Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 14 (2006) 8622–8625
Bioorganic & Medicinal Chemistry

Anti-cancer activities of 5-acyl-6-[2-hydroxy/benzyloxy-3-(amino)-propylamino]-1,3-dialkyl-1*H*-pyrimidin-2,4-diones

Palwinder Singh* and Kamaldeep Paul

Department of Chemistry, Guru Nanak Dev University, Amritsar-143005, India

Received 26 July 2006; revised 16 August 2006; accepted 17 August 2006
Available online 1 September 2006

Abstract—All the nine 1,3-dialkylated-pyrimidin-2,4-diones investigated are active against all the 59 human tumor cell lines. Compounds **2**, **3**, **4**, and **6** show significant anti-cancer activities at some specific cell lines while compounds **7** and **9** exhibit anti-cancer activities against more number of cell lines. The structure–activity relationship studies indicate that the presence of piperidine/pyrrolidine at the end of C-6 chain, benzoyl group at C-5, and benzyl groups at N-1, N-3 of the pyrimidine ring increases the anti-cancer activities of these molecules.

1. Introduction

Since 1940, when for the first time the nitrogen mustard[1] alkylating agents were used for the treatment of leukemia and lymphomas, various chemotherapeutic agents have been tested to have anti-cancer activities[2] and based upon the nature of the target biomolecule (mechanism of action),[2] each category of drugs has a characteristic pharmacophore. The difficulty to diagnose the disease at the earlier stage, the selectivity of anti-cancer agents toward cancerous cells without harming the normal cells[3] and ultimately the obstacle of multidrug resistance[4] has made cancer as number one cause of untimely deaths all over the world. The enzymes like topoisomerase II[5] (Topo II), thymidylate synthase,[6] (TS) thymidylate phosphorylase[7] (TP), etc., are the common targets of anthraquinone and pyrimidine based anti-cancer drugs[8] among which mitoxantrone[9] and 5-flurouracil[10] are in clinical use. Mechanistically, both these anti-cancer agents work at the DNA level; mitoxantrone intercalates with the DNA or DNA-Topo II complex through the hydrophobic interactions from anthraquinone nucleus and the polar interactions from the side arms while 5-fluorouracil acts as anti-metabolite of RNA/DNA via inhibition of TS. However, the narrow therapeutic index, multidrug resistance, and various side effects associated with anti-cancer agents have considerably reduced their efficacy and necessitated the search for more effective chemical entities.

Recently, we have synthesized a series of pyrimidine derivatives[11] and found them to interact[12] with P-glycoprotein (P-gp), the causative agent for multidrug resistance (MDR). Further, the structural features of these molecules, that is, the presence of a chain with two hydrogen donating sites (similar to the polar side arms of mitoxantrone) and a cyclic tertiary nitrogen base at C-6 along with a benzoyl group either in the C-6 chain or at C-5 of the pyrimidine moiety (C-5 occupied like 5-fluorouracil), which could contribute toward both the lipophilic and hydrophilic interactions of these compounds with the targets like DNA/DNA-Topo II complex or TS, encouraged us to investigate these pyrimidine derivatives for their anti-cancer activities. Nine compounds were subjected to 59 human tumor cell lines and some of these compounds show appreciable results. The structure–activity relationship studies highlight that the compounds with pyrrolidine and piperidine moieties at the end of C-6 chain along with the presence of one or two benzoyl groups in the molecule show better profile of their anti-cancer activities.

2. Results

2.1. Chemistry

From a series of recently synthesized pyrimidine derivatives,[11] nine compounds (**1**–**9**, Fig. 1) have been selected

Keywords: Pyrimidin-2,4-diones; Anti-cancer; In vitro; Human tumor cell lines.
* Corresponding author. Tel.: +91 183 2258802x3495; fax: +91 183 2258819; e-mail: palwinder_singh_2000@yahoo.com

