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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF NEW *N*-ACYLHYDRAZONE DERIVATIVES CONTAINING BENZOTHIAZOLE AND INDOLE BASED MOIETY

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Through a structure-based molecular hybridization strategy, a series of new *N*-acylhydrazone derivatives containing the benzothiazole and indole based moiety were designed, synthesized and screened for *in vitro* antiproliferative activity against Hep G2 cancer cell line. One compound (**7a**) exhibited excellent antiproliferative activity with IC_{50} values of 0.78 μM against Hep G2. In addition, C-5 substitutions of the indole ring of target compounds might be crucial for their cytotoxic activities. Additionally, the relative configuration of target compounds was confirmed as the *E* isomer. Further chemical manipulation of derivative 7a can make it possible to obtain new potential antitumor agents.

Keywords: synthesis; *N*-acylhydrazone; benzothiazole; indole based moiety; antiproliferative activity.

1. INTRODUCTION

Cancer is one of the most life-threatening diseases worldwide. Despite tremendous efforts to develop effective treatment strategies, satisfactory results have not yet been obtained, and the overall survival rate and disease-free survival rate of most cancers are still very low [1, 2]. Therefore, discovery of more effective and safer anticancer agents remains urgently needed.

Indole, as a typical *N*-heterocyclic aromatic compound is widespread in nature, It has been considered as an important structural component in many pharmaceutical agents including antidepressant [3], anticonvulsant [4], antifungal [5], antiviral [6] anti-inflammatory [7], and particularly in discovery of new antitumor agents [8, 9]. In recent years, more new indole derivatives have been developed as potent antitumor agents, including cediranib (**1**) [10], obatoclax (**2**) [11], D-24851 (**3**) and sunitinib (**4**) [12] (Fig. 1).

Benzothiazoles are heterocyclic compounds with diverse range of biological activities. Many studies reported that benzothiazoles derivatives exhibited various activities, including antimicrobial [13], anti-inflammatory [14], antifungal [15] anticancer [16], antidiabetic [17], anticonvulsant [18], antiviral [19], antitubercular [20], antimalarial [21], and antihelmintic [22]. Particularly, 2-substituted benzothiazoles are among privileged structures in medicinal chemistry [23, 24]. Recently, some 2-substituted benzothiazole derivatives have been evaluated as potential antitumor agents **5** [25] and **6** [26] (Fig. 2).

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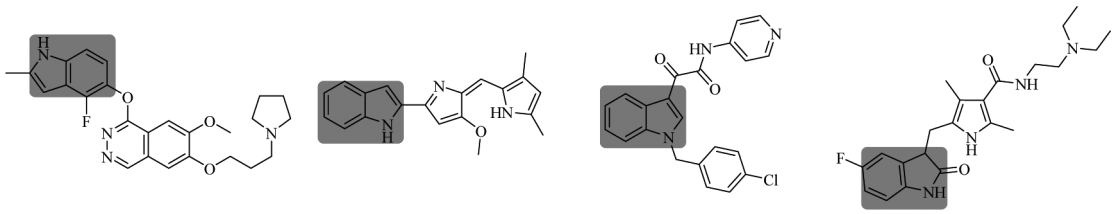


1 (cediranib) **2 (obatoclax)** **3 (D-24851)** **4 (sunitinib)**

Fig. 1. Structures of compounds bearing indole based moiety.

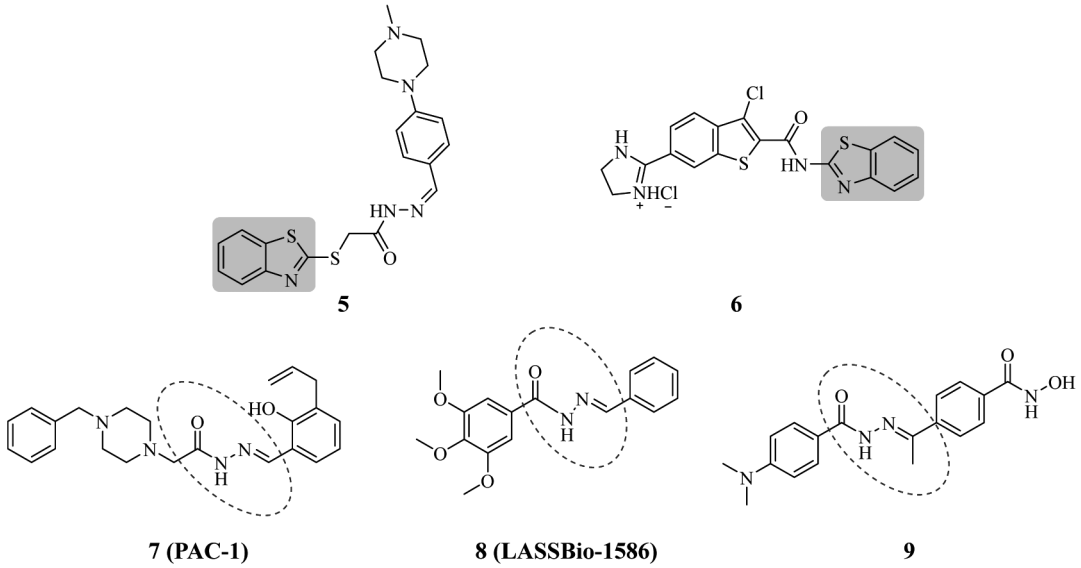


Fig. 2. Structures of benzothiazole and *N*-acylhydrazone derivatives.

