Bioorganic & Medicinal Chemistry Letters 23 (2013) 2974–2978

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis, cytotoxicity and topoisomerase II inhibitory activity of lomefloxacin derivatives

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ARTICLE INFO

Article history:
Received 7 January 2013
Revised 7 March 2013
Accepted 11 March 2013
Available online 20 March 2013

Keywords:
Lomefloxacin derivatives
Topoisomerase I and II inhibitory activity
Docking study

ABSTRACT

A novel series of amide derivatives of lomefloxacin were synthesized and evaluated for their topoisomerase I and II inhibitory activity as well as cytotoxicity against a panel of five human cancer cell lines. Of the compounds prepared compounds **9d** and **9g** exhibited strong inhibition against topoisomerase II at 100 μM. In addition, docking studies were performed to predict the inhibition mode.

DNA topoisomerases, generally classified as types I and II, are critical cellular enzymes necessary for cell proliferation by solving topological problems in the process of DNA replication.[1–3] Topoisomerase I produces a single strand break in DNA allowing relaxation of DNA supercoils. On the other hand, topoisomerase II controls DNA topology by transient cleavage of the DNA double helix.[4] Due to the critical role of these enzymes for the cell proliferative process, topoisomerases have become one of the major targets for the design of novel antitumor drugs.[5,6] To date, drugs from the camptothecin (CPT, **1**) family and etoposide (**2**) are clinically used to target topoisomerase I and topoisomerase II, respectively. Unfortunately, the therapeutic potential of **1** and its derivatives, topotecan (**3**) and irinotecan (**4**), is severely hindered due to their rapid inactivation through lactone ring hydrolysis at physiological pH (Scheme 1).[7] In order to overcome the E-ring-opening inactivation of camptothecins, metabolically stable non-CPT derivatives have been developed without a lactone ring in their skeleton compared with camptothecins.[8–11]

Previously we reported that a novel series of topoisomerase I inhibitors were designed and synthesized on the basis of **1** using scaffold modification strategy (Fig. 1).[12] The original framework of **1** was changed into a combination of the quinolone skeleton and benzimidazole group or its bioisoesters having a single carbon bond. The result of MTT test and topoisomerases inhibition test suggested that our scaffold modification approach based on **1** has yielded new generation of topoisomerase I inhibitors for cancer treatment.

In order to extend the scaffold of the anticancer drug candidates targeting topoisomerase I, we designed and synthesized a novel series of compounds based on the most active compound (**5**) in the previous work (Fig. 1). The two fluoro atoms and one 3-methylpiperazine group on the quinolone ring of **5** were retained. It is widely reported that the fluoro atom could improve the metabolic characteristics of compounds by prolonging the half-lives, and the piperazine could enhance the water solubility of compounds. Meanwhile, the scaffold hopping method is used in the modification of **5** by breaking the benzothiazole ring to aniline amide group. The amide group was expected to improve the solubility and rigidity of **5**. Substituents with different electronic properties were introduced onto the phenyl group of aniline to investigate the impact of their electronic effects on the biological profile. Furthermore, the effects of increased flexibility of central linkage and replacement of the phenyl group of aniline by other aromatic rings on biological activity were investigated, respectively. Total 12 new compounds (**9a**–**9l**, Scheme 2) were synthesized and evaluated for Top I and Top II inhibition activities and for cytotoxicity against five cancer cell lines (HCT-116, MDA-MB-231, A549, Bel7402 and KB).

