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Synthesis of 1,2,4-triazole derivatives and evaluation of their antioxidant activity

Hany A. M. El-Sherief[1,2], Mohamed Abdel-Aziz[3*], Hamdy M. Abdel-Rahman[1,4]

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Nahda University, Beni-suef, Egypt.
[2] Department of Pharmaceutical Chemistry, College of Pharmacy, Jouf University, Aljouf, Sakaka 2014, Saudi Arabia
[3] Department of Medicinal Chemistry, Faculty of Pharmacy, Minia University, 61519-Minia, Egypt.
[4] Department of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, 71526-Assiut, Egypt.

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Abstract

A series of 1,2,4-triazole scaffold containing alkoxy moiety **4a-b** and **9a-e** was synthesized by stirring compounds **3a-b** or **8a-d** at room temperature with different acid chlorides including 4-methoxy benzoyl chloride or 3,4,5-trimethoxybenzoyl chloride. The structure of the prepared compounds was confirmed using different spectroscopic techniques such as IR, ^{1}H NMR, ^{13}C NMR, Mass spectra and high resolution mass or elemental analysis. The synthesized compounds examined for their antioxidant activity using DPPH radical scavenging activity. Results indicated that most of the tested compounds exhibited moderate antioxidant activity. Compound **9b** exhibited remarkable antioxidant activity with DPPH radical scavenging rate of 49.4 % compared to trolox as a reference antioxidant agent at 10µM concentration.

Key words
1,2,4-Triazole , trolox , antioxidant activity

1. Introduction

Antioxidant agents have acquired a serious role in medicine due to their extensive preventive and therapeutic use in many diseases. Free radicals are continuously being formed either in the typical functioning of organs or in cases of exceptional oxidative stress [1]. Accordingly, the high level of free radicals can cause damage to biomolecules and consequently resulted in malignancy in lipids, enzymes, and in the DNA of bodily cells and tissue. It was recognized that a leading cause of cancer arises from DNA that is damaged because of oxidative stress [2]. The mutation of DNA was reported as an essential phase in carcinogenesis. Moreover, an elevated presence of oxidative DNA lesions was observed in various types of cancers. New perspectives on medicine have come from the realization that free radicals have an important role in cancer, as well as diabetes, cardiovascular, and auto-immune diseases, as well as neurodegenerative conditions and complications of aging [3]. The approach of limiting oxidative damage is proposed as a potential path to prevent these diseases by stopping the formation of free radicals or may possibly impede an oxidizing chain reaction [4]. Phenolic and polyhydroxy derivatives as gallocatechin **(I)**, epigallocatechin **(II)** and caffeic acid **(III)** (**Figure 1**) also were originated to illustrate a variety of useful properties such as radical scavenging [5], antiviral [6], antioxidant [7], anti-inflammatory [8], antiartherosclerotic activity [9], anticancer [10] and strong topoisomerase inhibiting activities [11]. During the last few decades, a considerable attention has been devoted to 1,2,4-triazole derivatives due to their wide spectrum of biological activities such as anticonvulsant [12], anticancer [13], antidepressant [14], antibacterial [15], antifungal [16], anti-inflammatory, analgesic [17] and antiviral activities [18]. Previously synthetic 1,2,4-triazole derivatives were found to exhibit important antioxidant activity (compounds **IV**, **V** and **VI**) (**Figure 2**) [19-21]. It has been reported that structural properties of triazoles, like moderate dipole character, hydrogen bonding capability, rigidity and stability under *in vivo* conditions are the main reasons for their superior pharmacological activities [22]. Promoted with the above mentioned data, this study was designed to develop some 1,2,4-triazole derivatives for the purpose of screening their radical scavenging activity, where the presence of 1,2,4-triazole ring would augment the bioavailability and chemical stability. The steric and electronic effects of different electron donating alkoxy groups on the benzene moiety of ring A and B were chosen to improve the physicochemical properties of these compounds.

