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Novel Quinoline-3,4-dihydropyrimidinone Hybrids Privileged Scaffolds: Design, Synthesis, and Cytotoxic Evaluation

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ABSTRACT

Biologically active quinoline-3,4-dihydropyrimidin-2(1*H*)-one hybrids as novel monastrol analogs, were designed *via* molecular hybridization and structural modification rational. The synthesis was easily accessible *via* classical Biginelli reaction in good yields. Investigation of *in vitro* cytotoxicity was achieved through MTT cell line assay. The results revealed that the target derivatives showed a spectrum of potential cytotoxic effects on HepG-2, HCT-116, MCF-7, and PC3 cell lines, taking 5-FU as a standard antitumor agent. Compounds **7e** (R = *tert*-butoxy) and **7f** (R = 2-methylpropyloxy) showed the greatest cytotoxic efficacy among the tested hybrids on the investigated cell lines; in which **7e** showed IC_{50} values range of (11.57 µM - 19.05 µM), while **7f** showed IC_{50} values range (14.16 µM - 19.96 µM), in comparison with the standard 5-FU (IC_{50} from 5.3 µM to 8.3 µM). The introduced quinoline-3,4-dihydropyrimidin-2(1*H*)-one hybrids with their readily straightforward structural modification, may serve as a potential cytotoxic antitumor candidate after suitable optimization.

Keywords: *Monastrol; Quinoline; 3,4-Dihydropyrimidin-2(1H)-one; Cytotoxicity*

1. Introduction

Cancer accounted for around 12% of deaths worldwide, making it the second most common cause of death behind cardiovascular and cerebrovascular illnesses (Xu et al., 2008). Even still, cancer remained a major cause of death, accounting for 9.6 million cancer-related deaths and an anticipated 18.1 million new cases (Bray et al., 2018). Thus, in order to contribute to the fight against cancer, researchers from many domains must work continuously and tirelessly.

Quinolines, as privileged heterocyclic scaffolds heterocycles have been developed for many medicinal applications, e.g. treatment of inflammatory conditions, cardiovascular diseases, and cancer (Fujioka et al., 1992). The antitumor activity was among the unique research areas of the quinoline heterocycle (Chu et al., 2019; Mansour, Bayoumi, El-Sayed, Abouzeid, & Massoud, 2022; Mansour, Henen, Bayoumi, El-Sayed, & Massoud, 2021; Mansour et al., 2023; Massoud, El-Sayed, Bayoumi, & Mansour, 2019).

On the other hand, monastrol derivatives and analogs (Figure 1) have been found a wide use in organic synthesis and medicinal chemistry (Razzaghi-Asl et al., 2019). It was shown that the cell-permeable compound monastrol (**1**) interferes with mitosis in mammalian cells that have monopolar spindles. Monastrol showed the mobility of the mitotic kinesin Eg5 motor spindle protein in vitro (Mayer et al., 1999).

Additionally, a series of 3,4-dihydropyrimidine-2-thiones functionalized with 4,5-diaryl groups was reported and tested for cytotoxic activity, with the strongest activity against breast cancer (MCF7) (Sośnicki et al., 2014). Furthermore, compounds **2**, **3a**, and **3b** were also classified as monastrol-type with anticancer activity, since their cytotoxic action is associated with the suppression of the kinesin Eg5 protein (Kettmann, Svetlik, & Veizerova, 2008) (Figure 1).

In an *in vitro* anticancer experiment using the hepatic carcinoma cell line (HepG-2), compound **4** demonstrated cytotoxic activity (IC_{50} = 17.90 ± 3.75 µM) comparable to that of cisplatin (IC50 = 15.00 ± 2.65 µM) (Creaven et al., 2010).

Quinolinone derivatives **5a-d** were tested for their anticancer potency by the National Cancer Institute (NCI). Compound **5b** exhibited an average growth percentage inhibition of 66.23 in a one-dose assay and its GI_{50} was found to range between 1.41–15.8 μ M (Salahuddin, Mazumder, & Shaharyar, 2014).

After careful consideration of the previous reported findings and literature survey, the design rational of the target hybrid derivatives **7a-i** was based on molecular hybridization of 2-hydroxyquinoline and tetrahydropyrimidinone moieties. The hybrid structure possesses a characteristic chiral center at C_4 of the pyrimidinone ring allowing a free rotation between the two ring systems that facilitate flexible alignment and orientation in interaction with enzyme receptors.

