Design, Synthesis and DNA Cleavage Studies of benzofuran-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives

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ABSTRACT

Purpose: *Benzofuran is a significant heterocyclic compound, constituting the structural context of bioorganic and medicinal noteworthy organic compounds. Therefore, in this study synthesis and evaluation of DNA cleavage activity of benzofuran fused thiadiazine derivatives* ***(IVa–h)*** *was discussed.*

Methodology: *Benzofuran fused thiadiazine derivatives were synthesized by the fusion of commercially available benzofuran-2-carboxylic acid* ***(I)*** *with thiocarbohydrazide* ***(II)*** *followed by condensation reaction with different phenacyl bromides* ***(a-h).*** *The synthesized compounds were characterized by phisico-chemical spectral analysis such as melting point, elemental analysis, FT-IR,* ^{13}C *NMR,* ^{1}H *NMR, and mass spectroscopy studies. The DNA cleavage efficacy was evaluated using agarose gel electrophoresis.*

Analysis/Results: *The DNA cleavage studies of all synthesized compounds* ***(IVa-h)*** *were photolyzed at 365 nm, at 50µM concentration in the presence of pUC19 DNA. The study shows that the compounds* ***IVa*** *and* ***IVb*** *showed a more intense streak, indicating better activity than that of other compounds.*

Originality/Value: *Synthesis and DNA cleavage evaluation of benzofuran fused thiadiazine derivatives were not explored. DNA cleavage studies, it is observed that some of the synthesized derivatives have exhibited better DNA cleavage activity. The obtained results recommend that these classes of compounds can be considered as new templates for further structural optimization to achieve better biological drug development.*

Type of Paper: *Experimental.*

Keywords: Benzofuran fused thiadiazine, Phenacyl bromides, pUC19 DNA, Thiocarbohydrazide

1. INTRODUCTION :

Benzofuran is an important moiety among the oxygen-containing heterocycles because presence in plenty of bioactive natural and synthetic compounds. Benzofuran and its substituted scaffolds have attracted great attention in the fields of pharmaceuticals, polymers, and agriculture, due to their noticeable applications. (Dawood, K.M. (2013). [1], Yeung, K.S. (2012). [2], Shamsuzzaman, K.H. (2015). [3], Aqsa, M. et al (2024) [4]). The various topics of the biological importance of benzofuran and its derivatives number articles has been published (Khodarahmi, G. et al (2015). [5], Radadiya, A., & Shah, A. (2015). [6], Hiremathad, A. et al (2015). [7], Bhargava, S., & Rathore. D. (2017).[8], Goyal, D. et al (2018). [9], Miao, Y.H. et al (2019). [10]). The different biological and pharmacological

applications of benzofuran derivatives such as antiplatelet, antioxidant, anticonvulsant, antitubercular, antimalarial, antidepressant, immunomodulatory and anti-inflammatory properties. (Abbas, A. A., & Dawood, K. M. (2022). [11]). More than thirty analogues of benzofuran have been approved as drug by Food and Drug administration in the market. (FDA) (Xu, Z. et al (2019).[12], Chand, K. et al (2017) [13], Nevagi, R. et al (2015). [14], Zelova, H.Z. et al (2014) [15]). Naturally occurring benzofurans were also merged in drug design and discovery. The extraction of benzofuran natural products from herbs, and plants were used for the treatment of antifeedant, immunosuppressive, antiarrhythmic antiviral, anticancer, antioxidant agents. (Naik, R. et al (2015) [16], Farhat, J. et al (2022) [17]). In addition to this, a variety of benzofuran natural occurring compounds have also showed dermprotecetive effects such as methoxalene, viniferin, and psoralene are employed in the treatment of psoriasis and other dermal diseases. (Baldisserotto, A. et al (2018). [18]). Review article of Dawood et.al. outlines an updated broad report on the recent developments of the biological and pharmacological significances of both synthetic and natural bioactive benzofuran compounds as pro-drugs or highly promising source of drug development for various diseases.

