RESEARCH ARTICLE

Synthesis and Characterization of (3-ethanethioate-4-N- cyclic imide-5-aryl)-1,2,4-triazole and Study Anticancer and Antioxidant

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ABSTRACT

New derivatives of 5-aryl-4-amino-3-thio- 1,2,4- triazole (a-d) were prepared from fusion subs. benzoic acids with thiocarbohydrazide. Then reacted with different cyclic anhydrides via stirring to give amic acid of 1,2,4-triazole (1-20). Compounds (1-20) convert to N-cyclic imides of subs. -1,2,4- triazole (21-40) by using acetic anhydride and anhydrous sodium as a water drawer agent (21-40).

Conclusion: Physical properties identified all prepared compounds, Fourier transform infrared spectroscopy (FTIR), ^{1}H-NMR for some of them, anticancer and antioxidant applications were measured**.**

Keywords: Anticancer and Antioxidant, Benzoic acid, FTIR, HNMR.

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Conflict of interest: None

INTRODUCTION

Cyclic imides are the current focus due to their wide spectrum of biological activities.[1-3] N-substituted cyclic imides have been recognized as one of the highly favorable scaffold, which contains the general structure (–CO–NH(R)–CO–) where (R) is a hydrogen atom, alkyl, or aryl and may be described as nitrogen analogous of anhydrides or as diacyl derivatives of ammonia or primary amines. They remain hydrophobic and neutral, and hence, they can cross biological membranes *in vivo*.[4]

The derivatives of 1,2,4-triazole always possess a wide range of biological properties such as insecticidal,[5] antifungal.[6] antimicrobial,[7] antiviral,[8] antitumors,[9] and anti-inflammatory.[10] Some derivatives have been widely used in agriculture and medicine, e.g., e, bromuconazole and fluconazole are being used as commercial anti fungicides for many years,[11] whereas anastrozole and vorozole have recently been developed and as anticancer drugs.[12] To explore the development of bioactive nitrogen consisting of heterocycles, we engaged in the design and efficient synthesis of imides of 1,2,4-triazole substructure, which would be expected to showcase important features because of the co-existence of two kinds of pharmacophore.

EXPERIMENTAL

Material

All used chemicals in this article were provided by Sigma aldrich. Fourier transform infrared radiation spectra were recorded on SHIMADZU fourier transform infrared spectroscopy (FTIR) - 8400 using KBr disc. ^{1}H nuclear magnetic resonance spectra were recorded on NMR spectrometer 400MHZ Avance 400 Bruker, Germany in the Central Laboratory of Isfahan University, Tahran, Iran, using tetramethylsilane as internal standard and DMSO-d6 as solvents. Melting points were measured on Gallen Kamp capillary melting point apparatus (University of Baghdad college of science) and were uncorrected. Antioxidant activity has been measured liver functions, and this study was carried out at the University of Babylon, Iraq.

Methods

General method for Synthesis of 3-thiol-4-Amino-5- aryl-1,2,4-triazole (a-d) [13, 14]

The mixture of substituted benzoic acid (0.01 mol) and thiocarbohydrazide (0.015 mol) was heated until it gets melted and then it was kept at this temperature for next 15-20 minutes. Further to that, after cooling, it was treated with a sodium bicarbonate solution in order to neutralize any possibility of unreacted carboxylic acid. Further to that, it was then cleaned with the help of water, collected by filtration method and recrystallized with ethanol and dioxane in ratio 1:1 to afford the title compounds.

General Method for Synthesis of amic acid of 1,2,4-triazole Dervatives (1-20)[15]

Dissolved (0.01 mmol) of compound (a-d) dissolved in (30 mL) of acetone and added to the solution of (0.01 mol) of cyclic

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anhydride (phthalic,3-nitro phthalic, itaconic, maleic and succinic) anhydride in (30 mL) of acetone with stirring the mixture for (3-4) hrs. The formed precipitate was filtered under observation, washed with ether, and then dried and purified by recrystallization with the help of a suitable solvent.

General method for Synthesis of 3- acetothio-4-(N- cyclic imide)-5- aryl- 1,2,4-triazole (21-40).

