Simple and Efficient Methods for the Synthesis of New Biologically Active Heterocycles

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Abstract

An efficient and simple synthesis of fused pyridazine derivatives has been developed. These fused pyridazines were used as key starting material for the preparation of new heterocyclic compounds. All the structures of the newly synthesized compounds were confirmed on the basis of FT-IR, ^{1}H and ^{13}CNMR, mass spectral techniques. Six of the new synthesized compounds (6_f , 8_{c-f} and 8_i) were selected and tested against a panel human tumor cell line HCT-116 (colon cancer) using Imatinib as standard drug. The cytotoxicity data of the tested compounds virus Imatinib in means of IC_{50} values were (6_f, 8_{c-f} and 8i) recorded as in (Figure I). The obtained data showed that the compounds 1-(3-(2-methoxyphenyl)-7-methoxyphenylisoxazolo[4,5-d]pyridazin-4-yl)hydrazine (8_i) and 1-(3-(2-methoxyphenyl)-1-phenyl-7-methoxyphenyl-1H-pyrazolo[4,3-d]pyridazin-4-yl)hydrazine (8_f) have high reactivity towards cell line HCT-116 (colon cancer) more than the standard drug Imatinib.

Keywords: Heterocyclic compounds, Fused pyridazines, Tetrazolo, Sulfonamide, Colon cancer.

1. Introduction

Nitrogen heterocycles are of special interest because they constitute an important class of natural and non-natural products, many of which exhibit useful biological activities.

Pyridazine is an important component of various biological active synthetic compounds which can leads to the development of new and safe medicinal agents **[Mohammed Asif et al, (2013)].**

Pyridazines are an important class of heterocycles, that are known for a wide range of biological activities **[Frank H et al, (1990) , Li CS et al, (2003) , Giblin GMP et al, (2007) , Dorsch D et al, (1997) , Nomoto Y et al, (1996) , Barbaro R et al, (2001)]** and have been the subject of extensive research **[Kandile N.G. et al, (2009)].**

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Pyridazines and fused pyridazines are an important class of heterocycles of considerable interest, which have been the subject of extensive research, particularly in the pharmaceutical and agrochemical areas, so their synthesis and applications have been comprehensively reviewed **[Haider et al, (2004), Woo et al, (2002), Cignarella et al, (2002), Kolar et al, (1999), Màtyus et al, (2004) and Gelain (2005)].**

Almost unlimited combinations of fused heterocyclic structures can be designed , resulting in novel polycyclic frameworks with the most diverse physical , chemical and biological properties .The fusion of several rings lead to geometrically well-defined rigid polycyclic structures and , thus , holds the promise of a high functional specialization resulting from the ability to orient substituents in three dimensional space **[Kaushik N.K. et al, (2013)].**

The therapeutic potential of isoxazoline derivatives is further evident from their antibacterial antifungal, analgesic, antiviral, anti-inflammatory, anti convulsant, COX-2 enzyme inhibitory activities, anticancer, anti HIV, GABA antagonist activity and anti-HIV activities **[Shreenivas M.T. et al, (2011)].**

Sulfonamides (sulfa drugs) were the first drugs largely employed and systematically used as preventive and chemotherapeutic agents against various diseases .

A serious of benzenesulfonamides bearing biologically active pyrazole, pyrimidine and pyridine moieties were prepared and evaluated for their anticancer activity **[Ghorab M.M. et al, (2014)].**

Tetrazoles represent a class of five-membered heterocyclic compounds with polynitrogen electron-rich planar structural features. This special structure makes tetrazole derivatives useful drugs, explosives, and other functional materials with a wide range of applications in many fields of medicine, agriculture, material science **[Cheng-Xi Wei et al, (2015)]**.

In continuation to our research interest in the synthesis of biologically active heterocycles **[Kandile N.G. et al, (2003), (2009), (2010), (2012), (2013), (2015), Zaky H.T. et al, (2004), (2014), Zaky H.T., (2002), (2006), (2007), Mohamed M.I. et al, (2003), (2004), (2013), Abdel-Sattar S. Hamad Elgazwy et al, (2003), (2006)]**, and in particular pyridazine derivatives **[Kandile N.G. et al, (2003), (2009), (2010), (2015), Zaky H.T. et al, (2014), Mohamed M.I. et al, (2003), (2004)].** In view of this facts and as a part of our recent research programme aiming a new rout of new fused pyridazines in order to obtain compounds possessing better biological activities.

2. Materials and Methods

Chemistry

All melting points are uncorrected and were determined on a Gallenkamp instrument. Infrared spectra of the new compounds were measured on a Perkin-Elmer spectrophotometer model 1430 using potassium bromide pellets and frequencies are reported in cm^{-1}. The ^{1}HNMR and ^{13}C-NMR were measured on a Varian genini-300 MHz spectrophotometer and chemical shifts (δ) are in ppm. The mass spectra (*m/z*) values were measured on mass spectrophotometer HP model GC MS-QPL000EX (Shimadzu) at 70 e-V.

Synthesis of 3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-ones (3_{a-c}), 3-(2-methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-ones (3_{d-f}) and 3-(2-methoxyphenyl)-7-arylisoxazolo-1H-pyrazolo[4,5-d]pyridazin-4(5H)-ones (3_{g-i})

Pyridazinone derivatives (3_{a-i}) were prepared via condensation reaction of 4-(2-methoxybenzoyl)-6-arylpyridazin-3(2H)-ones (2_{a-c}) (0.01mol) with appropriate nucleophile (hydrazine hydrate, phenyl hydrazine, hydroxylamine hydrochloride) (0.01mol) in presence of 10 drops of piperidine and was refluxed in ethanol for 35h. The reaction mixture was concenterated and left to cool. The separated solid was filtered off and recrystallized from ethyl alcohol to give the corresponding 3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-ones (3_{a-c}), 3-(2-methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-ones (3_{d-f}) and 3-(2-methoxyphenyl)-7-arylisoxazolo-1H-pyrazolo[4,5-d]pyridazin-4(5H)-ones (3_{g-i}) respectively. All the reactions followed by T.L.C.

3-(2-Methoxyphenyl)-7-phenyl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-one (3_a)

Pale yellow crystals, 90% yield, m.p. 192-194^{0}C, IR (KBr pellet): 2 bands at 3429-3251 for (NH) and 1650 for (C=O) cm^{-1}, MS: m/z 318 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 13.10 (s, 2H, 2NH), 7.71-6.87 (m, 9H, 2Ar-H), and 3.81-3.78 (s, 3H, OCH_3) ppm.

3-(2-Methoxyphenyl)-7-tolyl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-one (3_b)

Yellow crystals, 92% yield, m.p. 200-202^{0}C, IR (KBr pellet): 3434-3256 for (NH) and 1651 for (C=O) cm^{-1}, MS: m/z 332 (M^+).

3-(2-Methoxyphenyl)-7-methoxyphenyl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-one (3_c)

Yellowish crystals, 91% yield, m.p. 220-222^{0}C, IR (KBr pellet): 3395-3253 for (NH) and 1651 for (C=O) cm^{-1}, MS: m/z 350 (M^{+2}).

3-(2-Methoxyphenyl)-1,7-diphenyl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-one (3_d)

Dark yellow crystals, 93% yield, m.p. 184-186^{0}C, IR (KBr pellet): 3440 for (NH) and 1650 for (C=O) cm^{-1}, MS: m/z 394 (M^+).

3-(2-Methoxyphenyl)-1-phenyl-7-tolyl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-one (3_e)

Orange crystals, 90% yield, m.p. 197-198^{0}C, IR (KBr pellet): 3302 for (NH) and 1654 for (C=O) cm^{-1}, MS: m/z 408 (M^+) , ^{1}HNMR (DMSO, 300 MHz): δ 13.04 (s, 1H, NH), 7.60-6.88 (m, 13H, 3Ar-H), 3.80-3.73 (s, 3H, OCH_3) and 3.29 (s, 3H, CH_3) ppm, ^{13}CNMR (DMSO, 300 MHz): 160.62, 157.12, 143.78, 142.07, 138.58, 132.20, 130.33, 129.46, 128.10, 127.28, 125.34, 120.35, 110.90, 55.34, 40.36, 40.08, 39.81, 39.53, 39.25, 38.97, 38.69.

3-(2-Methoxyphenyl)-1-phenyl-7-methoxyphenyl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-one (3_f)

Dark orange crystals, 91% yield, m.p. 212-214^{0}C, IR (KBr pellet): 3297 for (NH) and 1651 for (C=O) cm^{-1}, MS: m/z 426 (M^{+2}).