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.08.025

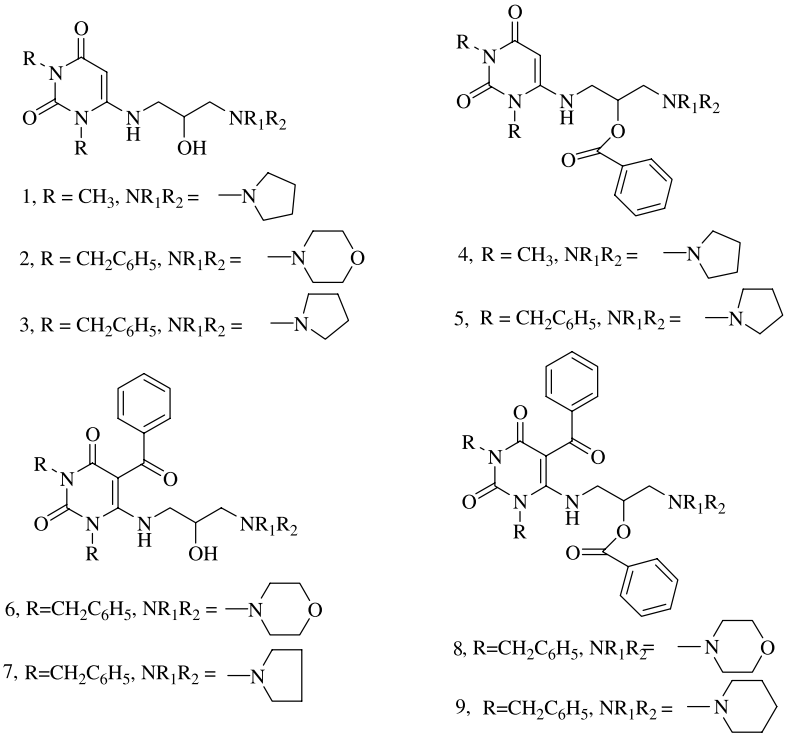

Figure 1.

on the basis of the nature of cyclic tertiary amine present at the end of C-6 chain (pyrrolidine, piperidine or morpholine), presence/absence of benzoyl group either in the C-6 chain or C-5 of pyrimidine or at both the places and the nature of N-1, N-3 substituents (CH_3, CH_2Ph).

2.2. Anti-cancer screening

The anti-cancer activities of all the nine compounds have been tested, utilizing 59 different human tumor cell lines representing leukemia, melanoma, and cancers of the lung, colon, brain, ovary, breast, prostate as well as kidney, following the standard procedure.[13] The results of these studies in terms of growth inhibition of 50% (GI_{50}) have been given in Table 1.

3. Discussion

From the results of Table 1, it has been noticed that all the tested compounds are active against all the cell lines. Some of these compounds have been found to be highly cell line specific and show appreciable inhibition of a particular tumor cell line at sub-micromolar concentration. Amongst compounds **1**, **2**, and **3** (no benzoyl group in the molecule), compound **3** (average GI_{50} over all cell lines 3.65×10^{-5} M) (with pyrrolidine moiety and benzyl groups) shows better anti-cancer activity in comparison to compounds **1** (average GI_{50} 10^{-4} M) and **2** (average GI_{50} 4.1×10^{-5} M). Compound **4**, with pyrrolidine moiety and one benzoyl group present in the C-6 chain, exhibits high specificity for CCRF-CEM cell line. Shifting of benzoyl group to C-5 (compounds **6** and **7**) has remarkably increased the anti-cancer activities of these compounds. Again compound **7** (average GI_{50} 1.38×10^{-5} M) with pyrrolidine moiety has slightly better anti-cancer activity in comparison to compound **6** (average GI_{50} 1.92×10^{-5} M). Between compounds **8** and **9** (with two benzoyl groups in the molecule), compound **9** (average GI_{50} 1.71×10^{-5} M) having piperidine residue at the end of C-6 chain exhibits better anti-cancer activity than compound **8** (average GI_{50} 5.1×10^{-5} M). The highly potent compounds **7** and **9** show slightly better anti-cancer activities than 5-fluorouracil (average GI_{50} 1.77×10^{-5} M).

Therefore, a suitable combination of the groups and their appropriate placement in the molecule significantly control the function of the molecule. In the present investigations, for a compound to show better results, the following preferences have been observed:

(i) Presence of pyrrolidine or piperidine moiety in the molecule is preferred over the morpholine.
(ii) Benzoyl group is preferred at C-5 of pyrimidine instead of its presence as benzyloxy group in the C-6 chain (compare the results of compound **5** with those of **7**).
(iii) Presence of two benzoyl groups in the molecule instead of one at C-5, to some extent, changes the specificity of the molecule from one cell line to the other (compare the results of compounds **7** and **9**).

