IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
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Human Journals
Research Article
October 2023 Vol.:28, Issue:3

Calcinated Egg Shells for the Synthesis of Newer Acetamide Derivatives and Their Antimicrobial Activity

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ISSN 2349-7203
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Submitted: 18 September 2023
Accepted: 23 September 2023
Published: 30 October 2023

Keywords: Acetamide, Antimicrobial, Coumarin, Thiazole, Minimum inhibitory concentration, zone of inhibition.

ABSTRACT

For the synthesis of a series of novel Coumarin derivatives, an efficient and easy approach has been designed. 1H-NMR and Fourier-transformed infrared (FTIR) are used to characterize the structures of freshly synthesized compounds, which are then evaluated for antibacterial activity in vitro by calculating zone of inhibition and minimum inhibitory concentration. The antibacterial and antifungal activity of 4-[(2-oxo-2-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]aminoethyl]amino]benzoic acid was comparable to that of ciprofloxacin and flucanazole. The reaming compounds had moderate to good activity.

INTRODUCTION

Antibiotics are among the most often prescribed antibiotics today, and their research and commercialization have saved countless lives. Multidrug-resistant bacteria cause a variety of bacterial diseases, including diarrhea, food poisoning, and rheumatic fever. Bacterial resistance to currently available antibiotics is rapidly increasing, posing a serious threat to human health[1].

Coumarin and its derivatives are one of the most active chemical classes, with a broad range of biological action. Antibacterial, antifungal, anti-inflammatory, anticoagulant, anti-HIV, and anticancer properties have been demonstrated for several of these substances. Various substituted coumarin analogues have antibacterial, anticonvulsant, acetylcholinesterase inhibitory, and aldehyde reeducate inhibitory properties. [2, 3, 4]

The design of novel compounds, as well as their vast range of pharmaceutical and medical uses, is a crucial study field for the expanding interest in thiazole chemistry. The thiazole ring is a fundamental structural component found in many natural bioactive substances such as marine alkaloids. Penicillin (wide range antibiotics), sulfathiazole (antimicrobial drug), ritonavir (antiretroviral drug), abafungin (antifungal drug), and tiazofurin (antifungal drug) all contain the thiazole ring (antineoplastic drug). [5, 6, 8, 9]

MATERIAL AND METHODS

Merck, Germany, and Loba Company supplied all chemicals and solvents. A Bruker Advance 500 MHZ equipment was used to obtain 1HNMR spectra. To determine the purity of the, thin-layer chromatography (TLC) was used. The melting points were determined in an open capillary using a Veego (model:-VMP-D) electronic instrument. The Shimadzu 8400 FT-IR Spectrophotometer was used to get IR spectra of the synthesized compounds.

Synthesis of 3-acetyl-2H-chromen-2-one

The solution was made up of 25.65mL (0.1mol) ethyl acetoacetate and 21.29mL (0.1mol) salicylaldehyde. In an ice bath, it was chilled. Rapid stirring was used to add 2ml (0.02mol) of piperidine to the liquid. The reaction mixture was held at a low temperature. The yellowish material was separated after 20 minutes, filtered, and washed with ethanol thereafter. It was recrystallized from ethanol from ethanol: water (7:3)[9].

Yield-85%, Melting point-110-114^{0}c, IR Spectra (KBr) - C=O (α, β unsaturated) at 1600 to 1700 cm^{-1} in addition to ketone C=O at 1745 to 1715 cm$^{-1,}$ aliphatic C-H 3000 to 2850 cm^{-1}, aromatic C-H 3150 to 3000 cm^{-1}.

Synthesis of 2-oxo-2H-chromene-3-carbonyl bromide

4.7gm (0.025mol) 3-acetycoumarin was taken in a beaker and dissolved in 20 ml of chloroform. 4gm (0.05mol) bromine was taken in the round bottom flask. 13 ml of chloroform was added to it. The two mixtures were mixed by slowly stirring and refluxing for 1hr. After 1hr reaction mixture was cooled, filtered and washed with petroleum ether. It was recrystallized from ethanol: chloroform (2:1)[4].

