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Synthesis of triazole Schiff bases: Novel inhibitors of nucleotide pyrophosphatase/phosphodiesterase-1

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ABSTRACT

A series of Schiff base triazoles **1**–**25** was synthesized and evaluated for their nucleotide pyrophosphatase/phosphodiesterase-1 inhibitory activities. Among twenty-five compounds, three compounds **10** (IC_{50} = 132.20 ± 2.89 μM), **13** (IC_{50} = 152.83 ± 2.39 μM), and **22** (IC_{50} = 251.0 ± 6.64 μM) were identified as potent inhibitors with superior activities than the standard EDTA (IC_{50} = 277.69 ± 2.52 μM). The newly identified inhibitors may open a new avenue for the development of treatment of phosphodiesterase-I related disorders. These compounds were also evaluated for carbonic anhydrase, acetylcholinesterase and butyrylcholinesterase inhibitory potential and were found to be inactive. The compounds showed non-toxic effect towards PC3 cell lines.

1. Introduction

Triazoles are five membered organic heterocyclic compounds having a di-unsaturated ring comprising of three nitrogen and two carbon atoms. 1,2,4-Triazoles are cyclic hydrazones with *H*-atom (or substitution) on either hydrazide *N*-atom or an amide *N*-atom. According to the literature, 1*H*-1,2,4-triazoles and their analogues have diverse biological activities, such as antiasthamatic,[1,2] antiviral,[3] antifungal,[4] antibacterial,[5] hypnotic,[6] pesticidal,[7] breast cancer preventive,[8,9] anticonvulsant, anticancer, antiinflammatory,[10–19] CNS depressant,[20] and antihypertensive[21,22] activities. 1,2,4-Triazole derivatives have been used as mimics and isosteres.[23–30] Vasodilatory,[31,32] psychotropic,[33,34] cyclooxygenase and 5-lipoxygenase inhibition,[35] hypoglycemic,[36] antitumor activities,[37] plant growth regulating and anticoagulant properties are also reported for this class of compounds.[38]

Easily attainable heterocyclic compounds of synthetic origin are ideal candidates for drug discovery and development against defined biological targets. Based on this hypothesis, we recently reported 1,3,4-oxadiazole-2(3*H*)-thiones and 1,3,4-thiadiazole-2(3*H*)-thiones (Fig. 1), as potent inhibitors of nucleotide pyrophosphatase/phosphodiesterase-I.[39] Due to close structural resemblance of 1,2,4-triazoles with above-mentioned compounds, we synthesized 1,2,4-triazoles as potential inhibitors of nucleotide pyrophosphatase/phosphodiesterase-1. Enzyme inhibition studies on 1,2,4-triazoles proved our hypothesis.

Nucleoside-5′-monophosphates are released by the catalytic action of nucleotide pyrophosphatase/phosphodiesterase-1 (NPP/PDEs) from a variety of nucleotides and their derivatives.[40] These PDEs/NPPs occur in both forms as soluble proteins in the body or membrane proteins with active site outside the body. These enzymes are widely distributed throughout the body cells, as well as in a variety of organs, such as liver cells, serum, mammalian intestinal mucosa, and snake venom.[41–43] Plasma cell membrane glycoprotein (NPP1/PC-1) is the major agent for bone calcification and related tissues. The hyperactivity of PDE1 results in chondrocalcirosis,[44] whereas down regulation of enzymes severes calcification in mice.[45,46] Human calcification syndrome is found to be associated with the down regulation of PDEs.

Only a few studies have been conducted on the inhibition of nucleotide pyrophosphatases/phosphodiesterases by natural or synthetic compounds.[47–53] Progress in the discovery and development of inhibitors slowed largely due to the unavailability of the three-dimensional structures of the target enzymes.[54]

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0968-0896/

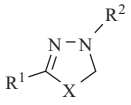


Figure 1. 1,3,4-Oxadiazole-2(3*H*)-thiones and 1,3,4-thiadiazole-2(3*H*)-thiones.

During the current study, triazole Schiff bases **1**–**25** were synthesized by using very simple and one step synthetic procedure keeping in mind the easy adoptability by pharma industries and cheap commercialization. All the synthetic Schiff bases **1**–**25** were screened for the inhibition of the snake venom nucleotide pyrophosphatase/phosphodiesterase-1. According to literature survey, these compounds are novel inhibitors of PDE1. This is the first report on the inhibitory potential of Schiff base of triazoles against PDE1/NPP1.

