INTRODUCTION

Oral squamous cell carcinoma (OSCC) developed from mucosal lining of oral cavity is ranked as 6th most common cancer worldwide. Adverse effects of available anticancer agents intended present work to carry out the synthesis, characterization and evaluation of new quinoline analogues (NQA) against OSCC cell lines. In present study, substituted quinoline (1) was treated with ethyl chloroacetate to offer ester derivative (2), which on treatment with hydrazine hydrate yielded hydrazide derivative (3), which was cyclized into oxadiazole derivative (4) when cyclized with 4-methoxy benzoic acid. Characterization of molecular structures of synthesized NQAs was done based on the FTIR, 1H NMR, 13C NMR and mass spectrometric data. The characterized NQAs were investigated for their anticancer potential. The anticancer studies involved antiproliferation study (IC50 determination) against OSCC cell lines (CAL-27), followed by cell cycle analysis. The results of antiproliferation study of NQAs revealed that among all, NQA 3 exhibited lowest IC50 (3.26 µg/mL). Also, the results of cell cycle analysis of all NQAs revealed that all NQAs caused cancer cells arrest in ‘S’ phase. The high anticancer activity of NQA 3 and ability of all the NQAs to cause CAL-27 cells arrest in ‘s’ phase supports their potential application in OSCC treatment. However, the synthesized NQAs must be additionally investigated for the in vivo and clinical studies.

Keywords: Quinoline analogues, Irinotecan, Synthesis, Oxadiazole, Hydrazide, Ester, Anticancer activity.
survival by up to 16% [5]. Local and regional recurrence of cancers accounts up to 90% of treatment failures of surgery and radiotherapy. Chemotherapeutic agents are known to activate the tumor cell apoptosis and preferentially act on proliferating “cycling” cancer cells, making malignant cells marginally susceptible to these agents [6], but significant limitation to chemotherapeutic agents is development of multidrug resistance (MDR) by human cancer cells [7].

Among available anticancer agents irinotecan has been widely used in the treatment of various types of cancers [8]. Facts suggest modest antitumor activity of irinotecan, the camptothecin derivative (quinoline analogue) as single agent in chemo-naive and previously treated head and neck cancer [9,10]. The use of such quinoline analogue is not generally considered in OSCC treatment, possibly due to lack of studies. Study so far only two camptothecin analogues (irinotecan and topotecan) the topoisomerase-1 inhibitors have been approved for cancer treatment [12]. Although several camptothecin derivatives (quinoline analogues) topoisomerase-1 inhibitors have been synthesized but very less in vitro studies are available on their potential against OSCCs.

There is evidence that enhancing their anticancer action by incorporating ester, hydrazide or oxadiazole moieties to the quinoline moiety [13-15]. Evidence suggests that the incorporation of esters, hydrazide and oxadiazole groups in the organic compounds enhances their anticancer potential [16,17]. Recent investigations mentioned the synthetic protocols to convert the phenolics into esters [16], esters into hydrazides [18] and hydrazides into oxadiazoles [19] using ethylchloroacetate, hydrazine hydrate and aromatic acids. Hence based on the problem of oral squamous cell carcinomas (OSCCs), scarcity of agents to target OSCCs and anticancer potential of quinolines, hydrazide, ester, and oxadiazole groups, in present study was designed to perform synthesis, characterization and in vitro anticancer activity of new quinoline analogues against oral squamous cell carcinoma.

**EXPERIMENTAL**

In this study, the chemicals and solvents to synthesize new quinoline analogues (NQAs) were procured from Sigma-Aldrich Co. (St Louis, USA), Merck KGaA (Darmstadt, Germany), Qrec Chemicals (Rawang, Malaysia) and HmbG® Chemicals (Hamburg, Germany) and Friedemann Schmidt Chemical (Washington, DC). The NQAs' 1H NMR and 13C NMR spectral data were recorded by JASCO FT/IR-6700 at wavelength ranged between 4000 and 400 cm⁻¹. Mass spectral data of synthesized NQAs were obtained using Direct Infusion IonTrap MS Full Scan (Thermo Scientific Q Exactive HF-X hybrid quadrupole-Orbitrap mass spectrometer). For the elemental analysis, Perkin-Elmer 240 B and 240 C instruments were used. The purity of the synthesized NQAs was checked by open capillary tube using SMP11 Analogue equipment and the melting points were calculated. Reaction monitoring was done using TLC over aluminum sheets (0.2 mm) with silica gel 60 F254 (Merck, Germany) using Sprechtrone® CM-26 UV chamber and solvent system of methanol:chloroform (9:1).

