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RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION AND BILOGICAL SCREENING OF SOME NOVEL INDOLE BASED 1,2,4-TRIAZOLO 1,3,4-THIADIAZINES

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ABSTRACTS:

A series of novel 3-(1*H*-indole-4-yl)-6-aryl-7*H*-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazines **(6a-f)** were synthesized by involving 1*H*-indole-4-carboxylic acid **(1)** as raw material and 1*H*-indole-4-carboxylic acid ethyl ester **(2)**, 1*H*-indole-4-carboxylic acid hydrazide **(3)**, 5-(1*H*-indole-4-yl)-[1,3,4]oxadiazole-2-thiol **(4)** and 4-amino-5-(1*H*-indol-4-yl)-4*H*-[1,2,4]-triazole-3-thiol **(5)**as intermediates. The chemical structures of the all newly synthesized compounds were elucidated by their IR, ^{1}H and ^{13} C NMR, mass spectral data and elemental analysis. Further, the target compounds were used to find their antifungal and nematicidal activity.

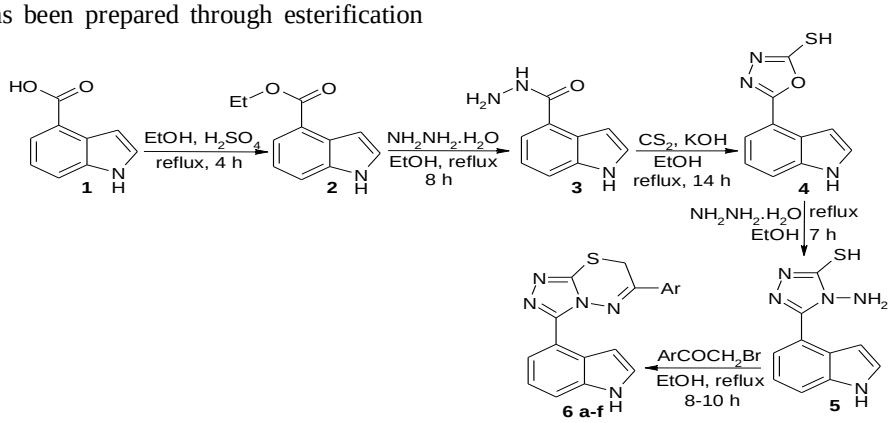
Key-Words: Indole, 1,2,4-Triazole, 1,3,4-Thiadiazines, Antifungal activity, Nematicidal activity.

INTRODUCTION:

Recently, it was reported that the heterocyclic moiety such as triazole based thiadiazoles and thiadiazines possess variety of pharmacological activities like antimicrobial [1] antiviral [2] antibacterial, [3] anti-inflammatory, [4] herbicidal [5] and anti-HIV-1.[6] On the other hand, it has been reported that certain compounds bearing a thiadiazole and 1,2,4-triazole nucleus possess significant anti-inflammatory activity. [7]

These initial reports stimulated us to integrate thiadiazine moiety in triazole frame work, since these systems possess well documented antimicrobial and nematicidal activity. The target compounds, 3-(1*H*-indole-4-yl)-6-aryl-7*H*-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazines **(6a-f)** have been prepared by using commercially available 1*H*-indole-4-carboxylic acid **(1)** as raw material and by involving 1*H*-indole-4-carboxylic acid ethyl ester **(2)**, 1*H*-indole-4-carboxylic acid hydrazide **(3)**, 5-(1*H*-indole-4-yl)-[1,3,4]oxadiazole-2-thiol **(4)** and 4-amino-5-(1*H*-indol-4-yl)-4*H*-[1,2,4]-triazole-3-thiol **(5)** as intermediates.

