. Moreover, among oxazole and thiazoles none of the synthesized compound displayed antibacterial activity.

Table 3: Antibacterial activity results

Bacterial Strains	Zone of Inhibition (mm) at 200 µg/ml													
	3a	3b	3c	3d	3e	3f	3g	3g	3h	3i	3j	3k	3l	3m
Salmonella Typhi	-	-	8	-	-	-	-	-	-	1	-	2	-	-
Streptococcus Faecalis	-	-	-	2	-	-	-	-	-	4	-	-	2	-
Staphylococcus aureus	-	-	1	10	-	-	-	-	-	-	-	-	2	-
Staphylococcus epidermidis	-	-	0	4	-	-	-	-	-	-	-	-	-	-
Cornybacterium diphthereae	-	-	8	18	-	-	-	-	-	-	-	-	-	-
Eschericia coli	-	-	2	2	-	-	-	-	-	-	-	-	-	-
Proteus mirabilis	-	-	2	10	-	-	-	-	-	-	-	-	2	-
Enterobacter aerogenes	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aerogenosa	-	-	1	1	-	-	-	-	-	-	-	-	1	-
Klebsiella pneumonaea	-	-	-	2	-	-	-	-	-	-	-	-	-	-

Conclusion

A series of imine derivatives (**3a-m**) including thio-semicarbazone, semicarbazone, thiazole and oxazole functional moieties were examined for anti-cancer activity *in-vitro* by MTT assay using glioblastoma cell line (U87). Among all compound **3m** was found most potent with IC_{50} value of 8.86 ± 0.15 µM. Cytotoxicity of compounds were evaluated using normal human embryonic kidney cell line i.e HEK-293. Only derivatives **3k** and **3m** were found cytotoxic, other derivatives did not show cytotoxicity against HEK-293. Moreover, antibacterial activity of synthesized derivatives was also evaluated by disc diffusion method. None of the compound showed significant antibacterial activity.

Experimental

Anti-cancer Activity

All the synthesized semicarbazone, thiosemicarbazone, oxazole and thiazole derivatives were examined for cytotoxic potential against a of two cell lines, HEK and U87 by MTT assay using a quinonoidal drug i.e., Doxorubicin as positive control. These cells were planted in 96 well plates at approximately 10,000 cells per well density and incubated for 24 hours to obtain a uniform single layer of cells. Test compounds were dissolved in DMSO and subsequently diluted with complete medium-CM to achieve different concentrations (1, 2.5, 5, 10, 15, 20, 25 µM). Each concentration of test compound was added in triplicate to the cell lines and incubated for 48 hours at 37°C in a carbon dioxide incubator. After the treatment completion 30 µl of MTT (5 mg/ml) solution was inoculated and incubated for 4 hours at 37°C. The absorbance of MTT formazan formed was measured on ELISA plate reader at 540 nm. From absorbances obtained, percentage cell viability and percentage cytotoxicity were determined using the following equation;

% cell viability = (A sample-Ablank)/ (Acontrol-A blank) × 100

Anti-bacterial Assay

Anti-bacterial activity of synthesized semicarbazones, thiosemicarbazones, oxazole and thiazoles was evaluated by using agar well diffusion method. Test organisms involved *Staphylococcus aureus, Enterococcus faecalis, Staphylococcus epidermidis, Proteus mirabilis, Eschericia coli, Enterobacter aerogenes, Corynebacterium diphtheriae, Salmonella typhi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* Bacterial suspension was prepared after inoculation of freshly grown culture into 5ml sterile saline and turbidity equivalent to 0.5 McFarland standard was adjusted visually. The suspension prepared was then uniformly swabbed on labeled Mueller Hinton agar plates. The plates were permitted to dry for 5 min prior to punching the wells by the help of sterile metallic borer (7 mm diameter). The

dilutions of compounds tested were inoculated in wells and permitted to diffuse at ambient temperature before incubation for 24 hours at 37°C. Anti-bacterial activity was evaluated by measuring the diameter of zones of growth inhibition (in mm). Growth inhibition was computed with reference to the positive control i.e., moxifloxacin.

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