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REVIEW ARTICLE

Multicomponent chemistry in the synthesis of carbonic anhydrase inhibitors

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Abstract

Carbonic anhydrase inhibitors (CAIs) are of growing interest since various isoforms of the enzyme are identified as promising drug targets for treatment of disease. The principal drawback of the clinically used CAIs is the lack of isoform selectivity, which may lead to observable side effects. Studies aiming at the design of isoform-selective CAIs entail generation and biological testing of arrays of compounds, which is a resource- and time-consuming process. Employment of multicomponent reactions is an efficient synthetic strategy in terms of gaining convenient and speedy access to a range of scaffolds with a high degree of molecular diversity. However, this powerful tool appears to be underutilized for the discovery of novel CAIs. A number of studies employing multicomponent reactions in CAI synthesis have been reported in literature. Some of these reports provide inspiring examples of successful use of multicomponent chemistry to construct novel potent and often isoform-selective inhibitors. On critical reading of several publications, however, it becomes apparent that for some chemical series designed as CAIs, the desired inhibitory properties are only assumed and never tested for. In these cases, the biological profile is reported based on the results of phenotypical cellular assays, with no correlation with the intended on-target activity. Present review aims at critically assessing the current literature on the multicomponent chemistry in the CAI design.

Keywords

Carbonic anhydrase inhibitors, lead discovery, multicomponent reactions

History

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Introduction

Carbonic anhydrase (CA EC 4.2.1.1) is a ubiquitous zinc enzyme, the crucial function of which is catalysis of reversible CO_2 hydration[1]. Sixteen isoforms of CA differing in their activity and subcellular localization have been characterized in humans. Many CA isoforms play critical role in both normal physiological processes and pathogenic mechanisms where bicarbonate and/or protons are essential. These isozymes gain attention as promising targets for treatment of a range of diseases[2]. In particular, inhibition of cytosolic isoform CA II is used in glaucoma treatment for intraocular pressure control[3]. Transmembrane isoforms CA IX and XII were found to be overexpressed in many hypoxic tumors[4,5]. Small molecules binding and inhibiting these isozymes can be used in tumor diagnostics as well as in therapy through suppression of extracellular acidification of cancer cells[6]. However, many of the clinically used carbonic anhydrase inhibitors (CAIs) do not possess significant selectivity with respect to a particular CA isoform and the associated side effects limit the use of these compounds. Therefore development of new, isoform-selective CAIs is highly desirable[7].

Any systematic quest for such new inhibitors is associated with creating and assaying of novel small molecule libraries[8]. Besides identifying new compounds possessing the classical CA inhibitory pharmacophore, primary sulfonamide, such efforts have resulted in identification of new chemical classes endowed with the ability to inhibit CAs[9]. These comprise recently reported inhibitors such as coumarins[10], phenols[11], polyamines[12], etc. Moreover, crystallography studies of enzyme–inhibitor complexes help elucidate the mechanism of inhibitory action of various chemotypes. For instance, sulfonamides were found to coordinate with the catalytic zinc ion; coumarins bind at the entrance of the active site, and polyamines as well as phenols are able to interact with the zinc-coordinated water molecule.

Generation of novel small molecules libraries is a labor-intensive and time-consuming process. In this context, multicomponent approaches manifested themselves as powerful synthetic tools, providing convenient access to a great diversity of novel promising drug candidates[13–15]. Multicomponent chemistry is one of the effective strategies in modern lead discovery, gaining growing attention of the drug discovery community due to its resource economy and high productivity.

In this review our goal we aim at drawing attention to the fact that multicomponent chemistry in the context of synthesizing CAIs is fairly undeveloped. To this end, we summarize a fairly limited number of appropriate cases reported in the literature. In addition, we have encountered a challenge related

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to the data quality. Unfortunately, only a handful of reported compounds have acceptable grounds to be considered true CAIs having known inhibitory level supported by credible testing data. In particular, in many cases inhibitory activity of compounds was assumed based on the presence of a known CA inhibitory pharmacophore (mostly, primary sulfonamide) and the subsequently determined *in vivo* bioactivity or *in vitro* determined cytotoxity, and not on the accurate measurement of on-target activity, for instance, via the well-validated CO_2-hydratase inhibition assay against an isolated recombinant protein. We chose to include such cases in the present review highlighting the synthetic aspects related to the use of multicomponent chemistry and underscoring the absence of on-target data for the resulting compounds.

Multicomponent synthesis of aminocyanopyrazole-based CAIs

In 2013 Allouche et al.[16] suggested convenient access to substituted 2-amino-3-cyanopyrazoles through the reaction of malononitrile **1** with orthoesters **2** and phenylhydrazine **3** under acidic conditions. Several types of CAIs with hypothetically novel mechanism of action were successfully generated using this multicomponent approach. In particular, reaction involving phenylhydrazines **3** was realized in ethanol under reflux in the presence of acetic acid and provided *N*-phenyl substituted aminocyanopyrazoles **4** (Scheme 1). Inhibitory properties of these compounds toward human carbonic anhydrase (*h*CA) were then evaluated via classical stopped flow CO_2 hydration assay.

Aminocyanopyrazoles **4** possessed micromolar and submicromolar inhibitory activity against cytosolic (*h*CA I and II) and transmembrane (*h*CA IX and XII). The authors hypothesized that compound **4** binds to the enzyme in a manner similar to the recently reported polyamine spermine inhibitors which anchor to the zinc-coordinated water/hydroxide ion[12]. However, the mechanism of inhibitory action of **4** must be confirmed by X-ray crystallography of inhibitor-protein complexes.

Furthermore, as suggested by Alp et al.[17], *N*-sulfonyl pyrazoles akin to **4** can act as CAIs by interacting with the coumarin-binding site at the entrance of the cavity. This mode of action was suggested for *N*-tosyl-substituted aminocyanopyrazoles **5**, synthesized by a similar reaction involving tosyl hydrazine (Scheme 2).

These compounds also demonstrated inhibitory properties against *h*CA I, II, IX and XII with K_i's in the range of 10^{-6}–10^{-7} μM. Their hypothetical mechanism of CA inhibition also requires further investigation.

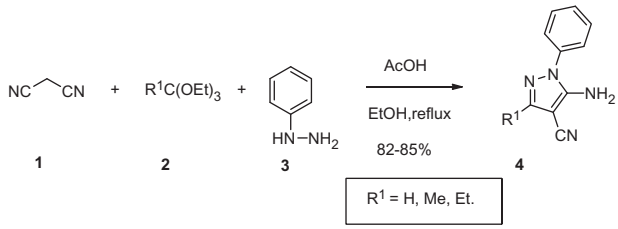
N-Sulfonamide derivatives **6** generated from **4** via the reaction with sulfamoyl chloride (Scheme 3) inhibited medicinally relevant isoforms of *h*CA most probably due to the canonical sulfonamide-Zn binding mechanism[18].

K_i values of sulfonamide derivatives **6** were found to be in the low nanomolar range. Best representatives of each CA inhibitor chemotype generated by Allouche et al.[16] are shown in Figure 1.

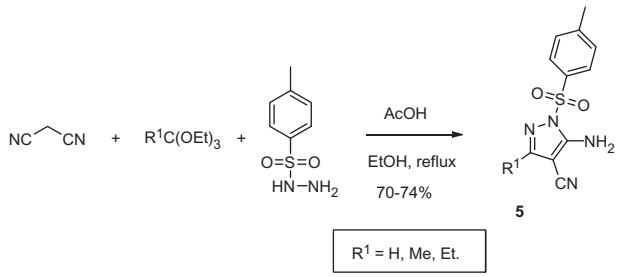
As it can be seen from these K_i values, the compounds synthesized via the multicomponent pyrazole synthesis demonstrated significant inhibitory activity, especially toward membrane-bound *h*CA isoforms IX and XII.

Dicarbonyl derivatives of methylaminobenzene-sulfonamide

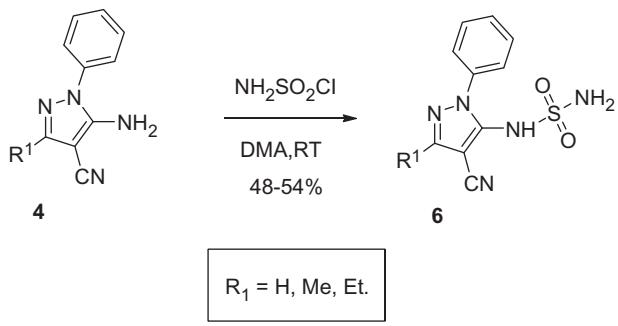
Dimirci and colleagues[19] synthesized a series of 1,3-dicarbonyl 4-(*N*-methylamino)benzene sulfonamide (sulfanilamide) derivatives **8** using multicomponent chemistry. Eight novel sulfonamides were prepared from sulfanilamide, triethyl orthoformate and dicarbonyl compounds **7**. Reaction in ethanol under heating furnished **8** in high yields (Scheme 4).



Scheme 1. Multicomponent synthesis of aminocyanopyrazoles **4**.



Scheme 2. Synthesis of *N*-tosyl-substituted aminocyanopyrazoles **5**.



Scheme 3. Preparation of *N*-sulfonamide derivatives **6**.

