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Synthesis and characterization of 1,2,4-triazole containing hydrazide-hydrazones derived from (*S*)-Naproxen as anticancer agents

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ABSTRACT: A novel series of new naproxen derivatives (S)-ethyl{[4-(4-fluorophenyl)-5-[(1-(6-methoxynaphtalen-2-yl)ethyl)]-4*H*-1,2,4-triazole-3-yl]sulphanyl}acetate (**5**), (S)-2-({5-[1-(6-methoxynaphtalen-1-yl)ethyl]-4-fluorophenyl-4*H*-1,2,4-triazole-3-yl}sulphanyl)acetohydrazide **(6),** 2-{[5-[1-(6-methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(substituted)methylidene]acetohydrazides (**7a-m**) were synthesized, in this study. The structures of compounds **5, 6** and **7a-m** were defined by spectral (^{1}H-NMR, ^{13}C-NMR, HR-MS and FT-IR) methods and their purity was proven by elemental analysis, thin layer chromatography and high pressure liquid chromatography. These compounds were evaluated for *in vitro* anticancer activity by using MTS method against PC-3 and DU-143 (androgen-independent human prostate cancer cell lines) and LNCaP (androgen-sensitive human prostate adenocarcinoma) prostate cancer cell lines. Cisplatin was used as the positive sensitivity reference standard. Compounds (**7a-m**) exhibited anticancer activity with IC_{50} values of 87.2-400 µM against prostate cancer cell lines.

KEYWORDS: Anticancer activity; MTS method; (S)-Naproxen; hydrazide-hydrazones.

1. INTRODUCTION

Cancer, defined as uncontrolled proliferation and spread of abnormal cells, is one of the most important health problems of our time. It is also a public health problem because of its frequent appearance and high mortality. The rapid rise of cancer cases in the world and in our country and the fact that they are among the first causes of deaths suggests that further studies on the development of new drugs and treatment methods should be conducted in this regard. According to the latest data of association in Turkey fight cancer, is the second highest incidence of cancer among men with prostate cancer was 37.6%.

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most used up medicinal drugs generally used as anti-inflammatory, antipyretic and analgesic drugs and their side-effects were worked involving gastrointestinal and cardiovascular risks [1]. (+)-(*S*)-2-(6-Methoxynaphthalen-2-yl)propanoic acid (Naproxen) is a non-steroidal anti-inflammatory drug which contains carboxylic acid funcionality with antipyretic and analgesic properties. It is thought to study by reducing the action of cyclooxygenase (COX) included in the production of prostaglandins that are manufactured in answer to certain diseases or injury and cause pain, inflammation and swelling [2]. As well as other NSAIDs, naproxen was reported to be efficient in the prevention of many cancers [3-5]. Hydrazide-hydrazones [6-21] and 1,2,4-triazole rings [22-26] have diverse biological activities.

In this study, 1,2,4-triazole containing hydrazide-hydrazones derived from naproxen have been synthesized. The purity of the synthesized compounds have been proven by thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC), elementel analysis and melting point assay. Their structures have been characterized by ^{1}H-NMR, ^{13}C-NMR, HR-MS and FT-IR spectroscopic methods. The anticancer activity of the synthesized compounds were studied against PC-3, DU-143 and LNCaP prostate cancer cell lines.

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2. RESULTS AND DISCUSSION

2.1. Chemistry

(+)-(*S*)-2-(6-Methoxynaphthalen-2-yl)propanoic acid (Naproxen) was used as the starting compound to design several new 1,2,4-triazole ring and hydrazide-hydrazones. Firstly, (*S*)-methyl 2-(6-methoxy-2-naphthyl)propanoate **(1)** was prepared by the reaction of naproxen and methanol in the presence of a few drops of concentrated sulfuric acid. Compound **1** and hydrazine-hydrate %80 was refluxed in ethanol in order to prepare (*S*)-2-(6-methoxy-2-naphthyl)propanoic acid hydrazide **(2)**. Equimolar 4-fluorophenylisothiocyanate and compound **2** was heated in butanolic medium to prepare (*S*)-*N*-(4-fluorophenyl)-2-[2-(6-methoxynaphtalen-2-yl)propanoyl]hydrazinecarbothioamide **(3)**. (*S*)-5-[1-(6-methoxynaphtalen-2-yl)ethyl]-4-fluorophenyl-4*H*-1,2,4-triazole-3-thione **(4)** was synthesized in 4N NaOH with reaction of compound **3** [31]. (*S*)-Ethyl {[4-(4-fluorophenyl)-5-[(1-(6-methoxynaphtalen-2-yl)ethyl)]-4*H*-1,2,4-triazole-3-yl]sulphanyl}acetate **(5)** was prepared in solution of acetone with equimolar ethyl bromoacetate and twice as much mol of potassium carbonate. (*S*)-2-({5-[1-(6-Methoxynaphtalen-1-yl)ethyl]-4-fluorophenyl-4*H*-1,2,4-triazole-3-yl}sulphanyl)acetohydrazide **(6)** was synthesized with compound **5** and hydrazine-hydrate (%80) in solution of ethanol. Our final compounds hydrazide-hydrazones **(7a-m)** was prepared with compound **6** and appropriate aromatic aldehydes in ethanolic medium in a few drops of glacial acetic acid. Compounds **5**, **6** and **7a-m** are original molecules in this study (Figure 1).

The structures of compounds **5, 6** and **7a-m** were confirmed by elemental analysis, HPLC, TLC and spectral techniques such as ^{1}H-NMR, ^{13}C-NMR, HR-MS and FT-IR.

Optical rotation angle analysis has been performed to prove that Naproxen was S-enantiomer as reported in the raw material declaration certificate and in the literature [27]. In this study, optical rotation angle measurement was performed on a Rudolph Autopol V Plus brand / model polarimeter. For this measurement according to European Pharmacopoeia (EP) monograph [28], 0.5 g of Naproxen was dissolved in 25 ml of pure ethanol. The measurement result (+59.246°) was in line with (S)-Naproxen's value which was reported by EP 7.0 as between +59° and +62° [28]. Therefore, we reported our target compounds as (S)-isomers.

