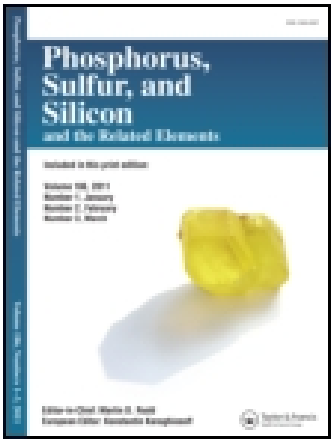

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Synthesis, Spectroscopic, and Antimicrobial Activity Studies of Novel 10-Substituted Camptothecin Phosphorothioate Analogs

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SYNTHESIS, SPECTROSCOPIC, AND ANTIMICROBIAL ACTIVITY STUDIES OF NOVEL 10-SUBSTITUTED CAMPTOTHECIN PHOSPHOROTHIOATE ANALOGS

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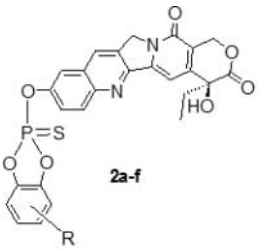
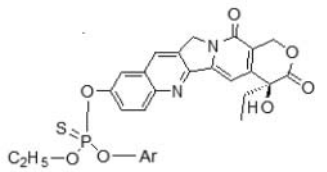
GRAPHICAL ABSTRACT



2a-f



3a-f

Abstract *A series of title compounds **2** and **3** were efficiently synthesized via the condensation of 10–hydroxycamptothecin with various symmetric (*O,O′*-monoaryl)-thiophosphoryl chlorides and asymmetric (*O*-ethyl-O′-aryl)-thiophosphoryl chlorides in sodium hydroxide powder and acetonitrile system. The structures of title compounds **2** and **3** were confirmed by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, $^{31}P[^{1}H]$ NMR,and mass spectral data. These symmetric [(O,O′-monoaryl)-thiophosphoryl)]-(20S)-camptothecin **(2a–f)** and asymmetric [(O-ethyl-O′-aryl)-thiophosphoryl)]-(20S)-camptothecin **(3a–f)** compounds were also tested for their in vitro antimicrobial activities against some bacterial strains, namely, S. aureus, B. Simplex, E. acetylicum, E. coli, P. aeruginosa, S. flexenari, S. aureus, S. typhi, and some fungal strains Aspergillus niger, Aspergillus flavus (molds), S. cerevisiae, C. albicans, T. longifucus, A. flavus, M. canis, F. solani, and C. glaberata (yeasts).*

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Keywords Synthesis; antimicrobial activity; camptothecine; phosphorothioate

INTRODUCTION

20(S)–Camptothecin (CPT), a pentacyclic alkaloid, originally isolated from the Chinese plant *Camptotheca acuminata* by Wall and Wani in 1966,[1] is one of the most important lead compounds in anticancer research. The antitumor activity of CPT is thought to be due to its ability to stabilize the reversible covalent DNA topoisomerase I (top 1) complex,[2–7] preventing the relegation step of the breakage/rejoining reaction mediated by the enzyme. The net result is that the drug causes fragmentation of chromosomal DNA, cell death and extensive sister chromatid exchange and chromosomal aberrations.[8–13] Elucidation of the specific target and mechanisms of camptothecin has stimulated intensive efforts to identify novel analogs that overcome the drawbacks of the natural camptothecin molecule, which include low solubility in water; severe and unpredictable toxicity, including hemorrhagic cystitis; reversibility of the drug–target interaction; lactone instability; and drug resistance.[14–16] One of the initial major strategies in this regard has been to improve the solubility of the natural camptothecin by chemical modification.[16,17] This approach has produced different series of water-soluble analogs or water-soluble pro-drugs, among which topotecan and irinotecan are the most successful. CPT topoisomerase I (top I) inhibitors are proving useful against a range of refractory tumors, most prominently against some colon and ovarian cancers.[18–20] Two of the CPTs, topotecan and CPT–11, have received Food and Drug Administration approval, and several others are in clinical trials.

