RESEARCH ARTICLE

Synthesis, Evaluation of Antiangiogenic Activity of Novel Coumarinyl 4-Thiazolidinone Derivatives

Ishwar Bhat K[1*], Vijaya Bhaskar K[2]

[1]*Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Deralakatte, Mangaluru, Karnataka, India.*
[2]*Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, India.*

Received: 16th March, 2024; Revised: 23rd May, 2024; Accepted: 02nd August, 2024; Available Online: 25th September, 2024

ABSTRACT

Cancer remains in the maximum percentage probability of death in spite of the latest advancement in prior diagnosis and different approaches to treatment. The major challenge in drug discovery for medicinal chemists is to develop new cytotoxic agents to cure cancer with fewer side effects. A new series of substituted coumarinyl 4-thiazolidinone derivatives (TZL1–TZL7) were prepared by treating different 4-hydroxy-3-(1-(phenylimino0ethyl)-2H-chromen-2-one with HSCH2COOH. The Schiff bases were obtained by treating 4-hydroxy coumarin with different aniline derivatives in the presence of C2H5OH. All the title compounds were purified and subjected to TLC to check their purity.

Further, it was characterized by using IR, ^{1}H-NMR, and mass spectral studies. Selected synthesized compounds (TZL1–TZL7) were screened for *in-vivo* antiangiogenic activity by using the Zebrafish model. TZL2 had the best antiangiogenic effect with an average vein count of 2.7. TZL4, TZL5, and TZL6 had an average vein count of 4.1, 3.9, and 3.4, respectively.

Keywords: Schiff bases, 4-Thiazolidinone, coumarins, Antiangiogenic, Zebrafish.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.3.41

How to cite this article: Bhat IK, Bhaskar VK. Synthesis, Evaluation of Antiangiogenic Activity of Novel Coumarinyl 4-Thiazolidinone Derivatives. International Journal of Drug Delivery Technology. 2024;14(3):1547-1552.

Source of support: Nil.

Conflict of interest: None

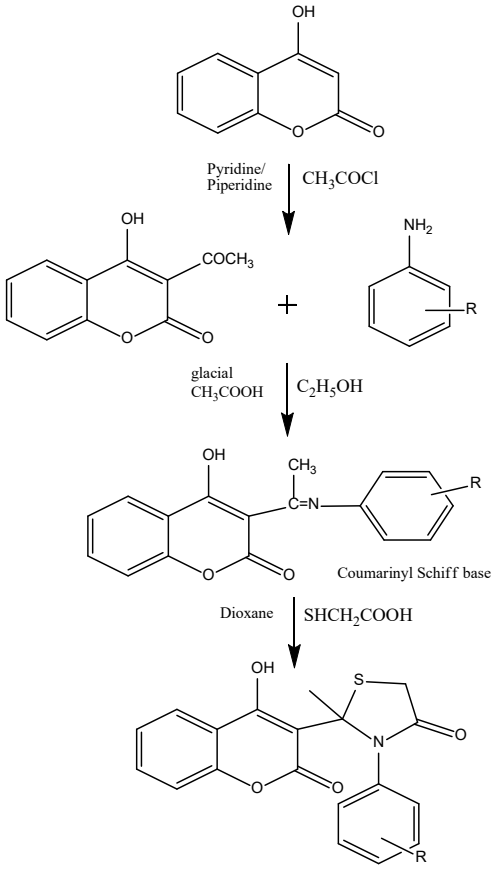
INTRODUCTION

Angiogenesis is mainly responsible for the development of lumps of solid tumors because they require blood supply if it has to grow beyond a certain size. This growth will cause blood supply by giving off chemical signals, which enhance angiogenesis. The growth of tumors can also induce the nearby cells to result in cancer signaling molecules. These newly developed blood vessels will help the growing tumors with rich nutrients and oxygen, which makes the solid tumors enlarge and invade the surrounding tissues, which spread throughout the body and cause the development of secondary malignant growth at a specific distance from the primary site of cancer which is referred to as metastases. Without blood supply, tumors cannot grow beyond a certain size and hence, angiogenic inhibitors are developed by scientists to block these tumors. These drugs are referred to as antiangiogenic agents, which prevent or slow down the growth of cancer by removing the blood supply. A major focus was attributed to chemo-preventive drugs involving coumarinyl thiazolidinone derivatives, which display a major role in various pharmacological activities.[1] They also produce apoptosis in cancer cell lines, which were thought to be involved in suppressing angiogenesis.