3-(2-Methoxyphenyl)-7-phenylisoxazolo[4,5-d]pyridazin-4(5H)-one (3_g)

Yellow crystals, 94% yield, m.p. 190-192^{0}C, IR (KBr pellet): 3299 for (NH) and 1651 for (C=O) cm^{-1}, MS: m/z 321 (M^{+2}), ^{1}HNMR (DMSO, 300 MHz): δ 13.14 (s, 1H, NH), 7.71-6.88 (m, 9H, 2Ar-H), and 3.81-3.78 (s, 3H, OCH_3) ppm.

3-(2-Methoxyphenyl)-7-tolylisoxazolo[4,5-d]pyridazin-4(5H)-one (3_h)

Dark yellow crystals, 92% yield, m.p. 168-170^{0}C, IR (KBr pellet): 3300 for (NH) and 1651 for (C=O) cm^{-1}, MS: m/z 335 (M^{+2}).

3-(2-Methoxyphenyl)-7-methoxyphenylisoxazolo[4,5-d]pyridazin-4(5H)-one (3_i)

Pale yellow crystals, 91% yield, m.p. 216-218^{0}C, IR (KBr pellet): 3297 for (NH) and 1651 for (C=O) cm^{-1}, MS: m/z 349 (M^+).

Synthesis of 4-Chloro-3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazines ($4_{a–c}$), 4-Chloro-3-(2-methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,3-d]pyridazines (4_{d-f}) and 4-Chloro-3-(2-methoxyphenyl)-7-arylisoxazolo-1H-pyrazolo[4,5-d]pyridazines (4_{g-i})

A mixture of (3_{a-i}) and $POCl_3$ (3ml) was refluxed for 1h at 60^{0}C, after cooling the reaction mixture was added to the crushed ice. The solids which separated were filtered off and crystallized from ethanol to give (4_{a-i}) respectively. All the reactions followed by T.L.C.

4-Chloro-3-(2-methoxyphenyl)-7-phenyl-1H-pyrazolo[4,3-d]pyridazine (4_a)

Pale yellow crystals, 95% yield, m.p. 154-155^{0}C, IR (KBr pellet): 3055 for (NH) and 763 for (C-Cl) cm^{-1}, MS: m/z 337 (M^+).

4-Chloro-3-(2-methoxyphenyl)-7-tolyl-1H-pyrazolo[4,3-d]pyridazine (4_b)

Grey crystals, 97% yield, m.p. 128-130^{0}C, IR (KBr pellet): 3037 for (NH) and 761 for (C-Cl) cm^{-1}, MS: m/z 351 (M^+).

4-Chloro-3-(2-methoxyphenyl)-7-methoxyphenyl-1H-pyrazolo[4,3-d]pyridazine (4_c)

Dark yellow crystals, 96% yield, m.p. 114-116^{0}C, IR (KBr pellet): 3063 for (NH) and 754 for (C-Cl) cm^{-1}, ^{1}HNMR (DMSO, 300 MHz): δ 5.4 (s, 1H, NH), 7.99-6.88 (m, 9H, 2Ar-H), and 3.81-3.77 (s, 3H, OCH_3) ppm. ^{13}CNMR (DMSO, 300 MHz): 158.19, 157.01, 156.17, 140.46, 134.84, 130.27, 129.17, 129.03, 128.70, 128.56, 126.96, 124.14, 120.54, 111.07, 110.90, 55.41 .

4-Chloro-3-(2-methoxyphenyl)-1,7-diphenyl-1H-pyrazolo[4,3-d]pyridazine (4_d)

Pale orange crystals, 96% yield, m.p. 152-154^{0}C, IR (KBr pellet): 1598 for (C=N) and 763 for (C-Cl) cm^{-1}, MS: m/z 412.5 (M^+).

4-Chloro-3-(2-methoxyphenyl)-1-phenyl-7-tolyl-1H-pyrazolo[4,3-d]pyridazine (4_e)

Yellow crystals, 94% yield, m.p. 140-142^{0}C, IR (KBr pellet): 1606 for (C=N) and 764 for (C-Cl) cm^{-1}, MS: m/z 427 (M^+) , ^{1}HNMR (DMSO, 300 MHz): δ 7.92-6.89 (m, 13H, 3Ar-H), 3.80-3.66 (s, 3H, OCH_3) and 3.31 (s, 3H, CH_3) ppm, ^{13}CNMR (DMSO, 300 MHz): 158.09, 157.01, 155.87, 142.06, 140.30, 140.15, 132.03, 130.16, 129.68, 129.43, 128.59, 128.08, 127.24, 126.66, 126.52, 125.32, 124.19, 120.47, 110.98, 55.34, 40.36 .

4-Chloro-3-(2-methoxyphenyl)-1-phenyl-7-metoxyphenyl-1H-pyrazolo[4,3-d]pyridazine (4_f)

Pale brown crystals, 98% yield, m.p. 122-124^{0}C, IR (KBr pellet): 1605 for (C=N) and 754 for (C-Cl) cm^{-1}, MS: m/z 441 (M^{-2}).

4-Chloro-3-(2-methoxyphenyl)-7-phenylisoxazolo[4,5-d]pyridazine (4_g)

Brown crystals, 97% yield, m.p. 144-146^{0}C, IR (KBr pellet): 1598 for (C=N) and 763 for (C-Cl) cm^{-1}, MS: m/z 337 (M^+).

4-Chloro-3-(2-methoxyphenyl)-7-tolylisoxazolo[4,5-d]pyridazine (4_h)

Dark brown crystals, 95% yield, m.p. 134-136^{0}C, IR (KBr pellet): 1602 for (C=N) and 761 for (C-Cl) cm^{-1}, MS: m/z 351 (M^+).

4-Chloro-3-(2-methoxyphenyl)-7-methoxyphenylisoxazolo[4,5-d]pyridazine (4_i)

Off white crystals, 94% yield, m.p. 126-128^{0}C, IR (KBr pellet): 1605 for (C=N) and 755 for (C-Cl) cm^{-1}, MS: m/z 368.5 (M^+).

Synthesis of 4-(3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino) benzenesulfonamides (5_{a-c}), 4-(3-(2-methoxyphenyl)-7-aryl-1-phenyl-1H-pyrazolo[4,3-d] pyridazin-4-ylamino)benzenesulfonamides (5_{d-f}), 4-(3-(2-methoxyphenyl)-7-arylisoxazolo[4,5-d]pyridazin-4-ylamino)benzenesulfonamides (5_{g-i}), 1-(4-(3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)phenylsulfonyl)guanidines (5_{j-l}), 1-(4-(3-(2-methoxy phenyl)-7-aryl-1-phenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)phenylsulfonyl)guanidines (5_{m-o}),1-(4-(3-(2-methoxyphenyl)-7-arylisoxazolo[4,5-d]pyridazin-4-ylamino)phenylsulfonyl) guanidines(5_{p-r}),1-(4-(3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino) phenylsulfonyl)sodium acetamides (5_{s-u}), 1-(4-(3-(2-methoxyphenyl)-1-aryl-7-tolyl-1H-pyrazolo [4,3-d]pyridazin-4-ylamino) phenylsulfonyl)sodiumacetamides (5_{v-x}) and 1-(4-(3-(2-methoxyphenyl)-7-arylisoxazolo[4,5-d]pyridazin-4-ylamino)phenylsulfonyl)sodium acetamides ($5_{y-z'}$)

A mixture of (4_{a-i}) (0.01mol) and different sulfonamides (sulfanilamide, sulfaguanidine, sod.- sulfaacetamide) (0.01mol) in glacial acetic acid (10ml) was refluxed for 2h. The reaction mixture was concentrated and left to cool , the solid separated was filtered off and recrystallized from ethanol to give ($5_{a-z'}$) respectively. All the reactions followed by T.L.C.

4-(3-(2-Methoxyphenyl)-7-phenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)benzenesulfonamide (5_a)

White crystals, 87% yield, m.p. 194-196^{0}C, IR (KBr pellet): 3368 for (2NH) , 3293-3208 for (NH of NH_2) and 1155 for sulfonamide cm^{-1}, MS: m/z 472 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 13.00 (s, 2H, 2NH) , 10.24 (s, 2H, NH_2), 7.76-6.87 (m, 13H, 3Ar-H), and 3.79-3.73 (s, 3H, OCH_3) ppm.

4-(3-(2-Methoxyphenyl)-7-tolyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)benzenesulfonamide (5_b)

Pale grey crystals, 85% yield, m.p. 174-176^{0}C, IR (KBr pellet): 3367 for (2NH), 3296-3208 for (NH of NH_2) and 1155 for sulfonamide cm^{-1}, MS: m/z 486 (M^+).

4-(3-(2-Methoxyphenyl)-7-methoxyphenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino) benzenesulfonamide (5_c)

Beig crystals, 80% yield, m.p. 208-210^{0}C, IR (KBr pellet): 3368 for (2NH), 3295-3206 for (NH of NH_2) and 1155 for sulfonamide cm^{-1}, MS: m/z 503 (M^{+1}).

