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In-vitro cytotoxic evaluation of newly designed ciprofloxacin-oxadiazole hybrids against human liver tumor cell line (Huh7)

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Abstract: Fluoroquinolones are targets of interest due to their broad spectrum antibacterial activity. Structure-activity relationship (SAR) of fluoroquinolones clearly indicates that substitution at C-7 position enhances the lipophilicity of these scaffolds resultantly affording pharmacologically significant compounds. Therefore, various ciprofloxacin-oxadiazole hybrids were synthesized and characterized by spectral analysis. Cytotoxic activity of these derivatives was assessed using human liver tumor cells (Huh7). One dose anticancer test results revealed moderate cytotoxicity of the newly synthesized compounds against this cell line. As the only compound 4a depicted comparatively lower cell viability value (81.91% using 100µg/mL concentration) than the other compounds.

Keywords: Cancer, fluoroquinolone, ciprofloxacin, human liver cell line, oxadiazole.

INTRODUCTION

Cancer incidence and death rates are progressing globally these days. One in four persons in the developing world is subjected to this stigma (Thun *et al.,* 2009; Hu *et al.,* 2012). It is a disorder resulting from uncontrolled growth of abnormal cells. Effective cancer treatment may include chemotherapy besides radiation and surgery (Lingaraju *et al.,* 2018). Most of the cytotoxic agents act by interfering the DNA function of malignant cells. Development of new cytotoxic agents to overcome the demerits (poor selectivity and multidrug resistance) of clinically available antitumor agents may play an important role in on going successful research (Meacham and Morrison, 2013; Fisher *et al.,* 2013; Farghaly *et al.,* 2020).

Fluoroquinolones provide a base line for developing a large number of pharmacologically active agents (Esfahani *et al.* 2019). These scaffolds effectively contribute for the treatment of tuberculosis and several other bacterial infections (Mohammadhosseini *et al.,* 2015; Anaya-Gonzalez *et al.,* 2019). Recently on structural modification of fluoroquinolones new compounds have emerged possessing potent activity against different enzymes and tumor cell lines (fig. 1). Addition of aromatic moieties at C-7 site of quinolone nucleus enhances their anticancer activity rather than antibacterial (Hu *et al.,* 2011; Akhtar *et al.,* 2016; Zahoor *et al.,* 2017).

Ciprofloxacin belonging to second generation of fluoroquinolones is a potent broad spectrum antibacterial agent effective for a number of infections. Its derivatives display anti-proliferative and apoptotic activities against colon, bladder, ovarian and lung cancer cells (Abdel-Aal *et al.,* 2019). Clinically their role as antifungal, antiprotozoal and antitubercular especially in multidrug resistance tuberculosis (MDR-TB) is well established (Azema *et al.,* 2009 and 2011; Suresh *et al.,* 2013).

Our research group is extensively involved in the development of new potent molecules with enhanced anticancer activity (Akhtar *et al.,* 2019; Hafeez *et al.,* 2021). In continuation of this research work, herein we report the synthesis of *N*-acylated ciprofloxacin hybrids and their cytotoxic estimation against Huh7 cell line. First, *N*-bromoacetamide of ciprofloxacin was synthesized and then treated with a variety of oxadiazoles to enhance the lipophilicity of the targeted analogues of ciprofloxacin.

MATERIALS AND METHODS

General experimental part

Reactions were carried out using bromoacetyl bromide (Alfa-Aesar), pyridine (Scharlau), phenols and carboxylic acids (Alfa-Aesar and Daejung) as the main chemicals. Targeted derivatives were purified via column chromatography technique and their melting points were determined through Gallenkamp equipment. Spectral analysis (^{1}H-NMR, ^{13}C-NMR and FT-IR) of these compounds were done by using Bruker spectrophotometer.

