Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3177–3180
Bioorganic & Medicinal Chemistry Letters

Synthesis and biological evaluation of chalcones and their derived pyrazoles as potential cytotoxic agents

B. A. Bhat,[a] K. L. Dhar,[a,*] S. C. Puri,[a] A. K. Saxena,[b] M. Shanmugavel[b] and G. N. Qazi[b]

[a]*Division of Natural Product Chemistry, Regional Research Laboratory (CSIR) Jammu 180001, India*
[b]*Division of Pharmacology, Regional Research Laboratory (CSIR) Jammu 180001, India*

Received 3 November 2004; revised 1 March 2005; accepted 3 March 2005
Available online 12 May 2005

Abstract—A series of substituted chalcones and their corresponding pyrazoles were synthesized and evaluated for in vitro cytotoxic activity against a panel of human cancer cell lines. Out of 93 compounds screened, 8 compounds, **1s**, **3i**,**j**,**n**, **4i**,**j**,**n** and **4s**, showed marked activity. Compounds **4j**,**n** and **4s** were found to be the most promising in this study. SAR is also discussed.

1. Introduction

Microtubules are essential components of cell structure and are involved in many cellular processes, including mitosis, morphogenesis, intracellular transport and secretion.[1] They are hollow tubes consisting of α- and β-tubulin heterodimers that polymerize parallel to the cylindrical axis. Tubulin binding molecules interfere with the dynamic instability of microtubules and thereby disrupt microtubule inducing cell cycle arrest in the M-phase, forming abnormal spindles and finally leading to apoptotic cell death.[2] A variety of natural compounds such as paclitaxol, vinblastin, combretastatin A-4 and colchicine attack microtubules by interfering with the dynamics of tubulin polymerization and depolymerization, resulting in mitotic arrest.[3]

For a structurally simple group of compounds, chalcones have displayed an impressive array of biological activities, among which anti-malarial,[4] anti-protozoal,[5] anti-inflammatory,[6] immunomodulatory,[7] nitric oxide inhibition,[8] tyronase inhibition,[9] cytotoxic[10] and anti-cancer[11] activities have been cited in the literature. These compounds obtained by convenient synthetic methods strongly inhibit the polymerization of tubulin by binding to the colchicine-binding site.[12,13] The relatively simple structure and high affinity of chalcones for the colchicine-binding site because of similarity of the two-aryl group placements in the two molecules has led to the synthesis and subsequent evaluation of a large number of chalcones.[14]

As a part of our drug discovery programme, we have been actively involved in determining the features important for anti-mitotic activity and in searching novel anti-cancer agents that show strong growth inhibitory activities against a panel of human cancer cell lines. Herein, we report the synthesis of four series of organic compounds, viz., chalcones, epoxy-chalcones, 3,5-diphenyl-4-hydroxy-4,5-dihydro,1*H*-pyrazoles and 3,5-diphenyl,1*H*-pyrazoles, and we determine their cytotoxic potential in an in vitro cell culture system. The cytotoxic potential of 3,5-diphenyl-4-hydroxy-4,5-dihydro,1*H*-pyrazoles and 3,5-diphenyl,1*H*-pyrazoles appears to be an unexplored field and needs attention. SAR studies revealed that the introduction of pyrazole nucleus between two aryl rings of chalcones played an integral role for the increase in cytotoxic potential.

2. Chemistry

Chalcones were synthesized by a base catalyzed Claisen–Schmidt condensation reaction[15] of appropriately substituted acetophenones and aldehydes. The method is attractive since it specifically generates (*E*)-isomer. From ^{1}H NMR spectra, all chalcones were geometrically pure and with trans-configuration (J Hα–Hβ = 15.50–15.60 Hz). Reacting chalcones with hydrogen peroxide in alkaline conditions at 0 °C afforded

Keywords: Chalcone analogues; In vitro cytotoxicity; Cell lines.
* Corresponding author. Tel.: +91 0191 2549051(O); fax: +91 0191 2548607; e-mail: dharkl@yahoo.com

