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Isatin: a privileged scaffold for the design of carbonic anhydrase inhibitors

Claudia Melis[a], Rita Meleddu[a], Andrea Angeli[b], Simona Distinto[a], Giulia Bianco[a], Clemente Capasso[c], Filippo Cottiglia[a], Rossella Angius[d], Claudiu T. Supuran[b] and Elias Maccioni[a]

[a]Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy; [b]Dipartimento NEUROFARBA, Sezione di Scienze Farmaceutiche, Università degli Studi di Firenze, Sesto Fiorentino, Florence, Italy; [c]Istituto di Bioscienze e Biorisorse, CNR, Napoli, Italy; [d]Laboratorio NMR e Tecnologie Bioanalitiche, Sardegna Ricerche, Pula, CA, Italy

ABSTRACT

The isatin scaffold is the constitutive fragment of several natural and synthetic bioactive molecules. Albeit several benzene sulphonamide-based carbonic anhydrase inhibitors (CAIs) have been reported, only recently isatin benzene sulphonamides have been studied and proposed as CAIs. In this study we have designed, synthesised, and evaluated the biological activity of a series of differently substituted isatin-based benzene sulphonamides which have been designed for the inhibition of carbonic anhydrase isoforms. The activity of all the synthesised compounds was evaluated towards human carbonic anhydrase I, II, IX, and XII isozymes. Our results indicate that the nature and position of substituents on the isatin ring can modulate both activity and isozyme selectivity.

Introduction

The isatin nucleus could be considered as a privileged scaffold for the design of biologically active agents. The discovery and optimisation of isatin-based therapeutic agents have consistently attracted the interest of medicinal chemists and the chemistry, the biological properties and the therapeutic potential of isatin-based agents has been recently reviewed[1]. Several biological activities could be achieved by decoration of the isatin scaffold such as anti-cancer[2–7], anti-oxidant[6], HIV reverse transcriptase inhibition[8,9], neuroprotective[10], anti-fungal[11,12], anti-bacterial[13,14] and anti-diabetic[15]. Moreover, the isatin ring has been pointed out as an essential part of anticancer hybrid molecules[16,17]. Recently, the design of isatin-based carbonic anhydrase inhibitors (CAIs) has been reported[10,11,18–20]. It is common knowledge that carbonic anhydrase isozyme family is involved in several physiological and/or pathological metabolic pathways[21–26]. They catalyse the simple reaction of the reversible hydration of carbon dioxide to bicarbonate and protons[27], which is essential for the regulation of the different chemical species connected with CO_2 in the body and its transport across biological membranes such as the inter-, intra- and extra-cellular spaces[25,28]. Not surprisingly several CAIs have been reported and their therapeutic potential has been directed towards different pathologies[29–31] (Figure 1).

Within CAIs, benzene-sulphonamides are widely represented[32–35] and their binding on carbonic anhydrase investigated[36]. Benzene-sulphonamides are versatile scaffold that can be efficiently substituted to achieve isozyme specificity[17,19,20,35,37–39]. In this respect, the specific targeting of the tumour associated CA isoforms IX and XII represents an innovative and specific approach for the treatment of tumours[23,40,41]. Furthermore, the identification of hybrid molecules, containing both the isatin scaffold and the benzene-sulphonamide moiety, with a multi-pharmacological

effect is an attractive target for medicinal chemists, for the treatment of multifactorial pathologies such as cancer[41–43]. On the basis of the above and with the aim to achieve structure-activity relationships on isatin derived CAIs, we have designed and synthesised a series of new 4-{[5–(2-oxo-2,3-dihydro-1H-indol-3-ylidene)-3-methyl-4-oxo-1,3-thiazolidin-2-ylidene]amino}benzene-1-sulphonamides as potential inhibitors of the tumour associated CA isoforms IX and XII.

Methods

Materials and apparatus

Starting materials and reagents were obtained from commercial suppliers and were used without purification. All melting points were determined on a Stuart SMP11 melting points apparatus (Stone, UK) and are uncorrected. Electron ionisation mass spectra were obtained by a Fisons QMD 1000 mass spectrometer (Danvers, MA) (70 eV, 200 mA, ion source temperature 200 °C). Samples were directly introduced into the ion source. Found mass values are in agreement with theoretical ones. Melting points, yield of reactions and the analytical data of derivatives are reported in Table 1.