Yield-78%, Melting point-140-144^{0}c, IR Spectra- C-Br at 756 cm^{-1}, lactone at 1700 to 1600 cm^{-1}, ketone C=O at 1745 to 1715 cm^{-1}, aromatic C=C at 2350 to 2100 cm^{-1}, aromatic C-H 3150 to 3000 cm^{-1}.

Synthesis of 3-(5-amino-1, 3-thiazol-2-yl)-2*H*-chromen-2-one

1gm of 3-bromo acetyl coumarin (1mol) and 1gm (1mol) of thiourea were taken in 250 ml of round bottom flask and dissolved in 20 ml of ethanol by slowly stirring for 15min. After 15 min 0.5ml of ammonia was added to it. The reaction mixture was heated under the reflux for 30min. After 30min the reaction mixture was poured in ice cold water (50ml), filtered and dried. It was recrystallized from ethanol[10].

Yield-85%, Melting point-220-222^{0}c, IR Spectra(KBr) - C-S at 635 to 500 cm^{-1}, aromatic C=N at 1660 to 1340 cm^{-1} ,lactone at 1700 to 1600 cm^{-1}, amine N-H at 3500 to 3100 $^{-1}$cm.

Synthesis of 2-chloro-*N*-[2-(2-oxo-2*H*-chromen-3yl)-1, 3-thizol-5-] acetamide

In round bottom flask, 0.7gm (0.005mole) of 3-(5-amino-1, 3-thiazol-2-yl)-2*H*-chromen-2-one, 0.5gm of anhydrous potassium carbonate and 1ml of triethyl amine were taken and dissolved into 65ml of dry chloroform with slowly stirring. The reaction mixture was refluxed for 4-5hr. After 5hr the reaction mixture was evaporated. The resulting solid was washed with cold water and dried. It was recrystallized from ethanol : water (80:20)[11].

Yield-85%%, Melting point-230-232^{0}c, IR Spectra (KBr**)** 591 (C-S), 643 (C-Cl), 1249 (C-O), 1319 (C-N), 1556(C=N), 1651(C=O), 1735(keto C=O), 1797(amide N-H), 2327(Ar C=C), 3078(C-H), 3140(Ar C-H), 3371(amine N-H)

Synthesis of 1-(substituted phenyl)-3-[4-(2-oxo-2*H*-chromen-3-yl)-1,3-thiazol-2-yl]acetamide

3gm (0.13mol) of 2-chloro-*N*-[2-(2-oxo-2*H*-chromen-3yl)-1, 3-thizol-5-] acetamide and substituted aniline were taken into the mortar. And calcinated egg shell to it which was first calcinated by heating in a muffle furnace at very high temperature. This reaction mixture was mix continues with pestle at room temperature for 2hr. After 2 hr the reaction mixture was kept for the TLC solid was separated, which was filtered and dried. It was recrystallized by using ethanol: water (80:20) [2].

(2-(4-chlorophenylamino)-N-(4-(2-oxo-2H-chromen-3-yl) thiazol-2-yl)acetamide)

IR Spectrum (KBr cm^{-1}) -600 (C-S), 832(C-S), 1187(C-O), C=N (1288), 1535(lactone C=O), 1666(ketone C=O), 2391(Ar C=C), 2978(C-H), 3294(amine N-H), 1424(Amide N-H)

^{1}H-NMR ($CDCl_3$, δ ppm) - a) 12.7 (NH, *1H*, S), b) 3.4 (CH, 1*H*, S), c) 4.4 (NH, 1*H*,t), d) 8.0 (Ar.CH,1*H*,d), e) 7.8 (Ar.CH,1*H*,d), f) 7.6 (Ar.CH,1*H*,t), g) 7.4 (Ar.CH,1*H*,d), h) 7.5 (Ar.CH,1*H*,t), i) 8.3 (Ar.CH,1*H*,d).

