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Original Article

SYNTHESIS OF SOME NOVEL 3,5,6-TRISUBSTITUTED-[1,2,4]TRIAZOLO[3,4-c][1,2,4] TRIAZINESAS DNA PHOTOCLEAVING AGENTS

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ABSTRACT

Objective: To develop an easy and non toxic method for synthesis of some novel triazolotriazines via oxidative transformation of triazinylhydrazones using iodobenzene diacetate and to evaluate their DNA photocleavage potential.

Methods: A series nineteen triazin-3-yl moiety linked hydrazones of various aryl and heteroaryl aldehydes has been prepared which on treatment with iodobenzene diacetate in dichloromethane gave novel triazolo[3,4-c]triazine derivatives under mild reaction conditions. DNA photocleavage activity of all these compounds was performed using agarose gel electrophoresis.

Results: Synthesis of some novel triazolotriazines has been successively achieved and structures of compounds were established on the basis of FT-IR, ^{1}H, ^{13}C NMR and mass spectral data. Most of the compounds such as **4m-n**, **4p-s**, as well as **3b**, **3d**, **3e**, **3h**, **3p** and **3q** exhibited admirable DNA photocleavage potential.

Conclusion: The employed approach to afford triazolo[3,4-c]triazines only because the Dimorth rearrangement occurred under catalytic amount of base and acid etc. The results indicated that both triazolotriazines and triazinylhydrazones exhibited promising DNA photocleavage activity. Moreover, compounds containing phenyl ring with electron-releasing substituent attached to pyrazole nucleus possessed increased DNA photocleavage potential.

Keywords: Pyrazole, Triazole, Triazine, Hypervalent Iodine, DNA photocleavage.

INTRODUCTION

Triazine nucleus is a well known bioactive and basic component of active ingredients such as Azaribine, Azanucleosides [1-3] etc. Literature survey reveals that substituted 1,2,4-triazines are an interesting class of heterocyclic compounds owing to their role in resistance to p38α MAP kinase [4] and as leukemia, lung, breast and CNS anticancer agents [5-12]. Azole derivatives on the other side are a class of five-membered heterocycles has also possessed a great biological significance [13-15]. More specifically, substituted pyrazoles [16, 17] and 1,2,4-triazoles have been used extensively as an important synthons in the field of heterocyclic chemistry and drug designing approach. They are known to possess a broad spectrum of pharmacological activities such as antimicrobial [18, 19], anticancer [20], antitumor [21], antitubercular [22, 23], antioxidant [24], anti-inflammatory [25] and antidepressant [26]. Many azoles are currently being used as active pharmaceutical ingredients such as Conazoles, Itaconazole, Fluconazole, Ravuconazole etc. [27-29]. DNA serves a primary site where most of the chemotherapeutic agents interact and result in DNA photocleavage which in turn leads to inhibition of growth of cancerous cells [30]. Therefore, compounds having binding or interacting ability with DNA structure could be used as probes for DNA structure, potential chemotherapeutic and diagnostic agents [31]. In recent years, more attention has been paid to evaluate the DNA photocleavage potential of triazoles [32-37]. In view of medicinal importance and in continuation of our research work related to synthesis of biologically potent novel azoles, herein we report synthesis and DNA photocleavage activity of the some triazolotriazines and triazinylhydrazones.

MATERIALS AND METHODS

Chemistry

Melting points of all synthesized compounds were determined in an open capillary using digital melting point apparatus and are uncorrected. FT-IR spectra were recorded as KBr discs on a Perkin-Elmer Spectrophotometer in a range 4000–450 cm^{-1}. Both ^{1}H and ^{13}C NMR spectra of the compounds were recorded on the Bruker Advance NMR Spectrophotometer at 400 MHz and 100 MHz, respectively. Chemical shifts were measured relative to an internal reference standard, tetramethylsilane (TMS) (δ=0) in $CDCl_3$ or DMSO-d_6 and were reported on δ scale (in ppm). Coupling constants (J) were given in Hz. Mass spectra were recorded on Agilent Mass Spectrometer. Carbon, Nitrogen, hydrogen contents were analyzed using LECO 9320 analyzer.

Synthesis of triazinylhydrazones (3a-s)

General procedure

A solution of substituted benzaldehyde (**2a-k**, 0.01 mol) or 4-formylpyrazole (**2l-s**, 0.01 mol) in dichloromethane was added to an ethanolic solution of 5,6-Diphenyl-3-hydrazino-1,2,4-triazine (**1**, 0.01 mol). The reaction mass was refluxed for 40-45 minutes after addition of about one drop of conc. sulfuric acid and the reaction was monitored by thin layer chromatography. An excess of the solvent was evaporated and resulting mass was cooled to room temperature. The obtained product was filtered on a buchner funnel, washed with alcohol and recrystallised from ethanol. Noted m. p. And the samples were submitted to analysis.

3-(2-(Benzylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3a**). Yield 92%; m. p. 240-241 °C; R_f = 0.49 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3216 (N-H str.), 1592 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.33-7.54 (m, 13H, Ph', Ph''-H & Ph'''-H), 7.75 (d, 2H, J = 8.3 Hz, 2''', 6'''-H), 8.29 (s, 1H, 7'''-H), 11.78 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 126.5, 128.0, 128.1, 128.2, 128.5, 128.9, 129.1, 129.3, 130.1, 134.7, 135.8, 136.0, 143.9, 150.6, 156.2, 158.5; MS (ESI) m/z: 352.3 (M^+ + 1); Anal. Calcd. for $C_{22}H_{17}N_5$: C, 75.15; H, 4.84; N, 19.93. Found: C, 75.14; H, 4.81; N, 19.92.

3-(2-(4'''-Methoxybenzylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3b**). Yield 91.5%; m. p. 233-234 °C; R_f = 0.32 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3233 (N-H str.), 1593

(C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.81 (4'''-OC**H**$_3$), 7.01 (d, 2H, J = 8.8 Hz, 3''', 5'''-H), 7.34-7.50 (m, 10H, Ph'-H & Ph''-H), 7.68 (d, 2H, J = 8.76 Hz, 2''', 6'''-H), 8.24 (s, 1H, 7'''-H), 11.79 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.2, 114.2, 119.8, 127.3, 128.1, 128.2, 128.2, 128.3, 128.9, 129.4, 130.1, 135.9, 136.1, 143.9, 150.5, 156.2, 160.3; MS (ESI) m/z: 382.4 (M^+ + 1); Anal. Calcd. for $C_{23}H_{19}N_5O$: C, 72.36; H, 4.98; N, 18.35. Found: C, 72.33; H, 4.96; N, 18.32.

3-(2-(2'''-Ethoxybenzylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3c**). Yield 90%; m. p. 196-197 °C; R_f = 0.60 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3228 (N-H str.), 1589 (C=N str.); ^{1}H NMR (CDCl$_3$, 400 MHz): δ 1.43 (t, 3H, C**H**$_3$), 4.07-4.12 (q, 2H, 2'''-OC**H**$_2$), 6.89 (d, 1H, J = 8.2 Hz, 3'''-H), 7.00 (t, 1H, J = 7.9 Hz, 4'''-H), 7.30-7.57 (m, 11H, Ph', Ph''-H & 5'''-H), 8.16 (d, 1H, J = 7.8 Hz, 6'''-H), 8.52 (s, 1H, 7'''-H), 9.24 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 20.7, 55.4, 114.0, 114.2, 114.7, 118.8, 120.5, 127.9, 128.0, 128.5, 128.9, 129.0, 130.0, 130.5, 130.8, 141.2, 142.9, 150.1, 156.4, 160.1; MS (ESI) m/z: 396.4 (M^+ + 1); Anal. Calcd. for $C_{24}H_{21}N_5O$: C, 72.84; H, 5.31; N, 17.70. Found: C, 72.82; H, 5.30; N, 17.68.