In the present paper, we would like to report the results on the antimicrobial activity investigation of twelve representatives of symmetric 10-[(*O,O′*-monoaryl)thiophosphoryl]-(20S)-camptothecin **(2a–f)** and asymmetric 10-[(*O*-ethyl-*O′*-aryl)thiophosphoryl-(20S)-camptothecin **(3a–f)**. The synthetic route is shown in Figure 1. Characterization was performed by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and mass spectral data. The biological activities of the synthesized comounds **(2a–f)** and **(3a–f)**, have been examined against some antimicrobial strains namely, *S. aureus, B. Simplex, E. acetylicum* (Gram-positive bacteria), *E. coli, P. aeruginosa, S. flexenari, S. aureus*, *S. typhi* (Gram-negative bacteria); and *A. niger, A. flavus* (molds), *S. cerevisiae, C. albicans, T. longifucus, A. flavus, M. canis, F. solani,* and *C. glaberata* (yeasts) for evaluation of antibacterial and antifungal activities of the synthesized chemical compounds. The results obtained were compared with standard antibiotic: *Ciprofloxacin* and the standard antifungal drug: *Amphotericin–B*.

RESULTS AND DISCUSSION

Chemistry

The present work was to design and synthesize some new symmetric (*O,O′*-monoaryl)-thiophosphoryl-(20S)-camptothecin and asymmetric [(*O*-ethyl-*O′*-aryl)-thiophosphoryl-(20S)-camptothecin derivatives (Figure 1) carrying the biologically active thiophosphoate moiety, and hence expected to show antimicrobial activity.

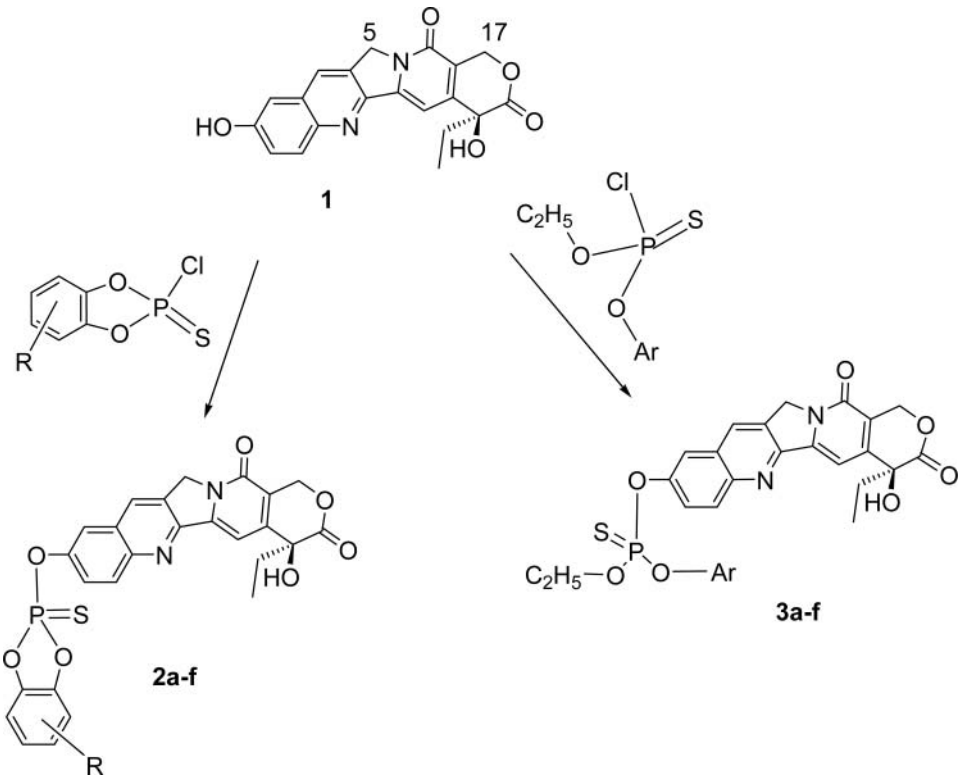

Figure 1 Scheme for synthesis of symmetric [(*O,O′*-monoaryl)-thiophosphoryl)]-(20S)-camptothecin **(2a–f)** and asymmetric [(*O*—ethyl-*O′*-aryl)-thiophosphoryl)]-(20S)-camptothecin **(3a–f)** compounds.