**Synthesis of 19-(2-ethoxy-2-oxoethoxy)-10,19-diethyl-14,18-dioxo-17-oxa-3,13-diazapentacyclo[11.8.0.0²,11.0₉,9.0₁₅,₂₀]henicosa-1(21),2,4,6,8,10,15(20)-heptaen-7-yl[1,4′-bipiperidine]-1′-carboxylate (4):** NQA 4 was synthesized as per the standard procedure given in the literature with some minor modifications [20-23]. Briefly, a mixture of 0.01 M of irinotecan (1) and ethyl chloroacetate (0.01 M) in acetonitrile was refluxed for 17 h to obtain NQA 2, which was treated with hydrazide hydrate to offer NQA 3. NQA 3 was further refluxed for 8 h with 4-methoxybenzoic acid in equimolar concentration to offer a crude product. The crude obtained was recrystallized with methanol using activated charcoal to yield pure NQA 4 (Scheme-I).

**19-(2-Ethoxy-2-oxoethoxy)-10,19-diethyl-14,18-dioxo-17-oxa-3,13-diazapentacyclo[11.8.0.0²,11.0₉,9.0₁₅,₂₀]henicosa-1(21),2,4,6,8,10,15(20)-heptaen-7-yl[1,4′-bipiperidine]-1′-carboxylate (2):** Light yellow solid, yield: 82%, m.p. 239 °C; FTIR (KBr, νmax, cm⁻¹): 3058 (=C-H str.), 2935 (C-H str.), 1709 (C=O str.), 1647 (C=C), 1589 (C=N); 1H NMR (DMSO-d₆, ppm): δ: 0.85 (3H, t, J = 7.6, H-25), 1.14 (3H, t, J = 7.6, H-29), 1.36 (6H, m, H-1″, 2″ & 6″), 1.43 (4H, m, H-2″, 6″), 1.81 (2H, q, J = 7.6 & 7.6, H-24), 2.44 (4H, t, J = 7.6, H-3″ & 4″), 2.59 (2H, t, J = 7.6, H-22), 3.39 (4H, t, J = 7.6, H′3 & 5″), 4.08 (2H, t, J = 7.6, H-28), 4.29 (2H, s, H-12), 4.39 (2H, s, H-26), 4.80 (2H, s, H-16), 6.53 (1H, s, H-21), 7.33-7.99 (3H, m, Ar-H5,6,8); 13C NMR (DMSO, ppm): δ: 8.82 (C25), 13.38 (C29), 22.69 (C23), 24.97 (C22), 26.47 (C1′), 27.73 (C24), 30.76 (C2″ & C6″), 39.29 (C2′ & C6′), 44.62 (C3′ & C5″), 45.92 (C3′ & C5′), 46.98 (C12), 52.63 (C15), 57.69 (C26), 61.77 (C28), 65.72 (C16), 72.85 (C19), 101.11, 106.02, 115.41, 119.35, 126.52, 127.52, 128.90, 131.40, 135.12, 145.70, 146.43, 150.48, 152.10 (Ar-C), 153.10 (C14), 157.32 (C7″), 179.24 (C18), 169.36 (C27); and Mass (m/z): 672. Anal. calcld. (found) % for C₄₅H₃₇O₆N₂S: C, 66.05 (66.11); H, 6.59 (6.71); N, 8.33 (8.29).