Synthetic method for ciprofloxacin-oxadiazole compounds 4a-j

A DCM solution containing 1.45 mmoles of *N*-bromoacetamide of ciprofloxacin 2 and 9 mmoles of base (pyridine) was stirred for 15 minutes at 25°C. After that oxadiazoles 3a-j (2.17mmoles) were added and this

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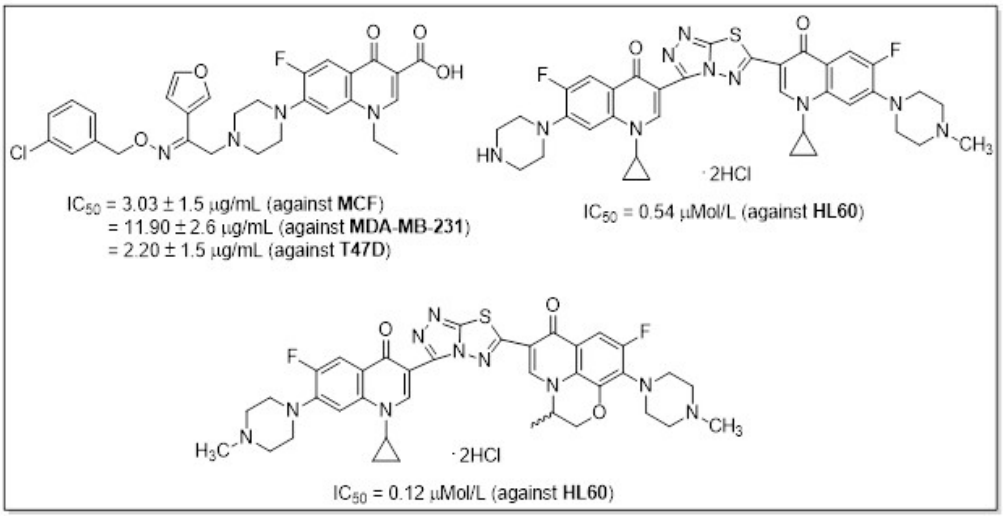


Fig. 1: Structures of some fluoroquinolones as anticancer agents.

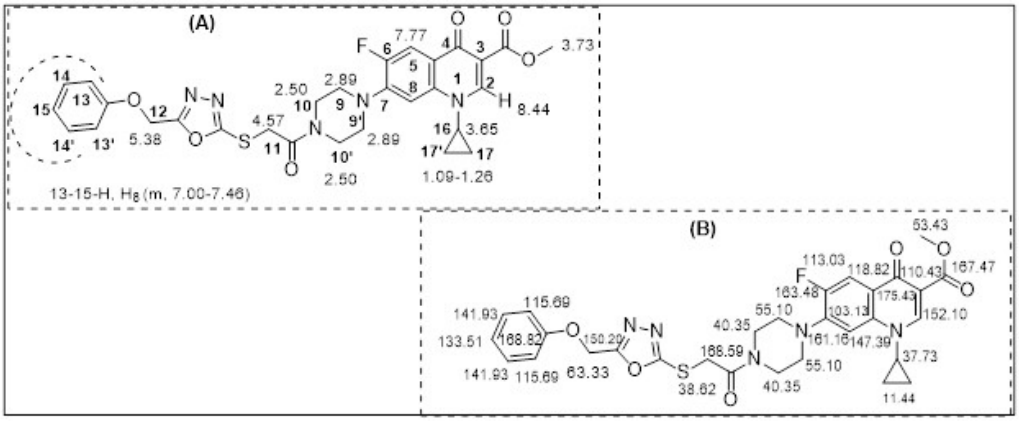


Fig. 2: ^{1}H (A) and ^{13}C-NMR (B) Chemical shifts of compound 4a.

mixture was further stirred for 72-96h. n-Hexane was used to obtain precipitates which were collected and purified with silica gel chromatography (CH_2Cl_2/MeOH).