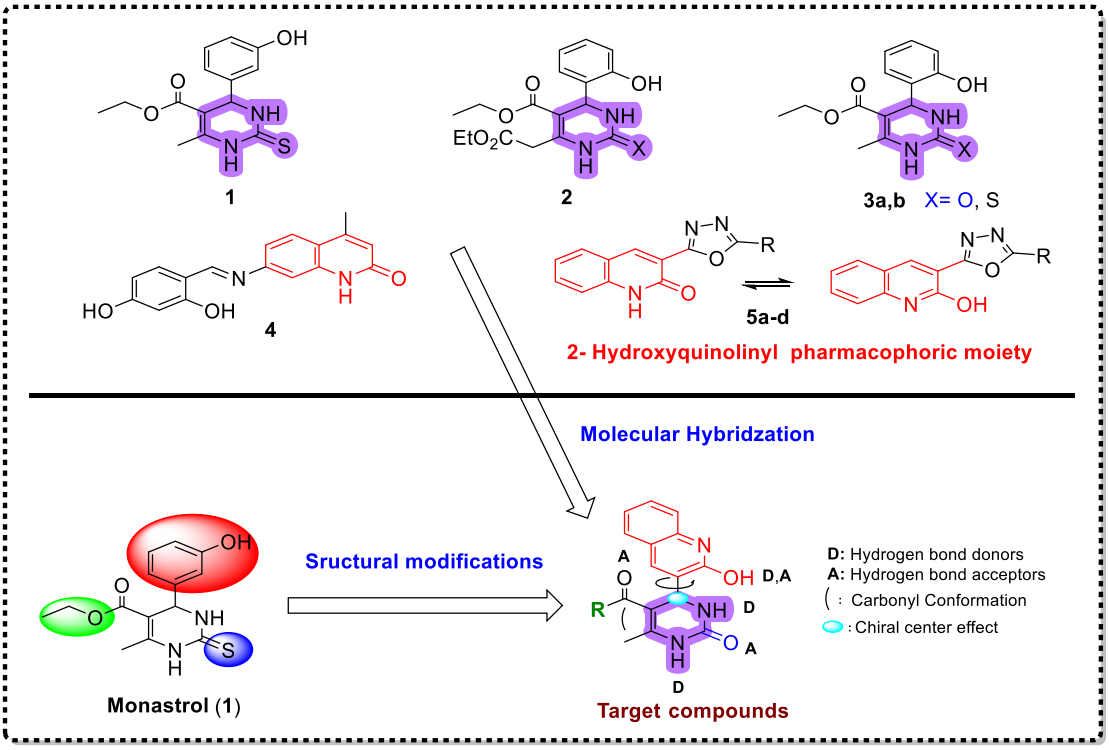


Figure 1. Rational design of the newly synthesized compounds **(7a-i)**

In addition, an allosteric structural modification for monastrol (**1**) was achieved to investigate how structural variation affects the antitumor activity. Another important structural feature in target derivatives **7a-i** is the R group located at the α-position to the carbonyl group, affording ester moiety (R = OR') or ketone moiety (R = R'). Variation of R will be vital for optimization of the cytotoxic activity and allow SAR prediction (Figure 1).

2. Material and methods

2.1. Chemistry

Stuart melting point apparatus was used to record the melting points (ºC), which are uncorrected. Using a potassium bromide disc, IR spectra (υ' in cm^{-1}) were acquired on a Shimadzu IR-470 spectrometer at the Faculty of Pharmacy, Mansoura University. Proton and carbon NMR were performed on Joel spectrophotometer (500 MHz-^{1}H and 125 MHz-^{13}C) in DMSO-d_6 with *TMS* as an internal standard at the Faculty of Science, Mansoura University. Reaction times were decided guided by TLC, and the spots were visualized by UV.

General procedure for preparation of 3,4-Dihydropyrimidin-2(1*H*)-one derivatives (**7a-i**)
A mixture of **6** (1.15 g, 6 mmol), the suitable 1,3-dicarbonyl compound (6 mmol), urea (0.36 g, 6 mmol), and 4 drops of conc. HCl in EtOH (25 mL, 95%). The reaction mixture was heated under reflux for about 5-6 h. After cooling to ambient temperature. The precipitated product was removed by filtration and washed with cooled ethanol (10 mL). Crystallization with ethanol affords pure products.