According to structure-based molecular hybridization strategy, researchers have attempted to combine benzothiazole and indole through different linkers to obtain new candidates with useful pharmacological activity [27, 28]. The *N*-acylhydrazone (NAH) scaffold is usually employed in the design of heterocyclic compounds, which endow molecules with good thermal, hydrolytic, and chemical stability, as well as the feasibility of changing chemical composition by various substituents [29, 30]. In recent years, some compounds containing NAH scaffold have been reported as antitumor agents, e.g., PAC-1 (**7**) [31], LASSBio-1586 (**8**) [32], and compound **9** [33] (Fig. 2), and it was indicated that introduction of NAH as a linker was an effective approach for developing more potent and broad spectrum antitumor agents.

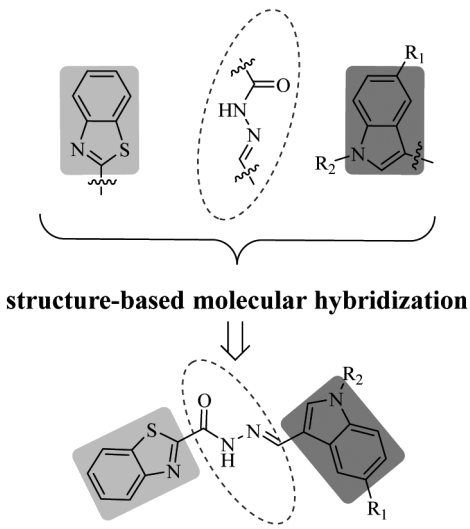


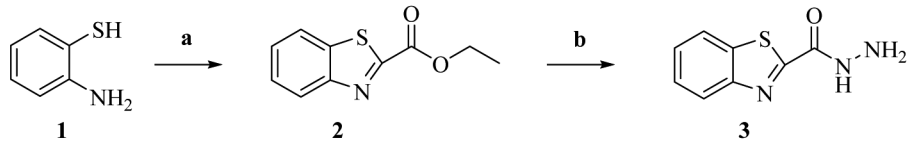
Fig. 3. The design of target compounds.

In this study, based on a structure-based molecular hybridization strategy – a common and effective approach to developing small molecules with multi-targeted molecular mechanism, we have designed and synthesized a series of new NAH derivatives containing the benzothiazole and indole-based moiety (Fig. 3). Herein, twelve new NAH derivatives and their antiproliferative activities *in vitro* against Hep G2 cancer cell line are reported. Additionally, the configuration of target compounds was confirmed as the *E* isomer. The proposed structures of target compounds were confirmed by ^{1}H NMR, ^{13}C NMR, NOESY NMR, and IR spectroscopy data.

2. EXPERIMENTAL CHEMICAL PART

2.1. Chemicals and Instruments

All chemicals and solvents were obtained from commercial sources and used without further purification. Reactions were monitored by thin layer chromatography (TLC) using glass plates precoated with a layer of silica gel (GF254) containing a fluorescent indicator. TLC plates were visualized by exposure to ultraviolet light (254 nm). The melting points of synthesized compounds were determined in an electric melting point apparatus and remained uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were measured on 500 MHz Bruker spectrometer in DMSO-d_6 using TMS as internal standard. Infrared (IR) spectra were recorded on Nicolet 370 FT-IR spectrometer.

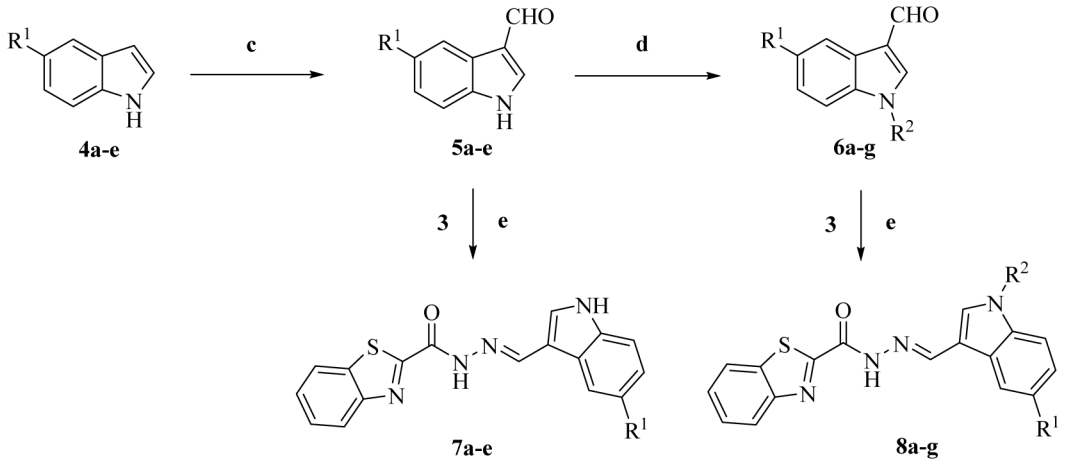


Scheme 1. Synthetic route to compounds **2** and **3**. Reagents and conditions: (a) diethyl oxalate, 140°C, 4 h; (b) 80% hydrazine hydrate, ethanol, 80°C, 9 h.

2.2. Synthetic Routes

An efficient method for the synthesis of linked *N*-acylhydrazone derivatives containing the benzothiazole and indole-based moiety (**7a – 7e**, **8a – 8g**) was developed as shown in Schemes 1 and 2. According to a convergent synthesis approach, we initially synthesized the key intermediates benzothiazole-2-carbohydrazide (**3**) and substituted 1*H*-indole-3-carbaldehydes (**5a – 5e**, **6a – 6g**) to afford the proposed acylhydrazones. As illustrated in Scheme 1, condensation of compound **1** with diethyl oxalate furnished compound **2**, which was reacted with hydrazine to obtain target hydrazides **3**.

