GÜFBED/*GUSTIJ* (2019) 9 (4): 645-654
DOI: 10.17714/gumusfenbil.528165 Araştırma Makalesi / *Research Article*

Synthesis and Some Reactions of Pyrazole-3-carboxylic acid Having Trifluoromethyl Unit

Triflorometil Grubuna Sahip Pirazol-3-karboksilik Asit Sentezi ve Bazı Tepkimeleri

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• Geliş tarihi / *Received*: 17.02.2019 • Düzeltilerek geliş tarihi / *Received in revised form*: 12.06.2019 • Kabul tarihi / *Accepted*: 19.06.2019

Abstract

4-benzoyl-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxylic acid **2** was obtained from 4-benzoyl-5-phenyl-2,3-furandione **1** and (4-(trifluoromethyl)phenyl) benzaldehyde-phenyl hydrazone **1a**. Obtained new pyrazole carboxylic acid derivative was converted into acid chloride form. Reaction of new derivatives between acid chloride and some of nucleophiles were progressed. The esters (**4a-c**) were obtained using Schotten Baumann method with the corresponding alcohols and acid chloride (**3**). Pyrazolopiridazinone derivatives (**8**, **9**, **10**) were obtained as a result of cyclization reactions between pyrazole carboxylic acid and various hydrazines. Additionally, reactions between an acide chloride **3** and certain amines and ureas were carried out to yield amides (**5a**, **5b-f**) and carbo-urea (**6a-c**) derivatives. The nitrile compound (**7**) was also synthesized from the amine compound via the removal of water with the use of $SOCl_2$ and DMF. All synthesized molecules were subjected to antibacterial activity to see their potential as unknown reagent. 6-nitro-3,4-diphenyl-2-(4-(trifluoromethyl)phenyl)-2,6-dihydro-7H-pyrazolo[3,4-d]pyridazin-7-one (**10)** showed antibacterial activity against only the Gram-positive bacteria, Bacillus cereus and Micrococcus luteus, with a MIC value of 32 and 128 µg/mL, respectively. This finding revealed that functional group is important for antibacterial activity. Compound 10 having a nitro group inhibited growing of bacteria more than positive control, streptomycin in two different types of bacteria. This might be a good clue for further investigation about antibacterial agents.

Keywords: Cyclic oxalyl compounds, Cyclization, Fluoride, Pyrazole

Öz

*4-Benzoil-5-fenil-2,3-furandion **1** ve (4-(triflorometil)fenil) benzaldehit-fenil hidrazonun **1a** reaksiyonundan 4-benzoil-5-fenil-1-(4-(triflorometil)fenil)-1H-karboksilik asit **2** elde edildi. Elde edilen yeni pirazol karboksilik asit (**2**) türevi, asit klorürüne dönüştürüldü ve çeşitli nükleofillerle asit klorür arasında yeni türevlerin reaksiyonları gerçekleştirildi. Esterler (**4a-c**), ilgili alkoller ve asit klorür (**3**) ile Schotten Baumann metodu kullanılarak elde edildi. Pirazolopiridazinon türevleri (**8**, **9**, **10**), pirazol karboksilik asit ve çeşitli hidrazinler arasındaki halkalanma reaksiyonları sonucunda elde edildi. Ek olarak, amidleri (**5a**, **5b-5f**) ve karbon-üre türevlerini (**6a-c**) elde etmek için asit klorür ile çeşitli aminler ve ürelerle reaksiyonlar gerçekleştirildi. Nitril bileşiği (**7**), $SOCl_2$ ve DMF kullanılarak amin bileşiğinden suyun uzaklaştırılması ile elde edildi. Sentezlenen tüm moleküller, potansiyel aktivitelerinden dolayı antimikrobiyal aktiviteye tabi tutuldu. Moleküllerden 6-nitro-3,4-difenil-2-(4-(triflorometil)fenil)-2,6-dihidro-7H-pirazolo[3,4-d]piridazin-7-on bileşiğinin (**10**) sadece Gram-pozitif bakterilere karşı antibakteriyal aktivite gösterdi. Bacillus cereus ve Micrococcus luteus, A MIC değerleri 32 ve 128 µg/mL aktif olduğu bulundu. Yapısında nitro grubu bulunduran ve aktivite gösteren 10 nolu molekülden dolayı fonksiyonel grubun aktivite üzerinde önemli bir etkisi olduğu söylenebilir. Ayrıca 10 nolu molekülün pozitif kontrol olan streptomycin antibiyotiğinden daha etkili bir zon oluşturduğu da gözlendi. Bu durumda etkisini gösterdiği bakteriler için ileri çalışmalar konusunda iyi bir antibiyotik adayı olabilir.*

Anahtar kelimeler: Siklik okzalil bileşikleri, Halkalanma, Florür, Pirazol,

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1. Introduction

Organic compounds containing fluorine atoms are used in daily life and in many different scientific fields. The majority of pharmaceutical and chemical products that possess fluorine atoms display biological activities and are often using in agriculture (Hagmann, 1994; Banks et al., 1994). Carbon−fluorine bonds are important pharmacophore units in medicine and are also found in naturally occurring organic molecules (Harper et al., 2003). There are two general approaches to the synthesis of fluorine-containing organic compounds; the first of which is the attachment of atomic fluorine directly to the ring, and the second is the attachment of a fluorine-containing functional group to the ring (Baasner et al., 2000; Chambers, 2004). Pyrazole skeletons possessing different functional groups have been utilized in various scientific fields such as biology (Çetin and Bildirici, 2018), medicine (Adnan et al., 2008; Badawey and Ashmawey, 1998), and agriculture (Thomson, 1997; Londershausen, 1996). A plethora of research worldwide has highlighted the importance of pyrazole derivatives. In particular, the synthesis of pyrazole possessing tetrasubstituted compounds from a reaction between 4-benzoyl-5-phenyl-2,3-furandione **1** and various hydrazines or hydrazones has been recently reported (Şener et al., 2007; Akçamur et al., 1997). The compound, 4-benzoyl-5-phenyl-2,3-furandione **1,** yields pyrazole-3-carboxylic acid and pyridazinone derivatives when reacted with hydrazine derivatives (Mengeş and Bildirici, 2017). In the present study, several novel compound derivatives possessing fluorine-containing functional groups attached to the pyrazole ring were synthesized.