10–hydroxycamptothecin **1** reacted with various thiophosphoryl chlorides to yield the target compounds **2, 3** in moderate yields. In order to optimize the reaction conditions, we scanned different bases and solvent systems, such as Et_3N/CH_2Cl_2, $Et_3N/CHCl_3$, Et_3N/dioxane, pyridine/dioxane, pyridine/$CHCl_3$, K_2CO_3/dioxane, K_2CO_3/CH_3CN, $NaHCO_3/H_2O$+THF, and NaOH(s)/CH_3CN.

Finally, we found that the reaction gave the best results in mild reaction conditions in the NaOH(s)/CH_3CN system, and no byproduct was detected by TLC (thin layer chromatography).

The structures of compounds **2a–f** and **3a–f** were deduced from their spectral data (IR, ^1H, ^{13}C, and ^{31}P NMR, MS) and elemental analyses. Selected spectra for **2a** and **3a** are shown in Figures S1–6 (Supplemental Materials). IR spectra of compounds **2** and **3** showed normal stretching absorption bands, indicating the existence of OH (∼3500 cm^{-1}),[21] C=O (∼1720 cm^{-1}),[22] C=N (∼1600 cm^{-1}),[23] Ar—H (∼1510, 1450 cm^{-1}),[21] P—O—C (∼1225, 1060, 1030 cm^{-1}),[24] and P=S (∼1000 cm^{-1}),[25,26] and in ^{31}P NMR spectra, the P signal in all of the title compounds was displayed as a singlet, in the chemical shift range of δ 60.4–76.6.[26,27]

The ^1H, ^{13}C, and ^{31}P NMR spectra of these novel camptothecin derivatives showed the correct characteristic proton peaks for their different subsistents. The mass spectra of obtained compounds showed molecular ion peaks that were consistent with their molecular formulae.

Biological Activity. In the present study, all chemically synthesized compounds were evaluated against three Gram-positive, five Gram-negative bacteria, and nine fungi (two mold and two strains). Minimum Inhibitory concentrations (MIC) of some of these synthesized compounds against some Gram-positive bacteria were determined by the method given by Andrews.[28] The structure activity relationship (SAR) studies revealed that the presence of an electron-withdrawing group (**2b, 2f, 3d, and 3f**) on the *O,O*-monophenyl ring **(2)** or *O*-ethyl*O*-phenyl ring **(3)** increased the antimicrobial activity, and activity decreased in the presence of electron-releasing atoms or groups.[29]

EXPERIMENTAL

Melting points (°C, uncorrected) were determined in open capillaries on a GallenKemp melting point apparatus (Sanyo GallenKemp, Southborough, UK).

The infrared spectra were recorded in KBr pellets on a Pye unicam SP 3300 and FTIR S101PC Shimadzu Infrared Spectrometers (Shimadzu, Tokyo, Japan).

NMR spectra were obtained in deuterated $CDCl_3$ or DMSO (dimethylformamide) on a Varian MERCURY (^{1}H: 300 MHz and ^{13}C: 75 MHz) spectrometer (Bruker, Flawil, Switzerland, δ ppm) using TMS as an internal reference. ^{31}P NMR spectra were measured on the same spectrometer using H_3PO_4 (85%) as external reference.

Electron impact Mass Spectra were recorded on a Shimadzu Gc−Ms−Qp 5000 instrument (Shimadzu, Tokyo, Japan).

Elemental analysis was performed on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany). The phosphorus content was determined gravimetrically as phosphoammoniummolybdate.[30]

TLC analysis was carried out on silica gel plates GF_{254}. Flash column chromatography was carried out on silica gel 300–400 mesh. HPLC analysis was performed on a Waters Alliance 2690 instrument with UV detection at 360 nm: column: Waters Symmetry–C_{18}.

All of the solvents and materials were reagent grade and purified are required. Camptothecin was purchased from Sigma Chemical (St. Louis, MO).