The synthetic routes for the target compounds are described in Scheme 2. The lomefloxacin hydrochloride (**6**) as starting material was commercially available. As shown in Scheme 2, the compound **6** was protected by Boc group using Boc_2O under NaOH condition to give compound **7** in 90.6% yield. Subsequently, **7** was condensed

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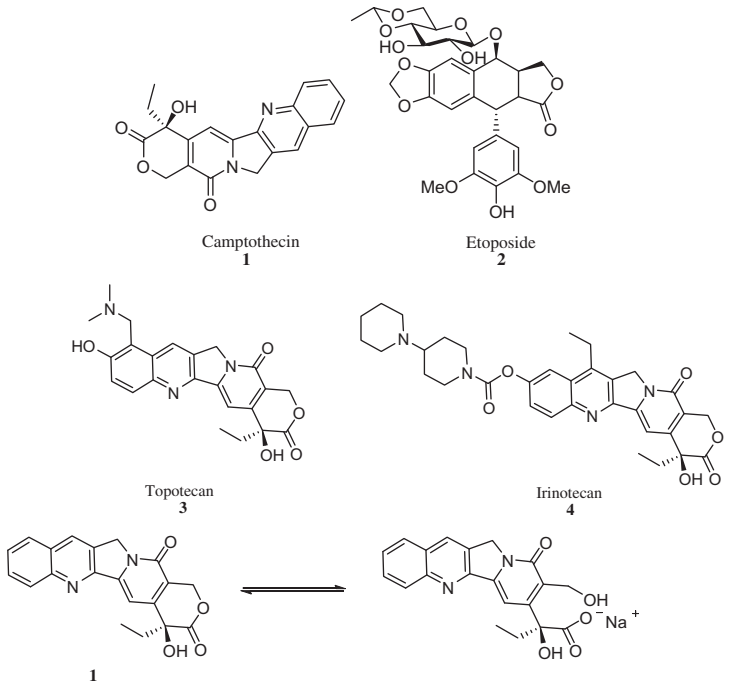
http://dx.doi.org/10.1016/j.bmcl.2013.03.037



Scheme 1. Lactone ring hydrolysis of **1**.

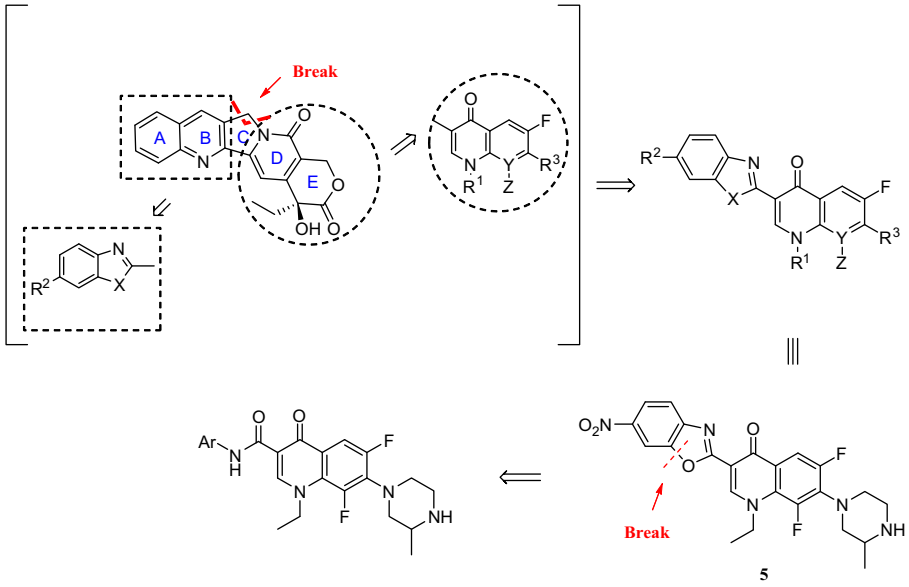
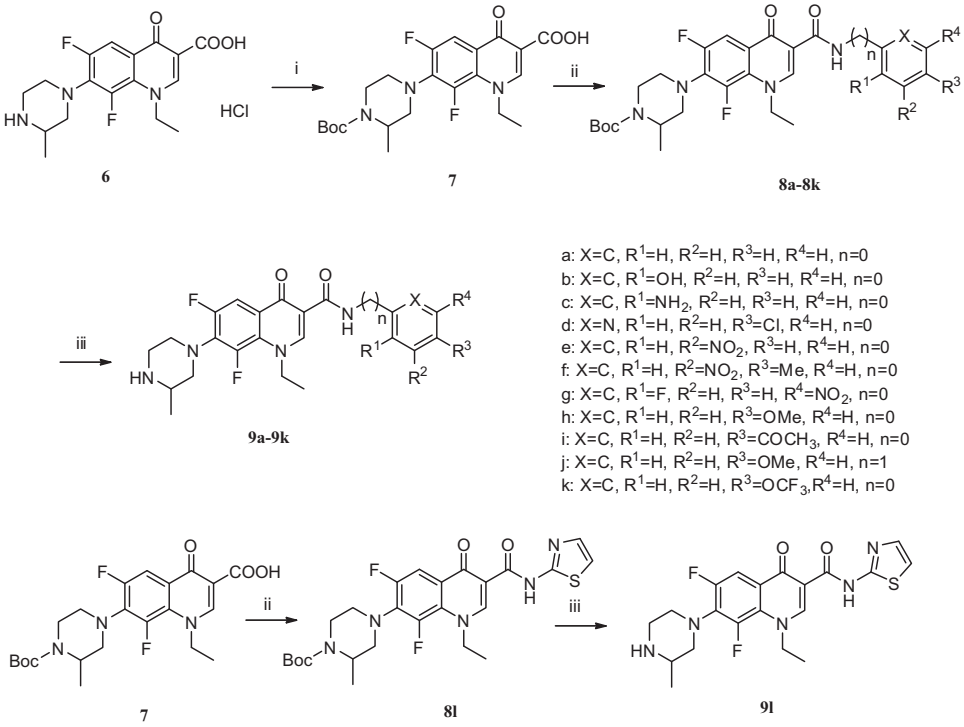


