CHEMICAL INHIBITORS TO RAS ONCOPROTEINS: CURRENT LANDSCAPE AND FUTURE OPPORTUNITIES

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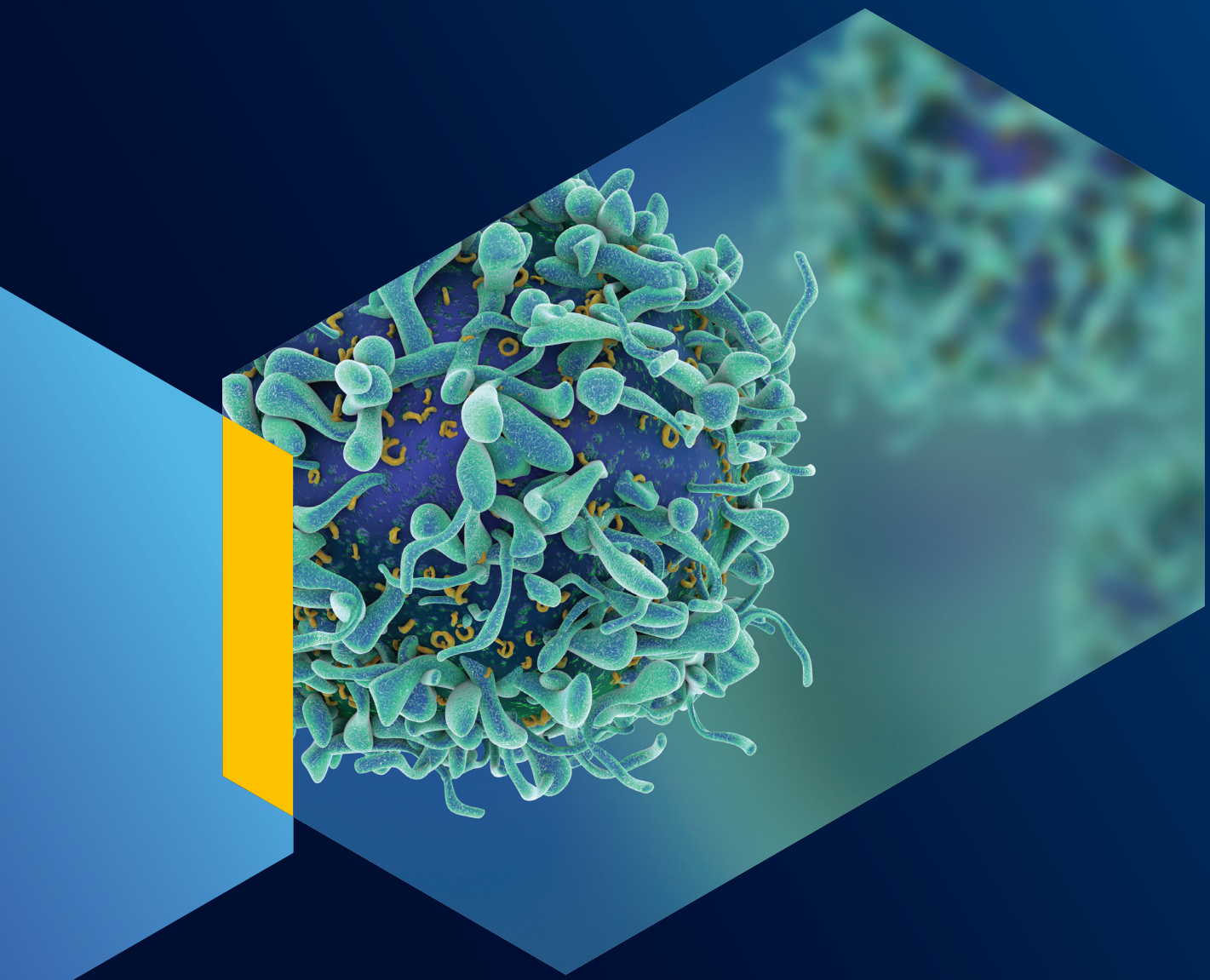
Introduction

RAS (KRAS, HRAS, and NRAS) is the most frequently mutated gene family in human cancers that have been considered "undruggable" due to the elusive nature of RAS inhibitors. Over the past few years, there have been significant findings in small-molecule binding pockets on RAS, resulting in a large collection of published chemical structures.

This comprehensive analysis of the RAS inhibitor structure landscape reveals insights that accelerate structure-based discovery of RAS inhibitors. The wide range of views into this landscape (structural similarity, patent assignees, and chemical properties) will enable your team to build on the progress of other publications more effectively.



RAS oncoproteins and emerging research trends for RAS inhibitors in cancer treatment

RAS is one of the most frequently mutated gene family in human cancers, with approximately 20% of all tumors harboring a mutation of at least one isoform.[1,2] RAS proteins are small switch-signaling guanosine triphosphatases (GTPases) that cycle between an active GTP-bound form and an inactive, guanosine diphosphate (GDP)-bound form. Under normal conditions, guanine exchange factors (GEF) mediate the GDP to GTP exchange and GTPase activating proteins (GAP) mediate GTP hydrolysis (Figure 1).[3] In a GTP-bound state, RAS interacts with several downstream signaling pathways such as mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways, which control a diverse range of cellular processes. Mutations in RAS can lock RAS into a constant active state (GTP-bound) continuously activating downstream signaling pathways, resulting in excessive cell proliferation and tumor growth.[1,2]

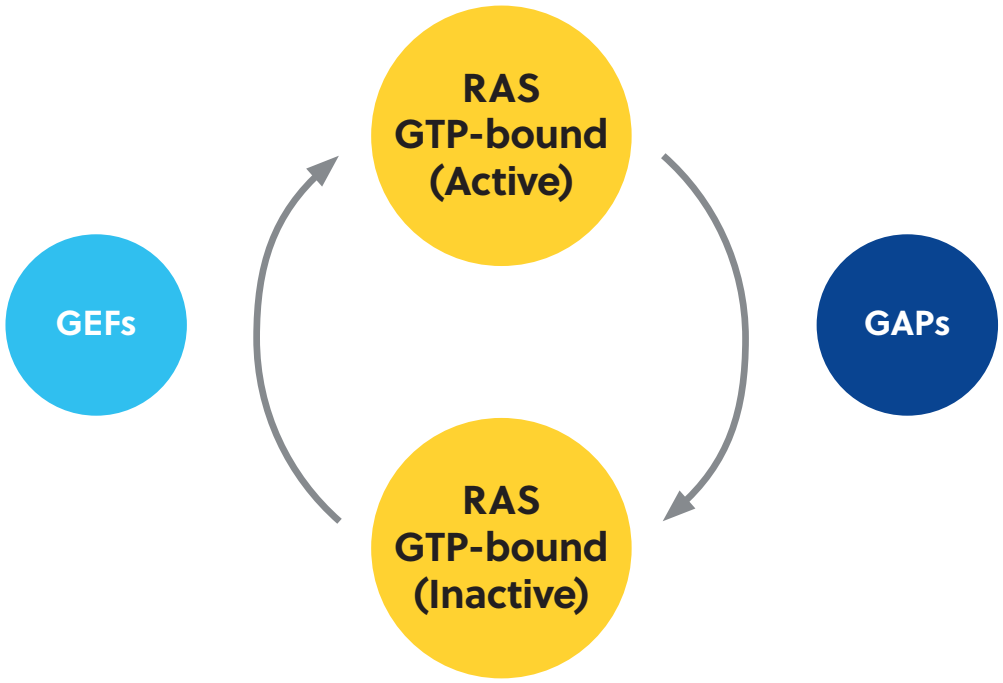


Figure 1: RAS activation cycle[3]

Critical binding regions that act during GTP binding and GTP hydrolysis include nucleotide binding pocket (P-loop), Switch II pocket, SwitchI/II pocket, and A59 site (Figure 2).[4] Examination of these pockets has led to the development of potential small molecule inhibitors, by looking at the binding affinity and activity of functional groups and scaffolds using structure activity relationship (SAR).

