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ARTICLE

Design and Synthesis of Novel Heterocyclic Curcumin Analogues as Anticancer Agents and Filarial Topoisomerase II Inhibitors

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Asian Journal of Organic & Medicinal Chemistry

Volume: 3 Year: 2018
Issue: 4 Month: October–December
pp: 164–170
DOI: https://doi.org/10.14233/ajomc.2018.AJOMC-P149

Received:
Accepted:
Published:

ABSTRACT

A series of substituted curcumin analogues have been designed and synthesized *via* condensation reaction of benzaldehydes and dehydroacetic acid. Synthesized molecules were further evaluated for their inhibitory activity against various cancer cell lines. Most of the synthesized compounds were significantly inhibited the growth of these cell lines. Ten most active compounds in the series were further screened to check their inhibitory effect against filarial topoisomerase II enzyme. All the compounds screened against topoisomerase II exhibited excellent inhibition upto percentage inhibition more than 95 %. Further, the structure-activity relationships of the evaluated compounds reveals that among the synthesized compounds, nitro substituted chalcones **5** and **8** were the most active compounds having IC_{50} value of 5.49 and 4.46 µM against A549 (*lung carcinoma*) cell lines, respectively and significantly inhibited *S. cervi* Topoisomerase II activity upto more than 95 %.

KEYWORDS

Curcumin, Anticancer, Topoisomerases II, Chalcones.

INTRODUCTION

In spite of the recent progress and development of cancer chemotherapy, there is still need for new compounds of therapeutic interest to bring this disease under control. A large number of modern drugs have been developed from natural sources, especially from plants [1]. Natural product derive or inspired molecules forms a large group of compounds with anticancer activity. A variety of naturally occuring compounds such as curcumin, paclitaxol, vinblastin, combretastatin A-4, desmosdumotin C and colchicine are well known anticancer agents.

Among them curcumin is the compound possessing a large number of biological activities and is most abundant in nature. Curcumin, is the yellow pigment extracted from the rhizoma of *Curcuma longa*, is the pharmacologically active substance of turmeric. By tradition, turmeric has been used for many ailments, particularly as an anti-inflammatory agent, and curcumin has been identified as the active principle of turmeric [2]. Curcumin is non-toxic and has a variety of positive pharmacological effects as anti-inflammatory, anti-oxidative and antiseptic properties have been reported and displayed good pharmacological effect in cardiovascular diseases, sporadic Alzheimer's

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disease, sarcopenia, type II diabetes, arthrosis and arthritis among others [3-5].

Despite its efficacy and safety, curcumin has not yet been approved as a therapeutic agent, and the relative bioavailability and solubility of curcumin has been highlighted as a major problem behind this issue [6]. Eventually this drawback render this molecule a possible anticancer drug, as both chemo preventive and chemotherapeutic. This evidence has strongly suggests that curcumin can be considered a promising tool for cancer therapy in recent years.

Among the various natural anticancer molecules, chalcones contains potentially important group of structurally effective feature bearing ketone functionality and an unsaturated group [7,8]. These are in conjugated arrangement among the various naturally occurring as well as synthetic anticancer agents. These class of compounds are well known for their tumor-reudcing and antiproliferative activities [9,10]. Any change in the three carbon propenone skeleton is known to lose the biological activities. Incorporating heterocyclic rings in chalcone core structure has been employed by medicinal chemists to enhance the pharmacological properties [11,12] *viz.* some coumarin-based compounds psorospermin (b), have been reported to display a good cytotoxic activity, exerting a good anticancer and topoisomerase II inhibitor activity [13].

DNA topoisomerase II is a enzyme that controls DNA topology by transient cleavage of DNA double helix. The non-covalent interaction of protein with DNA is the key step in the topoisomerase II catalytic cycle. Under physiological conditions, DNA replication, repair and transcription processes are significantly controlled by Topoisomerase II. Among the various enzymes identified as target against parasitic diseases, DNA topoisomerases have attracted medicinal chemists as a novel target for antifilarial drug development [14]. DNA topoisomerases are the enzymes required for the replication, transcription and recombination of DNA [15]. These enzymes play crucial roles in the organization of DNA within the cell nucleus as well as in its structure and function [16,17].

Now a days in the design of new drugs, the development of hybrid molecules through the combination of different pharmacophores may lead to compounds with interesting biological profiles [18,19]. This combination rationale has been used in our laboratory to synthesize 3-cinnamoyl-4-hydroxy-6-methyl-2H-pyran-2-one analogues by incorporating conjugated 1,3-dicarbonyl group from curcumin and pyran-2-one moiety from different biologically active natural product nucleus as novel class of cytotoxic agents (Fig. 1). We hypothesized to synthesize the chalcone analogue consisting of both the phrmacophore and to see their cytotoxic activity in various *in vitro* cancer cell lines and filarial topoisomerase-II activity.

