ABSTRACT
A series of new glucocorticoid oxadiazines (4–6) were synthesized by reacting glucocorticoids (1–3) with thiosemicarbazide and its derivatives. The structural assignment of products is confirmed on the basis of IR, 1H NMR, 13C NMR, MS and analytical data. The synthesized compounds (4–6) obeyed the Lipinski’s “Rule of Five” analysis based on a computational prediction of molecular and pharmacokinetic properties. The interaction studies of compounds (4–6) with DNA were carried out by employing single-cell gel electrophoresis (comet assay), UV-vis and fluorescence spectroscopy. Compounds (4–6) were found capable of cellular DNA degradation breakage in isolated normal human lymphocytes. Viscometric and steady-state measurements further correlated with the comet assay studies. Hence, it could be suggested that the glucocorticoid compounds bearing a core oxadiazine scaffold would be a potent biological agent. Molecular docking studies further characterize the interaction of the synthesized compounds with DNA.

1. Introduction
Steroidal compounds have shown tremendous potential as putative curative agents for cancers and other diseases. It has been shown in recent studies that incorporation of heteroatoms (N/O/S) enhances the biological activities of steroid molecules [1–5]. It was proved by various in vivo and in vitro assays which show significant antimicrobial, anti-inflammatory, hypotensive, hypocholesterolemic and diuretic activities [6–15]. Similarly, such effects have been shown by the most commonly used systemic glucocorticoids such as hydrocortisone, prednisolone and triamcinolone. The glucocorticoids have good oral bioavailability and are eliminated mainly by hepatic metabolism and renal excretion of the metabolites [16]. The beneficial effects of glucocorticoids in the treatment of chronic inflammatory diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease and autoimmune disorders have been appreciated for over 50 years [17–21]. A considerable research effort has been devoted to the structural modifications of some glucocorticoids, with a hope of increasing its potencies and bioavailability. Khan and Lee have reviewed the enormous literature with respect to the synthesis of derivatives of steroidal antedrugs [22]. Despite enormous literature precedence, there are several unexplored opportunities for generating the heterocyclic compounds. In spite of significant advances in the field of the biological chemistry
of glucocorticoids has been made, not many efforts have been made to obtain small heterocyclic fused ring products of the glucocorticoids moiety. It has been well established that the biological and hence therapeutic potential and consequent antedrug activities are drastically influenced by infusion of such a moiety.

2. Results and discussion

2.1. Chemistry

Highly functionalized molecules developed from simple building blocks has always fascinated the inquisitiveness of synthetic chemists. So, herein we report an expedient path for the synthesis of new derivatives (4–6) from a mixture of glucocorticoids (1–3) and thiosemicarbazide/4-phenylthiosemicarbazide/4-methyl-3-thiosemicarbazide/4-ethyl-3-thiosemicarbazide, (Table 1) in EtOH using few of drops conc. HCl under reflux condition for 24 h. On completion of reaction, the products were obtained in better yields (75–80%) in case of 4-phenylthiosemicarbazide because it generates a better leaving group (anilinium ion) than all. The mechanism for the formation of compounds (4–6) involves the nucleophilic attack of the hydroxyl oxygen on the thionyl carbon of thiosemicarbazide, 4-phenylthiosemicarbazide, 4-methyl-3-thiosemicarbazide and 4-ethyl-3-thiosemicarbazide making the ammonia, phenylamine, methylamine and ethylamine group to leave, which leads to the formation of six-membered heterocyclic moiety with a glucocorticoid skeleton as shown in Scheme 1. It has been confirmed by NMR studies that the major product will be the compounds (4–6) as it’s well known that simple ketones are more reactive than $\alpha,\beta$-unsaturated ketones for the nucleophilic nature at carbonyl carbon. The structures of the products were established by means of their IR, $^1$H NMR, $^{13}$C NMR, MS and analytical data.