The synthetic route to compounds **4a – 4e** is outlined in Scheme 2. Under Vilsmeier-Haack (DMF-$POCl_3$) conditions, compounds **4a – 4e** were transformed into the corresponding 3-carboxaldehyde functionalized indoles **5a – 5e**. The *N*-ethyl, *N*-isopropyl and *N*-phenylsulfonyl derivatives (**6a – 6g**) of indole-3-carboxaldehyde were obtained via reaction of **5a – 5c** with bromoethane, 2-bromopropane or ben-



Compd.	R^1	R^2
4a,5a,7a	H	
4b,5b,7b	5-OCH_3	
4c,5c,7c	5-Br	
4d,4d,7d	5-Cl	
4e,5e,7e	5-F	
6a,8a	H	Et
6b,8b	5-OCH_3	Et
6c,8c	5-Br	Et
6d,8d	H	*i*-Pr
6e,8e	5-OCH_3	*i*-Pr
6f,8f	5-Br	*i*-Pr
6g,8g	H	Phenylsulfonyl

Scheme 2. Synthetic route to compounds **5a –– 5e**, **6a – 6g** and target compounds **7a –– 7e** and **8a – 8g**. Reagents and conditions: (c) (i) $POCl_3$, DMF, 0°C, 20 min; (ii) 35°C, 1 h; (d) (i) 20% NaOH, DMSO, r.t, 15 min; (ii) R_2Br, 30°C, 5 h; (e) glacial acetic acid, ethanol, reflux 6 h.

Fig. 4. NOESY of representative compound **7a**.

zenesulfonyl chloride in the presence of sodium hydroxide (NaOH) in DMSO and water. This was the first time that DMSO and water were used as a mixed solvent for this reaction and such a high yield has not been reported before. Finally, the condensation of compound **3** with compounds **5a – 5e** or compounds **6a – 6g**, produced the target compounds **7a – 7e** and **8a – 8g**. In summary, this synthetic route for a series of *N*-acylhydrazone derivatives containing the benzothiazole and indole-based moiety shows significant advantages, such as mild conditions, high yields, and simple operation process. The results are summarized in Table 1.

TABLE 1. Main ^{1}H and ^{13}C NMR Signals for Target Compounds **7a – 7e** and **8a – 8g**

Compd.	^{13}C NMR (ppm) Acylhydrazone carbonyl	^{1}H NMR (ppm) Imine protons		M.p. (°C)	Yield (%)
		(*E*)	(*Z*)		
7a	164.51	7.86	-	278 – 280	76
7b	164.58	7.80	-	270 – 271	77
7c	164.35	7.91	-	311 – 313	82
7d	164.36	7.95	-	303 – 305	72
7e	164.40	7.94	-	294 – 296	76
8a	164.51	7.94	-	201 – 202	77
8b	164.57	7.87	-	172 – 174	83
8c	164.35	8.01	-	197 – 199	76
8d	164.54	7.94	-	251 – 252	84
8e	164.61	7.89	-	220 – 221	82
8f	164.37	8.10	-	185 – 187	84
8g	163.80	7.99	-	210 – 211	78

Due to the presence of an imino bond, all target compounds **7a – 7-e** and **8a – 8g** could exist in either *E* or *Z* isomeric form, hence, the next step in this work was to determine the relative configuration of the imino double bond in target compounds. By careful analysis of the ^{1}H-NMR spectra of compounds **7a – 7e** and **8a – 8g**, we only detected the presence of one imino hydrogen signal (Table 1). According to the literature [34], this was attributed to the presence of only (*E*)-diastereomers in *N*-acylhydrazone derivatives. Compound **7a** was further selected to identify the stereochemistry by undergoing NOESY NMR. Results (Fig. 4) showed that an evident NOE signal was observed between the H^1 (-CH=N-, $\delta = 7.86$ ppm) and H^2 (=N-NH-, $\delta =$ 11.69 ppm) in the *E* isomer (*E*-**7a**), owing to the large intramolecular H-H distance (*Z*-**7a**), NOE signal should not be observed in the putative *Z* isomer. Thus, target compounds **7a – 7e** and **8a – 8g** were clearly confirmed as *E* isomers.

2.3. Synthesis of Benzothiazole-2-carboxylate (2)

A mixture of 2-aminothiophenol (0.05 mol) and ethyl oxalate (0.1 mol) was stirred at 140 °C for 4 h. After completion of reaction as indicated by TLC, the reaction mixture was cooled to room temperature and poured into a solution containing 50 mL of hydrochloric acid, 150 mL of water and 70 mL of ethanol with stirring, the oil dissolved and a solid formed. Then the mixture was separated by filtering under vacuum and washed with water, dried to afford compound **2**: white solid; yield 95%; m.p. 68 – 69°C (lit.[35] 68 – 70°C); IR (KBr, cm^{-1}): 1748(C=O), 1500, 1097(C-O), 1012, 722; ^{1}H NMR (DMSO-d_6; δ, ppm): 8.29 – 8.19 (m, 2H, Ar$\underline{H}$), 7.72 – 7.61 (m, 2H, Ar$\underline{H}$), 4.46 (q, $J = 7.0$ Hz, 2H, -C$\underline{H}_2$-C=O), 1.38 (t, $J = 7.1$ Hz, 3H, -C$\underline{H}_3$).

