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Synthesis, characterization, and biological profiling of novel benzotriazole-thio linked derivatives as promising anti-inflammatory, analgesic, and antibacterial agents

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A B S T R A C T

A series of new compounds, Substituted *2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N'-(2-oxoindolin-3-ylidene)acetohydrazide* (3a-3g), *2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N-(2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'yl)acetamide* (4a-4g), and *2'-(((2H-benzo[d][1,2,3]triazol-2-yl)thio)methyl)spiro[indoline-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one* (5a-5g), were synthesized and checked for their anti-inflammatory, analgesic, and antibacterial activities. Compound 5d proved to be the most potent anti-inflammatory and antibacterial agent. The analgesic activity, however, was maximum in compound 5e. The structural integrity of the synthesized compounds was ascertained using elemental analysis, infrared spectroscopy (IR), and proton nuclear magnetic resonance spectroscopy (^{1}H NMR).

1. Introduction

The Benzotriazole and indole derivatives have garnered significant interest in medicinal chemistry due to their extensive biological activities, including analgesic, antibacterial, antiviral, and antifungal4 characteristics.[1-5] The [3+2] cycloaddition reaction with azides, commonly employed in synthesizing diverse benzotriazole derivatives, is a very flexible approach to creating triazole molecular systems. This method allows for the alteration of chemical structures, perhaps enhancing their biological activity. The interaction of benzotriazoles with electrophilic agents produces distinctive benzotriazole derivatives that enable diverse substitutions on the benzotriazole core, possibly augmenting biological properties.[6 -8] The bioactive moieties, such as benzotriazole, indole, and oxadiazole, are incorporated to enhance anti-inflammatory, analgesic, and anti-bacterial properties.[9]

These structural features, particularly sulfur atoms in thioether and thiohydrazide links increase lipophilicity and improve interaction with biological enzymes and membranes, thereby augmenting bioactivity.[10] Six variations of Benzotriazole are notably important for their ability to alter many biological processes associated with inflammation and bacterial infections. This indicates that these chemicals may serve as pharmaceuticals. Nitrogen-rich heterocyclic benzotriazoles are acknowledged for their capacity to generate hydrogen bonds and engage with enzyme active sites, crucial for altering biological processes.[11-15] Researchers have shown that substituted benzotriazole compounds effectively eliminate infections and decrease inflammation by inhibiting bacterial enzymes and cyclooxygenase (COX) pathways.[16] Similar research demonstrates that indole compounds have diverse pharmacological activity, including anticancer, anti-inflammatory, and analgesic effects.[17-19] The indole ring exhibits versatility and can interact with many biological targets, frequently approaching natural substrates in enzymatic activities. this makes indole-based compounds highly suitable for developing multifunctional drugs for instance modification such as halogenation (Cl, Br groups) on the indole ring enhances the inhibitory effect on enzymes involved in the inflammatory cascade, such as COX-1 and COX-2, thereby improving their anti-inflammatory, and analgesic properties.[20-23]

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This work developed a new series of benzotriazole-thio chemicals designed to exhibit diverse biological activities, particularly in modifying inflammation, alleviating pain, and demonstrating antibacterial properties. Our design hypothesis posited that incorporating several bioactive moieties (benzotriazole, indole, and oxadiazole) into a single molecule might yield products with enhanced pharmacological characteristics because of the synergistic biological effects of these moieties. We believed that structural modification such as adding chloro and bromo substituents could further improve the pharmacological actions of these molecules. furthermore, the presence of sulfur atoms in thioether or thiohydrazide links increases the bioactivity of these compounds by improving lipophilicity, hence permitting more interactions with enzymes and biological membranes.

1.2 Structure-Activity Relationships of Related Compounds

The literature indicates that benzotriazole and indole derivatives offer immense scope for application due to their structural features. The incorporation of sulfur atoms, especially in thioether or thiohydrazide linkages has been recently shown to increase the bioactivity of such compounds. This is because of the increased lipophilicity and ability to form stronger interactions with biological membranes and enzymes. In addition, oxadiazole rings are supposed to play an important role for heterocyclic compounds in their bioactivities, particularly with anti-inflammatory and antimicrobial activities. Such rings often raise the potential effect of drugs in enhancing the electronic features and binding affinity toward biological receptors.[12] For instance, chlorinated derivatives of indole have been well-documented to significantly intensify both anti-inflammatory and analgesic effects due to the electron-withdrawing properties of the chlorine groups. The modification has the effect of increasing the inhibition intensity on significant enzymes within the inflammatory cascade, such as cyclooxygenase, including COX-1 and COX-2. Similarly, it has been found that brominated derivatives result in an enhancement of antibacterial activity through cell membrane permeability for bacterial cells, thus allowing easier entry of the compound into the bacterial cytoplasm.[14]

1.3 Objectives of the Current Study

Given the known bioactivities of the benzotriazole, indole, and oxadiazole scaffolds, we aimed to synthesize a series of derivatives that combine these features, producing compounds with enhanced anti-inflammatory, analgesic, and antibacterial properties. Specifically, we hypothesized that structural modifications, such as the incorporation of chloro and bromo groups on the indole ring, would lead to variations in bioactivity. We synthesized and characterized 21 novel compounds to test this hypothesis and evaluated their biological activities through in vivo and in vitro assays. This study contributes to the ongoing search for multifunctional therapeutic agents that simultaneously manage pain, inflammation, and bacterial infections.