The selected suggestive bands in IR spectra of targeted compounds (4–6) provide valuable information for determining their structures. In their IR spectra, the presence of absorption bands in the range 3000–3300 cm$^{-1}$ can be expected due to the hydroxyl group of the glucocorticoid moiety, 2800–2900 cm$^{-1}$ for the alkyl groups of the thiosemicarbazide derivatives, and 1600–1700 cm$^{-1}$ for the carbonyl group of the glucocorticoid moiety.

Table 1. Thiosemicarbazide derivatives and % yield.

| Compound | H$_2$NNHCSNR' | Yield (%) |
|----------|---------------|-----------|
| R' = H   | 77            |           |
| R' = Ph  | 80            |           |
| R' = CH$_3$ | 50         |           |
| R' = C$_2$H$_5$ | 45    |           |
| R' = H   | 78            |           |
| R' = Ph  | 78            |           |
| R' = CH$_3$ | 46         |           |
| R' = C$_2$H$_5$ | 42    |           |
| R' = H   | 75            |           |
| R' = Ph  | 75            |           |
| R' = CH$_3$ | 44         |           |
| R' = C$_2$H$_5$ | 39    |           |

Table 1. Thiosemicarbazide derivatives and % yield.

Scheme 1. Schematic reaction pathway of glucocorticoid derivatives (4–6).
3427–3421 cm$^{-1}$ shows the presence of NH band. The absorption band in the region of 1651–1595 and 1061–1055 cm$^{-1}$ shows the presence of C = N and C–O, respectively. The absorption band at 1279–1249 cm$^{-1}$ were ascribed to C = S, respectively, for the compounds (4–6). The absorption band at 980 cm$^{-1}$ shows the presence of C–F in the compound (6). In $^1$H NMR study the downfield singlet at $\delta$11.74–10.48 was ascribed to NH while in $^{13}$CNMR study, the signals at $\delta$150.5–152.5 and $\delta$155.5–156.2 confirm the presence of C = S and C = N groups, respectively, in the compounds (4–6). Finally the presence of distinct molecular ion peak [M$^+$] at m/z: 416, 418 and 450 in the MS spectra also proved the formation of the compounds (4–6), respectively. The strategy can also be applied to varied glucocorticoids which may allow further modifications on the substituted heterocyclic moiety.

3. Rule of Five and bioactivity score

For biological activity the Lipinski’s rule was used as a filter to choose the reasonable scaffolds [2,23–26]. The physicochemical parameters which include octanol partition coefficients (C log P), Mw, HBD, HBA and TPSA were calculated using ChemBioOffice2015. Molinspiration server (http://www.molinspiration.com/cgi-bin/properties) was used to calculate the bioactivity score. The Lipinski’s rule which is widely used as a filter for drug-like properties states that most molecules with good membrane permeability have log P $\leq$ 5, molecular weight $\leq$ 500, number of hydrogen bond acceptors $\leq$ 10, number of hydrogen bond donors $\leq$ 5. This rule is widely used as a filter for drug-like properties. As can be seen in (Table 2) the synthesized compounds (4–6) showed no violation of Lipinski rules. The results displayed that all the compounds (4–6) having polar surface area less than 140 Å$^2$. Moreover, the synthesized compounds (4–6) acquired rotatable bonds less than 10.

The observed results showed that the synthesized compounds obeyed the Lipinski’s “Rule of Five” analysis based on a computational prediction of molecular and pharmacokinetic properties [27] and have good oral absorption. The exceptions to the Lipinski’s rule were recognized and involve anticancer drugs such as Doxorubicin [28,29]. The physicochemical properties of the synthesized compounds are sensible starting point for a drug discovery effort. The bioactivity scores of the synthesized compounds (4–6) were also calculated for six criteria, GPCR ligand activity, ion channel modulation, kinase inhibition activity, protease inhibitor, enzyme inhibitor and nuclear receptor ligand activity (Table 3).