EXPERIMENTAL

Unless otherwise specified all the reagents and catalysts were purchased from Sigma-Aldrich and used without further any purification. The common solvents were purchased from Ranbaxy. Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator. Chromatographic purification of products was accomplished using flash chromatography on 230-400 mesh silica gel. Reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates visualized under UV light, iodine or $KMnO_4$ staining. ^1H and ^{13}C NMR spectra were recorded on a Brucker DRX-200 MHz spectrometer. Chemical shifts (δ) are given in ppm relative to TMS and coupling constants (*J*) in Hz. IR spectra were recorded on a FTIR spectrophotometer Shimadzu 8201 PC and are reported in terms of frequency of absorption (cm^{-1}). Mass spectra (ESIMS) were obtained by micromass quattro II instrument.

General procedure for synthesis of chalcone analogues (3-22): Chalcone analogues (**3-22**) were synthesized *via* aldol condensation of substituted benzaldehyde and dehydroacetic acid. In dry chloroform, substituted benzaldehyde (1 mmol), dehydroacetic acid (1 mmol) in the presence of catalytic pyrrolidine (20 mol %) was taken. Reaction was stirred at room temperature for 2 h leading to generation of chalcone. Progress of reaction was monitored by TLC. After completion of reaction solvent was evaporated under the reduced pressure and residue was extracted with ethyl acetate and water. The organic layer was separated and dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated under vacuum on a rotary evaporator.

3-Cinnamoyl-4-hydroxy-6-methyl-2*H*-pyran-2-one (3): White solid, m.p. 146 ºC; IR (KBr, ν_{max}, cm^{-1}): 3424, 3083, 1725, 1626, 1326, 1236. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.36 (1H, d, *J* = 15.75 Hz); 8.01 (1H, d, *J* = 15 Hz); 7.27-7.69 (2H, m),

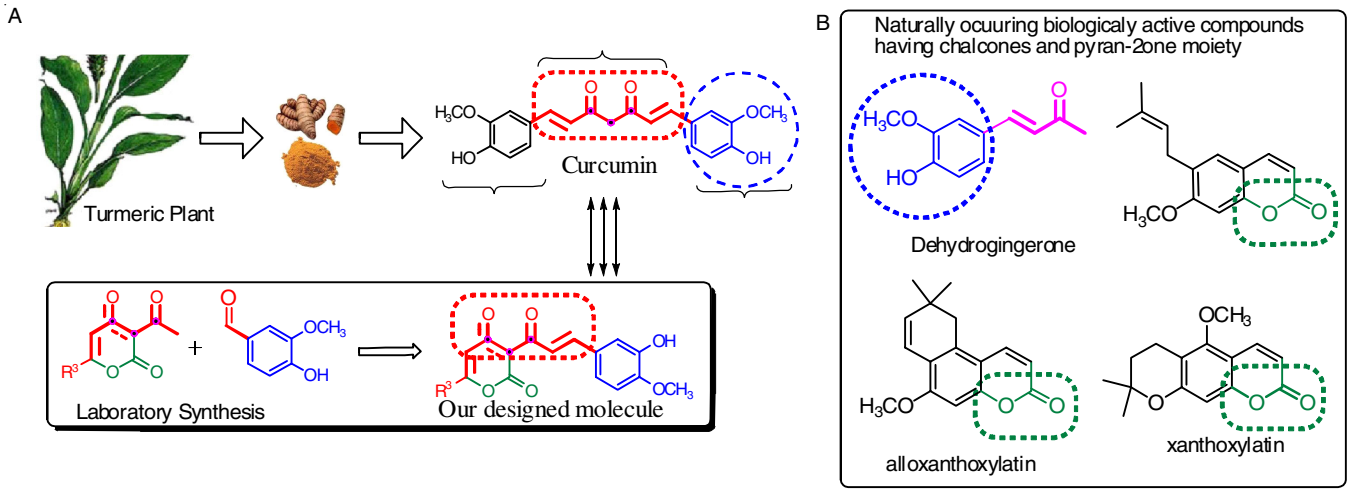


Fig. 1. (A) Designing of curcumin type chalcone analogues; (B) Some naturally occurring biologically important chalcones

7.45-7.42 (3H, m), 5.98 (1H, s), 2.30 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.0, 182.4, 166.5, 162.1, 150.5, 148.6, 131.6, 129.8, 127.5, 123.21 120.6, 116.1, 111.4, 103.1, 98.9, 20.7. MS (ES): *m/z* (%) = 257 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{15}H_{12}O_4$: C, 70.31 (70.28), H, 4.72 (4.69).

(*E*)-4-Hydroxy-3-(3-(4-methoxyphenyl)acryloyl)-6-methyl-2*H*-pyran-2-one (4): Yellow solid; m.p. 130 ºC; IR (KBr, ν_{max}, cm^{-1}): 3419, 3087, 1717, 1642, 1348, 1254. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.89 (1H, s), 7.86 (2H, d, *J* = 8.9 Hz), 7.03 (2H, d, *J* = 8.7Hz), 5.9 (1H, s), 6.97-6.88 (2H, m), 3.98 (3H, s), 2.28 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.6, 183.7, 166.4, 161.6, 150.5, 147.2, 137.4, 120.4, 115.6, 111.6, 104.1, 98.7, 55.6, 20.4; MS (ES): *m/z* (%) = 287 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{16}H_{14}O_5$: C, 67.13 (67.08); H, 4.93 (4.89).