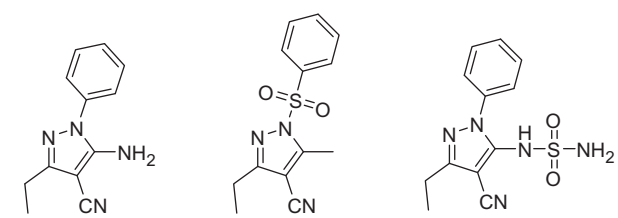
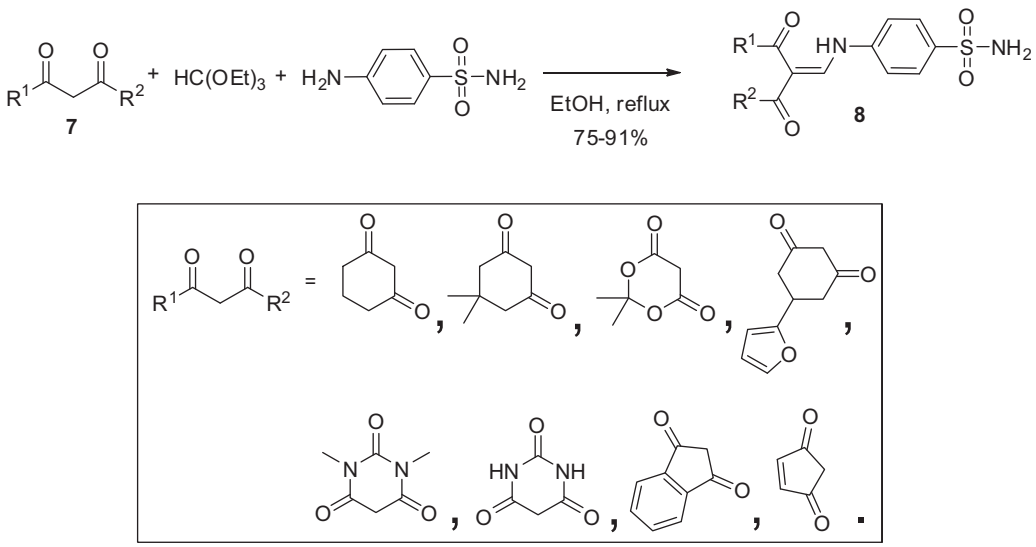


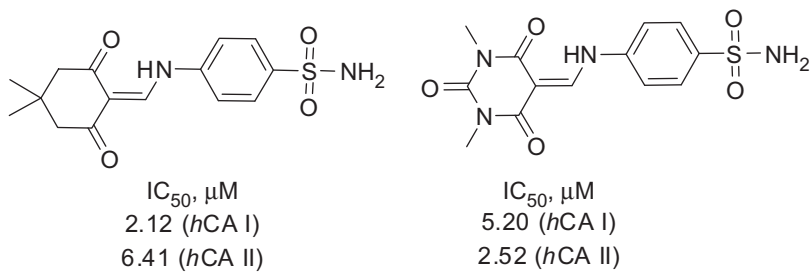
Figure 1. Most potent CA inhibitors reported by Allouche et al[16].

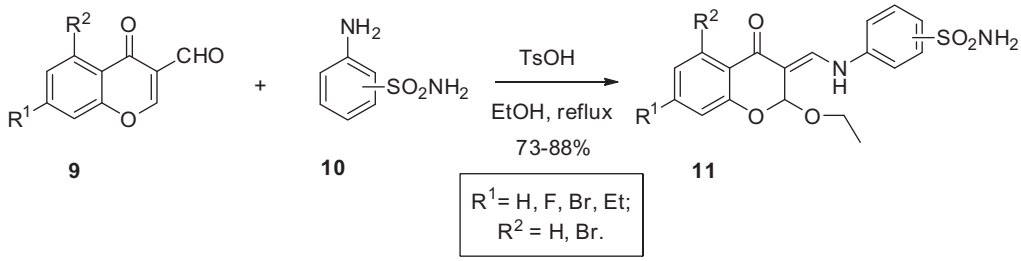
Sulfonamides **8** were assayed in CO_2 hydration assay and their IC_{50} values toward cytosolic *h*CA I and II isoforms were determined to be in micromolar region. The most active compounds derived from dimedone and *N,N*-dimethylbarbituric acid are shown in Figure 2.



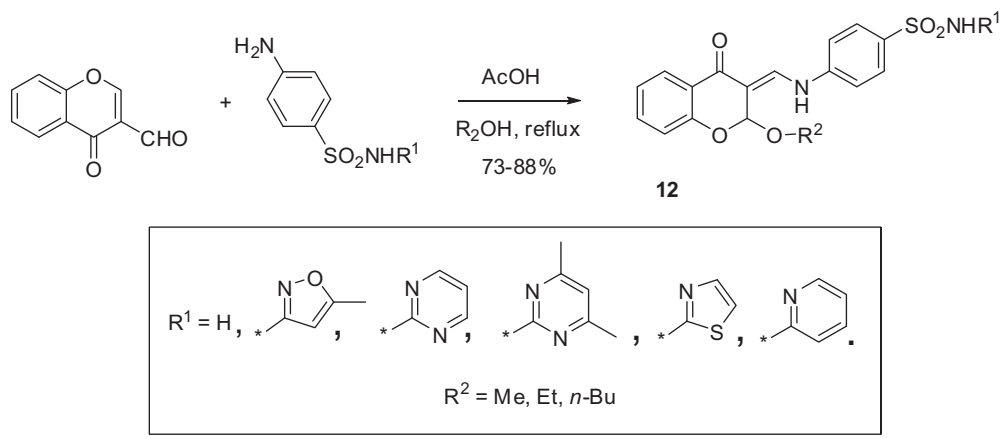
Scheme 4. Three-component synthesis of 1,3-dicarbonyl derivatives of sulfanilamide (**8**)*.

Figure 2. Most potent inhibitors of *h*CA I and *h*CA II, respectively, reported by Dimirci et al[19].





Scheme 5. Preparation of sulfonamide containing chromone derivatives **11**.



Scheme 6. Preparation of sulfonamide containing chromone derivatives **12**.

Chromone-based sulfonamides

Sulfonamides incorporating a chromone moiety were recently reported to possess considerable activity as CAIs by al-Rashida et al.[20] The authors reported the formation of enamine adducts **11** by condensation of 3-formylchromones **9** with 3- and 4-amino benzenesulfonamides **10** in refluxing ethanol (with incorporation of the latter in the product's molecule). Parallel to formation of the Shiff bases in reaction of amine with aldehyde group, nucleophilic attack at the electron-deficient C-2 atom took place resulting in 2-ethoxy-3-enamine-chromone derivatives in high yields (Scheme 5).

Inhibitory properties of **11** were assessed against bovine cytosolic CA. The assay was based on esterase activity of carbonic anhydrase (*p*-nitrophenyl acetate ester hydrolysis). Compound **11** exhibited high activity, being micromolar *b*CA inhibitors (i.e. comparable to the reference drug – acetazolamide displaying IC_{50} of 1.13 μM in the esterase assay). Although the CO_2 hydrating and the ester hydrolyzing sites of CA show some similarity, the inhibition of the CA esterase activity demonstrates poor CO_2 hydration activity. Therefore, leads showing activity in the ester hydrolysis assay should be cautiously considered as true CAIs. Taking this into account, we will only mention, that IC_{50}'s of compound **11** were determined to be between 4.13 and 29.12 μM for *b*CA esterase activity, which is quite weak.

In 2015, Awadallah et al.[21] reported a similar reaction performed in methanol, ethanol and *n*-butanol, resulting in corresponding 2-alkoxy derivatives **12**. Acetic acid was used as catalyst (Scheme 6).

Compounds **12** synthesized by this group were tested for inhibition of CA-catalyzed CO_2 hydration in the classical stopped-flow kinetics assay and demonstrated selective inhibition of transmembrane tumor-associated isozymes IX and XII, with most promising K_i's in the nanomolar range. Moreover, the most potent compounds from this series were tested *in vitro* against MCF-7 breast cancer and A-549 lung cancer cell lines and

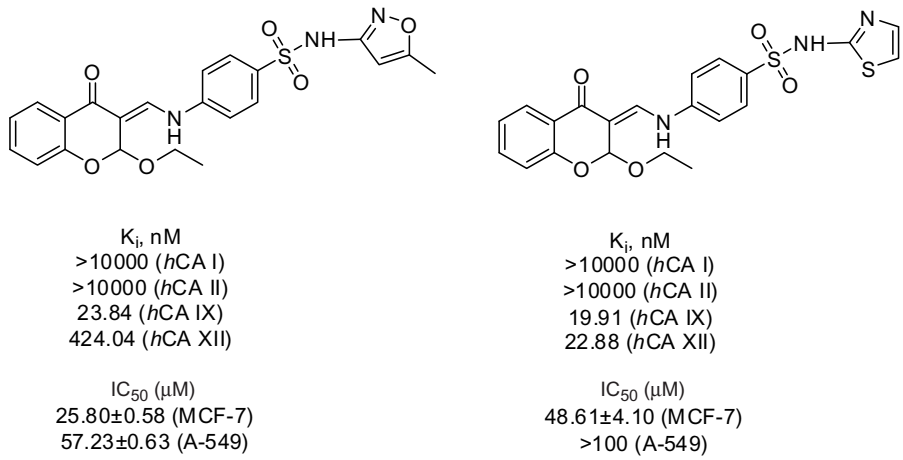
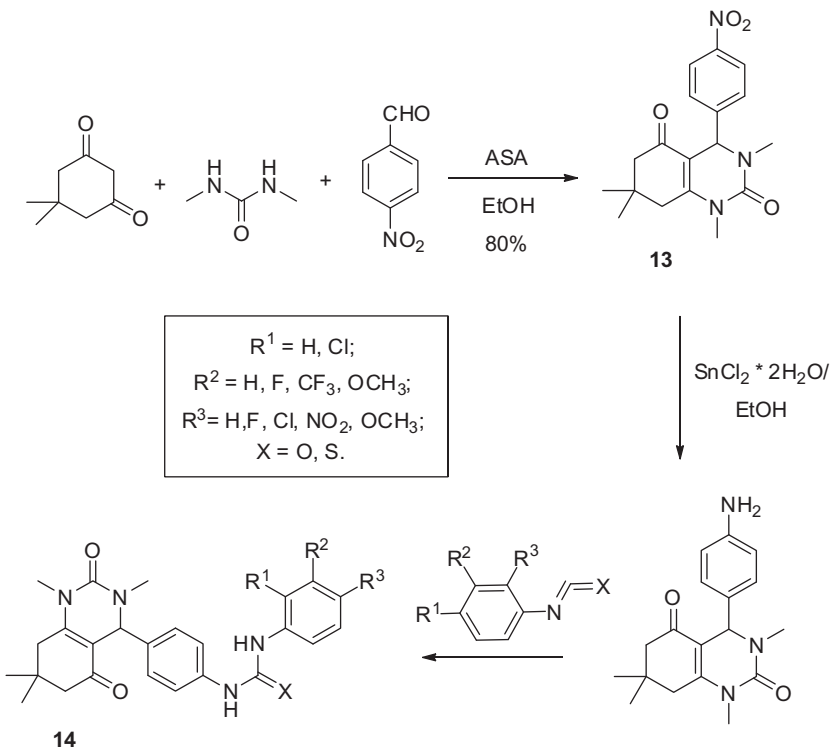


Figure 3. Biological activity data for the lead compound **12** reported by Awadallah et al[21].



