Novel compounds targeting the RNA-binding protein HuR. Structure-based design, synthesis and interaction studies.

Serena Della Volpe,[†,#] Rita Nasti,[†,#] Michele Queirolo, [†] M. Yagiz Unver,[‡,§] Varsha R. Jumde,[‡,§], Alexander Dömling,[∥] Francesca Vasile,[¶,*] Donatella Potenza,[¶] Francesca A. Ambrosio,[◆] Giosué Costa,[◆] Stefano Alcaro,[◆] Chiara Zucal,[⊥] Alessandro Provenzani,[⊥] Marcello Di Giacomo,[†] Daniela Rossi,[†] Anna K. H. Hirsch,[‡,§,*] Simona Collina.[†,*]

[†]Department of Drug Sciences, Medicinal Chemistry and Technology Section, University of Pavia, Via Taramelli 12, 27100, Pavia, Italy

[‡]Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) - Helmholtz Centre for Infection Research (HZI), Department of Drug Design and Optimization and Department of Pharmacy, Saarland University, Campus building E8.1, 66123, Saarbrücken, Germany

[§]Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, NL-9747 AG Groningen

[∥]Department of Drug Design, University of Groningen, A. Deusinglaan 1, Groningen, 9713 AV, Netherlands

[¶]Department of Chemistry, University of Milan, Via Golgi 19, 20133, Milano, Italy

[◆]Department of Health Sciences, University "Magna Græcia" of Catanzaro, Viale Europa, 88100, Catanzaro, Italy

[⊥]Department of CIBIO, University of Trento, Via Sommarive 9, 38123, Povo, TN, Italy

KEYWORDS. RNA-binding protein; HuR–RNA complexes; STD-NMR; Virtual screening; multi-component reactions.

ABSTRACT: The key role of RNA-binding proteins (RBPs) in regulating post-transcriptional processes and their involvement in several pathologies (*i.e.*, cancer and neurodegeneration) have highlighted their potential as therapeutic targets. In this scenario, Embryonic Lethal Abnormal Vision (ELAV) or Hu proteins and their complexes with target mRNAs have been gaining growing attention. Compounds able to modulate the complex stability could constitute an innovative pharmacological strategy for the treatment of numerous diseases. Nevertheless, medicinal-chemistry efforts aimed at developing such compounds are still at an early stage. As part of our ongoing research in this field, we hereby present the rational design and synthesis of structurally novel HuR ligands, potentially acting as HuR–RNA interferers. The following assessment of the structural features of their interaction with HuR, combining saturation-transfer difference NMR and *in silico* studies, provides a guide for further research on the development of new effective interfering compounds of the HuR–RNA complex.

RNA is an important regulatory element of many cellular processes and, thus, so-called RNA-binding proteins (RBPs), play a prominent role in affecting the fate of target messenger RNAs (mRNAs) coding for proteins pivotal in key cellular functions.[1-5] Therefore, it is not surprising that a dysregulation of RBPs may be related to the pathogenesis of several diseases, including neurological disorders and cancer.[2,4] Accordingly, various RBPs have been proposed as potential drug targets.[6] In 2018, the first candidate drug targeting RBPs, called H3B-8800, reached the clinical phase for the treatment of acute myelogenous leukemia and chronic myelomonocytic leukemia.[7]

Among RBPs, the family of ELAV (Embryonic Lethal Abnormal Vision) proteins is involved in controlling the functional activities of diverse RNA populations. In particular, HuR regulates splicing, stability and translation of thousands of coding and non-coding RNAs and is therefore considered a valid drug target for anticancer therapy.[8] HuR is a nuclear protein but, upon cell stress such as DNA damage, it shuttles into the cytoplasm where it regulates the fate of cargo mRNAs and determines the abundance of the encoded proteins. Over-expression of HuR or an aberrant nucleus/cytoplasm ratio are associated with tumor progression and poor prognosis in various cancer types.[8] For these reasons, there is a great interest in the scientific community to identify compounds able to bind HuR and to inhibit the formation of the HuR–RNA complex. So far, various high-throughput screening campaigns afforded several natural products (*i.e.*, MS-444[9] and dihydrotanshinone, DHTS[10-12]).

