Original Research Article

THEOPHYLLINE DERIVATIVES' IN-VIVO ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES

Abstract

The reaction of theophylline and chloro-acetyl chloride produced an exceptional series of substituted theophylline derivatives (3A-3D), followed by ammonium thiocyanate and substituted aromatic aldehyde, and these synthesized derivatives were screened to study their analgesic and anti-inflammatory activities. UV, IR, H-1 NMR, mass spectral data, and CHN activities were used to describe the compounds, which were shown to be considerably efficacious at 100 mg/kg p.o., as well as experimental results that were statistically significant at the p0.01 and p0.05 levels.

Keywords: Theophylline, analgesic, anti-inflammatory

INTRODUCTION

Asthma is a long-term inflammatory condition characterized by reversible airflow obstruction and airway hyper-responsiveness, the etiology of which is unknown [1]. Hyper-responsiveness arises as a result of a variety of stimuli, such as irritating chemicals, pollen grains, stimulant medicines, pollution, cold air, and so on. Asthma is the most common condition among children globally, according to the World Health Organization. This chronic condition affects a total of 339 million individuals worldwide. Bronchospasm, viscous and obstructive bronchial hypersecretion, inflammation, and edema of bronchial mucous are the physiological pathogenic components of these disorders [2-3]. Low- and lower-middle-income nations account for more than 80% of asthma-related fatalities. For the last two decades, glucocorticoids and,2-adrenergic agonists have been regarded first-line treatment. ,2-adrenergic agonists cure symptoms by inhibiting bronchoconstriction, while glucocorticoids slow disease progression by acting as anti-inflammatory agents [4]. Tachycardia, palpitations, headaches, and other significant side effects are often connected with this therapy method. Because of its importance in the synthesis of novel derivatives with high biological activity, 1,3-thiazolidin-2-yl-purine-2, 6-dione has been the subject of several recent studies. Theophylline and theophylline derivatives have been employed for conjugation with several high-molecular-weight poly (ethylene glycol)s, according to the

literature[5]. Among the xanthine derivatives, theophylline outperforms theobromine and caffeine in terms of DNA binding effectiveness. According to the research, theophylline creates hydrogen-bonding complexes with DNA and acts as a powerful antioxidant that protects DNA from harm [6].

By avoiding the depletion of the inhibitory I-B, theophylline and nuclear factor-B (NF-B) restrict the translocation of the proinflammatory transcription factor into the nucleus, possibly decreasing inflammatory gene expression in asthma and COPD [7]. Chemical manipulation of this scaffold has led to the discovery of novel physiologically active chemicals such bronchodilators [8, hypoglycemic agents [9], anticancer agents [10], and anti-inflammatory drugs [11]. Since 2011, a hypoglycemic agent, Linagliptin (Tradjenta®, Trajenta®), a DPP-4 inhibitor [12, 13], has been used in the United States for the treatment of diabetes mellitus type 2 [14], and its additional antioxidant properties have been shown to be very useful in managing the vascular complications of diabetes (macrovascular-myocardial infarction, angina pectoris, stroke, and microvascular- diabetic ne

In asthma and COPD, theophylline exhibits anti-inflammatory effects at plasma concentrations lower than those necessary for its bronchodilator actions [15]. In-vitro theophylline inhibits mediator release from mast cells and reactive oxygen species from neutrophils, but low-dose theophylline reduces the late response and airway eosinophil influx after inhaled allergen [16] and the number of eosinophils in bronchial biopsies, bronchoalveolar lavage, and induced sputum in patients with mild asthma [17]. In individuals with nighttime asthma, it also lowers bronchoalveolar lavage neutrophil influx [18]. Theophylline, unlike corticosteroids, reduces the number of neutrophils in generated sputum and the concentration of CXCL8, showing that it has an anti-inflammatory effect in COPD patients [19-21]. At high doses, theophylline inhibits the proliferation of CD4+ and CD8+ lymphocytes as well as the chemotactic response of T cells. Inhibition of PDE is responsible for these effects [22]. Low-dose theophylline treatment produces an increase in activated circulating CD4+ and CD8+ T lymphocytes but a reduction in these cells in the airways, indicating that it may limit the trafficking of activated T cells into the airways in asthma patients [23]. Because of the relevance of theophylline derivatives, it was thought that it would be useful to synthesis some new substituted theophylline derivatives and screen them for analgesic and anti-inflammatory effects as part of our continuing project effort.

