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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthetic utility of sydnones: synthesis of pyrazolines derivatized with 1,2,4-triazoles as antihyperglymic, antioxidant agents and their DNA cleavage study

Tasneem Taj · Ravindra R. Kamble · Pramod P. Kattimani · Bharati V. Badami

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Abstract Ring transformation of sydnone (**1a–i**) to 1,3,4-oxadiazoline-2-one (**2a–i**) was carried out using bromine in acetic anhydride. The compounds (**2a–i**) on heating with hydrazine hydrate gave 1,2,4-triazole (**3a–i**) in good yields. The structure of these unknown compounds was confirmed by IR, ^{1}H NMR, MS and elemental analysis. Further, these compounds were evaluated for the extent of penetration into biological membranes (clog*P*) drug likeliness and finally drug score was calculated. The title compounds were also screened for their antihyperglycemic, DNA cleavage and antioxidant activity.

Keywords 1,3,4-Oxadiazoles · 1,2,4-Triazoles · Drug score · Antihyperglycemic activity · DNA cleavage · Antioxidant activity

Introduction

Sydnones are representatives of mesoionic heterocyclic compounds which possess a broad spectrum of pharmacological activities and have been used as synthons in 1,3-dipolar cycloadditions to be transformed to various amide derivatives, 1,3,4-oxadiazole, pyrazole, phenyl indazole, pyrazoline and tetrazine (Taj *et al.*, 2011, Ohta and Kato, 1969; Mallur and Badami, 2000; Browne and Harrity, 2010; Wu *et al.*, 2010).

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1,3,4-Oxadiazole derivatives have a highlighted chemistry (Bloom and Murray, 1992; Barnes *et al.*, 1991; Fujiwara *et al.*, 1997). Several five-membered aromatic systems having three hetero atoms at symmetrical position such as 1,3,4-oxadiazoles have been synthesized with various substituted aromatic acids in presence of $POCl_3$ (Chudgar *et al.*, 1989). Typically, the synthesis was promoted by heat and reagents such as thionyl chloride, phosphorous pentoxide, triphenylphosphine and triflic anhydride (Al-Talib *et al.*, 1990; Theocharis and Alexandrou, 1990; Carlsen and Jorgensen, 1994; Brown *et al.*, 1997; Liras *et al.*, 2000).

The common synthetic approaches to oxadiazoles (Löffler and Schobert, 1997) also involve cyclisation of diacylhydrazines.

The azole moiety is important and much attention has been focused on 1*H*-1,2,4-triazole derivatives (Sweetman, 2004; Herbrecht *et al.*, 2002) for their broad-spectrum activities such as fungicidal, herbicidal, DNA cleavage, antioxidant, anticonvulsant and also used as plant growth regulators (Fig. 1) (Chai *et al.*, 1989; Stankovsky *et al.*, 1993; Ferrer *et al.*, 2004; Andreadou *et al.*, 2002; Ragenovic *et al.*, 2001).

Many synthetic studies have been focused on the design of analogs of oxadiazoles and biological properties of 1,2,4-triazoles in our laboratory (Kattimani *et al.*, 2011). In view of this we have designed the title compounds bearing 1*H*-1,2,4-triazole in a molecular framework and the synthesis involved the ring transformation of sydnone to 1,3,4-oxadiazole followed by nitrogen insertion to get the title compounds through the proposed reaction (Scheme 1).

Chemistry

3-[4-(5-Aryl-4,5-dihydro-1*H*-pyrazolin-3-yl)-phenyl] sydnone (**1a–i**) upon reaction with bromine in acetic anhydride

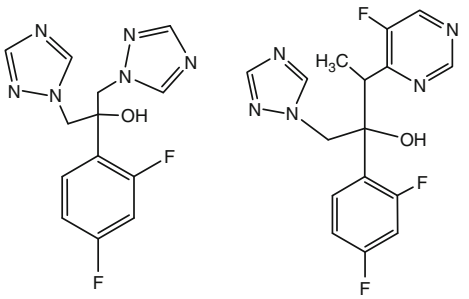


Fig. 1 Structurally related bioactive compounds

gave 3-[4-(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-phenyl]-5-methyl-3*H*-[1,3,4]-oxadiazol-2-one (**2a–i**) and further ring insertion with hydrazine hydrate yielded 2-[4-(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-phenyl]-4-amino-5-methyl-2,4-dihydro-[1,2,4]-triazol-3-one (**3a–i**).

The mechanism for the conversion of the compounds (**1a–i**) to the compounds (**2a–i**) and further ring insertion with hydrazine hydrate to final product (**3a–i**) proposed in (Scheme 2).

Results and discussion

The structures of the compounds (**2a–i**) and title compounds (**3a–i**) were confirmed by IR, ^{1}H NMR and mass spectral and elemental analysis. All the compounds (**2a–i**) have shown a common strong absorption band around 1,775–1,781 cm^{-1} and a medium intensity sharp band around 1,650–1,660 cm^{-1} due to carbonyl of acetyl group, sharp common band around 1,580–1,604 cm^{-1} appeared for C=N stretching frequencies of pyrazoline ring.

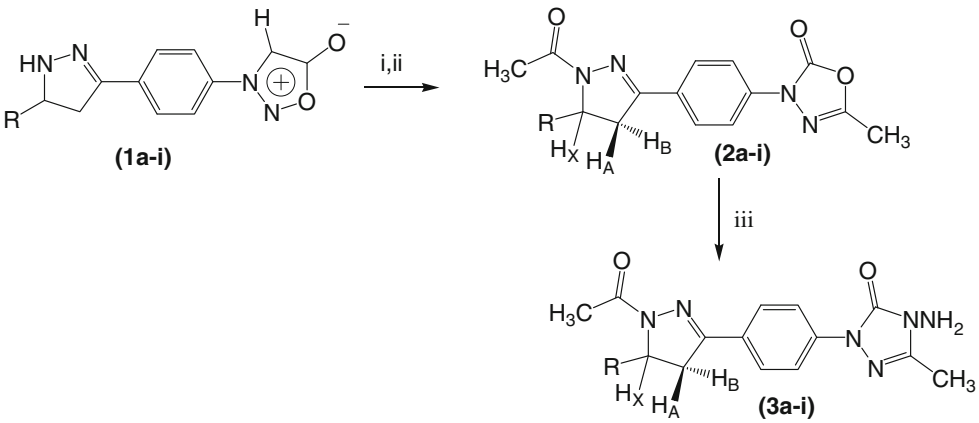
The final compounds (**3a–i**) have shown common strong absorption bands around 3,310–3,320 cm^{-1} (NH_2 group), 1,697–1,710 cm^{-1} (carbonyl of triazoline ring) and a medium intensity sharp band around 1,650–1,660 cm^{-1} (carbonyl of *N*-acetyl group). Another sharp band is observed in the range 1,580–1,604 cm^{-1} (C=N) stretching frequencies of pyrazoline and triazoline ring. In the compounds **2i** and **3i**, a broad band around 3,400 cm^{-1} appeared indicating the presence of OH group.

The ^{1}H NMR spectral analysis of all the compounds (**2a–i**) and (**3a–i**) showed a singlet around 2.12–2.45 ppm for three protons due to methyl protons of the *N*-acetyl group. Whereas, the compounds (**2a–i**) have shown a singlet for three protons in the range 2.38–2.55 ppm due to protons of the methyl group attached at C_5-carbon of the 1,3,4-oxadiazole ring. Similarly, the compounds (**3a–i**) have shown a singlet in the range 2.31–2.55 ppm attributed to the protons of the methyl group attached at the C_5-carbon of triazole ring.