10–hydroxy–camptothecin **(1)** was prepared by following the literature procedure.[31]

Symmetric thiophosphoric chlorides were prepared according to the procedure in the literature.[26]

Asymmetric thiophosphoric chlorides were prepared according to the procedures in the literature.[32,33]

Preparation of Compounds 2, 3: General Procedure

A mixture of 10—hydroxy-CPT (0.732 g, 2 mmol), NaOH powder (0.08 g, 2 mmol) and anhydrous acetonitrile (15 mL) were added into a three necked flask at 298 K. After vigorously stirring for 5∼10 min, then (*O,O′*-monoaryl) thiophosphoryl chloride or (*O*-ethyl-*O′*-aryl) thiophosphoryl chloride (2 mmol) in acetonitrile (5 mL) was added dropwise. TLC was carried out to detect the reaction throughout. After the mixture was stirred under N_2 atmosphere at 60 °C for 72∼ 75 h, it was added to certain water. The mixture was filtered and the solid was washed with water and acetone, and then dried. After the solvent was removed under reduced pressure, the residue was taken up in the mixed solution (eluent: $CHCl_3/CH_3OH$ 8:2) and purified by column chromatography (eluent: $CHCl_3/CH_3OH$ 100:1–100:3).

***O,O′*-monophenyl-[(20S)-camptothecin]-phosphorothionate (2a).** Yellow oil, yield: 77.3%, IR (KBr, cm^{-1})ν: 3500 (OH), 1721 (C=O), 1600 (C=N), 1510, 1454 (Ar), 1224, 1063, 1033 (P−O−C), 1000 (P=S). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 0.99 (3H, t, J = 7.5 Hz, H–18), 1.86 (2H, m, H–19), 4.73 (2H, s, H–5), 4.40 (2H, s, H–17), 2.54 (1H, s, 20−OH), 6.68–7.86 (8H, m, Ar−H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 7.9, 30.4, 51.6, 64.7, 72.5, 108.6, 115.2 × 2, 117.7, 118.8, 121.7 × 2, 129.9 × 2, 130.8, 136.4, 144.1, 145.4 × 2, 145.7, 148.4, 150.2, 153.4, 158.5, 172.7; ^{31}P NMR (121.5 MHz, DMSO–d_6) δ: 76.5 (P=S); MS *m/z* (%): 613 [M^+] (2.1), 76 (100). Anal. Calcd. for $C_{26}H_{18}BrN_2O_7PS$: C, 50.91; H, 2.96; Br, 13.03; N, 4.57; P, 5.05; S, 5.23. Found: C, 50.92; H, 2.95; N, 4.55; P, 5.02; S, 5.24.

***O,O′*-[mono-p-bromophenyl)-(20S)-camptothecin]-phosphorothionate (2b).** Yellow oil, yield: 76.3%, IR (KBr, cm^{-1})ν: 3505 (OH), 1716 (C=O), 1602 (C=N), 1513, 1452 (Ar), 1224, 1063, 1034 (P−O−C), 1005 (P=S). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 0.90 (3H, t, J = 7.5 Hz, H–18), 1.86 (2H,m, H–19), 2.35 (3H, t, J = 7.2 Hz, CH_3), 4.25 (2H, s, H–5), 4.76, 4.74 (2H, s, H–17), 2.55 (1H, s, 20−OH), 6.72–7.89 (7H, m, Ar−H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 7.9, 30.5, 51.7, 65.4, 72.7, 97.4, 108.3, 111.2, 115.3, 119.8, 120.2, 123.7, 126.7, 130.3 × 2, 130.9, 138.4, 144.2, 147.5, 145.7, 147.6, 149.4, 150.4, 153.2, 156.8, 172.4; ^{31}P NMR (121.5 MHz, DMSO–d_6) δ: 72.2 (P=S); MS *m/z* (%): 548 [M^+] (1.3), 83 (100). Anal. Calcd. for $C_{27}H_{21}N_2O_7PS$: C, 59.12; H, 3.86; N, 5.11; P, 5.65; S, 5.85. Found: C, 59.10; H, 3.84; N, 5.10; P, 5.63; S, 5.82.