METHODS AND MATERIALS

Chemistry

The compounds' IR spectra were recorded using a SHIMADZU FT-IR8400S with KBr pellets, and the inaccurate melting points were recorded on an X4-Data microscopic melting point equipment. Similarly, 1H-NMR spectra and chemical shifts from tetramethyl silane (TMS) were recorded on JNM-ECS400, 400 MHz, and given as parts per million down fields. On the LC–MS 2010A SHIMADZU equipment, mass spectra were obtained. UV–SPECORD® 50 PLUS-232H1004 UV-visible spectrophotometer was used for UV– visible spectroscopy analysis. A Euro ER elemental Analyzer was used to conduct the elemental evaluations. The purity of the compounds was determined by TLC on precoated silica gel G (E Merck).

Synthesis of 7-(chloro-acetyl)-1, 3-dimethyl-3, 4, 5, 7-tetrahydro-1*H*-purine-2, 6-dione (1) [24]

In a 250 ml round bottom flask, 1 mole theophylline and 2 mole chloro-acetyl chloride were mixed violently to obtain a homogenous mixture, then the mixture was heated and agitated until the temperature reached 1000C. Over the course of 5-8 hours, 125 ml of 1.6 (N) NaOH was added consistently while the temperature of the mixture was maintained at 1000C. Following the conclusion of the reaction, the reaction mixture was filtered to remove any precipitated NaCl, and the reaction mixture was concentrated further to yield colorless crystalline compound 1.

Molecular formula: $C_9H_{11}ClN_4O_3$,

Molecular Wt.: 258.66, %Yield: 92 %, mp: 261-263^{0}C, R_f : 0.56 [n-Hexane: ethyl acetate (8.5:1.5)], IR(cm-1): 1654.80 (C=N, st.); 1746.12 (C=O, st.), 1498.34 (C-N, st.), 1636 (C=C, st.), 769.27 (C-Cl), *(m/z)*: 258(M)$^+$, λ-max: 321, (CHN analysis) Calculated: 41.79, 4.29,21.66; Found: 41.52, 4.60, 21.69.

General method for synthesis of 1, 3-dimethyl-7-(4-oxo-1, 3-thiazolidin-2-yl)-3, 4, 5, 7-tetrahydro-1*H*-purine-2, 6-dione (2) [25].

In 35 ml ethanol, compound 1 (7 mmol) and ammonium thiocyanate (15 mmol) were refluxed for 3 hours and the reaction mixture was held overnight. To make compound 2, the result was filtered, dried, and recrystallized from ethanol-water. TLC was used to determine the purity of the compounds, using benzene:acetone (9:1) as the mobile phase.

Molecular formula: $C_{10}H_{13}N_5O_3S$, Molecular Wt: 283.30, %Yield: 85 %, mp: 210 - 211^{0}C, R_f : 0.51 [Ethanol: Acetone (9.5:0.5)], IR(cm-1): 3322 (NH), 1678(C=O), 1524 (C=N), 687.73

(C-S-C, st.),1649.95 (C=N, st.), *(m/z)*: 282(M-1)$^{+}$, λ-max: 330, (CHN analysis) Calculated: 42.39,4.63,24.72; Found: 42.43,4.45,24.80.

General method for synthesis of 7-[5-(substituted benzylidene)-4-oxo-1, 3-thiazolidin-2-yl]-1,3-dimethyl-3,4,5,7-tetrahydro-1*H*-purine-2,6-dione 3(A-D).

In 20 ml ethanol, 2 ml piperidine and 3 mmol substituted benzaldehyde were added to compound 2 (3 mmol). The mixture was then refluxed for 12-18 hours before being poured over broken ice and neutralized with HCl. The precipitate was filtered, washed with water, dried, and recrystallized from ethanol-DMF to get final product 3 (A-D), and the purity of the compound was determined by TLC with benzene:acetone (9:1) as the mobile phase.