2.4. Synthesis of Benzothiazole-2-carbohydrazide (3)

Compound **2** (0.01 mol) was dissolved in ethanol (60 mL). hydrazine hydrate (80%) (0.02 mol) was added to the solution dropwise with constant stirring and the reaction mixture was refluxed for 9 h. The reaction mixture was cooled to room temperature, filtered, washed with water, dried overnight and finally recrystallized from absolute ethanol, filtered and dried to afford compound **3:** light yellow solid; yield 87%; m.p. 173 – 174°C (lit.[36] 172 – 174°C); IR (KBr, cm^{-1}): 3310, 3205 (N-H), 1680 (C=O), 1501, 1201 (C-N), 1022 (N-N), 750; ^{1}H NMR (DMSO-d_6; δ, ppm): 10.46 (s, 1H, N-H), 8.22 (d, J = 7.9 Hz, 1H, ArH), 8.11 (d, J = 8.1 Hz, 1H, ArH), 7.62 (t, J = 7.6 Hz, 1H, ArH), 7.57 (t, J = 7.5 Hz, 1H, ArH), 4.72 (s, 2H, NH_2).

*2.5. Synthesis of Substituted 1H-Indole-3-carbaldehydes (**5a – 5e**)*

General procedure. $POCl_3$ (5mL) was dropped into DMF (15mL) in an ice bath and stirred for 30 min. After that, Indole (**4a – 4e**, 0.01mol) dissolved in 5ml DMF was added into the mixture and heated to 40°C for 1 h. The resulting reaction mixture was poured into 50 mL ice water and then adjusted to pH 8 using 20% NaOH aq. The solution was filtered and the residue was washed with water, dried and finally recrystallized from absolute ethanol, filtered and dried to afford pure compounds **5a – 5e** in 95 – 98% yields.

1*H*-Indole-3-carbaldehyde (5a): orange solid; m.p. 194 – 196°C (lit.[37] 196 – 198°C); ^{1}H NMR (DMSO-d_6; δ, ppm): 12.15 (s, 1H, N-H), 9.92 (s, 1H, CHO), 8.27 (s, 1H, ArH), 8.07 (d, J = 7.7 Hz, 1H, ArH), 7.50 (d, J = 8.0 Hz, 1H, ArH), 7.25 (t, J = 7.5 Hz, 1H, ArH), 7.20 (t, J = 7.3 Hz, 1H, ArH).

5-Methoxy-1*H*-indole-3-carbaldehyde (5b): white solid; m.p. 180 – 182°C (lit.[38] 181 – 182°C).

5-Bromo-1*H*-indole-3-carbaldehyde (5c): light pink solid; m.p. 206 – 207°C (lit.[37] 202 – 204°C).

5-Chloro-1*H*-indole-3-carbaldehyde (5d): light yellow solid; m.p. 217 – 218°C (lit.[37] 215 – 216°C).

5-Fluoro-1*H*-indole-3-carbaldehyde (5e): white solid; m.p. 164 – 165°C (lit.[39] 162 – 163°C).

*2.6. Synthesis of Substituted 1-Ethyl-1H-indole-3-carbaldehydes (**6a – 6c**), Substituted 1-Isopropyl-1H-indole-3-carbaldehydes (**6d – 6f**), and 1-(Phenylsulfonyl)-1H-indole-3-carbaldehyde (**6g**)*

General procedure. A mixture of compound **5a**, **5b** or **5c** (0.02 mol) , DMSO (30 mL) and 20% NaOH solution (10 mL) was stirred at r.t. for 15 min. After that, bromoethane, 2-bromopropane or benzenesulfonyl chloride was dropwise added into the mixture and heated to 35°C for 2 – 5 h. After completion of reaction as indicated by TLC, the reaction mixture was cooled to room temperature and poured into water (150 mL), stirring until a solid formed. Filtering under vacuum and washed with water. Recrystallization afforded compound **6a – 6g** with yields of 85–94 %.

1-Ethyl-1*H*-indole-3-carbaldehyde (6a): yellow solid; m.p. 97–99°C (lit.[40] 98–100°C); IR (KBr, cm^{-1}): 3102 (-CH_3), 2978 (-CH_2-), 1695 (C=O), 1447, 781, 748; ^{1}H NMR (DMSO-d_6; δ, ppm): 9.90 (s, 1H, CHO), 8.35 (s, 1H, ArH), 8.11 (d, J = 7.8 Hz, 1H, ArH), 7.64 (d, J = 8.2 Hz, 1H, ArH), 7.32 (t, J = 7.5 Hz, 1H, ArH), 7.26 (t, J = 7.4 Hz, 1H, ArH), 4.31 (q, J = 7.3 Hz, 2H, -CH_2-), 1.43 (dd, J = 9.3, 5.2 Hz, 3H, CH_3).

1-Ethyl-5-methoxy-1*H*-indole-3-carboxaldehyde (6b): light pink solid; m.p. 100 – 101°C (lit.[40] 97°C).

5-Bromo-1-ethyl-1*H*-indole-3-carbaldehyde (6c): white solid; m.p. 106 – 107°C (lit.[40] 98°C).

1-Isopropyl-1*H*-indole-3-carbaldehyde (6d): yellow solid; m.p. 81 – 83°C; IR (KBr, cm^{-1}): 3120 (-CH_3),2984 (CH aliphatic), 1655 (CHO), 1340, 790, 751; ^{1}H NMR (DMSO-d_6; δ, ppm): 9.92 (s, 1H, CHO), 8.47 (s, 1H, ArH), 8.11 (d, J = 7.8 Hz, 1H, ArH), 7.68 (d, J = 8.2 Hz, 1H, ArH), 7.33 – 7.29 (m, 1H, ArH), 7.26 (t, J = 7.4 Hz, 1H, ArH), 4.90 – 4.81 (m, 1H, CH aliphatic), 1.52 (d, J = 6.6 Hz, 6H, CH_3).

1-Isopropyl-5-methoxy-1*H*-indole-3-carbaldehyde (6e): yellow solid; m.p. 116 – 117°C.

5-Bromo-1-isopropyl-1*H*-indole-3-carbaldehyde (6f): light green solid; m.p. 117 – 119°C.