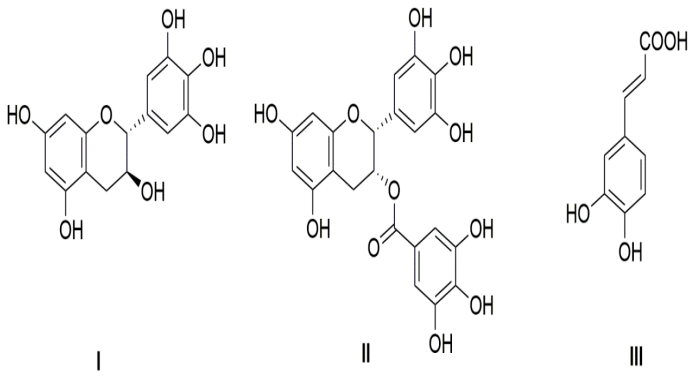


Figure 1: Chemical structure of gallocatechin (I), epigallocatechin (II) and caffeic acid (III).

**** Correspondence: M Abdel-Aziz***
Tel.: *+201003311327* ***; Fax:*** *+20 862369075*
Email Address: *abulnil@hotmail.com*

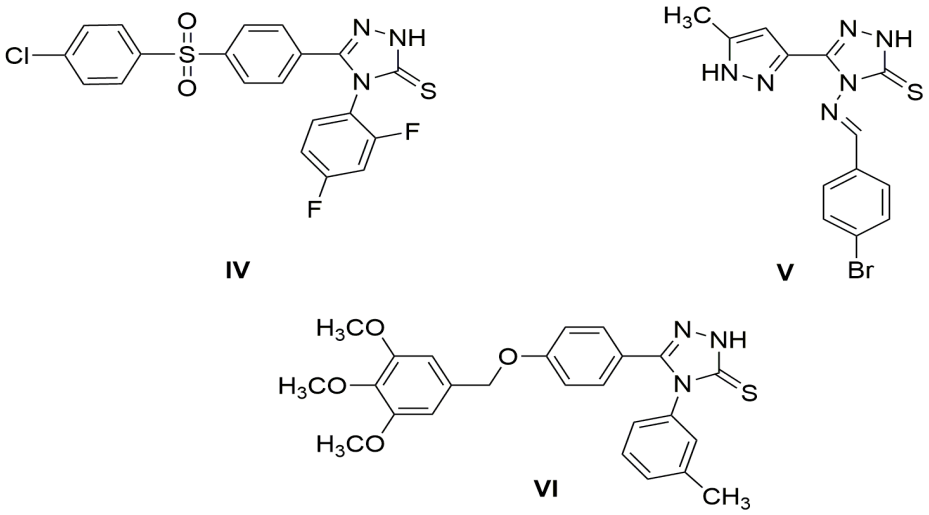


Figure 2: Chemical structure of some 1,2,4-triazoles with antioxidant activity (IV), (V) and (VI).

2. Experimental

2.1. Chemistry

2.1.1. General method for synthesis of (5-amino-3-(hetero) aroyl-1*H*-1,2,4-triazol-1-yl) (substituted phenyl) methanone 4a-b and 9a-e.

Substituted benzoyl chloride (1.1 mmol) was added in small portions to a stirred solution of the appropriate compounds **3a-b** or **8a-d** (1 mmol) in dry pyridine (10 mL) cooled to -5 ^{o}C. The reaction mixture was kept for 30 min at - 5 ^{o}C and then stirred overnight at room temperature. Reaction mixture was then poured into ice; the obtained precipitate was filtered off, dried and recrystallized from ethanol.

2.1.1.1. Synthesis of (5-amino-3-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazol-1-yl)(4-methoxyphenyl) methanone (9b) [23]

White powder (0.21 g, 55% yield), m.p. 189-191 ^{o}C, IR (KBr) υ max (cm^{-1}) 3150 (NH_2), 1695 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.72 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 3.89 (s, 3H, OCH_3), 7.14 (d, 2H, *J* = 8.8 Hz, OCH_3-Ar-H), 7.26 (s, 2H, NH_2), 7.79 (s, 2H, Ar-H), 8.29 (d, 2H, *J* = 8.8 Hz, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 55.88, 56.09, 56.50, 60.61, 104.21, 105.46, 114.07, 126.11, 134.15, 140.87, 153.50, 159.49, 163.69, 166.94; HRMS (ESI) m/z: calcd for $[M+H]^+$ $C_{19}H_{20}N_4O_5$: 385.1512; found: 385.1520.

