Bioorganic & Medicinal Chemistry Letters 19 (2009) 3054–3058

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and anticancer activities of hybrids of indole and barbituric acids—Identification of highly promising leads

Palwinder Singh *, Matinder Kaur, Pooja Verma

Department of Chemistry, Guru Nanak Dev University, Amritsar 143 005, India

ARTICLE INFO

Article history:
Received 27 February 2009
Revised 1 April 2009
Accepted 3 April 2009
Available online 9 April 2009

Keywords:
Indole
Barbituric acid
Hybrid molecules
Anticancer activities

ABSTRACT

By combining the structural features of indole and barbituric acid, new hybrid molecules were designed and synthesized. Evaluations of these molecules over 60 cell line panel of human cancer cells have identified two molecules with significant anticancer activities. Dockings of two active molecules in the active sites of COX-2, thymidylate synthase and ribonucleotide reductase indicate their strong interactions with these enzymes.

Cancer is continuing to be a major health problem in both developed and developing countries. Surpassing heart disease, it is taking the position of number one killer due to various worldwide factors. Several anticancer agents including taxol, vinblastine, vincristine, etoposide, camptothecin and its derivatives (topotecan and irinotecan),[1] mitoxantrone,[2] 5-fluorouracil,[3] indomethacin[4] etc. are in clinical use all over the world. However, these drugs suffer from various side effects like low blood pressure, bone marrow suppression, gastrointestinal toxicity, constipation and hair loss. Therefore, the lack of effective chemotherapy of cancer is continuously inciting the scientific community to explore new chemical entities for the effective and safe cure of cancer.

The pivotal role of small chemical entities (like amino acids, glucose, nucleobases) and the acceptability of their synthetic analogues in the biological systems may be the reason for the design and development of about 80% of the drugs based on small organic molecules.[5] Moreover, a suitable combination (keeping in mind Lipinski's rule of 5)[6] of the structural features of two small organic, biologically active molecules may result in the creation of new molecules (hybrid or conjugates)[5,7,8] which are proving to be more efficacious, economical and safe for their use as drugs. Hybrid molecules or conjugates, as defined by Mehta and Singh,[7] 'are the constructs of different molecular entities, natural or unnatural, to generate functional molecules in which the characteristics of various components are modulated, amplified or give rise to entirely new properties'.

Due to the biological significance of indoles, barbituric acids as anti-convulsant,[9] anti-hypnotic,[10] anticancer,[11] anti-inflammatory,[12] agents and also their use as agrochemicals,[13] we have chosen these molecular entities (**1** and **2**, Fig. 1) to suitably combine them through carbon–carbon bond formation for creating new hybrid molecules (**3**, Fig. 1). Investigations of new molecules (analogues of a cytotoxic marine isolate aplysinopsin[14] and carrying the components of indomethacin and 5-fluorouracil) for their tumour growth inhibitory activities over 60 cell line panel of human cancer cell lines have identified two promising candidates as leads for anticancer drugs.

The preparation of the target compounds was achieved by irradiating a finely ground mixture of two reactants in microwave

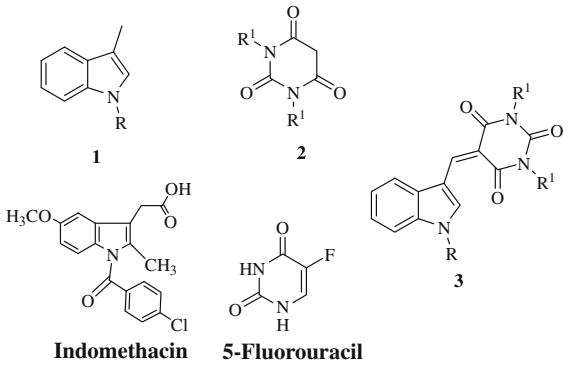
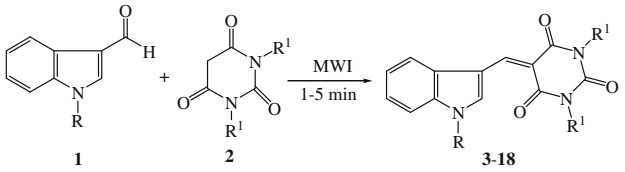


Figure 1. Design of new molecules.