Coumarin comes under the benzopyrone chemical class and they are well-known lactones which are aromatic in nature. Coumarin derivatives are vital pharmacophores that exhibit a wide spectrum of biological activities. These compounds are renowned for their use in photochemotherapy, particularly in the treatment of skin disorders. They are known to exhibit a wide spectrum of activities like antioxidant,[2] anti-inflammatory,[3] antimicrobial,[4] antitumor[5] and antidiabetic.[6]

4-Thiazolidinones are thiazolidinenone compounds characterized by a carbonyl group at the 4th position. They are synthesized by substituting sulfur on an imine carbon, followed by cyclization and the elimination of an H2O molecule. The Thiazolidinone ring system is quite significant because it is the main pharmacophore present in many drugs and possesses a number of biological activities like anticancer,[7] antiangiogenic,[8] antidiabetic,[9] anticonvulsant,[10] antihyperlipidemic,[11] antimicrobial,[12] antitubercular[13] and antioxidant[14] activities.

**Author for Correspondence: bhatishwark@nitte.edu.in*



Scheme 1: Scheme of synthesis of 4 thiazolidinone derivatives TZL 1 – TZL 7

In view of the synergic effect of the combination of two moieties, it was assumed to prepare some new molecules by integrating the two pharmacophores 4-thiazolidinone and coumarin in a single molecular framework and screen them for antiangiogenic activity using the zebrafish model.

MATERIALS AND METHODS

Detailed procedures for the preparation of coumarinyl 4-thiazolidinone derivatives (TZL1-TZL7) are outlined. About 0.01 mol of 4-hydroxycoumarin was dissolved in pyridine, a small amount of piperidine was added, and the mixture was cooled to 0 to 5°C. Then, 0.01 mol of CH3COCl was added, and the reaction mixture was shaken for two days. Then it was cooled and acidified with strong hydrochloric acid. The precipitates were filtered, washed with ice water, and recrystallized with C_2H_5OH, and pure 3-acetyl-4-hydroxycoumarin was obtained.[15] An equal amount of this and 0.01 mol of aromatic amine was dissolved in C_2H_5OH and refluxed for 5 hours. After cooling with ice, it was filtered, washed with H_2O, and recrystallized with C_2H_5OH, and a pure Schiff base was obtained[16] 0.001 mol of Schiff bases and 0.002 mol of thioglycolic acid were refluxed for 10 hrs in the presence of dioxane. Then it was cooled with ice and solid mass separated by filtration, finally recrystallized from C_2H_5OH and different substituted 2-(4-hydroxy-2-oxo-2H-chromen-3-yl)-2-methyl-3-phenylthiazolidin-4-one was obtained[17] which is given in Scheme 1.

Antiangiogenic activity

The antiangiogenic screening and toxicological evaluation of coumarinyl 4-thiazolidinone derivatives was performed by using the Zebrafish model as per standard protocol.[18]

Toxicological evaluation of coumarinyl 4-thiazolidinone on zebra fish for antiangiogenic screening

Fish maintenance and preparation of embryos

Matured zebrafishes (Wild type WT; KA strain) were maintained with 14 hours of light and 10 hours of dark photoperiod at 28ºC with proper supply of aeration and fresh water. The fishes were spawned in the morning and their eggs were collected and separated from the unfertilized ones by afternoon. It was then immediately cleaned and cultured in embryonic media (0.17 mM KCl, 5 mM NaCl, 0.33 mM $MgCl_2$, 0.33 mM $CaCl_2$, 1% methylene blue and pH 7.2) and incubated at 25 to 28°C. For exposure, the eggs were divided and put in a 12-well microtiter plate as untreated control and as triplicates of treated ones. It was then exposed to various concentrations of title derivatives.

Mode of exposure

In the present study, we have used four different title derivatives (A, B, C and D) at different concentrations such as 1, 3, 5, and 10 ppm of final concentration as per the literature reports. Test compounds solubilized in dimethyl sulfoxide as stock solutions (2500 and 1000 ppm) and diluted to the desired concentration for treatment. In the control group, the maximum concentration of DMSO was 0.25%. This derivative solution was available as the main stock with concentrations of 2500 and 1000 ppm.

