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Synthesis and Antibacterial Activity of Certain Novel 1-Cyclopropyl-6-fluoro-1,4-dihydro-7- 4-susbstituted-piperazin-1-yl-4-oxoquinoline-3-carboxylates

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Abstract

Fluoroquinolones are one of the most important classes among synthetic antibacterial agents. They have achieved significant improvement in terms of potency, spectrum and pharmacokinetic properties. Despite several advances, it still exists a continuous need for novel quinolones for better and targeted action and with fewer side effects. A series of 1-cyclopropyl-6-fluoro-1,4-dihydro-7-4-substiuted-piperazin-1-yl-4-oxoquinoline-3-carboxylates (V) have been synthesized and evaluated for antibacterial activity. Ciprofloxacin was reacted with thionyl chloride, to yield ciprofloxacin which was used immediately in the next step to react with respective alcohols to furnish the corresponding esters i.e. 1-cyclopropyl-6-fluoro-1,4-dihydro-7-piperazin-1-yl-4-oxoquinoline-3-carboxylates (III). Nicotinoyl chloride (IV), was synthesized by adopting reported procedures, reacted appropriately with previously synthesized esters (III) to yield the title compounds (V). The structures of synthesized compounds were established on the basis of analytical and spectral studies. All the synthesized compounds were evaluated for antibacterial activity against four different strains of bacteria. Compounds exhibited significant activities on molar basis.

Key Words: Fluoroquinolones, Ciprofloxacin, Nicotinoyl chloride, antibacterial activity

Introduction

Infectious diseases are one of the leading causes of death worldwide (WHO, 1999). During the past two decades, new infectious diseases have appeared and old ones previously thought to be controlled have reemerged (Cassell and Mekalanos, 2001). The reasons for increase in incidence of infectious diseases are not fully understood. Changes in human demographic and behavior (e.g. increasing use of day care facilities, a risk factor for otitis media); technology and industry; economic development and land use (possibly accounting for an increase in zoonotic disease); international travel and commerce; and breakdown of public health measures are thought to contribute to new infectious diseases and reemergence of infectious diseases thought to be have been controlled (National Academy Press, 1992). On the other hand, shortage of new antibacterial drugs and increasing resistance of bacteria to antimicrobial agents has been a major cause of concern (Pool, 2001; Thorsteinsson et al., 2003).

The synthetic antibiotics include the sulfa drugs, nitrofuran derivatives, pyridine-carboxylic acid analogs, fluoroquinolones and oxazolidinones. Quinolones have become a major class of antibacterial agents, which are under extensive clinical development. They have an important position because of their extremely potent activity, rapid bactericidal effects, safety, good

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tolerance, broad antibacterial spectrum and lower incidence of resistance (Appelbaum and Hunter, 2000; Mizuki et al., 1996; Ball , 2000; Snaz-Nebot et al., 1997; Rahul et al., 2005; Foroumadi et al., 2005).

Quinolones can cause certain adverse effects, such as CNS effects, phototoxicity, tendonitis, hypoglycemia, and serious cardiac dysrhythmias. Thus, despite many advances in the fluoroquinolone field, there exists continuous need for novel quinolones with better activity profile, targeted action at desired site, improved pharmacokinetic profile and tolerability to overcome the limitations of existing drugs (Foroumadi et al., 2006).

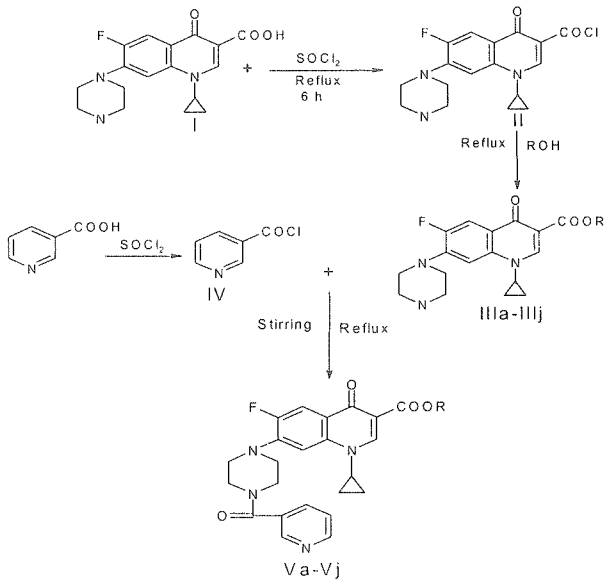
Ciprofloxacin, structurally 1-cyclopropyl-6-fluoro-1,4- dihydro-4-oxo-7-[1-piperazinyl]-3-quinolinecarboxylic acid, is a potent fluoroquinolone chemotherapeutic of the second-generation group of nalidixic acid derivatives, first commercially introduced in the 1980s. Due to the broad spectrum effect, systemic action and lack of cross reactivity with penicillins, cephalosporins and aminoglycosides (Fick and Reinscheid, 2006), it is widely used both in human and veterinary medicine to treat infectious diseases, caused particularly by Gram-negative and some Gram-positive bacteria. The target of highly selective action of ciprofloxacin is bacterial DNA gyrase, a type of topoisomerase II (Vybiralova et al., 2005). Thus, keeping in view the above facts it was felt worthwhile to synthesize and evaluate some novel derivatives of Ciprofloxacin and evaluate them for antibacterial activity.