2. Results and discussion

2.1. Chemistry

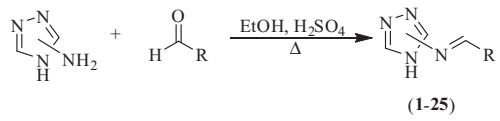
Triazole Schiff bases **1**–**25** were synthesized by refluxing different substituted benzaldehydes and amino triazole in equimolar proportion in ethanol as a solvent with a catalytic amount of H_2SO_4 for 4–10 h. Progress of reaction was periodically monitored by TLC. After completion of reaction, solvent was evaporated under reduced pressure. The solid mass was re-crystallized with ethanol, which gave pure compounds (Scheme 1). These resulting compounds were characterized by ^{1}H NMR and EI mass spectrometry. Elemental analyses of all the compounds were found satisfactory. Compounds **3**, **4**, **6**, **7**, **8**, **13**, and **25** were synthesized for the first time.

In vitro snake venom NPP1 inhibitory activity of all derivatives of triazole Schiff bases **1**–**25** was carried out, whereas EDTA was used as the positive control. Compounds **10**, **13**, and **22** were found to be most potent inhibitors against PDE1/NPP1 enzymes with IC_{50} values 132.20 ± 2.89, 152.83 ± 2.39, and 251.0 ± 6.64 μM, respectively, better than the standard EDTA (IC_{50} = 277.69 ± 2.52 μM). Compounds **23**, **21**, and **20** were found to be weakly active with IC_{50} values between 404 and 1164 μM. Nonetheless, all other derivatives were found to be inactive. The IC_{50} values of all the inhibitors against snake venom PDE1/NPP1 are shown in Table 1.

EDTA is used for the treatment of rheumatoid arthritis, osteoarthritis, diabetes, Parkinson's disease, and multiple sclerosis. Skin conditions, including scleroderma and psoriasis are also being treated with EDTA. As the PDEs hyperactivity is involved in a number of diseases such as arthritis, hypertension, and cardiovascular diseases,[55] therefore, it is usually used as standard for PDE1/NPP1 enzymes.

2.2. Structure–activity relationship

Structure–activity relationship (SAR) revealed that activity is mainly due to substitutions on benzene ring. Compound **10** having



Scheme 1. Synthesis of triazole Schiff bases **1**–**25**.

Table 1
Phosphodiesterase-I inhibition by Schiff bases of triazole **1**–**25**

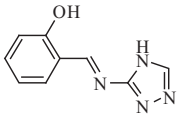
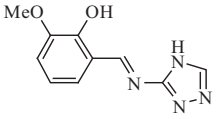
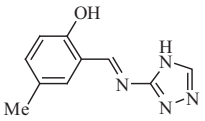
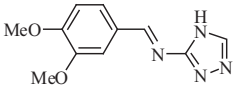
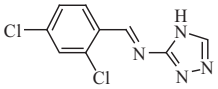
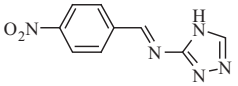
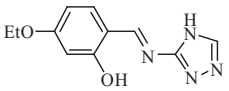
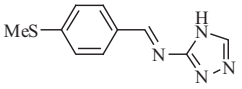
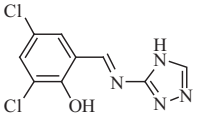
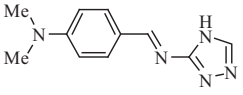
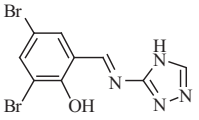
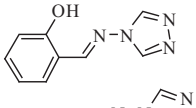
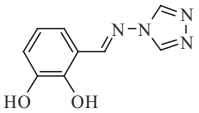
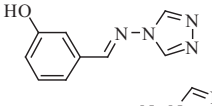
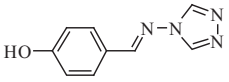
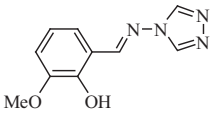
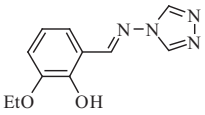
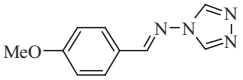
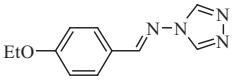
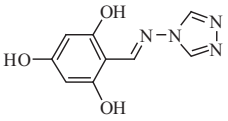
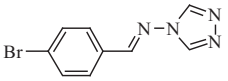
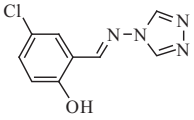
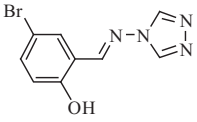
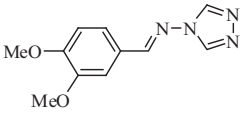
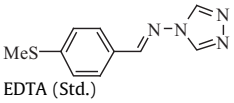
Compounds	Structures	IC_{50} ± SEM[a] (μM)
1		NA[b]
2		NA[b]
3		NA[b]
4		NA[b]
5		NA[b]
6		NA[b]
7		NA[b]
8		NA[b]
9		NA[b]
10		132.2 ± 2.89
11		NA[b]
12		NA[b]
13		152.83 ± 2.39
14		NA[b]
15		NA[b]
16		NA[b]