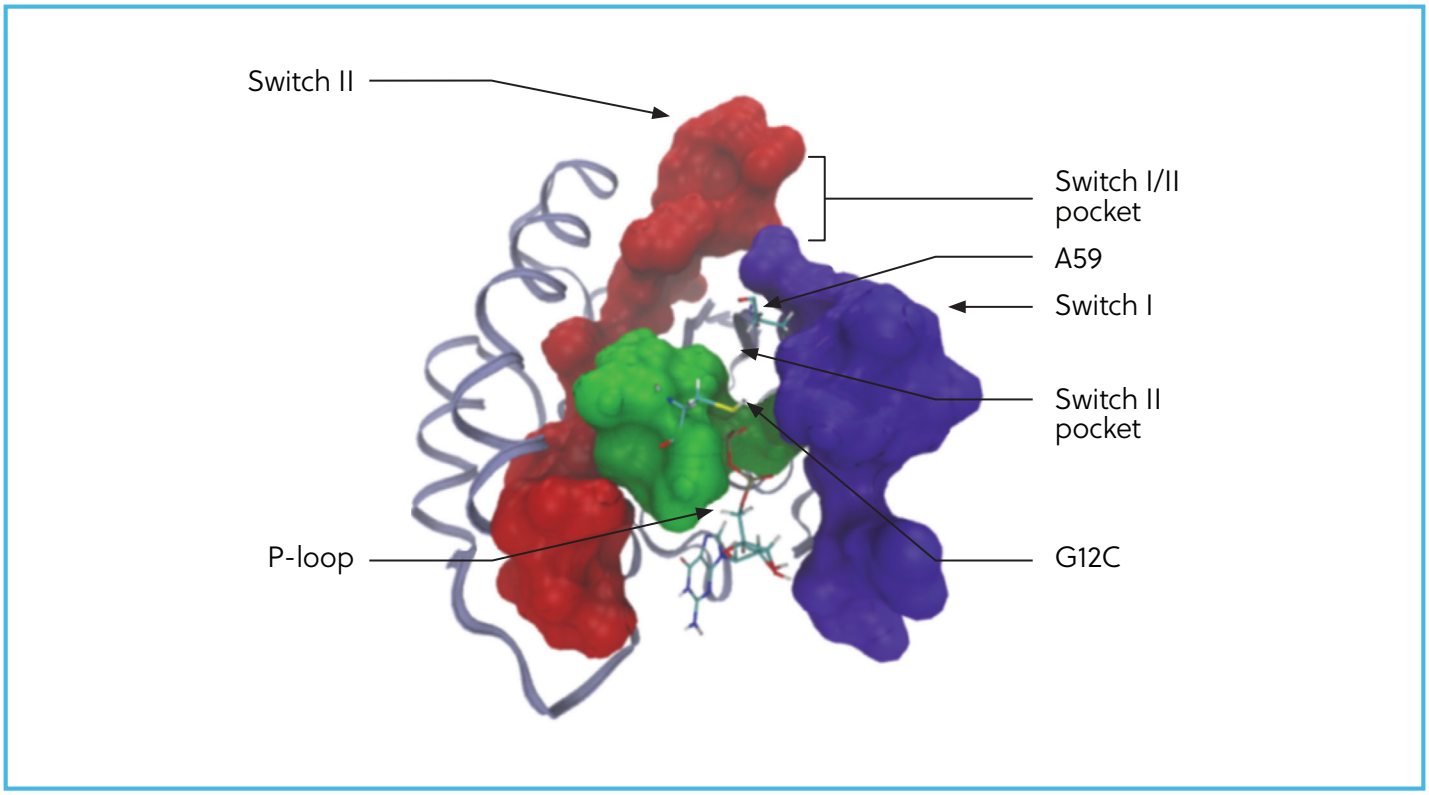


Figure 2: Crystal Structure of K-RAS G12C, GDP-bound[5]

Three human RAS genes *(KRAS, NRAS, and HRAS)* encode four RAS proteins (KRAS4A, KRAS4B, NRAS, and HRAS). The two KRAS isoforms (KRAS4A and KRAS4B) arise from alternative ribonucleic acid (RNA) splicing. The four isoforms are highly similar, sharing 82–90% amino acid sequence identity as well as structure and biochemical properties (e.g., GTP binging, hydrolysis, and prenylation).[6] Most oncogenic mutations in RAS isoforms are in codons G12, G13, or Q61. Isoforms differ in their frequency of alterations, for example in KRAS, G12, and G13 mutations predominate, whilst Q61 mutations are rare in KRAS, but common in NRAS. Mutated RAS isoforms, codon and amino acid substitution vary by tissue and cancer type (KRAS ~25% in lung cancer, ~90% in pancreatic cancer, ~50% in colorectal cancer; NRAS most common in melanoma; and HRAS most commonly mutated isoform in head and neck cancers).[2] Mutations in the KRAS isoform are the predominant mutations found in RAS-mutated human cancer (22%). Eighty percent (80%) of KRAS mutations occur at amino acid position 12 from glycine to other residues such as cysteine (G12C, 14%), aspartic acid (G12D, 36%), and valine (G12V, 23%) (Figure 3).[4]

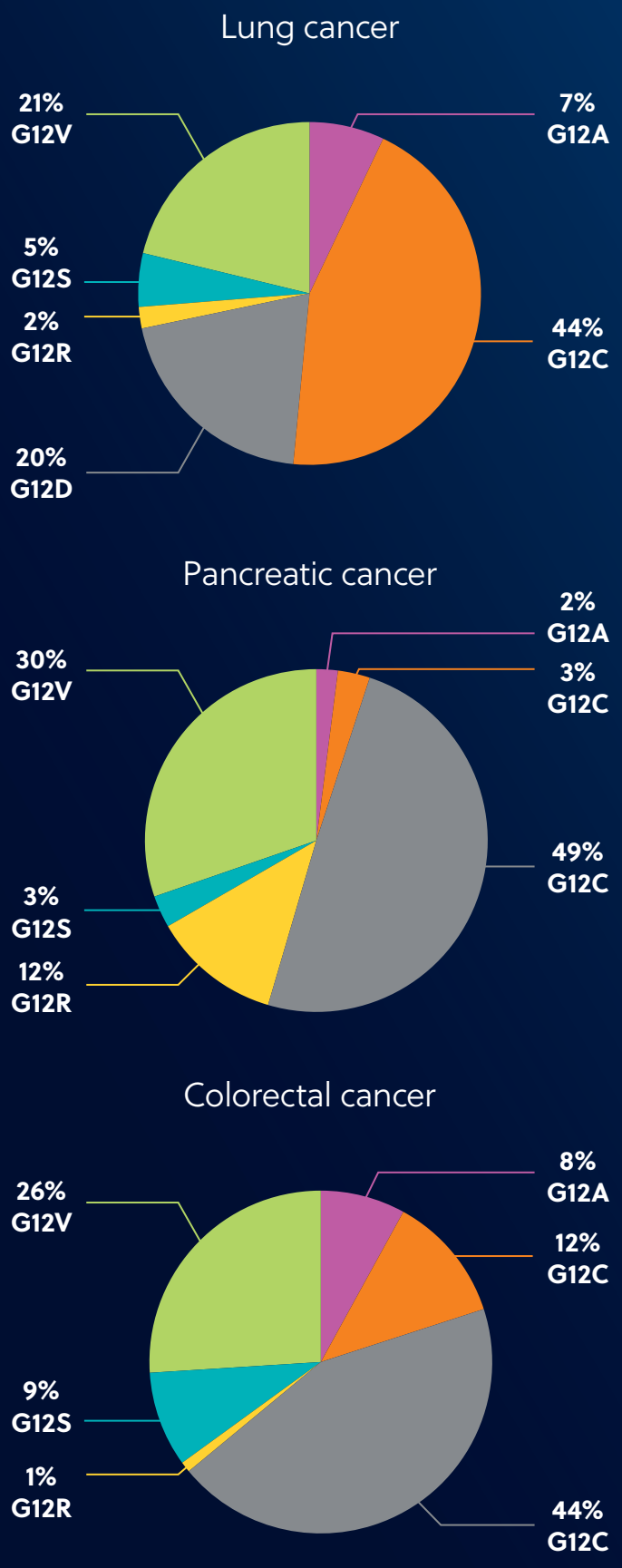


Figure 3: Profiles of KRAS mutations (codon 12) in lung, pancreatic, and colorectal cancer[4]

Despite being the most frequently mutated gene in cancer, there is a deficiency of RAS-targeted agents licensed for therapeutic use.[2] Early attempts to develop direct RAS GTP-competitive inhibitors failed due to the high micromolar cellular concentrations of GTP, the high picomolar affinity of GDP/GTP for RAS, and the lack of suitable hydrophobic pockets to allow high-affinity binding of small molecules.[2,7]

Research efforts have increased for RAS inhibitors in recent years with a variety of emerging research trends.[1] GDP derivatives harboring an electrophilic group on the beta-phosphate were shown to bind irreversibly to the oncogenic G12C mutant of RAS, overcoming the competing cellular levels and high binding affinity of GTP, renewing attention to this research topic.[7] A number of approaches have been developed to directly inhibit RAS, including covalent allele-specific inhibitors that bind to KRAS-G12C.[1] This approach led to FDA approval of the KRAS-G12C inhibitor AMG 510 for the treatment of non-small cell lung cancer (NSCLC).[8]

Insights into the world of RAS inhibitors

An analysis of RAS inhibitor chemical structures

CAS is a leader in scientific information solutions, accelerating breakthroughs by curating, analyzing, and connecting the world's scientific knowledge to reveal unseen relationships. The CAS Content Collection™ is the largest human-curated collection of published scientific knowledge. To identify RAS inhibitor chemical structures, we classified and quantified documents within the CAS Content Collection relating to direct RAS inhibitors between 2016–2021, with the aim of extracting and analyzing leading compound structures, including key functional group identification, structure similarity analysis, and structure classification.

We examined the number of patents per year to gain insight into the growth of RAS inhibitor discovery. 223 patents were identified relating to direct RAS inhibition from the analysis. Chemical substances with therapeutic or pharmacological roles in the direct RAS inhibitory space were manually assessed, and 26,598 structures extracted from the analysis. An increasing trend per year was observed for both patents and chemical substances, which relates to the significant rise in research effort towards RAS inhibitor discovery (Figure 4A and 4B).