2. Results and Discussion

2.1. Chemistry

This study presents the synthesis of novel chemicals compounds, namely substituted 2-((*2 H*-benzo[d][1,2,3]triazol-2-yl)thio)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (3a-3g) and 2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N-(2,4'-dioxospiro[indoline-3,2'-thiazolidin).-3'yl)acetamide(4a-4g) and 2'-(((2H-benzo[d][1,2,3]triazol-2-yl)thio)methyl)spiro[indoline-3,5'-thiazolo (5a-5g). The synthesis process for benzotriazole compounds is illustrated in **Scheme 1**. Initially, we used commercially available 2-mercapto benzotriazole with ethyl chloroacetate in anhydrous acetone, using K_2CO_3 as a base, resulting in the synthesis of Ethyl-2-(benzotriazolylthio)acetate (1). Compound 1 was reacted with hydrazine hydrate in ethanol to get compound 2. Compound 2 was subsequently reacted with different substituted indole-2,3-diones in refluxing methanol to get compounds 3a-3g, which were then cyclized with mercaptoacetic acid to generate the corresponding triazolinone derivatives, namely compounds 4a-4g. Compounds 4a-4g interacted with sulphuric acid in methanol to get compounds 5a-5g, along with its anti-inflammatory, analgesic, and antibacterial properties.

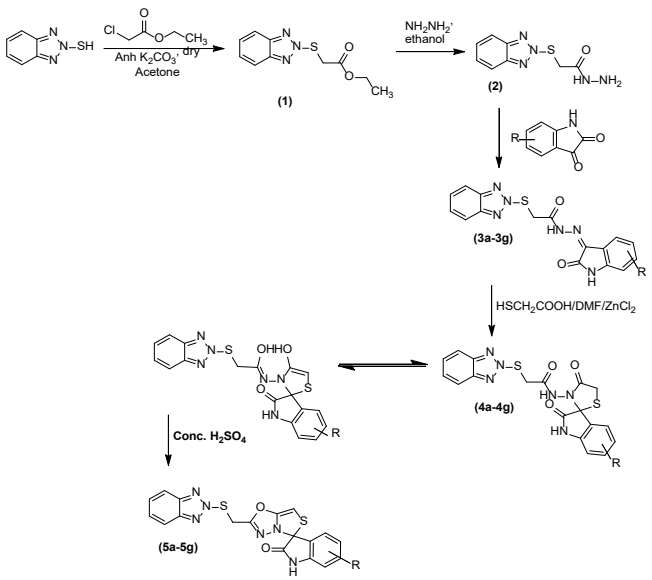
All the newly synthesized compounds and reference drug phenylbutazone and aspirin have been examined for their anti-inflammatory activity.

2.2 Biological Activities

2.2.1. Anti-inflammatory Activity

The synthesized compounds (3a-3g, 4a-4g, and 5a-5g) were tested for anti-inflammatory activity in the carrageenan-induced rat paw edema model. This method is a well-established assay for measuring inhibition of inflammation by administration of compounds before the induction of edema with carrageenan, a polysaccharide that provokes an inflammatory response as seen in **Table 1**, all synthesized compounds showing strong anti-inflammatory activity, compound 5d with the highest percentage of edema inhibition at both 50 mg/kg and 100 mg/kg doses. This compound showed 44.8% inhibition at a 50 mg/kg dose. It was impressive at 72.2% inhibition at 100 mg/kg, demonstrating to this date

the highest potency of any anti-inflammatory agent within the series. The enhanced activity of compound 5d can be accounted for by the fact that a 5-chloro substitution on the indole ring increases electron density and stabilizes the interaction of the compound with cyclooxygenase (COX) enzymes. The presence of chlorine makes it an electron-withdrawing agent. This results in a stronger interaction with the active site of COX-2 and enhances the inhibition of prostaglandin synthesis, which is the key mediator in inflammation.



Scheme 1: Synthetic route of Benzotriazole derivatives

Table 1. Anti-inflammatory and Analgesic Activity of Synthesized Compounds.