4-(3-(2-Methoxyphenyl)-1,7-diphenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)benzene sulfonamide (5_d)

yellow crystals, 80% yield, m.p. 202-204^{0}C, IR (KBr pellet): 3369 for (NH), 3293-3212 for (NH of NH_2) and 1156 for sulfonamide cm^{-1}, MS: m/z 548 (M^+) , ^{1}HNMR (DMSO, 300 MHz): δ 10.24 (s, 2H, NH_2) , 7.20 (s, 1H, NH), 7.76-7.70 (m, 18H, 4Ar-H), and 3.31 (s, 3H, OCH_3) ppm.

4-(3-(2-Methoxyphenyl)-7-tolyl-1-phenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)benzene sulfonamide (5_e)

Beig crystals, 80% yield, m.p. 193-194^{0}C, IR (KBr pellet): 3438 for (NH), 3304-3205 for (NH of NH_2) and 1159 for sulfonamide cm^{-1}, MS: m/z 564 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 13.06 (s, 2H, NH_2), 7.23 (s, 1H, NH), 7.60-6.88 (m, 17H, 4Ar-H), 3.69 (s, 3H, OCH_3) and 3.29-3.20 (s, 3H, CH_3) ppm, ^{13}CNMR (DMSO, 300 MHz): 160.62, 157.11, 143.78, 142.06, 138.59, 132.19, 130.32, 129.47, 128.10, 127.29, 125.34, 125.30, 120.35, 110.89, 55.34, 40.35, 40.07, 39.79, 39.52, 39.24, 38.96, 38.68.

4-(3-(2-Methoxyphenyl)-7-methoxyphenyl-1-phenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)benzenesulfonamide (5_f)

Dark orange crystals, 75% yield, m.p. 186-188^{0}C, IR (KBr pellet): 3368 for (NH), 3293-3209 for (NH of NH_2) and 1155 for sulfonamide cm^{-1}, MS: m/z 580 (M^+), ^{13}CNMR (DMSO, 300 MHz): 168.82, 160.53, 157.09, 143.59, 142.14, 138.04, 130.38, 130.29, 128.17, 128.03, 127.37, 127.26, 127.10, 126.89, 125.29, 120.38, 120.27, 118.38, 114.36, 114.22, 110.92, 110.77, 55.35, 55.27.

4-(3-(2-Methoxyphenyl)-7-phenylisoxazolo[4,5-d]pyridazin-4-ylamino)benzenesulfonamide (5_g)

Yellow crystals, 80% yield, m.p. 184-186^{0}C, IR (KBr pellet): 3368 for (NH) , 3295-3206 for (NH of NH_2) and 1156 for sulfonamide cm^{-1}, MS: m/z 473 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 13.13 (s, 1H, NH), 10.24 (s, 2H, NH_2), 7.73-6.88 (m, 13H, 3Ar-H), and 3.81-3.78 (s, 3H, OCH_3) ppm.

4-(3-(2-Methoxyphenyl)-7-tolylisoxazolo[4,5-d]pyridazin-4-ylamino)benzenesulfon amide (5_h)

Off white crystals, 83% yield, m.p. 218-220^{0}C, IR (KBr pellet): 3368 for (NH), 3292-3210 for (NH of NH_2) and 1155 for sulfonamide cm^{-1}, MS: m/z 487 (M^+).

4-(3-(2-Methoxyphenyl)-7-methoxyphenylisoxazolo[4,5-d]pyridazin-4-ylamino)-benzeneesulfonamide (5_i)

Orange crystals, 75% yield, m.p. 200-201^{0}C, IR (KBr pellet): 3368 for (NH), 3296-3204 for (NH of NH_2) and 1155 for sulfonamide cm^{-1}, MS: m/z 503 (M^+).

1-(4-(3-(2-Methoxyphenyl)-7-phenyl-1H-pyrazolo[4,3-d]pyridazin-4ylamino)phenyl sulfonyl)guanidine (5_j)

Brown crystals, 87% yield, m.p. 194-196^{0}C, IR (KBr pellet): 3456 for (NH), 3388-3298 for (NH of NH_2) and 1320 for (SO_2) cm^{-1}, MS: m/z 514 (M^+).

1-(4-(3-(2-Methoxyphenyl)-7-tolyl-1H-pyrazolo[4,3-d]pyridazin-4ylamino)phenylsulf onyl)guanidine (5_k)

Buff crystals, 88% yield, m.p. 202-204^{0}C, IR (KBr pellet): 3568 for (NH), 3432-3335 for (NH of NH_2) and 1317 for (SO_2) cm^{-1}, MS: m/z 528 (M^+).

1-(4-(3-(2-Methoxyphenyl)-7-methoxyphenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)-phenylsulfonyl)guanidine (5_l)

Pale brown crystals, 85% yield, m.p. 216-218^{0}C, IR (KBr pellet): 3568 for (NH), 3434-3335 for (NH of NH_2) and 1314 for (SO_2) cm^{-1}, MS: m/z 544 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 13.00 (s, 1H, NH), 10.19 (s, 2H, NH_2), 7.15-7.02 (s, 2H, 2NH), 6.98 (s, 1H, NH) , 7.66-6.90 (m, 12H, 3Ar-H), and 3.79-3.43 (s, 6H, 2OCH_3) ppm, ^{13}CNMR (DMSO, 300 MHz): 168.75, 160.66, 157.76, 157.11, 143.78, 142.16, 138.07, 134.93, 130.44, 128.99, 128.82, 128.22, 128.02, 127.45, 127.25, 120.42, 120.26, 118.27, 110.98, 110.78, 55.39 .

1-(4-(3-(2-Methoxyphenyl)-1,7-diphenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)-phenylsulfonyl)guanidine (5_m)

Dark yellow crystals, 85% yield, m.p. 196-198^{0}C, IR (KBr pellet): 3479-3301 for (NH , NH of NH_2) and 1296 for (SO_2) cm^{-1}, MS: m/z 594 (M^{+4}).

1-(4-(3-(2-Methoxyphenyl)--1-phenyl-7-tolyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino) phenylsulfonyl)guanidine (5_n)

Grey crystals, 83% yield, m.p. 204-206^{0}C, IR (KBr pellet): 3301-3131 for (NH , NH of NH_2) and 1294 for (SO_2) cm^{-1}, MS: m/z 606 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 13.05 (s, 2H, NH_2) , 7.28-7.27 (s, 2H, 2NH) , 6.92 (s, 1H, NH) , 7.59-6.87 (m, 17H, 4Ar-H), 3.80-3.46 (s, 3H, OCH_3) , and 3.44-3.41 (s, 3H, CH_3) ppm, ^{13}CNMR (DMSO, 300 MHz):. 160.63, 157.11, 143.78, 142.06, 138.57, 132.19, 130.32, 129.45, 128.09, 127.26, 125.33, 120.34, 110.87, 55.32, 40.35, 40.07, 39.79, 39.51, 39.24, 38.96 , 38.68 .

1-(4-(3-(2-Methoxyphenyl)-1-phenyl-7-methoxyphenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)phenylsulfonyl)guanidine (5_o)

Dark orange crystals, 82% yield, m.p. 214-216^{0}C, IR (KBr pellet): 3569-3127 for (NH , NH of NH_2) and 1313 for (SO_2) cm^{-1}, MS: m/z 621 (M^{+1}).

1-(4-(3-(2-Methoxyphenyl)-7-phenylisoxazolo[4,5-d]pyridazin-4-ylamino)phenyl-sulfonyl)guanidine (5_p)

Brown crystals, 87% yield, m.p. 190-192^{0}C, IR (KBr pellet): 3299-3252 for (NH), 3204-3082 for (NH of NH_2), 1603 for (C=N) and 1320-1296 for (SO_2) cm^{-1}, MS: m/z 515 (M^+).

1-(4-(3-(2-Methoxyphenyl)-7-tolylisoxazolo[4,5-d]pyridazin-4-ylamino)phenylsulfonyl) guanidine (5_q)

Pale grey crystals, 85% yield, m.p. 238-240^{0}C, IR (KBr pellet): 3473-3434 for (NH), 3373-3332 for (NH of NH_2), 1604 for (C=N) and 1320 for (SO_2) cm^{-1}, MS: m/z 529 (M^+).

