ABSTRACT

New drug candidates remained under consideration by the pharmaceutical industries to cure new arising diseases. To fulfill the requirement of new drug candidates, synthetic chemistry has been added much in this regard. Many bioactive compounds have been synthesized by different fields of Chemistry which emphasize the significance of synthetic chemistry in the field of pharmacy. The presented work is also about the synthesis of some new bioactive molecules by coupling different bioactive functionalities. The valuable biological activities of derivatives of thiazole and 1,2,4-triazole prompted us to synthesize some new compounds comprising amalgamation of these functionalities; and to demonstrate their enzyme inhibitory potentials supported by kinetic studies and computational docking simulations and find out their % hemolytic activities.

The presented work has been extended into six (6) different schemes. In first scheme the starting compound, Ethyl 2-(2-Amino-1,3-thiazol-4-yl) acetate (**1**) synthesis was geared up by refluxing compound (**1**) with methanol and hydrazine hydrate to get its acetohydrazide (**2**). The compound **2** was further refluxed with ethyl isothiocyante **(3)** in methanol to obtain the solid intermediate hydrazinecarbothioamide (**4**) and further cyclized to get 5-[(2-Amino-1,3-thiazol-4-yl) methyl]-4-ethyl-4*H*-1,2,4-triazole-3-thiol (**5**). The electrophiles (**8a-d,f-j,l-o)** were synthesized by reacting 3-(chloromethyl)benzoyl chloride **(7)** with un/substituted anilines (**6a-d,f-j,l-o**) in complementary set of reactions. Then nucleophile **(5)** dissolved in DMF and one pinch of LiH added to the mixture and stirred for 15 - 20 min to activate its mercapto position. Finally, the nucleophilic substitution reaction was carried out with equimolar amounts of different benzamides (**8a-d,f-j,l-o)** one in each reaction to achieve the targeted derivatives (3-[({5-[(2-amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)methyl]-N-(aryl)benzamides), **9a-m** (Scheme-1).

Eleven compounds were synthesized (4-[({5-[(2-amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)methyl]-N-(aryl)benzamides), (**12a-k**, Scheme-2) by the cyclized compound (**5**). The electrophiles (**11b-l**) were synthesized by reacting 4-(chloromethyl)benzoyl chloride (**10**) with un/substituted amine (**6b-l**) in complementary set of reaction. Finally, nucleophile **5** was dissolved in DMF and activated by adding a pinch of LiH and then reacted with equimolar quantity of electrophiles, **11b-l** (one in each reaction) to yield **12a-k** molecules. (Scheme-2) VA

Fourteen phenylated bi-heterocyclic benzamides, (3-[({5-[(2-amino-1,3-thiazol-4-yl)methyl]-4-phenyl-4H-1,2,4-triazol-3-yl}sulfanyl)methyl]-N-(aryl)benzamides), **16a-n** were designed by refluxing compound **2** with phenyl isothiocyante **(13)** in methanol to obtain the solid intermediate hydrazinecarbothioamide (**14**) which was further cyclized to get 5-[(2-Amino-1,3-thiazol-4-yl) methyl]-4-phenyl-4*H*-1,2,4-triazole-3-thiol (**15**). Then nucleophile **15** was dissolved in DMF and one pinch of LiH added to the mixture and stirred for 15 - 20 min to activate its mercapto position. Finally, the nucleophilic substitution reaction was carried out with equimolar amounts of different benzamides (**8a-j,l-o)** one in each reaction) to achieve the targeted derivatives (3-[({5-[(2-amino-1,3-thiazol-4-yl)methyl]-4-phenyl-4H-1,2,4-triazol-3-yl}sulfanyl)methyl]-N-(aryl)benzamides), **16a-n** (Scheme-3).
In Scheme 4, nucleophile **15** was dissolved in DMF and one pinch of LiH added to the mixture and was stirred for 15 - 20 min to activate its mercapto position. Finally, the nucleophilic substitution reaction carried out with equimolar amounts of different benzamides (**11b-l**, one in each reaction) to achieve the targeted derivatives (4-[({5-[(2-amino-1,3-thiazol-4-yl)methyl]-4-phenyl-4H-1,2,4-triazol-3-yl}sulfanyl)methyl]-N-(aryl)benzamides), **17a-k** (Scheme-4)
The designed multi-functional benzamides (3-[({5-[(2-amino-1,3-thiazol-4-yl)methyl]-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl}sulfanyl)methyl]-N-(aryl)benzamides), (**21a-l**) were produced by several steps through a convergent approach as designated in Scheme-5. The compound **2** was refluxed with 4-nitrophenyl isothiocyante **(18)** in methanol to obtain the solid intermediate hydrazinecarbothioamide (**19**) which was further cyclized to get 5-[(2-Amino-1,3-thiazol-4-yl) methyl]-4-(4-nitrophenyl)-4*H*-1,2,4-triazole-3-thiol (**20**). Then nucleophile **20** was dissolved in DMF and one pinch of LiH was added to the mixture and was stirred for 15 - 20 min to activate its mercapto position. Finally, the nucleophilic substitution reaction was carried out with equimolar amounts of different benzamides (**8a-o,g-j,m-o)** one in each reaction) to achieve the targeted derivatives (3-[({5-[(2-amino-1,3-thiazol-4-yl)methyl]-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl}sulfanyl)methyl]-N-(aryl)benzamides), **21a-l** (Scheme-5)
In Scheme 6, compound **20** was dissolved in DMF and one pinch of LiH was added and stirred for 15 - 20 min to activate its mercapto position. Finally, the nucleophilic substitution reaction was performed with equimolar amounts of different benzamides (**11b,c,e-l**, one in each reaction) to achieve the targeted derivatives (4-[({5-[(2-amino-1,3-thiazol-4-yl)methyl]-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl}sulfanyl)methyl]-N-(aryl)benzamides), **22a-j** (Scheme-6). VA

The structural characterization have been well supported by the spectral data of IR (Infra-Red), ^{1}H-NMR (Proton Nuclear Magnetic Resonance), ^{13}C-NMR (Carbon-13 Nuclear Magnetic Resonance), HMBC (Heteronuclear Multiple Bond Correlation), HMQC (Heteronuclear Multiple Quantum Coherence) and EIMS (Electron Impact Mass Spectrometry). Some of the1H-NMR, 13C-NMR, EIMS and IR spectra of synthesized compounds are also presented for the obvious perceptive of signals. The physical data of all the compounds was also provided which includes color, state, yield, melting points (in case of solids), molecular formula and molecular mass.

The enzyme inhibitory activity of these synthesized compounds against tyrosinase/carbonic anhydrase/alkaline phosphatase/urease/acetyl cholinesterase was done along with their kinetic studies to find out the mode of action inhibitors and inhibition constant. The enzyme inhibition data is also explicated in detail through molecular docking studies and found out their % hemolytic activity. The reference standards used were Kojic acid for tyrosinase, Acetazolamide for carbonic anhydrase, KH_2PO_4 for alkaline phosphatase, Thiourea for urease, Neostigmine methylsulfate for acetyl cholinesterase and Triton X-100 for % hemolytic evaluation.

All the compounds were found active and showed excellent activity. The biological activity data in comparison of each scheme with the reference standard drugs is presented in results and discussion section.