* Corresponding author. Tel.: +91 183 2258802 09x3495; fax: +91 183 2258819.
E-mail address: palwinder_singh_2000@yahoo.com (P. Singh).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.04.014

Table 1
Reaction time, percentage yields and melting points for compounds **3–18**



1 **2** **3-18**

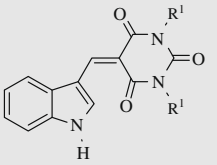
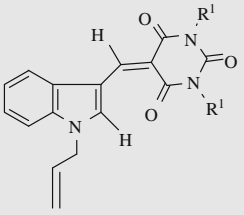
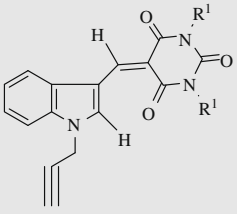
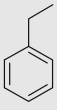
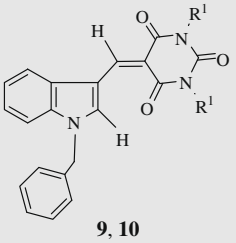
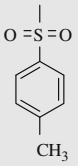
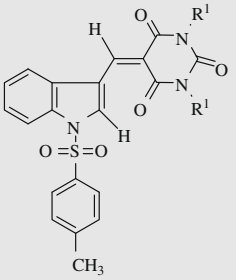
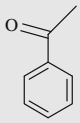
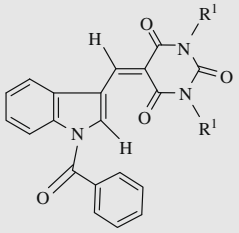
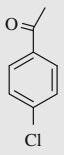
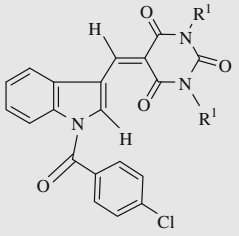
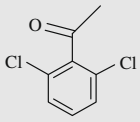
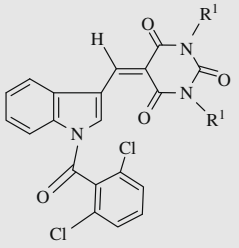
Entry	R	R_1	Product	Yield (%)	Mp (°C)
1	H	H, CH_3	 **3**, **4**	99, 93	>260, >260
2		H, CH_3	 **5**, **6**	93, 81	>250, 190
3		H, CH_3	 **7**, **8**	91, 86	>250, 235
4		H, CH_3	 **9**, **10**	90, 80	>250, 192
5		H, CH_3	 **11**, **12**	85, 82	>240, 246

Table 1 (*continued*)

Entry	R	R_1	Product	Yield (%)	Mp (°C)
6		H, CH_3	 **13**, **14**	75, 80	>240, >250
7		H, CH_3	 **15**, **16**	75, 88	>240, >250
8		H, CH_3	 **17**, **18**	75, 85	>240, >240

oven.[15] Indole-3-carboxaldehyde (**1**) (1 mmol) and barbituric acid (**2**) (1.2 mmol) on irradiating in a microwave oven for 5 min, after washing with diethyl ether gave a solid compound (99%, mp >260 °C, MS: (FAB) *m/z* 225), which from its NMR, IR and mass spectral data has been identified as compound **3** (R^1 = H). Following the same reaction procedure, other N-substituted indole-3-carboxaldehydes react with barbituric acids to give compounds **4–18** (Table 1) in quantitative yields.[16]

Therefore, the target compounds are synthesized in quantitative yields through Knoevenagal condensation using a very convenient synthetic methodology.