A mixture of amic acid of 1,2,4-triazole derivatives (1-20) (0.01 mole) were dissolved in (10 mL) acetic anhydride and (0.2 g) of anhydrous sodium acetate. The mixture was refluxed for 3 hours. after complete reflux, the obtained clear solution after cooling then poured into ice water with vigorous stirring. The product was filtered, washed with water several multiplications, dried and recrystallized from suitable solvent.

Assay of Antioxidant Activity

Animals: nine male rabbits with the body weight of (1000 ± 100) each were divided into three groups. The first group N served with control and received a normal diet along with 1ml of corn oil daily for one weak. The second and third groups are considered as a toxic control and received a normal diet with a daily dose of 2 mL/kg b.w of CCl_4 (i.p) with corn oil daily for one week; after that, these groups were given normal diet with a daily oral dose of the compounds (8,29 and 32) respectively in corn oil (100 mg/kg b.w) for one week. Then the blood samples were collected before and after giving the compounds (8,29 and 32) respectively and the obtained sera were ready for analysis of liver enzymes like (AST, ALT, Total protein and albumin) using chemical kits.

Maintenance of Cell Cultures

MCF-7 were maintained in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week, and further incubated at 37 °C.[16]

Cytotoxicity Assays

To determine the cytotoxic effect of x- substances, the MTT cell viability assay was done using 96-well plates. Cell lines were seeded at 1×10^4 cells/well. After 24 hours at 37°C, a stock solution of the compounds (14,30&38) have been prepared and diluted to desired concentrations (6.125, 12.5, 25, 50 and 100 µM) in a culture medium. Or a confluent monolayer was found. Cells were treated with tested compounds at different concentrations. Cell viability was measured 72 hours post-treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT stain and further incubating the cells for 2.5 h at a designated temperature of 37 °C. After removing the MTT solution, the remaining crystals were solubilized by adding 130 µL of DMSO (Dimethyl Sulphoxide) followed by proper 37°C incubation for 15 min with gentle shaking.[17] The absorbency was determined on a microplate reader at 492 nm and the assay was performed in triplicate. The following equation helped in calculation of inhibition rate of cell growth or in other terms the percentage of cytotoxicity: -

$$\text{Cytotoxicity} = A - B/A \times 100$$

Here (A) the optical density of control and (B) the optical density of Samples

Statistical Analysis

The data thus obtained here was statically analyzed considering an unpaired t-test with GraphPad Prism 6 and the values were put forward as the mean ± SEM of triplicate measurements.[18]

RESULT AND DISCUSSION

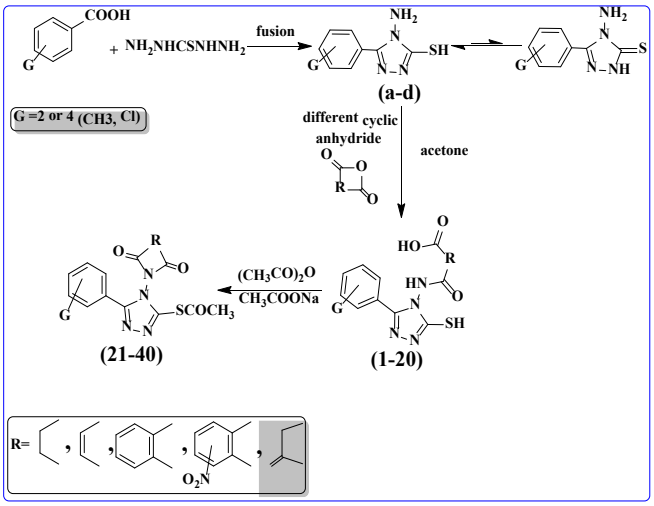
In this research 5-aryl-4-amino-3-thio-1,2,4- triazole (a-d) were prepared from fusion subs. benzoic acids with thiocarbohydrazide. Then reacted with different cyclic anhydrides via stirring to give amic acid of 1,2,4-triazole (1-20). Compounds (1-20) convert to N-cyclic imides of subs. -1,2,4-triazole (21-40) as shown in Scheme 1 and observed the rang of yield ratio was 76-93٪; the rang of measured melting point was [124-215], characterized by FT-IR spectra and ^{1}H-NMR spectra of some prepared compounds as shown in Table 1.