3-(2-(4'''-Fluorobenzylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3d**). Yield 85%; m. p. 262-263 °C; R_f = 0.45 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3384 (N-H str.), 1592 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.15-7.53 (m, 12H, 3''', 5''', Ph'-H & Ph''-H), 7.78 (d, 2H, J = 8.6 Hz, 2''', 6'''-H), 8.28 (s, 1H, 7'''-H), 11.86 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 114.1, 114.9, 119.8, 123.0, 127.6, 128.3, 129.2, 129.5, 129.8, 130.3, 130.5, 140.8, 142.1, 150.0, 156.1, 161.9; MS (ESI) m/z: 370.2 (M^+ + 1); Anal. Calcd. for $C_{22}H_{16}FN_5$: C, 71.51; H, 4.33; N, 18.96. Found: C, 71.48; H, 4.31; N, 18.95.

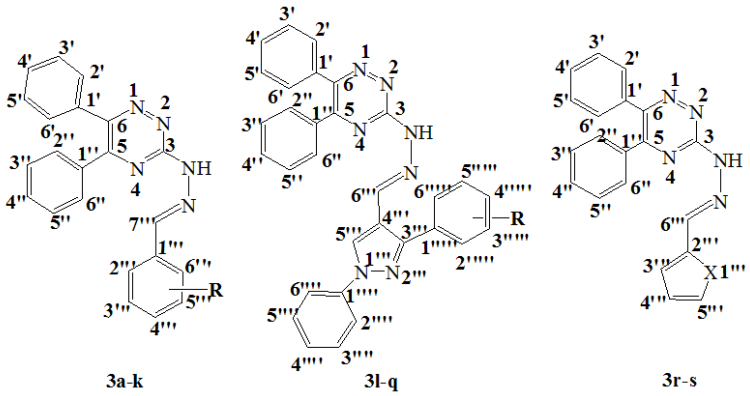


Fig. 1: Chemical structures of compounds 3a-k and 3l-s

3-(2-(4'''-Chlorobenzylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3e**). Yield 92%; m. p. 255-256 °C; R_f = 0.40 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3388 (N-H str.), 1599 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.34-7.52 (m, 12H, 3''', 5''', Ph'-H & Ph''-H), 7.75 (d, 2H, J = 8.6 Hz, 2''', 6'''-H), 8.27 (s, 1H, 7'''-H), 11.99 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 126.1, 128.2, 128.4, 128.5, 129.3, 129.6, 130.7, 130.9, 131.1, 135.1, 135.4, 136.2, 141.9, 143.2, 149.9, 155.9; MS (ESI) m/z: 386.7 (M^+ + 1), 388.1 (M^+ + 2) in the ratio showing typical chlorine isotope profile (3:1); Anal. Calcd. for $C_{22}H_{16}ClN_5$: C, 68.45; H, 4.15; N, 18.15. Found: C, 68.43; H, 4.13; N, 18.14.

3-(2-(4'''-Bromobenzylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3f**). Yield 88%; m. p. 249-250 °C; R_f = 0.39 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3392 (N-H str.), 1603 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.31-7.57 (m, 12H, 3''', 5''', Ph'-H & Ph''-H), 7.68 (d, 2H, J = 8.5 Hz, 2''', 6'''-H), 8.26 (s, 1H, 7'''-H), 11.93 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 125.9, 126.0, 128.0, 128.2, 128.3, 129.1, 129.6, 130.4, 130.9, 131.2, 134.2, 135.6, 142.1, 143.4, 150.2, 156.1; MS (ESI) m/z: 430.1 (M^+ + 1), 432.1 (M^+ + 2) in the ratio showing typical bromine isotope profile (1:1); Anal. Calcd. for $C_{22}H_{16}BrN_5$: C, 61.52; H, 3.73; N, 16.31. Found: C, 61.50; H, 3.72; N, 16.29.

3-(2-(4'''-Methylbenzylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3g**). Yield 91%; m. p. 251-252 °C; R_f = 0.44 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3244 (N-H str.), 1595 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.36 (s, 3H, 4'''-C**H**$_3$), 7.23 (d, 2H, J = 8.0 Hz, 3''', 5'''-H), 7.32-7.47 (m, 8H, Ph'-H & Ph''-H), 7.50 (d, 2H, J = 8.4 Hz, 2', 6'-H), 7.63 (d, 2H, J = 8.0 Hz, 2''', 6'''-H), 8.26 (s, 1H, 7'''-H), 11.84 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 21.0, 126.5, 128.0, 128.1, 128.2, 128.9, 129.2, 129.3, 130.1, 130.6, 132.0, 135.9, 136.0, 139.0, 144.1, 150.5, 156.3; MS (ESI) m/z: 366.2 (M^+ + 1); Anal. Calcd. for $C_{23}H_{19}N_5$: C, 75.58; H, 5.20; N, 19.17. Found: C, 75.55; H, 5.20; N, 19.16.

3-(2-(2''',4'''-Dichlorobenzylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3h**). Yield 89%; m. p. 242-243 °C; R_f = 0.79 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3398 (N-H str.), 1600 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.31-7.57 (m, 12H, Ph', Ph''-H & 5''', 6'''-H), 7.73 (s, 1H, 3'''-H), 8.28 (s, 1H, 7'''-H), 11.79 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 125.9, 127.1, 128.4, 128.6, 128.7, 130.4, 130.6, 130.9, 131.4, 131.9, 132.9, 135.5, 136.7, 138.1, 142.0, 143.1, 150.0, 156.2; MS (ESI) m/z: 421.1 (M^+ + 1); Anal. Calcd. for $C_{22}H_{15}Cl_2N_5$: C, 62.84; H, 3.57; N, 16.66. Found: C, 62.82; H, 3.56; N, 16.64.

3-(2-(4'''-Hydroxybenzylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3i**). Yield 85%; m. p. 236-237 °C; R_f = 0.28 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3421 (O-H str.), 3382 (N-H str.), 1595 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.80 (d, 2H, J = 8.0 Hz, 3''', 5'''-H), 7.34-7.55 (m, 11H, Ph', Ph''-H & 4'''-O**H**), 7.79 (d, 2H, J = 8.2 Hz, 2''', 6'''-H), 8.24 (s, 1H, 7'''-H), 11.75 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 116.5, 117.0, 129.4, 129.7, 128.9, 129.2, 130.1, 130.3, 130.7, 131.0, 136.3, 141.5, 142.6, 150.3, 156.1, 155.8; MS (ESI) m/z: 368.1 (M^+ + 1); Anal. Calcd. for $C_{22}H_{17}N_5O$: C, 71.92; H, 4.63; N, 19.07. Found: C, 71.89; H, 4.60; N, 19.06.