In vitro tumour growth inhibitory activities of these compounds were investigated at NCI, NIH, Bethesda on 60 cell line panel of human cancer cells using standard procedure. Compounds were first evaluated at 10^{-5} M concentration for the growth inhibitory activities at entire 60 cell line panel. Compounds with appreciable activities at 10^{-5} M concentration (as per the standard of NCI) were subjected to detailed tumour growth inhibitory studies at five concentrations viz. 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M and 10^{-8} M.

At 10^{-5} M concentration, along with the inhibition of tumour growth of other cell lines, remarkably, most of the compounds under present investigation show activity at various cell lines of renal cancer. Percent growth inhibition of RXF 393, TK-10 and UO-31 cell lines of renal cancer by compounds **3**, **4**, **9–16** is given in Table 2. Here, compounds **9** and **10** show 71% and 95% tumour growth inhibition at TK-10 cell line.

On the basis of preliminary investigations, compounds **9** and **10** were selected for detailed studies at five concentrations where they show highly encouraging results. The GI_{50} (50% growth inhibitory concentration) of compound **9** (average over

Table 2
Percent growth inhibition of renal cancer cells by compounds **3**, **4**, **9**–**16**

Compound	Percent growth inhibition for renal cancer		
	RXF 393	TK-10	UO-31
3	–	–	39
4	–	–	33
9	–	71	29
10	43	95	36
11	–	–	32
12	52	–	41
13	55	–	36
14	55	–	36
15	–	–	22
16	33	–	–

Table 3
GI_{50} (μM), total growth inhibitory concentration (TGI) and LC_{50} (μM) of compounds **9** and **10** at various cell lines

Cell lines	GI_{50} (μM)		TGI (μM)		LC_{50} (μM)	
	9	**10**	**9**	**10**	**9**	**10**
K-562	3.1	4.3	23	>100	>100	>100
HOP-92	1.5	8.3	15	50	58.8	>100
NCI-H226	5.4	3.4	28	27	>100	>100
NCI-H460	0.3	4.1	16.5	>100	63	>100
COLO 205	1.4	0.7	10.9	9.1	41.6	>100
SF-295	3.3	1.1	70.7	>100	>100	>100
SK-MEL-2	2.9	3.0	23.4	45.7	>100	>100
UACC-62	1.6	1.0	16.5	12.5	>100	>100
IGROV1	0.48	0.06	10.4	0.26	63	0.85
OVCAR-4	2.0	6.1	44	>100	>100	>100
OVCAR-5	0.3	0.1	3.8	0.67	39.8	>100
A498	0.03	0.2	0.26	0.64	1.09	2.29
CAKI-1	4.6	0.5	25.7	6.4	93	>100
TK-10	2.0	3.1	8.3	57.5	>100	>100
T-47D	4.5	5.0	22.3	>100	95	>100
MDA-MB-468	0.1	0.02	0.38	0.06	>100	0.69

all the 60 cell lines) is 7.5 μM while compound **10** exhibit GI_{50} as 13.8 μM. Interestingly, the GI_{50} values of both these compounds are better than indomethacin (GI_{50} 64.3 μM) and 5-fluorouracil (GI_{50} 17.7 μM).

Compounds **9** and **10** exhibit significant tumour growth inhibitory activities at various cell lines of non-small cell lung cancer (NSCLC), colon cancer, CNS cancer, ovarian cancer, renal cancer and breast cancer (Table 3).

It is apparent from the data given in Table 3 that compound **9** exhibits tumour growth inhibitory activities at micro/sub-micromolar concentrations. It exhibits GI_{50} 0.3 μM for NCI-H460, 0.48 μM for IGROV1, 0.3 μM for OVCAR-5, 0.03 μM for A498 and 0.1 μM for MDA-MB-468 cell lines of NSCLC, ovarian cancer, renal cancer and breast cancer, respectively. Remarkably, the total growth inhibitory concentrations (TGI) of compound **9** for A498 and MDA-MB-468 cell lines are 0.26 μM and 0.38 μM, respectively. Compound **10** shows more promising results with GI_{50} 0.06 μM, 0.1 μM, 0.2 μM and 0.02 μM for IGROV1, OVCAR-5, A498 and MDA-MB-468 cell lines, respectively. Respective TGI concentrations of compound **10** at these cell lines are 0.26 μM, 0.67 μM, 0.64 μM and 0.06 μM. LC_{50} values (Table 3) of compounds **9** and **10** are quite high in comparison to their GI_{50} values indicating their toxicity at high concentrations.

