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Biological activity of newly synthesised compounds of 2-(3-oxo-2, 3-dihydro-4H-benzo [b] [1,4] oxazin-4-yl)-N-substituted phenyl acetamide and its derivatives

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ABSTRACT

In vitro antiproliferative activity of the newly prepared synthetic compounds of 2 - (3 – oxo - 2, 3 – dihydro – 4 *H* – benzo [*b*] [1,4] oxazin – 4 - yl) - N-substituted phenyl acetamides (8a-j) were evaluated against four different human cancer cell lines, MIAPACA (pancreatic), HeLa (cervical), IMR32 (neuroblastoma) MDA-MB-231 (breast) and IMR32 (neuroblastoma) summarized in Table below. The compounds 8g and 8i were more active than the remaining compounds.

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INTRODUCTION

Quinoxaline and its moieties are a crucial class of nitrogen-containing Heterocycle in medicinal chemistry [1], possess a wide [2, 3] and diverse Spectrum of pharmacological properties [4, 5], such as antibiotic [6], antiviral [7], anti-cancer [8], and anti-inflammatory activities [1, 9]. The quinoxaline core moiety is also associated with multiple applications in semiconductors [2, 10], dyes [3], cavitands and dehydroannulenes [1].

MATERIALS AND METHODS

Maintenance, Cell Cultures, and Antiproliferative Evaluation. The cell lines, MIAPACA, HeLa, IMR 32 (cervical, pancreatic MDA MB 231, breast and neuroblastoma) used in this study were procured from American Type Culture Collection (ATCC), USA.

Effects of the Compounds on the Viability of Human Cancer Cells

In vitro antiproliferative activity of the designed compounds, 8a-j evaluated against four different human cancer cell lines, MIAPACA (pancreatic), HeLa (cervical), IMR32 (neuroblastoma), MDA-MB-231 (breast) and summarized in Table 1 and Figure 1. The compounds were picked for an assay against four human cancer cell lines at five different concentrations values (0.01, 0.1, 1, 10, 100 μM). GI_{50} (growth inhibitory activity) calculated and the values corresponded to the concentration compound causing fifty percent downfall in the net cell growth as compared with standard drugs, Doxorubicin and Paclitaxel. Results calculated for each and every of these parameters, if the level of activity was reached; however, if the effect was not achieved, the value was expressed as less (or) more than the minimum (or) maximum concentration tested.

At Table 1, the newly prepared compounds 8a-j showed more to moderate cancer cell growth inhibition with GI_{50} values ranging 0.1 to >100 μM. The effect of various derivatives on the 1,4 - benzoxazine structure was examined. The structure-activity rela-