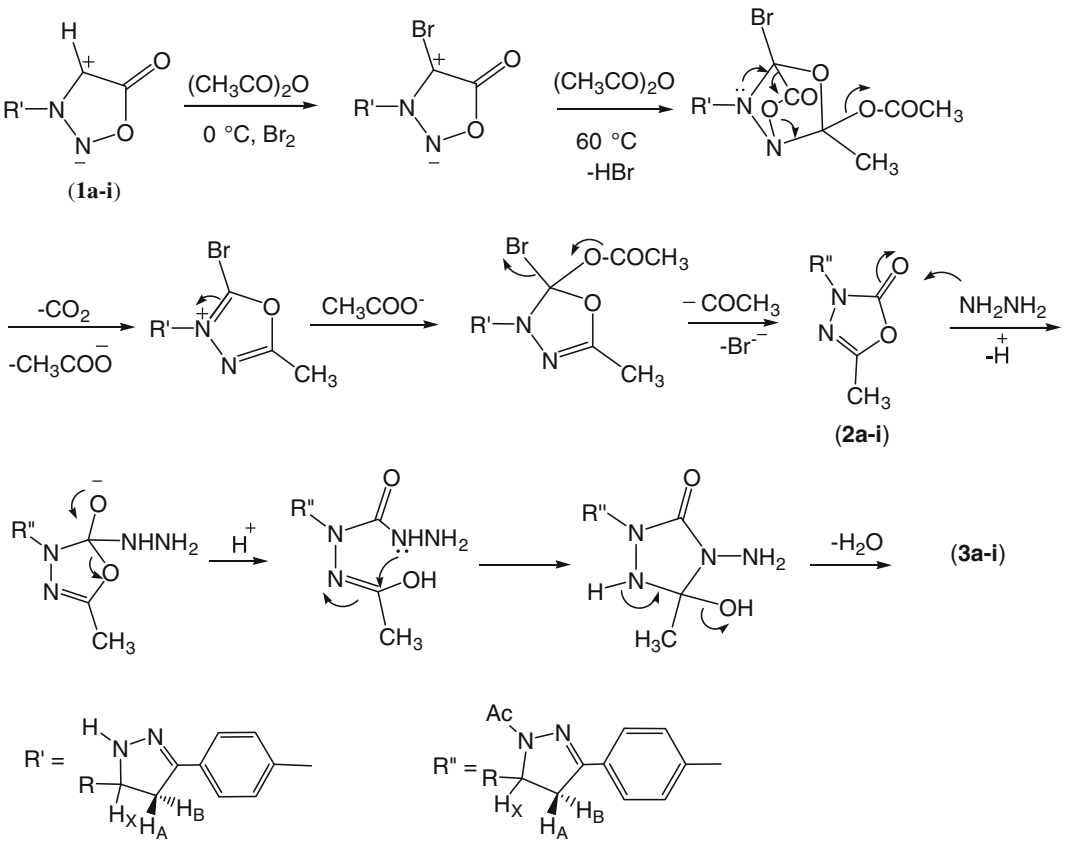
The protons of pyrazolin ring, i.e. a methine proton and the diastereotopic methylene protons show a characteristic feature. The methylene protons can be assigned as H_A, H_B and the methine proton as H_X. The H_A proton appears as doublet of doublet in the range δ 3.48–3.73 ppm. The H_B also appears as doublet of doublet at 3.08–3.47 ppm. The H_x always appears as four line spectrum in the range δ 4.32–4.92 ppm. Aromatic protons appear around 7.0–8.0 ppm. The compounds (**3a–i**) show a singlet (D-$_2$O exchanged) due to NH group in the range 6.80–8.10. The compounds **2i** and **3i** show a broad singlet (D_2O exchanged) at around 8.0 ppm due to OH proton.

Molecular osiris property explorer

A new molecule to qualify as a drug molecule, it has to be analysed for the parameters set by Lipinski's rule of five (Lipinski, 2004; http://www.organic-chemistry.org/prog/peo/; Emami *et al.*, 2011; Sangamwar *et al.*, 2007). This is a thumb rule to evaluate drug likeliness to determine whether a compound with a certain pharmacological activity has properties that make it a likely orally active drug in humans. It is important for drug development where pharmacologically active lead structure is optimised step wise for increased activity, selectivity, as well as drug-like properties. Also, these



Scheme 1 Ring transformation of sydnone (**1a–i**) to 1,3,4-oxadiazolin-2one (**2a–i**) followed by ring insertion of hydrazine hydrate to 1,2,4-triazole (**3a–i**). Reagents and conditions: (*i*) Br_2, Ac_2O 0–5°C, (*ii*) 60°C, (*iii*) $NH_2NH_2{\cdot}H_2O$, 6 h reflux. *a* Phenyl, *b* *o*-chlorophenyl, *c* *m*-chlorophenyl, *d* *p*-chlorophenyl, *e* *p*-nitrophenyl, *f* *p*-tolyl, *g* *p*-hydroxyphenyl, *h* *p*-anisyl, *i* 3,4-dimethoxy



Scheme 2 Proposed mechanism for the formation of compounds (**2a–i**) and (**3a–i**)

properties are important for a drug pharmacokinetics in human body, including its absorption, distribution, metabolism and excretion (ADME). The modification of the molecular structure often leads to drugs with higher molecular weight, more rings, more rotatable bonds and higher lipophilicity. The rule states that in general an orally active drug has no more than 5 hydrogen bond donors, and not more than 10 hydrogen acceptors. A molecular weight under 500 and partition coefficient (clog*P*) less than 5 do not qualify as a drug molecule. The title compounds do not violate the Lipinski rule and they fall well in the range. All the title compounds showed clog*P* well within the range and also molecular weights less than 500. The drug likeliness ranged from 3 to 7 where as the drug score ranged from 0.12 to 0.35. The *chloro* substituent in compounds **2b**, **2c**, **2d**, **3b**, **3c**, **3d** was shifted to different positions (*ortho*, *para*, *meta*) to study the change on the positional effect of substituent. Interestingly, these compounds showed almost the same drug score which indicating that there is no positional effect on these compounds. The compounds **2e** and **3e** which have electron withdrawing nitro group showed drug likeliness in a negative value and also the drug score was less. The compounds **2f**, **2h**, **3f** and **3h** which have electron donating groups show almost same drug score as depicted in (Table 1). The compounds **2g** and **3g** have also shown diversified effects, and **2i**, **3i** with two electron donating methoxy groups based on drug likeliness and drug score, but clog*P* values are well within the range as mentioned by the rule.

Amylase inhibition assay

The basic heterocyclic rings present in the various medicinal agents are 1,2,3-triazole and 1,2,4-triazole. A large volume of research has been carried out on triazole and their derivatives, which has proved the pharmacological importance of this heterocyclic nucleus (Rajeev *et al.*, 2011). This study is an attempt to study the antihyperglycemic activities of triazole (**3a–i**) derivatives (Fig. 2).

In this study, the compounds **3a** (phenyl), **3d** (*p*-chloro) and **3e** (*p*-nitro) have shown strong inhibitory activity. **3b** (*o*-chloro), **3g**, **3h**, **3i** (hydroxyl, $-OCH_3$, 3,4-dimethoxy) have exhibited good inhibitory activity against α-amylase compared with the control enzyme inhibitor (Fig. 3). Some of the derivatives like **3c** (*m*-chloro) and **3f** (*p*-tolyl) have shown moderate activity. The bar graph representation of comparative activities of the title compounds (**3a–i**) is shown in (Table 2).

Table 1 Pharmacological parameters for bioavailability of compounds (**2a–i**) and (**3a–i**)

Entry no.	clog*P*	Mol wt	Druglikeness	Drug score
2a	3.43	362	−5.22	0.35
2b	4.04	396	−5.53	0.28
2c	4.04	396	−6.02	0.28
2d	4.04	396	−4.33	0.28
2e	3.30	407	−16.08	0.31
2f	3.74	376	−7.33	0.31
2g	3.13	378	−5.66	0.36
2h	3.32	392	−5.81	0.34
2i	3.22	422	−5.63	0.33
3a	1.94	376	3.51	0.27
3b	2.55	410	3.19	0.23
3c	2.55	410	2.72	0.22
3d	2.55	410	4.43	0.23
3e	1.81	421	−7.32	0.12
3f	2.55	390	1.41	0.23
3g	3.16	392	3.06	0.27
3h	1.83	406	2.89	0.26
3i	1.73	436	3.08	0.25

It is apparent that the C_5 position of the pyrazoline ring is sterically sensitive and this overides any acidity contributions (e.g., from phenyl, 4-nitrophenyl). The lipophilicity range of active compounds is quite narrow and this parameter too can overide acidity through hindrance of oral absorption. It is also possible that a specific tautomeric form of the heterocycle is required for in vivo activity (Kees *et al.*, 1995), tailed significance and hence the compounds **3a** (phenyl) and **3e** (nitro phenyl) groups show strong inhibitory activity. Electron-withdrawing character, associated with a *chloro* or *bromo* group appears to be optimal for antihyperglycemic activity (Ellingboe *et al.*, 1993). Hence, compound **3d** with *p*-chloro phenyl moiety exhibits strong antihyperglycemic activity.