2. Experimental

2.1. Instrumentations

All reagents were used commercially available unless otherwise stated, and all solvents were used as received. Melting points were determined using an electrothermal Gallenkamp apparatus and remained uncorrected. Microanalyses were performed using a Carlo Erba elemental analyzer, model EA1108. The IR spectra were obtained in potassium bromide pellets using a Thermo Scientific Nicolet iS10 FTIR spectrometer. Varian XL-400 (400 MHz) and Varian XL-200 (50 MHz) spectrometers were used respectively, using TMS as an internal standard. All experiments were followed by TLC using Merck silica gel 60 F_{254} plates and a CAMAG TLC lamp (254/366 nm). Properties of molecules such as closed formula, molecule mass(g/mol), melting point (°C), product quantity (mg) and yield (%) are given Table 1.

2.2. 4-benzoyl-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxylic acid (2)

In the 1/1 molar ratio, dibenzoylmethane and oxalylchloride were reacted in the absence of solvent, under the drying head ($CaCl_2$). Two days later, the furandione (**1**) was obtained by washing with diethyl ether (Kollenz et al., 1976). (4-(trifluoromethyl)phenyl) benzaldehyde-phenyl hydrazone (**1a)** was obtained as followed: (4-(trifluoromethyl)phenyl) hydrazine (1.1 mmol) and benzaldehyde (1 mmol) were reacted in EtOH (10 mL). After 1 hour, precipitated product, (**1a)**, was filtered off and dried at room temperature using without any further purification method (Akçamur et al., 1997). Without any solvent, by adding 4-benzoyl-5-phenyl-2,3-furandione (**1)** (1 mmol) and (4-(trifluoromethyl)phenyl) benzaldehyde-phenyl hydrazone (**1a)** (1 mmol) into a beaker, the mixture was heated to 110°C for about 40 min,. The solid mixture was left cooling till it reaches room temperature and treated with mixture toluene- n-hexan, the obtained material was filtered and purified by crystallization from carbon tetra chloride (CCl_4) **2**; (Şener et al., 2007).; IR(cm^{-1}): 3059 (br, OH, COOH), 2763-2621 (Ar-H), 1689 (C=O, COOH), 1671 (C=O, benzoyl),1617, 1595, 1580, 1499, 1447, 1434(phenyl and pyrazole rings C-C,C-N), 1256(asm. C-O-C), 853, 829, 796, 771, 724(out-off-plane C-H bend), 688, 671 (out-off-plane rings C-C bend)(Badawey and Ashmawey, 1998).; ^{1}H-NMR (DMSO): δ =13.32 (s, 1H, OH), 7.81-7.21 (m, 14H, Ar-H); ^{13}C-NMR (DMSO): δ =191.3, 162.9, 143.8, 142.4, 138.2, 134.2, 130.4, 130.2, 129.8, 129.6, 129.3, 128.2, 127.0, 124.0; ^{19}F NMR (56.5MHz, DMSO); -61.50;Anal. Calcd. for $C_{24}H_{16}F_3N_2O_3$: C, 65.90; H, 3.69; N, 6.40. Found: C, 65.84; H, 3.68; N, 6.39

2.3. 4-benzoyl-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carbonyl chloride (3)

Compound 2a (1mmol) was soluted in excess of $SOCl_2$ (2mL). The reaction flask was stirred at room temperature for 1 day. This compound was used for further reaction without any purification due to its unstable form. Forming of the molecule was determined by FT-IR. IR (cm^{-1}): 3059(Ar-H), 1756 (C=O, acyl), 1663 (C=O, benzoyl).

2.4. Methyl-4-benzoyl-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxylate (4a)

General Procedure: The acid chloride (**3**) was dissolved in methanol and 2 drops of pyridine were added and refluxed for 3h. The crude product precipitated by addition of 12% HCl to the reaction mixture which was brought to room temperature was filtered and purified by crystallization from the same alcohol (**4a)**; IR(cm^{-1}): 3081 (Ar-H), 1734 (C=O, ester), 1669 (C=O); ^{1}H-NMR (DMSO): δ =7.83-7.20 (m, 14H, Ar-H), 3.61(s, 3H, OCH_3); ^{13}C-NMR (DMSO): δ =191.0, 161.9, 144.2, 142.5, 142.3,138.2, 134.3, 130.4, 130.3, 129.7, 129.4, 129.3, 127.9, 127.1, 125.8, 124.0, 123.1, 52.7. ^{19}F NMR (56.5 MHz, DMSO): -61.51. *Anal*. Calcd. For $C_{25}H_{18}F_3N_2O_2$: C, 69.96; H, 4.17; N.6.09. Found: C, 70.24; H, 4.15; N, 6.06.