**10,19-Diethyl-19-[((hydrazinecarbonyl) methoxy]-14,18-dioxo-17-oxa-3,13-diazapentacyclo[11.8.0.0²,11.0₉,9.0₁₅,₂₀]henicosa-1(21),2,4,6,8,10,15(20)-heptaen-7-yl[1,4′-bipiperidine]-1′-carboxylate (3):** Yellow solid, yield: 87%, m.p. 230 °C; FTIR (KBr, νmax, cm⁻¹): 3255 (N-H str.), 2959 (C-H str.), 3058 (=C-H str.), 1709 (C=O str.), 1589 (C=N); 1H NMR (DMSO-d₆, ppm): δ: 0.85(3H, t, J = 7.6, H-25), 1.35(6H, m, H-1″, 2″ & 6″), 1.43(4H, m, H-2″, 6″), 1.81(2H, q, J = 7.6 & 7.6, H-24), 2.24(4H, t, J = 7.6, H-3″ & 4″), 2.59(2H, t, J = 7.6, H-22), 3.38(4H, t, J = 7.6, H′3″ & 5″), 4.29(2H, s, H-12), 4.39(2H, s, H-26), 4.80(2H, s, H-16), 6.52(1H, s, H-21), 7.32-7.99(3H, m, Ar-H5,6,8), 8.83(1H, brs, NH); 13C NMR (DMSO, ppm): δ: 8.21 (C25), 22.68 (C23), 24.96 (C22), 26.45 (C1′), 27.73 (C24), 30.75 (C2″ & C6″), 39.28 (C2′ & C6′), 44.63 (C3′ & C5′), 45.90 (C3″ & C5″), 46.96 (C12), 52.62 (C1′), 57.68 (C26), 65.72 (C16), 72.85 (C19), 101.10, 106.01, 115.40, 119.34, 126.51, 127.51, 128.91, 131.41, 135.11, 145.71, 146.42, 150.47, 152.11 (Ar-C), 153.12 (C14), 157.31 (C7″), 169.37 (C27), 172.93 (C18); Mass (m/z):
658. Anal. calcd. (found) % for C_{37}H_{44}N_{4}O_{8}S: C, 63.81 (63.79); H, 6.53 (6.49); N, 12.76 (12.79).

10,19-Diethyl-19-[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-ylmethoxy]-14,18-dioxo-17-oxa-3,13-diazapentacyclo[11.8.0.0^{14},0^{15},0^{12},0^{18}]henicosa-1(21),2,4,6,8,10,15(20)-heptna-7-yl[1,4′-bipiperidine]-1′-carboxylate (4): Yellow solid, yield: 76%, m.p. 242 ºC; FTIR (KBr, ν max, cm–1): 3049 (＝C-H str.), 2938 (C-H str.), 1689 (C=O), 1591 (C=N); 1H NMR (DMSO-d$_6$, ppm) δ: 0.85 (3H, t, J = 7.6, H-25), 1.35 (6H, m, H-1″, 2″ & 6″), 1.43 (4H, m, H-2′, 6′), 1.81 (2H, q, J = 7.6 & 7.6, H-24), 2.24 (4H, t, J = 7.6, H-3″ & 4″), 2.59 (2H, t, J = 7.6, H-22), 3.39 (4H, t, J = 7.6, H3′ & 5′), 3.74 (3H, s, O-CH$_3$), 4.29 (2H, s, H-12), 4.39 (2H, s, H-26), 4.80 (2H, s, H-16), 6.52 (1H, s, H-21), 6.81-7.99 (8H, m, Ar-H); 13C NMR (DMSO, ppm) δ: 8.19 (C25), 22.66 (C23), 24.94 (C22), 26.44 (C1″), 27.77 (C24), 30.78 (C2″ & C6″), 39.26 (C2′ & C6′), 44.63 (C3′ & C5′), 45.90 (C3″ & C5″), 46.96 (C12), 52.62 (C1″), 57.68 (C26), 59.99 (Ar-O-C), 61.76 (C26), 65.72 (C16), 72.85 (C19), 101.12, 106.03, 114.49, 115.40, 119.34, 126.48, 127.51, 128.91, 131.41, 135.11, 145.71, 146.42, 150.39, 152.09 (Ar-C), 153.15 (C14), 157.29 (C7″), 164.21 & 165.31 (C=O), 172.95 (C18); Mass (m/z): 774. Anal. calcd. (found) % for C_{43}H_{46}N_{6}O_{8}: C, 66.65 (66.71); H, 5.98 (5.95); N, 10.85 (10.91).

Antiproliferation activity: In present study, in vitro anticancer potential of NQAs was determined against CAL27 cells (OSCCs) using standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT) assay given in the literature with minor modification [24]. Briefly, CAL27 cells (obtained from ATCC) were allowed to propagate in Dulbecco modified eagle medium (DMEM-Corning, USA) enriched with 5% inactivated fetal bovine serum (FBS) using incubator (Heal Force/HF90, China) maintained at 37 ºC, 5% CO$_2$ and 95% relative humidity. For the antiproliferation assay, the CAL27 cell were allowed to proliferate over 96-well culture plate with 1 × 10$^4$ cells density per well and incubate over-night (to attach cells). The synthesized NQAs and standard NQA1 (irinotecan) were subjected to serial dilution using DMEM and poured in each well of microplate to achieve the final concentrations of 3.9, 7.81, 15.62, 31.25, 62.5, 125, 250 and 500 µg/mL. Prepared microplates were further incubated for 24 h, at 37 ºC in 5% CO$_2$. Next, to each well of microplate 10 µL MTT solution (Merck, USA) was added and re-incubated for 4 h at 37 ºC in dark. From each well, the contents were pipetted out and to each well 100 µL of DMSO was added to dissolve formazan crystals. Finally, the absorbance at 570 nm was recorded using GloMax Multiple Detection System (Promega, USA), percen-
tage cytotoxicity was calculated using following expression and IC₅₀ of NQAs was calculated using non-linear regression analysis in GraphPad Prism (Boston, MA, USA).