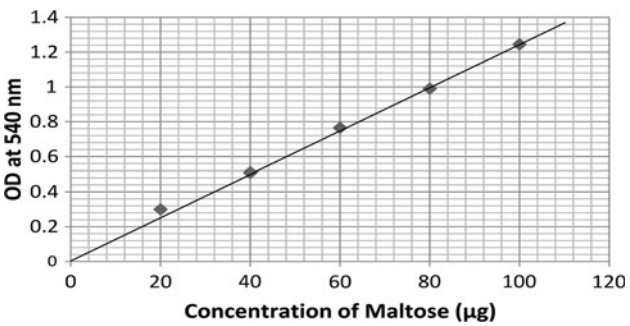


Fig. 2 Standard maltose curve

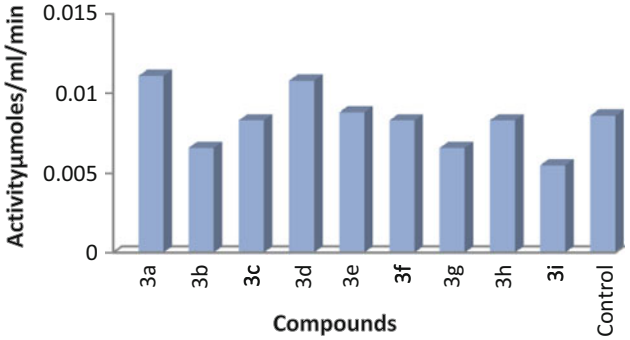


Fig. 3 Antihyperglycemic activity of the compounds (**3a–i**)

Table 2 Antihyperglycemic activity of compounds (**3a–i**)

Sample	OD 540 nm	*C* (µg)	Activity (µmoles/ml/min)	% Activity
3a	0.50	40	0.0111	129.07
3b	0.19	24	0.0066	76.74
3c	0.40	30	0.0083	96.51
3d	0.49	39	0.0108	125.58
3e	0.41	32	0.0088	102.33
3f	0.40	30	0.0083	96.51
3g	0.19	24	0.0066	76.74
3h	0.40	30	0.0083	96.51
3i	0.14	20	0.0055	63.95
Control	0.40	31	0.0086	100.00

C concentration of maltose liberated

T test analysis for amylase inhibition assay

T test analysis is carried out to check the level of confidence compared to the control drug (Landau and Everitt, 2004; SPSS, 2008[1]) which is done using the "Statistical Package of Programs" (SPSS). It is a package of programs for manipulating, analysing and presenting data; the package is widely used in the social and behavioural sciences. There are several forms of SPSS. The core program is called SPSS Base and there are a number of add-on modules that extend the range of data entry, statistical, or reporting capabilities. According to this statistical analysis, amylase inhibition assay indicated the two-tailed significance which lies at 0.595 and is well within the permissible value for the 95% confidence interval of the difference as compared to the control. Hence, it can be inferred that

[1] SPSS is a computer programme used for survey authoring and deployment (IBM data Collection), data mining (IBM SPSS Modeler), text analytics, statistical analysis and collaboration and deployment (batch and automated scoring services). The *t* test is used to test the null hypothesis that the means of two populations are the same, H_0: $\mu_1 = \mu_2$, when a sample of observations from each population is available. The observations made on the sample members must all be independent of each other. One sample *t* tests can be used to determine if the mean of a sample is different from a particular value.

antihyperglycemic activity is excellent for the compounds (**3a–i**) (given in Supplementary information).

Antioxidant activity (DPPH method)

Free radical scavenging is one of the best known mechanisms by which antioxidants inhibit lipid oxidation. The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method assay was developed to determine the reducing ability of biological fluids and aqueous solutions of pure compounds. Butylated hydroxyl anisole (BHA) was used as a standard. Although, the final dilution of the sample in the reaction mixture was high (100 µg), any influence of the solvent on the reaction was also checked. The values of absorbance at 700 nm compared to BHA with concentrations of 10, 50, 100 µg are given in Table 3 and are represented in Fig. 4.

The compounds **3d** and **3f** (*p*-chloro and *p*-tolyl) have shown very good activity compared to BHA at all the concentrations tested (Fig. 3). Compounds **3a**, **3b** and **3c** (phenyl, *o* and *m-chloro*) have shown moderate activity compared to the standard. Compound **3g** (hydroxyl) has shown less activity compared to the standard. Compounds **3e**, **3h** and **3i** (*p*-NO_2, *p*-OCH_3, 3,4-dimethoxy) have shown least activity compared to standard BHA. The antioxidant activity of these compounds may be related to their redox properties which allow them to act as reducing agents or hydrogen-atom donors, their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals. Such antioxidants function as free-radical scavengers and chain breakers, complexes of pro-oxidant metal ions and quenchers of singlet-oxygen formation (Scheme 3).

T test for free radical scavenging activity

One sample *t* test is used for DDPH activity for individual concentrations of 10, 50 and 100 µg. The two tailed significance lies at 0.29, 0.12 and 0.17 respectively. But the values are low as compared to the control BHA for the 95% confidence interval of the difference. Hence, it can be concluded that the compounds (**3a–i**) possess moderate to low activity (given in supplementary information).

DNA cleavage activity

A number of studies have shown that the clinical efficacies of many drugs correlate with their abilities to induce enzyme mediated DNA cleavage. The inhibitory potency of the test compounds was assessed by comparing the cleavage of DNA with the control used and presence of title compounds. The relative efficacy of the drugs to stimulate DNA cleavage varies considerably from one congener to another. A detailed comparison between the nine compounds (**3a–i**) tested allows us to make some

Table 3 Percentage of free radical scavenging activity

Entry no.	Test sample (µg)		
	10	50	100
3a	24.70	30.16	56.15
3b	20.00	67.83	78.38
3c	15.25	63.76	73.79
3d	21.66	69.87	81.48
3e	00.40	01.09	01.49
3f	23.00	70.10	82.45
3g	03.08	09.03	16.87
3h	00.60	02.28	02.40
3i	00.48	01.18	01.67
BHA	21.64	69.86	81.46

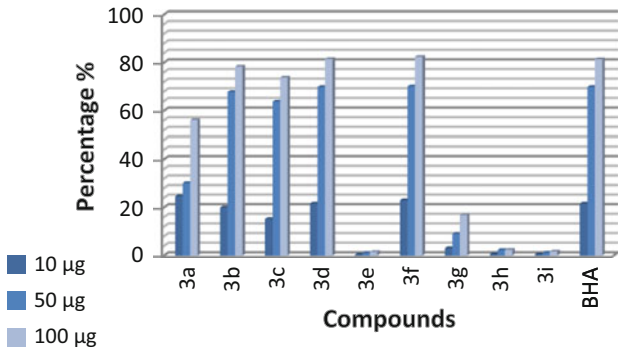


Fig. 4 Antioxidant activity of the compounds (**3a–i**)

significant observations. Studies show that 1,2,4-triazoles can be induced to undergo photoextrusion under conditions required for DNA cleavage, producing intermediates capable of hydrogen abstraction (Wender *et al.*, 1996).

After treatment with DNA samples **3g** and **3h** have cleaved the DNA completely. In **3g** lane (hydroxyl and methoxy substituent), whole band of DNA is missing which indicated the complete cleavage of DNA. DNA treated with remaining samples has shown partial cleavage (Fig. 5).