(E)-4-Hydroxy-3-(3-(3-methoxyphenyl)acryloyl)-6-methyl-2*H*-pyran-2-one (5): Yellow solid; m.p. 135 ºC; IR (KBr, ν_{max}, cm^{-1}): 3421, 3072, 1719, 1659, 1328, 1212. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.08 (1H, s), 8.22 (2H, d, *J* = 14 Hz), 7.97 (2H, d, *J* = 14 Hz), 6.98-6.94 (1H, m), 5.96 (1H, s), 3.98 (3H, s), 2.28 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.7, 182.6, 167.4, 161.2, 151.2, 148.6, 131.4, 127.4, 123.1, 121.3, 116.2, 111.6, 103.2, 97.8, 21.4. MS (ES): *m/z* (%) = 287 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{16}H_{14}O_5$: C, 67.13 (67.09); H, 4.93 (4.90).

(*E*)-3-(3-(3,4-Dimethoxyphenyl)acryloyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (6): Yellow solid; m.p. 145 ºC; IR (KBr, ν_{max}, cm^{-1}): 3466, 3112, 1722, 1629, 1316, 1252. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.11 (1H, s), 8.23 (1H, d, *J* = 15 Hz), 7.97 (1H, d, *J* = 15.7 Hz), 7.49-7.22 (2H, m), 7.01-6.89 (2H, m), 5.96 (1H, s), 3.86 (6H, s), 2.38 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.0, 181.4, 167.4, 162.1, 151.4, 148.6, 132.8, 127.7, 123.2, 121.0, 116.1, 112.5, 103.1, 98.9, 56.4, 21.3. MS (ES): *m/z* (%) = 317 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{17}H_{16}O_6$: C, 64.55 (64.51); H, 5.10 (5.06).

(*E*)-4-Hydroxy-6-methyl-3-(3-(4-nitrophenyl)acryloyl)-2*H*-pyran-2-one (7): Creamish solid; m.p. 182 ºC; IR (KBr, ν_{max}, cm^{-1}): 3697, 3020, 2924, 1725, 1596, 1216. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.13 (1H, s), 8.40-8.34 (2H, m), 8.26 (1H, d, *J* = 12.4Hz), 8.09 (1H, d, *J* = 12.4Hz), 7.98-7.94 (2H, m), 5.98 (1H, s), 2.28 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.1, 183.7, 167.4, 161.6, 152.8, 148.4, 131.6, 122.7, 114.2, 112.7, 103.2, 98.9, 20.5; MS (ES): *m/z* (%) = 302 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{15}H_{11}NO_6$: C, 59.80 (59.75); H, 3.68 (3.63).

(*E*)-4-Hydroxy-6-methyl-3-(3-(2-nitrophenyl)acryloyl)-2*H*-pyran-2-one (8): Yellow solid; m.p. 176 ºC; IR (KBr, ν_{max}, cm^{-1}): 3571, 3032, 1719, 1636, 1218. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.16 (1H, s), 8.43-8.35 (2H, m), 8.28 (1H, d, *J* = 12 Hz), 8.10-8.06 (2H, m), 7.58 (1H, d, *J* = 12 Hz), 6.00 (1H, s), 2.29 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.1, 182.6, 167.5, 161.2, 150.4, 148.6, 134.8, 127.7, 123.2, 120.4, 116.1, 111.7, 103.2, 98.9, 21.3. MS (ES): *m/z* (%) = 302 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{15}H_{11}NO_6$: C, 59.80 (59.74); H, 3.68 (3.66); N, 4.65 (4.67).

(*E*)-3-(3-(4-(Dimethylamino)phenyl)acryloyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (9): Dark maroon solid; m.p. 180 ºC; IR (KBr, ν_{max}, cm^{-1}): 3516, 3116, 1724, 1669, 1326, 1218. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.08 (1H, s), 8.22 (1H, d, *J* = 15 Hz), 8.11 (1H, d, *J* = 15Hz), 8.90 (2H, d, *J* = 9Hz,) 7.62 (1H, s), 6.72 (2H, d, *J* = 9 Hz), 5.98 (1H, s), 3.09 (6H, s), 2.27 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.3, 183.9, 167.4, 161.6, 152.7, 148.3, 131.8, 122.7, 116.4, 111.7, 103.2, 98.9, 40.1, 20.5. MS (ES): *m/z* (%) = 300 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{17}H_{17}NO_4$: C, 68.21 (68.18); H, 5.72 (5.67); N, 4.68 (4.71).

(*E*)-3-(3-(3-Chlorophenyl)acryloyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (10): Light yellow solid; m.p. 170 ºC; IR (KBr, ν_{max}, cm^{-1}): 3518, 3087, 1720, 1621, 1328, 1248. 1^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.87 (1H, s), 8.30 (1H, d, *J* = 14 Hz), 8.12 (1H, d, *J* = 14 Hz), 7.94-7.67 (3H, m), 7.63-7.55 (1H, m), 5.98 (1H, s), 2.28 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.1, 181.4, 167.5, 161.2, 150.4, 147.6, 134.8, 127.7, 122.2, 120.8, 116.3, 112.7, 103.2, 98.7, 21.1.; MS (ES): *m/z* (%) = 291 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{15}H_{11}O_4Cl$: C, 61.98 (61.91); H, 3.81 (3.84).