Scheme 7. Preparation of 1,4-dihydropyrimidinone substituted diaryl(thio)ureas **14**.

demonstrated fairly potent cytotoxic activity with IC_{50}'s in micromolar range (Figure 3).

Some of compounds reported in this study also showed proapoptotic activity. Taking these results into account, the central role of CA inhibition in the observed cytotoxic effect was proposed. The high selectivity of the lead compounds toward *h*CA IX and XII (and no activity toward off-target cytosolic *h*CA I and II isoforms) makes them promising leads for further investigation.

Biginelli reaction of urea derivatives in the synthesis of CAIs

Celik et al.[22] employed a Biginelli reaction to prepare aryl-substituted dihydropyrimidines in order to study their CA inhibitory properties. Reaction of dimedone with dimethylurea and *p*-nitrobenzaldehyde was carried out in the ethanol in the presence of aluminia sulfuric acid (ASA) at 90 °C and afforded **13** in 80% yield. The nitro group of compound **13** was reduced with tin (II) chloride in ethanol and the resulting aniline was treated with sets of isocyanates or isothiocyanates in toluene at 60 °C resulting in **14** (Scheme 7).

The inhibitory properties of **14** toward *h*CA I and II were evaluated via CO_2 hydration assay. IC_{50}'s were found to be in the 66–198 μM range. Possible mechanism of inhibitory action of **14** proposed by the authors involved binding to the Zn-coordinated water molecule/hydroxide ion. However, this hypothesis remains to be verified by X-ray crystallographic structures of the respective inhibitor–protein complexes. The most potent inhibitors identified among nine compounds prepared are shown in Figure 4.

In the follow-on study, Celik and coworkers employed *p*-cyanobenzaldehyde in a similar reaction with ethyl acetoacetate or dimedone (Scheme 8).[23] Resulting compounds **15** and **16** were transformed into tetrazolyl derivatives **17** and **18** via reaction with sodium azide in DMF. Subsequent treatment of **18** with acetic anhydride or benzoyl chloride at 150 °C provided 1,3,4-oxadiazolyl substituted compound **19**.

The IC_{50} values of compounds **15–19** toward *h*CA I were found to be in submillimolar region, i.e. the compounds displayed

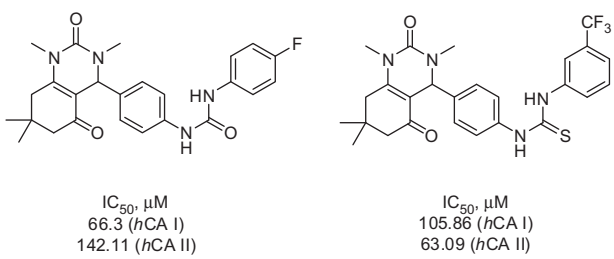


Figure 4. Inhibitory properties of exemplary urea and thiourea derivatives reported by Celic et al[22].

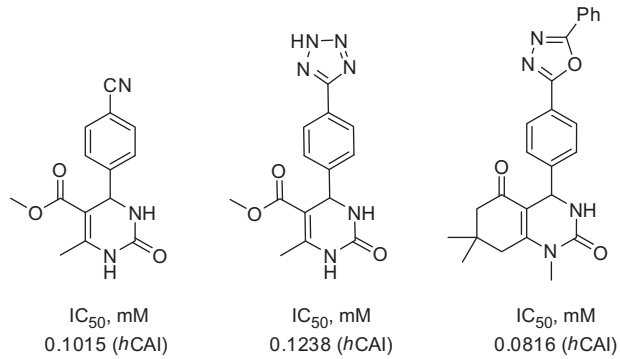
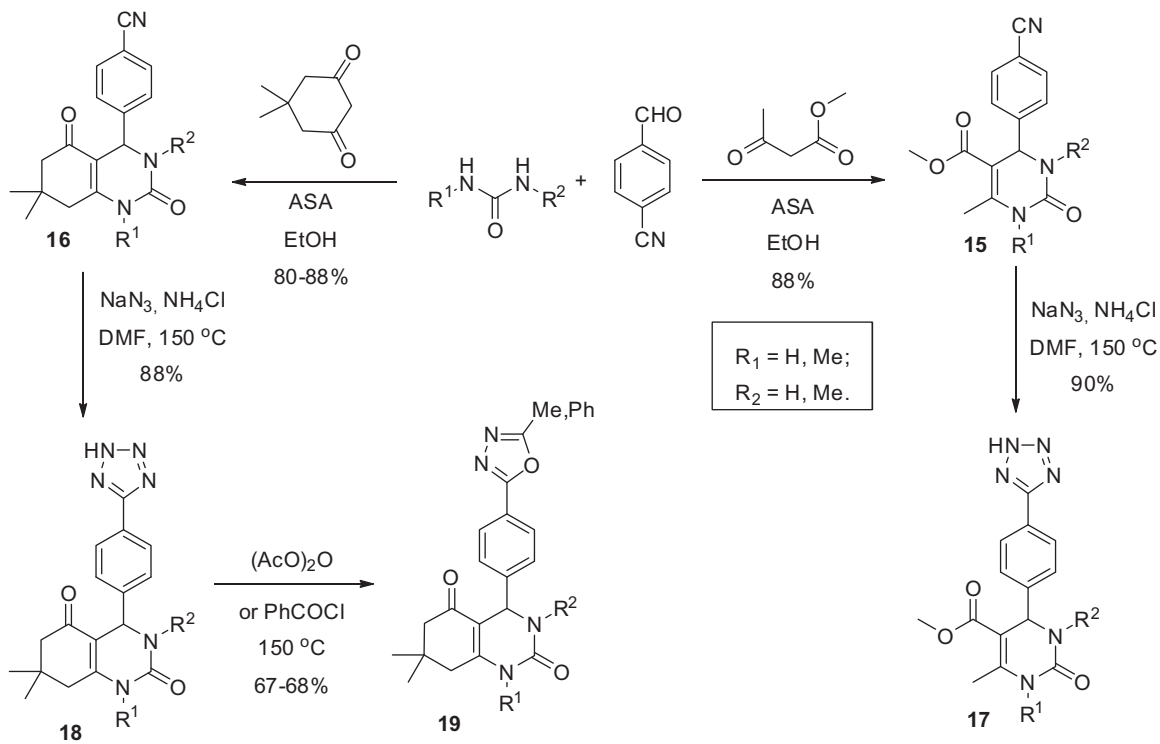
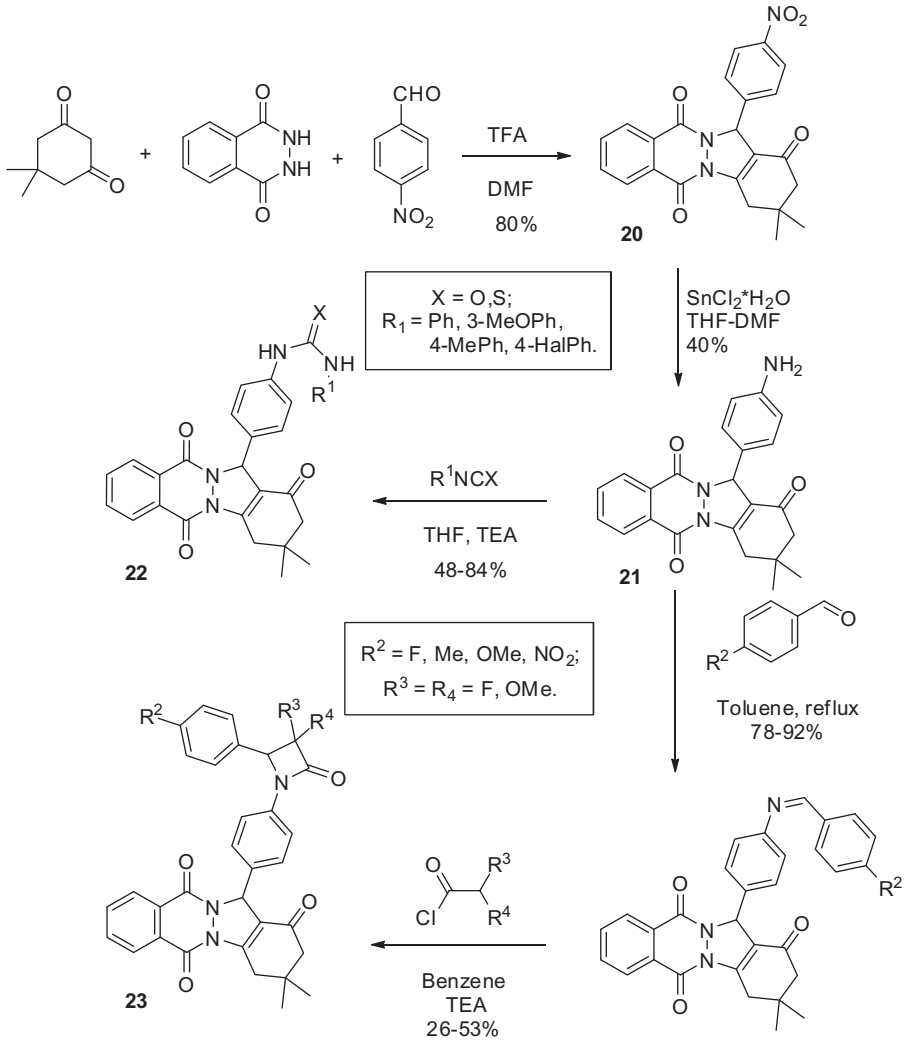


Figure 5. IC_{50}'s against *h*CA I for selected weak inhibitors reported by Celik et al.



