The renaissance of Ras

Milroy, L.G.; Ottmann, C.

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The Renaissance of Ras
Lech-Gustav Milroy and Christian Ottmann*

Laboratory of Chemical Biology and Institute of Complex Molecular Systems, Department of Biomedical Engineering, Technische Universiteit Eindhoven, Den Dolech 2, 5612 AZ Eindhoven, The Netherlands

ABSTRACT: Increased signaling by the small G protein Ras is found in many human cancers and is often caused by direct mutation of this protein. Hence, small-molecule attenuation of pathological Ras activity is of utmost interest in oncology. However, despite nearly three decades of intense drug discovery efforts, no clinically viable option for Ras inhibition has been developed. Very recently, reports of a number of new approaches of addressing Ras activity have led to the revival of this molecular target with the prospect of finally fulfilling the therapy promises associated with this important protein.

An important breakthrough in the Ras research field occurred last year with the publication of two papers in Nature reporting two new classes of Ras inhibitor compounds operating via distinct mode-of-actions. These discoveries are at the forefront of a Ras renaissance and serve as a clarion call for renewed efforts in Ras drug discovery bringing with them other exciting and contemporary developments in the field. It is pertinent to reflect on the timing of these papers, which begs the question: which technological developments have enabled these events to take place now, and therefore, what can be learned for future drug discovery efforts? Three reasons: first, these discoveries are the product of truly collaborative research efforts, combining techniques as diverse as high-throughput screening and X-ray crystallography through to chemical synthesis, medicinal chemistry, biochemistry, and cellular pharmacology; second, X-ray crystallography in particular has played a pivotal role in mapping the molecular architecture of Ras and its interaction with bespoke modulator compounds. Indeed one of the outstanding aspects of the two Nature contributions is the sheer quality and quantity of the structural data available (21 co-crystal structures were disclosed in these two articles alone!), which makes for irrefutable evidence of their respective modes-of-binding. Third, the judicious choice of screening strategies has been essential for “unpicking” previously unseen drug pockets at the dynamic Ras protein or increasing the success rate of screening large and structurally diverse compound libraries. For this, techniques such as MS and NMR spectroscopy will play an increasingly important role by providing complementary feedback on the protein’s native dynamic state (in contrast to “snap-shots” provided by X-ray crystallography, for example). Two excellent reviews have recently been published on small molecule modulation of Ras signaling. The purpose of this mini-review, therefore, is to put the Ras drug discovery field into fresh perspective, especially in light of other exciting developments, which sit alongside the two outstanding Nature contributions. For this, priority is given to examples that provide a clear structural view of small molecule binding by X-ray co-crystallography.

RAS AND RAS SIGNALING
Ras is a small GTPase switch, which regulates signaling pathways critical for the growth and differentiation of cells such as the RTK-Sos-Ras-Raf-MEK-ERK pathway (Scheme 1). The binding of growth factors to membrane-bound receptor tyrosine kinases (RTKs) induces phosphorylation of tyrosine residues which signals for recruitment of Ras-specific guanine nucleotide exchange factors (GEF) (e.g., Sos (Son of sevenless)). Sos-binding promotes the switch from an “inactive” to an “active” state through exchange of GDP for GTP, respectively. Once in the “active” state, Ras relays its signal through binding to effector proteins such as Raf. GTPase activating proteins (GAP) bind to Ras and catalyze the hydrolysis of Ras-bound GTP, thus switching Ras back to the GDP-bound “inactive” state. Four Ras isoforms of the Ras subfamily are known—HRas, NRas, KRas4A, and KRas4B—each of which contribute to oncogenic signaling. Ras mutations are one of the principle hallmarks of cancer, with Pan-Ras mutations occurring in 16% of human cancers according to a recent metric, and are most abundant in pancreatic and skin cancers, as well as cancers of the large intestine and biliary tract. Kras is the most recurrently mutated isoform of the Ras subfamily, with an incidence rate of >20%, and KRas(G12C) one of the most common KRas mutants, especially for lung adenocarcinomas. The Cys12 mutation renders Ras insensitive to GAP catalyzed GTP hydrolysis, thereby locking Ras in the GTP-bound “active” state. The
G12C mutation is highly significant in the view of recent developments in the Ras drug discovery field, as the proximity of the Cys mutation to the nucleotide binding site can be exploited for the development of oncogene-specific drug treatment through selective covalent modification of the cysteine’s sulfhydryl side chain \((\text{vide infra})\).