1-(Phenylsulfonyl)-1*H*-indole-3-carbaldehyde (6g): white solid; m.p. 157 – 158°C; ^{1}H NMR (DMSO-d_6; δ, ppm): 10.09 (s, 1H, CHO), 8.91 (s, 1H, phenylsulfonyl), 8.13 (t, J = 6.9 Hz, 3H, phenylsulfonyl), 7.98 (d, J = 8.3 Hz, 1H, phenylsulfonyl), 7.78 (t, J = 7.5 Hz, 1H, ArH), 7.67 (t, J = 7.9 Hz, 2H, ArH), 7.47 (dd, J = 11.5, 4.2 Hz, 1H, ArH), 7.41 (t, J = 7.5 Hz, 1H, ArH).

*2.7. Preparation of target compounds (**7a – 7e** and **8a – 8g**)*

General procedure: A mixture of compound **3** (0.002 mol) and compounds **5a – 5e** (**6a – 6g)** (0.0022 mol) and two drop of glacial acetic acid in ethanol (20 mL) was heated at reflux for 6 h and then cooled to room temperature, the precipitate was filtered and washed with diethyl ether. Finally recrystallized from absolute ethanol and dried in air to furnish pure compounds **7a – 7e** and **8a – 8g**.

***(E)-N′-[(1H*-Indol-3-yl)methylene]benzothiazole-2-carbohydrazide (7a):** yellow solid; yield 76%; m.p. 278 – 280°C; IR (KBr, cm^{-1}): 3292 (N-H aromatic), 1621 (C=O), 1607, 1544 (C=N), 1235 (C-N), 1127 (N-N), 745; ^{1}H NMR (DMSO-d_6; δ, ppm): 12.40 (s, 1H, N-H), 11.69 (s, 1H, =N-NH-), 8.82 (s, 1H, ArH), 8.30 (d, J = 7.9 Hz, 1H, ArH), 8.28 (d, J = 7.9 Hz, 1H, ArH), 8.20 (d, J = 8.1 Hz, 1H, ArH), 7.86 (d, J = 2.7 Hz, 1H, -CH=N-), 7.67 (t, J = 7.6 Hz, 1H, ArH), 7.62 (t, J = 7.5 Hz, 1H, ArH), 7.46 (d, J = 7.9 Hz, 1H, ArH), 7.23 (t, J = 7.3 Hz, 1H, ArH), 7.18 (t, J = 7.3 Hz, 1H, ArH). 13C NMR (DMSO-d_6; δ, ppm): 164.51 (C=O), 155.40, 152.80, 147.43 (C=N-NH-), 137.05, 135.98, 131.05, 127.14, 126.85, 124.33, 123.94, 122.98, 122.73, 121.96, 120.58, 111.87, 111.56.

***(E)-N′*-[(5-Methoxy-1*H*-indol-3-yl)methylene]benzothiazole-2-carbohydrazide (7b)**: yellow solid; yield 77%;

m.p. 270 – 271°C; IR (KBr, cm^{-1}): 3301 (N-H aromatic), 3261 (-CH_3), 1671 (C=O), 1608, 1544 (C=N), 1215 (C-N), 1022(N-N), 764; ^{1}H NMR (DMSO-d_6; δ, ppm): 12.37 (s, 1H, N-H), 11.55 (s, 1H, =N-NH-), 8.80 (s, 1H, ArH), 8.28 (d, J = 8.0 Hz, 1H, ArH), 8.20 (d, J = 8.1 Hz, 1H, ArH), 7.87 (s, 1H, ArH), 7.80 (d, J = 2.6 Hz, 1H, -CH=N-), 7.67 (t, J = 7.5 Hz, 1H, ArH), 7.61 (t, J = 7.6 Hz, 1H, ArH), 7.35 (d, J = 8.8 Hz, 1H, ArH), 6.87 (d, J = 8.8 Hz, 1H, ArH), 3.81 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6; δ, ppm): 164.58 (C=O), 155.35, 154.56, 152.82, 147.44 (C=N-NH-), 135.99, 132.01, 131.33, 127.12, 126.83, 124.99, 123.92, 122.98, 112.50, 112.36, 111.34, 104.20, 55.28 (O-C).

***(E)-N′*-[(5-Bromo-1*H*-indol-3-yl)methylene]benzothiazole-2-carbohydrazide (7c)**: yellow solid; yield 82%; m.p. 311 – 313°C; IR (KBr, cm^{-1}): 3204 (N-H aromatic), 1671 (C=O), 1618, 1534 (C=N), 1290 (C-N), 1131 (N-N), 757, 621 (C-Br); ^{1}H NMR (DMSO-d_6; δ, ppm): 12.46 (s, 1H, N-H), 11.85 (s, 1H, =N-NH-), 8.77 (s, 1H, ArH), 8.45 (s, 1H, ArH), 8.26 (d, J = 7.9 Hz, 1H, ArH), 8.18 (d, J = 8.1 Hz, 1H, ArH), 7.91 (d, J = 2.4 Hz, 1H, -CH=N-), 7.65 (t, J = 7.3 Hz, 1H, ArH), 7.60 (t, J = 7.6 Hz, 1H, ArH), 7.42 (d, J = 8.6 Hz, 1H, ArH), 7.33 (dd, J = 8.6, 1.8 Hz, 1H, ArH). ^{13}C NMR (DMSO-d_6; δ, ppm): 164.35 (C=O), 155.52, 152.80, 146.92 (C=N-NH-), 135.99, 135.80, 132.42, 127.16, 126.90, 125.96, 125.27, 124.15, 123.96, 123.01, 113.97, 113.29, 111.17.