Table 1 (*continued*)

Compounds	Structures	IC_{50} ± SEM[a] (μM)
17		NA[b]
18		NA[b]
19		NA[b]
20		1164 ± 100.45
21		823.1 ± 31.56
22		251.0 ± 6.64
23		404.56 ± 10.74
24		NA[b]
25		NA[b]
	EDTA (Std.)	277.69 ± 2.52

[a] Standard error of mean.
[b] NA = Not active.

N,N′-dimethyl substituent exhibited a potent activity with IC_{50} = 132.2 ± 2.89 μM, as compared to other analogues. Similarly in compound **13** with *ortho* hydroxyl groups, a comparable activity (IC_{50} = 152.83 ± 2.39 μM) was observed. Compound **22** (IC_{50} = 251.0 ± 6.64 μM) was found to be an excellent inhibitor with *ortho* hydroxyl and *meta* chloro groups. However, the presence of bromo group, as in compound **23** (IC_{50} = 404.56 ± 10.74 - μM) decreased the activity. It may be due to larger size of bromo substituent than a chloro group which developed steric and electronic hindrances for interaction with the active site residues of PDE1. Furthermore, the presence of chloro and hydroxyl groups in compound **22** enhanced the activity, as compared to the compound **23** which may be due to more electronegativity of chlorine than bromine. Compounds **21** (IC_{50} = 823.1 ± 31.56 μM), and **20** (IC_{50} = 1164 ± 100.45 μM) were found to be weak inhibitors with different substituents. Weak inhibitor **20** indicated that the presence of three hydroxyl groups caused a marked decrease in activity which may be due to steric hindrance making π-electrons non-available to chelate with the active site amino acid residues of the enzyme. In compound **21,** the presence of bromo residue enhanced the activity as compared to compound **20** which possessed three hydroxyl groups contributing in the steric hindrance.

In brief, this study has identified compounds **10**, **13**, and **22** as leads for further research towards the treatment of phosphodiesterase-I related disorders.

Table 2
Cytotoxcity of Schiff bases of triazole 1-25 against PC3 cell lines

Compounds	IC_{50} ± SEM (μM)	Compounds	IC_{50} ± SEM (μM)
1	>30	**14**	>30
2	>30	**15**	>30
3	>30	**16**	>30
4	>30	**17**	>30
5	>30	**18**	>30
6	>30	**19**	>30
7	>30	**20**	>30
8	>30	**21**	>30
9	>30	**22**	>30
10	>30	**23**	>30
11	>30	**24**	>30
12	>30	**25**	>30
13	>30	Doxorubicin Std.	0.912 ± 0.12

2.3. Cytotoxicity studies

All the compounds **1–25** were found to be inactive against the anticancer activity (PC3 Cell Line) (Table 2).

3. Conclusion

Three compounds **10**, **13**, and **22** were identified as potential lead compounds for nucleotide pyrophosphatase/phosphodiesterase-1 inhibition. These compounds were also evaluated for carbonic anhydrase and found to be inactive. The result illustrates that these synthetic compounds are selective towards nucleotide pyrophosphatase/phosphodiesterase-1 and may be useful for the treatment of disorders related to PDEs hyperactivity after further mechanistic studies.

4. Experimental

4.1. Material and methods

The phosphodiesterase-1 (Broth atrox crude dried snake venom) was purchased from Sigma–Aldrich, USA. The tris buffer was purchased from Scharlau, Spain Cat #TR0423, magnesium acetate was purchased from Sigma–Aldrich, USA Cat #M-0631, bis(*p*-nitrophenyl) phosphate was used as a substrate (Sigma–Aldrich, USA N-3002). 3-Amino-4(*H*)-1,2,4-triazoles, 4-amino-4(*H*)-1,2,4-triazoles, and different benzaldehydes of analytical grades were purchased from TCI, Japan. Ethanol was dried using magnesium turnings. ^{1}H NMR experiments were run on Avance Bruker AM 300 MHz instrument. Electron impact mass spectrometry (EI-MS) analyses were performed on a Finnigan MAT-311A (Germany) mass spectrometer. CHN analyses were performed on a Carlo Erba Strumentazione-Mod-1106, Milano, Italy. Thin layer chromatography (TLC) was monitored on precoated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). TLC Chromatograms were visualized under UV at wavelengths of 254 and 365 nm or by using iodine vapours spray.