Scheme 8. Preparation of novel 1,4-dihydropyrimidine derivatives **15–19**.

Scheme 9. Preparation of phtalazine derivatives **20**, **21**, **22** and **23**.



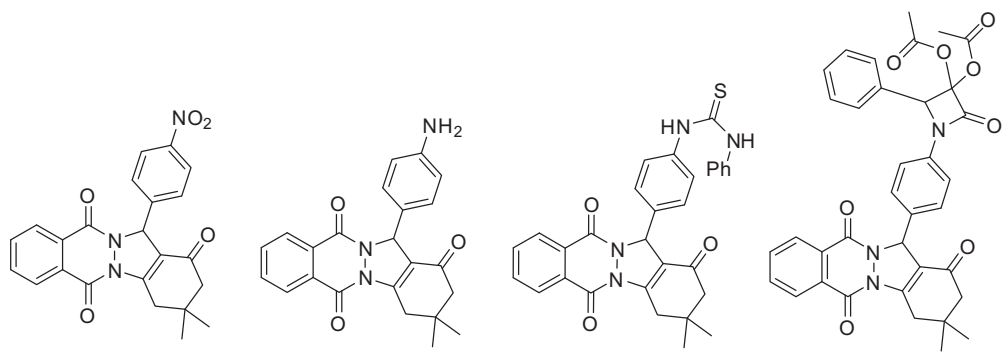
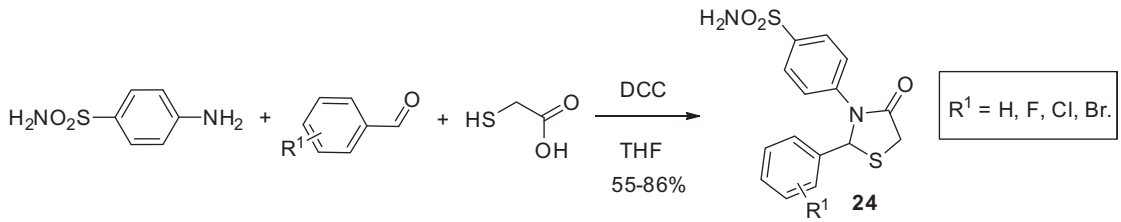
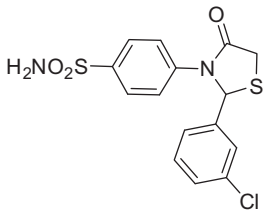


Figure 6. Most active compounds of 28 phtalazine derivatives reported by Beber et al[24,25].



Scheme 10. Synthesis of arylthiazolidine-substituted benzenesulfonamides **24**.



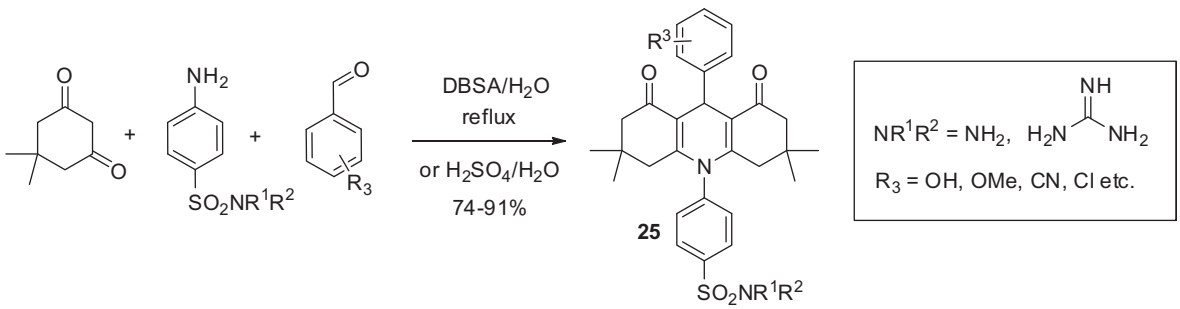
K_i, nM	IC_{50}, μg/ml
838.3 (*h*CA I)	5.03 (COLO 205)
135.5 (*h*CA II)	5.81 (MDA-MB_231)
2.2 (*h*CA IX)	23.93 (DU-145)

Figure 7. Leading compound reported by Suthar et al[28].

very low inhibitory activity (Figure 5). Authors proposed that the mechanism of CA inhibition could be similar to that of coumarins (i.e. anchoring to the binding site at the entrance of the catalytic cavity[10]). Again, such a hypothesis should be further substantiated by crystallography studies of the respective inhibitor-protein complexes. No inhibition data against other isoforms of *h*CA (besides *h*CA I) were reported for compounds **15**–**19**.

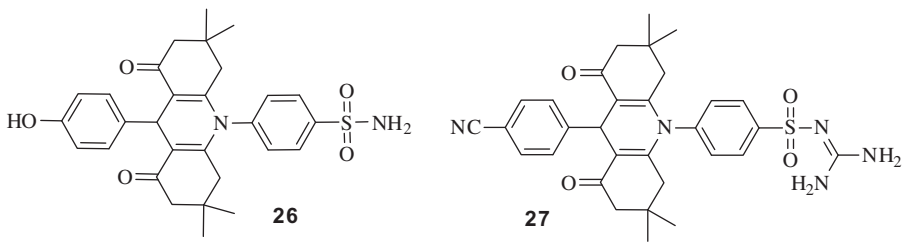
Biginelli-type reaction involving phtalazine in the synthesis of CAIs

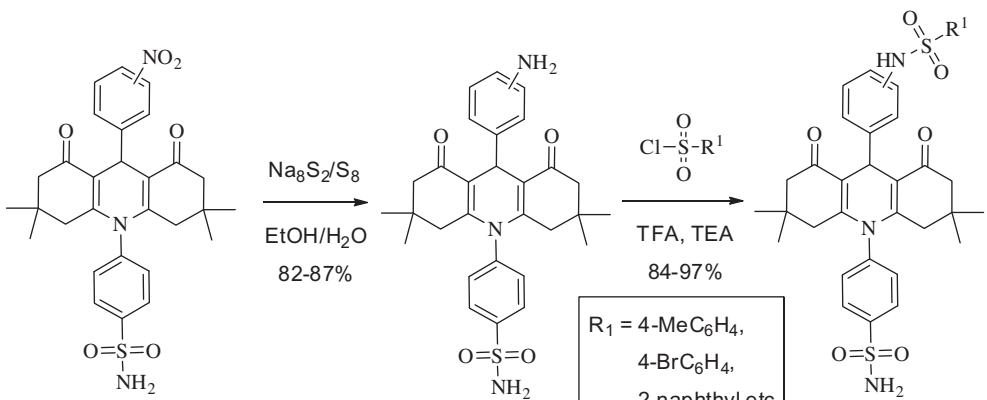
Beber and colleagues[24,25] from Sakaraya University reported the use of modified Biginelly-type reaction employing phtalazine as a 1,2-nucleophile in order to obtain indazolophtalazinetrione **20**.



Scheme 11. Preparation of acridine-based sulfonamides **25** via the Hantzsch reaction.

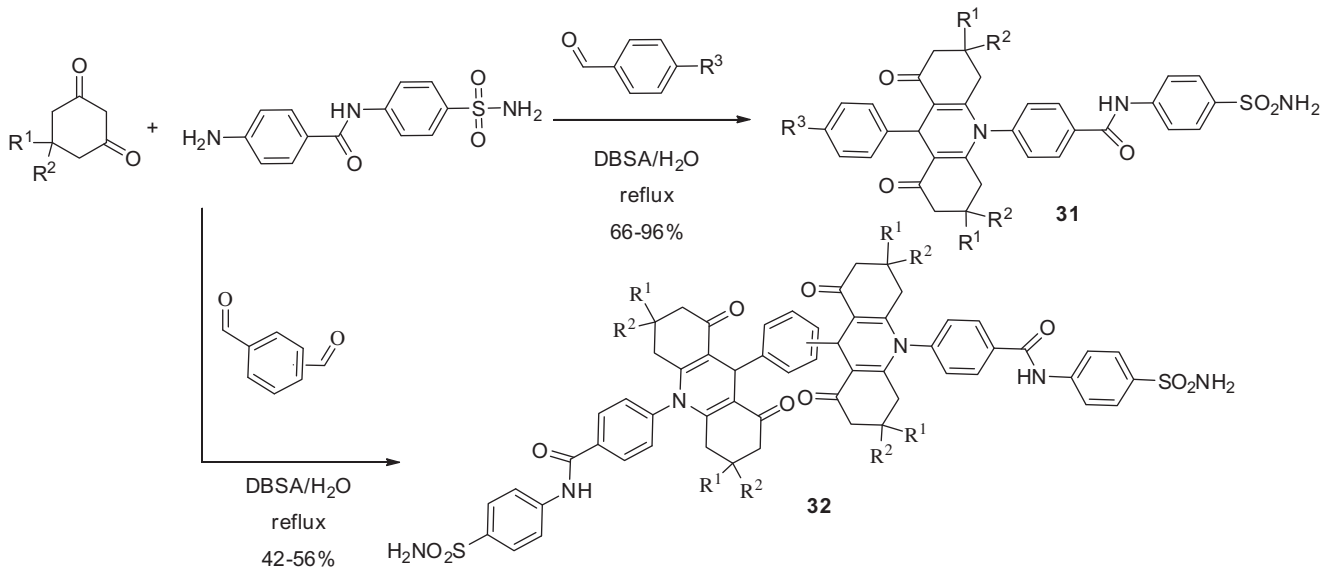
Figure 8. Examples of acridine monosulfonamide derivatives.