After studying the concept of druggability of ELAV proteins and related complexes with mRNA,[13-15] we analyzed the literature concerning the main findings on the ELAV–RNA complexes from a medicinal-chemistry standpoint and defined the interaction features of HuR and a small series of natural products.[16,17] These findings constituted the starting point for the identification of novel HuR ligands. In this letter, we report on the structure-based design and the synthesis of compounds with different "core struc-

tures" and the investigation of their interactions with HuR using a combination of saturation-transfer difference (STD)-NMR and *in silico* studies. While the interaction of HuR and RNA is mediated on the RNA side by AU-rich elements (AREs), on the protein side two RNA recognition motif-type (RRM) domains are involved. These two consecutive domains (RRM1 and RRM2) are located near the N-terminus and interact directly with target RNAs through highly conserved ribonucleoprotein (RNP1 and RNP2) sequences.[18,19] For the design of new compounds, we focused on a pocket-like region hosting small HuR ligands, formed by the two asymmetric units of the protein, which belong to RNP1 and RNP2 of RRM1. Inspection of the cocrystal structure of the HuR RRM1 and RRM2 domains in complex with the ARE sequence of RNA$^{c\text{-}fos}$ (PDB code 4ED5), confirms that this region corresponds to binding site of RNA uridine residues 8 and 9 (U8-U9). This narrow region is characterized by the presence of arginine and asparagine residues, rendering this cleft partially basic. By contrast, the rest of the ARE sequence is solvent-exposed and is mainly involved in electrostatic interactions with the surface of the protein (Figure 1a).

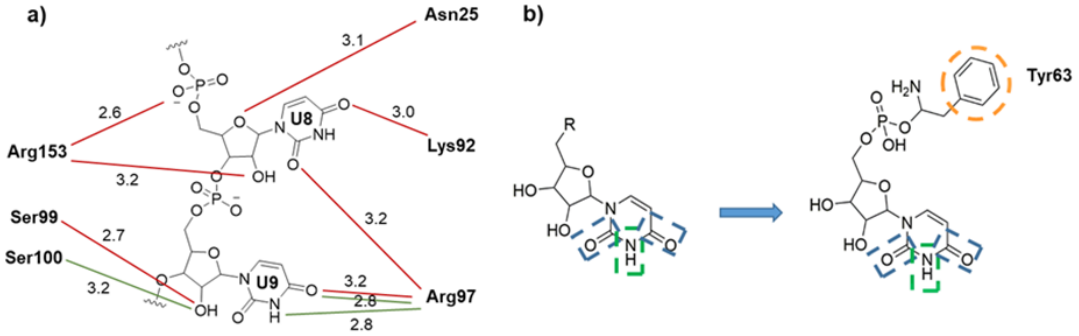


Figure 1. a) HuR–RNA contacts (PDB code 4ED5) for U8-U9. Red lines show side-chain contacts, green lines show main-chain contacts. Distances are expressed in Å;[20] b) Modification applied to the anchor to increase the predicted affinity. Dashed blue rectangles indicate hydrogen-bond acceptors and dashed green rectangles hydrogen-bond donors on both the original and modified anchor, while the dashed orange circle shows the anchor modification potentially allowing for additional hydrophobic interactions with the selected Tyr63 residue.

We based our search for new scaffolds for HuR ligands on the key interactions shown in Figure 1a and employed the free web-based virtual screening platform AnchorQuery™.[21-24] This program is specifically designed for targeting protein–protein interactions (PPIs) with small molecules by combining the anchor concept with one-pot multicomponent reaction (MCR) chemistry. Briefly, this platform is based on the critical role the anchor side chains play in PPI by targeting relatively stable pockets on the surface of the receptor. In fact, PPI inhibitors are characterized by specific moieties able to mimic amino acid side chains of the donor protein, called "anchor motifs". Since the contact surfaces involved in PPIs are typically large and flat, similar to protein–RNA interactions, we decided to use NucleoQuery, a nucleoside derivative of the web application AnchorQuery™ for the rational structure-based design of protein–RNA targeting compounds, focusing on HuR–RNA interfering compounds. We selected U8 and U9 as anchors given that they line the binding site, and investigated whether one of the two nucleotides may be a pharmacophore in terms of occupied position and interactions. To increase the probability of discovering new ligands, the anchors were modified so as to bear an additional phenyl group in order to establish a π–π-stacking interaction with Tyr63 (Figure 1b). As a result of four runs in NucleoQuery, a large library (800 molecules) featuring a wide range of structurally diverse derivatives was obtained. To select the candidates for synthesis, we relied on visual inspection and molecular-recognition studies. This way, we selected 17 compounds featuring piperidinones, aromatic heterocycles, *N,N*-disubstituted amides and sulfonamides as scaffolds (see SI, S1.2). For all compounds, the anchor shows good overlap with the corresponding uracil ring, preserving the same hydrogen bonds with Arg97 and Lys92, while the additional aromatic rings should afford the desired interaction with Tyr63. Moreover, selected compounds are engaged in additional interactions with the protein, including hydrophobic interactions with residues Ile23, Asn25, Phe65, Ile103, Ile133 (docking poses of exemplary compounds for each scaffold can be found in SI, S1.3). Taking into account the synthetic feasibility and commercial availability of the corresponding starting materials, we selected the most promising scaffolds and replaced the uracil moiety by an easy-to-handle starting material. To this aim, we evaluated several hydrophobic/aromatic portions as anchors, potentially able to interact with HuR in the position occupied by U8 and preserving its main contacts. As a result, we designed compounds **1**–**4** (Figure 2), characterized by different scaffolds and synthesized them for the experimental studies.