As a general rule, for a synthesized compound to be active, larger is the bioactivity score, higher is the probability. For organic molecules if the bioactivity score is ($>0.00$), then the compound is active, but if it is between $-0.50$ and $0.00$ then the compound is moderately active and if the compound ($<-0.50$), then the compound is inactive. The results are mentioned in (Table 3), showed that some compounds are biologically active, some are moderately active and some are inactive molecules.

3.1. DNA breakage by compounds 4–6 in normal cellular lymphocytes as measured by comet assay

The ability of compounds (4–6) to cause DNA strand breaks in a cellular system of normal human peripheral lymphocytes as measured by standard comet assay shown in Figure 1, although the compounds (4–6) tested cause significant breakage of cellular DNA in isolated normal lymphocytes. The degree of such breakage is found to be maximum in compound (6) and is found of the order of 6 $>5>4$. The increase in DNA damage suggested that glucocorticoids derivative induced dose-dependent fragmentation of chromosomal DNA leading to necrobiosis.

4. DNA binding experiments

4.1. Fluorescence spectroscopic studies

The various molecular interactions such as excited state reactions, molecular rearrangements, energy transfer and collision are studied by using the technique of fluorescent quenching [30]. In the present study, we have undertaken the fluorescent quenching technique to investigate the interaction of glucocorticoid oxadiazine derivatives (4–6) with CT DNA [31]. The compounds (4–6) were excited at their respective $\lambda_{max}$. Calf thymus DNA was added to exhibit a gradual increase in fluorescence emission intensity which implied that compounds (4–6) reveal strong interaction with DNA as illustrated in Figure 2. The increase in the emission intensity is greatly due to the extent to which the molecule is inserted into the hydrophobic environment of DNA. Since the surroundings inside the DNA helix is hydrophobic it lessens the accessibility of solvent

| Comp. | Mw | ClogP | HBD | HBA | TPSA | Rotatable bonds | No violations |
|-------|----|-------|-----|-----|------|-----------------|--------------|
| 4     | 416.54 | 2.33  | 3   | 6   | 91.15 | 1               | 0            |
| 5     | 418.56 | 2.35  | 3   | 6   | 91.15 | 1               | 0            |
| 6     | 450.53 | 1.40  | 4   | 7   | 111.38 | 1               | 0            |

Table 2. Estimated physicochemical properties of oxadiazine derivative of glucocorticoids (4–6).

Table 3. Bioactivity scores of oxadiazine derivative of glucocorticoids (4–6).

| Comp. | GPCR ligand | Ion channel | Kinase inhibitor | Protease inhibitor | Nuclear Receptor ligand | Enzyme inhibitor |
|-------|-------------|-------------|------------------|-------------------|-------------------------|-----------------|
| 4     | $0.13$      | $-0.21$     | $-0.89$          | $-0.01$           | $0.91$                  | $0.69$          |
| 5     | $0.05$      | $-0.26$     | $-1.02$          | $-0.17$           | $0.82$                  | $0.59$          |
| 6     | $0.12$      | $-0.12$     | $-0.68$          | $0.40$            | $1.15$                  | $0.72$          |
molecules at the binding site and thus precluding the quenching effect. The binding of the compound to the DNA helix could decrease the collisional frequency of solvent molecules with the compound which leads to the emission enhancement of the compound.

4.2. Absorption studies

The absorption spectra are used to determine the binding strength and the mode of DNA binding with small molecules [32]. The UV-vis spectra Figure 3 of compounds 4–6 with increasing concentrations of CT DNA exhibited an intense absorption band centred at 280 nm for compound (6). The ratio of the absorbance of Ct-DNA at 170 and 280 nm in 10 mM Tris-HCl buffer (pH 7.2) was taken. The concentration of Ct-DNA was determined from its absorption intensity at 260 nm with a molar extinction coefficient of 6600 M\(^{-1}\) cm\(^{-1}\) [33]. The experiments were performed by titrating increasing concentrations of DNA to a fixed concentration of each complex. The blanks were taken for each tube comprising an equal amount of Ct-DNA present in the sample without any complex. After baseline correction using DNA solution as a blank and the observed absorbance is due to the occurrence of the complex with DNA.