The purity of the synthesized compounds have been proven by TLC and HPLC. The purity of the compounds was controlled on TLC plates precoated with silica gel G in a solvent system comprising of petroleum ether: ethyl acetate (40:60, v/v) mixture as an eluent. The spots were located under UV light (254 nm) (t: 21 ^{o}C). HPLC data of synthesized compounds **5**, **6**, **7a-m** were performed on Agilent Technologies 1100 series using a reverse phase ZORBAX C8 column in gradient mode (Acetonitrile:double distilled water system was used as gradient system: 50:50 from 0 to 5 min; 75:25 to 50:50 from 5 to 7 min; 100:0 to 75:25 from 7 to 15 min).

FT-IR spectral data of novel hydrazide-hydrazones **7a-m** were observed hydrazone N-H, hydrazone C=O and hydrazone C=N streching data between 3080-3055, 1697-1674 and 1606-1602 cm^{-1}, respectively. The streching data of C=O were 1734 cm^{-1} for compound **5**, 1685 cm^{-1} for compound **6**, respectively.

In the ^{1}H-NMR spectra of compound **5**, characteristic signals for -CH_3 moiety have been detected 1.16 ppm and for –CH_2 moiety 1.24 ppm as signal protons. In the ^{1}H-NMR spectra of compound **6**, characteristic signals for –NH-NH_2 moiety have been detected 9.31 and 4.32 ppm as signal –NH and –NH_2 protons respectively.

^{1}H-NMR spectral data of compounds **7a-m** showed supporting evidence to identify their structures. The two singlet signals belonging to azomethine proton in eight compounds **7a, 7b, 7e, 7f, 7g, 7i, 7k** and **7l** were detected at 7.93-8.38 ppm and 8.11- 8.57 ppm, compounds **7c** and **7j** were detected singlet azomethine proton peaks at 8.24 and 8.29 ppm respectively, compounds **7d, 7h** and **7m** were detected azomethine proton at aromatic area. The -NH- proton of compounds **7a-m** was detected two peaks in the range of 11.48-11.98 ppm and 11.57-12.13 ppm. Only compound **7c** gave singlet peak at 11.89 ppm.

^{13}C-NMR spectral data of compounds **7a-m** showed the signals belonging to –C=O group and -N=CH- group were detected at 169.01-168.24 ppm and 147.04-142.40 ppm, respectively. Thiomethylene carbon (-S-CH_2-) was screened at 34.85-34.57 ppm. In thioesther compound **5** alifatic carbons (-S-CH_2-CO-O$\underline{C}H_2$-$\underline{C}H_3$) detected at 61.21 and 13.92 ppm respectively. These carbons disappeared in hydrazide compound **6**.

It is thought that all the thirteen molecules hold half or one mole of ethanol in their structure. It was proven with elemental analysis, FT-IR and ^{1}H-NMR spectrum. In FT-IR spectra O-H streching bands were screened at range of 3281-3201. In ^{1}H-NMR spectra, -CH_3 protons and –CH_2- protons were monitorized at

the range of 0.9-1.3 ppm and 1.2-1.9 ppm, respectively. The –OH protons were observed at the range of 4.0-5.6 ppm.

The HR-MS spectra of six novel naproxen derivatives were studied in this study. The ionization format of **7e** and **7j** was electron impact (EI); the ionization format of compounds **5, 6, 7b** and **7c** was electron spray impact (ESI). Compounds **7e** and **7j** gave the peak at their molecular weight. Other compounds gave the peak at [M+1] because of catching a hydrogen atom from medium. HR-MS spectrum of selected compound **7e** displayed molecular ion peak at m/z 379. The major fragmentation pathway appeared by the cleavage of -CH_2-CONHN=CH-Ar bonds of amide moiety (Figure 2).

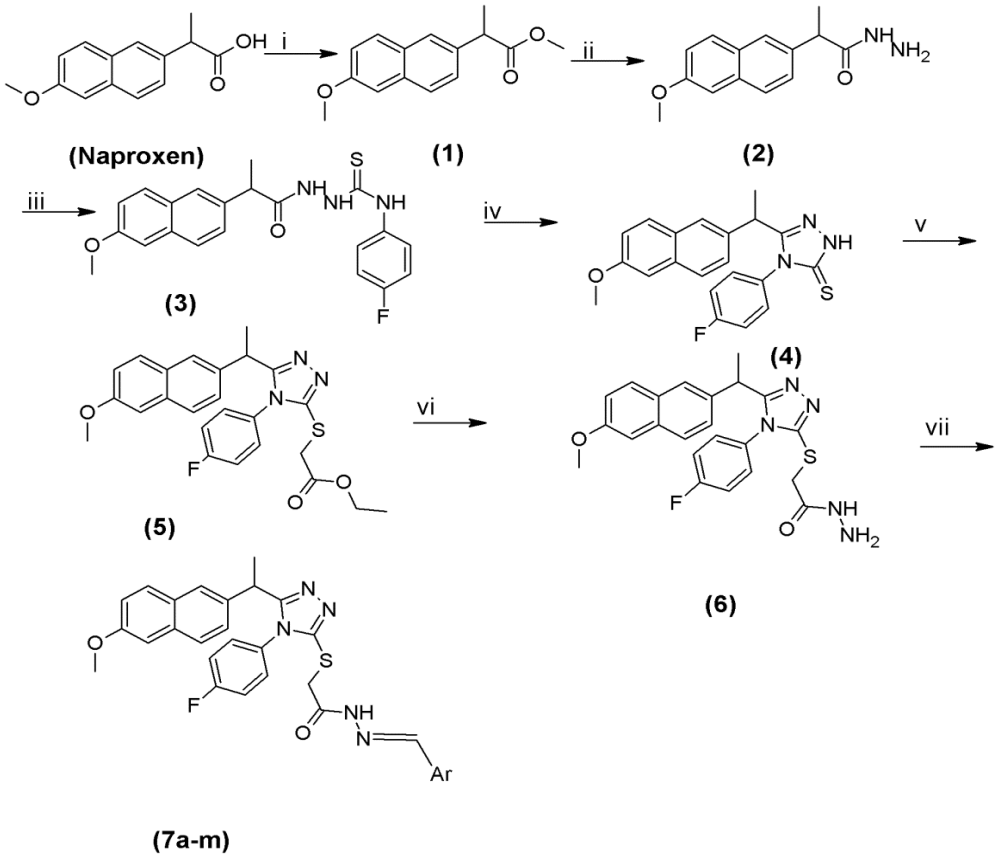

Figure 1. Synthetic route to naproxen derivatives **7a-m**.
***i*:** $CH_3OH/d.H_2SO_4$; ***ii*:** $NH_2NH_2.H_2O/C_2H_5OH$; ***iii*:** 4-F-C_6H_4-NCS/*n*-butanol; ***iv*:** 4N NaOH; ***v*:** Br-CH_2-$COOC_2H_5$/ K_2CO_3/Acetone; **vi:** $NH_2NH_2.H_2O$/EtOH; ***vii*:** EtOH/g. CH_3COOH/Ar-CHO.