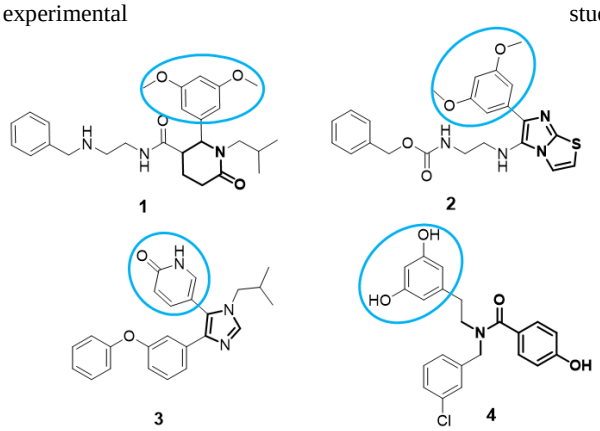
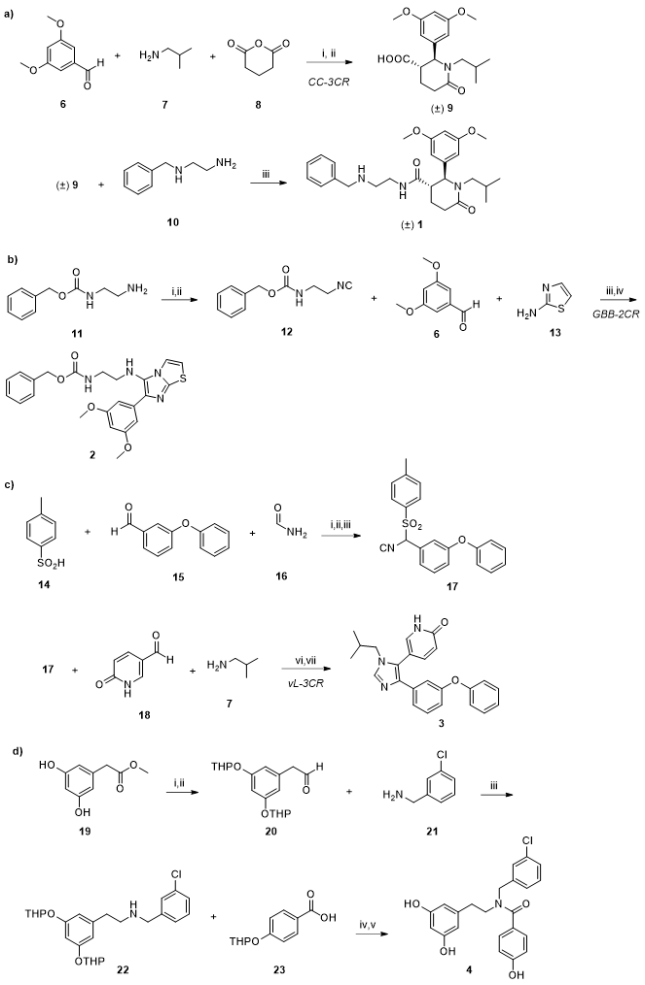


Figure 2. Structures of the four compounds selected for the experimental study. The modified anchors are highlighted in light blue. The different scaffolds (piperidinones, heterocycles, N,N-disubstituted amides) are highlighted in bold.

According to the AnchorQuery approach, the computationally predicted structures can be easily prepared by a short synthesis using multicomponent reactions or equally efficient processes. Thus, compound **1** could be synthesized by a Castagnoli-Cushman reaction (CC-3CR)[25] followed by amidation; **2** by a Groebke-Blackburn-Bienaymé (GBB-2CR);[26] **3** by a Van Leusen reaction (vL-3CR),[27] and **4** by a reductive amination[28] followed by acyla-

tion. Herein, we will briefly describe the synthetic strategies adopted to obtain compounds **1**–**4**. Further details on protocols employed and characterization of intermediates and final products are reported in the supporting information (SI, S2).

Amidation of the acid intermediate *trans* (±)-**9** with amine **10** afforded compound (±)-**1** as racemate.[29] The key intermediate *trans* 6-oxopiperidine-3-carboxylic acid (**9**) was obtained in good yield and diastereoselectivity, applying a microwave-aided one-pot CC-3CR on benzaldehyde (**6**), isobutylamine (**7**), and glutaric anhydride (**8**) (Scheme 1a).[25]



Scheme 1. Synthetic pathways of compounds 1–4. Reagents and conditions: a) Compound (±)-**1**: i) **6**, **7**, HCOOH, ACN, mw 120°C, 50 W, 30 min; ii) **8**, p-xylene, reflux, 10 h, yield 62%; iii) TBTU, DIPEA, THF, rt, 12 h, yield 71%. b) Compound **2**: i) Ethyl formiate, 10h; ii) TEA, $POCl_3$, DCM, rt, 4 h, yield 62%; iii) **6**, **13**, HCOOH, ACN, mw 120°C, 30 min; iv) **12**, $ZrCl_4$, 80 °C, 10 h, yield 21%. c) Compound **3**: i) H_2O, 30 min; ii) HCl, MTBE, 30 min; iii) **15**, **16** Me_3SiCl, ACN, Toluene, N_2, 50 °C, 5 h; iv) **14**, 16 h, yield 62%; v) $POCl_3$, triethylamine, THF, 10°C, 45 min, yield 85%; vi) **18**, **7** dry DMF, rt, 4 h; vii) **17**, K_2CO_3, rt, 72 h, yield 15%.

