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Synthesis of new coumarin derivatives containing aminobenzotriazole, triazole moieties and their antimicrobial activities

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ABSTRACT

Background: Coumarins are structural units of several natural products and feature widely in pharmacologically and biologically active compounds. Their derivatives are characterized by excellent chemical reactivity and different bioactivity. **Objective:** The present work aims to design and synthesise new coumarin derivatives bearing 2-aminobenzotriazole, 2-amino-5-mercapto-1,3,4-thiadiazole moieties. Moreover, this study also includes testing of target compounds in order to assay their antibacterial and antifungal activities. **Methods:** This includes the synthesis of coumarin-3-carboxylic acid (compound 2), 2-aminobenzotriazole (compound 3), 2-amino-5-mercapto-1,3,4-thiadiazole (compound 5) and followed by a challenge of coupling between steric heterocycles compounds (2 with 3) and (2 with 5). In this study, the chemical structures of these new coumarin derivatives were investigated and identified by their physicochemical properties, spectroscopic FTIR and CHNS &O elemental microanalysis techniques in France. **Results:** Coumarin-3-carboxylic acid, 2-aminobenzotriazole and 2-amino-5-mercapto-1,3,4-thiadiazole are the key intermediates required to prepare the target products. **Conclusion:** The development of bacterial resistance has led to the synthesis of newer, more potent, and complex coumarin derivatives. It was observed that when an amine group containing a heterocyclic compound was introduced to the carboxylic side, comparable antimicrobial activity against organisms was achieved from the levofloxacin nucleus.

INTRODUCTION

Coumarins are structural units of several natural products and feature widely in pharmacologically and biologically active compounds (Fylaktakidou, Hadjipavlou-Litina, Litinas, & Nicolaides, 2004; GÜRSOY & KARALİ, 2003). Their derivatives are characterized by excellent chemical reactivity and different bioactivity (Kadam, Bodke, Pushpavathi, Satyanarayan, & Nippu, 2023; Omar, Koparir, Sarac, Koparir, & Safin, 2023). As an important group of organic heterocycles, coumarin derivatives have been found to possess versatile pharmacological/biological activities, which can display anticancer (Nolan et al., 2007; Riveiro, Moglioni, et al., 2008; Riveiro, Vazquez, et al., 2008) anti-HIV (Spino, Dodier, & Sotheeswaran, 1998), antiviral, antimicrobial, anti-inflammatory and antioxidant activities. Their remarkable biological potential is the reason for synthesising many new products suitable for application in the modern therapy (Al-Azzawi & Hassan, 2014; Ghate, Manohar, Kulkarni, Shobha, & Kattimani, 2003). Besides, Benzotriazole acts as a precursor in many organic synthesis and has proven to be a fertile source of medicinal agents such as antimicrobial, anticonvulsant, anti-inflammatory, anti-tumour etc. It has been demonstrated that heterocyclic compounds containing nitrogen atoms are considered to be one of the most effective antimicrobial drugs used either as single agents or in combination for cancer therapy (Aguilera-Alcala, Morales-Reyes, Martin-Lopez, Moleon, & Sanchez-Zapata, 2020; Krasavin, Pershin, Larkin, & Kravchenko, 2005; Li et al., 2007; Scapin et al., 2003; Xu, Jian, Gao, & Zhu, 2003). Benzotriazole and its derivatives are versatile intermediates involved in producing antiseptic and anticoagulant agents, pesticide products and other interesting bioactive. Moreover, various benzotriazoles have been reported to inhibit the growth of some microorganisms, and some benzotriazole derivatives show anti-inflammatory properties (Bushuev et al., 2002). Several derivatives of benzotriazoles are reported as agonists of peroxisome proliferator-activated receptors. Synthesis and biological activity of 1Hbenzotriazole analogues as NT pase / helicase inhibitors and some related Flavivirade has been extensively investigated (Bretner et al., 2005). In addition, Touami et al. also reported that the conjugates of benzotriazole derivative photo nucleases and DNA minor groove binders exhibited enhanced cleavage efficiency and unique selectivity. A class of stable benzotriazole esters was also reported as mechanism-based in activators of SARS-3CLpro, which has been shown to be essential for the replication of the SARS virus. Benzotriazole moiety is a versatile lead molecule in pharmaceutical development, and owing to its versatile chemotherapeutical activities, significant research activity has been directed towards this class. After a survey of existing literature revealed that there were no reports describing the synthesis and activity of a heterocyclic system in which 2-amionbenzotriazole moiety has been linked with substituted coumarin nuclei at the C-2 position. Nitrogen-containing heterocycles, including 1,3,5-triazole derivatives, are important in the life science industry and many other industrial fields related to special and fine chemistry, representing a widely used lead structure with a multitude of interesting applications in the human field. Several derivatives showed antifungal, antimicrobial, herbicidal activity, and recently cytotoxic and antitumor3 activity have well elicited these derivatives for e.g., 2-aminobenzotriazole and 5-amino- 2-mercapto-1,3,4, thiadiazole. Looking at the importance of these compounds, the present