The recent developments of the biological and pharmacological significances of both synthetic and natural bioactive compounds of benzofuran as promising drug development for various diseases has reported by Dawood et.al. In some areas, also focused on providing details of the results of Structure-activity relationships (SAR) and pharmacological activities. The biological and pharmacological activities reported on benzofuran included antitumor, antimicrobial, antitubercular, antioxidant, herbicidal, antidepressant, antiviral, anti-alzheimer as well as enzyme inhibitory activities (Dawood, K. M. (2019). [19]).

Based on the above research results, and continuation of our research on heterocyclic compounds (Kumar, A.C. et al (2024) [20], (Rangaswamy, J. et al. (2017) [21], (Rangaswamy, J. et al (2016) [22]. In the present work a novel benzofuran fused thiadiazine derivatives was designed, synthesized, and assessed for their pUC19 DNA cleavage activity.

2. REVIEW OF LITERATURE :

Benzofurans have intrigued both pharmaceutical researchers and chemists owing to the medicinal usage of their derivatives against copious disease-causing agents (Aqsa, M., et al. (2024) [23]. Many review articles covering the adaptable synthetic approaches for benzofuran synthesis have been published by different researchers (Abu-Hashem et al (2014), [24] (Heravi, M.M et al. (2015) [25]. Later, Bhargava and Miao group published reviews on the synthetic paths toward the synthesis of medicinally important benzofurans and their derivatives. (Miao, Y. H. et al (2019) [26]. Furthermore, benzofuran are also biodynamic active scaffolds that can be preferred to design and develop new potent active therapeutic agents. Many scholars have reported compounds shows different biological efficacy such as anti-tumor, (Xu, X.L. et al (2017) [27] antibacterial, (Liang, Z. et al (2016) [28] (Kenchappa, R. et al (2017) [29] anti-oxidative, (Aswathanarayanappa, C. et al (2012) [30], (Marwa Abdel-motaal, E. et al (2017) [31] anti-AD, (Hiremathad, A. et al (2018) [32], anti-parasitic, (Thevenin, M. et al (2013) [33], anti-acetylcholine, (Ragab, H.M. et al 2008) [34] and anti-inflammatory activities (Xie, Y.S. et al (2014) [35]. Recently, in mid-2022, another review reporting the biological applications and synthetic strategies to achieve different benzofurans was published by Dwarakanath and Gaonkar (Dwarakanath, D., & Gaonkar, S.L., (2022) [36]. In addition, some of the 2-arylbenzofurans extracted from naturally available products also exhibited efficient biological activities, (Sharma, U. et al (2013) [37], such as anti-cancer, (Promchai, T. et al (2020) [38], anti-inflammatory (Chen, H. et al (2015) [39], anti-oxidative (Zelova, H., et al. (2014) [40] and antibacterial activities Kyekyeku, J.O. et al. (2016) [41], (Tan, Y.X. et al. (2012) [42]. Due to ever-increasing utilization of benzofurans as pharmaceutical agents persuaded the chemists to devise novel and facile methodological approaches to assemble the biologically potent benzofuran nucleus. In this article, we have synthesis of some benzofuran scaffolds, to study their DNA cleavage activity.

3. OBJECTIVES OF THE PAPER :

(1) To synthesize new benzofuran fused thiadiazine derivatives by using standard method.
(2) To characterize the above-synthesized compounds by employing various physicochemical spectral studies.

(3) To explore DNA cleavage efficacy of novel synthesized compounds by making use of gel electrophoresis assay.
(4) To make comparative studies of the cleaving ability of synthesized analogues with control.