A microwave-assisted GBB-2CR,[26,30] involving isocyanide **12**, aldehyde **6** and amino-thiazole **13** furnished compound **2**. Isocyanide **12** was obtained starting from amine **11**, which was converted into the corresponding formamide and subsequently dehydrated (Scheme 1b). A vL-3CR[27] on commercially available aldehyde **18**, isobutylamine (**7**) and substituted TosMIC derivative **17** led to compound **3**. This MCR allows to access functionally rich imidazoles in a single pot via cycloaddition of TosMIC reagents on imines, generated *in situ* from an aldehyde and an amine under mildly basic conditions. We synthesized **17** starting from *p*-toluenesulfinic acid (**14**), which afforded the intermediate formamide derivative upon reaction with commercially available 3-phenoxybenzaldehyde (**15**), formamide (**16**) and chlorotrimethylsilane; subsequent dehydration furnished isocyanide **17** (Scheme 1c).[31] To access compound **4**, a reductive amination on aldehyde **20** followed by an amide coupling with acid **23** was exploited. Finally, removal of the THP protecting groups afforded target compound **4** (Scheme 1d).[28] Prior to the interaction study, compounds **1**–**3** were converted into the corresponding hydrochloride salts, and their solubility in both the buffer and time-ranges required for STD-NMR experiments was evaluated. Under these conditions, compound **2** proved to be poorly soluble and had to be excluded from the interaction study.

The interaction of compounds **1, 3** and **4** with HuR was studied by STD-NMR spectroscopy. This technique, based on the nuclear Overhauser effect, is a well-established epitope-mapping methodology for studying the target–ligand interactions with a large range of affinities (K_D = 10^{-9}–10^{-3} M).[32] Briefly, the method relies on the selective irradiation of the protein, which allows magnetization to be transferred to the bound ligand; the observation of the ligand signals in the NMR spectrum provides an indication of the interaction. Those ligand protons that are nearest to the protein are more likely to become highly saturated, and therefore show the strongest signal in the mono-dimensional STD spectrum. Therefore, the intensity of the STD signal (expressed as absolute STD percentage) reflects the proximity of the ligand to the protein surface.[33-36] The group epitope mapping illustrates which chemical moieties of the ligand are key for molecular recognition in the binding site. The analysis of STD data shows that compounds **1** (Figure S3.2.1 and Table S3.2.1), **3** (Figure S3.2.2 and Table S3.2.2), and **4** (Figure 3 and Table S3.2.3) interact with the protein.

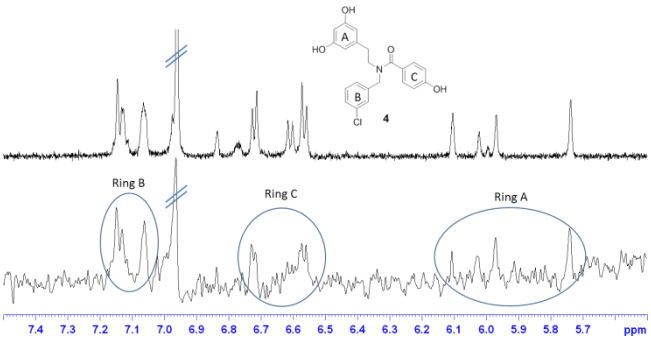


Figure 3. STD-NMR spectrum (bottom) and 1H-NMR (top) of compound **4**. The strong interaction observed for aromatic rings A, B and C is evidenced.

For ligand **1**, we observed the following contacts: the isopropyl group shows the strongest interaction (0.40 STD %), while the STD signal related to ring B is the least intense (0.03 STD %); ring A contributes to the interaction (0.20 STD %), while the protons of the amino chain (H14, H15 and H16) do not give STD signals. The binding of compound **3** to HuR is mediated by the pyridone anchor (1.20 STD %) and by the isopropyl moiety (0.30 STD %). For compound **4**, we observe STD signals of similar intensity for the three aromatic rings (0.40 % for ring A, 0.36 % for ring B and 0.25% for ring C, respectively), suggesting that they are positioned within a protein pocket.