2.5. Propyl-4-benzoyl-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxylate (4b)

IR (cm^{-1}): 3062-2970 (Ar-H), 1741 (C=O, ester), 1669 (C=O); ^{1}H-NMR (DMSO): δ =7.83-7.19 (m, 14H, Ar-H), 3.96 (t, J=6.2 Hz, 2H,OCH_2), 1.32 (hex, J=6.2 Hz, 2H,-CH_2-) 0.67 (t, J=6.2 Hz, 3H-CH_3); ^{13}C-NMR (DMSO): δ =191.0, 161.3, 144.2, 142.6, 142.4, 138.1, 134.4, 130.4, 130.3, 129.8, 129.4, 129.3, 127.9, 127.2, 127.1, 123.8, 67.1, 21.8, 10.8. *Anal*. Calcd. For $C_{27}H_{22}F_3N_2O_3$: C, 67.63 H, 4.62; N.5.89. Found: C, 67.42; H, 4.60; N, 5.91

2.6. Butyl 4-benzoyl-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxylate (4c)

IR(cm^{-1}): 3062 (Ar-H), 1728 (C=O, ester), 1666 (C=O); ^{1}H-NMR (DMSO): δ =7.83-7.19 (m, 14H, Ar-H), 4.01 (t, J=6.3 Hz 2H,OCH_2), 1.26 (pentet, J=6.3 Hz, 2H, -CH_2-), 1.07 (hextet, J=7.3 Hz 2H,-CH_2-), 0.72 (t, J=7.3 Hz 3H, -CH_3); ^{13}C-NMR (DMSO): δ =191.0, 161.3, 144.1, 142.6, 142.3, 138.1, 134.4, 130.4, 130.3, 129.8, 129.4, 129.3, 128.0, 127.2, 127.1, 127.0, 123.8, 65.3, 30.4, 19.1, 14.1. *Anal*. Calcd. For $C_{28}H_{24}F_3N_2O_3$: C, 68.32 H, 4.71; N.5.68. Found: C, 68.18; H, 4.68; N, 5.70

2.7. 4-benzoyl-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (5a)

General Procedure: Acid clorid (**3**) (1mmol) was dissolved in xylene. Aqueous ammonia (2 mmol) was added to reaction flask. The mixture was stirred in ice-water for 24h. Starting material was controlled with TLC method. After the starting material was disappeared, the mixture was filtered and purified by crystallization from methanol (**5a)**; IR(cm^{-1}): 3472-3285 (NH_2), 3161 (Ar-H), 1684 (C=O, benzoyl), 1671 (C=O, amide); ^{1}H-NMR (DMSO-d_6): δ =7.91 (s, 2H, -NH_2) 7.82-7.18 (m, 14H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ =191.4, 162.7, 147.0, 143.8, 142.5,138.5, 133.9, 130.3, 130.2, 130.0, 129.4, 129.3, 129.2, 128.3, 127.0, 126.9, 125.8, 122.8; *Anal*. Calcd. For $C_{24}H_{16}F_3N_3O_3$: C, 66.21; H, 3.70; N, 9.65. Found: C, 66.05; H, 3.71; N, 9.61.

2.8. 4-benzoyl-N-butyl-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (5b)

General Procedure: Acid chloride (**3**) (1mmol) was dissolved in xylene. Butyl amine (2mmol) was added to reaction flask. The mixture was refluxed for 4h. Then it was evaporated, organic layer was treated with dry ether and filtered. Then purified by crystallization from ethyl alcohol (**5b)**; IR(cm^{-1}): 3526 (NH), 2961 (Ar-H), 2932 (R-H), 1662 (C=O, amide), 1599 (C=O, benzoyl); ^{1}H-NMR (DMSO): δ =8.53 (1H, -NH), 7.82-7.17 (m, 14H, Ar-H), 3.12-3.07 (m, 2H-CH_2), 2.73-2.71 (m, 2H, CH_2-), 1.34-1.31 (m, 2H-CH_2-), 0.86-0.80 (m, 3H, CH_3); ^{13}C-NMR (DMSO): δ =191.3, 160.7,147.1, 143.8, 142.5, 138.5, 133.9, 130.8, 130.2, 129.6, 129.4, 129.1, 128.9, 128.3, 127.0, 126.8, 125.8, 122.5, 31.8, 29.8, 20.2, 14.5. *Anal*. Calcd. For $C_{28}H_{24}F_3N_3O_2$: C, 68.42; H, 4.92; N, 8.55. Found: C, 68.27; H, 4.94; N, 8.51.

2.9. 4-benzoyl-N-hexyl-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (5c)

IR(cm^{-1}): 3525 (NH), 3058 (Ar-H), 2955 (R-H), 1656 (C=O, amide), 1616 (C=O, benzoyl); ^{1}H-NMR (DMSO): δ =8.51(br, 1H, -NH), 7.82 -7.17 (m, 14H, Ar-H), 3.09-3.07 (m, 2H-CH_2), 2.71-2.47 (m, 2H, CH_2-), 1.54-1.38 (m, 2H, CH_2-), 1.23-1.19 (m, 4H, CH_2-), 0.84-0.79 (m, 3H, CH_3); ^{13}C-NMR (DMSO): δ =191.3, 160.7,147.1, 143.8, 142.5, 138.4, 133.9, 130.3, 130.1, 129.6, 129.3, 129.1, 128.3, 127.0, 122.6, 31.5, 29.7, 27.6, 26.5, 22.7, 14.6; ^{19}F NMR (56.5MHz, DMSO); -61.43*Anal*. Calcd. For $C_{30}H_{28}F_3N_3O_2$: C, 69.35; H, 5.43; N, 8.09. Found: C, 69.17; H, 5.40; N, 8.06.