4. METHODOLOGY :

All reagents, solvents and chemicals were procured from Avra synthesis and TCI, AR, LR grade and were used without further purification. Progress of the reaction by using Thin Layer Chromatography (TLC) on alumina sheets precoated with silica gel 60F-254 with visualization under UV at 254 nm. Purification of compounds by column chromatography, Silicon dioxide of 60-120 mesh was used as the stationary phase and Hexane: Ethyl acetate (8:2) as mobile phase. 100 MHz ^{13} C NMR and 400 MHz ^{1}H NMR were obtained using an AC Bruker spectrometer in the appropriate solvent Dimethyl sulfoxide (DMSO-d_6). Infra LUM FT-02 FT-IR spectrophotometer in the range 4000-400 cm^{-1} in KBr IR spectra were recorded. Melting point (M.P) were recorded on a Reichert thermopan apparatus, equipped with a thermometer (0-300^{o}C). The mass spectrum of all the compounds was attained using an Electron Impact mass spectrometer at 70 ev ionizing beam and using a direct insertion probe. Elemental analyses were performed on a PerkinElmer Series II2400 analyzer.

5. EXPERIMENTAL :

5.1 Synthetic procedure:

Synthesis of 4-amino-5-(benzofuran-2-yl)-4H-1,2,4-triazole-3-thiol (3).

A mixture of benzofuran-2-carboxylic acid (1) and thiocarbohydrazide (2) was taken in a RB flask, and the contents were mixed thoroughly. The reaction mixtures were slowly fused by raising by the temperature 110-120 ^{o}C. The molten reaction mass was kept at the same temperature for about 10-15 min while mixing thoroughly with constant stirring. Finally, the temperature of the reaction mass slowly decreased to room temperature (RT) and stirred with distilled water for a few minutes. The obtained product intermediate was filtered and dried in a vacuum.

Yield (56%), m.p. 185–187 °C, IR (KBr)ν_{max}(cm^{-1}): 3273(–NH_2), 1695(–C=N), ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 13.90 (s, 1H, –SH), 7.14 (s, 1H, B-furan-H), 7.32-7.38 (d, 2H, Bf-H), 7.60 (d, 1H, Bf-H), 7.85 (d, 1H, Bf-H) 2.51 (s, 3H, –CH_3), 5.80 (s, 2H, –NH_2); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 167.0, 156.0, 155.3, 150.5, 129.3, 124.5, 123.0, 120.9, 111.0, 102.5. MS (ESI) m/z: 232 (M^{+}); Anal. calcd. for $C_{10}H_8N_4OS$: C, 51.71; H, 3.47; N, 24.12; found: C, 51.67; H, 3.45; N, 24.15%

Synthesis of novel benzofuran-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives (IVa–h).

A mixture of 4-amino-5-(benzofuran-2-yl)-4H-1,2,4-triazole-3-thiol (1 mmol) and substituted phenacyl bromides **(a-h) (1 mmol)** was taken in ethanol. The reaction was refluxed at 70 ^{o}C with constant stirring until TLC showed complete consumption of two reactants into the product. After the accomplishment of the reaction, the mixture was cooled to RT and the obtained solid was separated by filtration. The compounds were recrystallized from the appropriate solvent.

3-(benzofuran-2-yl)-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (IVa)

Brown solid, m.p.: 170-172 ^{o}C. IR (KBr)ν_{max}(cm^{-1}): 3150-2980 (Ar-CH), 1695 (C=N); ^{1}H NMR (DMSO-d_6 400MHz) δ ppm: 7.10-7.90 (m, 10H, Ar-H), 4.50 (s, 1H, thiadiazine-H); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 164.0, 159.0, 156.0, 155.2, 151.0, 134.0, 131.0, 129.3, 128.5, 124.7, 123.0, 120.5, 111.0, 102.7, 36.3; MS (ESI) m/z: 332. (M^{+}); Anal.calcd. for $C_{18}H_{12}N_4OS$: C, 65.04; H, 3.64; N, 16.86; found: C, 64.98; H, 3.60; N, 16.89 %.

3-(benzofuran-2-yl)-6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (IVb)

Off white solid, m.p.: 156-158 ^{o}C. IR (KBr)ν_{max}(cm^{-1}): 3145-2975 (Ar-CH), 1695 (C=N); ^{1}H NMR (DMSO-d_6 400MHz) δ ppm: 7.12-7.85 (m, 9H, Ar-H), 4.45 (s, 1H, thiadiazine-H), 3.80 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 164.2, 159.5, 156.0, 155.0, 151.5, 134.1, 131.0, 129.3, 128.5, 128.0, 124.5, 123.0, 120.2, 111.5, 102.5, 55.5, 36.0; MS (ESI) m/z: 332. (M^{+}); Anal.calcd. for $C_{18}H_{12}N_4OS$: C, 65.04; H, 3.64; N, 16.86; found: C, 64.98; H, 3.60; N, 16.89 %.