1-(4-(3-(2-Methoxyphenyl)-7-methoxyphenylisoxazolo[4,5-d]pyridazin-4-ylamino)-phenylsulfonyl)guanidine (5_r)

Orange crystals, 85% yield, m.p. 254-256^{0}C, IR (KBr pellet): 3473-3252 for (NH), 3373-3082 for (NH of NH_2) and 1320-1296 for (SO_2) cm^{-1}, MS: m/z 545 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 13.01 (s, 1H, NH), 10.18 (s, 2H, NH_2), 7.67 (s, 1H, NH), 7.65-6.65 (m, 12H, 3Ar-H), and 3.79-3.73 (s, 6H, $2OCH_3$) ppm.

1-(4-(3-(2-Methoxyphenyl)-7-phenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)phenyl-sulfonyl)sodiumacetamide (5_s)

Brown crystals, 80% yield, m.p. 188-189^{0}C, IR (KBr pellet): 3341-3299 for (2NH), 1650 for (C=O) and 1321 for (SO_2) cm^{-1}, MS: m/z 536 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 13.01 (s, 1H, NH), 7.84 (s, 1H, NH), 7.81-6.88 (m, 13H, 3Ar-H), 3.79-3.77 (s, 3H, OCH_3) , and 3.30 (s, 3H, CH_3) ppm.

1-(4-(3-(2-Methoxyphenyl)-7-tolyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)phenyl-sulfonyl)sodiumacetamide (5_t)

Buff crystals, 78% yield, m.p. 196-198^{0}C, IR (KBr pellet): 3340-3304 for (2NH), 1651 for (C=O) and 1320 for (SO_2) cm^{-1}, MS: m/z 550 (M^+).

1-(4-(3-(2-Methoxyphenyl)-7-methoxyphenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)phenylsulfonyl)sodiumacetamide (5_u)

Dark yellow crystals, 76% yield, m.p. 216-218^{0}C, IR (KBr pellet): 3341-3201 for (2NH), 1651 for (C=O) and 1319 for (SO_2) cm^{-1}, MS: m/z 566 (M^+).

1-(4-(3-(2-Methoxyphenyl)-1,7-diphenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)-phenylsulfonyl)sodiumacetamide (5_v)

yellow crystals, 79% yield, m.p. 244-246^{0}C, IR (KBr pellet): 3342 for (NH), 1650 for (C=O) and 1323 for (SO_2) cm^{-1}, MS: m/z 600 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 13.05 (s, 1H, NH), 7.59-6.87 (m, 17H, 4Ar-H), 3.80-3.78 (s, 3H, OCH_3) ,and 3.28 (s, 3H, CH_3) ppm.

1-(4-(3-(2-Methoxyphenyl)-1-phenyl-7-tolyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)-phenylsulfonyl)sodiumacetamide (5_w)

White crystals, 76% yield, m.p. 199-200^{0}C, IR (KBr pellet): 3300 for (NH), 1652 for (C=O) and 1293 for (SO_2) cm^{-1}, MS: m/z 628 (M^+),^{13}CNMR (DMSO, 300 MHz):. 160.63, 157.11, 143.77, 142.06, 138.58, 132.20, 130.32, 129.46, 128.10, 127.28, 125.34, 125.30, 120.35, 110.89, 55.33, 40.07, 40.36, 39.52, 39.24, 38.96, 38.80, 38.69 .

1-(4-(3-(2-Methoxyphenyl)-7-methoxyphenyl-1-phenyl-1H-pyrazolo[4,3-d]pyridazin-4-ylamino)phenylsulfonyl)sodiumacetamide (5_x)

orange crystals, 77% yield, m.p. 210-212^{0}C, IR (KBr pellet): 3342 for (NH), 1651 for (C=O) and 1317 for (SO_2) cm^{-1}, MS: m/z 641 (M^{-1}).

1-(4-(3-(2-Methoxyphenyl)-7-phenylisoxazolo[4,5-d]pyridazin-4-ylamino)phenyl-sulfonyl)sodiumacetamide (5_y)

Brown crystals, 79% yield, m.p. 196-198^{0}C, IR (KBr pellet): 3340 for (NH), 1699 for (C=O) and 1323 for (SO_2) cm^{-1}, MS: m/z 537 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 13.13 (s, 1H, NH), 7.85-6.90 (m, 13H, 3Ar-H), 6.88 (s, 3H, CH_3) , and 3.81-3.72 (s, 3H, OCH_3) ppm.

1-(4-(3-(2-Methoxyphenyl)-7-tolylisoxazolo[4,5-d]pyridazin-4-ylamino)phenyl-sulfonyl)sodiumacetamide (5_z)

yellow crystals, 81% yield, m.p. 256-258^{0}C, IR (KBr pellet): 3341 for (NH), 1700 for (C=O) and 1324 for (SO_2) cm^{-1}, MS: m/z 551 (M^+).

1-(4-(3-(2-Methoxyphenyl)-7-methoxyphenylisoxazolo[4,5-d]pyridazin-4-ylamino)-phenylsulfonyl)sodiumacetamide ($5_{z'}$)

Pale orange crystals, 77% yield, m.p. 210-212^{0}C, IR (KBr pellet): 3341 for (NH), 1698 for (C=O) and 1298 for (SO_2) cm^{-1}, MS: m/z 567 (M^+).

Synthesis of 3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,5-d]tetrazolo[4,3-d] pyridazines (6_{a-c}), 3-(2-methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,5-d]tetrazolo[4,3-d]pyridazines (6_{d-f}), and 3-(2-methoxyphenyl)-7-arylisoxazolo[4,5-d]tetrazolo[4,3-d]pyridazines (6_{g-i})

A mixture of (4_{a-i}) (0.01mol) and NaN_3 (0.01mol) in DMF (10ml) was refluxed for 2h. The reaction mixture was concenterated and left to cool , the solid separated was filtered off to give (6_{a-i}) respectively. All the reactions followed by T.L.C.

3-(2-Methoxyphenyl)-7-phenyl-1H-pyrazolo[4,5-d]tetrazolo[4,3-d]pyridazine (6_a)

Beig crystals, 42% yield, m.p. 134-136^{0}C, IR (KBr pellet): 3404 for (NH) and 2078 for (N_3) cm^{-1}, MS: m/z 343 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 8.11 (s, 1H, 1NH), 8.10-6.87 (m, 9H, 2Ar-H), and 3.79-3.78 (s, 3H, OCH_3) ppm.

3-(2-Methoxyphenyl)-7-tolyl-1H-pyrazolo[4,5-d]tetrazolo[4,3-d]pyridazine (6_b)

Dark orange crystals, 40% yield, m.p. 219-220^{0}C, IR (KBr pellet): 3321 for (NH) and 2141 for (N_3) cm^{-1}, MS: m/z 356 (M^{-1}).

3-(2-Methoxyphenyl)-7-methoxyphenyl-1H-pyrazolo[4,5-d]tetrazolo[4,3-d]pyridazine (6_c)

Brown crystals, 43% yield, m.p. 182-184^{0}C, IR (KBr pellet): 3432 for (NH) and 2034 for (N_3) cm^{-1}, MS: m/z 373 (M^+).

3-(2-Methoxyphenyl)-1,7-diphenyl-1H-pyrazolo[4,5-d]tetrazolo[4,3-d]pyridazine (6_d)

Brown crystals, 40% yield, m.p. 152-154^{0}C, IR (KBr pellet): 1597 for (C=N) and 2065 for (N_3) cm^{-1}, MS: m/z 419 (M^+).

3-(2-Methoxyphenyl)-1-phenyl-7-tolyl-1H-pyrazolo[4,5-d]tetrazolo[4,3-d]pyridazine (6_e)

Pale grey crystals, 41% yield, m.p. 139-140^{0}C, IR (KBr pellet): 1601 for (C=N) and 2043 for (N_3) cm^{-1}, MS: m/z 421 (M^{+1}).

3-(2-Methoxyphenyl)-7-methoxyphenyl-1-phenyl-1H-pyrazolo[4,5-d]tetrazolo[4,3-d]pyridazine (6_f)

Dark orange crystals, 44% yield, m.p. 186-188^{0}C, IR (KBr pellet): 1603 for (C=N) and 2124 for (N_3) cm^{-1}, MS: m/z 441 (M^{+1}).

3-(2-Methoxyphenyl)-7-phenylisoxazolo[4,5-d]tetrazolo[4,3-d]pyridazine (6_g)

Pale brown crystals, 45% yield, m.p. 242-244^{0}C, IR (KBr pellet): 1598 for (C=N) and 2132 for (N_3) cm^{-1}, MS: m/z 344 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 8.53-6.89 (m, 9H, 2Ar-H), and 3.88-3.78 (s, 3H, OCH_3) ppm.

3-(2-Methoxyphenyl)-7-tolylisoxazolo[4,5-d]tetrazolo[4,3-d]pyridazine (6_h)

Dark orange crystals, 39% yield, m.p. 202-204^{0}C, IR (KBr pellet): 1600 for (C=N) and 2140 for (N_3) cm^{-1}, MS: m/z 358 (M^+).