Therefore, the screening of these ten rationally designed compounds for anticancer activities have identified two candidates which could be further refined to improve their anticancer activities and drug like properties. Moreover, the improved anticancer activities of compounds **9** and **10** over their component molecules (indomethacin and 5-fluorouracil) justify the design of these molecules.

Although the cellular target is not defined in the experimental anticancer investigations of these molecules, to look into the possible interactions of these compounds at the enzymatic level,[17] we performed the dockings of compounds **9** and **10** in the active sites of COX-2 (probable mode of action of indomethacin) and thymidylate synthase (cellular target of 5-fluorouracil). Docking programme[18] was validated by docking Sc-558 in the crystal structure of COX-2 (containing Sc-558, pdb ID 6COX). A close overlapping between the two ligands (docked Sc-558 and native Sc-558) was observed (Fig. 2).

Docking of compound **9** in the active site of COX-2 (Fig. 3) shows H-bond interactions between NH, CO of pyrimidine moiety and S353, Q192, H90 amino acid residues of the active site. The phenyl ring present at indole is oriented towards W387 and Y385 residues exhibiting hydrophobic interactions. Similar interactions of compound **10** with the active site amino acid residues of COX-2 were observed. Therefore, the bindings of compounds **9** and **10** in the active site of COX-2 indicate their indomethacin like mode of action.

Dockings of compounds **9** and **10** in the active site of thymidylate synthase (TS) shows a number of H-bond interactions between the three carbonyl groups of pyrimidine moiety of compounds **9** and **10** and amino acid residues of the enzyme (Fig. 4).

Additionally, compounds **9** and **10** were also docked in the active site of ribonucleotide reductase (RNR) (another enzyme involved in the propagation of cancer; synthesis of raw material for DNA replication). Here also compounds **9** and **10** interact with the active site amino acids through a number of H-bonds (Fig. 5).

Therefore, the dockings of these two compounds in the active sites of COX-2, TS and RNR indicate the probable mode of action of these compounds for anticancer activities.

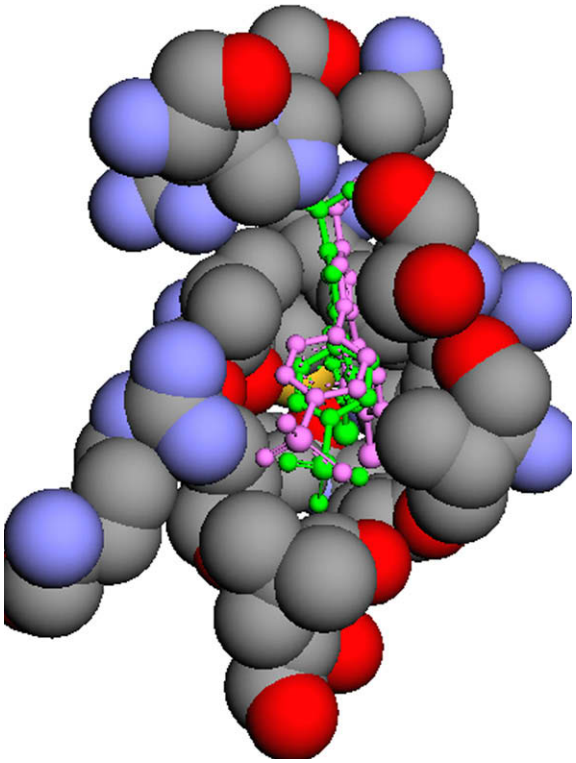


Figure 2. Sc-558 docked in the active site pocket of COX-2. A close overlapping between the docked molecule (pink) and one present as crystal (green) validates the docking programme.