Cytotoxic activity

Cytotoxic activity of synthesized derivatives 4a-j was checked by using Huh7 cell line. On account of this, viability of the cells was examined by MTT assay. Temperature was maintained at 37°C to culture human liver cells. 10% Fatal bovine serum was used with Dulbecco's Modified Eagle's Medium, penicillin (100 units/mL) and streptomycin (100μg/mL). Ciprofloxacin derivatives were first dissolved in dimethyl sulfoxide solvent (0.05% concentration of DMSO) and then treated with cells. After that incubated the cells for 4 h by adding 500 μg/mL MTT reagent. DMSO (150μL) was used to dissolve formazan crystals and at 570 nm absorbance was observed.

STATISTICAL ANALYSIS

All the tests were completed in triplicates and cell viability results are expressed as mean ± SD (standard deviation). Statistical study was carried out by using Microsoft Excel 2013.

RESULTS

Chemistry

First ciprofloxacin hydrochloride was condensed in the presence of methanol using catalytic amount of H_2SO_4, resultantly 70% yield of ciprofloxacin ester 1 was attained (Shaharyar *et al.,* 2007) that further treated with bromoacetyl bromide to obtain *N*-bromoacetamide of ciprofloxacin 2 (87%) (Faiz *et al.,* 2019, Shahzadi *et al.,* 2020). On the other hand, a variety of oxadiazoles were obtained by treating different carbohydrazides of phenols and carboxylic acids with CS_2/KOH (Kaplancikli, 2011). After attaining desired precursors, optimized conditions were set for *S*-alkylation of ciprofloxacin 2 with oxadiazoles 3a-j. For this purpose, reaction between *N*-bromoacetamide of ciprofloxacin 2 with oxadiazole 3a was accomplished in the presence of K_2CO_3/DMF and pyridine/DCM. Due to attaining lower yield of derivative 4a in the former method, pyridine/DCM was selected as suitable condition in which 4a was obtained in 67% yield.