The initial intermediate, 1*H*-indole-4-carboxylic acid ethyl ester **(2)** has been prepared through esterification by boiling of a mixture of 1*H*-indole-4-carboxylic acid **(1)** and sulfuric acid in ethanol for 4 h. The compound **2** was reacted with hydrazine hydrate in absolute ethyl alcohol at reflux for 8 h to get 1*H*-indole-4-carboxylic acid hydrazide **(3)**. The intermediate, 5-(1*H*-indole-4-yl)-[1,3,4]oxadiazole-2-thiol **(4)** for the synthesis of title compounds was prepared by the cyclization of compound **3** with carbon disulphide in the presence of potassium hydroxide in ethanol at reflux for 14 h followed by acidification. Further the compound **4** when reacted with hydrazine hydrate in ethanol at reflux for 6 h resulted 4-amino-5-(1*H*-indol-4-yl)-4*H*-[1,2,4]triazole-3-thiol **(5)**. Finally, the compound **5** has been condensed successively with a variety of phenacylbromides in ethyl alcohol under reflux for 8-10 h to get the title compounds, 3-(1*H*-indole-4-yl)-6-aryl-7*H*-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazines **(6a-f)**. The chemical structures of the all newly synthesized compounds were elucidated by their IR, ^{1}H and ^{13} C NMR, mass spectral data and elemental analysis. Further, the target compounds were used to find their antifungal and nematicidal activity.



Scheme 1: 6 Ar = (a) C_6H_5; (b) 4-$OCH_3C_6H_4$; (c) 4-ClC_6H_4; (d) 4-BrC_6H_4; (e) 4-$NO_2C_6H_4$; (f) 4-OHC_6H_4

 ISSN: 2250-1177 CODEN (USA): JDDTAO

Table 1: *In vitro* antifungal activity of compounds 6a-f (MIC in µg/mL)

Compound	*C. albicans*	*A. Fumigatus*	*T. Rubrum*	*T. Mentropyhte*
6a	25.0	12.5	>50.0	25.0
6b	25.0	25.0	25.0	25.0
6c	12.5	6.25	3.12	3.12
6d	25.0	12.5	6.25	12.5
6e	3.12	12.5	12.5	25.0
6f	50.0	25.0	12.5	12.5
Amphotericin B	6.25	3.12	3.12	3.12

Table 2: Median lethal dose (LD_{50}, ppm) of compounds 6a-f

Nematicide	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**Levamisole**
D. myceliophagus	170	270	950	430	570	190	170
C. elegans	190	220	870	200	610	780	180

ANTIFUNGAL ACTIVITY

Compounds **6a-f** were screened for their antifungal activity against four fungal organisms *viz.*, *Candida albicans*, *Aspergillus fumigatus*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* in dimethyl sulfoxide by broth dilution method.[8] The minimum inhibitory concentration (MIC, µg/mL) were measured and compared with the standard drug Aamphotericin B (Table 1). Among the screened compounds, **6c** is highly active against *T. rubrum*, *T. mentagrophytes*, **6e** is also active against only *C. albicans* and **6g** is highly active against *C. albicans*, *T. mentagrophytes* and the activity of these compounds are almost equal to the standard. All the compounds in this series exhibited either excellent or moderate activity towards different organisms. None of the compounds is inactive against any one of the organism. It is interesting to note that **6e** showed excellent antifungal activity towards *C. albicans* at the concentration of 3.12 µg/mL, which is less than the concentration of the standard.

NEMATICIDAL ACTIVITY

All the newly synthesized compounds **6 a-f** in this study were also assayed for their nematicidal activity against *Ditylenchus myceliophagus* and *Caenorhabditis elegans* by aqueous *in vitro* screening technique[9] at various concentrations. The results have been expressed in terms of LD_{50} *i.e.* median lethal dose at which 50% nematodes became immobile (dead), and compared with the standard drug levamisole. The screened data reveal that, **6a** is the most effective against *D. myceliophagus* and *C. elegans* with LD_{50} of 170 and 190 ppm, respectively. The compounds **6d** and **6f** are also most active against *C. elegans* with LD_{50} of 200 ppm and *D. myceliophagus* with LD_{50} of 190 ppm, respectively. The activity of **6a** is almost equal to the activity of the standard Levamisole. The other tested compounds showed moderate activity. The LD_{50} values of the compounds screened are presented in Table 2.