\[
\text{Cytotoxicity (\%)} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100
\]

**Cell cycle arrest:** The effect of all synthesized NQAs on CAL27 was determined based on the standard protocol of cell cycle arrest study using flow cytometry with minor modifications [25]. Briefly, the CAL27 cells were seeded into six-well plates (at a density of 2 x 10⁵ cells per well). After 24 h, the cells were treated with previously determined IC₅₀ of each NQA-1-4 respectively for another 24 h. Later, the cells were harvested, fixed and permeated with 70% cold ethanol for 30 min. The samples were washed twice with cold PBS and further incubated with propidium iodide (DNA fluorochrome) in a solution containing Triton X-100 as well as RNase at room temperature for 30 min prior to cell cycle analysis. Next effects of NQAs on cell cycle were determined using BD FACSCalibur Flow cytometer with ModFit LT software.

**RESULTS AND DISCUSSION**

The facts over OSCC, related morbidity and high anti-cancer potential of ester, hydrazide and oxadiazoles emphasize the need for oxadiazole synthesis. **Scheme-1** depicted the quinoline analogues (NQAs) synthesis was based on the standard protocols given in the literature [17,20] and offered all NQAs in good yield.

The present study offered NQA-4 (oxadiazole derivative) through treatment of NQA-3 (hydrazide analogue) with methoxy benzoic acid following cyclization reaction. Whereas NQA-3 (hydrazide derivative) was obtained by amination of NQA-2 (ester derivative of quinoline) using hydrazine hydrate. The precursor NQA-2, was synthesized by esterification of NQA-1 (quinoline derivative) using ethylchloroacetate. For esterification, NQA-1 was refluxed in dried ethanol using anhydrous potassium carbonate in equimolar concentration. The resultant crude ester was extracted with ether to offer NQA-2. During all synthesis experiments, total anhydrous conditions were maintained. The purification of all the synthesized NQAs was done through recrystallization of crude with methanol and activated charcoal. The purity of all synthesized NQAs was assessed based on the sharp melting point, single spot TLC pattern and elemental analysis. The elemental analysis of NQAs revealed that C, H and N elements were within ±0.4% of theoretical values. The structures of NQAs were characterized and confirmed based on the FTIR, ¹H NMR, ¹³C NMR and mass spectrometric data and were supported with the literary facts [20,21]. The presence of characteristic FTIR band at 2935 cm⁻¹ attributed to C-H stretching; ¹H NMR signal at δ value of 1.14 & 4.08 corresponding to CH₂-29 and CH₂-28 protons; disappearance of ¹³C NMR signals at δ value of 13.38 & 61.77 corresponding to C-29 & C-28 protons; and molecular ion peak at m/z value of 658, confirmed the structure of NQA-3; and appearance of FTIR bands at 1591 cm⁻¹ attributed to C=N stretching; appearance of ¹H NMR signal at δ value of 3.74 for O-CH₃ protons and disappearance of ¹³C NMR signals at δ value of 672, confirmed the structure of NQA-4.

**Biological activity:** The synthesized NQAs 1-4 were further evaluated for their in vitro anticancer potential against CAL27 cells using MTT assay method on 96-well culture plate with minor modification [26] and each antiproliferation experiment was performed in triplicate. The percentage cell cytotoxicity and IC₅₀ was determined as per the standard protocol [27]. The antiproliferation activity data for NQAs 1-4 given in Fig. 1, revealed that cytotoxicity activity of NQAs 1-4 increased with an increase in the concentration of NQAs 1-4 against CAL27. The cytotoxicity study results suggest that the synthesized NQAs 2-4 were effective when compared with standard irinotecan (NQA-1).

Among NQAs 1-4, NQA-2 and NQA-3 exhibited IC₅₀ lower than standard irinotecan (NQA-1). This is because when all NQAs were added to CAL27 cells at the dose of ranging from 500 to 3.26 µg/mL, NQA-2 and NQA-3 exhibited the IC₅₀ of 4.16 & 3.26, respectively (Table-1), which was lower than the IC₅₀ of irinotecan with 13.2 µg/mL, respectively. Relating the cytotoxicity study data and chemical structure of NQAs revealed that the substitution of electron donating group with NQA-3 exhibited highest cytotoxicity against CAL27. The IC₅₀ values of all the NQAs were calculated and found to be in the range of 3.26-125 µL/mL (Table-1).