Figure 1. Compound generation by scaffold modification.

with various aromatic amines in the presence of BOP to provide a series of amide derivatives (**8a**–**8l**) in 24.0–66.4% yield. Lastly, the removal of the Boc groups from compounds **8a–8l** using TFA afforded the target compounds (**9a**–**9l**) in 76.4–97.2% yield.[13] The final products were purified by column chromatography and analytical data of 4 representative compounds were described in the reference section.[14,15]

MTT assay[16] was performed to evaluate the cytotoxic activities of our synthesized compounds against five different cancer cell lines including A549 (human lung carcinoma cell line), HCT-116 (human colorectal cancer cell line), MDA-MB-231 (human breast cancer cell line), Bel7402 (human hepatocarcinoma cell line) and KB (oral human epidermoid carcinoma cancer cell line), with 10-OH CPT and compound **5** as positive controls. Most of these derivatives displayed potent cytotoxic activities against cancer cell lines tested (Table 1). Compound **9a**, which contains an unsubstituted aromatic benzene ring, showed moderate cytotoxic activity on MDA-MB-231, Bel7402 and KB cell lines, but weak cytotoxic activity on A549 and HCT-116 cell lines. In comparison with **9a**, compounds (**9b**, **9c**, **9h**) containing electron donating substituents on the benzene ring displayed better cytotoxicity on A549 and HCT-116 cell lines, indicating a different mode of action involved. It is noteworthy that the introduction of specific electron withdrawing groups into the benzene ring of **9a** (**9e**, **9g**, **9k**) could apparently enhance the in vitro cytotoxicity. When evaluated on the MDA-MB-231 cell line, compound **9k** showed a high cytotoxicity in the micromolar range (IC_{50} = 0.98 μM), higher than compound **5** (IC_{50} = 2.0 μM). Compounds **9e** and **9g** also showed



Scheme 2. Synthesis of lomefloxacin derivatives. Reagents and conditions: (i) THF/NaOH (aq), Boc_2O, rt; (ii) BOP/DMAP, TEA, CH_2Cl_2, rt; (iii) TFA, CH_2Cl_2, rt.