2.10. 4-benzoyl-N-octyl-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (5d)

IR(cm^{-1}): 3378 (-NH), 3063 (Ar-H), 2957 (R-H), 1654 (C=O, amide), 1616 (C=O, benzoyl); ^{1}H-NMR (DMSO): δ = 8.51-8.49 (m, 1H, -NH), 7.85-7.17 (m, 14H, Ar-H), 3.09-3.06 (m, 2H-CH_2), 2.48-2.47 (m, 2H, CH_2-), 1.39-1.19 (m, 10H, CH_2-), 0.87-0.81 (m, 3H-CH_3); ^{13}C-NMR (DMSO): δ =191.3, 160.7, 147.1, 143.8, 142.5, 138.5, 133.8, 130.3, 130.2, 129.6, 129.3, 129.1, 128.3, 127.0, 122.6, 31.9, 29.7, 29.3, 27.7, 27.0, 26.5, 22.8, 14.6; *Anal*. Calcd. For $C_{32}H_{32}F_3N_3O_2$: C, 70.19; H, 5.89; N, 7.67. Found: C, 70.03; H, 5.86; N, 7.64.

2.11. 4-benzoyl-N,N-diethyl-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (5e)

IR(cm^{-1}): 3531 (NH), 3060 (Ar-H), 2972 (R-H), 1655 (C=O, amide), 1640 (C=O, benzoyl); ^{1}H-NMR , (DMSO): δ =7.80-7.19 (m, 14H, Ar-H), 3.56-3.25 (m, 2H, CH_2), 3.23-3.20 (m, 2H-CH_2), 1.19-1.11 (m, 3H, CH_3), 0.83-0.77 (m, 3H, CH_3; ^{13}C-NMR (DMSO): δ =190.5, 162.3, 149.3, 144.7, 142.5, 138.6, 133.5, 130.8, 130.1, 129.6, 129.2, 128.9, 128.5, 127.8, 127.0, 125.8, 122.8, 114.8, 43.3, 41.9, 15.1, 12.6; *Anal*. Calcd. For $C_{28}H_{24}F_3N_3O_2$: C, 68.42; H, 4.92; N, 8.55. Found: C, 68.25; H, 4.90; N, 8.52.

2.12. 4-benzoyl-N-(tert-butyl)-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (5f)

IR(cm^{-1}): 3531-3294 (NH), 3131 (Ar-H), 2969-2932 (R-H), 1709 (C=O, amin), 1669 (C=O, benzoyl); ^{1}H-NMR (DMSO): δ =10.16 (s, 1H, -NH), 7.94-7.18 (m, 14H, Ar-H), 1.24 (s, 9H, CH_3); ^{13}C-NMR (DMSO): δ =190.8, 162.2, 151.4, 144.9, 144.4, 142.2, 137.8, 134.4, 130.4, 130.4, 129.9, 129.6, 129.4, 127.9, 127.0, 126.8, 125.8, 123.1, 50.8, 29.1. *Anal. Calcd.* for $C_{28}H_{24}F_3N_3O_2$: C, 68.42; H, 4.92; N, 8.55. Found: C, 68.29; H,4.81; N, 8.56.

2.13. 4-benzoyl-N-(methylcarbamoyl)-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (6a)

General Procedure: Acid chloride (**3**) (1mmol) was dissolved in xylene. Methyl-urea (1mmol) was added to reaction flask. The mixture was refluxed for 5h. Then it was evaporated, organic layer was treated with dry ether and filtered. The compound was crystallized from ethyl alcohol; IR(cm^{-1}): 3516-3342 (NH), 3067 (Ar-H), 1702 (C=O, urea), 1672 (C=O, benzoyl), 1631 (C=O); ^{1}H-NMR (DMSO): δ =10.30 (bs, H, NH), 8.50 (bs, 1H, NH), 7.99-7.17 (m, 14H, Ar-H), 2.49-2.34 (m, 3H, CH_3); ^{13}C-NMR (DMSO): δ =190.8, 161.7, 153.4, 146.8, 144.7, 144.4, 143.7, 142.2, 138.4, 137.8, 134.4, 133.9, 130.3, 129.5, 128.3, 127.5, 126.9, 125.8, 123.2, 122.6, 26.5. ^{19}F NMR (56.5MHz, DMSO): -61.42. *Anal.Calcd.* for $C_{26}H_{19}F_3N_4O_3$: C, 63.41; H, 3.89; N, 11.38. Found: C, 63.20; H, 3.91; N, 11.33.

2.14. 4-benzoyl-N-(ethylcarbamoyl)-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (6b)

IR(cm^{-1}): 3312 (NH), 3061 (Ar-H), 1701 (C=O, urea), 1665 (C=O, benzoyl), 1618 (C=O); ^{1}H-NMR (DMSO): δ =10.25 (br, 1H, -NH), 8.05-8.03 (m, NH-Et), 7.84-7.19 (m, 14H, Ar-H), 3.13-3.09 (m, 2H -CH_2), 1.00-0.95 (m, 3H, CH_3); ^{13}C-NMR (DMSO): δ =190.8, 161.8, 152.7, 144.7, 144.3, 142.2, 137.8, 134.4, 130.5, 130.4, 129.8, 129.4, 129.4, 127.9, 127.0, 127.0, 126.8, 123.2, 34.7, 15.5. *Anal. Calcd.* for $C_{27}H_{21}F_3N_4O_3$: C, 64.03; H, 4.18; N, 11.06. Found: C, 63.88; H, 4.20; N, 11.02.

2.15. 4-benzoyl-N-(benzylcarbamoyl)-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (6c)

IR(cm^{-1}): 3530-3306 (NH), 3062 (Ar-H), 1699 (C=O, urea), 1662 (C=O benzoyl), 1630 (C=O); ^{1}H-NMR (DMSO): δ =10.42 (s, 1H, NH), 8.51-8.47 (m, 1H, NH-CH_2), 7.84-7.20 (m, 14H, Ar-H), 4.33-4.31 (m, 2H, CH_2); ^{13}C-NMR (DMSO): δ =190.7, 161.9, 153.0, 144.8, 144.4, 142.2, 139.8, 137.8, 134.4, 130.4, 129.9, 129.6, 129.4, 129.3, 129.0, 127.9, 127.6, 127.0, 127.0, 126.8, 123.2, 39.8. *Anal. Calcd.* for $C_{31}H_{21}F_3N_4O_3$: C, 67.15; H, 3.82; N. 10,10. Found: C, 66.95; H, 3.84; N, 10.06.