5-Acetyl-4-(2-hydroxyquinolin-3-yl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (**7a**)
Canary-yellow crystalline solid; yield 65%, mp 241-243° C (Reported 241-243 °C. **IR** (KBr, υ'/cm^{-1}): 1548 (ArC=C), 1662 (amide carbonyl), 1722 (ketone carbonyl), 2979 (C-H aliphatic), 3080 (C-H aromatic), 3313 (NH), 3421 (OH).**^{1}H NMR** (500 MHz, DMSO-d_6): δ 1.19 (s, 3H, 6'-CH_3), 2.49 (s, 3H, $COCH_3$), 5.70 (s, 1H, 4'-H), 6.17 (d, 1H, *J* = 9.5 Hz, NH), 6.99 (d, 1H, *J* = 9.4 Hz, NH), 7.68 (m, 1H, 6-H), 7.83 (m, 1H, 7-H), 7.96 (d,1H, *J* = 8.4 Hz, 8-H), 8.17 (d, 1H, *J* = 8.0 Hz, 5-H), 8.54 (s, 1H, 4-H), 10.38 (s, 1H, OH). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 194.0, 160.3, 154.4, 150.6, 144.0, 131.8, 129.9, 128.6, 128.4, 124.5, 124.3, 123.9, 109.5, 44.6, 29.8, 19.1. .Anal. Calcd for $C_{16}H_{15}N_3O_3$ (297.31): C, 64.64; H, 5.09: N, 14.13. Found: C, 64.61; H, 5.11; N, 14.09.

Methyl 4-(2-hydroxyquinolin-3-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7b**)
Buff crystalline solid; yield 69%, mp > 300° C. **IR** (KBr, υ'/cm^{-1}): 1568 (ArC=C), 1658 (amide carbonyl), 1711 (ester carbonyl), 2950 (C-H aliphatic), 3107 (C-H aromatic), 3401 (NH). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.36 (s, 3H, 6'- CH_3), 3.53 (s, 3H, OCH_3), 5.37 (s, 1H, 4'-H), 7.10 (s, 1H, NH), 7.18 (t, 1H, *J* = 6.7 Hz, 6-H), 7.33 (d, 1H, *J* = 7.7 Hz, 7-H), 7.49 (d, 1H, *J* = 6.8 Hz, 8-H), 7.57 (s, 1H, 4-H), 7.72 (d, 1H, *J* =7.4 Hz, 5-H), 9.27 (s, 1H, NH), 11.93 (s, 1H, OH). **^{13}C NMR** (125 MHz, DMSO-d_6): δ 167.1, 160.2, 153.1, 148.14, 143.8, 131.7, 129.6, 128.3, 128.2, 124.3, 123.9, 123.2, 102.2, 51.7, 44.3, 19.0. Anal. Calcd for $C_{16}H_{15}N_3O4$ (313.31): C, 61.34; H, 4.83; N, 13.41. Found: C, 61.38; H, 4.80; N, 13.45.

Ethyl 4-(2-hydroxyquinolin-3-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7c**)
Pale-yellow crystalline solid, yield 73%, mp >300° C (Reported >300 °C). **IR** (KBr, υ'/cm^{-1}): 1566 (ArC=C), 1659 (amide carbonyl), 1713 (ester carbonyl), 2962 (C-H aliphatic), 3103 (C-H aromatic), 3397 (NH). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, *J* = 7.2 Hz, ester-CH_3), 2.33 (s, 3H, CH_3), 3.97 (q, 2H, *J* =7.2 Hz, ester-CH_2), 5.35 (s, 1H,4'-H), 7.08 (s, 1H, NH), 7.16 (t, 1H, *J* =7.6 Hz, 6-H), 7.30 (d, 1H, *J* = 8 Hz, 7-H), 7.47 (t, 1H, *J* =7.6 Hz, 8-H), 7.56 (s, 1H, 4-H), 7.69 (d, 1H, *J* = 8 Hz, 5-H), 9.20 (s, 1H, NH), 11.87 (s, 1H, OH). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 167.7, 160.6, 153.9, 148.22, 144.1, 132.3, 129.9, 128.6, 128.4, 124.5, 124.5, 123.3, 104.2, 60.2, 44.4, 19.0, 14.49. Anal. Calcd for $C_{17}H_{17}N_3O_4$ (327.34): C, 62.38; H, 5.23: N, 12.84. Found: C, 62.40; H, 5.24; N, 12.81.