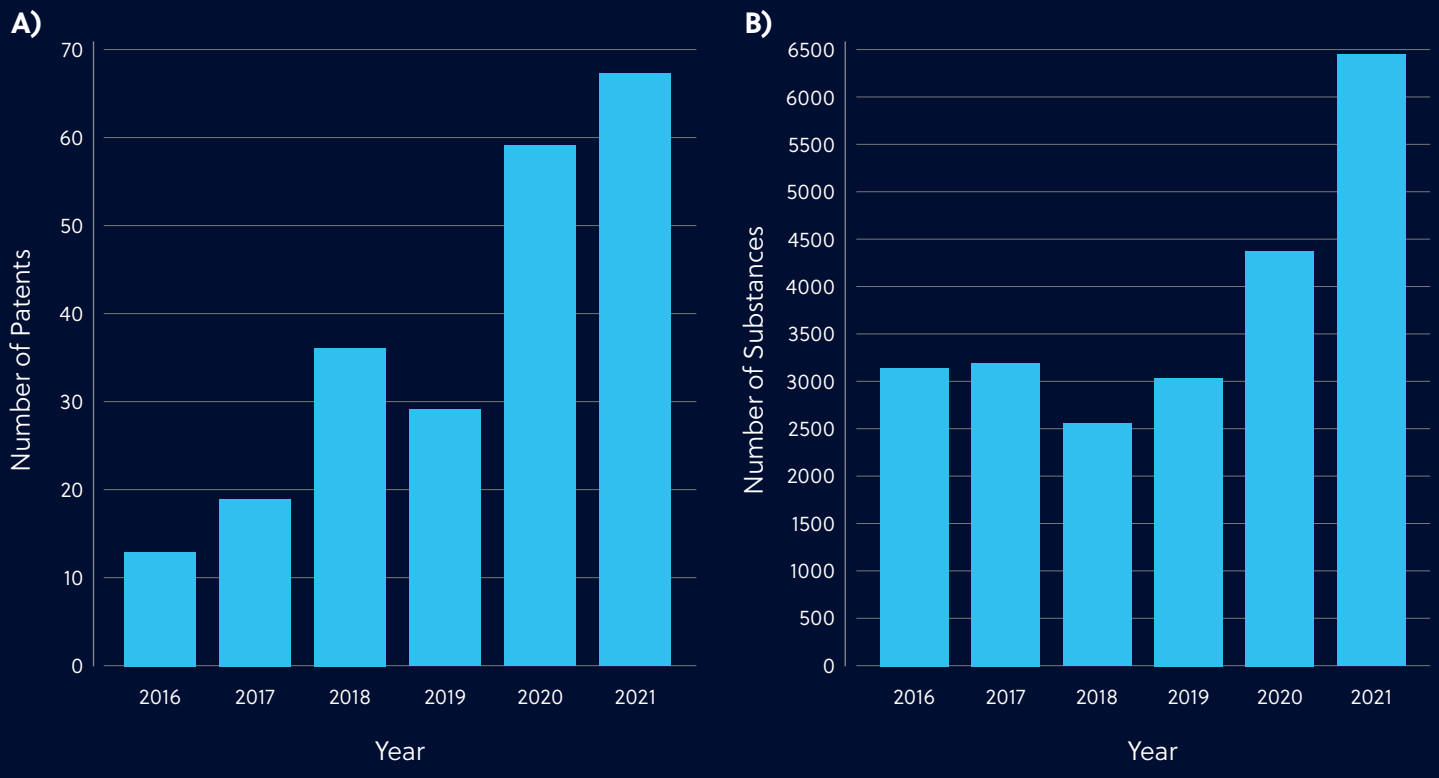


Figure 4: **A)** Number of patents relating to direct RAS inhibitors per year and **B)** number of chemical substances relating to direct RAS inhibition per year added to the CAS Content Collection in the years 2016–2021, presented as number of records per year

We further examined the number of patents per country (commercial and academic). USA has the highest number of commercial and academic patents followed by China, Japan, Germany, UK and South Korea (Figure 5).

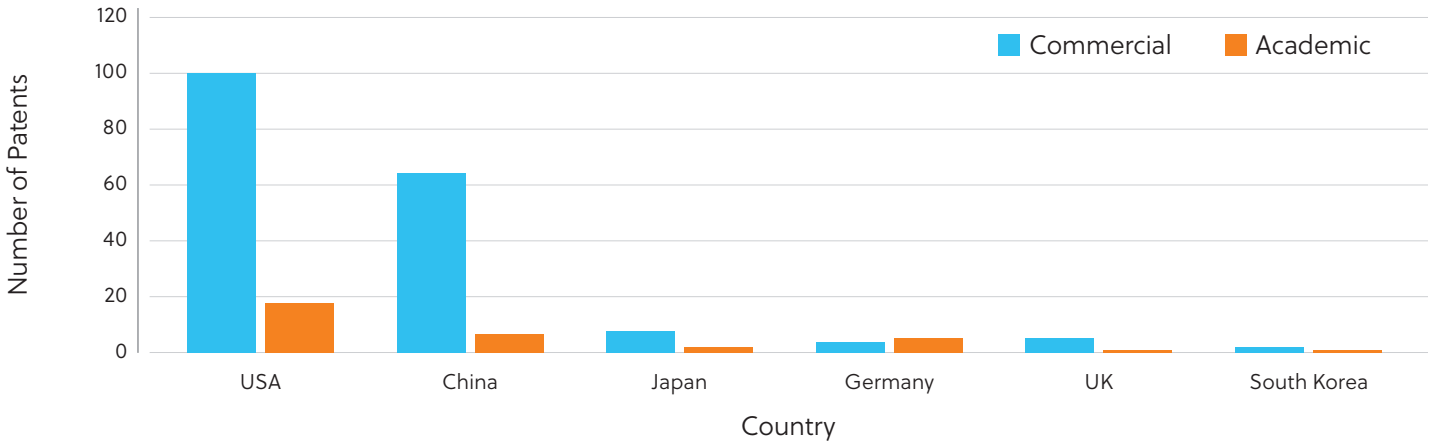


Figure 5: Number of commercial and academic patents added to the CAS Content Collection in the years 2016–2021, presented as number of records per country

Of the 26,598 extracted substances, the mode of the molecular weight was approximately 576 g mol^{-1}, although a small number of inhibitors exceed 1 kg mol^{-1} (Figure 6). This tight grouping demonstrates the similarity of most small-molecule RAS inhibitors that are in development. In the drug discovery process, skeleton scaffolds are slightly modified with a variety of functional groups to enhance activity and selectivity, and decrease toxicity, as can be seen in the the similar molecule weight distribution analysis.

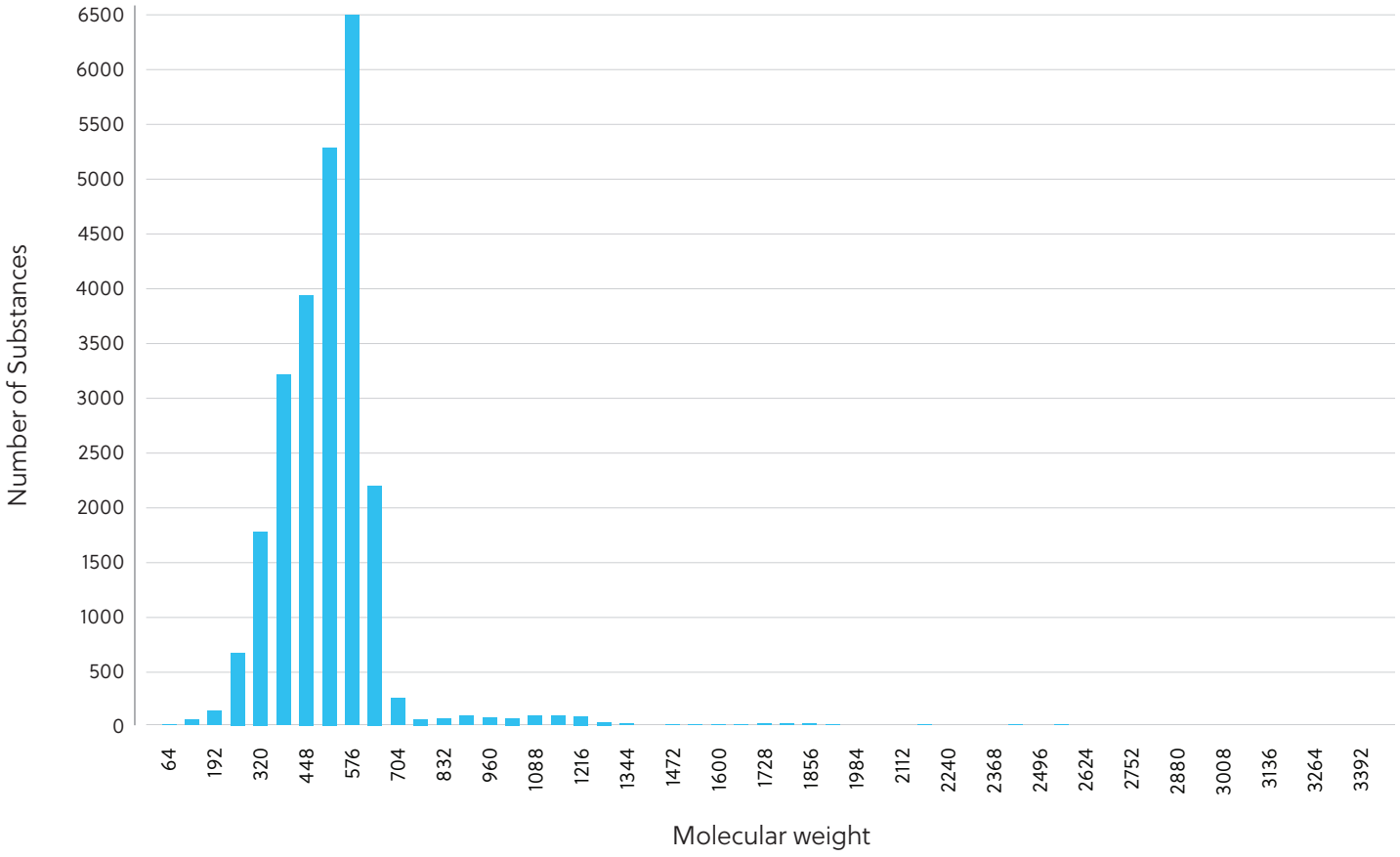


Figure 6: Number of records relating to direct RAS inhibitors by molecular weight, extracted from the CAS Content Collection in the years 2016–2021, presented as number of records per molecular weight