Four strategies for directly targeting Ras have been cited:\(^4\) namely, prevention of Ras-GTP formation (Strategy 1); inhibition of Ras-effector interactions (Strategy 2); impairment of Ras localization (Strategy 3); and GTPase activation (Strategy 4). This mini-review will focus exclusively on advances in the field since the beginning of 2012, which have primarily focused on Strategies 1–3. For the sake of consistency, we classify each compound class according to the same headings used in ref 4.

**PREVENTION OF RAS–GTP FORMATION**

Direct targeting of Ras with small molecules is extremely challenging given the picomolar binding affinities of GDP and GTP to Ras, and the lack of evident druggable pockets on the flat Ras protein. That said, a number of studies published in 2012 have clearly demonstrated the potential of the approach, specifically targeting Ras-binding to GTPase exchange factors (GEFs) such as Sos.\(^9,10\) In each of the examples below, high-throughput fragment screening was used to identify hit compounds and X-ray co-crystallographic studies of Ras to provide a clear atomistic-scale picture of the binding mode of each compound class. (Table 1 summarizes the co-crystal structures reported by studies referenced in this mini-review.)

Fang and Wang\(^9\) and Fesik\(^10\) have identified different structural classes of orthosteric inhibitors of Sos-catalyzed KRas activation, which nevertheless target the same binding site on the KRas protein—namely, a site located between the switch I and switch II regions. Fang and Wang used an NMR-based saturation transfer difference (STD) assay to screen a 3300-member compound library for binding to the recombinant Kras4B-G12D, KRas<sub>GM</sub> bound to GDP or GMPPCP (guanosine-5’[(βγ)-methylene]triphosphate).\(^9\) From there on, 240 primary hits were identified (7.2% hit rate), of which 25 were validated by 2D 1H-15N HSQC studies using 15N-labeled KRas<sub>GM</sub> protein (0.8% hit rate). Co-crystals of three of the validated fragment hits were obtained by soaking the fragments into KRas<sub>GM</sub> crystals: namely benzamidine, BZDN, benzimidazole, BZIM, and 4,6-dichloro-2-methyl-3-aminoethyl-indole, DCAI (Scheme 2). In the GDP-bound KRas apo structure, the hydroxyl functional group of residue Tyr71 is aromatic ring, which mimics the aromatic side chain of Tyr71. The structurally similar fragments, BZDN and BZIM, both adopt a similar Ras-binding mode, whereas the larger DCAI fragment causes an expansion of the opening to the hydrophobic binding pocket from 7 × 7 Å to 7 × 10 Å. More specifically, a reorientation of for example the side chain of Asp54 is needed to accommodate the 4-chloro group of DCAI (Scheme 2).

Fesik and colleagues used a NMR-spectroscopy-based technique to screen 11 000 fragments for binding to the GDP-bound KRas (G12D) mutant, resulting in 140 fragment hits with a success rate of 1.3%.\(^10\) Cmpd 1 is representative of these hit fragments (typical binding affinities to KRas (G12D) were ca. 1.3–2 mM) and was selected as a scaffold structure for further structure—activity relationship studies. The close structural analog, cmpd 4, with improved affinity and water solubility, was used for X-ray co-crystallography studies (Scheme 2). The indole moiety of 4 occupies the same hydrophobic binding pocket targeted by BZDN and DCAI. The indole NH of cmpd 4 forms a hydrogen bond with residue Asp54 similar to BZDN and BZIM but in contrast to DCAI, whose indole ring reorientates itself 65° relative to BZIM, resulting in alternative rotamer conformations of Asp54 and Arg41, which preserve a stabilizing salt bridge. The more extended structure of cmpd 4 enables additional interactions proximal to the hydrophobic pocket compared to BZDN, BZIM, and DCAI (Scheme 2). For example, the nitrogen atom at the 1-position of the imidazopyridine moiety of cmpd 4 interacts with side chain group of Ser39 via a bridging water molecule. The indole NH of cmpd 5 makes a direct hydrogen bond interaction with Ser39. The phenolic OH of cmpd 2 also makes hydrogen bonding interactions, this time with Asp54, alongside hydrophobic stacking of the pyrrolidine moiety of cmpd 2 against the aromatic side chain of Tyr71. Interestingly, the three nitrogen atoms of the 2,6-diaminopyridine moiety of cmpd 6 all engage in water-mediated hydrogen bonding with residues Ser39, Arg41, and Asp54. The Ras–Sos inhibitory activity of these compounds was demonstrated by fluorescence-based Sos-catalyzed nucleotide exchange assay. DCAI inhibited both nucleotide exchange (IC<sub>50</sub> = 342 μM) and release reactions (IC<sub>50</sub> = 155 μM) in fluorescence-based assays.