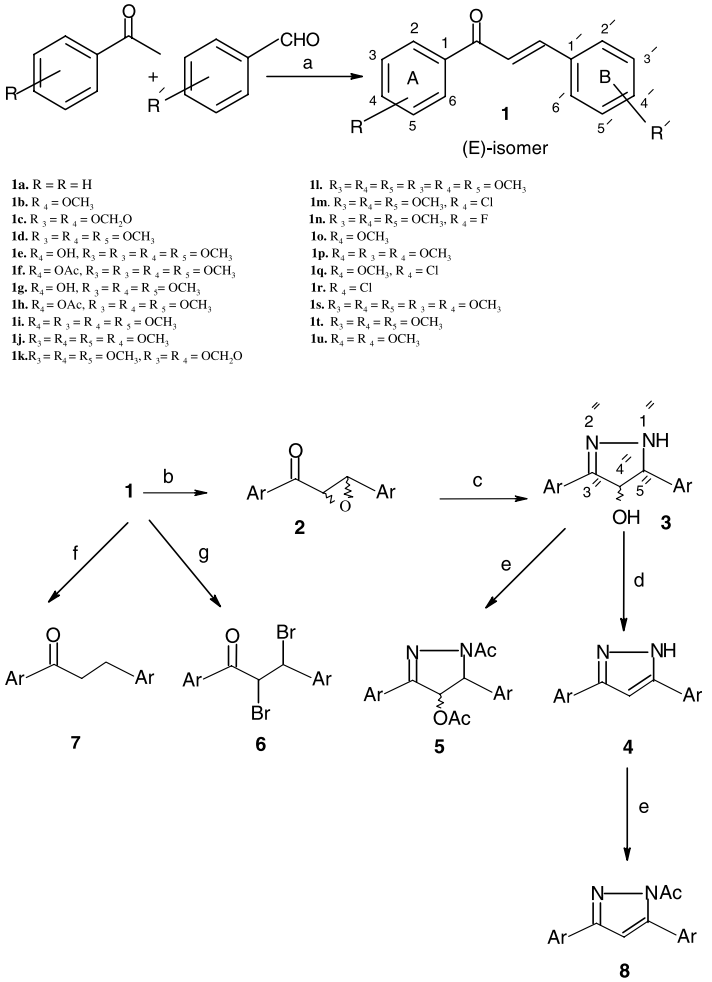
0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.03.121

the corresponding α,β-epoxide derivatives.[16] These epoxy derivatives were condensed with hydrazine hydrate to corresponding 4-hydroxy pyrazoles and pyrazoles.[17,18] In addition, compounds **1i–l** and **1s** were brominated with Br_2 to corresponding 2,3-dibromochalcones, and **1e** and **1s** reduced with H_2/Pd to 2,3-dihydrochalcones in order to observe the effect of double bond on activity (Scheme 1).

3. Biology

Chalcones and their derived analogues were assayed for in vitro cytotoxicity against 12 human cancer cell lines, HT-29, HCT-15, SW-620 (colon) A-549, HOP-62 (lung), Hep-2 (liver), SiHa (cervix), SKOV-3, OVCAR-5 (ovary), PC-3, DU-145 (prostate) and SNB-78 (CNS), using sulforhodamine B.[19] The cells were allowed to proliferate in presence of test material for 48 h and results are reported in terms of IC_{50} values (Table 1). From the IC_{50} values, it is clear that the compounds **4j**,**n** and **4s** showed significant cytotoxic activity especially against HT-29, HCT-15 and SW-620 cell lines. These three compounds were also evaluated for noncancerous cell line CV-1 (derived from African monkey kidney) and were found devoid of any cytotoxicity. Compound **4s**, which contained a 3,4,5-trimethoxy moiety at ring A and 3,4-dimethoxy moiety at ring B linked with pyrazole nucleus, showed significant cytotoxic activity, especially selective against HCT-15 and SW-620 cell lines.