Example RAS inhibitors and RAS protein binding mechanisms

Covalent inhibitors of KRAS G12C via the Switch-II pocket

ARS-1620 is a quinazoline-based compound which contains a fluorophenol hydrophobic binding moiety, piperazine bridge, and an acrylamide *warhead*.[9] The KRAS is trapped in the inactive form via a covalent bond between ARS-1620 and mutant cysteine within the Switch II pocket. The hydroxyl group of the fluorophenol moiety forms several water-mediated hydrogen bonds with KRAS, and the fluoro-group occupies a hydrophobic region (Figure 7).[10] Additionally, its quinazoline core engages in hydrogen bonding with the H95 residue side-chain. ARS-1620 has shown high bioavailability in mice and sufficient plasma stability in mice as well as in humans.[7] ARS-1620 was optimized into the later approved AMG 510 which is discussed below.[7]

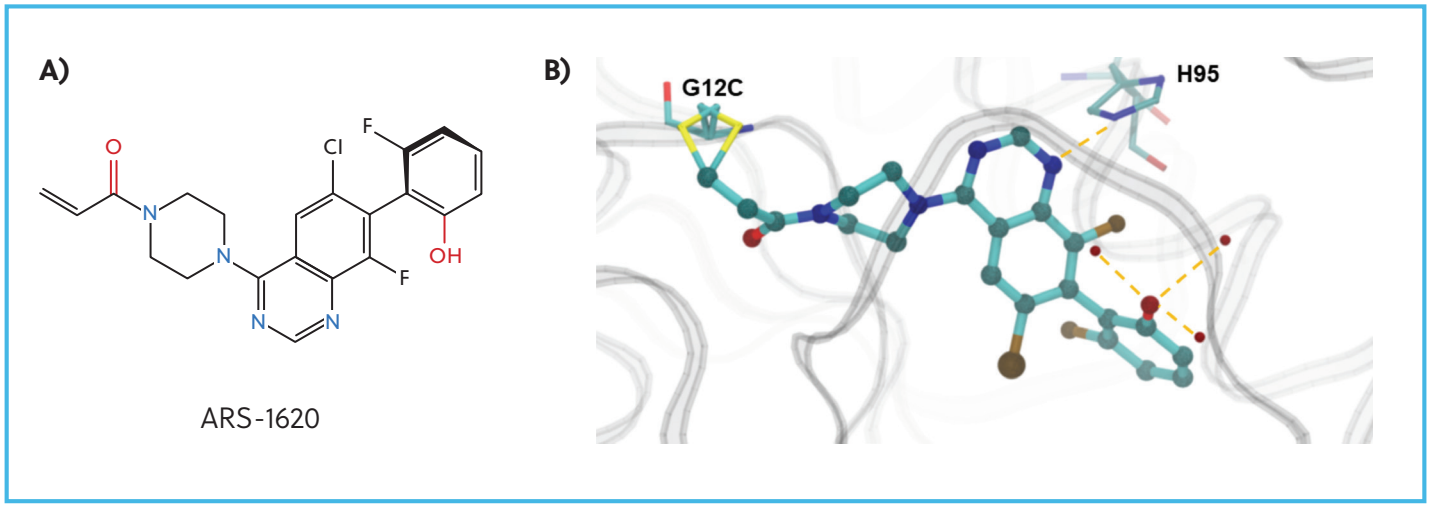


Figure 7: **A)** Chemical structure of ARS-1620 and **B)** Crystal structure of small molecule ARS-1620 covalently bound to KRAS G12C

AMG 510, developed by Amgen, was the first KRAS G12C inhibitor to enter a clinical trial for treatment of KRAS G12C mutated solid tumors (August 2018).[7,8] While studying the crystal structures of KRAS-inhibitor complexes, a groove on the surface of KRAS was discovered in the space between H95, Y96, and Q99. Screening efforts of compounds similar to ARS-1620 were directed towards accessing this groove, leading to the discovery of AMG 510 (Figure 8).[11] Combined with the additional hydrogen bonds afforded by its quinazolinone core, an isopropyl-methylpyridine substituent that occupied the cryptic groove led to a 10-fold improvement in potency in a biochemical assay compared to ARS-1620.[7] AMG 510 was recently approved by FDA for the treatment of adult patients with KRAS G12C mutated locally advanced or metastatic NSCLC, who have received at least one prior systemic therapy.[9] As a promising candidate for treatment, AMG 510 has inspired research towards similar compounds, and analysis of the landscape of AMG 510-like compounds is provided in the following *section*.

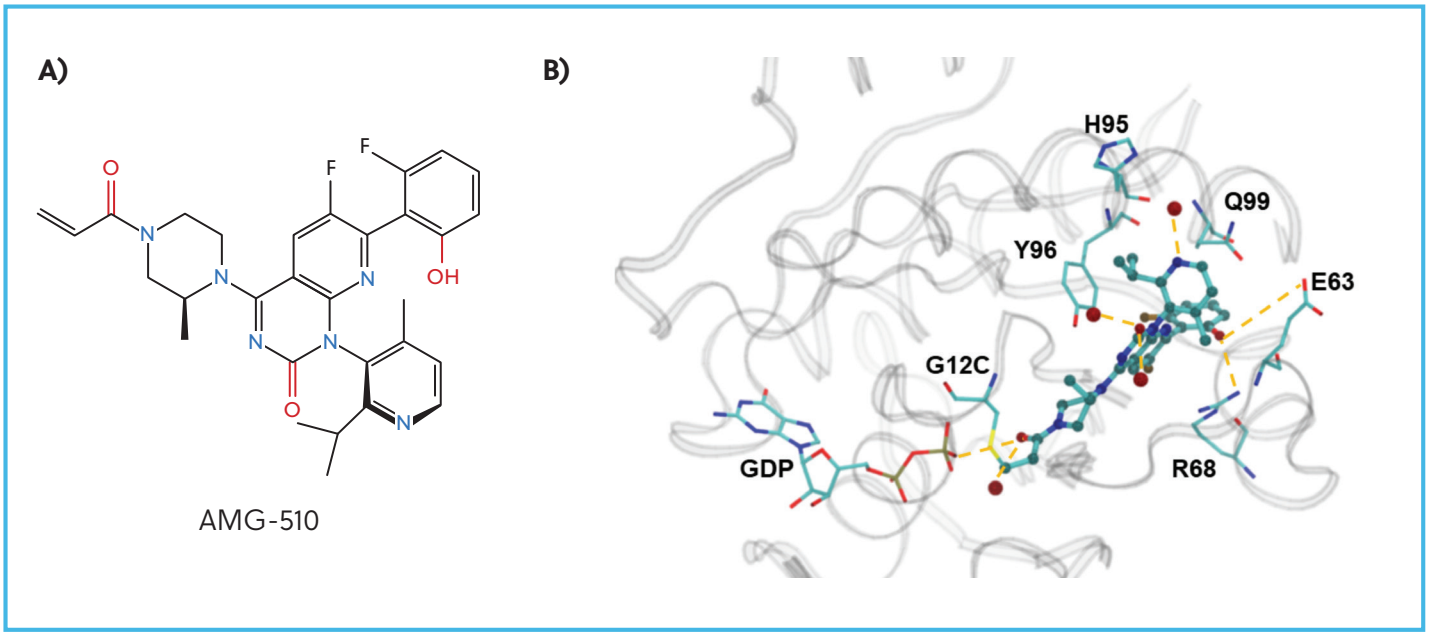


Figure 8: **A)** Chemical structure of AMG 510 and **B)** Crystal structure of human KRAS G12C covalently bound to AMG 510

MRTX849, developed by Mirati Therapeutics, was discovered by a structure-based drug design approach. The core structure was based on the ARS-1620 and AMG 510 scaffolds, with optimization with respect to favorable drug-like properties. 8-chloronaphtyl substituent, that replaced the fluorophenol head group, fills the lipophilic Switch II pocket via the mutant cysteine and interacts with Y64 on KRAS, trapping KRAS in an inactive conformation. To mitigate the metabolization of the acrylamide warhead by GST-mediated reaction with glutathione, an α-fluoro was substituted at the alpha-position of acrylamide *warhead* which led to an improved pharmacokinetic profile and lower reactivity towards glutathione (Figure 9A).[12] Key hydrogen bonds are formed between the cyanomethyl group and the backbone NH of Gly10, and between the pyrrolidine moiety and Glu62 (Figure 9B).[13] MRTX849 entered a human clinical trial in January 2019 for the treatment of solid tumor malignancies with KRAS G12C mutation, and earned FDA breakthrough therapy designation for KRAS G12C positive NSCLC in June 2021.[7,14,15] Analysis of the landscape of compounds similar to MRTX849 is provided in the following <u>section</u>.