***(E)-N′*-[(5-Chloro-1*H*-indol-3-yl)methylene]benzothiazole-2-carbohydrazide (7d)**: yellow solid; yield 72%; m.p. 303 – 305°C; IR (KBr, cm^{-1}): 3201 (N-H aromatic), 1671(C=O), 1604, 1544 (C=N), 1291 (C-N), 1131 (N-N), 757, 728(C-Cl); ^{1}H NMR (DMSO-d_6; δ, ppm): 12.48 (s, 1H, N-H), 11.87 (s, 1H, =N-NH-), 8.80 (s, 1H, ArH), 8.32 (s, 1H, ArH), 8.28 (d, J = 7.9 Hz, 1H, ArH), 8.20 (d, J = 8.0 Hz, 1H, ArH), 7.95 (d, J = 2.5 Hz, 1H, -CH=N-), 7.67 (t, J = 7.3 Hz, 1H, ArH), 7.62 (t, J = 7.5 Hz, 1H, ArH), 7.48 (d, J = 8.6 Hz, 1H, ArH), 7.24 (d, J = 8.6 Hz, 1H, ArH). ^{13}C NMR (DMSO-d_6; δ, ppm): 164.36 (C=O), 155.53, 152.80, 146.91 (C=N-NH-), 135.99, 135.54, 132.57, 127.16, 126.89, 125.33, 125.25, 123.95, 123.00, 122.72, 121.12, 113.50, 111.28.

***(E)-N′*-[(5-Fluoro-1*H*-indol-3-yl)methylene]benzothiazole-2-carbohydrazide (7e):** yellow solid; yield 76%; m.p. 294 – 296°C; IR (KBr, cm^{-1}): 3189 (N-H aromatic), 1680 (C=O), 1610, 1544 (C=N), 1317 (C-N), 1288 (C-F), 1044 (N-N), 764; ^{1}H NMR (DMSO-d_6; δ, ppm): 12.44 (s, 1H, N-H), 11.77 (s, 1H, =N-NH-), 8.80 (s, 1H, ArH), 8.28 (d, J = 7.9 Hz, 1H, ArH), 8.20 (d, J = 8.1 Hz, 1H, ArH), 8.01 (d, J = 9.7 Hz, 1H, ArH), 7.94 (s, 1H, -CH=N-), 7.67 (t, J = 7.5 Hz, 1H, ArH), 7.62 (t, J = 7.5 Hz, 1H, ArH), 7.47 (dd, J = 8.6, 4.5 Hz, 1H, ArH), 7.08 (t, J = 8.2 Hz, 1H, ArH). ^{13}C NMR (DMSO-d_6; δ, ppm): 164.40 (C=O), 155.52, 152.80, 146.98 (C=N-NH-), 135.99, 133.66, 132.69, 127.12, 126.85, 123.93, 122.96, 113.05, 111.70, 110.95, 110.75, 106.85, 106.66.

***(E)-N′*-[(1-Ethyl-1*H*-indol-3-yl)methylene]benzothiazole-2-carbohydrazide (8a):** yellow solid; yield 77%; m.p. 201 – 202°C; IR (KBr, cm^{-1}): 2998 (-CH_3), 1648 (C=O), 1608, 1544 (C=N), 1245 (C-N), 1188 (N-N), 762; ^{1}H NMR (DMSO-d_6; δ, ppm): 12.38 (s, 1H, =N-NH-), 8.81 (s, 1H, ArH), 8.31 (d, J = 7.8 Hz, 1H, ArH), 8.27 (d, J = 7.9 Hz, 1H, ArH), 8.20 (d, J = 8.1 Hz, 1H, ArH), 7.94 (s, 1H, -CH=N-), 7.67 (t, J = 7.6 Hz, 1H, ArH), 7.61 (t, J = 7.5 Hz, 1H, ArH), 7.57 (d, J = 8.2 Hz, 1H, ArH), 7.28 (t, J = 7.5 Hz, 1H, ArH), 7.22 (t, J = 7.4 Hz, 1H, ArH), 4.27 (q, J = 7.2 Hz, 2H, -CH_2-), 1.42 (t, J = 7.2 Hz, 3H, -CH_3). ^{13}C NMR (DMSO-d_6; δ, ppm): 164.51 (C=O), 155.38, 152.81, 146.95 (C=N-NH-), 136.60, 135.99, 133.15, 127.14, 126.86, 124.89, 123.93, 122.99, 122.76, 122.21, 120.82, 110.75, 110.28, 40.68, 15.19.

***(E)-N′*-[(1-Ethyl-5-methoxy-1*H*-indol-3-yl)methylene]-benzothiazole-2-carbohydrazide (8b):** yellow solid; yield 83%; m.p. 172 – 174°C; IR (KBr, cm^{-1}): 2997 (-CH_3), 1648 (C=O), 1608, 1544 (C=N), 1255 (C-N), 1185 (N-N), 758; ^{1}H NMR (DMSO-d_6; δ, ppm): 12.36 (s, 1H, =N-NH-), 8.78 (s, 1H, ArH), 8.27 (d, J = 8.0 Hz, 1H, ArH), 8.20 (d, J = 8.1 Hz, 1H, ArH), 7.87 (s, 2H -CH=N-), 7.67 (t, J = 7.6 Hz, 1H, ArH), 7.61 (t, J = 7.6 Hz, 1H, ArH), 7.48 (d, J = 8.8 Hz, 1H, ArH), 6.92 (d, J = 8.9 Hz, 1H, ArH), 4.22 (q, J = 7.3 Hz, 2H, -CH_2-), 3.82 (s, 3H, -CH_3), 1.40 (t, J = 7.2 Hz, 3H). ^{13}C NMR (DMSO-d_6; δ, ppm): 164.57 (C=O), 155.30, 154.79, 152.81, 146.94 (C=N-NH-), 135.99, 133.30, 131.71, 127.14, 126.84, 125.54, 123.92, 123.00, 112.22, 111.02, 110.34, 104.48, 55.32 (O-C), 40.83, 15.24.