FT-IR spectra of compounds (a-d) represented the appearance of some bands at [(3475- 3364) asy -(3359- 3315) sym, (2648-2549), (1652-1643) and (1454-1421)] belong to ν(-NH2), ν(S-H), ν(C=N) and ν(N-N) respectively and disappearance band of ν(C=O) carboxylic acid. These bands and other absorption bands.

FTIR spectra of amic acid of 1,2,4-triazole derivatives (1-20) represented disappearance of a band of ν(-NH2) and appearance of the bands at (3287-3126), (2642-2511) (1733-1706), (1695- 1685) cm^{-1} belong to ν(-NH amide), ν(O-H carboxylic acid) ν(C=O carboxylic acid) and ν(C=O amide) respectively. These bands and other absorption bands.

^{1}H-NMR spectrum data of compounds (3, 4, 7 and 9) δ ppm in (DMSO) represented disappearance of the signal of NH_2 and appearance of the signals δ= 13.7-13.6(s, 1H, SH), 12.4- 12.2 (s, 1H, OH), 10.2-7.0 (s, 1H, NH), 8.4-7.0(m, 7H, HAr), 2.6- 2.42(s, 1H, CH_3) as shown in table (1).

The FTIR spectra of imides of 1,2,4-triazole derivatives (21-40) represented the disappearance of band of ν(-NH), ν(-SH) and ν(-OH carboxylic acid). And the appearance of



Scheme 1: Preparation of compounds (1-40)

the bands at (1775-1683) cm^{-1} belong to ν (C=O imide) and of [(3099-3037), (3006-2823), (1656-1610) and (1610-1490)] cm^{-1} belong to ν (C-H aromatic), ν (C-H aliphatic), ν(C=N), and ν(C=C aromatic) respectively for compounds (21-40).

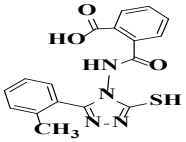
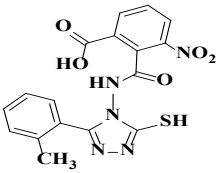
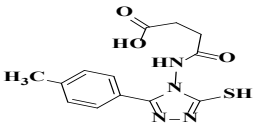
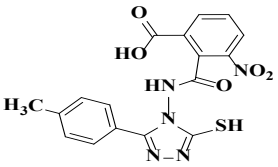
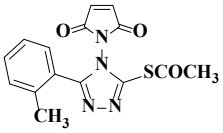
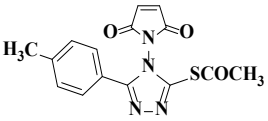
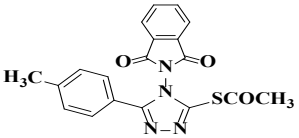
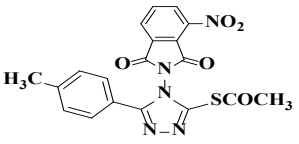
^{1}H-NMR spectrum data of compound (21) δ ppm in (DMSO) presented the appearance of the signals δ= 7.9(d, 1H, =CH), 8.4-7.3(m, 7H, HAr), 2.05(s, 3H, CH_3) and 1.37(s, 3H, $SCOCH_3$) and disappearance of signals of NH, OH and SH, as shown in Table 1.

^{1}H-NMR spectrum data of compound (26) δ ppm in (DMSO) presented the appearance of the signals of δ= 77.94 (d, 1H, =CH), 8.4-7.3(m, 7H, HAr), 2.48 (s, 3H, CH_3), 2.02(s, 3H, $SCOCH_3$) and disappearance of signals of NH, OH, and SH, as shown in Table 1.

^{1}H-NMR spectrum data of compound (28) δ ppm in (DMSO) presented an appearance of the signals of δ= 8.0-7.4(m, 7H, HAr), 2.47(s, 3H, $SCOCH_3$), 2.02 (s, 3H, CH_3) and disappearance of signals of NH, OH, and SH, as shown in Table 1.

^{1}H-NMR spectrum data of compound (29) δ ppm in (DMSO) presented an appearance of the signals of δ= 8.4-7.3(m, 7H, HAr), 2.4(s, 3H $SCOCH_3$), 2.02(s, 3H, CH_3) and disappearance of signals of NH, OH, and SH, as shown in Table 1.