3-(2-(4'''-Nitrobenzylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3j**). Yield 86%; m. p. 268-270 °C; R_f = 0.35 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3380 (N-H str.), 1589 (C=N str.), 1492 (NO_2 symmetric str.), 1389 (NO_2 asymmetric str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.29-7.56 (m, 10H, Ph', Ph''-H & 2''', 6'''-H), 7.94 (d, 2H, J = 8.0 Hz, 3''', 5'''-H), 8.32 (s, 1H, 7'''-H), 11.70 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 125.8, 127.0, 127.4, 127.5, 127.9, 129.5, 130.2, 130.3, 130.4, 136.1, 136.9, 140.9, 142.3, 146.2, 150.0, 156.4; MS (ESI) m/z: 397.1 (M + 1)$^+$; Anal. Calcd. for $C_{22}H_{16}N_6O_2$: C, 66.65; H, 4.04; N, 21.21. Found: C, 66.62; H, 4.02; N, 21.18.

3-(2-(4'''-Hydroxy-3'''-methoxybenzylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3k**). Yield 84.5%; m. p. 226-227 °C; R_f = 0.36 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3410 (O-H str.), 3310 (N-H), 1591 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.84 (3'''-OC**H**$_3$), 6.85 (d, 1H, J = 8.3 Hz, 5'''-H), 7.29-7.52 (m, 11H, Ph'-H, Ph''-H & 4'''-O**H**), 7.64 (d, 2H, J = 8.2 Hz, 2''', 6'''-H), 8.23 (s, 1H, 7'''-H), 11.61 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.3, 115.9, 119.9, 121.5, 122.8, 126.5, 127.1, 127.3, 127.7, 129.9, 130.4, 131.3,

131.7, 141.7, 143.5, 150.5, 156.6, 157.8, 159.2; MS (ESI) m/z: 398.2 (M^+ + 1); Anal. Calcd. for $C_{23}H_{19}N_5O_2$: C, 69.49; H, 4.78; N, 17.62. Found: C, 69.48; H, 4.76; N, 17.60.

3-(2-(1''',3'''-Diphenylpyrazol-4'''-yl)methylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3l**). Yield 91%; m. p. 252-254 °C; R_f = 0.47 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3386 (N-H str.), 1594 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.36-7.55 (m, 16H, Ph', Ph'', Ph''''-H & Ph'''''-H), 7.84 (d, 2H, J = 8.4 Hz, 2'''', 6''''-H), 8.01 (d, 2H, J = 8.0 Hz, 2''', 6'''-H), 8.47 (s, 1H, 6'''-H), 8.92 (s, 1H, 5'''-H), 11.78 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 116.7, 118.8, 126.9, 127.1, 127.4, 128.1, 128.5, 128.7, 128.8, 128.9, 129.3, 130.0, 130.2, 130.5, 131.7, 132.0, 133.7, 140.6, 141.8, 143.5, 151.2, 152.3, 156.3; MS (ESI) m/z: 494.3 (M^+ + 1); Anal. Calcd. for $C_{31}H_{23}N_7$: C, 75.41; H, 4.66; N, 19.87. Found: C, 75.40; H, 4.64; N, 19.86.

3-(2-(3'''-(4'''''-Nitrophenyl-1'''-phenylpyrazol-4'''-yl)methylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3m**). Yield 88%; m. p. 281-282 °C; R_f = 0.43 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3394 (N-H str.), 1592 (C=N str.) 1498 (NO_2 symmetric str), 1395 (NO_2 asymmetric str); ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): δ 7.39-7.58 (m, 13H, Ph', Ph''-H & Ph''''-H), 8.01 (d, 2H, J = 8.0 Hz, 2''', 6'''-H), 8.11 (d, 2H, J = 8.2 Hz, 2'''', 6''''-H), 8.36 (d, 2H, J = 8.3 Hz, 3''''', 5'''''-H), 8.56 (s, 1H, 6'''-H), 8.99 (s, 1H, 5'''-H), 11.98 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 116.9, 118.7, 124.5, 126.6, 126.9, 127.1, 127.6, 128.1, 128.7, 128.9, 129.5, 130.8, 131.3, 131.9, 133.8, 137.1, 140.4, 140.9, 143.0, 146.3, 150.6, 151.0, 156.7; MS (ESI) m/z: 539.2 (M^+ + 1); Anal. Calcd. for $C_{31}H_{22}N_8O_2$: C, 69.12; H, 4.09; N, 20.81. Found: C, 69.10; H, 4.07; N, 20.79.

3-(2-(3'''-(4''''-Methoxyphenyl-1'''-phenylpyrazol-4'''-yl)methylidene) hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3n**). Yield 89%; m. p. 259-260 °C; R_f = 0.41 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3390 (N-H str.), 1597 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.82 (s, 3H, 4''''-OC**H**$_3$), 7.05 (d, 2H, J = 8.8 Hz, 3''''', 5''''-H), 7.33-7.54 (m, 13H, Ph', Ph''-H & Ph''''-H), 7.80 (d, 2H, J = 8.6 Hz, 2''''', 6''''-H), 8.00 (d, 2H, J = 8.6 Hz, 2'''', 6''''-H), 8.45 (s, 1H, 6'''-H), 8.91 (s, 1H, 5'''-H), 11.80 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.3, 113.9, 116.6, 118.6, 119.5, 125.3, 126.9, 127.0, 127.5, 127.9, 128.1, 128.4, 128.6, 130.6, 131.0, 131.3, 133.6, 139.3, 140.5, 144.2, 150.7, 151.4, 156.5, 159.2; MS (ESI) m/z: 524.5 (M^+ + 1); Anal. Calcd. for $C_{32}H_{25}N_7O$: C, 73.35; H, 4.78; N, 18.72. Found: C, 73.33; H, 4.76; N, 18.69.

3-(2-(3'''-(4''''-Fluorophenyl-1'''-phenylpyrazol-4'''-yl)methylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3o**). Yield 83%; m. p. 262-264 °C; R_f = 0.57 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3399 (N-H str.), 1599 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.20-7.53 (m, 15H, 3''''', 5''''', Ph', Ph''-H & Ph''''-H), 7.95-7.98 (m, 4H, 2''''', 6'''''-H & 2'''', 6''''-H), 8.44 (s, 1H, 6'''-H), 8.93 (s, 1H, 5'''-H), 11.79 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 115.9, 116.7, 118.5, 127.1, 127.7, 127.9, 128.4, 128.5, 128.8, 129.0, 129.6, 130.0, 130.4, 131.2, 131.7, 135.8, 140.4, 142.9, 144.0, 150.6, 151.6, 156.8, 161.4; MS (ESI) m/z: 512.1 (M^+ + 1); Anal. Calcd. for $C_{31}H_{22}FN_7$: C, 72.78; H, 4.30; N, 19.17. Found: C, 72.76; H, 4.28; N, 19.15.