^{1}H-NMR (Table 2) were registered on a Bruker 400 MHz spectrometer (Billerica, MA) or on a Varian 500 MHz (Palo Alto, CA) (Table 2). All samples were measured in DMSO. Chemical shifts are reported referenced to the solvent in which they were measured. Coupling constants J are expressed in hertz (Hz). Elemental analyses were obtained on a Perkin–Elmer 240 B microanalyser (Waltham, MA). Analytical data of the synthesised compounds are in agreement within ±0.4% of the theoretical values. TLC chromatography was performed using silica gel plates (Merck F 254, Billerica, MA), spots were visualised by UV light.

CONTACT Rita Meleddu ✉ rita.meleddu@unica.it ◱ Department of Life and Environmental Sciences, University of Cagliari, Via Ospedale 72, Cagliari 09124, Italy; Claudiu T. Supuran ✉ claudiu.supuran@unifi.it ◱ Dipartimento NEUROFARBA, Sezione di Scienze Farmaceutiche, Università degli Studi di Firenze, Via Ugo Schiff, 6 50019 Sesto Fiorentino, Florence, Italy
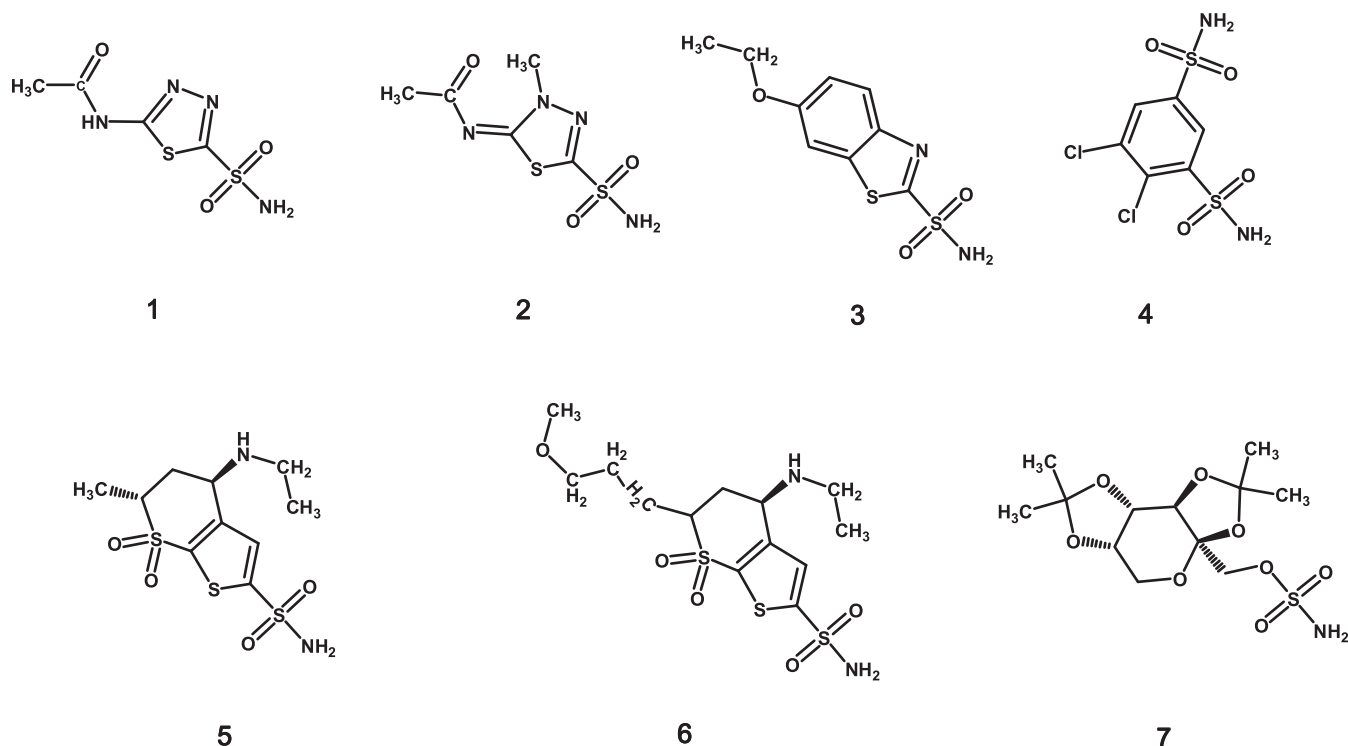
Figure 1. Carbonic anhydrase inhibitors in clinical use: (**1**) acetazolamide, (**2**) (methazolamide, (**3**) ethoxzolamide, (**4**) dichlorphenamide, (**5**) dorzolamide, (**6**) brinzolamide, and (**7**) topiramate.