Table 1: ^{1}H-NMR spectral data of some compounds [3,4,7,9,21,26,28 and 29]

Comp. No.	*Compound Structure*	*^{1}H-NMR spectral data δ ppm*
3		13.6(s, 1H, SH), 12.2(s, 1H, OH), 8.3(s, 1H, NH), 7.9-7.0(m, 7H, HAr), 2.6(s, 3H, CH_3).
4		13.6(s, 1H, SH), 12.2(s, 1H, OH), 10.2(s, 1H, NH), 8.4-7.3(m, 6H, HAr), 2.42(s, 3H, CH_3).
7		13.7(s, 1H, SH), 12.4(s,1H, OH), 7.0(s, 1H, NH), 8.3-7.3(m, 6H, HAr), 2.5(t, 2H, CH_2), 2.48(t, 2H, CH_2), 2.43(s, 3H, CH_3).
9		13.6(s, 1H, SH), 13.4(s, 1H, OH), 8.0(s, 1H, NH), 8.4-7.3(m, 8H, HAr), 2.6(s, 3H, CH_3).
21		7.9(d, 1H, CH), 8.4-7.3(m, 7H, HAr), 2.05(s, 3H, CH_3) 1.37(s, 3H, $SCOCH_3$).
26		7.9(d, 1H, CH), 8.4-7.3(m, 7H, HAr), 2.48(s, 3H, $SCOCH_3$), 2.02(s, 3H, CH_3).
28		8.0-7.4(m, 7H, HAr), 2.47(s, 3H, $SCOCH_3$), 2.02(s, 3H, CH_3).
29		8.4-7.3(m, 7H, HAr), 2.4(s, 3H, $SCOCH_3$), 2.0(s, 3H, CH_3).

Liver Function Tests (LFT)

The liver function parameters like (AST, ALT, TP and Alb) were measured at different times before and after treatment with ethanolic extract.

Liver Function Levels After Administration with CCl_4 for One Week.

All the liver function levels were measured after administration with CCl_4 for one week, as shown in Table 2.

In Table 2 G2, G3 and G4 were administrated with carbon tetrachloride (CCl_4), one of the oldest toxins utilized to induce hepatotoxicity in laboratory animals. CCl_4 is a selective hepatotoxic chemical agent and it is found to produce free radicals, which affect the cellular permeability of hepatocytes leading to elevated levels of liver enzymes.

While calculating the activities of serum marker enzymes like AST, ALT, total protein and albumin can assess liver function. When liver cell plasma membrane is damaged, various enzymes located in, are released into the bloodstream resulting in enhancing liver enzymes; it also explains the increase of the levels of AST and ALT significantly, while decrease of TP and Alb levels significantly, in G2, G3, and G4 that received CCl_4 for one week when they are compared with a control group (G1) as shown in Table 3.

The results in Tables (3) illustrated that levels of AST and ALT were decreased for G2, G3 and G4 after administration with the compounds (8, 29 and 32) compared with G2, G3 and G4 in Table 3 and G1 (control) in both Tables 3 and 4, while the levels of TP and Alb were increased as the control values of G1. Treatment with the compounds (8, 29 and 32) to CCl_4-intoxicated animals was found with significant hepatoprotective activity along with restoring the hepatocellular activity and stabilizing the plasma membrane that has been distributed by the effect of CCl_4. This is because the compounds (8, 29 and 42) have antioxidant properties affecting body defense system against reactive species accountable for developing many chronic and degenerative diseases.

Table 2: Liver function levels after administration with CCl4 for one Week to G2, G3 and G4 Compared with G1

Parameters	*Groups*	*Mean ± SD*
AST U/L	G1	80 ± 3.74
	G2	230 ± 21.16
	G3	251 ± 39.3
	G4	244 ± 26.5
ALT U/L	G1	74 ± 2.58
	G2	161 ± 24.49
	G3	180 ± 17.9
	G4	173 ± 20.5
TP g/dl	G1	6.6 ± 0.38
	G2	5.03 ± 0.21
	G3	5.25 ± 0.18
	G4	5.6 ± 0.23
Alb g/dl	G1	3.9 ± 0.52
	G2	2.51 ± 0.23
	G3	2.36 ± 0.28
	G4	2.26 ± 0.24

Application of Anticancer Cell Line

Combination Chemotherapy and Viral Cytotoxicity *in vitro*

The concentration-dependent manner used to study the potential interaction between the compounds (14, 30 and 38) with chemotherapy *in vitro* [287, 288]. The effectiveness of the combined treatment for several concentrations of the compounds (14, 30 and 38) with cell cancer have been used the breast cancer cells (MCF-7) and brain cancer cells (AMJM) various agglutination conditions were evaluated.