2.2. Evaluation of antioxidant activity

The reported DPPH method was applied to assess the scavenging ability of the compounds. The compounds were tested in the range of 0-25 µg/mL in methanol. To 2.5 ml of compound in 3 different concentrations, 1 mL of 0.3 mM DPPH ethanol solution was added. Then 1 mL of methanol was added to the solution and allowed to react for 30 min in the dark at room temperature. The change in the absorbance was read at 518 nm. The blank was comprised of 2.5 mL of test compound and 1 mL methanol, while the mixture of 1 mL DPPH and 2.5 mL of methanol served as negative control. The percentage antioxidant activity was calculated as follows:

$$\%\ Inhibition = \frac{A_A - A_B}{A_B} \times 100$$

Where: A_B: absorption of blank sample, A_A: absorption of test samples. The percentage inhibition value was calculated and compared with that of Trolox as a reference [29].

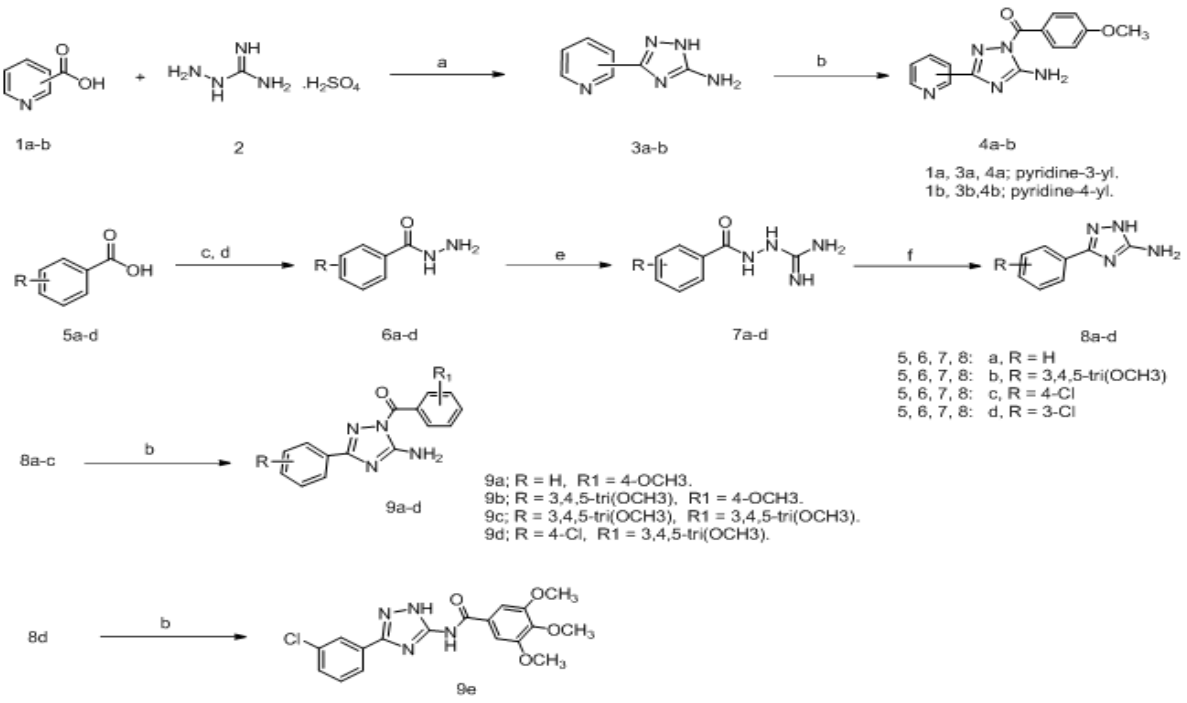
3. Result and discussion

3.1. Chemistry

The target compounds in Scheme 1 are selected from a group of 1,2,4-triazole derivatives synthesized in our laboratories and experienced significant anticancer activity against a panel of cancer cell lines. The results revealed that these compounds were discovered to be potent inhibitors of cancer cell proliferation and were also observed to be strong Tubulin inhibitors. The full details of their synthesis, spectroscopic and elemental characterization have been published recently [23].