4.3. Viscometric measurements

Viscosity measurements were performed on calf thymus DNA with the endeavour of clarifying the binding mode of the compounds (4–6) to DNA by varying the concentration of the compounds added. Due to the increase in separation of the base pairs at the intercalation sites a classical intercalative molecular interaction causes a significant increase in viscosity of the DNA solution and hence an increase in the overall DNA length was observed.

For compounds (4–6), the viscosity of calf thymus DNA decreases with increase in the ratio of compounds to calf thymus DNA, due to the interaction of compounds with DNA generating bends in the DNA strand. The values of relative viscosity (\(\eta/\eta_0\)), where \(\eta\) and \(\eta_0\) are the specific viscosities of DNA in the presence and absence of the compounds, were determined and plotted against values of 1/R (R = [Compound]/[DNA]) (Figure 4).

4.4. Steady-state binding experiment

A steady-state competitive binding experiment of the compounds (4–6) with an anionic quencher K\(_4\) [Fe(CN)\(_6\)]\(^{-1}\) which may afford further information about the binding of the compound to DNA was carried out. In the absence and presence of DNA, compounds (4–6) were proficiently quenched by K\(_4\) [Fe(CN)\(_6\)]\(^{-1}\) [34]. Stern–Volmer plot of I/I\(_0\) vs K\(_4\) [Fe(CN)\(_6\)]\(^{-1}\) for the three complexes are illustrated in Figure 5.

4.5. Molecular docking

Molecular docking has gained a growing interest in the investigation of binding interaction mechanism of biological macromolecules with small molecules. It plays an important role to understand the drug-DNA interactions for rational drug designing and discovery. Molecular docking studies of nucleic acids and their complexes can provide valuable information that is not available by other experimental techniques.
Figure 2. Fluorescence spectra of the compounds 4–6 with increasing concentration of calf thymus DNA in molar base pair ratio. [Tris-HCl buffer (0.01 M, pH 7.2)].

This method gives the possibility to study an active site in detail and can be used for hit identification, virtual screening and binding mode determination. In the present work, molecular docking studies were performed to predict the binding modes of compounds (4–6) with DNA duplex of sequence d(CGCGAATTCGCG)₂ (PDBID: 1BNA), and provide an energetically favourable docked structures Figure 6. The minimized conformation of compounds (4–6) sitting in the groove of the sequence d(CGCGAATTCGCG)₂ show hydrogen bonding with the oxygen of the oxadiazine ring and –OH group of glucocorticoid moiety in the docked pose of the dodecamer. Generally, the more negative the relative binding energy,
Figure 3. Absorption spectra of the compounds 4–6 with increasing concentration of calf thymus DNA in molar base pair ratio. [Tris-HCl buffer (0.01 M, pH 7.2)].

Figure 4. Effect of increasing amount of compounds 4–6 on the relative viscosity of calf thymus DNA.

Figure 5. Emission quenching curves of compounds 4–6 in the absence & presence of calf thymus DNA.

The stronger is the interaction between molecule and DNA and more stable is the compound formed. Thus, the compound formed between compound (6) and DNA with relative binding energy of $-8.6 \text{ kcal mol}^{-1}$ might be having highest stability than compound (5) ($-8.4 \text{ kcal mol}^{-1}$) and (4) ($-7.9 \text{ kcal mol}^{-1}$) in the order $6 > 5 > 4$. Irrespective of the absence of any net positive charge on compounds (4–6), negative values of the relative binding energies indicated a higher binding potential of these compounds with DNA. Thus, we can conclude that there is a mutual complement between spectroscopic techniques and molecular modelling, which can provide valuable information about the mode of interaction of the compounds with DNA and the conformation constraints for adduct formation.
5. Materials and methods