Table 1
Result of cytotoxicity of compounds synthesized against five cancer cells

Compounds/cells	IC_{50} (μM)				
	A549	HCT-116	MDA-MB-231	Bel7402	KB
10-OH CPT	0.07	0.3	0.06	0.2	0.6
5	0.9	1.1	2.0	1.6	0.9
9a	39.3	12.7	7.4	6.0	4.4
9b	24.8	5.37	7.43	>50	5.05
9c	5.9	2.7	9.5	4.8	2.8
9d	3.3	3.3	4.8	4.9	1.6
9e	3.24	2.71	3.25	1.0	1.7
9f	3.7	11.8	4.2	29.1	3.9
9g	1.7	2.6	6.9	2.9	3.2
9h	9.7	3.8	7.3	5.4	2.6
9i	4.0	4.6	5.6	>50	42.6
9j	7.0	1.4	25.3	10.3	1.63
9k	1.32	2.35	0.98	2.0	1.4
9l	20.2	8.66	>50	>50	43.5

comparable cytotoxic activity against cancer cell lines tested. These results indicated that the decrease of aromatic ring electron density was advantageous to the cytotoxicity. When benzene ring was replaced with pyridine or thiazole, the resulting compounds, **9d** and **9l**, demonstrated moderate or weak cytotoxicity on cancer cell lines, respectively. Compared with **9h**, compound **9j** with an additional CH_2 group showed comparable antiproliferative activity against HCT-116 and KB cell lines, but decreased cytotoxicity against MDA-MB-231 and Bel7402 cell lines.

In summary, we have prepared a series of lomefloxacin derivatives and conducted preliminary analysis of the impact of electron property on the in vitro cytotoxicity. Of the compounds prepared compounds **9d**, **9e**, **9g** and **9k** showed comparable cytotoxic activity against cancer cell lines tested.

Topoisomerases inhibition assays were used to assess the effects of all the 12 synthesized compounds on the catalytic activity of human topoisomerase I and II (Topogen), with camptothecin and etoposide as positive controls.[17] As shown in Figure 2A, the result clearly showed that none of tested compounds possessed inhibitory activity on topoisomerase I, which means benzo-heterocycle group is closely correlated with topoisomerase I inhibitory activity. In the topoisomerase II inhibition assay (Fig. 2B), supercoiled plasmid DNA was treated with human topoisomerase II in the presence of the reference compound (etoposide) or tested compounds at a concentration of 100 μM. Inhibition of topoisomerase II was clearly specifically detected with the reference compound (etoposide), which produced a marked level of DNA double stranded breaks, corresponding to linear DNA. Linear DNA bands were also observed for some of the tested compounds. From the enzyme assay, 6 active compounds with inhibitory effect on topoisomerase II enzyme were identified. Figure 2B shows that compounds **9d** and **9g** were active inhibitors on topoisomerase II enzyme with comparable extent to etoposide at a concentration of 100 μM, whereas compounds **9e**, **9i**, **9f** and **9k** appeared to be less active than etoposide. The result indicated that the introduction of various electron withdrawing groups into the benzene ring of **9a** could generate a series of derivatives with topoisomerase II inhibitory activity.

In summary, results of topoisomerases inhibition assays indicated that the new synthesized lomefloxacin derivatives exerted their antitumor activities through specific inhibition of human topoisomerase II activity without any human topoisomerase I inhibitory activity. However, there was no remarkable correlation between cytotoxicity and topoisomerase II inhibitory activity, which implied that we can not exclude the possibility that other targets can be involved in the mechanism of action of the active compounds.