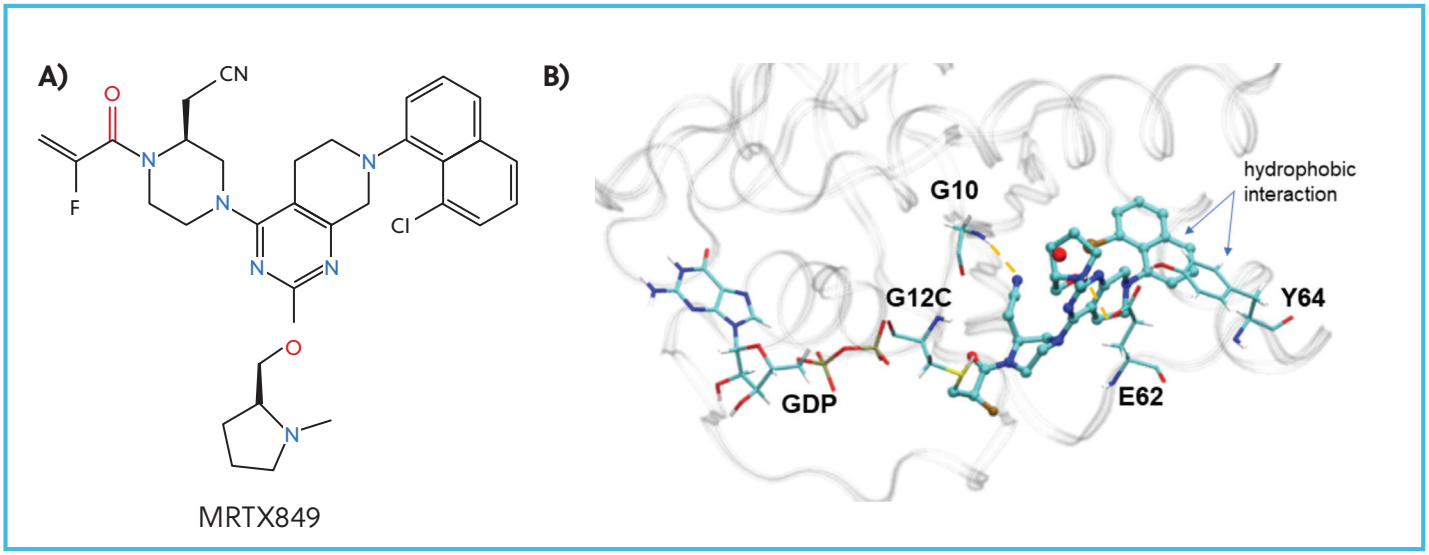


Figure 9: **A)** Chemical structure of MRTX849 and **B)** Crystal structure of KRAS G12C covalently bound to MRTX849

Inhibitors of protein-protein interactions via Switch I/II pocket

A more general non-covalent inhibitor with efficacy against non-G12C mutant is desirable to expand the breadth of direct RAS inhibitors. DCAI (4,6-dichloro-2-methyl-3-aminoehtyl-indole) is an indole-derivative that was identified using fragment-based lead discovery (FBLD).[16] DCAI is capable of binding to a small-molecule binding pocket located adjacent to the functionally important Switch I and II regions of KRAS (known as the Switch I/II pocket).[16,17] The chloro-substituent at the 6 position anchors the ligand into the hydrophobic pocket, and the 4- chloro group substituent is accommodated into the binding pocket through conformational change of the indole core (Figure 10A).[16] DCAI also disrupts the KRAS-SOS interaction by inhibiting nucleotide release by locking KRAS into its inactive GDP-bound form.[16]

Extensive studies on other small-molecule indole derivatives using FBLD and optimization of fragments that weakly bind to GCP-KRAS-G12D using structure-based design, led to the discovery of BI-2852 (Figure 10B). This small-molecule binds over a larger region of KRAS to inhibit SOS1-catlayzed exchange of GDP-KRAS to GTP-KRAS, and GAP-catalyzed exchange of GTP-to GDP-KRAS. Hydrogen bonding from the phenolic oxygen to E37 on Switch II is important for RAS binding to downstream effectors, GEF and GAP.[17]

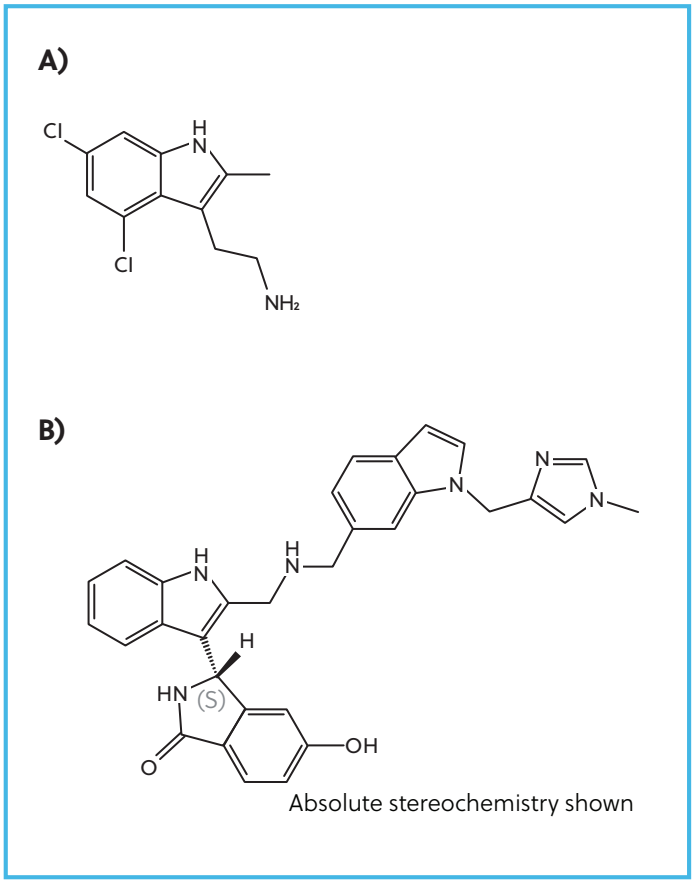


Figure 10: **A)** Chemical structure of DCAI and **B)** chemical structure of BI-2852[16,17]

Other strategies investigated for the development of RAS-binding small molecules includes the fusion of two chemical series to combine and improve upon functional mechanisms of disruption. Ch-3 was developed by fusion of protein-protein-interaction networks (PPIN) and antibody fragment (Abd) series, which led to a RAS protein-protein-interaction (PPI) inhibitor series (Figure 11).[15] With a low molecular weight, Ch-3 can be a starting point for further medicinal chemistry to improve drug-like properties and binding.[18]

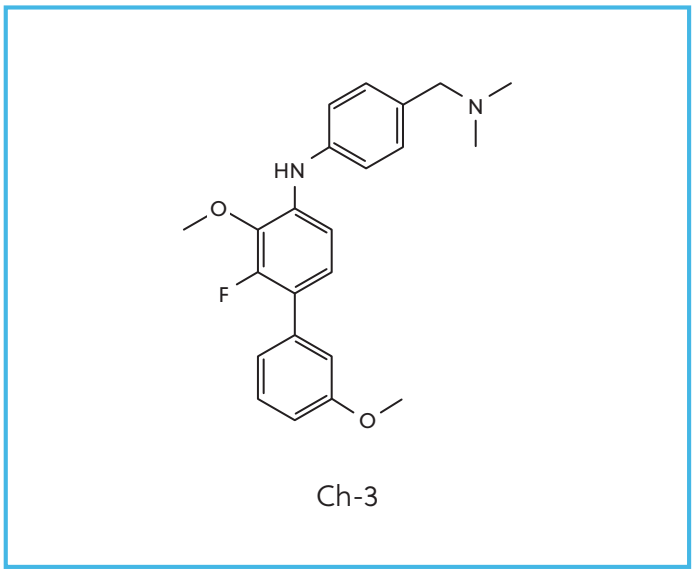


Figure 11: Chemical structure of Ch-3[18]

A59 inhibition

3144 is a novel indole derivative that has been shown to target multiple adjacent sites (A59, Y32 and D38) to create a multivalent inhibitor (Figure 12). Compound 3144 was able to induce selective lethality and signaling blockage in RAS-dependent cells while inhibiting tumor growth in G12D-mutated KRAS. The specificity was insufficient to prevent toxicity, and further optimization would be needed to create a more specific pan-RAS inhibitor.[19]

Figure 12: Chemical structure of 3144[19]

Classification of key inhibitors and similarity analysis

Tanimoto similarity values were assessed between several example compounds above and structures from the extracted data set (26,598 compounds). The subsequent analyses elucidate the substructural features most highly studied in covalent KRAS inhibitor development and thus point to their relative importance in drug function.

AMG 510

When compared to AMG 510, 2013 compounds in the dataset had a Tanimoto similarity score above 0.6 (Figure 13).