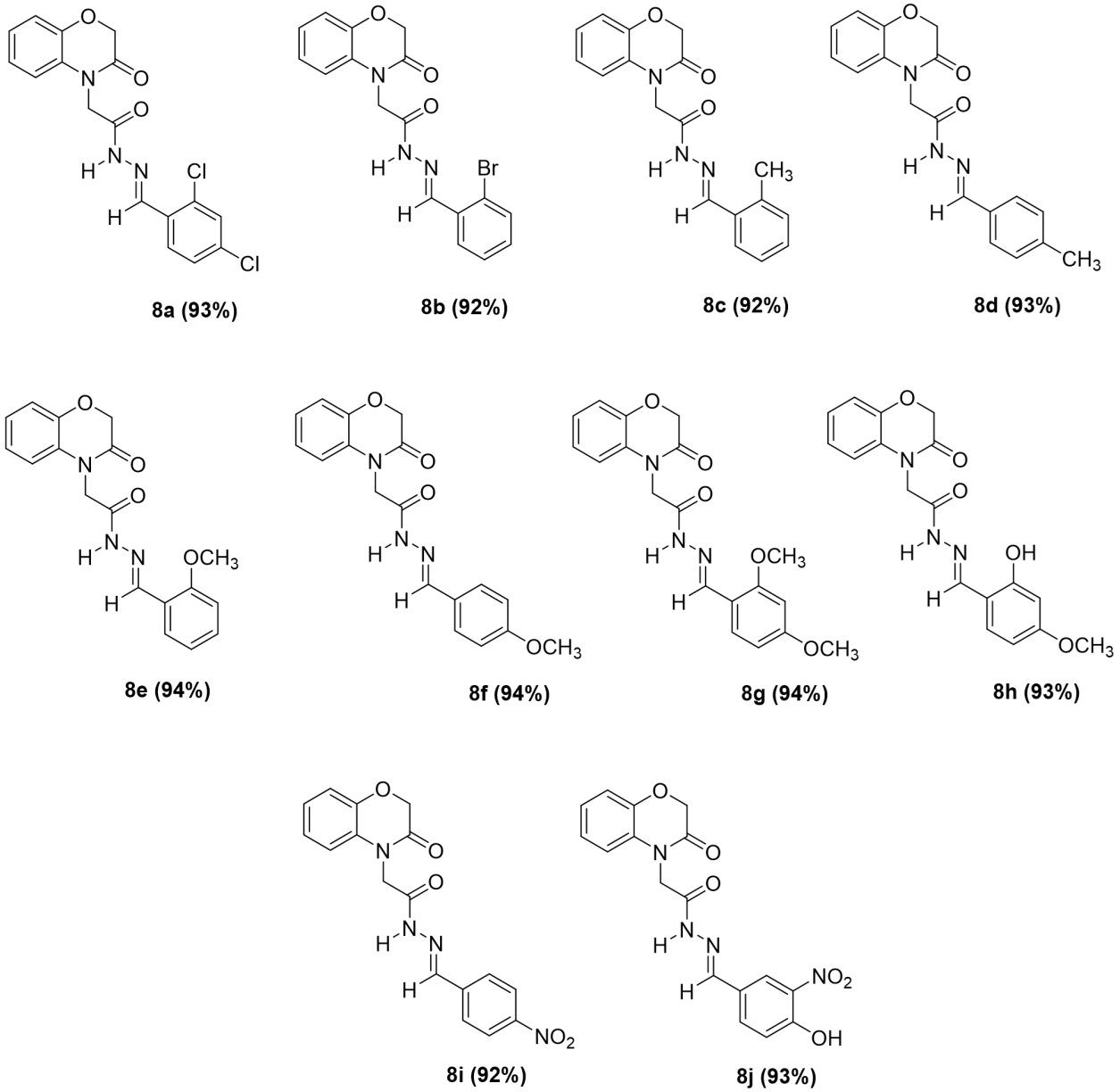

Figure 1: Newly prepared compounds of 2 - (3– oxo - 2, 3 – dihydro - 4H – benzo [b] [1,4] oxazin-4-yl) – N - substituted phenyl acetamides (8a-j)

tionship (SAR) study revealed that the orientation of substituent on phenyl ring is not only crucial but also required 1,4-benzoxazine along with an acetamide linker for inducing the anti-proliferative activity against these cancer cell lines. In particular, the compounds 8a, 8b, 8e, 8f, 8g, 8i and 8j showed promising anti-proliferative activity with GI_{50} values ranging from 0.1 to 1.1, 1.1 to 23.2, 0.1 to 12.5 and 1.2 to 2.5 μM respectively, also against the four human cancer cell lines. Compounds 8g and 8j showed high anti-proliferative activity against all the four human cancer cell lines. The substituents on 1, 4 - benzoxazine containing 2-methoxyphenyl (8e), nitro (8i) and 2 - hydro-4-methoxyphenyl (8h)] associated with a significant high in the growth inhibitory effect against MIAPACA, MDA - MB – 231 and IMR32 human cancer cell lines. In comparison, the structures 8g, 8i more active than the remaining compounds, in particular on HeLa, MIAPACA, MDA-MB-231 and IMR32 cancer cell lines. Finally, we observed that the emene linker 1, acetamide linker 2 and various substituted phenyl rings containing 1,4-benzoxazine moiety, played an important role in the anti-proliferative activity.