Table 1: Spectroscopic study of derivatives 4a-j

Ciprofloxacin derivative*	Melting point (°C)	FT-IR (KBr, cm^{-1})ν_{max}/^{1}H and ^{13}C-NMR (300/75 MHz, DMSO-d_6)/[M+H$^+$]
4a	165	1019 (C-F), 1256 (C-O), 1313 (C-N), 1429 (C=C), 1626 (C=O), 1690 (C=N), 1716 (C=O), 2950 (-CH_2)/δ_H = 1.09-1.26 (m, 4H, cyclopropyl ring), 2.50 (t, 4H, 6Hz, piperazine ring), 2.89 (t, 4H, 6Hz, piperazine ring), 3.65 (m, 1H, cyclopropyl ring), 3.73 (s, 3H, $COOCH_3$), 4.57 (s, 2H, CH_2 linker), 5.38 (s, 2H, CH_2), 7.00-7.46 (m, 5H (aryl-H), 1H (H_8 quinolone)), 7.77 (d, 1H, 12Hz, H_5 quinolone), 8.44 (s, 1H, H_2 quinolone)/δ_C = 11.42, 37.73, 38.62, 40.35, 53.43, 55.10, 63.33, 103.13, 110.43, 113.03, 115.69, 118.82, 133.51, 141.93, 147.39, 150.20, 152.10, 161.16, 163.48, 167.47, 168.59, 168.82, 175.43/[M+H$^+$]: calculated 594.1778, found 594.1820
4b	170	1015 (C-F), 1258 (C-O), 1315 (C-N), 1425 (C=C), 1627 (C=O), 1695 (C=N), 1706 (C=O), 2950 (-CH_2)/δ_H = 1.09-1.26 (m, 4H, cyclopropyl ring), 2.27 (s, 1H, CH_3), 2.50 (t, 4H, 6Hz, piperazine ring), 2.89 (t, 4H, 6Hz, piperazine ring), 3.34 (m, 1H, cyclopropyl ring), 3.73 (s, 3H, $COOCH_3$), 4.56 (s, 2H, CH_2 linker), 5.34 (s, 2H, CH_2), 6.80-7.45 (m, 4H (aryl-H), 1H (H_8 quinolone)), 7.76 (d, 1H, 15Hz, H_5 quinolone), 8.43 (s, 1H, H_2 quinolone)/δ_C = 11.44, 24.90, 38.61, 40.33, 49.08, 55.10, 57.26, 63.29, 94.09, 110.45, 113.04, 115.38, 115.72, 119.49, 126.46, 133.22, 141.94, 147.41, 152.09, 161.18, 167.51, 168.49, 168.59, 168.82, 175.44/[M+H$^+$]: calculated 608.1934, found 608.1984
4c	242	1017 (C-F), 1250 (C-O), 1347 (C-N), 1450 (C=C), 1620 (C=O), 1685 (C=N), 1760 (C=O), 2950 (-CH_2)/δ_H = 1.10-1.26 (m, 4H, cyclopropyl ring), 2.50 (t, 4H, 6Hz, piperazine ring), 2.89 (t, 4H, 6Hz, piperazine ring), 3.53 (m, 1H, cyclopropyl ring), 3.73 (s, 3H, $COOCH_3$), 4.56 (s, 2H, CH_2 linker), 5.58 (s, 2H, CH_2), 7.28 (d, 1H, 9Hz, H_8 quinolone), 7.43 (d, 2H, 9Hz, aryl-H), 7.75 (d, 1H, 15 Hz, H_5 quinolone), 8.22 (d, 2H, 12Hz, aryl-H), 8.43 (s, 1H, H_2 quinolone)/δ_C = 7.43, 34.61, 36.37, 45.05, 49.41, 51.09, 59.92, 106.39, 111.37, 111.68, 115.40, 120.48, 125.71, 137.92, 141.71, 143.24, 143.38, 148.08, 162.14, 162.69, 164.55, 164.78, 171.40/[M+H$^+$]: calculated 639.1629, found 639.1659