***O,O′*-[(mono-4-tolyl)]-[(20S)-camptothecin]-phosphorothi-onate (2c).** Yellow oil, yield: 65.8%, IR (KBr, cm^{-1})ν: 3508 (OH), 1717 (C=O), 1602 (C=N), 1513, 1452 (Ar), 1225, 1064, 1036 (P−O−C), 1002 (P=S). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 0.74 (3H, t, J = 7.5 Hz, H–18), 1.44 (2H,m, H–19), 2.27 (3H, t, J = 7.2 Hz, CH_3), 5.25 (2H, s, H–5), 5.43 (2H, s, H–17), 6.54 (1H, s, 20-OH), 7.17–7.89 (7H, m, Ar−H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 7.9, 11.5, 19.6, 30.7, 51.3, 56.9, 65.7, 73.0, 97.6, 108.3, 114.2, 119.8, 120.3, 122.7, 125.9, 130.2, 130.9, 132.7, 138.3 142.2, 145.3, 145.5, 149.8, 150.6, 153.3; ^{31}P NMR (121.5 MHz, DMSO–d_6) δ: 72.3 (P=S); MS *m/z* (%): 548 [M^+] (1.3), 83 (100). Anal. Calcd. for $C_{27}H_{21}N_2O_7PS$: C, 59.12; H, 3.86; N, 5.11; P, 5.65; S, 5.85. Found: C, 59.10; H, 3.84; N, 5.10; P, 5.63; S, 5.82.

***O,O′*-[(mono-3,4-dimethylphenyl)]-[(20S)-camptothecin] Phosphorothionate (2d).** Yellow oil, yield: 55.3%, IR (KBr, cm^{-1})ν: 3499 (OH), 1719 (C=O), 1602 (C=N), 1514, 1450 (Ar), 1220, 1060, 1035 (P−O−C), 1004 (P=S). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 0.88 (3H, t, J = 7.4 Hz, H–18), 1.86 (2H, m, H–19), 2.37 (3H, s, CH_3), 2.39 (3H, m, CH_3), 5.23 (2H, s, H–5), 5.42 (2H, s, H–17), 6.53 (1H, s, 20−OH), 7.18–7.89 (6H, m, Ar−H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 7.9, 11.5, 19.6, 30.7, 51.3, 56.9, 65.7, 73.0, 97.6, 108.3, 114.2, 119.8, 120.3, 122.7, 125.9, 130.2, 130.9, 132.7, 138.3 142.2, 145.3, 145.5, 149.8, 150.6, 153.3; ^{31}P NMR (121.5 MHz, DMSO–d_6) δ: 72.1 (P=S); MS *m/z* (%): 562 [M^+] (3.2), 104 (100). Anal. Calcd. for $C_{28}H_{23}N_2O_7PS$: C, 59.78; H, 4.12; N, 4.98; P, 5.51; S, 5.70. Found: C, 59.79; H, 4.11; N, 4.97; P, 5.50; S, 5.71.

***O,O′*-[(mono-4-tertiarybutylphenyl)]-[(20S)-camptothecin]-phosphorothionate (2e).** Yellow oil, yield: 76.5%, IR (KBr, cm^{-1})ν: 3500 (OH), 1718 (C=O), 1606 (C=N), 1514, 1453 (Ar), 1225, 1062, 1033 (P−O−C), 1001 (P=S). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 0.88 (3H, t, J = 7.2 Hz, H–18), 1.85 (2H, m, H–19), 2.03 [6H, d, CH $\underline{(CH_3)_2}$], 2.21 (H, m, $CH_3\underline{CH}CH_3$), 5.13 (2H, s, H–5), 5.24 (2H, s, H–17), 2.46 (1H, s, 20−OH), 6.98–7.78 (8H, m, Ar−H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 7.2, 30.3, 31. 5 × 3, 34.4, 51.3, 65.7, 72.8, 97.7, 108.4, 109.0, 119.6, 120.3, 121.3, 122.4, 130.3 × 2, 130.8, 138.2, 139.3, 141.2, 144.0, 144.6, 145.5, 149.4, 150.3, 153.8, 156.7, 172.6; ^{31}P

NMR (121.5 MHz, DMSO–d_6) δ: 71.7 (P=S); MS *m/z* (%): 576 [M^+] (0.8), 77 (100). Anal. Calcd. for $C_{29}H_{25}N_2O_7PS$: C, 60.41; H, 4.37; N, 4.86; P, 5.37; S, 5.56. Found: C, 60.40; H, 4.38; N, 4.87; P, 5.36; S, 5.57.