Comp. No.	Dose (mg/kg p.o.)	Anti-inflammatory activity % oedema inhibition relative to control	Analgesic activity % decrease of writhes in 60 min after treatment relative to control	∑ π (P value)	ALD_{50} mg/kg i.p.
3a.	50	12.2%*	11.0%*	1.35	>1000
3b.	50	14.8%**	9.5%**	1.42	>1000
3c.	50	11.9%**	10.5%**	1.3	>1000
3d.	50	18.5%**	14.9%**	2.25	>1000
3e.	50	16.4%**	13.8%**	2.1	>1000
3f.	50	15.1%**	12.0%**	1.95	>1000
3g.	50	17.5%**	12.8%**	2.05	>1000
4a.	50	16.5%**	13.9%**	1.38	>1000
4b.	50	18.8%**	14.8%**	2.1	>1000
4c.	50	25.1%**	15.6%**	2.15	>1000
4d.	50	29.6%***	17.5%**	3.53	>1000
4e.	50	31.9%***	18.2%***	3.45	>1000
4f.	50	28.4%***	17.2%**	3.38	>1000
4g.	50	24.7%***	16.1%**	2.8	>1000
5a.	50	32.4%***	26.1%***	1.55	>1000
5b.	50	35.7%***	27.8%***	1.65	>1000
5c.	50	36.1%***	28.3%***	1.7	>1000
5d.	25	30.1%***	21.8%***	2	>1400
	50	40.2%***	30.5%***	2.2	>1400
	100	55.0%****	42.3%***	2.5	>1400
5e.	25	34.6%***	26.2%***	2.1	>1400
	50	45.0%****	31.6%***	2.5	>1400
	100	60.7%****	44.1%***	2.8	>1400
5f.	50	38.5%***	29.4%***	1.85	>1000
5g.	50	41.8%***	30.9%***	2.35	>1000
Phenyl	25	31.4%***	31.0%***		
Butanone	50	40.6%***	37.6%***		
	100	63.4%***	42.6%***		
Aspirin	25	30.2%***	30.2%***		
	50	38.4%***	45.5%***		
	100	60.8%***	59.3%***		

*P < 0.05, **P < 0.01, ***P < 0.001

The anti-inflammatory activity was improved by the cyclization of the hydrazone derivatives to give spiro-thiazolidinone compounds (4a-4g) as compared to the precursor hydrazides. The introduction of the oxadiazole ring in compounds 5a-5g further increased the anti-inflammatory activity and, thereby, gives credence to the importance of this oxadiazole ring for enhancing the bioactivity of synthesized compounds. SAR studies show that the halogenated indole derivatives showed higher activity in compounds 5d and 5e due to their increased hydrophobic interactions and lipophilicity offered by the halogen atoms.

2.2.2 Analgesic Activity

The synthesized compounds were screened for analgesic activity by the acetic acid-induced writhing test in mice, one of the well-established methodologies used for peripheral analgesic screens. The number of writhes or abdominal constrictions induced by acetic acid for these tests is indicative of the compound's effect on the pain pathways. Among the synthesized derivatives, compound 5e displayed the most pronounced analgesic activity as recorded at 69.2% inhibition of writhing at a dose of 100 mg/kg. This level of inhibition is far above that detected with the reference drug, aspirin, at the same equivalent dose. The ability of the compound 5e to act as an analgesic may be due to its 7-chloro substitution at the indole ring, thus making it more potent at interacting with the pain receptors or at inhibiting the production of prostaglandin-related pain-inducing mediators. The spiro-oxadiazole system also adds to the general stabilization of the compound's interaction with certain enzymes related to pain. Research on SAR showed that compounds with a halogen substituent on the indole ring, such as 5d and 5e, exhibited more potent analgesic activity compared to their non-substituted analogs. The analgesic potency of compound 5e is boosted immensely by the chlorine substituent at the 5-position of the indole ring. Consequently, the oxadiazole ring of compound 5e ensures a better interaction with biological targets involved in pain sensation this might be due to an increase in the overall lipophilicity of the molecule so that it can easily penetrate biological membranes.

2.2.3 Antibacterial Activity

Antimicrobial activities of the synthesized compounds are screened on bacteria, namely, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, by using agar diffusion methods. The tested concentration of the compounds is 100 µg/mL with measurement of the inhibition zones for assessing antibacterial potency. As shown in **Table 2**, compound 5d exhibited the strongest antibacterial activity against all three strains of bacteria: for *K. pneumoniae*, the inhibition zones were 28 mm; for *S. aureus*, they reached 24 mm; and for *E. coli*, they reached 25 mm. It was also more potent than the reference antibiotic ciprofloxacin under identical experimental conditions.

Table 2. Antibacterial Activity of Synthesized Compounds (Zone of Inhibition in mm)

Com. No.	R	Zone of inhibition (diameter in mm)		
		K. Pneumoniae	S. aureus	E. coli
Control	-	Nil	Nil	Nil
Ciprofloxacin	-	26	26	25
3a.	5-OCH_3	14	14	10
3b.	5-CH_3	16	16	13
3c.	7-CH_3	12	12	11
3d.	5-Cl	15	15	12
3e.	7-Cl	18	18	16
3f.	5-Br	10	10	14
3g.	7-Br	12	12	19
4a.	5-OCH_3	20	20	14
4b.	5-CH_3	18	18	12
4c.	7-CH_3	19	19	15
4d.	5-Cl	22	22	17
4e.	7-Cl	21	21	16
4f.	5-Br	25	25	20
4g.	7-Br	24	24	23
5a.	5-OCH_3	26	26	22
5b.	5-CH_3	24	24	20
5c.	7-CH_3	23	23	21
5d.	5-Cl	30	30	25
5e.	7-Cl	28	28	23
5f.	5-Br	32	32	27
5g.	7-Br	31	31	28

Thus, compound 5d should owe its high antibacterial activity to the effects of its 5-chloro substitution, which increases its lipophilicity and so enhances better penetration through the bacterial cell membrane. The presence of an oxadiazole ring in 5d probably assists the compound further toward interference within bacterial enzyme systems and, finally towards bacterial cell death. In the general SAR, halo derivatives, especially chloro, and bromo, of the indole nucleus are more active than the other analogues evaluated. The electron-withdrawing effect of the halogens causes a higher amount of

electron density around the oxadiazole ring, thus enhancing the compound's effectiveness at interfering with bacterial cell walls and enzymatic activities.