(*E*)-3-(3-(4-Chlorophenyl)acryloyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (11): Light brown solid; m.p. 166 ºC; IR (KBr, ν_{max}, cm^{-1}): 3452, 3152, 1721, 1665, 1327, 1242. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.15 (1H, s); 8.26 (1H, d, *J* = 16 Hz), 7.95 (1H, d, *J* = 16 Hz), 7.51-7.18 (2H, m), 6.99-6.78 (2H, m), 5.98 (1H, s), 2.31 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.8, 183.9, 166.3, 161.6, 151.5, 147.2, 138.4, 121.7, 116.4, 112.6, 103.1, 98.9, 20.4. MS (ES): *m/z* (%) = 291 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{15}H_{11}O_4Cl$: C, 61.98 (61.94); H, 3.81 (3.79).

(*E*)-3-(3-(2-Chlorophenyl)acryloyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (12): Yellow solid; m.p. 188 ºC; IR (KBr, ν_{max}, cm^{-1}): 3517, 3084, 1720, 1626, 1328, 1248. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.83 (1H, s), 8.30-8.11 (m, 2H), 7.97-7.66 (3H, m), 7.61-7.55 (1H, m), 5.96 (1H, s), 2.27 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.4, 181.6, 167.9, 161.6, 150.1, 147.3, 134.5, 127.2, 122.2, 120.1, 116.3, 112.7, 103.2, 98.7, 21.4. MS (ES): *m/z* (%) = 291 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{15}H_{11}O_4Cl$: C, 61.98 (61.94); H, 3.81 (3.86).

(*E*)-4-Hydroxy-3-(3-(3-hydroxy-4-methoxyphenyl)-acryloyl)-6-methyl-2*H*-pyran-2-one (13): Yellow solid; m.p. 175 ºC; IR (KBr, ν_{max}, cm^{-1}): 3355, 3100, 1711, 1600, 1424, 1275. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.08 (1H, s), 8.21 (1H, d, *J* = 15.6Hz), 7.11 (1H, d, *J* = 15.6Hz), 7.28-7.22 (3H, m), 6.77 (1H, d, *J* = 8.1Hz), 5.96 (1H, s), 3.98 (3H, s), 2.28 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.1, 181.3, 167.4, 162.1, 151.4, 148.6, 132.8, 126.7, 123.1, 121.2, 116.4, 112.3, 103.1, 98.7, 56.4, 21.2; MS (ES): *m/z* (%) = 303 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{16}H_{14}O_6$: C, 63.57 (63.53); H, 4.67 (4.63).

(*E*)-3-(3-(4-(Benzyloxy)phenyl)acryloyl)-4-hydroxy-6-methyl-2H-pyran-2-one (14): Yellow solid; m.p. 164 ºC; IR (KBr, ν_{max}, cm^{-1}): 3436, 3021, 1724, 1635, 1343, 1217. ^{1}H NMR ($CDCl_3$, 300 MHz): δ10.18 (1H, s), 8.19 (1H, d, *J* = 15.6Hz), 7.68 (2H, d, *J* = 8.7Hz), 7.43-7.41 (5H, m), 7.42-7.03 (1H, m), 7.01 (1H, d, *J* = 8.7Hz), 5.95 (1H, s); 5.11 (2H, s), 2.27 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): 190.3, 187.2, 164.3, 166.2, 158.2, 142.7, 136.7, 130.2, 128.9, 127.6, 127.5, 124.4, 114.2, 70.8, 20.9. MS (ES): *m/z* (%) = 363 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{22}H_{18}O_5$: C, 72.92 (72.89); H, 5.01 (4.98).

(*E*)-4-Hydroxy-3-(3-(3-hydroxyphenyl)acryloyl)-6-methyl-2*H*-pyran-2-one (15): Orange solid; m.p. 195 ºC; IR (KBr, ν_{max}, cm^{-1}): 3536, 3172, 1722, 1657, 1378, 1220. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.04 (1H, s), 8.31 (1H, d, *J* = 15.8Hz), 7.89 (1H, d, *J* = 15.8Hz), 7.62 (2H, d, *J* = 6.68 Hz), 7.39 (1H, d, *J* = 6.9 Hz), 5.97 (1H, s), 2.29 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.3, 182.2, 167.5, 161.2, 150.1, 148.4, 134.6, 127.7, 123.2,

121.4, 116.1, 112.7, 103.1, 98.9, 21.1.; MS (ES): *m/z* (%) = 273 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{15}H_{12}O_5$: C, 66.17 (66.12); H, 4.44 (4.38).