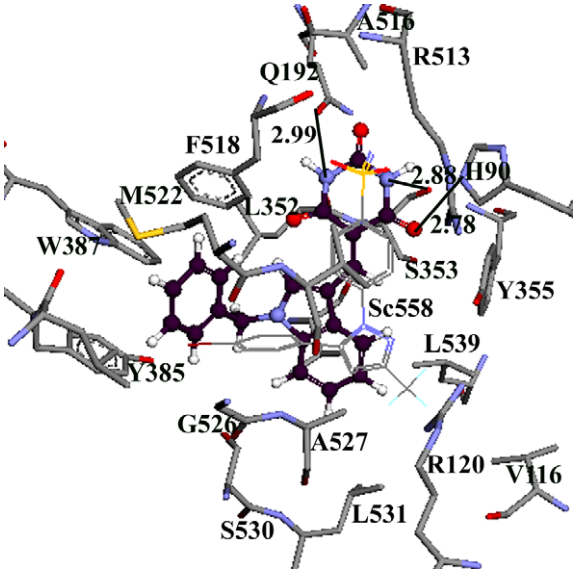


Figure 3. Compound **9** docked in the active site of COX-2. Hs' are omitted for clarity. H-bonds are visible between NH, CO groups of compound **9** and S353, Q192, H90 of COX-2. Carbon atoms of compound **9** are given different colour for clarity.

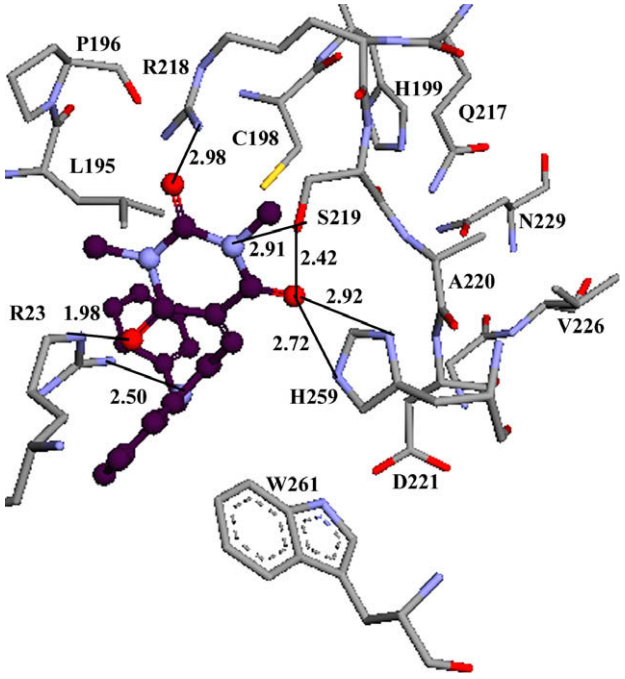


Figure 4. Compound **10** docked in the active site of TS. Carbon atoms of compound **10** are given different colour for clarity.

Remarkably, such correlations between the experimental data and the docking studies are useful for refining the structure of the molecules and improving their efficacies. Lipinski values of compounds **9** and **10** (Table 4) are also in favour of their drug like properties.

In conclusion, we have designed hybrid molecules on the basis of the biological significance of indole and barbituric acid and evaluated for their anticancer activities. Out of a set of 10 molecules, two compounds exhibit significant anticancer activities and could be used as leads for further investigations.

