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The work embodied in this thesis concerns with the synthesis of a number of fused 1,2,4-triazoles as well as 4-functionalized pyrazoles as carbonic anhydrase (CA). Another part of the thesis work pertains to the development of facile and efficient synthetic methodologies for 3-chloroacrylonitriles, propynenitriles and triazolothiones. The thesis is organized in the form of two sections consisting of six chapters in all. Section A, comprising of chapters 1-3, describes the general introduction to carbonic anhydrases (CAs) and their inhibitors, synthesis, characterization and biological testing results of fused 1,2,4-triazoles and 4-functionalized pyrazoles. Section B, comprising of chapters 6 and 7, presents facile and efficient synthetic methodologies for 3-chloroacrylonitriles, propynenitriles and triazolothiones.

The biological evaluation of fused triazoles and 4-functionalized pyrazoles as CA inhibitors reported in Section A has been carried out in the laboratories of our collaborator Prof. Claudiu T. Supuran at Laboratorio di chimica bioinorganica, University of Florence, Italy.

Chapter 1 of section A of the thesis describes a brief review of CAs while each of the chapters 2, 3, 4, 5 and 6 begins with a brief description highlighting the motivation for undertaking the present work. This is followed by a detailed discussion of the synthetic methodology used, and elucidation of the structures of the compounds thus synthesized through rigorous analysis of their IR, NMR as well as Mass spectral data. This is followed by biological evaluation (in case of chapter 2 and 3 only), conclusions and experimental details while adequate bibliography has been appended at the end of each chapter. Chapters 4 and 5 open up with a brief description of synthetic importance of 3-chloropropenenitriles and alkynenitriles respectively followed by a detailed discussion of the novel methodologies developed for their efficient synthesis. Chapter 6 starts with introduction to 1,2,4-triazoles followed by their biological importance and literature methods of preparation. Motivation for current work and optimization of protocol is discussed subsequently. For the sake of easy reference to work described in various chapters in the thesis, original numbers for the compounds, figures and schemes used in the chapters are retained in the summary. This arrangement may seem a bit out of place because of the discontinuity
of structure numbers, but would be helpful in finding the relevant section in the respective chapters in the thesis.

Chapter 1. Carbonic Anhydrase (CA) Inhibitors – A Brief Review

The carbonic anhydrase enzymes (CA, EC 4.2.1.1) are zinc containing metalloproteins which efficiently catalyse the reversible conversion of carbon dioxide to bicarbonate and release protons. The enzymes are essentially important for biological system and play crucial role in various physiological functions. There are 16 different isoforms of α class of CAs differing widely in their cellular localization and biophysical properties. Since their discovery, targeting CAs for clinical drug discoveries has been at the forefront of scientific research and many drugs based upon them have been developed and are in clinical use. hCA IX and XII are dimeric transmembrane human associated CA isoforms having extracellular active site and are marker for a broad spectrum of hypoxic tumor types. Thus specifically targeting the tumor associated isoforms hCA IX and XII over the main off-target isoforms hCA I and II, which have a physiological relevance, using specific inhibitors is considered to be a promising strategy in the cancer therapy. This chapter describes the overview of CAs, their mechanism of action as well as inhibition. This chapter also provides a brief review of selective inhibitors of hCA IX and XII isoforms of CAs over hCA I and II.

Chapter 2. Synthesis and Biological Evaluation of Triazolothiadiazines and Triazolothiadiazoles as Human CA Inhibitors, Anti-Inflammatory and Antimicrobial Agents

Carbonic anhydrases (CAs, EC, 4.2.1.1) are the omnipresent metalloenzymes, present throughout the living organisms and are encoded by five evolutionarily unrelated gene families: α, β, γ, δ and ζ-CAs. hCA I and II are cytosolic isoforms that are widespread throughout the human body while hCA IX and XII are dimeric transmembrane isoforms responsible for tumor progression. Thus using specific inhibitors of tumor associated isoforms hCA IX and XII over the main off-target isoforms hCA I and II is increasingly catching the fancy of researchers as a primary strategy in cancer therapy. Sulfonamides are the most widely investigated class of CA
inhibitors possessing significant inhibitory power against many isoforms of CA. The class of heterocyclic compounds containing 1,2,4-triazole scaffold has also been increasingly attracting the attention of researchers for a long time due to their wide spectrum of biological activities. These findings motivated us to synthesize benzenesulfonamide bearing triazolothiadiazines (3) and triazolothiadiazoles (4) as CA inhibitors, anti-inflammatory and antimicrobial agents (Figure 2.2).