3-(benzofuran-2-yl)-6-(4-nitrophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (IVc)
Light yellow solid, m.p.: 202-204 ºC. IR (KBr)ν_{max}(cm^{-1}): 3158-2982 (Ar-CH), 1690 (C=N); ^{1}H NMR (DMSO-d_6 400MHz) δ ppm: 7.15-7.90 (m, 9H, Ar-H), 4.50 (s, 1H, thiadiazine-H); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 164.9, 159.0, 156.0, 155.0, 151.8, 134.9, 131.5, 129.2, 128.8, 128.0, 124.5, 123.1, 120.5, 111.4, 102.4, 36.5; MS (ESI) m/z: 377. (M^+); Anal.calcd. for $C_{18}H_{11}N_5O_3S$: C, 57.29; H, 2.94; N, 18.56; found: C, 57.31; H, 2.90; N, 18.51%.

4-(3-(benzofuran-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-yl)phenol (IVd)
Light brown solid, m.p.: 218-220 ºC. IR (KBr)ν_{max}(cm^{-1}): 3165-2990 (Ar-CH), 1692 (C=N); ^{1}H NMR (DMSO-d_6 400MHz) δ ppm: 7.10-7.82 (m, 9H, Ar-H), 4.40 (s, 1H, thiadiazine-H); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 164.5, 159.3, 156.2, 154.9, 151.5, 134.9, 131.3, 129.5, 128.5, 128.0, 124.3, 123.0, 120.5, 111.4, 102.4, 36.0; MS (ESI) m/z: 348 (M^+); Anal.calcd. for $C_{18}H_{12}N_4O_2S$: C, 62.06; H, 3.47; N, 16.08; found: C, 62.05; H, 3.51; N, 16.03%.

4-(3-(benzofuran-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-yl)phenol (IVe)
Brown solid, m.p.: 187-189 ºC. IR (KBr)ν_{max}(cm^{-1}): 3160-2985 (Ar-CH), 1695 (C=N); ^{1}H NMR (DMSO-d_6 400MHz) δ ppm: 7.15-7.80 (m, 9H, Ar-H), 4.45 (s, 1H, thiadiazine-H); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 164.0, 159.1, 156.0, 154.5, 151.2, 134.5, 131.2, 129.4, 128.4, 128.0, 124.2, 122.5, 120.5, 110.8, 103.4, 36.5; MS (ESI) m/z: 377 (M^+); Anal.calcd. for $C_{18}H_{11}N_5O_3S$: C, 57.29; H, 2.94; N, 18.56; found: C, 57.24; H, 2.98; N, 18.59%.

3-(benzofuran-2-yl)-6-(4-chlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (IVf)
Dark Brown solid, m.p.: 192-194 ºC. IR (KBr)ν_{max}(cm^{-1}): 3145-2989 (Ar-CH), 1690 (C=N); ^{1}H NMR (DMSO-d_6 400MHz) δ ppm: 7.10-7.85 (m, 9H, Ar-H), 4.54 (s, 1H, thiadiazine-H); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 164.5, 159.0, 156.5, 154.0, 151.0, 134.3, 131.0, 129.2, 128.2, 127.0, 124.5, 122.4, 120.4, 110.5, 103.2, 36.0; MS (ESI) m/z: 366 (M^+); Anal.calcd. for $C_{18}H_{11}ClN_4OS$: C, 58.94; H, 3.02; N, 15.27; found: C, 58.94; H, 3.02; N, 15.27%.