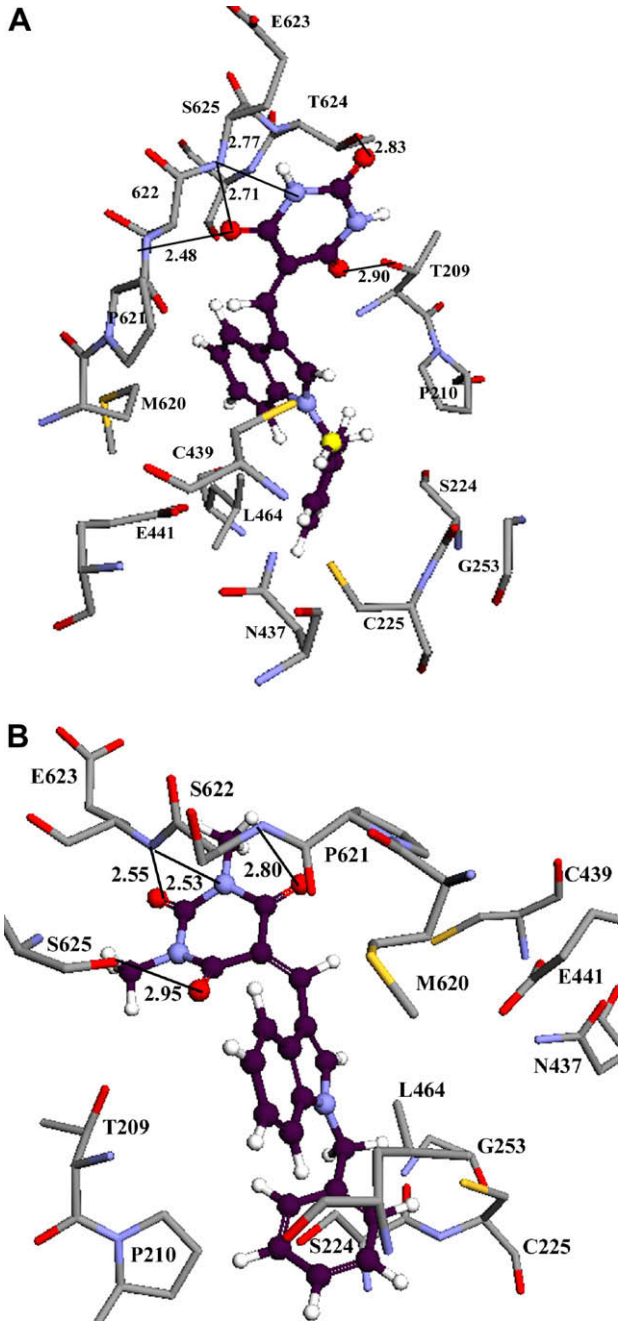


Figure 5. (A) Compound **9** docked in RNR. H-bonds between compound **9** and different amino acid residues are visible. (B) Compound **10** docked in RNR. H-bonds between compound **10** and different amino acid residues are visible. Carbon atoms of **9** and **10** are given different colour.

Table 4
Lipinski values for compounds **9** and **10**

Compound	Log *P*	TPSA (Å^2)	nON	nOHNH
9	2.10	87.2	6	2
10	2.24	66.01	6	0

Acknowledgements

Authors are grateful to CSIR, DST and UGC New Delhi for research grants. M.K. thanks UGC and P.V. thanks CSIR for fellowships. Dr. V. L. Narayanan and his team at NCI, NIH, Bethesda,

USA is gratefully acknowledged for screening anticancer activities. RSIC, CDRI Lucknow is acknowledged for recording mass spectra and CHN analysis.