Table 1. Chemical, analytical, and physical data of derivatives **EMAC 10020.**

Compound	R	R.F.*	C–H–N Calc.	C–H–N Found	M.P. °C	Yield %	Mass fragments
EMAC 10020 a	H	0.52	C, 52.16; H, 3.40; N, 13.52	C, 52.19; H, 3.39; N, 13.50	320d	38	414; 386; 373
EMAC 10020 c	5-Cl	0.38	C, 48.16; H, 2.92; N, 12.48;	C, 48.19; H, 2.90; N, 12.46;	320-1	71	448; 420; 407
EMAC 10020 d	5-F	0.42	C, 49.99; H, 3.03; N, 12.96;	C, 50.01; H, 3.00; N, 12.93;	310-2	66	432; 404; 391
EMAC 10020 l	5-CH₃	0.50	C, 53.26; H, 3.76; N, 13.08;	C, 53.29; H, 3.73; N, 13.05;	320-2	67	428; 400; 387
EMAC 10020 m	7-F	0.65	C, 49.99; H, 3.03; N, 12.96;	C, 50.00; H, 3.01; N, 12.95;	288d	43	432; 404; 391
EMAC 10020 n	5-CF₃	0.51	C, 47.30; H, 2.72; N, 11.61;	C, 47.33; H, 2.70; N, 11.59;	314d	41	482; 454; 441
EMAC 10020 o	7-Br	0.69	C, 43.82; H, 2.66; N, 11.36;	C, 43.85; H, 2.64; N, 11.33;	320d	75	492; 464; 451

*R.F. values were obtained on silica gel plates using a mixture of ethyl acetate/n-hexane 2/1.

Biological activity

Carbonic anhydrase inhibition assay

The purification of cytosolic CA isoenzymes (CA I and CA II) were previously described with a simple one-step method by a Sepharose-4B-L tyrosine-sulphanilamide affinity chromatography[44]. The protein quantity in the column effluents was determined spectrophotometrically at 280 nm. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied with a Bio-Rad Mini Gel system Mini-PROTEIN® system (Hercules, CA), Bio-Rad Laboratories, Inc., China after purification of both CA isoenzymes. Briefly, it was performed in acrylamide for the running (10%) and the stacking gel (3%) contained SDS (0.1%), respectively. Activities of CA isoenzymes were determined according to a method by Verporte et al.[45] The increase in absorbance of reaction medium was spectrophotometrically recorded at 348 nm. Also, the quantity of protein was determined at 595 nm according to the Bradford method[46]. Bovine serum albumin was used as standard protein. The IC_{50} values were obtained from activity (%) versus compounds plots[47]. For calculation of K_I values, three different concentrations were used. The Lineweaver–Burk curves were drawn and calculations were realised[47]. The biological data are reported in Table 3.

Table 2. ^{1}H NMR and ^{13}C NMR data of derivatives EMAC 10020.