work is aimed at the design and synthesis of new coumarin derivatives bearing 2-aminobenzotriazole, 2-amino-5-mercapto-1,3,4-thiadiazole moieties. Moreover, the study includes testing of target compounds for their antibacterial and antifungal activities. Hence it is interesting and worthwhile to synthesize and explore the activity of these new coumarin derivatives (Al-Abdeen & Qasir, 2014; Wu et al., 2006).

METHODS

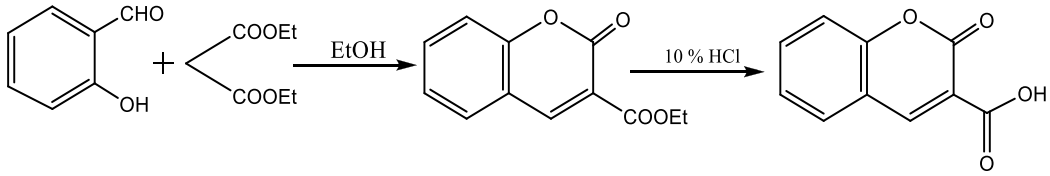
The parent compounds (salicylaldehyde, diethylmalonate, o-nitroaniline, o-phenylene diamine(OPD)) were purchased from Sigma and Fluka companies (Germany). All chemicals used in preparations were of Analar quality reagents, and the solvents were purified by distillation prior to use. All air- or moisture-sensitive materials operations were performed under a dry argon atmosphere using syringes, oven-dried glassware, and freshly dried solvents (Ethyl acetate, Cyclohexane, THF, toluene and CH_2Cl_2 were purified by passage through a solvent drying column and stored under argon over 3 Å molecular sieves). Air and moisture-sensitive liquids, reagents and solvents were transferred via syringe using standard techniques. All reagents were obtained from commercial sources and used without further purification unless otherwise stated. Unless otherwise noted, organic solutions were concentrated by rotary evaporation (house vacuum, ca. 40 Torr) at 30°C. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel 60 F254 (0.2 mm thickness) F5 pre-coated, 20 ×20 cm impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV, 366 nm, Whatmann, Merck, Germany)., and/or vanillin, followed by a brief (ca. 30 s) heating on a stream of hot air (ca. 300°C). The melting points were determined by electrothermal CIA 9300 melting point apparatus in open capillaries and were uncorrected. The ultraviolet spectra were obtained via Carrywinn U.V. Varian U.V. – visible spectrophotometer (Australia). Structures were drawn by Chemdraw Office 2008 software.IR spectra (KBr discs) were recorded on a Buck 500 FT/IR scientific spectrophotometer (France). The analysis was done in the microanalytical center (France). The purity of all compounds was established by a single spot on the Thin-layer chromatography (TLC) plastic sheets silica gel 60 F5 pre-coated, 20 ×20 cm, layer thickness 0.2 mm. The spots on the chromatograms were localized using U.V. light (366 nm) (Whatmann). (Merck, Germany). The CHNO&S analysis has been done by using Carlo Erba elemental analyzer (in the microanalytical center of France laboratories) for final products(6 and 7) was accomplished by Schimadzo atomic absorption instrument AA 670/GU-7 flame atomic absorption spectrophotometer(France laboratories).

Part I - synthesis of coumarin derivatives

Synthesis of 2-oxo-2H-chromene-3-carboxylic Acid Ethylester (compound 1). Typically, (13.88 mmol., 3.02 g.) of salicylaldehyde and (13.88 mmol) of diethylmalonate were dissolved in (50 ml) of ethanol, followed by adding 2.08 mL of diethylamine, and the mixture was refluxed for (24 hrs). After cooling the mixture, the solvent was evaporated and the residue was purified by column chromatography on silica gel (hexane-ethyl acetate (4:1) volume ratio) (Bretner et al., 2005).