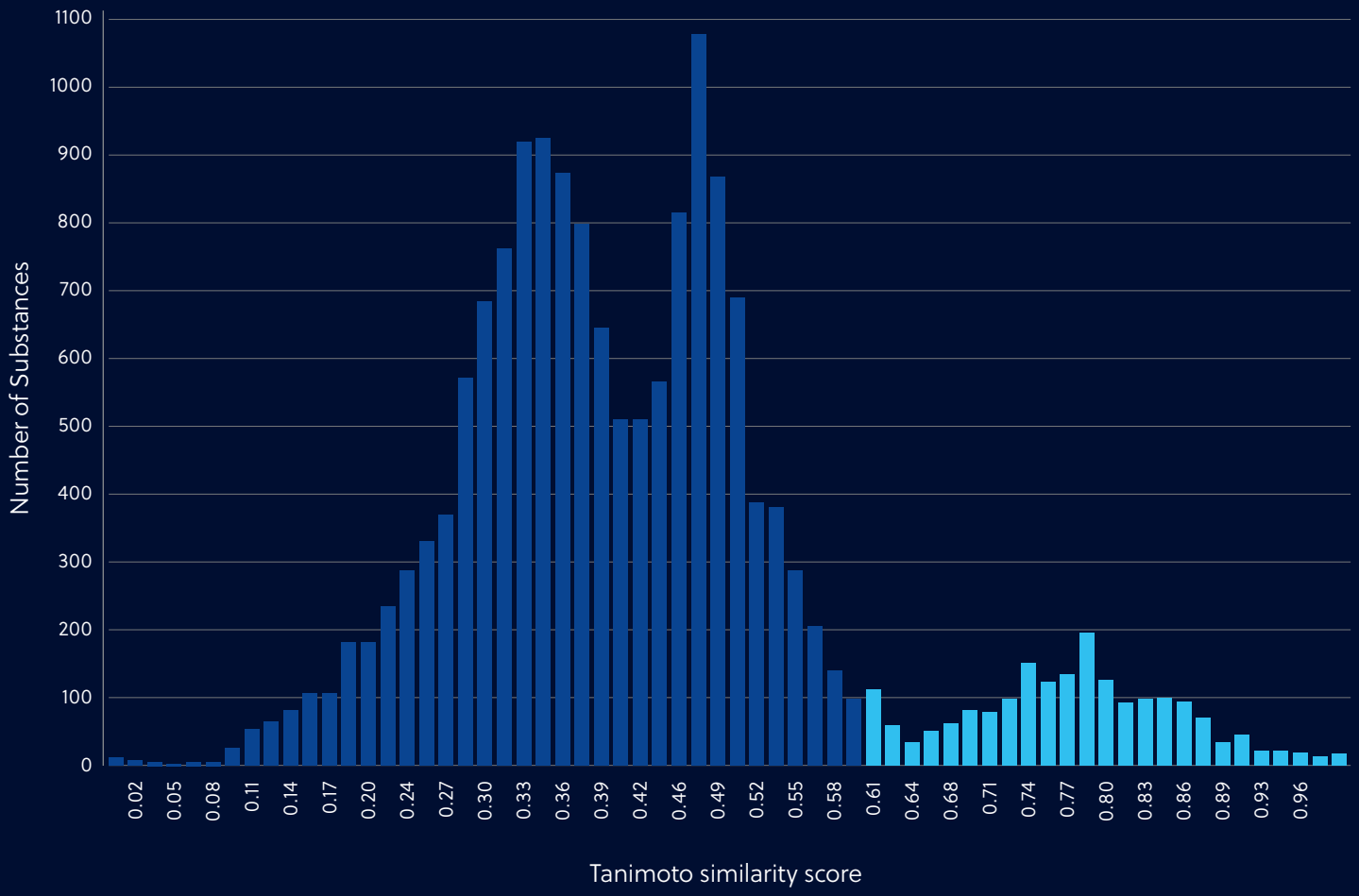


Figure 13: Similarity distribution using AMG 510 as a comparator structure

Data on the most common functional groups in AMG 510-like compounds reveal the most frequent areas of modification. Figure 14 illustrates the occurrence of pertinent substructural features of AMG 510 found in the dataset. Modification of key scaffolds with various functional groups is an important tool in drug discovery, and can be used to improve binding, pharmacokinetics/pharmacodynamics, and toxicity of chemical substances as seen above.[7,15-19]

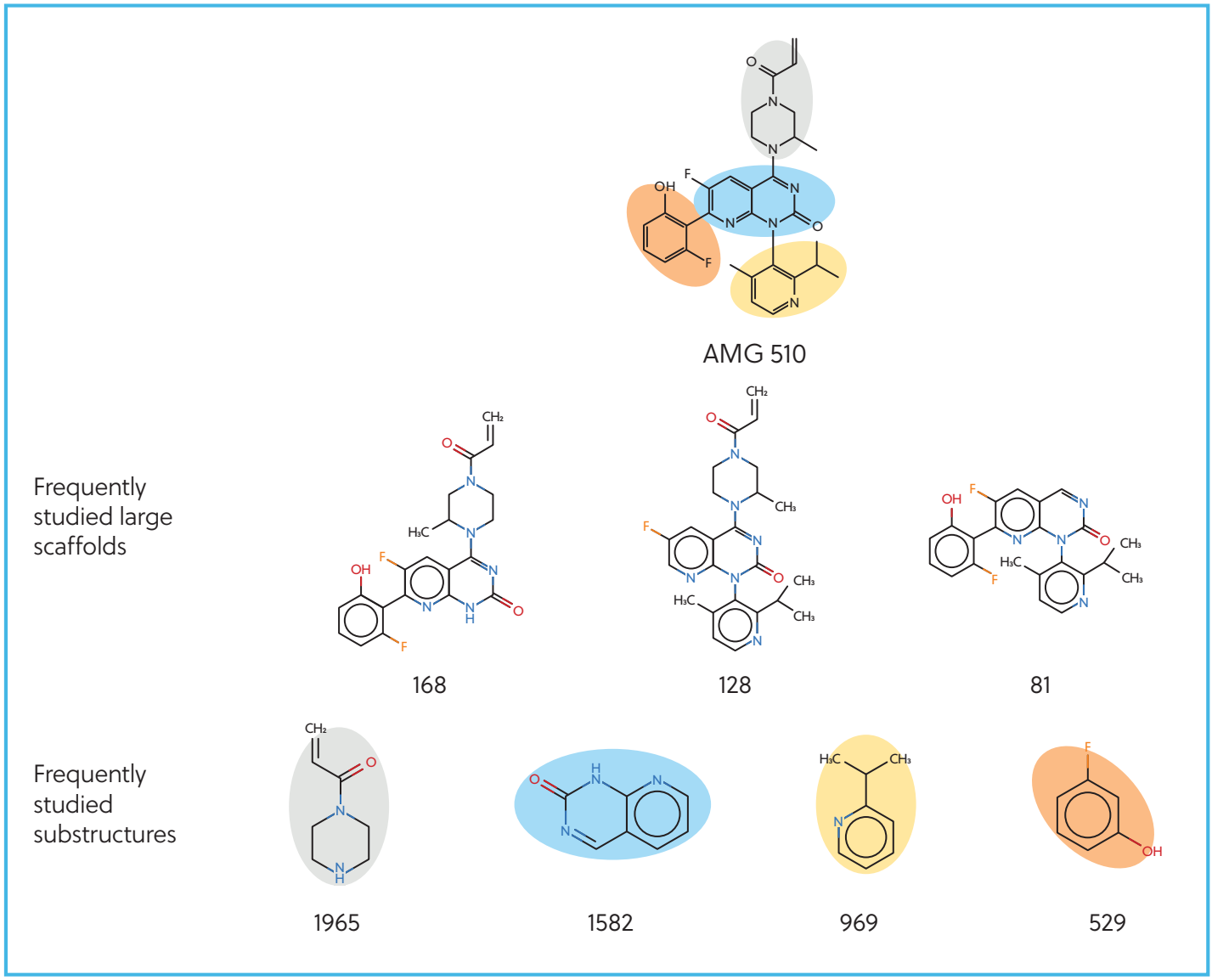


Figure 14: Substructural features in AMG 510 and their occurrence in similar compounds (Tanimoto similarity > 0.6). Highlights: blue=core, grey=warhead, yellow=cryptic pocket-binding, red=hydrogen bonding fragments

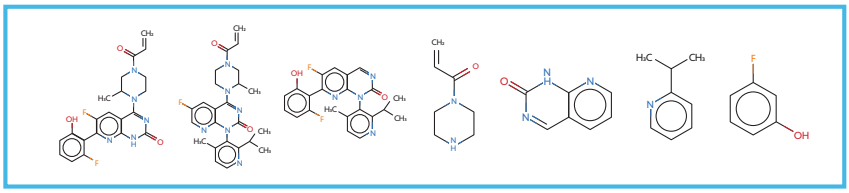
Of all structural variations in AMG 510-like compounds, the most obvious key substructure is the piperazineacrylamide fragment, highlighted in grey. Occuring in 1,965 of 2,013 similar compounds, this fragment acts as the covalent *warhead* binding to KRAS G12C and appears to be well-established in this function. Also of note is the core quinazolinone structure, occurring in 1,582 similar compounds. As discussed previously, substitution at this core structure can modulate key hydrogen bonding interactions with KRAS. It is important to consider the scope of intellectual property protections when assessing structural variations at such scaffold cores; this likely contributes to some degree of structural diversity in covalent KRAS inhibitors.

The assignees applying for patents containing AMG 510-like substances are shown in Table 1, alongside their most recent respective compound additions to CAS Registry®. As expected, Amgen has studied this class of compounds most extensively and continues to innovate on the structure. There are however a number of additional companies studying AMG 510-like compounds, many of which are highly recent. Table 2 gives a more detailed breakdown of the occurrence of key structural components of these compounds studied by their respective patent assignees.

Table 1: Patent assignees for AMG 510-like substances, including number of substances per assignee and newest substance year.