**Scheme 1. Summary of the RTK-Sos-Ras-Raf-MEK-ERK Pathway**

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*Reviewed by Fang and Wang*
Table 1. Summary of All Ras and PDEδ X-ray Co-Crystal Structures Reported by the Studies Highlighted in This Mini-Review

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<th>Ligand name</th>
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<th>Protein</th>
<th>Nucleotide</th>
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Table 1. continued

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Whereas cmpd 4 did not inhibit Sos-catalyzed nucleotide exchange, the more extended structures, as exemplified by cmpds 12 and 13 (Scheme 2), bound more strongly to GDP-KRas and were able to inhibit nucleotide exchange. The inhibitory activity of this compound class could be explained therefore in one of two ways: either due to steric blockade of Sos-binding to Ras, or via reorientation of side chains, which destabilizes the Ras–Sos interaction. For DCAI, the 2-methyl and 3-aminoethyl side chains would block Sos binding. Similarly, the more extended structure of cmpd 13 would also block Sos binding, aided by an additional hydrogen bond interaction with the Asp38 residue (Scheme 2). In summary, successful ligand binding at the transient Ras pocket characterized by Fang and Wang and Fesik—between the switch I and switch II regions—requires mimicry of the hydrophobic aromatic side chain of Tyr71 and disruption of the network of hydrogen-bond interactions that normally exist in the “closed” state between the phenolic OH group of Tyr71 and the polar side chains of residues Asp 38, Ser39, Arg41, and Asp54. Efficient inhibition of Sos-mediated activation depends on steric blockade of Sos binding to Ras or the formation of an inactive conformation of the Ras protein that disfavors Sos binding.

The well-characterized molecules of Wang and Fang, and Fesik, demonstrate the potential of directly targeting Ras with small molecules. The question remains, however, how to translate this early potential into higher affinity lead compounds for further drug development. Opening up and occupying more of the plastic hydrophobic pocket (e.g., BZDN → DCAI) or molecularly addressing more of the protein surface (e.g., cmpd 4 → cmpd 13) are both possibilities (see also ref 12). An alternative, complementary way to address more of the protein surface is to use short modified peptides with stabilized secondary structures, which mimic either of the two interacting
proteins. For example, orthosteric inhibitors of Ras-Sos and Rab-effector protein−protein interactions have recently been reported. On the subject of Ras-specificity, the compounds represented by cmpds 4 and 13 were reported to bind to KRas wt, mutants KRas (G12D) and KRas (G12 V), and HRas, and thus not to bind Ras in an isoform or mutant-specific manner (by analogy, the same could also be surmised for BZDN, BZIM, and DCAI). However, given that a clear mechanistic link between ligand binding and Sos-catalyzed activation has yet to be established, it is not clear yet whether these compounds can be engineered to inhibit Sos-mediated activation (and therefore Ras) in an isoform- and/or mutant-specific manner.
INHIBITION OF RAS–EFFEC TOR INTERACTIONS

An elegant solution to the selectivity quandary sketched above has been reported by Shokat and Wells in their work on irreversible KRas(G12C) mutant-specific inhibitors. In contrast to other screening strategies discussed elsewhere in this minireview, they used tethering—a disulfide-fragment-based screening approach based on the disulfide exchange reaction—combined with protein mass spectrometry (MS). Disulfide exchange is a widely studied reaction for dynamic combinatorial chemistry and drug discovery.15 Ligand binding to the KRas(G12C) mutant protein favors disulfide exchange with the Cys12 residue, which tethers the ligand to the protein surface. The stronger the binding, the more the protein becomes modified as detected by MS. The advantage of this combined fragment tethering/MS approach is the high sensitivity and detection accuracy of the MS instrument, thus necessitating only minute quantities of protein, combined with the speed and high-throughput nature of the technique. In this case, a library of 480 structurally diverse disulfide fragments were screened against GDP-bound KRas(G12C). From there, two fragments—6H05 and 2E07 (Scheme 3)—were found to modify the GDP-bound KRas(G12C) protein most. X-ray co-crystal data showed that both compounds underwent disulfide exchange with the sulfhydryl side-chain of residue Cys12 as well as binding to a pocket deep within the switch-II region of Ras (S-IIP), between the central β-sheet and the α2- and α3-helices—a pocket not evident in previously published Ras structures. Notably, the binding of 6H05 and 2E07 to S-IIP results in a reordering of the switch-II region, while the conformation of switch I and the co-ordination of the metal ion remain effectively unchanged (Scheme 3).