Figure 2.2. Triazolothiadiazines (3a-3g) and triazolothiadiazoles (4a-4g) as CA inhibitors, anti-inflammatory and antimicrobial agents.

The synthetic route adopted for the synthesis of target compounds is outlined in Scheme 2.1. Aminotriazole 8 was the key intermediate in the preparation of triazolothiadiazines (3) and triazolothiadiazoles (4), which was prepared from 4-aminosulfonylbenzoic acid hydrazide 6. The later was prepared by refluxing an aqueous suspension of p-toluenesulfonamide 5 with KMnO4 to generate the corresponding carboxylic acid followed by refluxing its methanolic solution firstly in acidic conditions and then in excess of hydrazine hydrate. Subsequently, the required dithiocarbazinate 7 was prepared by reacting hydrazide 6 with carbon disulfide and potassium hydroxide in anhydrous ethanol. The salt 7 underwent ring closure on refluxing with an excess of hydrazine hydrate to give aminotriazole 8. The compound 8 thus obtained was treated with variously substituted phenacyl bromides in ethanol under reflux conditions to obtain the final compounds triazolothiadiazines (3). To prepare triazolothiadiazoles (4), sulfonamide group was first protected as its formamidine 9 following a green methodology developed recently by our research group. Compound 9 was then reacted with aromatic acids under the conditions of reflux in POCl3 which resulted into successful conversion to fused bicyclic triazolothiadiazoles 10. The final compounds 4 were obtained by deprotection of sulfonamide group in 10 under acidic conditions.
Structures of all the target compounds and their precursors have been confirmed by rigorous analysis of their spectral data (IR, $^1$H NMR, $^{13}$C NMR and mass). Newly synthesized 14 target compounds viz. triazolothiadiazines 3 and triazolothiadiazoles 4 were evaluated as inhibitors against four isoforms of $\alpha$-class of human CAs comprising two cytosolic, ubiquitous forms hCA I and hCA II as well as the tumor associated transmembrane isoforms hCA IX and hCA XII. For the purpose of exploring biological applications, they were also evaluated as anti-inflammatory as well as antimicrobial agents. Triazolothiadiazines 3 were the most potent compounds exhibiting moderate to excellent inhibitory potential against all of the four hCA
isoforms. However, triazolothiadiazoles 4 were comparatively weaker inhibitors but highly selective against tumor associated isoforms over the off-target cytosolic isoforms. As it has been evidenced that hCA isoforms IX and XII are responsible for tumours and are also potential targets for diagnosis and treatment of cancers, discovery of potent selective hCA IX and XII inhibitors will be a promising step in the strategy for an effective cancer therapy.

Chapter 3. Synthesis and Biological Evaluation of 4-Functionalized Pyrazoles as Human CA Inhibitors, Anti-Inflammatory and Antimicrobial Agents

Small and simple thiazole as well as benzothiazole nuclei have made their presence felt in the drug arena as thiazole ring is present in established carbonic anhydrase inhibitor (CAI) drugs like ethoxzolamide (EZA) and some derivatives of benzothiazoles (compounds 1, 2 and 3) containing sulfonamide moiety have exhibited excellent inhibitory potential against many of the CA isoforms (Figure 3.1). Of late, 4-functionalized pyrazoles are also attracting considerable attention as potential CAIs besides being associated with a diverse range of biological activities. Further, heterocycles bearing primary sulfonamides are the most widely investigated class of CAIs that led to the development of several classes of pharmaceutical agents including clinically used CAI drugs like acetazolamide (AZA), methazolamide (MZA) etc. (Figure 3.1).