Synthesis of 2-oxo-2H-chromene-3-carboxylic Acid(compound 2)
First, (9.79 mmol, 2.83 g) of compound 1 was dissolved in (10 ml) of ethanol, to

which (20 ml) of (0.5 mol/l) NaOH solution was added. The resulting mixture was refluxed and stirred for (10 min). After cooling, the addition of 10% HCl until pH=2, the solid was filtered and washed with water (Scheme 1), yielding analytically pure carboxycoumarine derivative; the compound purity was established by TLC, and its result showed that only a single spot was observed. The percentage yield, physical appearance, melting point, and R_f value of this compound are listed in Table (1).



(Scheme 1): Synthesis of Coumarin3-carboxylic acid.

Synthesis of 2-aminobenzotriazole (compound 3):

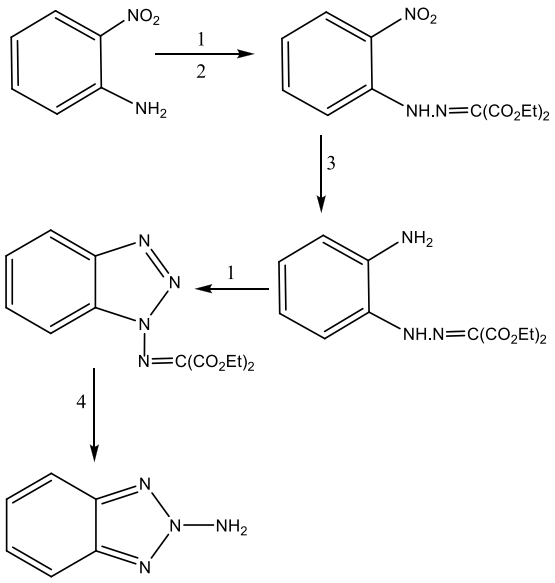
Two methods were used for its preparation. This includes a method (A), which implies four steps starting from o-nitroaniline (54%), and method (B), directly, by the amination of the previously prepared benzotriazole with hydroxylamine-O-sulphonic acid.

Method (A):

O-Nitroaniline (0.3 mol) was stirred with concentrated hydrochloric acid (90 ml.) until totally converted into hydrochloride. Water (200 ml.) was added, and to the well-stirred suspension at (0°C), sodium nitrite (0.35 mol) in water (50 ml.) was added. The resulting solution was filtered and added dropwise to a vigorously stirred emulsion of diethyl malonate (0.3 mol) in water (200 ml.) at (5°C). Sodium acetate (100 g.) was added in portions during the reaction to buffer the reaction medium. When the addition was complete, the mixture was stirred for an additional (1 hr.), and the solid was filtered off, washed with water, and recrystallised from ethanol (charcoal) to give diethyl mesoxalate O-nitrophenylhydrazone (II). The hydrazone (0.1 mol) was suspended in methanol (300 ml.) and hydrogenated in the presence of 10% Pd-C (1.5 g.). The reaction vessel was cooled intermittently in an ice-salt bath, and the theoretical amount of hydrogen was taken up in (3 hrs). The mixture was filtered, and the deep-red solution evaporated to one-third its volume. Sodium nitrite (0.11 mol) in water (25 ml.) was added to the methanolic solution, and this mixture was added dropwise to a stirred solution of concentrated hydrochloric acid (25 ml.) in water (100 ml.) at (5°C). The solid was filtered off, washed with water, and crystallized from ethanol (charcoal) to give diethyl (benzotriazol-l-y1)-iminomalonate (IV). Although the amine (III) was not normally isolated, evaporation of the methanolic solution following hydrogenation gave a viscous orange oil that was not crystalline after chromatography on basic alumina (ether as eluent), or after regeneration from it. The amine was converted into its picrate (from ethanol).

The finely powdered triazole (c) (0.05 mol) was shaken at room temperature with concentrated hydrochloric acid (80 ml.) until the solution was completely homogeneous (cu. 3 hrs). Water (100 ml.) was added, the solution was extracted with ether (3 x 50 ml. portions), and the ethereal solutions were discarded. The aqueous acid layer was

neutralised with solid sodium carbonate, and ether extraction gave a solid which crystallised from benzene petroleum (b.p.60-80°C) to give 2-aminobenzotriazole (Scheme 2).



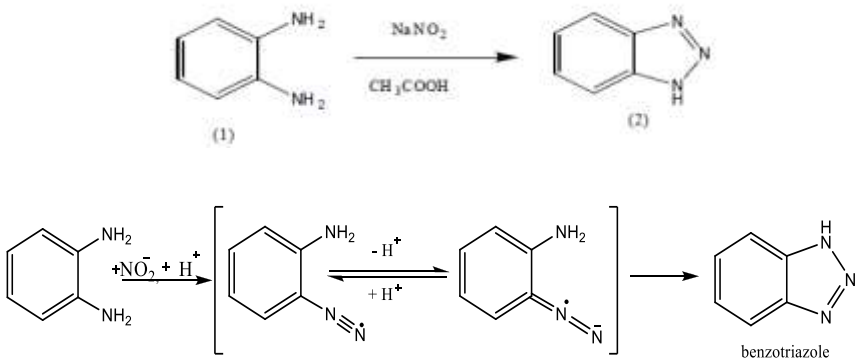
(Scheme 2): Reagents: 1, HONO; 2, $H_2C(CO_2Et)_2$; 3, 10% Pd-C, H_2; 4, HCl.