4d	225	1086 (C-F), 1247 (C-O), 1347 (C-N), 1478 (C=C), 1626 (C=O), 1690 (C=N), 1715 (C=O), 2950 (-CH_2)/δ_H = 1.08-1.25 (m, 4H, cyclopropyl ring), 2.91 (t, 4H, 6Hz, piperazine ring), 3.42 (t, 4H, 6Hz, piperazine ring), 3.59 (m, 1H, cyclopropyl ring), 3.73 (s, 3H, $COOCH_3$), 4.56 (s, 2H, CH_2 linker), 5.50 (s, 2H, CH_2), 7.24 (d, 1H, 9Hz, H_8 quinolone), 7.36-7.85 (m, 7H (aryl-H), 1H (H_5 quinolone)), 8.40 (s, 1H, H_2 quinolone)/δ_C = 7.41, 34.58, 36.33, 45.80, 49.46, 51.09, 59.46, 106.37, 107.85, 111.34, 111.65, 118.10, 120.51, 124.05, 126.50, 126.69, 127.43, 128.8, 133.79, 137.88, 143.22, 148.05, 155.00, 163.38, 164.58, 164.79, 171.39/[M+H$^+$]: calculated 644.1934, found 644.1976
4e	218	1019 (C-F), 1256 (C-O), 1313 (C-N), 1429 (C=C), 1626 (C=O), 1695 (C=N), 1716 (C=O), 2950 (-CH_2)/δ_H = 1.17-1.38 (m, 4H, cyclopropyl ring), 2.91 (t, 4H, 6Hz, piperazine ring), 3.42 (t, 4H, 6Hz, piperazine ring), 3.94 (s, 3H, $COOCH_3$), 4.01 (s, 2H, CH_2 linker), 4.23 (m, 1H, cyclopropyl ring), 5.39 (s, 2H, CH_2), 6.03 (d, 1H, 3Hz, H_8 quinolone), 6.65-8.31 (m, 7H (aryl-H), 1H (H_5 quinolone)), 8.69 (s, 1H, H_2 quinolone)/δ_C = 7.42, 33.48, 35.60, 45.80, 52.00, 53.00, 61.60, 105.30, 105.90, 111.34, 111.65, 115.80, 121.50, 122.30, 125.10, 125.60, 126.60, 135.60, 137.80, 145.50, 150.50, 155.03, 163.38, 165.58, 165.79, 170.10/[M+H$^+$]: calculated 644.1934, found 644.1976
4f	145	1017 (C-F), 1258 (C-O), 1318 (C-N), 1425 (C=C), 1629 (C=O), 1695 (C=N), 1726 (C=O), 2950 (-CH_2)/δ_H = 1.08-1.33 (m, 4H, cyclopropyl ring), 3.34 (t, 4H, 3Hz, piperazine ring), 3.58 (t, 4H, 3Hz, piperazine ring), 3.77 (s, 3H, $COOCH_3$), 3.81 (s, 2H, CH_2), 4.00 (s, 2H, CH_2 linker), 4.12 (m, 1H, cyclopropyl ring), 6.03 (d, 1H, 3Hz, H_8 quinolone), 7.23-7.33 (m, 5H, aryl-H), 8.01 (d, 1H, 3Hz, H_5 quinolone), 8.69 (s, 1H, H_2 quinolone)/δ_C = 7.41, 30.10, 34.50, 37.30, 47.80, 53.00, 54.50, 106.37, 111.20, 112.30, 116.80, 127.80, 129.80, 134.20, 134.50, 145.60, 147.50, 152.50, 166.40, 164.58, 164.79, 170.30/[M+H$^+$]: calculated 578.1829, found 578.1879
4g	186	1015 (C-F), 1258 (C-O), 1315 (C-N), 1430 (C=C), 1620 (C=O), 1697 (C=N), 1716 (C=O), 2950 (-CH_2)/δ_H = 1.08-1.33 (m, 4H, cyclopropyl ring), 3.34 (t, 4H, 3Hz, piperazine ring), 3.58 (t, 4H, 3Hz, piperazine ring), 3.77 (s, 3H, $COOCH_3$), 4.00 (s, 2H, CH_2 linker), 4.12 (m, 1H, cyclopropyl ring), 6.03 (d, 1H, 3Hz, H_8 quinolone), 7.40-8.05 (m, 5H (aryl-H), 1H (H_5 quinolone)), 8.69 (s, 1H, H_2 quinolone)/δ_C = 7.50, 35.80, 36.70, 45.80, 52.00, 53.30, 102.50, 111.80, 112.20, 115.80, 126.10, 127.50, 129.20, 128.70, 134.50, 142.60, 147.50, 152.50, 164.50, 167.30, 168.20, 171.20/[M+H$^+$]: calculated 564.1672, found 564.1702