2.16. 4-benzoyl-5-phenyl-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carbonyl cyanide (7)

The compound acid amide (**5**) (1mmol) was dissolved in a mixture of DMF (0.7mL) and $SOCl_2$ (0.15mL). The mixture was stirred in ice-water for 24h. Starting material was controlled with TLC method. After the starting material was disappeared, the reaction was stirred by pouring ice into the flask. The formed precipitates were filtrated and crystallized from methanol (**7**); IR(cm^{-1}): 2243 (CN), 1648 (C=O, benzoyl); ^{1}H-

NMR (DMSO): δ =7.84-7.16 (m, 14H, Ar-H); ^{13}C-NMR (DMSO): δ =188.8, 146.6, 141.8, 136.9, 134.1, 131.1, 130.6, 130.4, 130.1, 129.1, 128.9, 127.7, 127.2, 126.7, 125.2, 122.9, 113.4. *Anal*. Calcd. for $C_{24}H_{14}F_3N_3O$: C, 69.06; H, 3.38; N. 10.07. Found: C, 68.85; H, 3.39; N, 10.03.

2.17. 3,4,6-triphenyl-2-(4-(trifluoromethyl)phenyl)-2,6-dihydro-7H-pyrazolo[3,4-d]pyridazin-7-one (8)

General Procedure: The compound (**2**) (1 mmol) was dissolved in xylene at room temperature. Phenyl hyrazine (1mmol) was added to reaction flask. The mixture was refluxed for 5 h. Starting material was controlled with TLC method. After the starting material disappeared, the reaction was left for cooling. The white precipitates were filtrated and recrystallized from xylene (**8**); identical IR spectrum with literature compound, mp 271°C (Bildirici et al., 2009).; IR(cm^{-1}): 3210 (Ar-H), 1692 (C=O); ^{1}H-NMR (DMSO): δ =7.81-6.98 (m, 19H, Ar-H); ^{13}CNMR (DMSO-d6): δ=155.4, 144.5, 143.3, 142.6, 142.6, 142.3, 141.7, 134.2, 131.3, 130.2, 129.9, 129.7, 129.2, 128.7, 128.5, 128.2, 127.9, 127.0, 126.6, 125.7, 122.9, 117.2. *Anal*. Calcd. for $C_{30}H_{19}F_3N_4O$: C, 70.86; H, 3.77; N. 11.02. Found: C, 70.77; H, 3.74; N, 11.08.

2.18. 3,4-diphenyl-2-(4-(trifluoromethyl)phenyl)-2,6-dihydro-7H-pyrazolo[3,4-d]pyridazin-7-one (9)

IR(cm^{-1}): 3338-3056 (NH↔OH), 1682 (C=O); ^{1}H-NMR (DMSO): δ =12.66 (s, 1H, NH↔OH), 7.79-6.93 (m,14H, Ar-H); ^{13}C NMR (DMSO): δ=156.7, 144.3, 143.1, 142.7, 141.2, 137.9, 134.8, 131.3, 130.3, 130.1, 129.9, 129.6, 128.8, 128.6, 127.8, 126.9, 126.6, 117.5. *Anal*. Calcd. for $C_{24}H_{15}F_3N_4O$: C, 66.67; H, 3.50; N. 12.96. Found: C, 66.49; H, 3.42; N, 12.88.

2.19. 6-(4-nitrophenyl)-3,4-diphenyl-2-(4-(trifluoromethyl)phenyl)-2,6-dihydro-7H-pyrazolo[3,4-d]pyridazin-7-one (10)

IR(cm^{-1}): 3051 (Ar-H), 1713 (C=O); ^{1}H-NMR (DMSO): δ =7.81-7.18 (m, 18H, Ar-H); ^{13}C NMR (DMSO-d6):δ=162.9, 151.3, 144.4, 143.9, 142.8, 140.9, 139.5, 138.5, 138.3, 134.2, 130.3, 130.2, 129.8, 129.5, 129.3, 129.2, 128.9, 128.2, 127.3, 127.0, 126.6, 126.4, 115.9, 113.0. *Anal*. Calcd. for $C_{30}H_{18}F_3N_5O_3$: C, 65.10; H, 3.28; N. 12.65. Found: C, 65.01; H, 3.26; N, 12.57.

Table 1. Some physical properties of synthesized compounds

Compound	Closed Formula	Molecule Mass (g/mol)	Melting point (°C)	Product Quantity (mg)	Yield (%)
2	$C_{24}H_{16}F_3N_2O_3$	437	218-220	201	46
4a	$C_{25}H_{18}F_3N_2O_2$	435	161-163	305	70
4b	$C_{27}H_{22}F_3N_2O_3$	479	104-105	383	80
4c	$C_{28}H_{24}F_3N_2O_3$	493	103-105	394	80
5a	$C_{24}H_{16}F_3N_3O_3$	451	254-256	226	50
5b	$C_{28}H_{24}F_3N_3O_2$	491	186-188	392	80
5c	$C_{30}H_{28}F_3N_3O_2$	519	147-149	311	60
5d	$C_{32}H_{32}F_3N_3O_2$	547	199-200	301	55
5e	$C_{28}H_{24}F_3N_3O_2$	491	100-102	329	67
5f	$C_{28}H_{24}F_3N_3O_2$	491	217-218	275	56
6a	$C_{26}H_{19}F_3N_4O_3$	492	227-228	271	55
6b	$C_{27}H_{21}F_3N_4O_3$	506	190-191	304	60
6c	$C_{31}H_{21}F_3N_4O_3$	554	238-239	111	20
7	$C_{24}H_{14}F_3N_3O$	417	124-125	238	57
8	$C_{30}H_{19}F_3N_4O$	508	195-197	305	60
9	$C_{24}H_{15}F_3N_4O$	432	294-296	181	42
10	$C_{30}H_{18}F_3N_5O_3$	553	262-263	221	40