To verify the binding mode of lomefloxacin derivatives, compounds **9g** and **9a** were docked into the ATP-binding domain of human topoisomerase IIα, respectively. ATP-binding domain is the probable binding site for etoposide as reported earlier.[18] We carried out a docking study using CDOCKER in Discovery Studio 3.0 and the docking results are shown in Figure 3. An analysis of the docked compound **9a** to the ATP-binding domain showed that

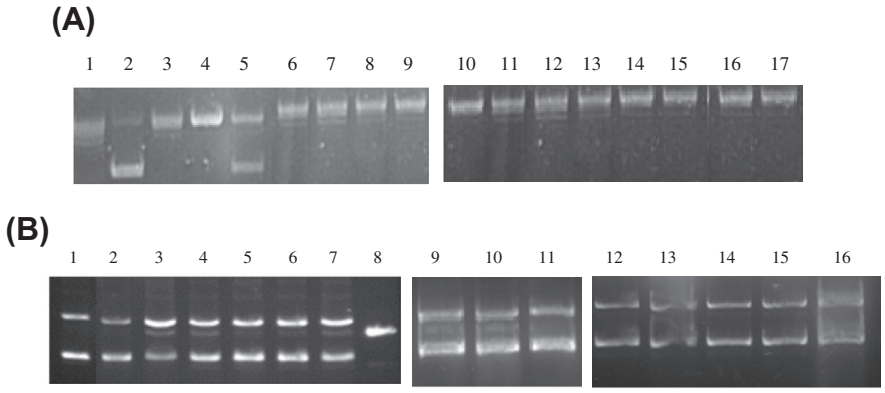


Figure 2. Topoisomerase I (A) and II (B) inhibitory activities of the tested compounds. All compounds were examined in a final concentration of 100 μM. (A) lane 1: relaxed DNA only, lane 2: ScDNA + 1% DMSO, lane 3: ScDNA + Topo I, lane 4: ScDNA + Topo I + camptothecin, lane 5: ScDNA + Topo I + compound **5**, lanes 6–17: ScDNA + Topo I + compounds in order of **9d**, **9k**, **9e**, **9g**, **9i**, **9f**, **9l**, **9a**, **9b**, **9c**, **9h** and **9j**. (B) lane 1: relaxed DNA only, lane 2: ScDNA + Topo II, lane 3: ScDNA + Topo II + etoposide, lanes 4–7 and 9–16: ScDNA + Topo II + compounds as the same as the order of (A), lane 8: linear DNA only.

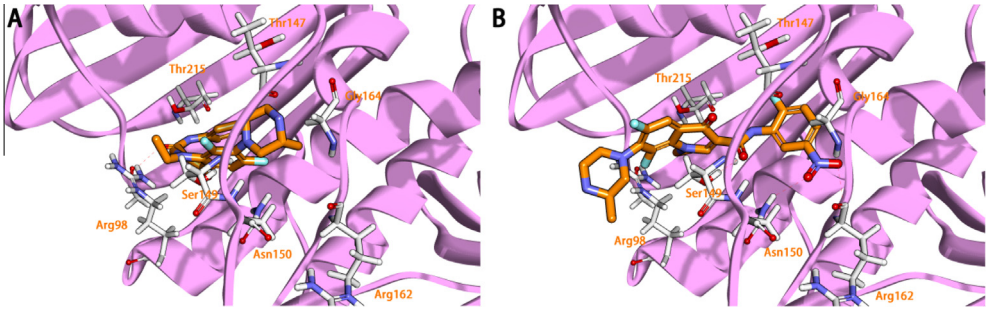


Figure 3. (A) Molecular docking between compound **9a** and ATP-binding domain of human topo IIα. Compound **9a** is depicted by stick. The key residues of the ATP-binding site of topo IIα are shown as sticks. The representation of the compound is colored by the atom type (carbon, orange; oxygen, red; nitrogen, blue; fluoro, cyan). (B) Molecular docking between compound **9g** and ATP-binding domain of human topo IIα. Compound **9g** in stick representation is described as above.

compound **9a** occupies the ATP-binding site in an unreasonable conformation, which would lead to increased internal energy. In contrast, molecular docking study revealed compound **9g** to have a stable binding pattern to the ATP-binding domain, which was matching with previously reported docking results of other topoisomerase II inhibitors.[19] Compound **9g** was found to has strong intermolecular contacts with Arg 98, Gly164 and Ser149. The nitro group attached to the benzene ring forms two hydrogen bonds with Asn 150 and Arg 162. In addition, the fluoro atom substituent forms a hydrogen bond with Thr 147 (Fig. 3B).