3-(benzofuran-2-yl)-6-(3,4-dichlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (IVg)
Gray solid, m.p.: 190-192 ºC. IR (KBr)ν_{max}(cm^{-1}): 3155-2990 (Ar-CH), 1695 (C=N); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 7.15-7.85 (m, 9H, Ar-H), 4.54 (s, 1H, thiadiazine-H); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 164.5, 159.0, 156.0, 154.5, 151.3, 134.5, 131.5, 129.4, 128.5, 127.3, 124.4, 122.0, 120.3, 110.4, 102.1, 36.2; MS (ESI) m/z: 400 (M^+); Anal.calcd. for $C_{18}H_{10}Cl_2N_4OS$: C, 53.88; H, 2.51; N, 13.96; found: C, 53.85; H, 2.50; N, 13.92%.

3-(benzofuran-2-yl)-6-(3-bromophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (IVh)
Yellow solid, m.p.: 160-162 ºC. IR (KBr)ν_{max}(cm^{-1}): 3150-2985 (Ar-CH), 1690 (C=N); ^{1}H NMR (DMSO-d_6 400MHz) δ ppm: 7.10-7.80 (m, 9H, Ar-H), 4.45 (s, 1H, thiadiazine-H); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm: 164.0, 159.0, 156.5, 154.0, 151.0, 134.4, 131.0, 129.2, 128.0, 127.0, 124.5, 122.0, 120.3, 110.4, 102.5, 36.0; MS (ESI) m/z: 409 (M^+); Anal.calcd. for C18H11BrN4OS: C, 52.57; H, 2.70; N, 13.62; found: C, 52.59; H, 2.68; N, 13.65%.

5.2 Biological investigation:

DNA cleavage study

The DNA cleavage studies were monitored by using agarose gel electrophoresis (Ravikumar Naik, T.R. et al (2009). [43]. Concentration of tested compounds (50 µM) along with supercoiled pUC19DNA (50 µM). The solutions of all compounds were irradiated for 2hrs in DMSO: Trisbuffer (2:8) at 20µM, PH-7.2, in 365 nm under UV light. A loading of buffer congaing bromophenol blue 25%, xylene 0.25% cyanol, and glycerol 30% was added and electrophoresis was performed at 50V for 3 hrs in Tris-borate-EDTA (TBE) buffer using 0.8% agarose gel containing 1.0µg/ml ethidium bromide. Bands were visualized using UV light and photographed. The cleavage efficiency was measured by defining the ability of the compounds to convert the supercoiled DNA (SC) to a nicked circular form (NC)

6. RESULT AND DISCUSSION :

6.1 Chemistry:

Synthesis of the target benzofuran fused thiadiazine derivatives **(IVa-h)** as depicted in **Scheme 1.**

The synthetic pathway for the synthesis of the target molecule **(IVa-h)** was the synthesis of an intermediate 4-amino-5-(benzofuran-2-yl)-4H-1,2,4 triazole-3-thiol **(III).** The intermediate **(III)** was synthesized by the reaction between commercially available benzofuran-2-carboxylic acid **(I)** with thiocarbohydrazide (TCH) (**II**). The structure was confirmed by IR, ^{1}H NMR and ^{13}C NMR spectral analysis.

The intermediate (III) 1H-NMR spectrum shows peaks at δ 14.05 due to SH rather than NH-C=S, δ 5.77 ppm due to NH_2 protons (integrating two protons), and other peaks were observed at appropriate chemical shift values. This structure is further supported by IR spectral studies. The title derivatives were prepared by the reported method (Krishnaiah, V. et al (2016). [44]), by the condensation with different substituted phenacyl bromides **(a-h)**, the intermediate was converted into the final product effectively under simple reaction conditions (**Scheme 1**). The characterization and structure of the target molecules by various physico-chemical spectral analysis techniques and further subjected to explore DNA cleavage activities.