Compound	^{1}H NMR δ (ppm)
EMAC 10020 a	^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.32- 3.36 (3H, s, CH$_3$, N-CH$_3$); 7.05- 7.09 (1H, s, CH, Isat.); 6.91-6.93 (1H, d, $J = 7.6$ Hz, CH, Isat.); 7.20 (2H, d, CH, $J = 8.8$ Hz, 4-SO$_2$NH$_2$ phenyl); 7.35- 7.39 (3H, m, CH + NH$_2$, Isat.+ SO$_2$NH$_2$)7.86- 7.89 (2H, d, CH, $J = 8.8$ Hz, 4-SO$_2$NH$_2$ phenyl); 8.84- 8.86 (1H, d, CH, $J = 8$ Hz, Isat.); 11.20 (1H, s, NH, Isat.). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 29.10, 109.93, 120.10, 121.46, 123.55, 127.19, 128.05, 124.30, 130.80, 132.37, 140.16, 140.98, 150.52, 154.32, 165.46, 168.40.
EMAC 10020 c	^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.29- 3.37 (3H, s, CH$_3$, N-CH$_3$); 6.93- 6.95 (1H, d, CH, $J = 8.5$ Hz, 5-Cl Isat.); 7.16- 7.20 (2H, d, CH, $J = 8.5$ Hz, 4-SO$_2$NH$_2$ phenyl); 7.33 (2H, s, NH$_2$, SO$_2$NH$_2$); 7.41- 7.43 (1H, d, CH, $J_o = 8.5$ Hz, $J_m = 2$, 5 Hz -Cl Isat.); 7.86- 7.88 (2H, d, CH, $J = 8.5$ Hz, 4-SO$_2$NH$_2$ phenyl); 8.89 (1H, d, CH, $J = 2$ Hz, 5-Cl Isat.); 11.32 (1H, s, NH, 5-Cl Isat.). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 28.88, 111.47, 121.23, 121.41, 123.79, 125.81, 127.10, 127.31, 131.08, 132.55, 140.32, 141.89, 150.32, 153.96, 165.44, 168.05.
EMAC 10020 d	^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.33 (3H, s, CH$_3$, N-CH$_3$); 6.88 (1H, m, CH, 5-F Isat.); 7.18- 7.22 (2H, d, CH, $J = 8.4$ Hz, 4-SO$_2$NH$_2$ phenyl); 7.22-7.25 (1H, d, CH, 5-F Isat.); 7.37 (2H, s, NH$_2$, SO$_2$NH$_2$); 7.87- 7.90 (2H, d, CH, $J = 8.8$ Hz, 4-SO$_2$NH$_2$ phenyl); 8.58 (1H, m, CH, 5-F Isat.); 11.20 (1H, s, NH, 5-F Isat.). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 28.91, 111.08, 114.54, 118.16, 120.70, 121.38, 124.46, 127.28, 132.35, 140.34, 150.35, 154.06, 156.30, 158.63, 165.58, 168.35.
EMAC 10020 l	^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.30 (3H, s, CH$_3$, 5-CH$_3$ Isat.); 3.30- 3.35 (3H, s, CH$_3$, N-CH$_3$); 6.79 (1H, d, CH, 5-CH$_3$ Isat.); 7.16-7.19 (3H, m, CH, 4-SO$_2$NH$_2$ phenyl +5-CH$_3$ Isat.); 7.33 (2H, s, NH$_2$, SO$_2$NH$_2$); 7.87 (2H, d, CH, $J = 8$ Hz, 4-SO$_2$NH$_2$ phenyl); 8.67 (1H, s, CH, 5-CH$_3$ Isat.); 11.05 (1H, s, NH, 5-CH$_3$ Isat.). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.14, 29.12, 109.94, 120.06, 121.47, 123.50, 127.20, 128.03, 130.12, 130.70, 132.36, 140.15, 140.97, 150.52, 154.32, 165.45, 168.39.
EMAC 10020 m	^{1}H NMR (500 MHz, Acetone-d_6) δ (ppm): 3.48 (3H, s, CH$_3$, N-CH$_3$); 7.13-7.15(1H, m, CH, 7-F Isat.); 7.21-7.23 (2H, d, CH, $J = 8.5$ Hz, 4-SO$_2$NH$_2$ phenyl); 7.25-7.29 (3H, m, CH + NH$_2$,7-F Isat.+ SO$_2$NH$_2$), 7.96 (2H, d, CH, $J = 8.5$ Hz, 4-SO$_2$NH$_2$ phenyl); 8.85 (1H, d, CH, $J = 8$ Hz, 7-F Isat.); 10.43 (1H, s, NH, 7-F Isat.). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 28.91, 111.10, 114.54, 118.16, 120.70, 121.38, 122.90, 125.90, 127.28, 132.35, 150.35, 154.06, 156.30, 162.90, 165.58, 168.35.
EMAC 10020 n	^{1}H NMR (400 MHz, Acetone-d_6) δ (ppm): 3.35 (3H, s, CH$_3$, N-CH$_3$); 7.04- 7.08 (1H, m, CH, 5-CF$_3$ Isat.); 7.12 (2H, d, CH, $J = 8.5$ Hz, 4-SO$_2$NH$_2$ phenyl); 7.27- 7.31 (1H, t, CH, $J = 8$ Hz, 5-CF$_3$ Isat.); 7.31 (2H, weak s, NH$_2$, SO$_2$NH$_2$); 7.99 (2H, d, CH, $J = 8.5$ Hz, 4-SO$_2$NH$_2$ phenyl); 8.67 (1H, d, CH, $J = 8$ Hz, 5-CF$_3$ Isat.); 11.67 (1H, NH, 5-CF$_3$ Isat.). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 29.13, 109.95, 120.07, 121.47, 123.50, 124.60, 127.20, 128.04, 130.12, 130.71, 132.36, 140.16, 140.97, 150.52, 154.32, 165.45, 168.39.
EMAC 10020 o	^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.34 (3H, s, CH$_3$, N-CH$_3$); 6.99- 7.02 (1H, t, CH, $J = 8$ Hz, 7-Br Isat); 7.19- 7.20 (2H, d, CH, $J = 8.5$ Hz, 4-SO$_2$NH$_2$ phenyl); 7.34 (2H, s, NH$_2$, SO$_2$NH$_2$); 7.54- 7.55 (1H, d, $J = 8$ Hz, CH, 7-Br Isat.); 7.87- 7.89 (2H, d, CH, $J = 8.5$ Hz, 4-SO$_2$NH$_2$ phenyl); 8.81- 8.83 (1H, d, CH, $J = 8$ Hz, 7-Br. Isat.); 11.43 (1H, s, NH, 7-Br Isat.). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 29.02, 102.69, 121.45, 121.68, 123.37, 124.44, 126.71, 127.26, 132.79, 134.27, 140.30, 142.17, 150.38, 153.97, 165.31, 168.26.