The spectral information of the representative compounds was as per the following-

7-[5-(2-hydroxybenzylidene)-4-oxo-1,3-thiazolidin-2-yl]-1,3-dimethyl-3,4,5,7-tetrahydro-1*H*-purine-2,6-dione (3A):

Molecular Formula:$C_{17}H_{17}N_5O_4S$, Molecular Wt.: 387.41; %Yield: 73%; mp: 222 - 224^{0}C; Rf: 0.50 [n-Hexane: ethyl acetate (8.5:1.5)]; IR(cm^{-1}):3451 (OH), 1592 (C=N),2819 (C-H Str), 1703 (C=O), 1654 (sec. NH), 1560 (Ar-C=C), 687.73 (C-S-C, st.), 1442 and 1319 (N-H def); ^{1}H-NMR(δ): 3.240(s, 3H, CH3); 3.446 (s, 3H, CH3);4.062–4.112 (m, 1H, CH); 7.76 (d, 2H, Ar-H), 7.15 (d, 2H, Ar-H); *(m/z)*: 386(M-1)$^{+}$; λ-max: 403; (CHN analysis) Calculated: 52.70, 4.42, 18.08; Found: 52.78, 4.39, 18.10

7-[5-(3-chlorobenzylidene)-4-oxo-1, 3-thiazolidin-2-yl]-1, 3-dimethyl-3, 4, 5, 7-tetrahydro-1*H*-purine-2, 6-dione (3B):

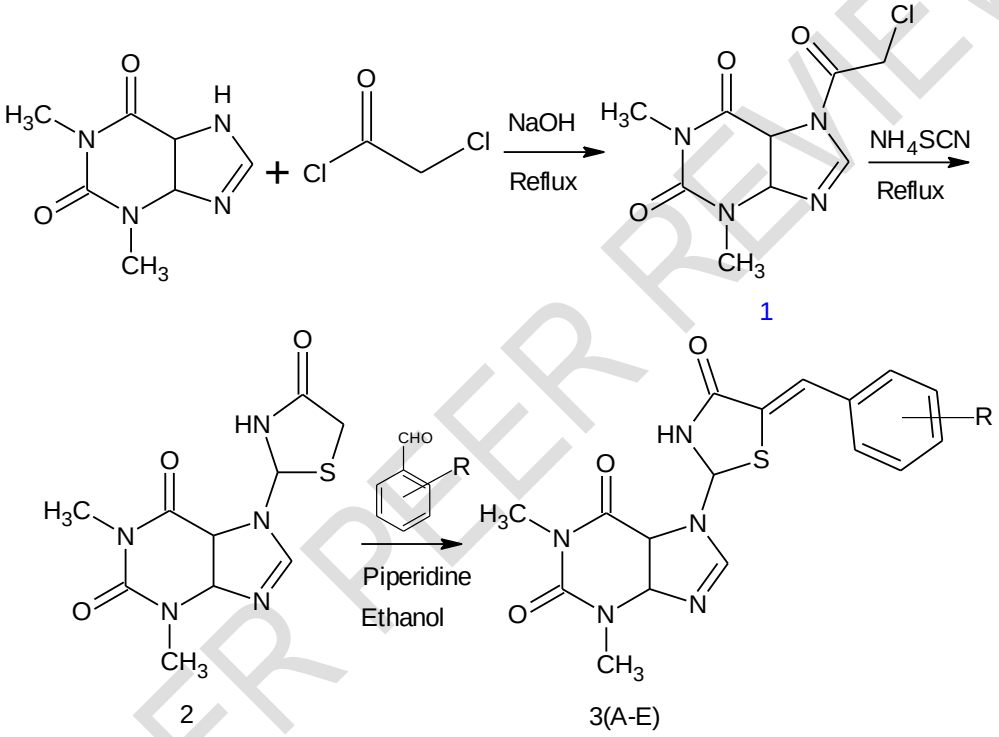
Molecular Formula: $C_{17}H_{16}ClN_5O_3S$, Molecular Wt.: 405.85; %Yield: 73%; mp: 260 - 263^{0}C; R_f :0.58 [n-Hexane: ethyl acetate (9.0:1.0)]; IR(cm^{-1}): 3316 (NH), 1777(C=O), 1525 (C=N); 682.50 (C-S-C, st.), 760.64, 786.10 (C-Cl); ^{1}H-NMR(δ): 3.229(s, 3H, CH3), 3.431 (s, 3H, CH3), 4.033–4.107 (m, 1H, CH), 4.391–4.436 (d, 2H, CH2), 7.92 (d, 2H, Ar-H), 7.48 (d, 2H, Ar-H); *(m/z)*: 406(M+1)$^{+}$; λ-max: 393; (CHN analysis) Calculated:50.31, 3.97, 17.26; Found: 50.35, 3.99, 17.32.