2.20. Determination of Antimicrobial Activity

A series of pyrazole-3-carboxylic acid **2** derivatives were synthesized and examined for antimicrobial activity according to the disc diffusion method proposed by Bauer et al., in 1966 (Bauer et al., 1966). To this end, 5-mm-diameter discs of Whatman® No. 1 filter paper were impregnated with 10 µL of each 25 µg pyrazole carboxylic acid derivative dissolved in dimethyl sulfoxide (DMSO), which was subsequently allowed to dry. Streptomycin (10 µg) and nalidixic acid (10 µg), and *Saccharomyces cerevisiae* were used as the control antibiotics and microorganism, respectively.

The turbidity of the microbial cultures produced in Mueller−Hinton broth and potato dextrose broth was adjusted to McFarland standard turbidity tube No. 10. *Bacillus cereus* ATCC 7064, *Escherichia coli* ATCC 4230, *Micrococcus luteus* ATCC 9345, and *Staphylococcus aureus* ATCC 6538 were plated on Mueller−Hinton agar using a glass spreader. *Saccharomyces cerevisiae* ATCC 9763 was plated on potato dextrose agar in the same manner.

The impregnated discs containing the pyrazole carboxylic acid derivatives were placed at regular intervals on the surface of the microbial-seeded agar, and the Petri dishes were allowed to incubate at 37°C for 24 hours. Following incubation, inhibition zones around the discs were evaluated as positive for antimicrobial activity. Minimum inhibitory concentration (MIC) values were also determined for the pyrazole carboxylic acid derivatives that were shown to be positive for antimicrobial activity. Solutions were prepared at concentrations of 8-250 µg/mL in Mueller-Hinton broth and potato dextrose broth, which were inoculated with 100 µl *B. cereus* or *M. luteus* solutions adjusted to McFarland standard turbidity tube No. 10, and allowed to incubate for 24 hours at 37°C. Following incubation, the tube series was examined, and the lowest concentration at which urea was not found was determined to be the MIC (Wayne et al., 2003; Jorgensen and Ferraro., 2009).

3. Results and Discussion

3.1. Chemistry

Solvent-free heating of 2,3-furandione **1** and hydrazone derivative **1a** gave pyrazole-3-carboxylic acid **2,** which was synthesized *via* different routes to obtain an approximate yield of 46% (**Figure 1**).

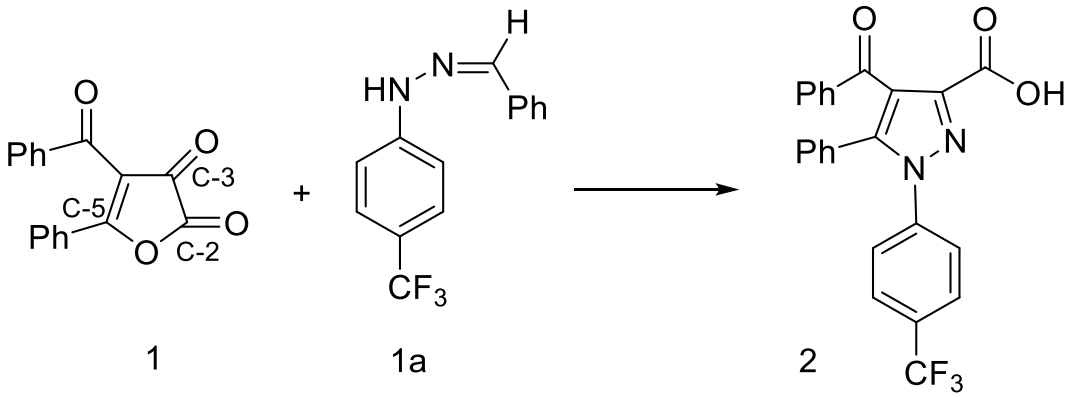

Figure 1. Synthesis of compound **2**.

From the chemical behaviour of 4-benzoyl-5-phenylfuran-2,3-dione **1**, it can be interpreted that the reaction resulted in a moderate yield. Different reactivity of 2,3-furandiones led to a dozen different compounds. The C-2, C-3, and C-5 atoms of 2,3-furandiones are susceptible to attack by nucleophiles (Yıldırım et al., 1995; Kappe et al., 1995). With this information, 2,3-furandione was reacted with hydrazine derivative **1a**. In **Figure 1**, a similar reaction pathway to that discussed for 4-benzoyl-5-phenyl-2,3-furandione (Şener et al., 2002) is plotted from furandione **1** to pyrazole carboxylic acid **2**.