General procedure for the synthesis of compound EMAC10020

Synthesis of 1-methyl-3–(4-sulphamoylphenyl)thiourea[1]

4-Aminobenzensulphonamide (1 eq.) was refluxed in 2-propanol until a clear solution is obtained. Then a solution of methylisothiocyanate (1 eq.) in 2-propanol was added dropwise. By adding the isothiocyanate the solution became yellowish. The mixture was stirred until reaction completion (5–6 h) monitored by TLC (ethyl acetate/n-hexane 2/1). The reaction is allowed to cool down at r.t. and the formation of a white foaming precipitate is observed, which was filtered and crystallised from ethanol. White crystals; MW: 245.32 g/mol; yield: 65%; Mp: °C 204–6

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.15 (3H, s, CH$_3$, N-CH$_3$); 7.08 (d, 2H, CH, $J = 8.4$, 4-SO$_2$NH$_2$ phenyl); 7.30 (2H, s, NH$_2$, SO$_2$NH$_2$); 7.80 (d, 2H, CH, $J = 8.4$, 4-SO$_2$NH$_2$ phenyl); 10.01 (s, 2H,NH, thiourea).

Synthesis of 4-(4-oxo-1,3-thiazolidin-2-ylidene)aminobenzene-1-sulphonamide2

An ethanol solution of **1** (1.70 eq.), ethyl bromoacetate (1.90 eq) and anhydrous sodium acetate (6.9 eq.) was refluxed under vigorous stirring till the completion of the reaction (16–20 h), TLC (ethyl acetate/n-hexane 2/1). Then the solution was cooled to 0 °C, and the formed precipitate filtered under vacuum and crystallised from water.