The activity of disulfide fragments 6H05 and 2E07 clearly demonstrates the utility of the tethering approach for Ras inhibition. However, disulfides are unstable in the reducing environment of the cell cytoplasm (where oncogenic Ras signaling takes place), and therefore ill-suited as drug compounds. By way of improvement, reversible disulfide exchange was replaced by 1,4-Michael addition as tethering strategy of choice using carbon-centered electrophiles such as vinyl sulfonamides and acrylamides, which both react with the sulfhydryl side-chain to form an irreversible carbon–sulfur bond (Scheme 3). A total of 100 analogs were screened for irreversible modification of the GDP-bound KRas(G12C) protein, and vinyl sulfonamide 8 and acrylamide 12 were identified as two of the most active analogs of the series. X-ray crystallography studies showed that both compounds covalently modify the Cys12 residue of Ras and bind to the same S-IIP pocket with a similar trajectory as the disulfide fragments 6H05 and 2E07. Most notably, the binding of 8 caused a more significant displacement of switch-II than the disulfide fragments, which translated into increased disorder in the switch-I region, with an additional loss in electron density typically assigned to the magnesium ion. The functional consequence of ligand binding is most intriguing in this case. For example, vinyl sulfonamide 8 caused a decrease in GTP binding affinity, resulting in a switch in binding preference for GDP and thus an accumulation of Ras in the inactive Ras-GDP state, as judged by a fluorescence-based competition assay. Furthermore, simultaneous inhibition of Ras-binding to exchange-factor proteins was also observed according to a fluorescence-based Sos-catalyzed nucleotide exchange, in addition to disruption of effector proteins such as C-Raf and B-Raf by coimmunoprecipitation, most likely, the result of increased ligand-induced disorder in the switch-I region as observed by X-ray crystallography. At the cellular level, acrylamide 12 caused a concentration-dependent decrease in cell viability with an associated increase in apoptosis induction, which was only observed for Ras-dependent lung cancer cells annotated with the Ras(G12C) mutation, and not for Ras-independent and (most importantly) Ras-dependent cell lines lacking the G12C mutation. Tellingly, the close structural analog 10 was significantly less active in the same cellular assay, while 17 was inactive, thus mirroring their structure—activity relationship in vitro. The ability to robustly and comprehensively disrupt Ras signaling, combined with their oncogene-specific behavior, suggests that irreversible inhibitors targeting the S-IIP will serve as extremely useful lead compounds for treating Ras-dependent tumors expressing the KRas(G12C) mutant. It remains to be seen whether the tethering strategy can be extended to include other active lesions, such as G12D and G12V.

Gray and co-workers have also developed a KRas inhibitor designed to specifically target the KRas G12C oncogene. In contrast to irreversible inhibitors 6 and 8, this inhibitor class—represented by SML-8-73-1 (SML)—targets the guanine nucleotide (GN) binding site and is descended from GDP (Scheme 4). SML was shown to selectively label KRas(G12C)
based on ESI-MS analysis. Incubation of the KRas(G12C) protein with SML for 2 h in phosphate buffer at a molar protein/ligand ratio of 1:10 resulted in formation of a single covalent adduct, whereas no labeling was observed for the wtKRas at a molar protein/ligand ratio of 1:50 and ligand concentration of 2.5 mM. A "caged" variant of SML was synthesized to improve cell permeability, alanine ester phosphoramidate SML-10-70-1, and was effective at inhibiting the binding of desthiobiotin-GTP to KRas(G12C) in H358 cells incubated with SML-10-70-1 for 6 h at a concentration of 100 μM. SML-10-70-1 exhibited a similar antiproliferative effect on KRas-dependent tumor cells annotated with the KRas(G12C) lesion (H23 and H358) as well as KRas independent tumor cells (A549). Nevertheless, subsequent X-ray co-crystal analysis of SML bound to KRas(G12C) confirmed that SML indeed covalently modifies the Cys12, with the sulfhydryl side chain adopting two conformations in the GN binding site (Scheme 4).22 The co-crystal structure also supports conclusions from earlier hydrogen exchange mass spectrometry (HX-MS) studies that the binding of SML locks the protein in an open "inactive" conformation with the effect of inhibiting KRas binding to effector proteins such as Raf. Important for the future development of this compound class was the evidence that SML selectively targets KRas(G12C) over other GTP-binding proteins, according to chemical proteomics profiling performed in MIA PaCa-2 cell lysates.22