Experimental

Melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded on Nicolet Impact 5200 USA FT IR spectrophotometer using KBr pellets. ^{1}H NMR spectra were recorded on FT NMR Spectrometer model: Avance 300 MHz Bruker superconducting with TMS as an internal standard. Mass spectra were recorded on Shimadzu Japan QP2010 S model spectrometer and elemental analyses were carried out using

Scheme 3 Schematic representation of quenching of DPPH radical

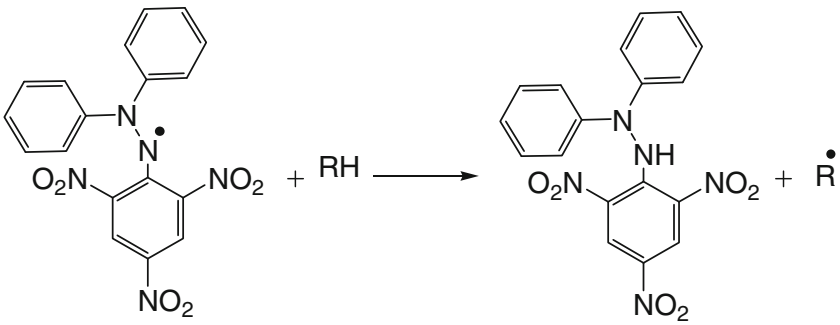
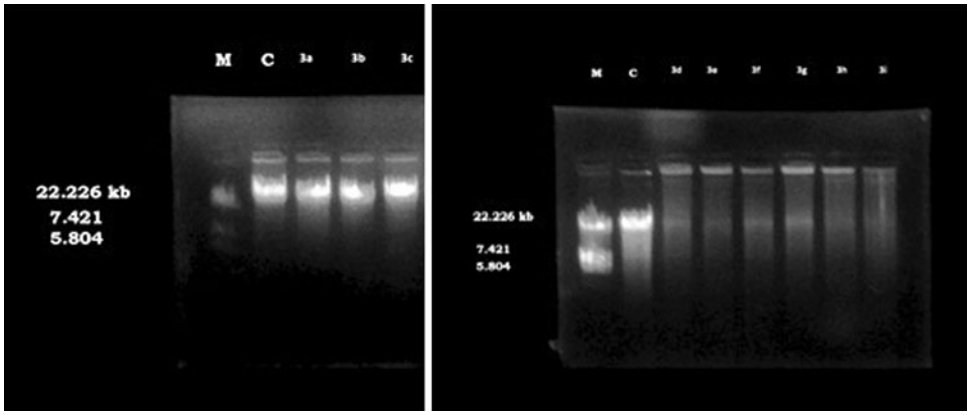


Fig. 5 DNA cleavage activity of the compounds (**3a–i**)



Heraus CHN rapid analyzer. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plate using benzene and ethyl acetate as eluent. The Osiris property explorer was used to evaluate clog*P* values, drug likeliness and drug score of the synthesised molecules. Antihyperglycemic, DNA cleavage and antioxidant activities of the synthesised molecules were carried out at Biogenics, Hubli, Karnataka, India.

3-[4-(5-Aryl-4,5-dihydro-1*H*-pyrazolin-3-yl)-phenyl]-sydnone (**1a–i**)

The compounds (**1a–i**) was prepared according to the literature method (Dambal *et al.*, 1984).

General procedure for the preparation of 3-[4-(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-phenyl]-5-methyl-3*H*-[1,3,4]-oxadiazol-2-one (**2a–i**)

The compound (**1a–i**) (0.01 mol) was suspended in acetic anhydride (5 ml) and bromine (0.5 ml) in acetic anhydride (5 ml) was added at 0–5°C within a period of 15 min. The reaction mixture was then stirred at room temperature (30 min), and warmed on water bath (60°C) till the evolution of CO_2 ceased and poured to ice cold water. Filtered and recrystallized using ethanol to yield the compound (**2a–i**) (yield 80–85%).

*3-[4-(1-Acetyl-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-phenyl]-5-methyl-3H-[1,3,4]-oxadiazol-2-one (**2a**)*

Brown amorphous solid (ethanol). Yield; 71%; R_f 0.87; mp; (°C); 95–96. IR (ν max in cm^{-1}); 1776, 1659, 1603. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.43 (s, 3H, CH_3 –C_5), 2.54 (s, 3H, CH_3 of $NCOCH_3$), 3.1 (*dd*, 1H, [H_A] CH_2), 3.6 (*dd*, 1H, [H_B] CH_2, $J = 4.65$ Hz), 4.56 (m, 1H, [H_X] CH_2,), 7.27–7.86 (m, 9H, Ar H). GCMS (*m/z*); 362 [M], 337 (12), 265 (10), 251 (60), 235 (30), 212 (40), 209 (30), 194 (35), 176 (35), 159 (55), 150 (30), 134 (100), 119 (75), 106 (40), 91(60), 74 (10), 65 (25), 54 (5), 49 (10). CHN analysis; calculated for $C_{20}H_{18}N_4O_3$: C, 66.25; H, 4.90; N, 15.42. Found: C, 66.29; H, 4.97; N, 15.46.

*3-{4-[1-Acetyl-5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-5-methyl-3H-[1,3,4]-oxadiazol-2-one (**2b**)*

Brown amorphous solid (methanol).Yield; 87%; R_f 0.90; mp; (°C); 154–155. IR (ν max in cm^{-1}); 1778, 1660, 1600. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.34 (s, 3H, CH_3–C_5), 2.41 (s, 3H, CH_3 of $NCOCH_3$), 3.11 (*dd*, 1H, [H_A] CH_2), 3.55 (*dd*, 1H, [H_B] CH_2, $J = 4.79$ Hz), 4.59 (m, 1H, [H_X] CH_2), 7.14–8.10 (m, 8H, Ar H). GCMS (*m/z*); 398 [M + 2, 12], 396 [M, 74], 362 (30), 337 (12), 265 (10) 251 (60), 235(30), 212 (40), 209 (30), 194 (35), 176 (35), 159 (55), 150 (30), 134 (80), 119 (75), 106 (40), 91(60), 74

(10), 65 (25), 54 (5), 49 (100). CHN analysis; calculated for $C_{20}H_{17}N_4O_3Cl$ C, 60.48; H, 4.21; N, 14.07. Found: C, 60.52; H, 4.28; N, 14.12.

*3-{4-[1-Acetyl-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-5-methyl-3H [1,3,4]-oxadiazol-2-one (**2c**)*

Brown amorphous solid (methanol). Yield; 80%; R_f 0.90; mp; (°C); 169–170. IR (ν max in cm^{-1}); 1780, 1660, 1600. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.23 (s, 3H, CH_3–C_5), 2.49 (s, 3H, CH_3 of $NCOCH_3$), 3.22 (*dd*, 1H, [H_A] CH_2), 3.59 (*dd*, 1H, [H_B] CH_2, $J = 4.60$ Hz) 4.47 (m, 1H, [H_X] CH_2), 7.20–7.91 (m, 8H, Ar H). GCMS (*m/z*); 398 [M + 2, 10], 396 [80], 362 (30), 338 (10), 267 (12), 251 (60), 235 (30), 209 (40), 205 (30), 194 (35), 176 (35), 159 (55), 150 (30), 134 (80), 109 (75), 106 (40), 91 (60), 74 (10), 65 (25), 54 (100), 49 (10). CHN analysis; calculated for $C_{20}H_{17}N_4O_3Cl$: C, 60.48; H, 4.21; N, 14.07. Found: C, 60.51; H, 4.27; N, 14.11.