3-(2-(3'''-(4''''-Chlorophenyl-1'''-phenylpyrazol-4'''-yl)methylidene)hydrazinyl-5,6-diphenyl-1,2,4-triazine (**3p**). Yield 87%; m. p. 256-257 °C; R_f = 0.53 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3402 (N-H str.), 1602 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.34-7.55 (m, 15H, 3''''', 5''''', Ph', Ph''-H & Ph''''-H), 7.977-8.00 (m, 4H, 2''''', 6'''''-H & 2'''', 6''''-H), 8.46 (s, 1H, 6'''-H), 8.92 (s, 1H, 5'''-H), 11.82 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 117.3, 118.6, 126.9, 127.3, 127.6, 128.5, 128.8, 129.0, 129.4, 129.7, 130.0, 130.2, 130.6, 130.8, 131.2, 133.5, 135.9, 140.6, 141.0, 143.4, 150.6, 151.0, 156.8; MS (ESI) m/z: 528.2 (M^+ + 1), 530.2 (M^+ + 2) in the ratio showing typical chlorine isotope profile (3:1); Anal. Calcd. for $C_{31}H_{22}ClN_7$: C, 70.56; H, 4.17; N, 18.59. Found: C, 70.54; H, 4.16; N, 18.57.

3-(2-(3'''-(4'''''-Bromophenyl-1'''-phenylpyrazol-4'''-yl)methylidene)hydrazinyl-5,6-diphenyl,2,4-triazine (**3q**). Yield 89%; m. p. 248-249 °C; R_f = 0.47 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3410 (N-H str.), 1606 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.36-7.55 (m, 15H, 3''''', 5''''', Ph', Ph''-H & Ph''''-H), 7.84 (d, 2H, J = 8.4 Hz, 2''''', 6'''''-H), 8.00 (d, 2H, J = 8.5 Hz, 2'''', 6''''-H), 8.46 (s, 1H, 6'''-H), 8.90 (s, 1H, 5'''-H), 11.82 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 116.8, 118.7, 123.9, 127.0, 127.4, 127.5, 127.9, 128.6, 128.9, 129.2, 129.6, 130.1, 130.4, 130.9, 131.5, 132.8, 133.8, 140.5, 141.5, 142.5, 150.8, 151.3, 156.0; MS (ESI) m/z: 572.2 (M^+ + 1), 574.4 (M^+ + 2) in the ratio showing typical bromine isotope profile (1:1); Anal. Calcd. for $C_{31}H_{22}BrN_7$: C, 65.13; H, 3.85; N, 17.16. Found: C, 65.11; H, 3.82; N, 17.14.

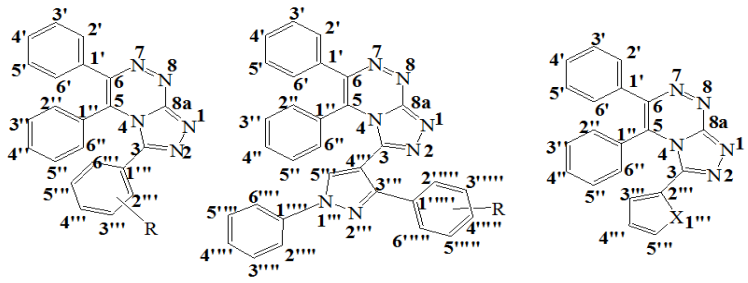
5,6-Diphenyl-3-((2-(thiophen-2'''-yl)methylidene)hydrazinyl)-1,2,4-triazine (**3r**). Yield 86.5%; m. p. 243-244 °C; R_f = 0.44 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3420 (N-H str.), 1595 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.09-7.47 (m, 10H, 3''', 4''', Ph'-H & Ph''-H), 7.49 (d, 2H, J = 8.2 Hz, 2', 6'-H), 7.54 (d, 1H, J = 5.1 Hz, 5'''-H), 8.48 (s, 1H, 6'''-H), 11.88 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 127.5, 127.6, 128.0, 128.1, 128.3, 128.9, 129.1, 129.3, 130.1, 135.8, 136.0, 139.1, 139.6, 150.6, 156.2, 158.2; MS (ESI) m/z: 358.2 (M^+ + 1); Anal. Calcd. for $C_{20}H_{15}N_5S$: C, 67.19; H, 4.20; N, 19.60. Found: C, 67.16; H, 4.19; N, 19.59.

3-((2-(Furfural-2'''-yl)methylidene)hydrazinyl)-5,6-diphenyl-1,2,4-triazine (**3s**). Yield 85%; m. p. 227-228 °C; R_f = 0.32 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3416 (N-H str.), 1594 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): 6.99-7.12 (m, 1H, 3'''-H), 7.31-7.46 (m, 9H, 4''', Ph'-H & Ph''-H), 7.46 (d, 2H, J = 8.2 Hz, 2', 6'-H), 7.51 (d, 1H, J = 5.2 Hz, 5'''-H), 8.47 (s, 1H, 6'''-H), 11.89 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 127.6, 127.9, 128.1, 128.4, 128.8, 129.1, 129.3, 129.6, 130.4, 135.8, 136.0, 138.8, 139.4, 150.4, 156.0, 157.8; MS (ESI) m/z: 342.1 (M^+ + 1); Anal. Calcd. for $C_{20}H_{15}N_5O$: C, 70.36; H, 4.40; N, 20.52. Found: C, 70.34; H, 4.39; N, 20.51.

Synthesis of 3,5,6-Trisubstituted-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (4a-s)

General procedure

IBD (0.011 mol) was added in a portion wise manner to the suspension/solution of triazinylhydrazones (**3a-s**, 0.01 mol) in dichloromethane under stirring. The reaction mass was further stirred for 1.0 h and reaction was monitored by TLC. After completion of the reaction, the solvent was evaporated and residues were triturated with petroleum ether twice to obtain the crude product which was recrystallised from ethanol.



4a-k **4l-q** **4r-s**

Fig. 1: Chemical structures of compounds 4a-k and 4l-s

3,5,6-Triphenyl-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4a**). Yield 90%; m. p. 222-223 °C; R_f = 0.29 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1577 (C=N str.); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.34-7.59 (m, 13H, Ph'', 3''', 4''', 5'''-H & Ph'-H), 8.54 (d, 2H, J = 8.6 Hz, 2''', 6'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 125.8, 127.1, 127.5, 128.1, 128.4, 129.1, 129.5, 129.7, 130.2, 130.5, 130.6, 136.0, 145.4, 148.2, 150.7, 155.8; MS (ESI) m/z: 350.2 (M^++ 1); Anal. Calcd. for $C_{22}H_{15}N_5$: C, 75.60; H, 4.30; N, 20.05. Found: C, 75.57; H, 4.29; N, 20.03.

3-(4'''-Methoxyphenyl)-5,6-diphenyl-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4b**). Yield 87.5%; m. p. 219-220 °C; R_f = 0.32 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1576 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.85 (s, 3H, 4'''-OC**H**$_3$), 6.60 (d, 2H, J = 8.0 Hz, 3''', 5'''-H), 6.98-7.22 (m, 5H, Ph''-H), 7.37-7.50 (m, 5H, Ph'-H); 8.35 (d, 2H, J = 8.9 Hz, 2''', 6'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.4, 112.9, 114.5, 118.1, 119.1, 126.9, 127.4, 128.3, 128.5, 129.6, 129.9, 130.3, 136.1, 143.2, 145.4, 155.3, 159.7; MS (ESI) m/z: 380.1 (M^+ + 1); Anal. Calcd. for $C_{23}H_{17}N_5O$: C, 72.80; H, 4.48; N, 18.46. Found: C, 72.78; H, 4.46; N, 18.46.