3-(2-Methoxyphenyl)-7-methoxyphenylisoxazolo[4,5-d]tetrazolo[4,3-d]pyridazine (6_i)

Brown crystals, 40% yield, m.p. 128-130^{0}C, IR (KBr pellet): 1604 for (C=N) and 2139 for (N_3) cm^{-1}, MS: m/z 374 (M^+).

Synthesis of 3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazine-4-thiols (7_{a-c}), 3-(2-methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,3-d]pyridazine-4-thiols (7_{d-f}) and 3-(2-methoxyphenyl)-7-arylisoxazolo-1H-pyrazolo[4,5-d]pyridazine-4-thiols ($7_{g–i}$)

A mixture of (4_{a-i}) (0.01mol) and thiourea (0.01mol) in ethyl alcohol (10ml) was refluxed for 2h. The reaction mixture was concenterated and left to cool , the solid separated was filtered off and recrystallized from alcohol to give (7_{a-i}) respectively. All the reactions followed by T.L.C.

3-(2-Methoxyphenyl)-7-phenyl-1H-pyrazolo[4,3-d]pyridazine-4-thiol (7_a)

Orange crystals, 88% yield, m.p. 190-192^{0}C, IR (KBr pellet): 3319 for (NH), 2836 for (SH) and 745 for (C-S) cm^{-1}, MS: m/z 336 (M^{+2}), ^{1}HNMR (DMSO, 300 MHz): δ 14.94 (s, 1H, SH), 7.73-6.92 (m, 9H, 2Ar-H), 6.89 (s, 1H, NH) and 3.78-3.30 (s, 3H, OCH_3) ppm.

3-(2-Methoxyphenyl)-7-tolyl-1H-pyrazolo[4,3-d]pyridazine-4-thiol (7_b)

Pale orange crystals, 90% yield, m.p. 182-184^{0}C, IR (KBr pellet): 3136-3113 for (NH), 2832 for (SH) and 750 for (C-S) cm^{-1}, MS: m/z 352 (M^{+4}).

3-(2-Methoxyphenyl)-7-methoxyphenyl-1H-pyrazolo[4,3-d]pyridazine-4-thiol (7_c)

Brown crystals, 91% yield, m.p. 170-172^{0}C, IR (KBr pellet): 3317-3227 for (NH), 2836 for (SH) and 753 for (C-S) cm^{-1}, MS: m/z 366 (M^{+2}).

3-(2-Methoxyphenyl)-1,7-diphenyl-1H-pyrazolo[4,3-d]pyridazine-4-thiol (7_d)

Pale orange crystals, 91% yield, m.p. 182-184^{0}C, IR (KBr pellet): 2835 for (SH) and 691 for (C-S) cm^{-1}, MS: m/z 410 (M^+).

3-(2-Methoxyphenyl)-1-phenyl-7-tolyl-1H-pyrazolo[4,3-d]pyridazine-4-thiol (7_e)

Orange crystals, 89% yield, m.p. 190-191^{0}C, IR (KBr pellet): 2834 for (SH) and 691 for (C-S) cm^{-1}, MS: m/z 424 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 14.93 (s, 1H, SH), 7.61-6.88 (m, 13H, 3Ar-H), 3.76-3.66 (s, 3H, OCH_3) and 3.44-3.35 (s, 3H, CH_3) ppm.^{13}CNMR (DMSO, 300 MHz): 179.21, 157.21, 151.15, 149.02, 139.72, 131.25, 130.55, 129.68, 128.32, 125.64, 124.98, 122.28, 120.42, 110.93, 55.34, 40.34, 40.06, 39.79, 39.51, 39.23, 38.95 .

3-(2-Methoxyphenyl)-7-methoyphenyl-1-phenyl-1H-pyrazolo[4,3-d]pyridazine-4-thiol (7_f)

Dark orange crystals, 90% yield, m.p. 176-178^{0}C, IR (KBr pellet): 2836 for (SH) and 655 for (C-S) cm^{-1}, MS: m/z 446 (M^{-3}).

3-(2-Methoxyphenyl)-7-phenylisoxazolo[4,5-d]pyridazine-4-thiol (7_g)

Dark yellow crystals, 92% yield, m.p. 186-187^{0}C, IR (KBr pellet): 2834 for (SH) and 690 for (C-S) cm^{-1}, MS: m/z 338 (M^{-2}).

3-(2-Methoxyphenyl)-7-tolylisoxazolo[4,5-d]pyridazine-4-thiol (7_h)

Dark orange crystals, 87% yield, m.p. 194-196^{0}C, IR (KBr pellet): 2833 for (SH) and 690 for (C-S) cm^{-1}, MS: m/z 351 (M^{+2}).

3-(2-Methoxyphenyl)-7-methoxyphenylisoxazolo[4,5-d]pyridazine-4-thiol (7_i)

Brown crystals, 90% yield, m.p. 162-164^{0}C, IR (KBr pellet): 2837-2833 for (SH) and 690-655 for (C-S) cm^{-1}, MS: m/z 365 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 14.84 (s, 1H, SH), 7.67-6.91 (m, 8H, 2Ar-H), and 3.78-3.77 (s, 6H, $2OCH_3$) ppm.

Synthesis of 1-(3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4-yl)hydrazines (8_{a-c}), 1-(3-(2-methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4-yl)hydrazines (8_{d-f}) and 1-(3-(2-methoxyphenyl)-7-arylisoxazolo-1H-pyrazolo[4,5-d]pyridazin-4-yl)hydrazines (8_{g-i})

A mixture of (7_{a-i}) (0.01mol) and hydrazine hydrate (0.01mol) in ethyl alcohol (10ml) was refluxed for 2h. The reaction mixture was concenterated and left to cool , the solid separated was filtered off and recrystallized from alcohol to give (8_{a-i}) respectively. All the reactions followed by T.L.C.

1-(3-(2-Methoxyphenyl)-7-phenyl-1H-pyrazolo[4,3-d]pyridazin-4-yl)hydrazine (8_a)

yellow crystals, 52% yield, m.p. 196-197^{0}C, IR (KBr pellet): 3440 for (NH) and 3141-3119 for (NH of NH_2) cm^{-1}, MS: m/z 332 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 14.81 (t, 1H, NH), 7.73 (s, 1H, NH), 7.72-6.89 (m, 9H, 2Ar-H), 3.77 (s, 3H, OCH_3) and 3.30 (d, 2H, NH_2) ppm.

1-(3-(2-Methoxyphenyl)-7-tolyl-1H-pyrazolo[4,3-d]pyridazin-4-yl)hydrazine (8_b)

Pale orange crystals, 50% yield, m.p. 172-174^{0}C, IR (KBr pellet): 3150 for (NH) and 3136-3113 for (NH of NH_2) cm^{-1}, MS: m/z 351 (M^{+5}).

1-(3-(2-Methoxyphenyl)-7-methoxyphenyl-1H-pyrazolo[4,3-d]pyridazin-4-yl)-hydrazine (8_c)

Dark yellow crystals, 53% yield, m.p. 184-186^{0}C, IR (KBr pellet): 3175 for (NH) and 3135-3111 for (NH of NH_2) cm^{-1}, MS: m/z 366 (M^{+4}).

1-(3-(2-Methoxyphenyl)-1,7-diphenyl-1H-pyrazolo[4,3-d]pyridazin-4-yl)hydrazine (8_d)

Yellow crystals, 51% yield, m.p. 190-192^{0}C, IR (KBr pellet): 3260 for (NH) and 3141-3118 for (NH of NH_2) cm^{-1}, MS: m/z 408 (M^+).

1-(3-(2-Methoxyphenyl)-1-phenyl-7-tolyl-1H-pyrazolo[4,3-d]pyridazin-4-yl)hydrazine (8_e)

Dark yellow crystals, 49% yield, m.p. 182-184^{0}C, IR (KBr pellet): 3190 for (NH) and 3136-3113 for (NH of NH_2) cm^{-1}, MS: m/z 363 (M^+).

1-(3-(2-Methoxyphenyl)-1-phenyl-7-methoxyphenyl-1H-pyrazolo[4,3-d]pyridazin-4-yl)hydrazine (8_f)

Brown crystals, 50% yield, m.p. 172-174^{0}C, IR (KBr pellet): 3260-3150 for (NH) and 3141-3111 for (NH of NH_2) cm^{-1}, MS: m/z 436 (M^{-2}), ^{1}HNMR (DMSO, 300 MHz): δ 14.84

(t, 1H, NH), 7.68-7.67 (d, 2H, NH_2), 7.65-6.89 (m, 13H, 3Ar-H), and 3.79-3.73 (s, 6H, $2OCH_3$) ppm.