(2-(4-nitrophenylamino)-N-(4-(2-oxo-2H-chromen-3-yl) thiazol-2-yl) acetamide)

IR spectrum (KBr, cm^{-1}) 640 (C-S), 1126(C-O), 1394 (NO_2), 1464 (Amide N-H), 1558 (C=N), 1651 (lacto C=O), 1712(keto C=O), 2075 (Ar C=C), 3294 (N-H)

^{1}H-NMR ($CDCl_3$, δ ppm) - a) 9.5 (NH, *1H*, S), b) 3.7 (CH, 1*H*, S), c) 4.0 (NH, 1*H*,t), d) 8.3 (Ar.CH,1*H*,s), e) 7.6 (Ar.CH,1*H*,d), f) 7.5 (Ar.CH,1*H*,t), g) 6.6 (Ar.CH,1*H*,d), h) 7.1 (Ar.CH,1*H*,t), i) 8.6 (Ar.CH,1*H*,d).

2-(3-nitrophenylamino)-N-(4-(2-oxo-2H-chromen-3-yl) thiazol-2-yl)acetamide)

IR spectrum (KBr, cm^{-1}) 600 (C-S), 1187(C-O), 1300 (NO_2), 1464 (Amide N-H), 1512 (C=N), 1535 (lacto C=O), 1666(keto C=O), 2391 (Ar C=C), 3294 (N-H)

^{1}H-NMR ($CDCl_3$, δ ppm) - a) 9.1 (NH, *1H*, S), b) 3.7 (CH, 1*H*, S), c) 4.0 (NH, 1*H*,t), d) 8.6 (Ar.CH,1*H*,s), e) 8 (Ar.CH,1*H*,d), f) 7.6 (Ar.CH,1*H*,t), g) 7.4 (Ar.CH,1*H*,d), h) 7.8 (Ar.CH,1*H*,t), i) 8.6 (Ar.CH,1*H*,d).

4-[(2-oxo-2-{[5-(2-oxo-2*H*-chromen-3-yl)-1,3-thiazol-2-yl]amino}ethyl)amino]benzoic acid

IR spectrum (KBr, cm^{-1}) 600 (C-S), 1180(C-O), 1464 (Amide N-H), 1280 (C-N), 1635 (lacto C=O), 1666(keto C=O), 3001 (Ar C=C), 3201 (N-H), 3230(C=O)

^{1}H-NMR ($CDCl_3$, δ ppm) - a) 10.4 (NH, *1H*, S), b) 3.7 (CH, 1*H*, S), c) 4.0 (NH, 1*H*,t), d) 7.4 (Ar.CH,1*H*,s), e) 7.8 (Ar.CH,1*H*,d), f) 7.4 (Ar.CH,1*H*,t), g) 6.6 (Ar.CH,1*H*,d), h) 7.6 (Ar.CH,1*H*,t), i) 7.5 (Ar.CH,1*H*,d), j) 12.3 (COOH, 1*H*,s).

***N*-[5-(2-oxo-2*H*-chromen-3-yl)-1,3-thiazol-2-yl]-2-(phenylamino)acetamide**

IR spectrum (KBr, cm^{-1}) 600 (C-S), 1126(C-O), 1303 (C-N), 1335 (lacto C=O), 1712(keto C=O), 2027 (Ar C=C), 3294 (N-H), 3009(C-H)

^{1}H-NMR ($CDCl_3$, δ ppm) - a) 9.5 (NH, *1H*, S), b) 3.4 (CH, 1*H*, S), c) 4.0 (NH, 1*H*,t), d) 8.3 (Ar.CH,1*H*,s), e) 7.8 (Ar.CH,1*H*,d), f) 7.5 (Ar.CH,1*H*,t), g) 6.4 (Ar.CH,1*H*,d), h) 7.6 (Ar.CH,1*H*,t), i) 8 (Ar.CH,1*H*,d), j) 7.5 (COOH, 1*H*,t).