The results of molecular docking indicated that the introduction of electron withdrawing substituents into the benzene ring may anchor in the ATP-binding domain of human topoisomerase IIα resulting in increased binding affinity, which lead to a higher topoisomerase II inhibitory activity.

In conclusion, we have designed and synthesized a series of lomefloxacin derivatives and evaluated their pharmacological activity. Of the compounds prepared compounds **9d**, **9e**, **9g**, **9k**, **9i** and **9f** could selectively impede topoisomerase II function without affecting topoisomerase I catalytic activity. Molecular docking study revealed compound **9g** to have a stable binding pattern to the ATP-binding domain of human topoisomerase IIα. This study may provide valuable information to researchers working on the development of novel antitumor agents targeting topoisomerase II.

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13. *General procedure for the synthesis of intermediates **8a–8l**:* To a solution of the Boc-protected Lomefloxacin (0.5 g, 1.1 mmol) in CH_2Cl_2 (20 mL) was added a catalytic amount of DMAP (54 mg, 0.44 mmol) followed by TEA (0.5 mL, 3.3 mmol) and BOP (1 g, 2.2 mmol). The reaction mixture was allowed to stir for 1 h at room temperature. After that a solution of amine (0.1 g, 1.1 mmol) in CH_2Cl_2 (10 mL) was added dropwise and slowly to the mixture. The reaction mixture was allowed to stir overnight at room temperature. Saturated ammonium chloride aqueous solution (30 mL) was added and the reaction was extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The product was then purified over silica gel eluting with PET/EtOAc (1:1) to give pure **8a** (0.29 g) in 50.1% yield. The compounds **8b–8l** were similarly produced as **8a**.
14. *General procedure for the synthesis of the target compounds **9a–9l**:* A solution of **8a** (0.2 g, 0.38 mmol) and CF_3COOH (5 mL) in dry CH_2Cl_2 (20 mL) was stirred at room temperature for 2 h. Saturated sodium bicarbonate solution (30 mL) was added and the reaction was extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude material was purified over silica gel eluting with CH_2Cl_2/MeOH (10:1) to give the pure **9a** (0.15 g) in 92.6% yield. The target compounds **9b–9l** were similarly produced as **9a**.
15. *The data of selected compound **9d**:* yield 91.5%; mp 229–232 °C; IR (KBr): 3462, 2969, 2845, 1680, 1605, 1563, 1471, 1376, 1318, 1296, 1022, 796, 649 cm^{-1}; EI-MS: 462 (M); ^1H NMR (300 MHz, $CDCl_3$): δ 12.56 (s, 1H, –NH-CO), 8.59 (s, 1H, Ar-H), 8.24 (d, J = 1.9 Hz, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.94 (d,

