DESIGN, FACILE SYNTHESIS, AND BIOLOGICAL EVALUATION OF NOVEL 1,3-THIAZINE DERIVATIVES AS POTENTIAL ANTICONVULSANT AGENTS

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ABSTRACT

Objective: Chalcones and their heterocyclic analogs represent an important class of small molecules having anticonvulsant activities. Therefore, in this study, the synthesis and anticonvulsant activity of some new chalcones and 1,3-thiazines were described.

Methods: The reaction of 1-acetylnaphthalene with substituted aromatic aldehydes in the presence of aq. NaOH afforded corresponding chalcones which upon further cyclization with thiourea resulted in 1,3-thiazine derivatives. The newly synthesized compounds were tested for anticonvulsant activity by pentylenetetrazole-induced seizures method using diazepam as standard.

Results: Most of the compounds showed good anticonvulsant activity but is less than diazepam. 1,3-thiazines were more potent than chalcones and among them, compound P4 containing 4-fluorophenyl substituents on the thiazine moiety was more potent as it has prolonged the onset of convulsions by 15.2 seconds.

Conclusion: We described the synthesis and anticonvulsant activity of novel chalcones and 1,3-thiazine derivatives. 1,3-thiazines are more active anticonvulsant agents than chalcones and in particular compounds with electron withdrawing substituents.

Keywords: Chalcone, 1,3-thiazine, Pentylenetetrazole.

INTRODUCTION

Thiazines are a class of six-membered heterocyclic organic compounds with one nitrogen and sulfur atoms situated in a 1, 2- 1,3-, 1,4- positions or as a part of phenothiazine ring structure (Fig. 1). Due to nitrogen thiazines are chemically basic, 1,3-thiazines are of great importance because they form part of the framework of cephalosporins (3,6-dihydro-2H-1,3-thiazine) [1] and also in some other medicinally important compounds such as xylazine (agonist at the α2-adrenergic receptor is used for sedation, anesthesia, muscle relaxation, and analgesia in animals) [2] and chloromezone (used as an anxiolytic and a muscle relaxant) [3].

They exhibit sundry of pharmacological activities including antimicrobial [4-11], anti-inflammatory [12-15], anticancer [16,17], antidiabetic [18], analgesic [19], immunotropic [20] and antitumor [21], anticonvulsant, and antianxiety [22]. Chalcones, on the other hand, are α, β-unsaturated enones with a broad range of biological activities and also acts as key synthon in the chemical synthesis of heterocyclic compounds [23]. One important pharmacological activity of chalcones is anticonvulsant property [24]. Most of the anticonvulsant agents in therapy comprise a hydrophobic group with urea or urea like functionality (Fig. 2). Hydrophobicity assists the molecule to reach the brain by crossing blood brain barrier and also to interact with the target site via the hydrophobic interactions whereas the urea or urea like functionality for the polar interactions. These structural features of the existing drugs have become the rationale for designing novel anticonvulsant agent-containing hydrophobic naphthyl and phenyl portions along with polar enone and thiourea moieties in chalcones and 1,3-thiazines, respectively.

In the present work, we reported the synthesis of novel hydrophobic chalcones and 1,3-thiazine derivatives containing electron withdrawing and electron-releasing substituents on the phenyl ring and without any modification on the naphthyl portion to study the effect of such substitutions on anticonvulsant activity.

METHODS

General Melting points were determined in an open capillary melting point apparatus and are uncorrected. 1H NMR was recorded in CDCl3 on Bruker WM 400 MHz spectrometer with TMS as internal standard. Infrared spectra were recorded (KBr) on Perkin-Elmer AC-1 spectrophotometer. Microanalyses were performed on Carloerba EA-1108 element analyzer. All the compounds have been purified by column chromatography performed on Silica gel-G and crystallized from a suitable solvent to get chalcones (A1-A6).