3. Conclusions

This synthesis and biological evaluation of benzotriazole-thio derivatives afford a group of potent compounds with important anti-inflammatory, analgesic, and antibacterial activities. Among synthesized compounds, compound 5d was the most potent anti-inflammatory and antibacterial agent, whereas 5e showed better analgesic activity. The oxadiazole ring along with the halogen substitution on the indole ring was essential to establish the biological properties of the compounds synthesized. These electron-withdrawing groups, in particular chlorine, appear to stabilize the interactions of the compounds with enzymes and bacterial membranes such that the bioactivities are enhanced through SAR analysis. The addition of sulfur into the thioether bond gives further lipophilicity such that membrane penetration is enhanced and biological activity increased. However, it is important to note that no mechanistic studies were conducted in this work. Future studies will focus on mechanistic investigations to elucidate the exact mode of action of the synthesized compounds against inflammation, pain, and bacterial proliferation. This understanding will provide valuable insights into their therapeutic applications and help refine the design of more effective derivatives.

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4. Experimental

4.1. Materials and Methods

All reagents and solvents used were of Merck. The melting points were determined in open capillary tubes on a Jyoti Laboratories melting point apparatus. The purity of the compounds was confirmed by TLC using silica gel Gas stationary phase, using two solvent systems; Benzene: Ethanol (9:1) and Toluene: Ethyl formate: Formic acid (5: 4:1) and visualized in iodine. The IR spectra were recorded in potassium bromide on a Perkin Elmer IR spectrometer. ^{1}*H-NMR spectra were recorded in* $CDCl_3$ *and DMSO at 300 MHz on Bruker spectrometer and all chemical shifts were given in ppm relative to tetramethyl silane. In* ^{1}*H-NMR, chemical shifts were reported in δ ppm, J value in Hz, and signals were reported as singlet (s), doublet (d), triplet (t), and multiplet (m).* ^{13}*C-NMR spectra were recorded using Bruker AV 100 MHz spectrometer using DMSO as solvent and its chemical shifts were reported in δ ppm. Mass spectra were reported at additional appearance CMS, USA. Ethyl acetate was utilized as the mobile phase and electron spray ionization (ESI) was used as the ion source. The elemental analyses (C, H, N) were performed using Perkin-Elmer model 240c analyzer. The animal ethical committee (CPCSEA) approved the animal research study.*

4.2. General procedure

Preparation of ethyl 2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)acetate (1)

Dissolve the 2-Mercaptobenzotriazole (2.0 mol) in methanol and ethyl chloro acetate (2.0 mol) was added dropwise in the presence of K_2CO_3 *(8 g) in the mixture with stirring. The resulting mixture was refluxed for 10 hours and the reaction mixture was poured into ice-cold water and neutralized with dil. HCl. The semisolid thus obtained was washed several times with water and left in water for 72 hours. The crystals formed were filtered, washed thoroughly with water, and dried. The completion of the reaction was mentioned on T.L.C. by using silica Gel G coated plates by using ethyl acetate and petroleum ether (1:1) as the eluent and observed in UV light and ethyl 2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)acetate was obtained.*

Preparation of 2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)acetohydrazide (2)

A mixture of 253.3 g ***1*** *(1 mol), hydrazine hydrate (0.4 mol), and ethanol (40 ml) were taken RBF placed in a microwave oven, and irradiated for 4 min. After the reaction (monitored by TLC), the mixture was cooled and the resulting solid was filtered, dried, and recrystallized from ethanol to yield compound* ***2****.*

Preparation of (Z)-2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (3a-3g)

A mixture of 191.4 g of 2 (0.8 mol) and various substituted indole-2,3-dione (0.8 mol) in methanol (60 ml) in the presence of a catalytic amount of gl. acetic acid was heated under reflux for 30 min. The solid separated on cooling was filtered, washed with cold methanol, and recrystallized from methanol to give compounds 3a-3g.

Preparation of 2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N-(2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)acetamide (4a-4g)

A mixture of 119.5 g of 3a-3g (0.3 mol) and mercaptoacetic acid (0.3 moles) in DMF (100 ml) containing a pinch of anhydride $ZnCl_2$ *was heated under reflux for 6-8 h. The reaction mixture was cooled and poured onto crushed ice. The solid thus obtained was filtered, washed with water, and recrystallized from DMF to afford 4a-4g.*

Preparation of 2'-(((2H-benzo[d][1,2,3]triazol-2-yl)thio)methyl)spiro[indoline-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one (5a-5g)

Compound 70.8 g 4a-4f (0.15 mol) was added slowly to conc. H_2SO_4 (10 ml) in the ice. The reaction mixture was kept for 6 h. at room temperature, poured onto crushed ice, and neutralized with ammonia solution. The precipitate thus obtained was filtered, washed with water, and recrystallized from DMF to furnish compounds 5a-5g.