4.2. Nucleotide pyrophosphatases/phosphodiesterases-1 inhibition assay

The activity of snake venom PDE1/NPP1 was carried out by the standard procedure as reported in literature[56] with some modifications, such as 33 mM tris–HCl as buffer at pH of 8.8, magnesium acetate at a concentration of 30 mM as a co-factor, and 7.42×10^{-4} units of PDE1 enzyme dissolved in cold de-ionized water. After 30 min of incubation at 37 °C, the reaction was monitored by the addition of substrate (bis(*p*-nitrophenyl)phosphate) at 0.33 mM of total concentration. The rate of *p*-nitrophenol release

was monitored at 410 nm in 96-well plate by spectrophotometer (Molecular Devices, CA, USA).

All the reactions were performed in triplicate, and the initial rates were measured as the rate of changes in the $V_{max/min}$ (maximal velocity/min) and used in subsequent calculations.

The formula for % inhibition is:

$$\%\text{Inhibition} = 100 - (V_{max} \text{ of test}/V_{max} \text{ of control}) \times 100$$

IC_{50} Values were calculated by using the EZ-fit enzyme kinetics software (USA).

4.3. General procedure for the synthesis of compounds 1–25

In a typical procedure, 3-arylimino-1,2,4-triazoles and 4-arylimino-1,2,4-triazoles **1**–**25** were synthesized by mixing 3-amino-1,2,4-triazole or 4-amino-1,2,4-triazole (2 mmol), substituted benzaldehydes (2 mmol) and H_2SO_4 (2 mL) in ethanol (15 mL). The mixtures were refluxed for 4–10 h, while progress of the reaction was monitored through thin layer chromatography. When reaction was completed, solvent was evaporated on a rotary evaporator under reduced pressure and residue was washed with hot hexane. Resulting compounds were crystallized by ethanol to give title compounds in moderate to good yields.

4.3.1. *N*-[(3′-Hydroxyphenyl)methylidene]-4*H*-1,2,4-triazol-3-amine (1)

Yield: 74%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.41 (s, 1H, N═CH—Ar), 8.41 (s, 1H, H-5), 7.79 (d, 1H, $J_{6',5'}$ = 7.6 Hz, H-6′), 7.44 (t, 1H, $J_{4',3',5'}$ = 7.6 Hz, H-4′), 6.98 (m, 2H, H-5′, H-3′); EI-MS *m*/*z* (% rel. abund.): 188 $[M]^+$ (48), 171 (100), 84 (31); Anal. Calcd for $C_9H_8N_4O$, C = 57.44; H = 4.28; N = 29.77; O = 8.50. Found: C = 57.42; H = 4.27; N = 29.78.

4.3.2. *N*-[(2′-Hydroxy-3′-methoxyphenyl)methylidene]-4*H*-1,2,4-triazol-3-amine (2)

Yield: 95%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.40 (s, N═CH—Ar), 8.42 (s, 1H, H-5), 7.38 (dd, 1H, $J_{6',5'}$ = 7.8 Hz, $J_{6',4'}$ = 1.3 Hz, H-6′), 7.35 (dd, 1H, $J_{4',5'}$ = 7.6 Hz, $J_{4',6'}$ = 1.2 Hz, H-4′), 6.91 (t, 1H, *J* = 7.8 Hz, H-5′), 3.82 (s, 3H, OCH_3); EI-MS *m*/*z* (% rel. abund.): 218 $[M]^+$ (47), 201 (100), 92 (24); Anal. Calcd for $C_{10}H_{10}N_4O_2$, C = 55.04; H = 4.62; N = 25.68. Found: C = 55.02; H = 4.61; N = 25.67.

4.3.3. *N*-[(2′-Hydroxy-5′-methylphenyl)methylidene]-4*H*-1,2,4-triazol-3-amine (3)

Yield: 65%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.35 (s, N═CH—Ar), 8.39 (s, 1H, H-5), 7.58 (s, 1H, H-6′), 7.26 (d, 1H, $J_{4',3'}$ = 8.0 Hz, H-4′), 6.88 (d, 1H, $J_{3',4'}$ = 8.0 Hz, H-3′), 4.01 (s, 1H, OH), 3.16 (s, 3H, CH_3), 2.26 (s, 3H, OCH_3); EI-MS *m*/*z* (% rel. abund.): 202 $[M]^+$ (72), 185 (100), 84 (86); Anal. Calcd for $C_{10}H_{10}N_4O$, C = 59.40; H = 4.98; N = 27.71; O = 7.91. Found: C = 59.41; H = 4.97; N = 27.70.