28	**29**	**30**
K_i, μM	K_i, μM	K_i, μM
0.7-5.1 (*h*CA I)	2.5-8.87 (*h*CA I)	2.09-8.91 (*h*CA I)
0.64-0.89 (*h*CA II)	0.50-0.65 (*h*CA II)	0.10-0.96 (*h*CA II)
0.42-1.02 (*h*CA IX)	0.92-1.00 (*h*CA IX)	0.09-1.12 (*h*CA IX)
0.7-5.1 (*h*CA XII)	0.03-0.61 (*h*CA XII)	0.53-1.02 (*h*CA XII)

Scheme 12. Acridine derivatives **28–30**.



Scheme 13. Preparation of **31** and bisacridines **32** via the Hantzsch reaction.

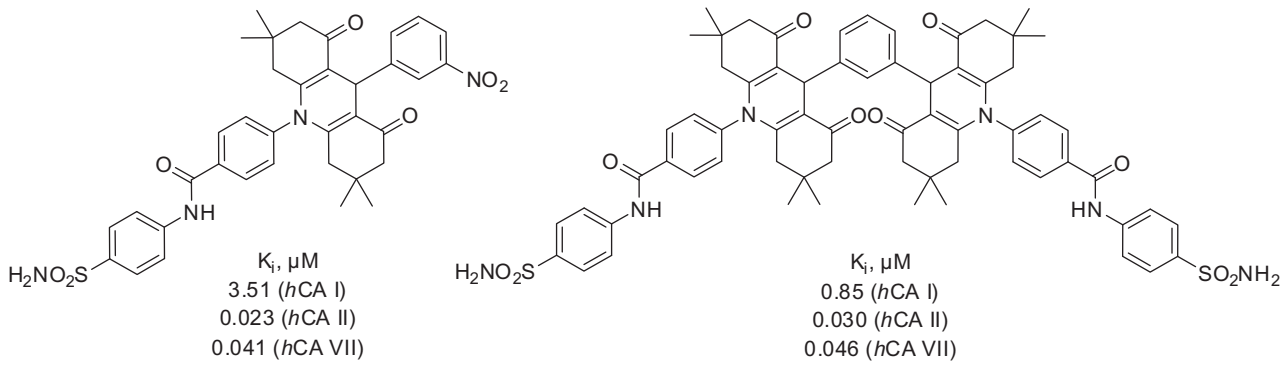
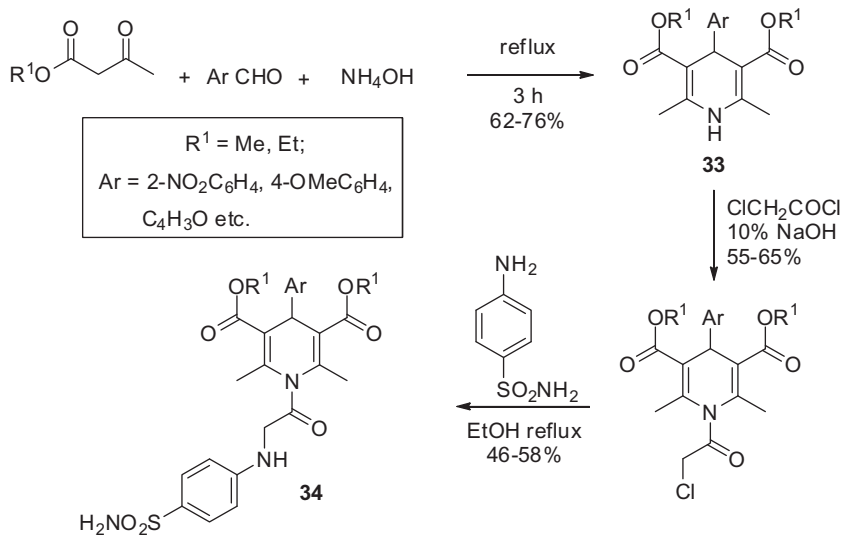


Figure 9. Most potent representatives of **31** and **32** and their K_i's against *h*CA I, II and VII.

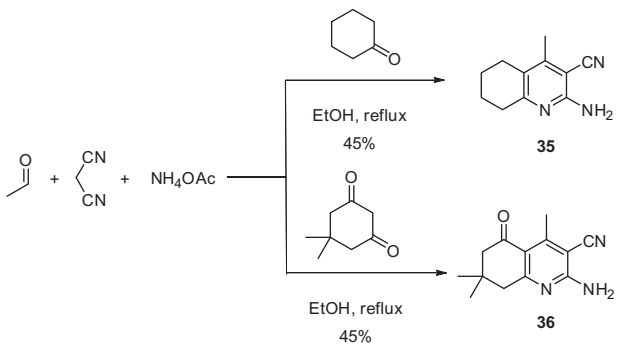
Scheme 14. Synthesis of 1,4-dihydropyridine-containing benzenesulfonamide derivatives **34**.



This scaffold provided convenient access to two types of CA inhibitor, such as (thio)urea derivatives **22** and β-lactam phtalazines **23**. The nitro group of **20** was reduced with tin (II) chloride. Resulting amino derivative **21** reacted with isocyanates or thioisocyanates (resulting in **22**). Alternatively, it was converted to Schiff bases on treatment with aromatic aldehydes following azeotropic removal of water and the imines were reacted with ketenes generated from acetyl or acetoxyacetyl chloride, which led to moderate yields of **23** (Scheme 9).

Compound **21** as well as its (thio)urea (**22**) and β-lactam (**23**) derivatives reported by Beber were evaluated in *h*CA I and II hydratase inhibition assay. IC_{50} values were found in the range from 6.13 to 24.84 μM. The most potent compounds from this series are shown in Figure 6.

Compounds **20–23** contain no traditional pharmacophores which are known to endow compounds with CA inhibitory activity. This suggested a novel mechanism of inhibition. It was hypothesized that the phtalazine scaffold is capable of binding at the entrance of CA catalytic cavity, similarly to coumarine derivatives. Urea and thiourea moieties had been reported to anchor Zn-coordinated water[26] and β-lactams supposed to be capable of binding directly to the catalytic Zn ion[27]. However, detailed mechanism of the CA inhibition by compounds **20–23** remains to be elucidated.



Scheme 15. Multicomponent synthesis of 2-amino-nicotinonitriles **35** and **36**.

Multicomponent synthesis of thiazolidine-based sulfonamides

Suthar and coworkers[28] reported an efficient synthesis of arylthiazolidine substituted benzenesulfonamides **24** via the reaction of sulfanilamide with aromatic aldehydes and thioglycolic acid in the presence of dicyclohexylcarbodiimide (DCC) in THF (Scheme 10). *h*CA I, II and IX inhibitory properties of the compounds thus generated were studied via the stopped flow CO_2 hydratase assay. Compound **24** demonstrated nanomolar inhibition of *h*CA IX and pronounced selectivity toward this transmembrane CA isoform over cytosolic isoforms *h*CA I and II. K_i values of studied compounds were in the range 112.3–1247.9 nM, 28.4–135.5 nM and 2.2–53.1 nM against *h*CA I, II and IX, respectively (Figure 7).

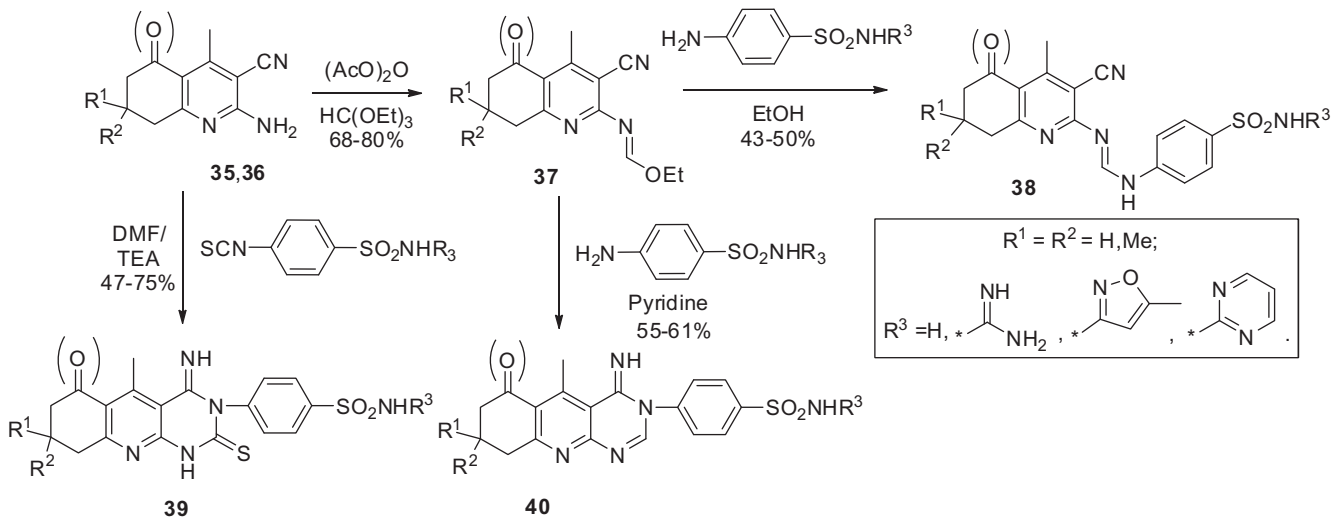
Upon evaluation of the compounds' inhibitory properties toward CAs, their cytotoxic activity was determined *in vitro* against COLO-205, MDA-MB-231 and DU-145 human carcinoma cell lines. For the most promising compounds, the anticancer activity was further investigated *in vivo*. Compound **24** displayed cytotoxic and *in vivo* antitumor activity. The mechanism of antitumor activity was assumed to involve carbonic anhydrase inhibition. This assumption was supported with CA inhibitory activity for the most cytotoxic compounds as well as molecular docking studies.