Materials and Methods

The melting points of synthesized compounds were determined in open capillary tubes using Veego VMP-1 melting point apparatus, expressed in ^{0}C and are uncorrected. The IR spectra of compounds were recorded on a Perkin Elmer Infra Red Spectrophotometer in KBr discs and absorption bands are expressed in cm^{-1}. ^{1}H NMR spectra were recorded on a Brucker Aveance 700 MHz NMR Spectrometer (Chemical shift in δ ppm) using TMS as internal standard. Reactions were monitored by thin layer chromatography on pre-coated silica gel G plates using different solvent systems. The purity of synthesized compounds was ascertained by TLC, using iodine vapors as visualizing agents.

Chemistry

The title compounds were prepared in following steps (Scheme 1).



Scheme 1

Synthesis of ciprofloxacin esters (IIIa-IIIj)

Ciprofloxacin (I) (0.01 mol) was heated under reflux with thionyl chloride (10 ml) for 6 h, which yielded ciprofloxacin acid chloride (II). Excess of thionyl chloride was distilled off under vacuum. Pale yellow crystals of acid chloride (II) thus obtained were used immediately in the next step by reacting suitably with respective alcohol to yield 1-cyclopropyl-6-fluoro-1,4-dihydro-7-(piperazin-1-yl)-4-oxoquinoline-3-carboxylates, the corresponding esters (III). After completion of the reaction (monitored by TLC) excess of solvent was removed by distillation and the resultant viscous mass was poured into ice water (100 ml) with vigorous stirring and left over night for complete precipitation. The resultant solid product was neutralized, filtered, washed with cold water, dried and recrystallized.

Synthesis of Nicotinoyl Chloride (IV)

Nicotinic acid (0.01 mol) was heated under reflux with thionyl chloride (10 ml) for 6 h. The solvent was evaporated under reduced pressure. Needle shaped, pale yellow crystals were formed and the product was used immediately for the next step.

Synthesis of title compounds (Va-Vj)

Ciprofloxacin esters (0.01 mol) (III) were dissolved in anhydrous pyridine (20 ml). The resulting suspension was reacted with nicotinoyl chloride (IV) in anhydrous pyridine by adding dropwise at 0^0 C. The mixture was first, stirred at room temperature for 6 h on magnetic stirrer followed by heating under reflux for 3 h. Afterwards, the reaction mixture was poured in 250 ml of distilled ice cold water and kept overnight. The precipitates of 1-cyclopropyl-6-fluoro-1,4-dihydro-7-piperazin-1-yl-4-susbstituted-4-oxoquinoline-3-carboxylates (Va-Vj) thus formed, were filtered off. Subsequent washings with cold water was done in order to remove the pyridine from the product. The product was collected and dried in vacuum dessicator. The product was recrystallized using an appropriate solvent.

Physical and analytical data of synthesized compounds is summarized in Table 1 and characterization data in Table 2.

Antibacterial Screening

All the compounds were screened for their antibacterial activity against two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram-positive strains (*Bacillus subtilis* and *Staphylococcus aureus*). Antimicrobial activity was assessed by serial two-fold dilution technique. Ciprofloxacin was used as a standard drug for antibacterial activity. The MIC of the compounds tested in this study was determined according to the method of Goto *et al* by a serial dilution technique (Goto *et al.*,1981). The inoculum size was approximately 10^6 colony-forming units (CFU/mL). The reference standard ciprofloxacin inhibited Gram-negative bacteria viz., *E. coli* and *P. aeruginosa* at a MIC of 0.03 mmol/mL and 0.75 mmol/mL respectively whereas against Gram positive bacteria viz., *S. aureus* and *B. subtilis* MIC was found to be 0.45 mmol/mL and 0.36 mmol/mL respectively. All synthesized compounds exhibited moderate to low antibacterial activity against all selected bacterial strains. Each experiment was done in triplicate and the average reading was taken. The results of antibacterial screening are summarized in Table 3.