***O,O′*-[(mono-4-chlorophenyl)]-[(20S)-camptothecin]-phos-phorothio-nate (2f).** Yellow oil, yield: 79.3%, IR (KBr, cm^{-1}) ν: 3500 (OH), 1725 (C=O), 1610 (C=N), 1515, 1455 (Ar), 1225, 1060, 1035 (P−O−C), 1005 (P=S). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 0.92 (3H, t, J = 7.3 Hz, H–18), 1.89 (2H, m, H–19), 4.58 (2H, s, H–5), 4.76,4.73 (2H, s, H–17), 2.63 (1H, s, 20−OH), 6.66–7.82 (8H, m, Ar−H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 8.2, 30.9, 51.4, 65.7, 72.6, 97.5, 108.8, 111.6, 115.5, 119.4, 120.8, 123.9, 126.6, 130.4 × 2, 131.2, 138.9, 144.8, 147.7, 145.9, 147.8, 149.7, 150.7, 153.6, 156.9, 172.8; ^{31}P NMR (121.5 MHz, DMSO–d_6) δ: 76.6 (P=S); MS *m/z* (%): 569 [M^+] (1.2), 76 (100). Anal. Calcd. for $C_{26}H_{18}ClN_2O_7PS$: C, 54.89; H, 3.19; Cl, 6.23; N, 4.92; P, 5.44; S, 5.64. Found: C, 54.87; H, 3.18; Cl, 6.24; N, 4.93; P, 5.43; S, 5.65.

***O*-ethyl-*O*-phenyl-[(20S)-camptothecin]-phosphorothionate (3a).** Yellow powder, yield: 65.0%, IR (KBr, cm^{-1}) ν: 3488 (OH), 1723 (C=O), 1612 (C=N), 1512, 1455 (Ar), 1227, 1065, 1034 (P−O−C), 1007 (P=S). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 0.86 (3H, t, J = 7.3 Hz, H–18), 1.86 (2H, m, H–19), 4.22 (2H, s, H–5), 4.42, 4.44 (2H, s, H–17), 2.05 (1H, s, 20–OH), 4.09 (2H, s, H–22), 1.29 (3H, s, H–23), 6.68–7.89 (8H, m, Ar−H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 6.8, 17.7, 30.2, 51.2, 63.5, 65.7,72.5, 78.5, 109.3, 120.2, 120.7 × 2, 121.4, 130.2 × 2, 130.6 × 2, 144.1, 145.5, 149.3, 150.5, 151.4, 153.6, 156.8, 174.0; ^{31}P NMR (121.5 MHz, DMSO–d_6) δ: 62.7 (P=S); MS *m/z* (%): 550 [M^+] (0.7), 83 (100). Anal. Calcd. for $C_{28}H_{27}N_2O_6PS$: C, 61.08; H, 4.94; N, 5.09; P, 5.63; S, 5.82. Found: C, 61.04; H, 4.92; N, 5.07; P, 5.61; S, 5.80.

***O*-ethyl-*O*-(4-Tolyl)-[(20S)–camptothecin)]-phosphorothi-onate (3b).** Yellow powder, yield: 86.3%, IR (KBr, cm^{-1})ν: 3500 (OH), 1719 (C=O), 1608 (C=N), 1519, 1454 (Ar), 1227, 1060, 1035 (P−O−C), 1009 (P=S). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 0.93 (3H, t, J = 7.0 Hz, H–18), 1.82 (2H, m, H–19), 2.33 (3H, s, Ar−CH_3), 4.28–4.34 (2H, m, $\underline{CH_2}CH_3$), 4.21 (2H, s, H–5), 4.42 (2H, s, H–17), 2.49 (1H, s, 20–OH), 6.92–7.94 (8H, m, Ar−H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 6.6, 17.4, 22.6, 30.5, 51.6, 63.7, 65.4, 72.3, 78.4, 109.4, 120.3, 118.7 × 2, 121.7, 130.4 × 2, 130.4 × 2, 144.3, 145.7, 149.7, 150.7, 151.7, 153.9, 156.7, 174.2; ^{31}P NMR (121.5 MHz, DMSO–d_6) δ: 60.4 (P=S); MS *m/z* (%): 564 [M^+] (1.7), 91 (100). Anal. Calcd. for $C_{29}H_{29}N_2O_6PS$: C, 61.69; H, 5.18; N, 4.96; P, 5.49; S, 5.68. Found: C, 61.64; H, 5.16; N, 4.93; P, 5.45; S, 5.64.

