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Anti-Proliferative Evaluation of Benzo[6,7] Cyclohepta[1,2-*b*]Pyridine Derivatives in Addition to *Anti-Inflammatory* Activity

Research Article

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Abstract

A variety of benzo[6,7]cyclohepta[1,2-*b*]pyridine derivatives have been evaluated for their *in vitro* anti-proliferative activity against four cancer cell lines such as HeLa (cervical), MIAPACA (pancreatic), MDA-MB-231 (breast) and IMR32 (neuroblastoma). Among tested compounds, a exhibited potent anti-proliferative activity against IMR32 with GI_{50} value less than 0.01 μM and compounds d, l and n exhibited promising anti-proliferative activity against IMR32 with GI_{50} values 0.1, 0.21 and 0.21 μM, respectively. This is the first report on *in vitro* anti-proliferative evaluation of benzo[6,7]cyclohepta[1,2-*b*]pyridine derivatives in addition to *anti-inflammatory* activity.

Keywords: Benzosuberone; Benzo[6,7]cyclohepta[1,2-*b*]pyridines; Anti-proliferative; Cell lines; Doxorubicin; Paclitaxel

Introduction

Benzosuberone motif has remarkable biological activities such as anti-inflammatory, anti-pyretic, anti-ulcer, CNS-depressant, CNS-stimulant and anticonvulsant activities. And some of the derivatives are also known for anti-tumor activity in murine p338 cell line tests [1]. In addition these derivatives widely used in pharmaceutical applications, such as tricyclic antidepressants containing dibenzosuberone moieties mostly effect the autonomic and central nervous systems, and traditional anti-depressants, like amitriptyline [2], imipramine [3] and noxiptiline [4] which continue to be used as first-line drugs in treating depressive disorders.

Pyridyl compounds are of interest to organic chemists in recent years owing to their wide spectrum of physiological activity [5-10]. The condensed derivatives of pyridines play significant role in bioactive molecules, especially in the form of benzo[5,6] cyclohepta[1,2-*b*]pyridines which are structural analogues to benzosuberone. The benzo[5,6]cyclohepta[1,2-*b*]pyridine is an important core biologically active compound with diverse biological activities, such as antihistamine as well as antitumor and anti-inflammatory activities [11-17]. It is a highly potent pharmacophore and widely used in drug molecular design. Because of the important aforementioned properties of benzocyclohepta[1,2-*b*]pyridines derivatives, preparation of this heterocyclic nucleus has gained great importance in organic synthesis.

Recently, we have published [18] synthesis and *anti-inflammatory* activity of benzo[6,7]cyclohepta [1,2-*b*]pyridines, in the present manuscript, presenting *in vitro* anti-proliferative activity of benzo[6,7]cyclohepta [1,2-*b*]pyridines against four cancer cell lines such as HeLa (cervical), MIAPACA (pancreatic), MDA-MB-231

(breast) and IMR32 (neuroblastoma) and a SRB cell proliferation assay to estimate viability or growth in addition to *anti-inflammatory* activity in continuation to our ongoing research activities [19-27], to discover and develop tumor growth inhibitors and apoptotic inducers as potential new anti-cancer agents.

Effects of the Compounds on the Viability of Human Cancer Cells

All the synthesized compounds of benzo[6,7]cyclohepta[1,2-*b*] pyridine derivatives (a-n) were screened for their *in vitro* anti-proliferative activity against four cancer cell lines such as HeLa (cervical), MIAPACA (pancreatic), MDA-MB-231 (breast) and IMR32 (neuroblastoma) summarized in Table 1.

The compounds were picked for an advanced assay against these four human cancer cell lines at five concentrations (0.01, 0.1, 1, 10, 100 μM). GI_{50} (growth inhibitory activity) was calculated and these values corresponded to the concentration of the compound causing 50% decrease in the net cell growth as compared with the standard drugs, doxorubicin and paclitaxel. Results were calculated for each of these parameters if the level of activity was reached; however, if the effect was not achieved, the value was expressed as greater or less than the maximum or minimum concentration tested.