### STABILIZATION OF ACTIVE AND INACTIVE RAS–PROTEIN COMPLEXES

All of the examples discussed so far involve small molecule inhibition of Ras binding to either effector or exchange factor proteins (or both in the case of irreversible inhibitors targeting S-IIP). Small molecule stabilization of Ras–protein complexes is a highly promising alternative strategy for disrupting Ras signaling.23 With this concept in mind, Fesik and colleagues have recently characterized small molecule stabilizers of the Ras/Sos/Ras ternary complex, resulting in activation of Ras signaling.12 In this case, the molecules bind to a hydrophobic pocket formed exclusively by Sos but which is conveniently located adjacent to the Switch-II region of Ras. Although the downstream effects of this PPI stabilization have still to be elucidated, these molecules nevertheless offer a unique opportunity to develop Ras-Sos modulator compounds that simultaneously address both proteins. The combined high-throughput fragment screening—X-ray co-crystallography strategy used by many of the research groups, arguably more attuned to the dynamic conformational behavior of Ras than structure-based design or in silico approaches—has enabled the identification of Ras inhibitors, which would otherwise have been impossible to detect. It is expected, therefore, that an improved understanding of the different conformational states of Ras will in the future lead to entirely new classes of Ras modulator compounds with different modes of action. To this end, Kalbitzer and co-workers have used high-pressure NMR spectroscopy to investigate the different conformational states of Ras,24 and 31P NMR spectroscopy to identify metal–ligand complexes that either stabilize25,26 or inhibit27 one conformational state of Ras—State 1(T).

### IMPAIRMENT OF RAS LOCALIZATION

Though clearly promising, the binding affinities of the current generation of inhibitors directly targeting Ras are too low for further drug development. This situation is representative of a common "bottleneck", not only in the Ras drug discovery field but also in PPI drug discovery as a whole. For Ras, the bottom
line remains though that despite intensive research efforts over the past three decades, there are still no drugs in the clinic that function through interference of Ras function. There is thus considerable interest in developing alternative approaches to disrupting oncogenic Ras signaling. A complementary, “Systems Biology” approach to solving this Ras conundrum (compared to the traditional “MedChem” approach delineated above) has been reported by Waldmann, Bastiaens, and Wittinghoer at the Max Planck Institute in Dortmund (Germany), which uses small molecules to disrupt the subcellular localization of oncogenic KRas. Stable binding of Ras to the plasma membrane depends on farnesylation and carboxymethylation at the C-terminal cysteine residue as well as an electrostatic interaction between a basic polylysine C-terminal sequence and the negatively charged inner leaflet of the plasma membrane. Binding of Ras to the prenyl binding protein PDEδ maintains proper cellular distribution of Ras, which is critical for correct Ras signaling. Disruption of Ras signaling by small molecule inhibition of farnesyltransferase activity is restored by geranylgeranylation of Ras for some Ras isoforms, while dual prenyltransferase inhibitors have exhibited intolerable toxicity in mice. Disruption of the degradomitoxygen machinery via small molecule inhibition of deplemitotylating thiosterases APT-1 and APT-2 may show promise as a basis for treating tumors driven by aberrant HRas or NRas signaling. For the KRas isoform, low-nanomolar affinity inhibitors of the KRas–PDEδ interaction (Scheme 5) have been developed, which disrupt KRas signaling and inhibit the proliferation of tumor cells dependent on PDEδ binding for membrane localization in vitro and in vivo—in this case, human pancreatic ductal adenocarcinoma cells. It is worth highlighting that by targeting the deep prenyl-binding pocket of PDEδ, this small molecule approach conveniently overcomes the aforementioned affinity “bottleneck” currently observed for alternative molecular approaches, which directly target the comparatively “flat” Ras protein surface.