3-(2'''-Ethoxyphenyl)-5,6-diphenyl-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4c**). Yield 90%; m. p. 148-149 °C; R_f = 0.26 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1570 (C=N str.); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.7 (t, 3H, -C**H**$_3$), 4.10 (q, 2H, 2'''-OC**H**$_2$), 7.06-7.37 (m, 9H, Ph'', 3''', 4''', 5'''-H & 4'-H), 7.42-7.54 (m, 4H, Ph'-H), 7.75 (d, 1H, J = 7.8 Hz, 6'''-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.8, 55.3, 113.9, 114.2, 114.6, 118.1, 119.3, 126.9, 127.1, 127.4, 128.3, 128.5, 129.6, 129.8, 130.0, 136.4, 143.2, 145.3, 155.1, 159.6; MS (ESI) m/z: 394.2 (M^+ + 1); Anal. Calcd. for $C_{24}H_{19}N_5O$: C, 73.25; H, 4.83; N, 17.80. Found: C, 73.23; H, 4.80; N, 17.78.

3-(4'''-Fluorophenyl)-5,6-diphenyl-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4d**). Yield 85%; m. p. 239-240 °C; R_f = 0.25 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1569 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.25 (d, 2H, J = 8.0 Hz, 3''', 5'''-H), 7.34-7.54 (m, 10H, Ph''-H & Ph'-H), 8.55 (d, 2H, J = 8.6 Hz, 2''', 6'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 114.1, 114.4, 118.0, 122.0, 126.8, 127.5, 128.4, 128.7, 129.7, 129.9, 130.4, 136.1, 143.2, 145.4, 155.3, 162.7; MS (ESI) m/z: 368.2 (M^+ + 1); Anal. Calcd. for $C_{22}H_{14}FN_5$: C, 71.90; H, 3.81; N, 19.06. Found: C, 71.88; H, 3.80; N, 19.04.

3-(4'''-Chlorophenyl)-5,6-diphenyl-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4e**). Yield 88.5%; m. p. 212-214 °C; R_f = 0.22 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1573 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.31-7.48 (m, 10H, 3''', 5''', Ph''-H & 3', 4', 5'-H), 7.67 (d, 2H, J = 8.4 Hz, 2', 6'-H); 8.43 (d, 2H, J = 8.4 Hz, 2''', 6'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 124.5, 127.3, 127.9, 128.0, 129.1, 129.6, 130.0, 130.2, 130.4, 134.0, 135.3, 135.8, 144.6, 148.2, 150.6, 155.6; MS (ESI) m/z: 384.1 (M + 1)$^+$, 386.1 (M + 2)$^+$ in the ratio showing typical chlorine isotope profile (3:1); Anal. Calcd. for $C_{22}H_{14}ClN_5$: C, 68.91; H, 3.65; N, 18.27. Found: C, 68.90; H, 3.65; N, 18.25.

3-(4'''-Bromophenyl)-5,6-diphenyl-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4f**). Yield 90.5%; m. p. 224-225 °C; R_f = 0.19 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1582 (C=N str.); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.30-7.51 (m, 10H, 3''', 5''', Ph''-H & 3', 4', 5'-H), 7.71 (d, 2H, J = 8.5 Hz, 2', 6'-H); 8.44 (d, 2H, J = 8.6 Hz, 2''', 6'''-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 124.4, 125.1, 127.2, 127.7, 128.2, 129.2, 129.8, 130.0, 130.3, 130.6, 133.7, 135.8, 144.7, 147.8, 150.9, 155.4; MS (ESI) m/z: 429.1 (M^+ + 1), 431.2 (M^+ + 2) in the ratio showing typical bromine isotope profile (1:1); Anal. Calcd. for $C_{22}H_{14}BrN_5$: C, 61.67; H, 3.27; N, 16.35. Found: C, 61.65; H, 3.26; N, 16.34.

5,6-Diphenyl-3-(4'''-methylphenyl)-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4g**). Yield 91%; m. p. 228-229 °C; R_f = 0.23 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1576 (C=N str.); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.42 (s, 3H, 4'''-C**H**$_3$), 7.36-7.50 (m, 12H, 3''', 5''', Ph''-H & Ph'-H), 8.30 (d, 2H, J = 8.4 Hz, 2''', 6'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 21.1, 123.0, 126.7, 128.0, 128.3, 128.4, 128.7, 129.5, 129.6, 129.9, 130.3, 135.9, 140.2, 145.5, 148.0, 150.4, 155.3; MS (ESI) m/z: 364.1 (M^+ + 1); Anal. Calcd. for $C_{23}H_{17}N_5$: C, 76.01; H, 4.68; N, 19.28. Found: C, 76.00; H, 4.66; N, 19.27.

3-(2''',4'''-Dichlorophenyl)-5,6-diphenyl-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4h**). Yield 90.5%; m. p. 235-236 °C; R_f = 0.49 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1578 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.34-7.51 (m, 10H, Ph''-H & Ph'-H), 7.57 (dd, 1H, J = 8.0 Hz, 5'''-H), 7.74 (s, 1H, 3'''-H), 7.81 (d, 1H, J = 8.3 Hz, 6'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 124.7, 126.3, 127.4, 128.0, 128.2, 129.3, 129.7, 130.0, 130.3, 130.7, 132.0, 135.8, 136.2, 137.2, 144.5, 148.2, 150.6, 155.6; MS (ESI) m/z: 419.1 (M^+ + 1); Anal. Calcd. for $C_{22}H_{13}Cl_2N_5$: C, 63.14; H, 3.11; N, 16.74. Found: C, 63.12; H, 3.11; N, 16.70.

5,6-Diphenyl-3-(4'''-hydroxyphenyl)-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4i**). Yield 88.5%; m. p. 221-222 °C; R_f = 0.13 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3418 (O-H str.), 1572 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.18-7.45 (m, 10H, 3''', 5''', Ph''-H & 3', 4', 5'-H), 7.72-7.77 (m, 3H, 2', 6'-H & 4'''-O**H**), 8.33 (d, 2H, J = 8.4 Hz, 2''', 6'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 115.9, 116.5, 128.1, 128.3, 128.6, 129.3, 129.6, 129.7, 129.9, 130.2, 136.1, 145.7, 147.8, 150.4, 155.2, 159.5; MS (ESI) m/z: 366.1 (M^+ + 1); Anal. Calcd. for $C_{22}H_{15}N_5O$: C, 72.31; H, 4.11; N, 19.17. Found: C, 72.30; H, 4.10; N, 19.16.

5,6-Diphenyl-3-(4'''-nitrophenyl)-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4j**). Yield 86.5%; m. p. 242-243 °C; R_f = 0.13 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1558 (C=N str.), 1464 (NO_2 symmetric str.), 1377 (NO_2 asymmetric str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.28-7.54 (m, 10H, Ph''-H & Ph'-H), 8.18 (d, 2H, J = 8.4 Hz, 3''', 5'''-H), 8.36 (d, 2H, J = 8.6 Hz, 2''', 6'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 124.4, 126.0, 126.7, 127.3, 127.5, 128.3, 129.5, 129.7, 129.8, 136.0, 137.2, 143.5, 146.7, 145.8, 150.4, 154.9; MS (ESI) m/z: 395.1 (M^++1); Anal. Calcd. for $C_{22}H_{14}N_6O_2$: C, 66.99; H, 3.55; N, 21.31. Found: C, 66.97; H, 3.53; N, 21.30.