***(E)-N′*-[(5-Bromo-1-ethyl-1*H*-indol-3-yl)methylene]-benzothiazole-2-carbohydrazide (8c):** yellow solid; yield 76%; m.p. 197 – 199°C; IR (KBr, cm^{-1}): 3102 (-CH_3), 1671 (C=O), 1608, 1544 (C=N), 1201 (C-N), 1022 (N-N), 757, 621 (C-Br); ^{1}H NMR (DMSO-d_6; δ, ppm): 12.46 (s, 1H, =N-NH-), 8.78 (s, 1H, ArH), 8.48 (s, 1H, ArH), 8.28 (d, J = 8.0 Hz, 1H, ArH), 8.20 (d, J = 8.1 Hz, 1H, ArH), 8.01 (s, 1H, -CH=N-), 7.67 (t, J = 7.5 Hz, 1H, ArH), 7.62 (t, J = 7.3 Hz, 1H, ArH), 7.59 (d, J = 8.7 Hz, 1H, ArH), 7.41 (d, J = 8.5 Hz, 1H, ArH), 4.26 (q, J = 7.1 Hz, 2H, CH_2), 1.40 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6; δ, ppm): 164.35 (C=O), 155.51, 152.79, 146.45 (C=N-NH-), 136.00, 135.41, 134.42, 127.17, 126.90, 126.39, 125.25, 124.36, 123.95, 123.01, 113.61, 112.50, 110.35, 40.93, 15.14.

***(E)-N′*-[(1-Isopropyl-1*H*-indol-3-yl)methylene]benzothiazole-2-carbohydrazide (8d):** yellow solid; yield 84%; m.p. 251 – 252°C; IR (KBr, cm^{-1}): 3190 (-CH_3), 1657 (C=O), 1648, 1544 (C=N), 1188 (C-N,), 1021 (N-N), 754; ^{1}H NMR (DMSO-d_6; δ, ppm): 12.38 (s, 1H, =N-NH-), 8.81 (s, 1H, ArH), 8.31 (d, J = 7.8 Hz, 1H, ArH), 8.27 (d, J = 7.9 Hz, 1H, ArH), 8.20 (d, J = 8.1 Hz, 1H, ArH), 7.94 (s, 1H, -CH=N-), 7.67 (t, J = 7.6 Hz, 1H, ArH), 7.61 (t, J = 7.5 Hz, 1H, ArH), 7.57 (d, J = 8.2 Hz, 1H, ArH), 7.28 (t, J = 7.5 Hz, 1H, ArH), 7.22 (t, J = 7.4 Hz, 1H, ArH), 4.27 (q, J = 7.2 Hz, 1H, CH), 1.42 (t, J = 7.2 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6; δ, ppm): 164.54 (C=O), 155.41, 152.81, 147.01 (C=N-NH-), 136.45, 135.98, 130.18, 127.14, 126.86, 124.84,

123.94, 122.99, 122.70, 122.24, 120.86, 111.07, 110.40, 47.04, 22.28.

***(E)-N′*-[(1-Isopropyl-5-methoxy-1*H*-indol-3-yl)methylene]benzothiazole-2-carbohydrazide (8e):** yellow solid; yield 82%; m.p. 220 – 221°C; IR (KBr, cm^{-1}): 3267 (-CH_3), 1680 (C=O), 1550, 1544 (C=N), 1203 (C-N), 1023 (N-N), 754; ^{1}H NMR (DMSO-d_6; δ, ppm): 12.40 (s, 1H, =N-N*H*-), 8.79 (s, 1H, Ar*H*), 8.27 (d, *J* = 7.9 Hz, 1H, Ar*H*), 8.20 (d, *J* = 8.1 Hz, 1H, Ar*H*), 7.95 (s, 1H, Ar*H*), 7.89 (d, *J* = 2.0 Hz, 1H, -C*H*=N-), 7.67 (t, *J* = 7.5 Hz, 1H, Ar*H*), 7.61 (t, *J* = 7.5 Hz, 1H, Ar*H*), 7.51 (d, *J* = 8.9 Hz, 1H, Ar*H*), 6.93 – 6.90 (m, 1H, Ar*H*), 4.76 (dd, *J* = 13.2, 6.5 Hz, 1H, C*H*), 3.82 (s, 3H, CH_3), 1.49 (d, *J* = 6.6 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6; δ, ppm): 164.61 (C=O), 155.34, 154.77, 152.82, 147.00 (C=N-NH-), 135.99, 131.54, 130.35, 127.13, 126.84, 125.48, 123.92, 122.99, 112.15, 111.11, 110.64, 104.50, 55.32 (O-C), 47.20, 22.31.

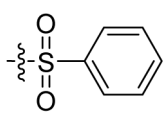
***(E)-N′*-[(5-Bromo-1-isopropyl-1*H*-indol-3-yl)methylene]benzothiazole-2-carbohydrazide (8f):** yellow solid; yield 84%; m.p. 185 – 187°C; IR (KBr, cm^{-1}): 3067 (-CH_3), 1648 (C=O), 1610, 1544 (C=N), 1197 (C-N), 1025 (N-N), 760, 625 (C-Br); ^{1}H NMR (DMSO-d_6; δ, ppm): 12.50 (s, 1H, =N-N*H*-), 8.79 (s, 1H, Ar*H*), 8.49 (s, 1H, Ar*H*), 8.28 (d, *J* = 7.9 Hz, 1H, Ar*H*), 8.20 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.10 (s, 1H, -C*H*=N-), 7.67 (t, *J* = 7.5 Hz, 1H, Ar*H*), 7.62 (t, *J* = 7.1 Hz, 2H, Ar*H*), 7.42 – 7.38 (m, 1H, Ar*H*), 4.81 (dt, *J* = 13.1, 6.6 Hz, 1H, C*H*), 1.50 (d, *J* = 6.6 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6; δ, ppm): 164.37 (C=O), 155.54, 152.80, 146.51 (C=N-NH-), 136.00, 135.23, 131.50, 127.15, 126.89, 126.33, 125.19, 124.40, 123.95, 123.00, 113.59, 112.53, 110.69, 47.42, 22.22.