Initially, a high-throughput (HTS) Alpha Screen composed of a biotinylated and farnesylated KRAS4B peptide and His-tagged PDEδ was performed. This screen identified a series of benzimidazole-derived inhibitor compounds (e.g., 1, Scheme 5), which could be further characterized using a host of biochemical techniques, including in a competitive fluorescence depolarization assay ($K_\text{D} = 166 \text{ nM for 1}$). The co-crystal structure of 1 bound to PDEδ was solved at 1.87 Å resolution (Scheme 5), and was an important early breakthrough for the project. Intriguingly, two molecules of 1 were found to bind to the PDEδ protein simultaneously: one bound deep into the hydrophobic tunnel in PDEδ typically occupied by the farnesyl group (as evidenced by overlay with the co-crystal structure of PDEδ in complex with farnesylated-RHEB), the second occupying a more solvent-exposed site proximal to the first. Though the two molecules do not make direct contact at the PDEδ–KRAS4B interface, their proximity to one another suggested that a permanent covalent linkage suggested would lead to higher affinity inhibitor molecules, and so, it proved that analog (S)-4, also referred to as Deltarasin, and (Rac)-5 were 4- and 17-fold more active than 1 in the same FP assay and adopted a similar binding mode to PDEδ as 1 (Scheme 5). This example once again highlights the benefit of using high-resolution X-ray co-crystal data early on in the design process in order to develop higher affinity molecules.

**COMMENT ON CLINICAL SIGNIFICANCE**

The therapeutic potential of targeting Ras with small molecules has recently been discussed. The direct targeting of Ras (G12C) with irreversible inhibitors (e.g., 6 and 8), which react selectively with the Cys12 residue, achieves oncogene-selective Ras inhibition. Certainly one of the most distinguishing features of this compound class is the ability to inhibit effector protein binding as well as disrupt GEF-catalyzed nucleotide exchange through a change in the GTP/GDP binding preference of Ras. The cellular efficacy of this compound class requires further investigation. Other compound classes are capable of inhibiting GEF-catalyzed nucleotide exchange reaction on Ras. However, inhibition of for example Ras–Sos currently lacks isoform selectivity and inhibition of Ras–GEF alone does not make good mechanistic sense for tumors driven by constitutively active Ras lesions such as Ras(G12V) and Ras(G12C). Furthermore, the benefit of inhibiting the Ras–GEF interactions as a basis for treating other tumor lines expressing aberrant RTK signaling remains unclear. The benzimidazole Ras–PDEδ inhibitor class has reached an affinity regime in vitro which might be considered ideal for further development of drug inhibitors of Ras–PDEδ, and Deltarasin has demonstrated a clear dose-dependent effect on tumor size and volume distribution in xenograft mice (Panc-Tu-1) dependent on Ras–PDEδ binding for oncogenic Ras signaling. That said, questions still need to be answered surrounding the in vivo selectivity and efficacy of this compound class, including their effects on the subcellular localization of other farnesylated GTPases other than Ras dependent on PDEδ-binding. Therefore, the development of a structurally diverse range of highly potent Ras–PDEδ inhibitors will help to decouple PDEδ-dependent from off-target effects in vivo. Looking to the future, therefore, the two approaches published in *Nature* can be considered complementary to one another and show the most potential to be developed into drug compounds in the future.

**AUTHOR INFORMATION**

**Corresponding Author**

*Email: c.ottmann@tue.nl.*

**Notes**

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**KEYWORDS**

**Ras:** A small GTPase switch which regulates signaling pathways critical for the growth and differentiation of cells.

**KRas:** One of the three main Ras isoforms besides HRas and NRas involved in oncogenic signaling.

**GAP:** GTPase activating protein which catalyzes the hydrolysis of Ras-bound GTP, thereby switching the Ras protein from an active to an inactive state.

**GEF:** Guanine nucleotide exchange factor which catalyzes the exchange of GDP for GTP, thereby promoting inactivation of the Ras protein.

**Sos:** Abbreviation for somatic mutation of sevenless, a guanine nucleotide exchange factor (GEF).

**PPI inhibitor:** a chemical entity, typically a low molecular weight synthetic ligand or peptide engineered to disrupt the binding of two or more proteins.

**PPI stabilizer:** a chemical entity engineered to enhance the binding affinity of two or more proteins.

**MedChem approach:** organic synthesis meets pharmacology meets X-ray crystallography to rationally develop small molecules.
molecule modulators of protein function as the basis for new drug therapies.

**Systems Biology approach**: goes further than the MedChem approach by taking into account the global impact of a chemical entity on the complex interactions within a biological system.

### REFERENCES