*3-[4-(1-Acetyl-5-p-tolyl-4,5-dihydro-1H-pyrazol-3-yl)-phenyl]-5-methyl-3H-[1,3,4]-oxadiazol-2-one (**2d**)*

Brown amorphous solid (methanol). Yield; 76%; R_f 0.80, mp; (°C); 218–219. IR (ν max in cm^{-1}); 1775, 1665, 1600. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.25 (s, 3H, CH_3), 2.52 (s, 3H, CH_3–C_5), 2.55 (s, 3H, CH_3 of $NCOCH_3$), 3.20 (*dd*, 1H, [H_A] CH_2), 3.65 (*dd*, 1H, [H_B] CH_2, H_B, $J = 4.60$ Hz), 4.68 (m, 1H, [H_X] CH_2), 7.0–8.0 (m, 8H, Ar H). GCMS (*m/z*); 376 [M], 337 (50), 335 (60), 265 (25), 251 (60), 235 (30), 212 (40), 209 (30), 194 (35), 176 (30), 159 (55), 150 (30), 134 (70), 119 (75), 106 (40), 91(80), 74 (10), 65 (25), 54 (5), 49 (100). CHN analysis; calculated for $C_{21}H_{20}N_4O_3$: C, 67.01; H, 5.36; N, 14.88. Found: C, 67.00; H, 5.37; N, 14.88.

*3-{4-[1-Acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-5-methyl-3H-[1,3,4]-oxadiazol-2-one (**2e**)*

Brown amorphous solid (ethanol). Yield; 75%; R_f 0.80, mp; (°C); 224–225. IR (ν max in cm^{-1}); 1767, 1660, 1602. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.35 (s, 3H, CH_3), 2.56 (s, 3H, CH_3–C_5), 2.60 (s, 3H, CH_3 of $NCOCH_3$), 3.21 (*dd*, 1H, [H_A] CH_2), 3.55 (*dd*, 1H, [H_B] CH_2, $J = 4.80$ Hz), 3.71 (s, 3H, CH_3 of OCH_3), 4.62 (m, 1H, [H_X] CH_2), 7.24–7.79 (m, 8H, ArH). GCMS (*m/z*); 392 [M], 387 (90), 364 (60), 361(10) 340 (80), 324 (65), 309 (15), 299 (10), 282 (70), 265 (87), 266 (25), 251 (60), 235 (40), 212 (40), 209 (30), 194 (35), 170 (35), 159 (55), 150 (30), 133 (75), 119 (75), 106 (40), 91(60), 75 (100), 65 (25), 54 (5), 49 (10). CHN analysis; calculated for $C_{21}H_{20}N_4O_4$: C, 64.28; H, 5.14; N, 14.28. Found: C, 64.27; H, 5.13; N, 14.30.

*3-{4-[1-Acetyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-5-methyl-3H- [1,3,4]-oxadiazol-2-one (**2f**)*

Brown amorphous solid (methanol). Yield; 79%; R_f 0.89, mp; (°C); 202–203. IR (ν max in cm^{-1}); 1780, 1659, 1604. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.39 (s, 3H, CH_3–C_5), 2.47 (s, 3H, CH_3 of $NCOCH_3$), 3.23 (*dd*, 1H, [H_A] CH_2), 3.56 (*dd*, 1H, [H_B] CH_2, $J = 4.58$ Hz), 4.55 (m, 1H, [H_X] CH_2), 7.22–8.14 (m, 8H, Ar H). GCMS (*m/z*); 398 [M + 2], 396 [M, 69], 362 (30), 337 (10), 265 (12), 251 (60), 235(30), 212 (40), 209 (30), 194 (35), 176 (100), 159 (55), 150 (30), 134 (60), 119 (75), 106 (40), 91(60), 74 (10), 65 (25), 54 (5), 49 (10). CHN Analysis; Calculated for $C_{20}H_{17}ClN_4O_3$: C, 60.53; H, 4.32; N, 14.12. Found: C, 60.50; H, 4.30; N, 14.10.

*3-{4-[1-Acetyl-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-5-methyl-3H- [1,3,4]-oxadiazol-2-one (**2g**)*

Brown amorphous solid (methanol). Yield; 86%; R_f 0.93, mp; (°C); 216–217. IR (ν max in cm^{-1}); 1781, 1659, 1604. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.44 (s, 3H, CH_3–C_5), 2.50 (s, 3H, CH_3 of $NCOCH_3$), 3.13 (*dd*, 1H, [H_A] CH_2), 3.56 (*dd*, 1H, [H_B] CH_2), $J = 4.70$ Hz), 4.70 (m, 1H, [H_X] CH_2), 7.00–7.95 (m, 8H, Ar H). GCMS (*m/z*); 407 [M], 397 [M], 362 (30), 336 (10), 265 (12), 251 (60), 230 (30), 212 (40), 209 (30), 194 (35), 176 (30), 159 (55), 150 (30), 134 (100), 119 (75), 106 (40), 90 (60), 74 (10), 65 (25), 50 (5), 49 (10). CHN analysis; calculated for $C_{20}H_{17}N_5O_5$: C, 58.97; H, 4.21; N, 17.19. Found: C, 58.95; H, 4.20; N, 17.20.

*3-{4-[1-Acetyl-5-(2,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-5-methyl-3H [1,3,4]-oxadiazol-2-one (**2h**)*

Brown amorphous solid (methanol). Yield; 75%; R_f 0.93, mp; (°C); 160–161. IR (ν max in cm^{-1}); 1779, 1659, 1604. ^1H NMR (300 MHz, δ ppm, $CDCl_3$- d_6): 2.20 (s, 3H, CH_3–C_5), 2.40 (s, 3H, CH_3 of $NCOCH_3$), 3.26 (*dd*, 1H, [H_A] CH_2), 3.54 (*dd*, 1H, [H_B] CH_2, $J = 4.58$ Hz), 3.71 (s, 6H, CH_3 of OCH_3), 4.79 (m, 1H, [H_X] CH_2), 7.2–8.0 (m, 8H, Ar H). GCMS (*m/z*); 422 [M], 407 (40), 397 (30), 362 (30), 337 (10), 265 (12), 251 (60), 235(30), 212 (40), 209 (30), 194 (35), 176 (35), 159 (55), 150 (100), 134 (80), 119 (75), 106 (40), 91(60), 74 (10), 65 (25), 54 (100), 49 (10). CHN analysis; calculated for $C_{22}H_{23}N_5O_3$: C, 58.95; H, 4.13; N, 17.15. Found: C, 58.96; H, 4.17; N, 17.19.

*3-{4-[1-Acetyl-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-5-methyl-3H-[1,3,4] oxadiazol-2-one (**2i**)*

Brown amorphous solid (ethanol). Yield; 70%; R_f 0.95, mp; (°C); 155–156. IR (ν max in cm^{-1}); 3400, 1780, 1659, 1604. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.31 (s, 3H, CH_3 of $NCOCH_3$), 2.38 (s, 3H, CH_3–C_5), 3.15 (*dd*, 1H, [H_A] CH_2), 3.62 (*dd*, 1H, [H_B] CH_2, $J = 4.65$ Hz) 3.71 (s, 3H, CH_3 of OCH_3), 4.81 (m, 1H, [H_X] CH_2), 7.0–8.1 (m, 8H Ar H), 8.4 (bs, 1H, OH). GCMS (*m/z*); 378 [M], 362 (30), 337 (10), 260 (12), 251 (60), 235 (35), 210 (40), 209 (30), 194 (35), 176 (30), 159 (55), 151 (30), 134 (90), 119 (75), 106 (40), 91(60), 74 (10), 65 (25), 54 (5), 49 (100). CHN analysis; calculated for $C_{20}H_{18}N_4O_4$: C, 63.48; H, 4.79; N, 14.81. Found: C, 63.45; H, 4.80; N, 14.80.

General procedure for the preparation of 2-[4-(1-acetyl-5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-phenyl]-4-amino-5-methyl-2,4-dihydro-[1,2,4]-triazolin-3-one (**3a–i**)

The compound (**2a–i**) (0.01 mol) was refluxed with hydrazine hydrate (0.01 mol) in ethanol (25 ml) for 6 h. The contents of the flask were poured in water to yield the crude product (**3a–i**) which was then filtered. Recrystallization from ethanol gave the yellow needles (yield 75–84%).