Table 1. Physical and analytical data of synthesized compounds

Comp. No.	R	M.P. (^{0}C)	Yield (%)	Mol Formula	Mol Wt	R_f value	R_m Value	CHN Analysis Calculated (Found)		
								%C	%H	%N
V_a	Methyl	230-231	52	$C_{24}H_{23}FN_4O_4$	450	0.49	-0.017	63.99 (66.97)	5.15 (5.19)	12.44 (12.42)
V_b	Ethyl	268-269	57	$C_{25}H_{25}FN_4O_4$	464	0.41	-0.15	64.32 (64.63)	5.29 (5.39)	12.25 (12.01)
V_c	n-Propyl	290-292	42	$C_{26}H_{27}FN_4O_4$	479	0.61	-0.20	64.64 (65.22)	5.42 (5.72)	12.04 (11.67)
V_d	Iso-Propyl	281-282	48	$C_{26}H_{27}FN_4O_4$	479	0.53	-0.124	64.80 (65.29)	5.49 (5.72)	11.97 (11.74)
V_e	Iso-butyl	265-266	44	$C_{27}H_{29}FN_4O_4$	493	0.58	-0.142	65.84 (65.82)	5.93 (5.90)	11.38 (11.34)
V_f	Tert butyl	270-271	46	$C_{27}H_{29}FN_4O_4$	493	0.49	-0.017	65.84 (65.81)	5.93 (5.89)	11.38 (11.33)
V_g	Pentyl	280-281	52	$C_{28}H_{31}FN_4O_4$	507	0.68	-0.327	66.03 (66.35)	6.01 (6.19)	11.27 (11.09)
V_h	Hexyl	261-262	44	$C_{29}H_{33}FN_4O_4$	521	0.72	-0.420	66.25 (66.91)	6.11 (6.41)	11.14 (10.79)
V_i	Heptyl	255-256	50	$C_{30}H_{35}FN_4O_4$	535	0.44	-0.120	67.40 (67.43)	6.60 (6.63)	10.48 (10.44)
V_j	Octyl	239-240	37	$C_{31}H_{37}FN_4O_4$	549	0.67	-0.356	67.63 (67.89)	6.70 (6.82)	10.34 (10.23)

Table 2. Spectral data of Synthesized Compounds

Compd	IR (KBr)cm^{-1}	H^1 N.M.R. ($CDCl_3$)
Va	Ar-C-H- 3135 C = 0- 1660, 1710, 1725 C = N – 1554 C – O -1225 C – F – 1168	δ = 9.07 (s, 1H, pyridine); 8.78 (d, 1H, pyridine); 8.22 (d, 1H, pyridine); 7.60(s, 1H, pyridone); 7.49 (t, 1H- pyridine); 7.01 (s, 1H – aromatic); 5.89 (s, 1H,aromatic); 3.67 (s, 3H, CH_3); 3.58 (t, 4H, CH_2-piperazine, N-C=O): 3.19 (t, 4H, CH_2-piperazine, N-Ar); 1.55 (m, 1H, CH-cyclopropane); 0.44 (q, 4 H, $2CH_2$-Cyclopropane)
Vb	Ar-C-H- 3115 C = 0- 1660, 1715, 1735 C = N – 1540 C – O -1230 C – F – 1152	δ = 9.11 (s, 1H, pyridine); 8.64 (d, 1H, pyridine); 8.32 (d, 1H, pyridine); 7.73 (s, 1H, pyridone); 7.46 (t, 1H- pyridine); 7.11 (s, 1H – aromatic); 5.69 (s, 1H,aromatic); 4.09 (q, 2H, CH_2); 3.41 (t, 4H, CH_2-piperazine, , N-C=O): 3.17 (t, 4H, CH_2-piperazine, N-Ar); 1.29 (m, 1H, CH-cyclopropane); 1.20 (t, 3H, CH_3); 0.41 (q, 4 H, $2CH_2$-Cyclopropane)
Vc	Ar-C-H- 3130 C = 0- 1660, 1710, 1730 C = N – 1535 C – O -1210 C – F – 1135	----------
Vd	Ar-C-H- 3135 C = 0- 1665, 1708, 1725 C = N – 1545 C – O -1212 C – F – 1150	δ = 9.37 (s, 1H, pyridine); 8.69 (d, 1H, pyridine); 8.23 (d, 1H, pyridine); 7.52 (s, 1H, pyridone); 7.39 (t, 1H- pyridine); 7.22 (s, 1H – aromatic); 5.84 (s, 1H,aromatic); 4.11 (m, 1H, CH); 3.86 (t, 4H, CH_2-piperazine, N-C=O,): 3.44 (t, 4H, CH_2-piperazine, N-Ar); 1.56 (d, 6H, CH_3); 1.35 (m, 1H, CH-cyclopropane); 0.47 (q, 4 H, $2CH_2$-Cyclopropane)
Ve	Ar-C-H- 3140 C = 0- 1660, 1710, 1725 C = N – 1545 C – O -1225 C – F - 1165	----------

Table 2 continued.