Table 1: Physicochemical data of compounds TZL1 – TZL7

Compound	*-R*	*Molecular formula*	*Molecular weight*	*Melting point (°C)*	*% yield*
TZL1	H	$C_{19}H_{15}NO_4S$	353.2	81-83	68
TZL2	2-NO_2	$C_{19}H_{14}N_2O_6S$	398.3	151-153	65
TZL3	2-CH_3	$C_{20}H_{17}NO_4S$	367.4	183-185	64
TZL4	4-Br	$C_{19}H_{14}BrNO_4S$	432.2	197-199	69
TZL5	2-Cl	$C_{19}H_{14}ClNO_4S$	387.3	103-105	70
TZL6	4-F	$C_{19}H_{14}FNO_4S$	311.2	147-149	62
TZL7	3-OCH_3	$C_{20}H_{17}NO_5S$	382.9	207-209	23

The desired working solution for 1, 3, and 5 ppm was prepared from 1000 ppm stock 1 (1-mL of main stock + 4 mL of DMSO) and 10 ppm was prepared from 2500 ppm stock 2 (5 mL of main stock + 5ml of DMSO). To obtain 1 ppm concentration, 1-µL of stock 1 was added to 999 µL of embryonic rearing media and for obtaining 3 ppm concentration, 3 µL of stock 1 was added to 997 µL of media. Likewise, 5 ppm was also obtained by adding 5 µL of stoc 1 to 995 µL of media. The higher concentration 10 ppm was obtained by adding 4 µL of stock 2 to 996 µL of media. At 10 hours post fertilization (hpf) all the embryos were exposed and monitored at four different time points such as 24, 48, 72 and 120 hpf. The media was changed, and the test solution was renewed twice a day.

Morphological observation

Exposed embryos were assessed for various developmental toxicity endpoints at different stages of development. The endpoint scoring was done in a binary fashion (present/absent) and the observations were recorded as the percent incidence of each endpoint relative to total embryos at a particular concentration. Data from three replicates were recorded for each endpoint and compared to the control (unexposed) embryos. The embryos were examined under a stereomicroscope for various lethal and sub-lethal phenotypes.

Morphological observation

Concentration: Toxicity was tested for every four concentrations (1, 3, 5, and 10 ppm) for the respective compounds (A, B, C, D). In triplicate experiments were performed with 25 embryos each.

Morphological changes in the embryos were recorded over five days, with water changes and compound renewal occurring twice daily. The growth stage at the first exposure was 10 hours post-fertilization (hpf). Various toxicological assays, including developmental toxicity, cardiotoxicity, neurotoxicity, and hepatotoxicity, were conducted. The assessment time points were as follows: Developmental toxicity on day 1, cardiotoxicity on day 2, neurotoxicity on day 3, and hepatotoxicity on day 5.

The embryos were grown in an embryonic medium and incubated at 28ºC in 12-well microtiter plates. Morphological endpoints were made at the above-mentioned time points and data was recorded for various toxicological endpoints.

Survivability

Exposure at 1-ppm the survival rate of all four compounds (A, B, C and D) was 100%. As the concentration and time points increased, the survivability was marginally decreased to 96% in chalcone B and C at the end of day 5, whereas, in chalcone A and D at the concentration of 10 ppm, survivability was 0%.

Hatchability

The percentage of hatchability was checked by monitoring the ability of the embryos to come out of the chorions on the third day of development. The embryos treated with Chalcone A did not show any delayed hatching at concentrations of 1, 3, and 5 ppm, but at 10 ppm, it showed a delay of 66%. Likewise, chalcone B also did not show any delayed hatching in all three concentrations except 10 ppm in which it was delayed in 50% of the embryos. In chalcone C, at concentrations of 3 and 5 ppm one or two of the embryos in one of the replicates showed delayed hatching, whereas in chalcone D, at a concentration of 10 ppm, only one embryo among the replicates showed delayed hatching.

Heart rate

In Heart rate analysis, treated embryos showed fluctuating heart rates compared to the control. Both the heart rate and rhythmicity of the resting heart were not constant in all different compounds at different concentrations. The embryos were subjected to this analysis on the second day of post-fertilization.