4.3.4. *N*-[(3′,4′-Dimethoxyphenyl)methylidene]-4*H*-1,2,4-triazol-3-amine (4)

Yield: 69%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.92 (s, 1H, H-4), 9.11 (s, N═CH—Ar), 8.22 (s, 1H, H-5), 7.58 (s, 1H, H-2′), 7.53 (d, 1H, $J_{6',5'}$ = 8.4 Hz, H-6′), 7.11 (d, 1H, $J_{5',6'}$ = 8.4 Hz, H-4′), 3.84 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3); EI-MS *m*/*z* (% rel. abund.): 232 $[M]^+$ (99), 231 (100), 84 (9); Anal. Calcd for $C_{11}H_{12}N_4O_2$, C = 56.89; H = 5.21; N = 24.12; O = 13.78. Found: C = 56.87; H = 5.20; N = 24.11.

4.3.5. *N*-[(2′,4′-Dichlorophenyl)methylidene]-4*H*-1,2,4-triazol-3-amine (5)

Yield: 56%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.50 (s, N═CH—Ar), 8.42 (s, 1H, H-5), 7.82 (d, 1H, $J_{3',5'}$ = 1.8 Hz, H-3′), 7.20 (d, 1H, $J_{6',5'}$ = 8.4 Hz, H-6′), 7.59 (dd, 1H, $J_{5',6'}$ = 8.4 Hz, $J_{5',3'}$ = 1.8 Hz, H-5′); EI-MS *m*/*z* (% rel. abund.): 242 $[M+2]^+$ (6), 240 $[M]^+$ (10), 207 (37), 205 (100); Anal. Calcd for $C_9H_6Cl_2N_4$, C = 44.84; H = 2.51; Cl = 29.41; N = 23.24. Found: C = 44.82; H = 2.52; N = 23.23.

4.3.6. *N*-[(4′-Nitrophenyl)methylidene]-4*H*-1,2,4-triazol-3-amine (6)

Yield: 62%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.35 (s, N═CH—Ar), 8.55 (s, 1H, H-5), 8.36 (d, 2H, $J_{3',2'} = J_{5',6'}$ = 8.8 Hz, H-3′, H-5′), 7.82 (d, 2H, $J_{2',3'} = J_{6',5'}$ = 8.8 Hz, H-2′, H-6′); EI-MS *m*/*z* (% rel. abund.): 217 $[M]^+$ (74), 76 (13); Anal. Calcd for $C_9H_7N_5O_2$, C = 49.77; H = 3.25; N = 32.25. Found: C = 49.76; H = 3.24; N = 32.26.

4.3.7. *N*-[(4′-Ethoxy-2′-hydroxyphenyl)methylidene]-4*H*-1,2,4-triazol-3-amine (7)

Yield: 77%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.39 (s, N═CH—Ar), 8.43 (s, 1H, H-5), 7.35 (d, 1H, $J_{6',5'}$ = 8.0 Hz, H-6′), 7.15 (d, 1H, $J_{5',6'}$ = 8.0 Hz, H-5′), 6.90 (m, 1H, H-3′), 4.08 (q, 2H, OCH_2), 1.35 (t, 3H, CH_3); EI-MS *m*/*z* (% rel. abund.): 232 $[M]^+$ (94), 215 (100), 175 (40); Anal. Calcd for $C_{11}H_{12}N_4O_2$, C = 56.89; H = 5.21; N = 24.12; O = 13.78. Found: C = 56.87; H = 5.20; N = 24.11.

4.3.8. *N*-[4′-(Methylsulfanyl)phenyl]methylidene-4*H*-1,2,4-triazol-3-amine (8)

Yield: 57%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.99 (s, 1H, H-4), 9.15 (s, N═CH—Ar), 8.22 (s, 1H, H-5), 7.91 (d, 2H, $J_{2',3'} = J_{6',5'}$ = 8.4 Hz, H-2′, H-6′), 7.38 (d, 2H, $J_{3',2'} = J_{5',6'}$ = 8.4 Hz, H-3′, H-5′), 3.32 (s, 3H, SCH_3); EI-MS *m*/*z* (% rel. abund.): 218 $[M]^+$ (95), 217 (100), 149 (77); Anal. Calcd for $C_{10}H_{10}N_4S$, C = 55.02; H = 4.62; N = 25.67; *S* = 14.69. Found: C = 55.03; H = 4.61; N = 25.66.