(*E*)-4-Hydroxy-3-(3-(4-hydroxyphenyl)acryloyl)-6-methyl-2*H*-pyran-2-one (16): Bright yellow solid; m.p. 159 ºC; IR (KBr, ν_{max}, cm^{-1}): 3541, 3167, 1724, 1658, 1346, 1268. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.04 (1H, s), 8.39 (1H, d, *J* = 16 Hz), 7.92 (1H, d, *J* = 16 Hz), 7.76 (2H, d, *J* = 7.81 Hz), 7.51 (1H, d, *J* = 7.78Hz), 5.98 (1H, s), 2.28 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.3, 181.2, 166.5, 162.1, 151.3, 147.2, 129.6, 126.4, 123.2, 111.5, 103.1, 97.6, 21.0.; MS (ES): m/z (%) = 273 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{15}H_{12}O_5$ C, 66.17; H, 4.44; Found: C, 66.12; H, 4.41%.

(*E*)-3-(3-(4-Fluorophenyl)acryloyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (17): Pale yellow solid; m.p. 127 °C; IR (KBr, ν_{max}, cm^{-1}): 3546, 3162, 1724, 1657, 1268. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.03 (1H, s), 8.42 (1H, d, *J* = 16 Hz), 7.98 (1H, d, *J* = 16 Hz), 7.71 (2H, d, *J* = 7.81 Hz), 7.68 (1H, d, *J* = 7.78 Hz), 5.95 (1H, s), 2.29 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.2, 181.4, 166.6, 160.6, 150.6, 146.1, 130.4, 126.4, 122.1, 110.8, 103.6, 96.4, 21.3. MS (ES): *m/z* (%) = 275 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{15}H_{11}O_4F$: C, 65.69 (66.64); H, 4.04 (4.06).

(*E*)-4-Hydroxy-3-(3-(2-methoxyphenyl)acryloyl)-6-methyl-2*H*-pyran-2-one (18): Yellow solid; m.p. 146 ºC; IR (KBr, ν_{max}, cm^{-1}): 3422, 3072, 1716, 1642, 1348, 1253. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.86 (1H, s); 7.81 (2H, d, *J* = 8.6 Hz), 7.08 (2H, d, *J* = 8.7Hz), 5.94 (1H, s), 6.99-6.84 (2H, m), 3.97 (3H, s), 2.28 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.0, 182.7, 164.4, 161.6, 151.2, 147.8, 137.2, 121.1, 115.5, 111.6, 104.1, 98.1, 55.6, 21.3; MS (ES): *m/z* (%) = 287 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{16}H_{14}O_5$: C, 67.13 (67.10); H, 4.93 (4.85).

(*E*)-3-(3-(2-Chlorophenyl)acryloyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (19): Yellow solid; m.p. 165 ºC; IR (KBr, ν_{max}, cm^{-1}): 3448, 3127, 1720, 1664, 1338, 1228. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.04 (1H, s), 8.41 (1H, d, *J* = 15.6Hz), 8.26 (1H, d, *J* = 15.6Hz), 8.04-7.87 (3H, m), 7.65-7.58 (1H, m), 5.98 (1H, s); 2.28 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.0, 181.6, 166.5, 161.2, 150.4, 148.6, 133.5, 126.8, 123.1, 121.4, 116.1, 110.6, 102.6, 97.6, 20.6. MS (ES): *m/z* (%) = 291 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{15}H_{11}O_4Cl$: C, 61.98 (3.81); H, 3.81 (3.83).

(*E*)-3-(3-(3-Bromo-4-methylphenyl)acryloyl)-4-hydroxy-6-methyl-2H-pyran-2-one (20): Brown solid; m.p. 156 ºC; IR (KBr, ν_{max}, cm^{-1}): 3572, 3022, 1722, 1656, 1217. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.25 (1H, s); 8.06 (1H, d, *J* = 14.8 Hz), 7.50 (1H, d, *J* = 14.8 Hz), 7.39-7.28 (2H, m); 5.94 (1H, s), 2.67 (3H, s), 2.27 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.2, 181.4, 168.4, 162.1, 153.2, 147.4, 137.8, 131.6, 124.1, 120.2, 114.2, 112.1, 102.1, 98.7, 23.2, 21.0.; MS (ES): m/z (%) = 350 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{16}H_{13}O_4Br$: C, 55.04 (55.01); H, 3.75 (3.71).

(*E*)-3-(3-(2,5-Dimethoxyphenyl)acryloyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (21): Yellow solid; m.p. 126 ºC; IR (KBr, ν_{max}, cm^{-1}): 3468, 3121, 1725, 1629, 1316, 1252. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.10 (1H, s); 8.21 (1H, d, *J* = 15 Hz), 7.94 (1H, d, *J* = 15.7 Hz), 7.50-7.24 (2H, m), 7.04-6.86 (2H, m), 5.94 (1H, s), 3.84 (6H, s), 2.32 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.0, 181.6, 167.2, 162.9, 151.6, 148.4, 131.8, 126.6, 123.2, 121.2, 116.4, 112.5, 103.8, 98.6, 56.4, 21.3. MS (ES): *m/z* (%) = 317 (100) $[M+1]^+$. Anal. calcd. (found) % for C17H16O6: C, 64.55; H, 5.10; Found: C, 64.50; H, 5.13%.