2-[(3-nitrophenyl) amino]-*N*-[5-(2-oxo-2*H*-chromen-3-yl)-1,3-thiazol-2-yl]acetamide

IR spectrum (KBr, cm^{-1}) 632 (C-S), 1141(C-O), 1581 (C-N), 1435 (lacto C=O), 1563 (AmideN-H) 1743(keto C=O), 1643 (Ar C=C), 3363 (N-H), 2862(C-H), 3101(Ar CH)

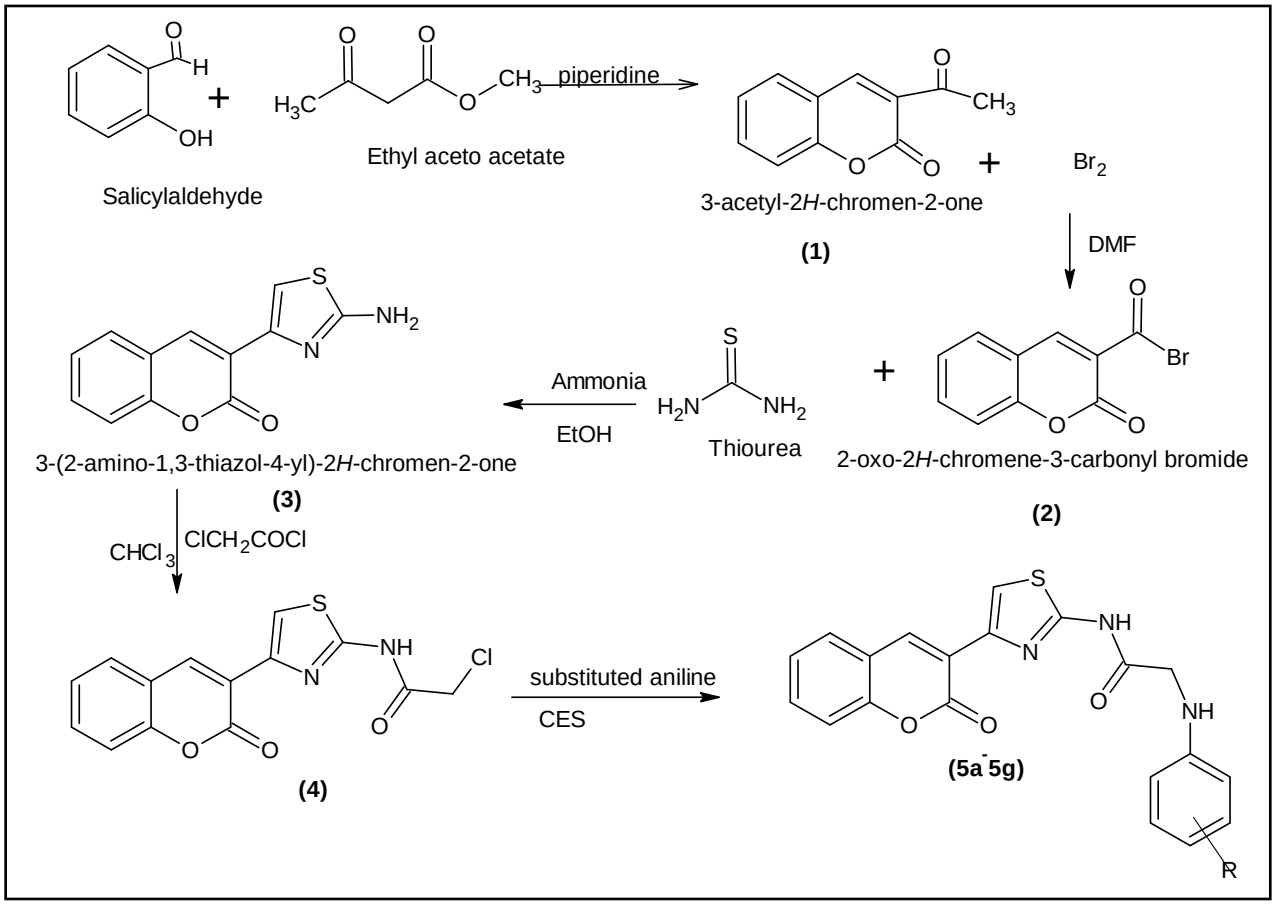
^{1}H-NMR ($CDCl_3$, δ ppm) - a) 12.7 (NH, *1H*, S), b) 4 (CH, 1*H*, S), c) 4.4 (NH, 1*H*,t), d) 8.3 (Ar.CH,1*H*,s), e) 7.8 (Ar.CH,1*H*,d), f) 7.4 (Ar.CH,1*H*,t), g) 6.5 (Ar.CH,1*H*,d), h) 7.6 (Ar.CH,1*H*,t), i) 8 (Ar.CH,1*H*,d), j) 3.4 (CH, 1*H*,s).