EXPERIMENTAL

All the reagents and solvents were used as purchased without further purification. Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer BX serried FTIR 5000 spectrometer using KBr pellet. NMR spectra were recorded on a Varian 300 MHz spectrometer for ^{1}H–NMR and 100 MHz for ^{13}C–NMR. The chemical shifts were reported as ppm down field using TMS as an internal standard. Mass spectra were recorded on a VG-Micromass 7070H spectrometer operating at 70 eV.

1*H*-Indole-4-carboxylic acid ethyl ester (2) To the solution of 1*H*-indole-4-carboxylic acid **(1)** (0.01 mol) in absolute ethyl alcohol (15 ml), conc. H_2SO_4 (2 ml) was added. The mixture was refluxed for 4 h. After completion of the reaction (monitored by TLC), the solvent was removed under reduced pressure and the obtained residue was recrystallized with petroleum ether to get pure 1*H*-indole-4-carboxylic acid ethyl ester **(2)**.

1*H*-Indole-4-carboxylic acid hydrazide (3) A mixture of 1*H*-indole-4-carboxylic acid ethyl ester **(2)** (0.01 mol) and hydrazine hydrate (0.025 mol) in ethanol (20 ml) was refluxed for 8 h. After completion of the reaction (monitored by TLC), the mixture was cooled to room temperature and filtered. The crude product was recrystallized from ethanol to give 1*H*-indole-4-carboxylic acid hydrazide **(3)** in pure form.

5-(1*H*-Indole-4-yl)-[1,3,4]oxadiazole-2-thiol (4) A mixture of 1*H*-indole-4-carboxylic acid hydrazide **(3)** (0.01 mol), potassium hydroxide (0.02 mol) and carbon disulfide (0.03 mol) in ethanol (100 mL) was heated under reflux with stirring for 14 h. The solvent was distilled *in vacuo*, the residual mass was poured over crushed ice and neutralized the alkaline solution with 10% hydrochloric acid. The precipitated crude product was filtered, washed with water, dried and recrystallized from ethanol to get the pure compound 5-(1*H*-indole-4-yl)-[1,3,4]oxadiazole-2-thiol **(4)**.

4-Amino-5-(1*H*-indol-4-yl)-4*H*-[1,2,4]-triazole-3-thiol (5) To a warm solution of 5-(1*H*-indole-4-yl)-[1,3,4]-oxadiazole-2-thiol **(4)** (0.01 mol) in ethanol (20 mL), 80% hydrazine hydrate (0.03 mol) was added drop wise and the reaction mixture was heated under reflux for 7 h. The solvent was distilled off *in vacuo*, cooled and the solid separated were filtered, washed with cold ethanol and recrystallized from chloroform to give pure compound 4-amino-5-(1*H*-indol-4-yl)-4*H*-[1,2,4]-triazole-3-thiol **(5)**.

3-(1*H*-indole-4-yl)-6-aryl-7*H*-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazines (6a-f) A mixture of 4-amino-5-(1*H*-indol-4-yl)-4*H*-[1,2,4]-triazole-3-thiol **(5)** (0.01 mol) and corresponding phenacylbromide (0.02 mol) in absolute ethanol (20 mL) was refluxed for 8–10 h. The reaction mixture was concentrated and cooled to room temperature, and the remaining solvent was removed under reduced pressure, then diethyl ether (25 mL) was added and the reaction mixture was left at 0 ^{o}C for overnight. The precipitated solid was filtered off. The crude product thus obtained was purified by column chromatography on silica gel with hexane-ethyl acetate as eluent to afford pure 3-(1*H*-indole-4-yl)-6-aryl-7*H*-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazines **(6a-f)**.