1,3-dimethyl-7-[5-(4-nitrobenzylidene)-4-oxo-1,3-thiazolidin-2-yl]-3,4,5,7-tetrahydro-1*H*-purine-2, 6-dione (3C):

Molecular Formula: $C_{17}H_{16}N_6O_5S$, Molecular Wt.:416.41; %Yield: 88%; mp: 242 - 244^{0}C; Rf: 0.52 [n-Hexane: ethyl acetate (8.5:1.5)]; IR(cm^{-1}): 3291 (NH), 1757 (C=O), 1556 (C=N), 1554 (NO_2), 1416 (aryl C=C, st.), 678 (C-S-C, st.); ^{1}H NMR (δ): 3.238(s, 3H, CH3); 3.456 (s, 3H, CH3); 3.776–3.815 (m, 2H, CH2);4.033–4.107 (m, 1H, CH); 7.62-7.27 (m, 4H, Ar-H) ;*(m/z)*: 416(M)$^{+}$; λ-max: 384; (CHN analysis) Calculated:49.03,3.87,20.18; Found: 49.83, 3.89, 20.00

7-[5-(4-methoxybenzylidene)-4-oxo-1,3-thiazolidin-2-yl]-1,3-dimethyl-3,4,5,7-tetrahydro-1*H*-purine-2,6-dione (3D):

Molecular Formula:$C_{18}H_{19}N_5O_4S$, Molecular Wt.: 401.43; %Yield: 91%; mp: 230-232^{0}C; Rf: 0.56 [Ethanol: Acetone (9.5:0.5)]; IR(cm^{-1}):3322 (NH), 1678(C=O), 1524 (C=N), 1650 (sec. NH), 1575 (Ar-C=C), 681 (C-S-C, st.); ^{1}H NMR (δ): 3.212(s, 3H, CH3); 3.460 (s, 3H, CH3); 3.775–3.823 (m, 2H, CH2);4.040–4.126 (m, 1H, CH); 7.56–7.76 (m, 2H, Ar-H); *(m/z)*: 400(M-1)$^+$; λ-max: 313; (CHN analysis) Calculated: 53.85, 4.77, 17.45; Found: 53.96, 4.70, 17.50.



R=2-OH, 3-Cl, 4-NO_2, 4-OCH_3

SCHEME

BIOLOGICAL ACTIVITY

Experimental animals

Experimental animals were adult Swiss albino mice (20-25 gm) and albino rats (150-200 gm) of either sexe. They were housed in groups of 4-8 per cage at a temperature of 25°C and a relative humidity of 45–55 percent while doing the studies, and they were kept in a 12 hour dark/12 hour light cycle. Animals were allowed unlimited access to food and water. The standards of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animals Ethics Committee (IAEC) were followed throughout the experiment for animal care (AdtU/ IAEC /2017 /07).

Acute toxicity studies

The acute toxicity tests were performed on six Swiss albino mice weighing 20-25 grams that were fasted overnight and given the test substances orally. Orally, the dose ranged from 100 to 1000 mg/kg body weight. The Institutional Animal Ethics Committee, Faculty of Pharmaceutical Science, Assam Downtown University, Guwahati, Assam, India, approved all animal research.