Method (B):

This method includes the synthesis of benzotriazole followed by the amination with hydroxylamine-o-sulphonic acid as the following:

Synthesis of benzotriazole (compound 4):

OPD (0.1 mol, 10.8 gm) was dissolved in a mixture of (0.2 mol; 12 gm, 11.5 ml) of glacial acetic acid and (30 ml) water content in a (250 ml) beaker. The clear solution was cooled to (15°C) and stirred magnetically, and then added a solution of (0.11 mol, 7.5 gm) of NaNO2 was in (15 ml) of water in one portion. The reaction mixture becomes warm within (2-3 min.) and reaches a temp of about (85°C) and begins to cool while the colour changes from deep red to pale brown continue stirring for (15 min.), by which time the temp will have dropped to (35-40°C) and then thoroughly chilled in an ice water bath for (30 min.). Then the solid compound was separated and washed with (30 ml) portion of ice-cold water, the compound purity was established by TLC, and its result showed that only a single spot was observed (Scheme 3). The percentage yield, physical appearance, melting point, and R_f value of this compound are listed in Table (1).

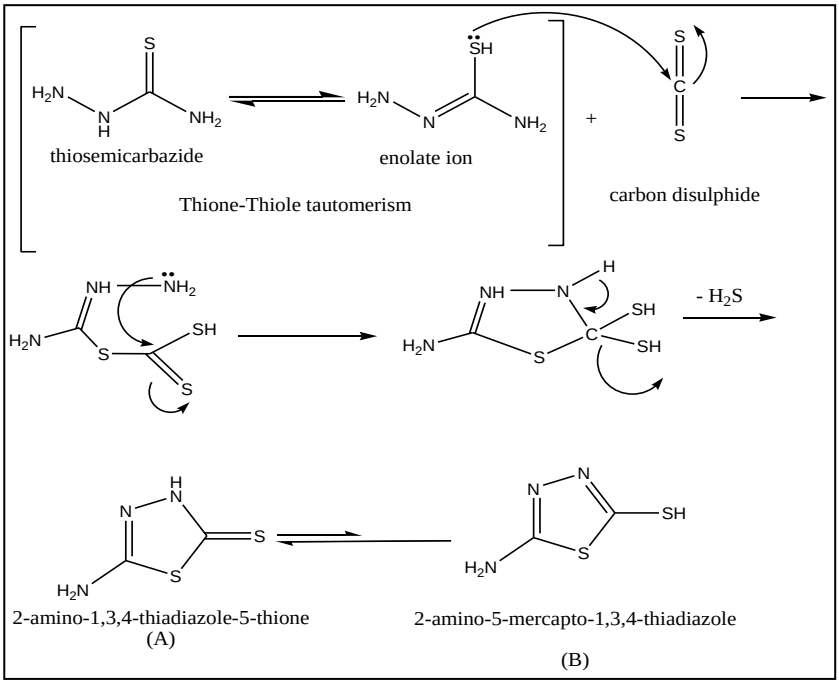


(Scheme 3): Mechanism of diazotization of one of the amine groups.

Hydroxylamine-o-sulphonic acid was prepared (76%) by the method of GÕSL and Meuwsen and dried in vacuo before use. Benzotriazole (0.1 mol) was dissolved in a solution of potassium hydroxide (0.5 mol) in water (100 ml.) at (60°C). Solid hydroxylamine-o-sulphonic acid (0.2 mol.) was added in portions during (1 hr.), and the temperature was maintained at (70-75°C). The mixture was then stirred for (1 hr.) at (70°C), cooled, and filtered. The alkaline solution was extracted with ether (3 x 100 ml.); the precipitated potassium sulphate was washed with ether, and the combined ether extracts and washings were dried and evaporated. The resulting solid (two components by (TLC) was chromatographed on silica gel (150 g.; 200-300 mesh). Petroleum (b.p. 40-60°C)-ether (2:1) eluted 2-aminobenzotriazole (11%), the compound purity was established by TLC, and its result showed that only a single spot was observed. The percentage yield, physical appearance, melting point, and R_f value of this compound are listed in Table(1).