J = 12.0 Hz,1H, Ar-H), 7.59 (dd, *J* = 11.3 Hz, 1H, Ar-H), 4.38 (m, 2H, N-*CH*$_2$–CH$_3$), 3.24 (m, 3H, N-CH$_2$, N-CH), 2.94 (m, 4H, 2 × N-CH$_2$), 1.49 (t, *J* = 13.6 Hz, 3H, –CH$_2$–*CH*$_3$), 1.06 (d, *J* = 6.0 Hz, 3H, –CH–*CH*$_3$); ^{13}C NMR (75 MHz, CDCl$_3$): 15.8, 18.9, 45.9, 48.8, 50.8, 53.6, 58.0, 107.9, 109.2, 112.8, 114.7, 120.4, 125.7, 128.8, 133.6, 136.9, 146.3, 149.8, 152.8, 156.0, 162.4, 173.6. Anal. Calcd for $C_{22}H_{22}N_5O_2F_2Cl$: C, 57.21; H, 4.80; N, 15.16. Found: C, 56.78; H, 5.19; N, 15.12. *Compound* ***9e****:* yield 91.0%; mp 216–219 °C; IR (KBr): 3469, 2967, 2825, 1681, 1603, 1527, 1351, 1320, 1241, 798, 738 cm^{-1}; EI-MS: 471 (M); ^{1}H NMR (300 MHz, CDCl$_3$): δ 12.51 (s, 1H, –NH-CO), 8.77 (s, 1H, Ar-H), 8.72(s, 1H, Ar-H), 8.00 (d, *J* = 12.8 Hz, 2H, Ar-H), 7.94 (s, 1H, Ar-H), 7.50 (t, *J* = 8.0 Hz, 1H, Ar-H), 4.49 (m, 2H, N-*CH*$_2$–CH$_3$), 3.35 (m, 3H, N-CH$_2$, N-CH), 3.05 (m, 4H, 2 × N-CH$_2$), 1.59 (t, *J* = 5.4 Hz, 3H, –CH$_2$–*CH*$_3$), 1.15 (d, *J* = 4.1 Hz, 3H, –CH–*CH*$_3$); Anal. Calcd for $C_{23}H_{23}N_5O_4F_2$: C, 58.60; H, 4.92; N, 14.85. Found: C, 58.22; H, 5.17; N, 15.28. *Compound* ***9g****:* yield 96.23%; mp 287–289 °C; IR (KBr): 3422, 2979, 1677, 1604, 1560, 1534, 1474, 1384, 1348 cm^{-1}; EI-MS: 489 [M]$^+$; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.05 (d, *J* = 6 Hz, 3H, –CH–*CH*$_3$), 1.45 (t, 3H, –CH$_2$–*CH*$_3$), 2.88 (m, 6H, 3 × –N-CH$_2$), 4.56 (m, 3H, –N-CH–CH$_2$–N), 7.61 (t, *J* = 18 Hz, 1H, 3′-H), 7.83 (d, *J* = 9 Hz, 1H, 6′-H), 8.00 (dd, *J* = 13.5 Hz, 1H, 5-H), 8.88 (s, 1H, 2-H), 9.36 (dd, *J* = 9 Hz, 1H, 5′-H), 12.71 (s, 1H, –N-H); Anal. Calcd for $C_{23}H_{22}F_3N_5O_4$: C, 56.44; H, 4.53; N, 14.31. Found: C, 56.01; H, 4.54; N, 14.18. *Compound* ***9k****:* yield 95.8%; mp 210–214 °C; IR (KBr): 3469, 2955, 2825, 1679, 1626, 1555, 1537, 1473, 1263, 1200, 1022, 799, 656 cm^{-1}; EI-MS: 510(M); ^{1}H NMR (300 MHz, CDCl$_3$): δ 12.17 (s, 1H, –NH-CO), 8.73 (s, 1H, Ar-H), 8.04 (d, *J* = 11.7 Hz, 1H, Ar-H), 7.78 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.21 (d, *J* = 8.3 Hz, 2H, Ar-H), 4.48 (m, 2H, N-*CH*$_2$–CH$_3$), 3.51 (m, 3H, N-CH$_2$, N-CH), 3.25 (m, 4H, 2 × N-CH$_2$), 1.57 (t, *J* = 13.4 Hz, 3H, –CH$_2$–*CH*$_3$), 1.33 (d, *J* = 5.0 Hz, 3H, –CH–*CH*$_3$); Anal. Calcd for $C_{24}H_{23}N_4O_3F_5$: C, 55.49; H, 4.66; N, 10.79. Found: C, 55.92; H, 4.74; N, 10.79.

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