4.3.9. *N*-[(3′,5-Dichloro-2′-hydroxyphenyl)methylidene]-4*H*-1,2,4-triazol-3-amine (9)

Yield: 40%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.27 (s, 1H, H-4), 13.80 (s, 1H, OH), 9.41 (s, N═CH—Ar), 8.63 (s, 1H, H-5), 7.94 (d, 1H, $J_{4',6'}$ = 1.1 Hz, H-4′), 7.78 (d, 1H, $J_{6',4'}$ = 1.1 Hz, H-6′); EI-MS *m*/*z* (% rel. abund.): 258 $[M+2]^+$ (41), 256 $[M]^+$ (62), 243 (12), 241 (66), 239 (100); Anal. Calcd for $C_9H_6Cl_2N_4O$, C = 42.05; H = 2.35; Cl = 27.58; N = 21.79; O = 6.22. Found: C = 42.04; H = 2.34; N = 21.78.

4.3.10. *N*-[4′-(Dimethylamino)phenyl]methylidene-4*H*-1,2,4-triazol-3-amine (10)

Yield: 51%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.75 (s, 1H, H-4), 8.99 (s, N═CH—Ar), 8.04 (s, 1H, H-5), 7.78 (d, 2H, $J_{2',3'} = J_{6',5'}$ = 8.5 Hz, H-2′, H-6′), 7.38 (d, 2H, $J_{3',2'} = J_{5',6'}$ = 8.5 Hz, H-3′, H-5′), 3.03 (s, 6H, 2 × NCH_3); EI-MS *m*/*z* (% rel. abund.): 215 $[M]^+$ (100), 214 (100), 146 (85); Anal. Calcd for $C_{11}H_{13}N_5$, C = 61.38; H = 6.09; N = 32.54. Found: C = 61.37; H = 6.10; N = 32.53.

4.3.11. *N*-[(3′,5′-Dibromo-2′-hydroxyphenyl)methylidene]-4*H*-1,2,4-triazol-3-amine (11)

Yield: 51%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 14.24 (bs, 2H, H-4, OH), 9.38 (s, N═CH—Ar), 8.61 (s, 1H, H-5), 8.07 (d, 1H, $J_{4',6'}$ = 2.4 Hz, H-4′), 7.96 (d, 1H, $J_{6',4'}$ = 2.4 Hz, H-6′); EI-MS *m*/*z* (% rel. abund.): 348 $[M^+{+}4]$ (51), 346 $[M+2]^+$ (100), 344 $[M]^+$ (56), 225 (26); Anal. Calcd for $C_9H_6Br_2N_4O$, C = 31.24; H = 1.75; Br = 46.19; N = 16.19; O = 4.62. Found: C = 31.22; H = 1.74; N = 16.17.

4.3.12. *N*-[(2′-Hydroxyphenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (12)

Yield: 64%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.15 (s, 3H, H-3, H-5, N═CH—Ar), 7.77 (d, 1H, $J_{6',5'}$ = 7.8 Hz, H-6′), 7.40 (t, 1H, $J_{4',3',5'}$ = 8.4 Hz, H-4′), 7.0 (d, 1H, $J_{3',4'}$ = 8.4 Hz, H-3′), 6.92 (t, 1H, $J_{5',4',6'}$ = 7.5 Hz, H-5′); EI-MS *m*/*z* (% rel. abund.): 188 $[M]^+$ (99),

119 (44), 84 (100); Anal. Calcd for $C_9H_8N_4O$, C = 57.44; H = 4.28; N = 29.77, O = 8.50. Found: C = 57.42; H = 4.27; N = 29.78.

4.3.13. *N*-[(2′,3′-Dihydroxyphenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (13)

Yield: 72%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.71 (s, 2H, 2 × OH), 9.15 (s, 3H, H-3, H-5, N=CH—Ar), 7.22 (dd, 1H, $J_{6',5'}$ = 7.8 Hz, $J_{6',4'}$ = 1.5 Hz, H-6′), 6.98 (dd, 1H, $J_{4',5'}$ = 7.8, $J_{4',6'}$ = 1.5 Hz, H-4′), 6.76 (t, 1H, $J_{5',6',4'}$ = 7.8 Hz, H-5′); EI-MS *m*/*z* (% rel. abund.): 204 $[M]^+$ (100), 135 (84), 107 (51); Anal. Calcd for $C_9H_8N_4O_2$, C = 52.94; H = 3.95; N = 27.44; O = 15.67. Found: C = 52.93; H = 3.93; N = 27.43.

4.3.14. *N*-[(3′-Hydroxyphenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (14)

Yield: 46%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.86 (s, 1H, OH), 9.12 (s, 2H, H-3, H-5), 8.99 (s, N=CH—Ar), 7.35 (m, 1H, H-5′), 7.35 (m, 1H, H-6′), 7.25 (m, 2H, H-4′, H-2′); EI-MS *m*/*z* (% rel. abund.): 188 $[M]^+$ (100), 84 (93); Anal. Calcd for $C_9H_8N_4O$, C = 57.44; H = 4.28; N = 29.77; O = 8.50. Found: C = 57.42; H = 4.27; N = 29.78.