Synthesis of 2-amino-5- mercapto-1,3,4-thiadiazole (compound 5):

To thiosemicarbazide (0.1mol, 10g) suspended in anhydrous ethanol (40 ml), anhydrous sodium carbonate (5.82 gm) was added, and carbon disulphide (0.12 mol, 10.1 gm). The reaction mixture was heated with stirring under reflux for (1 hr) and then heated in the steam bath for 4 hrs. The solvent was largely removed by a rotary evaporator, and the residue was dissolved in water (44 ml), and then acidified with concentrated hydrochloric acid (8.8 ml) to give the pure product (Figure 5) after recrystallization from ethanol/water. Physical appearance, melting point, and Rf values are listed in Table 1. compound 5 was synthesized through the reactions of thiosemicarbazide with carbon disulphide under basic conditions according to the following mechanism (Scheme 4).

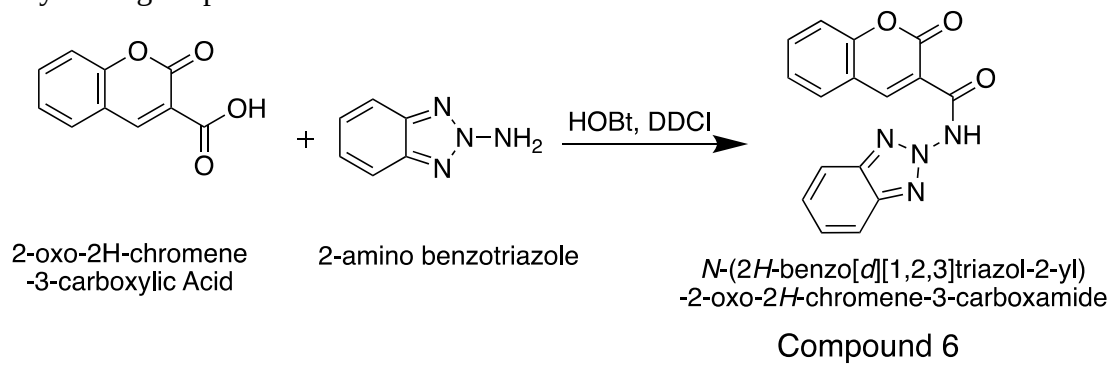


(Scheme 4): Mechanism of synthesis of 2-amino-5-mercapto-1,3,4-thiadiazole.

Synthesis of N-(2H-benzo[d][1,2,3]triazol-2-yl) -2-oxo-2H-chromene-3-carboxamide (compound 6):

Conventional Solution method (Al-Abdeen & Qasir, 2014) was used as a coupling method between the (coumarin-3-carboxylic acid) and 2-aminobenzotriazole for synthesizing the compound 6. Dicyclohexylcarbodiimide (DCC) was used as a coupling reagent in amide bond formation, while 1-hydroxybenzotriazole(HOBT) and N-hydroxysuccinamide (HOSu) were used to increase the yields of the product and to suppress racemization. To a stirred solution of coumarin-3-carboxylic acid (1mmole, 0.19 gm.) in (3 ml.) DMF, NMM (1 mmol, 0.11 ml) was added, followed by stirring for (10 min). Solution of 2-aminobenzotriazole (1mmole, 0.13 gm.) in (3 ml.) DMF was added to the reaction mixture. The mixture was then cooled to (-15 ºC), followed by DCC (1mmol, 0.23 gm.) addition with stirring, which was continued for (72 hrs.) at (0 ºC) and for (48 hrs.) at ambient temperature (20 ºC). Ethyl acetate (10 ml.) was added to the reaction mixture, which was then filtered to get rid of N, N-dicyclohexylurea (DCU). The filtrate was then evaporated to dryness under vacuum, and the residue was re-dissolved in ethyl acetate (10 ml.); the excess DCU, which was still adhesive on the amide residue, was precipitated out and filtered. The clear filtrate was washed twice with (5 ml.) of (0.1 N) HCL solution, once with (10 ml.) D.W. and with (10 ml.) saturated NaCl solution using the separatory funnel. The

ethyl acetate layer was then dried under a vacuum by a rotary evaporator; the remaining ethyl acetate was dried using anhydrous magnesium sulphate. The product was re-crystallized from (ethanol: n-pentane) mixture to get the pure product 5scheme 5); the compound purity was established by TLC, and its result showed that only a single spot was observed. The percentage yield, physical appearance, melting point, and R_f value of this compound are listed in Table (1).