Morphological observation

The images were taken using Leica Stereomicroscope at lower (2.5X) and higher magnification (4X). At different time points (day 1, day 2, day 3, and day 5) for different concentrations observations were taken according to the toxicological endpoints.

RESULTS AND DISCUSSION

Synthesis of substituted 4-Thiazolidinone derivatives using coumarinyl Schiff bases and thioglycolic acid in the presence of dioxane was the main focus of the present investigation given in Scheme 1. The physicochemical data of the title compounds are listed in Table 1. Confirmation of all the title compounds was done by using FTIR, ^{1}H-NMR, and mass spectral studies enclosed in Table 2.

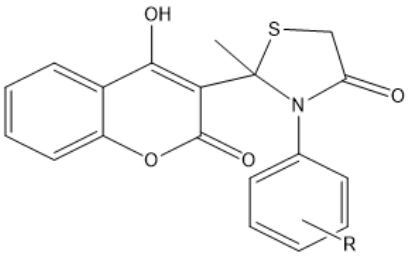
Authentification of various functional group changes in 4-thiazolidinone derivatives was performed by analyzing the stretching and bending frequencies in FTIR. The O-H stretching frequency was observed in 3345 to 3375 cm^{-1} range in all derivatives. In the range 1085-1116 cm^{-1} C-O-C stretching frequency appeared across all the compounds. C-S-C stretching frequency appeared within the range of 672 to 689 cm^{-1} in all the compounds. Aliphatic C-H stretching frequency appeared in the range of 2845 to 2881 cm^{-1} in all derivatives. An aromatic stretching peak was observed in the range 3072 to 3085 cm^{-1} across all the compounds. Aromatic C=C bonds were found within the region 1510 to 1560 cm^{-1}. Stretching bands of C-F, C-Cl, C-Br were observed in the region 795 to 905 cm^{-1} in products TZL6, TZL5, and TZL4.

Confirmation of all the protons was performed by using ^{1}H-NMR of all the final products. All aromatic protons were observed as multiplets in the region δ 7.1 to 7.8 ppm in all the title products. Aromatic OH protons were observed as singlets in the range δ 4.1 to 4.9 ppm across all the compounds. Two protons of CH_2 of thiazolidinone appeared as doublet in the region of δ 1.23 to 1.29 ppm. One proton of methene appeared as a singlet in the range of δ 5.2 to 5.3 ppm in all the products. Three protons of OCH_3 were seen as singlet at δ 3.8 ppm in TZL7.

Molecular mass confirmation was performed by using MS data. Molecular ion peaks appeared for 4-thiazolidinone derivatives TZL1, TZL2, TZL3, TZL4, TZL6, and TZL7 at