*2-[4-(1-Acetyl-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-phenyl]-4-amino-5-methyl-2,4-dihydro-[1,2,4]-triazolin-3-one (**3a**)*

Pale yellow amorphous solid (ethanol). Yield; 65%; R_f 0.95, mp; (°C); 12–121. IR (ν max in cm^{-1}); 3321, 1712, 1649, 1605. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.18 (s, 3H, CH_3–C_5), 2.31 (s, 3H, CH_3 of $NCOCH_3$), 3.10 (*dd*, 1H, [H_A] CH_2), 3.8 (*dd*, 1H, [H_B] CH_2 $J = 4.65$ Hz), 4.52 (m, 1H, [H_X] CH_2), 7.22–7.95 (m, 9H, Ar H), 8.22 (s, 2H, NH_2). GCMS (*m/z*); 377 [M + 1, 6], 376 [M], 362 (30), 337 (10), 265 (12), 251 (60), 235(30), 212 (40), 209 (30), 194 (35), 176 (35), 159 (55), 150 (30), 134 (100), 119 (75), 106 (40), 91(60), 74 (10), 65 (25), 54 (5), 49 (10). CHN analysis; calculated for $C_{20}H_{20}N_6O_2$: C, 63.82; H, 5.36; N, 22.33. Found: C, 63.80; H, 5.35; N, 22.30.

*2-{4-[1-Acetyl-5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-4-amino-5-methyl-2,4-dihydro-[1,2,4]triazolin-3-one (**3b**)*

Pale brown amorphous solid (ethanol). Yield; 70%; R_f 0.90, mp; (°C); 210–211. IR (ν max in cm^{-1}); 3321, 1712, 1645, 1605. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.33 (s, 3H, CH_3–C_5–), 2.40 (s, 3H, CH_3 of $NCOCH_3$), 3.20 (*dd*, 1H, [H_A] CH_2), 3.35 (*dd*, 1H, [H_B] CH_2, $J = 4.70$ Hz), 4.67 (m, 1H, [H_X] CH_2), 7.22–7.90 (m, 8H, Ar H), 8.2 (s, 2H, NH_2). GCMS (*m/z*); 412 [M + 2, 16] 410 [M, 84], 392 (50), 387 (90), 377, 376, 362 (30), 330 (10), 265 (12), 251 (60), 235(30), 212 (40), 210 (30), 195 (35), 176 (30), 159 (55), 150 (30), 134 (60), 119 (75), 106 (40), 91(60), 74 (10), 65 (25), 54 (5), 49 (100). CHN analysis; calculated for $C_{20}H_{19}ClN_6O_2$: C, 60.53; H, 4.66; N, 20.45. Found: C, 60.50; H, 4.64; N, 20.43.

*2-{4-[1-Acetyl-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-4-amino-5-methyl-2,4-dihydro-[1,2,4]-triazolin-3-one (**3c**)*

Pale brown solid (ethanol). Yield; 71%; R_f 0.90, mp; (°C); 212–213. IR (ν max in cm^{-1}); 3319, 1709, 1649, 1605. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.35 (s, 3H, CH_3–C_5), 2.42 (s, 3H, CH_3 of $NCOCH_3$), 3.10 (*dd*, 1H, [H_A] CH_2), 3.40 (*dd*, 1H, [H_B] CH_2, $J = 4.70$ Hz), 4.60 (m, 1H, [H_X] CH_2), 7.23–7.88 (m, 8H, Ar H), 8.2 (s, 2H, NH_2). GCMS (*m/z*); 412 [M + 2, 12], 410 [M, 79], 392 [M], 387 (90), 377 (30), 370 (45), 362 (30), 337 (10), 265 (12), 251 (60), 235(30), 212 (40), 210 (30), 190 (35), 175 (35), 159 (55), 150 (30), 134 (90), 119 (75), 106 (40), 91(60), 74 (10), 65 (25), 54 (100), 49 (10). CHN analysis; calculated for $C_{20}H_{19}ClN_6O_2$: C, 60.53; H, 4.66; N, 20.45. Found: C, 60.51; H, 4.63; N, 20.42.

*2-[4-(1-Acetyl-5-p-tolyl-4,5-dihydro-1H-pyrazol-3-yl)-phenyl]-4-amino-5-methyl-2,4-dihydro-[1,2,4]-triazolin-3-one (**3d**)*

Brown amorphous solid (ethanol). Yield; 76%; R_f 0.90, mp; (°C); 218–219. IR (ν max in cm^{-1}); 3325, 1712, 1649, 1605. ^1H NMR (300 MHz, δ ppm, $CDCl_3$- d_6): 2.27 (s, 3H, CH_3), 2.34 (s, 3H, CH_3–C_5), 2.50 (s, 3H, CH_3 of $NCOCH_3$), 3.19 (*dd*, 1H, [H_A] CH_2), 3.56 (*dd*, 1H, [H_B] CH_2, $J = 4.58$ Hz) 4.62 (m, 1H, [H_X] CH_2), 7.24–7.85 (m, 8H, Ar H), 8.25 (s, 2H, NH_2). GCMS (*m/z*); 390 [M], 387 (90), 377 (40), 376 (10), 362 (30), 337 (10), 265 (12), 251 (60), 240 (30), 212 (40), 209 (30), 194 (35), 176 (35), 159 (55), 150 (30), 134 (86), 119 (75), 106 (40), 91(60), 74 (10), 65 (25), 54 (5), 49 (100). CHN Analysis; Calculated for $C_{21}H_{22}N_6O_2$: C, 64.60; H, 5.68; N, 21.52. Found: C, 64.59; H, 5.67; N, 21.50.

*2-{4-[1-Acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-4-amino-5-methyl-2,4-dihydro-[1,2,4]-triazolin-3-one (**3e**)*

Pale green amorphous solid (ethanol). Yield; 80%; R_f 0.91, mp; (°C); 235-236. IR (ν max in cm^{-1}); 3318, 1710, 1649, 1605. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.21 (s, 3H, CH_3–C_5), 2.41 (s, 3H, CH_3 of $NCOCH_3$), 3.15 (*dd*, 1H, [H_A] CH_2), 3.3 (*dd*, 1H, [H_B] CH_2, $J = 4.65$ Hz), 3.72 (s,

3H, CH_3 of OCH_3), 4.73 (m, 1H, [H_X] CH_2), 6.80–8.08 (m, 9H, Ar H), 9.27 (s, 2H, NH). GCMS (*m/z*); 408 (30), 405 (10), 390 (40), 385 (90), 364 (70), 360 (12), 340 (80), 324 (65), 309 (15), 300 (10), 282 (70), 265 (87), 266 (25), 251 (60), 235 (40), 212 (40), 209 (30), 194 (35), 176 (35), 159 (55), 150 (30), 134 (70), 119 (75), 106 (40), 91(60), 74 (10), 65 (25), 54 (100), 49 (10). CHN analysis; calculated for $C_{21}H_{22}N_6O_3$: C, 62.06; H, 5.46; N, 20.68. Found: C, 62.07; H, 5.43; N, 20.65.

*2-{4-[1-Acetyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-4-amino-5-methyl-2,4-dihydro-[1,2,4]-triazolin-3-one (**3f**)*

Pale brown amorphous solid (ethanol). Yield; 80%; R_f 0.90, mp; (°C); 210–211. IR (*v* max in cm^{-1}); 3318, 1710, 1649, 1605. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.45 (s, 3H, CH_3–C_5), 2.55 (s, 3H, CH_3 of $NCOCH_3$), 3.22 (*dd*, 1H, [H_A] CH_2), 3.45 (*dd*, 1H, [H_B] CH_2, $J = 4.70$ Hz), 4.65 (m, 1H, [H_X] CH_2), 6.15 (s, 2H, NH), 7.02–8.10 (m, 8H, Ar H). GCMS (*m/z*); 412 [M + 2, 18], 410 [M, 96], 403 (10), 385 (10), 361 (10), 345 (15), 329 (15), 316 (40), 305 (20), 291 (15), 273 (10), 251 (10), 237 (10), 219 (20), 206 (10), 191 (35), 175 (26), 159 (15), 147 (55), 131 (15), 119 (20), 105 (20), 91 (25), 78 (60), 57 (80), 44 (100), 41 (30). CHN analysis; calculated for $C_{20}H_{19}ClN_6O_3$: C, 60.53; H, 4.66; N, 20.45. Found: C, 60.50; H, 4.64; N, 20.43.