Number of Substances	Patent Assignees	Newest Substance Year
981	Amgen Inc.	2021
251	Guangdong Newopp Biopharmaceuticals Co., Ltd.	2020
212	Jiangsu Hansoh Pharmaceutical Group Co., Ltd.; Shanghai Hansoh Biomedical Co., Ltd	2020
140	Inventisbio Shanghai Ltd.	2020
127	Guangzhou BeBetter Medicine Technology Co., Ltd.	2021
109	Araxes Pharma LLC	2018
98	WIGEN Biomedicine Technology (Shanghai) Co., Ltd.	2020
75	Jiangxi Jemincare Group Co., Ltd.; Shanghai Jiyu Pharmaceutical Technologh Co., Ltd.	2021
27	GenFleet Therapeutics (Shanghai) Inc.; Zhejiang GenFleet Therapeutics Co., Ltd.	2021
26	Array BioPharma, Inc.; Mirati Therapeutics, Ltd.	2020
19	Nanjing Ruijie Pharmaceutical Technology Co., Ltd.	2021
16	Betta Pharmaceuticals Co., Ltd.	2021
	Jinfu Pharmaceutical (Suzhou) Co., Ltd	2021
14	Shouyao Holdings (Beijing) Co., Ltd.	2021
5	Suzhou Sinovent Pharma Co., Ltd	2020
3	Jacobio Pharmaceuticals Co., Ltd.	2021
	Jiangsu Hengrui Medicine Co., Ltd.; Shanghai Hengrui Pharmaceutical Co., Ltd.	2021
1	F. Hoffmann-La Roche AG; Genentech, Inc.	2020
	Turning Point Therapeutics Inc.	2021

Table 2: Key structural components of compounds shared by AMG 510 studied by respective patent assignees.



Patent Assignees							
Amgen Inc.	52	50	25	953	739	300	189
Araxes Pharma LLC	0	0	0	109	109	5	43
Array BioPharma, Inc.; Mirati Therapeutics, Ltd.	7	0	1	26	26	1	12
F. Hoffmann-La Roche AG; Genentech, Inc.	0	0	0	1	1	0	0
GenFleet Therapeutics (Shanghai) Inc.; Zhejiang GenFleet Therapeutics Co., Ltd.	1	20	0	21	21	20	1
Guangdong Newopp Biopharmaceuticals Co., Ltd.	29	25	16	239	198	195	78
Guangzhou BeBetter Medicine Technology Co., Ltd.	6	2	2	76	13	72	20
Inventisbio Shanghai Ltd.	35	7	32	131	101	132	70
Jacobio Pharmaceuticals Co., Ltd.	0	0	0	3	0	0	2
Jiangsu Hansoh Pharmaceutical Group Co., Ltd. Shanghai Hansoh Biomedical Co., Ltd	19	15	2	212	207	183	44
Jiangsu Hengrui Medicine Co., Ltd.; Shanghai Hengrui Pharmaceutical Co., Ltd.	0	0	0	3	1	1	2
Shouyao Holdings (Beijing) Co., Ltd.	0	0	0	13	0	0	14
Suzhou Sinovent Pharmaceuticals Co., Ltd	0	0	0	5	5	0	0
WIGEN Biomedicine Technology (Shanghai) Co., Ltd.	10	6	1	97	96	35	25

MRTX849

When compared to MRTX849, 1,841 compounds in the dataset had a Tanimoto similarity score above 0.6 (Figure 15).

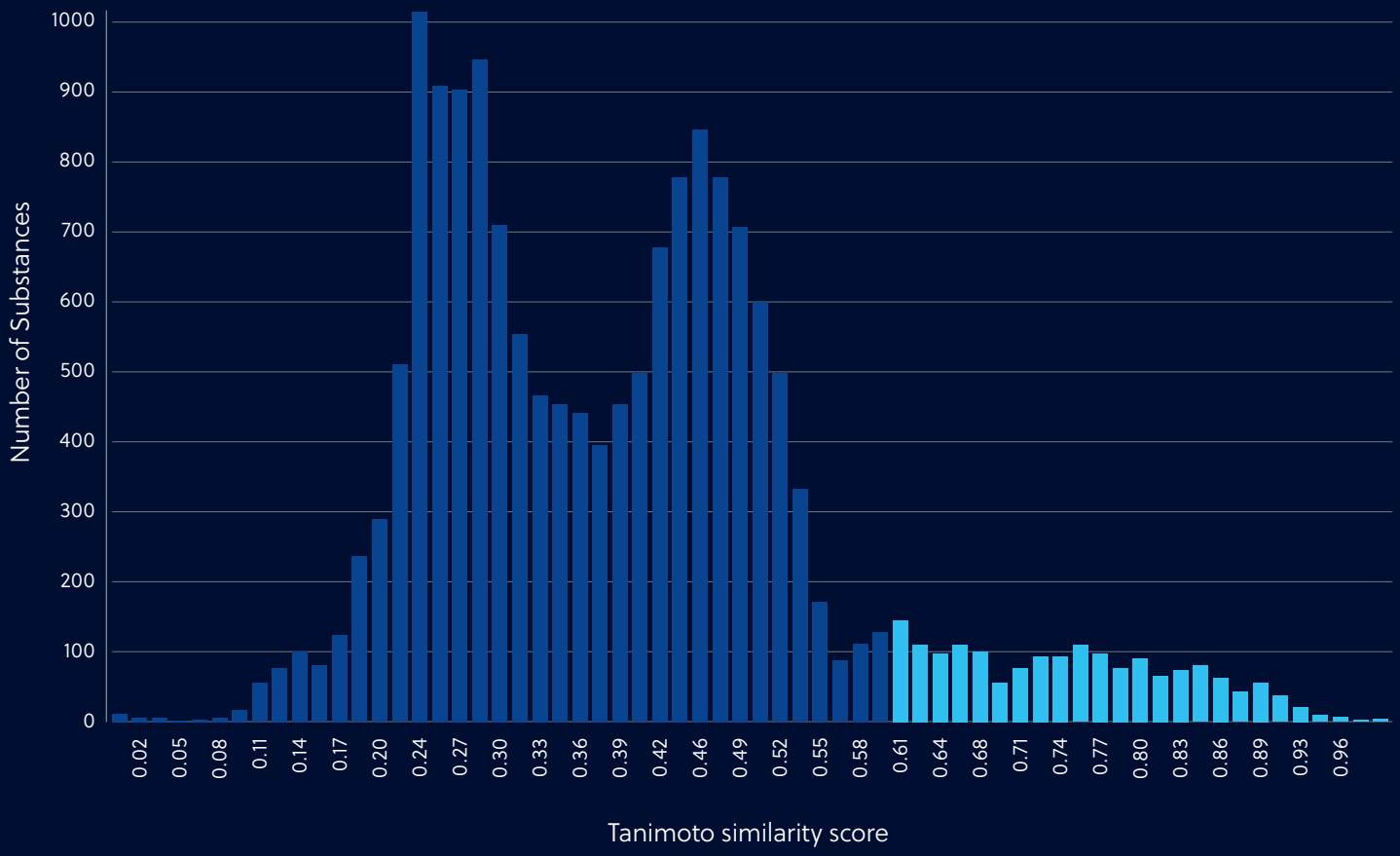


Figure 15: Similarity distribution using MRTX849 as a comparator structure

Among MRTX-like compounds, the most common key substructure is the 2-cyanomethylpiperazineacrylamide *warhead* with or without the fluoro substituent on the acrylamide, as shown below (Figure 16). This is again followed by the core ring structure, which is in this case 5,6,7,8-tetrahydropyrido[3,4-d]pyrimidine, and then by functional groups connected to the core structure.