***(E)-N′*-[(1-(Phenylsulfonyl)-1*H*-indol-3-yl)methylene]-benzothiazole-2-carbohydrazide (8g):** yellow solid; yield 78%; m.p. 210 – 211°C; IR (KBr, cm^{-1}): 3292, 3055, 1621 (C=O), 1607, 1544 (C=N), 1290 (S=O), 1195 (C-N), 1022 (N-N), 745; ^{1}H NMR (DMSO-d_6; δ, ppm): 12.82 (s, 1H, =N-N*H*-), 8.84 (s, 1H, Ar*H*), 8.42 (d, *J* = 5.7 Hz, 2H, Ar*H*), 8.28 (d, *J* = 7.9 Hz, 1H, Ar*H*), 8.22 (d, *J* = 8.1 Hz, 1H, Ar*H*), 8.09 (d, *J* = 7.7 Hz, 2H, Ar*H*), 7.99 (d, *J* = 8.2 Hz, 1H, -C*H*=N-), 7.74 (t, *J* = 7.5 Hz, 1H, Ar*H*), 7.68 (t, *J* = 7.2 Hz, 1H, Ar*H*), 7.63 (td, *J* = 7.6, 4.3 Hz, 3H, Ar*H*), 7.46 (t, *J* = 7.4 Hz, 1H, Ar*H*), 7.41 (t, *J* = 7.5 Hz, 1H, Ar*H*). ^{13}C NMR (DMSO-d_6; δ, ppm): 163.80 (C=O), 156.10, 152.73, 144.79 (C=N-NH-), 136.64, 136.04, 134.96, 134.72, 130.53, 129.98, 127.24, 127.07, 126.89, 126.84, 125.95, 124.39, 124.06, 123.29, 123.04, 118.02, 113.08.

3. EXPERIMENTAL BIOLOGICAL PART

By using MTT assay, all target compounds were evaluated for their *in vitro* antiproliferative activity against Hep G2 (human liver cancer) cells, and sunitinib (a multi-targeted tyrosine kinase inhibitor possessing anti-angiogenic and antitumor activity against a variety of advanced solid tumors) was used as positive controls. The results presented as IC_{50} values are summarized in Table 2, where the values show average of at least two independent experiments. As can be seen from Table 2, the results indicated that part of the synthesized target compounds showed moderate to excellent cytotoxic activity against Hep G2 cancer cell line. In general, when the C-5 position of indole was substituted by electron-donating group, this was not beneficial to the antiproliferative activity. Notably, compound **7a** exhibited the best antiproliferative activity with IC_{50} values of 0.78 μM for Hep G2 cells, and thus was more potent than the positive control compound sunitinib. Furthermore, electronic and steric effects of the *N*-1 position of indole were studied by introduction of various R^2 groups. The results revealed that increasing size of substituent at the N-1 position of indole clearly decreased the antiproliferative activity (**7a** vs. **8a**, **8d**), which suggested that steric hindrance of the group in this region of R^2 groups exhibited a negative effect on the cytotoxic activity. On the whole, it seemed that the C-5 sub-

TABLE 2. Antiproliferative Activity of Target compounds **7a – 7e** and **8a – 8g** against Hep G2 Cancer Cell Line

Compd.	R_1	R_2	IC_{50} (μmol/L)
			Hep G2
7a	H		0.78 ± 0.26*
7b	5-OCH_3		>50
7c	5-Br		>50
7d	5-Cl		>50
7e	5-F		35.15 ± 10.28
8a	H		16.79 ± 4.08*
8b	5-OCH_3		>50
8c	5-Br		>50
8d	H		20.93 ± 3.47
8e	5-OCH_3		>50
8f	5-Br		>50
8g	H		>50
sunitinib[a]			3.22 ± 0.52

* $p < 0.05$ compared to the value for sunitinib; [a] positive control.

stitution of the indole ring of compounds might be crucial for their cytotoxic activities.

In view of above observation, these cytotoxic activity results provided a valuable leading compound **7a** with excellent antiproliferative activity, and highlighted the potential for further development of new *N*-acylhydrazone derivatives as potent antitumor agents.

4. RESULTS AND DISCUSSION

In this study, a series of *N*-acylhydrazone derivatives containing the benzothiazole and indole-based moiety were designed, synthesized and evaluated for the IC_{50} values against Hep G2 cancer cell line. The relative configuration of target compounds was confirmed as the *E* isomer. Additionally, we optimized the synthesis of *N*-substituted indoles to reach a high yield that has not been reported before.

Part of the synthesized target compounds showed moderate to excellent cytotoxic activity. In general, it seemed that the C-5 substitutions of the indole-ring of these compounds might be crucial for their cytotoxic activity. Notably, compound **7a** showed better activity against Hep G2 cancer cell line than the positive control drug sunitinib. Thus, compound **7a** emerged as a valuable lead for further structural modifications. Further studies on structure optimization are underway in our laboratory. Overall, the structurally new *N*-acylhydrazone derivatives provide us promising lead compounds for future anticancer drug discovery.

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