Most doses exhibited enhancement cytotoxicity for the combination of cell cancer with the compounds (14, 30 and 38). The percentages of viable cells were calculated and compared with untreated cells (289).

Inhibition of proliferation of human breast cancer cells (MCF-7) cells by the compounds (14, 30 and 38):

The investigation and comparison of the effects of the compounds (14,30&38) against cancer cell growth. Human breast cancer cells (MCF-7) cells were treated with the compounds (14,30 and 38) at different concentrations 6.125, 12.5, 25, 50 and 100 µg/mL for 72 hours., followed by 3-(4,5-dimethyltriazol2-yl)−2,5diphenyltetrazolium bromide (MTT) colorimetric assay (290). The cells were treated with the solvent DMSO which was used as control. Found that the compounds (14,30 and 38) were growth-inhibitory potency in a dose-dependent manner at most concentrations, as shown in Table 4.

In (MCF-7) cells, a breast cancer with the compound (14) resulted (55.86%) inhibition rate (IR) at concentration 100 µg/mL and the proliferation rate (PR = 7.91%) was

Table 3: Liver function levels after administration with compounds (8, 29 and 32) for one Week to G2, G3 and G4 Compared with G1

Parameters	*Groups*	*Mean ± SD*
AST U/L	G1	87 ± 4.94
	G2	104 ± 6.69
	G3	95 ± 9.26
	G4	103 ± 7.66
ALT U/L	G1	76 ± 5.57
	G2	96 ± 10.4
	G3	89 ± 7.86
	G4	93 ± 8.64
TP g/dL	G1	6.2 ± 0.61
	G2	5.2 ± 0.23
	G3	5.53 ± 0.25
	G4	5.91 ± 0.22
Alb g/dL	G1	3.8 ± 0. 18
	G2	2.63 ± 0.28
	G3	2.8 ± 0.26
	G4	2.7 ± 0.25

Table 4: Cytotoxicity assays of (MCF- 7) cells for compounds [14,31 and 50]

Comp. No.	*IR%, (C) µg/mL*	*PR%, (C) µg/mL*	*Other effect cytotoxicity%, (C) µg/mL*		
14	55.86(100)	7.91(6.125)	12.82(12.5)	20.70(25)	37.54(50)
30	41.85(100)	4.95(6.125)	10.21(12.5)	22.17(25)	28.34(50)
38	68.45(100)	13.65(6.125)	22.78(12.5)	30.12(25)	45.87(50)

statistically significant at concentration C = 6.125 µg/mL, while the effect of cytotoxicity at concentration 12.5µg/mL was 12.82%. At concentration 25 µg/mL cytotoxicity effect was 20.70% and in concentration 50 µg/mL cytotoxicity effect was 37.54 % as shown in Table 4.

In (MCF-7) cells, a breast cancer with the compound (30) resulted (41.85%) inhibition rate (IR) at concentration 100 µg/mL and the proliferation rate (PR = 4.95%) was statistically significant at concentration C = 6.125 µg/mL, while the effect of cytotoxicity at concentration 12.5µg/mL was 10.21%. At concentration 25 µg/mL cytotoxicity effect was 22.17% and in concentration 50 µg/mL cytotoxicity effect was 28.34 % as shown in Table 4.

In (MCF-7) cells, a breast cancer with the compound [38] resulted (68.45%) inhibition rate (IR) at concentration 100 µg/mL and the proliferation rate (PR = 13.65%) was statistically significant at concentration (C=6.125) µg/mL, while the effect of cytotoxicity at concentration 12.5µg/mL was 22.78%. At concentration 25 µg/mL cytotoxicity effect was 30.12% and in concentration 50 µg/mL cytotoxicity effect was 45.87% as shown in Table 4.

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