Based on Table 1, the synthesized compounds a-n showed potent to significant cancer cell growth inhibition with GI_{50} values ranging from <0.01 to >100 μM. Among tested compounds a exhibited potent anti-proliferative activity against IMR32 with GI_{50} value less than 0.01 μM, which was more active than standards Doxorubicin and Paclitaxel. Compound d showed anti-proliferative activity against IMR32 with GI_{50} value 0.1 μM, which was close to the standard Paclitaxel. Five compounds l, n, m, k and b exhibited promising anti-proliferative activity against IMR32 with GI_{50} values 0.21, 0.21, 0.69, 0.98 and 1.1 μM. The Structure-Activity Relationship (SAR) revealed that. The presences of ester group/cyclic group on pyridine ring in combination with methyl group on C-2 position appear to be crucial for observed activity. Further studies are underway to optimize the lead molecule (Figure 1).

SRB Proliferative Assay Method

The cell lines, HeLa, MIAPACA, MDA MB 231 and IMR 32 (cervical, pancreatic, breast and neuroblastoma) which were used in this study were procured from American Type Culture Collection (ATCC), USA. The synthesized test compounds were evaluated for their *in vitro* anti-proliferative 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 Dulbecco'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 T25 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 microtiter plates were incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs and were incubated for 48 h with different doses (0.01, 0.1, 1, 10, 100 μM) of prepared derivatives. After 48 h incubation at 37 °C, the cell monolayers were fixed by the addition of 10% (wt/vol) cold trichloroacetic acid and incubated at 4 °C for 1 h and were 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 absorbance 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

[(Ti-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)] x 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

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	a	15.3 ± 0.9	72.1 ± 0.6	12.1 ± 0.3	< 0.01
2	b	13.2 ± 0.5	53.3 ± 0.67	5.3 ± 0.3	1.1 ± 0.06
3	c	> 100	6.1 ± 0.1	2.6 ± 0.08	1.8 ± 0.07
4	d	91.2 ± 0.3	97.1 ± 0. 9	5.1 ± 0.05	0.1 ± 0.03
5	e	71.3 ± 0.2	14.1 ± 0.4	3.5 ± 0.03	4.2 ± 0.05
6	f	51.0 ± 0.8	52.4 ± 0.8	1.9 ± 0.06	9.3 ± 0.06
7	g	> 100	> 100	2.5 ± 0.08	6.0 ± 0.02
8	h	> 100	23.3 ± 0.9	> 100	4.9 ± 0.3
9	i	23.5 ± 0.31	1.9 ± 0.7	16.5 ± 0.3	3.0 ± 0.05
10	j	10.0 ± 0.14	> 100	3.5 ± 0.03	2.5 ± 0.1
11	k	> 100	7.1 ± 0.6	> 100	0.98 ± 0.01
12	l	> 100	36.5 ± 0.1	66.7 ± 0.3	0.21 ± 0.05
13	m	> 100	23.4 ± 0.07	4.2 ± 0.3	0.69 ± 0.03
14	n	18.5 ± 0.8	18.3 ± 0.9	0.53 ± 0.07	0.21 ± 0.05
Doxorubicinb		0.08 ± 0.001	0.091 ± 0.003	0.075 ± 0.002	0.031 ± 0.0012
Paclitaxelb		0.032 ± .0012	0.059 ± .0032	0.089 ± 0.0052	0.073 ± 0.005

a) GI50: 50% Growth inhibition, concentration of drug (in μM) resulting in a 50% reduction in net protein increase compared with control cells.
b) Positive controls.

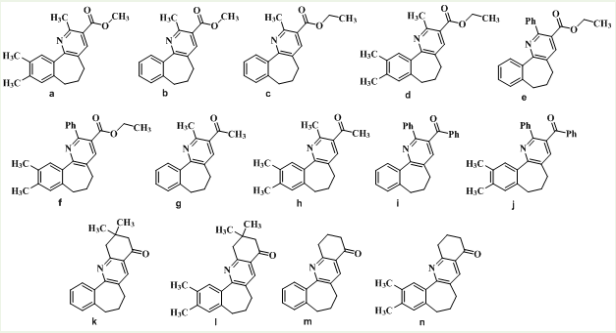

Figure 1: Structures of anti-proliferative compounds.

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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 maximum or minimum concentration tested.

Conclusion

Taken together, we have synthesized a series of benzo[6,7] cyclohepta[1,2-*b*]pyridine derivatives (a-n) in good yields and performed there *in vitro* anti-proliferative activity against four different human cancer cell lines, HeLa, MIAPACA, MDA-MB-231 and IMR32. Compound **a** exhibited potent anti-proliferative activity against IMR32 with GI_{50} value less than 0.01 μM, which was more active than standards Doxorubicin and Paclitaxel in addition to *anti-inflammatory* activity.

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