These findings motivated us to synthesize a series of 1,3-diarylpyrazole-4-carboxamides (4) as CA inhibitors as well as antimicrobial agents (Figure 3.2). Excellent results of CA inhibition as well as antimicrobial activity profiles exhibited by pyrazole-4-carbaxamides (4) prompted us to further explore some 4-functionalized
pyrazoles (5, 6, 7 and 8) containing 6-aminosulfonylbenzothiazole moiety at position-1 of the pyrazole ring (Figure 3.2). The benzothiazole ring was introduced with the purpose to provide a space linker between sulfonamide group and the pyrazole motif and the newly synthesized compounds were evaluated for CA inhibition as well as anti-inflammatory – antimicrobial activity profile.

**Figure 3.2.** Design of 4-functionalized pyrazoles (4, 5, 6, 7 and 8) as CAIs.

The synthetic route adopted for the synthesis of pyrazole-4-carboxamides (4) is illustrated in Scheme 3.1.

**Scheme 3.1.** Synthesis of pyrazole-4-carboxamides (4). (i) a. NaNO₂/HCl, b. SnCl₂/HCl; (ii) p-substituted acetophenones, Ethanol-water, 1-2 drops of glacial acetic acid, reflux; (iii) POCl₃/DMF; (iv) NaOH/THF/MeOH; (v) THF/NH₃/I₂, stir; (vi) THF, NaOH/H₂O₂, stir.
Synthesis of 4-hydrazinobenzensulfonamide (10) from sulfanilamide (9) was achieved following the literature procedure. The hydrazones 11 were prepared by condensation reaction of hydrazine 10 with suitably substituted acetophenones in ethanol containing catalytic amount of glacial acetic acid. Vilsmeier-Haack reaction of the hydrazones 11 resulted into the sulfonamide protected 4-formylpyrazoles 12 which were deprotected under basic conditions yielding 4-formylpyrazoles 13 with free sulfonamide moiety. 4-Formylpyrazoles 13 were efficiently converted into 4-cyanopyrazoles 14 using milder and ecofriendly reaction conditions i.e molecular iodine and aqueous ammonia in THF as solvent. Finally, hydrolysis of 4-cyanopyrazoles 14 using hydrogen peroxide under basic conditions resulted into corresponding pyrazole-4-carboxamides 4 in good yields.

The synthetic route adopted for the synthesis of target compounds 5-8 is outlined in Scheme 3.8. 6-Aminosulfonyl-2-hydrazinobenzothiazole 30 was the basic requirement for realizing the synthesis of final compounds and was obtained by a methodology reported recently by our group. Condensation of hydrazine 30 with variously substituted acetophenones 31 in DMF acidified with glacial acetic acid afforded corresponding hydrazones which upon subsequent reaction under Vilsmeier-Haack conditions afforded 4-formylpyrazoles 32 with protected sulfonamide group. Deprotection of 32 was carried out under acidic conditions which resulted in complete conversion of 32 to 5. Further, oxidation of 4-formylpyrazoles 5 to carboxylic acids 6 was carried out using oxone as the oxidizing agent. On the other hand, transformation of formylpyrazoles (5) to the corresponding 4-cyanopyrazoles (7) was achieved in excellent yield in a single step following our earlier adopted methodology. The formyl group of pyrazoles 5 was next converted into oxime (8) by refluxing the former with hydroxylamine hydrochloride in ethanol affording predominantly E-isomer along with 11-30% formation of Z-isomer.