Hantzsch-type reaction in synthesis of acridine-based sulfonamides

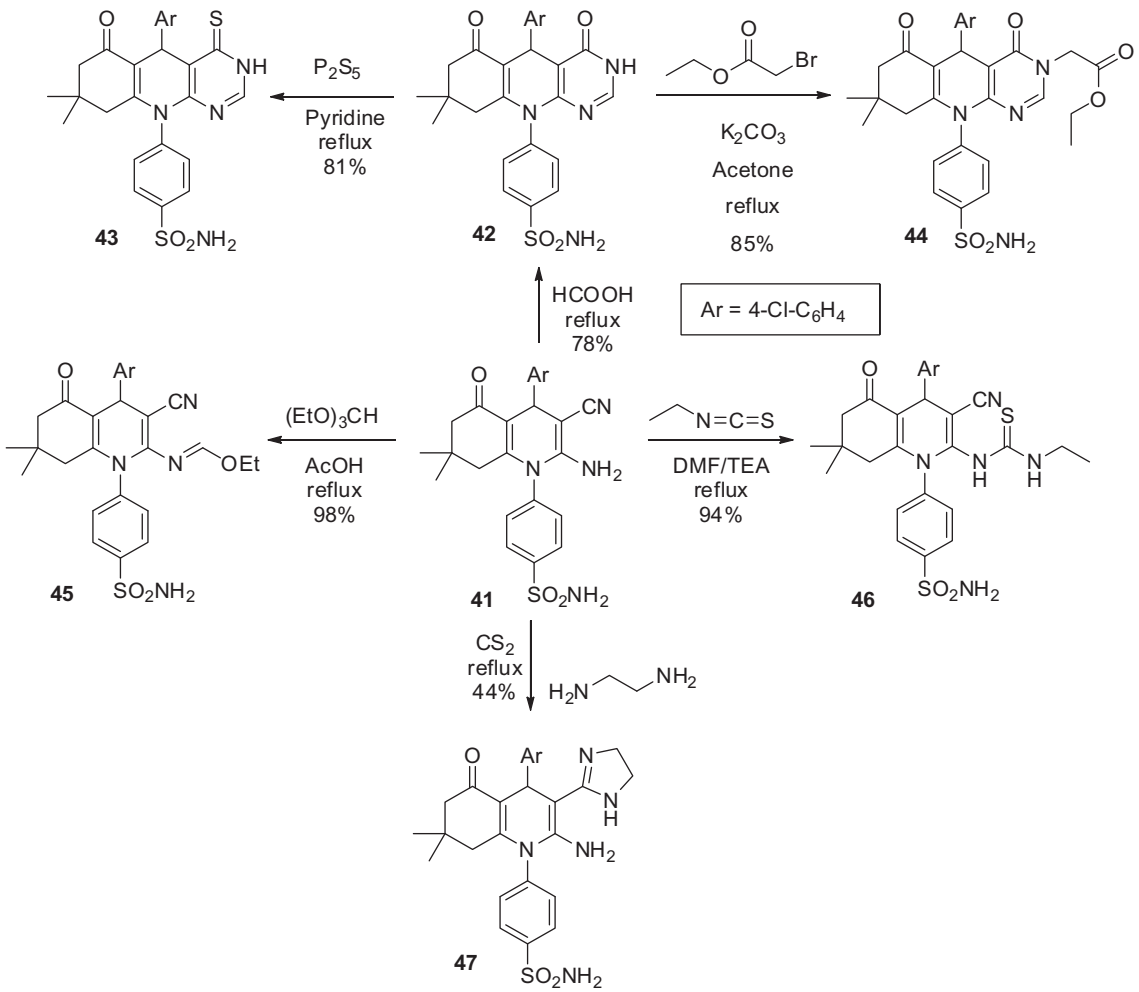
The Kaya group from Dumlupinar University investigated acridine-based sulfonamides **25** synthesized via a multicomponent Hantzsch reaction[29–32] (Scheme 11) as CAIs.

The exemplary compounds from this series (**26** and **27**) clearly are rather bulky compounds and they did not exert significant inhibition of the CA-catalyzed CO_2-bicarbonate interconversion[29,30] and only suppressed the esterase activity of *h*CA I and *h*CA II (in their earlier studies this group used *p*-nitrophenylacetate based esterase assay in order to test inhibitory properties of compounds) (Figure 8). Based on these data, the authors drew a conclusion that these acridine-based sulfonamides have affinity to the enzyme.

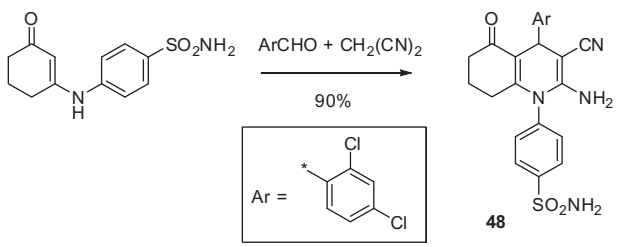
In subsequent studies[31], closely related 3- and 4-nitrophenyl-substituted acridine derivatives **28** were reduced to give the respective anilines **29** and ensuing treatment with sulfonyl



Scheme 16. Preparation of quinoline and pyrimidoquinoline sulfonamide derivatives **38–40**.



Scheme 17. Examples of derivatization of 2-amino-3-cyano-1,4-dihydropyridine moiety in sulfonamide **41**.



Scheme 18. Synthesis of 2-aminonicotinonitrile **48** via a Hantzsch-type reaction.

chlorides afforded corresponding bis-sulfonamides **30**. Compounds **28–30** were tested via classical stopped flow CO_2-hydratase assay and were found to be micromolar *h*CA I and low nanomolar *h*CA II, IX and XII inhibitors (Scheme 12). Interestingly, potent CA inhibitors **28–30** appear quite similar to compounds **25** which did not inhibit the CA activity in the CO_2 hydration assay. Although the reasons for the striking difference is not clear in the case of nitrophenyl (**28**) and aminophenyl (**29**) derivatives, the presence of the pharmacophoric secondary sulfonamide moiety[18] in **30** was likely responsible for the observed high potency of the latter.

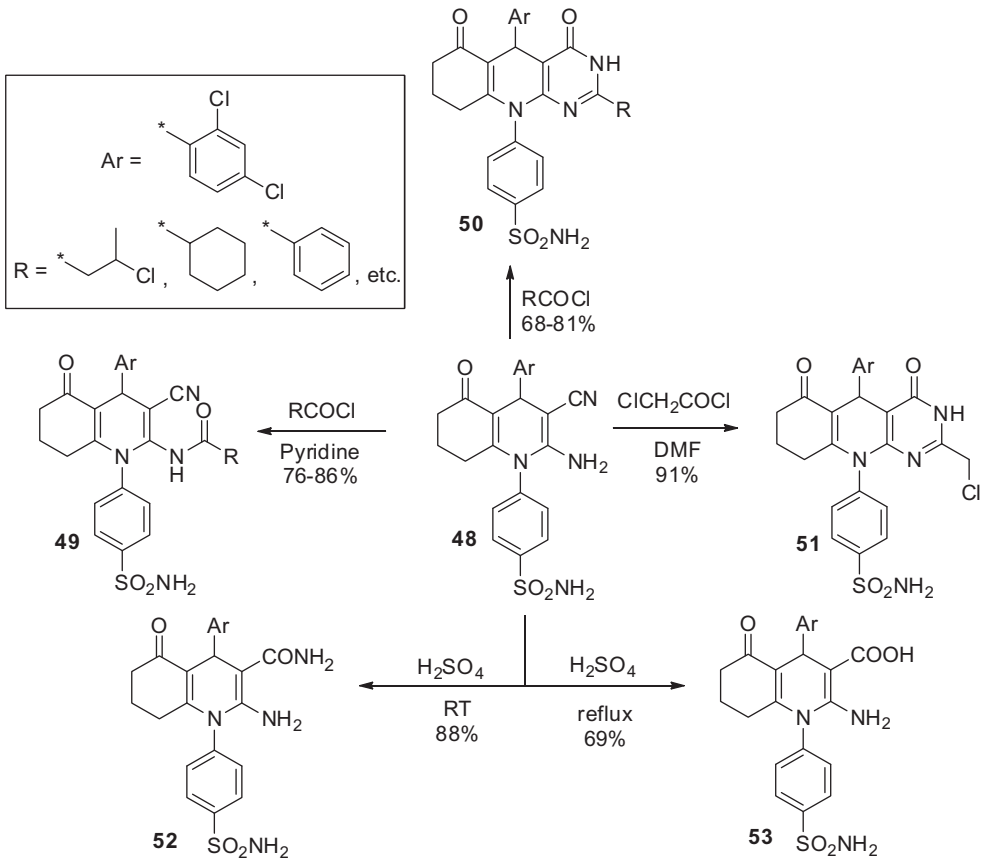
Compound **31** incorporating an amide linker between the benzenesulfonamide and the acridine moieties was prepared using a similar Hantzsch methodology[32]. When *m*- and *p*-phthalic dialdehyde was used (in combination with 2 equivalent of other components), bis-acridines **32** were obtained in good yields (Scheme 13).