Stirring at room temperature of compounds **3a-b** or **8a-d** with different acid chlorides afforded the target compounds **4a-b** and **9a-e**, respectively (**Scheme 1**). Acylation of aminotriazoles with acid chlorides at room temperature yielded a ring-acylated product rather than the amino-acylated one [24].

Interestingly, the 1-benzoylated-1,2,4-triazole derivatives underwent thermal rearrangement either in an organic inert solvent, e.g., sulfolane, dimethylformamide, dimethylsulfoxide or without solvent at a temperature of 200-250 ^{o}C to give the 5-benzamido-1,2,4-triazole derivatives [24]. Acylation of 5-amino-3-substituted-1,2,4-triazoles with acyl chlorides to yield either a ring-acylated product A or its 5-acylamino isomer B was intensively studied in the past 50 years by many authors [24-26] (**Figure 3**).



Scheme 1. Synthesis of the target compounds 4a-b and 9a-e.

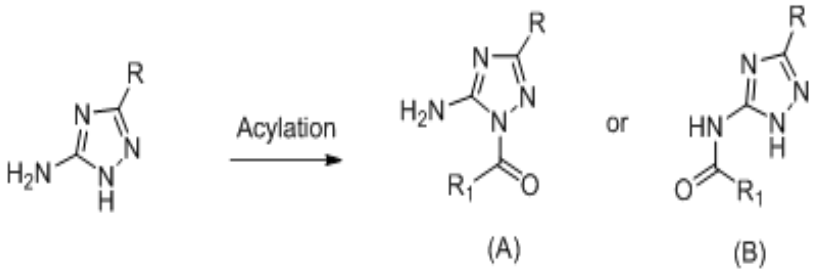


Figure 3: Isomeric structures of monoacylated 5-amino-1,2,4-triazole derivatives.

Nevertheless, structural elucidation of the obtained compounds was in many cases ambiguous. Reiter *et al*. [26] reported a detailed study on the isomeric and tautomeric structures of the monoacylated 5-amino-1,2,4-triazole derivatives with the help of the spectroscopic data. Studying the isomeric structures of type A and B monoacylated 5-amino-1,2,4-triazole derivatives with the help of their spectroscopic data resulted in the following conclusions. In the IR spectra of the type A derivatives, the NH_2 bands (regardless on the type of R and R_1) appeared in the region between 3400 and 3465 cm^{-1}.

On the other hand, the corresponding NH bands of the type B derivatives appeared as broad bands never exceeding the value of 3300 cm^{-1} giving a good possibility for the differentiation between them. The range of the carbonyl frequencies of type A derivatives (1680-1700 cm^{-1}) practically overlapped the range of those of the corresponding type B derivatives (1665-1685 cm^{-1}).

However, the carbonyl frequency of the type A derivatives was in all cases higher than that of the corresponding type B derivatives. In the ^1H NMR spectra of type A derivatives, the NH_2 protons appeared as singlet between 7.8 and 8.3 ppm. On the other hand, the two broad well-separated NH signals of type B derivatives ($NHCOR_1$ and N_1-H) appeared between 12.3-12.8 ppm ($NHCOR_1$) and 13.2-14.3 ppm (N_1-H), respectively.

This again makes it possible to differentiate between ring-acylated products A and their 5-acylamino isomers B. It was also reported that the annular acetylation of 5-amino-1*H*-[1,2,4]triazole is very fast and gives the monoacetylated kinetic product: 1-acetyl-3- amino-1*H*-[1,2,4]triazole, that undergoes isomerisation into the acetylamino derivative [27].