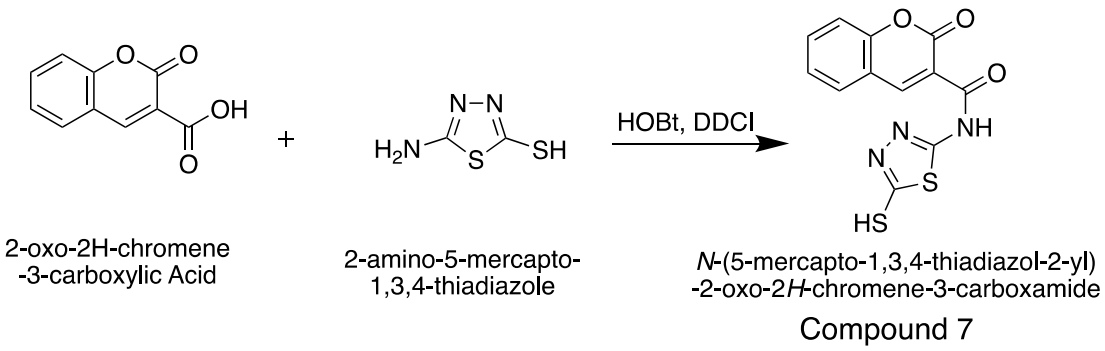


Compound 6

(Scheme 5): Synthesis of compound 6.

Synthesis of N-(5-mercapto-1,3,4-thiadiazol-2-yl)-2-oxo-2H-chromene-3-carboxamide (compound 8):

The conventional Solution method (Al-Abdeen & Qasir, 2014) was used as a coupling method between the coumarin-3-carboxylic acid and 2-amin-5-mercapto-1,3,4-thiadiazole for synthesizing the compound 8. Dicyclohexylcarbodiimide (DCC) was used as a coupling reagent in amide bond formation, while 1-hydroxybenzotriazole (HOBT) and N-hydroxysuccinamide (HOSu) were used to increase the yields of the product and to suppress racemization. To a stirred solution of coumarin-3-carboxylic acid (1mmole, 0.19 gm.) in (3 ml.) DMF, NMM (1 mmol, 0.11 ml) was added, followed by stirring for (10 min). Solution of 2-amino-5-mercapto-1,3,4-thiadiazole (1mmole, 0.13 gm.) in (3 ml.) DMF was added to the reaction mixture. The mixture was then cooled to (-15 ºC), followed by DCC (1mmol, 0.23 gm.) addition with stirring, which was continued for (72 hrs.) at (0 ºC) and for (48 hrs.) at ambient temperature (20 ºC). Ethyl acetate (10 ml.) was added to the reaction mixture, which was then filtered to get rid of N, N-dicyclohexylurea (DCU). The filtrate was then evaporated to dryness under vacuum, and the residue was re-dissolved in ethyl acetate (10 ml.); the excess DCU, which was still adhesive on the amide residue, was precipitated out and filtered. The clear filtrate was washed twice with (5 ml.) of (0.1 N) HCL solution, once with (10 ml.) D.W. and with (10 ml.) saturated NaCl solution using the separatory funnel. The ethyl acetate layer was then dried under vacuum by a rotary evaporator; the remaining ethyl acetate was dried using anhydrous magnesium sulphate. The product was re-crystallized from (ethanol: n-pentane) mixture to get the pure product, the compound purity was established by TLC, and its result showed that only a single spot was observed. The percentage yield, physical appearance, melting point, and R_f value of this compound are listed in Table (1).



(Scheme 6): Synthesis of compound 7.

Table (1): Physical appearance, yield percentage, melting points and R_f values of intermediate and final products.

Compound No.	Compound name	Yield percent (w/w)	Physical appearance	Observed meting point(°C)	R_f values	Solvent system
1	2-oxo-2H-chromene-3-carboxylic acid Ethylester	70.6	yellow crystals	….	0.78	Ethyl acetate/Cyclohexane 8/2
2	2-oxo-2H-chromene-3-carboxylic acid	86.9	Pale yellow crystals	180	0.64	Ethyl acetate /Cyclohexane 8/2
3	2-aminobenzotriazole	88	White needles	121-122	0.89	Ethyl acetate /Cyclohexane 8/2
4	2-amino-5-mercapto-1,3,4-thiadiazole	89	Yellow crystals	72-74	0.87	Ethyl acetate /Cyclohexane 8/2
6	N-(2H-benzo[d][1,2,3]triazol-2-yl) -2-oxo-2H-chromene-3-carboxamide	88	White cystals	109-110	0.80	Ethyl acetate /Cyclohexane 8/2
7	N-(5-mercapto-1,3,4-thiadiazol-2-yl)-2-oxo-2H-chromene-3-carboxamide	90	Pale yellow crystals	89-91	0.9	Ethyl acetate /Cyclohexane 8/2