Analgesic activity by Tail-flick method in mice [26]

Tail-flick technique was used to test analgesic activity in Swiss albino mice. Heat is employed as a cause of pain in this procedure. Adult male Swiss albino mice, weighing between 20 and 25 grams, were collected in groups of six for the study and fasted overnight in a healthy setting. The animals were confined to a tiny cage with a tail entrance in the back wall. The proximal section of the tail was exposed to a light beam, which generated radiant heat. The mice's tail tips were individually placed on the radiant heat source at a constant temperature of 55°C with a 15-second cut-off response time to prevent tissue injury. At 0 hr, 0.50 hr, 1 hr, 2 hrs, and 4 hrs following treatment with test medications, the tail flick response was assessed using a digital analgesiometer (INCO, India). The reference treatment was pentazocine (5 mg/kg, i.p.), whereas the test groups got synthetic Theophylline derivatives at 100 mg/kg p.o.

Anti-inflammatory activity by Carrageenan-induced rat paws edema method [27]

A carrageenan-induced rat paw edema model was used to assess the test compounds' anti-inflammatory efficacy. One hour before the 1 percent w/v solution injection of 0.1 ml carrageenan into the plantar area of the left hind paw, rats of either sex were given Theophylline derivatives (100 mg/kg p.o.) and the conventional medicine Diclofenac sodium (20 mg/kg p.o.). In each animal of both groups, the marking was done slightly below the tibia-tarsal junction of the left hind paw (knee joint). Plethysmometer (Model 520, IITC, Life sciences, USA) was used to assess paw volume at 0-hr, 1 hr, 3 hours, and 4 hrs following carrageenan injection. The difference between the paw volume at 4 hours and the 0 hour measurement was used to calculate the edema volume. The percentage inhibition in the paw was calculated using the formula percentage inhibition=100 (1-Vt/Vc), where Vt is the mean increase in paw volume in the test and Vc is the mean increase in paw volume in the control.

RESULTS AND DISCUSSION

Chemistry

IR, 1H-NMR, and MASS analyses were used to structurally describe all of the compounds. The C=N, C=O, NO2, –C-S-C, Functional group might be ascribed to the IR absorption band of the compounds 3A-3D about 1524-1592cm-1, 1678-1777 cm-1, 1554cm-1, 678-687 cm-1. O-H stretching vibration causes the band at 3451-3464 cm-1. CH3, the CH proton signal, which showed at 4.033-4.126 in all produced compounds, is responsible for the singlet in 1H NMR at 3.212-3.460. Singlet, doublet, and multiplet peaks were seen in the spectra 3.775–3.834 (m, 2H, CH2) and 7.15-7.76 (m, 4H, Ar-H), indicating the presence of methylene and aromatic protons, respectively, in all produced compounds. These compounds' mass spectra revealed molecular ion peaks that corresponded to their chemical formula.

Pharmacologicalactivities

Analgesic Activity: Tail-Flick Method

Table 1 reveals that the Maximum possible effect (MEP) values of compounds 3C and 3D were equivalent (P > 0.05) at 50 minutes, but those of 3A and 3B were statistically substantially smaller (P 0.05). The mean MPE of 3C was highest at 1 and 2 hours, and it was substantially higher than the other four groups (P 0.05). When compared to 3D, 3A, and 3B at 4 hours, MPE of 3C was equivalent and considerably higher (P 0.05). Among the varied time periods, compound 3D had the highest MPE at 4 hours, which was equal to compound 3C (P > 0.05). [Table 1]

Anti-inflammatory activity:Carrageenin induced paw edema

The baseline mean paw volume was similar in all groups, as shown in Table 2. When compared to the control group, the mean paw volume in all of the investigated chemical and standard medication treated groups was statistically substantially reduced at 3 and 4 hours (P 0.01). At all time intervals, the percentage inhibition of acute inflammation in the compound 3C group was higher than in the 3A, 3B, and 3D groups. At all time intervals, the percentage inhibition of acute inflammation in the Ibuprofen group was higher than in the 3C group. At all time intervals, the percentage inhibition in the 3D group was higher than in the 3A and 3B groups. [Table 2]

Finally, compound 3C out of the five compounds tested for analgesic and anti-inflammatory activity exhibited significant analgesic and anti-inflammatory action when compared to the conventional one. After a thorough examination of the synthesized compounds, it was discovered that compounds with nitro and methoxy groups on the distant phenyl ring have

high potency in the Tail-Flick and Carrageenin induced paw edema methods, whereas compounds with hydroxy and chloro groups on the distant phenyl ring have lower analgesic and anti-inflammatory activity. Compound 3C's higher activity might be linked to the presence of a p-nitro phenyl substitution, which could be responsible for improved penetration or binding affinity towards the receptor binding site owing to the compound's increased lipophilic nature.