To understand in which phase the anticancer agents arrest the cancer cell cycle, it is very important to carry out the cell cycle analysis [28]. For present study, the propidium iodide

![Fig. 1. Antiproliferation activity of NQA1-4](image320x258 to 549x392)
(PI) stained CAL27 cells arrest by NQAs in various phases of cell cycle was analyzed by flow cytometry [29]. The various DNA distribution histograms for CAL27 cells in the presence and absence of NQAs 1-4 at respective IC₅₀ are presented in Fig. 2. Each histogram presents CAL27 cells arrest by respective NQAs 1-4 in specific growth phase against fluorescence emission. The histogram (A) of PI stained CAL27 cells revealed that cell content percentage in the G0/G1, G2M and S phase was 36.55%, 17.86% and 45.64%. The histogram (B) of PI

<table>
<thead>
<tr>
<th>IC₅₀</th>
<th>NQA1</th>
<th>NQA2</th>
<th>NQA3</th>
<th>NQA4</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/mL</td>
<td>13.2</td>
<td>4.16</td>
<td>3.26</td>
<td>&lt;125</td>
</tr>
</tbody>
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Table 1: Anticancer Activity (IC₅₀) of NQAs Against CAL27

Fig. 2. Histograms representing CAL27 cell cycle arrest with and without NQA1-4
stained CAL27 cells (after treatment with NQA-1 with IC50 of 13.2 µg/mL) revealed that cell content percentage in the G0/G1, G2M and S phase was 16.86%, 9.64% and 73.5%. The histogram (C) of PI stained CAL27 cells (after treatment with NQA2 with IC50 of 4.16 µg/mL) revealed that cell content percentage in the G0/G1, G2M and S phase was 8.34%, 3.96% and 87.7%. The histogram (D) of PI stained CAL27 cells (after treatment with NQA-3 with IC50 of 3.26 µg/mL) revealed that cell content percentage in the G0/G1, G2M and S phase was 11.67%, 10.95% and 77.38%. The histogram (E) of PI stained CAL27 cells (after treatment with NQA-3 with IC50 of 125 µg/mL) revealed that cell content percentage in the G0/G1, G2M and S phase was 33.79%, 0.27% and 66.23%. It is well observed that after treatment of CAL27 cells with NQAs there was an increase in the cell content percentage in S-phase. So, on comparing the IC50 and S phase content percentage of all NQAs, it was found that although NQA-1 (IC50 of 125 µg/mL) exhibits maximum cell cycle arrest in S and G2M phase, however, it can be well analyzed that NQA-2 & NQA-3 (IC50 of 4.16 and 3.26 µg/mL) also exhibits good cell cycle arrest in S and G2M phase which is at much lower dose when compared with NQA-1 & NQA-4. Also, all the NQAs exhibit maximum cell cycle arrest in S-phase. So, the results of cell cycle arrest study confirms that all NQAs arrest the cell cycle in S-phase maximum. The MTT based antiproliferation study and cell cycle analysis not only determines the anticancer potential but also determines the phase in which any anticancer agent generally acts [29]. Similarly, in present study also, the MTT assay based antiproliferation study and cell cycle arrest study data of NQAs not only supports their high efficacy but also determines that synthesized NQAs exhibits the cell cycle arrest maximum in S-phase (which does not allow the OSCC to grow further), however, the synthesized NQAs must be further evaluated for the in vivo preclinical and clinical significance.

Conclusion

The present study involved successful synthesis of new quinoline analogues (NQAs 2-4) from NQA-1 (irinotecan) via esterification, hydrazination and cyclization reactions. The structures of synthesized NQAs were further confirmed based on the single spot TLC, sharp melting point, IR, NMR and mass spectrometric data. Present study concludes that all the synthesized NQAs exhibits maximum cell cycle arrest in S-phase and among all synthesized NQAs, NQA-2 and NQA-3 having ester group and hydrazide group, respectively at position C16 exhibits high anticancer potential against CAL27 cell lines. Hence, the synthesized NQAs are proven to be an effective anticancer against for the treatment of oral squamous cell carcinoma, however, additional in vivo and clinical studies are required to further establish the safety and efficacy of quinoline analogues in the treatement of oral squamous cell carcinoma.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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