Chemicals were purchased from Merck and Sigma-Aldrich as analytical grade and used without further purification. Melting points were determined on a Bio-gen digital auto melting point apparatus. The IR spectra were recorded on KBr pellets with PyeUnicam SP3-100 Spectrophotometer and values are given in cm$^{-1}$. $^1$H and $^{13}$C NMR spectra were run in CDCl$_3$ on a Bruker Avancell 400 NMR Spectrometer (operating at 400 MHz for $^1$H and at 100 MHz for $^{13}$C NMR) with TMS as internal standard and values are given in parts per million (ppm) ($\delta$). Mass spectra were recorded on a JEOL D-300 Mass spectrometer. Elemental analysis was recorded on Perkin Elmer 2400 CHN Elemental Analyzer. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapours to check the homogeneity as well as the progress of the reaction. Sodium sulphate (anhydrous) was used as a drying agent.
Agarose, Calf thymus DNA, low melting point agarose (LMPA), RPMI 1640, Trypan blue, Histopaque 1077 and phosphate buffered saline (PBS) Ca²⁺ and Mg²⁺ free were purchased from Sigma (St. Louis, MO). Compounds (4–6) were dissolved in 2 mM dimethyl sulfoxide before use as a stock of 1 mM solution. When added to reaction mixtures, in the presence of buffers concentrations talk about, all the compounds employed stayed in solution. The quantity of stock solution added did not initiate significant change in the pH of reaction mixtures.

5.1. Synthesis of oxadiazine derivative of glucocorticoids (4–6)

To a boiling solution of glucocorticoid (1–3) (1.38 mmol) in EtOH (7.5 mL), a few drops of conc. HCl were added followed by thiosemicarbazide and its derivatives (1.34 mmol) in EtOH (4.5 mL) with stirring. The reaction mixture was refluxed and stirred for about 24 h. After completion of reaction (monitored by TLC), the heavy precipitate was formed which was suspended in water (30 mL), filtered using suction and air dried. For further purification, the compounds were recrystallized from EtOH to give desirable products (4–6).

5.2. 3′,6′-Dihydro-2H-1′,3′,4′-oxadiazine-2′-thione-5′(17)-prednisolone (4)

Yellow powder, mp 183–184°C, yield: (80%). IR (KBr) $\nu_{max}/cm^{-1}$ 3424 (NH), 1651 (C = N), 1061 (C–O) and 1249 (C = S). $^1$H NMR (400 MHz, CDCl₃): $\delta$ 10.48 (s, 1H, NH), 6.14 (s, 1H, C₁₇–OH), 4.12 (m, 1H, C₁₁–αH), 1.26 (s, 3H, C₁₀–CH₃), 0.88 (s, 3H, C₁₃–CH₃). $^{13}$C NMR (100 MHz, CDCl₃): $\delta$ 153.55 (C₁), 130.32 (C₂), 180.21 (C₃), 120.40 (C₄), 160.24 (C₅), 39.15 (C₆), 39.32 (C₇), 37.63 (C₈), 48.51 (C₉), 54.93 (C₁₀), 78.32 (C₁₁), 46.21 (C₁₂), 79.05 (C₁₃), 49.71 (C₁₄), 37.62 (C₁₅), 39.80 (C₁₆), 40.10 (C₁₇), 15.11 (C₁₈), 16.90 (C₁₉), 155.55 (C₅′), 70.91 (C₆′), 150.55 (C₂′). MS (EI): m/z 416 [M⁺]. Anal. Calcd. (%) for C₂₂H₂₇FN₂O₅S (450): C, 58.65, H, 6.04, N, 6.22, found: C, 58.61, H, 6.02, N, 6.25.