Both **31** and **32** manifested themselves as submicromolar to micromolar inhibitors of *h*CA I (K_i range 0.16–9.64 μM) and also inhibited *h*CA II and brain-associated *h*CA VII with the K_i range of 0.004–0.499 μM. The remarkable potency of representative compounds from this series (Figure 9) compared to that of **28–30** well illustrated the efficiency of tail approach in designing novel sulfonamide CA inhibitors[33].

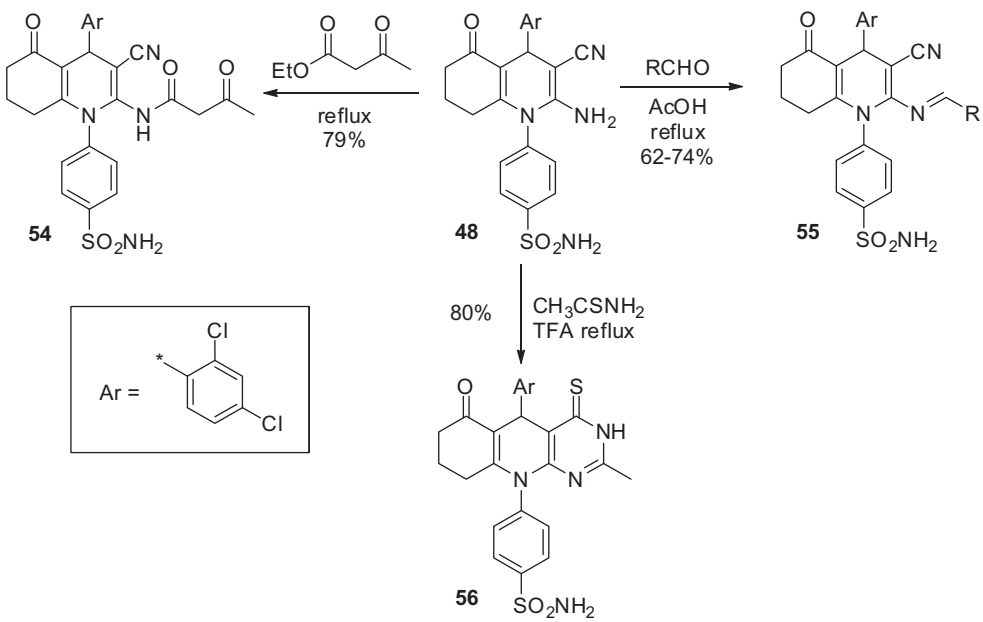
In summary, the Kaya group evaluated CA inhibitory properties of 55 acridine-based sulfonamides, and reported a range of novel inhibitors possessing micromolar to nanomolar-level potency.

The use of a similar reaction of 1,3-dicarbonyl compounds with aromatic aldehydes and ammonia hydroxide was reported by Subudhi and coworkers[34]. The process was conducted in refluxing ethanol furnishing 1,4-diydropyridines **33** in 67–76% yields. Several of the resulting 1,4-dihydropyridines **33** were decorated with a benzenesulfonamide moiety through the amide linkage, via sequential treatment with chloroacetyl chloride under basic conditions followed by sulfanilamide in refluxing ethanol (Scheme 14).

Unfortunately, the Subudhi team did not undertake evaluation of compound **34** for their CA inhibitory properties. The compounds were tested for anticonvulsant activity in mice. The observed anticonvulsant properties of **34** were assumed to be due to carbonic anhydrase inhibition, based on the presence of the primary sulfonamide pharmacophore. However, this conclusion should be taken with a due degree of caution since the compounds were not tested against carbonic anhydrase.

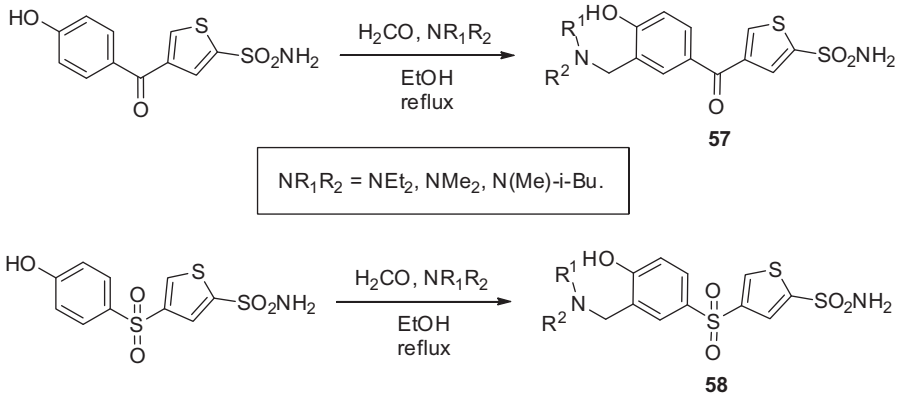


Scheme 19. Derivatization of **48**.



Scheme 20. Further derivatization of core building block **48**.

Scheme 21. Aminomethylation of furan and thiophene sulfonamide derivatives (no yields reported).



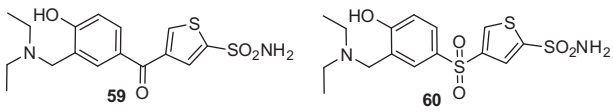


Figure 10. Most active Mannich adducts, reported by Hartman et al[39].

Nonsymmetrical Hantzsch-type reaction in synthesis of CAIs

A nonsymmetrical Hantzsch reaction (i.e. a multicomponent reaction employing two different carbonyl components) was used in the synthesis of CAIs. In particular, a series of novel sulfonamides was synthesized from substituted 2-aminonicotinonitriles **35** and **36** by Ghorab and coworkers (Scheme 15)[35,36].

The multicomponent reaction of acetaldehyde with malononitrile, cyclohexanone (or dimedone) and ammonia acetate proceeded in refluxing ethanol affording **35** or **36** in modest yields. 2-Aminonicotinonitriles **35** and **36** were subjected to further modification in order to generate sulfonamide derivatives **38–40** (Scheme 16).

Sulfonamide **41** was obtained in a similar Hatzsch-type reaction using sulfanilamide *in lieu* of ammonia. It was used as a core building block for further modifications at the 2-amino-3-cyano-1,4-dihydropyridine moiety as illustrated by the examples in Scheme 17.

Cytotoxic activity of sulfonamides thus synthesized was evaluated *in vitro* against human breast cancer cell line and the respective IC_{50} values were determined. Again, it was only hypothesized that the observed cytotoxic action was due to the compounds being CA inhibitors. However, **38–47** were not assayed against carbonic anhydrase, and their CA inhibitory properties remain to be substantiated by obtaining solid biochemical data. Cytotoxic (cytostatic or cytocydic) activity of small molecules can be exerted via a myriad of different targets and mechanisms[37] and compounds **38–47**, too, can act via a mechanism completely different from CA inhibition (this possibility is perhaps underscored by the similar cytotoxic activity of primary and secondary sulfonamides, whereas the former are known to be a lot more potent CA inhibitors compared to the latter.

Attesting to the popularity of the 1,4-dihydropyridine scaffold in the CAI design, core 2-aminonicotinonitrile **48** was synthesized on multigram scale via a Hantzsch reaction by al-Said et al.[38] (Scheme 18).

Treatment of **48** with various reagents afforded a wide range of novel sulfonamide derivatives. In particular, reaction of **48** with acid chlorides in pyridine afforded **49**, while in the absence of the basic solvent, a cyclization took place, furnishing **50**. A similar cyclization occurred when **48** was treated with chloroacetyl chloride, affording **51**. Treatment of **48** with concentrated sulfuric acid at room temperature provided primary carboxamide derivative **52**, while raising the reaction temperature to reflux led to a complete hydrolysis and formation of carboxylic acid **53** (Scheme 19).

Several other benzenesulfonamide derivatives **54–56** were synthesized through treatment of **48** with various carbonyl compounds as well as thioacetamide or ethyl acetoacetate (Scheme 20).

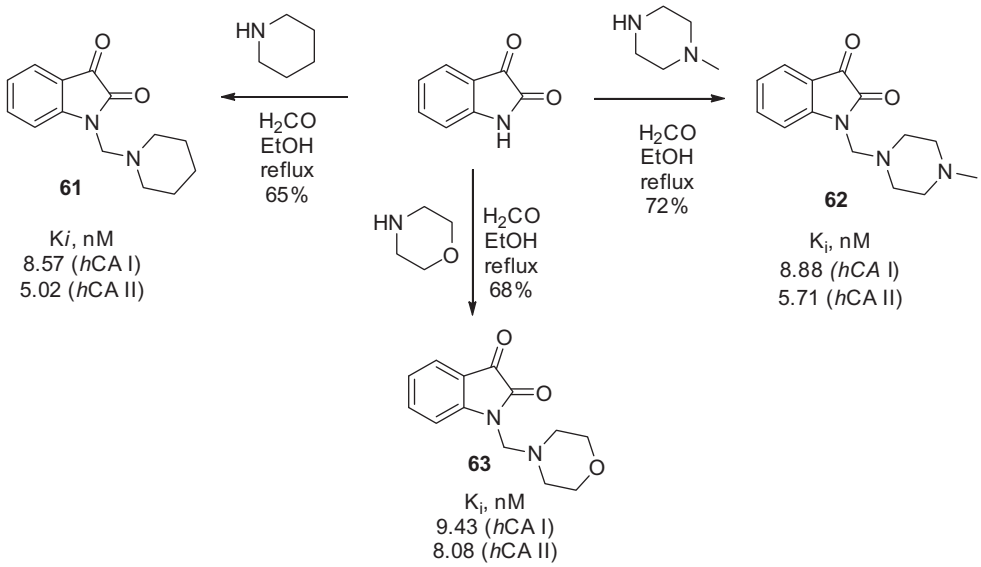
In vitro cytotoxic activity of 22 synthesized compounds was evaluated and the principal mechanism of the observed cytotoxic action was assumed to involve CA inhibition. However, this assumption was only supported by computer modeling data, and the CA inhibitory activity of reported sulfonamides was not evaluated in a biochemical assay.