4.3.15. *N*-[(4′-Hydroxyphenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (15)

Yield: 76%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.34 (s, 1H, OH), 9.05 (s, 2H, H-3, H-5), 8.91 (s, N=CH—Ar), 7.69 (d, 2H, $J_{2',3'}$ = $J_{6',5'}$ = 8.8 Hz, H-2′, H-6′), 6.95 (d, 2H, $J_{3',2'}$ = $J_{5',6'}$ = 8.8 Hz, H-3′, H-5′); EI-MS *m*/*z* (% rel. abund.): 188 $[M]^+$ 100, 106 (95); Anal. Calcd for $C_9H_8N_4O$, C = 57.44; H = 4.28; N = 29.77; O = 8.50. Found: C = 57.42; H = 4.27; N = 29.78.

4.3.16. *N*-[(2′-Hydroxy-3′-methoxyphenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (16)

Yield: 61%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.86 (s, 1H, OH), 9.16 (s, 3H, H-3, H-5, N=CH—Ar), 7.37 (dd, 1H, $J_{6',5'}$ = 7.8, $J_{6',4'}$ = 1.2 Hz, H-6′), 7.14 (dd, 1H, $J_{4',5'}$ = 7.8, $J_{4',6'}$ = 1.2 Hz, H-4′), 6.89 (t, 1H, $J_{5',6',4'}$ = 7.8 Hz, H-5′), 3.85 (s, 3H, OCH_3); EI-MS *m*/*z* (% rel. abund.): 218 $[M]^+$ (100), 149 (100), 106 (83); Anal. Calcd for $C_{10}H_{10}N_4O_2$, C = 55.04; H = 4.62; N = 25.68; O = 14.66. Found: C = 55.02; H = 4.61; N = 25.67.

4.3.17. *N*-[(3′-Ethoxy-2′-hydroxyphenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (17)

Yield: 48%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.85 (s, 1H, OH), 9.16 (s, 3H, H-3, H-5, N=CH—Ar), 7.35 (dd, 1H, $J_{6',5'}$ = 8.1 Hz, $J_{6',4'}$ = 1.2 Hz, H-6′), 7.14 (dd, 1H, $J_{4',5'}$ = 8.1 Hz, $J_{4',6'}$ = 1.2 Hz, H-4′), 6.88 (t, 1H, $J_{5',6',4'}$ = 7.8 Hz, H-5′), 4.09 (s, 2H, OCH_2), 1.36 (s, 3H, CH_3); EI-MS *m*/*z* (% rel. abund.): 232 $[M]^+$ (100), 164 (36), 135 (100); Anal. Calcd for $C_{11}H_{12}N_4O_2$, C = 56.89; H = 5.21; N = 24.12; O = 13.78. Found: C = 56.87; H = 5.23; N = 24.10.

4.3.18. *N*-[(4′-Methoxyphenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (18)

Yield: 49%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 2H, H-3, H-5), 8.98 (s, N=CH—Ar), 7.79 (d, 2H, $J_{2',3'}$ = $J_{6',5'}$ = 8.7 Hz, H-6′, H-2′), 7.11 (d, 2H, $J_{3',2'}$ = $J_{5',6'}$ = 8.7 Hz, H-5′, H-3′), 3.84 (s, 3H, OCH_3); EI-MS *m*/*z* (% rel. abund.): 202 $[M]^+$ (100), 120 (73), 105 (25); Anal. Calcd for $C_{10}H_{10}N_4O$, C = 59.40; H = 4.98; N = 27.71; O = 7.91. Found: C = 59.41; H = 4.97; N = 27.70.

4.3.19. *N*-[(4′-Ethoxyphenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (19)

Yield: 70%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.07 (s, 2H, H-3, H-5), 8.98 (s, N=CH—Ar), 7.78 (d, 2H, $J_{2',3'}$ = $J_{6',5'}$ = 9.0 Hz, H-6′, H-2′), 7.11(d, 2H, $J_{3',2'}$ = $J_{5',6'}$ = 9.0 Hz, H-5′, H-3′) 4.11 (s, 2H, OCH_2), 1.35 (s, 3H, CH_3); EI-MS *m*/*z* (% rel. abund.): 216 $[M]^+$ (100), 188 (41), 106 (84); Anal. Calcd for $C_{11}H_{12}N_4O$, C = 61.10; H = 5.59; N = 25.91; O = 7.40. Found: C = 61.11; H = 5.57; N = 25.92.