PHYSICAL AND SPECTRAL DATA

1*H*-Indole-4-carboxylic acid ethyl ester (2) Yellow solid; yield 78%; bp 342-343 °C; IR (KBr) cm^{-1}: 3212 (N-H), 3024 (Ar-H), 2962 (C-H, CH_3), 1699(C=N), 1588 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J = 5.6 Hz, CH_3), 4.00 (2H, q, J = 5.6 Hz, CH_2), 7.42 (1H, d, J = 7.4 Hz, Ar-H), 7.39-7.62 (3H, m, Ar-H), 7.85 (1H, d, J = 7.4 Hz, Ar-H), 11.12 (1H, s, NH); ^{13}C-NMR ($CDCl_3$) δ: 13.6, 59.1, 106.3, 112.5, 119.4, 121.7, 122.8, 126.3, 130.4, 132.5, 165.4; MS *m*/*z*: 189 (M^+); Elemental analysis calculated for $C_{11}H_{11}NO_2$: C-69.83, H-5.86, N-7.40, O-16.91. Found: C-67.36, H-5.42, N-7.06, O-15.98.

1*H*-Indole-4-carboxylic acid hydrazide (3) Brown solid; yield 81%; mp 187-189 °C; IR (KBr) cm^{-1}: 3318 (NH_2), 3218 (N-H), 3065 (Ar-H), 1645 (C=N), 1548 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 5.30 (2H, s, NH_2), 7.38 (1H, d, J = 7.2 Hz, Ar-H), 7.42-7.64 (3H, m, Ar-H), 7.70 (1H, s, NH), 7.79 (1H, d, J = 7.2 Hz, Ar-H), 11.06 (1H, s, NH); ^{13}C-NMR ($CDCl_3$) δ: 105.7, 114.2, 121.0, 123.7, 124.4, 127.3, 132.7, 135.3, 162.3; MS *m*/*z*: 175 (M^+); Elemental analysis calculated for $C_9H_9N_3O$: C-61.70, H-5.18, N-23.99, O-9.13. Found: C-60.12, H-4.89, N-22.17, O-8.89.

5-(1*H*-Indole-4-yl)-[1,3,4]oxadiazole-2-thiol (4) Pale yellow solid; yield 74%; mp 185-187 °C; IR (KBr) cm^{-1}: 3236 (N-H), 3028 (Ar-H), 2610 (S-H), 1648 (C=N), 1559 (C=C), 1155; ^{1}H-NMR ($CDCl_3$) δ: 3.81 (1H, s, SH), 7.35 (1H, d, J = 7.6 Hz, Ar-H), 7.41-7.58 (3H, m, Ar-H), 7.79 (1H, d, J = 7.6 Hz, Ar-H), 11.24 (1H, s, NH); ^{13}C-NMR ($CDCl_3$) δ: 102.4, 116.3, 120.7, 123.4, 125.8, 128.4, 136.1, 139.8, 145.6, 158.9; MS *m*/*z*: 217 (M^+); Elemental analysis calculated for $C_{10}H_7N_3OS$: C-55.29, H-3.25, N-19.34, O-7.69, S-14.76. Found: C-53.69, H-3.12, N-18.45, O-7.02, S-13.38.

4-Amino-5-(1*H*-indol-4-yl)-4*H*-[1,2,4]-triazole-3-thiol (5) White solid; yield 72%; mp 147-149 °C; IR (KBr) cm^{-1}: 3248 (N-H), 3018 (Ar-H), 2648 (S-H), 1662 (C=N), 1552 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 3.65 (1H, s, SH), 3.85 (2H, s, NH_2), 7.37 (1H, d, J = 7.3 Hz, Ar-H), 7.45-7.74 (3H, m, Ar-H), 7.80 (1H, d, J = 7.3 Hz, Ar-H), 11.21 (1H, s, NH); ^{13}C-NMR ($CDCl_3$) δ: 105.6, 110.8, 116.7, 119.4, 127.6, 129.4, 132.4, 137.6, 142.3, 152.7; MS *m*/*z*: 231 (M^+); Elemental analysis calculated for $C_{10}H_9N_5S$: C-51.93, H-3.92, N-30.28, S-13.86. Found: C-50.12, H-3.45, N-29.65, S-12.98.