In parallel, we performed docking studies on the crystal structure of HuR RRM1-2 domains according to the approach we published.[17] We used STD-NMR in combination with *in silico* studies (applied to the "closed" HuR conformations, see SI) to enable a more detailed description of the ligand–protein behavior in solution. Molecular docking simulations were performed in order to elucidate the binding mode and the interactions between compounds **1**, **3**, and **4** with HuR protein. In particular, we relied on Maestro tools[37] for a visual inspection and an analysis of protein–ligand interactions. We observed that the anchor moiety of each compound is predicted to be superimposed with U8, establishing pivotal interactions with the HuR protein. In detail, for compound **1** we found that aromatic ring A is involved in hydrophobic interactions with Tyr63, Ile23, Asn25 and Leu61 of HuR. The carboxyl group of the piperidone ring establishes a double hydrogen bond with Arg97 and hydrophobic interactions with the same residues. The isopropyl group (that has the strongest STD signal) establishes strong hydrophobic interactions with HuR (specifically, residues Ile103, Phe154, Asp155, Arg153 and Ile133) (Figure S4.2). Regarding compound **3**, the anchor moiety establishes π–π-stacking interactions with Tyr26 and additional hydrophobic interactions with Arg153, Ile133, Tyr26 and Asn25. We observed that the aromatic and imidazole rings establish several hydrophobic interactions (Figure S4.2.2). The isopropyl group is involved in hydrophobic interactions with Val93, Ser94, Ile133, Asn134, Lys92 and Asn82. As for compound **4**, we report strong hydrophobic interactions for all aromatic rings as evidenced by STD data. The amide carbonyl group establishes double hydrogen bonds with Arg97 and hydrophobic interactions with the same residue and Ile103 (Figure 4). Regarding ring B, it establishes a π–π-stacking interaction with Phe65 and hydrophobic interactions with Arg97, Pro98 and Phe65. Ring C is involved in a double hydrogen bond with Arg153 and Ile103 and different hydrophobic interactions. The zoomed-in view in Figure 4 highlights the full overlap of U8 and the anchor motif (ring A) of compound **4**.

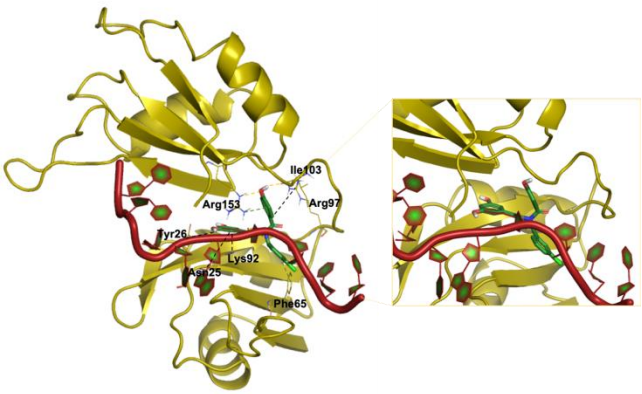


Figure 4: 3D representation of HuR–4 interactions. Hydrogen bonds and hydrophobic interactions are displayed as yellow and dark dashed lines, respectively; a π–π-stacking interaction is shown as a blue dashed line. RNA and HuR are represented as a brown and yellow cartoon, respectively. Compound **4** is shown in green sticks.

In this letter, we report for the first time on the structure-based design and synthesis of potential HuR ligands. The structure-based design strategy used in the present study enabled the identification of three novel compounds that interact with HuR, establishing contacts with the RNP regions of RRM1 and RRM2 domains of the protein. Targeting this region is pivotal for the inhibition of the protein activity and so the identification of compounds able to bind to this site plays a critical role in the development of new potential HuR inhibitors. Biophysical experiments (STD-NMR) are in agreement with computational results, confirming that the designed compounds **1**, **3**, and **4** are are indeed binders of HuR. Our approach led to the identification of new chemical scaffolds compared to previously reported HuR binders. Furthermore, theoretical experiments carried out by molecular dynamics and docking studies confirm that compound **4** is the best binder of HuR protein, in terms of theoretical binding affinity (SI, S4.2). The identified hit compounds will be be further optimized in forthcoming studies, building an *in silico* focused library. Our findings represent an important step in the discovery of novel compounds interfering with the Hu–RNA complex as new potential pharmacological tools in the treatment of several pathologies such as cancer, inflammation, and neurodegeneration, in which ELAV–RNA complexes play a pivotal role.

Experimental Procedures

Design of new scaffolds. We report all protocols in the SI, S1.
Synthesis of target compounds. Detailed synthetic procedures are reported in the SI, S2.
Protein expression and purification. Protein expression, purification, and purity assessment for HuR aliquots utilized in the STD-NMR study were performed as already described.[10,38]
Interaction study with HuR (STD-NMR and in silico combined approach). STD-NMR experiments, Molecular dynamics simulations and docking studies were carried out as reported in the SI (S3 and S4, respectively) and according to a previously described procedure.[17]

ASSOCIATED CONTENT

Supporting Information

Detailed protocols for compound design, synthesis and characterization, and interaction studies (STD-NMR and *in silico*) are reported in the supporting information.
The Supporting Information is available free of charge on the ACS Publications website.