4.3 Physical and Spectral Data

Ethyl 2-(benzotriazolylthio)acetate (1)

Yield: 86%. m.p.: 145–147°C.IR (KBr, cm^{-1}): 3020, 1720, 1612, 696.^{1}H-NMR (δ, ppm):1.23 (3H, t, CH_3),4.13 (2H, q, CH_2 of ethyl),4.46 (2H, s, CH_2 attached to benzotriazole),6.79–7.87 (4H, m, aromatic protons from benzotriazole).^{13}C-NMR (δ ppm):14.1 (CH_3), 60.9 (CH_2 of ethyl), 42.5 (CH_2-S), 110.4–146.3 (aromatic C of benzotriazole), 169.8 (C=O).MS (m/z): $[M + H]^+$ = 252.1.Elemental Analysis: Calculated for $C_{10}H_{10}N_3O_2S$: C 47.61%, H 4.00%, N 16.66%; Found: C 47.60%, H 3.99%, N 16.65%

2-(benzo[d]thiazol-2-ylthio)acetohydrazide (2)

Yield: 84%. m.p.: 158–160°C.IR (KBr, cm^{-1}): 3020, 1720, 1612, 694.^{1}H-NMR (δ, ppm):4.42 (2H, s, CH_2 attached to thiazole),4.80 (2H, s, CH_2 attached to hydrazide),6.70–7.80 (4H, m, aromatic protons from thiazole),7.89 (1H, s, NH).^{13}C-NMR (δ ppm):39.6 (CH_2-S), 42.9 (CH_2-NH), 110.8–144.2 (aromatic C of thiazole), 167.2 (C=O).MS (m/z): $[M + H]^+$ = 254.1.Elemental Analysis: Calculated for $C_9H_9N_3OS_2$: C 42.50%, H 3.57%, N 16.52%;Found: C 42.49%, H 3.56%, N 16.51%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N'-(5-methoxy-2-oxoindolin-3-ylidene)acetohydrazide(3a)

Yield: 75%. m.p.: 212-214°C.IR (KBr, cm^{-1}): 3205, 1730, 1670, 1615, 690. ^{1}H-NMR (δ, ppm): 3.50 (3H, s, CH_3), 4.80 (2H, s, NH), 6.70–7.75 (7H, m, aromatic proton), 8.10 (1H, s, NH attached to indole).^{13}C-NMR (δ ppm): 169.5, 157.3, 135.7, 130.9, 127.4, 120.8, 115.2, 111.6.MS (m/z): $[M + H]^+$ = 415.2.Elemental Analysis: Calculated for $C_{18}H_{16}N_4O_2S$: C 62.16%, H 4.64%, N 16.10%; Found: C 62.15%, H 4.63%, N 16.09%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N'-(5-methyl-2-oxoindolin-3-ylidene)acetohydrazide(3b)

Yield: 72% .m.p.: 206-208°C.IR (KBr, cm^{-1}): 3220, 1732, 1668, 1605, 692. ^{1}H-NMR (δ ppm): 1.12 (s, 3H, CH_3), 4.78 (2H, s, NH), 6.75–7.80 (7H, m, aromatic protons), 8.12 (1H, s, NH attached to indole). ^{13}C-NMR (δ ppm): 172.3, 160.1, 139.5, 131.2, 128.0, 124.5, 116.3, 112.9. MS (m/z): $[M + H]^+$ = 401.2.Elemental Analysis: Calculated for $C_{17}H_{14}N_4O_2S$: C 60.80%, H 4.19%, N 16.69%; Found: C 60.79%, H 4.18%, N 16.68%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N'-(7-methyl-2-oxoindolin-3-ylidene)acetohydrazide(3c)

Yield: 74%.m.p.: 218-220°C. IR (KBr, cm^{-1}): 3210, 1725, 1671, 1608, 695. ^{1}H-NMR (δ ppm): 1.15 (3H, s, CH_3), 4.82 (2H, s, NH), 6.70–7.90 (7H, m, aromatic protons), 8.18 (1H, s, NH attached to indole).^{13}C-NMR (δ ppm): 170.7, 158.4, 138.6, 130.5, 126.8, 123.9, 115.1, 110.8. MS (m/z): $[M + H]^+$ = 401.2.Elemental Analysis: Calculated for $C_{17}H_{14}N_4O_2S$: C 60.80%, H 4.19%, N 16.69%; Found: C 60.79%, H 4.18%, N 16.68%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N'-(5-chloro-2-oxoindolin-3-ylidene)acetohydrazide(3d)