4. Conclusions

Out of the nine compounds investigated in the present contribution, two compounds (**7** and **9**) show significant

Table 1. Concentrations of compounds **1**–**9** resulting in growth inhibition of 50% ($\log_{10}$ GI_{50}) of in vitro human tumor cell lines

Panel/cell line	Compound									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	5-Fluorouracil[10]
Leukemia										
CCRF-CEM	>−4.0	−4.43	−4.55	−7.12	−4.63	−4.30	−4.61	−4.47	−5.09	−4.5
HL-60 (TB)	>−4.0	−5.00	−5.11	>−4.0	nd	nd	nd	−4.51	−5.13	−4.7
K-562	>−4.0	−4.51	−4.60	>−4.0	−4.04	−4.32	−4.62	−4.49	−5.13	−4.7
MOLT-4	>−4.0	−4.56	−4.61	>−4.0	>−4.0	−4.32	−4.71	nd	nd	−4.9
RPMI-8226	>−4.0	−4.55	−4.65	>−4.0	−4.53	−4.44	−4.71	−4.57	−5.16	−5.3
SR	>−4.0	−5.00	−6.07	>−4.0	nd	nd	nd	nd	nd	−5.4
Non-small cell lung cancer										
A549/ATCC	>−4.0	−4.27	>−4.0	>−4.0	>−4.0	−4.42	−4.49	−4.31	−4.30	−5.7
EKVX	>−4.0	−4.15	>−4.0	>−4.0	>−4.0	−4.63	−4.71	−4.28	−4.42	−3.5
HOP-62	>−4.0	−4.47	−5.03	>−4.0	>−4.0	−4.03	−4.66	−4.26	−4.75	−4.7
HOP-92	>−4.0	−4.66	−5.13	>−4.0	nd	nd	nd	−4.53	nd	−3.8
NCI-H226	>−4.0	−4.25	>−4.0	>−4.0	−4.09	−4.51	−4.73	−4.30	−4.17	−3.6
NCI-H23	>−4.0	−4.37	−4.04	>−4.0	>−4.0	−7.43	−4.70	−4.36	−4.73	−4.9
NCI-H322M	>−4.0	>−4.0	−4.39	>−4.0	>−4.0	−4.31	−4.70	−4.25	−4.67	−4.7
NCI-H460	>−4.0	−4.35	−4.40	>−4.0	>−4.0	−4.42	−4.70	−4.34	−4.67	−6.0
NCI-H522	>−4.0	−4.55	−4.10	>−4.0	>−4.0	−4.38	−4.70	−4.33	−4.68	−4.4
Colon cancer										
COLO 205	>−4.0	−4.63	−5.01	>−4.0	>−4.0	−5.72	−5.09	−4.33	−5.01	−5.2
HCC-2998	>−4.0	−4.46	−4.59	>−4.0	>−4.0	−7.19	−4.69	−4.32	−5.22	−5.8
HCT-116	>−4.0	−4.46	−5.17	>−4.0	−4.25	−4.47	−5.12	−4.39	−5.13	−5.4
HCT-15	>−4.0	−4.34	−4.29	>−4.0	>−4.0	−4.49	−5.07	−4.41	−5.17	−5.2
HT29	>−4.0	−4.73	−5.00	>−4.0	−4.46	−4.74	−5.06	nd	nd	−5.2
KM12	>−4.0	−4.44	−5.13	nd	>−4.0	−4.39	−5.10	−4.38	−5.00	−5.0
SW-620	>−4.0	−4.20	4.39	>−4.0	>−4.0	−4.30	−5.11	−4.12	−4.45	−4.6
CNS cancer										
SF-268	>−4.0	−4.20	−4.32	>−4.0	>−4.0	−4.20	−5.01	>−4.0	−4.62	−4.3
SF-295	>−4.0	−4.38	−4.33	>−4.0	>−4.0	−4.50	−5.05	−4.38	−4.66	−4.3
SF-539	>−4.0	−4.22	>−4.0	>−4.0	>−4.0	−4.33	−5.01	−4.04	−4.66	−5.9
SNB-19	>−4.0	>−4.0	>−4.0	>−4.0	>−4.0	−5.27	−4.20	−4.15	−4.52	−3.9
SNB-75	>−4.0	−4.50	−4.60	>−4.0	−4.78	−5.17	−5.07	nd	nd	−3.7
U251	>−4.0	−4.32	−4.36	>−4.0	>−4.0	−4.51	−5.09	−4.37	−4.82	−4.4
Melanoma										
LOX IMVI	>−4.0	−4.42	−4.73	>−4.0	>−4.0	−4.38	−5.10	−4.23	−5.19	−5.2
MALME-3M	>−4.0	−4.57	−4.65	−5.06	>−4.0	−4.29	nd	>−4.0	−5.03	−4.7
M14	nd	nd	nd	nd	nd	nd	−5.06	nd	−5.09	−4.3
SK-MEL-2	>−4.0	>−4.0	>−4.0	>−4.0	>−4.0	>−4.0	−5.09	−4.42	−5.02	−3.4
SK-MEL-28	>−4.0	−4.54	−4.78	>−4.0	>−4.0	−4.39	−5.15	−4.25	nd	−4.3
SK-MEL-5	>−4.0	−4.72	−4.77	>−4.0	>−4.0	−4.57	−5.20	−4.42	−5.04	−4.9
UACC-257	>−4.0	−4.52	−4.50	>−4.0	>−4.0	−4.09	−5.08	−4.11	−5.12	−4.0
UACC-62	>−4.0	−4.64	−4.68	>−4.0	−4.05	−4.37	−5.06	−4.41	−5.08	−4.9
Ovarian cancer										
IGROV1	>−4.0	−4.48	−4.58	>−4.0	>−4.0	−4.29	−4.67	−4.19	−4.76	−4.9
OVCAR-3	>−4.0	−4.25	>−4.0	>−4.0	>−4.0	−4.73	−4.74	−4.47	−4.75	−4.6
OVCAR-4	>−4.0	−4.40	−4.23	>−4.0	>−4.0	−4.69	−4.62	nd	nd	−4.2
OVCAR-5	>−4.0	>−4.0	>−4.0	>−4.0	>−4.0	−6.28	−4.53	>−4.0	−4.61	−3.8
OVCAR-8	>−4.0	−4.14	>−4.0	>−4.0	>−4.0	−4.24	−4.73	−4.19	−4.74	−4.7
SK-OV-3	>−4.0	>−4.0	>−4.0	>−4.0	>−4.0	−5.05	−4.47	>−4.0	−4.43	−3.8
Renal cancer										
786-0	>−4.0	−4.38	−4.49	>−4.0	>−4.0	−4.20	−4.72	−4.15	−4.83	−4.9
A498	>−4.0	−4.31	>−4.0	>−4.0	>−4.0	−4.69	−5.64	−4.43	−4.49	−5.0
ACHN	>−4.0	−4.30	>−4.0	>−4.0	>−4.0	−4.42	−4.65	−4.33	−4.52	−5.0
CAKI-1	>−4.0	−4.07	>−4.0	>−4.0	>−4.0	−4.55	−4.61	−4.36	−4.74	−5.4
RXF 393	>−4.0	−4.52	−4.34	>−4.0	−4.12	−4.60	−4.67	−4.46	−4.83	−4.3
SN12C	>−4.0	−4.37	>−4.0	>−4.0	>−4.0	−4.36	−4.71	−4.29	−4.71	−4.6
TK-10	>−4.0	>−4.0	>−4.0	>−4.0	>−4.0	−4.55	−4.71	−4.16	−4.63	−3.9
UO-31	>−4.0	−4.56	−4.34	>−4.0	>−4.0	−4.38	−4.73	−4.28	−4.78	−5.3
Prostate cancer										
PC-3	>−4.0	−4.47	−4.57	>−4.0	>−4.0	−4.22	−4.76	−4.41	−4.88	−4.3
DU-145	>−4.0	−4.29	−4.06	>−4.0	−5.72	−4.75	−4.75	>−4.0	−4.55	−5.0