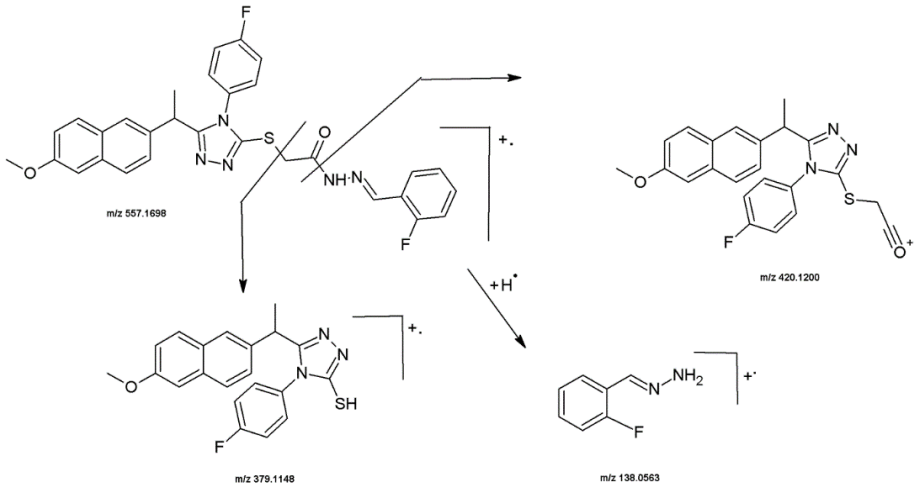

Figure 2. MS fragmentation pathway for the compound **7e**.

2.2. Biological activity

The anticancer activity of naproxen, the synthesis starting compound, has been reported. On the light of foregoing, we aimed to investigate the anticancer activity of (S)-naproxen hydrazide-hydrazones (**7a-m**). The anticancer activity of all compounds was evaluated in Faculty of Pharmacy, Erciyes University. The sensitivity of the proatate cancer cell lines towards the compounds was evaluated from MTS method [29]. The anticancer activity of the compounds were evaluated against three cancer cell lines. The cell lines used were PC-3 and DU-143 (androgen-independent human prostate cancer cell lines) and LNCaP (androgen-sensitive human prostate adenocarcinoma) prostate cancer cell lines. Cisplatin was used as the positive sensitivity reference standard for cell lines. Compounds **7a-m** were found with IC_{50} values of 87.2-400 µM according to MTS assay. The results of anticancer activity of synthesized compounds **7a-m** are given Table 1.

Table 1. IC_{50} values of compounds **7a-m**.

Compd.	Lab Code	A	IC_{50} (µM)		
			PC-3	DU-145	LNCaP
Cisplatin			39.9	9.6	20.7
7a	**SGK-558**	2-chloro-6-fluoro	>400	>400	>400
7b	**SGK-560**	2,4-dichloro	400≤	400≤	400≤
7c	**SGK-561**	2,6-dichloro	>400	>400	>400
7d	**SGK-563**	3,4-dichloro	400≤	244,5	400≤
7e	**SGK-564**	2-fluoro	>400	>400	>400
7f	**SGK-567**	2-chloro	400≤	400≤	400≤
7g	**SGK-568**	4-chloro	115,1	87,2	>400
7h	**SGK-569**	3-bromo	170,0	97,71	263,3
7i	**SGK-570**	4-bromo	>400	235,7	>400
7j	**SGK-571**	4-nitro	>400	>400	>400
7k	**SGK-573**	4-methyl	400≤	400≤	220,6
7l	**SGK-577**	4-methoxy	400≤	400≤	400≤
7m	**SGK-578**	3,5-bis trifluoromethyl	129,8	159,1	400≤

A computational study for prediction of ADME properties of the molecules was applied by determination of lipophilicity, topological polar surface area (TPSA), absorption (% ABS) and simple molecular descriptors used by Lipinski in formulating his "rule of five". Calculations were performed using Molinspiration on-line property calculation toolkit (http://www.molinspiration.com) [30] (Table 2). Percentage of absorption (% ABS) was estimated using the equation: % ABS = 109 – (0.345 × TPSA) [31].

Table 2. Predicted ADME, Lipinski parameters and molecular properties of the synthesized compounds **7a-m**.

Compound	% ABS	TPSA	n-ROTB	n-ON acceptors	n-OHNH donors	mi LogP	Formula Weight	n violations
7a	80	81.41	9	7	1	6.90	592.07	2
7b	80	81.41	9	7	1	7.43	608.53	2
7c	80	81.41	9	7	1	7.41	608.53	2
7d	80	81.41	9	7	1	7.43	608.53	2
7e	80	81.41	9	7	1	6.27	557.63	2
7f	80	81.41	9	7	1	6.78	574.08	2
7g	80	81.41	9	7	1	6.83	574.08	2
7h	80	81.41	9	7	1	6.94	618.53	2
7i	80	81.41	9	7	1	6.96	618.53	2
7j	65	127.24	10	10	1	6.11	584.63	2
7k	80	81.41	9	7	1	6.60	553.66	2
7l	77	90.65	10	8	1	6.21	569.66	2
7m	80	81.41	11	7	1	7.87	675.63	2
Naproxen	92	46.53	3	3	1	3.38	230.26	0

3. CONCLUSION

Naproxen containing 1,2,4-triazole derivatives **5**, **6** and **7a-m** were synthesized and evaluated for their anticancer activity in this study. All of the compounds tested (compounds **7a-m**) were found to be no or weak activity against PC-3, DU-145 and LNCaP cancer cell lines. Cisplatin was used as the positive sensitivity reference standard for cell lines.