(E)-4-Hydroxy-6-methyl-3-(3-(2,3,5-trimethoxyphenyl)-acryloyl)-2*H*-pyran-2-one (22): Orange solid; mp 161 ºC; IR (KBr, ν_{max}, cm^{-1}): 3428, 3144, 1722, 1656, 1346, 1262. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.84 (1H, s), 8.17 (1H, d, *J* = 15 Hz), 8.04 (1H, d, *J* = 15 Hz), 7.24 (1H, s), 7.13 (1H, s), 5.91 (1H, s), 4.29 (3H, s), 4.18 (3H, s), 3.87 (3H, s,), 2.28 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.8, 181.4, 168.4, 162.1, 153.2, 147.4, 136.8, 133.6, 132.8, 128.4, 124.2, 112.4, 103.1, 96.4, 56.4, 21.0. MS (ES): *m/z* (%) = 346 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{18}H_{18}O_7$ C, 62.42 (62.38); H, 5.24 (5.21).

(*E*)-4-Hydroxy-6-methyl-3-(3-(3-nitrophenyl)acryl-oyl)-2H-pyran-2-one (23): Pale yellow solid; m.p. 42 ºC; IR (KBr, ν_{max}, cm^{-1}): 3531, 3021, 1720, 1639, 1538, 1217. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.86 (1H, s), 8.46-8.37 (3H, m), 8.28 (1H, d, *J* = 14Hz), 8.06 (1H, d, *J* = 14Hz), 7.98-7.94 (1H, m), 5.96 (1H, s), 2.27 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 190.1, 181.6, 167.5, 160.2, 150.4, 147.6, 135.3, 127.7, 123.1, 120.4, 116.1, 111.4, 103.6, 98.8, 20.8. MS (ES): *m/z* (%) = 302 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{15}H_{11}NO_6$: C, 59.80 (59.76); H, 3.68 (3.64).

(*E*)-3-(3-(4-(benzyloxy)-3-methoxyphenyl)acryloyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (24): Light brown solid; mp 59 ºC; IR (KBr, ν_{max}, cm^{-1}): 3700, 3325, 3062, 2968, 1722, 1656, 1265. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.84 (1H, s), 8.21 (1H, d, *J* = 16Hz), 7.93 (1H, d, *J* = 16.7Hz), 7.46-7.35 (5H, m), 7.34-7.01 (2H, m), 7.01 (1H, d, *J* = 8.4Hz), 5.92 (1H, s), 5.25 (2H, s), 3.95 (3H, s), 2.27 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.0, 181.2, 167.4, 162.4, 150.8, 148.6, 136.4, 132.8, 130.3, 128.4, 127.8, 127.1, 123.4, 121.1, 114.3, 112.5, 103.1, 98.9, 56.4, 21.3; MS (ES): *m/z* (%) = 393 (100) $[M+1]^+$. Anal. calcd. (found) % for $C_{23}H_{20}O_6$ C, 70.40 (70.36); H, 5.14 (5.10).

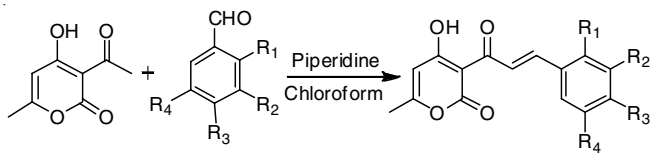
Anticancer activity: Screening for anticancer activity was done using 5 cell lines. The human cancer cell line KB (oral carcinoma), MCF-7 (breast carcinoma) and C33A (cervical carcinoma) were maintained in EMEM medium; A549 (lung carcinoma) in F-12 medium and mouse embryonic fibroblast line NIH/3T3 (used as control) was maintained in DMEM medium. The cells were seeded in 96 well cultured plates (1-3 × 10^4 cells/180 µL/well, depending on the cell line) and incubated (37 ºC, 5 % CO_2 in humidified atmosphere) for 24 h. The diluted test samples were added to cell monolayers in duplicate wells (20 µL per well). For each plate, positive control (doxorubicin) and vehicle (DMSO) controls were included. Drug or test sample treated cells were again incubated for 48 h (5 % CO_2, 37 ºC). Cell viability was estimated by sulphorhodamine-B (SRB) assay using a slight modification of the published protocol [1,2]. In brief, cell attached to subatratum of plate were fixed adding 50 % trichloroacetic acid (50 µL per well) on top of medium and incubated for 1 h (4 ºC). Later, the plate was gently washed five times to remove trichloroacetic acid, growth medium and dead cells allowed to dry in air. Sulphorhodamine-B (50 µL of 0.4 % (w/v)) dissolved in 1 % acetic acid was added to each well and left for 30 min. At the end of staining period, unbound SRB was removed by washing 4 times with 1 % acetic acid.

Plates were again air dried and 150 µL of 10 mM tris base was added to each well in order to solubilize the cell bound dye. Plates were shaken for 15-30 min on gyratory shaker followed by reading the absorbance at 550 nm in microplate spectrophotometer.