White powder; MW: 285.34 g/mol; yield: 86%; Mp: 179–180 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.17 (3H, s, CH$_3$, N-CH$_3$); 4.06 (s, 2H, CH$_2$, thiazol.); 7.10 (d, 2H, CH, $J = 8.4$, 4-SO$_2$NH$_2$ phenyl); 7.31 (2H, s, NH$_2$, SO$_2$NH$_2$); 7.80 (d, 2H, CH, $J = 8.4$, 4-SO$_2$NH$_2$ phenyl).

Synthesis of 4-(3-methyl-4-oxo-5-(2-oxoindolin-3-ylidene)thiazolidin-2-ylideneamino)benzene-sulphonamides EMAC 10020

A mixture of 4-(3-methyl-4-oxothiazolidin-2-ylideneamino)benzene-sulphonamide (1 eq), the opportune isatin derivative (1 eq), acetic anhydride (1,5 eq), and sodium acetate (2 eq) was refluxed overnight in acetic acid. Once the reaction has come to completion TLC (ethyl acetate/n-hexane 2/1) the hot suspension was filtered. The obtained red/orange solid was washed with water. Compounds **EMAC 10020** were purified by column chromatography on silica gel (ethyl acetate/n-hexane 2/1) to obtain the desired compounds whose data are reported in Tables 1 and 2.

Results and discussion

As a part of our ongoing research in the field of CAIs[37] and to achieve a better understanding of the structural requirements for the selective inhibition of the different CA isoforms, we have synthesised a series of 4-(3-methyl-4-oxo-5-(2-oxoindolin-3-ylidene)thiazolidin-2-ylideneamino)-benzenesulphonamides indicated as compounds **EMAC 10020 a, c, d, l, m, n,** and **o**. All the synthesised compounds bear a differently substituted isatin scaffold linked, by the interposition of a thiazolidinone spacer, to a benzene-sulphonamide moiety, as zinc binder group. The synthesis of compounds **EMAC 10020** was performed as illustrated in Figure 2.

The procedure consists of two steps. The first step is the synthesis of the 4-sulphamoylphenyl-thiourea derivative (**1** of Figure 2) by simple reaction of the 4-aminobenzensulphonamide with methylisothiocyanate. The second step of the synthetic route consists of the formation of the thiazolidinone spacer[2] which can be obtained by reacting **1** with ethyl-bromoacetate. Desired compounds were obtained by reacting compound **2** with the appropriate isatin. We attempted to perform step two and three in a one step one pot reaction, but our efforts only gave poor yields with respect to the three step procedure which was therefore preferred. Compounds **EMAC 10020** were submitted to enzymatic assay to evaluate their activity and selectivity towards human CA (hCA) isozymes I, II, IX, XII. The results are illustrated in Table 3. As shown in Table 3 some of the tested compounds could be considered as hCA IX preferential inhibitors. However, the nature and position of the substituents on the isatin scaffold played a crucial role in

Table 3. Inhibition data towards hCA I, II, IX, and XII of compounds EMAC 10020.

K_I (nM)