5,6-Diphenyl-3-(3'''-methoxy-4'''-hydroxyphenyl)-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4k**). Yield 84.5%; m. p. 205-207 °C; R_f = 0.21 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 3412 (O-H str.), 1576 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.78 (s, 3H, 3'''-OC**H**$_3$), 7.10-7.57 (m, 11H, 5''', Ph'' & Ph'-H), 7.78-7.835 (m, 2H, 4'''-O**H** & 2'''-H), 8.32-8.34 (d, 1H, J = 8.0 Hz, 6'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.4, 115.6, 118.6, 120.1, 121.5, 125.9, 126.2, 126.5, 126.9, 128.8, 130.2, 130.7, 131.5, 136.9, 142.9, 145.7, 155.2, 159.3, 159.5; MS (ESI) m/z: 396.1 (M^+ + 1); Anal. Calcd. for $C_{23}H_{17}N_5O_2$: C, 69.86; H, 4.30; N, 17.71. Found: C, 69.82; H, 4.30; N, 17.70.

3-(1''',3'''-Diphenylpyrazol-4'''-yl)-5,6-diphenyl-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4l**). Yield 92%; m. p. 232-233 °C; R_f = 0.21 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1578 (C=N str.); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.88 (d, 2H, J = 8.4 Hz, 3'', 5''-H), 7.36-7.55 (m, 16H, Ph', Ph'', Ph''''-H & Ph''''-H), 7.80 (d, 2H, J = 8.0 Hz, 2'''', 6''''-H), 8.70 (s, 1H, 5'''-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 106.6, 119.6, 125.8, 127.3, 127.4, 127.9, 128.2, 128.3, 128.7, 129.2, 129.7, 129.9, 130.0, 130.6, 131.0, 131.6, 133.6, 142.5, 143.8, 147.8, 150.1, 152.1, 155.4; MS (ESI) m/z: 492.2 (M + 1)$^+$; Anal. Calcd. for $C_{31}H_{21}N_7$: C, 75.73; H, 4.28; N, 19.95. Found: C, 75.71; H, 4.27; N, 19.94.

3-(3'''-(4''''-Nitrophenyl)-1'''-phenylpyrazol-4'''-yl)-5,6-diphenyl-[1,2,4]

triazolo[3,4-c][1,2,4]-triazine (**4m**). Yield 91%; m. p. 262-263 °C; R_f = 0.21 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1570 (C=N str.); 1474 (NO_2 symmetric str.), 1382 (NO_2 asymmetric str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.42-7.60 (m, 13H, Ph', Ph''-H & Ph''''-H), 8.06 (d, 2H, J = 8.2 Hz, 2'''', 6''''-H), 8.32-8.356 (m, 4H, Ph''''-H), 8.82 (s, 1H, 5'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 106.6, 119.0, 124.6, 125.8, 126.3, 126.8, 128.4, 128.8, 128.9, 129.4, 129.9, 130.0, 130.1, 131.6, 133.6, 137.2, 139.6, 142.2, 146.3, 147.8, 150.5, 151.8, 155.6; MS (ESI) m/z: 537.2 (M^+ + 1); Anal. Calcd. for $C_{31}H_{20}N_8O_2$: C, 69.38; H, 3.73; N, 20.89. Found: C, 69.36; H, 3.71; N, 20.88.

3-(3'''-(4''''-Methoxyphenyl)-1'''-phenylpyrazol-4'''-yl)-5,6-diphenyl-[1,2,4]

triazolo[3,4-c][1,2,4]-triazine (**4n**). Yield 89.8%; m. p. 238-239 °C; R_f = 0.21 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1577

(C=N str.); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.79 (s, 3H, 4''''-OC**H**$_3$), 6.94 (d, 2H, J = 8.6 Hz, 3'''', 5''''-H), 7.02 (d, 2H, J = 7.8 Hz, 3'', 5''-H), 7.22-7.51 (m, 11H, Ph', Ph''-H & Ph''''-H), 7.71 (d, 2H, J = 8.6 Hz, 2'''', 6''''-H), 7.81 (d, 2H, J = 7.9 Hz, 2'''', 6''''-H), 8.71 (s, 1H, 5'''-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 55.3, 106.1, 113.9, 119.4, 119.5, 124.2, 125.6, 127.3, 127.9, 128.4, 128.5, 129.6, 129.9, 130.1, 130.4, 131.1, 133.6, 139.5, 142.5, 147.6, 150.3, 152.0, 155.6, 160.1; MS (ESI) m/z: 522.2 (M^+ + 1); Anal. Calcd. for $C_{32}H_{23}N_7O$: C, 73.68; H, 4.41; N, 18.80. Found: C, 73.66; H, 4.40; N, 18.79.

3-(3'''-(4''''-Fluorophenyl)-1'''-phenylpyrazol-4'''-yl)-5,6-diphenyl-[1,2,4]

triazolo[3,4-c][1,2,4]-triazine (**4o**). Yield 84.5%; m. p. 245-246 °C; R_f = 0.21 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1579 (C=N str.); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.01-7.53 (m, 12H, 3'''', 5''''-H & Ph', Ph''-H), 7.57-7.84 (m, 5H, 3'''', 4'''', 5''''-H & 2'''', 6''''-H), 7.99 (d, 2H, J = 8.0 Hz, 2'''', 6''''-H), 9.22 (s, 1H, 5'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 106.1, 115.3, 119.0, 126.6, 127.3, 128.1, 128.2, 129.5, 129.6, 129.7, 130.1, 130.2, 130.4, 130.8, 132.4, 135.9, 141.2, 143.5, 147.8, 149.3, 150.3, 155.9, 162.3; MS (ESI) m/z: 510.2 (M^+ + 1); Anal. Calcd. for $C_{31}H_{20}FN_7$: C, 73.06; H, 3.93; N, 19.25. Found: C, 73.05; H, 3.91; N, 19.24.

3-(3'''-(4''''-Chlorophenyl)-1'''-phenylpyrazol-4'''-yl)-5,6-diphenyl-[1,2,4]

triazolo[3,4-c][1,2,4]-triazine (**4p**). Yield 86.6%; m. p. 234-235 °C; R_f = 0.21 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1580 (C=N str.); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.78-7.44 (m, 15H, 3'''', 5'''', Ph', Ph''-H & Ph''''-H), 7.66-7.72 (m, 4H, 2'''', 6''''-H & 2'''', 6''''-H), 8.70 (s, 1H, 5'''-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 106.6, 119.5, 125.9, 127.5, 128.5, 128.6, 128.9, 129.5, 129.6, 129.8, 129.9, 130.0, 130.5, 131.0, 131.6, 133.6, 135.3, 139.4, 142.1, 147.7, 150.4, 151.0, 155.5; MS (ESI) m/z: 527.1 (M^+ + 1), 529.2 (M^+ + 2) in the ratio showing typical chlorine isotope profile (3:1); Anal. Calcd. for $C_{31}H_{20}ClN_7$: C, 70.71; H, 3.80; N, 18.63. Found: C, 70.69; H, 3.78; N, 18.62.