Reagents and reaction conditions: (a) heating at 210°c, 2 h; (b) substituted benzoyl chloride, pyridine, stirring for 30 min. at -5 °C, then at rt for 24 h; (c) H_2SO_4/CH_3OH, reflux, 4-8 h; (d) NH_2NH_2. H_2O, EtOH, reflux for 2-4 h; (e) S-methylthiourea, NaOH, stirring at rt for 72 h; (f) H_2O, reflux for 4 h.

Table 1: DPPH Radical scavenging activity of compounds 4a-b and 9a-e.

	Antioxidant activity %		
Compound	**100µM**	**50µM**	**10µM**
4a	82.7	53.3	24.6
4b	79.5	41.7	13.7
9a	88.5	57.3	29.5
9b	82.1	62.5	49.4
9c	60.4	39.3	19.7
9d	67.5	49.4	31.5
9e	79.2	52.6	39.3
Trolox	96.7	80.2	75.4

3.2. Evaluation of antioxidant activity using DPPH radical scavenging activity

The antioxidant capacity of the tested compounds **4a-b** and **9a-e** was studied through their scavenging activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The bleaching of DPPH was monitored at an absorbance of 518 nm. The percentage of DPPH radical scavenging rates was calculated using trolox (6-hydroxy-2,5,7,8-chroman-2-carboxylic acid) as a reference antioxidant at three different concentration 100 µM, 50 µM and 10 µM (**Table 1**) [28]. Seven compounds exhibited noticeable antioxidant activity with DPPH radical scavenging rates ranging from 19.7 to 49.4% at the concentration of 10 µM. Among the tested compounds, compound **9b** exhibited remarkable antioxidant activity among the tested compounds with DPPH radical scavenging rate of 49.4 %, the antioxidant activity of compound **9b** equal about 65.5 % the activity of the reference drug trolox at 10 µM. Compounds **9e** and **9d** exhibited good antioxidant activity with DPPH radical scavenging rate of 39.3 % and 31.5 %, respectively at 10 µM. Compounds **9a** and **4a** exhibited good antioxidant activity with DPPH radical scavenging rate of 29.5 % and 24.6 %, respectively at 10 µM. Several conclusions could be deduced from the above mentioned results; (1) the presence of phenyl moiety or substituted phenyl moiety in general at R position has a remarkable antioxidant activity compared to pyridine moiety (**9b**, **9e**, **9d** and **9a** versus **4a** and **4b**). (2) The presence of substituted phenyl moiety either with electron donating (OCH_3) or electron withdrawing (Cl) at R position is associated with the increase in the DPPH radical scavenging activity than of the unsubstituted phenyl compounds **9b**, **9e** and **9d** versus **9a**. (3) Increasing the number of methoxy groups as in compound **9c** is associated with the decreasing of their DPPH radical scavenging activity.

The new synthetic 1,2,4-triazole containing compounds, prepared in this study exhibited noticeable antioxidant activity, in the same time most of these tested compounds showed noteworthy antiproliferative effects against four human cancer cell lines including human pancreas cancer cell line (Panc-1), pancreatic carcinoma cells (PaCa-2), colon cancer cells (HT-29) and lung cancer cells (H-460) using the propidium iodide (PI) fluorescence assay with IC_{50} values < 2.0 µM [24]. And this may support the idea that the antioxidant activity of these 1,2,4-triazole derivatives may be one of the mechanisms of action of these compounds as antiproliferative agents.

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References

[1] M. Valko, C.J. Rhodes, J. Moncol, M. Izakovic, M. Mazur, Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.* 2006, 160, 1-40.

[2] W. Droge, Free radicals in the physiological control of cell function. *Physiol. Rev*. 2002, 82, 47-95.

[3] D.V. Ratnam, D.D. Ankola, V. Bhardwaj, D.K. Sahana, M.N. Kumar, Role of antioxidants in prophylaxis and therapy: a pharmaceutical perspective. *J. Contr. Release*. 2006, 113, 189-207.