AUTHOR INFORMATION

Corresponding Author

*S.C. e-mail: simona.collina@unipv.it
*F.V. e-mail: francesca.vasile@unimi.it
*A.K.H.H. email: Anna.Hirsch@helmholtz-hzi.de

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. #S.D.V and R.N. contributed equally to the work.

Present Addresses

R. N.: Department of Environmental Science and Policy (ESP), University of Milan, via C. Golgi 19, 20133 Milano-Italy, Building 5

M.Q.: Agro business Park 10, 6708 PW Wageningen, The Netherlands

Funding Sources

A.K.H.H. gratefully acknowledges funding from the Helmholtz-Association's Initiative and Networking Fund.

ACKNOWLEDGMENT

F.V. and S.C. gratefully acknowledge Federico Sala for his collaboration in the NMR laboratory. S.D.V. and S.C. thankfully recognize Scuola di Alta Formazione Dottorale of University of Pavia for the mobility research scholarship provided. R.N. and M.Q. acknowledge the Erasmus Traineeship program for carrying out a work experience placement at the Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, NL-9747 AG Groningen.

ABBREVIATIONS

ELAV, Embryonic lethal abnormal vision; RBP, RNA-binding protein; STD-NMR, saturation transfer difference-NMR; RRM, RNA recognition motif-type; RNP, ribonucleoprotein; MCR, multi-component reaction; CC-3CR, Castagnoli-Cushman reaction; GBB-2CR, Groebke-Blackburn-Bienaymé reaction, vL-3CR, Van Leusen reaction.