3-(3'''-(4''''-Bromophenyl)-1'''-phenylpyrazol-4'''-yl)-5,6-diphenyl-[1,2,4]

triazolo[3,4-c][1,2,4]-triazine (**4q**). Yield 90%; m. p. 227-228 °C; R_f = 0.21 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1594 (C=N str.); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38-7.54 (m, 15H, 3'''', 5'''', Ph', Ph''-H & Ph''''-H), 7.58-7.62 (m, 4H, 2'''', 6''''-H & 2'''', 6''''-H), 8.72 (s, 1H, 5'''-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 106.5, 119.6, 124.1, 126.1, 127.8, 128.0, 128.8, 128.9, 129.1, 129.6, 129.8, 129.9, 130.1, 130.2, 131.5, 132.6, 133.8, 139.5, 142.3, 147.6, 150.3, 150.9, 155.5; MS (ESI) m/z: 571.3 (M^+ + 1), 573.1 (M^+ + 2) in the ratio showing typical bromine isotope profile (1:1); Anal. Calcd. for $C_{31}H_{20}BrN_7$: C, 65.23; H, 3.51; N, 17.18. Found: C, 65.21; H, 3.50; N, 17.16.

5,6-Diphenyl-3-(thiophen-2'''-yl)-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4r**). Yield 81.9%; m. p. 198-199 °C; R_f = 0.21 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1575 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.18-7.55 (m, 11H, 5''', Ph'-H & Ph''-H), 7.819-7.834 (m, 1H, 4'''-H), 8.17-8.18 (t, 1H, 3'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 125.9, 126.3, 126.9, 127.5, 128.0, 129.1, 129.6, 130.0, 130.2, 130.3, 134.0, 135.8, 142.4, 147.6, 150.7, 155.5; MS (ESI) m/z: 356.1 (M + 1)$^+$; Anal. Calcd. for $C_{20}H_{13}N_5S$: C, 67.59; H, 3.66; N, 19.71. Found: C, 67.57; H, 3.65; N, 19.69.

5,6-Diphenyl-3-(furan-2'''-yl)-[1,2,4]triazolo[3,4-c][1,2,4]-triazine (**4s**). Yield 82%; m. p. 205-206 °C; R_f = 0.21 [ethylacetate: petroleum ether (3:7)]; FT-IR (KBr, cm^{-1}): 1576 (C=N str.); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.01-7.49 (m, 12H, 5''', 4''', Ph'-H & Ph''-H), 7.89-7.92 (t, 1H, 3'''-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 126.4, 127.5, 127.9, 128.1, 128.9, 129.0, 129.5, 130.0, 130.5, 131.3, 134.6, 136.0, 142.3, 147.5, 150.6, 155.4; MS (ESI) m/z: 340.1 (M^++1); Anal. Calcd. for $C_{20}H_{13}N_5O$: C, 70.78; H, 3.83; N, 20.64. Found: C, 70.76; H, 3.81; N, 20.62.

Biological activity

DNA photocleavage studies

DNA photocleavage experiment was performed by taking 10 µl solution containing plasmid DNA in TE (Tris 10 mM, EDTA 0.01 mM, pH 8.0) buffer in presence of 40 µg of the synthesized compounds. The sample solution held in caps of polyethylene microcentrifuge tubes were placed directly on the surface of a trans-illuminator (8000 mW/cm) at 360 nm and were irradiated for 30 minutes at room temperature. After irradiation, samples were further incubated at 37°C for 1h. Irradiated samples were mixed with 6X loading dye containing 0.25% bromophenol blue and 30% glycerol. The samples were then analyzed by electrophoresis on a 0.8% agarose horizontal slab gel in Tris-acetate EDTA buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH: 8.0). Untreated plasmid DNA was maintained as a control in each run of gel electrophoresis and carried out at 5V/cm for 2.0 h. Gel was stained with ethidium bromide (1 µg/mL) and photographed under UV light [37-38] for further step.

RESULT AND DISCUSSION

Chemistry

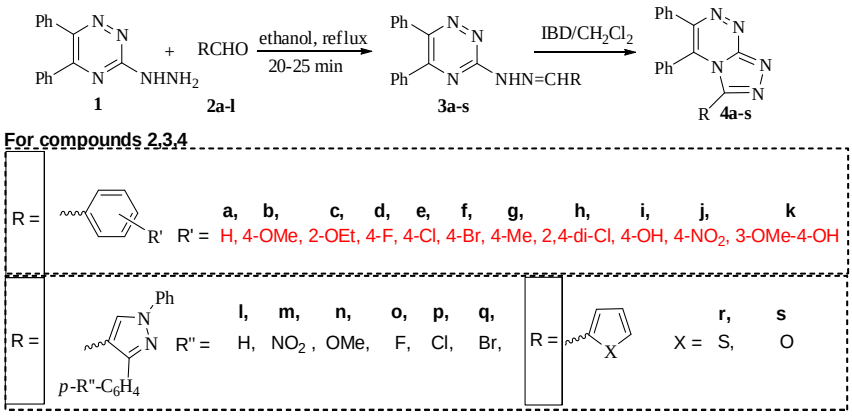
Recently, organic synthesis acquired various advantages such as a shorter reaction time, higher regio-selectivity [39], use of greener solvents or reagents with low toxicity profile etc. Because of non-toxic and eco-friendly nature, organoiodine (III) reagents are well known for their selectivity in organic synthesis [40, 41] and have extensively been used in construction of a large number of heterocycles. Viewing a wide range of pharmaceutical activities of triazine and azoles, it was decided to synthesize some novel aryl/heteroaryl substituted triazine based triazoles under mild reaction conditions and to explore their biological potential by evaluating DNA photocleavage activity in comparison to plasmid DNA.

In this study, the triazolotriazines have been synthesized by the oxidative cyclization of triazinylhydrazones using IBD in dichloromethane under mild conditions with high purity and good yields. In the present investigation, total thirty eight novel compounds were prepared as summarized in **Scheme 1** and characterized on the basis of FT-IR, ^{1}H, ^{13}C NMR and mass spectral data. To achieve the target, initially we prepared the key substrate, 5,6-Diphenyl-3-hydrazino-1,2,4-triazine **1** which involves in the synthesis of 3,5,6-trisubstituted-[1,2,4]triazolo[3,4-c][1,2,4]triazines. Reactant **1** was synthesized by the reaction of benzil with thiosemicarbazide [42, 43] followed by the successive reactions with different reagents [44]. Another starting material, 4-formylpyrazole was also prepared according to the literature method [45]. A series of nineteen triazinylhydrazones **(3a-s)** was obtained by the condensation of **1** with various substituted aldehydes (**2a-k)** and 3-aryl-1-phenyl-1H-pyrazole-4-carbaldehydes (**2l-s)** in ethanol under reflux in presence of a catalytic amount of concentrated H_2SO_4. Further, the oxidative cyclization of triazinylhydrazones has been achieved in the presence of 1.1 equivalent of IBD [46] in dichloromethane at room temperature to give the desired products successfully with 82-92% yields.