Compound	Structure	hCA I	hCA II	hCA IX	hCA XII
EMAC 10020 a		95.5	40.7	40.1	841
EMAC 10020 c		958	8300	55.7	865
EMAC 10020 d		50.3	274	50.7	85.0
EMAC 10020 l		87.6	79.1	3.0	440
EMAC 10020 m		44.3	139	4.1	90.7
EMAC 10020 n		945	5720	2660	873
EMAC 10020 o		877	8780	126	824
Acetazolamide		250	12	25	5.7
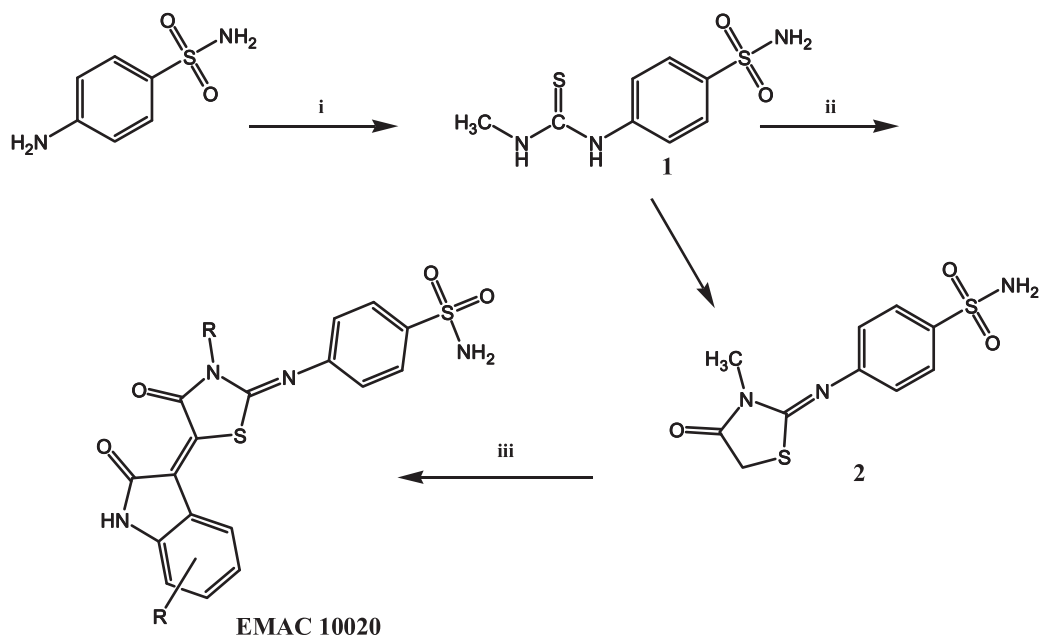
Figure 2. Synthetic pathway to compounds EMAC 10020. Reagents and conditions: (i) 2-propanol, methyl isothiocyanate; (ii) ethanol, ethyl bromoacetate, dry sodium acetate; (iii) R-isatin, acetic anhydride, dry sodium acetate, acetic acid.

determining the activity and the isozyme selectivity. In the case of compound **EMAC 10020 a**, bearing an un-substituted isatin, almost no selectivity can be observed, but for a very poor activity towards XII isozyme. With respect to isozyme IX, the introduction of an electron withdrawing (EW) group (compounds **EMAC 10020 c**, **EMAC 10020 d**, and **EMAC 10020 n**) in the position 5 of the isatin lead to a decrease of activity, albeit some selectivity towards hCA IX could be observed for compound **EMAC 10020 c**. However, the isosteric replacement of the chlorine atom and of the trifluoro-methyl moiety by a methyl group lead to an increase of activity (K_I 3.0 nM) and selectivity. According to these preliminary results, in the case hCA IX, the presence of an EW group in the position 5 of the isatin is not tolerated while the introduction of an electron donating substituent in the same position is beneficial for the activity and selectivity. Also in the case of compound **EMAC 10020 m**, bearing a fluorine atom in the position 7 of the isatin ring, a good activity (K_I 4.1 nM) and selectivity towards hCA IX was observed. Conversely the introduction of a bulkier EW group in the position 7 of the isatin scaffold, as for compound **EMAC 10020 o**, leads to a decrease of activity towards all isozymes. All together these data corroborate the hypothesis that the hybridisation of isatin scaffold with the benzene-sulphonamide moiety could be advantageous for the design of new therapeutic agents targeting the tumour associated hCA IX isoform.

Conclusions

We have synthesised a small library of isatin/benzene-sulphonamides hybrids for the inhibition of hCA isoforms. The activity of the newly synthesised derivatives has been evaluated towards hCA I, II, IX, and XII. Compounds **EMAC 10020 c**, **EMAC 10020 l**, and **EMAC 10020 m** could be considered as preferential hCA IX inhibitors. Our data indicated that the nature and the position of the substituents play a crucial role in tuning activity and selectivity towards hCA isozyme. Overall these data support the hypothesis that isatin hybrid molecules could represent a valuable starting point for the design of active and selective hCAIs.

Acknowledgements

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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