Isopropyl 4-(2-hydroxyquinolin-3-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7d**)
Beige crystalline solid, yield 88%, mp >300° C. **IR** (KBr, υ'/cm^{-1}): 1565 (ArC=C), 1660 (amide carbonyl), 1714 (ester carbonyl), 2974 (C-H aliphatic), 3103 (C-H aromatic), 3396 (NH). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 0.93 (d, 3H, *J* = 6.0 Hz, isopr-CH_3), 1.14 (d, 3H, *J* = 6.0 Hz, isopr-CH_3), 2.33 (s, 3H, 6'-CH_3), 4.81 (m, 1H, CH), 5.73 (d, 1H, *J* = 2.7 Hz, 4'-H), 7.10 (s, 1H, NH), 7.17 (t, 1H, *J* =7.6 Hz, 6-H), 7.32 (d, 1H, *J* = 8.2 Hz, 7-H), 7.48 (t, 1H, *J* =7.2 Hz, 8-H), 7.57 (s, 1H, 4-H), 7.70 (d, 1H, *J* =7.6 Hz, 5-H), 9.18 (s, 1H, NH), 11.86 (s, 1H, OH). **^{13}C NMR** (125 MHz, DMSO-d_6): δ 166.8, 160.2, 153.2, 149.0, 143.8, 131.7, 129.6, 128.3, 128.2, 124.3, 123.9, 123.1, 103.8, 68.3, 44.5, 22.0 (2C), 19.0. Anal. Calcd for $C_{18}H_{19}N_3O_4$ (341.37): C, 63.33; H, 5.61; N, 12.31. Found: C, 63.34; H, 5.57; N, 12.32.

tert-Butyl 4-(2-hydroxyquinolin-3-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7e**)
Buff crystalline solid; yield 69%, mp 269-271° C**. IR** (KBr, υ'/cm^{-1}): 1567 (ArC=C), 1657 (amide carbonyl), 1710 (ester carbonyl), 2970 (C-H aliphatic), 3105 (C-H aromatic), 3401 (NH). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.28 (s, 9H, $(CH_3)_3$-C), 2.34 (s, 3H, 6'-CH_3), 5.37 (d, 1H, *J* = 9.4 Hz, 4'-H), 6.58 (d, 1H, *J* = 21.6 Hz, NH), 7.17 (dd, *J* = 71.3, 42.5 Hz, 3H, 6-H, 7-H, 8-H), 7.53 (d, 1H, *J* = 29.5 Hz, 4-H), 7.75 (d, 1H, *J* = 25.6 Hz, 5-H), 9.17 (d, 1H, *J* = 49.3 Hz, NH), 11.89 (s, 1H, OH). **^{13}C NMR** (125 MHz, DMSO-d_6): δ 165.4, 160.2, 153.2, 149.2, 143.8, 131.7, 129.6, 128.3, 128.2, 124.3, 123.9, 123.1, 103.7, 81.1, 44.6, 28.1 (3C), 19.0. Anal. Calcd for $C_{19}H_{21}N_3O_4$ (355.39): C, 64.21; H, 5.96; N, 11.82. Found: C, 64.22; H, 5.99; N, 11.80.

Isobutyl 4-(2-hydroxyquinolin-3-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7f**)
Buff crystalline solid, yield 79%, mp >300° C. **IR** (KBr, υ'/cm^{-1}): 1566 (ArC=C), 1659 (amide carbonyl), 1708 (ester carbonyl), 2960 (C-H aliphatic), 3103 (C-H aromatic), 3392 (NH). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 0.71 (dd, 6H, *J*=2.5 Hz, *J* = 6.5 Hz, isobut- CH_3), 1.72 (td, 1H, *J* = 6.5 Hz, *J*=13.0 Hz, CH), 2.38 (s, 3H, CH_3), 3.69 (dd, 1H, *J* = 6.1 Hz, *J* = 10.6 Hz,, COOCH), 3.78 (dd, 1H, *J* = 6.4 Hz, *J* = 10.5 Hz, COOCH), 5.42 (d, 1H, *J* = 2.5

Hz, 4'-H), 7.11 (s, 1H, NH), 7.17 (t, 1H, *J* = 7.2 Hz, 6-H), 7.31 (d, 1H, *J* = 8.2 Hz, 7-H), 7.48 (t, 1H, *J* = 7.5 Hz, 8-H), 7.58 (s, 1H, 4-H), 7.71 (d, 1H, *J* = 7.8 Hz, 5-H), 9.25 (s, 1H, NH), 11.91 (s, 1H, OH). **^{13}C NMR** (125 MHz, DMSO-d_6): δ 167.3, 160.2, 153.1, 148.5, 143.8, 131.7, 129.6, 128.3, 128.2, 124.3, 123.9, 123.2, 101.9, 71.9, 44.5, 28.0, 19.2 (2C), 19.0. Anal. Calcd for $C_{19}H_{21}N_3O_4$ (355.39): C, 64.21; H, 5.96; N, 11.82. Found: C, 64.18; H, 5.97; N, 11.84.