1-(3-(2-Methoxyphenyl)-7-phenylisoxazolo[4,5-d]pyridazin-4-yl)hydrazine (8g)

Dark yellow crystals, 53% yield, m.p. 198-200^{0}C, IR (KBr pellet): 3150 for (NH), 3130-3119 for (NH of NH_2) and 1603 for (C=N) cm^{-1}, MS: m/z 333 (M^+).

1-(3-(2-Methoxyphenyl)-7-tolylisoxazolo[4,5-d]pyridazin-4-yl)hydrazine (8_h)

Orange crystals, 48% yield, m.p. 186-188^{0}C, IR (KBr pellet): 3150 for (NH), 3133-3110 for (NH of NH_2) and 1603 for (C=N) cm^{-1}, MS: m/z 347 (M^+), ^{1}HNMR (DMSO, 300 MHz): δ 14.89 (t, 1H, NH), 7.61 (d, 2H, NH_2), 7.59-6.89 (m, 8H, 2Ar-H), 3.83-3.77 (s, 3H, OCH_3) , and 3.30 (s, 3H, CH_3) ppm.

1-(3-(2-Methoxyphenyl)-7-methoxyphenylisoxazolo[4,5-d]pyridazin-4-yl)hydrazine (8_i)

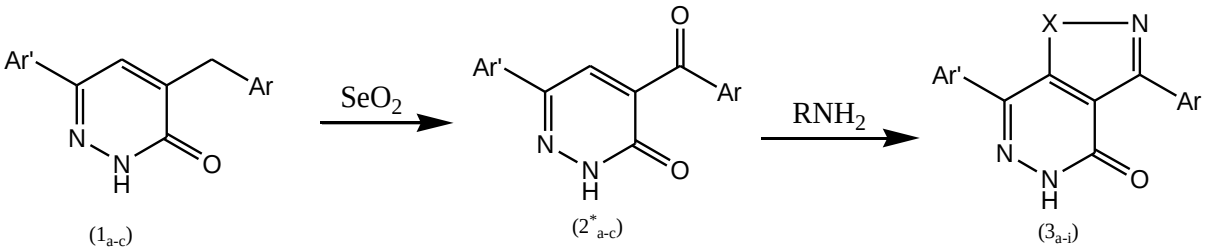
Yellow crystals, 51% yield, m.p. 172-174^{0}C, IR (KBr pellet): 3429 for (NH), 3156-3112 for (NH of NH_2) and 1606 for (C=N) cm^{-1}, MS: m/z 363 (M^+).

3. Results and Discussion

3.1 Chemistry

4-(2-Methoxybenzoyl)-6-arylpyridazin-3(2H)-ones ($2_{a–c}$) were achieved from the reaction of 4-(2-methoxybenzyl)-6-arylpyridazin-3(2H)-ones ($1_{a–c}$) with selenium dioxide according to the method reported in our previous work **[Mohamed M.I. et al, (2004)]**. The synthetic route used to synthesize compounds ($2_{a–c}$) is outlined in (Scheme 1).

The synthesis of new fused pyridazine derivatives of expected biological activity derivative incorporating pyridazine moiety were achieved from the reaction of compounds ($2_{a–c}$), towards different nucleophiles such as hydrazine hydrate , phenyl hydrazine or hydroxylamine hydrochloride. When compounds (2_{a-c}) were refluxed with hydrazine hydrate, phenyl hydrazine and hydroxylamine hydrochloride in ethanol under basic conditions, the products were 3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-ones($3_{a–c}$),3-(2-methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-ones($3_{d–f}$) and 3-(2-methoxyphenyl)-7-arylisoxazolo-1H-pyrazolo[4,5-d]pyridazin-4(5H)-ones ($3_{g–i}$). The structures of ($3_{a–i}$) were established



(1_{a-c}) (2^{*}_{a-c}) (3_{a-i})

Ar' , a = C_6H_5
b = C_6H_4-CH_3 (p)
c = C_6H_4-OCH_3(p)

Ar = C_6H_4-OCH_3 (o)

X , a = NH
b =N C_6H_5
c = O

Scheme 1. Synthesis of the target compound (3_{a-i})

Table 1. Pyridazne derivatives (3_{a-i})

No. of Comp.	X	Ar'
3a	NH	C_6H_5
3b	NH	$C_6H_4CH_3$
3c	NH	$C_6H_4OCH_3$
3d	NC_6H_5	C_6H_5
3e	NC_6H_5	$C_6H_4CH_3$
3f	NC_6H_5	$C_6H_4OCH_3$
3g	O	C_6H_5
3h	O	$C_6H_4CH_3$
3i	O	$C_6H_4OCH_3$

2^{*}_{a-c} :[M.I. Mohamed, et al (2004)]

from FT-IR, mass, ^{1}HNMR, and ^{13}CNMR spectra. The ^{1}HNMR spectra of compounds ($3_{a–c}$) displayed the protons of the (NH) groups which disappeared on addition of D_2O. 4-Chloro-3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazines($4_{a–c}$),4-Chloro-3-(2-methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,3-d]pyridazines($4_{d–f}$) and 4-Chloro-3-(2-methoxyphenyl)-7-arylisoxazolo-1H-pyrazolo[4,5-d]pyridazines ($4_{g–i}$) produced from the reaction of phosphorus oxychloride with 3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4(5H)-ones($3_{a–c}$),3-(2-methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,3-d] pyridazin-4(5H)-ones($3_{d–f}$) and 3-(2-methoxyphenyl)-7-arylisoxazolo-1H-pyrazolo[4,5-d]pyridazin-4(5H)-ones ($3_{g–i}$) as shown in (Scheme 2). The ^{1}HNMR spectrum of compound (4_e) shows the following δ 7.92-6.89 (m, 13H, 3Ar-H), 3.80-3.66 (s, 3H, OCH_3) and 3.31 (s, 3H, CH_3) ppm.

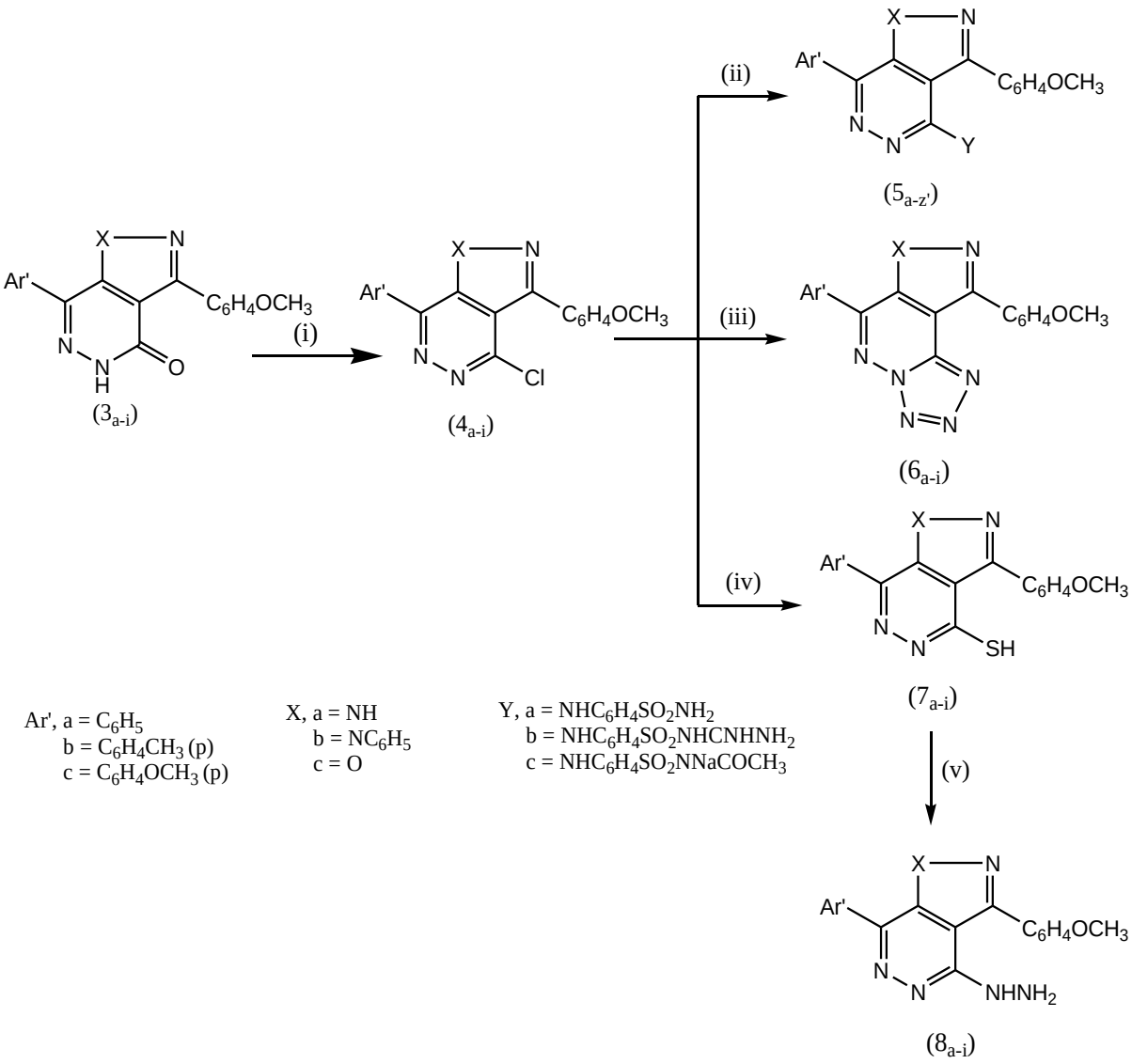
With a view of introducing biologically active moieties such as sulfonamides, 4-chloro-3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazines($4_{a–c}$),4-chloro-3-(2-

methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,3-d]pyridazines($4_{d–f}$) and 4-chloro-3-(2-methoxyphenyl)-7-arylisoxazolo-1H-pyrazolo[4,5-d]pyridazines ($4_{g–i}$) reacted with different sulfonamides such as sulfanilamide, sulfaguanidine and sodium sulfaacetamide by refluxing in glacial acetic acid for 2h to give the corresponding sulfonamide derivatives ($5_{a–z'}$) respectively as shown in (Scheme 2) and (Table 2). The ^{13}CNMR spectrum of (5_n) shows the following δ 160.63, 157.11, 143.78, 142.06, 138.57, 132.19, 130.32, 129.45, 128.09, 127.26, 125.33, 120.34, 110.87, 55.32, 40.35, 40.07, 39.79, 39.51, 39.24, 38.96, 38.68 .

The poly nitrogen electron rich tetrazolo derivatives ($6_{a–i}$) has been formed by reaction of 4-chloro-3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazines($4_{a–c}$),4-chloro-3-(2-methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,3-d]pyridazines($4_{d–f}$) and 4-chloro-3-(2-methoxyphenyl)-7-arylisoxazolo-1H-pyrazolo[4,5-d]pyridazines ($4_{g–i}$) with sodium azide in dimethylformamide. The IR spectrum of (6_b) shows bands at 3321 for (NH) and 2141 for (N_3) cm^{-1}.

The thiol derivatives namely by 3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazine-4-thiols(7_{a-c}),3-(2-methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,3-d]-pyridazine-4-thiols (7_{d-f}) and 3-(2-methoxyphenyl)-7-arylisoxazolo-1H-pyrazolo[4,5-d]pyridazine-4-thiols (7_{g-i}) obtained via reaction of the chloro derivatives ($4_{a–i}$) with thiourea (Scheme 2). The ^{1}HNMR spectrum of (7_a) shows the following: δ 14.94 (s, 1H, SH), 7.73-6.9 (m, 9H, 2Ar-H), 6.89 (s, 1H, NH), and 3.78-3.30 (s, 3H, OCH_3) ppm, where SH and NH bands disappeared on addition of D_2O.

Finally, for the importance of hydrazino derivatives **[Mohamed M.I., et al (2004)]**, we synthesized the hydrazine derivatives by reaction of thiol derivatives (7_{a-i}) with hydrazine hydrate as shown in (Scheme 2) to give the corresponding hydrazino derivatives namely by1-(3-(2-methoxyphenyl)-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4-yl)hydrazines(8_{a-c}),1-(3-(2-methoxyphenyl)-1-phenyl-7-aryl-1H-pyrazolo[4,3-d]pyridazin-4-yl)hydrazines(8_{d-f}) and1-(3-(2-methoxyphenyl)-7-arylisoxazolo)-1H-pyrazolo[4,5-d]pyridazin-4-yl)hydrazines(8_{g-i}) (Table 2) obtained. The structure of (8g) revealed a molecular ion peak at m/z 333 (M^+).



Scheme 2: Synthesis of the target compounds 4, 5, 6, 7, and 8

Reagents and conditions: (i) $POCl_3$, reflux, 60^0C, (ii) appreciate sulfonamide, CH_3COOH, reflux., (iii) NaN_3, D.M.F, reflux., (iv) NH_2CSNH_2, EtOH, reflux , (v) NH_2NH_2, EtOH, reflux.

Table 2. Pyridazine derivatives (4_{a-i})-(8_{a-i})

No. of Comp.	X	Ar'	Y
4a	NH	C_6H_5	
4b	NH	$C_6H_4CH_3$	
4c	NH	$C_6H_4OCH_3$	
4d	NC_6H_5	C_6H_5	
4e	NC_6H_5	$C_6H_4CH_3$	
4f	NC_6H_5	$C_6H_4OCH_3$	
4g	O	C_6H_5	
4h	O	$C_6H_4CH_3$	
4i	O	$C_6H_4OCH_3$	
5a	NH	C_6H_5	$NHC_6H_4SO_2NH_2$
5b	NH	$C_6H_4CH_3$	$NHC_6H_4SO_2NH_2$
5c	NH	$C_6H_4OCH_3$	$NHC_6H_4SO_2NH_2$
5d	NC_6H_5	C_6H_5	$NHC_6H_4SO_2NH_2$
5e	NC_6H_5	$C_6H_4CH_3$	$NHC_6H_4SO_2NH_2$
5f	NC_6H_5	$C_6H_4OCH_3$	$NHC_6H_4SO_2NH_2$
5g	O	C_6H_5	$NHC_6H_4SO_2NH_2$
5h	O	$C_6H_4CH_3$	$NHC_6H_4SO_2NH_2$
5i	O	$C_6H_4OCH_3$	$NHC_6H_4SO_2NH_2$
5j	NH	C_6H_5	$NHC_6H_4SO_2NHCNHNH_2$
5k	NH	$C_6H_4CH_3$	$NHC_6H_4SO_2NHCNHNH_2$
5l	NH	$C_6H_4OCH_3$	$NHC_6H_4SO_2NHCNHNH_2$
5m	NC_6H_5	C_6H_5	$NHC_6H_4SO_2NHCNHNH_2$
5n	NC_6H_5	$C_6H_4CH_3$	$NHC_6H_4SO_2NHCNHNH_2$
5o	NC_6H_5	$C_6H_4OCH_3$	$NHC_6H_4SO_2NHCNHNH_2$
5p	O	C_6H_5	$NHC_6H_4SO_2NHCNHNH_2$
5q	O	$C_6H_4CH_3$	$NHC_6H_4SO_2NHCNHNH_2$
5r	O	$C_6H_4OCH_3$	$NHC_6H_4SO_2NHCNHNH_2$
5s	NH	C_6H_5	$NHC_6H_4SO_2NNaCOCH_3$
5t	NH	$C_6H_4CH_3$	$NHC_6H_4SO_2NNaCOCH_3$
5u	NH	$C_6H_4OCH_3$	$NHC_6H_4SO_2NNaCOCH_3$
5v	NC_6H_5	C_6H_5	$NHC_6H_4SO_2NNaCOCH_3$
5w	NC_6H_5	$C_6H_4CH_3$	$NHC_6H_4SO_2NNaCOCH_3$
5x	NC_6H_5	$C_6H_4OCH_3$	$NHC_6H_4SO_2NNaCOCH_3$
5y	O	C_6H_5	$NHC_6H_4SO_2NNaCOCH_3$
5z	O	$C_6H_4CH_3$	$NHC_6H_4SO_2NNaCOCH_3$
5z'	O	$C_6H_4OCH_3$	$NHC_6H_4SO_2NNaCOCH_3$
6a	NH	C_6H_5	
6b	NH	$C_6H_4CH_3$	
6c	NH	$C_6H_4OCH_3$	
6d	NC_6H_5	C_6H_5	
6e	NC_6H_5	$C_6H_4CH_3$	
6f	NC_6H_5	$C_6H_4OCH_3$	
6g	O	C_6H_5	
6h	O	$C_6H_4CH_3$	
6i	O	$C_6H_4OCH_3$	
7a	NH	C_6H_5	
7b	NH	$C_6H_4CH_3$	
7c	NH	$C_6H_4OCH_3$	
7d	NC_6H_5	C_6H_5	
7e	NC_6H_5	$C_6H_4CH_3$	
7f	NC_6H_5	$C_6H_4OCH_3$	
7g	O	C_6H_5	
7h	O	$C_6H_4CH_3$	
7i	O	$C_6H_4OCH_3$	
8a	NH	C_6H_5	
8b	NH	$C_6H_4CH_3$	
8c	NH	$C_6H_4OCH_3$	
8d	NC_6H_5	C_6H_5	
8e	NC_6H_5	$C_6H_4CH_3$	
8f	NC_6H_5	$C_6H_4OCH_3$	
8g	O	C_6H_5	
8h	O	$C_6H_4CH_3$	
8i	O	$C_6H_4OCH_3$	

3.2 Biological evaluation

Cell line:

The cell lines were obtained from the American type culture collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37^0C in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week.