By comparing the cytotoxic potential of compounds with different substitution as well as at different positions, the following conclusions were drawn: (a) Presence of 3,4,5-trimethoxy moiety on either ring is essential; introducing it at ring A (**1j**,**t**) led to enhanced cytotoxic activity than when attached to ring B (**1d**,**i**). Acetylation of –OH in **1e** and **1g** to **1f** and **1h**, respectively, also increased the activity. (b) Introducing the 4′-methoxy, 3′,4′-dimethoxy, 3′,4′-methylenedioxy and 3′,4′,5′-trimethoxy (**1j**,**k**,**l**,**s**) led to enhanced cytotoxic activity compared with B ring unsubstituted compound (**1t**) and introducing 4′-flouro (**1n**) also enhanced the activity in comparison with 4′-chloro- (**1m**). (c) Converting α,β-unsaturated ketones (**1a–d**,**i–u)** to corresponding epoxides (**2a–d**,**i–u)** dramatically reduced the cytotoxicity (IC_{50} value $\geqslant 100\ \mu M$), in spite of the fact that the



Scheme 1. Reagents and conditions: (a) KOH/EtOH, 0 °C; (b) H_2O_2, 5% NaOH, 0–4 °C; (c) $NH_2NH_2{\cdot}H_2O$/EtOH reflux; (d) AcOH/H_2SO_4; (e) Ac_2O/pyridine; (f) H_2/5% Pd/C; (g) Br_2/CCl_4.

Table 1. IC_{50} values (μM) of **1d**–**n**,**s**,**t**, **3i**–**n**,**s**, **4i**–**n**,**s**, **8s** and **5k**

Compound	HT-29	HCT-15	SW-620	A-549	Hop-62	Hep-2	SiHa	PC-3	DU-145	SKOV-3	OVCAR-5	SNB-78
1d	14.41	8.77	16.01	21.3	20.49.	7.55	36.5	41.12	25.4	21.01	8.9	22.8
1e	20.15	7.75	NT[a]	19.15	20.25	6.01	40.4	NT	NT	NT	NT	NT
1f	10.50	7.75	NT	15.6	9.2	6.95	33.04	NT	NT	8.35	NT	NT
1g	12.01	26.00	NT	52.01	160.41	NT	15.61	NT	NT	NT	NT	NT
1h	8.37	7.75	NT	12.03	56.15	13.06	13.30	NT	NT	NT	NT	NT
1i	21.31	22.23	NT	NT	13.75	8.62	64.60	13.43	25.4	NT	25.01	28.70
1j	7.08	4.90	NT	8.07	10.07	13.71	10.91	11.62	14.93	NT	9.00	10.01
1k	NT	23.71	NT	NT	9.61	10.1	12.83	11.68	19.8	NT	5.65	16.73
1l	NT	6.55	NT	NT	10.00	7.75	8.55	NT	NT	NT	9.23	NT
1m	NT	24.00	NT	NT	26.94	12.01	40.02	24.06	28.05	NT	16.81	25.41
1n	NT	11.73	NT	NT	24.72	10.02	7.92	11.65	13.32	NT	18.32	13.78
1s	6.64	7.95	5.80	6.90	8.12	6.15	9.35	7.85	9.21	6.09	8.3	22.81
1t	12.01	15.80	8.45	8.96	16.64	11.86	20.81	NT	NT	56.51	NT	NT
3i	5.95	7.85	7.35	7.2	8.1	NT	NT	28.04	NT	NT	7.05	NT
3j	0.96	0.98	2.67	1.07	0.94	7.81	20.13	15.17	NT	NT	13.01	NT
3k	5.89	NT	6.75	6.74	20.27	40.00	43.03	34.52	34.7	NT	6.81	53.68
3l	7.41	8.35	13.58	15.55	20.45	NT	83.00	108.01	94.07	NT	20.55	NT
3m	11.08	9.45	8.41	15.08	20.41	NT	56.41	34.55	NT	NT	8.65	NT
3n	6.01	8.45	5.85	5.72	6.55	NT	NT	NT	NT	NT	7.15	NT
3s	8.43	12.01	8.63	10.04	10.71	NT	NT	NT	NT	NT	8.76	NT
4i	2.95	10.12	5.15	4.35	4.81	12.45	15.91	15.81	24.00	NT	9.54	21.03
4j	0.62	0.46	3.41	1.01	0.65	0.81	14.47	11.07	26.99	NT	7.85	50.03
4k	6.01	15.55	9.25	8.85	13.45	34.01	10.01	9.55	8.56	NT	33.01	74.04
4l	5.62	6.01	10.01	7.58	NT	6.43	25.04	13.35	35.08	NT	9.95	22.04
4m	8.09	6.45	4.96	15.82	12.34	NT	NT	NT	NT	NT	8.61	14.04
4n	4.75	0.48	5.9	3.8	0.56	3.81	1.08	8.93	3.87	NT	6.45	22.45
4s	0.47	0.25	0.23	0.87	0.93	8.41	3.63	2.64	NT	NT	4.40	NT
8s	10.44	16.00	16.05	10.04	22.05	NT	54.81	NT	NT	NT	16.71	NT
5k	15.64	8.90	NT	NT	18.45	61.60	58.10	18.05	48.03	39.06	NT	32.97