Table 2: Spectral data

Compound	*FTIR KBr (cm^{-1})*	*^{1}H-NMR (DMSO-d_6, ppm) δ*	*MS (m/z)*
TZL1	3360 (O-H), 1107 (C-O-C), 1025 (C-N), 680 (C-S-C), 1650 (C=O), 2850 (Aliphatic C-H), 3075 (Aromatic C-H), 1522 (Aromatic C=C)	7.3–7.8 (9H, m, Ar-H), 4.73 (1H, s, Ar-OH), 1.29 (2H, d, CH_2 of thiazolidinone), 5.32 (1H of methene, s)	353.2 (M+)
TZL2	3375 (O-H), 1106 (C-O-C), 1015 (C-N), 685 (C-S-C), 1665 (C=O), 2810 (Aliphatic C-H), 3072 (Aromatic C-H), 1545 (Aromatic C=C)	7.1–7.6 (8H, m, Ar-H), 4.9 (1H, s, Ar-OH), 1.27 (2H, d, CH_2 of thiazolidinone), 5.31 (1H of methene, s)	398.3 (M+)
TZL3	3369 (O-H), 1116 (C-O-C), 1030 (C-N), 683 (C-S-C), 2845 (Aliphatic C-H), 3095 (Aromatic C-H), 1535 (Aromatic C=C)	7.4–7.9 (8H, m, Ar-H), 4.6 (1H, s, Ar-OH), 1.23 (2H, d, CH_2 of thiazolidinone), 5.2 (1H of methene, s)	367.4 (M+)
TZL4	3362 (O-H), 1108 (C-O-C), 1024 (C-N), 682 (C-S-C), 1650 (C=O), 2881 (Aliphatic C-H), 905 (C-Br), 3085 (Aromatic C-H), 1548 (Aromatic C=C)	7.3–7.6 (8H, m, Ar-H), 4.4 (1H, s, Ar-OH), 1.27 (2H, d, CH_2 of thiazolidinone), 5.17 (1H of methene, s)	432.2 (M+)
TZL5	3371 (O-H), 1116 (C-O-C), 1017 (C-N), 689 (C-S-C), 2876 (Aliphatic C-H), 842 (C-Cl), 3080 (Aromatic C-H), 1540 (Aromatic C=C)	7.2–7.5 (8H, m, Ar-H), 4.2 (1H, s, Ar-OH), 1.27 (2H, d, CH_2 of thiazolidinone), 5.16 (1H of methene, s)	387.3 (M+2)
TZL6	3345 (O-H), 1098 (C-O-C), 1016 (C-N), 672 (C-S-C), 2879 (Aliphatic C-H), 795 (C-F), 3079 (Aromatic C-H), 1526 (Aromatic C=C)	7.1–7.5 (8H, m, Ar-H), 4.8 (1H, s, Ar-OH), 1.3 (2H, d, CH_2 of thiazolidinone), 5.23 (1H of methene, s)	311.2 (M+)
TZL7	3352 (O-H), 1085 (C-O-C), 1032 (C-N), 677 (C-S-C), 2868 (Aliphatic C-H), 3078(Aromatic C-H), 1515 (Aromatic C=C)	7.2–7.6 (9H, m, Ar-H), 4.9 (1H, s, Ar-OH), 1.34 (2H, d, CH_2 of thiazolidinone), 5.26 (1H of methene, s), 3.8 (3H, s, OCH_3)	382.9 (M+)



4-Thiazolidinone	*Code*
TZL-2	A
TZL -4	B
TZL -5	C
TZL -6	D

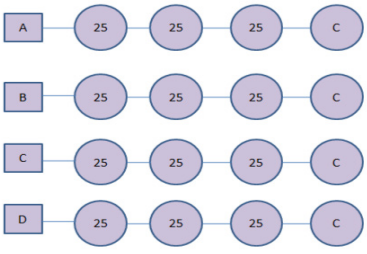


Figure 1: Distribution of the number of embryos in microtitre plate

353.2, 398.3, 367.4, 432.2, 311.2, and 382.9, respectively. In TZL5 an M+2 peak was seen due to chlorine isotopes. All the observed values are in agreement with the calculated mass.

Toxicological evaluation of Coumarinyl 4-Thiazolidinone on zebra fish for antiangiogenic screening

The embryos were distributed in the microtitre plates as shown in Figure 1 and were treated with 4 different concentrations (1, 3, 5, and 10) at different time points (24, 48, 72, and 120 hpf).