***O*-ethyl-*O*-[(4-methoxyphenyl)]-[(20S)-camptothecin]-phosphorothio-nate (3c).** Yellow powder, yield: 78.9%, IR (KBr, cm^{-1})ν: 3485 (OH), 1719 (C=O), 1602 (C=N), 1511, 1452 (Ar), 1223, 1062, 1030 (P−O−C), 1002 (P=S). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 0.89 (3H, t, J = 7.0 Hz, H–18),

1.86 (2H, m, H–19), 3.83 (3H, s, $ArOCH_3$), 4.27–4.36 (2H, m, $\underline{CH_2}CH_3$), 4.43 (2H, s, H–5), 4.48 (2H, s, H–17), 2.50 (1H, s, 20–OH), 6.78–7.87 (9H, m, Ar–H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 6.83, 17.4, 30.5, 51.6, 55.6, 63.7, 65.4, 72.3, 78.4, 109.4, 115.5 × 2, 117.5, 120.3, 122.5, 124.6, 130.3 × 2, 144.3, 145.7, 149.7, 150.3, 151.2, 153.4, 156.7, 173.4; ^{31}P NMR (121.5 MHz, DMSO–d_6) δ: 60.7 (P=S); MS *m/z* (%): 580 [M^+] (2.3), 45 (100). Anal. Calcd. for $C_{29}H_{29}N_2O_7PS$: C, 59.99; H, 5.03; N, 4.83; P, 5.33; S, 5.52. Found: C, 59.97; H, 5.06; N, 4.87; P, 5.35; S, 5.54.

***O*-ethyl-*O*-[(4-chlorophenyl)]-[(20S)-camptothecin]-phosphorothio-nate (3d).** Yellow powder, yield: 77.5%, IR (KBr, cm^{-1})ν: 3502 (OH), 1720 (C=O), 1600 (C=N), 1510, 1458 (Ar), 1224, 1063, 1032 (P−O−C), 1000 (P=S). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 0.87 (3H, t, J = 6.5 Hz, H–18), 1.82 (2H, m, H–19), 4.78–4.97 (2H, m,

CH_2CH_3), 4.25 (2H, s, H–5), 4.47 (2H, s, H–17), 2.52 (1H, s, 20—OH), 6.66–7.73 (8H, m, Ar—H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 7.7, 17.7, 22.8, 30.7, 51.5, 63.8, 65.9, 72.8, 78.6, 109.7, 120.6, 118.6 × 2, 121.9, 125.7 × 2, 132.4 × 2, 130.7 × 2, 145.2, 145.9, 149.5, 150.8, 152.2, 154.0, 157.8, 174.7; ^{31}P NMR (121.5 MHz, DMSO–d_6) δ: 67.3 (P=S); MS *m/z* (%): 585 [M^+] (3.1), 111 (100). Anal. Calcd. for $C_{28}H_{26}ClN_2O_6PS$: C, 57.49; H, 4.48; Cl, 6.06; N, 4.79; P, 5.29; S, 5.48. Found: C, 57.44; H, 4.42; Cl, 6.03; N, 4.76; P, 5.26; S, 5.43.

***O*-ethyl-*O*-[(4-nitrophenyl)]-[(20S)-camptothecin]-phosphorothionate (3e).** Yellow powder, yield: 82.6%, IR (KBr, cm^{-1})ν: 3502 (OH), 1720 (C=O), 1600 (C=N), 1510, 1458 (Ar), 1224, 1063, 1032 (P—O—C), 1010 (P=S). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 0.96 (3H, t, J = 7.2 Hz, H–18), 1.89 (2H, m, H–19), 4.74–4.95 (2H, m, CH_2CH_3), 4.46 (2H, s, H–5), 4.47 (2H, s, H–17), 2.65 (1H, s, 20—OH), 6.69–7.96 (8H, m, Ar—H); ^{13}C NMR (75 MHz, DMSO–d_6) δ: 7.6, 17.4, 22.6, 30.3, 51.4, 63.5, 65.3, 72.4, 78.3, 109.2, 120.4, 121.3 × 2, 125.7 × 2, 130.8, 132.4 × 2, 140.3, 141.5, 145.7, 149.8, 150.7, 152.2, 154.0, 156.7, 157.2, 174.7; ^{31}P NMR (121.5 MHz, DMSO–d_6) δ: 65.7 (P=S); MS *m/z* (%): 595 [M^+] (5.6), 122 (100). Anal. Calcd. for $C_{28}H_{26}N_3O_8PS$: C, 56.47; H, 4.40; N, 7.06; P, 5.20; S, 5.38. Found: C, 56.44; H, 4.42; N, 7.04; P, 5.21; S, 5.33.