REFERENCES

1) Pascale, A.; Govoni, S. The complex world of posttranscriptional mechanisms: Is their deregulation a common link for diseases? Focus on ELAV-like RNA-binding proteins. *Cell. Mol. Life Sci.* **2012**, *69*, 501–517.
2) Talman, V.; Amadio, M.; Osera, C.; Sorvari, S.; Boije af Gennäs, G.; Yli-Kauhaluoma, J.; Rossi, D.; Govoni, S.; Collina, S.; Ekokoski, E.; Tuominen, R. K.; Pascale, A. The C1 domain-targeted isophthalate derivative HMI-1b11 promotes neurite outgrowth and GAP-43 expression through PKCα activation in SH-SY5Y cells. *Pharmacol. Res.* **2013**, *73*, 44–54.
3) Campos-Melo, D.; Droppelmann, C. A.; Volkening, K.; Strong, M. J. RNA-binding proteins as molecular links between cancer and neurodegeneration. *Biogerontology*. **2014**, *15*, 587–610.
4) König, J.; Zarnack, K.; Luscombe, N. M.; Ule, J.; Protein-RNA interactions: new genomic technologies and perspectives. *Nat. Rev. Genet.* **2012**, *18*, 77–83.
5) Doxakis, E. RNA binding proteins: a common denominator of neuronal function and dysfunction. *Neurosci. Bull.* **2014**, *1*, 610–626.
6) Hong, S. RNA Binding Protein as an Emerging Therapeutic Target for Cancer Prevention and Treatment. *J Cancer. Prev.* **2017**, *22*, 203–210.
7) Seiler, M.; Yoshimi, A.; Darman, R.; Chan, B.; Keaney, G.; Thomas, M.; Agrawal, A. A.; Caleb, B.; Csibi, A.; Sean, E.; Fekkes, P.; Karr, C.; Klimek, V.; Lai, G.; Lee, L.; Kumar, P.; Lee, S. C.; Liu, X.; Mackenzie, C.; Meeske, C.; Mizui, Y.; Padron, E.; Park, E.; Pazolli, E.; Peng, S.; Prajapati, S.; Taylor, J.; Teng, T.; Wang, J.; Warmuth, M.; Yao, H.; Yu, L.; Zhu, P.; Abdel-Wahab, O.; Smith, P. G.; Buonamici, S. H3B-8800, an orally available small-molecule splicing modulator, induces lethality in spliceosome-mutant cancers. *Nat. Med.* **2018**, *24*, 497-504.
8) Filippova, N.; Yang, X.; Ananthan, S.; Sorochinsky, A.; Hackney, J.; Gentry, Z.; Bae, S.; King, P.; Nabors, L. B. Hu antigen R (HuR) multimerization contributes to glioma disease progression. *J Biol. Chem.* **2017**, *292*, 16999–17010.
9) Meisner, N. C.; Hintersteiner, M.; Mueller, K.; Bauer, R.; Seifert, J. M.; Naegeli, H. U.; Ottl, J.; Oberer, L.; Guenat, C.; Moss, S.; Harrer, N.; Woisetschlaeger, M.; Buehler, C.; Uhl, V.; Auer, M. Identification and mechanistic characterization of low-molecular-weight inhibitors for HuR. *Nat. Chem. Biol.* **2007**, *3*, 508−515.
10) D'Agostino, V. G.; Lal, P.; Mantelli, B.; Tiedje, C.; Zucal, C.; Thongon, N.; Gaestel, M.; Latorre, E.; Marinelli, L.; Seneci, P.; Amadio, M.; Provenzani, A. Dihydrotanshinone-I interferes with the RNA-binding activity of HuR affecting its posttranscriptional function. *Sci. Rep*. **2015**, *5*, 1−15.
11) Lal, P.; Cerofolini, L.; D'Agostino, V. G.; Zucal, C.; Fuccio, C.; Bonomo, I.; Dassi, E.; Giuntini, S.; Di Maio, D.; Vishwakarma, V.; Preet, R.; Williams, S. N.; Fairlamb, M. S.; Munk, R.; Lehrmann, E.; Abdelmohsen, K.; Elezgarai, S. R.; Luchinat, C.; Novellino, E.; Quattrone, A.; Biasini, E.; Manzoni, L.; Gorospe, M.; Dixon, D. A.; Seneci, P.; Marinelli, L.; Fragai, M.; Provenzani, A. Regulation of HuR structure and function by dihydrotanshinone-I. Nucleic Acids Res. 45, 9514–9527 (2017).
12) Manzoni, L.; Zucal, C.; Di Maio, D.; D'Agostino V. G.; Thongon, N.; Bonomo, I.; Lal, P.; Miceli, M.; Baj, V.; Brambilla, M.; Cerofolini, L.; Elezgarai, S.; Biasini, E.; Luchinat, C.; Novellino, E.; Fragai, M.; Marinelli, L.; Provenzani, A.; Seneci, P. Interfering with HuR-RNA interaction: Design, synthesis and biological characterization of Tanshinone mimics as novel, effective HuR inhibitors. *J Med. Chem.* **2018**, *61*, 1483–1498.
13) Rossi, D.; Amadio, M.; Carnevale Baraglia, A.; Azzolina, O.; Ratti, A.; Govoni, S.; Pascale, A.; Collina, S. Discovery of small peptides derived from Embryonic Lethal Abnormal Vision proteins structure showing RNA stabilizing properties. *J. Med. Chem.* **2009**, *52*, 5017–5019.
14) Amadio, M.; Pascale, A.; Govoni, S.; Laurini, E.; Pricl, S., Gaggeri, R.; Rossi, D.; Collina, S. Identification of peptides with ELAV-like mRNA-stabilizing effect: an integrated in vitro/in silico approach. *Chem. Biol. Drug. Des*. **2013**, *81*, 707–714.
15) Vasile, F.; Rossi, D.; Collina, S.; Potenza, D. Diffusion-Ordered Spectroscopy and Saturation Transfer Difference NMR spectroscopy studies of selective interactions between ELAV protein fragments and an mRNA target. *Eur. J. Org. Chem.* **2014**, *2014*, 6399–6404.
16) Nasti, R.; Rossi, D.; Amadio, M.; Pascale, A.; Unver, M. Y.; Hirsch, A.K.H.; Collina, S. Compounds Interfering with Embryonic Lethal Abnormal Vision (ELAV) Protein-RNA Complexes: An Avenue for Discovering New Drugs. *J Med. Chem.* **2017**, *60*, 8257–8267.
17) Vasile, F.; Della Volpe, S.; Ambrosio, F. A.; Costa, G.; Unver, M. Y.; Zucal, C.; Rossi, D.; Martino, E.; Provenzani, A.; Hirsch, A. K. H.; Alcaro, S.; Potenza, D.; Simona, C. Exploration of ligand binding modes towards the identification of compounds targeting HuR: a combined STD-NMR and Molecular Modelling approach. *Sci. Rep*. published online September 23, 2018; DOI: 10.1038/s41598-018-32084-z.
18) Nagai, K.; Oubridge, C.; Jessen, T. H.; Li, J.; Evans, P. R. Crystal structure of the RNA-binding domain of the U1 small nuclear ribonucleoprotein A. *Nature*. **1990**, *348*, 515–520.
19) Wang, X.; Tanaka Hall, T. M. Structural basis for recognition of AU-rich element RNA by the HuD protein. *Nat. Struc. Biol*. **2001**, *8*, 141–145.
20) Wang, H.; Zeng, F.; Liu, Q.; Liu, H.; Liu, Z.; Niu, L.; Teng, M.; Li, X. The structure of the ARE-binding domains of Hu antigen R (HuR) undergoes conformational changes during RNA binding. *Acta Crystallogr*. **2013**, *69*, 373–380.
21) http://anchorquery.csb.pitt.edu/
22) Koes, D.; Khoury, K.; Huang, Y.; Wang, W.; Bista, M.; Popowicz, G. M.; Wolf, S.; Holak, T. A.; Dömling, A.; Camacho, C. J. Enabling large-scale design, synthesis and validation of small molecule protein-protein antagonists. *PLoS ONE*, published online March 12, 2012; DOI: 10.1371/journal.pone.0032839.
23) Abdelraheem, E. M. M.; Camacho, C. J.; Dömling, A. Focusing on shared subpockets - New developments in fragment-based drug discovery. *Expert Opinion on Drug Discovery*. **2015**, *10*, 1179–1187.
24) Koes, D.R.; Dömling, A.; Camacho, C.J. AnchorQuery: Rapid online virtual screening for small-molecule protein–protein interaction inhibitors. *Prot. Sci.* **2018**, *27*, 229–232.
25) Kroon, E.; Schulze, J. O.; Süß E.; Camacho, C. J.; Biondi, R. M., A. Dömling Discovery of a Potent Allosteric Kinase Modulator by Combining Computational and Synthetic Methods. *Angew. Chem. Int. Ed.* **2015**, *54*, 13933–13936.
26) Groebke, K.; Weber, L.; Mehlin, F. A fast heterocyclic three component synthesis of imidazo[1,2-a]annulated pyridines, pyrazines, pyrimidines and thiazoles under microwave conditions. *Synlett.* **1998**, *47*, 661−663.
27) Gracias, V.; Gasiecki, F.A; Djuric, S.W. Synthesis of Fused Bicyclic Imidazoles by Sequential Van Leusen/Ring-Closing Metathesis Reactions. *Org. Lett.* **2005**, *7*, 3183–3186.

28) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. Reductive Amination of Aldehydes and Ketones with Sodium Triacetoxyborohydride. Studies on Direct and Indirect Reductive Amination Procedures. *J. Org. Chem.* **1996**, *61*, 3849–3862.
29) Rossi D.; Marra A.; Picconi P.; Serra M.; Catenacci L.; Sorrenti M.; Laurini E.; Fermeglia M.; Pricl S.; Brambilla S.; Almirante N.; Peviani M.; Curti D.; Collina S. Identification of RC-33 as a potent and selective σ1 receptor agonist potentiating NGF-induced neurite outgrowth in PC12 cells. Part 2: g-Scale synthesis, physicochemical characterization and in vitro metabolic stability. *Bioorg. Med. Chem.* **2013**, *21*, 2577–2586.
30) Shaaban, S.; Abdel-Wahab, B. F. Groebke–Blackburn–Bienaymé multicomponent reaction: emerging chemistry for drug discovery. *Mol. Divers.* **2016**, *20*, 233–254.
31) Sisko, J.; Mellinger, M.; Sheldrake, P.W.; Baine, N.H. *α-Tosylbenzyl isocyanide;* Organic Syntheses, Coll. 10, 2004; p 692; 77, 2000; p 198.
32) Mayer, M.; Meyer, B. Characterization of ligand binding by saturation transfer difference NMR spectroscopy. *Angew. Chem. Int. Ed.* **1999**, *38*, 1784–1788.
33) Vasile, F.; Gubinelli, F.; Panigada, M.; Soprana, E.; Siccardi, A.; Potenza, D. NMR interaction studies of Neu5Ac-α-(2,6)-Gal-β-(1-4)-GlcNAc with influenza-virus Hemagglutinin expressed in transfected human cells. *Glycobiology*. **2018**, *28*, 42–49.
34) Dapiaggi, F.; Pieraccini, S.; Potenza, D.; Vasile, F.; Macut, H.; Pellegrino, S.; Aliverti, A.; Sironi, M. Computer aided design and NMR characterization of an oligopeptide targeting Ebola virus VP24 protein. *New J Chem*. **2017**, *41*, 4308–4315.
35) Guzzetti, I.; Civera, M.; Vasile, F.; Arosio, D.; Tringali, C.; Piarulli, U.; Gennari, C.; Pignataro, L.; Belvisi, L.; Potenza, D. Insights into the binding of cyclic RGD peptidomimetics to α5β1 integrin by live cell NMR and computational studies. *Chem. Open*. **2017**, 6, 128–136.
36) Vasile, F.; Menchi, G.; Lenc,i E.; Guarna, A.; Potenza, D.; Trabocchi, A. Insight to the binding mode of triazole RGD-peptidomimetics to integrin-rich cancer cells by NMR and molecular modeling. *Bioorg. Med. Chem*. **2016**, *24*, 989-994.
37) Maestro, Schrödinger, LLC, New York, NY, 2018.
38) Kundu, P.; Fabian, M. R.; Sonenberg, N.; Bhattacharyya, S. N.; Filipowicz, W. HuR protein attenuates miRNA-mediated repression by promoting miRISC dissociation from the target RNA. *Nucleic Acids Res*. **2012**, *40*, 5088–100.