2-Methoxyethyl-4-(2-hydroxyquinolin-3-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7g**)
Pale-buff crystalline solid, yield 86%, mp 293-295° C. **IR** (KBr, υ'/cm^{-1}): 1566 (ArC=C), 1660 (amide carbonyl), 1717 (ester carbonyl), 2962 (C-H aliphatic), 3102 (C-H aromatic), 3383 (NH). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.34 (s, 3H, CH_3), 3.08 (s, 3H, OCH_3), 3.40 (t, 2H, *J* = 5.6 Hz, $COOCH_2$), 4.06 (dt, 2H, *J* =3.6 Hz, *J*=5.2 Hz, methoxy-CH_2), 5.37 (d, 1H, *J* = 2.8 Hz, 4'-H), 7.12 (s, 1H, NH), 7.17 (t, 1H, *J* =7.6Hz, 6-H), 7.32 (d, 1H, *J* = 8 Hz, 7-H), 7.48 (t, 1H, *J* =7.6 Hz, 8-H), 7.56 (s, 1H, 4-H) , 7.69 (d, 1H, *J* =7.6 Hz, 5-H), 9.25 (s, 1H, NH), 11.88 (s, 1H, OH). **^{13}C NMR** (125 MHz, DMSO-d_6): δ 166.9, 160.2, 153.1, 148.5, 143.8, 131.7, 129.6, 128.3, 128.2, 124.3, 123.9, 123.2, 103.5, 70.5, 64.8, 59.1, 44.5, 19.0. Anal. Calcd for $C_{18}H_{19}N_3O_5$ (357.37): C, 60.50; H, 5.36; N, 11.76. Found: C, 60.51; H, 5.33; N, 11.72.

5-Benzoyl-4-(2-hydroxyquinolin-3-yl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (**7h**)
Bright-buff crystalline solid, yield 82%, mp >300° C. **IR** (KBr, υ'/cm^{-1}): 1566 (ArC=C), 1657 (amide carbonyl), 1720 (ketone carbonyl), 2966 (C-H aliphatic), 3064 (C-H aromatic), 3378 (NH). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.76 (s, 3H, CH_3), 5.37 (d, 1H, *J* =2 Hz, 4'-H), 7.17 (t, 1H, *J* =7.6 Hz, 6-H), 7.29 (d, 1H, *J* = 8.2 Hz, 7-H), 7.35 (s, 1H, NH), 7.46 (t, 1H, *J* = 7.6 Hz, 8-H), 7.50 (t, 2H, *J* = 8 Hz, 3"-H, 5"-H), 7.53 (t, 1H, *J* =7.2 Hz, 4"-H), 7.56 (d, 2H, *J* = 8 Hz, 2"-H, 6"-H), 7.61 (s, 1H, 4-H), 7.71 (d, 1H, *J* =7.6 Hz, 5-H), 9.16 (s, 1H, NH), 11.86 (s, 1H, OH). **^{13}C NMR** (125 MHz, DMSO-d_6): δ 191.1, 160.0, 153.9, 148.6, 143.8, 137.9, 132.5, 131.3, 129.6, 128.3, 128.2, 128.0 (2C), 127.4 (2C), 124.6, 123.9, 123.3, 108.9, 46.0, 19.1. Anal. Calcd for $C_{21}H_{17}N_3O_3$ (359.39): C, 70.18; H, 4.77; N, 11.69. Found: C, 70.20; H, 4.81; N, 11.66.