RESULTS

The reaction of 1-acetylnaphthalene with substituted aromatic aldehydes in the presence of aq. NaOH afforded corresponding chalcones and among them, compound P4 containing 4-fluorophenyl substituents on the thiazine moiety was more potent as it has prolonged the onset of convulsions by 15.2 seconds.

CONCLUSION

We described the synthesis and anticonvulsant activity of novel chalcones and 1,3-thiazine derivatives. 1,3-thiazines are more active anticonvulsant agents than chalcones and in particular compounds with electron withdrawing substituents.

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reaction mixture was poured into ice-cold water; the solid product obtained was filtered, washed with water and crystallized from a suitable solvent to gain 1,3-thiazines (P1-P6). The purity of all the compounds was established by TLC using a mixture of hexane and ethyl acetate as a mobile phase. All the compounds are purified by column chromatography.

(E)-1-[napthalen-5-yl]-3-phenylprop-2-en-1-one (A1) yellowish solid; Yield: 81%; m.p. 151-154°C; infrared (IR) (KBr, cm\(^{-1}\)): 1669.24 (-CO-), 1518.12 (-CH=CH-), 3047.79 (Ar-CH stretching); \(^1\)H NMR (400 MHz, CDCl\(_3\), ppm): 7.56-7.60 (1H, d, J=16, Ar-CH=), 7.90-7.92 (1H, d, J=8.8, -CO-CH=C-), 7.42-7.88 (m, C-2', 3', 4', 5', 6', 7', Ar-H), 9.19 (1H, d, C-8', Ar-H).

(E)-3-(4-methylphenyl)-1-napthalen-5-yl)prop-2-en-1-one (A2) yellowish solid; Yield: 53%; m.p. 175-177°C; IR (KBr, cm\(^{-1}\)): 1669.24 (-CO-), 1515.66 (-CH=CH-), 2921.36 (Ar-CH stretching); \(^1\)H NMR (400 MHz, CDCl\(_3\), ppm): 2.35 (3H, s, CH\(_3\)), 7.58-7.62 (1H, d, J=16, Ar-CH=), 7.92-7.94 (1H, d, J=8.8, -CO-CH=C-), 7.40-7.84 (m, C-2', 3', 4', 5', 6', 7', Ar-H), 9.14 (1H, d, C-8', Ar-H), 7.16-7.26 (m, C-2', 3', 5', 6', Ar-H).

(E)-3-(4-chlorophenyl)-1-napthalen-5-yl)prop-2-en-1-one (A3) yellowish solid; Yield: 59%; m.p. 189-192°C; IR (KBr, cm\(^{-1}\)): 1667.95 (-CO-), 1520 (-CH=CH-), 2920.30 (Ar-CH stretching), 1064.38 (C-Cl); \(^1\)H NMR (400 MHz, CDCl\(_3\), ppm): 7.56-7.60 (1H, d, J=16, Ar-CH=), 7.90-7.92 (1H, d, J=8.8, -CO-CH=C-), 7.42-7.78 (m, C-2', 3', 4', 5', 6', 7', Ar-H), 9.25 (1H, d, C-8', Ar-H), 7.22-7.24 (m, C-2', 3', 5', 6', Ar-H).

(E)-3-(4-fluorophenyl)-1-napthalen-5-yl)prop-2-en-1-one (A4) fluorescent yellowish solid; Yield: 70%; m.p. 167-169°C; IR (KBr, cm\(^{-1}\)): 1663.12 (-CO-), 1518.51 (-CH=CH-), 3030.66 (Ar-CH stretching), 1047.70 (C-F); \(^1\)H NMR (400 MHz, CDCl\(_3\), ppm): 7.52-7.58 (1H, d, J=16, Ar-CH=), 7.94-7.96 (1H, d, J=8.8, -CO-CH=C-), 7.46-7.92 (m, C-2', 3', 4', 5', 6', 7', Ar-H), 9.09 (1H, d, C-8', Ar-H), 6.92-7.28 (m, C-2', 3', 5', 6', Ar-H).