Evaluation of the antitumor activity:

The antitumor activity was evaluated on carcinoma cell lines at the Regional center for Mycology & Biotechnology , Al-Azhar University , Cairo, Egypt. Briefly , the cell lines were grown as monolayers in growth medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The monolayers of 10,000 cells adhered at the bottom of the wells in a 96-well microtiter plate (Falcon, NJ, USA)incubated for 24h at 37^0C in a humidified incubator with 5% CO_2 . The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 µI from different dilutions of tested compound in fresh maintenance medium and incubated at 37^0C . A control of untreated cells was made in the absence of the tested compound. Three wells were used for each concentration of the test sample. Every 24h the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet followed by cell lysing using 33% glacial acetic acid and read the absorbance at 590nm using ELISA reader after well mixing. The absorbance values from untreated cells were considered as 100% proliferation and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested compounds and ODc is the mean optical density of untreated cells.**[Mosmann T (1983), Gangadevi. V., et al (2007) and Wilson, A. P. (2000)].**

Six of the new synthesized compounds (6_f , 8_{c-f} and 8_i) were selected and tested against a panel human tumor cell line HCT-116 (colon cancer) using Imatinib as standard drug.**[Morsy S.M., et al (2009)].** The cytotoxicity data of the tested compounds (6_f , 8_{c-f} and 8_i) virus Imatinib in means of IC_{50} values were recorded as in (Figure I). The obtained data showed that the compounds 1-(3-(2-methoxyphenyl)-7-methoxyphenylisoxazolo[4,5-d]pyridazin-4-yl)hydrazine (8_i) and 1-(3-(2-methoxyphenyl)-1-phenyl-7-methoxyphenyl-1H-pyrazolo[4,3-d]pyridazin-4-yl)hydrazine (8_f) have high reactivity towards cell line HCT-116 (colon cancer) more than the standard drug Imatinib.

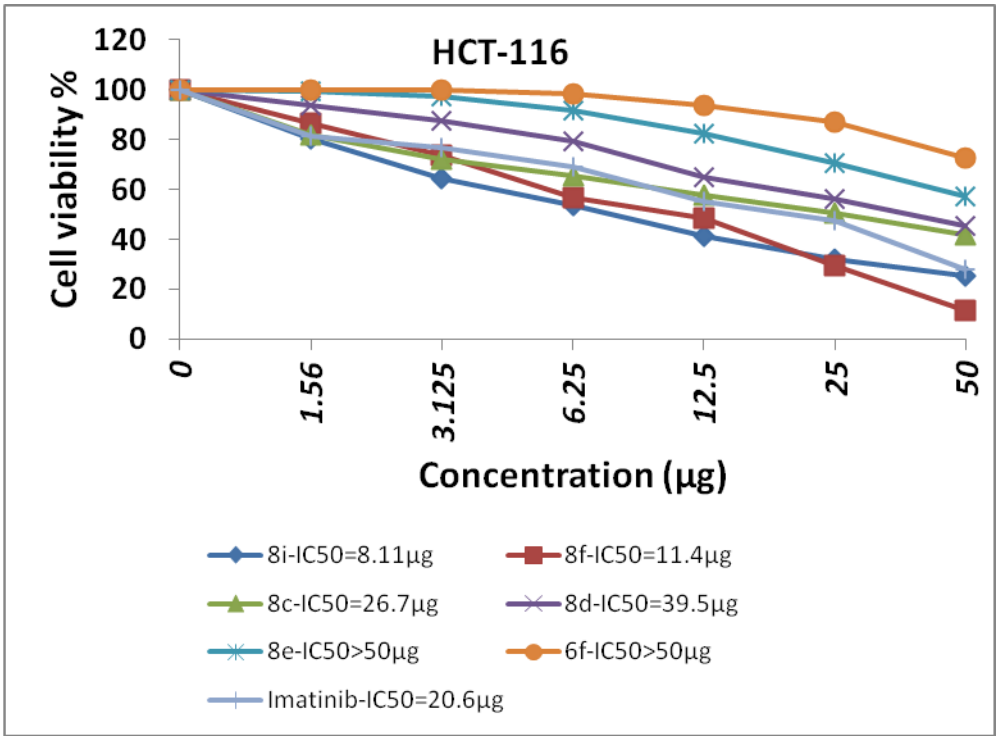


Fig. I

4. Conclusion

In the present work, we report synthesis of new series of fused pyridazine compounds which is used as the key material for further transformation. The present study illustrating that pyridazine moiety reactivity towards some nucleophiles with the aim of preparing new biologically active compounds. Some of the new compounds tested against a panel human tumor cell line HCT-116 (colon cancer). Compounds (8_f) and (8_i) showed high reactivity towards cell line HCT-116 (colon cancer) more than the standard drug (Imatinib).

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الملخص باللغة العربية

طرق بسيطة وفعالة لتشيد مركبات غير متجانسة الحلقة جديدة نشطة بيولوجيا

رنا عبد العزيز الزاملى، هويدا طلعت زكي ، نادية غريب قنديل
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فى هذه الدراسة تم تحضير مشتقات جديدة من البيريدازين والمتوقع ان يكون لها نشاط بيولوجى كما تم دراسة فاعلية هذه المركبات تجاه بعض النيكليوفيلات.

يتفاعل 2-ميثوكسى بنزالدهيد مع 6-اريل-4,5-ثنائى هيدروبيريدازين 3(2H)-اونات فى وجود قاعدة كعامل حفاز لتعطى المشتقات المقابلة من 6-اريل-4-(2-ميثوكسى بنزيل) بيريدازين-3(2H)-اونات (1_{a-c}).

كما تم اكسدة (1_{a-c}) بواسطة ثانى اكسيد السيلينيوم ليعطى الناتج المقابل المسمى ب 6-اريل-4-(2-ميثوكسى بنزويل) بيريدازين- 3(2H)- اونات (2^*_{a-c}). كما تم دراسة تاثير بعض النيكليوفيلات مثل هيدرات الهيدرازين، فينيل الهيدرازين وهيدروكسيل أمين هيدروكلوريد تحت ظروف قاعدية ليعطى 3-(2-ميثوكسى فينيل)- 7-اريل-H1-بيرازولو[4,3-d]بيريدازين-4(5H)- اونات (3_{a-c}) و 3-(2-ميثوكسى فينيل)- 1- فينيل-7-اريل-H1-بيرازولو [4,3-d] بيريدازين -4(5H)- اونات (3_{d-f}) و 3-(2-ميثوكسى فينيل)-7-اريل ايزواوكزازولو -H1-بيرازولو[4,3-d] بيريدازين -4(5H)- اونات (3_{g-i}) على التوالى وعند مفاعلة هذه البيريدازينات مع اوكسى كلوريد الفوسفور تم الحصول على 4-كلورو بيريدازينات (4_{a-i}) المقابلة على التوالى.

ايضا تم دراسة تفاعل مركبات الكلورو (4_{a-i}) مع سالفونااميدات مختلفة مثل سالفانيلاميد و سالفاجوانيدين و سالفااسيتاميد الصوديوم فى وجود حمض الخليك المركز لتعطى مشتقات السالفوناميد المقابلة ($5_{a-z'}$).

وشملت الدراسة ايضا تفاعل هذه البيريدازينات (4_{a-i}) مع ازيد الصوديوم فى وجود ثنائى ميثيل الفورماميد ليعطى مشتقات التترازولو المقابلة (6_{a-i}) على التوالى.

كما امتدت الدراسة لتشمل تفاعل كل من البيريدازينات (4_{a-i}) مع الثايويوريا فى وجود الكحول الايثيلى لتكوين مركبات الثايول المقابلة (7_{a-i}) على التوالى ولقد تم الحصول على الهيدروزنات (8_{a-i}) من خلال تفاعل مركبات الثايول (7_{a-i}) مع هيدرات الهيدرازين فى وجود الكحول الايثيلى.

وقد تم اختبار بعض من المركبات المحضرة لتقيم فاعلتها ضد سرطان القولون باستخدام الخلية السرطانية HCT- 116.

وقد اثبتت جميع التراكيب الكيميائية للمركبات الجديدة بواسطة التحليل الطيفى باستخدام طيف الكتلة و تاثير الاشعة تحت الحمراء و الرنين النووى المغناطيسى لكل من الهيدروجين والكربون .