4h	229	1019 (C-F), 1256 (C-O), 1313 (C-N), 1429 (C=C), 1626 (C=O), 1690 (C=N), 1716 (C=O), 2950 (-CH_2)/δ_H = 1.10-1.26 (m, 4H, cyclopropyl ring), 2.78 (t, 4H, 3Hz, piperazine ring), 3.02 (t, 4H, 3Hz, piperazine ring), 3.45 (m, 1H, cyclopropyl ring), 3.80 (s, 3H, $COOCH_3$), 4.01 (s, 2H, CH_2 linker), 7.32 (d, 1H, 6Hz, H_8 quinolone), 7.55-7.73 (m, 4H, aryl-H), 7.83 (d, 1H, 6Hz, H_5 quinolone), 8.56 (s, 1H, H_2 quinolone)/δ_C = 11.40, 37.80, 38.50, 42.35, 53.40, 54.40, 103.30, 110.50, 112.00, 115.60, 124.20, 127.90, 128.20, 132.30, 132.50, 141.90, 145.50, 153.50, 160.50, 165.30, 166.30, 170.30/[M+H$^+$]: calculated 599.1219, found 599.1261
4i	230	1017 (C-F), 1258 (C-O), 1315 (C-N), 1423 (C=C), 1627 (C=O), 1695 (C=N), 1726 (C=O), 2950 (-CH_2)/δ_H = 1.08-1.33 (m, 4H, cyclopropyl ring), 3.34 (t, 4H, 3Hz, piperazine ring), 3.58 (t, 4H, 3Hz, piperazine ring), 3.77 (s, 3H, $COOCH_3$), 4.00 (s, 2H, CH_2 linker), 4.12 (m, 1H, cyclopropyl ring), 4.46 (s, 2H, CH_2-NHCO), 6.03 (d, 1H, 3Hz, H_8 quinolone), 8.01 (d, 1H, 3Hz, H_5 quinolone), 7.63-8.03 (m, 5H (aryl-H), 1H (NH)), 8.69 (s, 1H, H_2 quinolone)/δ_C = 10.50, 37.80, 38.50, 42.90, 45.80, 52.20, 54.50, 103.50, 112.20, 113.20, 114.80, 127.50, 128.80, 132.10, 134.20, 134.50, 142.60, 147.50, 152.50, 163.20, 167.30, 168.20, 172.30/[M+H$^+$]: calculated 621.1887, found 621.1929
4j	175	1015 (C-F), 1259 (C-O), 1313 (C-N), 1429 (C=C), 1620 (C=O), 1696 (C=N), 1720 (C=O), 2950 (-CH_2)/δ_H = 1.08-1.33 (m, 4H, cyclopropyl ring), 3.34 (t, 4H, 3Hz, piperazine ring), 3.58 (t, 4H, 3Hz, piperazine ring), 3.77 (s, 3H, $COOCH_3$), 4.00 (s, 2H, CH_2 linker), 4.12 (m, 1H, cyclopropyl ring), 6.03 (d, 1H, 3Hz, H_8 quinolone), 7.58 (t, 1H, 3Hz, pyridine), 8.01 (d, 1H, 3Hz, H_5 quinolone), 8.43 (d, 1H, 6Hz, pyridine), 8.69 (s, 1H, H_2 quinolone), 8.70 (s, 1H, pyridine), 9.24 (s, 1H, pyridine)/δ_C = 7.42, 37.80, 38.50, 47.90, 53.20, 54.30, 102.50, 110.50, 111.30, 116.80, 124.00, 124.50, 135.00, 135.50, 140.50, 146.50, 147.00, 153.50, 153.40, 165.50, 167.20, 168.50, 170.50/[M+H$^+$]: calculated 565.1625, found 565.1667.