Part II – antimicrobial activity

A preliminary antibacterial and antifungal activity has been carried out according to Well Diffusion Method:

The prepared compounds (6 and 7) have been studied for their antimicrobial activities in vitro against three tested bacteria (Staphylococcus aureus, Streptococcus aureus, as gram-positive bacteria, and Proteus spp. As gram-negative bacteria) and two fungi (Aspergillus spp., and Candida spp.) were clinically activated and

maintained on nutrient agar medium for testing antibacterial activity and sabouraud agar medium for antifungal activity. Levofloxacin was use used as a standard drug for antibacterial activity, and ketoconazole was used as a reference standard drug for antifungal activity.

Sensitivity Assay

All the synthesized tested compounds were prepared by making a stock solution with a final solution of (120 µg/ml), and by using the equation [C_1V_1 X C_2V_2], the other concentrations (2, 5, 20, 50 µg/ml) were performed using (1% DMSO) as diluents. Well, diffusion assay was carried out by using bacterial suspensions of about ($1.5x10^6$ CFU/ml) obtained from the McFarland turbidity standard (number 0.5). This was inoculated by swabbing the surface of Muller Hinton agar 5MHA) plates. Excess liquid was air-dried under a sterile hood, and the impregnated discs were applied at equidistant points on top of the agar medium. Five wells were made in each agar plate of tested bacteria, and (30 µl) of each concentration was added to it. The plates were incubated at 30 °C for 72 hours (fungi spp.) or 37 °C for 24 hours (bacteria), and the antimicrobial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the disc in mm. The assessment of antibacterial activity was based on the measurement of the diameter of the inhibition zone formed around the well and showed that the zone increased with the increasing concentration of the tested compounds (4 and 5). The pattern of a result of the antifungal activity of the tested compounds is different from their antibacterial activity, compounds (4 and 5) showed maximum activity against Aspergillus spp. And medium activity by compound 5, while compound 4 showed the least activity against the same fungi. For Candida spp., compounds 4 and 5 showed maximum activity compared to the standard reference compound levofloxacin.

RESULTS

The reaction was carried out under the conventional coupling method. Coumarin-3-carboxylic acid, 2-aminobenzotriazole and 2-amino-5-mercapto-1,3,4-thiadiazole are the key intermediates required to prepare the target products. The IR spectra of the synthesized target compounds showed a characteristic band of absorption by which they were identified. FTIR data help identify the final compounds and are advantageous to follow up the reactions depending on the appearance or disappearance of specific group frequencies.

Table (2): IR data of compound (6); main characteristic IR bands.

Band (cm^{-1})	Interpretation
3327.27	NH stretching.
3034.28	Aromatic CH stretching.
2928.91 – 2850.65	Asymmetric and symmetric aliphatic C-H stretching of CH_2 and CH groups respectively.
1625.99	C=N of heterocyclic ring, C=O of amide (amide I band).
1574.98-1536.52	Aromatic C=C stretching and N-H bending.
1448.80 – 1436.30	Aliphatic CH bending.
1087.97 1068.78 1045.80	Aromatic C-H in plane bending.
966.65	Skeletal vibration of aromatic ring.

89236 842.47	
640.93	Aromatic C-H out of plane bending.

Table (3): IR data of compound (7); main characteristic IR bands.

Band (cm^{-1})	Interpretation
3327.27	OH stretching.
3034.28	Aromatic CH stretching.
2928.91 – 2850.65	Asymmetric and symmetric aliphatic C-H stretching of CH_2 and CH groups respectively.
1625.99	C=N of heterocyclic ring, C=O of amide (amide I band).
1574.98-1536.52	Aromatic C=C stretching and N-H bending of amide band.
1087.97 1068.78 1045.80	Aromatic C-H in plane bending.
966.65 89236 842.47	Skeletal vibration of aromatic ring.
640.93	Aromatic C-H out of plane bending.

Elemental microanalysis

The data of the elemental microanalysis of the synthesized compounds (6 and 7) obtained serving as a basis for determining their empirical formulas, and the results of the Karl Fisher analysis are presented in Table (3). There is good agreement between the calculated and the found values.

Table (4): Elemental analysis of final target products; compounds (6 and 7).