*2-{4-[1-Acetyl-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-4-amino-5-methyl-2,4-dihydro-[1,2,4]-triazolin-3-one (**3g**)*

Pale yellow amorphous solid (ethanol). Yield; 81%; R_f 0.92, mp; (°C); 220–221. IR (*v* max in cm^{-1}); 3330, 1697, 1646, 1610. ^1H NMR (300 MHz, δ ppm, $CDCl_3$- d_6): 2.25 (s, 3H, CH_3–C_5), 2.46 (s, 3H, CH_3 of $NCOCH_3$), 3.29 (*dd*, 1H, [H_A] CH_2), 3.49 (*dd*, 1H, [H_B] CH_2, $J = 4.60$ Hz), 4.80 (m, 1H, [H_X] CH_2), 6.83–8.05 (m, 8H, Ar H), 9.7 (s, 2H, NH). GCMS (*m/z*); 421 [M], 403 (10), 385 (10), 361 (10), 345 (15), 329 (15), 316 (40), 305 (20), 291 (15), 273 (10), 251 (10), 237 (10), 219 (20), 206 (10), 191 (35), 175 (26), 159 (15), 147 (55), 131 (15), 119 (20), 105 (20), 91 (25), 78 (60), 57 (80), 44 (100), 41 (30). CHN analysis; calculated for $C_{20}H_{19}ClN_7O_4$: C, 57.00; H, 4.54; N, 23.27. Found: C, 57.01; H, 4.53; N, 23.25.

*2-{4-[1-Acetyl-5-(2,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-4-amino-5-methyl-2,4-dihydro-[1,2,4]-triazolin-3-one (**3h**)*

Brown amorphous solid (ethanol). Yield; 81%; R_f 0.90, mp; (°C); 200–201. IR (*v* max in cm^{-1}); 3330, 1700, 1646, 1610. ^1H NMR (300 MHz, δ ppm, $CDCl_3$-d_6): 2.34 (s, 3H, CH_3–C_5), 2.40 (s, 3H, CH_3 of $NCOCH_3$), 3.25 (*dd*, 1H, [H_A] CH_2), 3.57 (*dd*, 1H, [H_B] CH_2, $J = 4.65$ Hz), 3.75 (s, 6H, CH_3 of OCH_3), 4.60 (m, 1H, [H_X] CH_2), 7.26–7.92 (m,7H, Ar H), 8.84 (s, 2H, NH). GCMS (*m/z*); 436, [M], 427 (10), 423(15), 421 (20), 403 (10), 385 (10), 361 (10), 345 (15), 329 (15), 316 (40), 305 (20), 291 (15), 273 (10), 251 (10), 237 (10), 219 (20), 206 (10), 191 (35), 175 (26), 159 (15), 147 (50), 131 (15), 119 (20), 105 (20), 91 (25), 78 (60), 57 (80), 44 (50), 41 (100). CHN analysis; calculated for $C_{22}H_{24}N_6O_4$: C, 60.54; H, 5.54; N, 19.25. Found: C, 60.52; H, 5.51; N, 19.19.

*2-{4-[1-Acetyl-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-4-amino-5-methyl-2,4-dihydro-[1,2,4]-triazolin-3-one (**3i**)*

Brown amorphous solid (ethanol). Yield; 81%; R_f 0.89, mp; (°C); 210–211. IR (*v* max in cm^{-1}); 3438, 3328, 1700, 1658, 1606. ^1H NMR (300 MHz, δ ppm, $CDCl_3$- d_6): 2.12 (s, 3H, C_5–CH_3), 2.33 (s, 3H, CH_3 of $NCOCH_3$), 3.33 (*dd*, 1H, [H_A] CH_2), 3.66 (*dd*, 1H, [H_B] CH_2, $J = 4.70$ Hz), 4.55 (m, 1H, [H_X] CH_2), 5.5 (s, 2H, NH), 6.98–7.46 (m, 7H, Ar H), 8.0 (s, 1H, OH). GCMS (*m/z*); 392 [M], 383 (10), 369 (10), 334 (10), 319 (10), 290 (10), 278 (10), 261 (10), 249 (10), 236 (10), 211 (10), 196 (15), 183 (15), 168 (15), 155 (15), 137 (20), 125 (30), 111 (40), 100 (45), 85 (60), 71 (70), 57 (90), 47 (100), 41 (50). CHN analysis; calculated for $C_{20}H_{20}N_6O_3$: C, 61.21; H, 5.14; N, 21.42. Found: C, 61.20; H, 5.10; N, 21.40.

Pharmacological evaluation

The title compounds were analysed for drug likeliness and drug score by Osiris molecular property explorer and subjected to antihyperglycemic, DNA cleavage and antioxidant assay.

Amylase inhibition assay (Sadasivam and Manickam 1996)

The antihyperglycemic assay (Himedia, Mumbai) was carried out with different concentrations of samples. Sodium phosphate buffer (control) (1.4 ml, 50 mM, pH 7.0-7.3) was added to the test tubes. Different volumes of solvent, starch (0.5 ml in buffer) and enzyme (0.1 ml) were also added to the sample in buffer (1 mg/ml). The tubes were incubated at 37°C for 10 min followed by addition of dinitrosalicylic acid (DNS; 1 ml). The blank (control) tube was added with DNS (1 ml) before the addition of enzyme. The tubes were then incubated in boiling water bath for 10 min, cooled and recorded the absorbance at 540 nm against blank. The maltose liberated was determined with the help of standard maltose curve and activities were calculated according to the following formula.Activity was expressed as μmoles/ml/min.

$$\text{Activity} = \frac{\text{Conc. of maltose liberated} \times \text{Vol of enzyme used (ml)} \times \text{Dilution factor}}{\text{Mol wt of maltose} \times \text{Incubation time (min)}}$$

The inhibitory/induction property shown by the sample was compared with that of control and expressed as percent induction/inhibition. This was calculated according to the following formula.

$$\%\ \text{Inhibition} = \frac{\text{Activity in presence of compound}}{\text{Control activity}} \times 100$$

Antioxidant activity [free radical scavenging activity by 1,1-diphenyl-2-picryl hydrazyl (DPPH method; Singh *et al.*, 2002)]

Different concentrations (10, 50 and 100 μg) of sample in DMSO and butylated hydroxy anisole (BHA), a synthetic antioxidant were taken in different test tubes. The volume was adjusted to 500 μl by adding methanol. Methanolic solution (0.1 mM, 5 ml) of DPPH was then added to tubes and were shaken vigorously. A control without the test compound was maintained. The tubes were allowed to stand at room temperature for 20 min. The absorbance of the samples was measured at 517 nm. Radical scavenging activity was calculated using the following formula

$$\%\ \text{Radical scavenging activity} = \frac{(\text{Control absorbance} - \text{Sample absorbance})}{\text{Control absorbance}} \times 100$$

DNA cleavage activity (Sambrook *et al.*, 1989)

Nutrient broth was used for the growth of the organism. The media (50 ml) was prepared, autoclaved for 15 min at 121°C, 15 lb pressure. The autoclaved media were inoculated with the seed culture and incubated at 37°C for 24 h.

Isolation of DNA

DNA was isolated using the procedure as reported (Singh *et al.*, 2002). The synthesised compounds (100 μg) were added separately to the DNA sample. The sample mixtures were incubated at 37°C for 2 h. Following the treatment of DNA samples, the electrophoresis of the samples was done according to the procedure.

SPSS analysis

The statistical analysis was carried out using SPSS (Landau and Everitt, 2004; SPSS, 2008). This analysis which includes the *t* test is done in order to understand the level of confidence of the title compounds in comparison with the control. This test is applied to antihyperglycemic and antioxidant assay.