4. MATERIALS AND METHODS

(S)-Naproxen was liberally ensured Abdi İbrahim. All aldehydes were purchased from Fluka and Aldrich. All other chemicals were purchased from Merck. Melting points were taken on Schmelzpunktbestimmer 9300 SMP II apparatus and are uncorrected. Synthesis of these compounds were carried out in Memmert WNB14 instrument and Heidolph MR Plug radley. Merck silica gel 60 F254 plates were used for analytical TLC. The purity of the compounds was controlled on TLC plates precoated with silica gel G in a solvent system comprising of petroleum ether: ethyl acetate (40:60, v/v) mixture as an eluent. The spots were located under UV light (254 nm) (t: 21 ^{o}C). HPLC data were performed on Agilent Technologies 1100 series. The separation was performed at ambient temperature using a reverse phase ZORBAX C8 column. All experiments were performed in gradient mode. Acetonitrile:bidistillee water system was used as gradient system: 50:50 from 0 to 5 min; 75:25 to 50:50 from 5 to 7 min; 100:0 to 75:25 from 7 to 15 min; the flow rate was 1.0 mL/min with monitoring at 254 nm (M1). Chromatographic data were collected and processed using Agilent Chemstation Plus software. Elemental analyses were performed on VarioMICRO V1.5.7. instrument. FT-IR spectra were run on Schimadzu FTIR-8400S spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a BRUKER AVANCE-DPX 300 instrument (Elementel analysis, ^{1}H-NMR and ^{13}C-NMR spectrum exist in İnönü University Scientific and Technological Research Center). MALDI-TOF HR-MS spectra using the EI and ESI ionization techniques were performed using a Jeol JMS-700 instrument. Optical rotation angle measurement for Naproxen was performed on a Rudolph Autopol V Plus brand / model polarimeter.

4.1. Chemistry

4.1.1. Preparation of (S)-methyl 2-(6-methoxy-2-naphthyl)propanoate (1, CAS Number: 26159-35-3)

In a few drops of concentrated sulfuric acid, Naproxen (0.05 mol) and methanol (50 mL) were refluxed for 8 h. The contents of the flask were cooled and neutralized by using $NaHCO_3$ (5%). The resulting precipitate was filtered, dried, and recrystallized from ethanol. Yield 75%; M.p. 100-101 °C (lit 88 °C) [32, 33].

4.1.2. Preparation of (S)-2-(6-methoxy-2-naphthyl)propanoic acid hydrazide (2, CAS Number: 57475-91-9)

To a ethanolic solution of compound (**1**) (20 mL, 0.01 mol) was added hydrazine-hydrate (80%, 20 mL) and refluxed for 4 h. The reaction mixture was then cooled, diluted with water and allowed to stand overnight. The precipitated solid was washed with water, dried, and recrystallized from ethanol. Yield 70%; M.p. 137 °C (lit 94) [33].

4.1.3. Preparation of (S)-N-(4-fluorophenyl)-2-[2-(6-methoxynaphtalen-2-yl)propanoyl]hydrazinecarbothio amide (3, CAS Number: 1003001-33-9)

A solution of compound (**2**) (0.01 mol) and equimolar amount of 4-fluorophenyl isothiocyanate in *n*-butanol (20 mL) was heated on radley for 4 h. After the mixture was cooled at room temperature and *n*-butanol was evaporated. The product was dried and recrystallized with ethanol. Yield 75%; M.p. 158-160 °C (lit 162°C) [33].

4.1.4. Preparation of (S)-5-[1-(6-methoxynaphtalen-2-yl)ethyl]-4-fluorophenyl-4H-1,2,4-triazole-3-thione (4, CAS Number: 1003001-45-3)

A solution of compound (**3**) (0.01 mol) in 4N sodium hydroxide solution (30 mL) was heated under radley for 16 h. After cooling to room temperature, the solution was adjusted to pH 6 by glacial acetic acid. The crude product was precipitated, filtered and washed with distilled water. The product was get by recrystallization from ethanol. Yield 60%; 145-146 °C (lit 144 °C) [33].

4.1.5. Synthesis of (S)-ethyl{[4-(4-fluorophenyl)-5-[(1-(6-methoxynaphtalen-2-yl)ethyl)]-4H-1,2,4-triazole-3-yl]sulphanyl}acetate (5)

Compound (**4**) (0.001 mol) was dissolved in 30 mL of acetone (30 mL) and potassium carbonate (0.002 mol) was added to solution. Then, ethyl bromoacetate (0.0011 mol) were added to the solution to divide four times at 15 minutes intervals. The content of the flask was stirred at room temperature for 20 h. The mixture was filtered of inorganic compounds. Then, aceton was evaporated and the precipitated compound was filtered, dried and recrystallized from methanol.

Yield: 84%; Brown solid; M.p. 100-101 °C; HPLC t_R (min.): 8.07 (M1); FT-IR (υ_{max}, cm^{-1}) 2982, 2933, 1734 (C=O), 1635, 1508, 1485, 1448, 1263, 1211, 1122 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H ppm: 1.16 (t, 3H, C$\underline{H}_3$), 1.24 (q, 2H, C$\underline{H}_2$), 1.64 (d, 3H, CH-C$\underline{H}_3$), 3.85 (s, 3H, OC$\underline{H}_3$), 3.99 (s, 2H, SC$\underline{H}_2$) 4.09 (q, 1H, C$\underline{H}$-CH$_3$), 7.01-7.65 (m, 10H, Ar-$\underline{H}$); ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C ppm: 168.04 (C=O), 163.91, 160.63, 158.08, 157.07, 136.83, 133.01, 129.81, 129.69, 128.95, 128.90, 128.18, 126.97, 125.69, 125.24, 118.66, 116.68, 116.36, 105.63, 61.21, 55.09, 36.09, 33.98, 20.67, 13.92; HR-MS (ESI) m/z: Calcd. for [$C_{25}H_{24}FN_3O_3S.H^+$] 466.1595 found 466.1601; Anal. Calcd. for $C_{25}H_{24}FN_3O_3S$. CH_3OH: C, 62.76, H, 5.67, N, 8.44, S, 6.44; found C, 62.28, H, 5.31, N, 8.31, S, 6.00.

4.1.6. Synthesis of (S)-2-({5-[1-(6-methoxynaphtalen-1-yl)ethyl]-4-fluorophenyl-4H-1,2,4-triazole-3-yl} sulphanyl)acetohydrazide (6)

To a mixture of compound (**5**) (0.001 mol) and hydrazine hydrate (0.003 mol, %80), absolute ethanol (50 mL) was added and it was refluxed for 6 h on a water bath. The mixture was concentrated, cooled, and poured into cold water. It was kept for 24h at fridge and the solid mass separated out was filtered, dried, and recrystallized from ethanol.