The FT-IR spectra of compounds **3a-s** showed absorption band for –NH stretching in a range at 3216-3420 cm^{-1} and thus indicated the formation of hydrazones. The two singlets in the range at δ 8.90-8.99 and 8.23-8.56 were appeared in ^{1}H NMR spectrum of triazinylhydrazones (**3l-s**) due to 5'''-H of pyrazole ring and N=CH, respectively. In ^{1}H NMR spectra of hydrazones **3a-s**, the characteristic downfield signal at δ 11.62-11.99 ppm was attributed to NH proton and rest of the protons exhibited multiplets in the aromatic region. Chemical shifts at δ 156.2-156.8 correspond to CH=N carbon in ^{13}C NMR spectra of hydrazones.

The structures of final products (**4**) were established by comparing the spectral data of hydrazones (**3a-s**) with **4**. The FT-IR spectra of **4a-s** were transparent in region of NH stretching and thus confirmed the successful oxidation of **3** into **4**. Disappearance of chemical shifts at δ 8.23-8.56 (N=CH) and 11.62-11.99 (NH) in ^{1}H NMR spectra of products (**4a-s**) also confirmed the oxidative transformation of triazinylhydrazones into 3,5,6-Trisubstituted-[1,2,4]triazolo[3,4-c][1,2,4]triazine. The ^{13}C NMR spectra showed peaks at around δ 155.3-155.6 (C-3) for triazole carbon and other signals at δ 107.0 and 139.3 corresponding to pyrazole ring carbons.

In ^{13}C NMR spectra, disappearance of signal at δ 156.2-156.77 supported the formation of titled compounds (**4**). The signal at δ 107.0 was appeared due to the pyrazole carbon attached to triazole ring.



Scheme 1: Synthesis of 3,5,6-trisubstituted-[1,2,4]triazolo[3,4-c][1,2,4]triazine (4a-s)

Biological activity

DNA photocleavage activity

The DNA photocleavage study was performed using agarose gel electrophoresis method and results are presented in **Fig. 3** and **4**. It has been observed that intensity of DNA was significantly decreased in the case of triazinylhydrazones and triazoles in comparison to DNA as control. Decrease in intensity of plasmid DNA in case of triazinylhydrazones **3b**, **3d**, **3e**, **3h**, **3i**, **3p**, **3q**, **3r** (Lane 3, 5, 6, 9, 10, 18, 19 and 20) as compared with control (Lane 1) indicated the cleavage of DNA forms. As shown in **Fig. 3**, the compounds **3b**, **3d-e**, **3h**, **3p**, **3q** and **3r** were responsible for the complete disappearance of supercoiled (form- I) DNA. However, compounds **3c**, **3f**, **3g**, **3i**, and **3o** (lane 4, 7, 8, 10 and 17, respectively) were responsible to decrease the intensity of supercoiled DNA.

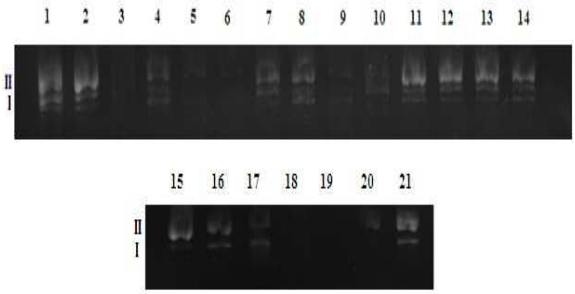


Fig. 3: DNA photocleavage study of triazinylhydrazones (3a-s)

Fig. 3: Lane 1: Control plasmid DNA + UV + DMSO, Lane 2: DNA + 40µg 3a, Lane 3: DNA + 40µg 3b, Lane 4: DNA + 40µg 3c, Lane 5: DNA + 40µg 3d, Lane 6: DNA + 40µg 3e, Lane 7: DNA + 40µg 3f, Lane 8: DNA + 40µg 3g, Lane 9: DNA + 40µg 3h, Lane 10: DNA + 40µg 3i, Lane 11: DNA + 40µg 3j, Lane 12: DNA + 40µg 3k, Lane 13: DNA + 40µg 3l, Lane 14: DNA + 40µg 3m, Lane 15: Control plasmid DNA + UV + DMSO, Lane 16: DNA + 40µg 3n, Lane 17: DNA + 40µg 3o, Lane 18: DNA + 40µg 3p, Lane 19: DNA + 40µg 3q, Lane 20: DNA + 40µg 3r, Lane 21: DNA + 40µg 3s.

Triazole derivatives **4m-n**, **4p-s** (lane 14, 16 18, 19, 20 and 21) on irradiation with UV light were found to show complete cleavage of DNA forms. Compounds **3b**, **3d**, **3e**, **3h**, **3p, 3q** and **4m-n**, **4p-s** were found to show excellent DNA cleaving activity in case of triazinylhydrazones and triazolotriazine, respectively. It was found that both hydrazones as well as triazole derivatives enhance the DNA cleavage activity significantly and may be used to serve the basis of some bioactive heterocycles in the future.

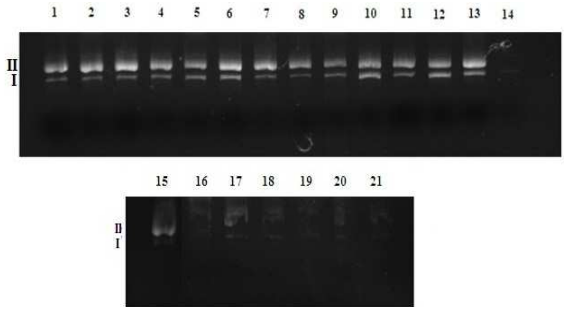


Fig. 4: DNA photocleavage study of triazolotriazines (4a-s)

Fig. 4: Lane 1: Control plasmid DNA + UV + DMSO, Lane 2: DNA + 40µg 4a, Lane 3: DNA + 40µg 4b, Lane 4: DNA + 40µg 4c, Lane 5: DNA + 40µg 4d, Lane 6: DNA + 40µg 4e, Lane 7: DNA + 40µg 4f, Lane 8: DNA + 40µg 4g, Lane 9: DNA + 40µg 4h, Lane 10: DNA + 40µg 4i, Lane 11: DNA + 40µg 4j, Lane 12: DNA + 40µg 4k, Lane 13: DNA + 40µg 4l, Lane 14: DNA + 40µg 4m, Lane 15: Control plasmid DNA + UV + DMSO, Lane 16: DNA + 40µg 4n, Lane 17: DNA + 40µg 4o, Lane 18: DNA + 40µg 4p, Lane 19: DNA + 40µg 4q, Lane 20: DNA + 40µg 4r, Lane 21: DNA + 40µg 4s.

CONCLUSION

In the present investigation, the synthesis of novel triazolotriazine derivatives via oxidative cyclization of new triazinylhydrazones using IBD is reported. The structures of the products were established on the basis of FT-IR, ^{1}H, ^{13}C NMR and mass spectral data. It has been observed that both classes of compounds **4m-n, 4p-s** as well as **3b**, **3d**, **3e**, **3h**, **3p, 3q** were acting as good photocleaving agents. In case of triazolotriazines, the para substitution on phenyl ring attached to pyrazole moiety with electron-releasing group increases the DNA photocleavage ability whereas electron-withdrawing group decreases to some extent. Further, triazinylhydrazones containing phenyl ring attached to pyrazole moiety having electron-releasing group also enhance the DNA photocleavage potential.

CONFLICT OF INTERESTS

Declared None.

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