Benzyl 4-(2-hydroxyquinolin-3-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7i**)
Pale-yellow crystalline solid; yield 69%, mp 297-299° C. **IR** (KBr, υ'/cm^{-1}): 1566 (ArC=C), 1662 (amide carbonyl), 1715 (ester carbonyl), 2958 (C-H aliphatic), 3033 (C-H aromatic), 3391 (NH). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.37 (s, 3H, CH_3), 4.99 (d, 1H, *J* =12.6 Hz, benzyl-CH), 5.10 (d, 1H, *J* = 12.4 Hz, benzoyl-CH), 5.44 (s, 1H, 4'-H), 7.15 (s, 1H, NH), 7.24 (m, 7H, phenyl-H, 6-H, 7-H), 7.53 (m, 3H, 8-H, 4-H, 5-H). 9.31 (s, 1H, NH), 11.90 (s, 1H, OH). **^{13}C NMR** (125 MHz, DMSO-d_6): δ 166.8, 160.2, 153.2, 148.6, 143.8, 135.6, 131.7, 129.6, 128.5 (2C), 128.3, 128.2, 128.1, 128.0 (2C), 124.3, 123.9, 123.1, 103.9, 66.8, 44.5, 19.0. Anal. Calcd for $C_{22}H_{19}N_3O_4$ (389.41): C, 67.86; H, 4.92; N, 10.79. Found: C, 67.85; H, 4.93; N, 10.77.

2.2. Cytotoxicity assay

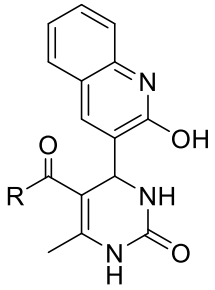
Cell line
For the in vitro cytotoxicity assessment, the following selected human tumor cells were utilized: human prostate carcinoma (PC3), hepatocellular carcinoma (HePG-2), colorectal carcinoma (HCT-116), and mammary gland breast cancer (MCF-7. The Holding Company for Biological Products and Vaccines (VACSERA), Cairo, Egypt, facilitated the acquisition of the cell lines from ATCC.

Chemical reagents
The reagents included fetal bovine serum, MTT, RPMI-1640 medium, DMSO, and 5-FU which was utilized as a typical positive control.

MTT assay
The MTT assay, which was carried out in triplicate, was performed to ascertain the cytotoxic effects of target derivatives on cell development using the distinct cell lines previously indicated (Denizot & Lang, 1986; Mosmann, 1983). The idea behind this assay is to measure (colorimetry) the transformation of yellow-colored tetrazolium bromide (MTT) by succinate dehydrogenase enzyme inside mitochondrial living cells into a purple color of formazan derivative. 10% Fetal bovine serum was added to the RPMI-1640 medium in which the cells were grown. At 37° C in an incubator with 5% CO_2, 100 units/mL of penicillin and 100µg/mL of streptomycin were introduced as antibiotics. The cells were seeded at 1.0x104 cells/well in a 96-well plate under 5% CO_2 for 48 hr at 37° C (Mauceri et al., 1998). Following the incubation period, the cells were subjected to various concentrations of the examined target compounds and left for a full day of incubation. 20 µL of 5 mg/mL MTT solution was added, after

which it was incubated for four hours after the drug treatment had been going on for 24 hours. Each well received 100 µL of DMSO for dissolving the developed purple-colored formazan. Colorimetric absorbance measurement was performed at 570 nm using a plate reader EXL 800. the IC_{50} is shown in Table 1.

Table 1. Cytotoxic activity of hybrids **7a-i**



Comp. No.	R	In *vitro* Cytotoxicity IC_{50} (µM)			
		HePG2	MCF-7	PC3	HCT-116
5-FU	--	7.9±0.17	5.4±0.20	8.3±0.35	5.3±0.32
7a	methyl	57.68±2.7	47.46±2.7	42.59±2.5	30.32±2.4
7b	methoxy	82.36±3.9	77.46±4.2	83.07±3.8	68.52±3.2
7c	ethoxy	23.41±1.7	39.86±2.0	30.99±1.9	18.68±1.6
7d	isopropyloxy	77.70±3.6	63.60±3.9	70.99±3.4	54.93±2.7
7e	*tert*-butoxy	**12.25±1.2**	**19.05±1.2**	**13.66±1.1**	**11.57±0.9**
7f	2-methylpropyloxy	**17.97±1.5**	**19.96±1.5**	**16.51±1.1**	**14.16±1.1**
7g	2-methoxyethoxy	41.30±2.3	43.63±2.6	36.96±2.0	22.12±1.8
7h	phenyl	64.19±3.1	50.79±3.1	64.64±3.3	34.22±2.3
7i	benzyloxy	>100	87.49±4.4	96.54±4.3	93.07±4.6