Yield: 75%; White solid; M.p. 178-179 °C; HPLC t_R (min.): 6.37 (M1); FT-IR (υ_{max}, cm^{-1}) 3041 (N-H), 3009, 2931, 1685 (C=O), 1508, 1489, 1448, 1259, 1220, 1126 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H ppm: 1.64 (d, 3H, CH-C$\underline{H}_3$), 3,80 (s, 2H, SC$\underline{H}_2$), 3.84 (s, 3H, OC$\underline{H}_3$), 4.14 (q, 1H, C$\underline{H}$-CH$_3$), 4.32 (s, 2H, CO-NH-N$\underline{H}_2$) 6.97-7.65 (m, 10H, Ar-$\underline{H}$), 9.31 (s, 1H, CO-N$\underline{H}$-NH$_2$); ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C ppm: 166.00 (C=O), 163.90, 160.35, 157.99, 157.06, 136.85, 133.01, 132.51, 129.89, 129.77, 128.98, 128.19, 126.97, 125.72, 125.24, 118.65, 116.64, 116.28, 105.63, 55.09, 36.10, 34.24, 20.69; HR-MS (ESI) m/z: Calcd. for [$C_{23}H_{22}FN_5O_2S.H^+$] 452.1551 found 452.1558; Anal. Calcd. for $C_{23}H_{22}FN_5O_2S$: C, 61.18, H, 4.91, N, 15.51; found C, 61.69, H, 4.23, N, 15.40.

4.1.7. General Procedure for the synthesis of (S)-2-{[5-[1-(6-methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4H-1,2,4-triazole-3-yl]sulphanyl}-N'-[(substituted phenyl) methylidene]acetohydrazides (7a-m)

Compound (**6**) (0.001 mol) was dissolved in absolute ethanol. A few drop glacial acetic acid were added to solution. Equimolar amount of the appropriate aromatic aldehyde were added and refluxed for 8 h. The flask content was allowed to cool, and the filtered and dried precipitate were recrystallized from ethanol.

(*S*)-2-{[5-[1-(6-Methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(2-chloro-6-fluorophenyl)methylidene]acetohydrazide (7a)

Yield: 86%; Pale brown solid; M.p. 152-154 °C; HPLC t_R (min.): 8.38 (M1); FT-IR (υ_{max}, cm^{-1}) 3064 (N-H), 2976, 2913, 1687 (C=O), 1604 (C=N), 1543, 1508, 1448, 1417, 1267, 1213, 1120 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H ppm: 1.63 (d, 3H, CH-C$\underline{H}_3$), 3.84 (s, 3H, OC$\underline{H}_3$), 4.14 (q, 1H, C$\underline{H}$-CH$_3$) 3.99, 4.33 (2s, 2H, SC$\underline{H}_2$), 7.01-7.65 (m, 13H, Ar-$\underline{H}$) 8.23, 8.39 (2s, 1H, N=C$\underline{H}$), 11.83, 11.96 (2s, 1H, CO-N$\underline{H}$); ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C ppm; 168.72 (C=O), 163.50, 160.60, 157.94, 157.05, 150.18, 146.87 (C=N), 136.89, 136.64, 133.45, 132.99, 131.60, 129.86, 129.74, 129.06, 129.02, 128.18, 126.96, 126.33, 125.70, 125.23, 120.15, 118.63, 116.60, 116.28, 105.62, 55.08, 36.11, 34.57, 20.68; Anal. Calcd. for $C_{30}H_{24}ClF_2N_5O_2S$. ½ C_2H_5OH.; C, 60.86, H, 4.09, N, 11.83, S, 5.42; found C, 60.48, H, 4.38, N, 11.38, S, 5.20.

(*S*)-2-{[5-[1-(6-Methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(2,4-dichlorophenyl)methylidene]acetohydrazide (7b)

Yield: 69%; White solid; M.p. 118-120 °C; HPLC t_R (min.): 9.06 (M1); FT-IR (υ_{max}, cm^{-1}) 3078 (N-H), 2997, 2935, 1680 (C=O), 1604 (C=N), 1550, 1508, 1485, 1440, 1265, 1219, 1118 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H ppm: 1.64 (d, 3H, CH-C$\underline{H}_3$), 3.84 (s, 3H, OC$\underline{H}_3$), 4.13 (q, 1H, C$\underline{H}$-CH$_3$), 3.99, 4.40 (2s, 2H, SC$\underline{H}_2$), 7.01-7.95 (m, 13H, Ar-$\underline{H}$) 8.32, 8.51 (2s, 1H, N=C$\underline{H}$), 11.85, 12.02 (2s, 1H, CO-N$\underline{H}$); ^{13}C-NMR (75 MHz, DMSO-

d$_6$) δ_C ppm; 168.73 (C=O), 163.59, 160.54, 157.99, 157.06, 146.10 (C=N), 138.70, 136.87, 133.82, 133.65, 133.00, 131.55, 130.27, 129.88, 129.76, 129.37, 128.96, 128.18, 127.94, 126.96, 125.69, 125.24, 118.64, 116.59, 116.29, 105.64, 55.09, 36.11, 34.64, 20.68; HR-MS (ESI) m/z: Calcd. for [$C_{30}H_{24}Cl_2FN_5O_2S.H^+$] 608.1084 found 608.1096; Anal. Calcd. for $C_{30}H_{24}ClF_2N_5O_2S$. ½ H_2O. ½ C_2H_5OH; C, 58.13, H, 4.41, N, 10.93, S, 5.01; found C, 57.59, H, 3.88, N, 10.61, S, 4.59.