After having optimized conditions in hand, required *S*-alkylated ciprofloxacin derivatives 4a-j were obtained in 65-87% yield range (Scheme 1, table 2).

Anticancer activity

Anticancer activity of 4a-j was assessed by using MTT assay. Firstly, one dose response was analyzed against human liver tumor cell line (Huh7) and results presented in table 3.

Table 2: Synthesized ciprofloxacin derivatives 4a-j.

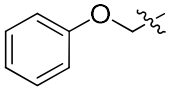
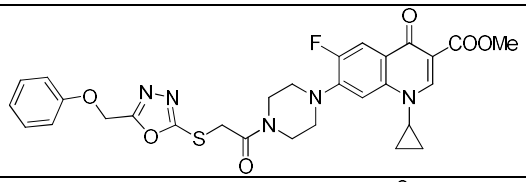
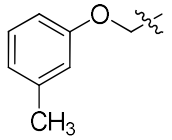
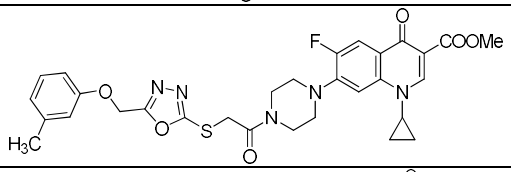
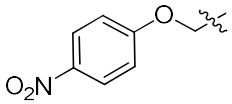
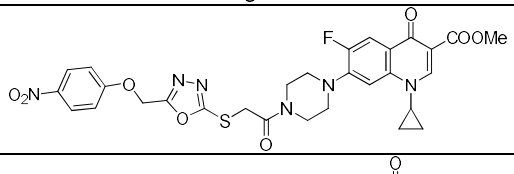
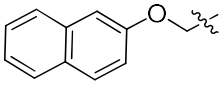
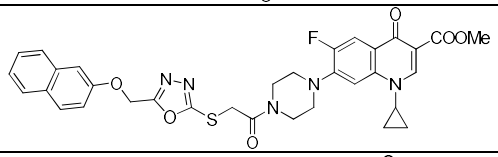
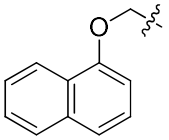
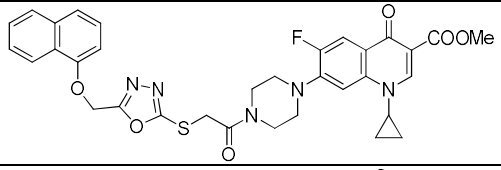
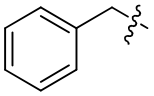
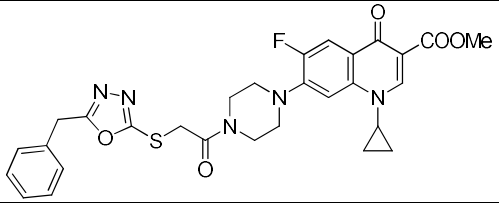
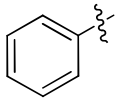
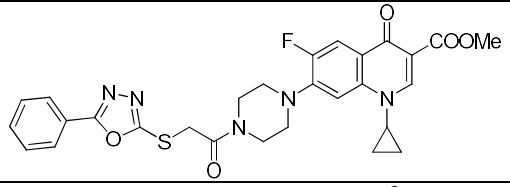
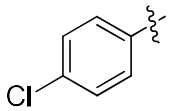
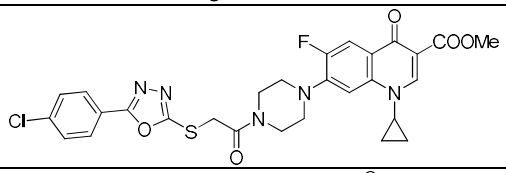
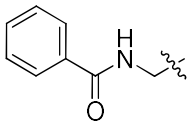
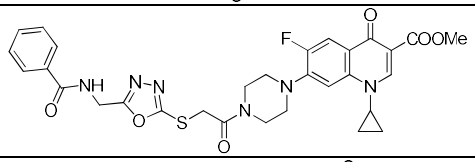
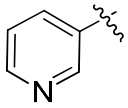
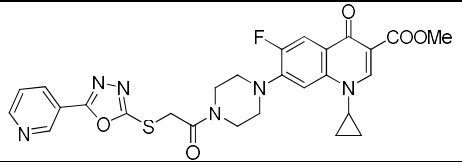
Sr. #	Compound	Substrate (R)	Product	Yield (%)
1.	4a			67
2.	4b			70
3.	4c			65
4.	4d			85
5.	4e			77
6.	4f			80
7.	4g			73
8.	4h			87
9.	4i			66
10.	4j			68

Table 3: Anticancer results of derivatives 4a-j against Huh-7 cell line

Compounds	Huh7 [a]% Cell viability ± SD
4a	81.91 ± 4.05
4b	112.80 ± 7.50
4c	196.40 ± 7.43
4d	128.39 ± 5.30
4e	96.12 ± 0.94
4f	148.87 ± 3.04
4g	114.53 ± 5.45
4h	83.82 ± 4.90
4i	162.32 ± 6.36
4j	133.10 ± 3.84
[b]Control	100 ± 0

[a]Cell viability: (Mean ± SD, n = 3 in triplicate), [b]Control = DMSO dissolved cells

DISCUSSION

Spectral studies of compound 4a

Compound 4a was characterized by infrared, proton and carbon NMR spectroscopic techniques (fig. 2). IR spectrum showed absorption bands at 1019 for carbon-fluorine bond, 1313 for carbon-nitrogen bond, 1429 for C=C, 1690 for carbon double bond with nitrogen, 1256 for carbon-oxygen bond, 1626 and 1716 cm^{-1} for carbonyl groups of ester and amide, respectively. ^{1}H-NMR of 4a depicted singlet peak for OCH_3 protons at 3.73ppm. Multiplet signal appeared at 1.09-1.26ppm and 3.65ppm for cyclopropyl protons.