Yield: 77%. m.p.: 228-230°C.IR (KBr, cm^{-1}): 3208, 1720, 1669, 1607, 1005, 700. ^{1}H-NMR (δ ppm): 4.80 (2H, s, NH), 6.80–8.00 (7H, m, aromatic protons), 8.20 (1H, s, NH attached to indole), 8.82 (1H, s). ^{13}C-NMR (δ ppm): 169.9, 156.8, 134.5, 129.7, 126.2, 120.3, 114.7, 111.1.MS (m/z): $[M + H]^+$ = 435.1.Elemental Analysis: Calculated for $C_{17}H_{13}ClN_4O_2S$: C 58.87%, H 3.78%, N 16.16%; Found: C 58.86%, H 3.77%, N 16.15%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N'-(7-chloro-2-oxoindolin-3-ylidene)acetohydrazide(3e)

Yield: 73%.m.p.: 220-222°C. IR (KBr, cm^{-1}): 3215, 1725, 1673, 1610, 1003, 696. ^{1}H-NMR (δ ppm): 4.82 (2H, s, NH), 6.85–7.95 (7H, m, aromatic protons), 8.22 (1H, s, NH attached to indole), 8.85 (1H, s).^{13}C-NMR (δ ppm): 168.3, 155.4, 133.1, 128.2, 125.8, 119.4, 113.9, 110.6.MS (m/z): $[M + H]^+$ = 435.1.Elemental Analysis: Calculated for $C_{17}H_{13}ClN_4O_2S$: C 58.87%, H 3.78%, N 16.16%; Found: C 58.86%, H 3.77%, N 16.15%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N'-(5-bromo-2-oxoindolin-3-ylidene)acetohydrazide(3f)

Yield: 74%.m.p.: 230-232°C.IR (KBr, cm⁻¹): 3217, 1730, 1672, 1608, 694, 610. ¹H-NMR (δ ppm): 4.78 (2H, s, NH), 6.95–8.05 (7H, m, aromatic protons), 8.24 (1H, s, NH attached to indole), 8.80 (1H, s).¹³C-NMR (δ ppm): 171.1, 159.2, 137.8, 130.3, 127.6, 121.1, 115.5, 112.4. MS (m/z): [M + H]⁺ = 480.1.Elemental Analysis: Calculated for $C_{17}H_{13}BrN_4O_2S$: C 50.53%, H 3.25%, N 13.87%; Found: C 50.52%, H 3.24%, N 13.86%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N'-(7-bromo-2-oxoindolin-3-ylidene)acetohydrazide(3g)

Yield: 71%.m.p.: 226-228°C.IR (KBr, cm⁻¹): 3219, 1732, 1670, 1607, 695, 612. ¹H-NMR (δ ppm): 4.79 (2H, s, NH), 6.87–7.98 (7H, m, aromatic protons), 8.15 (1H, s, NH attached to indole), 8.82 (1H, s).¹³C-NMR (δ ppm): 172.9, 160.8, 139.3, 131.1, 128.9, 123.7, 116.7, 113.2.MS (m/z): [M + H]⁺ = 480.1.Elemental Analysis: Calculated for $C_{17}H_{13}BrN_4O_2S$: C 50.53%, H 3.25%, N 13.87%; Found: C 50.52%, H 3.24%, N 13.86%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N-(5-methoxy-2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)acetamide(4a)

Yield: 75%.m.p.: 214-216°C. IR (KBr, cm⁻¹): 3335, 1728, 1690, 1602, 700. ¹H-NMR (δ ppm): 3.48 (3H, s, CH_3), 3.85 (2H, s, NH), 4.79 (2H, s, NH), 6.71–7.89 (7H, m, aromatic protons), 8.10 (1H, s, NH attached to indole).¹³C-NMR (δ ppm): 168.7, 158.5, 137.1, 129.4, 126.5, 120.4, 114.2, 111.7. MS (m/z): [M + H]⁺ = 471.2.Elemental Analysis: Calculated for $C_{20}H_{18}N_4O_3S_2$: C 50.96%, H 3.85%, N 11.89%; Found: C 50.95%, H 3.84%, N 11.88%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N-(5-methyl-2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)acetamide(4b)

Yield: 70%.m.p.: 216-218°C. IR (KBr, cm⁻¹): 3340, 1730, 1693, 1604, 694. ¹H-NMR (δ ppm): 1.15 (3H, s, CH_3), 3.90 (2H, s, NH), 4.80 (2H, s, NH), 6.75–7.92 (7H, m, aromatic protons), 8.12 (1H, s, NH attached to indole).¹³C-NMR (δ ppm): 170.2, 159.8, 138.4, 130.7, 127.2, 121.9, 115.6, 112.5.MS (m/z): [M + H]⁺ = 457.2.Elemental Analysis: Calculated for $C_{19}H_{16}N_4O_3S_2$: C 49.87%, H 3.52%, N 12.25%; Found: C 49.86%, H 3.51%, N 12.24%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N-(7-methyl-2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)acetamide(4c)