(*S*)-2-{[5-[1-(6-Methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(2,6-dichlorophenyl)methylidene]acetohydrazide (7c)

Yield: 81%; Cream colour solid; M.p. 125-127 °C; HPLC t_R (min.): 8.78 (M1); FT-IR (υ_{max}, cm^{-1}) 3055 (N-H), 2976, 2933, 1680 (C=O), 1604 (C=N), 1543, 1508, 1448, 1423, 1265, 1224, 1120 (C-F); ^{1}H-NMR (300 MHz, DMSO-*d*$_6$) δ_H ppm: 1.64 (d, 3H, CH-C<u>H</u>$_3$), 3.84 (s, 3H, OC<u>H</u>$_3$), 4.10 (q, 1H, C<u>H</u>-CH$_3$) 4.03, 4.35 (2s, 2H, SC<u>H</u>$_2$), 7.01-7.65 (m, 13H, Ar-<u>H</u>) 8.24 (s, 1H, N=C<u>H</u>), 11.89 (s, 1H, CO-N<u>H</u>); ^{13}C-NMR (75 MHz, DMSO-*d*$_6$) δ_C ppm; 168.82 (C=O), 163.56, 160.57, 157.93, 157.05, 143.98 (C=N), 142.50, 138.76, 136.88, 133.87, 133.00, 131.12, 129.86, 129.74, 129.43, 129.37, 129.01, 128.97, 128.18, 126.96, 125.70, 125.23, 118.64, 116.59, 116.28, 105.63, 55.08, 36.11, 34.66, 20.68; HR-MS (ESI) m/z: Calcd. for [$C_{30}H_{24}Cl_2FN_5O_2S.H^+$] 608.1084 found 608.1099; Anal. Calcd. for $C_{30}H_{24}Cl_2FN_5O_2S$. ½ H_2O. ½ C_2H_5OH; C, 58.13, H, 4.41, N, 10.93, S:5.01; found C, 57.59, H, 3.88, N, 10.61, S, 4.59.

(*S*)-2-{[5-[1-(6-Methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(3,4-dichlorophenyl)methylidene]acetohydrazide (7d)

Yield: 84%; White solid; M.p. 132-134 °C; HPLC t_R (min.): 8.55 (M1); FT-IR (υ_{max}, cm^{-1}) 3066 (N-H), 2985, 2935, 1680 (C=O), 1604 (C=N), 1550, 1508, 1483, 1440, 1265, 1219, 1120 (C-F); ^{1}H-NMR (300 MHz, DMSO-*d*$_6$) δ_H ppm: 1.64 (d, 3H, CH-C<u>H</u>$_3$), 3.84 (s, 3H, OC<u>H</u>$_3$), 4.13 (q, 1H, C<u>H</u>-CH$_3$), 4.00, 4.40 (2s, 2H, SC<u>H</u>$_2$), 7.01-8.15 (m, 13H, Ar-<u>H</u>; s, 1H, N=C<u>H</u>), 11.79, 11.92 (2s, 1H, CO-N<u>H</u>); ^{13}C-NMR (75 MHz, DMSO-*d*$_6$) δ_C ppm; 168.76 (C=O), 163,62, 160.59, 157.97, 157.06, 144.32 (C=N), 141.09, 136.89, 134.75, 133.00, 132.10, 131.69, 131.02, 129.88, 129.76, 128.96, 128.18, 126.96, 126.79, 126.71, 125.69, 125.23, 118.64, 116.61, 116.29, 105.63, 55.08, 36.12, 34.65, 20.68; Anal. Calcd. for $C_{30}H_{24}Cl_2FN_5O_2S$. C_2H_5OH; C, 58.72, H, 4.62, N, 10.70, S, 4.90; found C, 58.35, H, 3.99, N, 10.66, S, 4.82.

(*S*)-2-{[5-[1-(6-Methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(2-fluorophenyl)methylidene]acetohydrazide (7e)

Yield: 69%; White solid; M.p. 124-127 °C; HPLC t_R (min.): 7.88 (M1); FT-IR (υ_{max}, cm^{-1}) 3064 (N-H), 2976, 2933, 1680 (C=O), 1604 (C=N), 1564, 1550, 1508, 1490, 1265, 1230, 1136 (C-F); ^{1}H-NMR (300 MHz, DMSO-*d*$_6$) δ_H ppm: 1.63 (d, 3H, CH-C<u>H</u>$_3$), 3.84 (s, 3H, OC<u>H</u>$_3$), 4.14 (q, 1H, C<u>H</u>-CH$_3$) 3.99, 4.40 (2s, 2H, SC<u>H</u>$_2$), 7.02-7.89 (m, 14H, Ar-<u>H</u>) 8.20, 8.40 (2s, 1H, N=C<u>H</u>), 11.73, 11.87 (2s, 1H, CO-N<u>H</u>); ^{13}C-NMR (75 MHz, DMSO-*d*$_6$) δ_C ppm; 168.62 (C=O), 163.40, 160.62, 158.97, 157.96, 157.06, 146.67 (C=N), 136.90, 136.57, 133.00, 131.95, 129.89, 129.77, 128.97, 128.19, 126.95, 126.19, 125.70, 125.24, , 121.39, 118.64, 116.61, 116.29, 116.15, 115.87, 105.64, 55.09, 36.13, 34.66, 20.70; HR-MS (EI) m/z: Calcd. for [$C_{30}H_{25}F_2N_5O_2S$] 557.1697 found 557.1698; Anal. Calcd. for $C_{30}H_{25}F_2N_5O_2S$. ½ C_2H_5OH; C, 64.07, H, 4.82, N, 12.05, S, 5.34; found C, 63.98, H, 5.07, N, 12.01, S, 5.33.

(*S*)-2-{[5-[1-(6-Methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(2-chlorophenyl)methylidene]acetohydrazide (7f)

Yield: 75%; White solid; M.p. 152-155 °C; HPLC t_R (min.): 8.29 (M1); FT-IR (υ_{max}, cm^{-1}) 3072 (N-H), 2997, 2935, 1680 (C=O), 1604 (C=N), 1543, 1508, 1485, 1435, 1265, 1219, 1116 (C-F); ^{1}H-NMR (300 MHz, DMSO-*d*$_6$) δ_H ppm: 1.63 (d, 3H, CH-C<u>H</u>$_3$), 3.84 (s, 3H, OC<u>H</u>$_3$), 4.14 (q, 1H, C<u>H</u>-CH$_3$), 3.98, 4.41 (2s, 2H, SC<u>H</u>$_2$), 7.07-7.95 (m, 14H, Ar-<u>H</u>) 8.38, 8.57 (2s, 1H, N=C<u>H</u>), 11.81, 11.98 (2s, 1H, CO-N<u>H</u>); ^{13}C-NMR (75 MHz, DMSO-*d*$_6$) δ_C ppm; 168.68 (C=O), 163.49, 160.59, 157.96, 157.06, 142.87 (C=N), 139.71, 136.90, 133.13, 133.00, 131.61, 131.40, 131.15, 129.90, 129.77, 129.10, 128.97, 128.19, 127.65, 126.96, 125.70, 125.24, 118.64, 116.59, 116.29, 105.63, 55.09, 36.12, 34.70, 20.70; Anal. calcd. for $C_{30}H_{25}ClFN_5O_2S$. C_2H_5OH; C, 61.98, H, 5.04, N, 11.29, S, 5.17; found C, 61.08, H, 4.24, N, 10.76, S, 4.81.