Comp. No.	Comp.name	Calculated/Observed (%)				
		C	H	N	O	S
6	$C_{13}H_{14}N_4O_4$	62.74 63.99	3.29 3.99	18.29 19.93	15.67 16.78	… …
7	$C_{12}H_7N_3O_3S_2$	47.21 48.93	2.31 2.39	13.76 13.29	15.72 16.02	21.00 21.12

So, the IR spectra, elemental analysis data together with Elemental micro CHNS &O elemental microanalysis data supported the proposed structure for our final target compound, Table 3.

The overall activity profile of compounds (6 and 7) against microorganisms revealed

these derivatives are more than Levofloxacin in zone of inhibition values.

Table (5): Antibacterial and antifungal activities of final target products; compounds (6 and 7).

Comp. No.	Zone of inhibition in mm				
	Staphylococcus aureus	Streptococcus aureus	Proteus spp	Aspergillus spp.	Candida spp.
Compound 6 at (50 µg/ml)	15	12	7	19	22
Compound 7 at (50 µg/ml)	16	14	9	9	7
Levofloxacin (as a reference for antibacterial activity) at (50 µg/ml)	16	17	16	…	…
Ketoconazole (as a reference for antibacterial activity) at (50 µg/ml)	…	…	…	20	30

Regarding the structure-activity relationship, the antibacterial activity profile against all bacterium was altered by adding heterocyclic intermediates containing amino groups in coumarin molecules. This activity seems to be due to better interaction of molecules with target enzymes or penetration into these bacteria, as shown in Table 4.

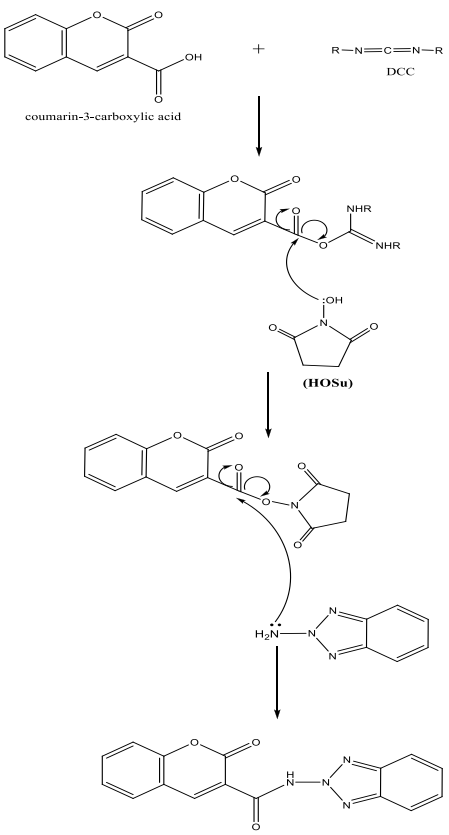
DISCUSSION

In the present work, the method used was the direct coupling between the synthesized coumarin-3-carboxylic and (2-aminobenzotriazole, 2-amino-5-mercapto-1,3,4-thiadiazole moieties) by using DCC/HOBt or DCC/ HOSu as a coupling reagent. The use of DCC is particularly convenient since it can be simply added to the solution containing the amine and carboxyl components to be coupled. It reacts rapidly with the free carboxylic acid present to form an "active ester" intermediate (isourea), which may react with the amine component directly or may proceed through an intermediate (symmetrical anhydride). These Coupling reactions mediated by DCC can be also modified by the addition of other reagents, such as HOBt and HOSu. This addition will lead to the formation of HOBt and HOSu active esters, which in turn may(99):

i. Accelerate coupling reactions.
ii. Suppress by-product formation.
iii. Suppress racemization.

So, the overall method of DCC/ HOBt is simple and efficient, with no racemization and leads to good yield at room temperature (107).

The mechanism by which coupling proceeds occurs as follows:



(Scheme 7): Mechanism of activation of the carboxylic acid of coumarin for coupling with amine-containing heterocycles.

The product ester (resulting from the reaction between the carboxyl group and DCC) is activated because substitution with any nucleophile expels this very stable urea as leaving group. Coupling of the 2-aminobenzotriazole with coumarin-3-carboxylic acid was done using DCC/ HOSu. The HOSu accelerates the coupling reaction and suppresses the by-product formation and racemization like HOBt.

CONCLUSION

The development of bacterial resistance has led to the synthesis of newer, more potent, and complex coumarin derivatives. As detailed above, two coumarin derivatives have been designed, synthesized, characterized and evaluated for their biological activities in vitro in order to discover potent agents against Gram-positive, Gram-negative bacteria and fungi. It was observed that when an amine group containing a heterocyclic compound was introduced to the carboxylic side, comparable antimicrobial activity against

organisms was achieved from the levofloxacin nucleus.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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