Experimental

Cell lines, MIAPACA, HeLa, MDA MB 231 and IMR 32 (cervical, pancreatic, breast and neuroblastoma) used in this study were procured from American Type Culture Collection (A T C C), USA. The prepared

Table 1: (GI_{50})[a] values of the tested compounds against four human cancer cell lines

S.No	Compounds	HeLa	MIAPACA	MDA – MB - 231	IMR 32
1	8a	6.01±0.7	14.0±0.5	4.9±0.6	0.33±0.02
2	8b	4.8 ±0.85	16.0±0.5	1.32 ±0.2	6.6 ±0.09
3	8c	12.0±0.5	10.0±0.5	4.0±0.7	>100
4	8d	>100	>100	8.0±0.6	1.8±0.05
5	8e	09.0±0.5	6.4±0.3	>100	0.11±0.03
6	8f	4.2±0.2	>100	21.5±0.1	>100
7	8g	0.10±0.2	0.15±0.09	1.1±0.08	0.51±0.04
8	8h	>100	>100	4.8±0.5	2.6±0.06
9	8i	>100	6.2±0.5	3.0±0.7	0.56±0.04
10	8j	3.3±0.5	1.00±0.6	5.7±0.2	2.5±0.3
	Doxorubicin[b]	0.078±0.001	0.096±0.002	0.088±0.001	0.024±0.002
	Paclitaxel[b]	0.032±0.001	0.059±0.002	0.097±0.005	0.076±0.003

[a] GI_{50} :50% Growth inhibition, concentration of drug (in μM) resulting in a 50% reduction in net protein increase compared with control cells; [b] Positive controls

compounds evaluated for their *in - vitro* antiproliferative activity in these four different human cancer cell lines. A protocol of 48 h continuous drug exposure was used and an SRB cell proliferation assay was used to estimate cell viability or growth. All the cell lines were grown in Dulbe cco's modified Eagle's medium (containing 10% FBS in a humidified atmosphere of 5% CO_2 at 37 °C). Cells were trypsinized when sub–confluent from T 25 flasks / 60 mm dishes and seeded in 96-well plates in 100 μL aliquots at plating densities depending on the doubling time of individual cell lines. The microtitre plates were incubated at 37°C, 100% relative humidity for, 5% CO_2, 95% air and 24 h prior to the addition of experimental drugs and were incubated for 48 hrs with different doses (0.01, 0.1, 1, 10, 100 μM) of prepared compounds. After incubation at 37°C for 48 hours, the cell monolayers were fixed by the addition of 10% (wt/vol) chilled trichloroacetic acid and incubated at 4 °C for 1 hour, then stained with 0.057% SRB dissolved in 1% acetic acid for 30 min at room temperature. Unbound SRB was washed with 1% acetic acid. The protein–bound dye was dissolved in 10 mM Tris base solution for OD determination at 510 nm using a microplate reader (Enspire, Perkin, Elmer, USA). Using the seven measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

[(Ti – Tz) / (C - Tz)] x 100 for concentrations for which Ti > / = Tz

[(T i- Tz) / Tz] x 100 for concentrations for which Ti < Tz.

The dose response parameter, growth inhibition of 50% (GI_{50}) was calculated from [(Ti – Tz) / (C - Tz)] $\times$ 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Values were calculated for this parameter if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter was expressed as greater or less than the high or low concentration tested.

CONCLUSION

All the newly prepared compounds evaluated for their antiproliferative activity and some of structures shown significant activity when compared with human cell lines (HeLa, MIAPACA, MDAMB-231 & IMR 32).

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Conflict of Interest

The authors declare that there is no conflict of interest.

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