

ABSTRACT

To medicate new ascending diseases, innovative drug molecules remained continuously under investigation by the medicinal industries. Synthetic chemistry has played much important role in this respect for the development of new drug molecules. The significance of synthetic chemistry in the field of pharmacy can be guessed by increasing number of synthetic bioactive compounds day by day. The existing effort of coupling different bioactive functionalities is also about the synthesis of some new bioactive candidates. The esteemed biological activities of derivatives of 1,2,4-triazole triggered us to synthesize some novel scaffolds to establish their enzyme inhibitory potentials and computational docking imitations and find out their cytotoxic activities.

This effort has been stretched into different eight (8) schemes. The preliminary compound, *p*-anisic acid (4-methoxybenzoic acid) (1) used to synthesize ethyl-4-methoxybenzoate (2) which was geared up by refluxing compound (2) in methanol and hydrazine hydrate to get its 4-methoxybenzohydrazide (3). Here 4-methoxybenzohydrazide (3) was divided into two parts. One of the parts 3 was refluxed with phenyl isothiocyanate (4) in methanol to obtain the solid intermediate hydrazinecarbothioamide (5) which was further cyclized to get 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol (6). This nucleophile 6 was dissolved in DMF and one pinch of LiH was added to the mixture which further stirred for 15 - 20 min. to activate its thiol position. The nucleophilic substitution reaction was carried out with equimolar amounts of different aralkyl/alkyl halides 7(a-s), one in each reaction to achieve the targeted derivatives; *S*-aralkylated/alkylated 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols 8(a-s) (Scheme-17).

The electrophiles 11(a-k) were synthesized by reacting 2-bromoacetyl bromide (10) with un/substituted amines (9) in complementary set of reactions. Lastly, nucleophile 6 was dissolved in DMF and activated by adding a pinch of LiH and then reacted with equimolar quantity of electrophiles 11(a-k); one in each reaction to yield eleven compounds as *N*-(substitutedphenyl)-2-(5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-ylthiol)acetamides 12(a-k) (Scheme-18) by the cyclized compound (6).

The electrophiles 14(a-d) were synthesized by reacting 3-bromopropanoyl chloride (13) with un/substituted amines (9) in complementary set of reactions. In the end, nucleophile 6 was dissolved in DMF and activated by adding a pinch of LiH and then reacted with equimolar quantity of electrophiles 14(a-d); one in each reaction to synthesis four compounds

as *N*-(substitutedphenyl)-3-(5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-ylthiol)propanamides **15(a-d)** (**Scheme-19**) by the cyclized compound (**6**).

The electrophiles **17(a-k)** were synthesized by reacting 4-chlorobutanoyl chloride (**16**) with un/substituted amines (**9**) in complementary set of reactions. The nucleophile **6** was dissolved in DMF and activated by adding a pinch of LiH and then reacted with equimolar quantity of electrophiles **17(a-k)**; one in each reaction to yield eleven compounds as *N*-(substitutedphenyl)-4-(5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-ylthiol)butanamides **18(a-k)** (**Scheme-20**) by the cyclized compound (**6**).

Other part of 4-methoxybenzohydrazide (**3**) precipitates were refluxed with ethyl isothiocyanate (**19**) in methanol to obtain an intermediary compound, *N*-ethyl-2-(4-methoxybenzoyl)hydrazinecarbothioamide (**20**) which was cyclized to obtain a solid nucleophile, 4-ethyl-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol (**21**). This heterocyclic nucleophile (**21**) was then treated with equimolar amounts of various aralkyl/alkyl halides **7b**, **7(f-i)**, **7(k-o)**, **7(q-t)** acting as electrophiles, to acquire the targeted hybrid molecules *S*-alkyl/aralkylated 4-ethyl-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiols **22(a-n)** (**Scheme-21**).

Sixteen heterocyclic acetamides; *N*-(substitutedphenyl)-2-(4-ethyl-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-ylthiol)acetamides **23(a-p)** were designed by coupling compound **21** with different electrophiles **11(a-p)** in DMF using LiH as an activator and outlined in **Scheme-22**.

Three heterocyclic propanamides, *N*-(substitutedphenyl)-3-(4-ethyl-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-ylthiol)propanamides **24(a-c)** were designed by coupling compound **21** with different electrophiles, **14(a-c)** in DMF using LiH as an activator and outlined in **Scheme-23**.

Twelve heterocyclic butanamides; *N*-(substitutedphenyl)-4-(4-ethyl-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-ylthiol)butanamides **25(a-l)** were designed by coupling compound **21** with different electrophiles **17(a-l)** in DMF using LiH as an activator and outlined in **Scheme-24**.

The physical statistics of all synthesized compounds are determined which included color, state, yield, melting points (in case of solids), molecular formula and molecular mass. FTIR (Fourier Transformer Infra-Red), ¹H-NMR (Proton Nuclear Magnetic Resonance), ¹³C-NMR (Carbon-13 Nuclear Magnetic Resonance) and EIMS (Electron Impact Mass Spectrometry) has been used for structure evaluation for these synthesized scaffolds. Some

of the $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, EI-MS and FTIR spectra of synthesized compounds are also presented here as representative of a series.

To find out the mode of action of synthesized scaffolds and IC_{50} values, the enzyme inhibition activity of these synthesized compounds against acetyl/butyryl cholinesterase was done. The enzyme inhibition statistics is also explicated in detail through molecular docking studies and finally found out their % cell viability (cytotoxicity). The reference standards used were Eserine for AChE/BChE inhibition and DMSO for % cell viability (cytotoxicity) evaluation. Most of the compounds were found active and showed excellent or good results. The biological activity data in comparison of each scheme with the reference standard drugs is presented in results and discussion section. Molecular docking was done for each compound to relate and ascertain structure and activity relationship of synthesized scaffolds.