[a] NT = Not tested.

epoxy group might also act as an alkylating moiety[20] at the same time it disturbs the conformation of two aryl rings. In addition, hydrogenation and bromination across carbon–carbon double bond also reduced the activity markedly (IC_{50} value $\geqslant 60$ μM). This result demonstrated that the conformation of the two aryl rings in the molecule has an essential role to play for the activity of chalcones and the double bond seems to be a crucial moiety as reported earlier.[10e] (d) Converting epoxides to hydroxy pyrazoles resulted in the re-appearance of the activity significantly (**3i**–**m**,**n**,**s**) compared with the corresponding epoxy chalcones. On dehydration to corresponding pyrazoles, the cytotoxic activity was enhanced to its maximum, in fact more than the corresponding chalcones (**4i**–**m**,**n**,**s**). This result demonstrated that introduction of pyrazole nucleus between two aryl rings enhanced the rigidity besides polarity and solubility in the molecule which played an integral role for their increase in cytotoxic potential. Pyrazoles with appropriate substitution (**4i**,**j**,**l** and **4s**) are more active than their hydroxy pyrazoles, confirming that –OH at position-4″ in pyrazole nucleus has either no or minimal role to play. When these hydroxypyrazoles and pyrazoles were converted into corresponding diacetates and acetates, respectively, the activity was markedly reduced (**8s**). This result demonstrated that the hydroxy pyrazoles and pyrazoles are also reacting via *N*-1″ of pyrazole nucleus and it might be the active binding site which gets deactivated by acylation. Finally, by comparing the activity of chalcones, 2,3-dibromochalcones, 2,3-dihydrochalcones, epoxy-chalcones, 4-hydroxy pyrazoles and pyrazoles, it is concluded that pyrazoles are more active than hydroxy pyrazoles, which in turn, are generally more active than their corresponding chalcones while the activity of other derivatives was insignificant.

In conclusion, we have discovered a novel class of chalcone-derived pyrazoles such as 3,5-diphenyl,1*H*-pyrazoles as potential cytotoxic agents. The position and size of substituents, rigidity and solubility of the molecule and finally the conformation of ring A and ring B seems to be crucial for the cytotoxicity. Compounds **4j**,**n** and **4s** were found to be most promising in this study. SAR studies and evaluation of more potent analogues are continuing.

Acknowledgements

The authors are grateful to the instrumentation division for elemental and spectral analysis and one of the authors, B. A. Bhat, is thankful to CSIR (India) for fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.03.121.