(E)-3-(4-dimethylaminophenyl)-1-napthalen-5-yl)prop-2-en-1-one (A5) yellowish solid; Yield: 91%; m.p. 231-233°C; IR (KBr, cm\(^{-1}\)): 1660.35 (-CO-), 1513.33 (-CH=CH-), 2922.91 (Ar-CH stretching); \(^1\)H NMR (400 MHz, CDCl\(_3\), ppm): 2.85 (6H, s, Ar-N(CH\(_3\))\(_2\)), 7.60-7.64 (1H, d, J=16, Ar-CH=), 7.94-7.96 (1H, d, J=8.8, -CO-CH=C-), 7.40-7.86 (m, C-2', 3', 4', 5', 6', 7', Ar-H), 9.23 (1H, d, C-8', Ar-H), 6.56-7.14 (m, C-2', 3', 5', 6', Ar-H).

(E)-3-(4-hydroxyphenyl)-1-napthalen-5-yl)prop-2-en-1-one (A6) Yellowish solid; 69%; m.p. 194-196°C; IR (KBr, cm\(^{-1}\)): 1663.99 (-CO-), 1515.76 (-CH=CH-), 3047.79 (Ar-CH stretching), 3443.23 (hydroxy benzene); \(^1\)H NMR (400 MHz, CDCl\(_3\), ppm): 5.1 (1H, s, 4'-OH), 7.56-7.60 (1H, d, J=16, Ar-CH=), 7.90-7.92 (1H, d, J=8.8, -CO-CH=C-), 7.42-7.88 (m, C-2', 3', 4', 5', 6', 7', Ar-H), 9.19 (1H, d, C-8', Ar-H), 6.68-7.13 (m, C-2', 3', 5', 6', Ar-H).

Fig. 1: Structures of different types of thiazines

Scheme 1: Synthesis of chalcones (A1-A6) and 1,3-thiazines (P1-P6); (i) substituted aromatic aldehydes, ethanol, aq. NaOH; (ii) Thiorea, ethanolic KOH

Fig. 2: Design of hydrophobic group linked 1,3-thiazine analogues for their anticonvulsant activity hydrophobic portion shown in blue whereas as polar urea or urea like portion in red
6-(4-chlorophenyl)-4-(naphthalen-5-yl)-2H-1,3-thiazin-2-amine (P3) pale yellow solid; Yield: 65%; m.p. 157-158°C; IR (KBr, cm⁻¹): 3412.97 (NH), 1642.85 (C=N), 703 (C−S), 1575.45 (C=C); H NMR (400 MHz, CDCl₃ ppm): 7.31 (1H,s, C-6 Ar-H), 7.38-7.75 (7H, m, C-2',3',4',5',6',7',8' Ar-H); Anal. Calcd for: C₂₀H₁₆N₄S: C, 76.33; H, 5.49; N, 8.48; Found: C, 76.44; H, 5.57; N, 8.49.

6-(4-fluorophenyl)-4-(naphthalen-5-yl)-2H-1,3-thiazin-2-amine (P4) pale yellowish solid; Yield: 68%; m.p. 188-190°C; IR (KBr, cm⁻¹): 3424.68 (NH), 1621.35 (C=N), 683 (C−S), 1528.36 (C=C); H NMR (400 MHz, CDCl₃ ppm): 6.95 (1H,s, C-6 Ar-H), 7.46 (2Hd, J=7Hz, C-3',5' Ar-H), 7.38-7.75 (7H, m, C-2',3',4',5',6',7',8' Ar-H); Anal. Calcd for: C₂₀H₁₆N₄F: C, 71.83; H, 4.52; N, 8.38; Found: C, 71.88; H, 4.59; N, 8.49.