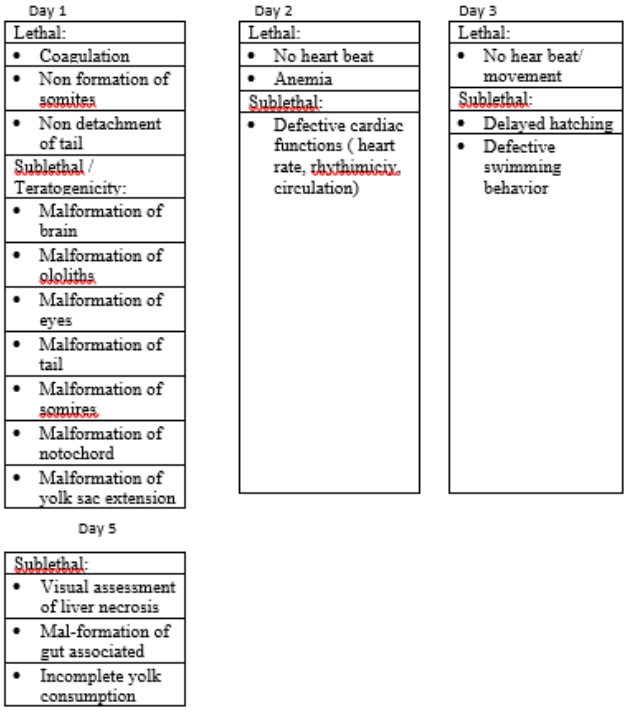


Figure 2: Toxicological endpoints for the morphological observation

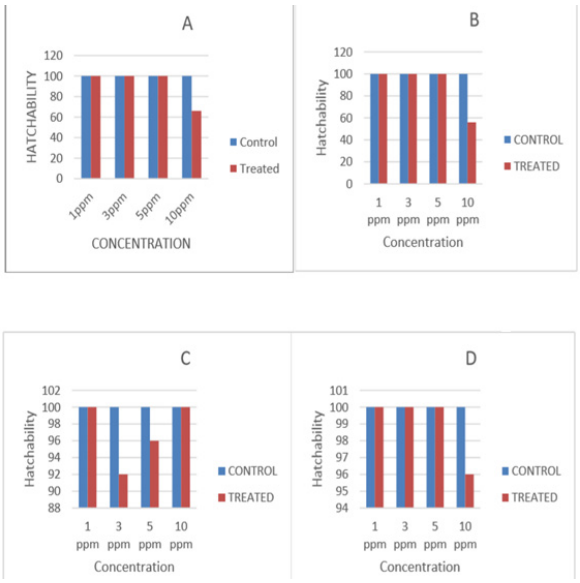


Figure 3: Graphs representing the hatching ability of embryos at different concentrations

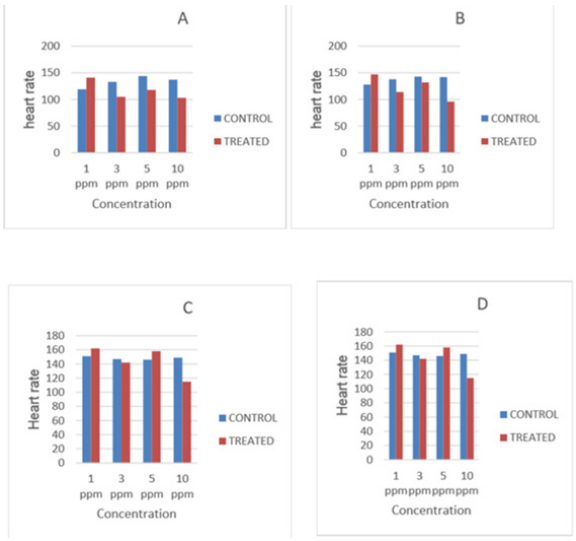


Figure 4: Graphs representing the heart rate of Coumarinyl 4-Thiazolidinone A, B, C and D at different concentrations

Table 3: Antiangiogenic activity of coumarinyl 4-thiazolidinone on transgenic zebra fish by studying the formation of SIV's (subintestinal veins) through exposure method-II (12–72 hpf)

Dosage (ppm)	*Number of SIV's*				
	Vehicle (0.12%)	*A*	*B*	*C*	*D*
0	8.6 ± 0.3				
0.1		8.8 ± 0.1	8.1 ± 0.2	9.4 ± 0.4	8.9 ± 0.6
1.0		5.9 ± 0.5	6.8 ± 0.4	6.4 ± 0.4	5.5 ± 0.2*
5.0		2.7 ± 0.2**	4.1 ± 0.6*	3.9 ± 0.5*	3.4 ± 0.4**

Data are the mean ± S.D. of twenty embryos from three independent experiments (n = 60 in each group). A t-test, with a 0.05 significance level, was carried out. The results as shwn in Table 3 indicated that the number of SIV in all experimental groups was significantly different from that in the vehicle group. (*: $p < 0.001$, **: $p < 0.0001$).