3. Results and Discussion

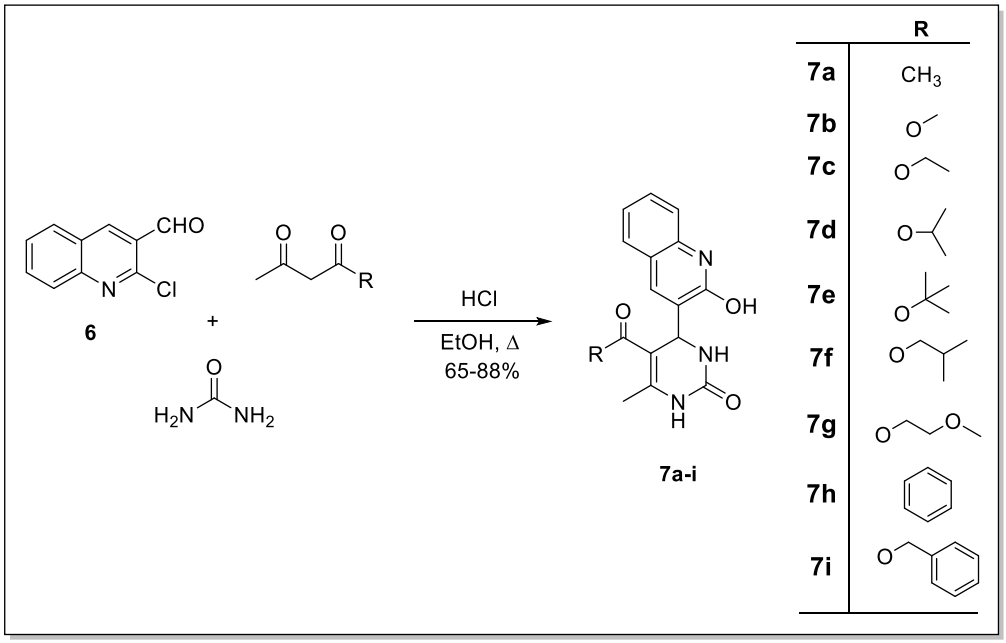
3.1. Chemistry

Construction of quinoline-3,4-dihydropyrimidinone hybrids (**7a-i**) is depicted in Scheme 1. The synthesis of Biginelli base (3,4-dihydropyrimidin-2(1*H*)-one) was first developed by Pietro Biginelli (Biginelli, 1893) in 1893, through refluxing benzaldehyde, EAA, and urea in alcohol. The reaction is catalyzed by HCl. The mechanism through which the reaction could proceed took a century to understand. Many proposals were conducted to comprehend the postulated mechanism of the Biginelli reaction, the first proposal was conducted by Folker*et al*. (Folkers & Johnson, 1933) in 1933, and the second proposal was presented by Sweet *et al*. (Sweet & Fissekis, 1973) and the latest proposal was offered by Kappe *et al*. (Kappe, 1997) in 1997. The first step in the procedure is the condensation of the urea and aldehyde through Mannich condensation. The formed iminium intermediate functions as an electrophile for the addition of the ketoesterenol, and the carbonyl group of the resultant adduct condenses with urea NH_2 to achieve cyclization. The poor to moderate yield is a major disadvantage of the classical Biginelli reaction, specifically when employing substituted aromatic aldehydes. Many modifications for this reaction were developed, including solid phase synthesis of Biginelli compounds (Wipf & Cunningham, 1995), Fluorous-Phase modifications (Studer, Jeger, Wipf, & Curran, 1997), and using inorganic catalysts like Indium(III) chloride (Ranu, Hajra, & Jana, 2000) and heavy metals (Ma, Qian, Wang, & Yang, 2000). Recently, the use of Lewis acids (Creaven et al., 2010; Fujioka et al., 1992; Jayakumar Swamy, Praveen, & Pramod, 2012; Mansour et al., 2023; Salahuddin et al., 2014), lanthanide compounds (Lu, Bai, Wang, Yang, & Ma, 2000; Ma et al., 2000),

and silica sulfuric acid (Salehi, Dabiri, Zolfigol, & Fard, 2003) also gave improved yields. Among these modifications, the synthesis of DHPMs is promoted by chlorotrimethylsilane, through the reaction of *N*-substituted urea or thiourea with EAA and appropriate aldehyde (Ryabukhin, Plaskon, Ostapchuk, Volochnyuk, & Tolmachev, 2007).