4.3.20. *N*-[(2′,4′,6′-Trihydroxyphenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (20)

Yield: 42%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.62 (s, 2H, H-3, H-5), 10.26 (s, N=CH—Ar), 9.10 (s, 2H, 2xOH), 9.02 (s, 1H, OH), 5.89 (s, 2H, H-3′, H-5′); EI-MS *m*/*z* (% rel. abund.): 220 $[M]^+$ (2), 151 (41), 44 (100); Anal. Calcd for $C_9H_8N_4O_3$, C = 49.09; H = 3.66; N = 25.45; O = 21.80. Found: C = 49.08; H = 3.67; N = 25.43.

4.3.21. *N*-[(4′-Bromophenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (21)

Yield: 45%; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.12 (s, 2H, H-3, H-5), 9.08 (s, N=CH—Ar), 7.78 (s, 4H, H-2′, H-3′, H-5′, H-6); EI-MS *m*/*z* (% rel. abund.): 252 $[M+2]^+$ (94), 250 $[M]^+$ (100), 89 (88); Anal. Calcd for $C_9H_7BrN_4$, C = 43.05; H = 2.81; Br = 31.82; N = 22.31. Found: C = 43.04; H = 2.80; N = 22.30.

4.3.22. *N*-[(5′-Chloro-2′-hydroxyphenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (22)

Yield: 48%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.17 (s, 2H, H-3, H-5), 9.09 (s, N=CH—Ar), 7.75 (d, 1H, $J_{6',4'}$ = 2.7 Hz, H-6′), 7.44 (dd, 1H, $J_{4',3'}$ = 9.0, $J_{4',6'}$ = 3.0 Hz, H-4′), 7.00 (d, 1H, $J_{3',4'}$ = 9.0 Hz, H-3′); EI-MS *m*/*z* (% rel. abund.): 224 $[M+2]^+$ (21), 222 $[M]^+$ (60), 153 (100); Anal. Calcd for $C_9H_7ClN_4O$, C = 48.55; H = 3.17; Cl = 15.92; N = 25.17; O = 7.19. Found: C = 48.54; H = 3.16; N = 25.16.

4.3.23. *N*-[(5′-Bromo-2′-hydroxyphenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (23)

Yield: 60%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.80 (s, 1H, OH), 9.17 (s, 2H, H-3, H-5), 9.08 (s, N=CH—Ar), 7.89 (d, 1H, $J_{6',4'}$ = 2.4 Hz, H-6′), 7.55 (dd, 1H, $J_{4',3'}$ = 8.7 Hz, $J_{4',6'}$ = 2.4 Hz, H-4′), 6.95 (d, 1H, $J_{3',4'}$ = 8.7 Hz, H-3′); EI-MS *m*/*z* (% rel. abund.): 268 $[M+2]^+$ (5), 266 $[M]^+$ (5), 199 (100); Anal. Calcd for $C_9H_7BrN_4O$, C = 40.47; H = 2.64; Br = 29.92; N = 20.98; O = 5.99. Found: C = 40.48; H = 2.63; N = 20.97.

4.3.24. *N*-[(3′,4′-Dimethoxyphenyl)methylidene]-4*H*-1,2,4-triazol-4-amine (24)

Yield: 73%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 3H, H-3, H-5), 9.97 (s, N=CH—Ar), 7.42 (d, 1H, $J_{2',6'}$ = 1.8 Hz, H-2′), 7.37 (dd, 1H, $J_{6',5'}$ = 8.4 Hz, $J_{6',2'}$ = 1.8 Hz, H-6′), 7.13 (d, 1H, $J_{5',6'}$ = 9.0 Hz, H-5′), 3.84 (s, 1H, OCH_3), 3.82 (s, 1H, OCH_3); EI-MS *m*/*z* (% rel. abund.): 232 $[M]^+$ (100), 166 (27), 135 (40); Anal. Calcd for $C_{11}H_{12}N_4O_2$, C = 56.89; H = 5.21; N = 24.12; O = 13.78. Found: C = 56.87; H = 5.20; N = 24.11.

4.3.25. *N*-[4′-(Methylthio)phenyl]methylidene-4*H*-1,2,4-triazol-4-amine (25)

Yield: 76%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.10 (s, 2H, H-3, H-5), 9.07 (s, N=CH—Ar), 7.76 (d, 1H, $J_{2',3'}$ = 8.4 Hz, H-2′, H-6′), 7.40 (d, 1H, $J_{3',2'}$ = 8.4, H-3′, H-5′), 1.13 (s, 3H, CH_3); EI-MS *m*/*z* (% rel. abund.): 218 $[M]^+$ (91), 149 (100), 122 (70), 84 (56); Anal. Calcd for $C_{10}H_{10}N_4S$, C = 55.02; H = 4.62; N = 25.67; S = 14.69. Found: C = 55.03; H = 4.61; N = 25.66.

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