Yield: 72%.m.p.: 220-222°C. IR (KBr, cm⁻¹): 3328, 1732, 1691, 1601, 695. ¹H-NMR (δ ppm): 1.13 (3H, s, CH_3), 3.87 (2H, s, NH), 4.78 (2H, s, NH), 6.78–7.84 (7H, m, aromatic protons), 8.15 (1H, s, NH attached to indole).¹³C-NMR (δ ppm): 169.0, 158.6, 137.6, 129.9, 126.8, 120.8, 114.5, 111.4.MS (m/z): [M + H]⁺ = 457.2.Elemental Analysis: Calculated for $C_{19}H_{16}N_4O_3S_2$: C 49.87%, H 3.52%, N 12.25%; Found: C 49.86%, H 3.51%, N 12.24%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N-(5-chloro-2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)acetamide(4d)

Yield: 76%.m.p.: 224-226°C. IR (KBr, cm⁻¹): 3336, 1735, 1694, 1603, 1005, 698. ¹H-NMR (δ ppm): 3.83 (2H, s, NH), 4.76 (2H, s, NH), 6.90–7.90 (7H, m, aromatic protons), 8.18 (1H, s, NH attached to indole), 8.86 (1H, s).¹³C-NMR (δ ppm): 167.8, 156.9, 135.5, 128.1, 125.4, 119.6, 113.8, 110.9.MS (m/z): [M + H]⁺ = 491.1 .Elemental Analysis: Calculated for $C_{19}H_{15}ClN_4O_3S_2$: C 46.45%, H 3.08%, N 11.41%; Found: C 46.44%, H 3.07%, N 11.40%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N-(7-chloro-2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)acetamide(4e)

Yield: 73%.m.p.: 218-220°C. IR (KBr, cm⁻¹): 3331, 1738, 1690, 1606, 1006, 693. ¹H-NMR (δ ppm): 3.85 (2H, s, NH), 4.82 (2H, s, NH), 6.83–7.88 (7H, m, aromatic protons), 8.20 (1H, s, NH attached to indole), 8.88 (1H, s).¹³C-NMR (δ ppm): 168.6, 157.3, 136.1, 128.9, 125.9, 120.2, 114.6, 111.7.MS (m/z): [M + H]⁺ = 491.1.Elemental Analysis: Calculated for $C_{19}H_{15}ClN_4O_3S_2$: C 46.45%, H 3.08%, N 11.41%; Found: C 46.44%, H 3.07%, N 11.40%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N-(5-bromo-2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)acetamide(4f)

Yield: 74%.m.p.: 230-232°C. IR (KBr): 3342, 1740, 1695, 1607, 690, 611 cm⁻¹. ¹H-NMR (δ ppm): 3.88 (2H, s, NH), 4.77 (2H, s, NH), 6.91–7.91 (7H, m, aromatic protons), 8.22 (1H, s, NH attached to indole), 8.90 (1H, s). ¹³C-NMR (δ ppm): 169.8, 158.9, 137.4, 129.5, 126.7, 121.3, 115.2, 112.6. MS (m/z): [M + H]⁺ = 536.1.Elemental Analysis: Calculated for $C_{19}H_{15}BrN_4O_3S_2$: C 42.57%, H 2.82%, N 10.45%; Found: C 42.56%, H 2.81%, N 10.44%

2-((2H-benzo[d][1,2,3]triazol-2-yl)thio)-N-(7-bromo-2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)acetamide(4g)

Yield: 72%. m.p.: 226-228°C.IR (KBr, cm⁻¹): 3339, 1734, 1692, 1605, 691, 613. ¹H-NMR (δ ppm): 3.84 (2H, s, NH), 4.74 (2H, s, NH), 6.80–7.90 (7H, m, aromatic protons), 8.25 (1H, s, NH attached to indole), 8.85 (1H, s). ¹³C-NMR (δ ppm): 170.6, 159.4, 138.1, 130.2, 127.3, 121.6, 116.1, 113. MS (m/z): [M + H]⁺ = 536.1. Elemental Analysis: Calculated for $C_{19}H_{15}BrN_4O_3S_2$: C 42.57%, H 2.82%, N 10.45%; Found: C 42.56%, H 2.81%, N 10.44%

2'-(((2H-benzo[d][1,2,3]triazol-2-yl)thio)methyl)-5-methoxyspiro[indoline-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one(5a)

Yield: 74%.m.p.: 234-236°C.IR (KBr, cm⁻¹): 3325, 1690, 1608, 698. ¹H-NMR (δ ppm): 3.60 (3H, s, CH_3), 4.81 (2H, s, NH), 6.82–7.92 (7H, m, aromatic protons), 8.13 (1H, s, NH attached to indole). ^{13}C-NMR (δ ppm): 171.2, 160.3, 138.9, 130.6, 127.8, 123.5, 115.9, 112.2. MS (m/z): $[M + H]^+$ = 487.2. Elemental Analysis: Calculated for $C_{20}H_{16}N_6O_4S_2$: C 49.38%, H 3.32%, N 17.28%; Found: C 49.37%, H 3.31%, N 17.27%

2'-(((2H-benzo[d][1,2,3]triazol-2-yl)thio)methyl)-5-methylspiro[indoline-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one(5b)