Estimation of DNA topoisomerase II inhibitory activity of compounds: The reaction catalyzed by DNA topoisomerase II was estimated as reported earlier [1]. The reaction mixture in a final volume of 20 µL contained 50 mM tris HCl (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 1 mM ATP, 0.1 mM EDTA, 0.5 mM dithiothreitol (DTT), 30 µg/mL bovine serum albumin (BSA), 0.25 µg pBR322 DNA and enzyme protein. The reaction was carried out at 37 ºC for 30 min. and stopped by adding 5 µL stop buffer. The samples were subjected to electrophoresis on 1 % agarose gel in tris-acetate buffer for 18 h at 20 V. Gels were stained with ethidium bromide (0.5 µg/mL) and visualized and photographed on a GDS 7500 UVP Trans illuminator (Ultraviolet Products, UK). The effect of inhibitors on enzyme activity was measured by incubating enzyme with the inhibitor for 10 min at 37 ºC and starting the reaction by addition of pBR322 DNA. The percentage inhibition was measured by micro densitometry of gel with gel base/gel blot Progel analysis software program.

RESULTS AND DISCUSSION

In present work, the synthesis as well as cytotoxicity and filarial topoisomerase II inhibitory activity of 22 substituted natural products inspired chalcone type analogues are described. Synthesized analogues bearing different functional groups at phenyl ring in an attempt to optimize the anticancer activity and potentially gain insight into the structure activity relationship of 3-cinnamoyl-4-hydroxy-6-methyl-2*H*-pyran-2-one and its derivatives. The reaction scheme for the synthesis of designed analogue is shown schematically in **Scheme-I**. Compounds (**3-22**) were synthesized from dehydroacetic acid (**1**) and substituted benzaldehydes (**2**) *via* aldol condensation reaction using pyridine as base. Both the reactants were mixed in anhydrous chloroform and reaction was allowed to reflux for appropriate time period upto completion of reaction. After completion of reaction desired products were isolated by only filtration. Negligible or no chromatography required for purification. All the compounds synthesized were confirmed by spectroscopic (^{1}H and ^{13}C NMR) and spectrometric (mass) data [17].



Scheme-I: Synthesis of substituted 3-cinnamoyl-4-hydroxy-6-methyl-2*H*-pyran-2-one

Anticancer activity: All the synthesized compounds were evaluated for their *in vitro* anticancer activity using Sulforhodamine-B assays. The growth-inhibitory effects were undertaken in four human cancer cell lines, KB (oral squamous cell carcinoma), C33A (cervical carcinoma), MCF-7 (breast adenocarcinoma), A549 (lung) and one normal fibroblast NIH3T3 (mouse embryo fibroblast) in order to determine their cyto-selective nature. The results are presented in Table-1. IC_{50} values were based on dose-response curves.

Each test compound displayed a concentration-dependent cytotoxic profile in all four cell lines. Out of all the compounds

TABLE-1
SYNTHESIS AND *in vitro* ANTICANCER ACTIVITY OF SYNTHESIZED 3-CINNAMOYL-4-HYDROXY-6-METHYL-2*H*-PYRAN-2-ONE DERIVATIVES

Compd.	R^1	R^2	R^3	R^4	Yield[g]	IC_{50} values (µM)				
						KB[a]	C-33A[b]	MCF-7[c]	A549[d]	NIH3T3[e]
3	H	H	H	H	88	NA[f]	NA	NA	NA	NA
4	H	H	OCH_3	H	91	24.8	21.76	30.4	9.31	43.18
5	H	OCH_3	H	H	90	6.83	5.92	10.21	5.41	17.15
6	H	OCH_3	OCH_3	H	90	11.69	14.69	10.89	11.17	>50
7	H	H	NO_2	H	89	9.05	10.41	8.96	6.82	18.19
8	NO_2	H	H	H	83	7.5	5.14	7.05	4.46	25.89
9	H	H	N,N-$(CH_3)_2$	H	92	NA	NA	NA	NA	NA
10	H	Cl	H	H	91	NA	NA	NA	NA	NA
11	H	H	Cl	H	92	NA	NA	NA	NA	NA
12	H	Cl	H	3-Cl	90	NA	NA	NA	NA	NA
13	H	OH	OCH_3	H	86	NA	NA	NA	NA	NA
14	H	H	OCH_2Ph	H	87	15.85	29.21	21.63	19.95	>50
15	H	OH	H	H	84	NA	NA	NA	NA	NA
16	H	H	OH	H	82	17.89	31.76	24.41	25.05	>50
17	H	H	F	H	94	14.41	12.71	15.66	10.6	36.04
18	OCH_3	H	H	H	90	8.73	9.16	10.44	11.19	22.31
19	3-Cl	H	H	H	92	12.72	10.13	16.08	9.61	27.18
20	H	Br	H	H	90	17.34	14.46	23.96	13.27	>50
21	OCH_3	H	H	OCH_3	88	35.23	26.44	44.08	16.19	>50
22	OCH_3	OCH_3	H	OCH_3	86	26.43	21.04	38.69	17.42	28.16
23	H	NO_2	H	H	91	13.07	19.43	26.48	13.56	24.91
24	H	OCH_3	OCH_2Ph	H	90	22.91	23.62	33.50	14.41	38.69

[a]KB (oral squamous cell carcinoma), [b]C33A (cervical carcinoma), [c]MCF-7 (breast adenocarcinoma), [d]A549 (lung) [e]NIH3T3 (mouse embryo fibroblast), [f] NA= inactive [g]= % yield of purified fractions.