(*S*)-2-{[5-[1-(6-Methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(4-chlorophenyl)methylidene]acetohydrazide (7g)

Yield: 58%; White solid; M.p. 141-142 °C; HPLC t_R (min.): 8.03 (M1); FT-IR (υ_{max}, cm^{-1}) 3057 (N-H), 2976, 2902, 1697 (C=O), 1606 (C=N), 1543, 1508, 1490, 1417, 1271, 1213, 1134 (C-F); ^{1}H-NMR (300 MHz,

DMSO-d_6) δ_H ppm: 1.64 (d, 3H, CH-C<u>H</u>$_3$), 3.84 (s, 3H, OC<u>H</u>$_3$), 4.14 (q, 1H, C<u>H</u>-CH$_3$), 3.99, 4.40 (2s, 2H, SC<u>H</u>$_2$), 7.01-7.73 (m, 14H, Ar-<u>H</u>) 7.98, 8.17 (2s, 1H, N=C<u>H</u>), 11.70, 11.81 (2s, 1H, CO-N<u>H</u>); ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C ppm; 168.58 (C=O), 163.40, 160.59, 157.94, 157.06, 142.40 (C=N), 136.90, 134.37, 133.00, 132.86, 131.13, 129.89, 129.77, 128.97, 128.89, 128.73, 128.46, 128.19, 127.62, 126.95, 125.71, 125.24, 118.64, 116.60, 116.28, 105.63, 55.08, 36.12, 34.75, 20.71; Anal. Calcd. for $C_{30}H_{25}ClFN_5O_2S$. C_2H_5OH; C, 61.98, H, 5.04, N, 11.29, S, 5.17; found C, 62.69, H, 4.42, N, 11.36, S, 4.68.

(*S*)-2-{[5-[1-(6-Methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(3-bromophenyl)methylidene]acetohydrazide (7h)

Yield: 79%; White solid; M.p. 170-172 °C; HPLC t_R (min.): 8.55 (M1); FT-IR (υ_{max}, cm^{-1}) 3066 (N-H), 2985, 2933, 1680 (C=O), 1604 (C=N), 1558, 1508, 1483, 1438, 1263,1211, 1120 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H ppm: 1.64 (d, 3H, CH-C<u>H</u>$_3$), 3.84 (s, 3H, OC<u>H</u>$_3$), 4.14 (q, 1H, C<u>H</u>-CH$_3$), 3.98, 4.39 (2s, 2H, SC<u>H</u>$_2$), 7.02-8.15 (m, 14H, Ar-<u>H</u>, s, 1H, N=C<u>H</u>), 11.71, 11.84 (2s, 1H, CO-N<u>H</u>); ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C ppm; 168.70 (C=O), 163.43, 160.60, 157.98, 157.06, 145.27 (C=N), 141.98, 136.89, 136.36, 133.00, 131.69, 130.96, 130.85, 129.89, 129.76, 128.97, 128.18, 126.97, 125.69, 125.23, 122.17, 119.88, 118.65, 116.61, 116.29, 105.63, 55.09, 36.11, 34.69, 20.68; Anal. Calcd. for $C_{30}H_{25}BrFN_5O_2S$. H_2O. C_2H_5OH; C, 56.31, H, 4.87, N, 10.26, S, 4.70; found C, 56.12, H, 3.96, N, 10.05, S, 4.56.

(*S*)-2-{[5-[1-(6-Methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(4-bromophenyl)methylidene]acetohydrazide (7i)

Yield: 86%; Pale brown solid; M.p. 133-135 °C; HPLC t_R (min.): 8.57 (M1); FT-IR (υ_{max}, cm^{-1}) 3057 (N-H), 2974, 2933, 1687 (C=O), 1604 (C=N), 1543, 1508, 1485, 1448, 1267, 1220, 1120 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H ppm: 1.64 (d, 3H, CH-C<u>H</u>$_3$), 3.84 (s, 3H, OC<u>H</u>$_3$), 4.14 (q, 1H, C<u>H</u>-CH$_3$), 3.99, 4.37 (2s, 2H, SC<u>H</u>$_2$), 7.01-7.84 (m, 14H, Ar-<u>H</u>) 7.96, 8.15 (2s, 1H, N=C<u>H</u>), 11.70, 11.81 (2s, 1H, CO-N<u>H</u>); ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C ppm; 168.59 (C=O), 163.43, 160.59, 158.05, 157.69, 157.06, 145.76 (C=N), 142.54, 136.88, 136.85, 133.18, 133.00, 131.79, 129.89, 129.76, 128.96, 128.69, 128.18, 126.97, 125.69, 125.24, 123.17, 118.65, 116.61, 116.29, 105.63, 55.09, 36.12, 34.74, 20.68; Anal. Calcd. for $C_{30}H_{24}BrFN_5O_2S$. C_2H_5OH; C, 57.83, H, 4.70, N, 10.54, S,4.82; found C, 57.38, H, 4.03, N, 10.68, S, 4.18.

(*S*)-2-{[5-[1-(6-Metoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(4-nitrophenyl)methylidene]acetohydrazide (7j)

Yield: 77%; Orange solid; M.p. 167-170 °C; HPLC t_R (min.): 7.78 (M1); FT-IR (υ_{max}, cm^{-1}) 3057 (N-H), 2958, 2931, 1691 (C=O), 1602 (C=N), 1572, 1508, 1483, 1438, 1265, 1209, 1134 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H ppm: 1.64 (d, 3H, CH-C<u>H</u>$_3$), 3.84 (s, 3H, OC<u>H</u>$_3$), 4.14 (q, 1H, C<u>H</u>-CH$_3$), 4.02, 4.43 (2s, 2H, SC<u>H</u>$_2$), 7.02-7.97 (m, 14H, Ar-<u>H</u>) 8.29 (s, 1H, N=C<u>H</u>), 11.92, 12.03 (2s, 1H, CO-N<u>H</u>); ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C ppm; 168.93 (C=O), 163.82, 160.57, 157.99, 157.06, 147.73, 144.50 (C=N), 141.36, 140.37, 140.18, 136.88, 133.00, 129.89, 129.77, 128.97, 128.18, 128.03, 127.76, 126.96, 125.70, 125.24, 124.03, 118.65, 116.61, 116.31, 105.62, 55.08, 36.11, 34.62, 20.70; HR-MS (EI) m/z: Calcd. for [$C_{30}H_{25}FN_6O_4S$] 584.1642 found 584.1630; anal. calcd. for $C_{30}H_{25}FN_6O_4S$. ½ C_2H_5OH; C, 61.28, H, 4.61, N, 13.83, S, 5.27; found C, 60.28, H, 4.21, N, 13.70, S, 5.16.