Mannich reaction in synthesis of CAIs

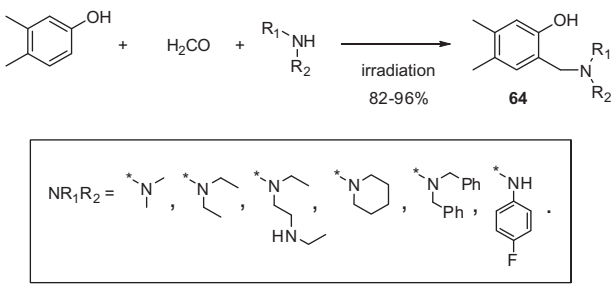
Mannich aminomethylation reaction has been amply used in synthesis of different types of CA inhibitors. Alkylamino groups can substantially influence inhibitor's affinity to the enzyme active site and result in potent inhibitors. In addition to that, such ''solubilizing side chains'' can endow the resulting inhibitors with pronounced isoform selectivity.

The aspect of increased potency is nicely illustrated by Mannich adducts **57** and **58** that were synthesized from corresponding 4-hydroxybenzoyl and 4-hydroxybenzenesulfonyl thiophene-2-sulfonamides by Hartman et al.[39] (Scheme 21). Compounds **57** and **58** (as well as their furan analogs) demonstrated inhibitory activity against *h*CA II and their IC_{50} values were found to be in the nanomolar range. Synthesized compounds also possessed remarkable results being tested *ex vivo* for their ability to penetrate the albino rabbit eye and to inhibit CA II in a homogenate of the iris-ciliary body. In addition, when evaluated *in vivo* in a rabbit model of ocular hypertension, most potent compounds (**59** and **60**) exhibited pronounced activity as intraocular pressure lowering agents, while, expectedly, demonstrating improved solubility compared to the starting phenolic compounds (Figure 10). Compound **59** was found to be a lead candidate for further development as a potent topical agent for glaucoma treatment.

Ozgun and coworkers[40] recently reported isatin Mannich bases **61–63**, bearing piperidine, morpholine or *N*-methylpiperazine appendages (Scheme 22). Compounds **61–63** were found to possess nanomolar inhibitory activity against cytosolic *h*CA I and *h*CA II isoforms.



Scheme 22. Preparation and K_i's of isatin Mannich bases **61–63**.



Scheme 23. Aminomethylation of 3,4-dimethylphenole.

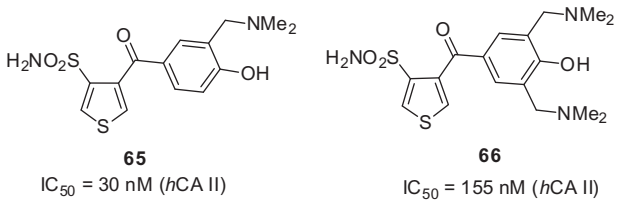


Figure 11. Mannich adducts reported by Chow et al[42].

Büyükkidan et al.[41] synthesized 3,4-dimethylphenole Mannich derivatives **64** (Scheme 23) and investigated their inhibitory properties toward CA.

While none of the compounds (**64**) investigated inhibited CA-catalyzed CO_2-to-bicarbonate interconversion, some of them were found to selectively inhibit the esterase activity of *h*CA II over *h*CA I. The observed CA esterase activity suppression was relatively weak, with K_i values ranging between 6.3 and 96.4 μM. Interestingly, the esterase activity was found to be potentiated, and not inhibited, by some of the analogs from series **64**.

Among the CA inhibitors synthesized by Chow and colleagues[42], two particularly potent Mannich bases **65** and **66** were reported. These compounds were found to strongly inhibit *h*CA II inhibition with IC_{50} values in nanomolar range (Figure 11).

In contrast to compounds **59–60** where introduction of the aminomethyl side chains into the phenolic sulfonamide core resulted in an increased potency and solubility, Mannich derivatives **65** and **66**, disappointingly, turned out to be less potent than the starting phenol-containing sulfonamides and, therefore, were not advanced into further *ex vivo* studies.

A case where introduction of the aminomethyl appendage did not seem to significantly influence inhibitory properties of the starting (phenolic) compound is illustrated by a series of phenolic Mannich bases including mono- and bis-dialkylamino dervatives such as **68** or **69**, reported by Yamali et al.[43] These compounds possessed similar or slightly lower inhibitory properties compared to starting **67** as expressed in the single-concentration data reported (Figure 12).

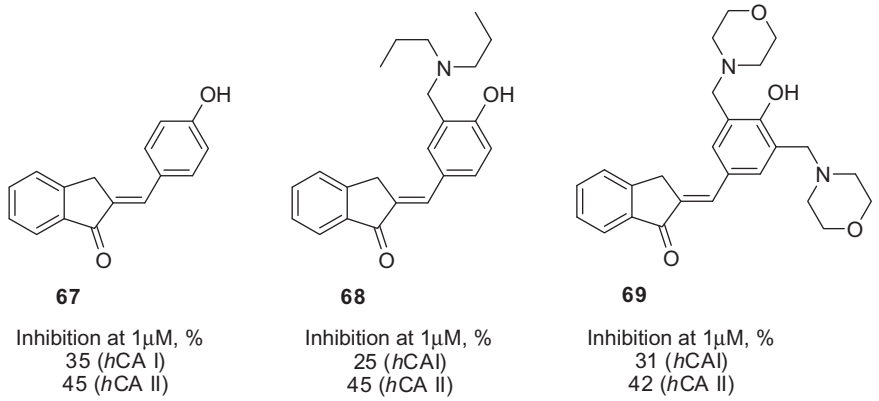
Similarly to compound **64** (*vide supra*), several thymol derivatives **70–71** were recently prepared via the Mannich reaction and evaluated for CA inhibition by Gul et al.[44]. The position of the aminomethyl side chain was influenced by the nature of the secondary amine employed in the Mannich reaction and the resulting compounds were isolated in rather modest yields (Scheme 24).

The seven compounds synthesized within series **70–71** were found to possess substantial cytotoxic properties but demonstrated only a weak inhibition of *h*CA I and II (Figure 13). Therefore, in their cytotoxic properties observed in the course of *in vitro* cell viability studies were assumed to be due to cellular mechanisms are than CA inhibition.

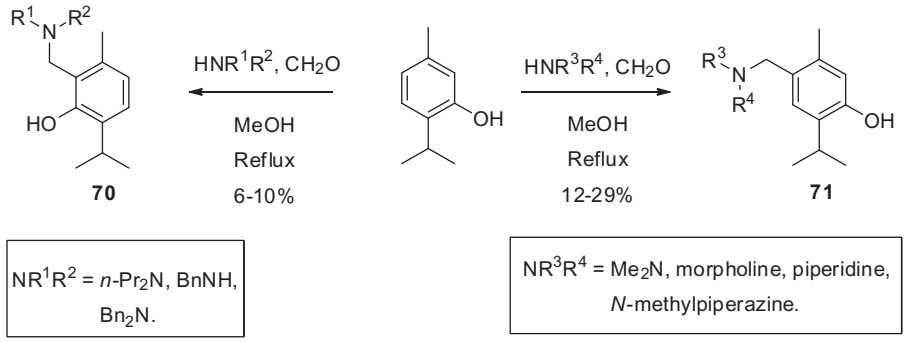
Conclusion

Multicomponent chemistry (primarily, the Biginelli, Hantzsch and Mannich reactions) is a powerful tool for generating arrays of novel compounds for biological evaluation. It has found significant utility in the construction of novel inhibitors of carbonic anhydrase. However, it is clear that a large body of literature reports scaffolds amenable via multicomponent approach, which contain the zinc-binding primary sulfonamide pharmacophore. For some of the compounds reported, CA inhibition was confirmed in a biochemical assay (through observation of the esterase or CO_2 hydratase activity). However, with disappointing frequency, compounds are evaluated in phenotypical cellular assays (such cancer cell viability) and the CA inhibition is simply assumed to be the principal mechanism of the observed biological

Figure 12. Percentage of inhibition data for progenitor compound **67** and its most potent Mannich derivatives **68–69**.



Scheme 24. Mannich aminomethylation in *ortho*- or *para*-position of thymol.



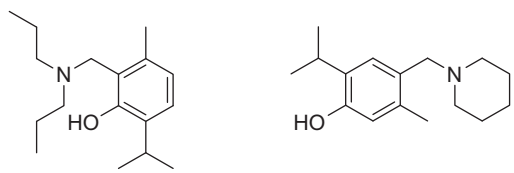


Figure 13. Inhibition percentage of most potent 2- and 4-methylamino derivatives of thymol.

action. Such ''CAIs'' should be considered with a due degree of caution and should be reevaluated in an accurate assay using an isolated CA target. Likewise, it is clear that systematic studies of the structure-activity relationships around scaffolds amenable by the modern multicomponent reactions (such as the isocyanide-based Ugi reaction,[45] the Castagnoli–Cushman lactam synthesis[46] and related reactions) represent a significant knowledge void in the area of CA inhibitors. Directing research efforts toward this area of medicinal chemistry research will be a timely, prudent and much needed undertaking.

Acknowledgement

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Declaration of interest

The authors declare no conflict of interest.

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