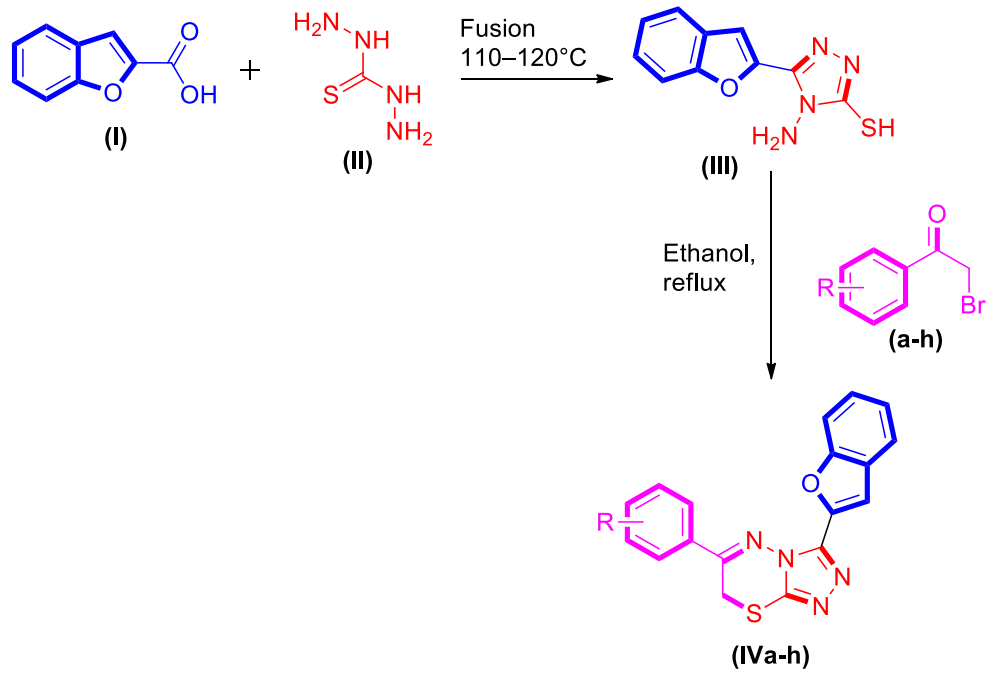
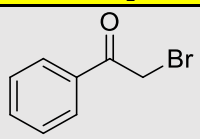
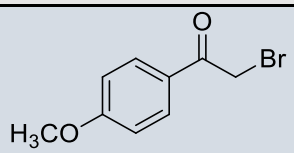
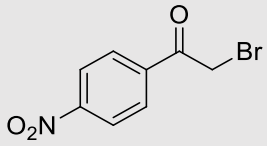
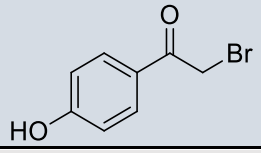
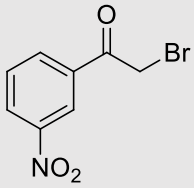
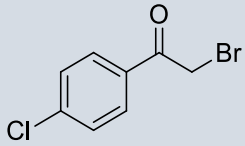
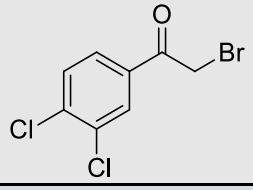
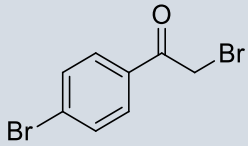


Fig. 1: Scheme 1: Synthesis pathway for benzofuran-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives (**IVa-h**).

Table 1: Chemical structure and yield (%) of benzofuran-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives(**IVa-h**).

S. No.	Compound No	$PhCOCH_2Br$	Yield (%)
1	IVa		77.50
2	IVb		59.30

3	**IVc**		67.55
4	**IVd**		68.70
5	**IVe**		81.00
6	**IVf**		78.15
7	**IVg**		72.20
8	**IVh**		55.00

6.2 Biological investigation:

***DNA cleavage activity*:**

The synthesized benzofuran fused thiadiazine analogues **(IVa-h)** were subjected to a test for DNA cleavage studies. The compounds were photolyzed at 365nm at 50µM concentration. All the solutions were irradiated for 2 hrs in DMSO: Trisbuffer (2:8), at 20µM, pH-7.2. Cleavage capacity was determined quantitatively by the efficiency in converting nicked circular (Form II) from supercoiled plasmid DNA (Form I) Fig. 1 at the 50µM concentration. The control experiment did not show any apparent cleavage of pUC19 DNA (Lane 1). The results exhibited that the tested benzofuran derivatives may be induced to photoextrusion under situations essential for DNA cleavage proceeding intermediates capable of hydrogen abstraction.