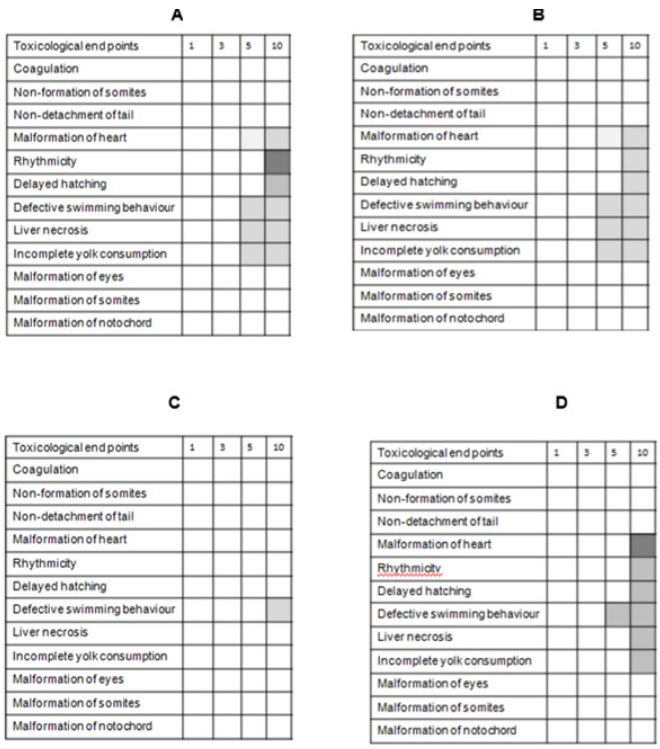


Figure 5: Heat maps showing 3-scale severity of coumarinyl 4-thiazolidinone A, B, C and D at different time points

The Figure 2 presents the toxicological endpoints, showcasing the morphological observations of embryos exposed to varying concentrations of Coumarinyl 4-Thiazolidinone derivatives. Key abnormalities or changes in the embryos' development are noted, indicating the potential toxicity at different concentrations.

Graphs in this Figure 3 depict the hatching ability of embryos when exposed to different concentrations of Coumarinyl 4-Thiazolidinone compounds (A, B, C, and D). The data indicates how each concentration influences the rate of hatching, providing insights into the impact of the compounds on embryonic development.

This Figure 4 shows graphs representing the heart rate of embryos exposed to Coumarinyl 4-Thiazolidinone compounds (A, B, C, and D) at varying concentrations. The heart rate is measured and compared to control groups, illustrating how each derivative affects cardiac function in embryos at different exposure levels.

In Figure 5 the Heat maps are used to represent the 3-scale severity (mild, moderate, severe) of toxic effects caused by Coumarinyl 4-Thiazolidinone compounds (A, B, C, and D) over different time points. These maps visually display the progression of toxicity, providing a clear overview of the severity and time-dependent effects of the compounds.

CONCLUSION

The present investigation revealed the synthesis of coumarinyl 4-thiazolidinone derivatives (TZL1-TZL7) and all of them were obtained in good percentages. All the synthesized compounds

were screened for antiangiogenic activity using the Zebrafish model. Compounds TZL2, TZL4, TZL5, and TZL6 were proven to be potential anticancer agents and, further, can be tested for their *in-vitro* and *in-vivo* clinical investigation.

ACKNOWLEDGMENT

The authors express their gratitude to the management of NITTE (Deemed to be University) and the NGSM Institute of Pharmaceutical Sciences for providing the necessary facilities and financial assistance to conduct this work.