TABLE-2
TOPOISOMERASE II INHIBITORY ACTIVITY OF TEST COMPOUNDS AGAINST FILARIAL PARASITE *Setaria cervi*

Lane No.	Lane contents	Inhibition (%)
1	pBR 322 DNA only (Control)	–
2	pBR 322 DNA only + *S. Cervi* DNA pol. II (Experimental)	–
3	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 7 (40 µg)	95
4	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 4 (40 µg)	50
5	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 6 (40 µg)	50
6	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 8 (40 µg)	95
7	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 5 (40 µg)	95
8	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 9 (40 µg)	75
9	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 18 (40 µg)	95
10	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 14 (40 µg)	30
11	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 19 (40 µg)	30
12	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 23 (40 µg)	90

evaluated, most of the compounds exhibited significant IC_{50} value ranging from 4.46 to 16.19 µM. The compounds having IC_{50} value more than 50 µM were considered inactive. A closure look into the structure activity relationship indicates that among the series of synthesized chalcone analogues (**3-22**), the compounds consisting of NO_2 group at 2- and 4-position (compounds **7** and **8**) of phenyl ring were the most active compounds of series with IC_{50} value of 6.82 µM and 4.46 µM in A549 (lung carcinoma) cell line. These compounds were also exhibiting significant inhibition in all the four human cancer cell lines KB (oral squamous cell carcinoma), C33A (cervical carcinoma), MCF-7 (breast adenocarcinoma), A549 (lung carcinoma) with IC_{50} value ranging between 4.46 µM to 10.41 µM. Furthermore, it is evident from Table-1 that compounds having halogen substitution at any position of phenyl ring were failed to show any significant activity and considered to be inactive.

Topoisomerase-II inhibitory activity: Ten compounds (**4**, **5**, **6**, **7**, **8**, **14**, **16**, **17**, **18** and **19**) selected on the basis of maximum inhibitory effect against cancer cell lines were further biologically screened against *S. cervi* Topoisomerase II enzyme. Results indicated that compounds screened were found to be active against Topoisomerase II enzyme (Fig. 2) with percentage inhibition in range of 30-95 %. Inhibition of Topoisomerase II activity was examined by studying the enzyme-mediated supercoiled pBR322 relaxation (Table-2).

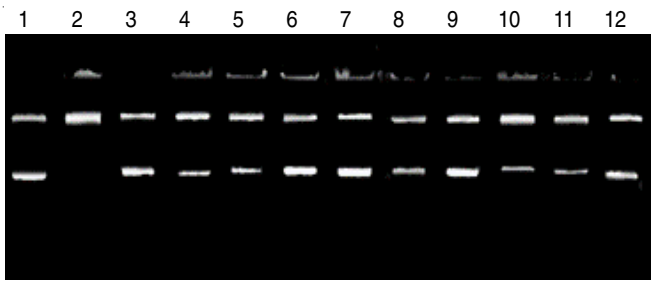


Fig. 2. Gel mobility shift assay of *S. cervi* topoisomerase II. Lane 1: pBR322 (0.25 µg) alone; lane 2: pBR322 + *S. cervi* Topo II; Lane 3: pBR322 + *S. cervi* Topo II + Comp. 7 (40 µg); Lane 4: pBR322 + *S. cervi* Topo II + Comp. 4 (40 µg); Lane 5: pBR322 + *S. cervi* Topo II + Comp. 6 (40 µg); Lane 6: pBR322 + *S. cervi* Topo II + Comp. 8 (40 µg); Lane 7: pBR322 + *S. cervi* Topo II + Comp. 5 (40 µg); Lane 8: pBR322 + *S. cervi* Topo II + Comp. 9 (40 µg); Lane 9: pBR322 + *S. cervi* Topo II + Comp. 18 (40 µg); Lane 10: pBR322 + *S. cervi* Topo II + Comp. 14 (40 µg); Lane 11: pBR322 + *S. cervi* Topo II + Comp. 19 (40 µg); Lane 12: pBR322 + *S. cervi* Topo II + Comp. 23 (40 µg). Table-2: DNA inhibition

Conclusion

We have designed and synthesized 22 chalcone type analogues as cinnamoyl-4-hydroxy-6-methyl-2*H*-pyran-2-one deriva-tives and evaluated their pharmacological activity. Most of the compounds showed promising cytotoxicity and topoisomerase II inhibitory activity. Compound **5** exhibited excellent cytotoxic activity in all the tested cell lines. Ten coumpounds which were active against cancer cell lines also tested against filarial topoisomerase-II and found to be promising active with percentage inhibition of more than 95 %. Present findings suggest that presence of nitro group of phenyl ring of chalcone moiety is prime factor for the pharmacological activity of compounds. To elaborate optimization of chalcone structure with nitro and methoxy substitution can produce potential anticancer drug compounds.

ACKNOWLEDGEMENTS

The authors are thankful to CSIR, New Delhi, India for financial support. The authors also acknowledge to SAIF-CDRI, Lucknow, India for providing all the spectral, analytical and biological activity data.

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