***O*-ethyl-*O*-[(2,4-dichlorophenyl)]-[(20S)-camptothecin]-phosphoroth-ionate (3f).** Yellow powder, yield: 79.8%, IR (KBr, cm^{-1})ν: 3500 (OH), 1721 (C=O), 1601 (C=N), 1512, 1455 (Ar), 1225, 1062, 1037 (P—O—C), 1009 (P=S). ^{1}H NMR (300 MHz, DMSO–d_6) δ: 1.03 (3H, t, J = 7.0 Hz, H–18), 1.86 (2H, m, H–19), 4.78 (2H, m, CH_2CH_3), 4.22 (2H, s, H–5), 4.75, 4.78 (2H, s, H–17), 2.62 (1H, s, 20–OH), 6.78–7.97 (8H, m, Ar—H); ^{13}C NMR (75 MHz, DMSO–d_6) 8.4, 18.6, 22.4, 30.6, 52.5, 63.7, 65.7, 73.2, 78.9, 109.8, 120.5, 122.3, 14.8, 129.4, 132.3, 132.3 × 2, 136.7, 142.1, 143.5, 145.6, 150.3, 150.9, 151.7, 153.7, 155.4, 156.3, 173.2; ^{31}P NMR (121.5 MHz, DMSO–d_6) δ: 66.8 (P=S); MS *m/z* (%): 619 [M^+] (3.2), 146 (100). Anal. Calcd. for $C_{28}H_{25}Cl_2N_2O_6PS$: C, 54.29; H, 4.07; Cl, 11.45; N, 4.52; P, 5.00; S, 5.18. Found: C, 54.28; H, 4.03; Cl, 11.43; N, 4.50; P, 5.02; S, 5.15.

Pharmacology

Test Microorganisms. Sixteen microbial strains (four bacterial and four fungi) were selected on the basis of their clinical importance in causing diseases in humans. *S. aureus, B. Simplex, E. acetylicum* (Gram-positive bacteria), *E. coli, P. aeruginosa, S. Flexenari, S. typhi* (Gram-negative bacteria), and *A. niger*, *A. flavus* (molds), *S. cerevisiae, C. albicans, T. longifucus, A. flavus, M. canis, F. solani, C. glaberata* (yeasts) were screened for evaluation of antibacterial and antifungal activities of the synthesized chemical compounds.

In vitro Antimicrobial Activity

Antibacterial and Antifungal Assays. The agar diffusion method[34] was followed for antibacterial and antifungal susceptibility tests.

Determination of Minimum Inhibitory Concentration (MIC). The MIC of the chemically synthesized compound was tested against bacterial and yeast strains through a macro dilution tube method.[27]

In vitro Antimicrobial Activity (for Molds)[35]. All the newly synthesized compounds were evaluated for their antimicrobial activity (molds) by poison food technique.[36] All the test molds were grown on Sabouraud dextrose agar (SDA) at 25 °C for 7–8 days.

One-week-old culture of the mold was used as inoculum for evaluating antifungal activity of chemical compounds. The molten SDA (45 °C) was poisoned by the addition of 100 mL volume having concentration of 1.0 mg/mL of each compound reconstituted in the DMSO and poured into the sterile Petri plates. The prepared SDA plates containing the test compound were inoculated with fungal plugs (8 mm diameter) obtained from the actively growing margins of the fungal plates. Plates were incubated at 25 °C for 7 days.[37] The medium with DMSO as solvent was used as a negative control whereas media with Amphotericin-B (standard antifungal drug) were used as positive control.

The experiments were performed in triplicates. Diameter of fungal colony was measured and expressed as percentage inhibition and determined by the formula given below:

Percentage inhibition of mycelial growth $= (dc - dt)/dc \times 100$
dc = average fungal colony diameter in control sets
dt = average fungal colony diameter in treatment sets

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