References and notes

1. Han, Y.; Malak, H.; Chaudhary, A. G.; Chordia, M. D.; Kingston, D. G.; Bane, S. *Biochemistry* **1998**, *37*, 6636.
2. Jordan, A.; Hadfield, J. A.; Lawrence, N. J.; McGown, A. T. *Med. Res. Rev.* **1998**, *18*, 259.
3. Hamel, E. *Med. Res. Rev.* **1996**, *16*, 207–231.
4. Liu, M.; Wilairat, P.; Cropft, S. L.; Tan, A. L. C.; Go, M. L. *Bioorg. Med. Chem.* **2003**, *11*, 2729–2738.
5. (a) Nielson, S. F.; Christensen, S. B.; Cruciani, G.; Kharazmi, A.; Liljefors, T. *J. Med. Chem.* **1998**, *41*, 4819; (b) Li, R.; Kenyon, G. L.; Cohen, F. E.; Chen, X.; Gong, B.; Dominguez, J. N.; Dvidson, E.; Kurzban, G.; Miller, R. E.; Nuzum, E. O.; Rosenthal, P. J.; Mckerrow, J. H. *J. Med. Chem.* **1995**, *38*, 5031; (c) Liu, M.; Wilairat, P.; Go, M. L. *J. Med. Chem.* **2001**, *44*, 4443.
6. (a) Ballesteros, J. F.; Sanz, M. J.; Ubeda, A.; Miranda, M. A.; Iborra, S.; Paya, M.; Alcaraz, M. *J. Med. Chem.* **1995**, *38*, 2794; (b) Hsieh, H. K.; Lee, T. H.; Wang, J. P.; Wang, J. J.; Lin, C. N. *Pharm. Res.* **1998**, *15*, 39.
7. Barford, L.; Kemp, K.; Hansen, M.; Kharazmi, A. *Int. Immunopharm.* **2002**, *2*, 545.
8. Rajas, J.; Paya, M.; Dominguez, J. N.; Ferrandiz, M. L. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1951.
9. Nerya, O.; Musa, R.; Khatib, S.; Tamir, S.; Jacob, O. *Phytochemistry* **2004**, *65*, 1389.
10. (a) Dimmock, J. R.; Kandepu, N. M.; Hetherington, M.; Quail, J. W.; Pugazhenthi, U.; Sudom, A. M.; Chamankhah, M.; Rose, P.; Pass, E.; Allen, T. M.; Halleran, S.; Szydlowski, J.; Mutus, B.; Tannous, M.; Manavathu, E. K.; Myers, T. G.; Clercq, E. D.; Balzarini, J. *J. Med. Chem.* **1998**, *41*, 1014; (b) Yit, C. C.; Das, N. P. *Cancer Lett.* **1994**, *82*, 65; (c) Satomi, Y. *Int. J. Cancer* **1993**, *55*, 506; (d) Edwards, M. L.; Stemerick, D. M.; Sunkara, P. S. *J. Med. Chem.* **1990**, *33*, 1948; (e) Yang, Y. Z.; Xia, P.; Bastow, K. F.; Nakanishi, Y.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 699.
11. (a) Wattenberg, L. W.; Coccia, J. B.; Galhaith, A. R. *Cancer Lett.* **1994**, *83*, 165; (b) Dinkova-Kostova; Abeygunawardana, C.; Talalay, P. *J. Med. Chem.* **1998**, *41*, 5287.
12. Sunkara, P. S.; Zwolshen, J. H.; Stemerick, D. M.; Edwards, M. L. *Am. Assoc. Cancer Res.* **1991**, *32*, 329.
13. Peyrot, V.; leynadier, D.; Sarrazin, M.; Briand, C.; Menendez, M.; Laynez, J.; Andreu, J. M. *Biochemistry* **1992**, *31*, 11125.