Conclusions

We have successfully ring transformed sydnone to 1,3,4-oxadiazolin-3-one and further to triazolin-3-one moiety. Ring transformation and ring insertion with ease and procedural simplicity are the key aspects of the synthesis. The result of the antihyperglycemic screening reveals that among all the title compounds, the compounds **3b**, **3g**, **3h** and **3i** showed strong inhibition while the other compounds **3c**, **3e**, **3f**, **3a** and **3d** displayed moderate to low inhibition. *T* test analysis for amylase Inhibition Assay lies at 0.595 and hence the antihyperglycemic activity is excellent for the title compounds. Antioxidant activity revealed three compounds **3d** and **3f** have exhibited good activity. *T* test for free radical scavenging activity lies at 0.29, 0.12 and 0.17, respectively, and the values are low as compared to the control BHA and the title compounds show moderate to low activity. In case of DNA cleavage activity it was observed that compounds **3g** and **3h** exhibited complete cleavage of DNA and other compounds have shown partial cleavage.

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References

Al-Talib M, Tastoush H, Odeh N (1990) Some carboxaldazines and s-triazoles of the anthraquinone series. J Org Chem 25:572–575

Andreadou I, Tasouli A, Bofilis E, Chrysselis M, Rekka E, Kakoulidou AT, Iliodromitis E, Siatra T, Kremastinos DT (2002) Antioxidant activity of novel indole derivatives and protection of the myocardial damage in rabbits. J Heterocycl Chem 33:165–168

Barnes PF, Blotch AB, Davidson PT, Snider DE (1991) Tuberculosis and HIV infection. Engl J Med 26:1882–1884

Bloom BR, Murray CJL (1992) Tuberculosis: commentary on a re-emergent killer. Science 257:1055–1064

Brown P, Best DJ, Broom NJP, Cassels R, O'Hanlon PJ, Mitchell TJ, Osborne NF, Wilson JM (1997) The chemistry of pseudomonic acid. Heterocyclic replacement of the α,β-unsaturated ester: Synthesis, molecular modeling, and antibacterial activity. J Med Chem 40(16):2563–2570

Browne DL, Harrity JPA (2010) Recent development in chemistry of sydnones. Tetrahedron 66:553–568

Carlsen PHJ, Jorgensen KB (1994) Synthesis of unsymmetrically substituted 4H-1, 2, 4-triazoles. J Heterocycl Chem 31:805–807

Chai B, Qian X, Cao S, Liu H, Song G (2003) Synthesis and insecticidal activity of 1,2,4-triazole derivatives. Arkivoc 2: 141–145

Chudgar NK, Shah SN, Vora RA (1989) Mesogenic semicarbazones and amino oxadiazoles-I. Mol Cryst Liq Cryst 172:51–56

Dambal DB, Pattanashetti PP, Tikare RK, Badami BV, Puranik GS (1984) Reactions of 3-(*p*-acetylphenyl)sydnones. Part-III. Indian J Chem 23B:186–190

Ellingboe JW, Lombardo LJ, Alessi TR, Nguyen TT, Guzzo F, Guinosso CJ, Bullington J, Browne EN, Bagli JF, Wrenn J, Steiner K, McCaleb ML (1993) Antihyperglycemic activity of novel naphthalenyl 3H-1,2,3,5-oxathiadiazole-2-oxides. J Med Chem 36:2486–2493

Emami S, Kebriaeezadeh A, Ahangar N, Khorasani R (2011) Imidazolylchromanone oxime ethers as potential anticonvulsant agents: Anticonvulsive evaluation in PTZ-kindling model of epilepsy and SAR study. Bioorg Med Chem Let 21:655–659

Ferrer S, Ballesteros R, Sambartolom A, Gonz G, Alzuet J, Borr M, Liu (2004) Syntheses, crystal structures, and oxidative DNA cleavage of some Cu(II) complexes of 5-amino-3-pyridin-2-yl-1,2,4-triazole. J Inorg Biochem 98:1436–1446

Fujiwara AU, Cook SV, Rutherford CM, Crawford JT, Glickman SE, Kreiswirth BN, Sachdev PS, Osahan SS, Ebrahimzadeh A, Frieden TR (1997) A continuing survey of drug-resistant tuberculosis New York City. Arch Intern Med 157:531–536

Herbrecht R, Denning D, Patterson T, Bennett J, Greene R, Oestmann J, Kern W, Marr K, Ribaud P, Lortholary O, Sylvester R, Rubin R, Wingard J, Stark P, Durand C, Caillot D, Thiel E, Chandrasekar P, Hodges M, Schlamm H, Troke P, de Pauw B (2002) Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med 347:408–415

Kattimani PP, Raikar SV, Kamble RR, Kariduraganavar MY, Hunnur RK (2011) An expeditious synthesis of 1,2,4-triazolinones appended to 1,3-thiazoles using zinc triflate as catalyst. Main Group Chem 10:165–167

Kees KL, Caggiano TJ, Steiner KE, Fitzgerald JJ, Kates MJ, Christos TE, Kulishoff JM, Moore RD, McCaleb ML (1995) Studies on new acidic azoles as glucose-lowering agents in obese, Diabetic db/db mice. J Med Chem 38:617–628

Landau S, Everitt BS (2004) A handbook of statistical analysis using SPSS. Chapman & Hill, Boca Raton

Lipinski CA (2004) Lead and drug-like compounds: the rule-of-five revolution. Drug Discov Today 1:337–341

Liras S, Allen MP, Segelstein BE (2000) A mild method for the preparation of 1,3,4-oxadiazoles: triflic anhydride promoted cyclization of diacylhydrazines. Synth Commun 30:437–443

Löffler J, Schobert R (1997) Synthesis of 1,3,4-oxadiazoles from carboxylic hydrazides and of 1,2-oxazin-6-ones from α-(hydroxyimino)carboxylic esters with keteneylidene triphenylphosphorane. Synth lett 28:283–284

Mallur SG, Badami BV (2000) Transformation of the sydnone ring into oxadiazolinones. A convienient one-pot synthesis of 3-aryl-5-methyl-1,3,4oxadiazolin-2-ones from 3-aryl sydnones and their antimicrobial activity II. Farmaco 55:65–67

Ohta M, Kato H (1969) Nonbenzenoid aromatics. Academic Press, New York, p 117

Ragenovic KC, Dimova V, Kakurinov V, Molnar DG, Buzarovska A (2001) Synthesis, antibacterial and antifungal activity of 4-substituted-5-aryl-1,2,4-triazoles. Molecules 6:815–824

Rajeev K, Sharma, Chander P, Yar, Shahar M (2011) Pharmacological significance of triazole scaffold. J Enzy Inhib Med Chem 20. doi:10.3109/14756360903524304

Sadasivam S, Manickam A (1996) Biochemical methods, 2nd edn. New Age International (P) limited, New Delhi, pp 124–126

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. A laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Sangamwar AT, Deshpande UD, Pekamwar SS, Vadvalkar SM (2007) Improving decision making for drug candidates: a computational approach for benzothaizoles as antifungal. Ind J Biotechnol 6:389–396

Singh RP, Murthy KNC, Jayaprakasha GK (2002) Studies on antioxidant activity of pomegranate peel and seed extracts using invitro models. J Agric Food Chem 50:81–86

SPSS (2008) Version 17.0, www.spss.com, SPSS Inc., Chicago, IL

Stankovsky S, Jedlovska E, Spirkova K (1993) Synthesis of some triazolyl acetanilides. Collect Czech Chem Commun 25: 2211–2214

Sweetman S (2004) Martindale: the complete drug reference, 34 edn. Pharmaceutical Press, London. ISBN 0-85369-550-4

Taj T, Raikar SV, Kamble RR (2011) Synthetic utility of sydnones to couple pharmacologically important heterocycles for antitubercular activity. Arab J Chem. doi:10.1016/j.arabic.2011.01.029

Theocharis AB, Alexandrou NE (1990) Synthesis and spectral data of 4,5-bis[5-aryl-1,3,4-oxadiazol-2-yl]-1-benzyl-1,2,3-triazoles. J Heterocycl Chem 27:1685–1688

Wender PA, Touami SM, Alaryac C, Philipp UC (1996) Triazole photonucleases: a new family of light activatable DNA cleaving agents. J Am Chem Soc 118:6522–6523

Wu C, Fang Y, Larock RC, Shi F (2010) Synthesis of 2H-indazoles by the [3 + 2] cycloaddition of arynes and sydnones. Org Lett 12:2234–2237