Structures of all the target compounds and their precursors have been confirmed by rigorous analysis of their spectral data (IR, $^1$H NMR, $^{13}$C NMR and mass). The newly designed and synthesized 32 4-functionalized pyrazoles 4, 5, 6, 7 and 8 were evaluated as inhibitors against four isoforms of α-class of human CAs comprising two cytosolic, ubiquitous forms hCA I and hCA II as well as the tumor
-associated transmembrane isoforms hCA IX and hCA XII. For the purpose of exploring their biological potential, some of the compounds were also evaluated as anti-inflammatory as well as antimicrobial agents. Pyrazole-4-carboxamides 4 were the most potent compounds exhibiting excellent inhibitory potential against all of the four hCA isoforms. Five compounds of this series 4b, 4d, 4e, 4f and 4h were better inhibitors against all the tested hCA isoforms as compared to the standard drug AZA. However, other pyrazoles containing 6-aminosulfonylbenzothiazole moiety were comparatively weaker inhibitors of hCA isoforms as compared to pyrazole-4-carboxamides but have shown high degree of selectivity for hCA IX and XII over hCA I. All the compounds showed excellent to moderate inhibitory potential against hCA II and some of the compounds were even better inhibitors than the standard drug.
AZA. Pyrazole-4-carboxylic acid 6e showed excellent inhibition against tumor associated isoform hCA IX exhibiting nearly 9 fold better profile than the reference drug AZA. Against other tumor associated isoform hCA XII, the profile was much better for four compounds 6e, 7a, 7b and 7d showing inhibition comparable to the standard drug AZA. Since all the compounds have exhibited moderate to excellent inhibitory profile against hCA II, such compounds may constitute interesting candidates for the development of novel antiglaucoma, antiepileptic, antiedema, or antialtitude sickness drugs. Further, their selectivity for both tumor associated isoforms over hCA I may point out towards their potential as interesting candidates for the development of more selective and potent novel hypoxia induced cancer drug therapeutics.

Chapter 4. Development of Novel Synthetic Methodology for 3-Chloroacrylonitriles

Heterocyclic compounds constitute the largest classical division of organic chemistry making up more than half of the known organic compounds, and are present in a wide array of drugs, most vitamins, many natural products, biomolecules and biologically active compounds against HIV, tumor, inflammation, microbes, malaria, insects etc. Along with the isolation of heterocycles from natural sources as lead compounds in drug development, most of these are synthesized in laboratory using readily available precursors. Therefore, considerable attention has been paid to develop new and efficient methods to synthesize heterocycles and their precursors. 3-Haloacrylonitriles have been identified as versatile building blocks for the synthesis of many heterocyclic motifs such as thiophene, selenophene, furan, isoxazole, pyrazole and some fused pyridines which are associated with many interesting chemical and biological properties. Their importance as valuable precursors for the synthesis of a diverse array of heterocyclic compounds has encouraged organic chemists to devote persistent efforts for the development of improved methods for the preparation of these halopropenenitriles.

Surprisingly, there is only one method available for the synthesis of 3-chloropropenenitriles. The original three step process utilizes acetophenones as starting material and involves Vilsmeier-Haack reaction to give 3-chloro-2-propeniminium salts or 3-chloropropenals which are treated with hydroxylamine
hydrochloride to afford corresponding aldoximes followed by dehydration resulting into the formation of 3-chloropropenenitriles. There has been slight variation to this three step process by way of changing the dehydration reagent from trichlorophosphate to acetic anhydride or phosphorous pentachloride, attempting it as a one pot process or achieving the synthesis by a continuous flow method.

![Scheme 4.1](image)

Use of toxic reagents, difficult isolation procedures and low yields limit the methodology. Therefore, development of a novel efficient approach for the synthesis of these versatile heterocyclic precursors under mild reaction conditions should be a welcome step. In view of the synthetic importance of these heterocyclic precursors, here in this chapter, we have reported a novel, simple and facile approach for the synthesis of 3-chloropropenenitriles 2 from chloropropenals 1 using molecular iodine and aqueous ammonia.

Our methodology is better as the transformation from chloropropenals to chloropropenenitriles is directly achieved in excellent yields under milder aqueous conditions using equimolar molecular iodine without involving a harsh dehydration step. Furthermore, the β-chlorocinnamalonitriles obtained after simple work-up are sufficiently pure thus negating the need for flash chromatography.

**Chapter 5. One Pot Facile Synthesis of Propynenitriles from 3-Chloropropenals.**

The chemistry of variously substituted acetylenes has felt a renaissance in the past decade not only due to their importance in biochemistry and material science, but
also as building blocks as well as versatile intermediates for synthesis of a vast array of heterocyclic moieties of potential medicinal interest. The carbon-carbon triple bond of alkynes is one of the constitutional functional groups in organic chemistry exhibiting the fundamental reactions of synthetic utility. Furthermore, cyano group has always been envisaged as a fountainhead of functionalization in organic medicinal chemistry due to its facile transformation to functional groups of medicinal significance such as amines, amides, amidines, aldehydes, ketones, carboxylic acids esters etc. Combination of these two versatile functionalities, viz. alkyne and cyano, in alkynenitriles make these as much sought after intermediates of potential synthetic as well as medicinal importance. Alkynenitriles serve as essential structural units for synthesis of a variety of five or six membered carbocycles and heterocycles such as cyclopentadienones, 1,2,3-triazoles, isoxazoles, iminopyrimidines, benzo[b]phospholes, benzopyranes, thiophenes, benzofurans, pyrroles, indoles, etc.