Table 1 (*continued*)

Panel/cell line	Compound									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	5-Fluorouracil[10]
Breast cancer										
MCF7	>−4.0	−4.38	−4.72	>−4.0	>−4.0	−4.26	−4.37	−4.32	−4.68	−5.8
NCI/ADR-RES	>−4.0	−4.18	>−4.0	>−4.0	>−4.0	−6.88	−5.01	−4.32	−4.75	−4.4
MDA-MB-2321/ATCC	>−4.0	−4.72	−4.58	>−4.0	−4.12	−4.47	−5.11	−4.37	−4.76	−3.3
HS 578T	>−4.0	−4.42	−4.43	>−4.0	>−4.0	−4.31	−5.09	−4.40	−4.38	−3.6
MDA-MB-435	>−4.0	−4.22	−4.57	>−4.0	>−4.0	−4.44	−5.06	−4.06	−4.73	−5.0
BT-549	>−4.0	−4.34	−4.21	>−4.0	−6.11	−4.64	−5.18	−4.38	−4.59	−4.0
T-47D	>−4.0	−4.21	−4.28	>−4.0	−4.72	−7.43	−4.67	>−4.0	>−4.0	−4.1

nd, not done.

inhibition of tumor cells at various cell lines. Some other compounds like **2**, **3**, **4**, and **6** also exhibit growth inhibition of particular cell lines at sub-micromolar concentrations. These studies highlight the role of various groups along with the placement in the molecule toward their tumor cell growth inhibitory properties and could be useful in the further tailoring of the molecules for improving their anti-cancer properties.