[4] C. C. Danta, P. Piplani, The discovery and development of new potential antioxidant agents for the treatment of neurodegenerative diseases. *Expert Opin. Drug Discov.* 2014, 9, 1205-22.

[5] T. Yokozawa, C.P. Chen, E. Dong, T. Tanaka, G-I Nonaka, I. Nishioka, Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2-picrylhydrazyl radical. *Biochem. Pharmacol.* 1998, 56, 213-22.

[6] T. Okuda, Systematics and health effects of chemically distinct tannins in medicinal plants. *Phytochemistry.* 2005, 66: 2012-31.

[7] A. Torres de Pinedo A, P. Penalver, J. C. Morales, New phenolicbased antioxidants: structure-activity relationships. *Food Chem.* 2007, 103: 55-61.

[8] N. Nazir, S. Koul, M. A. Qurishi, S. C. Taneja, S. F. Ahmad, S. Bani S, G. N. Qazi, Immunomodulatory effect of bergenin and norbergenin against adjuvant-induced arthritis: a flow cytometric study. *J. Ethnopharmacol***.** 2007, 112, 401-5.

[9] F. Visioli, G. Bellomo, G. Montedoro, C. Galli, Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. Atherosclerosis. 1995, 117, 25-32.

[10] G. S. Oh, H. O. Pae, H. Oh, S. G. Hong, I. K. Kim, K. Y. Chai, Y. G. Yun, T. O. Kwon, H. T. Chung, In vitro anti-proliferative effect of 1,2,3,4,6-penta-O-galloyl-beta-D-glucose on human hepatocellular carcinoma cell line, SK-HEP-1 cells. *Cancer Lett.* 2001, 174, 17-24.

[11] M. Abdel-Aziz, K. Matsuda, M. Otsuka, M. Uyeda, T. Okawara, K. Suzuki, Inhibitory activities against topoisomerase I and II by polyhydroxybenzoyl amide derivatives and their structure-activity relationship. *Bioorg. Med. Chem. Lett.* 2004, 14, 1669-72.

[12] V.K. Kamboj, P.K. Verma, A. Dhanda, S. Ranjan, 1,2,4-triazole derivatives as potential scaffold for anticonvulsant activity. *Cent. Nerv. Syst. Agents Med. Chem.* 2015, 15, 17-22.