(2-(4-methoxyphenylamino)-N-(4-(2-oxo-2H-chromen-3-yl) thiazol-2-yl)acetamide)

IR spectrum (KBr, cm^{-1}) 594 (C-S), 1180(C-O), 1390 (C=N), 1657 (lacto C=O), 1535 (AmideN-H) 1666(keto C=O), 3201 (N-H), 3001(Ar CH).

^{1}H-NMR ($CDCl_3$, δ ppm) - a) 12.7 (NH, *1H*, S), b) (OCH_3 3.5), c) 4.4 (NH, 1*H*,t), d) 8.3 (Ar.CH,1*H*,s), e) 7.8 (Ar.CH,1*H*,d), f) 7.4 (Ar.CH,1*H*,t), g) 6.5 (Ar.CH,1*H*,d), h) 7.6 (Ar.CH,1*H*,t), i) 8 (Ar.CH,1*H*,d), j) 3.4 (CH, 1*H*,s).

RESULTS:



The synthesized compounds were subjected to antimicrobial screening by the Cup plate method for zone of inhibition and Minimum Inhibitory Concentration. The Antibacterial activity was tested against various gram-positive and Gram-negative bacteria and anti-fungal activity against various fungal strains compared with standard drugs.

Table No. 1: Zone of inhibition of target compounds (5a-5g) for bacteria and fungi

Sr. No.	Compound	Conc. (µg/ml)	Zone of inhibition (mm)			
			S. aureus	*B. subtilis*	*E. coli*	*P.aeruginosa*
1	5a	50	9±1.73	12±1.68TG	7±1.25	10±1.63
		100	10±1.45	14±1.37	9±1.62	12±1.65
		150	12±1.36	15±1.15	11±1.55	15±1.18
2	5b	50	10±1.32	14±1.18	11±1.18	14±1.34
		100	12±1.67	16±1.17	15±1.27	15±1.16
		150	18±1.06	22±1.19	18±1.51	20±1.45
3	5c	50	8±1.34	12±1.34	10±1.10	12±1.38
		100	12±1.65	14±1.36	12±1.73	13±1.34
		150	14±1.46	15±1.65	14±1.52	15±1.16
4	5d	50	6±1.43	8±1.35	8±1.18	11±1.45
		100	7±1.82	10±1.36	10±1.74	13±1.28
		150	9±1.67	12±1.67	12±1.50	15±1.48
5	5e	50	8±1.43	11±1.48	5±1.45	9±1.84
		100	9±1.32	13±1.17	6±1.34	10±1.67
		150	10±1.10	14±1.04	7±1.72	12±1.47
6	5f	50	7±1.16	11±1.36	6±1.78	9±1.67
		100	10±1.25	13±1.58	18±1.23	12±1.43
		150	12±1.06	14±1.14	10±1.18	13±1.17
7	5g	50	8±1.36	8±1.17	8±1.67	11±1.12
		100	10±1.25	9±1.34	10±1.56	12±1.45
		150	12±1.16	12±1.17	12±1.15	12±1.48
Standard	**Ciprofloxacin**	**150**	**26±1.36**	**29±1.54**	**24±1.35**	**28±1.33**