6-(4-hydroxyphenyl)-4-(naphthalen-5-yl)-2H-1,3-thiazin-2-amine (P5) pale yellow solid; Yield: 68%; m.p. 116.5±0.61°C; IR (KBr, cm⁻¹): 3341.52 (NH), 1670/cm and 1510 and 1520/cm, whereas the bands in the IR analysis. Construction of the chalcones is confirmed by characteristic carbonyl and olefinic IR absorption bands in between 1660 and 1670/cm and 1510 and 1520/cm, whereas the bands in the IR

Table 1: Results of the anticonvulsant activity of chalcones (A1-A6) and 1,3-thiazines (P1-P6)

| Group | Number of animals used | Onset of convulsions (seconds) | Number of animals died | Percentage mortality |
|-------|------------------------|-------------------------------|------------------------|----------------------|
| PTZ   | 6                      | 89±2.16                       | 6                      | 100                  |
| STD+PTZ | 0                      | 0                              | 0                      | 0                    |
| PTZ+A1 | 6                      | 126.8±1.85                    | 2                      | 33.33                |
| PTZ+A2 | 6                      | 116.5±0.61                    | 3                      | 50                   |
| PTZ+A3 | 6                      | 131.2±1.83                    | 3                      | 50                   |
| PTZ+A4 | 6                      | 142.3±2.27                    | 1                      | 16.66                |
| PTZ+A5 | 6                      | 120±0.93                      | 3                      | 50                   |
| PTZ+A6 | 6                      | 116.2±0.79                    | 3                      | 50                   |
| PTZ+P1 | 6                      | 143.5±1.25                    | 2                      | 33.33                |
| PTZ+P2 | 6                      | 138.5±1.25                    | 3                      | 50                   |
| PTZ+P3 | 6                      | 151±1.52                      | 3                      | 50                   |
| PTZ+P4 | 6                      | 155.2±1.42                    | 2                      | 16.66                |
| PTZ+P5 | 6                      | 144.3±0.88                    | 2                      | 33.33                |
| PTZ+P6 | 6                      | 138.5±0.76                    | 3                      | 50                   |

Values are expressed as mean±SEM of each group (n=6) and are significant when done One-way ANOVA with Tukey’s post hoc test. ***p<0.001 when compared with disease control. PTZ: Pentylentetrazole, SEM: Standard error of mean

Anticonvulsant activity
According to neurological theory, epilepsy is a paroxysmal, self-limited cerebral dysrhythmia. It is accompanied by abnormal patterns on the electroencephalograph, and severe seizures may cause a loss of consciousness. It may or may not be associated with body movements or hyperactivity of the autonomic nervous system. All the experimental protocols and procedures described hereupon were prior approved by the Institutional Animal Ethics Committee. The method followed for activity is PTZ-induced Seizures [25-28]. Diazepam (10 mg/kg) and PTZ were dissolved in normal saline. The healthy albino rats (Wistar, 100-150 g, 5-6 weeks) were kept under standard laboratory conditions (room temperature: 23±2°C; relative humidity: 60±5%); illumination: 12 hrs light/dark cycle) and had freely access to food pellets and fresh water except for the short time duration when animals were removed for pharmacological testing. All experiments were performed between 9.00 AM and 2.00 PM. The animals were divided into 14 groups of six animals each for two test drugs. Group 1 is treated as a negative control and injected subcutaneously with PTZ at a dose of 85 mg/kg body weight. Group 2 serves as standard and treated with diazepam injected intraperitoneally at a dose of 10 mg/kg body weight. Groups 3–6 and 7–14 were treated with test compounds A1–A6 and P1–P6, respectively, at a dose of 100 mg/kg ip. Animals were pretreated with the test drug 30 minutes and diazepam 15 minutes before administration of PTZ 85 mg/kg subcutaneously. The onset of total duration as well as the frequency of clonic seizures was measured within a 30 minutes period and % mortality was measured, and the results are summarized in Table 1.

Table 1: Results of the anticonvulsant activity of chalcones (A1-A6) and 1,3-thiazines (P1-P6)
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