5.3. 3′,6′-Dihydro-2H-1′,3′,4′-oxadiazine-2′-thione-5′(17)-hydrocortisone (5)

Yellow powder, mp 146–148°C, yield: (78%). IR (KBr) $\nu_{max}/cm^{-1}$ 3427 (NH), 1595 (C = N), 1055 (C–O) and 1279 (C = S). $^1$H NMR (400 MHz, CDCl₃): $\delta$ 11.74 (s, 1H, NH), 3.84 (s, 1H, C₁₁–OH), 5.70 (s, 1H, C₁₇–OH), 3.58 (m, 1H, C₁₁–αH), 1.29 (s, 3H, C₁₀–CH₃), 0.86 (s, 3H, C₁₃–CH₃). $^{13}$CNMR (100 MHz, CDCl₃): $\delta$ 34.52 (C₁), 32.21 (C₂), 186.25 (C₃), 125.11 (C₄), 172.23 (C₅), 31.55 (C₆), 32.35 (C₇), 30.31 (C₈), 57.72 (C₉), 38.61 (C₁₀), 67.54 (C₁₁), 42.22 (C₁₂), 48.02 (C₁₃), 49.51 (C₁₄), 22.44 (C₁₅), 29.53 (C₁₆), 82.11 (C₁₇), 16.45 (C₁₈), 19.55 (C₁₉), 155.83 (C₅′), 81.11 (C₆′), 151.51 (C₂′). MS (EI): m/z 418 [M⁺]. Anal. Calcd. (%) for C₂₂H₃₀N₂O₄S (418): C, 63.13, H, 7.22, N, 6.69, found: C, 63.17, H, 7.19, N, 6.72.

5.4. 3′,6′-Dihydro-2H-1′,3′,4′-oxadiazine-2′-thione-5′(17)-triamcinolone (6)

Yellow powder, mp 195–197°C, yield: (75%). IR (KBr) $\nu_{max}/cm^{-1}$ 3421 (NH), 1596 (C = N), 1055 (C–O), 980 (C–F) and 1278 (C = S). $^1$H NMR (400 MHz, CDCl₃): $\delta$ 10.66 (s, 1H, NH), 4.36 (s, 1H, C₁₁–OH), 6.00 (s, 1H, C₁₆–OH), 6.26 (s, 1H, C₁₇–OH), 3.51 (m, 1H, C₁₁–αH), 3.93 (brs, 1H, C₁₆–CH₂), 1.40 (s, 3H, C₁₀–CH₃), 0.88 (s, 3H, C₁₃–CH₃). $^{13}$CNMR (100 MHz, CDCl₃): $\delta$ 154.21 (C₁), 130.25 (C₂), 178.22 (C₃), 129.13 (C₄), 177.80 (C₅), 29.91 (C₆), 25.44 (C₇), 35.65 (C₈), 98.22 (C₉), 47.51 (C₁₀), 71.25 (C₁₁), 30.25 (C₁₂), 35.53 (C₁₃), 40.73 (C₁₄), 29.61 (C₁₅), 75.53 (C₁₆), 86.32 (C₁₇), 17.22 (C₁₈), 20.15 (C₁₉), 156.20 (C₅′), 60.51 (C₆′), 152.51 (C₂′). MS (EI): m/z 450 [M⁺]. Anal. Calcd. (%) for C₂₂H₂₇FN₂O₅S (450): C, 58.65, H, 6.04, N, 6.22, found: C, 58.61, H, 6.02, N, 6.25.

6. Isolation of lymphocytes

From a single healthy donor heparinized blood samples (2 mL) were achieved by venepuncture and diluted suitably in Ca²⁺ and Mg²⁺ free PBS. Histopaque 1077 (Sigma) was used to isolate lymphocytes from blood and the cells were finally overhanging in RPMI 1640. For all the experiments a single donor donated the blood.

7. Viability assessment of lymphocytes

Trypan Blue Exclusion Test [35] was used to check the viability of lymphocytes before and after the reaction.