(*S*)-2-{[5-[1-(6-Methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(4-methylphenyl)methylidene]acetohydrazide (7k)

Yield: 76%; White solid; M.p. 120-122 °C. HPLC t_R (min.): 8.23 (M1); FT- IR (υ_{max}, cm^{-1}) 3078 (N-H), 2985, 2935, 1674 (C=O), 1604 (C=N), 1554, 1508, 1483, 1442, 1265, 1224, 1120 (C-F); ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H ppm: 1.64 (d, 3H, CH-C<u>H</u>$_3$), 3.84 (s, 3H, OC<u>H</u>$_3$), 4.13 (q, 1H, C<u>H</u>-CH$_3$), 3.97, 4.38 (2s, 2H, SC<u>H</u>$_2$), 7.02-7.65 (m, 14H, Ar-<u>H</u>) 7.95, 8.13 (2s, 1H, N=C<u>H</u>), 11.54, 11.64 (2s, 1H, CO-N<u>H</u>); ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C ppm; 168.38 (C=O), 163.18, 160.59, 158.04, 157.94, 157.06, 147.04 (C=N), 143.82, 139.99, 139.78, 136.90, 133.00, 131.27, 129.89, 129.77, 129.40, 129.06, 128.98, 128.19, 126.97, 125.70, 125.23, 118.64, 116.58, 116.29, 105.63, 55.08, 36.12, 35.08, 34.79, 20.69; Anal. Calcd. for $C_{31}H_{28}FN_5O_2S$. ½ C_2H_5OH; C, 66.65, H, 5.42, N, 12.14, S, 5.56; found C, 66.97, H, 5.00, N, 11.68, S, 5.21.

(*S*)-2-{[5-[1-(6-Methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(4-methoxyphenyl)methylidene]acetohydrazide (7l)

Yield: 74%; White solid; M.p. 128-130 °C. HPLC t_R (min.): 7.63 (M1); FT-IR (υ_{max}, cm^{-1}) 3066 (N-H), 2974, 2933, 1674 (C=O), 1602 (C=N), 1573, 1508, 1485, 1440,1249, 1222, 1120 (C-F); ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ_H ppm: 1.64 (d, 3H, CH-C<u>H</u>$_3$), 3.80 (s, 3H, OC<u>H</u>$_3$), 3.84 (s, 3H, OC<u>H</u>$_3$), 4.14 (q, 1H, C<u>H</u>-CH$_3$), 3.96,

4.37 (2s, 2H, SC<u>H</u>$_2$), 6.98-7.64 (m, 14H, Ar-<u>H</u>) 7.93, 8.11 (2s, 1H, N=C<u>H</u>), 11.48, 11.57 (2s, 1H, CO-N<u>H</u>); ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ_C ppm; 168.24 (C=O), 163.01, 160.70, 158.03, 157.93, 157.05, 150.11, 146.88 (C=N), 143.59, 136.90, 132.99, 129.89, 129.77, 129.11, 129.07, 128.97, 128.18, 126.96, 125.70, 125.23, 118.64, 116.58, 116.28, 114.48, 114.28, 105.63, 55.26, 55.08, 36.13, 34.82, 20.69; Anal. Calcd. for $C_{31}H_{28}FN_5O_3S$. ½ C_2H_5OH; C, 64.85, H, 5.27, N, 11.82, S, 5.41; found C, 64.71, H, 4.84, N, 11.25, S, 5.15.

(*S*)-2-{[5-[1-(6-Methoxynaphtalen-2-yl)ethyl]-4-(4-fluorophenyl)-4*H*-1,2,4-triazole-3-yl]sulphanyl}-*N*'-[(3,5-bistrifluoromethylphenyl)methylidene]acetohydrazide (7m)

Yield: 42%; White solid; M.p. 138-139 °C; HPLC t_R (min.): 9.18 (M1); FT-IR (υ_{max}, cm^{-1}) 3080 (N-H), 2989, 2932, 1681 (C=O), 1604 (C=N), 1543, 1508, 1485, 1442, 1276, 1211, 1132 (C-F); ^{1}H-NMR (300 MHz, DMSO-*d*$_6$) δ_H ppm: 1.63 (d, 3H, CH-C<u>H</u>$_3$), 3.84 (s, 3H, OC<u>H</u>$_3$), 4.14 (q, 1H, C<u>H</u>-CH$_3$), 4.04, 4.44 (2s, 2H, SC<u>H</u>$_2$), 7.01-8.35 (m, 13H, Ar-<u>H</u>; s, 1H, N=C<u>H</u>), 11.98, 12.13 (2s, 1H, CO-N<u>H</u>); ^{13}C-NMR (75 MHz, DMSO-*d*$_6$) δ_C ppm; 169.01 (C=O), 163.96, 160.57, 158.08, 157.06, 145.97 (C=N), 140.54, 136.88, 136.78, 133.00, 131.38, 130.97, 130.59, 129.87, 129.74, 129.39, 128.96, 128.18, 127.21, 127.06, 126.97, 126.21, 125.68, 125.23, 118.64, 116.64, 116.25, 105.62, 55.08, 36.10, 34.85, 20.66; Anal. Calcd. for $C_{32}H_{24}F_7N_5O_2S$. ½ C_2H_5OH; C, 56.73, H, 3.90, N, 10.02, S, 4.59; found C, 56.44, H, 3.61, N, 10.36, S, 4.86.

4.2. Anticancer Activity

The anticancer activity of all compounds were evaluated in Faculty of Pharmacy, Erciyes University. The sensitivity of the cell lines towards the compounds was evaluated from MTS method. The anticancer activity of the compounds were evaluated against three cancer cell lines. The cell lines used were PC-3, DU-145 and LNCaP. Anticancer activities of the compounds tested against that cell lines based on MTS assay **[29]**. Cell proliferative activity was measured using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay kit (Promega, Madison, WI, USA). Cisplatin was used as the positive sensitivity reference standard for cell lines.

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