Vf	Ar-C-H- 3142 C = 0- 1663, 1719, 1732 C = N – 1551 C – O -1229 C – F – 1176	δ = 9.22 (s, 1H, pyridine); 8.72 (d, 1H, pyridine); 8.36 (d, 1H, pyridine); 7.87 (s, 1H, pyridone); 7.69 (t, 1H- pyridine); 7.20 (s, 1H – aromatic); 5.63 (s, 1H,aromatic); 3.43 (t, 4H, CH_2-piperazine N-C=O): 3.23 (t, 4H, CH_2-piperazine, N-Ar); 1.51 (s, 9H, $3CH_3$); 1.30 (m, 1H, CH-cyclopropane); 0.40 (q, 4 H, $2CH_2$-Cyclopropane)
Vg	Ar-C-H- 3151 C = 0- 1658, 1716, 1749 C = N – 1550 C – O -1230 C – F – 1132	δ = 9.45 (s, 1H, pyridine); 8.34 (d, 1H, pyridine); 8.02 (d, 1H, pyridine); 7.63 (s, 1H, pyridone); 7.39 (t, 1H- pyridine); 7.01 (s, 1H – aromatic); 5.67 (s, 1H,aromatic); 4.40 (t, 2H, CH_2); 3.61 (t, 4H, CH_2-piperazine, N-C=O): 3.19 (t, 4H, CH_2-piperazine, N-Ar); 1.57 (m, 2H, CH_2); 1.35 (m, 1H, CH-cyclopropane); 1.33 (m, 2H, CH_2); 1.29 (m, 2H, CH_2); 0.96 (t, 3H, CH_3); 0.49 (q, 4 H, $2CH_2$-Cyclopropane)
Vh	Ar-C-H- 3127 C = 0- 1666, 1717, 1741 C = N – 1523 C – O -1226 C – F – 1139	δ = 9.11 (s, 1H, pyridine); 8.39 (d, 1H, pyridine); 8.20 (d, 1H, pyridine); 7.81 (s, 1H, pyridone); 7.43 (t, 1H- pyridine); 7.21 (s, 1H – aromatic); 5.66 (s, 1H,aromatic); 4.01 (t, 2H, CH_2); 3.61 (t, 4H, CH_2-piperazine, N-C=O): 3.19 (t, 4H, CH_2-piperazine, N-Ar); 1.68 (m, 2H, CH_2); 1.48 (m, 2H, CH_2); 1.35 (m, 1H, CH-cyclopropane); 1.30 (m, 4H, 2 CH_2); 0.91 (t, 3H, CH_3); 0.39 (q, 4 H, $2CH_2$-Cyclopropane)
Vi	Ar-C-H- 3158 C = 0- 1678, 1728, 1715 C = N – 1549 C – O -1236 C – F – 1140	----------
Vj	Ar-C-H- 3162 C = 0- 1674, 1706, 1730 C = N – 1532 C – O -1263 C – F – 1127	δ = 9.21 (s, 1H, pyridine); 8.67 (d, 1H, pyridine); 8.12 (d, 1H, pyridine); 7.79 (s, 1H, pyridone); 7.43 (t, 1H- pyridine); 7.01 (s, 1H – aromatic); 5.83 (s, 1H,aromatic); 4.26 (t, 2H, CH_2); 3.55 (t, 4H, CH_2-piperazine, N-C=O): 3.08 (t, 4H, CH_2-piperazine, N-Ar); 1.72 (m. 2H, CH_2), 1.49 (m, 8H, 4 CH_2); 1.35 (m, 1H, CH-cyclopropane); 1.28 (m, 2H, CH_2); 0.88 (t, 3H, CH_3); 0.42 (q, 4 H, $2CH_2$-Cyclopropane)

Table 3. *In vitro* antibacterial activity of compounds III_a-III_j (MIC mmol/ml)

Compound	Gram negative bacteria		Gram positive bacteria	
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
V_a	0.05	0.81	0.50	0.41
V_b	0.04	0.85	0.57	0.49
V_c	0.05	0.83	0.54	0.54
V_d	0.06	0.92	0.69	0.44
V_e	0.04	0.98	0.62	0.76
V_f	0.07	0.85	0.71	0.63
V_g	0.06	0.99	0.82	0.62
V_h	0.05	1.20	0.91	0.59
V_i	0.06	1.30	0.63	0.55
V_j	0.07	1.44	0.99	0.73
Ciprofloxacin (Standard drug)	0.03	0.75	0.45	0.36

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