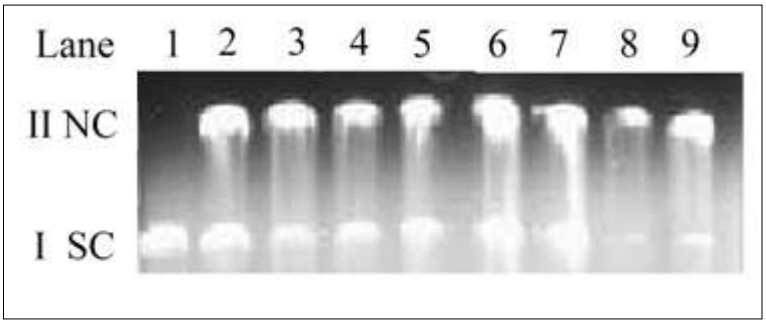


Fig. 2: Photo cleavages of DNA by benzofuran bearing C-2,4,6-substituted derivatives **(IVa-h)** Supercoiled (SC) DNA runs at I position and nicked coiled (NC) DNA at II position.

The amount of (Form I) of pUC19 DNA diminishes gradually, whereas (Form II) increases indicating effective cleavage capability of compounds. From the fig, the gel electrophoresis evidently revealed that the tested compounds did not act on the DNA as little tailing in the bands can be observed in the tested samples. The difference was detected in bands of all the treated compounds compared to the control DNA. This shows DNA alone didn't exhibit any apparent cleavage.
Lane 1: control DNA (without sample), Lane 2: 50 µM **(IVa)**; Lane 3: 50 µM **(IVb)**; Lane 4: 50 µM **(IVc)**; Lane 5: 50 µM **(IVd)**; Lane 6: 50 µM **(IVe)**; Lane 7: 50 µM **(IVf)**; Lane 8: 50 µM **(IVg)**; Lane 9: 50 µM **(IVh)**;
In order to make clear the cleavage studies of supercoiled DNA were entirely converted to nicked circular DNA in the presence of compound **IVa** (Lane 2) and **IVb** (Lane 3) showed a stronger streak, indicating the highest activity. However, compound **IVd** (Lane 5) also showed significant DNA cleavage activity, but in the same run the nitro and halogen analogs (**IVf-h**) are not completely transformed into nicked circular they displayed tailing in the bands. This result suggested that the synthesized benzofuran fused thiadiazine derivatives are capable of hastening the cleavage of DNA intensely, which might be the presence of the electron-donating groups (EDGs) such as hydroxyl and methoxy groups. The EDGs are highly reactive radicals and these free radicals abstract hydrogen atoms efficiently at C-40 of 2-deoxyribose in B-DNA. The analogs substituted with nitro and halogen groups are not as much of active toward the DNA cleavage.

7. CONCLUSION :

In the present studies, we have described the synthesis of benzofuran-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**IVa-h**) analogs by selecting proper standard experimental conditions and subjected investigating for their cleavage activity of DNA with the hope of noticing new structure primes serving as potent active pharmacological agents. In DNA cleavage study reveals, compounds (**IVb**) and (**IVd**) better cleaved the DNA as no traces of DNA were found. Whereas, the rest of the compounds, were nearly completely inactive towards the cleaved DNA. Meanwhile, structure-activity relationship studies discovered the important role of methoxy and hydroxy functional group in the target compounds at 4-position at phenacyl bromide phenyl ring that showed better cleavage ability as no traces of supercoiled DNA. This would be the reason, compounds (**IVb**) and (**IVd**) were demonstrated significant DNA Cleavage efficacy compared to other derivatives.

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