CONCLUSION

Novel 4-oxo-1, 3-thiazolidin-2-yl] compounds The Tail-Flick Technique and Carrageenin-induced paw edema method were used to investigate the potential analgesic and anti-inflammatory properties of -1,3-dimethyl-3,4,5,7-tetrahydro-1H-purine-2,6-dione. The findings revealed that the majority of the compounds had strong analgesic and anti-inflammatory properties. 1,3-dimethyl-7-[5-(4-nitrobenzylidene)-4-oxo-1,3-thiazolidin-2-yl]-1,3-dimethyl-7-[5-(4-nitrobenzylidene)-4-oxo-1,3-thiazolidin-2-yl]-1,3-dimethyl-7-[5-(4-nitro The most active chemical was -3,4,5,7-tetrahydro-1H-purine-2, 6-dione (3C), which had a great activity when compared to the normal one. This new information might be useful in the creation of substituted theophylline derivatives as new analgesics and anti-inflammatory drugs in the future.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1: Analgesic activity of the synthesized compounds after oral administration

Group	Dose	Pre test	Reaction time in second			
			0.5hr	1hr	2hrs	4hrs
Control	10 mg/kg	6.67±0.82	------	------	------	------
Pentazocine	5 mg/kg	7.24±0.17	13.23±0.07*	14.28±0.06*	15.40±0.13*	14.98±0.07*
3A	100mg/kg	5.32±0.07*	7.01±0.65	7.90±0.5**	8.95±0.03	9.65±0.02
3B	100mg/kg	5.32±0.21	8.10±0.09	8.54±0.18	9.39±0.45	9.76±0.65
3C	100mg/kg	6.12±0.18	11.13±0.17	12.20±0.08	13.66±0.21	13.45±0.5**
3D	100mg/kg	5.00±0.82	9.02±0.11	9.78±0.03	10.06±0.05	11.65±0.02
One-way ANOVA	P	>0.05	<0.05	<0.05	<0.05	<0.05

Values are mean ± SEM, n=6 in each group; *P<0.05 when compared to control group; **P<0.05 when compared to Pentazocine group

Table 2:Effect of different drugs on paw volume in carrageenin induced paw edema in rats

Compound No.	Paw volume. ± SD				% inhibition ± SD		
	0 hr (Basal)	After 1 hr	After 3 hrs	After 4 hrs	After 1 hr	After 3 hrs	After 4 hrs
3A	0.84 ±0.07	1.32 ± 0.07	1.73 ±0.11	1.79 ±0.11	13.72 ±7.41	25.75 ±6.36	28.69 ± 6.21[a]
3B	0.85 ±0.06	1.26 ± 0.07	1.64 ±0.08	1.71 ± 0.09	17.65 ±7.86	29.61 ±4.55	31.87 ± 4.38[a]
3C	0.84 ±0.04	1.16 ± 0.04	1.22 ±0.08	1.26 ± 0.10	24.18 ±1.89	47.64 ±3.35	49.80 ± 4.10
3D	0.87 ± 0.05	1.21 ± 0.09	1.65 ±0.11	1.68 ± 0.11	20.91 ±2.92	29.18 ±4.32	33.86 ± 3.74[a]
Ibuprofen	0.85 ± 0.06	0.99 ± 0.04	1.04 ± 0.04	1.06 ± 0.04	35.29 ± 0.90	55.36 ± 1.42	57.77 ± 1.66
Control	0.87 ± 0.08	1.53 ± 0.09	2.33 ± 0.07	2.51 ± 0.08	---------	---------	---------

Data were given in mean ± SD and analyzed by ANOVA followed by Dunnett's multiplecomparison test, (n = 6). [a]$p < 0.01$ compared to the standard drug (ibuprofen).