3-(1*H*-Indole-4-yl)-6-phenyl-7*H*-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazine (6a) Pink solid; yield 75%; mp 158-160 °C; IR (KBr) cm^{-1}: 3245 (N-H), 3018 (Ar-H), 2965 (C-H, CH_2), 1638 (C=N), 1548 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 1.86 (2H, s, CH_2), 7.32 (1H, d, J = 6.8 Hz, Ar-H), 7.41-7.74 (8H, m, Ar-H), 7.79 (1H, d, J = 6.8 Hz, Ar-H), 10.98 (1H, s, NH); ^{13}C-NMR ($CDCl_3$) δ: 33.7, 104.3, 116.7, 121.0, 125.4, 126.3, 128.7 (2), 129.7, 130.2, 131.7 (2), 132.7, 133.1, 138.4, 144.2, 148.7, 162.3; MS *m*/*z*: 331 (M^+); Elemental analysis calculated for $C_{18}H_{13}N_5S$: C-65.24, H-3.95, N-21.13, S-9.68. Found: C-63.25, H-3.64, N-20.28, S-8.95.

3-(1*H*-Indole-4-yl)-6-(4-methoxy-phenyl)-7*H*-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazine (6b) Pink solid; yield 80%; mp 162-164 °C; IR (KBr) cm^{-1}: 3239 (N-H), 3024 (Ar-H), 2960 (C-H, CH_2), 1642 (C=N), 1568 (C=C), 1145 (C-O); ^{1}H-NMR ($CDCl_3$) δ: 1.74 (2H, s, CH_2), 1.24 (3H, s, CH_3), 7.29 (1H, d, J = 7.0 Hz, Ar-H), 7.32 (2H, d, J = 7.2 Hz, Ar-H), 7.40 (2H, d, J = 7.2 Hz, Ar-H), 7.42-7.81 (3H, m, Ar-H), 7.85 (1H, d, J = 7.0 Hz, Ar-H), 10.84 (1H, s, NH); ^{13}C-NMR ($CDCl_3$) δ: 30.3, 44.7, 106.7, 118.4, 123.4, 126.3, 128.7, 129.6 (2), 130.7, 132.7, 133.2 (2), 134.1, 135.2, 137.4, 146.7, 149.5, 163.1; MS *m*/*z*: 361 (M^+); Elemental analysis calculated for $C_{19}H_{15}N_5OS$: C-63.14, H-4.18, N-19.38, O-4.43, S-8.87. Found: C-61.23, H-3.84, N-18.28, O-4.12, S-7.84.

3-(1*H*-Indole-4-yl)-6-(4-chloro-phenyl)-7*H*-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazine (6c) Yellow solid; yield 82%; mp 126-128 °C; IR (KBr) cm^{-1}: 3240 (N-H), 3028 (Ar-H), 2958 (C-H, CH_2), 1654 (C=N), 1595 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 1.68 (2H, s, CH_2), 7.26 (1H, d, J = 7.4 Hz, Ar-H), 7.30 (2H, d, J = 7.0 Hz, Ar-H), 7.38 (2H, d, J = 7.0 Hz, Ar-H), 7.46-7.76 (3H, m, Ar-H), 7.79 (1H, d, J = 7.4 Hz, Ar-H), 10.92 (1H, s, NH); ^{13}C-NMR ($CDCl_3$) δ: 32.3, 104.2, 116.2, 119.7, 122.0, 124.2, 125.3 (2), 128.4, 136.3, 139.2 (2), 140.2, 141.0, 142.7, 148.7, 150.2, 165.7; MS *m*/*z*: 365 (M^+); Elemental analysis calculated for $C_{18}H_{12}ClN_5S$: C-59.09, H-3.31, Cl-9.69, N-19.14, S-8.76. Found: C-57.16, H-3.14, Cl-8.84, N-18.56, S-7.48.