Table No. 2: Zone of inhibition of compounds target compounds (5a-5g) against fungus species

Sr. No	Compound	Conc. µg/ml	Zone of inhibition (mm)	
			A.niger	***C. albicans***
1	**5a**	50	9±1.18	10±1.36
		100	10±1.28	11±1.45
		150	12±1.15	15±1.18
2	**5b**	50	11±1.36	11±1.17
		100	13±1.39	12±1.23
		150	15±1.11	14±1.18
3	**5c**	50	14±1.48	10±1.23
		100	15±1.46	12±1.34
		150	17±1.12	14±1.15
4	**5d**	50	9±1.38	12±1.54
		100	10±1.38	14±1.53
		150	12±1.10	17±1.18
5	**5e**	50	10±1.30	10±1.36
		100	12±1.63	12±1.52
		150	14±1.72	14±1.17
6	**5f**	50	11±1.73	12±1.19
		100	12±1.45	13±1.53
		150	14±1.08	14±1.28
7	**5g**	50	10±1.11	9±1.23
		100	11±1.37	10±1.73
		150	13±1.15	12±1.16
Standard	**Fluconazole**	**150**	**25±1.35**	**27±1.33**

Table No. 3: Minimum Inhibitory Concentration (MIC)

Sr. No.	Compound code	*S. aureus*	*B. subtilis*	*E. coli*	*P. auerginosa*	*A. Niger*	*C. albicans*
1	5a	20	30	40	30	30	40
2	5b	20	20	30	20	30	20
3	5c	40	30	30	40	40	30
4	5d	20	30	30	30	30	40
5	5e	30	20	30	40	30	30
6	5f	40	50	40	40	40	50
7	5g	30	30	30	30	30	40
Standard	**Ciprofloxacin**	50	50	40	-	-	-
Standard	**Fluconazole**	-	-	-	40	50	50

The compound 5b has shown good antibacterial activity against *S. aureus* at MIC of 20µg/ml, *B. subtilis* at MIC of 20µg/ml.

The compound 5a has shown good antibacterial activity against *E. coli* at MIC 30µg/ml, *P. aeruginosa* at MIC of 20µg/ml.

The compound 5b has shown good antifungal activity against *A. niger* and *C. albicans* at MIC of 30 and 20µg/ml respectively.

DISCUSSION:

The antibacterial and antifungal properties of a series of new coumarin derivatives against stevdord medicines were investigated Compound 5b has proved to be effective against Ciprofloxacin at varied concentrations. The actions of the remaining derivatives were moderate.

Compound 5a and 5b have shown good antifungal activities against Fluconazole.

The present scheme is innovative and the derivatives obtained are novel. Few compounds have shown promising antibacterial and antifungal activities. But with suitable molecule modification of the scheme of the derivatives, we may expect best results.

CONCLUSION:

The present work describes the synthesis and biological evaluation of antibacterial and antifungal activities of new coumarin derivatives. The in vitro antimicrobial screening results of compounds indicate that the compound **5b, 5c, 5d,** and **5f** showed moderate activity.

Compounds **5e** and **5g** showed least activity against bacteria as well as fungus. Compound **5a**showed comparable antibacterial and antifungal activity to that of the standard. Thus, we conclude that the synthesized compounds have a new scaffold that can be used to as a lead in the development of novel antimicrobial agents.

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