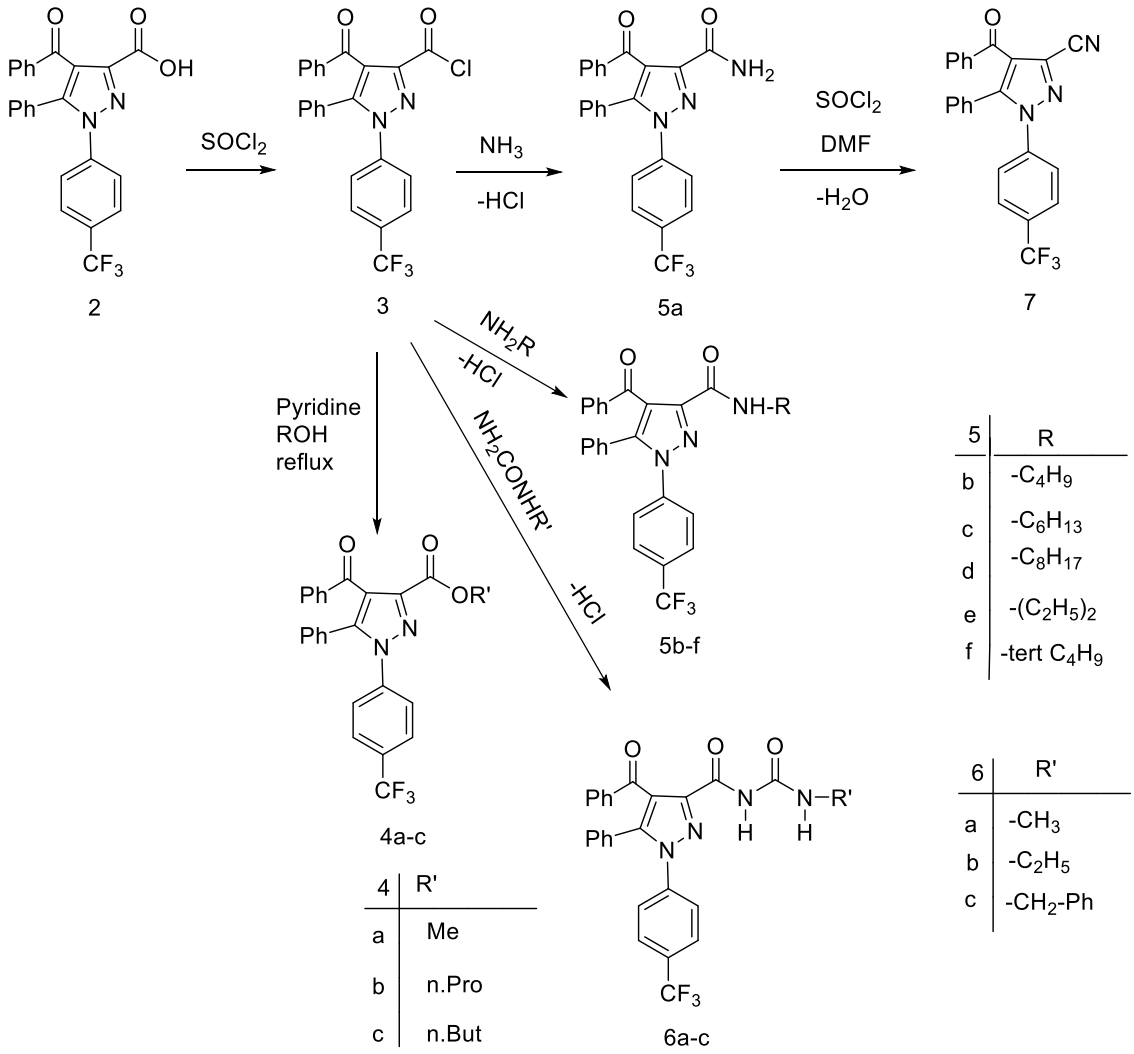

Figure 2. Reactions of pyrazole carboxylic acid with nucleophiles.

Compound **2** was determined from analytical and spectral data (**see Experimental**). Moreover, the acid **2** could be turned into the corresponding acid chloride **3**, which is known (Mennozzi et al., 1987; Scherowsky and Franke, 1974), ester **4a−c,** amide **5a−f,** and nitrile **7** derivatives by the common chemical methods (**Figure 2**). Acid chloride **3** was reacted with primary and secondary amines, resulting in **5a−f**. Furthermore, acid chloride **3** was reacted with urea and its derivatives to obtain **6a−c.**

The carboxylic acid functional group on C-3 and the benzoyl group on C-4 of pyrazole can be reacted with hydrazines to give rise to the pyrazolopyridazin(on)e system (Akbas and Berber, 2005). Pyrazole carboxylic acid **2** was therefore cyclized with hydrazine hydrate to obtain an approximate 42% yield of pyrazolopyridazinone **9 (Figure 3)**, of which both itself and its tautomeric form are known (Shawali, 1977).

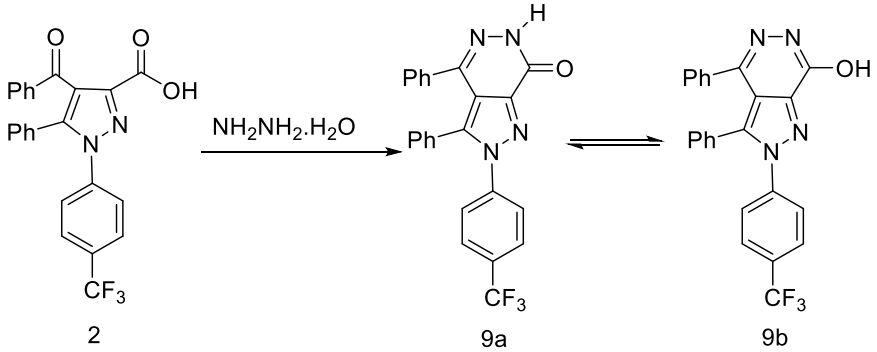

Figure 3. Reaction between pyrazole carboxylic acid and hydrazine hydrate.

In addition, the acid compound was reacted with various hydrazines to produce pyrazolopyridazinones **8** and **10** (**Figure 4**). Structural determination of the synthesized pyrazole derivatives acquired from **2** were validated by analytical and FT-IR ^{1}H- and ^{13}C-NMR spectroscopic data (**see Experimental**).