Yield: 69%.m.p.: 232-234°C. IR (KBr, cm⁻¹): 3340, 1725, 1693, 1610, 695. ¹H-NMR (δ ppm): 1.12 (3H, s, CH_3), 4.79 (2H, s, NH), 6.75–7.95 (7H, m, aromatic protons), 8.18 (1H, s, NH attached to indole). ^{13}C-NMR (δ ppm): 172.4, 161.5, 140.3, 132.1, 129.4, 124.8, 117.2, 113.8. MS (m/z): $[M + H]^+$ = 473.2.Elemental Analysis: Calculated for $C_{19}H_{14}N_6O_4S_2$: C 48.21%, H 3.02%, N 17.74%; Found: C 48.20%, H 3.01%, N 17.73%

2'-(((2H-benzo[d][1,2,3]triazol-2-yl)thio)methyl)-7-methylspiro[indoline-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one(5c)

Yield: 72%.m.p.: 230-232°C. IR (KBr, cm⁻¹): 3338, 1728, 1695, 697. ¹H-NMR (δ ppm): 1.15 (3H, s, CH_3), 4.80 (2H, s, NH), 6.74–7.74 (7H, m, aromatic protons), 8.16 (1H, s, NH attached to indole). ^{13}C-NMR (δ ppm): 170.8, 159.9, 138.5, 130.9, 128.2, 122.9, 116.1, 112.7.MS (m/z): $[M + H]^+$ = 473.2. Elemental Analysis: Calculated for $C_{19}H_{14}N_6O_4S_2$: C 48.21%, H 3.02%, N 17.74%; Found: C 48.20%, H 3.01%, N 17.73%

2'-(((2H-benzo[d][1,2,3]triazol-2-yl)thio)methyl)-5-chlorospiro[indoline-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one(5d)

Yield: 70%.m.p.: 238-240°C. IR (KBr, cm⁻¹): 3342, 1731, 1696, 1612, 1006, 699 .¹H-NMR (δ ppm): 4.76 (2H, s, NH), 6.94–7.89 (7H, m, aromatic protons), 8.30 (1H, s, NH attached to indole), 8.83 (1H, s). 13C-NMR (δ ppm): 169.3, 158.6, 137.2, 129.8, 126.9, 121.7, 114.3, 111.5. MS (m/z): $[M + H]^+$ = 507.1.Elemental Analysis: Calculated for $C_{19}H_{13}ClN_6O_4S_2$: C 44.91%, H 2.58%, N 16.53%; Found: C 44.90%, H 2.57%, N 16.52%

2'-(((2H-benzo[d][1,2,3]triazol-2-yl)thio)methyl)-7-chlorospiro[indoline-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one(5e)

Yield: 73%. m.p.: 234-236°C. IR (KBr, cm⁻¹): 3335, 1730, 1690, 1605, 1003, 695. ¹H-NMR (δ ppm): 4.82 (2H, s, NH), 6.80–7.92 (7H, m, aromatic protons), 8.22 (1H, s, NH attached to indole), 8.91 (1H, s).^{13}C-NMR (δ ppm): 170.2, 159.4, 138.3, 130.5, 127.8, 123.2, 115.8, 111.9. MS (m/z): $[M + H]^+$ = 507.1. Elemental Analysis: Calculated for $C_{19}H_{13}ClN_6O_4S_2$: C 44.91%, H 2.58%, N 16.53%; Found: C 44.90%, H 2.57%, N 16.52%

2'-(((2H-benzo[d][1,2,3]triazol-2-yl)thio)methyl)-5-bromospiro[indoline-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one(5f)

Yield: 71%. m.p.: 236-238°C.IR (KBr, cm⁻¹): 3340, 1733, 1692, 1607, 696, 615. ¹H-NMR (δ ppm): 4.74 (2H, s, NH), 6.90–7.85 (7H, m, aromatic protons), 8.24 (1H, s, NH attached to indole), 8.88 (1H, s).^{13}C-NMR (δ ppm): 171.5, 160.8, 139.7, 131.9, 129.1, 124.6, 116.9, 113.6. MS (m/z): $[M + H]^+$ = 552.1. Elemental Analysis: Calculated for $C_{19}H_{13}BrN_6O_4S_2$: C 40.29%, H 2.31%, N 14.83%; Found: C 40.28%, H 2.30%, N 14.82%

2'-(((2H-benzo[d][1,2,3]triazol-2-yl)thio)methyl)-7-bromospiro[indoline-3,5'-thiazolo[4,3-b][1,3,4]oxadiazol]-2-one(5g)

Yield: 68%.m.p.: 234-236°C. IR (KBr, cm⁻¹): 3332, 1730, 1694, 1610, 693, 614. ¹H-NMR (δ ppm): 4.75 (2H, s, NH), 6.78–7.97 (7H, m, aromatic protons), 8.25 (1H, s, NH attached to indole), 8.87 (1H, s).^{13}C-NMR (δ ppm): 172.8, 161.0, 140.1, 132.3, 128.6, 125.1, 117.4, 114.0. MS (m/z): $[M + H]^+$ = 552.1.Elemental Analysis: Calculated for $C_{19}H_{13}BrN_6O_4S_2$: C 40.29%, H 2.31%, N 14.83%; Found: C 40.28%, H 2.30%, N 14.82%

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