References and notes

1. Shoeb, M. *Bangladesh J. Pharmacol.* **2006**, *1*, 35. and references cited therein.
2. (a) Zee-Cheng, R. K. Y.; Cheng, C. C. *J. Med. Chem.* **1978**, *21*, 291; (b) Cheng, K. Y.; Zee-Cheng, R. K. Y.; Podrebarac, E. G.; Menon, C. S.; Cheng, C. C. *J. Med. Chem.* **1979**, *22*, 501.
3. NIH database, NSC 19893.
4. NIH database, NSC 77541.
5. Meunier, B. *Acc. Chem. Res.* **2008**, *41*, 69. and references therein.
6. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
7. Mehta, G.; Singh, V. *Chem. Soc. Rev.* **2002**, *31*, 324.
8. Morphy, R.; Rankovic, Z. *J. Med. Chem.* **2005**, *48*, 6523.
9. Goodman, L. S.; Gilman, A. In *The Pharmacological Basis of Therapeutics*; Mc Graw-Hill: New Delhi, 1991; pp 358–360.
10. (a) Andrews, G. In *Medical Pharmacology*; The CV Mosby Company: Saint louis, 1976; p 243; (b) Foye, W. O. *Principles of Medicinal Chemistry*; Lea & Febiger: London, 1989. 159.
11. Guerin, D. J.; Mazeas, D.; Musale, M. S.; Naguib, N. F. M.; Al Safarjalani, O. N.; el Kouni, M. H.; Panzica, R. P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1477. and references cited therein.
12. (a) Badawey, E.; El-Ashmawey, I. M. *Eur. J. Med. Chem.* **1998**, *33*, 349; (b) Radwan, M. A. A.; Ragab, E. A.; Sabry, N. M.; El-Shenawy, S. M. *Bioorg. Med. Chem.* **2007**, *15*, 3832. and references therein.
13. (a) Lee, D.; Carter, C. (Stauffer Chemical Co.), U.S. Patent 4, 797, 147, 1989; *Chem. Abstr.* **1989**, *111*, 148889c.; (b) Kratt, G.; Salbeck, G.; Bonin, W.; Duewel, D. Ger. Offen. DE 3, 903, 404, 1990; *Chem. Abstr.* **1991**, *114*, 23984k.; (c) Sundberg, R. J. *Indoles*; Academic Press: San Diego, 1996.
14. (a) Kazlauskas, R.; Murphy, P. T.; Quinn, R. J.; Wells, R. J. *Tetrahedron Lett.* **1977**, 61–64; (b) Hollenbeak, K. H.; Schmitz, F. J. *Lloydia* **1977**, *40*, 479.
15. Reddy, C. S.; Nagaraj, A.; Jalapathi, P. *Indian J. Chem, Sect. B* **2007**, *46*, 660.
16. Experimental data for selected compounds. *Compound* **9**: 5-((1-Benzyl-1*H*-indol-3-yl)methylene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione. Yield 90%; Yellow solid; mp >260 °C; IR (KBr, cm^{-1}): 1375, 1650 (C=O), 3505 (NH); ^1H NMR (300 MHz, $CDCl_3$ + DMSO): δ 5.39 (s, 2H, CH_2), 7.18–7.39 (m, 9H, ArH), 7.39 (s, 1H, CH), 10.00 (s, 1H, indole2-H); Anal. Calcd for $C_{20}H_{15}N_3O_3$: C, 69.56; H, 4.38; N, 12.17. Found: C, 69.39; H, 4.49; N, 12.24; MS (FAB) *m/z* 345 (M^+).
Compound **10**: 5-(1-Benzyl-1*H*-indol-3-ylmethylene)-1, 3-dimethyl-pyrimidine-2,4,6-trione. Yield 80%; Light Lime solid; mp 192 °C; IR (KBr, cm^{-1}): 1388, 1660, 1662 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ 3.41 (s, 3H, CH_3), 3.42 (s, 3H, CH_3), 5.47 (s, 2H, CH_2), 7.18–7.39 (m, 8H, ArH), 8.00 (d, *J* = 7.8 Hz, 1H, ArH,), 9.03 (s, 1H, =H), 9.69 (s, 1H, indole2-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.02, 28.81, 51.75, 108.57, 110.28, 111.34, 112.3, 118.77, 122.14, 123.06, 123.33, 124.20, 126.82, 127.20, 128.35, 129.08, 130.77, 134.96, 136.71, 142.60, 146.33, 151.78, 162.22, 163.71; Anal. Calcd for $C_{22}H_{19}N_3O_3$: C, 70.76; H, 5.13; N, 11.25. Found: C, 70.85; H, 5.09; N, 11.39; MS (FAB) *m/z* 374 (M+1).
17. Singh, P.; Bhardwaj, A. *Mini-Rev. Med. Chem* **2008**, *8*, 388.
18. Docking studies were performed as per our previous protocol: Singh, P.; Bhardwaj, A.; Kaur, S.; Kumar, S. *Eur. J. Med. Chem.* **2009**, *44*, 1278.