14. (a) 10d–e; (b) Ducki, S.; Forrest, R.; Hadfield, J. A.; Kendall, A.; Lawrence, N. J.; Mcgown, A. T.; Rennison, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1051.
15. (a) Schmidt, J. G. *Ber. Dtsch. Chem. Ges.* **1880**, *13*, 2342; (b) Claisen, L.; Claparede, A. *Ber. Dtsch. Chem. Ges.* **1881**, *14*, 349; (c) Kohler, E. P.; Chadwell, H. M. *Org. Synth.* **1922**, *2*, 1.
16. (a) Payne, G. B. *J. Am. Chem. Soc.* **1959**, *81*, 4901; (b) Payne, G. B. *J. Org. Chem.* **1959**, *24*, 2048; (c) Adams, R.; Johnson, J. R.; Wilcox, C. F. In *Laboratory Experiments in Organic Chemistry*, 5th ed.; The Macmillan Company: New York, 1963; p 381.
17. Bhat, B. A.; Puri, S. C.; Qurishi, M. A.; Dhar, K. L.; Qazi, G. N. *Synth. Commun.* **2005**, *35*, 1135.
18. All the compounds were characterized by analytical and spectral analysis. Selected spectroscopic data of **4j**,**n** and **4s**. ^{1}H NMR was recorded in 200 MHz NMR with TMS as internal reference.Compound **4j**: ^{1}H NMR ($CDCl_3$): δ 3.84 and 3.89 (s, $4 \times OCH_3$), 6.70 (s, 1H, H-4″), 6.89 (d, 2H, $J = 8.7$ Hz, H-3′ and H-5′), 6.92 (s, 2H, H-2 and H-6), 7.61 (d, 2H, $J = 8.7$ Hz, H-2′ and H-6′); ^{13}C NMR ($CDCl_3$): δ 55.1, 55.7, 60.8, 99.5, 102.5, 114.0, 122.9, 126.7, 127.6, 137.9, 146.5, 150.0, 153.3, 159.5. Anal. Calcd for $C_{19}H_{20}N_2O_4$: C, 67.04; H, 5.92; N, 8.23. Found: C, 67.38; H, 6.42, N, 8.31. MS (M^+) 340.Compound **4n**: ^{1}H NMR ($CDCl_3$): δ 3.81 and 3.87 (s, $3 \times OCH_3$), 6.71 (s, 1H, H-4″), 6.91 (s, 2H, H-2 and H-6), 7.04 (m, 2H, H-3′ and H-5′), 7.66 (m, 2H, H-2′ and H-6′); ^{13}C NMR ($CDCl_3$): δ 50.6, 56.1, 61.1, 99.8, 103.0, 115.9, 126.8, 127.5, 138.3, 148.0, 149.0, 153.7, 161.8, 163.8. Anal. Calcd for $C_{18}H_{17}N_2O_3F$: C, 65.84; H, 5.23; N, 8.53. Found: C, 66.04; H, 5.83; N, 8.34. MS (M^+) 314.Compound **4s**: ^{1}HNMR ($CDCl_3$): δ 3.82 and 3.92 (s, $5 \times OCH_3$), 6.67 (s, 1H, 4-H″), 6.84 (d, 1H, $J = 8.75$ Hz, H-5′), 6.94 (s, 2H, H-2 and H-6), 7.22 (s, 1H, H-2′), 7.23 (d, 1H, $J = 8.75$ Hz, H-5′); ^{13}C NMR (DMSO): δ 56.0, 56.1, 56.5, 60.6, 99.7, 103.1, 109.5, 112.5, 118.2, 137.7, 149.1, 149.4, 163.7. Anal. Calcd for $C_{20}H_{22}N_2O_5$: C, 64.85; H, 5.98; N, 7.56. Found: C, 64.94; H, 6.28; N, 8.31. MS (M^+) 370.
19. Skehan, P.; Storeng, R.; Seudiero, D.; Monks, A.; Memahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
20. Lee, K. H.; Hall, I. H.; Starness, C. D.; Elgebaly, S. A.; Waddel, T. G.; Hadgraft, R. T.; Ruffner, C. G.; Weidner, I. *Science* **1997**, *196*, 533.