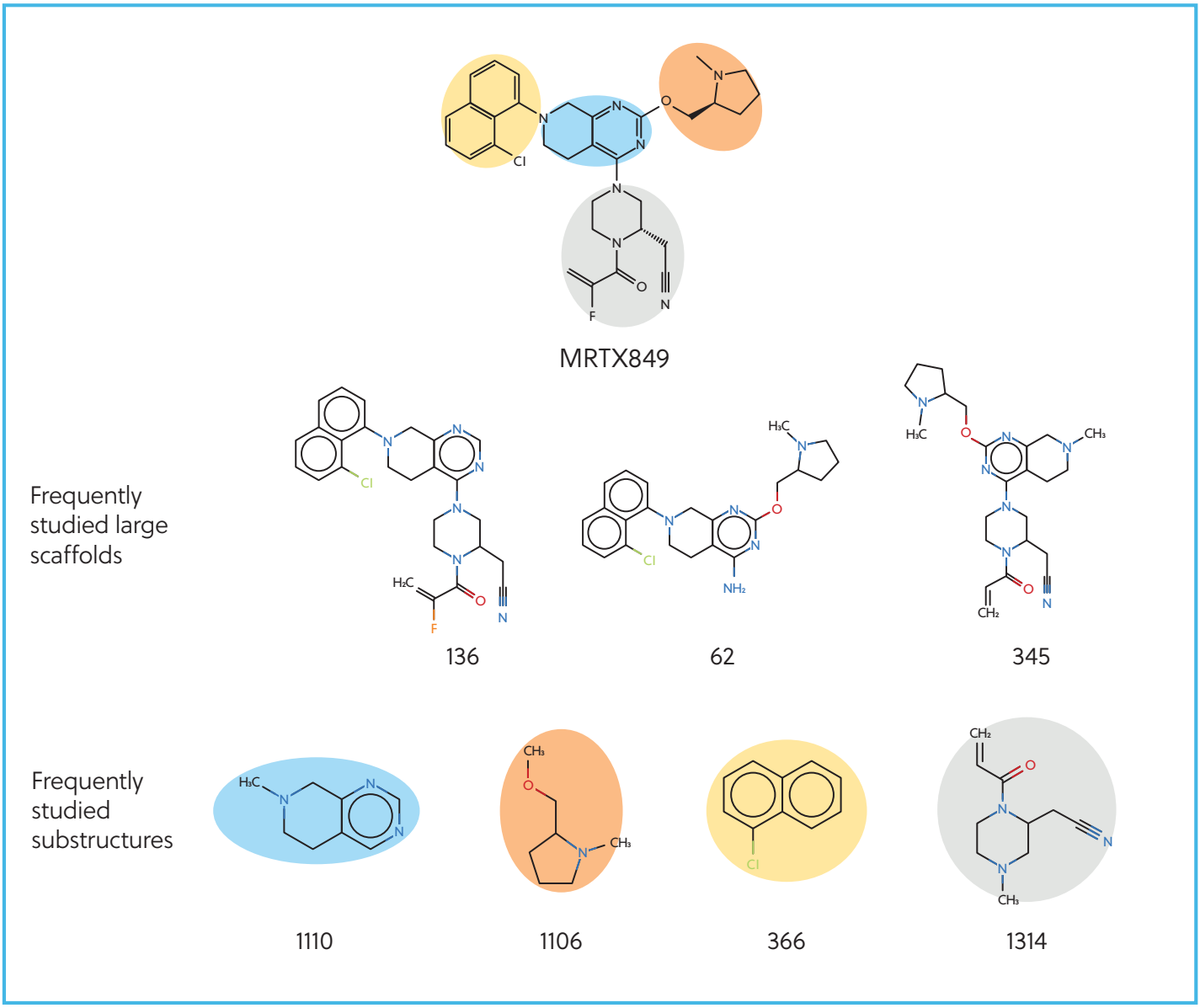


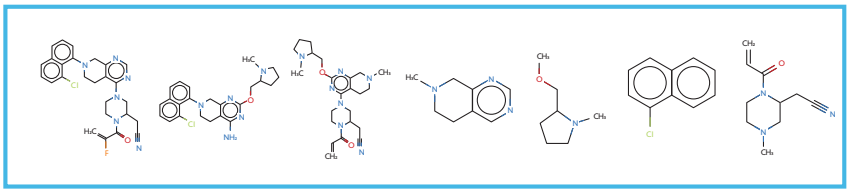
Figure 16: Substructural features in MRXT849 and their occurrence in similar compounds (Tanimoto similarity > 0.6). Highlights: blue=core, grey=warhead, yellow=lipophilic pocket-binding, red=hydrogen bonding fragments

As was also the case in AMG 510-like compounds, work on new MRTX849-like compounds continues among several commercial entities (Table 3). The principal company pursuing MRTX849 itself as a therapeutic, Mirati Therapeutics, Inc., accounts for the bulk of these variations. However, a number of companies have submitted patents on compounds sharing significant substructural features with MRTX849, for example, Jacobio Pharmaceuticals (Table 4). Moreover, MRTX849 appears to have influenced both the choices of scaffolds and side functional groups, as shown by the popularity of its methylpyrrolidine-containing side-chain.

Table 3: Patent assignees for MRT849-like substances, including number of substances per assignee and newest substance year.

Number of Substances	Patent Assignees	Newest Substance Year
1,572	Array BioPharma, Inc.	2020
596	Mirati Therapeutics, Inc.	2020
205	Jacobio Pharmaceuticals Co., Ltd.	2021
173	Nangene Biomedical Co., Ltd.	2021
159	F. Hoffmann-La Roche AG; Genentech, Inc.	2020
151	Array Biopharma, Inc.; Mirati Therapeutics, Ltd.	2020
90	BeiGene, Ltd.	2021
87	Chia Tai Tianqing Pharmaceutical Group Co., Ltd.; Nanjing Shunxin Pharmaceutical Co., Ltd.	2021
80	Jiangsu Hengrui Medicine Co., Ltd.; Shanghai Hengrui Pharmaceutical Co., Ltd.	2020
78	Innovent Biologics (Suzhou) Co., Ltd.	2021
28	Sichuan Haisco Pharmaceutical Co., Ltd.	2021
17	Jiangsu Hansoh Pharmaceutical Group Co., Ltd.; Shanghai Hansoh Biomedical Co., Ltd	2020
14	Shandong Xuanzhu Pharma Co., Ltd.	2020
	WIGEN Biomedicine Technology (Shanghai) Co., Ltd.	2020
13	Shanghai Zelgen Pharma. Tech Co., Ltd.; Suzhou Zelgen Biopharmaceuticals Co., Ltd.	2021
	Shanghai Zelgen Pharma. Tech Co., Ltd.; Suzhou Zelgen Biopharmaceuticals Co., Ltd.	2021
11	Jiangsu Simcere Pharmaceuticals Co., Ltd.	2020
8	Jinfu Pharmaceutical (Suzhou) Co., Ltd.	2021
3	Beijing JacoBio Pharmaceuticals Co., Ltd.	2021
	Shanghia De Novo Pharmatech Co., Ltd.	2021
2	Araxes Pharma LLC	2018
	Turning Point Therapeutics, Inc.	2021
1	BrightGene Bio-Medical Technology (Sizhou) Co., Ltd.	2021

Table 4: Key structural components of compounds shared by MRXT849 studied by respective patent assignees.



Patent Assignees							
Araxes Pharma LLC	0	0	0	0	2	0	0
Array BioPharma, Inc.; Mirati Therapeutics, Ltd.	218	103	615	1,572	888	389	1,136
BeiGene, Ltd.	0	0	0	0	79	3	86
Beijing JacoBio Pharmaceuticals Co., Ltd.	0	0	0	0	3	1	0
BrightGene Bio-Medical Technology (Sizhou) Co., Ltd.	0	0	0	0	1	0	1
Chia Tai Tianqing Pharmaceutical Group Co., Ltd.; Nanjing Shunxin Pharmaceutical Co., Ltd.	0	0	0	0	77	7	79
F. Hoffmann-La Roche AG; Genentech, Inc.	0	0	0	0	157	0	46
Innovent Biologics (Suzhou) Co., Ltd.	2	0	0	47	33	30	73
Jacobio Pharmaceuticals Co., Ltd.	11	1	5	205	5	20	205
Jiangsu Hansoh Pharmaceutical Group Co., Ltd.; Shanghai Hansoh Biomedical Co., Ltd	0	0	0	2	15	0	2
Jiangsu Hengrui Medicine Co., Ltd.; Shanghai Hengrui Pharmaceutical Co., Ltd.	3	2	13	80	27	17	45
Jiangsu Simcere Pharmaceuticals Co., Ltd.	0	0	9	11	10	0	10
Jinfu Pharmaceutical (Suzhou) Co., Ltd.	0	0	0	8	8	0	0
Mirati Therapeutics, Ltd.	28	39	274	596	337	75	389
Nangene Biomedical Co., Ltd.	0	0	0	0	164	39	78
Shandong Xuanzhu Pharma Co., Ltd.	0	0	0	0	14	0	14
Shanghia De Novo Pharmatech Co., Ltd.	0	0	0	0	3	0	3
Shanghai Zelgen Pharma. Tech Co., Ltd.; Suzhou Zelgen Biopharmaceuticals Co., Ltd.	0	0	0	0	13	1	13
Sichuan Haisco Pharmaceutical Co., Ltd.	6	0	4	28	4	6	28
Turning Point Therapeutics, Inc.	1	1	2	2	2	1	2
WIGEN Biomedicine Technology (Shanghai) Co., Ltd.	0	0	0	0	0	12	14

Opportunities, future growth and applications

A critical step in development is the use of X-ray crystallography with compounds after crystal soaking or co-crystallization to identify where molecules bind to the target protein.[18] As more of these binding pockets are being identified on RAS proteins, it is hopeful that more small molecule therapeutics may be developed.

As previously seen with current RAS inhibitors under development, gradual improvement in chemical structures can optimize binding and improve the drug effect and reduce toxicity.[7,16-20] By understanding the structure activity relationship (SAR) data available from this analysis, further advancements in RAS mutated cancer drug development can be achieved.

Opportunities include expanding the type of amino acids that can be targeted by covalent inhibitors such as G12D and G12V; varying approaches are needed from the current G12C inhibitors due to the lack of reactive cysteine within the proteins.[2,21] G12D is an important chemotherapy drug target, as a commonly observed mutation in colorectal and pancreatic cancers.[4] For the development of inhibitors against KRAS-G12D, ionic interactions can be used to bind at KRAS G12D via amine hydrogen-bond donors, provided the rest of the structure has suitable interactions within the binding pocket. One example includes the use of piperazine-substituted pyrido[3,4-d]pyrimidine derivatives, analogous to the covalent G12C inhibitors, that have recently been developed exploiting aspartate-amine interactions.

Optimization of current G12C inhibitor *warheads* can enhance ligand binding mode and labeling rate to KRAS. Recent work has also shown that aziridines and stabilized diazo electrophilic functional groups (warheads) preferentially react with carboxylates over thiols in the binding pocket.[21]

Cyclic peptides have also been investigated for binding in the Switch II groove of KRAS. KD2 was identified using a high-throughput selection platform and showed preferential binding to the GTP-bound state of KRAS-G12D (blocking RAS-RAF interactions), via ionic bonding between threonine of KD2 and mutant aspartate on KRAS.[22]

The use of biological inhibitors of RAS oncoproteins are also under investigation. Small-interfering RNAs (siRNAs) have shown promising therapeutic potential, but limitations include their short serum and intra-cellular half-life. This approach is currently being employed in an ongoing Phase II clinical trial of siRNA in combination with chemotherapy in patients with locally advanced pancreatic cancer. Other opportunities include the use of adoptive cellular therapy, RAS-specific vaccines and combination strategies.[2]

Conclusion

We have used the CAS Content Collection to analyze the literature and landscape of direct RAS inhibitors, manually assessing and extracting 26,598 active structures for analysis, including classification according to identified scaffolds, identification of common chemical moieties, and insights into the core scaffolds utilized by different organizations.

There are several opportunities for future growth and development in the RAS inhibitor space. By gaining a deeper understanding of the leading structures available, more therapeutics with modified binding affinity to RAS isoforms and mutant proteins can be developed for application in cancer therapy.

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