7.1. Treatment of lymphocytes and evaluation of DNA breakage by alkaline single-cell gel electrophoresis (Comet assay)

The method employed by Singh et al. [36] was used to perform comet assay with minor modifications. Fully frosted slides precoated with 1.0% normal melting agarose were applied. After treatment with compounds (4–6) about 10,000 cells were mixed with 90 µL of 1.0% LMPA to form a cell suspension and were pipetted over the first layer and covered with a cover slip. To solidify the agarose the slides were kept on an ice pack for 10 min. The coverslips were smoothly taken out and a third layer of 0.5% LMPA was pipetted over the first layer and covered with a cover slip. The slides were again covered with cover slips and were placed on ice packs to solidify. The slides were immersed in ice-cold lysis buffer for an hour after removing the cover slips. After lysis, the DNA was allowed to unwind in alkaline electrophoretic solution (300 mM NaOH, 1 mM EDTA, pH > 13). Electrophoresis was carried out in a field strength of 0.7 V/cm and 300 mA current at 4°C. The slides were neutralized with...
8. Statistics

The statistical analysis was performed as described by Tice et al. [37] and is expressed as mean ± SEM/SD of three independent experiments. Variance analysis was performed using ANOVA, p-values < .05 were considered statistically significant.

9. DNA binding experiments

9.1. Absorption and fluorescence spectroscopy

The absorption and fluorescence spectroscopy was done using the standard methods reported in the literature [38, 39]. The absorption spectra of the complexes (4–6) were recorded on UV-1800 Shimadzu spectrophotometer. All experiments were carried out by fluorometric titration using a Shimadzu spectrophotometer-5301 (Japan) equipped with constant temperature holder attached with Neslab RTE-110 water bath with an accuracy of ±0.1°C. The excitation was done at 240 nm while the emission spectra (280–380 nm) was obtained for all complexes with the widths of both slits (excitation and emission) were set to 5 small fluorescence studies. The fluorescence experiment was performed using complex (10 µM) with the varying range of concentrations of Ct-DNA.

9.2. Viscosity measurements

Viscosity measurements confirmed to the standard methods when DNA binding studies were carried out and practices previously explained in the literature [40–43]. Viscosity measurements were performed from observed flow time of CT-DNA containing a solution (t > 100 s) corrected for the flow time of buffer alone (t0), using Ostwald’s Viscometer at 25 ± 0.01°C. Digital stopwatch was used to measure flow time.

9.3. Molecular docking studies

Molecular docking studies were carried out using Autodock 4.0 to study the interaction of compounds (4–6) with B-DNA. The Lamarckian genetic algorithm used for calculations were employed in Autodock 4.0 which was proven to be more reliable, sensitive and effective [44–46]. The crystal structure of B-DNA dodecamer d(CGCAAATTTGCC)2 (PDB ID: 1BNA) were downloaded from the protein data bank (http://www.rcsb.org/pdb). The three-dimensional structure of compounds (4–6) were drawn using Chem Draw 12.0 and the structures were saved in.sdf format which was converted to.pdb format by Avogadro’s 1.01 [47]. All water molecules were removed before performing docking and Kollmann charges were introduced along with the polar hydrogen atoms. The size of the grid was set to 52, 64 and 102 along the x-axis, y-axis and z-axis respectively, with a spacing of 0.542 Å having centre of grid at x = 14.7, y = 20.9, z = 8.8. Out of different conformers docked, conformer with the minimum energy was taken into account. Docked conformation analysis was done using Accelrys Discovery Studio 4.0.

10. Conclusions

The effectively developed, expedient and operationally simple strategy for improved synthesis of glucocorticoid oxadiazines was successful. The synthesized compounds were subjected to DNA binding studies, and the results show that compounds (4–6) were found to be of order 6 > 5 > 4. Necrobiosis and genotoxicity were depicted by comet assay. Both absorbance and fluorescence studies showed the interaction of compounds (4–6) with DNA suggesting the stabilization of the energy levels of the compounds in the presence of DNA. Further, the relative viscosity index and Stern–Volmer indices were found to be maximum for compound (6) followed by (5) and (4), respectively, suggesting the significance of a structural feature of the glucocorticoids. The cleavage and molecular docking studies carried out in the present work which are in total agreement with the primary intercalative interactions can also be argued. Hence, the present work has shown that these synthesized compounds can be used as a template for future development by designing more potent and selective cytotoxic agents.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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