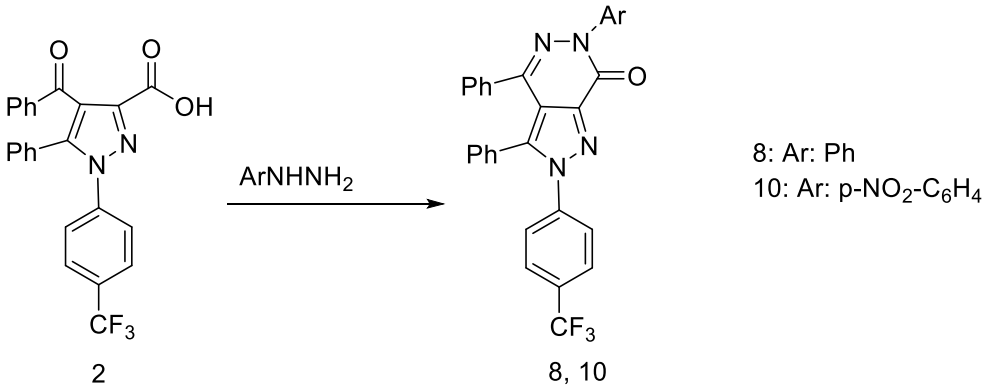

Figure 4. Reaction of pyrazole carboxylic acid with substituted hydrazines.

We have analyzed spectral data of all synthesized molecules. It is worth to say that; When the spectra of IR, ^{1}H-NMR and ^{13}C-NMR of the synthesized pyrazole carboxclic acid, ester, amide and urea derivatives are examined, the carbonyl groups show generally and respectively vibration at 3059 cm^{-1}, 1734 cm^{-1}, 1671 cm^{-1} and 1702 cm^{-1} (Şener et al., 2004). Chemical shift observed at 13.32 ppm for compound 2 belongs to OH group. While OCH_2 group of ester (**4a-c**) derivatives resonate between 3.61 and 4.01 ppm, NH protons of amide group (5a-f) resonated between 7.91 and 10.16 ppm. Furthermore, NH protons of urea (**6a-c**) appear to be resonance at 10.30 ppm, 10.25 ppm, 10.42 ppm. Also carbon of the carbonyl group which attached to the phenyl group appears to be at 191.0 ppm and the carbonyl group attached to the acid group appears to be at 161.9 ppm. The carbonyl carbons of the ester, amide and urea derivatives generally and respectively appear to be at 161.0, 162.0 and 153.0 ppm (Genç et al., 2019). Spectral data are in good agreement with literature.

3.2. Antimicrobial Activity

In recent years, it has been shown that pyrazole derivatives display antimicrobial effects (Abunada et al., 2008; Sarma et al., 2010). Results of the disk diffusion and MIC experiments show that only compound **10** possessed antimicrobial activities (Table 2), in which there is an identical nitro group attached to the phenyl ring. Furthermore, compound **10** showed antibacterial activity against only the Gram-positive bacteria, *B. cereus* and *M. luteus*, with a MIC value of 32 and 128 µg/mL, respectively. This finding revealed that functional group is important for antibacterial activity.

Table 2. Antimicrobial activity of the title compounds

Zone of inhibition, mm						MIC, µg/mL				
Compd.	*B. cereus*	*E. coli*	*M. luteus*	*S. aureus*	S. cerevisiae	*B. cereus*	*E. coli*	*M. luteus*	*S. aureus*	S. cerevisiae
2	S	S	S	S	S	-	-	-	-	-
4a	S	S	S	S	S	-	-	-	-	-
4b	S	S	S	S	S	-	-	-	-	-
4c	S	S	S	S	S	-	-	-	-	-
5a	S	S	S	S	S	-	-	-	-	-
5b	S	S	S	S	S	-	-	-	-	-
5c	S	S	S	S	S	-	-	-	-	-
5d	S	S	S	S	S	-	-	-	-	-
5e	S	S	S	S	S	-	-	-	-	-
5f	S	S	S	S	S	-	-	-	-	-
6a	S	S	S	S	S	-	-	-	-	-
6b	S	S	S	S	S	-	-	-	-	-
6c	S	S	S	S	S	-	-	-	-	-

Table 2. continued

Zone of inhibition, mm						MIC, µg/mL				
Compd.	*B. cereus*	*E. coli*	*M. luteus*	*S. aureus*	S. cerevisiae	*B. cereus*	*E. coli*	*M. luteus*	*S. aureus*	S. cerevisiae
7	S	S	S	S	S	-	-	-	-	-
8	S	S	S	S	S	-	-	-	-	-
9	S	S	S	S	S	-	-	-	-	-
10	9	S	9	S	S	32	-	128	-	-
Streptomycin	24	20	12	16	-[a]	15	-	-	-	-
Nalidixic acid	-	-	-	-	14	-	-	-	-	-
S: strength, a:not active by 256 µg/mL										

4. Conclusions

In the present study, a pyrazole carboxylic acid compound possessing a trifluoromethyl group was synthesized, and its acid chloride derivative was subjected to nucleophilic substitution reactions resulting in amide, ester, and urea derivatives possessing a trifluoromethyl group. These molecules may be very important due to the fluoride atom, which has unique properties in protein binding. Moreover, all the synthesized molecules were tested to elucidate a good candidate for an antibacterial. The successful antibacterial molecule had a nitro-phenyl unit and A MIC value of 32 and 128 µg/mL against the Gram-positive bacteria, *B. cereus* and *M. luteus*, respectively. This result indicated that nitro group on pyrazolopyrazinone has an effect on bacteria. We have seen that nitro group is a versatile and unique functional group in medicinal chemistry (Nepali et al., 2019). With this information, this type of molecules might be considered as antibacterial candidate for further studies.

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