Few other methods can afford Biginelli compounds, but they are usually restricted to certain reactants and are of limited synthetic. Among them, acetoacetate derivatives can react with urea with the elimination of methanethiol to produce dihydropyrimidine (Böhme & Mundlos, 1953). The pyrimidine might also be hydrogenated with H_2/Pt to yield the same molecule (Bergmann & Johnson, 1933).

In the present study, the synthesis of the target Biginelli hybrids (**7a-i**) was achieved through the traditional method. The yield and purity of products were found satisfactory. The defining signal of the singlet proton of 4'-H at about (δ = 5.35-5.73 ppm) is the most significant spectral pattern of the target derivatives **7a-i**. Classical aromatic protons in the ^{1}HNMR charts were noticed. Additionally, the three protons singlet peak of 6'-CH_3 appeared at about (δ = 1.19-2.38 ppm). The ^{13}C NMR spectra revealed the characteristic carbonyl of ester in **7b-g** and **7i** signals at (δ = 165.4-167.3ppm) and at (δ = 190.5-191.1 ppm) for ketonic carbonyl in **7a** and **7h**. Another distinctive signal of C_4 of the pyrimidinone ring was found at (δ = 101.9-109.5 ppm). In addition, the infrared spectrum showed the typical absorption band region for amide and ester carbonyls at (1657–1662 cm^{-1}) and (1708 -1717 cm^{-1}); respectively.



Scheme 1. Synthesis of target hybrids **7a-i**

3.2. Cytotoxicity assay

The target candidates (**7a-i**) were assessed for their cytotoxic activities *via* the well-known MTT assay (Denizot & Lang, 1986; Mosmann, 1983). A set of four human tumor cell lines were used for the assay. 5-FU was adopted as a reference standard antitumor agent. The results were expressed as IC_{50} (Table 1)**.**

Examination of the cytotoxic activity for target hybrids **7a-i** revealed that tested derivatives displayed a wide spectrum of cytotoxicity against different types of cell lines. The most important structural variable in target derivatives **7a-i** is the R group located at the α-position to the carbonyl group, affording ester moiety (R = OR') or ketone moiety (R = R'). Therefore, the effect of variation of the R group will be vital in the alteration of the cytotoxic activity of **7a-i**. Compounds **7e** (R = *tert*-butoxy) and **7f** (R = 2-methylpropyloxy) showed the most activity in comparison to all examined cell lines. Compound **7e** showed IC_{50} values ranging from 11.57 µM to 19.05 µM, while **7f** showed IC_{50} values ranging from 14.16 µM to 19.96 µM, in comparison with the standard 5-FU (IC_{50} from 5.3 µM to 8.3 µM). The rest of the compounds showed moderate to low cytotoxicity. It is obvious that branching of the R side chain with *tert*-butoxy group in **7e** and 2-methylpropyloxy group in **7f** resulted in potentiation of the cytotoxic activity. The cytotoxicity is markedly decreased when R is either a small group as in **7a** (R = methyl, IC_{50} = 30.32-57.68 µM) and **7b** (R = methoxy, IC_{50} = 68.52-83.07 µM) and or carrying an aromatic nucleus as in **7h** (R = phenyl, IC_{50} = 34.22-64.64 µM) and **7i** (R = benzyloxy, IC_{50} = 87.49->100 µM).

Compound **7c** (R = ethoxy) and **7g** (R = 2-methoxyethoxy) showed moderate cytotoxicity against the tested four cell lines: (**7c**, IC_{50} of 18.68 μM-39.86 μM) and (**7g**, IC_{50} of 22.12 μM-43.63 μM). Whereas substitution with aromatic functionality as phenyl in **7h** or benzyloxy in **7i** causes the cytotoxic activity to decrease against all cell examined lines.

4. Conclusion

A unique flexible facile construction of quinoline-3,4-dihydropyrimidin-2(1*H*)-one hybrids scaffold, as novel monastrol analogs, was designed *via* molecular hybridization and structural modification rational through a straightforward Biginelli reaction. The evaluation of *in vitro* cytotoxicity *via* MTT assay unveiled that two target hybrids **7e** and **7f** showed potential cytotoxic effects against all screened cell lines. The synthesized privileged hybrid structures of the target derivatives were introduced with an easily modifiable R group located at the carbonyl α-position, as a key of structural optimization. In this study, the introduced hybrids **7a-i** can be considered a versatile candidate for future development and optimization of antitumor activity.

Disclosure

The author reports no conflicts of interest in this work.

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