5. Experimental

The anti-cancer activities of all the compounds were tested by screening unit of NCI at NIH Bethesda, USA, following their standard procedure (www.dtp.nci.nih.gov/). The test compounds have been evaluated at five concentrations viz. 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M. The percentage growth of tumor cells was calculated at each cell line for each concentration of the compound. The results are expressed as growth inhibition of 50% (GI_{50}) which is the concentration of the compound causing 50% reduction in the net protein increase (as measured by SRB staining) in control cells during drug incubation. However, in these studies the particular cellular target of the compounds has not been identified.

Acknowledgments

CSIR (Grant No. 01(1735)/02/EMR-II) and DST (Grant No. SR/FTP/CS-20/2001), New Delhi, are gratefully acknowledged for financial assistance. Authors thank Dr. V. L. Narayanan and his group at NIH, Bethesda, USA, for anti-cancer screening.

References and notes

1. (a) Teicher, B. A., Ed.; *Cancer Therapeutics*; 1996; p 23. ISBN: 0896034607; (b) Torrence, P. F., Ed.; *Biomedical Chemistry: Applying Chemical Principles to the understanding and Treatment of Diseases*; 2000; p 164. ISBN: 047132633X.
2. NIH, USA has classified the anticancer drugs according to their mechanism of action. See NIH website: www.dtp.nci.nih.gov/.
3. Hanch, C.; Sammes, P. G.; Taylor, J. B. *In Comprehensive Medicinal Chemistry* **1990**, *2*, 753.
4. (a) Teodori, E.; Dei, S.; Scapecchi, S.; Gualtieri, F. *IL Farmaco* **2002**, *57*, 385; (b) Krishna, R.; Mayer, L. D. *Eur. J. Pharma. Sci.* **2000**, *11*, 265.
5. (a) Nitiss, J. L. *Biochem. Biophys. Acta* **1998**, *1400*, 63; (b) Wang, J. C. *Nat. Rev. Mol. Cell. Biol* **2002**, *3*, 430.
6. Lehninger, A. L.; Nelson, D. L.; Cox, M. M. In *Principles of Biochemistry*, 2nd ed.; CBS publishers and distributors, India, 1992; p 726.
7. (a) Furukawa, T.; Yoshimura, A.; Sumizawa, T.; Haraguchi, M.; Akiyama, S.; Fukui, K.; Ishikawa, M.; Yamada, Y. *Nature* **1992**, *356*, 668; (b) Haraguchi, M.; Miyadera, K.; Uemura, K.; Sumizawa, T.; Furukawa, T.; Yamada, Y.; Akiyama, S. *Nature* **1994**, *368*, 198; (c) Matsushita, S.; Nitanda, T.; Furukawa, T.; Sumizawa, T.; Tani, A.; Nishimota, K.; Akiba, S.; Miyadera, K.; Fukushima, M.; Vamada, Y.; Yoshida, H.; Kanzaki, T.; Akiyama, S. *Cancer Res.* **1999**, *59*, 1911.
8. (a) Cole, C.; Reigan, P.; Gbaj, A.; Adwards, P. N.; Douglas, K. T.; Stratford, I. J.; Freeman, S.; Jaffar, M. J. *Med. Chem.* **2003**, *46*, 207; (b) D'Arpa, P.; Liu, L. F. *Biochim. Biophys. Acta* **1989**, *989*, 163; (c) Lansiaux, A.; Laine, W.; Baldeyrou, B.; Mahieu, C.; Wattez, N.; Vezin, H.; Martinez, F. J.; Pineyro, A.; Bailly, C. *Chem. Res. Toxicol.* **2001**, *14*, 16.
9. (a) Koeller, J.; Eble, M. *Clin. Pharmacy* **1988**, *7*, 574; (b) Shenkenberg, T. D.; Von Hoff, D. D. *Ann. Int. Med* **1986**, *105*, 67.
10. NCI data base (www.dtp.nci.nih.gov), NSC 19893.
11. Singh, P.; Paul, K. D.; Holzer, W. *Org. Biomol. Chem* **2005**, *21*, 3958.
12. Singh, P.; Paul, K. D. *Bioorg. Med. Chem.* **2006**, doi: doi:10.1016/j.bmc.2006.06.060.
13. (a) Ally, M. C.; Scudiero, D. A.; Monks, P. A.; Hursy, M. L.; Czerwinski, M. J.; Fine, D. A.; Abbott, B. J.; Mayo, J. G.; Showmaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589; (b) Grever, M. R.; Schepartz, S. A.; Chabner, B. A. *Semin. Oncol.* **1992**, *19*, 622; (c) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91.