[13] I. Ali , J. Lee, A. Go, G. Choi, K. Lee, Discovery of novel [1,2,4]triazolo[4,3-a]quinoxaline aminophenyl derivatives as BET inhibitors for cancer treatment. *Bioorg. Med. Chem. Lett.* 2017, 27, 4606-13.
[14] S. Jubie S, P. Sikdar, S. Antony, R. Kalirajan, B. Gowramma, S. Gomathy, K. Elango, Synthesis and biological evaluation of some schiff bases of [4-(amino)-5-phenyl-4H-1, 2,4-triazole-3-thiol]. *Pak. J. Pharm. Sci.* 2011, 24, 109-12.
[15] T. Karabasanagouda, A.V. Adhikari, N.S. Shetty, Synthesis and antimicrobial activities of some novel 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles and 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines carrying thioalkyl and sulphonyl phenoxy moieties. *Eur. J. Med. Chem.* 2007, 42, 521-29.
[16] K. Sztanke, T. Tuzimski, J. Rzymowska, K. Pasternak, M. Kandefer-Szerszen´, Synthesis, determination of the lipophilicity, anticancer and antimicrobial properties of some fused 1,2,4-triazole derivatives. *Eur. J. Med. Chem.* 2008, 43, 404-19.
[17] D. Sarigol, A. Uzgoren-Baran, B.C. Tel, E.I. Somuncuoglu, I. Kazkayasi, K. Ozadali-Sari, O. Unsal-Tan, G. Okay, M. Ertan, B. Tozkoparan, Novel thiazolo[3,2-b]-1,2,4-triazoles derived from naproxen with analgesic/anti-inflammatory properties: Synthesis, biological evaluation and molecular modeling studies. *Bioorg. Med. Chem.* 2015, 23, 2518-28.
[18] I. Küçükgüzel, E. Tatar, S.G. Küçükgüzel, S. Rollas, E. De Clercq, Synthesis of some novel thiourea derivatives obtained from 5-[(4-aminophenoxy)methyl]-4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thiones and evaluation as antiviral/anti-HIV and anti-tuberculosis agents. *Eur. J. Med. Chem.* 2008, 43, 381- 92.
[19] S-F Barbuceanu, D. C. Ilies, G. Saramet, V. Uivarosi, C. Draghici, V. Radulescu, Synthesis and antioxidant activity evaluation of new compounds from hydrazinecarbothioamide and 1,2,4-triazole class containing diarylsulfone and 2,4-difluorophenyl moieties. *Int. J. Mol. Sci.* 2014, 15, 10908-925.
[20] K. Karrouchi a,c, L. Chemlal, J. Taoufik, Y. Cherrah, S. Radi, M. El Abbes Faouzi, M. Ansar, Synthesis, antioxidant and analgesic activities of Schiff bases of 4-amino-1,2,4-triazole derivatives containing a pyrazole moiety. *Ann. Pharm. Fr.* 2016, 74, 431-8.
[21] H. S. Kareem, N. Nordin, T. Heidelberg, A. Abdul-Aziz, A. Ariffin, Conjugated Oligo-Aromatic Compounds Bearing a 3,4,5-Trimethoxy Moiety: Investigation of Their Antioxidant Activity Correlated with a DFT Study. *Molecules.* 2016, 21, 224-42.
[22] J. Stefanska, D. Szulczyk, A. E. Koziol, B. Miroslaw, E. Kedzierska, S. Fidecka, B. Busonera, G. Sanna, G. Giliberti, P. La Colla, M. Struga. Disubstituted thiourea derivatives and their activity on CNS: Synthesis and biological evaluation. *Eur. J. Med. Chem.* 2012, 55, 205-13.
[23] H. A. M. El-Sherief, B. G. M. Youssif, M. Abdel-Aziz, H. M. Abdel-Rahman, Novel 1,2,4-triazole derivatives as potential anticancer agents: Design, synthesis, molecular docking and mechanistic studies. *Bioorg. Chem.* 2018, 76, 314-25.
[24] N.P. Peet, L.E. Baugh, S. Sunder, J.E. Lewis, E.H. Matthews, E.L. Olberding, D.N. Shah, 3-(1H-Tetrazol-5-yl)-4(3H)-quinazolinone sodium salt (MDL 427): a new antiallergic agent. *J. Med. Chem.* 1986, 29, 2403-09.
[25] A. Dzygiel, B. Rzeszotarska, E. Masiukiewicz, P. Cmoch, B. Kamienski, Synthesis, structure and properties of N-acetylated derivatives of methyl 5-amino-1H-[1,2,4]triazole-3-carboxylate. *Chem. Pharm. Bull.* 2004, 52, 192-198.
[26] J. Reiter, L. Pongo, P. Dvortsak, On triazoles. VI. The acylation of 5-amino-1,2,4-triazoles. *J. Heterocycl. Chem.* 1987, 24, 127-42.
[27] A. Dzygiel, E. Masiukiewicz, B. Rzeszotarska, Acetylation of 5-Amino-1H-[1,2,4]triazole Revisited. *J. Agric. Food Chem.* 2002, 50, 1383-88.
[28] M.S. Blois, Antioxidant determinations by the use of a stable free radical. *Nature*, 1958, 181, 1199-200.
[29] S.N. Bukhari, I. Jantan, V.H. Masand, D.T. Mahajan, M. Sher, M. Naeem-ul-Hassan, M.W. Amjad, Synthesis of alpha, beta-unsaturated carbonyl based compounds as acetylcholinesterase and butyrylcholinesterase inhibitors: characterization, molecular modeling, QSAR studies and effect against amyloid beta-induced cytotoxicity. *Eur. J. Med. Chem.* 2014, 83, 355-65.