REFERENCES

1. Cacić M, Molnar M, Sarkanj B, Has-Schön E, Rajković V. Synthesis and antioxidant activity of some new coumarinyl-1,3-thiazolidine-4-ones. Molecules. 2010;15 (10): 6795-809. doi:10.3390/molecules15106795.
2. Todorov L, Saso L, Kostova I. Antioxidant Activity of Coumarins and Their Metal Complexes. Pharmaceuticals (Basel). 2023 ;16(5):651. doi: 10.3390/ph16050651
3. Hamid SJ, Salih T. Design, Synthesis, and Anti-Inflammatory Activity of Some Coumarin Schiff Base Derivatives: In silico and in vitro Study. Drug Des Devel Ther. 2022;16:2275-2288. doi: 10.2147/DDDT.S364746.
4. Cheke RS, Patel HM, Patil VM, Ansari IA, Ambhore JP, Shinde SD, Kadri A, Snoussi M, Adnan M, Kharkar PS, Pasupuleti VR, Deshmukh PK. Molecular Insights into Coumarin Analogues as Antimicrobial Agents: Recent Developments in Drug Discovery. Antibiotics (Basel). 2022;11(5):566. doi: 10.3390/antibiotics11050566.
5. Wu Y, Xu J, Liu Y, Zeng Y, Wu G. A Review on Anti-Tumor Mechanisms of Coumarins. Front Oncol. 2020;10:592853. doi: 10.3389/fonc.2020.592853.
6. Ranđelović S, Bipat R. A Review of Coumarins and Coumarin-Related Compounds for Their Potential Antidiabetic Effect. Clin Med Insights Endocrinol Diabetes. 2021;14:11795514211042023. doi: 10.1177/11795514211042023.
7. Roszczenko P, Holota S, Szewczyk OK, Dudchak R, Bielawski K, Bielawska A, Lesyk R. 4-Thiazolidinone-Bearing Hybrid Molecules in Anticancer Drug Design. Int J Mol Sci. 2022 Oct 28;23(21):13135. doi: 10.3390/ijms232113135.
8. Sharma A, Sharma D, Saini N, Sharma SV, Thakur VK, Goyal RK, Sharma PC. Recent advances in synthetic strategies and SAR of thiazolidin-4-one containing molecules in cancer therapeutics. Cancer Metastasis Rev. 2023;42(3):847-889. doi: 10.1007/s10555-023-10106-1.
9. Kaminskyy D, Kryshchyshyn A, Lesyk R. 5-Ene-4-thiazolidinones - An efficient tool in medicinal chemistry. Eur J Med Chem. 2017;140:542-594. doi: 10.1016/j.ejmech.2017.09.031.
10. Siddiqui N, Arshad MF, Khan SA, Ahsan W. Sulfonamide derivatives of thiazolidin-4-ones with anticonvulsant activity against two seizure models: synthesis and pharmacological evaluation. J Enzyme Inhib Med Chem. ;25(4):485-91. doi: 10.3109/14756360903282833.
11. Nampurath GK, Mathew SP, Khanna V, Zachariah RT, Kanji S, Chamallamudi MR. Assessment of hypolipidaemic activity of three thiazolidin-4-ones in mice given high-fat diet and fructose. Chem Biol Interact. 2008;171(3):363-8. doi: 10.1016/j.cbi.2007.10.006.
12. Tratrat C, Petrou A, Geronikaki A, Ivanov M, Kostić M, Soković M, Vizirianakis IS, Theodoroula NF, Haroun M. Thiazolidin-4-Ones as Potential Antimicrobial Agents: Experimental and In Silico Evaluation. Molecules. 2022;27(6):1930. doi: 10.3390/molecules27061930.
13. Bhat MA, Al-Omar MA, Naglah AM, Khan AA. Synthesis of Novel Sulfamethaoxazole 4-Thiazolidinone Hybrids and Their Biological Evaluation. Molecules. 2020;25(16):3570. doi: 10.3390/molecules25163570.
14. Apotrosoaei M, Vasincu I, Constantin S, Buron F, Routier S, Profire L. Synthesis, characterization and antioxidant activity of some new thiazolidin-4-one derivatives. Rev Med Chir Soc Med Nat Iasi. 2014;118(1):213-8.
15. Moaz M. Abdou. 3-Acetyl-4-hydroxycoumarin: Synthesis, reactions and application. Arabian J of Chemistry.2007;10: S3664-3675. doi.org/10.1016/j.arabjc.2014.04.005
16. M. V. Girgaonkar and S. G. Shirodkar. Synthesis, characterization and Antimicrobial activity of some new Schiff's bases derived from 3-acetyl-4-hydroxy-2H-chromen-2-one and primary aromatic amines. J Chem Pharm Res, 2012, 4(1):260-264.
17. Pushkal Samadhiya, Ritu Sharma, S.K. Srivastava, S.D. Srivastava. Synthesis and biological evaluation of 4-thiazolidinone derivatives as antitubercular and antimicrobial agents. Arabian J Chemistry.2014:7(5): 657-65. doi.org/10.1016/j.arabjc.2010.11.015
18. Seo EJ, Kuete V, Kadioglu O, Krusche B, Schröder S, Greten HJ, Arend J, Lee IS, Efferth T. Antiangiogenic activity and pharmacogenomics of medicinal plants from traditional korean medicine. Evid Based Complement Alternat Med. 2013:131306. doi: 10.1155/2013/131306.