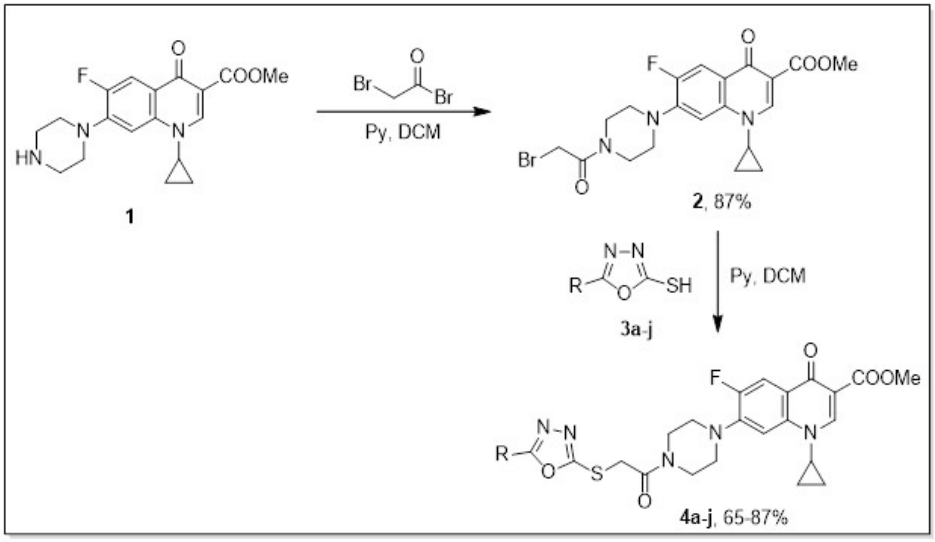
2 Doublet signals at 7.77 and 7.00 ppm and 1 singlet signal at 8.44ppm displayed for H_5, H_8 H_2 protons of quinolone ring, respectively. Triplet signal appeared at 2.50-2.89 ppm for piperazine ring and CH_2 (linker) gave chemical shift at 4.57ppm. At 5.38 ppm, CH_2-O showed singlet peak and multiplet signal at 7.00-7.46 ppm reserved for aryl ring. ^{13}C-NMR spectrum of 4a exhibited signals at 103.13-175.43ppm for quinolone ring. Whereas at 11.44ppm and 37.73ppm signals appeared for cyclopropyl ring. Carbons of piperazine showed chemical shifts at 40.35ppm and 55.10 ppm. 167.47 and 168.59ppm chemical shifts were assigned for carbonyl carbons of ester and amide respectively, and CH_2 (linker) peaks appeared at 38.62 ppm and 63.33 ppm. While carbons of phenyl ring depicted peaks from 115.69-141.93 ppm and 168.82 ppm.

Screening of anticancer activity

Results presented in table 3 indicate that these *S*-alkylated ciprofloxacin derivatives 4a-j could not attain the desired cytotoxic results. Among all the synthesized derivatives, better anticancer activity was observed of compound 4a against this cancer cell line. This compound (4a) exhibited comparatively lower cell viability value (81.91% at 100 µg/mL concentration) than the other compounds. Results clearly revealed that oxadiazoles which were used to improve the lipophilicity of the targeted molecules could not enhance the anticancer activity.

CONCLUSION

Fluoroquinolones primarily used as broad spectrum antibiotics also play a vital role to treat a variety of cancerous cells. Literature study reveals that piperazine moiety of FQs affords enough structural flexibility to obtain new potent targeted drugs. In present study, we synthesized a number of ciprofloxacin-oxadiazole hybrids and checked their cytotoxic activity by using human liver tumor cells (Huh7). However, significant results were not perceived against this cell line as only compound 4a gave comparatively better cell viability value (81.91% at 100 µg/mL concentration) than the other derivatives.



Scheme 1: Synthesis of analogues of *N*-acetamide derivatives of ciprofloxacin 2.

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