3-(1*H*-Indole-4-yl)-6-(4-bromo-phenyl)-7*H*-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazine (6d) Brown solid; yield 81%; mp 132-134 °C; IR (KBr) cm^{-1}: 3258 (N-H), 3032 (Ar-H), 2962 (C-H, CH_2), 1664 (C=N), 1565 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 1.54 (2H, s, CH_2), 7.16 (1H, d, J = 7.3 Hz, Ar-H), 7.26 (2H, d, J = 7.3 Hz, Ar-H), 7.34 (2H, d, J = 7.5 Hz, Ar-H), 7.40-7.68 (3H, m, Ar-H), 7.72 (1H, d, J = 7.5 Hz, Ar-H), 10.86 (1H, s, NH); ^{13}C-NMR ($CDCl_3$) δ: 36.3, 107.4, 112.0, 115.7, 118.6, 120.8, 122.3 (2), 124.7, 132.6, 134.8 (2), 138.7, 140.2, 145.7, 146.3, 152.7, 163.8; MS *m*/*z*: 410 (M^+); Elemental analysis calculated for $C_{18}H_{12}BrN_5S$: C-52.69, H-2.95, Br-19.47,

 ISSN: 2250-1177 CODEN (USA): JDDTAO

N-17.07, S-7.89. Found: C-50.36, H-2.65, Br-18.84, N-16.47, S-9.89.

3-(1*H*-Indole-4-yl)-6-(4-nitro-phenyl)-7*H*-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazine (6e) White solid; yield 79%; mp 154-156 °C; IR (KBr) cm^{-1}: 3262 (N-H), 3027 (Ar-H), 2945 (C-H, CH_2), 1652 (C=N), 1556 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 1.58 (2H, s, CH_2), 7.26 (1H, d, J = 6.8 Hz, Ar-H), 7.31 (2H, d, J = 6.8 Hz, Ar-H), 7.38 (2H, d, J = 7.2 Hz, Ar-H), 7.45-7.72 (3H, m, Ar-H), 7.78 (1H, d, J = 7.2 Hz, Ar-H), 11.08 (1H, s, NH); ^{13}C-NMR ($CDCl_3$) δ: 33.7, 108.9, 114.7, 117.4, 119.6, 122.3, 124.7 (2), 126.8, 133.7, 135.8 (2), 139.7, 142.4, 148.7, 149.6, 153.7, 164.9; MS *m*/*z*: 376 (M^+); Elemental analysis calculated for $C_{18}H_{12}N_6O_2S$: C-57.44, H-3.21, N-22.33, O-8.50, S-8.52. Found: C-55.98, H-3.12, N-21.28, O-7.87, S-7.95.

3-(1*H*-Indole-4-yl)-6-(4-hydroxy-phenyl)-7*H*-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazine (6f) Pale solid; yield 77%; mp 139-141 °C; IR (KBr) cm^{-1}: 3256 (N-H), 3042 (Ar-H), 2938 (C-H, CH_2), 1665 (C=N), 1548 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 1.46 (2H, s, CH_2), 5.02 (1H, s, OH), 7.22 (1H, d, J = 7.0 Hz, Ar-H), 7.35 (2H, d, J = 7.0 Hz, Ar-H), 7.40 (2H, d, J = 7.4 Hz, Ar-H), 7.48-7.78 (3H, m, Ar-H), 7.84 (1H, d, J = 7.4 Hz, Ar-H), 11.10 (1H, s, NH); ^{13}C-NMR ($CDCl_3$) δ: 30.3, 112.8, 115.4, 116.3, 121.4, 123.7, 126.8 (2), 128.7, 135.7, 138.7 (2), 140.2, 143.6, 145.7, 147.8, 151.6, 165.6; MS *m*/*z*: 347 (M^+); Elemental analysis calculated for $C_{18}H_{13}N_5OS$: C-62.23, H-3.77, N-20.16, O-4.61, S-9.23. Found: C-60.54, H-3.42, N-19.65, O-4.21, S-8.85.

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 ISSN: 2250-1177 **CODEN (USA): JDDTAO**