In view of their importance as intermediates in organic synthesis, many methods for preparation of alkynenitriles have been documented in literature. Most of these methods involve the use of toxic reagents like cyanogen halides or other organo based cyanating agents, dehydrating agents, oxidizing agents etc. Further, the utility of these methods is limited by the formation of side products, difficult preparation of starting precursors and low yields. Fascinating profile of these starting units coupled with our ongoing interest in developing better methods for heterocyclic precursors, we envisioned to develop a simple and efficient one pot methodology for their preparation. Here, in this chapter, we report an efficient one pot procedure for the preparation of alkynenitriles (2) starting from readily available chloropropenals (1) (Scheme 5.1).

![Scheme 5.1](image)

This newly developed approach is an efficient and facile methodology to prepare conjugated alkyne nitriles in one pot manner directly from 3-chloroacrylaldehydes using economically benign reaction conditions and non-toxic reagents such as molecular iodine, aqueous ammonia and sodium hydroxide. It is
noteworthy that no side products are formed in the reaction and yield of reaction is excellent and at the same time, the alkylnitriles thus formed do not need further purification.

Chapter 6. Efficient One Pot Access to Triazolothiones From Amines and Acyl Hydrazides

Heterocyclic compounds are widely distributed in nature and are also important building-blocks for new materials possessing interesting electronic, mechanical or biological properties. Heterocyclic compounds isolated from natural sources are known to act as lead compounds for the development of new drugs of therapeutic applications. Thus, the synthesis of heterocyclic compounds continues to receive a strong impetus necessitating a consistent interest in research on developing new and efficient routes to various heterocyclic scaffolds. Triazoles constitute an interesting class amongst five membered heterocyclic compounds and the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention owing to their potent therapeutic properties. They exhibit interesting biological activities such as antimicrobial, anti-inflammatory, analgesic, antitubercular, antioxidant, antiproliferative, anticonvulsant, carbonic anhydrase inhibition etc. The importance of 1,2,4-triazoles and their derivatives has also been realized as ligands in organometallic compounds, as ionic liquids as well as corrosion inhibitors. A variety of methods exist for the synthesis of substituted 1,2,4-triazole-3-thiones however, to the best of our knowledge, there is no report in literature providing direct access to 1,5-diaryl-1,2,4-triazole-3-thiones from anilines in one pot manner. Moreover, the existing methods of their preparation suffer from serious limitations such as formation of side products, use of hazardous reagents, typical reaction and isolation procedures etc. The most common method used for the preparation of 1,5-diaryl-1,2,4-triazole-3-thiones involves the condensation of arylisothiocyanates with aryl acid hydrazides followed by oxidative cyclization. This method is simple and practical but one of the reactants i.e. arylisothiocyanates used in this method needs to be prepared from aromatic amines which involves the use of hazardous reagents such as lead nitrate, acetic anhydride, thiophosgene etc. In view of the synthetic as well as biological importance of these heterocyclic scaffolds and the limitations associated with the existing methods of their synthesis, developing novel
and efficient methodology for their synthesis would certainly be a welcome step. In this chapter, we have envisioned and developed a simple and facile one pot approach for the synthesis of triazolothiones from readily available aromatic amines and aryl acid hydrazides using carbon disulfide and sodium hydroxide.

In the present methodology, aromatic amines were first converted to their corresponding dithiocarbamate salts using carbon disulfide as a reagent as well as solvent in presence of triethylamine. The \textit{in situ} generated salts were treated as such in the next step with aromatic acid hydrazides in the same pot. The methodology developed is practical and requires simple and easily available starting materials.